INTERACTIONS OF NITROGEN AND PHOSPHORUS ON THEIR UPTAKE BY MIXED CULTURES IN THE BIODEGRADATION OF ACETIC ACID

A Thesis

Presented to

the Faculty of the Department of Civil Engineering

The University of Houston

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

---

by

Oscar Saenz, Jr.

August 1968

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INTERACTIONS OF NITROGEN AND PHOSPHORUS ON THEIR UPTAKE BY MIXED CULTURES IN THE BIODEGRADATION OF ACETIC ACID

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ABSTRACT

A study was conducted on the interactions of nitrogen and phosphorus on their uptake by mixed cultures in the biodegradation of acetic acid.

Ammonium sulfate and sodium hexametaphosphate were supplied as nutrient sources for nitrogen and phosphorus, with the N:P ratios varying throughout each experiment. The effect of these variations was observed in the final uptake of nitrogen and phosphorus, the biological growth, and the T_bAC:C ratios.

It was found that nitrogen is the significant controlling factor in both the uptake of nitrogen and possibly phosphorus by the culture.

It was also found that cultures are able to assimulate other phosphorus sources other than ortho phosphates.

A new approach for measuring biological growth by the use of the modified Beckman Carbonaceous Analyzer was investigated.

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INTRODUCTION

The elution of compounds containing phosphorus and nitrogen is causing serious problems in rivers, lakes, estuaries, and other bodies of water.

Eutrophication of a body of water is caused by an accumulation of high concentration of nitrogen and phosphorus. These materials provide a rich nutrient environment for algal growth and unless they are eliminated or controlled in discharged effluents will lead to serious ecological inbalances in the receiving water. This condition will render a natural body of water undesirable from various standpoints. The water becomes turbid, highly colored, toxic as in the case of blue green algae growth, and deficient in bacteria. Even though pathogens are partially eliminated, the deficiency of bacteria is not desirable. Bacteria play a vital role in the total ecology of the natural receiving water. Besides providing a source of food for other forms of life, bacteria also assimulate biodegradable organic compounds thus stabilizing the stream or river. Eutrophication also renders a body of water useless for recreational activities, shellfish production, etc. Therefore, this condition is not good from an economic point of view. It is therefore necessary that nitrogen and phosphorus discharges into natural bodies of water be eliminated or properly controlled to low enough levels so as to not overproduce algal populations.

OBJECTIVES

The purposes of this project are as follows:

1) To study the interactions of NH_3-N and $(NaPO_3)_6$-P and their uptake by mixed biological cultures in the biodegradation of acetic acid. It is desired to establish some conclusions from the experimental data concerning the uptake of these nutrients by mixed cultures when the nutrient concentrations N:P ratios are varied.

2) To establish minimum N and P dosages in the total biodegradation of acetic acid to obtain a nutrient free effluent. This factor is of prime importance particularly in the biodegradation of chemical wastes where the operator desires to add nutrients to the system so that the effluent is free of nitrogen and phosphorus.

3) To introduce a tentative mode of studying biological growth in the biodegradation of an organic substrate.

BACKGROUND INFORMATION

In recent years investigators have conducted work on pollution abatement of phosphorus and nitrogen compounds. Various methods have been investigated. These methods are as follows:

1) Coagulation and sedimentation for phosphate removal.

2) Biological removal of phosphorus and nitrogen.

3) Air stripping of ammonia nitrogen.

4) Ion exchange techniques for nitrogen and phosphorus removal.

The "Tahoe" process[4] for nutrient removal uses the coagulation and air stripping methods for both phosphorus and nitrogen removal respectively. The results of this process have been encouraging.

The biological uptake of phosphorus and nitrogen has been studied somewhat by biologists. However, a number of questions remained unanswered particularly in employing this technique for the removal of nitrogen and phosphorus from waste waters. Echkenfelder[6] has studied some of the difficulties encountered in the biological removal of nitrogen. Connell and Vacker[3] conducted some investigations recently on the effect of a number of variables on the removal of phosphate from a domestic waste using activated sludge. The parameters under study were culture age, concentration of suspended solids, aeration rates,

and BOD loadings. It was concluded that for optimum removal of phosphate by activated sludge the following requirements are essential:

1) Culture must be in the declining growth or bioflocculant phase. Uptake may be as high as 20-25 mg $\equiv PO_4$/100 mg solid formed.

2) Adequate aeration will promote the removal to less than 1 mg/l $\equiv PO_4$ in the aeration tanks if the effluent contains sufficient BOD and utilizable cations. BOD to $\equiv PO_4$ ratio in the influent is the limitation factor of metabolic uptake.

3) Excessive aeration to high DO levels in the bioflocculation phase promotes endogenous lysing of cells thereby returning phosphate to solution.

4) Prolonged retention of sludge solids in final clarifiers results in the anaerobic release of $\equiv PO_4$.

A. W. Busch[1] has also presented an excellent summary of work conducted at Rice University. Some discussion is made concerning stoichiometric ammonia-nitrogen requirements in the biodegration of glucose by mixed cultures. A fresh approach using the carbonaceous analyzer as a tool for the determination of biological growth as well as the T_bAC is outlined.

Among Busch's references are included the work of N. Myrick entitled "BOD Progression in Soluble Substrates", and L. L. Hiser's

work entitled "An 8-Hour Biological Oxygen Demand Test Using Mass Culture Aeration and COD".

This information is quite relevant in the progress of this project. A recent publication in Environmental Science and Technology[8] brings to light the tremendous problems encountered in our natural bodies of water by the addition of phosphorus. Methods are outlined for the removal of $\equiv PO_4$ from effluents.[7]

EXPERIMENTAL PROTOCOL

Preparation of Substrate (Table 2)

An aqueous solution containing 3.78 grams acetic acid/l was prepared. This yielded a concentration containing 1.5 mg carbon/ml. The solution was then standardized by the use of the carbonaceous analyzer which measures total carbon.

Preparation of Stock Nutrient Solutions (Table 2A)

1) Ammonium sulfate $(NH_4)_2\ SO_4$.

   After being oven dried at 103° C and desiccated for 30 minutes, an aqueous solution containing 47.2 grams of ammonium sulfate/l was prepared. This yielded a concentration containing 10 mg nitrogen/ml.

2) Sodium hexametaphosphate $(NaPO_3)_6$.

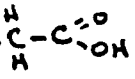
   The material was oven dried for one hour at 103° C and desiccated for 30 minutes. An aqueous solution containing 3.28 grams $(NaPO_3)_6$/l was prepared. This yielded a concentration containing 1 mg phosphorus/ml.

Preparation of Dilution Water

The dilution water was prepared by adding a few crystals of Na_2SO_3 to five gallons of tap water. The Na_2SO_3 reduced the chlorine to chloride. The alkalinity of the dechlorinated tap water was then reduced

PREPARATION OF SOLUTIONS

Substrate *

SUBSTRATE	MW	STRUCTURE	% CARBON	VAPOR PRESSURE mm Hg	B.P. °C	STOCK SOLUTION gms/ℓ	CARBON CONC. mg/ml
Acetic acid	60.1	 (liquid)	40.0	40@43°C	118	3.78	1.50

* In acclimation and experimental steps, 100 ml of stock solution diluted to 1000 ml in Imhoff cones will yield a solution that contains 150 mg/l of organic carbon.

Table 2

Stock Nutrient Solutions

NUTRIENT COMPOUND	M.W.	FORMULA	GRAV. FACTORS	REQUIRED CONC. mg/ml	gm/ℓ	STOCK SOLUTION gms/ℓ	FINAL SOLUTION mg/ml
Ammonium Sulphate	132.14	$(NH_4)_2SO_4$	4.72	10 mg N	10	47.2	10 NH_3-N
Sodium Meta-phosphate	611.80	$(NaPO_3)_6$	3.28	1 mg P	1	3.28	1 P

Table 2A

Nutrient Requirements

	NITROGEN						PHOSPHORUS					
NUTRIENT COMPOUNDS	N_1		N_2		N_3		P_1		P_2		P_3	
	C/2.5 mg/ℓ	ml/ℓ	C/5 mg/ℓ	ml/ℓ	C/10 mg/ℓ	ml/ℓ	C/10 mg/ℓ	ml/ℓ	C/20 mg/ℓ	ml/ℓ	C/30 mg/ℓ	ml/ℓ
$(NH_4)_2SO_4$	60	6	30	3	15	1.5						
$(NaPO_3)_6$							15	15	7.5	7.5	5.0	5.0

Table 2B

C (Substrate Carbon) = 150 mg/l

to $\approx$ 100 mg/l as $CaCO_3$ which is equivalent to 12 mg inorganic carbon/liter.

The desired alkalinity was obtained by adding concentrated HCl to the dechlorinated water according to the equation

Volume of concentrated HCl/l of dilution water = 0.0016 (A-X)

A = total alkalinity in mg/l $CaCO_3$
(obtained by titration)

X = desired residual alkalinity in mg/l $CaCO_3$
normally 100 mg/l $CaCO_3$ (12 mg/l carbon)

Assumptions

Assay of concentrated HCl = 37.4%

Density of concentrated HCl = 1.19

After the addition of the calculated HCl volume, the dilution water was aerated to remove the excess carbonates.

It was decided to use tap water as the source for a proper dilution water because it contains desired nutrients for the proper growth of the organisms. These nutrients include the essential trace minerals that are necessary for the synthesis of cells.

Acclimation of Culture

One hundred milliliters (1.50 mg organic carbon/ml) of the stock acetic acid solution was placed in a 250 ml beaker. The following nutrient concentrations were added:

1) Ammonium sulfate $(NH_4)_2 SO_4$ -
6 ml (60 mg NH_3-N)

2) Sodium metaphosphate $(NaPO_3)_6$ -
15 ml (15 mg Phosphorus)

The pH of this solution was adjusted to 6.0 with the addition of 1.0 N NaOH. A pH meter was utilized in this step.

The pH adjusted solution containing the substrate (150 mg organic carbon) and nutrients was then poured into a 1 liter Imhoff cone. The volume was brought up to 1 liter with dilution water. The sample was then mixed and the pH noted. The pH was buffered at 7.0 - 7.5 by the dilution water. Aeration was adjusted at 1 scfh. Five ml of the clear supernatant from a freshly acquired sewage seed were placed in the cone.

The mixture was allowed to aerate until a definite growth was visible. This normally took place within 24 to 30 hours. At the end of this time, the aeration was discontinued. A filtered sample was obtained and the soluble organic carbon was determined by the use of the carbonaceous analyzer. The carbon depletion was noted. The culture was allowed to settle and 150 ml of the supernatant was discarded. A new pH adjusted, nutrient containing substrate was again added to the cone. Aeration was again started and another filtered sample was obtained for carbon analysis. At the end of the next 24 to 30 hours, the aeration was again stopped. The culture was again allowed to settle and soluble organic carbon was again run on the mixture. The supernatant was

discarded and the settled culture was washed twice with dilution water. New feedings were again repeated with the acclimated culture until it was determined that the culture was thoroughly acclimated to the substrate. The guidelines that were used in making this decision were:

1) Rate of carbon depletion of the substrate

2) Flocculant appearance of the culture

The acclimated culture was continually fed until it was utilized.

Apparatus

A battery of eleven 1 liter Imhoff cones were set up. Individual air flow meters and aeration tubes were designated for each cone. Air diffusion stones bubblers were not used for aeration but rather 3 mm ID glass tubes. See Figures 1 and 2. It was felt that aeration could be accomplished successfully by using open tubes only. A Beckman pH meter equipped with long line glass., calomel and temperature compensation electrodes was used to monitor pH's. The long lines facilitated the pH measurements at the Imhoff cones rather than taking a small sample. This also eliminated possible contamination errors by transferring the liquid to and from the pH meter.

Sampling equipment included eleven 50 ml beakers which were each designated for each cone. By using the same beaker for each cone, contamination errors were eliminated. Other sampling equipment included two-inch test tubes which were used for sampling for total

TYPICAL EXPERIMENTAL "SET UP"

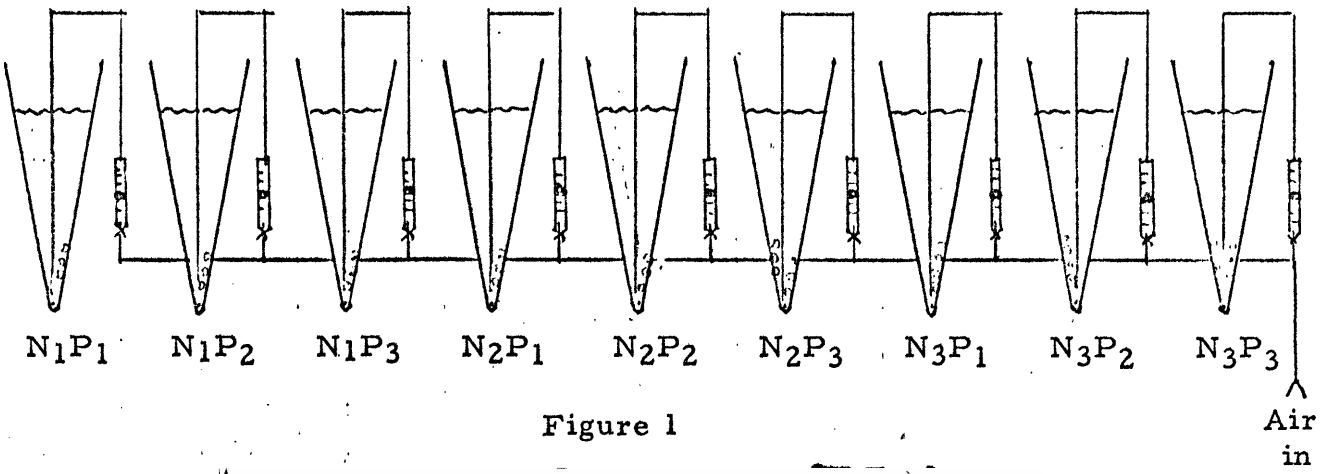


Figure 1

Figure 2

Apparatus

1) Nine 1000 ml Imhoff cones
2) Nine air flow meters
3) 3 mm ID glass tubing (aerators)
4) Tygon tubing
5) Air supply

Operating Conditions

Temperature - room
Air Flow Rate - 1 SCFH

unfiltered carbon analysis (TUC). This will be discussed in the analytical procedure section.

Filtering equipment included millipore assemblies which contained filter holders and flasks. Millipore filters with a 0.45μ pore size were used in the filtration of the sample. Associated equipment with filtration included 60 ml culture tubes which were employed to collect the filtrates.

Analytical apparatus which includes the carbonaceous analyzer, spectrophotometer, etc., will be discussed in the analytical procedure section.

Setting Up Experiment

Nine 100 ml aliquots of acetic acid stock solution (1 ml = 1.50 mg C) were placed in each of nine 250 ml beakers. Each beaker was designated according to Table 1.

The proper volume of nutrients as are specified in Table 2B were added to each beaker to give the proper C:N and C:P ratios desired. The pH of the solution in each beaker was adjusted to 6.0 with 1.0 N NaOH. The contents of each beaker were then placed into each of nine Imhoff cones. The volume was brought up to 1 liter with dilution water. This provided a mixture containing 150 mg/l acetic acid carbon. Concurrently two extra cones were included. These were control samples and included the following:

Control 1 = dilution water + substrate + N_1P_1 and no seed

Control 2 = dilution water + nutrients (N_1P_1) and no seed

Beaker No.	Beaker Desig.	C:N Weight	C:N Mole	C:P Weight	C:P Mole	N:P Weight
1	N_1P_1	2.5/1	2.9/1	10/1	26/1	4/1
2	N_1P_2	2.5/1	2.9/1	20/1	52/1	8/1
3	N_1P_3	2.5/1	2.9/1	30/1	78/1	12/1
4	N_2P_1	5/1	5.8/1	10/1	26/1	2/1
5	N_2P_2	5/1	5.8/1	20/1	52/1	4/1
6	N_2P_3	5/1	5.8/1	30/1	78/1	6/1
7	N_3P_1	10/1	11.6/1	10/1	26/1	1/1
8	N_3P_2	10/1	11.6/1	20/1	52/1	2/1
9	N_3P_3	10/1	11.6/1	30/1	78/1	3/1

(C = substrate carbon)

Table 1

It was desired to do an air stripping experiment with acetic acid and also an ammonia stripping experiment with the second control sample. The pH of the ammonia control was adjusted to pH 8.0.

