

A STUDY OF PHOSPHORUS UPTAKE BY ACTIVATED
SLUDGE AT VARIOUS COD:P RATIOS

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1976

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
December, 1978



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SLUDGE AT VARIOUS COD:P RATIOS

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ACKNOWLEDGMENTS

I wish to extend my sincere appreciation to Dr. Marcia H. Bates, my major thesis adviser, for her guidance, patience, and encouragement during the course of this study. I would like to express my gratitude to Dr. A. F. Gaudy, Jr. and Dr. Don F. Kincannon for serving as committee members and for their valuable instruction. I also wish to thank Mr. P. Reddy and Dr. Manickam for their help and encouragement during this study. Special thanks are extended to Mrs. Grayce Wynd for accurately typing this thesis.

My most sincere appreciation and gratitude are extended to my parents, Mohammad and Akram Torabian for their support, love, and understanding throughout the years of my studies.

Appreciation is extended to my colleagues for their friendship and cooperation.

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CHAPTER I

INTRODUCTION

Carbon is one of the important nutrients for sustaining microorganisms in the environment. When carbon is present in sufficient amounts along with other nutrients such as phosphorus and nitrogen, the enrichment of the biomass in the environment takes place unhindered. However, this enrichment, if permitted to proceed in lakes and streams, may affect the quality of life by increasing primary productivity, decreasing the dissolved oxygen concentration and eventually rendering the body of water useless.

In order to limit the concentration of these nutrients it is necessary that they be removed from wastewater before it is discharged into the receiving stream. The activated sludge process, among other methods, is one of the most important biological processes that has been proven effective in removing these nutrients from wastewater prior to discharge. In this process, microorganisms utilize carbon, phosphorus and nitrogen in the presence of oxygen for metabolism and growth. However, for growth to be balanced and for maximum removal of nutrients, the ratio in which these nutrients are available to the activated biomass is very important. Nutrients should be available in optimum proportions. Lack of these nutrients may cause severe impairment in the ability of organisms to remove organic matter. During the course of this study it was intended to gain an insight into the phosphorus

requirement of activated sludge for efficient removal of the organic matter from the waste. The purpose of this study was to investigate the optimum COD to phosphorus ratio for achieving good soluble organic carbon removal using the activated sludge process.

CHAPTER II

LITERATURE REVIEW

The activated sludge process is one of the most commonly used unit processes in water pollution control. It is a biological process wherein air is blown through the wastewater to supply the necessary oxygen level in the system and to keep the biological solids in a state of suspension. Biological solids, in turn, utilize the organic matter in the wastewater for synthesis of cell components and for energy. However, for balanced growth, the microorganisms require necessary nutrients as well as oxygen to utilize the organic carbon in the wastewater effectively. The nutrients generally required and which are essential for balanced growth and replication are nitrogen, phosphorus, sulfur, and other trace elements. Lack of these essential nutrients in the ecosystem may cause a reverse strain on the ability of microorganisms to remove the organic matter effectively from wastewater.

Various researchers have studied the optimum level of phosphorus required for efficient removal of organic matter by the activated sludge process. Lea and Nichols (1) as early as 1937 studied the ability of the sludge to remove organic matter in the presence and/or absence of both nitrogen and phosphorus, and found that it is essential that these nutrients be in sufficient amounts in order to measure the oxygen uptake of the sewage.

According to Greenberg et al. (2), the phosphorus requirement of

the activated sludge system is

. . . the minimum concentration of phosphorus permitting maximum BOD removal without causing an impairment of the sludge settling characteristics. This requirement depends primarily on the rate of sludge growth which, in turn, is dependent on BOD loading and aeration solids (p. 277).

They also studied the physical and chemical characteristics of the sludge due to phosphorus deficiency and phosphorus incorporation. Deficiency of phosphorus in the system resulted in poor settling characteristics and a shift in the population to filamentous organisms. They further demonstrated that in the complete absence of phosphorus, the development of activated sludge may not be possible and phosphorus deficiency caused reduced nitrogen utilization. They also found that phosphorus incorporation influenced the growth of sludge.

Helmets et al. (3) reported that the average maximum phosphorus requirement for stabilization of industrial waste in the activated sludge is .85 to one percent/100 lbs BOD removal.

Phosphorus Utilization in Activated Sludge

It is necessary to note several physical and biochemical parameters which influence the utilization of phosphorus in activated sludge. Melbury et al. (4) reported that all treatment plants which have a high phosphorus removal efficiency are plug flow reactors. In the investigation of the Baltimore Black River Plant, the effect of the flow plan was studied by these researchers using a plug flow reactor having a theoretical hydraulic detention time of 6.5 hours. Phosphorus removal efficiencies ranged from 30 to 90 percent. Using a step aeration flow design, the removal efficiency dropped to 40 percent. As a result, a plug flow process was reported to attain a greater percent

removal. Beer (5) suggests that the activated sludge must be taken through a period of great metabolic activity while under anaerobic conditions to encourage luxury uptake of phosphorus. These conditions exist at the head of a plug flow tank.

Hydraulic Detention Time

Melbury et al. (4) reported the hydraulic detention time does not affect phosphorus removal. Spiegel and Forrest (6) reported that any detention time is satisfactory; however, Witherow et al. (7) believe that phosphate reduction in the aeration tank is time-dependent and requires a model detention time of 2.5 hours or more.

Aeration Tank Suspended Solids Concentrations

Aeration tank suspended solids is one of many parameters studied by many investigators. In the Baltimore study (4), a range of suspended solids from 1400-3000 mg/l was used, with no effect on removal efficiency. Convery (8) reported to the Environmental Protection Agency that the recommended mixed liquor suspended solids range be limited to 2500-4000 mg/l. Witherow (7) stated that phosphorus removal increased with increasing MLSS concentration to an optimum level. The optimum level varied between 2500-4000 mg/l.

Dissolved Oxygen Level and Rate of Aeration

According to Nesbitt (9), the dissolved oxygen concentration should be at least 2 mg/l at the middle point of the tank, and 5 mg/l at the outlet for good removal efficiency. Melbury (4) reported a release of phosphate at the Baltimore Black River Plant when the DO

decreased from 3 to 2 mg/l at the outlet of the aeration basin. Phosphorus leakage as a result of low dissolved oxygen levels was confirmed by Wells (10) and Levin and Shapiro (11). Witherow (7) in his study of the San Antonio treatment plant found that phosphorus removal did not occur in the tanks with 0.2 to 0.4 mg/l DO, but 70 percent removal occurred in the tank with 1.5 to 5.3 mg/l DO. Levin and Shapiro (11) stated that the rate at which oxygen is applied affects the orthophosphate uptake capacity of the sludge organisms considerably. Menar and Jenkins (12) concluded that the operating parameter such as DO has no effect upon enhanced removal of phosphate by activated sludge. Gurmeet et al. (13) reported that aeration rates high enough to ensure aerobic conditions and a high degree of turbulence are considered to be the most effective means of biological phosphate removal.

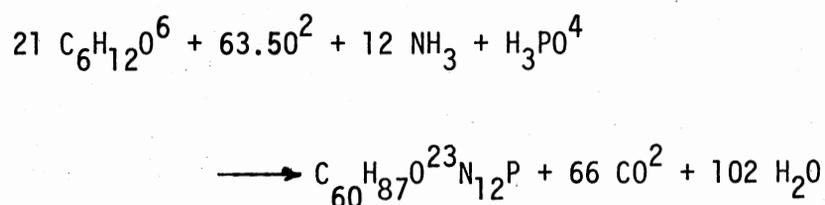
pH

To study the effect of pH on phosphate uptake, Levin and Shapiro (11) used a series of batch units with pH values of 5, 6, 8, and 9, and concluded that phosphate uptake is a function of pH. The greatest uptake took place at a pH range of 7 to 8. A pH of 9 resulted in a significant decrease in phosphate uptake and, furthermore, at pH values of 5 and 6, a rapid release of phosphate from microorganisms was reported.

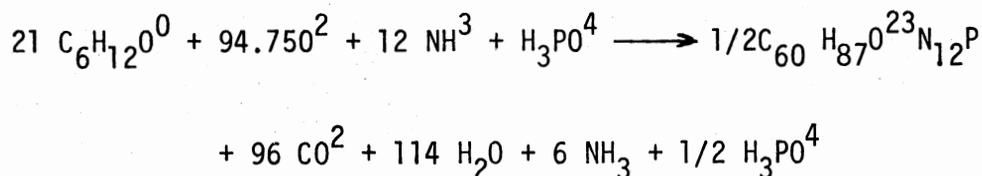
Mean Cell Residence Time and Stoichiometric Relationship

The effect of solids production on nutrient removal, especially phosphorus removal, was studied by Vacket et al. (14), who reported

that a higher growth rate is desired for maximum phosphorus removal. Mulbarger et al. (15) found that maximum removal efficiency occurred at a mean cell residence time of approximately 3.5 days. Decreasing removal efficiency took place at lower mean cell residence times due to the increase in effluent biological solids. Sherrard et al. (16) demonstrated that for a given cell and carbon source, phosphorus incorporation increased as the solids production increased. They found a stoichiometric equation describing the biological growth process. They reported that for a 2-day cell residence time when other nutrients are in excess, 100 percent phosphorus removal is possible. This is expressed in the following equation:



For the same amount of material at a 14-day residence time, the equation would be



As a result, it was concluded that phosphorus removal varies directly with sludge production. Stoichiometry of substrate has a significant effect in the phosphorus removal of the system. Sherrard et al. (16) in his studies showed at five days θ_c and COD:P ratios of 130:1 and 65:1 removal of phosphorus for the 130:1 ratio was higher than for

the 65:1 ratio.

