THE EFFECT OF VARYING FOOD-TO-MICROORGANISM RATIOS ON PHOSPHATE UPTAKE IN AN ACTIVATED SLUDGE ENVIRONMENT

by

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TABLE OF CONTENTS

		Page
	ACKNOWLEDGMENTS	ii
	LIST OF TABLES AND FIGURES	iv
I.	INTRODUCTION	1
II.	LITERATURE REVIEW	4
III.	EXPERIMENTAL METHODS AND MATERIALS	9
IV.	EXPERIMENTAL RESULTS	18
V.	DISCUSSION OF EXPERIMENTAL RESULTS	36
VI.	CONCLUSIONS	41
	BIBLIOGRAPHY	42
	APPENDIX	45
	VITA	48
	ABSTRACT	

LIST OF TABLES AND FIGURES

Table — Page

I Synthetic Sewage Substrate Composition. 13

II Data and Results From Batch Study No. 1 28

III Data and Results From Batch Study No. 2 29

IV Orthophosphate Analysis of Activated Sludge Cells - Batch Study No. 1 46

V Orthophosphate Analysis of Activated Sludge Cells - Batch Study No. 2 47

Figure

1 Pictorial Representation of the Laboratory Setup. 11

2 Flow Scheme for Orthophosphate Analysis, Technicon Auto Analyzer 16

3 Variation in pH, Soluble COD and Soluble PO_4 vs. Time - Batch Study No. 1. 20

4 Variation in pH, Soluble COD and Soluble PO_4 vs. Time - Batch Study No. 1. 21

5 Variation in pH, Soluble COD and Soluble PO_4 vs. Time - Batch Study No. 1. 22

6 Variation in pH, Soluble COD and Soluble PO_4 vs. Time - Batch Studies No. 1 and 2. 23

7 Variation in pH, Soluble COD and Soluble PO_4 vs. Time - Batch Study No. 2. 24

8 Variation in pH, Soluble COD and Soluble PO_4 vs. Time - Batch Study No. 2. 25

9 Variation in pH, Soluble COD and Soluble PO_4 vs. Time - Batch Study No. 2. 26

10 Sludge Total Phosphorus Content vs. Time - Batch Study No. 1 32

Figure Page

11 Sludge Total Phosphorus Content vs. Time - Batch Study No. 2 33

12 Adsorption Isotherm Analysis of Phosphate Uptake. 35

13 Variation in Initial Sludge Phosphate Content vs. Initial pH. 38

14 Variation in Initial Increase Sludge Phosphate Content vs. Initial pH. 39

I. INTRODUCTION

Eutrophication of lakes and reservoirs receiving waste treatment plant discharges has created serious economic and aesthetic problems which confront the sanitary engineer. This artificial introduction of nutrients results in algal blooms which degrade the quality of a lake or reservoir by giving the water an offensive appearance, odor and taste. It results in the consumption of the dissolved oxygen from the lower water strata, forcing fish to leave, and it can result in large fish kills if not corrected. The prolific algal growth renders boating difficult and discourages other recreational benefits.

It is widely accepted that of the main nutrients (carbon, nitrogen, phosphorus) required by algae for growth, phosphorus is the most practical factor to control. Carbon is readily available as carbon dioxide and carbonates in the water and algae produce CO_2 as an end product in the absence of sunlight. Nitrogen can be fixed from the atmosphere by certain algae species. Thus the problem generally associated with eutrophication control is to limit the concentration of phosphorus to a level which will not stimulate algae growth. There is much controversy on what the lowest allowable limit should be, but a figure that is constantly mentioned in the literature is 0.03 mg/l as orthophosphate (PO_4) after dilution by the stream (18).

Since the influent concentration of soluble phosphate to a wastewater treatment facility treating domestic sewage may range from 20 to 50 mg/l PO_4, significant reduction must be accomplished by the treatment plant to control eutrophication. However, sewage treatment

plants as presently designed and operated are usually concerned with removal of biologically oxidizable organics and not such nutrients as nitrogen and phosphorus. As a result only about 20 to 30 percent of the phosphorus present in sewage is removed by conventional treatment plants. Clearly, modified or additional processes are needed if adequate phosphorus removal is to be achieved.

Several chemical processes have been proposed for this purpose but they are too costly for general adoption and, in search for more economical methods, considerable attention has been focused on phosphorus control by the utilization of biological systems, in particular by the activated sludge process. Unfortunately, this attention has resulted in much confusion and controversy over the mechanisms and removal capacity of the activated sludge process.

It is generally concluded from several investigations that, when operated under certain conditions, the activated sludge process can remove large quantities of phosphorus. One relationship which was found to be typical of all plants is that soluble phosphorus concentration tends to decrease during aeration or organic substrate removal, and increase during final settling. Thus, to obtain maximum phosphate removals at the treatment plants, or minimum soluble phosphate concentration in the final effluent, it is necessary to maximize the amount of uptake and then to delay the release until the solids can be separated from the liquid (8). However, the mechanisms of uptake and release and the controls that result in optimum conditions for uptake and release are poorly understood and are subjects of considerable

dispute. Several researchers (8, 15, 16, 20, 21) have directed their attention to the mechanisms of soluble phosphate release, but not much work has been directed toward the understanding of soluble phosphate uptake.

Based on the need for more information, it was the purpose of this research to investigate and observe some of the factors that influence soluble phosphate uptake in an activated sludge environment and, if possible, to gain insight concerning mechanisms(s) of uptake. Special emphasis was placed on the relationship between uptake and food-to-microorganism ratio.

II. LITERATURE REVIEW

The fate of phosphate in the activated sludge process has been extensively studied but it has resulted in a vast amount of conflicting data. Many researchers have concentrated their work on phosphate release in the activated sludge environment (8, 16, 20, 21), resulting in some being concerned with soluble phosphate as an indication of the activated sludge condition (5, 6). Uptake of phosphate by activated sludge has been the main interest of only a few researchers but it has frequently been the subtopic of other articles.

Since wastewaters usually are relatively low in sources of carbon compared to the other nutrients, microbial growth in the activated sludge microflora is limited by carbon (30), and any significant amount of biological phosphorus removal would have to be of the enhanced or luxury type (31). Luxury uptake implies the ability of the sludge microorganisms to remove and store phosphorus in excess of that actually required for growth. Such uptake has been reported by numerous investigators (2, 4, 7, 10, 16, 23, 26).

There are essentially two schools of thought pertaining to the possibility of enhanced biological phosphorus uptake. One school believes that uptake is predominately biological in nature, and that in addition to normal uptake, luxury uptake by the microorganisms can and does occur. The other school believes that the biological fraction of removal is insignificant and that the removal process is essentially chemical in nature. Thus the main point of controversy and disagreement

among investigators is the existence of luxury uptake by biological mechanisms.

Many investigators studied the biological nature of phosphate uptake in the activated sludge processes and have obtained data that indicates luxury uptake. Borchardt and Azad (2) reported that organisms were capable of removing phosphate from solution in quantities greater than that required for growth. They also observed the fact that growth promoting temperatures produced a profound increase in the rate of phosphorus adsorption which tends to emphasize the metabolic nature of the phosphate uptake process. Srinath, et al., (23) pointed out the biological nature of phosphate uptake by demonstrating a marked decrease in phosphate removal upon addition of mercuric chloride, a metabolic poison, and upon heating the sludge at 40°C. Feng (5) reported on the ability of activated sludge to remove concentrations of orthophosphate much higher than those generally achieved in practice. Levin and Shapiro (10) supported the biological theory by showing that the addition of 2,4-dinitrophenol will inhibit oxidative but not substrate phosphorylation. They also indicated that uptake was not associated with growth, which would be expected if luxury uptake was predominant. Yall, et al., (31) further supported the biological nature of phosphate uptake by showing that the ability of sludge to remove ^{32}P was inhibited greatly by 2,4-dinitrophenol. Also, their experiments using ^{45}Ca indicated that calcium phosphate precipitation (chemical theory) plays a minor role in phosphate removal. In addition, Varma and Reid (27) have reported that excess uptake may occur due to

adsorption by microbial slimes and Moore, et al., (12) have provided evidence of storage of polymetaphosphates in activated sludge cells.

A parameter that is closely involved in enhanced biological phosphate removal is the dissolved oxygen concentration. Hall and Engelbrecht (7) observed that phosphorus removal is dependent on oxygen concentration and concluded that dissolved oxygen levels of at least 2 mg/l are necessary for optimum soluble phosphorus uptake. Levin and Shapiro (10) believed that phosphorus uptake is a function of aeration rates rather than the total quantity of air. Feng (5) reported better phosphate removal at high aeration rates. However, Ryckman and Rains (17) stated that mixing was more important and pointed out that all Levin and Shapiro (10) demonstrated was a linear relationship between orthophosphate removal and increased aeration rate. Another investigator who supports the ideas of Ryckman and Rains (17) was Wells (28). He concluded that maintaining a high dissolved oxygen concentration in the mixed liquor is not necessary, but that high aeration rates enhance the mixing and are beneficial to phosphate uptake. Yet, Vacker, et al., (26) found that the main difference in phosphorus removal for three San Antonio sewage treatment plants was the fact that the plant with the higher phosphorus removal rate had a significantly higher dissolved oxygen concentration at the end of the aeration tank compared to the other two plants.

A parameter which might have a profound effect on the possibility of enhanced biological phosphate uptake is the food-to-microorganism ratio. Several investigators have indicated that phosphate uptake is

a function of the loading rate. Hall and Engelbrecht (7) used a rather high food-to-microorganism ratio compared to normal plant operation, and it seems reasonable that their unusual data was due to this factor. Feng (5) reported better phosphate removals at higher food-to-microorganism ratios. Srinath, et al., (23), Levin and Shapiro (10) and Connell, et al., (4) observed that soluble phosphate uptake is enhanced by an increase in initial substrate concentration. More recently, Beer (1) has postulated that luxury biological phosphate uptake occurs only if the activated sludge organisms are subjected to a period of intense metabolic activity (log growth) which can be induced only by high substrate concentration (food-to-microorganism ratio) in an aerobic environment. This observation is also consistent with the conclusions of Moore, et al., (12) who used a correlation between cellular phosphate and enzyme activity. It is interesting to note that Randall (15) observed only one food-to-microorganism ratio high enough to produce logarithmic growth and the phosphorus uptake per unit solids during that experiment was considerable higher than any other result. This would appear to confirm Beer's hypothesis although much more evidence is needed to establish the point.

Menar and Jenkins (11), Sawyer (18), and Sekikawa, et al., (20) represent a few of the authors who do not believe that high phosphate removal is the result of enhanced biological phosphorus uptake, but instead is more of a chemical phenomenon. They maintain that sludge microorganisms remove no more phosphorus than that required for cell synthesis. Menar and Jenkins (11) have postulated that any additional

removal is in the form of a fine precipitate of calcium phosphate which becomes enmeshed into the activated sludge floc. If so, the degree of precipitation would be controlled by the pH conditions existing during the treatment process. Thus, they concluded that phosphate removal is proportional to the net sludge growth. Contrary to the beliefs of the biologically oriented authors on phosphate uptake, these investigators reported that the amount of phosphate incorporated biologically into activated sludge is not affected by the growth rate of the sludge, or by operating parameters such as organic loading, mixed liquor suspended solids concentration, aeration rate or mixed liquor dissolved oxygen concentration. For example, Menar and Jenkins (11) stated that the increased aeration rate effect on phosphorus removal, as reported by Levin and Shapiro (10) and by Vacker, et al., (26) was due to a decrease in CO_2 concentration at the end of the aeration unit caused by air stripping, which resulted in an increase in the pH of the mixed liquor and, therefore, greater removal.

