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In recent years considerable interest has developed in the fate of phosphorus during the wastewater treatment process. Some investigators, including Feng (1) and Sekikawa *et al.* (2), look to the behavior of phosphorus as an indicator of the character and quality of the activated sludge while others, such as Levin and Shapiro (3), Srinath *et al.* (4), and Vacker *et al.* (5) are interested in using phenomena such as the uptake of phosphate by the sludge organisms to reduce the concentration of phosphate in the effluent of activated sludge plants. During a study, directed at the latter goal, of phosphate uptake in the city of Baltimore activated sludge unit, the writers became aware that, although soluble phosphate was indeed reduced during aeration, the effluent from the secondary clarifier frequently contained higher concentrations of phosphate than did the effluent from the aeration basin. This paper deals with field and laboratory observations concerning this phenomenon.

Field Observations

Figure 1 shows the results of sampling at the Baltimore plant on three

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occasions. On the first two occasions, samples were taken essentially simultaneously at all points, and on the third, samples were taken at intervals such that the same slug of wastewater was being sampled. In each case, while uptake by the sludge reduced the dissolved phosphate concentration, subsequent release allowed the dissolved phosphate to increase. Thus, in the first case uptake reduced the concentration to 50 percent of initial, but release raised the effluent concentration back to 73 percent. The second and third cases showed uptake to 73 and 85 percent of the initial values but release back to 83 and 115 percent, respectively.

The same thing happens in the District of Columbia wastewater treatment plant, from which some data are shown in Table I. In three of the samples there was either no significant change or even a slight decline in phosphate during settling. However, in the other three cases there were significant increases of as much as 60 percent, suggesting that the phosphate taken up during aeration was leaking out of the cells during settling.

Since this phenomenon is obviously disadvantageous to the production of low-phosphate effluents, a study was begun of the settling basins to determine the type of environment under which such release occurs. The first step was aimed at determining the factors which occur in the settling basins which are responsible for the release there. A device was built which consists of a Plexiglas box through which

water is drawn by an electric pump. Inserted into the box through rubber stoppers and tubes are a thermometer, dissolved oxygen probe, conductivity electrode, calomel reference electrode, glass electrode, and platinum electrode. The sample is drawn in through a $\frac{5}{8}$ -in.- (1.6-cm-) ID garden hose, the end of which is weighted and lowered to the desired depth. Thus, simultaneous determinations of temperature, dissolved oxygen, conductivity, pH, and redox potential may be made at any point in the settling basin. In addition, the stream issuing from the pump outlet at the surface may be used to sample other parameters, in this case dissolved orthophosphate.

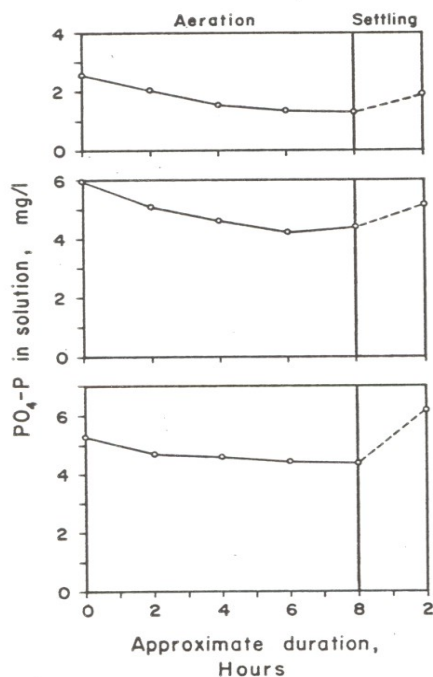


FIGURE 1.—Dissolved phosphate phosphorus during aeration and settling in the Baltimore Treatment Plant. Top, simultaneous sampling, March 26, 1964; middle, simultaneous sampling, March 31, 1964; bottom, sequential sampling, March 31, 1964.

TABLE I.—Dissolved $\text{PO}_4\text{-P}$ in Influent to and Effluent from Final Clarifiers, Blue Plains Treatment Plant, Washington, D. C.