Luxury Uptake Theory

Luxury uptake has been the subject of many papers, and it is defined by Levin and Shapiro (11, p. 800) as "the uptake of dissolved orthophosphate in the absence of growth." They concluded that an extra quantity of phosphorus could be incorporated into activated sludge due to DO concentration and pH. A high level of DO increases the rate of uptake, and low level DO was found to cause a leakage of phosphorus. Maximum results were obtained in a pH range of 7-8 (11). Mulberg (15) believed that since "volutin" granules have been observed in the cell and have been found to contain a considerable amount of stored phosphate in the form of metaphosphate, cells might have six to eight percent phosphate. Jenkins and Menar (12) used the works of Sawyer (17) and Hall and Engelbrecht (18) to conclude that the activated sludge mass can incorporate only about two to three percent biologically bound phosphorus by weight of volatile suspended solids. Any additional phosphorus in the sludge is due to chemical precipitation of phosphate. This theory was explained on the basis that phosphate reacts with calcium and forms a precipitate that becomes incorporated in the sludge floc and is later removed from the process with the waste sludge. They also concluded that the amount of incorporated phosphorus is proportional to net microbial growth. However, Gurmeet et al. (13) stated that calcium precipitation theories cannot explain the phosphate removal by enhanced aeration. He believed that the luxury uptake removal mechanism may depend on the available surface area.

Carberry and Tenney (19) studied the phenomenon of luxury uptake

in activated sludge with respect to various parameters, and concluded that luxury uptake does not occur by chemical precipitation, and their studies indicated that it may be caused by a biological mechanism causing the phosphate to be transported across the cell membrane. Two possible mechanisms by which phosphate could be transported into the cell would be the diffusion-controlled mechanisms driven by a positive concentration gradient between the outside and inside of the cell, or the active transport mechanisms in which energy derived from the metabolic process inside the cell drives the transport of phosphorus into the cell against a negative concentration gradient.

Borchardt and Azad (20) studied the removal of phosphorus in algae and extended their conclusion to bacterial reactions. Their conclusion was that luxury uptake in biological systems occurred only after periods of phosphate starvation.

If phosphorus removal is a real luxury uptake phenomenon, then application of the process to any activated sludge operating at the conditions for luxury uptake must produce high performance. Unfortunately, this has not proved to be true. Menar and Jenkins (12) found that a pilot plant operating under the same conditions (organic loading, hydraulic detention time, mean residence time, and DO) as the Rilling plant at the time of phosphate removal could not produce such high removal during those conditions. The activated sludge phosphorus content average was two to three percent of suspended solids.

Phosphorus Removal Efficiency in Activated Sludge

An activated sludge normally can remove 20 to 30 percent influent phosphorus by biological means (12). Higher phosphate removals, greater

than 70 percent, have been reported at municipal wastewater plants in San Antonio, Texas (4), and Baltimore, Maryland (5). Some reports (12, 17) have shown that the amount of phosphorus incorporated by activated sludge can be as high as seven percent by weight. Hatting (21) studied the effect of low sludge phosphorus content and stated that with a sludge having less than one percent phosphorus, 95 percent removal is possible at a substrate BOD:P ratio of 100:0.24.

Greenberg et al. (2) studied the minimum required sludge phosphorus content for good growth using batch laboratory units. When the substrate had a BOD:P ratio of 100:0.42, they reported that a BOD removal greater than 90 percent occurred with a sludge phosphorus content of one percent. However, below this value, BOD removal decreased. As they increased the concentration of phosphorus in the feed (BOD:P ratio of 100:2.5), the sludge phosphate increased to 1.6 percent.

Morgan et al. (22) concluded that the minimum required sludge phosphorus content for normal growth (85 to 95 percent COD removal) is approximately one percent, and occurs with an influent COD:P ratio of 100:0.15.

Wells (10) showed that there is a difference in the phosphate uptake rates because some sludges have greater ability to remove phosphorus from solution, while other sludges can remove the same organic material at much lower rates. He also showed that phosphate-acclimated sludge always showed a high total phosphate composition of six to eight percent phosphorus by dry weight of suspended solids. The non-acclimated sludge showed a much lower phosphate, i.e., about three to four percent.

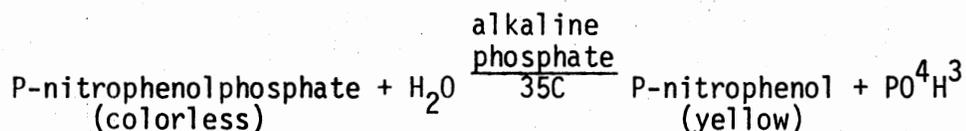
Jenkins and Menar (12) studied phosphorus removal through primary sedimentation in several plants with wastewater hardness ranging from

120 to 400 mg/l CaCO₃, and found that phosphorus removal is related directly to suspended solids removal. They also operated a pilot plant activated sludge system with an organic loading higher than those normally used in practice, and found that regardless of the loading, the cell phosphorus content was 2.6 percent of the suspended solids and that biological suspended solids and phosphorus removal was 20 percent.

Vacker et al. (14) reported up to 96 percent removal of total phosphate has been attained in an activated sludge plant where digester liquors were not returned to the system.

An Enzymatic Technique to Recognize Excess Phosphorus Uptake

The alkaline phosphatase technique utilizing P-nitrophenolphosphate as the only source of phosphate is used as a quantitative measurement of phosphorus assimilation by the microorganisms. P-nitrophenolphosphate is colorless, and when it loses its phosphate group, produces a yellow color. The reaction takes place as follows:



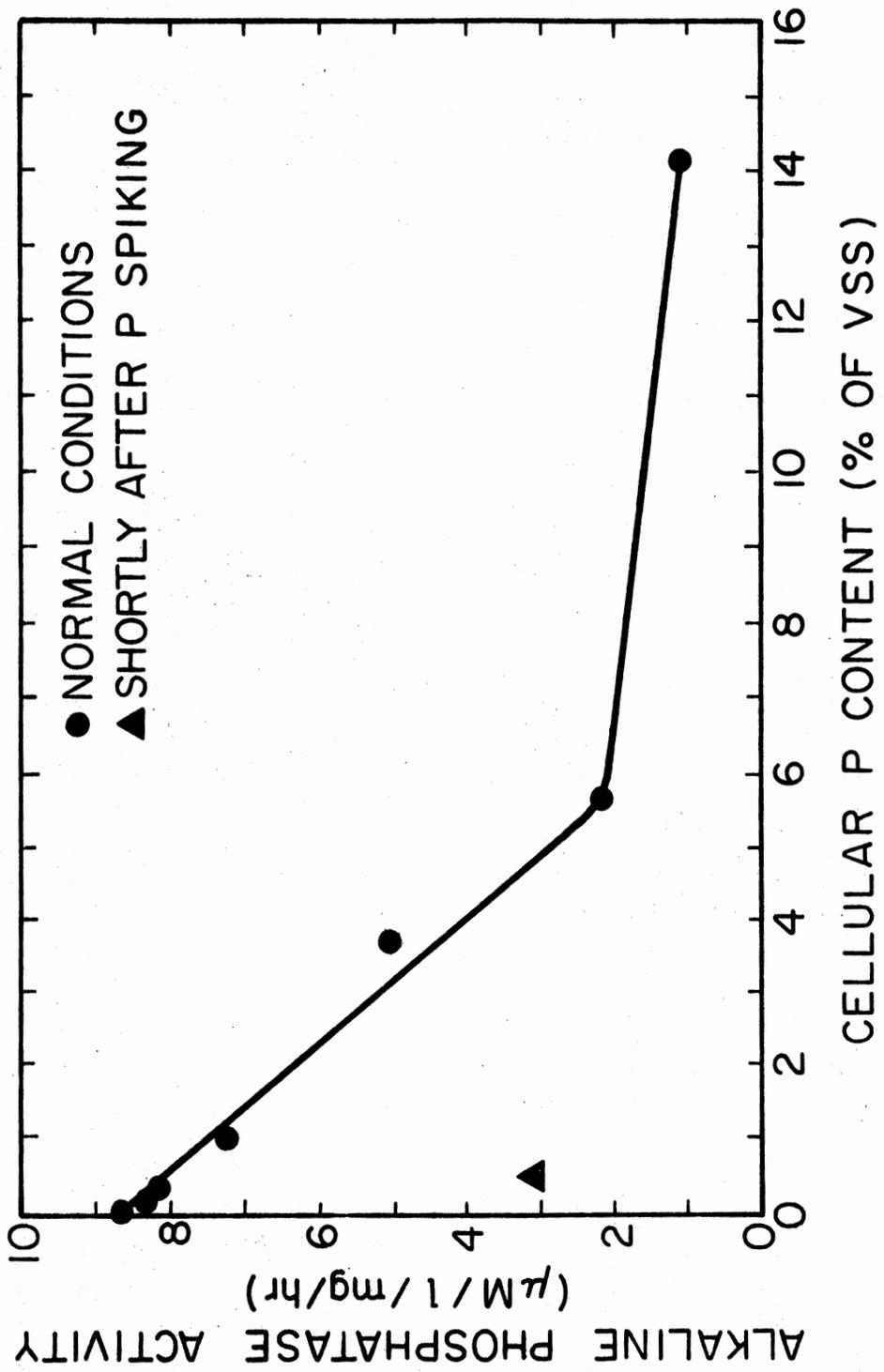
Liberated P-nitrophenol can be related to alkaline phosphate activity when P-nitrophenolphosphate is the only source of phosphorus.

Fitzgerald and Nelson (23) developed an alkaline phosphate bio-assay method for recognizing surplus metabolic uptake of phosphorus by algae. They found that there is a significant difference in the alkaline phosphate activity of algae when phosphorus is not limited

compared with limiting conditions.

Moore et al. (24) studied the relationship between alkaline phosphate activity and the cell phosphate content, and found that the alkaline phosphate activity decreases with increases in the cell phosphorus content. Some of these results are presented in Figure 1.

Figure 1. Relationship of Enzyme Activity and Cellular Phosphorus Content in Dilute Bacterial Cultures (after Moore et al.)



