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A STUDY OF PHOSPHATE INDUCED ALGAL GROWTH IN ORDER TO SUPPRESS OR ELIMINATE THIS PHENOMENON

Technical Completion Report
Project No. A-035-NMEX

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NEW MEXICO WATER RESOURCES RESEARCH INSTITUTE

Las Cruces, New Mexico 88001

TECHNICAL COMPLETION REPORT

Project No. A-035-NMEX

A STUDY OF PHOSPHATE INDUCED ALGAL GROWTH IN ORDER TO SUPPRESS OR ELIMINATE THIS PHENOMENON

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ABSTRACT

The kinetics of the radioactive isotope P^{32} uptake by the fresh water algae, chlorella, have been investigated in soil-water cultures, natural systems, and synthetic culture media. These laboratory investigations were done at three different pH values, 4, 7 and 10, and maintained at constant temperature of 20 ± 2°C.

Portions of reagent grade sodium pyrophosphate were irradiated with a fast neutron flux converting a portion of the sample into the radioisotope P^{32}. (P^{32} emits a 1.7 MeV beta with a half-life of 14.31 days.) Tagging was done at a rate sufficient to produce an activity of 0.03 microcuries/gram. Solutions were then prepared containing the tagged pyrophosphate. These phosphate solutions were used to spike chlorella cultures. The uptake of the isotope was then monitored as a function of time. After various times the cultures were samples, filtered and counted for P^{32} activity.

The data show that the kinetic interpretation of P^{32} uptake must allow for first order kinetics with respect to P^{32} at a pH of 10, and a second order kinetic scheme at pH 7 and 4. This evidence indicates that algal systems only utilize phosphorus in the orthophosphate form. These data also give phosphorus exchange rates with soil and give additional information about degradation pathways.

Two experiments, one with an oxygen rich environment and the second with a carbon dioxide rich environment, were carried out with sodium pyrophosphate-chlorella, soil-water and chemical cultures. In the first case, the oxygen experiment, P^{32} removal was eliminated in the chemical cultures; removal was slowed for the soil-water cultures. CO_2 enrichment greatly accelerated the removal.

INTRODUCTION

Algal growth constitues one of the more visible types of pollution in surface waters. Excessive algal populations have triggered much interest and concern since these formations (algal blooms) cause rapid degradation of surface water quality and markedly hinder this resource for both agricultural and recreational uses (1). Algal growth is known to be stimulated by excessive nutrient concentrations and by excessive sunlight. The Rio Grande Valley of New Mexico qualifies in both these categories (2). Thus, it is in both regional and national interest to explore those factors which influence algal growth.

Much controversy exists over the affects of phosphates upon an algal growth (3). Convincing evidence exists that would indicate that phosphorus is not the rate limiting step in algal growth. For instance, algal blooms have been found in waters where only trace amounts of phosphorus can be utilized while some other waters, even though they contain excessive phosphorus concentrations (Elephant Butte Reservoir is in this category (2)),tend to resist algal degradation. Then, the weight of phosphorus incorporated into growing algal systems is only a small fraction of the weight of carbon and nitrogen in the living plant (4). This also would lead to the inference that phosphorus is not the limiting factor. On the other hand, some investigators have shown that algal growth is limited by phosphate concentration and certainly this limit must exist since phosphates play a distinct role in the growth process. Currently,several cities are removing phosphates chemically from their sewerage effluents (5). These removal steps are high in cost. (Estimated cost for a city the size of Albuquerque (population 300,000) is $750/day). Relatively little quantitative information exists about the degradation mechanisms occurring when phosphates are consumed by the environment.

This problem has been magnified in recent years by the incorporation of phosphate esters as builders in detergent formulations. Annually, millions of tons of phosphorus are discharged into municipal sewerage plants. The detergent formulations use phosphate esters, pyrophosphate, tripolyphosphate, etc., instead of orthophosphate since the sodium salt orthophosphate, Na_3PO_4, reacts with water to form hazardously basic solutions. The hydrolysis of condensed phosphates goes with known energetics; the process is, under conditions which approach those found in the environment, extremely slow (6,7,8). However, catalysts exist which include H^+ and a variety of inorganic and organic entities. Under normal conditions for use, pH 9-11, little of the condensed phosphate is hydrolyzed to orthophosphate and the effluent discharge then contains a mixture of ortho and mainly the higher phosphate species. The acidities of the phosphate species have been well characterized so that it is relatively simple, given the pH, to deduce the exact concentration of the solution under study (3).

There are several possible degradation pathways for the discharged phosphate. They may precipitate as inorganic phosphates, $Ca_3(PO_4)_2$, etc., and be effectively removed. This is a reasonably improbable event since there is no reason to expect the availability of sufficient metallic ions. Then, they can be incorporated into living suspended systems, such as algae, where they can then enter the food chain. Alternatively, they can be used as fertilizers and also be consumed in a growth process. Exchange rates between inorganic and organic phosphates have been measured (9-17), and several reports have appeared describing the incorporation of phosphorus into naturally occurring systems (18,19). These previous studies have been instrumental in showing that phosphorus is consumed from an aqueous environment and is utilized by the growth process in plants. Each showed a regular decrease in the phosphorus concentration with time. No systematic model evolved from these works, however.

The present study grew out of a realization that really little was known of the mechanistic pathways which consume phosphorus from aqueous solution. It was decided to monitor the rate of phosphorus consumption from laboratory simulations by adding known amounts of phosphorus that had been spiked with a tagging fraction of the radioactive isotope of phosphorus, P^{32}. In this way, the removal of phosphorus could be readily monitored and the removal rate could be followed as the pH, temperature and nutrient concentrations were varied.

Specifically, answers to the following questions were sought:

1. What factors control the rate of phosphorus uptake in biological degradation?
2. How effective are algal systems in converting phosphate polymers into monomeric units, pyro to ortho phosphate?
3. What is the phosphate balance, i.e., how much is consumed by algal systems and how much is left on walls, soil and in solution?

EXPERIMENTAL

P^{32} Removal Studies: Studies were made of a typical fresh water algae, chlorella, to explore those factors which influence the uptake of phosphorus. These were performed by adding controlled amounts of tagged (radioactive) phosphorus to carefully controlled laboratory cultures and then observing the utilization of this phosphorus nutrient.

1. Preparation of the Irradiated Material: Samples of reagent grade sodium pyrophosphate decahydrate and sodium dihydrogen phosphate, Fisher Scientific Company, Certified, were activated by neutron irradiation. A 2 to 4 gram quantity was placed in the neutron flux in the annular core reactor operated by Sandia Laboratories, Albuquerque. Initially, these samples were irradiated in polyethylene vials but these were found unsuitable due to rather high surface contamination. Later irradiations were done in sealed quartz ampoules. (Since the P^{32} isotope has a relatively short half-life, about two weeks, irradiations were done on a monthly schedule.) The irradiation also produced radioactive sodium; fortunately this isotope has a half-life on the order of a few hours and the material could be handled outside of a glove box area one day after irradiation. A thermal neutron flux of approximately 10^{14} neutrons/second was utilized to convert a fraction of the naturally occurring phosphorus isotopes into P^{32} (20). P^{32} emits a beta ray with an energy of 1.71 MeV and a half-life of 14.31 days. Tagging yields produced activity approximating 0.03 microcuries per gram, quite adequate for the proposed experiments but not too hazardous to handle in an open laboratory situation. These isotopes were obtained with the cooperation of Dr. Bernard Kenna, Sandia Laboratories and were monitored by the office of the Radiological Safety Officer, Wilbur Tabor, at the University of New Mexico. The cooperation and assistance of these individuals was instrumental in these studies.