Date 1963	$\text{PO}_4\text{-P}$ in Effluent Mixed Liquor (mg/l)	$\text{PO}_4\text{-P}$ in Final Clarifier Effluent (mg/l)
Feb. 5	4.7	4.8
Feb. 27	4.1	3.6
Sept. 16	4.7	4.4
Oct. 22	5.5	8.8
Nov. 29	4.0	5.2
Dec. 31	4.7	6.7

Figure 2 shows the results of such a study on one of the circular secondary settling basins of the activated sludge unit of the Baltimore wastewater treatment plant on April 17, 1964. The samples for phosphate were filtered through HA Millipore filters within seconds after their recovery. Conductivity, which was unchanged with depth, is omitted from the figure. Several peculiarities of phosphate distribution stand out:

1. The dissolved phosphate is more concentrated near the bottom than elsewhere.
2. The bottom samples have a greater concentration of dissolved phosphate at the center of the basin than at its periphery.
3. The mid-depth and surface samples at the periphery of the basin have more dissolved phosphate than do these samples at the center of the basin.
4. The surface samples have more dissolved phosphate than the mid-depth samples.

The basins at Baltimore are of the type in which the influent enters at the center and the settled sludge is pushed by plows toward the center for withdrawal. The results therefore suggest that the following is happening: As the influent enters it moves peripherally and downward while the sludge settles out of it. As the sludge nears the bottom it begins to release its phosphate into solution. The dissolved phosphate concentration near the bottom is greater at the center of

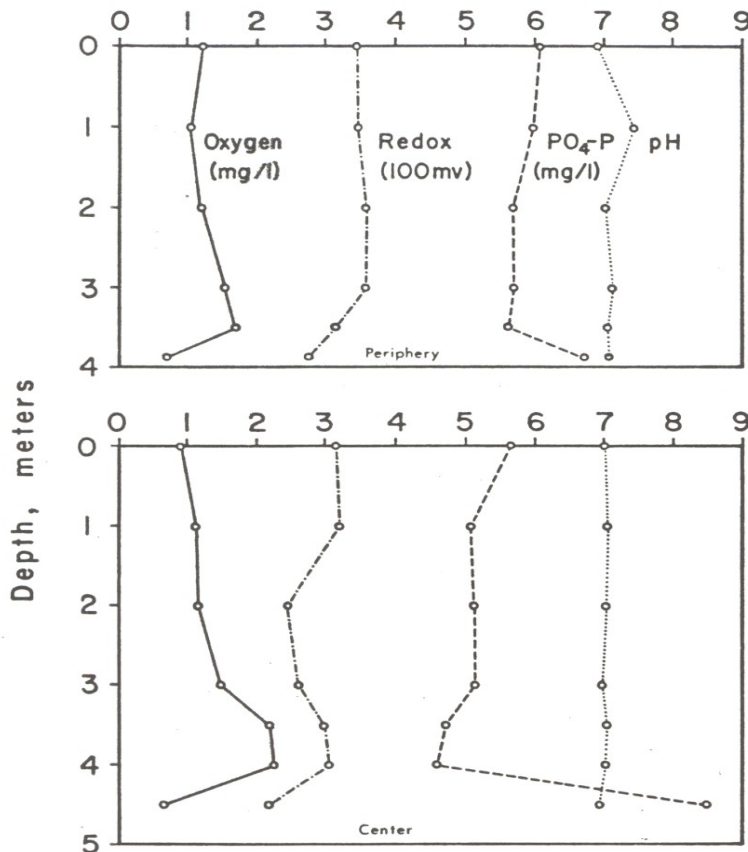


FIGURE 2.—Vertical profiles of oxygen, redox potential, phosphate, and pH in the final clarifier of the Baltimore activated sludge unit, April 17, 1964.

the basin presumably because it is here that the sludge is older and in greater concentration. As the plows move through the sludge they cause some turbulence and mixing upward. The sludge settles again but the phosphate, being in solution, does not. Thus the dissolved phosphate content somewhat above the bottom may be increased. As the clarified liquor moves peripherally it sweeps some of this dissolved phosphate with it and as it reaches the sides of the tank it moves vertically until it reaches the surface.

