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MICROBIAL RELEASE OF SOLUBLE PHOSPHATE IN AN ACTIVATED SLUDGE ENVIRONMENT

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PREFACE

The rapid increase in population and technology associated with modern civilization has resulted in increased water usage and, through the accumulation of waste products, increased deterioration of the quality of water that can be obtained from natural resources. One obvious manifestation of the change in water quality has been the overgrowth of algae and other aquatic plants. This effect has been attributed to the presence of increased concentrations of inorganic nutrients, particularly nitrogen and phosphorus, in the waterways.

A major source of fertilizer elements is treated sewage effluents, since nitrogen and phosphorus are incompletely or, in most cases, insignificantly removed in sewage treatment plants as conventionally operated. However, a profile of phosphorus concentrations through most plants will show that significant quantities of phosphorus are sorbed during biological treatment, but are then desorbed back to solution before the biological solids are removed. It has also been demonstrated that as much as 90% of the phosphorus can be removed by conventional treatment under certain, but as yet undefined, conditions.

The research reported herein was undertaken to increase the general understanding of the phosphorus removal mechanisms of activated sludge and to investigate ways that desorption of soluble phosphorus can be reduced or prevented in conventional treatment processes. It was the intention to provide designers and operators with knowledge that could be used to attain economical phosphorus removal.

Clifford W. Randall

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INTRODUCTION

One of the major problems confronting the water pollution control industry today is the eutrophication of surface waters receiving treated wastes. This nutrient enrichment from waste treatment plant discharges results in both economic and aesthetic problems of major proportions. The stream or lake may become clogged with aquatic plants which impair recreational benefits and interfere with the flow. The floating, malodorous masses of dead algae put a severe stress on all the aquatic species, not to mention the stress placed on the olefactory senses of the local residents. As the dead plants decay, the dissolved oxygen (DO) of the stream is depleted and this can result in large fish kills if not corrected.

It is widely accepted that, in the vast majority of cases, eutrophication can be controlled by limiting the concentration of phosphorus. Researchers disagree on what the lowest allowable limit should be, but a frequently mentioned figure is 0.03 mg/l as PO_4 after the effluent is mixed with the stream.[1] Since the influent concentration of soluble phosphate to a municipal treatment plant usually ranges from 20 to 50 mg/l as PO_4, a significant reduction must be accomplished by waste treatment to control eutrophication. However, sewage treatment plants as presently designed and operated achieve very little phosphorus removal at best, and in many cases accomplish no removal at all.[2] Clearly, process modifications or additional processes are needed if phosphorus control is to be achieved. Several chemical processes have been proposed for this purpose, but they are costly. Consequently, in the search for more economical methods, considerable attention has been focused on the utilization of biological systems for phosphorus control. Unfortunately, this attention has resulted in considerable controversy with respect to the ability of biological processes to remove phosphorus in significant amounts, and with respect to the mechanisms of removal involved. Most of the controversy has centered around the activated sludge process.

It has been theorized by several experts that, because of the unfavorable ratio of carbon, nitrogen, and phosphorus in sludge microorganisms as compared to that of raw sewage, it is not possible to remove significant amounts of phosphorus from domestic sewage by biological processes.[2,3,4,5] This viewpoint has been referred to as the Carbon Limitation Theory, and it is supported by the fact that in normal sewage treatment practice little phosphorus is removed. However, despite this unfavorable ratio, several investigators have demonstrated, using both full scale and laboratory units, that under the proper conditions the activated sludge process can remove

significant amounts of phosphorus from sewage.[3,4,5,6,7,8] Unfortunately, the conditions required are not understood sufficiently for design purposes, and the mechanisms of removal involved are a matter of basic disagreement. The main controversy has centered around whether the excess removals that have been observed were the result of biological activity, i.e. "luxury uptake," or were the result of peculiar chemical environments that caused phosphate precipitation. In support of luxury uptake, Moore, et al.[9] have recently demonstrated the storage of polyphosphates in activated sludge cells. On the other hand, Menar and Jenkins[10] have reported considerable data which imply that high removals occur because of the chemical precipitation of phosphorus and subsequent entrapment of the precipitate in the matrix of the biological floc. The first mechanism is entirely biological and would logically be controlled by biological parameters, whereas the second mechanism is entirely chemical and should logically be controlled by chemical parameters, particularly pH.

While it has been observed that most activated sludge plants accomplish very little phosphorus removal, this is not necessarily incompatible with the idea of luxury uptake. One relationship which is reasonably typical of all plants is that the soluble phosphorus concentration tends to decrease during organic substrate removal and then tends to increase again during subsequent treatment, particularly final settling.[3,7,8] Thus, the removal efficiency of the plant may be significantly lowered by the desorption of phosphorus back to solution before the activated sludge solids are removed from the effluent stream. Regardless of the mechanism of uptake, it is obvious that the phosphorus removal efficiency of most activated sludge plants could be improved if the release of soluble phosphate was prevented until after the solids were removed from the liquid effluent. As with uptake, however, the mechanisms of release and the conditions that control such release are poorly understood and are the subject of considerable dispute.

Postulated Mechanisms of Phosphate Release

As with excess uptake, the dispute concerning activated sludge phosphate release has centered around whether the phenomenon is basically biological or chemical in nature. Since the phosphate release phenomenon has most frequently been observed during final clarification, it was initially concluded that such release was biological and occurred because of an absence of dissolved oxygen (anoxic conditions).[3,5] However, Shapiro, et al.[11] concluded from full-scale plant studies that anoxic release occurred because of change in the redox potential rather than change in the DO concentration. They further stated that phosphate release was primarily the result of leakage

from viable cells rather than cell lysis. This conclusion was based on the observation that the increase in soluble phosphate under anoxic conditions was not accompanied by a corresponding increase in soluble carbohydrate, BOD, or dissolved Kjeldahl nitrogen.

Menar and Jenkins[10] have also reported that activated sludge phosphate release is not a direct function of the DO concentration. Consistent with their postulation that excess uptake is the result of chemical action, they have concluded that release under anoxic conditions is caused primarily by a decrease in pH which results in the resolubilization of phosphate precipitates. Thus, they have concluded that phosphate release is predominately chemical in nature.

Sekikawa, et al.[12], and Levin and Shapiro[7] have reported on the effect of pH on soluble phosphate release. The former reported that the values of 5.5 and 8.5 had very little effect compared to neutral conditions, but a pH of 4.0 greatly stimulated release. By contrast, the latter obtained results showing a drastic secretion of phosphate at pH values of 5.0 and 6.0. Both sets of investigators concluded that the observed release was the result of biological stress rather than chemical action.

Hall and Engelbrecht[13] observed no phosphate release under anoxic conditions even after several hours. However, they used a considerably higher food-to-microorganism ratio than would normally be found in actual operation, and it is possible that this was responsible for the unusual results they obtained. Vacker, et al.[4] also concluded that no release occurred with zero DO at the San Antonio Rilling Road plant, but their return sludge data shows significant leakage of phosphate from the solids indicating that there was simply a lag in release.

PROCEDURES

Laboratory-scale units were used for all experimental purposes, and most of the experiments were conducted in a constant temperature room at 20°C. Where possible, analyses were conducted in accordance with Standard Methods.[14] If modification of the procedures of Standard Methods were necessary, they are so noted during the discussion of the particular experiment concerned.

The activated sludge used in the experiments was obtained from the Roanoke, Virginia, sewage treatment plant, a conventional activated sludge installation treating a mixture of domestic sewage and industrial waste. Following collection, the sludge was maintained in a 16-liter stock unit at a temperature of 20°C using a feed substrate of raw sewage.

In general, each experiment consisted of an aerobic phase and an anoxic (anaerobic) phase. The desired volume of sludge was removed from the stock unit, settled or diluted to obtain the desired solids concentration, and then mixed with a synthetic substrate and aerated in a Plexiglas reaction vessel (Figure 1) for a period of time up to 8 hours. The composition of the substrate used is shown in Table I.

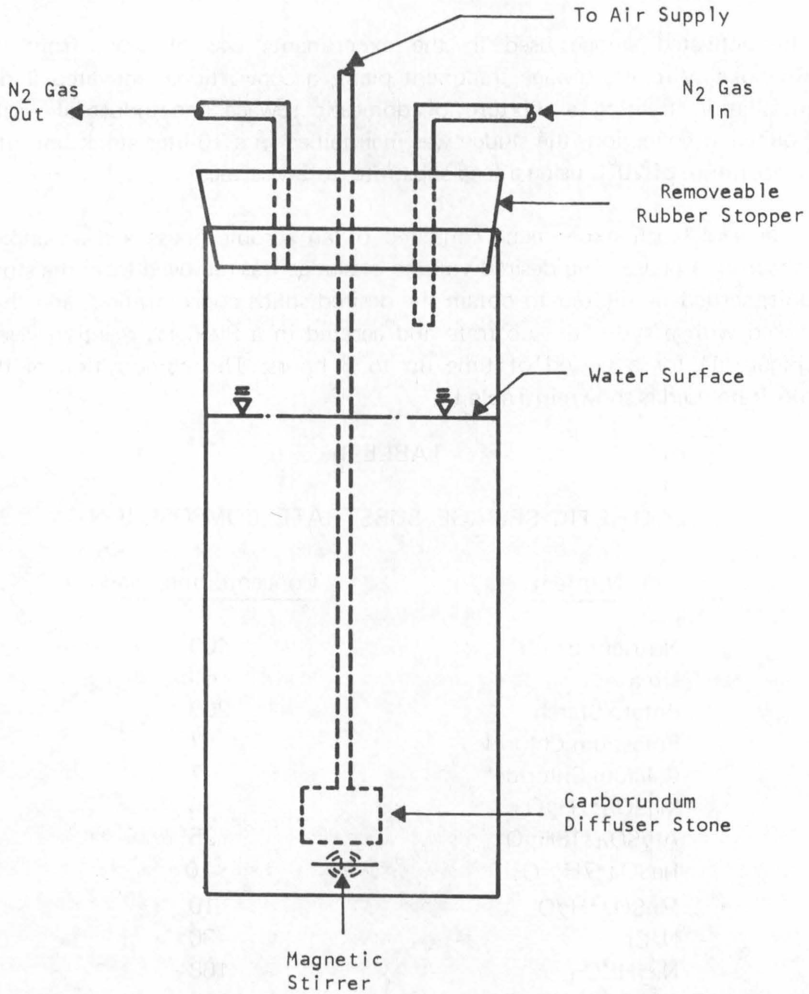
TABLE I

SYNTHETIC SEWAGE SUBSTRATE COMPOSITION

Nutrient	Concentration, mg/l
Nutrient Broth	400
Urea	60
Potato Starch	200
Potassium Chloride	7
Calcium Chloride	7
$MgSO_4 \cdot 7H_2O$	5
$Al_2SO_4 \cdot 18H_2O$	25
$FeSO_4 \cdot 7H_2O$	10
$MnSO_4 \cdot H_2O$	10
NaCl	30
Na_2HCO_3	168
KH_2PO_4	58
COD	400 mg/l

FIGURE 1

PLEXIGLAS REACTION VESSEL.