2. Preparation of Culture Media: Two different types of media were prepared. Initial experiments were done in the presence of soil and the later ones were done entirely with aqueous media.

   a. Soil-water Cultures: A sample of garden soil was collected from the Albuquerque area. Ten grams of calcium carbonate were placed in a 500-ml Erlenmeyer flask. 100 ml of soil was then added and then 400 ml of distilled water was carefully poured on the soil. The requisite amount of tagged phosphorus solution was then added along with a small amount of algae. All experiments used algae from the same culture; no attempt was made to keep this system bacteria free nor was the soil sterilized before use (21). These solutions were then adjusted to the desired pH by the addition of either sodium hydroxide or nitric acid, both approximately 0.1 M. Monitoring of pH by insertion of a Fisher Microprobe Combination Electronic System into the culture showed a constant pH (± 0.2 pH) throughout an experimental run.

   b. Chemical Cultures: Chemical culturing media was prepared using the following formula:

Compound	grams/liter
$Ca(NO_3)_2$	0.040
$MgSO_4 \cdot 7H_2O$	0.025
Na_2CO_3	0.020
Na_2SiO_3	0.025
Ferric ammonium citrate	0.003
Citric acid	0.003

This is similar to a media reported by Chu (Chu No. 10) (22). with the substitution of ferric ammonium citrate and the deletion of potassium phosphate. (The only phosphorus available to the algal system was that added in tagged amounts.) 400 ml of this culturing

media (termed here "chemical media") was poured into a 500-ml Erlenmeyer flask, the requisite amount of tagged phosphorus solution was added, the pH adjusted, and finally a seed amount of algae was added. Here again, monitoring of pH showed little or no change during any experiment.

3. Phosphorus Removal Experiments: The solutions prepared were then transferred into a large, open water bath maintained at constant temperature, 20 ≠ 2°C; the flasks were submerged to about one inch from the neck. Into each was inserted a gas probe connected to a gas manifold. All gases were first led through a water scrubbing apparatus to purify them and assure that they are saturated and finally passed through a particle filter. The gases, air, CO_2 and O_2, were then led into the gas manifold and then into each culturing flask. Each culturing media was illuminated with a long wave UV lamp. These conditions of constant temperature, gas flow and radiant flux were maintained for sufficient time to determine the fate of the tagged phosphorus.

   After a requisite amount of time had passed, a sample was removed (pipet) from the experiment under study; this was filtered and both the filter paper and the filtrate were counted for P^{32} activity, providing a material balance.

4. Counting and Data Analysis: A scintillation counter was used for all the analytical measurements. All samples were counted to a total of at least 5,000 c/min. Since this well type of counter is not the usual type of detector for beta radiation, initial experiments with each phosphate sample included an estimation of the decay rate. These data are shown in Figure I which shows that the decay measured went with the same half-life as previously reported for P^{32}.

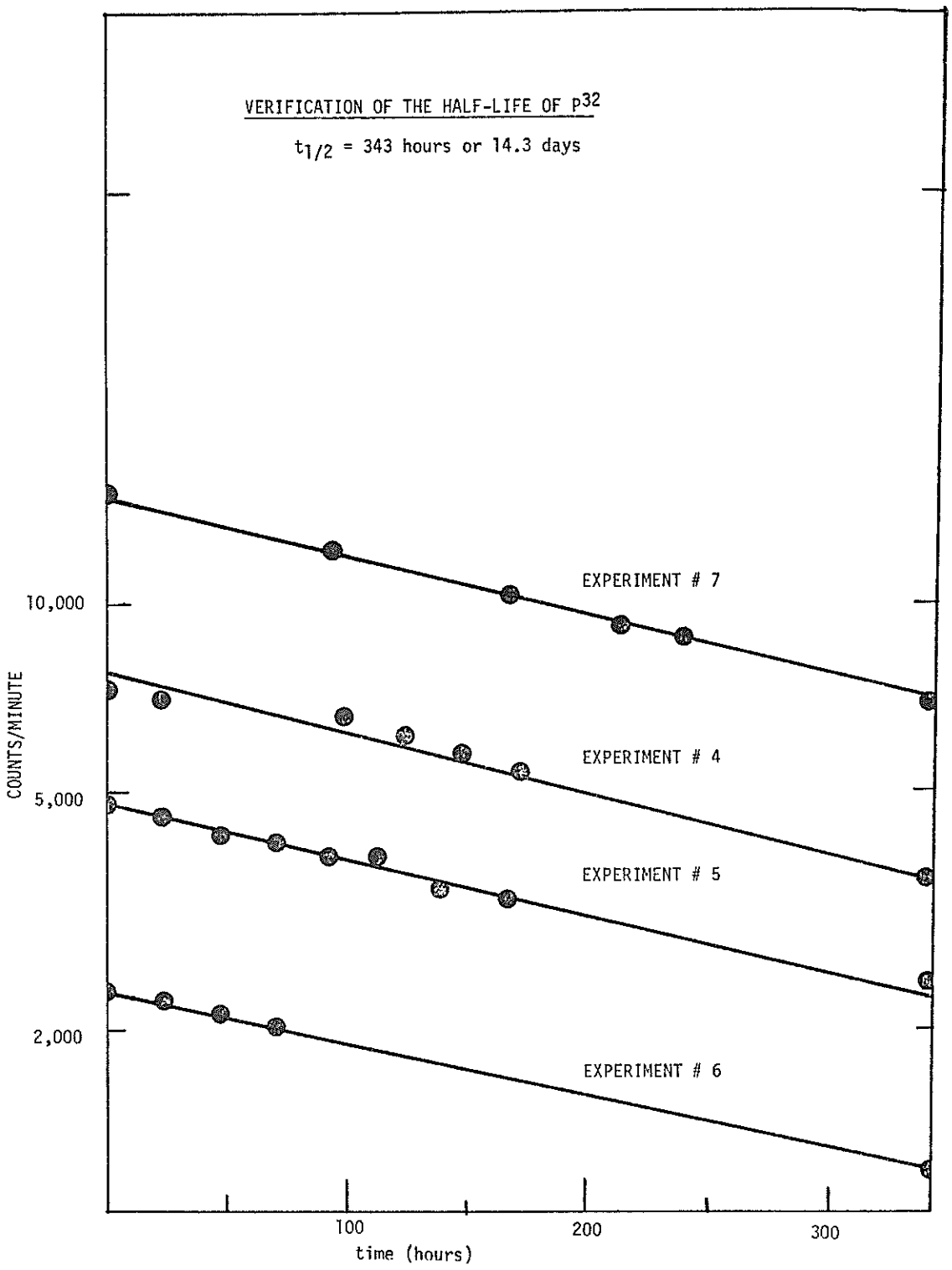

Figure I: VERIFICATION OF THE HALF-LIFE OF P^{32} ACTIVITY MEASURED DURING SEVERAL SERIES OF EXPERIMENTS ($t_{1/2}$ = 343 hours of 14.3 days)

Knowing this it was possible to determine the amount of activity that would disappear from the aqueous solutions due simply to this decay process; phosphorus removal determined in these experiments refers to the decrease in activity over that predicted by this decay process.

Initially, experiments were performed to determine if the chemical composition of the phosphate was altered by exposure to the intense neutron flux. The sample chosen for study was sodium pyrophosphate decahydrate; X-ray diffraction pattern analysis indicated that the only discernable change was a possible dehydration. This is predictable if one considers the relatively high temperatures found in close proximity to the reactor core.

5. Instrumentation: The following instrumentation was used for these studies.

   a. pH Determination: Corning Model 12 pH Meter

   b. Radioactive Counting:

      1) Hewlett/Packard S201L Scaler-Timer and High Voltage Power Supply coupled with a NaI crystal well scintillation detector.

      2) For counting dried and weighted filter papers, a Scaler Model SC-2, CMR Instrumentation Section (Los Alamos Scientific Laboratory) and a Model PA-6, CMB Division, Pulse Amplifier with associated Gieger tube.

RESULTS AND DISCUSSION

This experimental study on phosphate induced algal growth, as mentioned previously, was instigated with the intent to examine the rate of P^{32} removal from solutions of filtered, aqueous spiked sodium pyrophosphate solutions under controlled conditions of acidity, temperature, luminant flux and nutrients. The algal systems were unsterilized and were designed to be representative of natural systems. Thus, the majority of the experiments were done in the presence of local soil although several series of experiments were done using nutrients entirely dissolved in aqueous solution, "chemical media".

Typical raw experimental data are shown in Figure II. Here, the upper data set represents the decay of the standard, P^{32}, with a half-life of 343 hours while the other three curves, pH 10, 7 and 4 show the raw data for phosphorus removal from solution. The final data of removal is taken by subtracting the standard curve from each of the experimental plots.

It is important to first consider the relationships between counting rates and concentrations of radiation species. Due to the monomer-dimer equilibrium being explored,

$$2\ PO_4^{-3} = P_2O_7^{-4},$$

this relationship between the concentration of pyrophosphate and orthophosphate appears complicated. Sodium pyrophosphate is activated and that irradiation can be done statistically in several ways:

1. One phosphorus atom can be activated, $P\text{-}O\text{-}P^*$, in two ways, or
2. Two phosphorus atoms can be activated, $P^*\text{-}O\text{-}P^*$

Hydrolysis of this mixture yields the ordinary simple 2:1 relationship between ortho and pyrophosphate.