In summation, the evidence of previous work seems to indicate that cationic precipitation does at times account for a recognizable amount of phosphate removal, but it was generally observed that phosphorus removal is primarily biological in nature and concluded that biological luxury uptake does occur. However, the basic fundamentals of this uptake and the important parameters involved are not understood well enough to prove useful. The goal of the following research was to clarify the importance of the food-to-microorganism ratio and to evaluate the nature of phosphate uptake in the activated sludge environment.

III. EXPERIMENTAL METHODS AND MATERIALS

The experimental work described in this thesis was directed toward determining the effect of varying food-to-microorganism ratio on the biological uptake of soluble phosphate in an activated sludge environment. The work was divided up into two laboratory scale, batch-type operations. The purpose of this chapter is to describe the experimental procedures employed and all techniques utilized in the laboratory investigation.

A. Batch Tests Procedure

The batch studies were conducted in the constant temperature room at 20° C. Each batch study consisted of four two-liter graduated cylinders designed to have an activated sludge mixed liquor suspended solids concentration of 1000 mg/l. Different volumes of a synthetic substrate were added to the cylinders so that the food-to-microorganism ratios varied as follows: 0.20 in cylinder No. 1, 0.75 in cylinder No. 2, 1.25 in cylinder No. 3, and 2.00 in cylinder No. 4. Distilled water was used to bring the liquid volume of each cylinder up to the two-liter mark. Each cylinder was aerated by a carborundum diffuser stone at a rate of 12 mls/sec/l. In the first batch study, phosphates were incorporated within the synthetic substrate so that the soluble phosphate concentration varied from cylinder to cylinder in accordance with the varying volumes of synthetic substrate used. In the second batch study, phosphates were not incorporated within the synthetic substrate but were added to each cylinder in equal amounts so that the

soluble phosphate concentration was the same regardless of the food-to-microorganism ratio. This second batch study was run in order to determine if the phosphate uptake experienced in the first batch study was due to adsorption by the cells caused by the different concentrations of phosphates present or due to biological reactions in the cells. The soluble phosphate concentration used in the second batch was that used in cylinder No. 4 in the first batch study. Thus, the results found for the food-to-microorganism ratio of 2.00 was the same for both batch study runs. A pictorial representation of the batch studies laboratory set-up is shown in Figure 1.

B. Sample Collection

Samples were collected from each cylinder at basically hourly intervals until release of phosphate by the activated sludge cells was assumed to have occurred. The samples were grab samples and were immediately tested for pH and filtered. The filtrates were placed in small glass bottles, frozen, and accumulated until the batch run was completed. The residues of particular samples were stored on metal weighing pans in a desiccator for a one to two week period before analyzing for phosphates. Soluble chemical oxygen demand and soluble orthophosphate (PO_4) analysis were performed on the filtrates immediately after thawing to prevent any additional biological action upon the samples.

C. Information on Activated Sludge

All activated sludges used in the experiments was obtained from the activated sludge sewage treatment plant at the Interstate 81

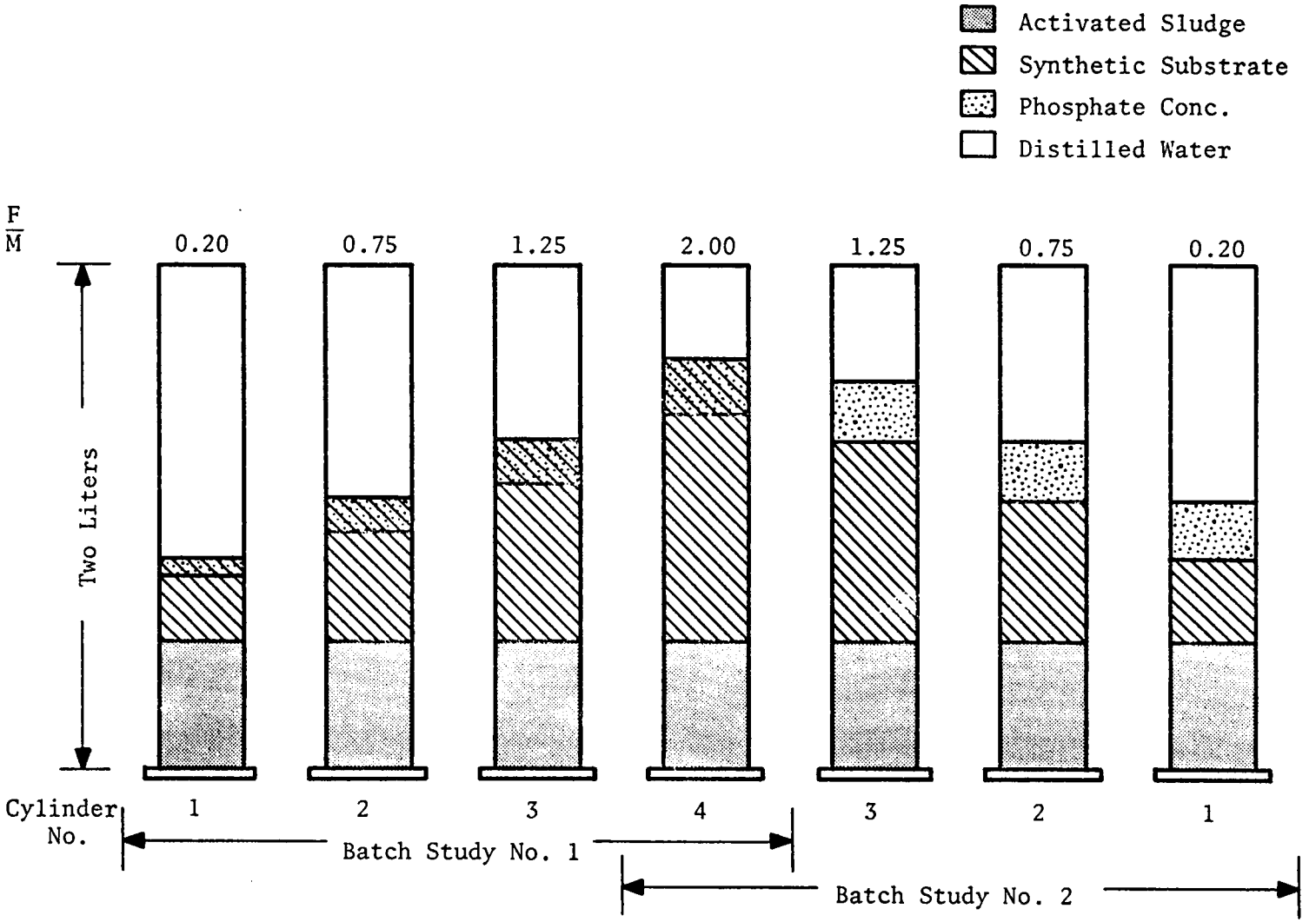

FIGURE 1. PICTORIAL REPRESENTATION OF THE LABORATORY SETUP

Reststop near Radford, Virginia. The activated sludge had a high mixed liquor suspended solids concentration of 4800 mg/l and a volatile suspended solids concentration of 3750 mg/l. The soluble chemical oxygen demand and the soluble orthophosphate concentration were found to be 16 mg/l and 27.9 mg/l, respectively. The pH was found to be 7.2, and the milligram orthophosphate per milligram dry weight of sludge was found to be approximately 22.8. Only 47 milliliters of activated sludge were used in each two-liter cylinder in order to achieve a 1000 mg/l mixed liquor suspended solids concentration.

D. Composition and Preparation of Synthetic Substrate

The basic composition of the synthetic sewage substrate used is shown in Table I. This medium is basically the same as that used by other researchers (8, 16) except all concentrations were increased ten-fold and the nutrients $Al_2SO_4 \cdot 7H_2O$ and NaCl were deleted.

A two-liter mixture of synthetic substrate was prepared and autoclaved for 35 minutes. In the first batch study run, KH_2PO_4 (anhydrous potassium dihydrogen phosphate) was incorporated into the original mixture. In the second batch study run, KH_2PO_4 was left out until after the mixture had been autoclaved. The equivalent amount of KH_2PO_4 found in cylinder No. 4, (food-to-microorganism ratio of 2.00) in the first batch study experiment was then added directly to cylinders No. 1, 2, and 3 (food-to-microorganism ratios of 0.20, 0.75, and 1.25, respectively). The synthetic substrate had a pH of 7.4 and a chemical oxygen demand of 6000 mg/l.

TABLE I

SYNTHETIC SEWAGE SUBSTRATE COMPOSITION

Nutrient	Concentration, mg/l
Nutrient Broth	4000
Urea	600
Potato Starch	2000
Potassium Chloride	70
Calcium Chloride	70
$MgSO_4 \cdot 7\ H_2O$	50
$FeSO_4 \cdot 7\ H_2O$	100
$MnSO_4 \cdot H_2O$	100
Na_2HCO_3	1680
KH_2PO_4	58
COD	6000 mg/l
pH	7.4

E. Methods of Laboratory Analyses

1. Cylinder Dissolved Oxygen Determination: A PCL Oxygen Analyzer was used to determine the dissolved oxygen concentration in each cylinder. This determination was necessary in order to maintain a constant D.O. for each food-to-microorganism ratio studied.

2. Suspended and Volatile Solids: All suspended and volatile solids analyses performed on the activated sludge were done as follows: Samples of 10 ml volume were filtered through glass-fiber discs placed in Gooch crucibles. The crucible and disc were first dried at 103° C for one hour, followed by cooling in a desiccator. The weight of the crucible and disc was then recorded as (A). The sample was then filtered through the crucible using a vacuum. The crucible and disc were then dried at 103° C for one hour and cooled in a desiccator. This weight was recorded as (B). The crucible and disc were finally ignited at 600° C for 20 minutes and then cooled in a desiccator. This weight was recorded as (C). Suspended and volatile solids concentrations were calculated as follows:

$$\text{mg/l Suspended Solids} = \frac{(B - A)(1000)}{\text{mls sample}}$$

$$\text{mg/l Volatile Solids} = \frac{(B - C)(1000)}{\text{mls sample}}$$

3. Sample Filtration: All samples were filtered through a 0.45μ millipore filter using a vacuum of at least 15 inches of mercury. The millipore filtering apparatus was thoroughly rinsed with

distilled water after each filtration. This procedure was followed each time in order to standardize conditions between filtrations.

4. Sample pH: All pH determinations were made with a Beckman Expandomatic pH meter. The meter was standardized with standard pH buffer solution, prepared by Fisher Scientific Company, before each batch study run.

5. Sample Orthophosphate Analysis: The Technicon Auto Analyzer was used for all orthophosphate determinations. The analysis was performed utilizing the method as described in the Technicon Auto Analyzer Methodology. The method along with the reagents used are shown in Figure 2. A standard curve was prepared each time a series of samples were run. Standard solutions were made by diluting a stock solution containing 1000 mg/l PO_4. The stock solution was prepared by dissolving 1.433 grams of KH_2PO_4 along with one milliliter of concentrated H_2SO_4 in distilled water diluted to 1000 milliliters.