CHAPTER III

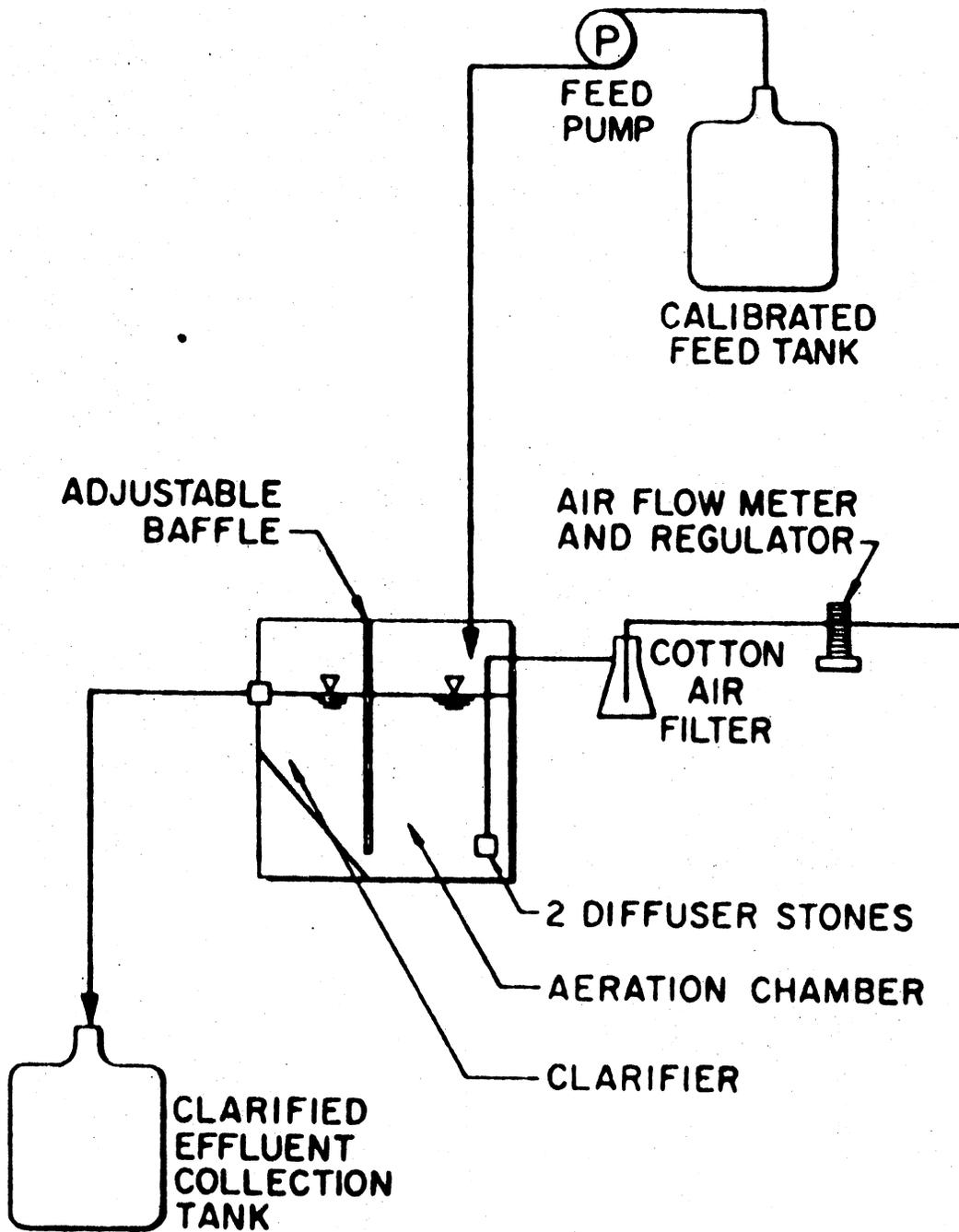
MATERIALS AND METHODS

To study the effect of the COD:P ratio on the activated sludge system, a laboratory bench scale unit was operated under controlled conditions. A description of the laboratory apparatus, the feed solution, initial startup, daily protocol, analytical procedure, and methods of data analyses are given below.

Laboratory Apparatus

A diagram of the laboratory apparatus used in this investigation is shown in Figure 2. A 4.5-liter plexiglass reactor with internal recycle of bacterial cells was used as the aeration tank and secondary clarifier. The aeration and settling compartment were separated by an adjustable plexiglass baffle. The aeration and clarifier volumes were 2.83 and 1.67 liters, respectively. A feed rate of 5.8 ml/min provided a hydraulic detention time of eight hours in the aeration tank and 4.8 hours in the clarifier. Air was supplied to the aeration compartment through two sintered glass diffusers at a rate of 3.5 liters/minute to provide an adequate oxygen supply to the biological solids for complete mixing of the aeration tank solids. The airflow rate was measured through a Gelman airflow meter. A positive displacement pump (Mini-pump, Masterflex) was used to provide a continuous flow to the system. Plastic tubing was used for both the suction side

Figure 2. Experimental Activated Sludge Unit With Internal Recycle



and the discharge side of the pump. Feedlines were cleaned by pumping one percent Clorox and water to prevent bacterial growth. The pumping rate was checked daily, using a graduated cylinder and timer. The effluent flowed by gravity from the settling compartment to the holding tank, where it was collected.

Feed Solution

Listed in Table I is the chemical composition of the synthetic wastewater used in this study. Glucose was used as the carbon and energy source. The synthetic waste was designed to have a chemical oxygen demand (COD) of 1000 mg/l. Other required nutrients were provided in the concentrations as shown in Table I. Potassium phosphate monobasic (KH_2PO_4) was used to supply phosphorus for the system. When varying the COD:P ratio of the feed, the COD was held constant and the phosphorus concentration was changed. Phosphorus concentrations of 1, 2, 3, 5, 7, 9, 10, 20, 35, and 50 mg/l were used. A bicarbonate buffer was employed to control the pH of the system. Tap water was used to dilute the concentrated stock solutions to the final volume of 20 liters. The feed solution pH was around 9. The feed solution was prepared by placing five liters of water in the glass bottle and then stock solutions of glucose, magnesium, manganese sulfate, ferric chloride, calcium chloride, potassium phosphate monobasic (KH_2PO_4) and sodium bicarbonate (NaHCO_3) were added in that order and thoroughly mixed in the original five liters of water. The feed solution was then diluted to final volume of 20 liters. Each time the feed was prepared to last for two days. The feed container was cleaned with chromic acid cleaning solution and rinsed several times with water before every feed

preparation.

TABLE I
COMPOSITION OF SYNTHETIC WASTEWATER

Constituents	Stock Concentration per ml (grams)	Final Concentration per 20 l (ml)
Glucose	400	100
MgSO ₄ · 7H ₂ O	20	200
MnSO ₄ · H ₂ O	2	200
(NH ₄) ₂ SO ₄	200	100
KH ₂ PO ₄	8.82	*
NaHCO ₃	60	100
FeCl ₃ · 5H ₂ O	0.1	200

* Depending upon COD:P ratio

Initial Startup

The original seed of microorganisms was taken from an activated sludge unit operated by T. Manickam in the Oklahoma State University bioengineering laboratories. These cells were cultivated in a batch reactor using synthetic waste. After growing a sufficient quantity of cells, the unit was switched to continuous flow operating conditions.

Daily Procedure

The synthetic waste was prepared every other day according to the proportions shown in Table I. A 20-ml sample of the fresh feed was removed for chemical oxygen demand determinations. A second 20-ml sample was removed for total feed phosphorus analysis. The pH of the feed was checked, and the feed supply was connected to the clean feed lines. A 20-ml sample of filtered effluent was used for chemical oxygen demand analysis. A 25-ml sample of the aeration basin mixed liquor was filtered for determination of the mixed liquor suspended solids. The effluent line was plugged, the baffle removed, and the contents were mixed thoroughly. A 25-ml sample was removed and filtered. This sample provided data for the total system suspended solids concentration. After mixing the reactor contents thoroughly, a part of the mixed liquor of the system was recovered and wasted. Care was taken in wasting the sludge so that the system always remained at a steady state (constant mean cell residence time of approximately six days) irrespective of the magnitude of effluent solids leakage. After wasting, the baffle was replaced and the plug removed. The unit was then put back on continuous flow operation. The pH of the aeration basin was monitored daily and recorded. Microscopic observations were made periodically.

Analytical Procedure

After the system reached steady state conditions, sampling was started to provide the necessary data for this investigation. The chemical oxygen demand, phosphorus concentration, aeration basin

biological solids concentration, volatile suspended solids, alkaline phosphate activity, and pH measured for five days. The following is a brief description of the methods and equipment used to measure these parameters.

Feed COD determinations were made in accordance with Standard Methods (25). Effluent COD determination used the dilute method as given in Standard Methods. Biological solids concentration was determined by filtering the appropriate volume through membrane filters (p.45 μ pore size, Millipore Filter Corp., Bedford, Mass.). The filter papers were placed in an oven and dried at 103⁰C for two hours. After reaching room temperature, initial weights were determined. All weights were obtained by using a Mettler Instrument Corporation balance (No. 1-910). Phosphorus determinations were made in accordance with procedures used by Morgan and Fruh (22). pH was determined by use of a Beckman Expandomatic SS-2 pH meter. Volatile suspended solids were measured according to Standard Methods (25). To measure enzymatic alkaline phosphatase activity, a 25-ml sample was taken from the aeration tank, filtered through a pre-washed 0.45 μ membrane filter, transferred to a 250-ml Erlenmeyer flask containing 100 ml P-nitrophenolphosphate (PNP). pH was maintained at 9, and the sample was incubated at 35⁰C. The initial concentration of PNP was 500 mg/l. After various incubation periods, the concentration of P-nitrophenol liberated by the action of alkaline phosphatase activity was determined by measuring the transmittance with a Bausch and Lomb Spectronic 20 at a wave length of 410 m μ and referring to a standard curve for P-nitrophenol. The analytical procedures for protein and carbohydrate tests, for which samples were taken from the aeration tank, placed in glass vials and frozen for

analysis were found in M-2 (Manual 2, Bioenvironmental Engineering Department, Oklahoma State University)(26).

Phosphorus in the cells was measured by taking a 50-ml sample from the aeration basin. A 25-ml sample out of the 50 ml was used for measuring the total phosphate concentration in the system. The rest was filtered through a Millipore filter and then used for measuring the soluble phosphorus in the system. The amount of phosphate in the cells was calculated by taking the difference between the total and soluble phosphate value. For each ratio, similar experimental protocol was strictly maintained after allowing the system to reach a new steady state.