Consider the case when a solution containing radioactive pyrophosphate is instantaneously hydrolyzed to orthophosphate. Let:

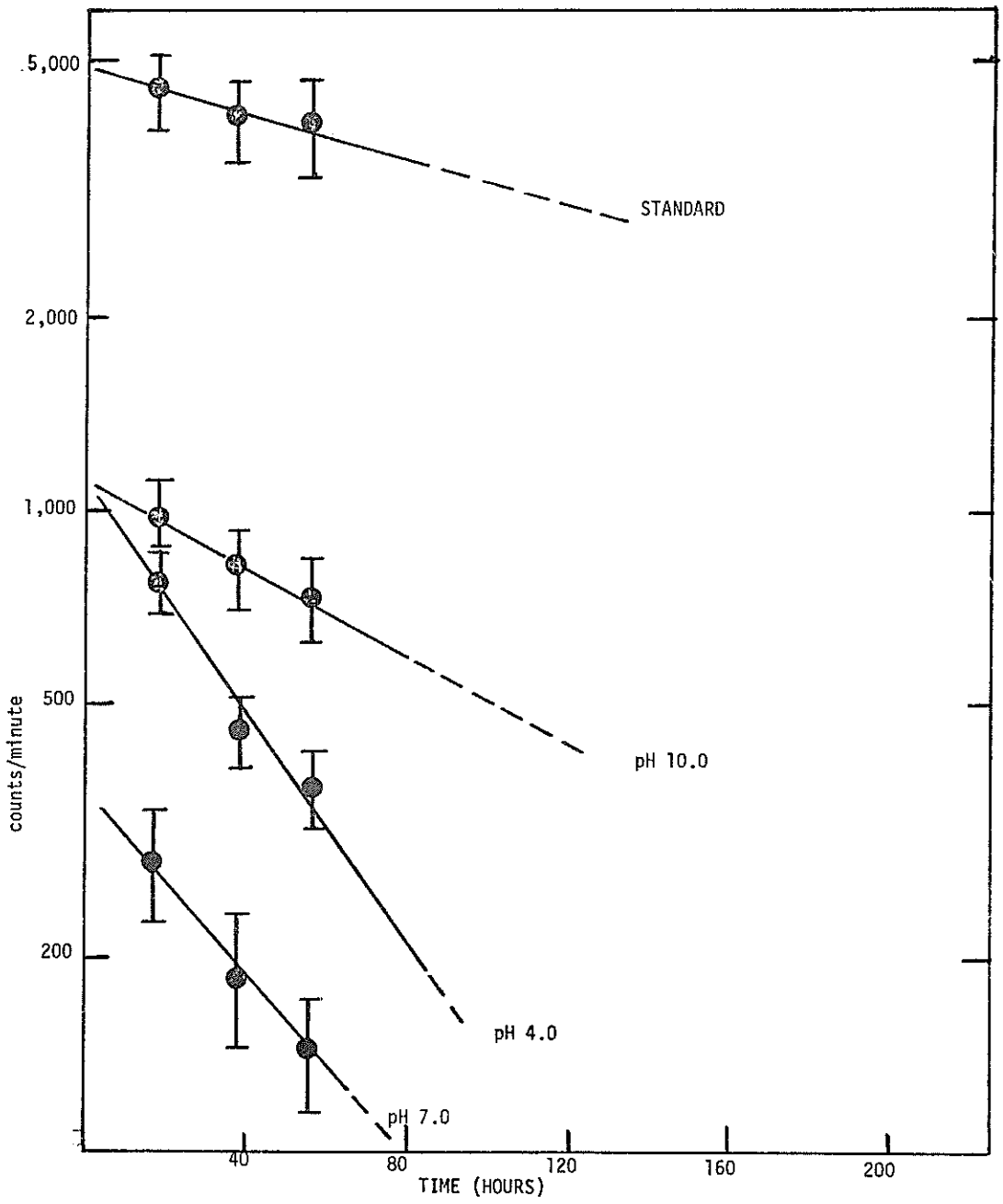

Figure II: TYPICAL EXPERIMENTAL DATA OBTAINED FOR THE REMOVAL OF PHOSPHORUS FROM CULTURE MEDIA

Upper Curve: Decay of Bulk P^{32}

Lower Curves: Solution Activity at 3 pH Values

C_{2p} = analytical concentration of $P_2O_7^{-4}$

C_p = analytical concentration of PO_4^{-3} resulting from instantaneous hydrolysis of $P_2O_7^{-4}$ of concentrated C_{2p}; $2C_{2p} = C_p$

f_1 = fraction of pyrophosphate activated with one radioactive P.

f_2 = fraction of pyrophosphate with two radioactive P atoms; from statistical arguments, assuming no interaction between P^{32} atoms, $f_1 = 4f_2$

f_3 = fraction of orthophosphate that is radioactive after instantaneous hydrolysis

P = probability of decay of a radioactive atom/minute

The activity/volume of the pyrophosphate solution will be given by:

$$a_{2p} = PC_{2p}(f_1 + 2f_2)$$

while the activity of the resulting orthophosphate solution will be simply:

$$a_p = PC_pf_3$$

Since some pyrophosphate yields two radioactive atoms and some a single radioactive atom, f_3 can be written in terms of f_1 and f_2

$$f_3 = (f_1 + 2f_2)$$

Note that both a_{2p} and a_p are directly proportional to concentration of $P_2O_7^{-4}$ and $PO_4^{\equiv}$, respectively. However, if we compare activities, we see the activity ratio

$$\frac{a_p}{a_{2p}} = \frac{2PC_{2p}(f_1 + 2f_2)}{PC_p(f_1 + 2f_2)} = \frac{2C_{2p}}{C_p}$$

directly corresponds to the mass balance equation for the hydrolysis of pyrophosphates. Thus, a process that depends upon utilization or consumption of pyrophosphate will show first order kinetics, since $a_{2p} \propto C_{2p}$. While a process that consumes $PO_4^{\equiv}$ (after hydrolysis) will have twice the concentration of $PO_4^{\equiv}$ than that available as $P_2O_7^{-4}$.

The kinetic data of pH 10.0 listed in Table 1 and shown in Figure III were first order with respect to the disappearance of P^{32} from the aqueous phase of

TABLE 1

EXPERIMENTAL RESULTS, P^{32}, REMOVAL SOIL-WATER CULTURES, AQUEOUS SODIUM PYROPHOSPHATE CHLORELLA EQUILIBRATION AT pH 10, 20 ± 2°C

Number	Time (hrs)	Concentration P^{32} c/min (C)	log C
4	0	916	2.961
	24	869	2.937
	78	701	2.846
	102	638	2.804
	126	558	2.746
	150	477	2.679
	173	444	2.647
5	0	1026	3.010
	24	980	2.990
	48	880	2.944
	72	787	2.895
	94	764	2.882
	114	715	2.854
	141	591	2.771
	167	580	2.763