Following aeration for a specified time, mixed liquor was either removed and placed into 1-liter capacity Plexiglas reaction vessels to achieve anoxic conditions, or permitted to go anoxic in the original Plexiglas reaction unit. For the former case, aeration of the remaining mixed liquor was continued past substrate depletion in order to study phosphate release under extended aeration conditions. During aeration and anoxia, parameters such as pH, dissolved oxygen (DO), redox potential (ORP), chemical oxygen demand (COD), total organic carbon (TOC), orthophosphate concentration (PO_4), mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), alkalinity, aeration rate, and occasionally other variables were measured with time to achieve experimental objectives. Normal procedure was to hold as many variables constant as possible, and vary the one of interest. Typical aeration phase and anoxic phase procedures are described as follows.

Aeration Phase

The contents of the Plexiglas reaction vessel were aerated at the desired rate, and samples were withdrawn as necessary for analysis. Samples for orthophosphate and COD determination were withdrawn from the container by means of an open mouth pipette and then filtered through a 0.45-micron filter to remove all solids. These parameters were monitored every half hour for the first two hours and then every hour thereafter until aeration was discontinued, usually in six to eight hours. While the samples were filtering, the DO and pH of the mixed liquor were determined and recorded along with the time that the samples were taken. Additional parameters of MLSS, MLVSS, and alkalinity were measured at the beginning and end of the aeration period. The contents of the reaction vessel were continually stirred by means of a magnetic stirrer during both the aeration and anoxic phases.

Anoxic Phase

After six to eight hours of aeration, the aeration device was removed and a rubber stopper fitted with glass tubing was placed in the Plexiglas reaction vessel. Nitrogen gas was then passed over the surface to exclude all air. The magnetic stirrers were continued in operation. This procedure was shown to be necessary by the results of Shapiro, et al.[11] Samples were taken as before, except that DO was monitored every ten minutes until it reached zero, and COD analyses were not made.

It was necessary to remove the rubber stopper in order to take a sample. This procedure, along with the measurement of pH and DO took less than a minute, and the error introduced by exposure to air for this period of time was considered to be insignificant. Samples were taken every half hour for two hours, and every hour thereafter for eight hours. After eight hours the vessels were sealed under nitrogen gas, and they remained that way until twenty-four hours after the experiment was initiated. At this time samples were again taken for orthophosphate, suspended solids, and alkalinity. DO and pH were also recorded.

A Technicon auto-analyzer was used for all PO_4 and COD determinations. All DO and pH measurements were made using electrodes. A Beckman Model 915 Carbon Analyzer was used for TOC determinations.

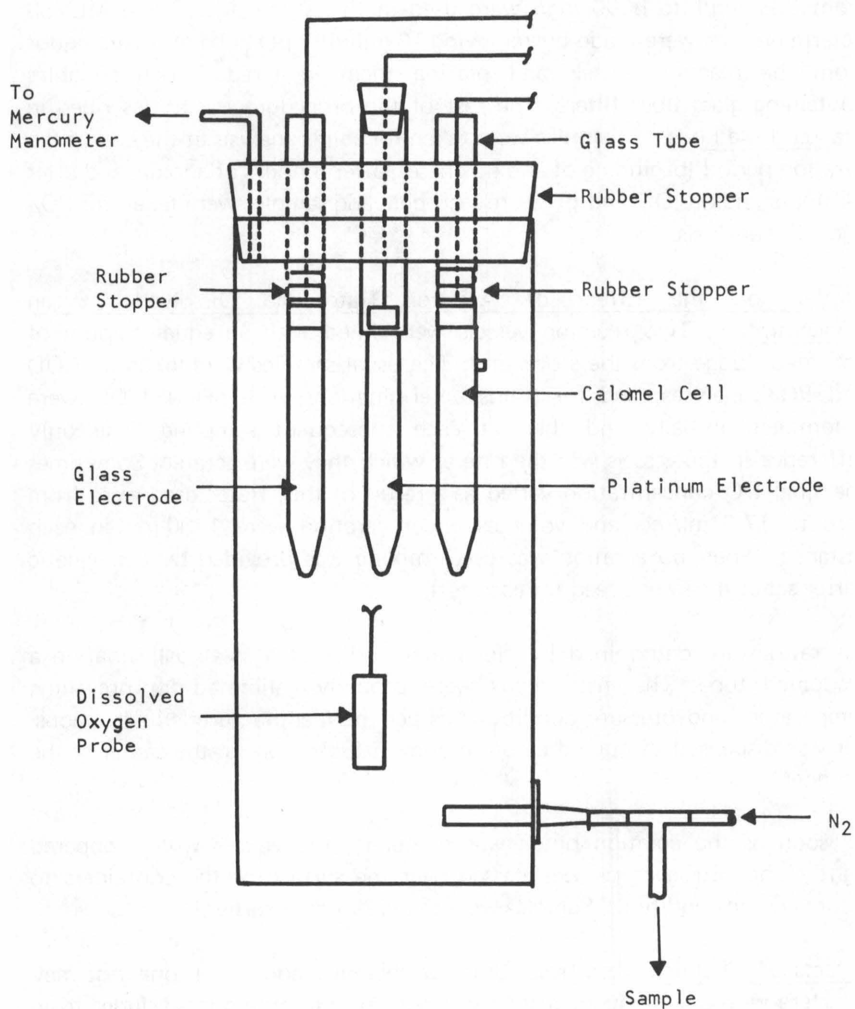
Specific Methods for Studying Various Parameters

Extended Aeration and ORP Studies. Activated sludge was first placed in a large flask and aerated for 8 hours at the rate of 10 ml per liter in the presence of the synthetic substrate to accomplish phosphate uptake. Following uptake, the mixed liquor was removed and placed into two, 1-liter capacity, sealed, Plexiglas cylinders (Figure 2) to study phosphate release under anoxic conditions. Aeration of the mixed liquor remaining in the large flask was continued so that the effect of extended aeration on phosphate release could be studied. The investigation consisted of seven such experiments which differed only in the anoxic phase where various concentrations of either Na_2SO_4 or $MgSO_4$ were added to one anoxic cylinder. These sulfate salts were chosen because they contain chemically bound oxygen which affects ORP, and because it has been reported that both compounds will completely inhibit lysis of pure cultures of microorganisms when present in 2% solution.[15] The concentrations used during this study varied from 0.007 moles per liter to 0.166 moles per liter (2%).

DO concentration, ORP, and PO_4 concentration were measured throughout the aeration and anoxic periods. Soluble COD was also monitored throughout aeration. A saturated calomel electrode was used as the standard half-cell for ORP measurements. During an experiment, the anoxic reaction vessels were opened to the atmosphere only when it was necessary to remove the ORP platinum electrode for cleansing. At those times nitrogen was bubbled through the cylinder to minimize oxygen infiltration. The contents of the cylinder were constantly mixed by a magnetic stirrer.

FIGURE 2

ONE LITER REACTION VESSEL.



Prior to each test, the ORP system was calibrated by immersing the electrodes in a solution of hydroquinone and 0.05 M potassium hydrogen pthalate. To minimize error, the same platinum electrode was used in each system throughout the study, and rigorous cleaning standards were observed. After each instantaneous measurement, the tip of the platinum electrode was placed in hot nitric acid for several minutes, then rinsed and stored in distilled water.

Procedures for Solids Studies. Mixed liquor suspended solids levels ranging from 300 mg/l to 8000 mg/l were used in this study. MLSS and MLVSS determinations were made by removing 10-milliliter portions of mixed liquor from the reaction vessels and placing them in tared Gooch crucibles containing glass fiber filters. The rest of the procedure was as described in Standard Methods.[14] Samples were taken for solids analysis at the end of the aeration period (beginning of anoxic phase), after 8 hours of anoxia, and after 24 hours anoxia. DO and pH were recorded, and samples were taken for PO_4 and COD analysis.

Studies on the Effect of Aeration Rate and Dissolved Oxygen Concentration. Two reaction vessels were filled with an equal amount of activated sludge from the stock unit. The usual samples were taken for COD and PO_4 analyses, and for solids determinations. The pH and DO were determined initially and then at each subsequent sampling. The only difference in the vessels was the rate at which they were aerated. Sometimes the final DO concentration varied as a result of this. Rates used were from zero to 17.6 ml/sec, and volumes under aeration were 1400 ml in each instance. When no aeration was used, mixing was provided by a magnetic stirrer set at the same speed for each test.

Air rates were determined by flowmeters utilizing a steel ball float in a graduated tube. The instruments were properly calibrated for prevailing temperature and pressure conditions as per the manufacturer's instructions. Air was dispensed through a carborundum diffuser stone at the center of the cylinder.

As soon as the aeration phase was complete, the vessels were stoppered tightly and nitrogen gas was passed over the surface of the containers to insure anoxic conditions. Samples were taken as stated earlier.