6. Chemical Oxygen Demand and Sludge Orthophosphate Analyses: The chemical oxygen demand was determined following the procedure outlined in Standard Methods (24). Total phosphorus concentration of the sludge samples was determined as orthophosphate after digestion of samples by the persulfate method given in Standard Methods (24) and as described below. The total phosphorus of the sludge samples was determined by placing the samples in cleaned flasks and adding 100 ml distilled water and the persulfate digestion mixture. After digestion, the samples were neutralized to

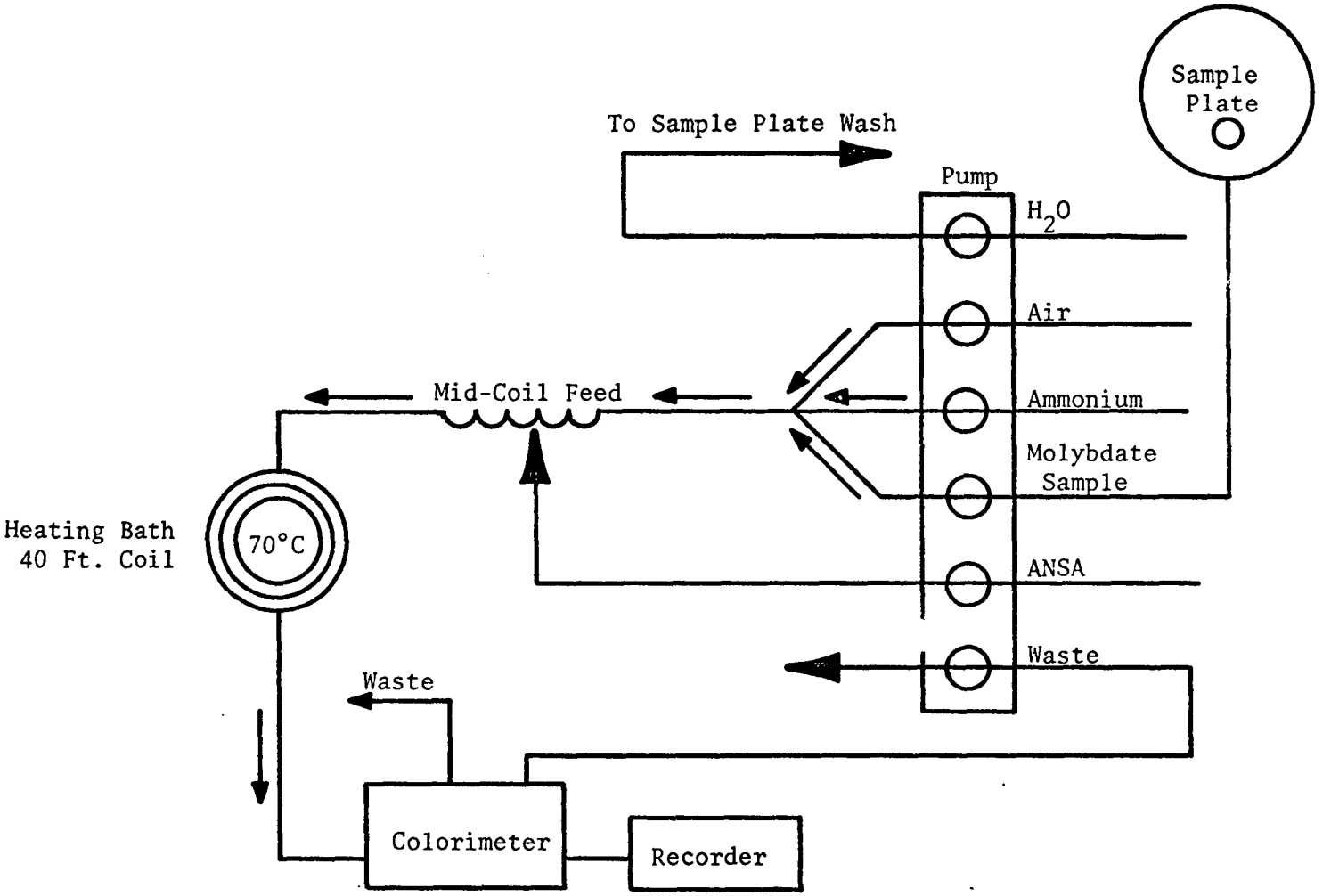

FIGURE 2. FLOW SCHEME FOR ORTHOPHOSPHATE ANALYSIS, TECHNICON AUTO ANALYZER

the phenolphthalein end point. The samples were then diluted to an exact volume with distilled water, and the orthophosphate concentration of this solution was determined using the Technicon Auto Analyzer. The phosphorus content of the sludge samples was calculated by the following formula:

$$\frac{\text{mg } PO_4}{\text{mg dry wt. of sludge}} = \frac{(P)(V)}{\text{mg dry weight}}$$

where P = orthophosphate concentration in final solution in mg/l.

V = volume of final solution in liters.

IV. EXPERIMENTAL RESULTS

The investigation of phosphate uptake with varying food-to-microorganism ratios was carried out in two batch study phases. In the first phase the initial phosphate concentration varied for each experiment while in the second phase the initial phosphate concentration was constant for each different food-to-microorganism (F/M). The studies were designed to observe the variation in such parameters as pH and soluble chemical oxygen demand along with the soluble PO_4 concentration with aeration time. A study was also made of the sludge total phosphorus content to determine any significant trends. An adsorption isotherm type of analysis was employed to help verify the probable nature of the phosphate uptake.

The Effect of Varying F/M on pH

In order to detect any significant changes in chemical conditions that might warrant a closer look at the theory postulated by Menar and Jenkins (11), pH measurements were made of all samples collected from both batch study runs.

In the first batch study, no significant changes occurred in the values of pH throughout the aeration time for each food-to-microorganism ratio, and thus, no correlation could be found between pH and phosphate concentration. The average pH values were 7.4, 8.1, 8.3, and 8.4 for food-to-microorganism ratios of 0.20, 0.75, 1.25, and 2.00, respectively (Figures 3, 4, 5 and 6). However, it was noted that the average pH values increased as the F/M values increased. One possible explanation for this trend is the fact that the synthetic substrate used in the

batch study had a pH greater than 7.0 (7.3). Thus, when more substrate was used to achieve a higher food-to-microorganism ratio, it potentially would also cause the pH to rise. A more probable explanation of this increasing pH trend with increasing F/M could be attributed to some chemical phenomenon.

In the second batch study a significant change of pH occurred with the F/M of 0.20 where the values ranged from 7.4 to 5.6 (Figure 7). A possible explanation for this variation in pH might be due to the fact that aerobic conditions occurred in the cylinder thus causing the pH to drop. The major change in pH occurred during phosphate release and after maximum phosphate uptake has been obtained. However, no correlation could be found between this pH change and the phosphate concentration, which leads to the conclusion that this pH change was not large enough to produce enough stress on the microbial cells to cause phosphate release. No significant changes occurred in the values of pH for the other food-to-microorganism ratios. The average pH values found were 6.9, 7.8 and 8.3 for food-to-microorganism ratios of 0.20, 0.75 and 1.25, respectively (Figures 7, 8 and 9). The trend of increasing pH with higher F/M was again observed in the second batch study and was probably due to the same reasons as concluded in the first batch study.

The Effect of Varying F/M on Substrate Utilization and Soluble PO_4 Uptake

The utilization of substrate and the uptake and release of phosphate during aeration are illustrated in Figures 3, 4, 5 and 6 for Batch Study No. 1 and Figures 6, 7, 8 and 9 for Batch Study No. 2.