One ml of previously washed acclimated culture was added to each of the cones, excluding the control samples. Aeration was started and adjusted at a flow rate of 1 scfh.

Sampling

The sample cones were sampled at various time intervals. "O" time samples were taken three minutes after the experiment was started.

The sampling time varied for each experiment. Normally sampling was conducted at "O", 3 hours, 9 hours, 15 hours, 24 hours, 30 hours, 40 hours, etc. An experimental run ran as long as 40 hours.

Sampling procedures were quite simple. At the time of sampling, the volumes in each cone were readjusted to the mark, the difference representing evaporation losses during Δ time. While aeration continued, a 30 ml grab sample was taken from each cone with a 50 ml beaker. Concurrently, small samples were taken in the small test tubes. These samples were used to determine total unfiltered carbon (TUC). Control samples were also taken without additional samples for total unfiltered carbon determinations. This parameter was not measured in the control samples.

After the samples were taken, aeration was stopped, pH's were noted and a new liquid level mark was placed on the glass surface of each cone. Aeration was again set at 1 scfh and started.

The 30 ml samples were filtered through a 0.45μ millipore filter. The filtrates were collected in culture tubes and saved for analysis.

ANALYTICAL PROCEDURES

Total Unfiltered Carbon (TUC)

Total unfiltered carbon was run on samples collected in the small test tubes. This analysis had to be done immediately after sample collection since the biological processes do not stop. The SwRI modified Beckman carbonaceous analyzer was used to determine total carbon in the unfiltered samples. Each sample was shaken vigorously and a 20 microliter sample was taken with a microsyringe and injected into the previously calibrated instrument. The peak height was compared with a calibration curve and values obtained for total carbon.

Total Filtered Carbon (TFC)

Total carbon was concurrently determined on the filtered samples. Again the same technique was employed as in the unfiltered samples.

Total Biological Carbon (TBC)

The difference between the total carbon from the unfiltered and filtered samples constitutes the carbon attributed to the biological growth.

The assumption made here is that all the suspended carbon is organic.

$$TBC = TUC - TFC$$

Inorganic Filtered Carbon (IFC)

A completely new inorganic carbon analyzer was used in this project. The old technique for eliminating the inorganic carbonates,

MODIFIED BECKMAN CARBONACEOUS ANALYZER

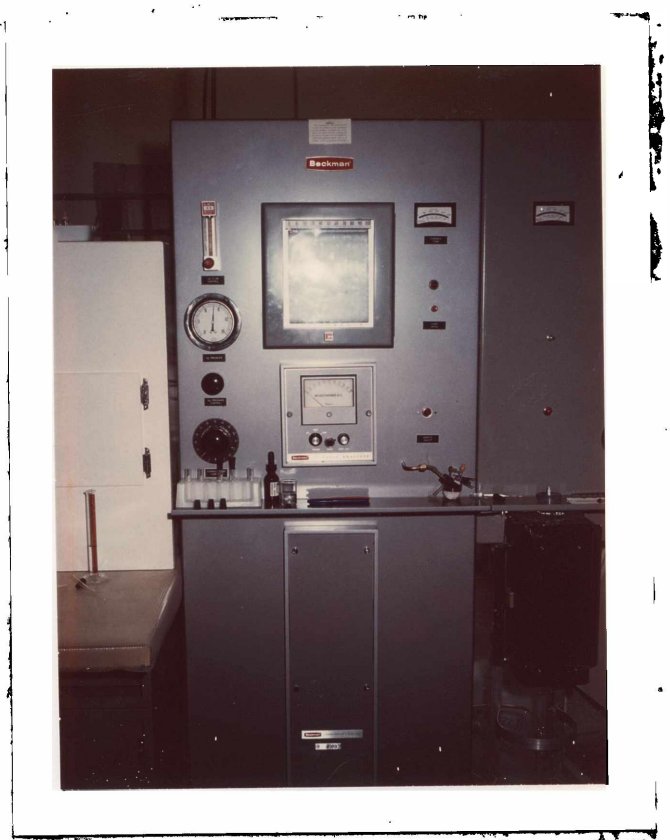


Figure 3

which of course provides a source of error in determining organic carbon, depend on either acid stripping or precipitation with $BaCl_2$. Both of these methods have proven inadequate. In the first case where the CO_2 is stripped there is the danger of stripping any volatile organic compounds. $BaCO_3$ precipitation is never totally complete and is very much pH sensitive.

The new approach does not eliminate the carbonates but provides a tool for actually measuring them. This method proved quite useful since it was possible to measure $H\overset{-}{C}O_3 + \overset{=}{C}O_3$ buildup in the system as the biodegradation of the substrate progressed.

Inorganic carbon was only determined in the filtered samples.

The mechanics of the system will not be discussed since possible employer patents and/or publications are pending.

Organic Filtered Carbon (OFC)

The difference between the total and inorganic carbon in the filtered samples provided the organic carbon in the sample. This parameter represents the substrate carbon.

$$OFC = TFC - IFC$$

Ammonia Nitrogen

Ammonia nitrogen in the filtered samples was determined by the Nesslerization Method.[10] The distillation step was omitted. The only modification was a millipore filtration step after color development.

Absorbances were read on a Spectronic-20 spectrophotometer. Readings were compared to a calibration curve.

Phosphorus

Stannous chloride method for Ortho Phosphate was used.[10] The nutrient used in the project was not an ortho posphate, so that it had to be converted to the proper form to be able to measure it. This was done as per standard methods. The procedure involved acidifying the samples and heating for a period of one hour. This step converted the sodium hexametaphosphate $(NaPO_3)_6$ to a $\equiv PO_4$ form which was then analyzed.

Nitrate ($\bar{N}O_3$)

Nitrate ion was analyzed as per standard methods.[10] In almost all cases it was not necessary to analyze for this parameter. When the method was used it was merely as a qualitative tool rather than a quantitative one.

Nitrite ($\bar{N}O_2$)

Nitrite ion being an intermediate product of nitrification was measured in most cases where it appeared necessary to do so. The Saltzman method[9] was used to measure $\bar{N}O_2$. Again, it was used as a qualitative tool.

Mixed Liquor Suspended Solids (MLSS)

In the early experiments MLSS were measured. However, it was not practical to use this method to measure biological growth. Further discussion will be presented.

MLSS were determined by the millipore filtration method. The sample aliquots were filtered through 0.45μ filters which had previously been tared. After filtration, the filters were dried at 103° C for 30 minutes and desicated for 30 minutes. Change in weight constituted the MLSS.

Calculation

$$\text{MLSS}_{mg/\ell} = \frac{\text{wt. filter + sample - tare filter (1000)}}{\text{volume of sample in ml}}$$

Total Biological Available Carbon (T_bAC)[1]

As was stated earlier, the difference between the total carbon and the inorganic carbon in the filtered samples represents the soluble organic carbon.

The T_bAC represents the total soluble organic carbon that may be metabolized by microorganisms. From the available organic carbon data from each experiment it was possible to calculate the T_bAC.

Calculation

T_bAC = initial organic filtered carbon - final residual organic filtered carbon at ΔT in mg/l

Biodegradable Fraction (BF)

Fraction of organic filtered carbon that is utilized by the microorganisms.

$$BF = \frac{T_bAC}{\text{Total organic filtered carbon}}$$

DATA PREPARATION

All analytical data obtained from each sample was tabulated in specially prepared data work sheets. In addition, all data was plotted on graph paper to observe progression results (Figures 7-15). Examples of data work sheets are presented in Tables 19-20 of the Appendix.

At the conclusion of the experimental work, a complete tabulated summary of all the obtained data was made (Tables 7-15 of the Appendix). From this summary, certain parameters such as nitrogen and phosphorus depletion in the system were extracted.

These values were tabulated after readjusting the "O" times to the initial log growth phase of the cultures (Figures 16-18). The final result of this tabulation was in obtaining averages. These averages for N and P depletion in most cases, represented the result of five experiments.

The tabulated averages for N and P depletions were transferred to Tables 4-5A. This time the tabulation was based on levels of phosphorus and nitrogen. It was necessary to perform this task in order to obtain a better relationship on the interactions of the variables. The data from these tables were plotted (Figures 4-5A).

Biological carbon data from Experiment 3 were extracted from Figures 7-15. This data was tabulated, once again based on levels of nitrogen and phosphorus (Tables 6A-6B). The results of Table 6A were plotted (Figure 6A).

From the nitrogen and phosphorus depletion data found in Tables 4-5A, a statistical analysis of variance between N-P, N-N, and P-P interactions was conducted. The statistical method used was the Sum of Squares method (Tables 3-3C).

Total Biological Available Carbon (T_bAC) and T_bAC/C was calculated and tabulated for Experiment 3 only (Figures 7-15).

STATISTICAL ANALYSIS OF NITROGEN AND PHOSPHORUS INTERACTIONS

Uptake of Phosphorus

Level of N.[1]	Level of P.[2]			Total
	15 P_1	7.5 P_2	5.0 P_3	
N_1 60	3.1	2.0	1.4	6.5
N_2 30	2.5	2.5	2.3	7.3
N_3 15	2.0	2.3	1.8	6.1
Total	7.6	6.8	5.5	19.9

[1] Level of N represent initial nitrogen concentrations in mg/l
[2] Figures under level of P represent maximum P uptakes in mg/l
Table 3

1. Total crude sum of squares =

$$\left[(3.1)^2+(2.0)^2.\text{---}(1.8)^2\right] = 45.89$$

2. Correction due to mean = $(19.9)^2/9 = 44.00$

   Total corrected sum of square = $45.89 - 44.00 = 1.89$

3. Corrected sum of squares between different N↓

$$(6.5^2+7.3^2+6.1^2)/3 - 19.9^2/3 = .25$$

4. Corrected sum of squares between P →

$$7.6^2+6.8^2+5.5^2/3 - 19.9^2/9 = .75$$

5. Interaction sum of squares

$$1.89 - .25 - .75 = .89$$

Analysis of Variance
Summary - Uptake of Phosphorus

Source of Variation	Sum of square	Degrees of Freedom	Mean square	Variance Ratio	F at 1%	F at 5%
Between nitrogen	.25	2	.12	.54	18.0	6.9
Between phosphorus	.75	2	.38	1.8	18.0	6.9
Remainder	.89	4	.22			
Total	1.89	8				

Table 3A

Uptake of Nitrogen

Level of P^1	Level of N^2			Total
	60 N_1	30 N_2	15 N_3	
P_1 15	37	23	15	75
P_2 7.5	26	22	15	63
P_3 5	32	17	15	64
Total	95	62	45	202

[1] Level of P represent initial P concentrations in mg/l
[2] Figures under level of N represent maximum N uptake in mg/l

Table 3B

1. Total crude sum of squares =

$$[37^2 + 23^2 - - - 15^2] = 5046$$

2. Correction due to mean = $(202)^2/9 = 4533.8$

Total corrected sum of squares =

No 1 - No 2 = 512.2

3. Corrected sum of squares between different level of P↓ =

$$[75^2 + 63^2 + 64^2]/3 - 202^2/9 = 29.5$$

4. Corrected sum of squares between nitrogen level N ⟶

$$[95^2 + 62^2 + 45^2]/3 - 202^2/9 = 430.9$$

5. Interaction sum of squares

$$512.2 - 29.5 - 430.9 = 51.8$$

Analysis of Variance
Summary - Uptake of Nitrogen

Source of Variation	Sum of square	Degrees of Freedom	Mean square	Variance Ratio	F at 1%	F at 5%
Between phosphorus	29.5	2	14.8	1.1	18.0	6.9
Between nitrogen	430.9	2	215.5	16.7	18.0	6.9
Remainder	51.8	4	12.9			
Total	512.2	8				

Table 3C

DISCUSSION AND RESULTS

The initial objectives of the project were to study interactions of N-P, N-N, and P-P in their uptake by aerobic microorganism when the initial of NH_3-N and PO_4-P were varied. The substrates under consideration were p-toluic acid and acetic acid. The variable of temperature was also to be considered. However, temperature was held constant and only the biodegradation of acetic acid was studied. Another change that was made was the use of sodium hexametaphosphate $(NaPO_3)_6$ as the phosphorus source instead of an ortho phosphate ($\equiv PO_4$). The use of this compound was necessary because of the possibility of $CaPO_4$ precipitation by using an ortho phosphate. The $(NaPO_3)_6$ compound will not precipitate calcium but possibly form a soluble complex.

The conditions under investigation were as follows:

Desig.	C:N		C:P		N:P
	Weight	Mole	Weight	Mole	weight
1) N_1P_1	2.5/1	2.9/1	10/1	26/1	4/1
2) N_1P_2	2.5/1	2.9/1	20/1	52/1	8/1
3) N_1P_3	2.5/1	2.9/1	30/1	78/1	12/1
4) N_2P_1	5/1	5.8/1	10/1	26/1	2/1
5) N_2P_2	5/1	5.8/1	20/1	52/1	4/1
6) N_2P_3	5/1	5.8/1	30/1	78/1	6/1
7) N_3P_1	10/1	11.6/1	10/1	26/1	1/1
8) N_3P_2	10/1	11.6/1	20/1	52/1	2/1
9) N_3P_3	10/1	11.6/1	30/1	78/1	3/1

(C = substrate carbon)

The nitrogen and phosphorus concentration were varied three times. In N_1, the initial nitrogen concentrations were held constant while the phosphorus varied P_1, P_2 and P_3. For N_2 and N_3 the same approach was used. This made a total of nine Imhoff cones in addition to two control samples for each experiment. A total of five experiments were conducted for N_1, while only two experiments for N_2 and N_3 conditions. These experiments were designated A, B, C, 2 and 3. The results for the N_2 and N_3 conditions correlated well with the N_1 conditions so that it was felt that these data was perhaps sufficient to obtain the desired conclusions. In all cases, the initial acetic carbon concentration was set up at $\approx$ 150 mg/l organic carbon.

In the first three experiments (A, B, C) for the N_1 condition, mixed liquor suspeded solids (MLSS) were determined. These values were to be used as a measure of biological growth. The approach was changed in the other experiments because of the difficulty in measuring MLSS. It was extremely difficult to weigh MLSS especially since we were dealing with very minute weight changes. Absorption of water on the filters produced error of as much as 50 percent. The reason for the minute weights is that we started with essentially zero weight of (MLSS) at "O" time and finished with weights in the order of 2-3 mgs for 25 ml samples taken at termination time. It was not possible to filter larger samples because of filter plugging problems. Perhaps this method for

measuring biological growth is adequate when measuring large weights so that the error due to water absorption is reduced, but in our case, this method proved ineffective.

In the last two experiments, biological growth was measured by the use of the carbon analyzer. The difference between total carbons for the unfiltered (TUC) and filtered (TFC) samples constituted the biological carbon. In experiments designated 2 and 3, this approach was used. The progression curve points attributed to biological carbon turned out quite well in each individual experiment (Figure 7-15). However, there was some variation in total biological carbon between the two experiments (Tables 10-15 of the Appendix). It is quite possible that different mixed cultures were involved in each of the last two experiments. The mixed liquor in experiment 2 had a yellowish appearance while experiment 3 was white. No attempt was made to identify the cultures. Generally, the difference in total biological carbon (TBC) in these two experiments was 10 mg/l.