Methods for Data Analysis

Treatment efficiency or COD removal was calculated according to the expression

$$E = \frac{100(A_0 - A)}{A_0}$$

where

E = COD removal efficiency, percent

A_0 = influent COD, mg/l

A = effluent COD, mg/l

Phosphorus removal efficiency was computed by using the following equation:

$$E_p = \frac{100 (P_I - P_E)}{P_I}$$

where

E_p = phosphorus removal efficiency, percent

P_I = influent phosphorus concentration, mg/l

P_E = effluent filtered phosphorus concentration, mg/l

The phosphorus content of the cells was calculated by the following equation:

$$\%P = \frac{(A - B) \times 100}{C}$$

where

$\%P$ = phosphorus content of a cell on dry weight percent

A = total phosphorus concentration of unfiltered sample, mg/l

B = total phosphorus concentration of filtered sample, mg/l

C = volatile suspended solids, mg/l

Alkaline phosphatase activity was calculated by using standard curve of P-nitrophenol and concentration of volatile suspended solids.

$$N = \frac{K}{V \times M}$$

where

N = alkaline phosphatase activity, $\mu\text{m}/\text{l}/\text{mg VSS}/\text{hr}$

K = concentration of P-nitrophenol liberated, $\mu\text{m}/\text{l}$

V = volatile suspended solids concentration, mg/l

M = time in which sample was in incubator, hr

CHAPTER IV

RESULTS AND DISCUSSION

A laboratory activity sludge unit was operated for a period of seven months. The influent COD was maintained at near 1000 mg/l and the phosphorus concentration was varied. The detention time in the aeration tank was eight hours, and mean cell residence time was maintained at approximately six days. A summary of steady state data for each of ten ratios is shown in the Appendix. The following is a description of the nature of the sludge and its characteristics for each ratio which was noted from visual assessments and various microscopic observations.

The initial system was operated with a COD to phosphorus ratio of 100:0.1 until it reached steady state. As can be seen from the summary of data, the experimental value of COD and phosphorus differed slightly from the theoretical value expected. The biomass showed a tendency for bulking, but the effluent was not turbid. Microscopic observations confirmed the presence of fungi and filamentous organisms during this run. After collection of data, the phosphorus concentration was changed to 2 mg/l in the feed, and the system took nearly 21 days to come to a steady state. During steady state it had a poor settling, with leakage of solids in the effluent. Microscopic observation showed that the shape of the organisms changed and it also confirmed the presence of different species of filaments. In brief, a change in predominance was

noted.

Poor settling sludge and a turbid effluent were seen in the COD to phosphorus ratio of 100:0.3. In response to the change in the phosphorus concentration from 3 to 5 mg/l, the amount of solids in the effluent decreased considerably, and settleability of the sludge changed and improved. The color of the biomass changed to orange. The biomass settleability improved in the COD to phosphorus ratio of 100:0.7, and the effluent was clear. Color of the biomass changed to yellow. For high phosphorus concentrations (9, 10, 20, 35, and 50 mg/l), the sludge had good settling and the effluent was clear. Microscopic observation did not show any detectible differences in the nature of the organisms. During the phosphorus-limiting conditions, filamentous organisms seemed to have predominated, but as the phosphorus in the feed was increased (0.9 percent of feed concentration), the percent of filaments with respect to the total biomass decreased.

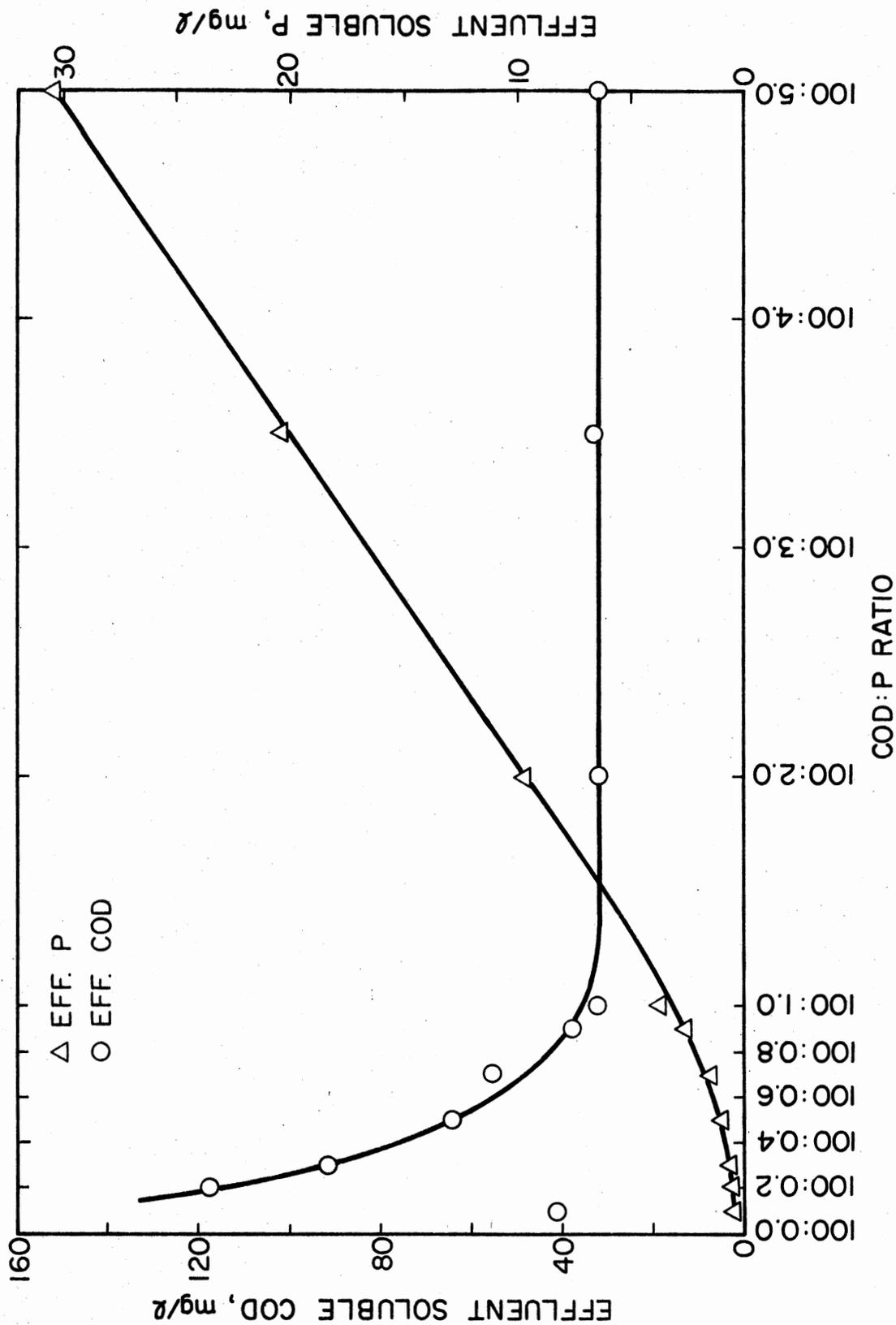
Table II shows the summary of the various parameters that have been measured for different COD:P ratios under steady state conditions. The values given in the table are the average values only. The raw data is presented in the Appendix. For comparison purposes, arithmetic plots have been made, using different characteristics to depict the nature of the system in relation to the basic ratios, their effect on the quality of the biomass and treatment efficiency of the activated sludge system.

Figure 3 shows the relationship between the COD:P ratio and the effluent COD concentration. Also shown in the figure is the effluent phosphorus concentration. It can be observed from Figure 3 that the COD in the effluent is greatest (117.6 mg/l) when the phosphorus

TABLE II
AVERAGE OF RAW DATA

Ratio	COD			Phosphorous Concentration					A.P.A. μM/mg VSS/hr	Protein %	Carbo. %	Biolog. Solids SS mg/l
	Feed mg/l	Eff. mg/l	Remov. Effec. %	Feed mg/l	So1. Eff. mg/l	Phos. Removed mg/l	Remov. Effec. %	Cell Phos. %				
100:0.1	1002.6	41.2	95.8	1.00	.4	.6	60.0	.16	1.11	42.0	13.3	3134
100:0.2	1082.6	117.6	89.1	1.99	.39	1.6	80.4	.19	3.33	45.4	12.5	1221
100:0.3	1007	91.6	90.9	2.99	.5	2.49	83.3	.35	1.57	48.6	12.9	1495
100:0.5	1043.5	64.2	93.8	5.14	.97	4.17	81.0	.43	.79	54.2	13.5	2227
100:0.7	1071	55.2	94.8	7.16	1.42	5.74	80.0	.55	.45	58.0	13.9	3719
100:0.9	1087	37.9	96.5	9.0	2.59	6.41	71.0	.7	.38	61.0	14.7	3824
100:1	1067	32.1	97.0	10.4	3.62	6.78	65.2	.84	.15	62.0	15.5	3862
100:2	1031	32.5	96.8	19.9	9.67	10.23	51.0	1.11	.03	63.4	15.8	3561
100:3.5	1066	33.4	96.8	35.48	20.34	15.14	42.7	1.27	.005	63.7	16.0	3256
100:5	1016	33.1	96.7	49.0	30.2	18.8	38.4	1.3	.004	63.8	16.2	3434