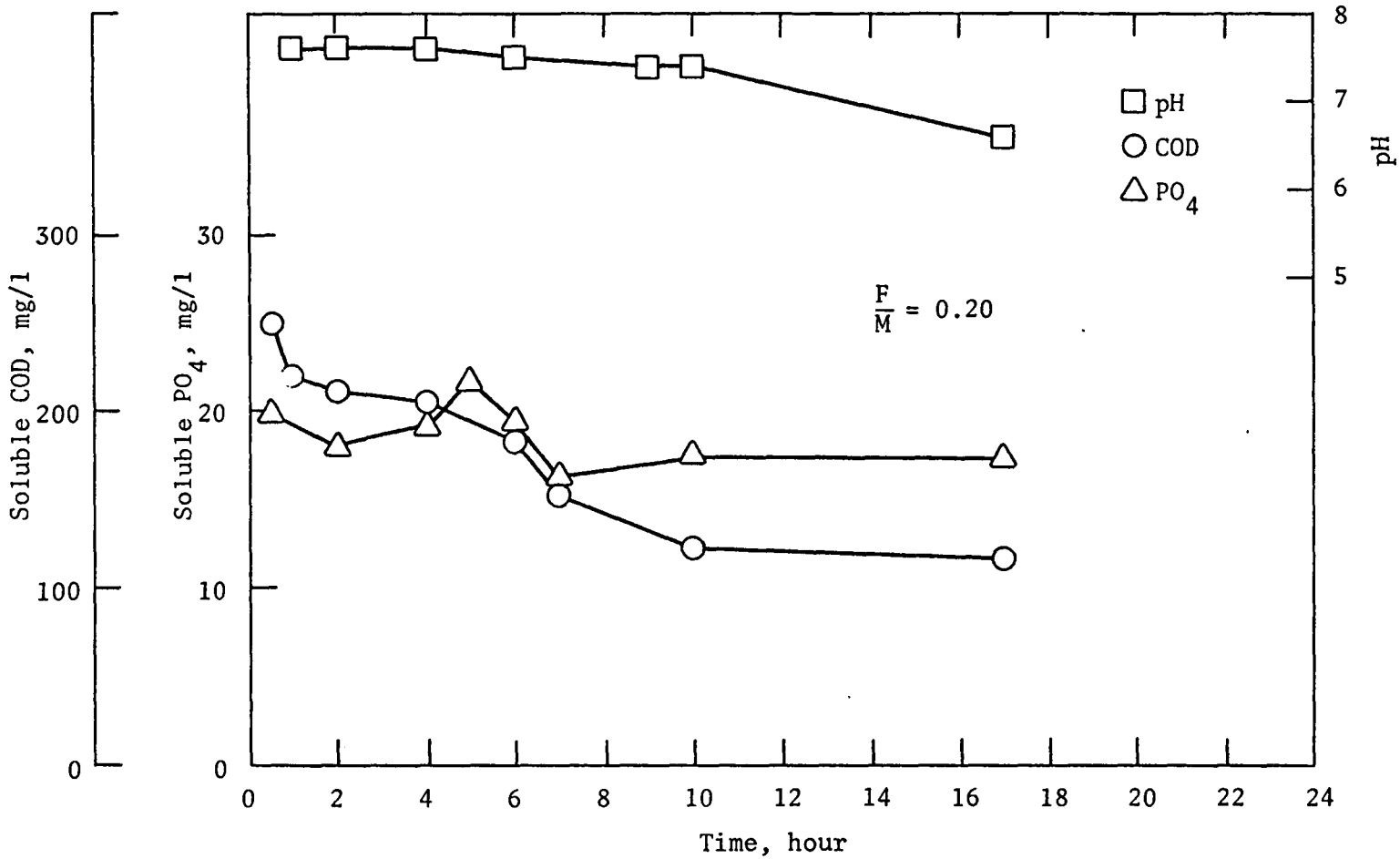

FIGURE 3. VARIATION IN pH, SOLUBLE COD AND SOLUBLE PO_4 VS. TIME - BATCH STUDY NO. 1

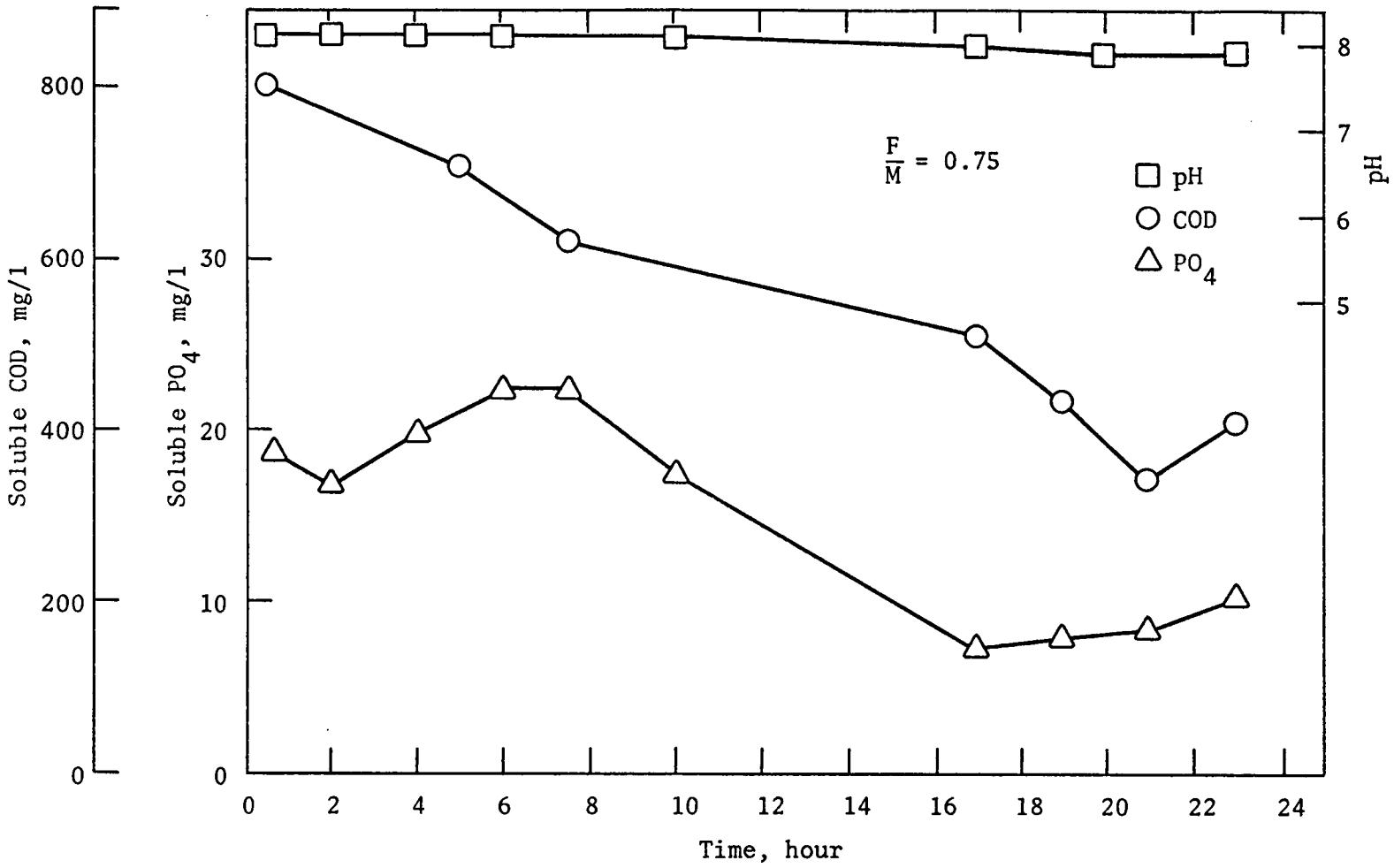

FIGURE 4. VARIATION IN pH, SOLUBLE COD AND SOLUBLE PO_4 VS. TIME - BATCH STUDY NO. 1

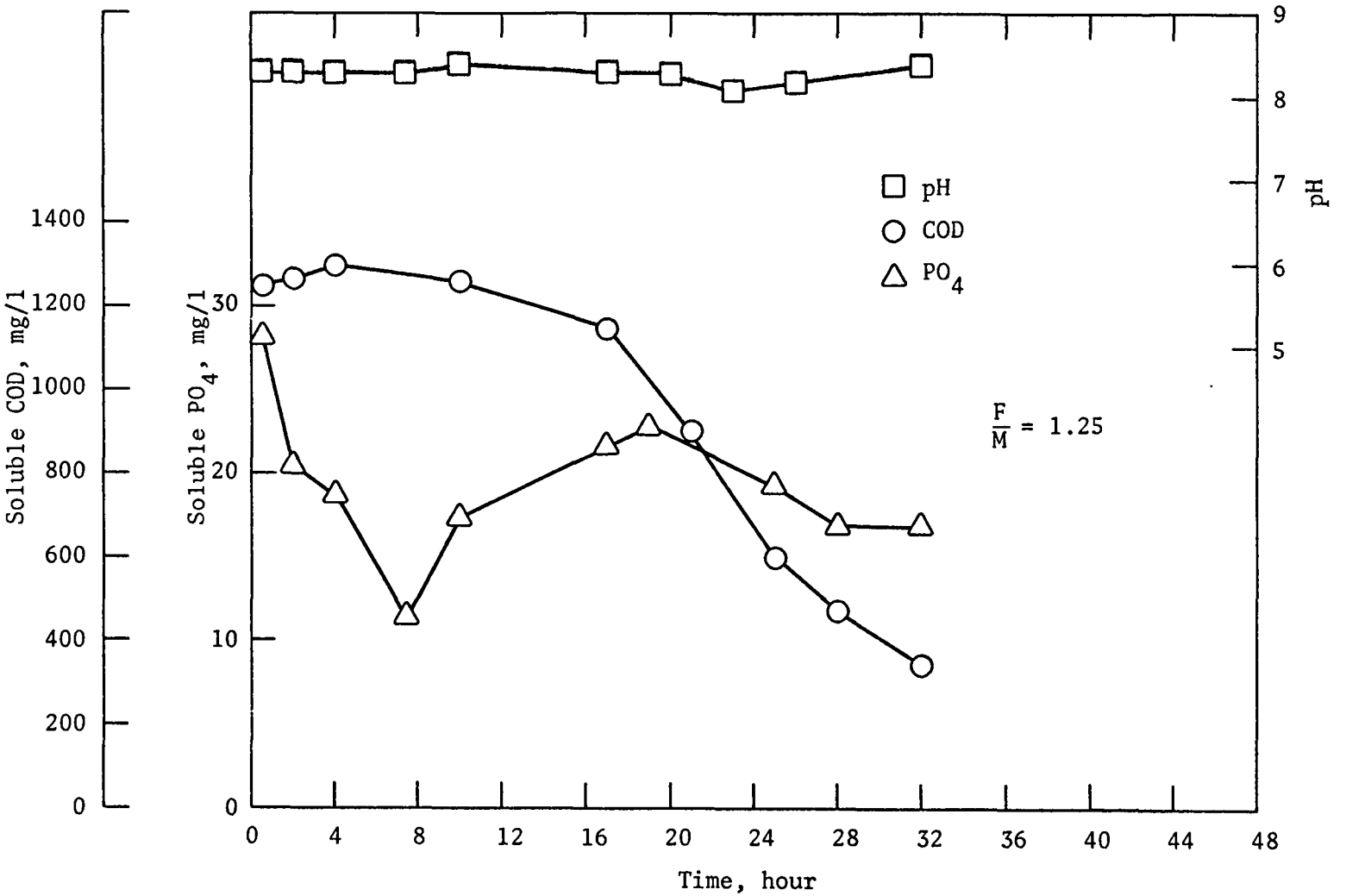

FIGURE 5. VARIATION IN pH, SOLUBLE COD AND SOLUBLE PO_4 VS. TIME - BATCH STUDY NO. 1

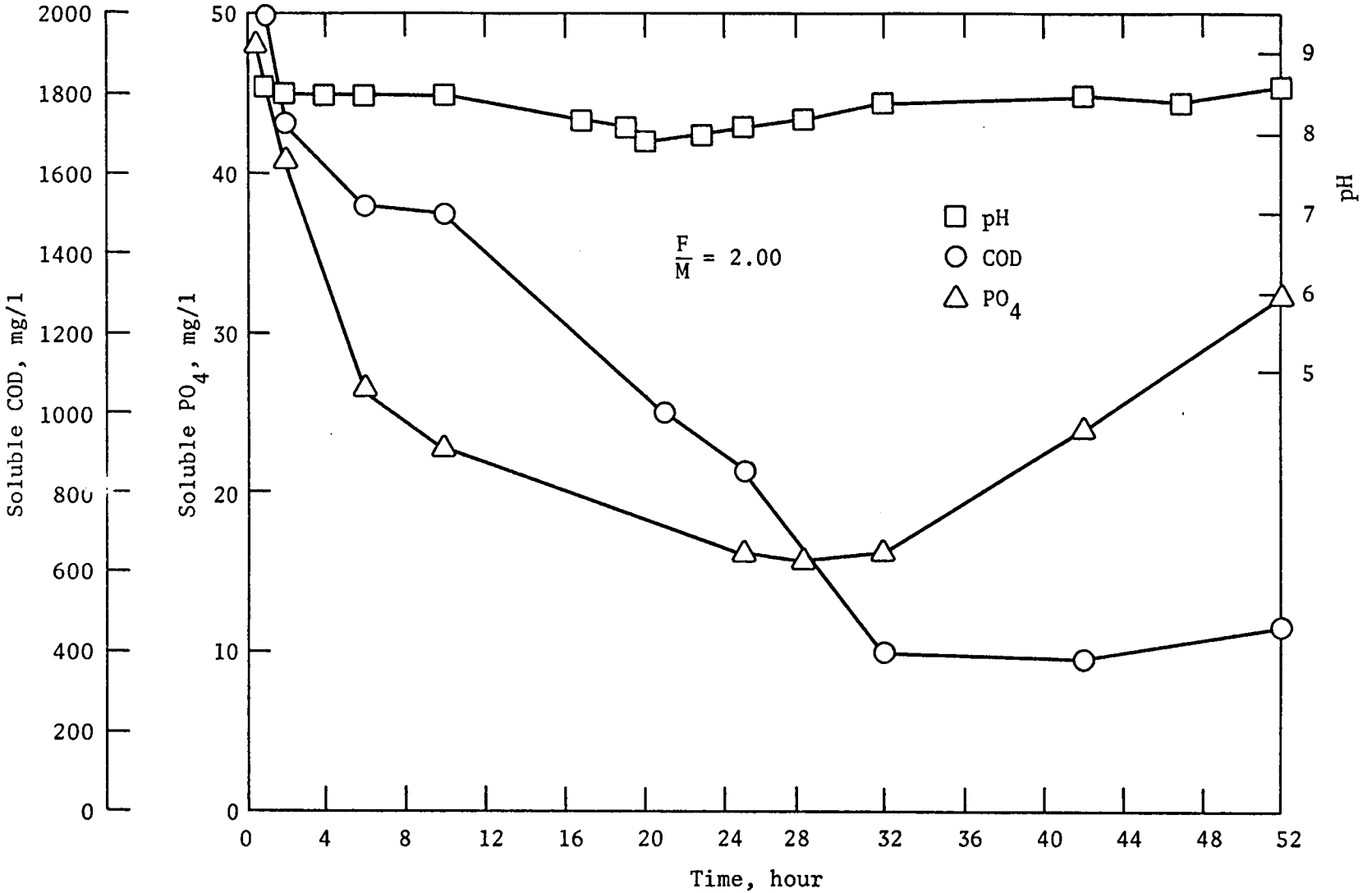

FIGURE 6. VARIATION IN pH, SOLUBLE COD AND SOLUBLE PO_4 VS. TIME - BATCH STUDIES NO. 1 AND 2

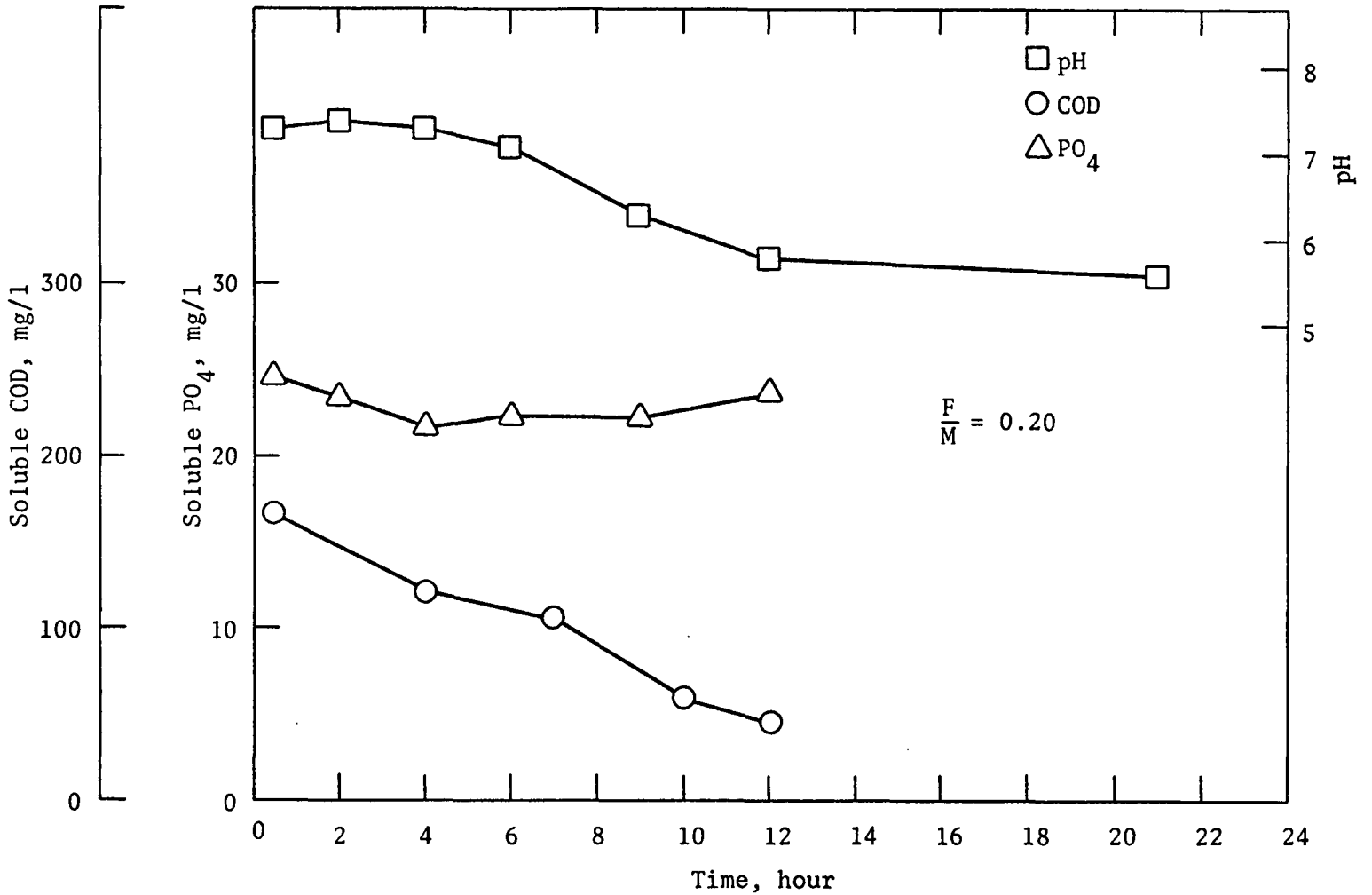

FIGURE 7. VARIATION IN pH, SOLUBLE COD AND SOLUBLE PO_4 VS. TIME - BATCH STUDY NO. 2

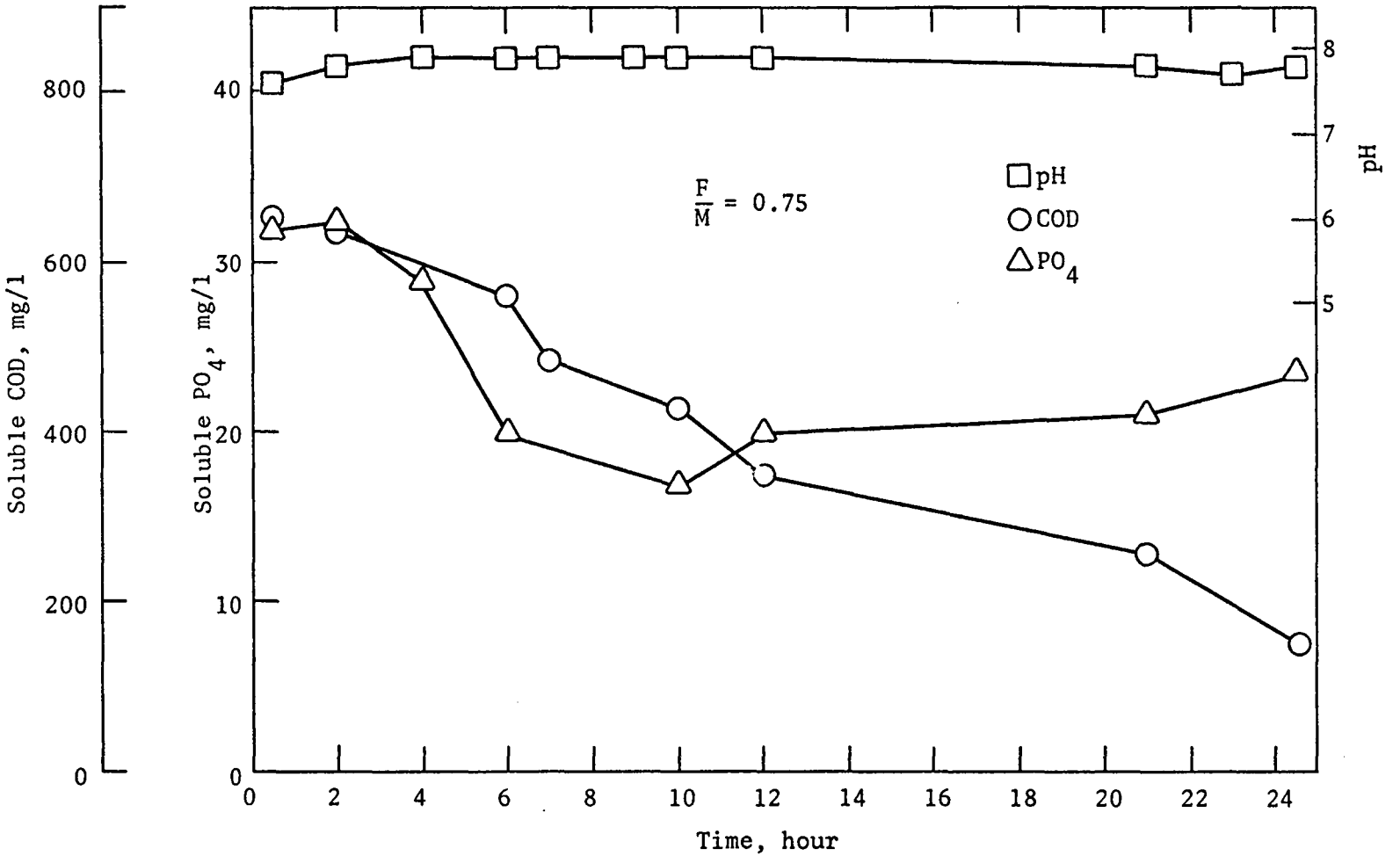

FIGURE 8. VARIATION IN pH, SOLUBLE COD AND SOLUBLE PO_4 VS. TIME - BATCH STUDY NO. 2

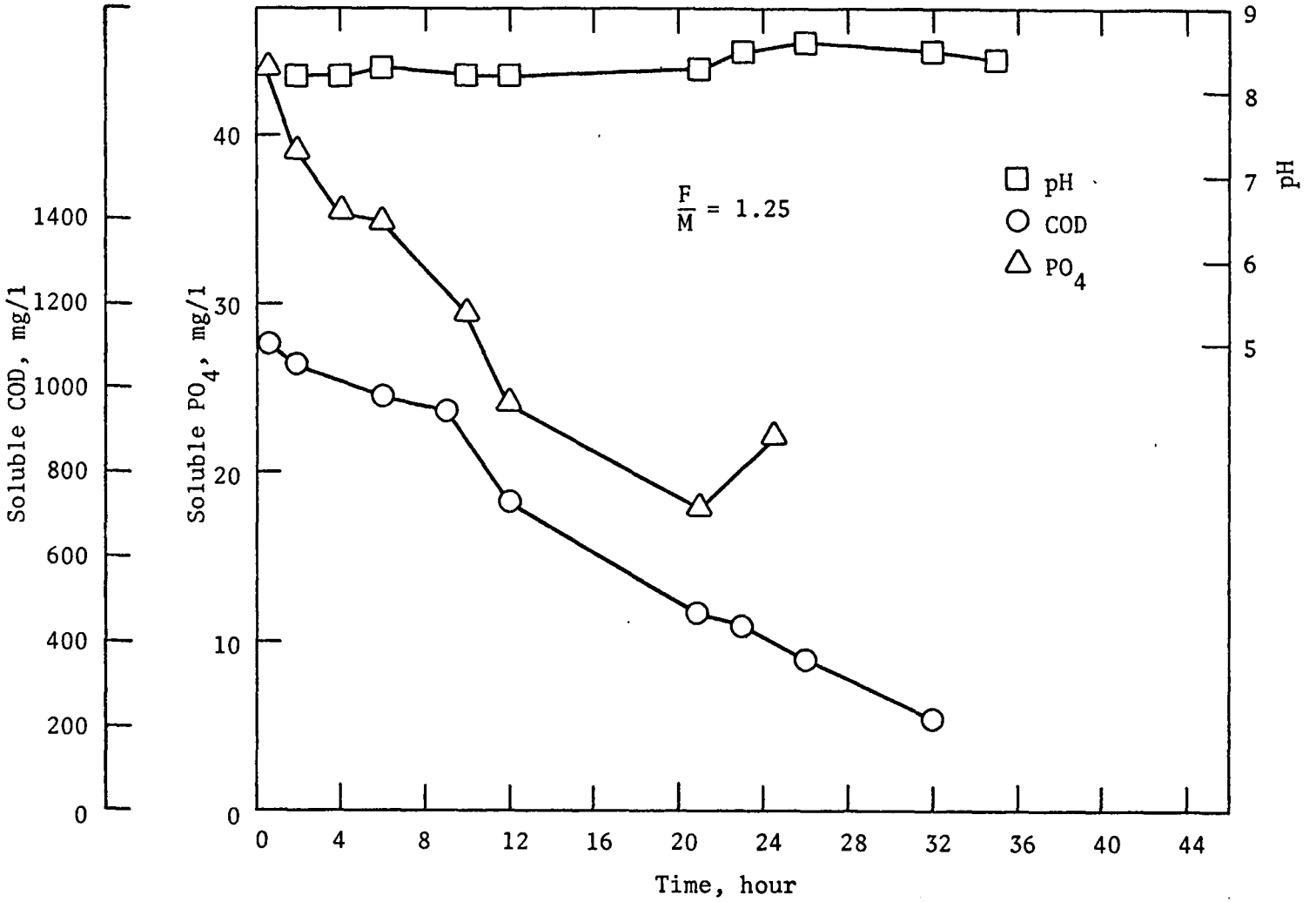

FIGURE 9. VARIATION IN pH, SOLUBLE COD AND SOLUBLE PO_4 VS. TIME - BATCH STUDY NO. 2

In the first batch study, using initial values, the total uptake of COD (mg/l) was 140, 461, 904 and 1620 for food-to-microorganism ratios of 0.20, 0.75, 1.25 and 2.00, respectively (Table II). In the second batch study, the total uptake of COD (mg/l) was 122, 511 and 886 mg/l for food-to-microorganism ratios of 0.20, 0.75 and 1.25, respectively (Table III). COD uptake generally occurred as a downward trend with increasing aeration time until stabilization was essentially complete. However, it was observed that the higher the F/M, the greater the amount of aeration time required to achieve complete stabilization. The aeration times required generally averaged 11, 21, 32 and 36 hours for food-to-microorganism ratios of 0.20, 0.75, 1.25 and 2.00, respectively.

The total uptake of soluble PO_4 in mg/l for the various food-to-microorganism ratios are indicated in Table II for Batch Study No. 1 and Table III for Batch Study No. 2. The theoretical initial PO_4 concentrations (mg/l) for the first batch study were 3.9, 14.6, 24.3 and 39.0 for food-to-microorganism ratios of 0.20, 0.75, 1.25 and 2.00, respectively. In the second batch study, the theoretical initial PO_4 concentration (mg/l) was maintained at 39.0 for all food-to-microorganism