An interesting observation in all experiments was the increase in soluble inorganic carbon in the system. This increase in all cases paralleled the biological growth (Figures 7-15). This, of course, is not unusual, since one of the products of bio-oxidation of the substrate is CO_2. Of course, since we are aerating with air it would seem as if the $\overline{HCO_3}$ - $\overline{\overline{CO_3}}$ would naturally increase, however, control samples showed no increase

of carbonate, biocarbonate over a period of an experiment (Figure 16). The increase of the $\overline{\overline{CO_3}}$ - $\overline{HCO_3}$ is therefore attributed directly or indirectly to CO_2 evolution from the reaction. The uptake of the substrate by the microorganism leaves an inbalance in the system. This inbalance is an excess of $\overset{+}{Na}$. Therefore, CO_2 either from the bio-oxidation process or from the aeration media immediately balances the system thus increasing the $\overline{HCO_3}$ - $\overline{\overline{CO_3}}$ in the system. It might be an interesting approach to use the parameter of $\overline{\overline{CO_3}}$ - $\overline{HCO_3}$ alkalinity increase in a bio-oxidation system to establish the end of a biochemical reaction.

Air stripping experiments showed that there was substantially no loss of the acetic acid substrate over a period of 40-50 hours at the operating pH and aeration rate (Figure 17). At levels N_1 there was some loss of NH_3 over a period of 30 hours at operating conditions. However, it did not appear substantial, since most of the experimental runs were complete before the 30 hours. See Figure 16. Air stripping controls of NH_3 were not set up at levels N_2 and N_3.

The depletion of nitrogen and phosphorus was quite interesting.

The statistical analysis of variance (Tables 3-3C) indicated the following conclusions:

1) Phosphorus uptake at F 1% level

   a) Nitrogen levels have virtually no effect on the phosphorus uptake.

b) Phosphorus levels have also no effect on the uptake of phosphorus.

2) Nitrogen uptake at F 1% level

a) Phosphorus levels have virtually no effect on the nitrogen uptake

b) Nitrogen levels almost have a significant effect on the uptake of nitrogen.

c) More testing is required.

To further verify the statistical conclusions it was necessary to do a graphical study. A series of tables and their respective graphs were prepared. It was necessary to tabulate and plot the nitrogen and phosphorus depletion data on all possible combination of interactions. (Tables 4-5A and Figures 4-5A). Once this task was performed conclusions were made as to what the interaction between these two parameters were. This required mostly visual observations of tabulated data and graphs.

From these tables and graphs the following conclusions were arrived:

1) Effect of Varying Initial Phosphorus Levels on Nitrogen Depletion (Table 4 - Figure 4)

a) Phosphorus levels have no effect on the uptake of nitrogen.

b) Nitrogen levels have a significant effect on the uptake of nitrogen. Level N_3 was limiting.

2) Effect of Varying Initial Nitrogen Levels on Nitrogen Depletion (Table 4A - Figure 4A)

a) Nitrogen levels have significant effect on the uptake of nitrogen.

3) Effect of Varying Initial Nitrogen Levels on Phosphorus Depletion (Table 5 - Figure 5)

a) Inconclusive - does not appear to be significant.

4) Effect of Varying Initial Phosphorus Levels on Phosphorus Depletion

a) Phosphorus levels do not appear to affect the uptake of phosphorus.

b) Nitrogen levels may have an effect on the uptake of phosphorus.

More testing is needed.

Conclusion - Nitrogen appears to be the controlling factor not only on its uptake but possibly the uptake of phosphorus.

As one can see the graphical interpretation correlated well with the statistical approach.

If the nitrogen levels are significant on the uptake of nitrogen, then the only probable route for the nitrogen is in the synthesis of cells.

A further study was conducted to establish if indeed more cells

were produced at higher nitrogen levels. In this case, the biological carbon data from Experiment 3 was used.

Tables 6A and 6B were used in tabulating the data. From this tabulation, it was once again verified that the nitrogen levels are significant in the increase of biological growth. The data was plotted on Figure 6A. This illustration confirmed that the greater uptake of nitrogen at higher initial nitrogen levels went into producing a larger cell growth.

The uptake of nitrogen and phosphorus by the cells was as follows:

$$\text{Level } N_1 = \frac{53 \text{ mg biological carbon produced}}{32 \text{ mg } NH_3\text{-N taken}} = \frac{1.9}{1} = \frac{C}{N} \text{ (molar)}$$

$$\text{Level } N_2 = \frac{47 \text{ mg biological carbon produced}}{22 \text{ mg } NH_3\text{-N taken}} = \frac{2.4}{1} = \frac{C}{N} \text{ (molar)}$$

$$\text{Level } N_3 = \text{Limiting}$$

$$\text{Level } N_1 = \frac{53 \text{ mg biological carbon produced}}{2.3 \text{ mg P taken}} = \frac{59}{1} = \frac{C}{P} \text{ (molar)}$$

$$\text{Level } N_2 = \frac{47 \text{ mg biological carbon produced}}{2.2 \text{ mg P taken}} = \frac{57}{1} = \frac{C}{P} \text{ (molar)}$$

$$\text{Level } N_3 = \text{Limiting}$$

The obtained values do not appear to agree at all with the classical formula for a cell which is:

$$C_5H_7NO_2$$

This formula indicates that the C:N molar ratio is 5:1. Our results show a molar ratio of 1.9:1 and 2.4:1 for two levels of nitrogen.

This difference would tend to indicate that we might have a loss of NH_3-N. Air stripping experiments (Figure 16) overrules this possibility. Another possibility is the analytical method of measuring NH_3-N. However, great care was taken in determining this parameter. The other possibility is that perhaps the classical formula for cells does not always hold true in mixed culture sustems. We are more inclined to believe the last possibility. However, we cannot completely confirm this deduction, since the cell mass was not analyzed.

Level N_3 was limiting in the experiments. The P levels did not become limiting in the biodegradation of acetic acid in any of the experiments. Good examples of what actually occurs in a limiting situation are observed in Figures 13-15. The T_bAC values drop. Likewise T_bAC/C which represents the fraction of biodegradable substrate decreases. Biomass only grows to the extent at which it has utilized all the nutrient or all the substrate. In a nutrient limiting situation, food is still available, but cells are no longer synthesized. Therefore, the net result is the biodegradable fraction still available is not utilized.

One last observation that was noted in this work was the remaining residual carbon. It is possible that this residual carbon is not attributed

to the initial substrate, but may be attributed to a metabolic product of the culture. To confirm this statement it would be necessary to analyze the sample for acetate ion (Figures 7-15).

EFFECT OF VARYING INITIAL PHOSPHORUS

LEVELS OF NITROGEN DEPLETION

NH_3-N mg/l

Time Hours	N_1 Level			N_2 Level			N_3 Level		
	N_1P_1	N_1P_2	N_1P_3	N_2P_1	N_2P_2	N_2P_3	N_3P_1	N_3P_2	N_3P_3
0	0	0	0	0	0	0	0	0	0
10	13	11	7	12	8	7	13	8	12
20	23	18	20	16	14	14	15	14	15
30	30	23	28	18	17	17	15	15	15
40	35	26	31	20	17	17	15	15	15
50	37	26	32		22				

TABLE 4

EFFECT OF VARYING INITIAL NITROGEN

LEVELS ON NITROGEN DEPLETION

Time Hours	P_1 Level			P_2 Level			P_3 Level		
	N_1P_1	N_2P_1	N_3P_1	N_1P_2	N_2P_2	N_3P_2	N_1P_3	N_2P_3	N_3P_3
0	0	0	0	0	0	0	0	0	0
10	13	12	13	11	8	8	7	7	12
20	23	16	15	18	14	14	20	14	15
30	30	18	15	23	17	15	28	17	15
40	35	20	15	26	17	15	31	17	15
50	37			26	22		32		

TABLE 4A

(Data on these tables is found on tables 16-18)

EFFECT OF VARYING INITIAL PHOSPHORUS

LEVELS ON NITROGEN DEPLETION

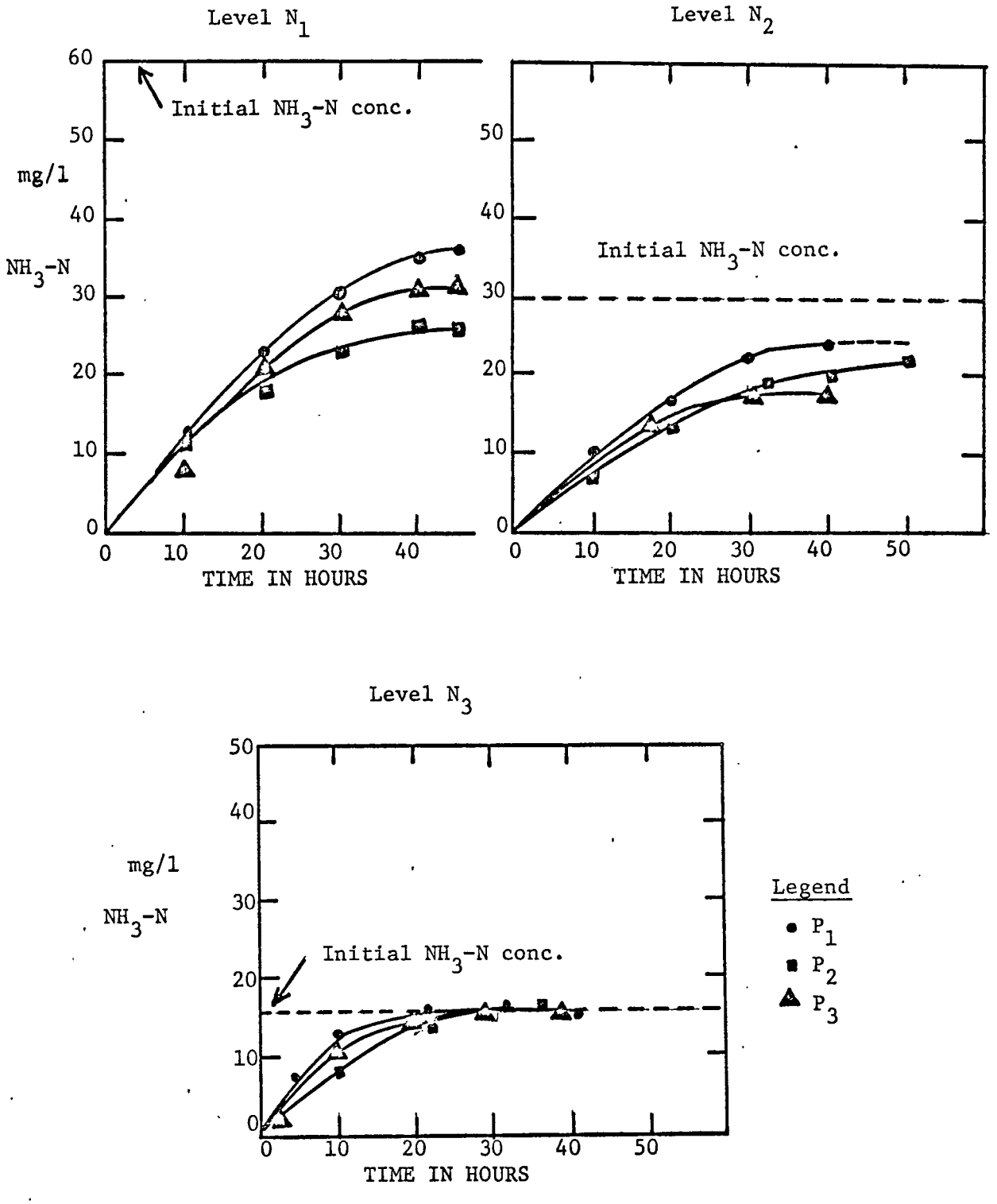


FIGURE 4

EFFECT OF VARYING INITIAL PHOSPHORUS

LEVELS ON PHOSPHORUS DEPLETION

$(NaPO_3)_6$ -P mg/l

Time Hours	N_1 Level			N_2 Level			N_3 Level		
	N_1P_1	N_1P_2	N_1P_3	N_2P_1	N_2P_2	N_2P_3	N_3P_1	N_3P_2	N_3P_3
0	0	0	0	0	0	0	0	0	0
10	.9	.6	.5	1.8	.8	1.7	1.7	1.1	1.0
20	2.0	1.1	1.0	2.4	2.1	2.3	2.0	1.5	1.5
30	3.1	1.8	1.3	2.5	2.5	2.3	2.0	2.3	1.8
40	3.0	2.0	1.4		2.5	2.1	2.0		
50	3.1	1.9	1.4						

TABLE 5A

(Data on these tables is found in tables 16-18)

EFFECT OF VARYING INITIAL NITROGEN

LEVELS ON PHOSPHORUS DEPLETION

Time Hours	P_1 Level			P_2 Level			P_3 Level		
	N_1P_1	N_2P_1	N_3P_1	N_1P_2	N_2P_2	N_3P_2	N_1P_3	N_2P_3	N_3P_3
0	0	0	0	0	0	0	0	0	0
10	.9	1.8	1.7	.6	.8	1.1	.5	1.7	1.0
20	2.0	2.4	2.1	1.1	2.1	1.5	1.0	2.3	1.5
30	3.1	2.5	2.0	1.8	2.5	2.3	1.3	2.3	1.8
40	3.0		2.0	2.0	2.5		1.4	2.1	
50	3.1			1.9			1.4		