Here it spreads laterally, some going over the weir and some moving toward the center, becoming diluted as it moves.

The pH data are too indefinite to support or refute the argument but the oxygen and redox information appear to support it. In both cases the measurements at the bottom are lower but less so near the periphery. Similarly, the surface oxygen values are lower than those at intermediate depths, suggesting either interpolation of water higher in dissolved oxygen at these

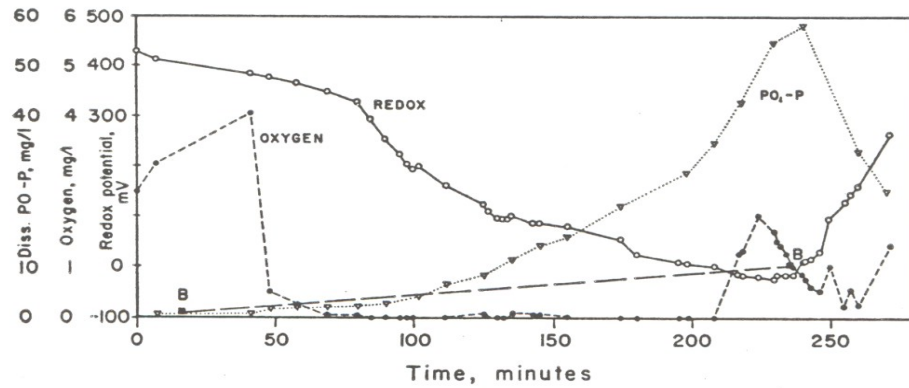


FIGURE 3.—Release of phosphate from settled sludge as a function of redox and dissolved oxygen. The points B and B show the small amount of release in a similar sample under continuous aeration.

depths, or overflow at the surface of water lower in dissolved oxygen, or both.

Laboratory Observations

The indications are that the release of phosphate is stimulated by low dissolved oxygen or low redox or both. This is not surprising, since it has been shown (3) that the uptake is biological and stimulated by high concentration of dissolved oxygen. To determine more closely the conditions under which leakage occurs, experiments were performed in which a quantity of effluent mixed liquor was settled to approximate the solids concentration of return sludge and was placed in a vessel equipped with a magnetic stirrer and a cover through which passed electrodes to measure redox potential and dissolved oxygen, tubes for nitrogen and air bubbling, and a siphon for sampling. The results of such an experiment are shown in Figure 3. The redox curve is erratic because air was bubbled periodically through the sludge in an attempt to delay release long enough to permit correlation of the release with one or another of the factors. Thus, even after 90 min, although the dissolved oxygen had been allowed to decline to the point where

the oxygen electrode showed no response, the release of phosphate was negligible, and in fact was not significantly different from the values represented by the line B-B, the latter showing the phosphate levels in a bottle of identical sludge under continuous aeration. It would seem then that it is not the absence of oxygen *per se* which initiates the release. During the time that the dissolved oxygen was kept low the redox potential had been falling and the point at which the release of phosphate began in earnest corresponded to a redox value of about 150 mv. The slope of the release curve seemed to increase again when the redox potential fell to zero. At 207 min a leak in the cover seal allowed air to enter. Despite this, phosphate release continued. At 230 min the air was turned on but the release of phosphate continued for another few minutes. Only when the redox potential rose did the phosphate leakage stop and uptake begin. It would appear, therefore, that the release is more closely related to redox conditions than to dissolved oxygen concentration. Again, in a similar experiment using unconcentrated mixed liquor, the slope of the phosphate re-

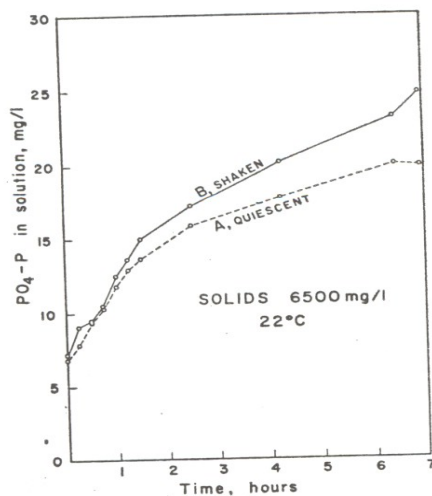


FIGURE 4.—Effect of perodic mixing on anoxic release of phosphate by sludge.

lease curve changed at the point where the redox potential reached 150 mv.