ratios. Any concentration of PO_4 above those theoretical values for the F/M indicated was assumed to be due to the release of phosphates from the activated sludge as a result of some mild stress imposed upon the microbial cells. This stress was possibly caused by the sudden introduction of substrate to the cells followed by immediate aeration with no acclimation period allowed. This could also be the

TABLE II

DATA AND RESULTS FROM BATCH STUDY NO. 1

$\frac{F}{M}$	0.20	0.75	1.25	2.00
Synthetic Substrate (mls)	67	250	417	667
Initial MLSS(mg/l)	1000	1000	1000	1000
D.O. Conc.(mg/l)	7.8	7.8	7.8	7.8
USING INITIAL [Highest] VALUES				
Maximum Uptake of Soluble PO_4 (mg/l)	3.6	11.4 [15.2]	16.8	32.4
Total Uptake of COD(mg/l)	140	461	904 [956]	1620
COD Uptake/PO_4 Uptake	39	40 [30]	53 [57]	50
Percent Phosphate Uptake	19	61 [68]	60	67
Theo. Initial PO_4 Conc.(mg/l)	3.9	14.6	24.3	39.0

TABLE III

DATA AND RESULTS FROM BATCH STUDY NO. 2

$\frac{F}{M}$	0.20	0.75	1.25	2.00
Synthetic Substrate	67	250	417	667
Initial MLSS(mg/l)	1000	1000	1000	1000
D.O. Conc.(mg/l)	7.8	7.8	7.8	7.8
USING INITIAL [Highest] VALUES				
Maximum Uptake of Soluble PO_4(mg/l)	3.0	15.0 [15.6]	26.4	32.4
Total Uptake of COD(mg/l)	122	511	886	1620
COD Uptake/PO_4 Uptake	41	34 [33]	34	50
Percent Phosphate Uptake	12	47 [48]	57	67
Theo. Initial PO_4 Conc.(mg/l)	39.0	39.0	39.0	39.0

reason why a "hump" appears in some of the PO_4 uptake curves (Figures 3 and 4).

The data also shows that the rate of phosphate uptake declined as aeration progressed until a point was reached where continued aeration resulted in a steady leakage of soluble phosphate back to solution. This point occurred after 4-8, 8-16, 8-20 and 24-32 hours of aeration for food-to-microorganism ratios of 0.20, 0.75, 1.25 and 2.00, respectively. Using initial values, the percent of phosphates that was taken up by the cells in Batch Study No. 1 was 19, 61, 60 and 67 and in Batch Study No. 2 was 12, 47 and 57 for the food-to-microorganism ratios indicated in Table II and Table III, respectively.