TABLE 5

EFFECT OF VARYING INITIAL NITROGEN

LEVELS ON PHOSPHORUS DEPLETION

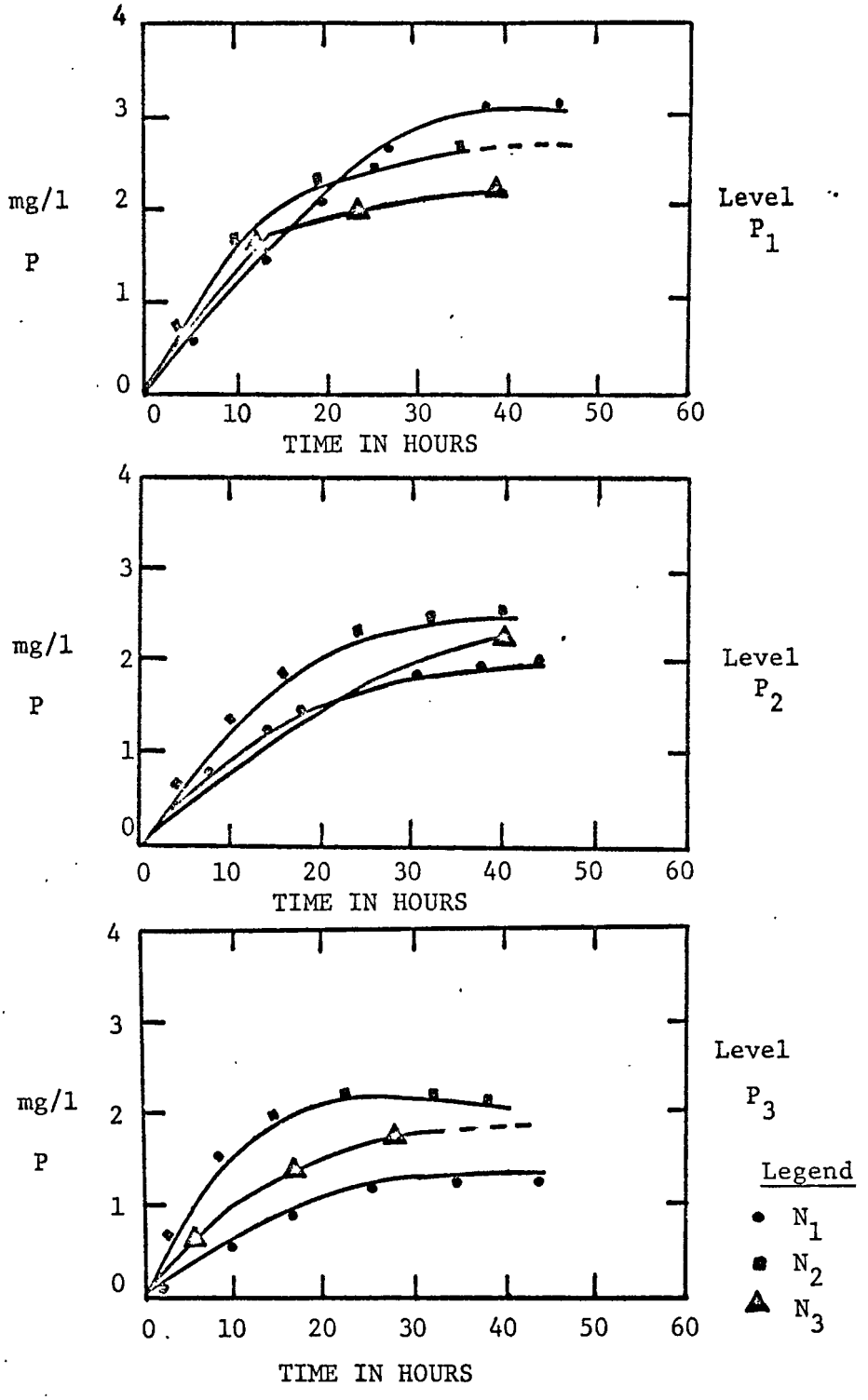


FIGURE 5

EFFECT OF VARYING INITIAL PHOSPHORUS
LEVELS ON PHOSPHORUS DEPLETION

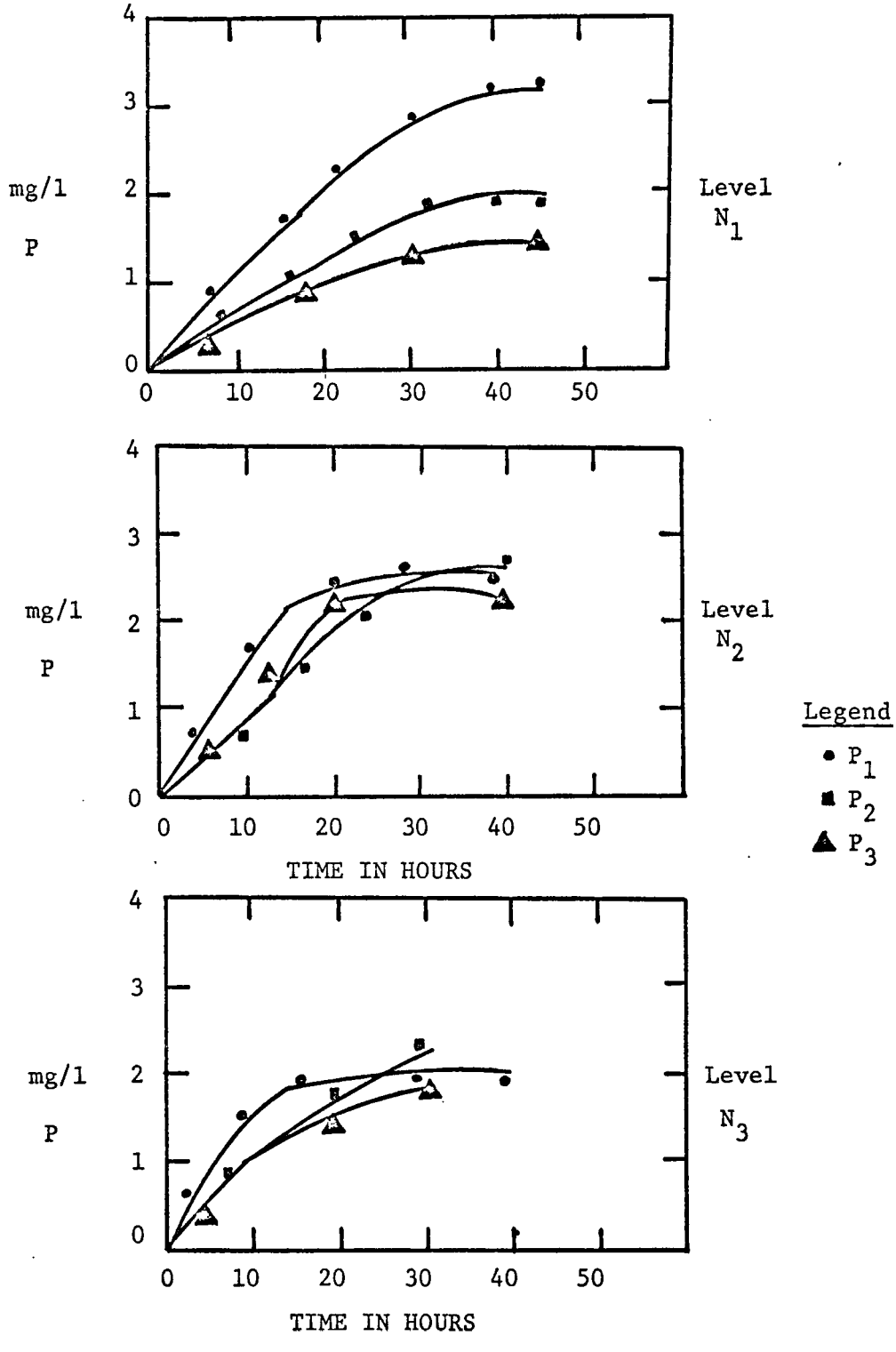
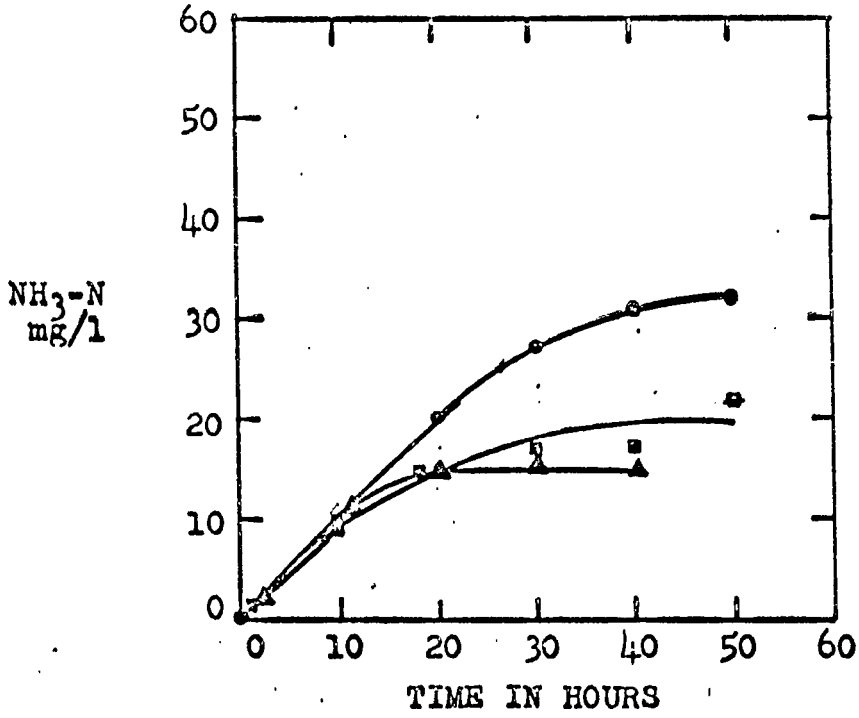


FIGURE 5A

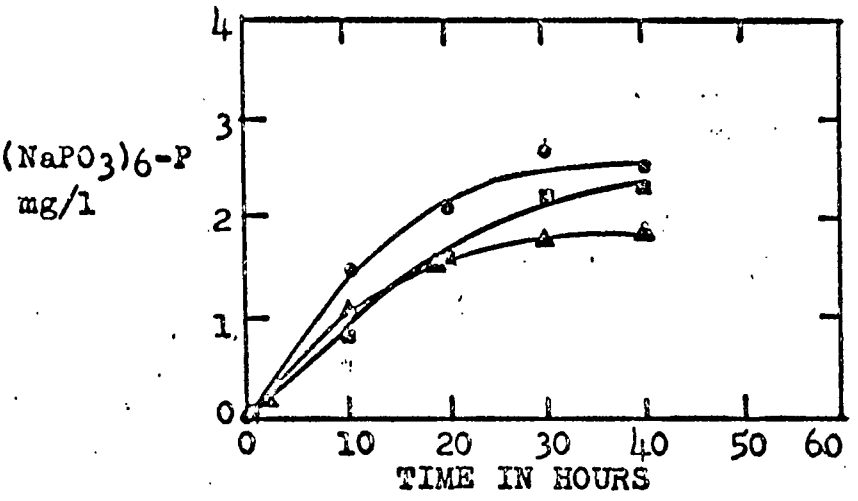
S U M M A R Y

THE EFFECT OF VARYING INITIAL LEVELS

ON THE DEPLETION OF NITROGEN AND PHOSPHORUS



(Refer to Table 4 averages)



(Refer to Table 5A averages)

FIGURE 6

THE EFFECT OF VARYING INITIAL PHOSPHORUS

LEVELS ON BIOLOGICAL GROWTH-EXP. NO. 3.

Biological Carbon mg/1

Time Hours	N_1 Level			N_2 Level			N_3 Level		
	N_1P_1	N_1P_2	N_1P_3	N_2P_1	N_2P_2	N_2P_3	N_3P_1	N_3P_2	N_3P_3
0	0	0	0	0	0	0	0	0	0
10	20	18	15	20	20	10	25	38	30
20	36	34	36	36	42	40	42	45	44
30	48	48	51	42	48	50	41	43	46
40	52	53	54	38	48	46			
50									

TABLE 6A

THE EFFECT OF VARYING INITIAL NITROGEN

LEVELS ON BIOLOGICAL GROWTH

Biological Carbon mg/1

Time Hours	P_1 Level			P_2 Level			P_3 Level		
	N_1P_1	N_2P_1	N_3P_1	N_1P_2	N_2P_2	N_3P_2	N_1P_3	N_2P_3	N_3P_3
0	0	0	0	0	0	0	0	0	0
10	20	20	25	18	20	38	15	10	30
20	36	36	42	34	42	45	36	40	44
30	48	42	41	48	48	43	51	50	46
40	52	38		53	48		54	56	
50									

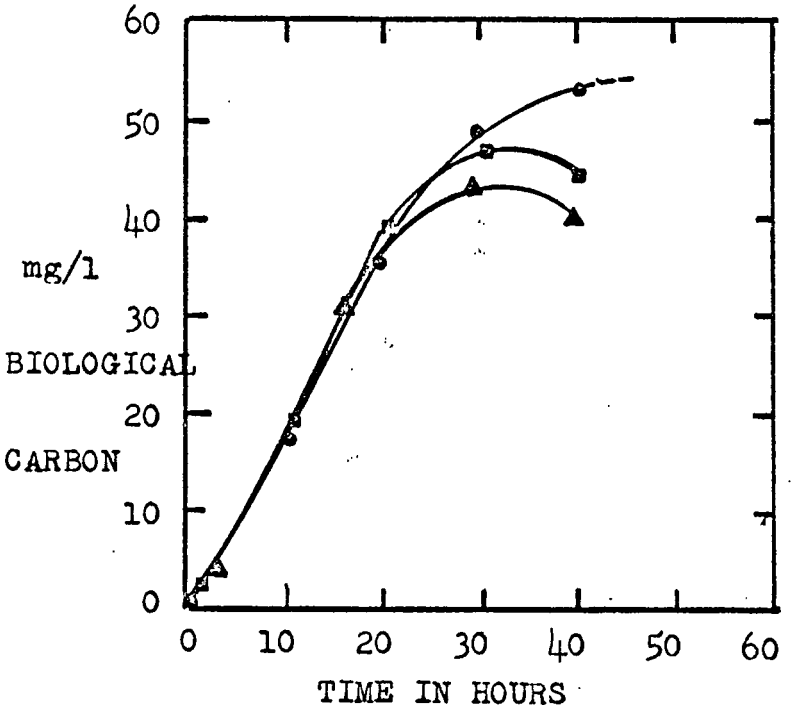
TABLE 6B

(Data on these tables obtained from figure 7-15)

THE EFFECT OF VARYING INITIAL

LEVELS OF NITROGEN

ON BIOLOGICAL GROWTH



(Refer to Table 6A averages)

FIGURE 6A

BIODEGRADATION OF ACETIC ACID

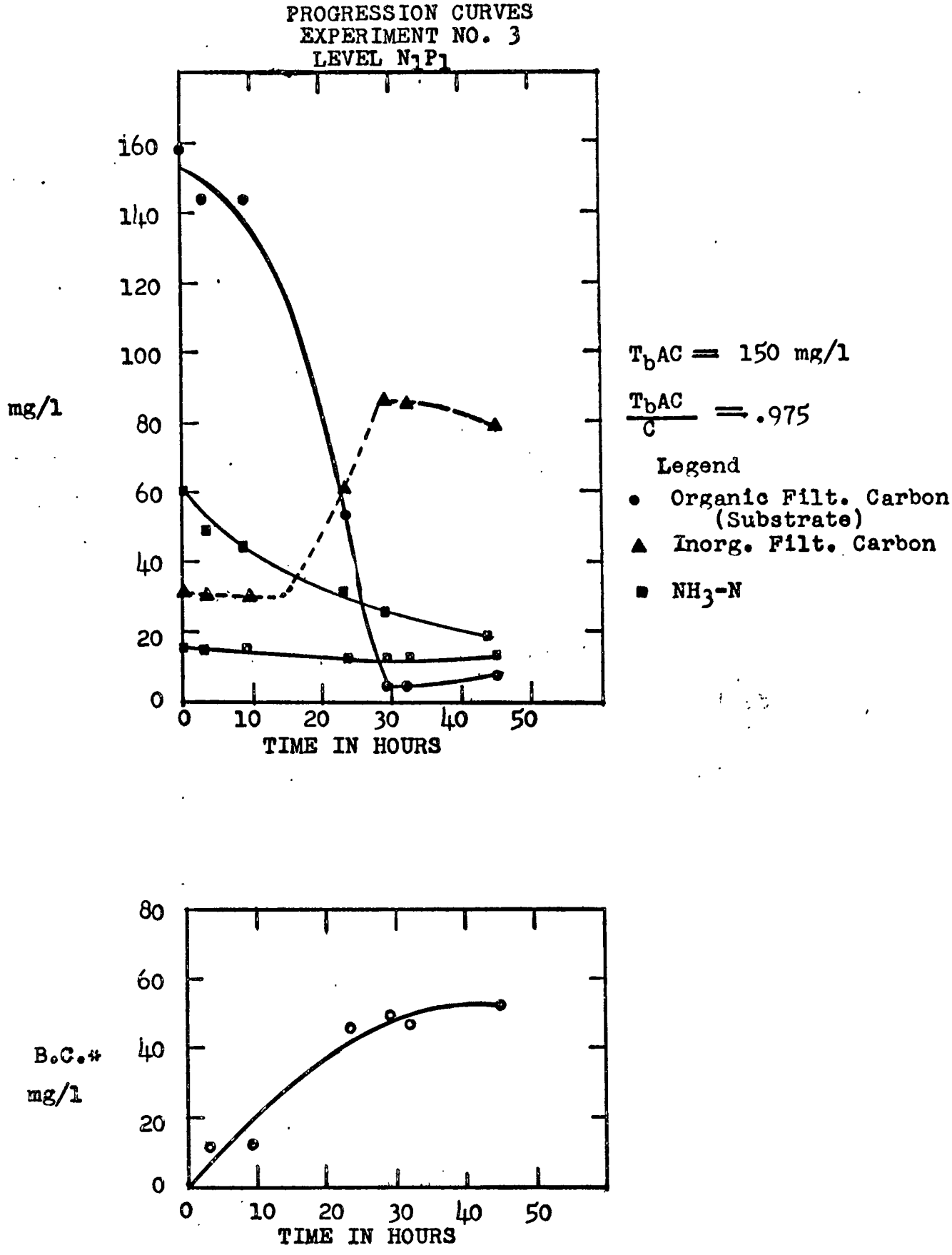
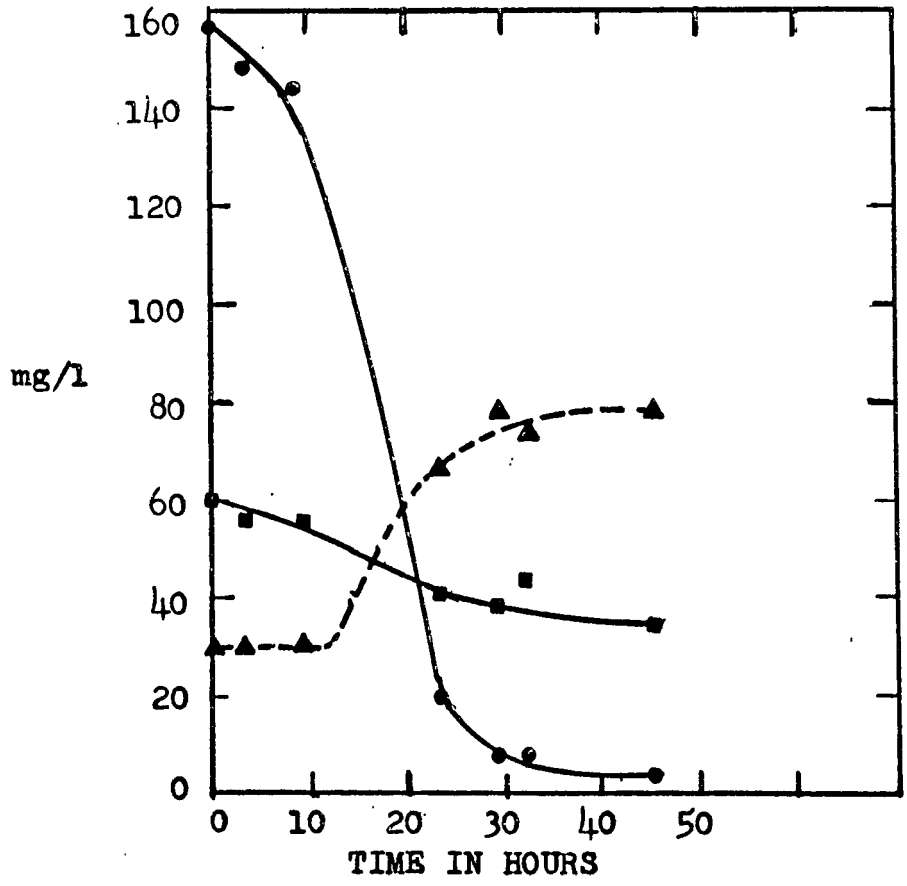