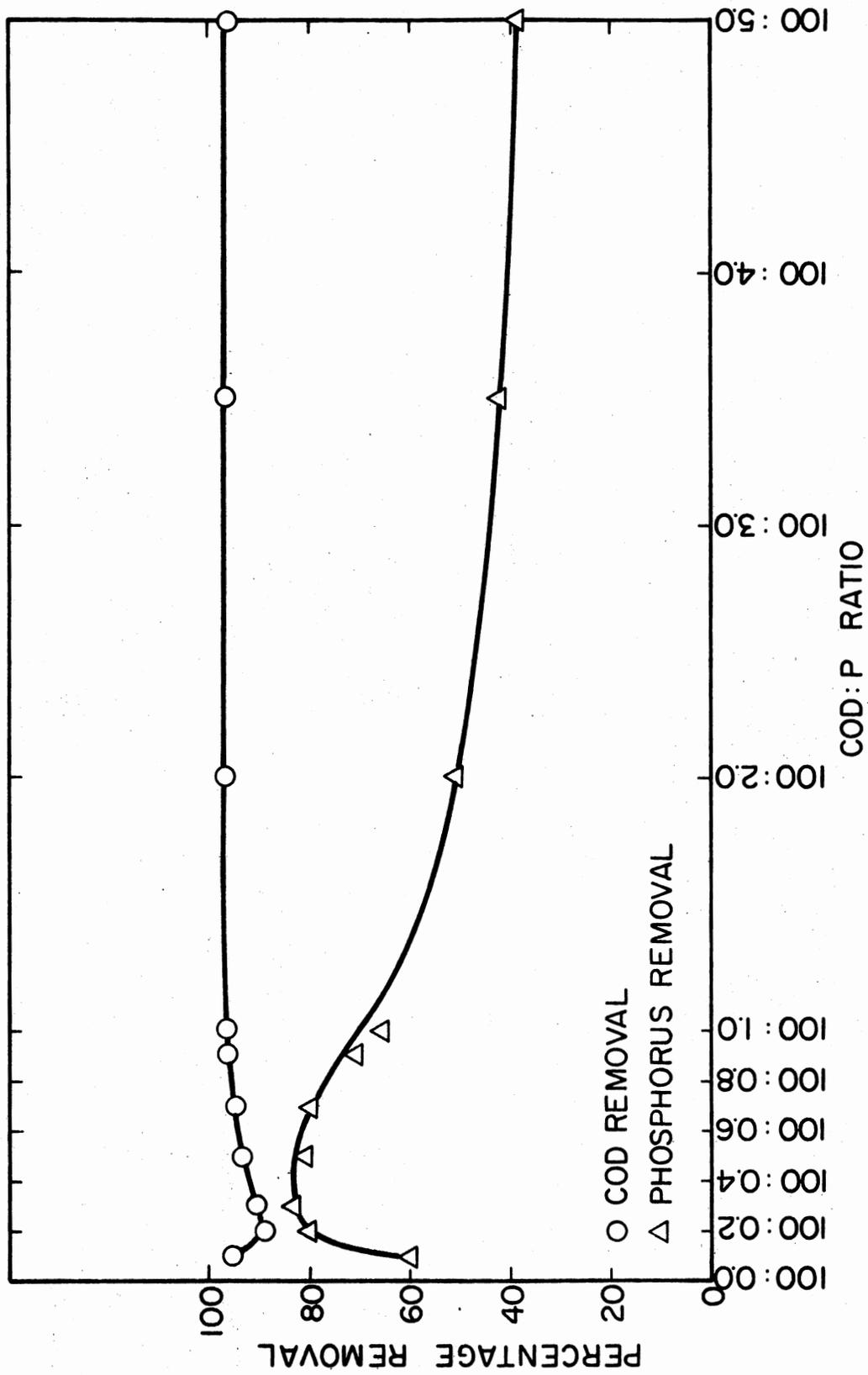
Figure 3. Effect of COD:P Ratio on Effluent Soluble COD and Soluble Phosphorus



concentration in the feed is low (1.99 mg/l) and decreases to a value of 32.1 mg/l at the 100:1 ratio. Any further increase in the phosphorus concentration did not improve the effluent quality. Also, the effluent phosphorus at low ratios is minimal but increases with higher phosphorus concentration in the feed. From COD:P ratios of 100:1 to 100:5, the interval during which the effluent COD remained constant, the effluent, P, increased significantly, indicating that increasing the P content of the feed above the 100:1 ratio did provide for enhanced COD removal but resulted in a deterioration of effluent quality with respect to phosphorus.

Figure 4 is a plot of the removal efficiency for COD and phosphorus against various feed phosphorus concentrations, keeping the influent glucose concentration constant at approximately 1000 mg/l. The COD removal efficiency was low at 89 percent when the feed phosphorus was limiting, but increased to a value of 97 percent with increase in feed phosphorus concentration. Further increase in the feed phosphorus beyond one percent glucose concentration did not increase the COD removal efficiency, and it remained at a relatively constant value even though the feed phosphorus concentration was increased in relation to the carbon source. The percentage removal of phosphorus showed the opposite behavior. The percentage phosphorus removal was high at about 83 percent under phosphorus-limiting conditions, but it increased more rapidly between the ratios of COD:P of 100:0.7 to 100:2 to a value of 50 percent. Further increase in the phosphorus value in the feed did not affect the phosphorus removal capacity appreciably. It only showed a gradual decrease in the percentage of the phosphorus removal for the ratios studied beyond the 100:2 ratio.

Figure 4. Percent of COD and Phosphorus Removal Efficiency
vs COD:P Ratio



A plot of percent of phosphorus in the cell vs the COD:P ratio is shown in Figure 5. It shows that the cell phosphorus content increased with increasing phosphorus content in the feed. The percentage of cell phosphorus was relatively constant from COD:P ratio of 100:3.5 to 100:5. The percentage of cell phosphorus increased to a value of 1.3 percent at 5 percent of the feed phosphorus concentration. The rate of phosphorus incorporated in the cell was higher during the ratios of COD:P of 100:0.1 to 100:1, but when the concentration of phosphorus was more than one percent in the feed, the percentage of feed phosphorus incorporated in the cell decreased and caused a higher percentage of feed phosphorus leaking into the effluent. Figure 4 clearly supports this observation when the available phosphate for the sludge was at a minimum, 60 to 84 percent of feed phosphorus was removed, but when the available phosphorus for the sludge was maximum, a higher amount of phosphorus was leaked into the effluent and only 38 percent of the feed phosphorus was removed. This is in general agreement with Jenkins and Menar (12). The activated sludge normally can remove 30 percent influent phosphorus. Jenkins and Menar (12) reported that the maximum phosphate incorporated in the cell would be on the order of two to three percent; however, our data showed the maximum phosphorus incorporated in the activated sludge was only 1.3 percent for the COD:P ratio of 100:5.

Figure 6 shows alkaline phosphate activity ($\mu_m/1/mg$ VSS/hr) vs the COD:P ratio. Figure 7 shows alkaline phosphate activity vs cellular phosphate content (percent of volatile suspended solids). The alkaline phosphate activity decreased with an increase in the availability of the phosphorus for the sludge. This result is in agreement

Figure 5. Percent Phosphorus Content of Sludge vs COD:P Ratio

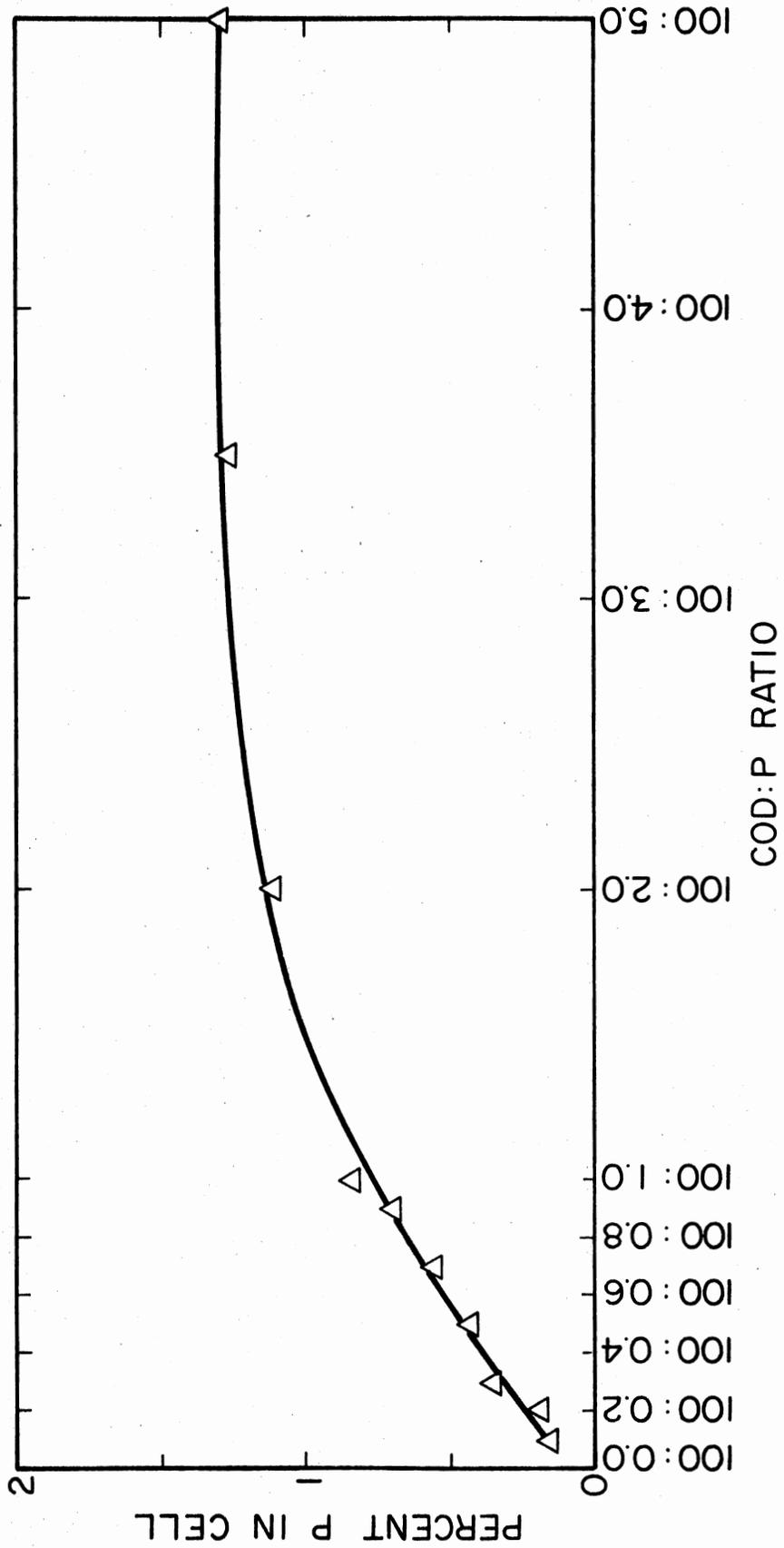


Figure 6. Alkaline Phosphatase Activity ($\mu\text{M}/1/\text{mg VSS}/\text{hr}$) vs
COD:P Ratio