Other parameters affecting the rate of release include stirring, suspended solids concentration, and temperature. The results of some studies on these have been published elsewhere (6) but are summarized below. All of these experiments were done using sludge prepared by settling effluent mixed liquor from the Baltimore or other wastewater treatment plants. The samples were brought to the laboratory under continuous aeration and used within one hour after removal from the plant. Dissolved phosphate was measured, as in the field studies, after dilution, by the molybdenum blue-stannous chloride method on samples passed through a Millipore (0.45- μ) filter, after brief centrifuging if necessary.

Effect of Stirring on Release of Phosphate

In this experiment, as in subsequent ones, the basic procedure was to protect the settled sludge from contact with the air and to make periodic mea-

surements of the dissolved orthophosphate. In order to determine whether stirring had a significant effect on the rate of phosphate release, 2 series of 11 BOD bottles each were filled with sludge, stoppered, and placed at 22°C. Bottles from series B were shaken often enough so that the sludge never showed appreciable settling. Bottles from series A were allowed to stand quietly and were shaken only before being used for phosphate measurement. The results are shown in Figure 4. Two things are to be noted. There was an immediate and dramatic release of orthophosphate into the solution, and this release was facilitated by mixing of the sludge. The release was rapid enough so that the initial phosphate phosphorus concentration doubled after only 1 hr and reached a concentration of 15 mg/l after only 2 hr. Thereupon the rate of release declined but was positive even after six hours. The effect of stirring is obvious and the implication is clear. The secondary settling tanks, in this case those of the Baltimore wastewater treatment plant, providing two hours of detention with occasional partial stirring by the scraper plow, provide excellent conditions for phosphate release. Thus, the increase in dissolved phosphate during passage through these tanks is understandable.

In view of these results all further experiments were performed using either magnetic stirring or nitrogen bubbling, which gave identical results. Also, the experiments were conducted by periodically withdrawing samples from one container kept at the desired conditions, rather than by using many separate containers.

Effect of Temperature on Phosphate Release

In order to determine the role of temperature in the phenomenon an experiment was performed in which 5 bottles of freshly settled sludge were maintained at carefully controlled tem-

peratures varying from 10° to 30°C. The results are shown in Figure 5. Clearly, temperature is an important factor in the release. The Q_{10} calculated for the period 60–180 min is, for 10°–20°C, 2.5; for 15°–25°C, 2.6; and for 20°–30°C, 2.1.

Effect of Solids Concentration on Phosphate Release

Figure 6 shows the results of using different solids concentrations. (At the time this sludge was collected from the Baltimore wastewater treatment plant the return sludge solids concentration was 6,150 mg/l.) The main feature of the curves is the increase in the rate of release at the higher concentrations. However, if the rates for the period 60–175 min are divided by the solids concentrations they yield about the same quotient, being for the cases from 1,700 to 15,800 mg/l, 2.9, 3.8, 2.6, and 3.1, respectively, i.e., the highest concentration of sludge released its phosphate at almost exactly the same rate per unit of solids as the lowest sludge concentration. This suggests that rate of release is independent of, and therefore not limited by, the concentration of phosphate in the medium.

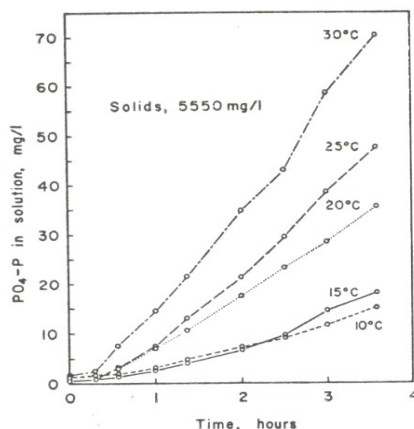


FIGURE 5.—Effect of temperature on anoxic release by phosphate by sludge.