FIGURE 7

BIODEGRADATION OF ACETIC ACID

PROGRESSION CURVES
EXPERIMENT NO. 3

LEVEL N_1P_2

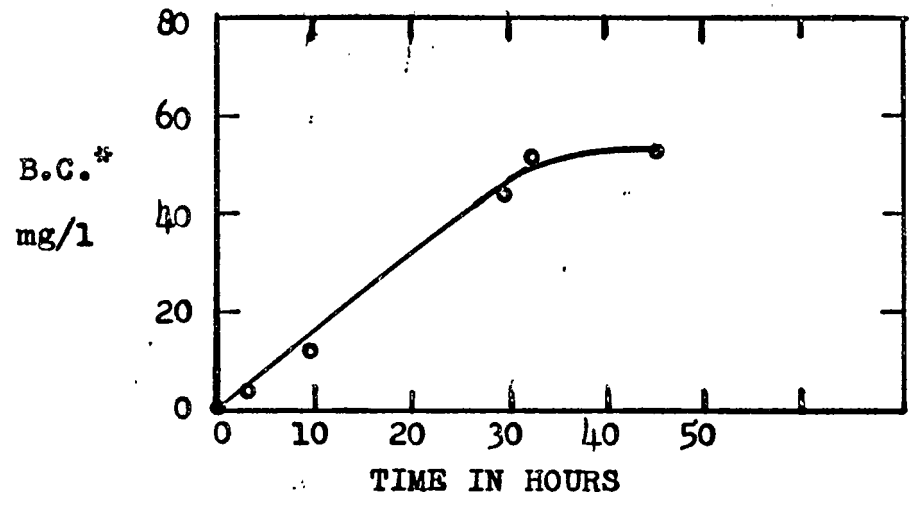


$T_bAC = 152$ mg/l

$\frac{T_bAC}{C} = .975$

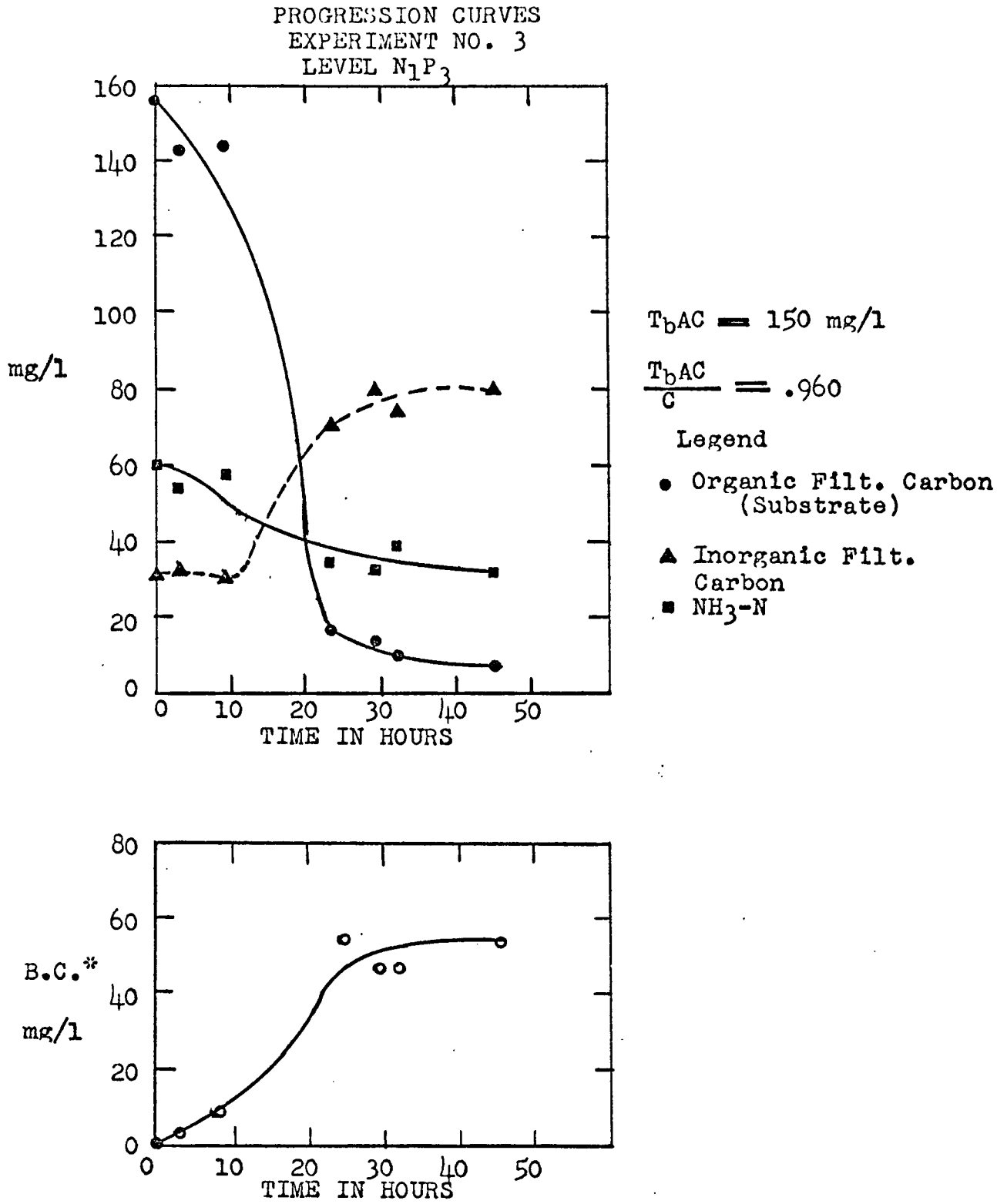
Legend

- Organic Filt. Carbon (Subs.)
- Inorg. Filt. Carbon
- NH_3-N



* Biological Carbon

FIGURE 8

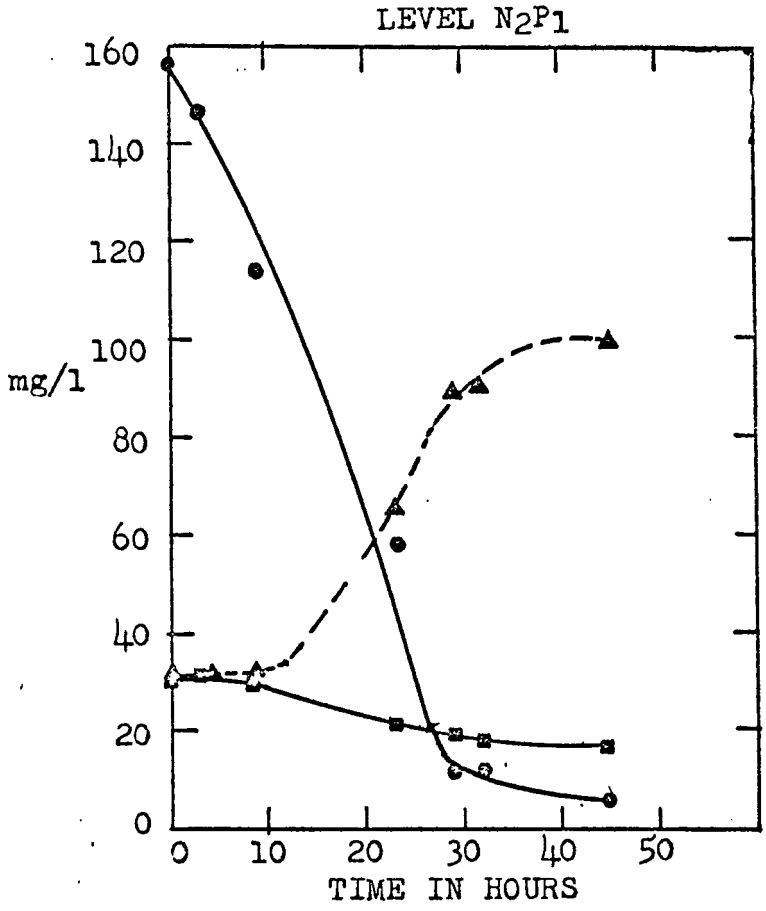


* Biological Carbon

FIGURE 9

BIODEGRADATION OF ACETIC ACID

PROGRESSION CURVES
EXPERIMENT 3

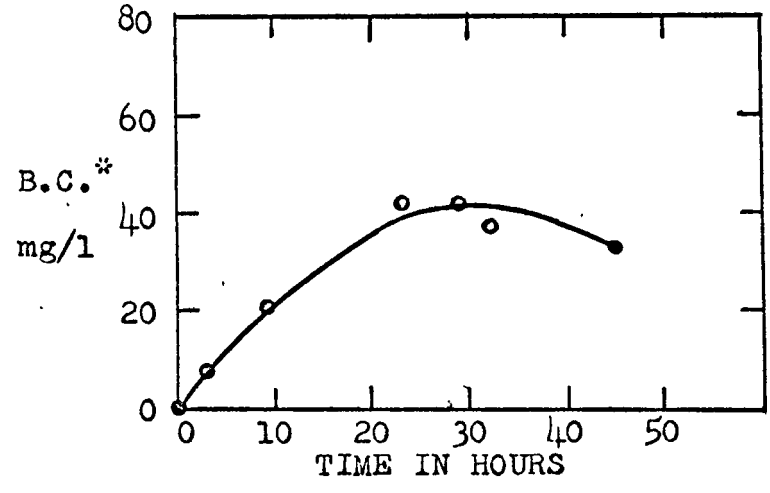


$T_bAC = 150$ mg/l

$\frac{T_bAC}{C} = .960$

Legend

- ● Organic Filt. Carbon (Subs.)
- ▲ Inorg. Filt. Carbon
- ■ NH_3-N



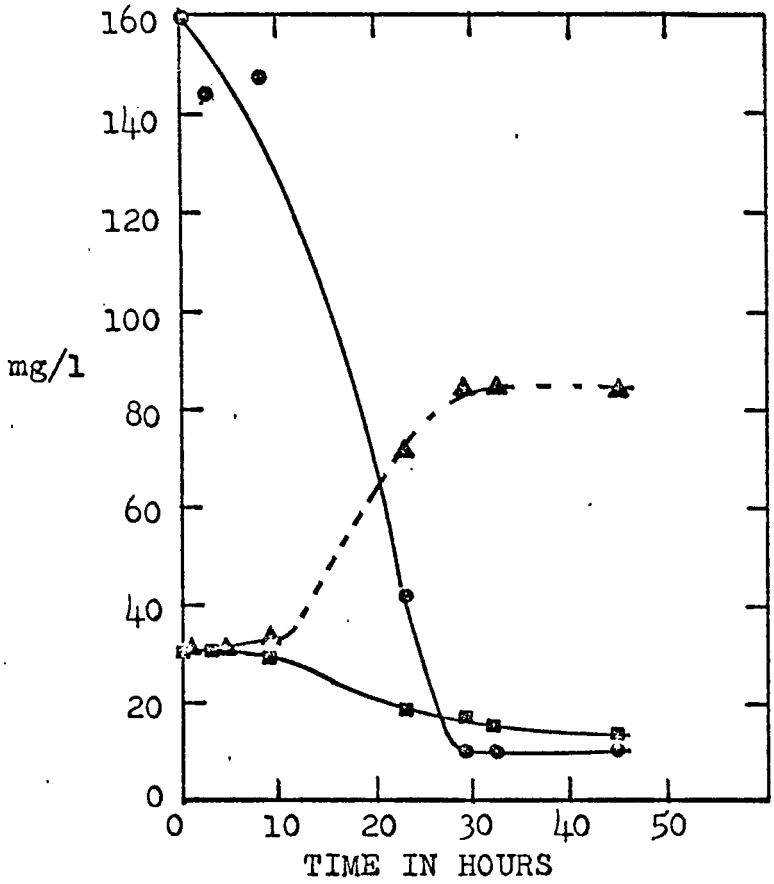
* Biological Carbon

FIGURE 10

BIODEGRADATION OF ACETIC ACID

PROGRESSION CURVES
EXPERIMENT NO. 3

LEVEL N_2P_2



T_bAC — 150 mg/l

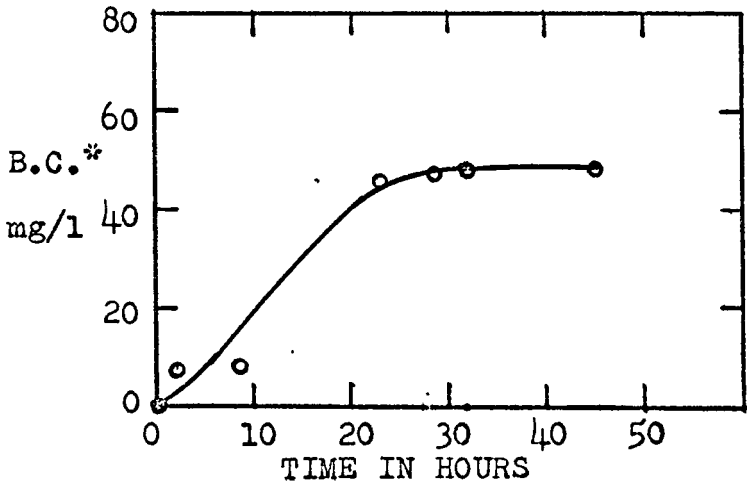
$\frac{T_bAC}{C} = .938$

Legend

● Organic Filt. Carbon (Substrate)