Phosphate uptake had a very wide range during this study, ranging from 3 mg/l for a F/M of 0.20 to 32.4 mg/l for a F/M of 2.00. Stated differently, the ratio of initial COD uptake to PO_4 uptake had average initial values of 40, 37, 43 and 50 for food-to-microorganism ratios of 0.20, 0.75, 1.25 and 2.00, respectively (Tables II and III). The ratios obtained are considerably less than those commonly reported for activated sludge, and if the uptakes were purely biological, they would indicate luxury uptake. The percent of total phosphates that was taken up had values as shown in Tables II and III for Batch Studies No. 1 and 2, respectively. The averaged percent of phosphate uptake had a range of 16, 54, 59 and 67 for food-to-microorganism ratios of 0.20, 0.75, 1.25 and 2.00, respectively.

The Effect of Varying F/M on Sludge PO_4 Content

The effect of varying F/M on sludge PO_4 content is shown graphically by Figure 10 for Batch Study No. 1 and by Figure 11 for Batch Study No. 2. A trend that is quite evident is the fact that as the food-to-microorganism ratio increases the PO_4 content in the sludge increases. In Batch Study No. 1, the total phosphorus content in the sludge measured as mg PO_4 per mg dry weight had average values of 42, 58, 77 and 100 for food-to-microorganism ratios of 0.20, 0.75, 1.25 and 2.00, respectively. In Batch Study No. 2 the average values were 34, 38 and 45 for food-to-microorganism ratios of 0.20, 0.75 and 1.25, respectively.

An important note to be made here is the wide difference in average sludge PO_4 content obtained for the same F/M in the two batch studies. A possible explanation might be that the two batch studies used activated sludge that variated widely in total phosphorus content. Because there was at least a month between activated sludge collections from the treatment plant for the batch study runs, this explanation seems plausible.

Adsorption Isotherm Analysis of PO_4 Uptake

In order to help clarify the possible nature of phosphate uptake and to determine what type of effect the increasing F/M had on uptake, an adsorption isotherm analysis was included in the results. If phosphate uptake was primarily due to an adsorption mechanism, the isotherm would plot as a straight line at approximately a 45° angle