Effects of Chemical Additives. Since a chemical added for one test may interfere with a subsequent test, it was necessary to obtain fresh sludge from the Roanoke facility for each chemical to be tested. After obtaining the

sludge and returning it to the constant temperature room of the Sanitary Engineering Laboratory, aeration was begun in the stock unit. Eight liters of sludge were placed in the unit and two liters of the synthetic sewage were added. The mixture was then aerated overnight in order to acclimate the microorganisms to the substrate. The following morning, aeration was discontinued and the sludge was permitted to settle. Next, four liters of supernatant were siphoned from the unit, and replaced by two liters of synthetic sewage. Aeration was resumed, and after three minutes a sample was taken for PO_4 analysis and solids determination. Aeration was continued for four hours at 20 ml/sec and another sample was taken for orthophosphate analysis. This procedure was repeated to standardize conditions between experiments. DO and pH were also determined at the end of the four-hour period. Following aeration, sludge was removed from the unit and four Plexiglas reaction vessels were filled with 1200 ml each of the mixed liquor. Vessel 1 acted as a control unit. It was stoppered and all air was excluded. Vessels 2 through 4 each contained a different concentration of the particular chemical additive under study.

Before sealing the vessels under anoxic conditions, a portion from each vessel was placed in a Warburg flask to determine if the chemical had an effect on subsequent oxygen uptake. Standard Warburg Procedures were used.[16]

Food to Microorganism Studies. For these studies, 1200 milliliters of thoroughly mixed sludge was distributed among each of 4 vessels from the stock unit. Again, vessel 1 acted as a control. To vessels 2, 3, and 4, different concentrations of glucose and mineral salts were added. A stock solution of glucose and distilled water with a final COD of 10,000 ppm was prepared following the procedure as outlined in Standard Methods.[14] Dilutions were then prepared to give concentrations of 5000 and 1000 ppm. Next, 120 milliliters of each dilution were placed in a beaker and 1 milliliter of nutrients and buffer were added. The nutrients and buffer used were as specified for the BOD test in Standard Methods.[14]

In order to make the control sample comparable to the others, a 120-ml aliquot of distilled water with nutrients and buffer added was used in place of the glucose solution. When the four beakers containing 0, 1000, 5000, and 10,000 ppm glucose were added to the 1200 ml of activated sludge in vessels 1 through 4, respectively, final concentrations of approximately 0, 100, 500, and 1000 ppm of glucose were obtained in the reaction vessels. This procedure was not performed more accurately since the sludge already contained some unknown amount of organic matter; and, for purposes of this experiment, the differences between vessels was of more significance than the

absolute value of the concentration. After the organic matter was added, the vessels were sealed and nitrogen gas was passed over the surface. Samples and readings were then taken as stated earlier.

Studies on the Effect of pH. It has been reported by Menar and Jenkins[10] that the increase in soluble orthophosphate in the secondary clarifiers of activated sludge plants is due mainly to the dissolution of a calcium phosphate precipitate formed at the end of the aeration period. They stated that this dissolution is caused by the decrease in pH which normally occurs in the clarifier. Other investigators have disputed this as the primary mechanism.[7]

In order to study the effects of pH, activated sludge was removed from the stock unit and placed in two Plexiglas containers. Samples and readings were taken as stated under general procedure. Vessel 1 was held at a constant pH by adjusting with NaOH or HCl. Vessel 2 was allowed to seek its own pH level. It was reasoned that the effect of pH on phosphate release could be observed by comparing the release obtained into two units. In addition to this experiment, pH was monitored closely during all the experiments, during both uptake and release. Therefore, considerable data concerning the effects of pH in the operating range of conventional activated sludge units were obtained.

Oxygen Uptake Studies. After chemicals had been added to four vessels as described under Effects of Chemical Additives, two 10-ml samples were removed from each vessel and placed in Warburg Respirometer flasks. The purpose was to determine if the metabolism of the microorganisms had been altered to any extent. The flasks also contained 10 ml of synthetic sewage as a substrate and 1 ml of a 20% KOH solution in the center well. The flasks and manometers had previously been calibrated at this volume, so the flask constants were known. The flasks were paired so that flasks 1 and 2 contained sludge from vessel 1, flasks 3 and 4 from vessel 2, and so on. Flask 9 contained 20 ml of distilled water and acted as a thermobarometer to compensate for changes in temperature and pressure.

Readings were taken periodically over a 3-day period. Results were recorded, and the cumulative oxygen uptake was computed at a later date. Computations were done by an IBM 360 computer which was equipped with a plotter capability. Plots of oxygen uptake vs. time were made to show the rates of uptake for each experiment.

RESULTS

It was the objective of this research to provide information that could be used to improve the activated sludge process from a phosphorus removal standpoint. To achieve this purpose, it was necessary to first define the nature of activated sludge phosphate uptake and release and then to elucidate the mechanisms and parameters that cause release. Therefore, the results initially presented are designed to develop evidence pertinent to the chemical-biological release controversy. Following these, data relating to the effects of operational and environmental parameters such as aeration rate, DO concentration, ORP, and organic loading on soluble phosphate release are presented. As a final step, data pertaining to the effects of various inorganic salts on activated sludge phosphate release are given.

Phosphate Uptake

It is generally agreed that phosphate uptake occurs only when a carbon source is being metabolized. by the microorganisms. Excess or luxury phosphorus uptake occurs when the amount of phosphorus removed from solution per unit carbon uptake is considerably in excess of the corresponding ratio in normal cell protoplasm. By the carbon limitation theory, which assumes no luxury uptake, it is calculated that the ratio of phosphorus uptake as P per unit COD uptake will be approximately 1 to 100. Therefore, according to the theory, to demonstrate luxury uptake it is necessary to achieve a COD:P ratio considerably less than 100:1. Since microbial populations are very variable and the previously mentioned ratio is only approximate, a ratio of 80:1, for example, would not be considered as excess uptake. A ratio of 50:1 or less would.

The results of several phosphorus uptake experiments are shown in Table II. These data were obtained from batch units where various parameters such as initial phosphate concentration, biological solids concentration, food-to-microorganism ratio, and aeration rate were varied. Using the COD:P ratio as a criterion, excess phosphorus removal occurred in 8 of 13 experiments. The 64:1 ratio is considered as excess. While the P uptake per unit COD removed varied from less than 2 to 1 to greater than 3 to 1 above what is considered to be normal, it is interesting to note that the P uptake ratio per unit initial solids varied from 0.0032:1 to 0.0056:1, but not necessarily in order with the COD:P uptake ratio. A reasonably common ratio for the other experiments was 0.0020:1 although there were two exceptions

TABLE II

SOLUBLE PHOSPHORUS UPTAKE

Initial Phosphorus Conc. mg/l	Total Phosphorus Uptake mg/l	Ratio P Uptake: Initial Solids	Ratio Initial COD:P Uptake	Ratio COD Removed: P Uptake	Ratio Substrate: Initial Solids	Aeration Rate mg/sec/liter
18.3	7.8	0.0040:1	50:1	38:1	0.2:1	10
19.2	11	0.0056:1	36:1	27:1	0.2:1	10
17	9.3	0.0048:1	42:1	32:1	0.2:1	10
16.3	6.2	0.0032:1	64:1	47:1	0.2:1	10
19	9.8	0.0050:1	40:1	30:1	0.2:1	10
14.7	7.5	0.0038:1	52:1	39:1	0.2:1	10
14.5	7.4	0.0038:1	52:1	40:1	0.2:1	10
7.2	2.4	0.0020:1	180:1	130:1	0.35:1	21
15.6	3.3	0.0102:1	123:1	93:1	1.25:1	21
21.8	1.6	0.0029:1	300:1	188:1	0.4:1	20
14.7	2.5	0.0035:1	91:1	64:1	0.32:1	21
14.2	4.15	0.0020:1	151:1	130:1	0.3:1	13
12.4	0.7	0.00034:1	890:1	750:1	0.3:1	4.7
20	8.3	0.0020:1				
15.3	2.4	0.0010:1				18

on the high side. The two exceptions had the highest COD to solids loadings of all the experiments.

It has been postulated by Beer[17] that luxury biological phosphate uptake occurs only if the activated sludge organisms are subjected to a period of intense metabolic activity as the result of a high food-to-microorganism ratio. This conclusion is also consistent with the data of Moore, et al.[9] For the data reported here, it is interesting to note that only once was the F:M ratio high enough to produce logarithmic growth and, further, that the P uptake per unit solids during that experiment was considerably higher than any other result. This would seemingly confirm Beer's conclusion, although the amount of data is insufficient to establish the point. If the conclusion is true, however, the results would indicate that P uptake per unit solids is a better measure of luxury uptake than the COD:P uptake ratio.

The data reported in Table II also indicate that P uptake is a function of aeration rate with intermediate levels such as 10 ml/sec/l being best and low rates such as 4.7 ml/sec/l being worst. It would appear that high rates retard uptake, but this effect can be overcome by a high F:M ratio.

The Nature of Phosphate Release

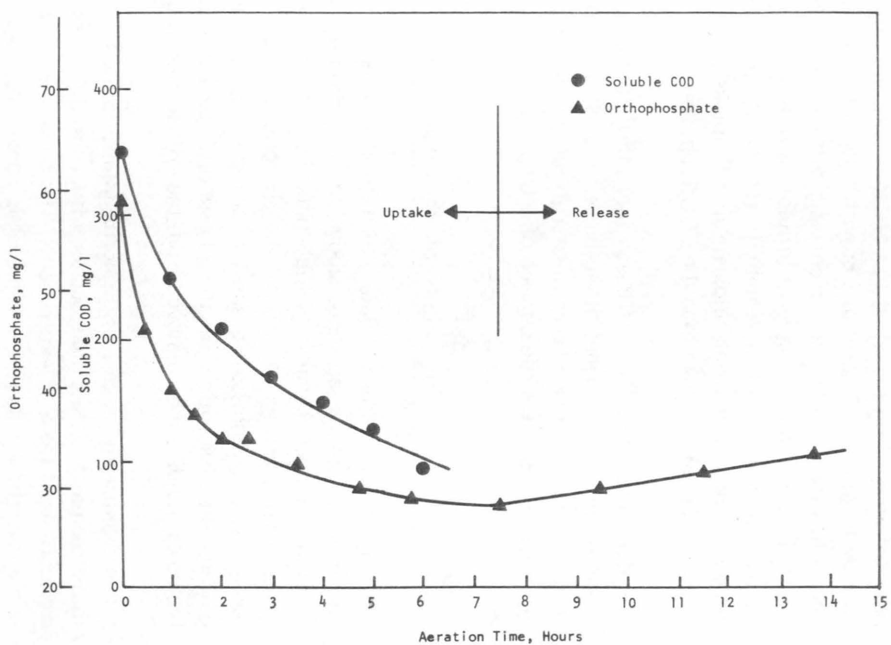
The previously discussed data illustrate that even under normal uptake conditions, a typical activated sludge concentration of 2000 mg/l biological solids will remove 4 mg/l of soluble phosphorus from solution (0.002 x 2000 mg/l). That accounts for 40% of the phosphorus typically contained in raw sewage, and it represents a substantial reduction. The problem, then, is to prevent the desorption of the phosphorus back to solution before the biological solids can be removed from the effluent supernatant.