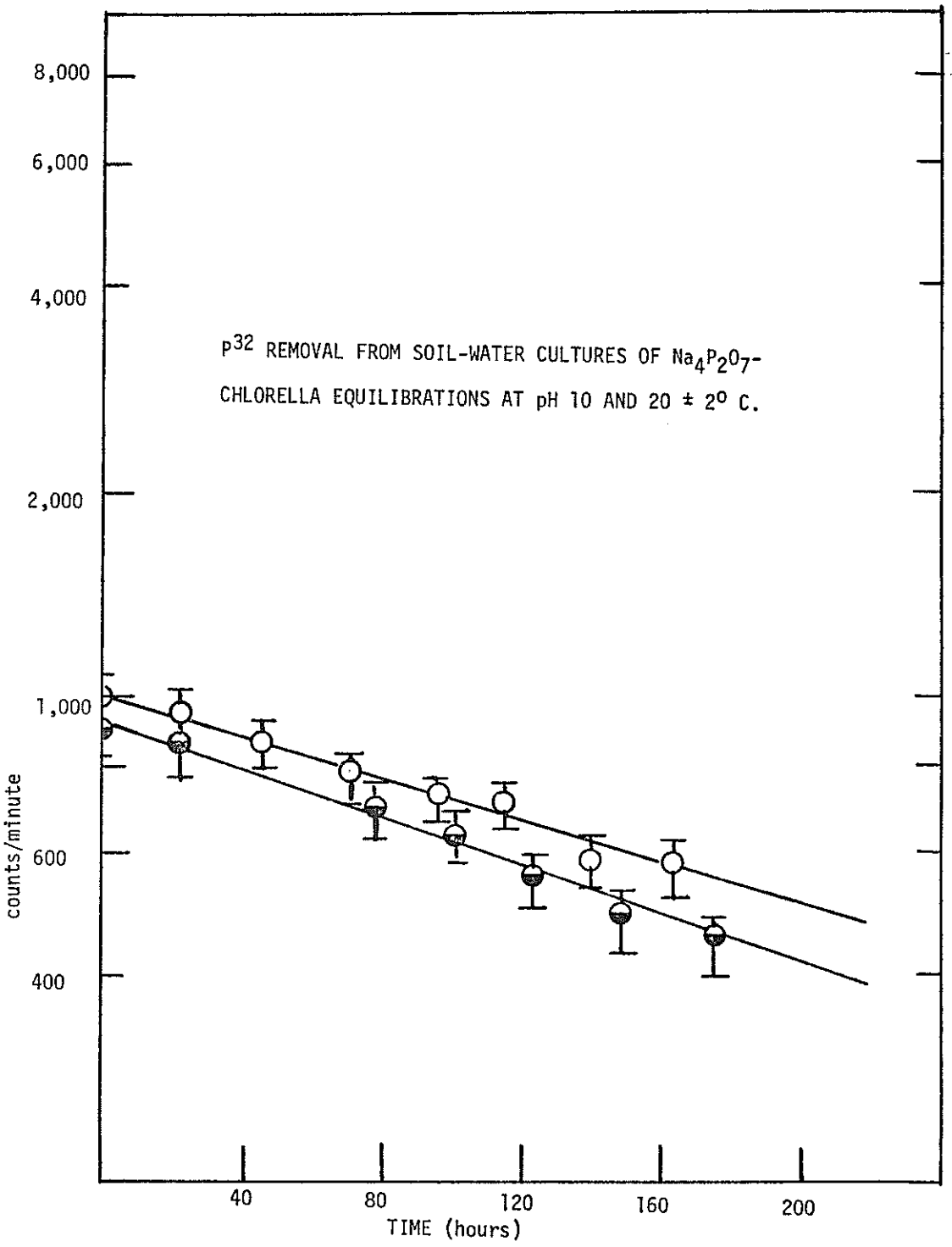

Figure III: FIRST ORDER KINETIC REPRESENTATION FOR PHOSPHORUS REMOVAL FROM SOIL-WATER CULTURES AT pH 10 MEASURED AT 20 ≠ 2°C

$k_1 = 3.52 \times 10^{-3}$ hours^{-1}

sodium pyrophosphate-chlorella soil-water cultures. These rate data show first order behavior with respect to pyrophosphate, P_2O_7, and suggest that the rate determining step is the hydrolysis of $P_2O_7^{=}$:

$$P^*_2O_7^{-4} + H_2O = 2HP^*O_4^{=} \quad (1)$$

subsequently the hydrogen phosphate, at pH 10, is consumed by the algal system:

$$HP^*O_4^{=} + \text{chlorella}_1 = \text{chlorella}_2 \quad (2)$$

Data could also be explained by assuming that the radioactive phosphorus was exchanging with the soil; this process, too, would give apparent first order behavior.

To explore the effects of soil upon these experiments, experiments were done in the "chemical media". A chemical culturing solution similar to that reported by Chu (Chu No. 10, Modified) was used (22). These P^{32} removal data are given in Table 2 and in Figure IV. As can clearly be seen here, too, the system shows first order kinetics for removal although, at 20°C, the rate is slower by a factor of ten:

$k_1 = 3.53 \times 10^{-3}$ hours^{-1} for the soil-water cultures

and

$k_2 = 3.01 \times 10^{-4}$ hours^{-1} in the case of chemical culture media.

Thus, with or without soil, the phosphorus removal is first order at the pH (10) although having soil present apparently promotes the rate of removal by more than one order of magnitude.

The rate of removal of phosphorus was also determined microscopically by measuring the rate of algal growth by assay under a microscope. The rate of growth closely approximates first order behavior although the calculated rate constant, at 20°C, is a power of ten larger for the microscopic counting method than that measured by monitoring residual activity. This, perhaps, is not too surprising since no attempt was made to estimate the average size of the algal species and if these results are to be made significant, distinctions must be

TABLE 2

EXPERIMENTAL RESULTS, P^{32} REMOVAL, MODIFIED CHU #10 CULTURE MEDIA, AQUEOUS SODIUM PYROPHOSPHATE-CHLORELLA EQUILIBRATIONS AT pH 10 AND 20 ± 2°C

Number	Time (hrs)	Concentration P^{32} c/min (C)	log c
9 & 10	0	461	2.663
	23	441	2.644
	55	444	2.647
	81	426	2.629
	89	449	2.652
	114	390	2.592
	137	434	2.636
	153	428	2.630
	161	450	2.652
	200	446	2.649
	225	417	2.620
	250	410	2.612

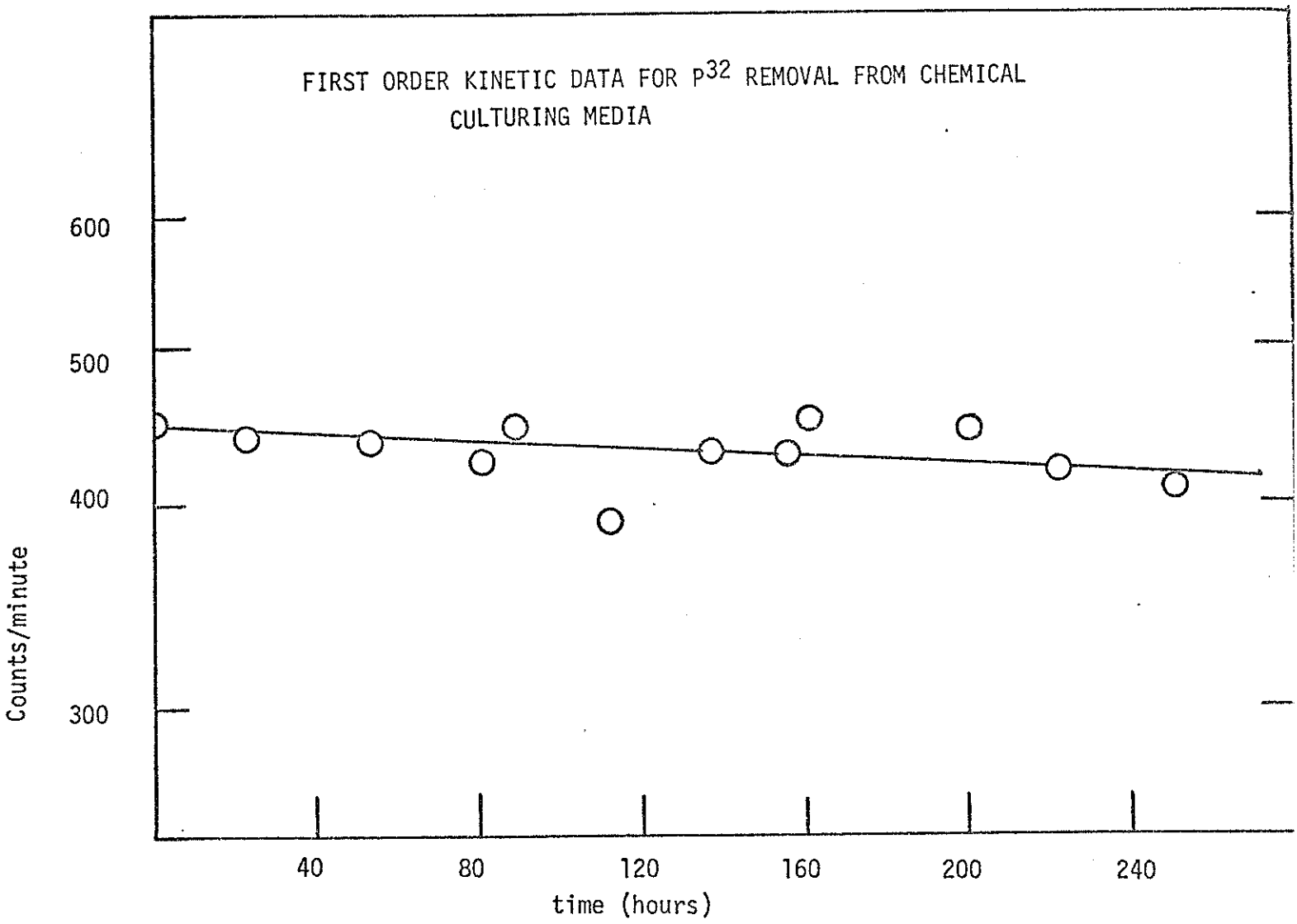

FIGURE IV: FIRST ORDER KINETIC REPRESENTATION FOR PHOSPHORUS REMOVAL FROM CHEMICAL CULTURES (MODIFIED CHU NO. 10) AT pH 10 AND 20 ± 2°C.