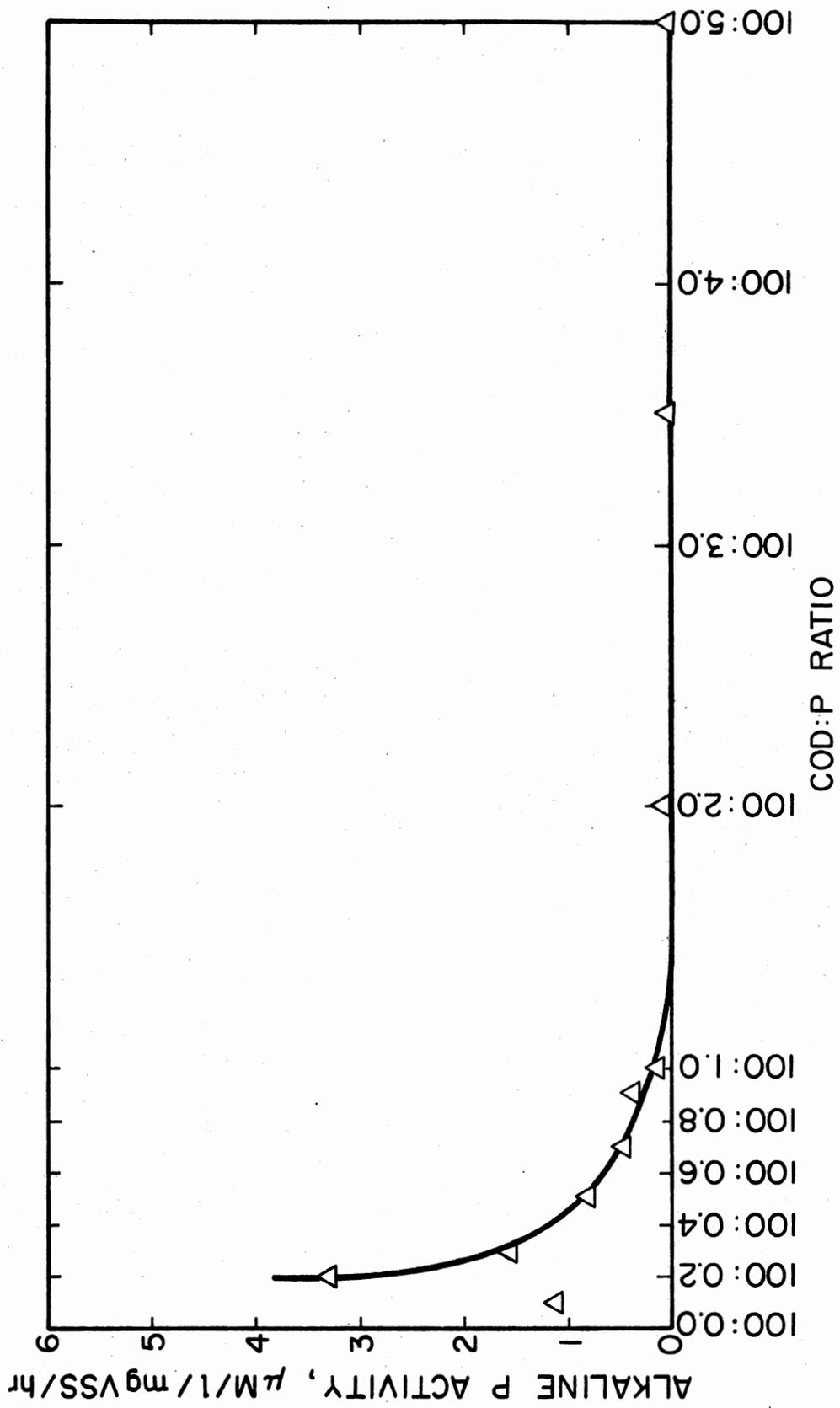
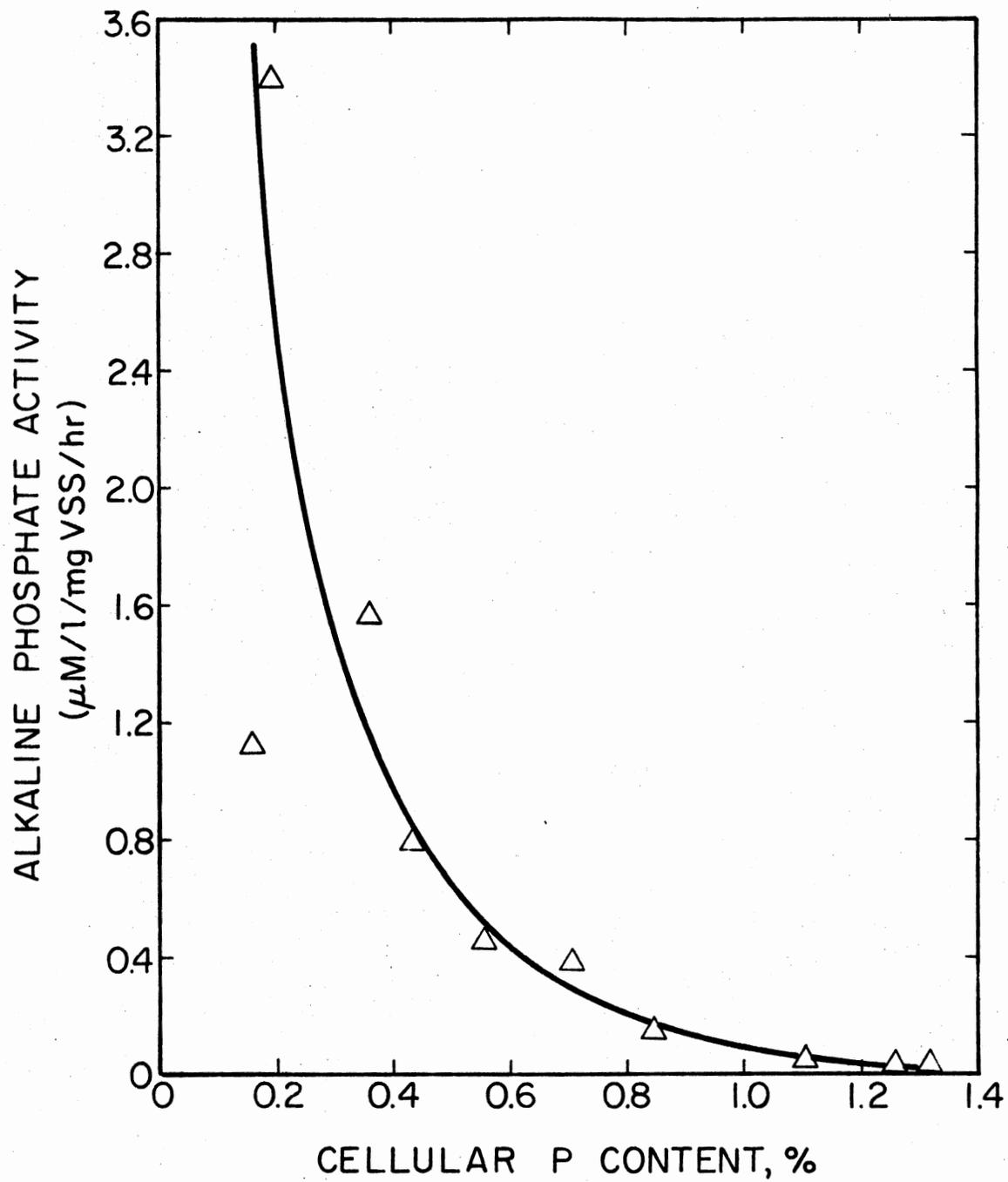


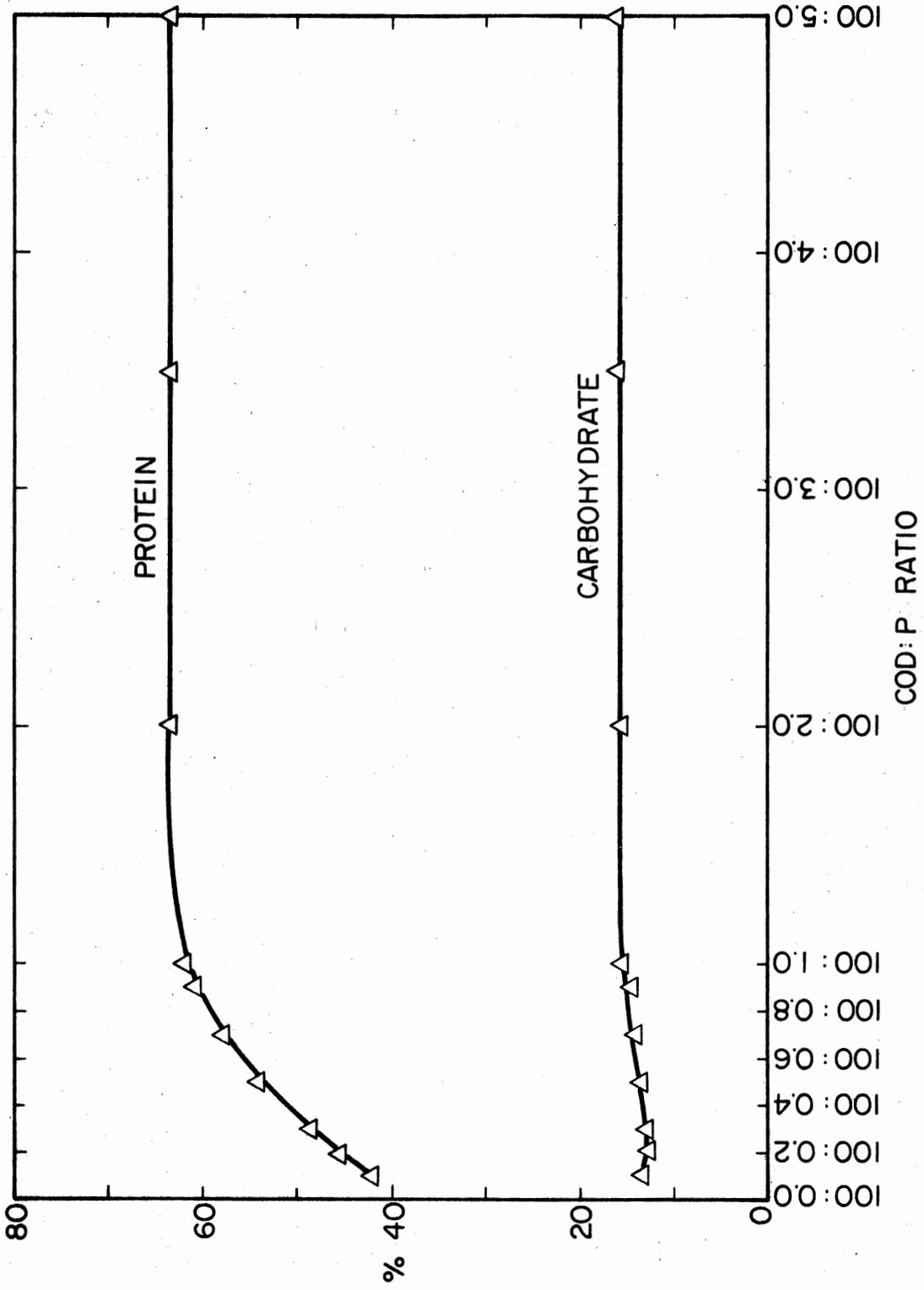
Figure 7. Alkaline Phosphatase Activity ($\mu\text{M}/1/\text{mg VSS}/\text{hr}$) vs Cellular P Content (%)