Figure 3 illustrates typical phosphorus uptake during substrate utilization and the subsequent release that occurs with continued aeration. Similar results were obtained from all experiments. The curve clearly shows that if aeration is continued after substrate removal is complete, desorption of phosphorus from the sludge will occur. For the purpose of this discussion such desorption will be termed aerobic release.

During extended aeration, the soluble phosphate concentration always increased as a straight-line function of aeration time, indicating a steady release that would continue until the supply was exhausted. Since extended aeration imposes starvation conditions on the microorganisms, it is

FIGURE 3

VARIATION IN COD AND PHOSPHATE CONCENTRATION WITH AERATION.



noteworthy that such conditions would cause a slow, steady die-away of organisms very similar to the observed phosphate release. Seemingly, the phosphate release relfects die-away conditions, thus indicating that release is biological and that it is the result of cell disruption.

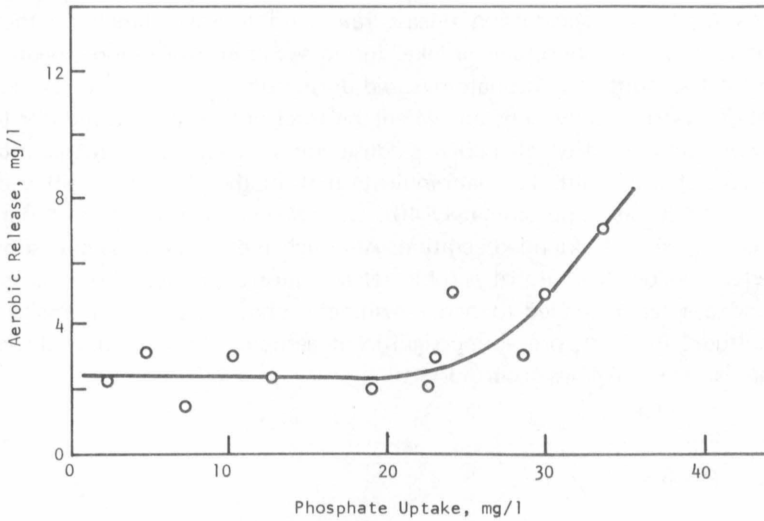
While aerobic phosphate release occurred at a steady rate during the individual experiments, a comparison of the release data from all studies shows that total release and release rate tend to vary directly with the magnitude of the phosphate uptake during substrate utilization (Figure 4). The total amount of phosphate released during the first 4 hours of extended aeration was relatively constant when the total uptake was 23 mg/l or less. However, the quantity released was considerably increased when the uptake exceeded that amount. The data indicate that, of the phosphate removed in excess of 23 mg/l, approximately 40% was released back to solution during the early stages of extended aeration. Although the total release data show a threshold value, the rate of aerobic release during the first three hours of extended aeration tended to increase with phosphate uptake regardless of the magnitude. In short, preventing significant aerobic release is more difficult when excess uptake has occurred.

Anoxic Release

During the studies, soluble phosphate release under anoxic conditions was much greater and much more rapid than that observed during extended aeration. While aerobic release was as high as 30% of the uptake only when uptake was very low (less than 5 mg/l), anoxic release averaged 60% of the total uptake. In addition, release under anoxic conditions occurred exponentially with time rather than as a linear function (Figure 5). Typically, rapid release of phosphate began when the DO concentration dropped to zero, and release was virtually complete within 90 minutes. However, a lag phase did occur in a few instances. Again, since many activated sludge organisms are obligate aerobes, it is logical to assume that a rapid die-away of cells would occur with the inception of anoxic conditions. Such a die-away would be reflected by a sudden increase in soluble phosphate similar to that actually observed if release were biological in nature. It is also consistent with cell disruption as a mechanism of release. In addition the data show that the amount of phosphate released under anoxic conditions is a strong function of the magnitude of uptake that precedes anoxia (Figure 5). This further emphasizes the difficulty of controlling desorption following excess uptake.

FIGURE 4

AEROBIC RELEASE VERSUS UPTAKE.



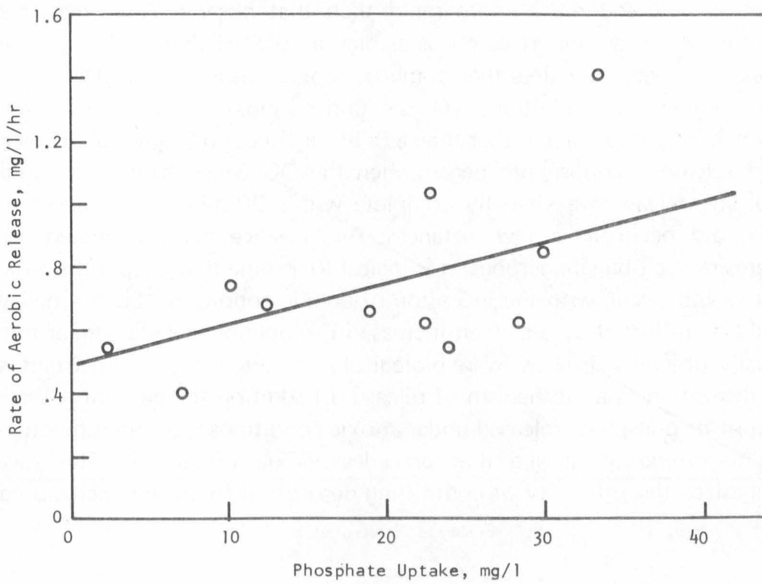
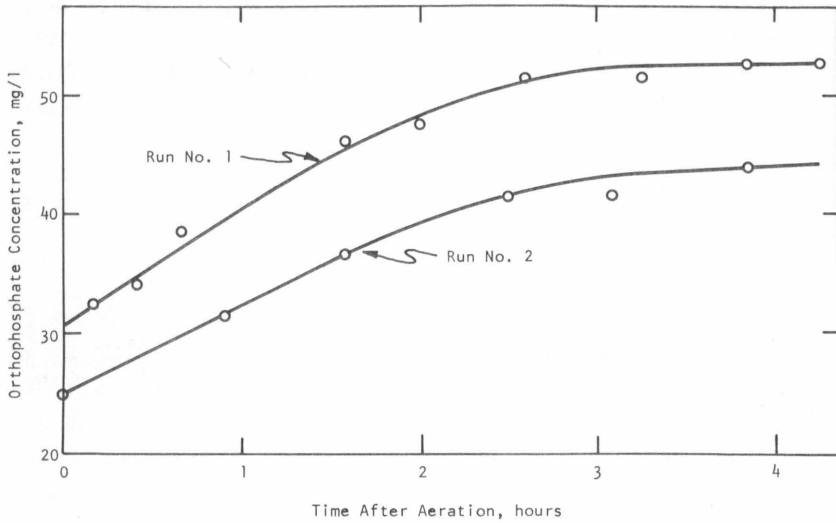
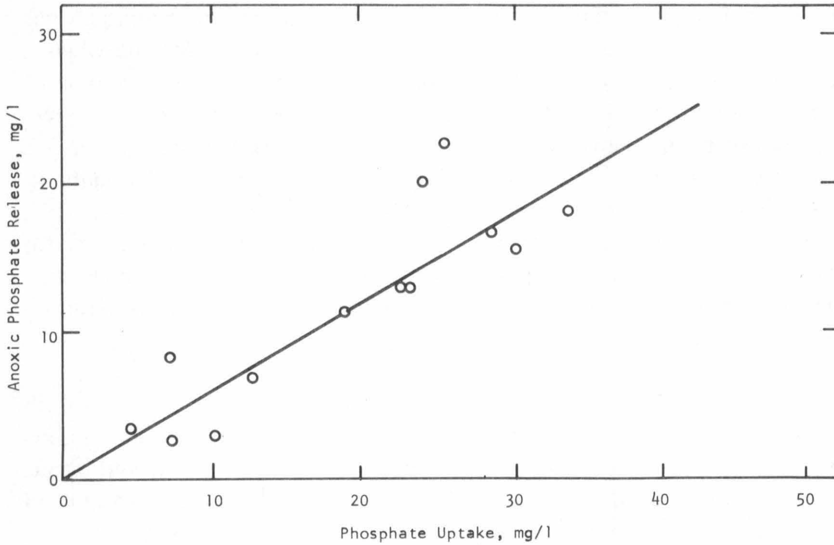


FIGURE 5

ANOXIC PHOSPHATE RELEASE.





Environmental Factors that Affect Anoxic Release

Since the amount of phosphorus desorbed under anoxic conditions greatly exceeds aerobic release, an understanding of the environmental factors and operational parameters that affect the mangitude of such release is vitally important if biological phosphorus control is to be achieved. It was of particular interest during the studies to determine whether release was predominantly a biological or chemical phenomenon. Therefore, it was essential that the relationship between pH, ORP, DO, and soluble phosphate release under anoxic conditions be established.

The Effect of pH

According to the chemical theory, a change in pH that results in resolubilization of phosphate precipitates is necessary for phosphate release to occur. Thus, a drop in pH would increase the soluble phosphate concentration, whereas a rise in pH would prevent an increase and possibly achieve a reduction.