$k_1 = 3.01 \times 10^{-4}$ hours^{-1}

made between the number of plants and the average size of a plant. Importantly, however, these experiments do show that the systems were experiencing growth and thus, the phosphorus removal can be attributed to this growth process. The experimental data show that a second order kinetic scheme must be used at pH 7.0 and 4.0. These data are summarized in Tables 3, 4 and 5. Here the rate constants determined from this information, shown graphically in Figures V, VI, VII and VIII, differ by a factor of 100;

for soil-water cultures, pH 7.0,

$$k_2 = 1.44 \times 10^{-5} \text{ hours}^{-1} \ (P^{32} \text{ counts/minute})^{-1}$$

and

for chemical cultures, pH 7.0,

$$k_2 = 8.09 \times 10^{-7} \text{ hours}^{1} \ (P^{32} \text{ counts/minute})^{-1}$$

while at a pH of 4.0, the rate constants were determined as

$$k_2 = 2.08 \times 10^{-5} \text{ hours}^{-1} \ (P^{32} \text{ counts/minute})^{-1}$$

and

$$k_2 = 7.49 \times 10^{-7} \text{ hours}^{-1} \ (P^{32} \text{ counts/minute})^{-1}$$

for soil-water and chemical cultures, respectively. As can be seen, at pH 7 and 4, removal was significantly faster than that measured at pH of 10.

These data do give some insight into the ways in which phosphorus is removed from aqueous solution. However, they are complicated in the fact that bacteria coexists with these algal growth and the growth of these bacteria also can consume phosphorus from an aqueous environment.

There is ample experimental and deductive evidence that chlorella utilizes carbon dioxide for life support and releases oxygen. Bacteria, coexisting with these plants, utilize oxygen, in part that produced by the metabolic process of the algae, and in part that dissolved from atmospheric gases. Although this algae-bacteria mutually sympathetic process has been appreciated for years (4), little has been done to explore the factors that influence the two simultaneous growth process.

TABLE 3

EXPERIMENTAL RESULTS, P^{32} REMOVAL, SOILWATER CULTURES, AQUEOUS SODIUM PYROPHOSPHATE CHORELLA EQUILIBRATION AT pH 7, 20 ± 2°C

Number	Time (hrs)	Concentration P^{32} c/min (C)	$1/c \times 10^{-3}$ $(c/min)^{-1}$	Remarks
4&5	0	299	3.35	3.56 average
		275	3.63	
		270	3.71	
	24	262	3.81	
		285	3.50	
	48	200	4.99	
	81	228	4.39	
	133	212	4.73	
	153	223	4.49	
	225	141	7.11	
	250	250	7.27	

TABLE 4

EXPERIMENTAL RESULTS, P^{32} REMOVAL, SOIL-WATER CULTURES, AQUEOUS SODIUM PYROPHOSPHATE-CHLORELLA EQUILIBRATIONS AT pH 4, 20 ± 2°C

Number	Time (hrs)	Concentration P^{32} c/min (C)	$1/c \times 10^{-3}$
4	0	805	1.24
	24	647	1.54
	78	347	2.88
	102	245	3.91
	126	231	4.33
5	0	822	1.23
	24	781	1.08
	48	499	2.00
	72	400	2.50
	94	335	2.98
	114	291	3.43
	141	256	3.91
	167	222	4.50

TABLE 5

EXPERIMENTAL RESULTS, P^{32} REMOVAL, MODIFIED CHU #10 CULTURE MEDIA, AQUEOUS SODIUM PYROPHOSPHATE-CHLORELLA EQUILIBRATIONS AT pH 7, 20 ± 2°C

Number	Time (hrs)	Concentration P^{32} c/min (C)	$1/c \times 10^{-3}$
10	0	461	2.170
	23	441	2.269
	55	440	2.270
	89	428	2.332
	114	428	2.336
	137	423	2.360

EXPERIMENTAL RESULTS, P^{32} REMOVAL, MODIFIED CHU #10 CULTURE MEDIA, AQUEOUS SODIUM PYROPHOSPHATE-CHLORELLA EQUILIBRATIONS AT pH 4, 20 ± 2°C

Number	Time (hrs)	Concentration P^{32} c/min (C)	$1/c \times 10^{-3}$
10	0	439	2.279
	23	418	2.390
	55	440	2.290
	89	406	2.460
	114	428	2.336
	137	402	2.481
	161	360	2.729
	200	446	2.239

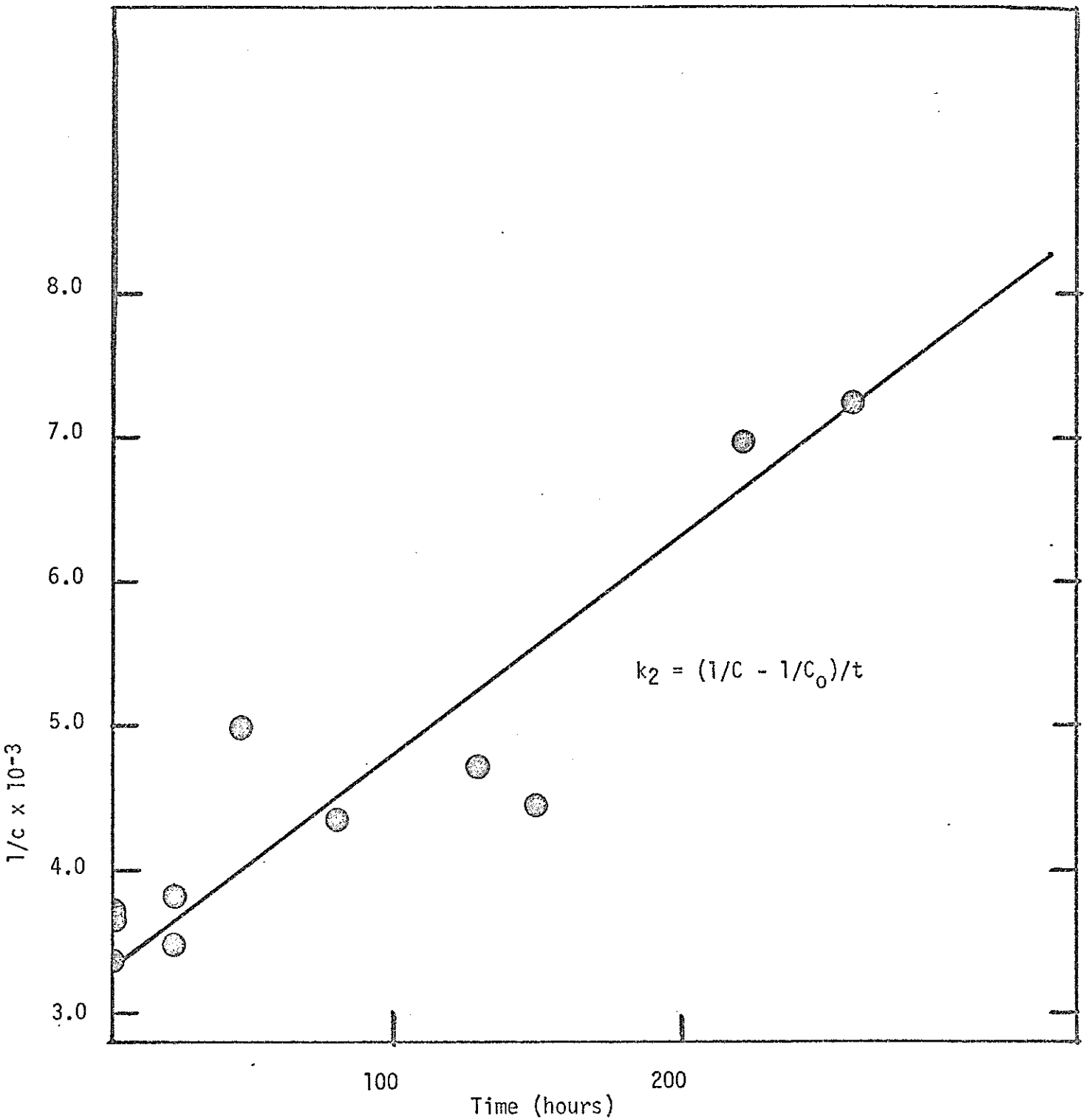

Figure V: SECOND ORDER KINETIC REPRESENTATION FOR PHOSPHORUS REMOVAL FROM AQUEOUS SOIL-WATER CULTURES AT pH 7 AT 20 ± 2°C.