▲ Inorg. Filt. Carbon

■ NH_3-N



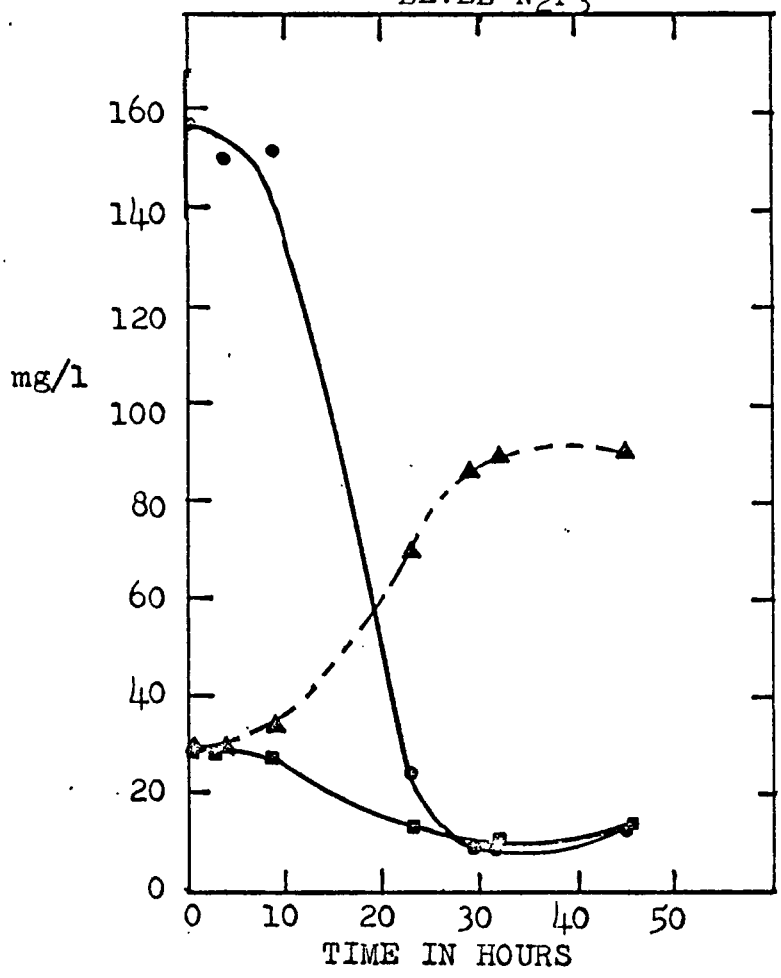
* Biological Carbon

FIGURE 11

BIODEGRADATION OF ACETIC ACID

PROGRESSION CURVES
EXPERIMENT NO. 3

LEVEL N_2P_3

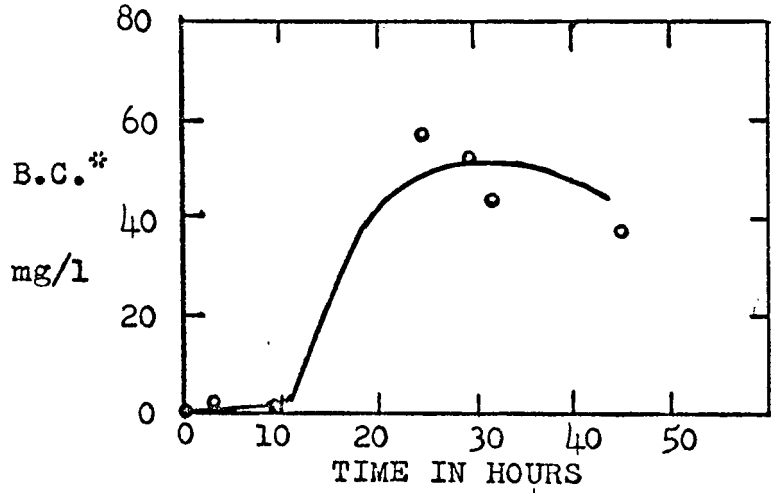


$T_bAC = 148$ mg/l

$\frac{T_bAC}{C} = .950$

Legend

- ● Organic Filt. Carbon (Substrate)
- ▲ Inorg. Filt. Carbon
- ■ NH_3-N



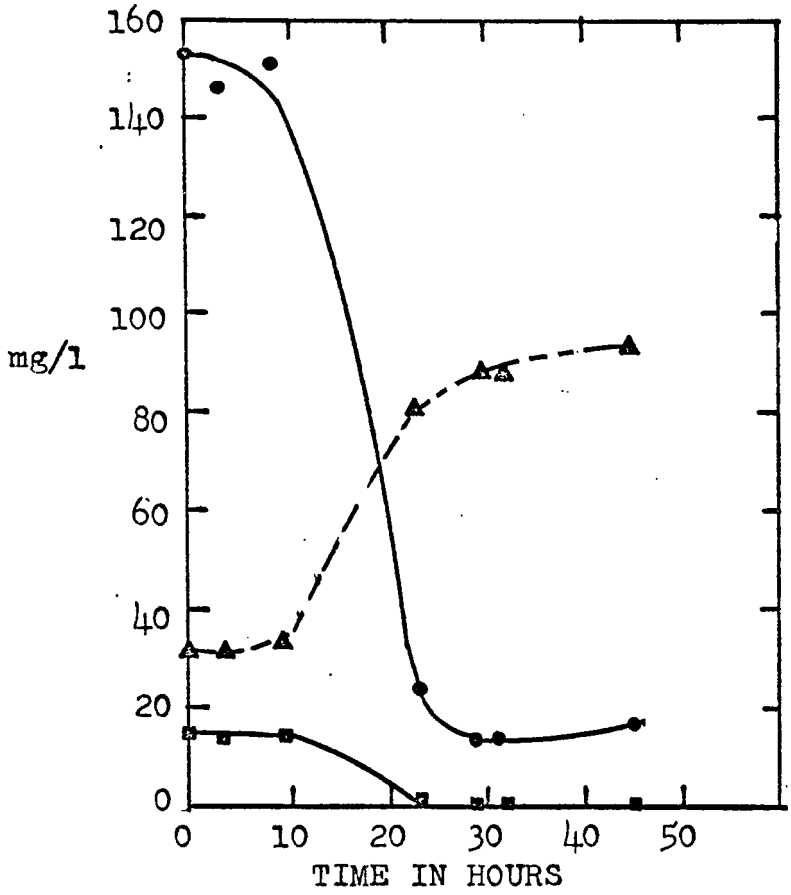
* Biological Carbon

FIGURE 12

BIODEGRADATION OF ACETIC ACID

PROGRESSION CURVES
EXPERIMENT NO. 3

LEVEL N_3P_1

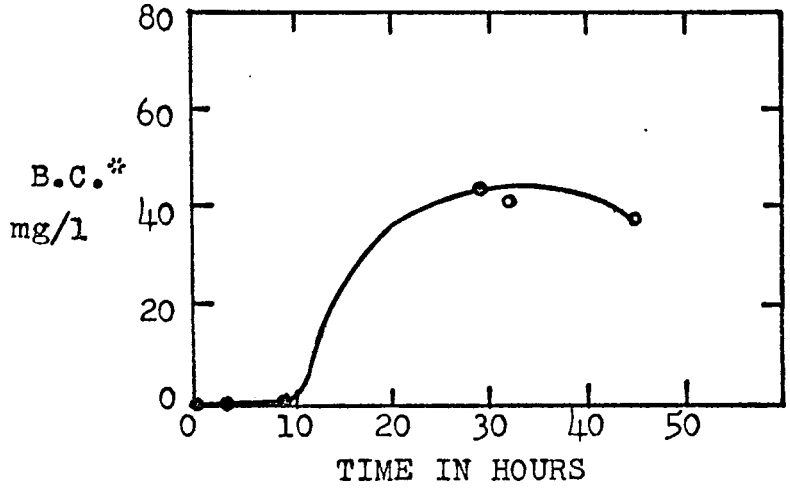


$T_bAC = 141$ mg/l

$\frac{T_bAC}{C} = .915$

Legend

- ● Organic Filt. Carbon (Substrate)
- ▲ Inorg. Filt. Carbon
- ■ NH_3-N



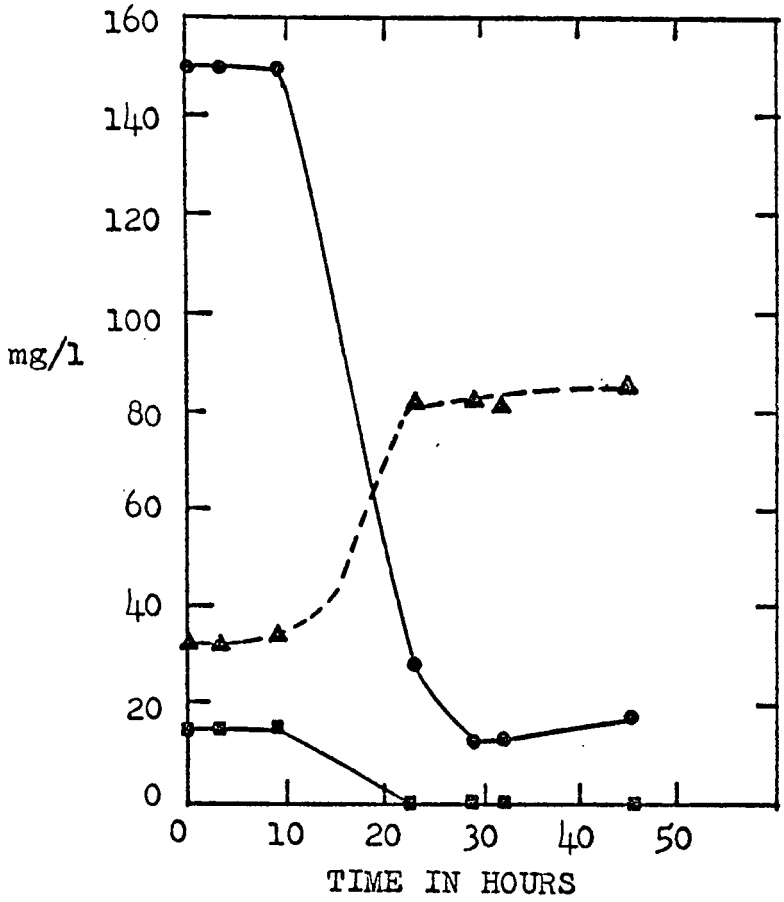
* Biological Carbon

FIGURE 13

BIODEGRADATION OF ACETIC ACID

PROGRESSION CURVES
EXPERIMENT NO. 3

LEVEL N_3P_2

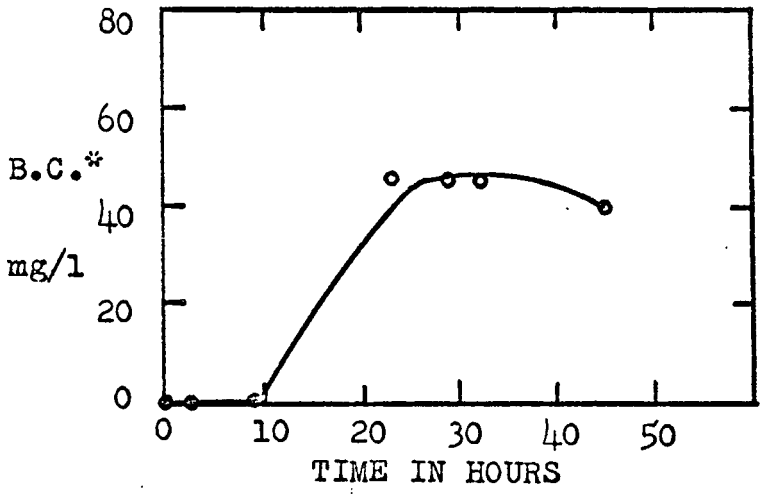


$T_bAC = 138$ mg/l

$\frac{T_bAC}{C} = .918$

Legend

- ● Organic Filt. Carbon (Substrate)
- ▲ Inorg. Filt. Carbon
- ■ NH_3-N



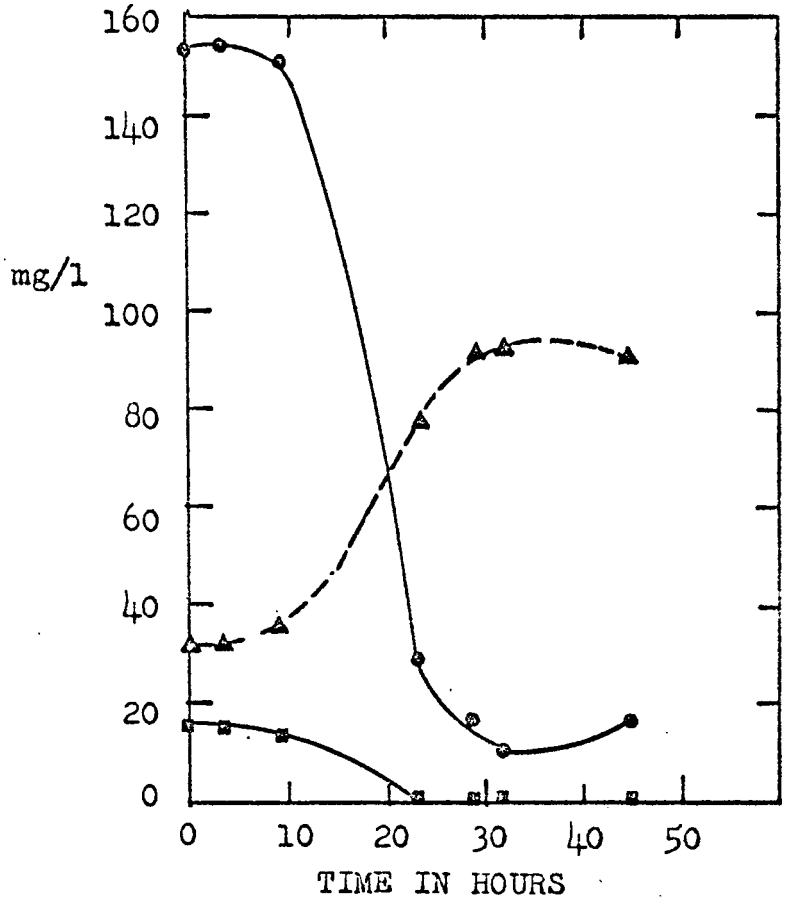
* Biological Carbon

FIGURE 14

BIODEGRADATION OF ACETIC ACID

PROGRESSION CURVES
EXPERIMENT NO. 3

LEVEL N_3P_3

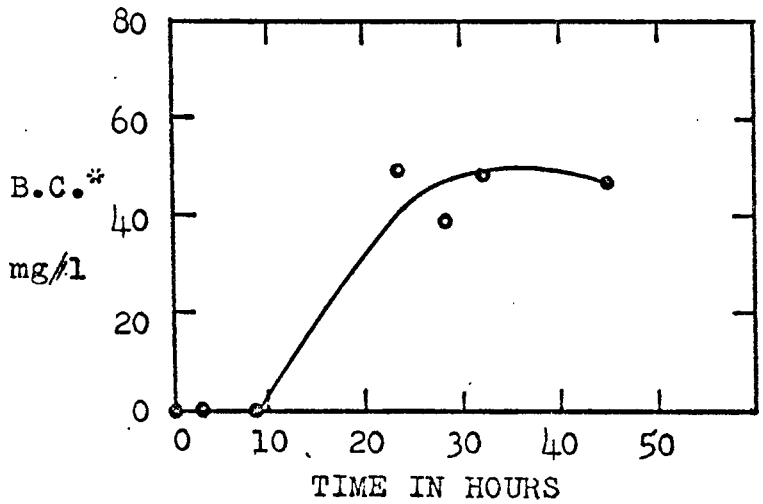


T_bAC = 141 mg/l

$\frac{T_bAC}{C}$ = .926

Legend

- Organic Filt. Carbon (Substrate)
- Inorg. Filt. Carbon
- NH_3-N



* Biological Carbon

FIGURE 15

AIR STRIPPING
OF AMMONIA

PROGRESSION CURVE
EXPERIMENT NO. 3

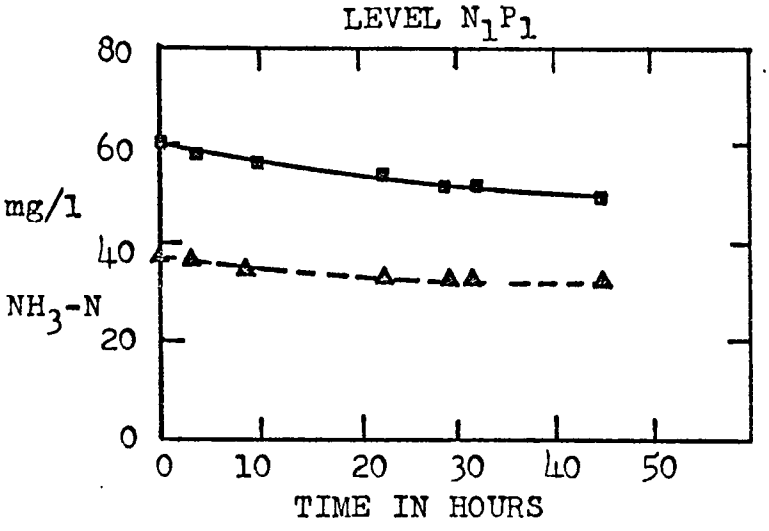


FIGURE 16

OPERATING	CONDITIONS
Air Flow	1 scfh
pH	8.0-8.5

AIR STRIPPING OF ACETIC ACID

PROGRESSION CURVE
EXPERIMENT NO. 2

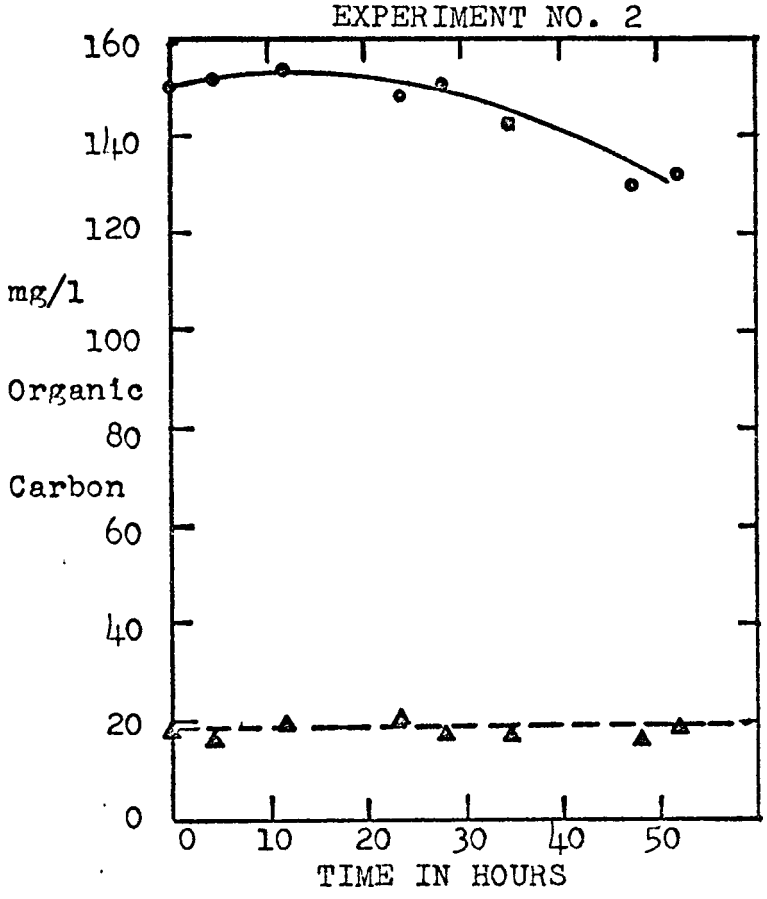


FIGURE 17

Legend

- ● Organic Filt. Carbon (Substrate)
- ▲ Inorg. Filt. Carbon
- ■ NH_3-N

CONCLUSIONS

1) Phosphorus Uptake at F 1% Level (statistical) Tables 3-3C

   a) Nitrogen levels have virtually no effect on the phosphorus uptake.

   b) Phosphorus levels have also virtually no effect on the uptake of phosphorus.

   Above conclusions are also confirmed graphically.

2) Nitrogen Uptake at F 1% Level (statistical) Tables 3-3C

   a) Phosphorus levels have virtually no effect on the nitrogen uptake.

   b) Nitrogen levels are almost significant on the uptake of nitrogen.

   More testing is needed.

   These conclusions are also verified graphically.

3) Cultures can utilize $(NaPO_3)_6$ as a phosphorus source.

4) With further experimentation it will be possible to utilize the modified carbonaceous analyzer to measure biological growth in a bio-oxidation system. Figure 7-15.

5) It has been found that the classical formula for a cell, $C_5H_7NO_2$ may not be completely accurate in mixed culture systems. The C:N molar ratios found in this work were in the order of 1.9:1 and 2.4:1 instead of 5:1 as is stipulated by the above formula.

Maximimum Nitrogen Uptake:

$$\frac{0.6 \text{ grams } NH_3\text{-N}}{\text{gram Biological Carbon}}$$

6) C:P molar ratio was found to be 59:1

Phosphorus Uptake:

$$\frac{.0432 \text{ grams P}}{\text{gram Biological Carbon}}$$

Note: C represents Biological Carbon

REFERENCES

1 Busch, A. W., "Energy, Total Carbon and Oxygen Demand", Water Resources Research, Vol. 2, No. 1, First Quarter 1966, pp. 59-69.

2 Busch, A. W., and Hiser, L. L., "An 8-Hour Biological Oxygen Demand Test Using Mass Culture Aeration", Journal of Water Pollution Control Federation, 36(4) 505, 1964.

3 Connell, C. H., and Vacker, D., "Parameters of Phosphate Removal by Activated Sludge", An Abstract, Seventh Annual Industrial Water and Waste Conference, Texas Water Pollution Control Association, University of Texas, Austin, June 1967, pp. II-1 - II-3.

4 Culp, Russel L., "The Tahoe Process for Nutrient Removal", Seventh Annual Industrial Water and Waste Conference, Texas Water Pollution Control Association, University of Texas, Austin, June 1967, pp. I-3 - I-24.

5 Davies, L. Owens, Design and Analysis of Industrial Experiments, Hafner Publishing Company, N.Y., 1956, Chapter 7, pp. 247-289.

6 Eckenfelder, W. Wesley, Jr., "Opening Remarks", Seventh Industrial Water and Waste Conference, Texas Water Pollution Control Association, University of Texas, Austin, June 1967, pp. I-1 - I-2.

7 "Phosphate Removal Process Prove Practical", Environmental Science and Technology, Vol. 2, No. 3, March 1968, pp. 182-185.

8 "A Nonmyopic Approach to the Problem of Excess Algae Growth", Environmental Science and Technology, Vol. 2, No. 3, March 1968, pp. 188-193.

9 Saltzman, Bernard E., "Determination of Nitrogen Dioxide and Nitric Oxide", Selected Methods for the Measurement of Air Pollutants, HEW, Public Health Service Publication No. 999-AP-11, May 1965, p. G-1.