The results of four experiments are shown in Figure 6. It is obvious from the data that pH change did not correlate with phosphate release. A rapid change in soluble phosphate usually did not coincide with pH change, and when it did, it was frequently the reverse of what would be calculated on the basis of chemical solubilities. This effect is further emphasized by the results of two simultaneous experiments where the pH in one unit was held constant at 7.5 while it was permitted to drop from 7.3 to 6.2 in the other unit (Figure 7). As the data show, the phosphate release was greater at the high pH, completely the reverse of what would occur with chemical release. No correlation whatever between pH and phosphate release could be established when pH variation was too low to cause cell death. Clearly, chemical release played no part in the observed results.

On the other hand, Figure 6 shows that phosphate release did not occur until the DO level dropped to near zero in all cases. It would appear that anoxia was one of the necessary conditions before substantial release would occur. However, since two of the plots show considerable lag between zero DO and phosphate release, it is obvious that other factors are also involved.

FIGURE 6

ORTHOPHOSPHATE VARIATION WITH pH AND DISSOLVED OXYGEN.

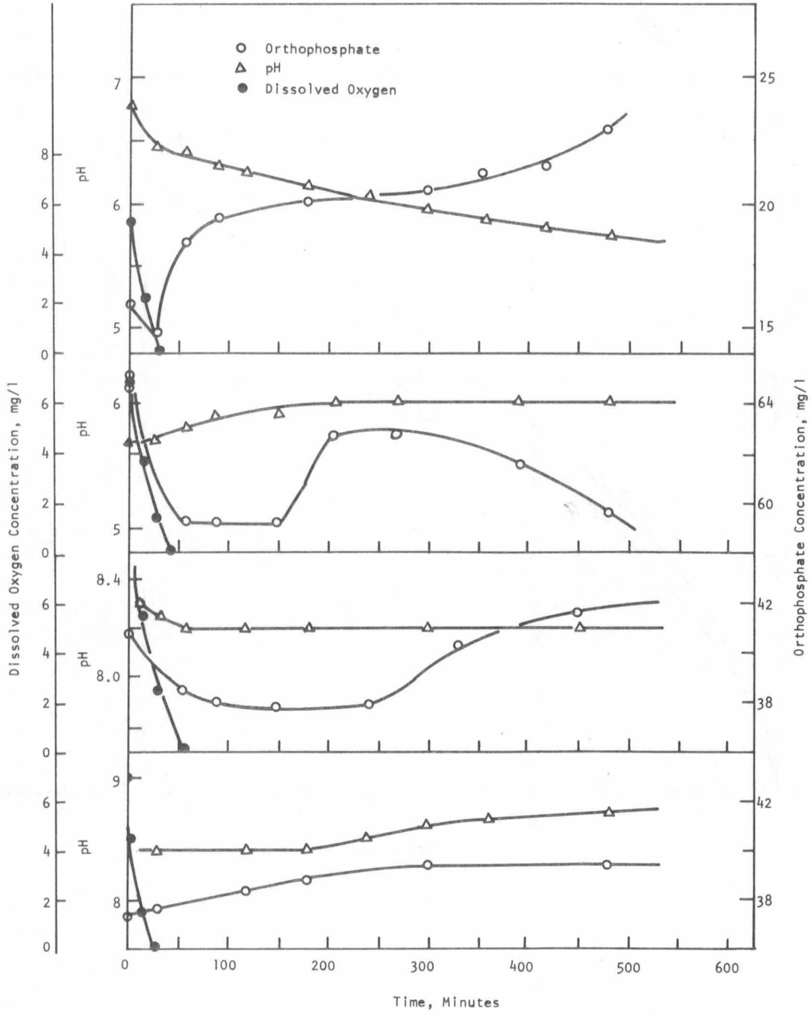
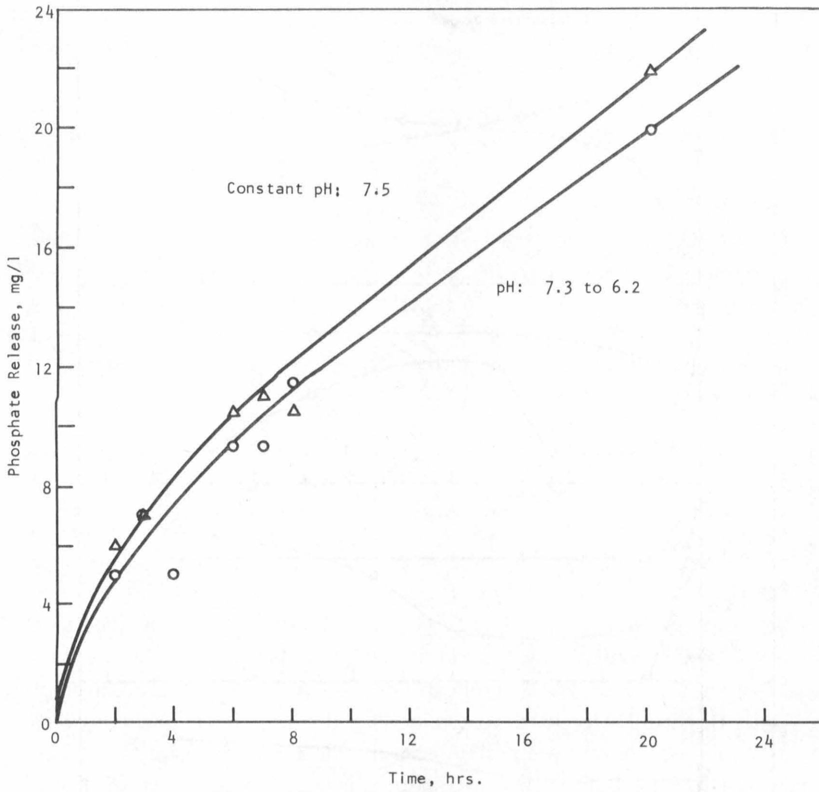


FIGURE 7

ANOXIC PHOSPHATE RELEASE VERSUS pH CHANGE.



Redox Potential and Phosphate Release

To establish whether the lags shown in Figure 6 were the results of slow changes in ORP and to investigate the claim of Shapiro, et al.[11] that anoxic release is caused by a change in ORP, several experiments were conducted wherein redox potential was carefully monitored throughout the course of the experiment. The data revealed that, in all cases, orthophosphate release began before any significant change in ORP had occurred (Figure 8). Rather than being a cause of phosphate release, it appears that ORP change is the result of such release or the conditions that caused the release. In fact, it has been previously reported by other investigators that microbial lysis is accompanied by a temporary decrease in ORP.[18] Thus, it seems likely that the decrease in ORP observed by Shapiro, et al.[11] was the result of lysis rather than the cause. This further implicates cell disruption as a mechanism of release, although in one instance release was not followed by a decrease in ORP.

Once again, the data show that phosphate release did not occur until after the DO had dropped to zero in every instance, and for these experiments release began simultaneously with zero DO. The results suggest that the observed soluble phosphate release was the result of biological stresses caused by the absence of DO. This conclusion seems particularly valid since pH remained constant throughout each of these studies, thereby ruling out any possibility of phosphate release by solubility change.

Anoxic Release as Related to Suspended Solids

If activated sludge phosphate release is primarily biological in nature, the magnitude of anoxic release should be a direct function of the solids concentration when the DO reaches zero. This would not necessarily be true for chemical mechanisms. A plot of data from this study, plus that observed by Shapiro, et al.[11], shows that phosphate release is a linear function of initial solids (Figure 9). In other words, activated sludge releases a relatively constant amount of phosphate per unit solids under anoxic conditions. The average value for the data shown in Figure 9 is 0.00474 grams/gram. For a chemical mechanism, the amount released should be a stronger function of the initial phosphate concentration (before uptake) than of the solids concentration.

If anoxic release is the result of cell lysis rather than leakage from viable organisms, then the amount released should be a direct function of the solids

FIGURE 8

ORTHOPHOSPHATE VARIATION WITH ORP AND DISSOLVED OXYGEN.