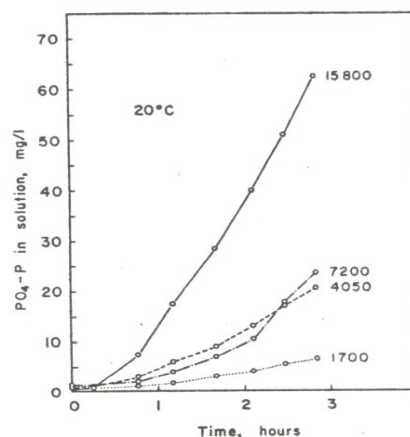


FIGURE 6.—Anoxic release of phosphate by different concentrations of sludge. The numbers refer to dry weight of suspended solids in mg/l.

Nature of the Release

The nature of the phosphate release has been discussed elsewhere in detail (6) but the chief results may be summarized as follows: Release of phosphate is not due to decomposition of the sludge or to lysis of the microorganisms. The phenomenon is completely reversible, the phosphate being taken up during aeration as rapidly as it was lost. Although as much as 40 percent of the total phosphorus may be lost from the sludge in a matter of hours, there is no increase in soluble carbohydrate or BOD and only a slight rise in the concentration of dissolved Kjeldahl nitrogen. These results suggest that the release is restricted to phosphorus, and comparison of soluble phosphate with total soluble phosphorus analyses shows that the phosphorus is probably released as orthophosphate. Fractionation (7) of the phosphorus compounds of the sludge before and after release suggests two sources for the phosphate, the acid-extractable phosphorus and the phosphorus bound in nucleic acids, although the former seems to make a greater contribution.

The effect of poisons varies with their concentration and nature. Cyanide at 10^{-3} M inhibits release but at 10^{-2} M it has the opposite effect. Mercuric chloride at 10^{-3} M stimulates release, but formaldehyde at 10 percent inhibits it. Low pH also causes release to occur (3). Uptake after release has occurred is different in that it is inhibited by poisons as well as by specific antimetabolites such as 2, 4 dinitrophenol.

Discussion

One of the most interesting facets of the phenomenon of release, as described, is its rapidity. Upon becoming anoxic the sludge begins to release phosphate immediately and can lose a significant percentage of its phosphorus in as little as two hours. Since final clarification frequently requires at least this period of time it is important, in trying to produce effluents low in phosphate, to find a way to circumvent this problem. One approach lies in the use of suction-type sludge collectors, several models of which are commercially available. A preliminary study at the York, Pa., treatment plant, which has a suction-type unit as well as a more conventional plow-type clarifier, showed less phosphate accumulating at the bottom in the former than in the conventional type. This is probably due to the fact that the suction-type collectors pass over a given spot in from 15 to 30 min, whereas in more conventional systems the sludge may be two hours old or older before it leaves the clarifier.

Westgarth (8) has shown that the initial reasoning behind the design of suction clarifiers—the prevention of “anaerobic degradation” of return activated sludge—is fallacious. His work showed that in conventional or high-rate treatment many hours of enforced anaerobiosis had no deleterious effect. The results of the present study, however, indicate other benefits from use of such equipment.

A second approach to preventing phosphate release would be to insure that anoxia did not occur during settling, i.e., that the dissolved oxygen content of the mixed liquor entering the clarifier was sufficient to insure against leakage. This has been shown to be effective during a plant-scale experiment at the District of Columbia plant (3).

Still another approach would be to separate the sludge from the effluent by an air-flotation process. In this manner anoxia would be prevented and no leakage would occur. The main objection at present is one of cost.

Another possible confirmation of the prevention of leakage through high dissolved oxygen concentrations during settling comes from the studies in Texas of Vacker *et al.* (5). In all three of the plants which they studied, the clarifier effluent had a DO concentration in excess of one milligram per liter and the effluent phosphate was essentially the same as that entering the basins. Their further experiments using mixed liquor suggested that even a 24-hr settling period would not cause phosphate release, but this is contradicted by their data on return sludge, which shows significant leakage of phosphate from the solids. Possibly the mixed liquor samples contained sufficient oxygen to prevent release.