with the work of Moore et al. (24) and shows that the cellular requirement for growth and energy was not met sufficiently when the phosphorus in the feed was limiting. However, when the feed phosphorus content reached a value of two percent of the glucose concentration, the enzymatic activity did not register any additional requirement for phosphate by the cell. In other words, the phosphorus requirement of the cell was met by the phosphorus present in the synthetic feed.

Figure 8 shows the relationship between COD:P ratio of the feed vs the percentage of protein and carbohydrate content of the activated sludge. It shows that the carbohydrate content was 13.3 percent initially at a feed phosphorus content of 0.1 percent of glucose, but the value increased to about 16 percent when the phosphorus content of the feed was 3.5 percent. The protein concentration was low at 43 percent at 0.1 percent feed phosphorus. The protein content showed a gradual but steady rise until it reached a value of 63 percent at a COD:P ratio of 100:2, and this value increased only slightly for the remainder of the ratios investigation. This plot shows an increase in the protein and carbohydrate content of the cell with an increase in the phosphorus content in the feed. The protein increased 32 percent, whereas the carbohydrate increased only 19 percent. This difference may have been due to the higher energy requirement for the synthesis of protein as compared to carbohydrate. When phosphorus is limiting in the system, there might not be sufficient energy produced in the cell to synthesize the needed protein. This difference may not be deduced conclusively from the data, but the rates of increases for carbohydrate and protein content are strikingly different.

Figure 8. Percentage of Protein and Carbohydrate Content of Sludge vs COD:P Ratio



During the phosphorus-limiting conditions, most filamentous organisms predominated, but their numbers were definitely reduced as the phosphorus content in the feed was increased. The types of organisms present had a profound influence on the settling characteristic of the sludge in the clarifier. This aspect was clearly visible at the lowest concentration of phosphorus supplied at a COD:P ratio of 100:0.1. The behavior of the sludge at this ratio was entirely different. Almost a complete predominance of filaments was observed. Fungi were also observed under these very low phosphorus conditions in the feed. During this ratio, the COD removal efficiency was very high, and it is difficult to give an adequate explanation for this deviation from the general trend of the data. However, the total predominance of filamentous fungi might have contributed to the higher COD removal and the low phosphorus utilization by the sludge. Also, at this ratio, the concentration of the biomass was very high (3143 mg/l) compared to a low solids concentration of 1221 mg/l at a ratio of 100:0.2, which may also have possibly influenced the higher organic matter removal efficiencies. Another interesting observation at this ratio is that the cell carbohydrate content was relatively high at 13.3 compared to 12.5 and 12.9 percent at COD:P ratios of 100:0.2 and 100:0.3, respectively.

A high quality wastewater effluent is characterized by a low suspended solids concentration and a low concentration of soluble organic matter. From the data shown in Figure 4 it can be seen that greater than 90 percent COD removal was obtained with all of the ratios except the 100:0.2 ratio. However, settling was generally poor for the ratios less than 100:0.7 except for the 100:0.1 ratio, which has been discussed previously. Neglecting the 100:0.1 ratio, the first ratio at which high

COD removal was obtained, concurrently with a clear effluent was the COD:P ratio of 100:0.7 where 94.8 percent COD removal was achieved. At the COD:P ratio of 100:1 COD removal, protein and carbohydrate approached near maximum while the alkaline phosphate activity reached its minimum. Increasing the COD:P ratio above 100:1 provided for little increase in the above mentioned parameters; therefore the ratio of 100:1 was found to be the optimum ratio for achieving a good quality effluent. Figures 3, 4, 5, 6, and 8 support this conclusion in that deflection points in the curves occurred at the COD:P ratio of 100:1. Maximum cellular phosphorus content of 1.3 percent was reached at the 100:5 ratio, and the maximum protein (63.8 percent) and carbohydrate (16.2 percent) concentration were also reached at the 100:5 ratio. Although the maximum values for cellular phosphorus, protein and carbohydrate contents were reached at the 100:5 ratio, it should be noted that the numbers observed at the 100:1 ratio differed only very slightly from the COD:P ratio of 100:5.

The alkaline phosphatase technique experimentally predicted the phosphate requirement of the cell. As supported by other authors (24), the results of the alkaline phosphatase analyses do exhibit a correlation between enzymatic activity and the cellular phosphorus content. Alkaline phosphatase activity was at a minimum when the cellular phosphorus content reached its maximum value. In addition, when the alkaline phosphatase activity was high, the sludge was characterized by poor settling and COD removals were somewhat lower than those achieved at minimum and near-minimum alkaline phosphatase activity.

CHAPTER V

CONCLUSIONS

From the previous experimental data and observations, the following conclusions may be drawn:

1. Phosphorus-limiting causes significant changes in the physical and chemical characters of the sludge and produce a poor settling sludge that is predominantly filamentous organisms.

2. The phosphorus concentration of one percent of the organic matter (measured as COD) is essential for good organic matter removal efficiency.

3. The alkaline phosphatase technique gave a good correlation between the cellular phosphorus content and enzymatic activity.

CHAPTER VI

SUGGESTIONS FOR FUTURE WORK

1. The minimum amount of phosphorus required for maximum removal efficiency at various cell residence times should be studied.

2. The effect of phosphorus limitation on the cell physiology, settling characteristics, and predominance changes should be more thoroughly studied.

3. Further understanding of the luxury uptake theory should be undertaken more thoroughly, and application of the luxury uptake theory to remove phosphorus from the wastewater should be studied.

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APPENDIX

RAW DATA FOR EACH OF TEN RATIOS AND
STEADY STATE DATA

TABLE III
 RAW DATA FOR COD:P = 100:0.1

COD		Biolog. Sol.			Phosphorus Concentration							Alkal. Phos. Activ.		
Feed mg/l	Eff. mg/l	pH	Total SS mg/l	Total VSS mg/l	Feed mg/l	Total Eff. mg/l	Sol. Eff. mg/l	Total Aer. Bas. mg/l	Sol. Aer. Bas. mg/l	Sludge P mg/l	% Phos- phorus in Cell	μ m/l		
												15 min.	30 min.	1 hr.
998	41	7.27	3136	3074	1.02	.51	.39	5.4	.5	4.9	.16	60	75	88
1002	44	7.2	2950	2895	1	.53	.4	5.6	.49	5.1	.17	59	72	85
1012	39	6.9	3250	3185	1.01	.52	.42	5.4	.49	4.91	.15	61	76	87
1005	42	7.2	3125	3063	1	.58	.38	5.5	.5	5	.16	60	74	85
996	40	7.29	3210	3146	.98	.54	.4	5.3	.48	4.82	.15	58	73	84
1002.6	41.2		3134	3071	1	.54	.4	5.4	.49	4.95	.16	59.6	74	85

TABLE IV
RAW DATA FOR COD:P = 100:0.2

COD		Biolog. Sol.			Phosphorus Concentration							Alkal. Phos. Activ.		
Feed mg/l	Eff. mg/l	pH	Total SS mg/l	Total VSS mg/l	Feed mg/l	Total Eff. mg/l	Sol. Eff. mg/l	Total Aer.Bas. mg/l	Sol. Aer.Bas. mg/l	Sludge P mg/l	% Phos- phorus in Cell	μ m/l		
												15 min.	30 min.	1 hr.
1001	108	7.45	1258	1021	1.95	1.74	.37	2.2	.36	1.84	.18	55	70	85
1010	115	7.54	1261	1018	1.89	1.95	.39	2.4	.46	1.94	.19	54	72	84
1126	119	7.35	1184	1022	2.05	2	.41	2.5	.42	2.08	.2	55	73	86
1135	121	7.4	1210	966	2	1.9	.38	2.3	.38	1.92	.19	45	60	74
1141	125	7.51	1195	935	2.08	1.85	.4	2.46	.42	2.04	.2	53	72	85
1082.6	117.6		1221	992	1.99	1.89	.39	2.37	.41	1.96	.19	52.4	69.4	82.8

TABLE V
RAW DATA FOR COD:P = 100:0.3

COD		Biolog. Sol.			Phosphorus Concentration							Alkal. Phos. Activ.		
Feed	Eff.	pH	Total	Total	Feed	Total	Sol.	Total	Sol.	Sludge	% Phos-	μ m/l		
mg/l	mg/l		SS	VSS		mg/l	Eff.	Eff.	Aer.Bas.			Aer.Bas.	P	phorus
			mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	in Cell	min.	min.	hr.
1019	98	7.5	1570	1559	3	1.78	.51	6	.51	5.49	.35	44	51	60
1005	97	7.45	1472	1463	2.95	1.85	.49	6.5	.45	6.05	.41	42	50	57
1006	88	7.4	1485	1473	3	1.2	.48	5.6	.5	5.1	.34	39	48	55
1012	85	7.5	1510	1499	3.01	1.92	.5	5.5	.48	5.02	.33	45	53	61
995	90	7.45	1440	1425	3	1.3	.52	5.4	.49	4.91	.34	40	47	58
1007	91.6		1495	1482	2.99	1.61	.5	5.8	.49	5.31	.35	42	49.8	58.2