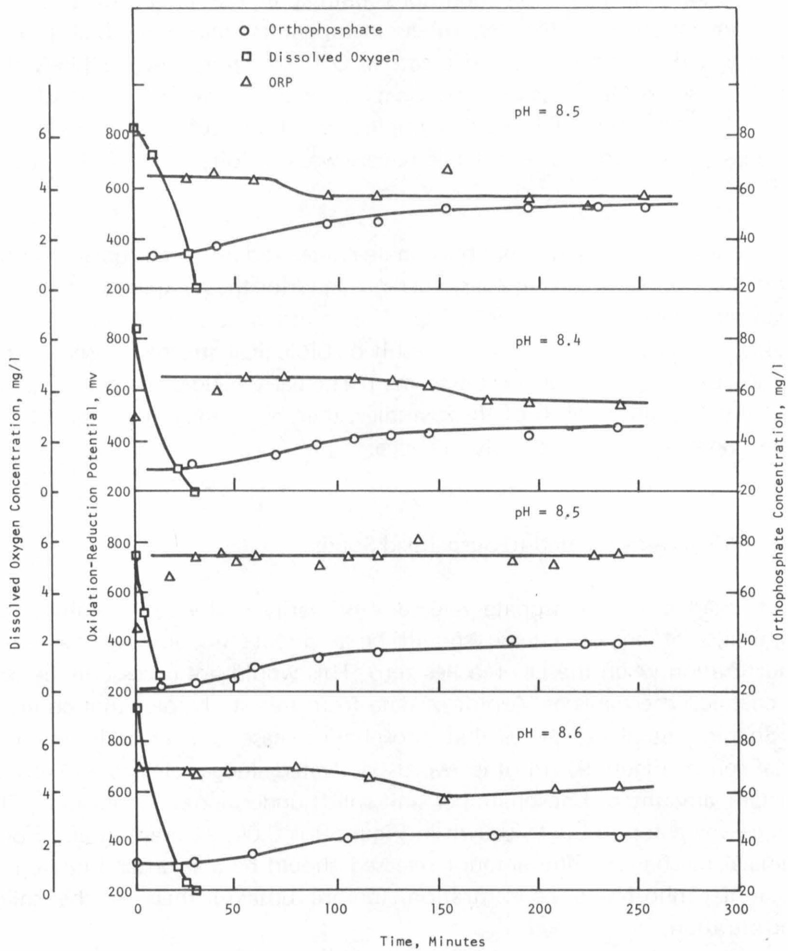
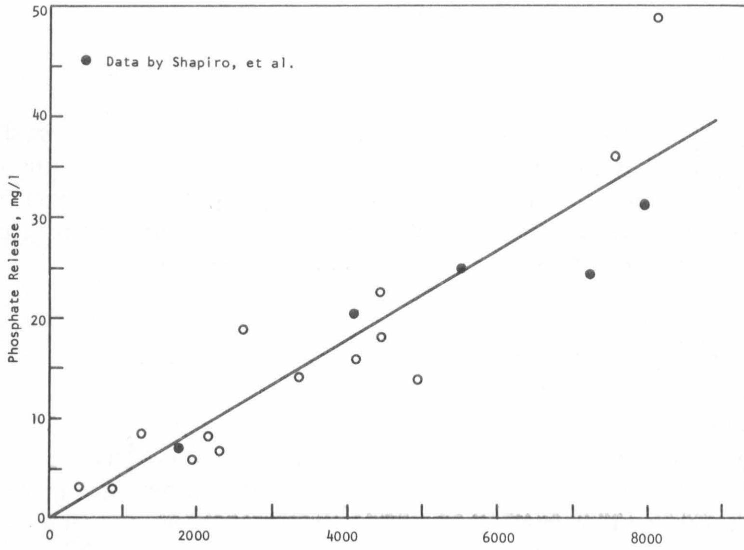
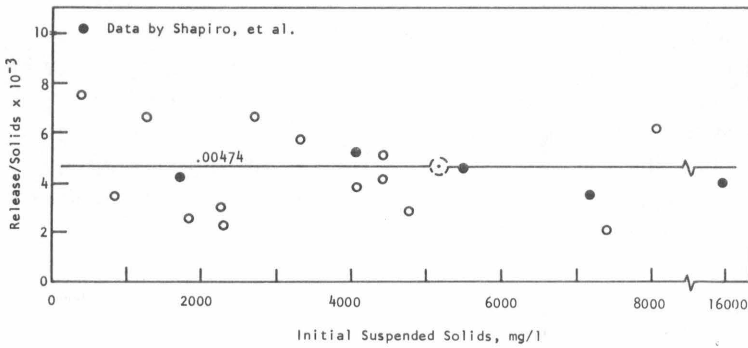


FIGURE 9

INITIAL SOLIDS VERSUS ANOXIC PHOSPHATE RELEASE.





destruction that takes place during release. A plot of the data, however, shows that this relationship is fairly complex. When these figures are plotted with the data of Sekikawa, et al.[12], the destroyed solids seemingly released phosphates in distinctly different amounts (Figure 10). Nevertheless, the amount released does seem to be a direct function of the solids destruction. There was not sufficient data to determine whether the change in the amount of phosphate released per unit solids destroyed was a function of the phosphate uptake during substrate utilization.

Operational Factors That Affect Anoxic Release

Campbell and Horton[2] from their study using a full-scale activated sludge plant concluded that the mode of plant operation was one of the most important factors in accomplishing maximum phosphorus removal. One of the objectives of these studies was to define ways plant operation procedures could be changed to improve phosphate removal efficiency.

Anoxic Release as a Function of Food-To-Microogranism Ratio

Sekikawa, et al.[11] reported that phosphate leakage occurs in an activated sludge process during aeration when the F:M ratio is low. That observation has been confirmed by data previously presented. On the other hand, Hall and Engelbrecht used a high F:M ratio during aeration and observed no phosphate release during subsequent anoxia.

Data accumulated during the course of this series of investigations were plotted to determine if anoxic release was delayed by the presence of high concentrations of organic substrate (Figure 11). As the data show, a high F:M ratio at the start of anoxia will significantly decrease the phosphate release rate. Thus, the failure to completely stabilize the organic substrate during aeration has a double benefit from a phosphorus removal standpoint – it prevents aerobic release and retards or prevents anoxic release.

Anoxic Release as a Function of Aeration Rate

Levin and Shapiro[7], from their experiments, related aeration rate to phosphorus uptake. Wells[19] also related aeration rate to phosphorus uptake, but he concluded that the increased uptake with higher air rates was the result of better mixing rather than the maintenance of high DO concentrations.

FIGURE 10

PHOSPHATE RELEASE WITH SOLIDS DESTRUCTION.

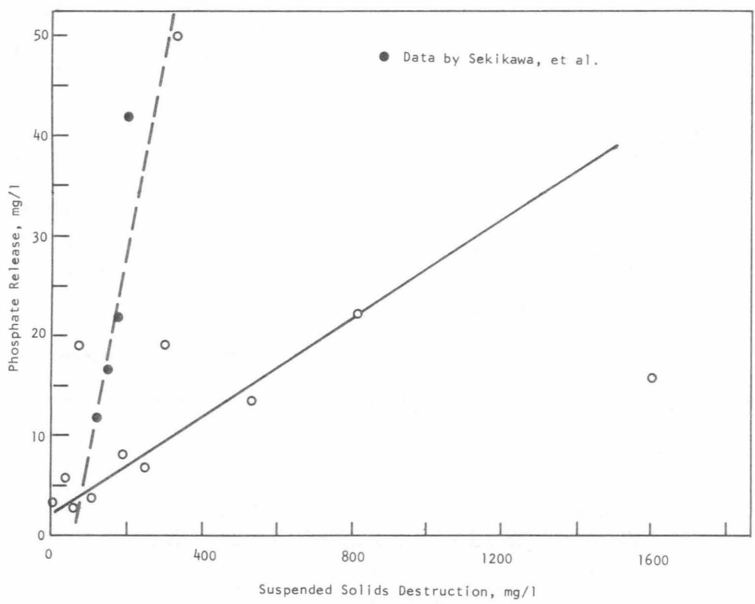
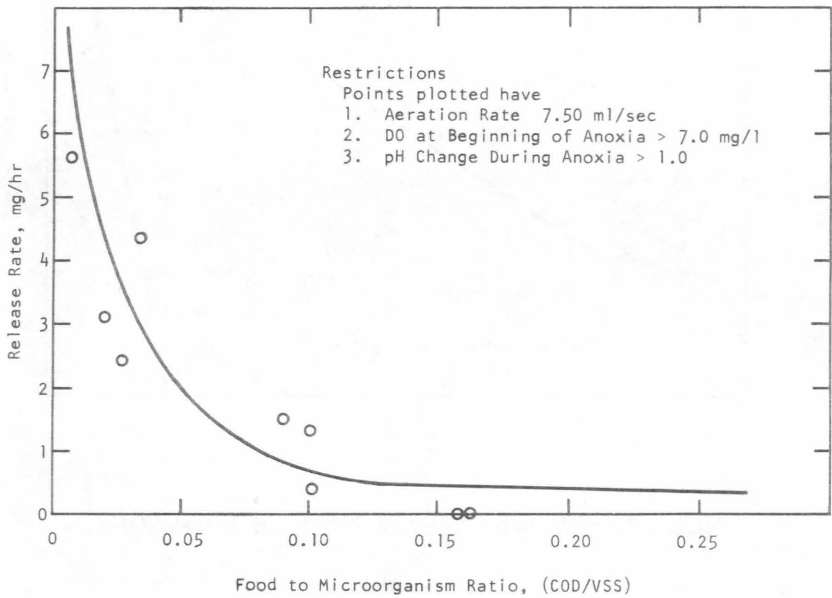


FIGURE 11

ANOXIC RELEASE RATE VERSUS F:M RATIO.



Since aeration rate affects phosphorus uptake and since it is an operational parameter that can be easily adjusted, its effect on subsequent anoxic release was thoroughly studied. The results of these studies indicate that anoxic release is a function of the aeration rate that is maintained during uptake (Figure 12). The top plot in Figure 12 shows that the rate of anoxic phosphate release was strongly retarded when high aeration rates were used. The data also show that low aeration rates resulted in a rapid release of phosphate at the start of anoxia, while there was a wide range of intermediate aeration values that produced similar effects. The aeration rates shown are for a volume of 1400 ml.

Since previous experiments demonstrated that anoxic release is a function of biological solids and also the magnitude of the phosphate uptake, the aeration rate data were corrected for these factors and plotted (Figure 12). This plot seemingly indicates that both low and high aeration rates retard anoxic release; however, as the top graph shows, such is not the actual case. When low aeration rates were used, phosphate uptake was retarded and, therefore, less phosphate per unit solids was available for release during anoxia. By contrast, the conclusion that high aeration rates do retard subsequent release seems to be valid even though it is tempered somewhat by the increased auto-oxidation that occurs at high aeration rates. The effect of such aerobic release is shown by the data in Table II.