The phenomenon of phosphate release under anoxic conditions has a bearing on the activated sludge process' production of effluent low in phosphate from both the practical and theoretical points of view. From the practical standpoint, if an activated sludge plant is being operated to remove soluble phosphate (3), it is advantageous to produce as little waste sludge as possible since disposal of this unstable material through digestion would produce problems similar to those being solved. On the other hand, if a considerable amount of sludge is not run to waste, then phosphate will begin to increase in the

effluent because of the limited phosphorus-holding capacity of the sludge. The alternative is to strip phosphate from the sludge before returning the sludge and to tie up the phosphate in a non-objectionable form such as calcium phosphate. In a previous paper (3) stripping through pH adjustment was proposed. However, the anoxic stripping procedure would be less complicated and probably less expensive.

In addition, Westgarth (8) has shown that an enforced period of anoxia for the return sludge results, at high-rate loadings, in the production of only about half the waste sludge and of sludge with better compaction characteristics—both factors advantageous to the phosphate-removal scheme.

On the theoretical side, objections have been raised by Wuhrmann (9), and Stumm and Morgan (10), among others, to the use of activated sludge to remove phosphates because of the alleged imbalance in C/P ratios of sludge and sewage. However, the results of the experiments reported here show that, even in a given situation, the C/P ratio in sludge is not fixed but can vary considerably, and within a short time. For example, in one experiment anoxic release was allowed to occur for 8 hr and then the air was turned on until 23 hr total time had elapsed. Samples of sludge were taken at intervals, dried, and analyzed for C, N, and P. Table II shows the results. During the release about 36 percent of the phosphorus was lost and during aeration all of this, plus 8 percent more, was taken up. During this period the carbon content changed only slightly. Thus the C/P ratio changed by a factor of almost two. It is suggested that this change is possible because of the presence in the sludge organisms of a pool of "disposable phosphate" which permits the organisms to lose or gain phosphorus without the necessity of a stoichiometric quantity of carbon and nitrogen.

TABLE II.—Elemental Analyses of Sludge Solids at Various Times during Anoxic Release and Aerobic Uptake of Phosphate

Condition	Time (hr)	% C*	% N*	% P*	Molar Ratio C:N:P
Anoxic	0	38.5	7.58	3.99	24.9:4.2:1
	3	40.4	7.53	2.77	37.9:6.0:1
	8	40.3	7.43	2.56	40.5:6.4:1
Air	23	37.3	7.03	4.31	22.4:3.6:1

* Results in terms of dry weight.

As this has been shown to be the case in algae and in other bacteria by Goldberg (11) and Kamen and Gest (12) it is not unlikely that the sludge organisms behave in the same way.

Finally, it should be made clear that the phosphate release described here is to be distinguished from that brought about through excessively long aeration of the sludge (2). This phenomenon also has been noted by the authors, but it has not been studied in detail.

Conclusions

The studies reported herein give rise to the following conclusions:

1. Although phosphate may be removed from solution during aeration of activated sludge it may be released back to solution during settling.
2. This release appears to be caused by conditions of lack of oxygen and low redox potential which were found to exist in the bottoms of clarifiers.
3. Laboratory studies suggest that the phosphate release is controlled more by the redox potential than by the concentration of dissolved oxygen. The phenomenon is reversible.
4. Stirring stimulates phosphate release, as does elevation of temperature. The Q_{10} is about 2.1–2.6. Release is independent of external phosphate concentration.
5. Only orthophosphate is released, and apparently mostly from an acid-soluble fraction of the sludge cells. High concentrations of cyanide and mercury stimulate the release.

6. Avoidance of release is to be sought through maintaining high oxygen and redox levels during clarification or by using mechanisms which quickly separate the sludge and supernatant from each other.

7. The anoxic release phenomenon may be applied to phosphate removal by providing a way to concentrate absorbed phosphate into a small volume before returning the phosphate-depleted sludge to the aeration basins. This, according to Westgarth's studies, would also reduce the amount of waste sludge produced and would result in sludge having better settling properties.

8. On the theoretical side, this work illustrates that the C/P ratio of sludge organisms is not an inviolable number but may be changed by almost twofold.

Acknowledgments

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