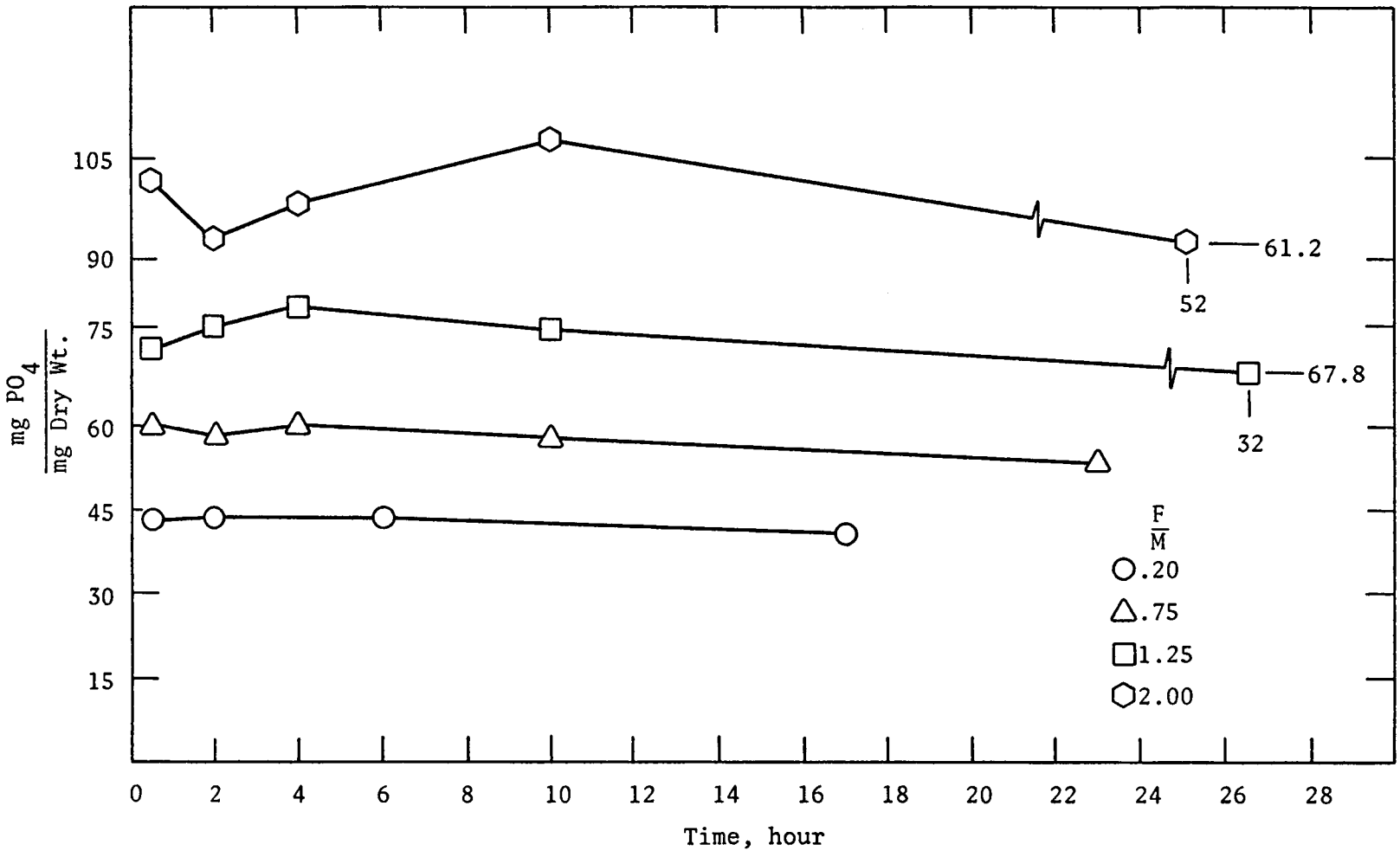

FIGURE 10. SLUDGE TOTAL PHOSPHORUS CONTENT VS. TIME - BATCH STUDY NO. 1

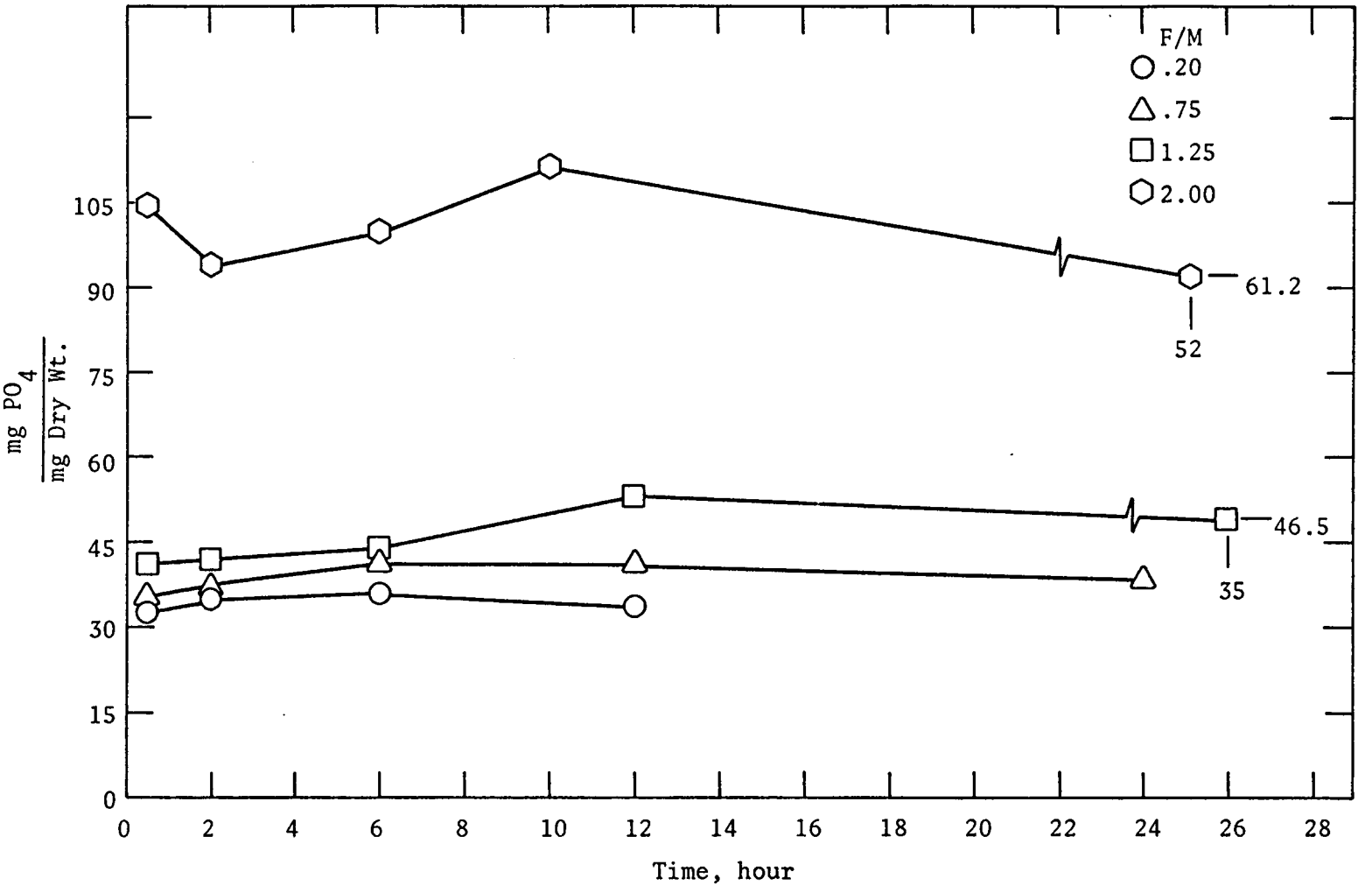

FIGURE 11. SLUDGE TOTAL PHOSPHORUS CONTENT VS. TIME - BATCH STUDY NO. 2

from left to right. However, as indicated in Figure 12, the plot of the actual data is generally a straight line from bottom to top. Therefore, the adsorption phenomenon was not the dominant mechanism of phosphate uptake and it might be concluded that the effect of increasing F/M on uptake was primarily of a biological nature.

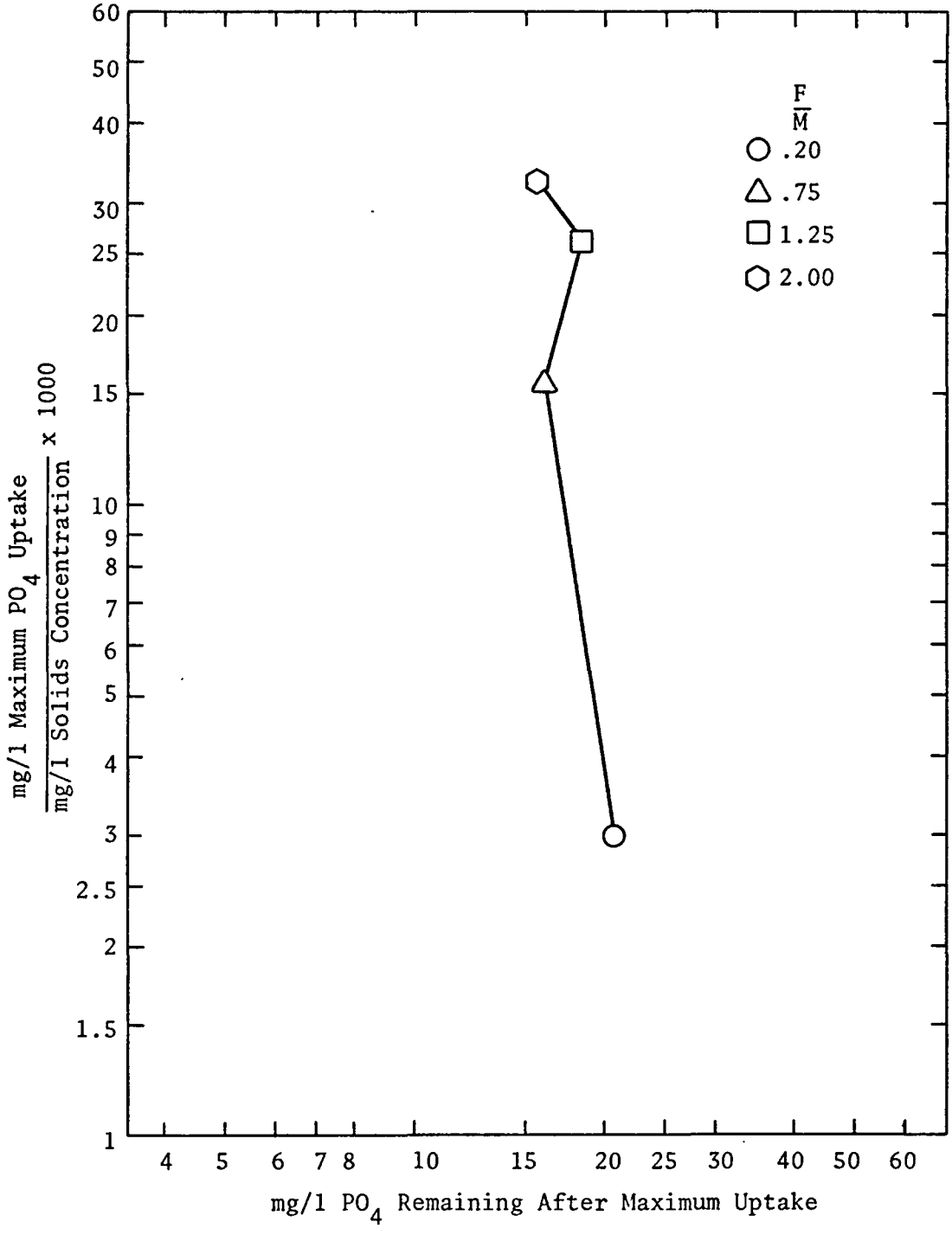

FIGURE 12. ADSORPTION ISOTHERM ANALYSIS OF PHOSPHATE UPTAKE

V. DISCUSSION OF EXPERIMENTAL RESULTS

This discussion will be mainly directed toward interpreting the data with respect to the mechanisms responsible for phosphate uptake in the activated sludge environment.

One of the major goals of this research was to attempt to shed some light over the controversy on whether the primary cause of orthophosphate uptake by the activated sludge organisms is mainly a biological or chemical process. The biological nature of phosphate uptake was stressed by the following indications: the graphs (Figures 3 - 9) showing no correlation between pH and soluble phosphate concentration throughout the aeration times required to reach substrate stabilization; the fact that the COD uptake per PO_4 uptake ratios were lower than usual indicating luxury uptake if the biological nature of uptake was dominant; and the curve in Figure 12 showing that the adsorption phenomenon was not the dominant mechanism of phosphate uptake.

The fact that a logical explanation could not be found that could give reason why there was an increasing pH with increasing F/M brought to light the possibility of a chemical mechanism of phosphate uptake. To further explore this phenomenon, a plot was made of the initial sludge PO_4 content with initial pH (Figure 13). The curve representing Batch Study No. 1 would not be expected to indicate much because of the phosphate concentrations that varied in accordance with the amount of synthetic substrate used. The curve representing Batch Study No. 2

shows a very interesting trend. A fairly straight line appears between the points representing food-to-microorganism ratios of 0.20 and 1.25 indicating some sort of correlation between initial sludge PO_4 content and pH which would be expected with increasing substrate. However, at pH 8.2 the curve rises sharply to the point represented by the F/M of 2.00. The probable reason for this sharp rise is due to the fact that hydroxyapatite precipitates out at pH around 8.4 which thus enmeshes the phosphates into the activated sludge floc causing a substantial increase in sludge PO_4 content. The plot of initial increase in sludge PO_4 content with initial pH (Figure 14) was obtained by subtracting the activated sludge PO_4 content (22.8 mg/l) from the initial sludge PO_4 content. The fact that these graphs show the importance the chemical parameter pH has on phosphate removal tends to support the hypothesis that luxury phosphate uptake is of a chemical nature.

It is obvious that both the chemical and biological processes exert an influence on phosphate uptake which leads to the conclusion that biochemical phenomena are responsible for phosphate removal in an activated sludge environment.