$k_2 = 1.44 \times 10^{-5}$ hours^{-1} (counts P^{32}/minute)$^{-1}$

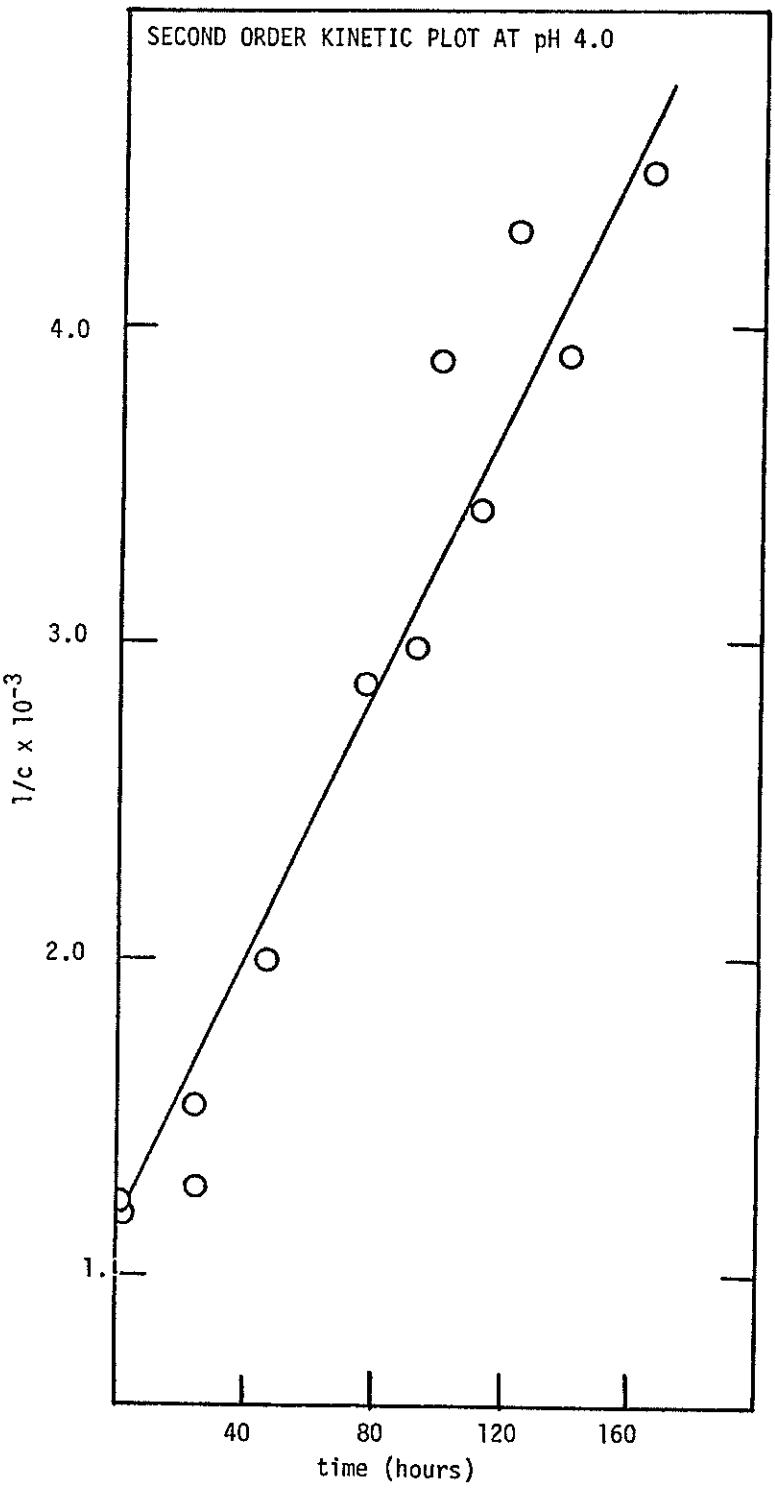

Figure VI: SECOND ORDER KINETIC REPRESENTATION FOR PHOSPHORUS REMOVAL FROM AQUEOUS SOIL-WATER CULTURES AT pH 4 AT 20 ± 2°C.

$k_2 = 2.08 \times 10^{-5}$ hours^{-1} (P^{32} c/min)$^{-1}$

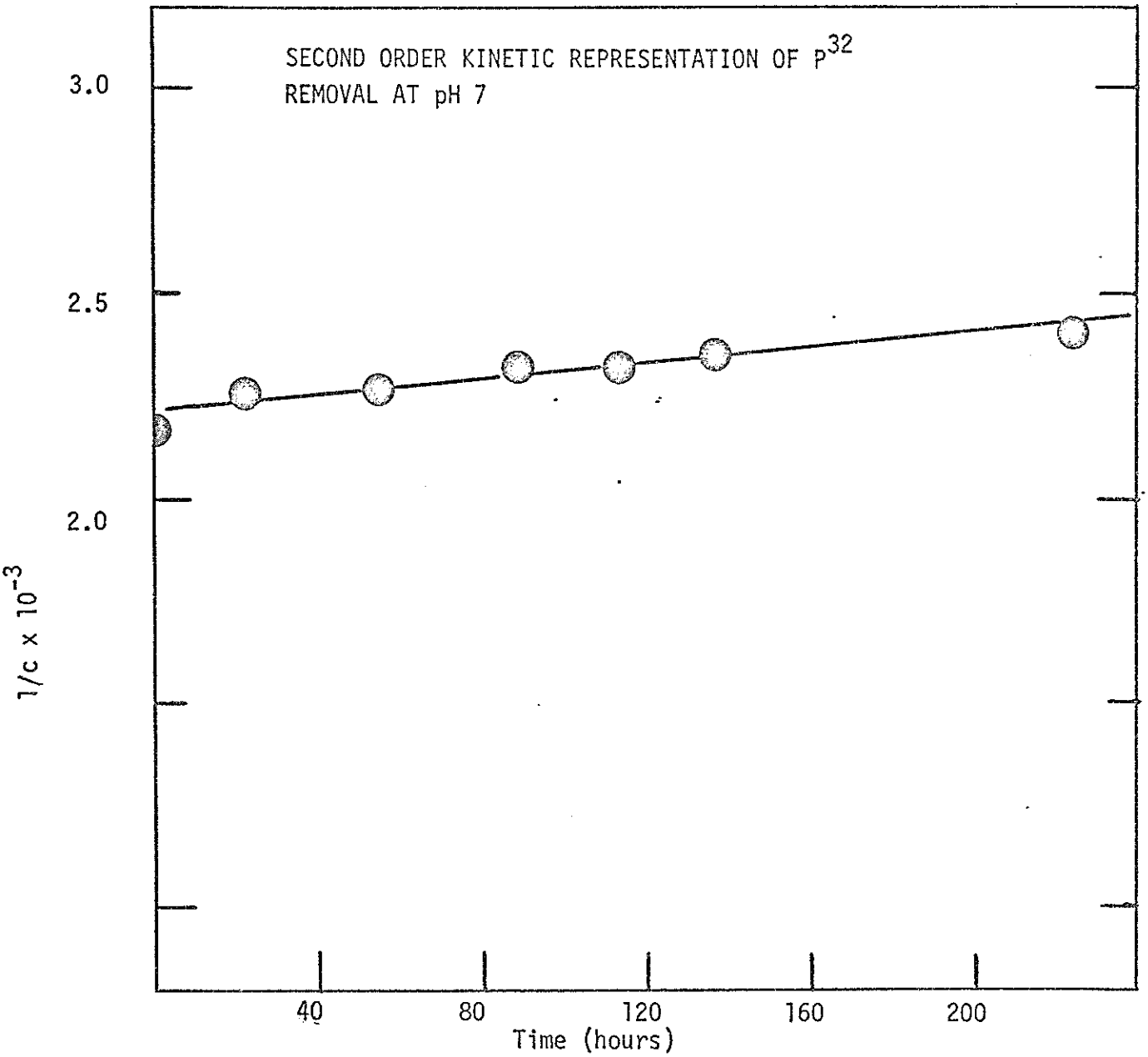

Figure VII: SECOND ORDER KINETIC REPRESENTATION FOR PHOSPHORUS REMOVAL FROM AQUEOUS CHEMICAL CULTURES AT pH 7 AND 20 ± 2°C.