10 Standard Methods for the Examination of Water and Wastewater, 12th Edition, APHA, AWWA, WPCF, 1965, pp. 193 and 234.

A P P E N D I X

Sample Designation	Exp. Desig.	pH	Temp. °C	Aeration Time Hours	Parameters mg/l					Δ Parameters/Time				
					OFC	IFC	TBC	NH_3-N	$PO_4^{\equiv}$-P	ΔOFC	ΔIFC	ΔTBC	Δ NH_3-N	Δ $PO_4^{\equiv}$-P
N_1P_1	A	7.25		0	147	25		60	13.60	0	0		0	0
		8.20		2.5	152	20		52.5	13.65				7.5	0
		8.25		20.0	96	43		40.0	13.00	51	18		20.0	.6
		8.80		26.5	30	63		30.0	10.52	117	38		30.0	3.1
		8.88		43.0	1.0	75		19.5	11.32	146	50		40.5	2.3
		8.85		51.0	60	72		20.0	11.40	141	47		40.0	2.2
N_1P_2	A	7.26		0	152	25		49	6.4	0	0		0	0
		8.26		2.5	153	19		54	6.0					.4
		8.48		20.5	136	23		39	6.0	16			10	.4
		8.55		26.5	113	26		37	5.9	39	1		12	.5
		8.36		42.5	36	63		23.5	5.0	116	38		25.5	1.4
		8.78		51.0	2	76		22.5	4.2	150	51		26.5	2.2
N_1P_3	A	7.35		0	144	24		70	2.8	0	0		0	0
		8.26		2.5	139	22		63	2.9	5	-		7	0
		8.45		20.5	120	29		59	1.9	24	5		11	.9
		8.56		26.5	80	43		43	2.0	64	19		27	.8
		8.80		42.5	6	72		37	1.3	138	48		33	1.5
		8.80		51.0	1	70		39	1.3	143	46		31	1.5

OFC - Organic Filtered Carbon
IFC - Inorganic Filtered Carbon
TBC - Total Biological Carbon

RESEARCH PROJECT TABULATION OF DATA - Table 7

Sample Designation	Exp. Desig.	pH	Temp. °C	Aeration Time Hours	Parameters mg/l					Δ Parameters/Time				
					OFC	IFC	TBC	NH_3-N	$PO_4^{\equiv}$-P	Δ OFC	ΔIFC	ΔTBC	Δ NH_3-N	Δ $PO_4^{\equiv}$-P
N_1P_1	B	8.00		0	153	35		60	12.5	0	0		0	0
		8.15		3	157	30		55	14.6				5	-
		8.25		6	152	26		59	13.6	1			1	-
		8.20		22	1	90		36.5	10.5	152	55		23.5	2.0
		8.35		27.5	1	82		36	9.2	152	47		24	3.3
		8.32		30.5	3	78		29	8.8	150	43		31	3.7
		8.30		48.5	7	74		28.5	9.4	146	41		31.5	3.1
		8.42		53.5	12	69		27.0	10.3	141	35		33	2.2
N_1P_2	B	7.90		0	144	39		60	5.2	0	0		0	0
		8.18		3	158	25		60	5.5	-	-		0	-
		8.28		6	145	25		58	5.3	-	-		2	-
		8.35		22	37	63		27	4.3	107	24		33	.9
		8.32		27.5	1	77			2.5	143	38			2.7
		8.35		30.5	6	70		23	2.5	138	31		37	2.7
		8.48		48.5	6	70		23	3.0	138	31		37	2.2
		8.47		53.5	7	69		27	3.2	137	30		33	2.0
N_1P_3	B	7.85		0	153	25		60	2.0	0	0		0	0
		8.18		3	155	23		58	2.0	-	-		2	0
		8.24		6	177	23		58	2.2	-	-		2	-
		8.20		24	58	51		31	1.3	95	26		29	.7
		8.28		28	37	63		29	1.2	116	38		31	.8
		8.40		31.5	20	63		27	1.8	133	38		33	.2
		8.52		48.5	11	63		20	.9	142	38		40	1.1
		8.55		53.5	11	63		20	.8	142	38		40	1.2

OFC - Organic Filtered Carbon
IFC - Inorganic Filtered Carbon
TBC - Total Biological Carbon

RESEARCH PROJECT TABULATION OF DATA - TABLE 8

Sample Designation	Exp. Desig.	pH	Temp. °C	Aeration Time Hours	Parameters mg/l					Δ Parameters/Time				
					OFC	IFC	TBC	NH_3-N	$PO_4^{\equiv}$-P	ΔOFC	Δ IFC	Δ TBC	Δ NH_3-N	Δ $PO_4^{\equiv}$-P
N_1P_1	C	7.65		0				59	12.7				0	0
		8.18		3.3				45	12.0				14	.7
		8.32		19.3				31	9.7				28	3.0
		8.41		21.5				26	9.3				33	3.4
		8.54		23.8				31	6.7				28	6.0
		8.64		26.8				27	9.0				32	3.7
		8.65		49.3				27	9.3				32	3.4
N_1P_2	C	7.65		0				52	4.4				0	0
		8.18		3.3				46	4.2				6	.2
		8.35		19.3				36	3.4				16	1.0
		8.38		21.5				30	2.6				22	1.8
		8.54		23.8				32	--				20	--
		8.61		26.8				30	3.0				22	1.4
		8.68		49.3				29	3.8				23	.6
N_1P_3	C			0				52	1.4				0	0
				3.3				51	1.1				1	.3
				19.3				41	.6				11	.8
				21.5				36	.5				16	.9
				23.8				37	.3				15	1.1
				26.8				32	.4				20	1.0
				49.3				25	.4				27	1.0

OFC-Organic Filtered Carbon
IFC - Inorganic Filtered Carbon
TBC - Total Biological Carbon

RESEARCH PROJECT TABULATION OF DATA - TABLE 9

Sample Designation	Exp. Desig.	pH	Temp. °C	Aeration Time Hours	Parameters mg/l					Δ Parameters/Time				
					OFC	IFC	TBC	NH_3-N	$PO_4^{\equiv}$-P	ΔOFC	ΔIFC	ΔTBC	Δ NH_3-N	Δ $PO_4^{\equiv}$-P
N_1P_1	2	6.55		0	140	20	0	60	15	0	0	0	0	0
		7.72		4.5	144	19	0	72	15	-4		0		0
		7.50		11.5	155	15	0	56	15.3	-15		0	4	
		7.30		23.5	148	15	0	73	16.4	-8		0		
				28.0	153	15	0	59	16.4	-13		0	1	
		7.50		34.5	114	21	0	67	15.3	26	1	0		
				47.5	3	55	36	50	14.0	137	35	36	10	1.0
		8.62		52.0	1	60	33	47	13.7	139	40	33	13	1.3
N_1P_2	2	6.70		0	132	24	0	60	7.5	0	0	0	0	0
		7.70		4.5	146	18	0	58	7.0	-14		0	2	.5
		7.60		11.5	157	18	7	51	7.2	-25		7	9	.3
		7.65		23.5	48	54	32	44	6.2	84	30	32	16	1.3
				28.0	4	74	32	40	5.6	128	50	32	20	1.4
		8.20		34.5	1	60	29	33	5.6	131	36	29	27	1.4
				47.5	1	60	34	36	5.2	131	36	34	24	1.8
		8.50		52.0	1	62	33	36	5.2	131	38	33	24	1.8
N_1P_3	2	6.70		0	140	24	0	60	5.0	0	0	0	0	0
		7.80		45	146	18		42	5.3	-6				
		7.68		11.5	163	15	8	59	4.5	-23		8	1	.5
		7.68		23.5	79	39	16	52	4.8	61	15	16	8	.2
				28.0	36	62	16	50	4.7	104	38	16	10	.3
		8.22		34.5	1	60	29	39	4.0	145	36	29	21	1.0
				47.5	1	60	30	42	4.2	145	36	30	18	.8
		8.65		52	1	62	29	49	4.6	145	38	29	11	.4

OFC-Organic Filtered Carbon
IFC - Inorganic Filtered Carbon
TBC - Total Biological Carbon