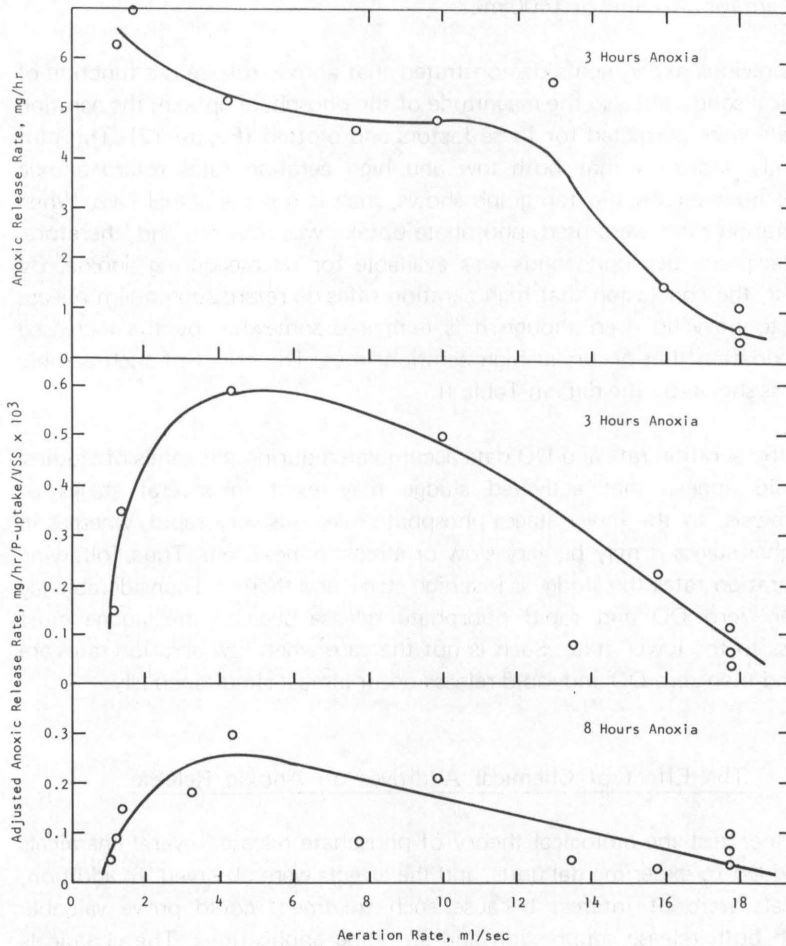
From the aeration rate and DO data accumulated during this series of studies, it would appear that activated sludge may exist in several stages of anaerobiosis. In the lower stages phosphate release is very rapid, whereas in the higher stages it may be very slow or almost nonexistent. Thus, following high aeration rates the sludge is in a high stage, and there is a considerable lag between zero DO and rapid phosphate release because the sludge must progress to the lower state. Such is not the case when low aeration rates are used and then zero DO and rapid release occur almost simultaneously.

The Effect of Chemical Additives on Anoxic Release

To further test the biological theory of phosphate release, several chemicals were added to experimental units, and the effects were observed. In addition, chemicals were of interest because such treatment could prove valuable through both release suppression and stripping applications. The chemicals used were classified according to the observed results as either toxic or inhibitive.

FIGURE 12

ANOXIC PHOSPHATE RELEASE
VERSUS AERATION RATE.



Toxic Chemicals

Chemicals added that caused a toxic effect on the activated sludge were sodium carbonate and sodium chloride. The data pertaining to these two chemicals are shown in Table III.

Since sodium carbonate is the salt of a strong base and a weak acid, its addition caused an increase in pH. According to chemical theory, an increase in pH would decrease the solubility of phosphate and retard release. Instead, as pH increased the amount of phosphate in solution steadily increased. Further, the magnitude of release was directly proportional to the degree of toxicity caused by the chemical as measured by comparative Warburg oxygen uptake runs. Clearly, the rate of phosphate release was a function of the biological stress placed on the microbial system.

Results of a similar nature were obtained when NaCl was added to the biological units. While the addition of NaCl did not cause a change in pH, the Warburg results showed that it did exert a toxic effect on the microorganisms, apparently sodium toxicity. As with the sodium carbonate, the amount of release was directly proportional to the degree of toxicity. The relationship between biological stress and phosphate release is clearly illustrated by Figure 13.

Inhibitive Chemicals

Komiyama[15] reported that the lysis of bacteria is inhibited by a 2% concentration of either sodium sulfate or magnesium sulfate due to the induction of plasmolysis. Thus, those chemicals were added to units to see if anoxic release could be inhibited by reducing bacteriolysis. The results (Figure 14) show that anoxic phosphate release was inhibited by the addition of such chemicals. However, the suppressive effect obtained appeared to be more closely related to the cation added rather than to the chemical or anion concentration in the unit. A virtual straight-line relationship between molar concentration and inhibitive effect was obtained with the magnesium salt. By contrast, sodium sulfate concentrations less than 0.21 molar had no inhibitive effect at all, and the 9% reduction observed with a 0.63 molar concentration could be attributed to statistical variation. Superficially, the difference in suppressive effect seems to indicate that the addition of magnesium caused phosphate precipitation. Further, a plot of sulfate salt concentration versus soluble phosphate remaining in solution after release was complete yielded parallel straight-line relationships for both chemicals up to a 1% solution. This

TABLE III

TOXIC CHEMICAL EFFECT ON PHOSPHATE RELEASE

Chemical	Conc. %	Anoxic Period Hours	Phosphate Release mg/l	Ratio of Release to Control	pH	% of Control O_2 Uptake
Na_2CO_3	0	6.5	2.5	1.0	7.4	100
Na_2CO_3	0.5	6.5	7	2.8	9.9	32
Na_2CO_3	1	6.5	11	4.4	10.4	22
Na_2CO_3	2	6.5	17	6.8	10.7	10
NaCl	0	6	12	1.0	7.7	100
NaCl	0.1	6	12	1.0	7.6	87
NaCl	0.5	6	16	1.33	7.5	83
NaCl	1	6	20	1.67	7.7	75
NaCl	0	6	12	1.0		100
NaCl	2	6	22	1.83		78
NaCl	3.5	6	25	2.09		65
NaCl	5	6	19	1.58		59

FIGURE 13

PHOSPHATE RELEASE AS A FUNCTION OF TOXICITY.

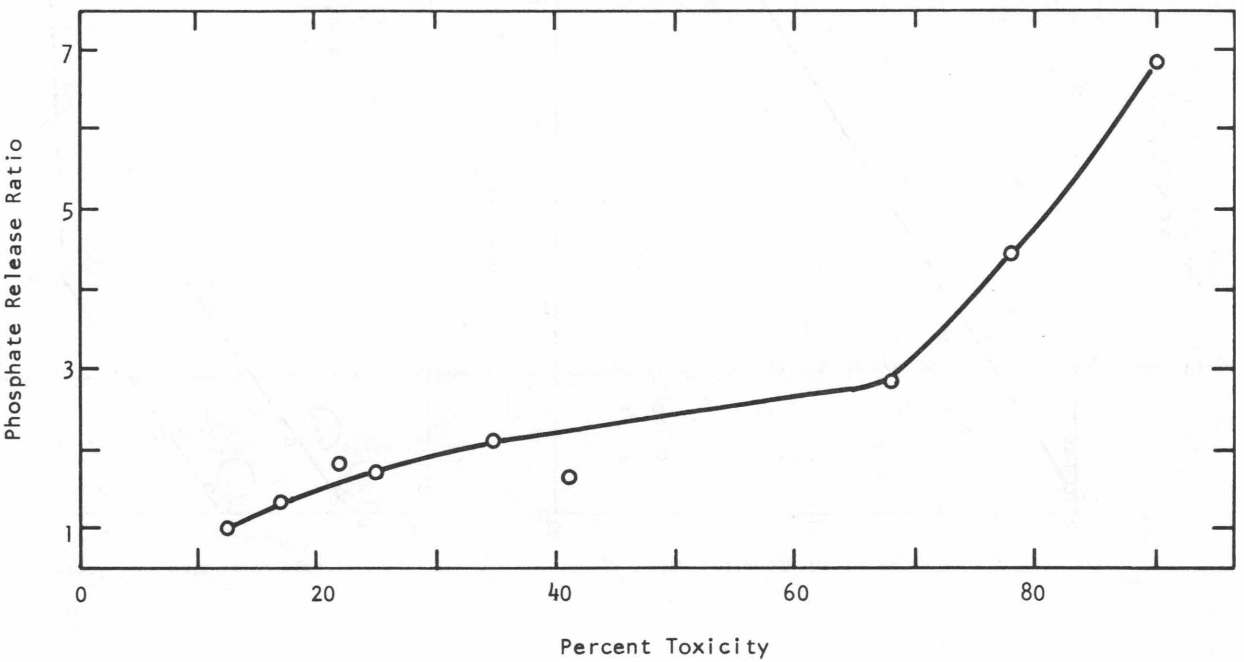
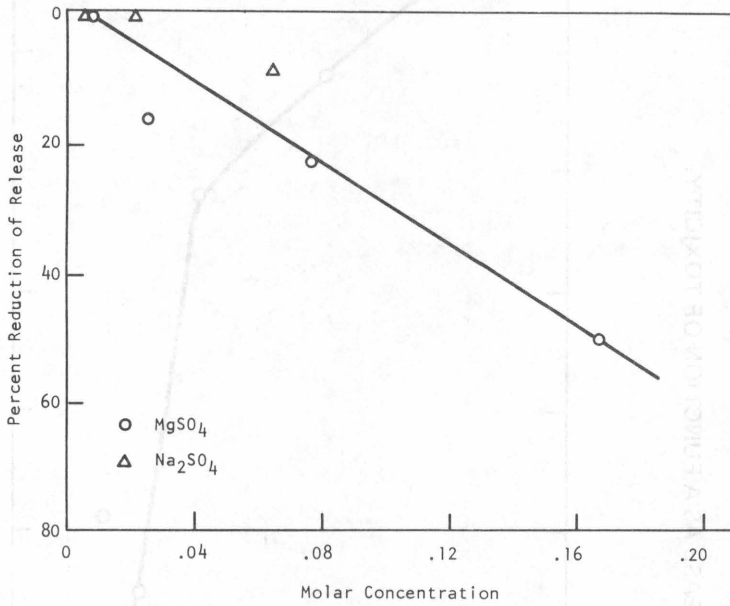
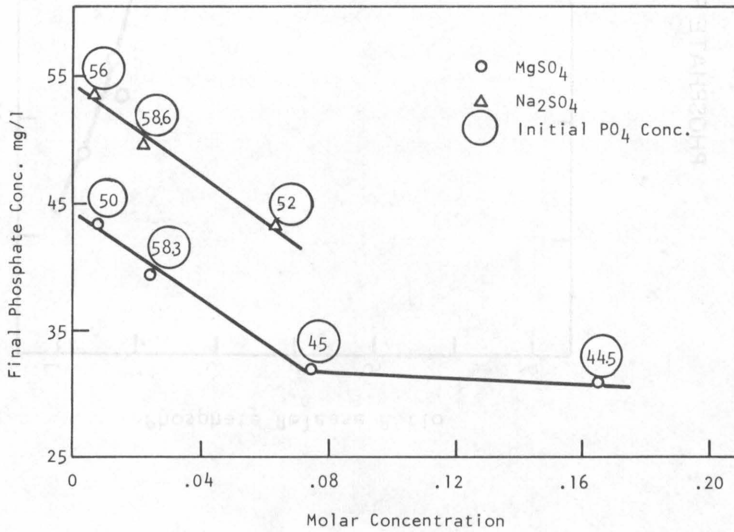


FIGURE 14

CHEMICAL INHIBITION OF
PHOSPHATE RELEASE.