A noticeable result that occurred in the graphs was the fact that the aeration times required to reach substrate stabilization and maximum soluble phosphate uptake increased as the F/M increased. This would seem logical because as more food becomes available to the activated sludge organisms, more time would be required for the organisms to metabolize this increase in substrate. Since substrate utilization corresponds with phosphate removal, the aeration times

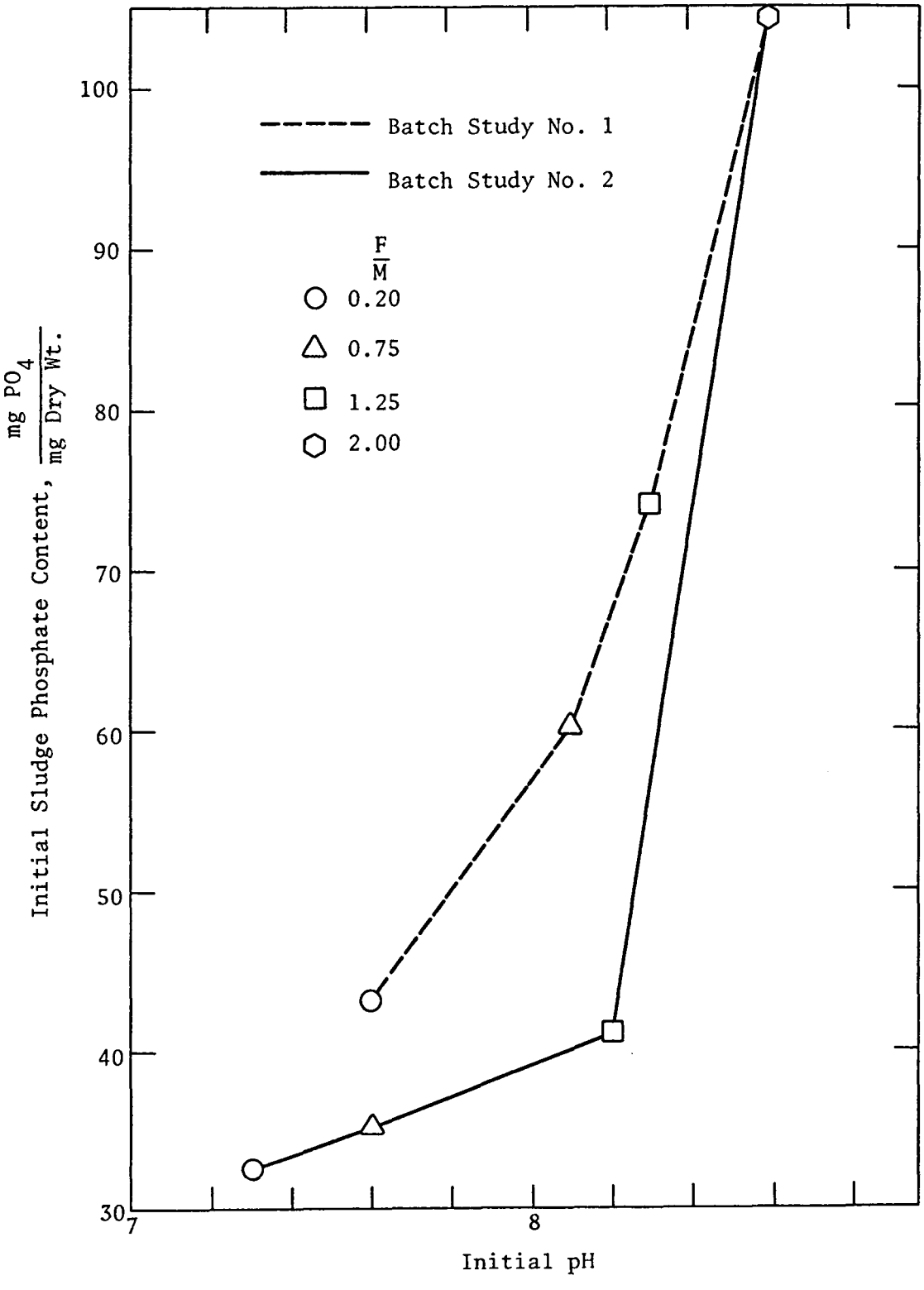

FIGURE 13. VARIATION IN INITIAL SLUDGE PHOSPHATE CONTENT VS. INITIAL pH

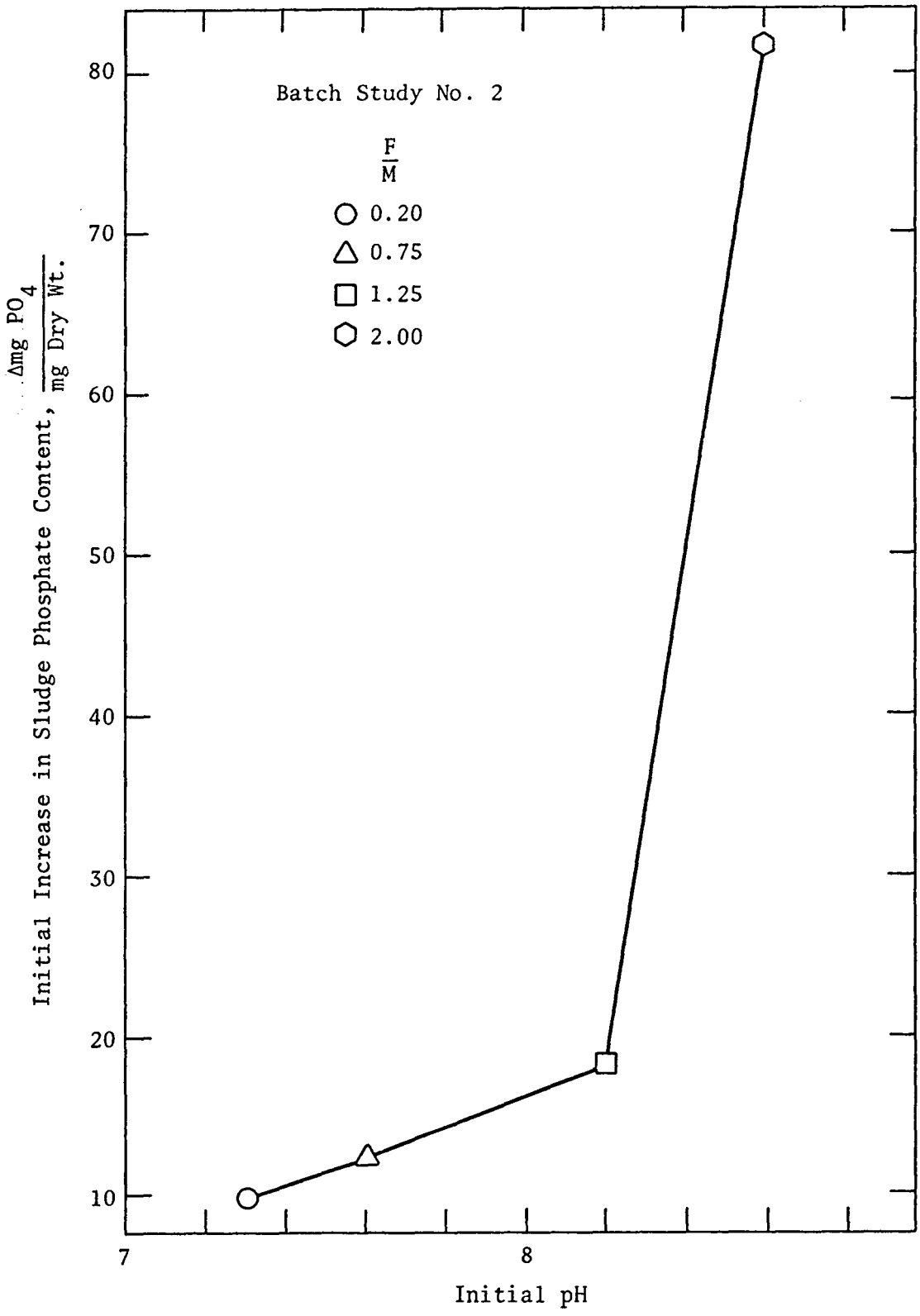

FIGURE 14. VARIATION IN INITIAL INCREASE SLUDGE PHOSPHATE CONTENT VS. INITIAL pH

required to reach maximum phosphate uptake would also be expected to increase.

In terms of COD uptake per PO_4 uptake, the best soluble phosphate removal occurred when the food-to-microorganism ratio ranged anywhere from 0.20 to 1.25, a wide range indeed. When this result was combined with the percent of total phosphate that was taken up, the F/M of 0.20 was eliminated as an acceptable loading rate because of its very low percent of phosphate uptake. From combining both batch study runs, the averaged percent of phosphates that were taken up by food-to-microorganism ratios of 0.75 (54%) and 1.25 (59%) were both found to be fairly close to each other. Because the F/M of 0.75 had the better COD uptake per PO_4 uptake ratio and because the F/M of 1.25 had the better percent phosphate uptake value, the best phosphate removal for practical purposes was assumed to occur in the range between these two food-to-microorganism ratios.

V. CONCLUSIONS

From studying the effects of varying food-to-microorganism ratios on phosphate uptake by activated sludge, the following conclusions have been drawn:

1. Both the biological and chemical processes have roles in phosphate uptake, but it is concluded that phosphate removal in an activated sludge environment is predominately biochemical in nature.
2. The total phosphorus content in the activated sludge cells increases as the food-to-microorganism ratio increases.
3. Physical adsorption is not the primary mechanism of phosphate uptake in an activated sludge environment.
4. The aeration times required to reach substrate stabilization and maximum soluble phosphate uptake increases as the food-to-microorganism ratio increases.
5. In terms of COD uptake per PO_4 uptake and percent of phosphate taken up, the best soluble phosphate removal occurs when the food-to-microorganism ratio ranges between 0.75 and 1.25.
6. The initial sludge phosphate content is pH dependent.

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APPENDIX

Table IV. Orthophosphate Analysis of Act. Sludge Cells

Batch Study I

$\frac{F}{M}$ = 0.20

Time, hrs.	1/2	2	6	17
$\frac{mg\ PO_4}{mg\ Dry\ wt.}$	43.3	43.8	43.5	40.7

$\frac{F}{M}$ = 0.75

Time, hrs.	1/2	2	6	10	23
$\frac{mg\ PO_4}{mg\ Dry\ wt.}$	60.2	58.4	60.2	58.4	53.6

$\frac{F}{M}$ = 1.25

Time, hrs.	1/2	2	6	10	32
$\frac{mg\ PO_4}{mg\ Dry\ wt.}$	74.0	78.2	81.6	77.7	67.8

$\frac{F}{M}$ = 2.00

Time, hrs.	1/2	2	6	10	52
$\frac{mg\ PO_4}{mg\ Dry\ wt.}$	104.7	94.2	100.4	111.6	61.2

Table V. Orthophosphate Analysis of Activated Sludge Cells

Batch Study II

$\frac{F}{M}$ = 0.20

Time, hrs.	1/2	2	6	12
$\frac{mg\ PO_4}{mg\ Dry\ wt.}$	32.6	34.8	36.0	34.1

$\frac{F}{M}$ = 0.75

Time, hrs.	1/2	2	6	12	24 1/2
$\frac{mg\ PO_4}{mg\ Dry\ wt.}$	35.1	37.2	41.4	41.1	38.1

$\frac{F}{M}$ = 1.25

Time, hrs.	1/2	2	6	12	35
$\frac{mg\ PO_4}{mg\ Dry\ wt.}$	41.1	42.0	43.5	53.3	46.5

$\frac{F}{M}$ = 2.00

Time, hrs.	1/2	2	6	10	52
$\frac{mg\ PO_4}{mg\ Dry\ wt.}$	104.7	94.2	100.4	111.6	61.2

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THE EFFECT OF VARYING FOOD-TO-MICROORGANISM RATIOS ON PHOSPHATE UPTAKE IN AN ACTIVATED SLUDGE ENVIRONMENT

by

Michael Robin Collins

(ABSTRACT)

An economical process for the removal of nutrients to receiving waters has become a difficult problem of major proportions. It was the purpose of this study to clarify the importance of the food-to-microorganism ratio (F/M) and to evaluate the nature of phosphate uptake in the activated sludge environment. The investigation consisted of two batch studies which allowed the F/M to vary as follows: 0.20, 0.75, 1.25 and 2.00. The phosphate concentration (PO_4) was allowed to vary in the first batch study and was kept constant in the second batch study. Measurements of pH, soluble COD and soluble PO_4 were made on all samples taken. Certain samples were measured for sludge total phosphorus content.

Results obtained showed that both the biological and chemical processes have roles in phosphate uptake, but it was concluded that phosphate removal is predominately biochemical in nature. It was also found that physical adsorption was not the primary mechanism of phosphate uptake in an activated sludge environment, and the best overall soluble phosphate removal occurred when the food-to-microorganism ratio ranged between 0.75 and 1.25.