RESEARCH PROJECT TABULATION OF DATA - TABLE 10

Sample Designation	Exp. Desig.	pH	Temp. °C	Aeration Time Hours	Parameters mg/l					Δ Parameters/Time				
					OFC	IFC	TBC	NH_3-N	$PO_4^{\equiv}$-P	ΔOFC	Δ IFC	ΔTBC	Δ NH_3-N	Δ ≡ PO_4-P
N_2P_1	2	6.70		0	136	24	0	30	15	0	0	0	0	0
		7.75		4.5	146	18	0	30	15	0	0	0	0	0
		7.62		11.5	156	18	8	28	15.6	0	0		2	0
		7.42		23.5	144	16	8	28	16.0	12	0		2	0
				28.0	146	18	0	29	15	10			1	0
		7.75		34.5	97	29	8	23	15	59	5	8	7	0
				47.5	1	62	36	10	12.7	155	38	36	20	2.3
		8.78		52.0	1	66	38	9	12.3	155	42	38	21	2.7
N_2P_2	2	6.90		0	132	24	0	30	7.5	0	0		0	0
		7.80		4.5	146	18	0	22	7.4	0			8	.1
		7.65		11.5	161	21	0	23	7.4	0			7	.1
		7.72		23.5	62	52	20	12	6.1	89	28		18	1.4
				28.0	20	70	24	11	5.3	141	46		19	2.2
		8.22		34.5	4	64	30	8	5.3	157	40		22	2.2
				47.5	4	60	34	16	5.2	157	36		--	2.3
		8.70		52	1	68	34	4	5.3	160	44		26	2.2
N_2P_3	2	6.90		0	134	22	0	30	5.0	0	0		0	0
		7.85		4.5	149	15	0	24	5.5	0			6	--
		7.70		11.5	150	18	14	29	5.1	0			1	--
		7.68		23.5	84	42	12	22	6.0	66	20		8	--
				28.0	46	56	8	17	4.3	104	34		13	.7
		8.15		34.5	2	62	34	16	4.2	148	40		14	.8
				47.5	2	59	33	13	4.7	148	37		17	.3
		8.75		52.0	1	66	36	14	4.6	149	44		16	.4

OFC-Organic Filtered Carbon
IFC - Inorganic Filtered Carbon
TBC - Total Biological Carbon

RESEARCH PROJECT TABULATION OF DATA - TABLE 11

Sample Designation	Exp. Desig.	pH	Temp. °C	Aeration Time Hours	Parameters mg/l					Parameters/Time				
					OFC	IFC	TBC	NH_3-N	$PO_4^{\equiv}$-P	ΔOFC	ΔIFC	ΔTBC	Δ NH_3-N	Δ $PO_4^{\equiv}$-P
N_3P_1	2	6.90		0	134	22	0	15	15					0
		7.92		4.5	137	19	-	10.3	15					0
		7.69		11.5	151	19	-	9.2	15					0
		7.40		23.5	141	19	0	11.4	15					0
				28.0	140	28	0	13.0	15.4					0
		7.62		34.5	124	18	0	13.8	15					0
				47.5	117	18	0	13.4	15					0
		8.25		52.0	130	22	0	8.8	15.3					0
N_3P_2	2	7.00		0	136	16	0	15	7.5	0	0		0	0
		7.80		4.5	138	18	-	13.2	7.4	0	0		1.8	.1
		7.65		11.5	160	18	-	14.8	7.6	0	0		.2	0
		7.35		23.5	142	18	-	15.2	7.4	18	0		---	.1
				28.0	133	27	-	14.2	7.4	27	9	8	.8	.1
		7.50		34.5	90	32	8	12.8	6.6	70	14	32	2.2	.9
				47.5	1	62	32	1.0	5.6	159	44	28	14.0	1.9
		8.70		52.0	1	70	28	2.6	5.6	159	52		12.4	1.9
N_3P_3	2	6.95		0	138	18	0	15	5.0	0	0	0	0	0
		8.00		4.5	142	18	0	11	4.5	0	0	0	4	.5
		7.69		11.5	160	18	0	14.2	4.5	0	0	0	.8	.5
		7.35		23.5	132	28	0	12.4	4.9	28	10	0	2.6	.1
				28	148	18	0	12.2	4.5	12	0		2.8	.5
		7.55		34.5	123	19	5	10.6	4.6	37	1	5	4.4	.4
				47.5	6	58	42	6.0	3.3	154	40	42	9.0	1.7
		8.68		52	4	68	26	3.4	3.2	156	50	26	11.6	1.8

OFC-Organic Filtered Carbon
IFC - Inorganic Filtered Carbon
TBC - Total Biological Carbon

RESEARCH PROJECT TABULATION OF DATA - TABLE 12

Sample Designation	Exp. Desig.	pH	Temp. °C	Aeration Time Hours	Parameters mg/l					Δ Parameters/Time				
					OFC	IFC	TBC	NH_3-N	$PO_4^{\equiv}$-P	ΔOFC	ΔIFC	ΔTBC	Δ NH_3-N	Δ $PO_4^{\equiv}$-P
N_1P_1	3	7.90		0	158	32	0	60	15	0	0	0	0	0
		8.50		3	144	30	12	49	15	14	-2	12	11	0
		8.52		9	144	30	12	43	15	14	-2	12	17	0
		8.50		23	54	60	46	31	13.4	104	28	46	29	1.6
		8.74		29	4	86	49	25	13.0	154	54	49	35	2.0
		8.80		32	4	84	47	34	12.7	154	52	47	26	2.3
		8.80		45	8	78	53	19	13	150	46	53	41	2.0
N_1P_2	3	7.98		0	156	30	0	60	7.6	0	0	0	0	0
		8.50		3	148	30	4	55	7.6	8	0	4	5	0
		8.55		9	144	30	12	59	7.6	12	0	12	1	0
		8.58		23	20	66	66	40	4.8	136	36	66	20	2.8
		8.82		29	8	78	44	38	4.8	148	48	44	22	2.9
		8.85		32	8	74	52	43	4.7	148	44	52	17	2.9
		8.82		45	4	78	52	34	5.1	152	48	52	26	2.5
N_1P_3	3	7.98		0	156	30	0	60	5.0	0	0	0	0	0
		8.48		3	142	32	4	54	4.5	14	-2	4	6	.5
		8.55		9	144	30	8	48		12	0	8	2	
		8.55		23	16	70	60	34	3.0	140	40	60	26	2.0
		8.84		29	14	80	46	32	2.8	142	50	46	28	2.2
		8.88		32	10	74	46	39	2.6	146	44	46	21	2.4
		8.82		45	6	80	53	32	3.1	150	50	53	28	1.9

OFC-Organic Filtered Carbon
IFC - Inorganic Filtered Carbon
TBC - Total Biological Carbon

RESEARCH PROJECT TABULATION OF DATA - TABLE 13

Sample Designation	Exp. Desig.	pH	Temp. °C	Aeration Time Hours	Parameters mg/l					Δ Parameters/Time				
					OFC	IFC	TBC	NH_3-N	$PO_4^{\equiv}$-P	ΔOFC	ΔIFC	Δ TBC	Δ NH_3-N	Δ $PO_4^{\equiv}$-P
N_2P_1	3	8.10		0	156	30	0	30	15	0	0	0	0	0
		8.54		3	146	32	8	31	14.7	10	2	8	0	.3
		8.59		9	114	32	20	30	14.7	42	2	20	0	.3
		8.60		23	58	65	42	20.5	13	98	35	42	9.5	2.0
		8.80		29	11	89	42	19.5	13	145	59	42	10.5	2.0
		8.88		32	12	90	37	17.5	12.7	144	60	37	12.5	2.3
		8.90		45	6	100	33	17.0	12.7	150	70	33	13.0	2.3
N_2P_2	3	8.10		0	159	30	0	30	7.6	0	0	0	0	0
		8.55		3	145	33	8	30	7.4	14	3	8	0	.3
		8.60		9	152	34	8	29	7.4	7	4	8	1	.3
		8.60		23	42	72	46	19	4.6	117	42	46	11	2.8
		8.85		29	10	84	48	17	4.6	149	54	48	13	2.8
		8.90		32	10	84	48	14.5	4.6	149	54	48	15.5	2.8
		8.90		45	10	84	48	13.0	4.6	149	54	48	17.0	2.8
N_2P_3	3	8.10		0	156	30	0	30	5.0	0	0	0	0	0
		8.55		3	148	30	4	27	3.9	8	0	4	3	1.1
		8.59		9	152	34	0	27	4.3	4	4	0	3	.7
		8.71		23	24	70	62	11	2.3	132	40	62	19	2.7
		8.92		29	8	86	52	8.5	2.3	148	56	52	21.5	2.7
		8.92		32	8	90	41	10.0	3.2	148	60	41	20.0	1.8
		8.90		45	12	90	37	13.5	3.0	144	60	37	16.5	2.0

OFC-Organic Filtered Carbon
IFC - Inorganic Filtered Carbon
TBC - Total Biological Carbon

RESEARCH PROJECT TABULATION OF DATA - TABLE 14

Sample Designation	Exp. Desig.	pH	Temp. °C	Aeration Time Hours	Parameters mg/l					Δ Parameters/Time				
					OFC	IFC	TBC	NH_3-N	$PO_4^{\equiv}$-P	ΔOFC	Δ IFC	ΔTBC	Δ NH_3-N	Δ $PO_4^{\equiv}$-P
N_3P_1	3	8.10		0	154	32	0	15	15	0	0	0	0	0
		8.60		3	146	32	8	13	15	8	0	8	0	0
		8.60		9	156	34	0	15	15	-2	2	0	0	0
		8.68		23	24	82	54	.6	13	130	50	54	14.4	2.0
		8.90		29	13	89	44	.6	13	141	57	44	14.4	2.0
		8.92		32	15	87	40	0	13	139	55	40	15	2.0
		8.90		45	17	93	36	0	13	137	61	36	15	2.0
N_3P_2	3	8.15		0	150	32	0	15	7.6	0	0	0	0	0
		8.60		3	150	32	0	15	7.6	0	0	0	0	0
		8.62		9	152	34	0	15	7.4	-2	2	0	0	.2
				23	28	82	46	1	4.6	122	50	46	14	3.0
		8.88		29	12	82	45	0	5.0	138	50	45	15	2.6
		8.92		32	14	80	45	0	5.0	136	48	45	15	2.6
		8.90		45	17	85	40	0	5.4	133	53	40	15	2.2
N_3P_3	3	8.20		0	152	32	0	15	5.0	0	0	0	0	0
		8.60		3	154	32	0	14.5	4.2	-2	0	0	.5	.8
		8.61		9	151	35	0	14.0	5.0	1	3	0	1.0	0
		8.75		23	29	77	54	.2	4.4	123	45	54	14.8	.6
		8.86		29	17	92	36	0	4.0	135	60	36	15.0	1.0
		8.92		32	10	92	50	0	3.4	142	60	50	15.0	1.6
		8.92		45	16	90	46	0	4.1	136	58	46	15.0	.9

OFC-Organic Filtered Carbon
IFC - Inorganic Filtered Carbon
TBC - Total Biological Carbon

RESEARCH PROJECT TABULATION OF DATA - TABLE 15

	* Time Hours	Δ NH_3-N Depletion mg/l Experiments						Δ $(NaPO_3)_6$ -P Depletion mg/l Experiments					
		A	B	C	2	3	Aver.	A	B	C	2	3	Aver.
N_1P_1	0	0	0	0		0	0	0	0	0		0	0
	10	11	11	17		12	13	.3	.6	1.8		1.0	.9
	20	21	21	27		22	23	1.4	1.9	3.1		1.7	2.0
	30	28	28	33		30	30	3.1	3.3	3.7		2.2	3.1
	40	35	33	25		38	35	2.9	3.3	3.7		2.2	3.0
	45	38	34	25		40	37	2.7	3.1	3.6		3.1	3.1
N_1P_2	0	0	0	0	0	0	0	0	0	0	0	0	0
	10	12	10	12	7	12	11	.1	.3	.8	.6	1.0	.6
	20	20	28	19	15	19	18	.4	.7	1.4	1.0	2.2	1.1
	30	25	36	23	22	23	23	.6	2.7	1.4	1.4	2.9	1.8
	40	29	37	25	25	25	26	1.2	2.6	1.1	1.6	2.7	2.0
	45	28	36	25	25	26	26	1.6	2.5	.8	1.8	2.2	1.9
N_1P_3	0	0	0	0	0	0	0	0	0	0	0	0	0
	10	10	9	3		7	7	.3	.2	.6	.4	1.2	.5
	20	21	24	13		20	20	.7	.6	1.0	.9	1.8	1.0
	30	29	33	23		28	28	1.2	.9	1.2	1.0	2.2	1.3
	40	33	27	26		31	31	1.4	1.1	1.1	.9	2.1	1.4
	45	33	29	27		32	32	1.5	1.1	1.1	.9	1.9	1.4

* "0" Time represents microorganisms in the initial log growth phase.

Table 16

COMPUTATION OF AVERAGES FOR NITROGEN AND PHOSPHORUS DEPLETIONS

	* Time Hours	Δ NH_3-N Depletion mg/l Experiments						Δ $(NaPO_3)_6$ -P Depletion mg/l Experiments					
		A	B	C	2	3	Aver.	A	B	C	2	3	Aver.
N_2P_1	0				0	0	0				0	0	0
	10				15	8	12				1.9	1.6	1.8
	20				21	11	16				2.7	2.1	2.4
	30				33	13	23				2.7	2.4	2.5
	40					14							
N_2P_2	0				0	0	0				0	0	0
	10				6	10	8				1.0	.6	.8
	20				14	14	14				2.1	2.2	2.1
	30				20	16	18				2.3	2.8	2.5
	40				23	17	20				2.3	2.8	2.5
	50				25	18	22						
N_2P_3	0				0	0	0					0	0
	10				5	8	7					1.7	1.7
	20				10	17	14					2.3	2.3
	30				13	20	17					2.3	2.3
	40				15	19	17					2.1	2.1
	50				16								

* "0" Time represents microorganisms in the initial log growth phase.

Table 17

COMPUTATION OF AVERAGES FOR NITROGEN AND PHOSPHORUS DEPLETIONS

	* Time Hours	Δ NH_3-N Depletion mg/l Experiments						Δ $(NaPO_3)_6$ -P Depletion mg/l Experiments					
		A	B	C	2	3	Aver.	A	B	C	2	3	Aver.
N_3P_1	0					0	0					0	0
	10					13	13					1.7	1.7
	20					15	15					2.0	2.0
	30					15	15					2.0	2.0
	40					15	15					2.0	2.0
N_3P_2	0				0	0	0				0	0	0
	10				3	12	8				.9	1.2	1.1
	20				12	15	14				1.8	2.2	1.5
	30				15	15	15				1.9	2.6	2.3
	40				15	15	15						
N_3P_3	0				0	0	0				0	0	0
	10				1	12	12				.7	1.3	1.0
	20				4	15	15				1.3	1.7	1.5
	30				7	15	15				1.7	1.8	1.8
	40				12	15	15						

* "0" Time represents microorganisms in the initial log growth phase.

Table 18

COMPUTATION OF AVERAGES FOR NITROGEN AND PHOSPHORUS DEPLETIONS

Table 19

RESEARCH PROJECT

Run No: Substrate: Date:

	Description	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2
	D.O. mg/l pH										
NH_3-N	Volume Sx DF Spec Read. μgNH_3-N mg/lNH_3-N										
$PO_4^{\equiv}$-P	Volume Sx DF Spec. Read. μgPO_4-P mg/l PO_4-P										
NO_3^--N	Volume Sx DF Spec. Read. μgNO_3-N mg/l NO_3-N										
NO_2^--N	Volume Sx DF Spec. Read. μl NO_2-N mg/l NO_2-N										
C A R B O N	Tot Unfil. Car. Reading mg/l										
	Total Filt Car. Reading mg/l										
	Filt. Inorg. Carb. Reading mg/l										
	Filtered Organic										

Calculations:

1. To convert μgNH_3-N, $PO_4^{\equiv}$-P, NO_3^-N to mg/l = $\frac{mg}{\text{sample size ml.}}$

2. To convert ul NO_2^--N to mg/l = $\frac{(\text{ml } NO_2^-\text{-N})(2.05 \times 10^{-6})}{\text{sample size}}$

RESEARCH PROJECT

Work Sheet - NH_3-N and PO_4P Analyses

2/25/68

Sample No.	Desig-nation	Sx ml NH_3	Sx ml PO_4	Spec. Read. NH_3-N	µg NH_3-N	mg/l NH_3-N	Spec. Read. PO_4-P	µg PO_4-P	mg/l PO_4-P	

Table 20