TABLE VI

RAW DATA FOR COD:P = 100:0.5

COD		Biolog. Sol.			Phosphorus Concentration							Alkal. Phos. Activ.		
Feed mg/l	Eff. mg/l	pH	Total SS mg/l	Total VSS mg/l	Feed mg/l	Total Eff. mg/l	Sol. Eff. mg/l	Total Aer.Bas. mg/l	Sol. Aer.Bas. mg/l	Sludge P mg/l	% Phos- phorus in Cell	μ m/l		
												15 min.	30 min.	1 hr.
1050	65	7.2	2916	2276	5.24	1.9	.95	11	1.35	9.65	.42	35	40	45
1049	64	7.15	2789	2174	5.21	2.02	.92	10.98	1.25	9.73	.44	34	39	42
1045	65	7.12	2830	2205	5.08	2.3	1.02	11.1	1.4	9.7	.43	36	41	44
1039	63	7.18	2845	2212	5.1	1.8	.98	10.75	1.12	9.55	.43	36	38	43
1035	64	7.14	2905	2267	5.08	2.28	1.0	11.2	1.25	9.95	.42	35	39	42
1043.5	64.2		2857	2227	5.14	2.06	.97	11	1.27	9.7	.43	35.2	39.6	43.8

TABLE VII
RAW DATA FOR COD:P = 100:0.7

COD		Biolog. Sol.			Phosphorus Concentration							Alkal. Phos. Activ.		
Feed mg/l	Eff. mg/l	pH	Total SS mg/l	Total VSS mg/l	Feed mg/l	Total Eff. mg/l	Sol. Eff. mg/l	Total Aer.Bas. mg/l	Sol. Aer.Bas. mg/l	Sludge P mg/l	% Phos- phorus in Cell	μ m/l		
												15 min.	30 min.	1 hr.
1071	55	7.1	3712	3562	7.1	3.65	1.45	21	1.51	19.49	.54	24	38	41
1065	56	7.11	3735	3585	7.09	3.7	1.38	21.5	1.45	20.05	.55	23	37	40
1069	54	7.09	3756	3609	7.21	3.54	1.51	21.8	1.55	20.25	.56	24	39	37
1074	57	7.05	3691	3543	7.13	3.69	1.39	22	1.45	20.55	.55	25	38	39
1079	54	7.1	3702	3570	7.25	3.72	1.35	21.5	1.51	19.99	.55	24	37	43
1071	55.2		3719	3574	7.16	3.66	1.42	21.56	1.51	20.7	.55	24	37.8	40

TABLE VIII

RAW DATA FOR COD:P = 100:0.9

COD		Biolog. Sol.			Phosphorus Concentration							Alkal. Phos. Activ.		
Feed mg/l	Eff. mg/l	pH	Total SS mg/l	Total VSS mg/l	Feed mg/l	Total Eff. mg/l	Sol. Eff. mg/l	Total Aer.Bas. mg/l	Sol. Aer.Bas. mg/l	Sludge P mg/l	% Phos- phorus in Cell	μ m/l		
												15	30	1
												min.	min.	hr.
1089	39	7.38	3850	3520	9.1	5.9	2.6	31	4.85	26.15	.74	15	25	33
1089	38	7.29	3825	3518	9.05	5.65	2.55	29.8	5.1	24.7	.70	16	24	35
1075	37.5	7.35	3798	3495	8.75	6.5	2.65	28.7	4.9	23.8	.68	17	25	32
1075	37	7.32	3810	3479	8.9	6.3	2.60	28.5	5.25	23.25	.67	15	24	34
1107	38	7.3	3839	3560	9.2	6.6	2.55	29	4.5	24.5	.69	18	23	35
1087	37.9		3824	3514	9	6.19	2.59	29.4	4.92	24.48	0.7	16.2	24.2	33.8

TABLE IX
RAW DATA FOR COD:P = 100:1.0

COD		Biolog. Sol.			Phosphorus Concentration							Alkal. Phos. Activ.		
Feed mg/l	Eff. mg/l	pH	Total SS mg/l	Total VSS mg/l	Feed mg/l	Total Eff. mg/l	Sol. Eff. mg/l	Total Aer.Bas. mg/l	Sol. Aer.Bas. mg/l	Sludge P mg/l	% Phos- phorus in Cell	μ m/l		
												15 min.	30 min.	1 hr.
1081	33	7.5	3875	3675	10.4	8	3.5	38	6.8	31.2	.84	9	10	15
1080	32	7.6	3864	3680	10.5	7.5	3.55	38.5	6.5	32	.86	8	11	14
1054	32.5	7.5	3840	3641	10.3	7.4	3.75	37.8	6.9	30.9	.84	7.8	12	15
1054	32	7.4	3864	3654	10.6	7.2	3.85	37	6.6	30.4	.82	8	15	14
1070	31	7.5	3870	3675	10.2	7.5	3.45	38	6.4	31.6	.86	7	13	12
1067	32.1		3862	3665	10.4	7.54	3.62	37.86	6.64	31.22	0.84	7.96	12.2	14

TABLE X
RAW DATA FOR COD:P = 100:2.0

COD		Biolog. Sol.			Phosphorus Concentration							Alkal. Phos. Activ.		
Feed mg/l	Eff. mg/l	pH	Total SS mg/l	Total VSS mg/l	Feed mg/l	Total Eff. mg/l	Sol. Eff. mg/l	Total Aer.Bas. mg/l	Sol. Aer.Bas. mg/l	Sludge P mg/l	% Phos- phorus in Cell	μ m/l		
												15	30	1
												min.	min.	hr.
987	32.9	7.28	3546	3274	19.5	10.65	9.24	49	12	37	1.13	0	0	2
991	32.5	7.3	3570	3280	19.4	11.8	9.37	48.5	11.85	36.65	1.1	0	0	3
1054	32.8	7.25	3605	3224	20.2	11.2	9.85	51	12.02	38.99	1.12	0	0	2.5
1060	32	7.27	3554	3283	19.98	11.4	10.02	50	11.64	38.36	1.1	0	0	2.9
1064	32.3	7.26	3530	3268	20.42	10.34	9.85	50.5	11.5	39	1.11	0	0	3
1031	32.5		3561	3265	19.9	11.1	9.67	49.8	11.80	38	1.11	0	0	2.68

TABLE XI
RAW DATA FOR COD:P = 100:3.5

COD		Biolog. Sol.			Phosphorus Concentration							Alkal. Phos. Activ.		
Feed mg/l	Eff. mg/l	pH	Total SS mg/l	Total VSS mg/l	Feed mg/l	Total Eff. mg/l	Sol. Eff. mg/l	Total Aer.Bas. mg/l	Sol. Aer.Bas. mg/l	Sludge P mg/l	% Phos- phorus in Cell	μ m/l		
												15 min.	30 min.	1 hr.
1075	33.5	6.95	3365	3128	35.2	24.4	19.5	62.2	22.5	39.7	1.26	0	0	.54
1076	33.6	7.2	3295	3068	34.9	23	20.5	61.8	22.7	39.1	1.27	0	0	.48
1045	34	7.1	3125	2909	35.6	22.8	21	59.8	23	36.8	1.26	0	0	.5
1046	33	6.9	3314	2991	35.5	23.5	20.7	62	22.3	39.7	1.29	0	0	.4
1092	32.9	7.2	3285	3074	36.2	23.8	20	62.3	22.5	39.8	1.28	0	0	.39
1066	33.4		3256	3034	35.48	23.5	20.34	61.62	22.6	39.02	1.27	0	0	.46

TABLE XII
 RAW DATA FOR COD:P = 100:5.0

COD		Biolog. Sol.			Phosphorus Concentration							Alkal. Phos. Activ.		
Feed mg/l	Eff. mg/l	pH	Total SS mg/l	Total VSS mg/l	Feed mg/l	Total Eff. mg/l	Sol. Eff. mg/l	Total Aer. Bas. mg/l	Sol. Aer. Bas. mg/l	Sludge P mg/l	% Phos- phorus in Cell	μ m/l		
												15	30	1
												min.	min.	hr.
998	33.1	7.23	3485	3294	47.6	34.1	28.4	74.3	33.5	40.8	1.3	0	0	.2
997	33.3	7.21	3425	3235	47.8	35.3	28.9	74.5	33.2	41.3	1.3	0	0	.3
1050	33.2	7.2	3395	3212	50.3	34.5	31	75	32.9	42.1	1.3	0	0	.4
1052	33.1	7.22	3415	3218	50.2	34.9	31.5	74.8	33.4	41.4	1.3	0	0	.2
984	33	7.25	3452	3262	49.5	35	31	75	33.4	41.6	1.3	0	0	.3
1015	33.1		3434	3244	49	34.8	30.2	74.72	33.28	41.44	1.3	0	0	.28

TABLE XIII
STEADY STATE DATA

Ratio	Solids, mg/l	COD, mg/l
100:.1	1324-1560-2465-2960-3105	53-49-47-45-44
100:.2	875-1054-1348-1175-1260	155-162-160-131-109
100:.3	830-1190-1254-1324-1419	138-112-108-103-99
100:.5	1750-2052-2570-2592-2834	81-80-79-67-68
100:.7	2970-3514-3691-3715-3698	63-59-58-55-56
100:.9	3615-3703-3630-3712-3851	53-47-43-38-37
100:1	3614-3701-3630-3824-3738	37-36-35.5-35-34.8
100:2	3612-3503-3605-3390-3512	34-33.5-33-34-33.7
100:3.5	3328-3250-3198-3325-3218	34-33.9-34-32-33.3
100:5	3204-3239-3122-3309-3442	33.9-33-35-33.9-32

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