$k_2 = 8.09 \times 10^{-7}$ hours^{-1} (P^{32} c/min)$^{-1}$

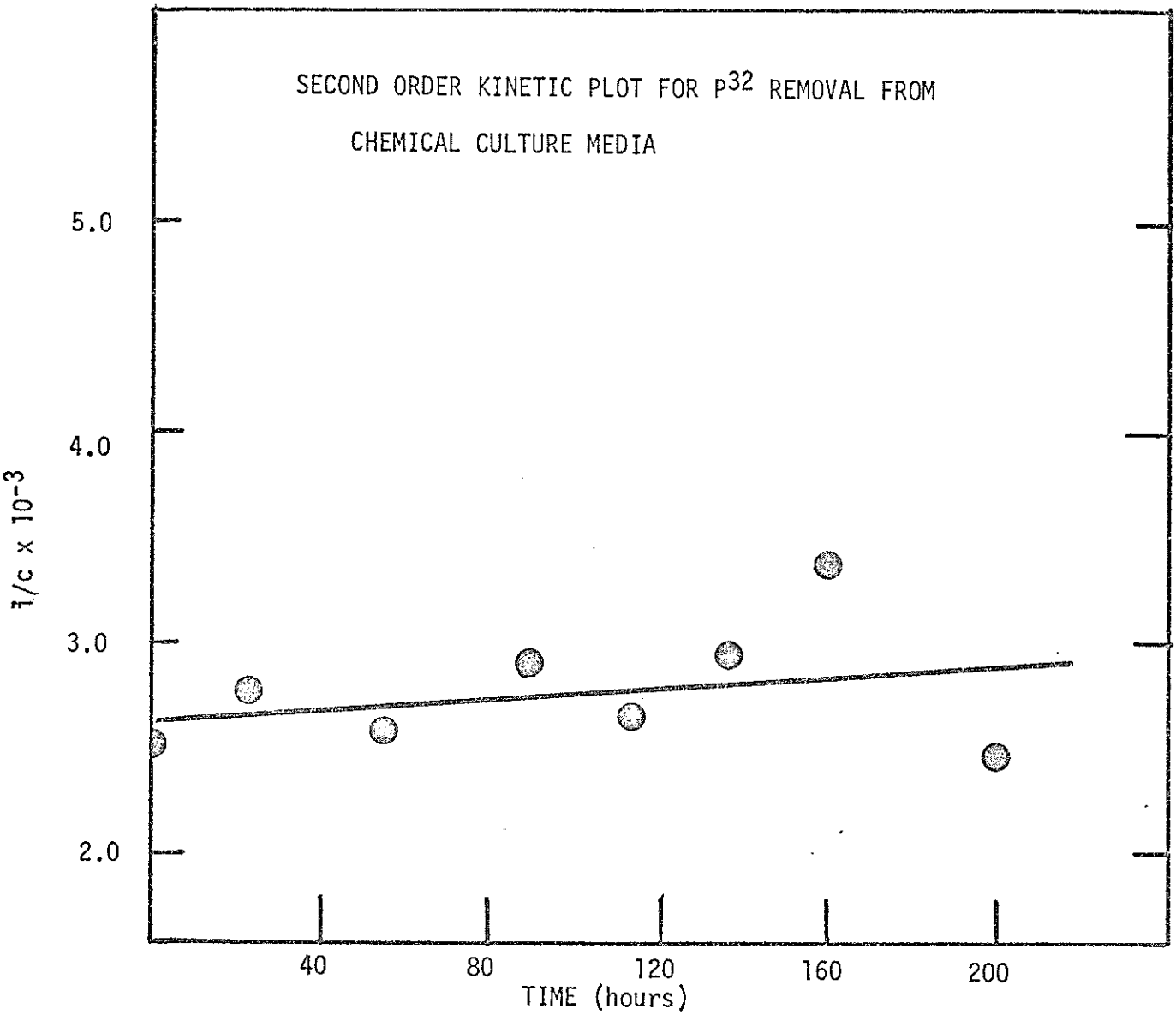

Figure VIII: SECOND ORDER KINETIC REPRESENTATION FOR PHOSPHORUS REMOVAL FROM AQUEOUS CHEMICAL CULTURES AT pH 4 AND 20 ± 2°C.

$k_2 = 7.49 \times 10^{-7}$ hours^{-1} (P^{32} c/min)$^{-1}$

In order to explore this situation more completely, two additional series of experiments were run. The first utilized an atmosphere of high purity oxygen while the second substituted this for high purity carbon dioxide. These gases were used for stirring as before (air was used then) and flowed through with a rapid enough rate to ensure that the systems under study would remain saturated with either O_2 or CO_2. This data is shown in Figure IX and Tables 6 and 7.

The data obtained for the oxygen rich experiments in the chemical culture data show little change at all pH values studied, again 10, 7 and 4 (Figure IX). This is suggestive that no appreciable algal growth occurred, a nonsurprising result, but also shows that other processes for phosphorus removal, adsorption, etc., are not highly significant since these oxygen would hardly hinder these degradation pathways. Moreover, these data suggest that bacteria growth, which should be accelerated under these conditions (4) is not significant. The data obtained for soil-water cultures at pH 10, 7 and 4, under oxygen conditions, are also shown in Figure XI. Here a decrease did occur with the rate increasing with increasing acidity. The data all show apparent first order behavior. The process occurring here, most probably, is exchange of the phosphorus with the soil. (Experiments counting the soil to prove this fact were not as yet run.) The indications are, however, that soil, also, exchanges phosphorus in the ortho-phosphate form and that the rate of exchange is accelerated by increases in acidity.

Data obtained for the carbon dioxide experiments are not conclusive since the activity used in this series was quite low. All series, both chemical media and soil-water, showed rapid P^{32} uptake with the soil-water systems stripping effectively all activity from the solution in a relatively short time, less than 4 days. These data are highly suggestive of rapid algal growth under these conditions and suggest, again, that bacteria (which would be retarded now) are not really effective in phosphorus removal from aqueous media.