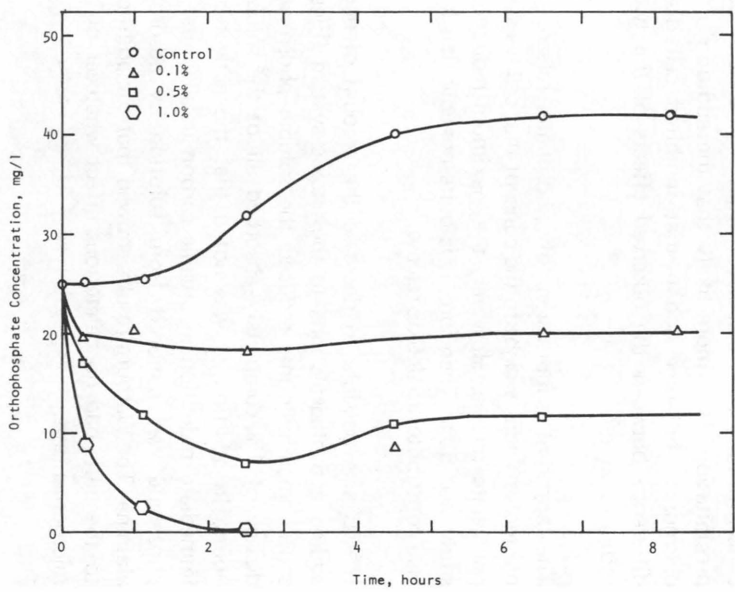
relationship was obtained despite considerable variation in the initial soluble phosphate concentration. At first glance, this result also seems to indicate a chemical equilibrium based on the solubility product of the cation added and phosphate. If this were true, then the greater effect of the magnesium salt could be explained by a difference in solubility products. In fact, however, such a chemical explanation is not logical when the relative solubilities of magnesium phosphate and sodium phosphate are considered. While it is possible that magnesium phosphate precipitation could occur with concentrations used, sodium phosphate precipitation is not at all feasible. Further, the lack of consistent additional precipitation when the magnesium concentration was increased from 1 to 2% belies simple chemical precipitation. It is more likely that the addition of sulfate salts did cause plasmolysis or some action that inhibited cell disruption, and that the difference between the observed effects of the two salts was caused by sodium toxicity.

The apparent suppression of phosphate release by the induction of plasmolysis adds weight to the concept that cell lysis is a major mechanism of phosphate release. However, the maximum reduction obtained during this study was 50%. Therefore, it is quite possible that leakage from viable cells also contributes to anoxic release.

It was also possible to decrease the amount of phosphate in solution by adding activated alumina to the anoxic system (Figure 15). As the results show, small amounts reduced the soluble phosphate considerably, and a dosage of 1% completely absorbed all of the soluble phosphate after 2.5 hours. In addition, it was noted that the activated alumina produced an immediate reduction in organic carbon in all units. Obviously, the soluble phosphate was removed from solution by adsorption on the activated alumina. The Warburg results showed that the addition of activated alumina to the units had no deleterious effect whatever on the metabolism of the microorganisms.

FIGURE 15

PHOSPHATE ADSORPTION BY ACTIVATED ALUMINA.



CONCLUSIONS

On the basis of previously described laboratory experiments, the following conclusions have been derived:

1. The release of soluble phosphorus (phosphate) from activated sludge occurs under both aerobic and anoxic conditions; but the amount released during anoxia is considerably greater, and it is released much more rapidly. Rate of release corresponds closely to the expected death rate of the microorganisms.

2. Activated sludge phosphate release is relatively independent of the chemical environment, notably pH, within the normal range of activated sludge operation. The primary effect of pH change is to cause biological stress on the microorganisms resulting in phosphate release proportional to the amount of stress. pH effects cannot be explained by chemical solubilities.

3. Anoxic phosphate release is not a function of ORP change. Instead, ORP change consistently follows such release.

4. Anoxic phosphate release is closely related to the concentration of DO maintained during the aeration period and to the time of zero DO during anoxia. Rapid release does not occur until the DO concentration drops to zero. The time between zero DO and rapid release can be prolonged by preceding anoxia with high aeration rates.

5. Rapid anoxic phosphate release is retarded by the presence of organic substrate at the start of anoxia.

6. Anoxic phosphate release is closely related to the activated sludge suspended solids concentration. Release is a linear function of MLSS, but the relationship with MLVSS is more complex. However, release does increase as the MLVSS concentration increases. Data from this study and that obtained by a previous researcher indicate that anoxic conditions produce a release of approximately 0.0045 grams of phosphate per gram of suspended solids initially present.

7. The amount of phosphate released and the rapidity of release from activated sludge under any conditions is proportional to the degree of biological stress imposed on the microbial system. Such stress may

result from starvation conditions, from the onset of anaerobic conditions, from excessive pH change, from the addition of toxic materials, and/or from alterations of the cell membrane by acid and alcohol end products.

8. Microbial cell disruption is a major cause of activated sludge phosphate release. Such release can be inhibited by sulfate salts due to the induction of plasmolysis.

9. Activated alumina is a very desirable material for removing phosphate from wastewater because of its high adsorption capacity for phosphate and because it exerts no toxic effect on the microorganisms.

RECOMMENDATIONS

It is recognized that with the present imbalance of phosphorus and organic carbon that exists in domestic sewage, the activated sludge process cannot be used to achieve complete phosphorus removal through biological sorption. However, it is believed that phosphorus removal by activated sludge can be significantly enhanced by proper design and operation. Such enhancement may well be sufficient to meet pollution control criteria in many cases and would almost certainly decrease the cost of subsequent chemical removal in any situation. On the basis of the previously described research, it is believed that the following recommendations can be used to maximize phosphorus removal by the activated sludge process:

1. The sewage retention time in the activated sludge unit should be shortened so that incomplete removal of the soluble organics will be obtained, thus avoiding aerobic release because of starvation conditions. The high food-to-microorganism ratio entering the clarifier will further assist by suppressing anoxic release. The design would be very similar to that used for contact stabilization.

2. The rate of aeration should be as high as practicable, and the DO level should be above 2.0 mg/l in all parts of the aeration tank.

3. Prolonged settling and thickening periods in the secondary clarifier must be avoided. The sludge should be removed from the unit as rapidly as possible with a maximum retention time of 1.5 hours.

4. The thickened activated sludge should be stabilized by further aeration and then forced to go anoxic for a considerable period of time (until rapid phosphate release has been completed). A thickener would be ideal for the latter purpose. Sludge wastage before stabilization is acceptable only if supernatant obtained from the waste sludge during further processing is not returned to the sewage flow without some type of phosphorus removal.

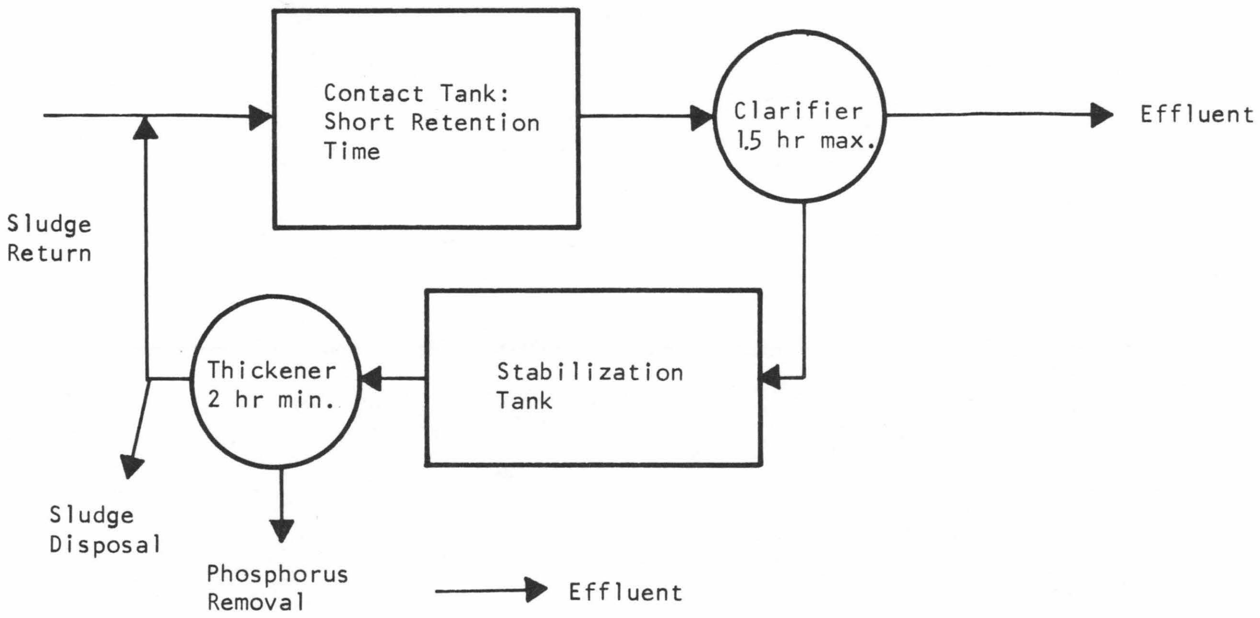
5. The desorbed sludge must be returned to the aeration tank to maintain the biological solids balance, whereas the thickener liquid must be treated for phosphorus removal by some means or disposed of without being recycled back to the influent sewage flow.

6. Activated alumina columns may be used for phosphorus removal from the liquid volumes.

The resulting flow diagram that would be obtained from these recommendations is shown in Figure 16. If extremely high organic removal is needed, the clarifier effluent could be brought back into contact with the desorbed sludge in a second aeration tank before it is released to the receiving water. An additional clarifier would also be needed.

FIGURE 16

PHOSPHORUS REMOVAL FLOW DIAGRAM.



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