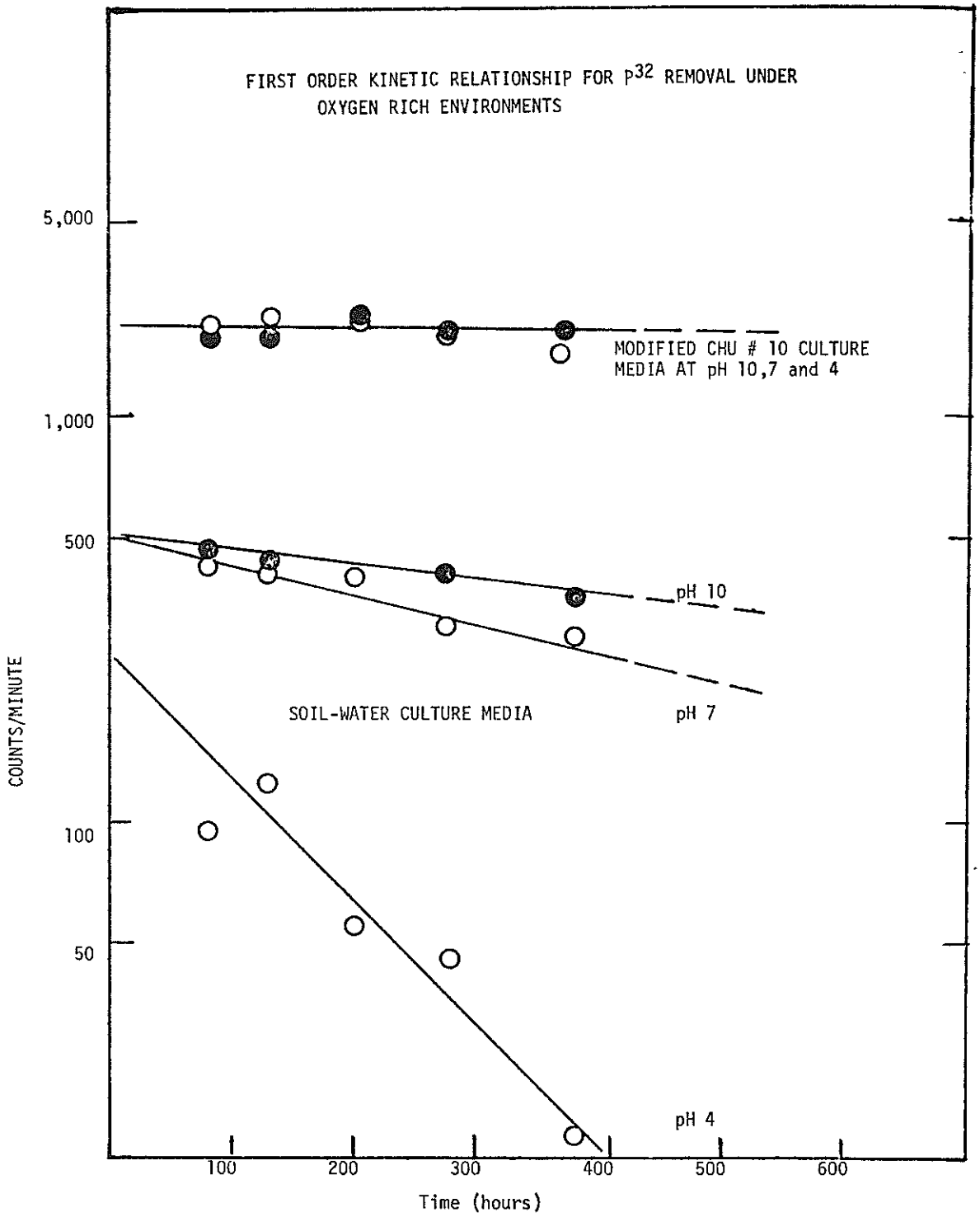

Figure IX: FIRST ORDER KINETIC REPRESENTATION FOR PHOSPHORUS REMOVAL UNDER OXYGEN RICH ATMOSPHERES

Upper Curves: Composite behavior of 3 pH values in chemical cultures

Lower Curves: Behavior of soil-water cultures

TABLE 6

P^{32} REMOVAL FROM AQUEOUS SODIUM PYROPHOSPHATE PHASE

MAINTAINED AT 20 ≠ 2°C UNDER OXYGEN ATMOSPHERE

Culture Media	pH	Time	Corrected P^{32} (counts/minute)
Chemical Media	10	82 hours	1589
		131	1675
		204	1700
		276	1550
		372	1430
Chemical Media	7	82	1589
		131	1620
		204	1799
		276	1630
		372	1645
Chemical Media	4	82	1680
		131	1780
		204	1700
		276	1670
		372	1645
Soil-Water	10	82	480
		131	452
		204	na
		276	418
		372	362
Soil-water	7	82	435
		131	412
		204	405
		276	305
		372	294
Soil-water	4	82	97
		131	114
		204	56
		276	47
		372	17

TABLE 7

P^{32} REMOVAL FROM AQUEOUS SODIUM PYROPHOSPHATE PHASE

MAINTAINED AT 20 ≠ 2°C UNDER CARBON DIOXIDE ATMOSPHERE

Culture Media	pH	Time	Corrected P^{32} (counts/minute)
Chemical Media	10	81	295
		129	105
		164	116
	7	81	363
		129	166
		164	98
	4	81	423
		129	104
		164	159
Soil-water	10	81	58
		129	0
	7	81	33
		129	0
	4	81	126
		129	0

CONCLUSIONS

These first year studies have led to a better understanding of the ways in which phosphorus is consumed after it is discharged into the environment. There are several possible processes which can contribute to the removal of phosphorus; these are summarized in Exhibits A and B. This present work was concerned with the activity of P^{32} remaining in aqueous solution of a spiked chlorella culture following filtration to remove all filterable particles, including the algal species.

Two mechanisms apparently control the phosphorus uptake and these processes can be interchanged by a simple pH variation. At a pH of 10, the removal rate is quite clearly first order with respect to P^{32} while the rate increases and changes order at pH 7 and 4. These data can be explained by assuming that the hydrolysis process is rate determining at pH of 10 and that the growth process is the rate determining step at pH 7 and 4. This explanation is sensible since the hydrolysis rate is accelerated by increases in acidity: the slow, at pH 10, process is accelerated so that it becomes rapid compared to the growth process at pH of 7. This explanation leads to the conclusion that algal systems do not consume pyrophosphate for growth nor are they particularily effective in accelerating the hydrolysis of pyrophosphate.

The enriched atmosphere experiments fit into this model with little difficulty. In fact, they greatly strengthen it since no attempt has previously been made in the literature to sort out the effects of bacterial growth and there remains a tacit understanding that bacterial metabolic processes do account for an appreciable fraction of phosphorus uptake. This evidence says that this is not so. Under oxygen environments, the only active process for P^{32} removal was that of exchange with the soil and this rate was accelerated by acidity; the inference here is that, again, orthophosphate is exchanging, not pyrophosphate nor phosphorus and that the hydrolysis rate is the rate determining step of the exchange process. (If this were not so, one could not easily account for the

EXHIBIT A: PROBABLE PHYSICAL, CHEMICAL AND BIOLOGICAL PROCESSES RESPONSIBLE FOR THE REMOVAL OF P^{32} FROM THE AQUEOUS PHASE OF SOIL-WATER CULTURES

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1. Diffusion of Phosphate Ion, Containing P^{32}, Through Aqueous Phase.
2. Diffusion of Phosphate Molecule, Containing P^{32}, Through Solid Phase.
3. Exchange of Phosphorus, P^{32}, with Nonradioactive Phosphorus at the Soil-Water Interface.
4. Capillary Action in the Solid Soil Phase.
5. Adsorption of Radioactive Phosphorus Ions on the Solid Interface.
6. Adsorption of Phosphate on the Walls of the Containing Vessel.
7. Adsorption of Phosphate on the Surfaces of the Algal Growth.
8. Incorporation of the Radioactive Phosphorus into the Phospholipid Layer Forming Biological Membranes.
9. Incorporation of the Radioactive Phosphorus into Bacteria Metabolic Processes.
10. Bacterial Activity Leading to a Large Particle Size, Filterable Composite Incorporating Radioactive Phosphorus.

EXHIBIT B: PROBABLE PHYSICAL, CHEMICAL AND BIOLOGICAL PROCESSES RESPONSIBLE FOR THE REMOVAL OF P^{32} FROM THE AQUEOUS PHASE OF CHEMICAL CULTURING MEDIA

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1. Diffusion of Phosphate ion Containing P^{32} Through the Aqueous Phase of the Culture.
2. Adsorption of Radioactive Phosphate on Walls of the Containing Vessel.
3. Adsorption of Radioactive Phosphate on Algal Surfaces.
4. Incorporation of the Radioactive Phosphorus into the Phospholipid Layer Forming Biological Membranes.
5. Bacterial Action Forming a Filterable Species.

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increased rate of P^{32} disappearance as the pH is varied from 7 to 4.)

This argument could be countered by claiming that bacterial growth, in the soil-water cultures, is contributing to removal. No special efforts were made to exclude bacteria from the chemical cultures, however, and one would have expected this removal process to be effective in the chemical cultures if it were operating in the soil-water cultures.

The very rapid removal of phosphorus under CO_2 environment is also a highly significant finding. Although this is described by only a single experiment, done during the tail end of the contract period, it leads to a possibly effective strategy for the removal of phosphorus from surface waters. CO_2 is an inexpensive chemical available from industrial processes as a waste product and, of course, readily available from numerous combustion sites. Phosphate stripping would be easily accomplished under a CO_2 rich environment. (Algal growths could be used as a food source.)

This report began with three questions. The answers are apparent from the above:

1. The rate of P^{32} uptake is controlled by pH and by the rate of the growth process.
2. Algal systems are not particularly effective in the hydrolysis of phosphate esters.
3. Almost all of the phosphorus in an aqueous solution can be stripped by the algal growth process.

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