METABOLIC UPTAKE OF PHOSPHORUS BY WASTEWATER ORGANISMS

Gilbert V. Levin and Joseph Shapiro

Several contemporary factors have combined to create serious pollution problems through sewage eutrophication of surface waters. These factors include the exponential growth of population, the trend toward urbanization, the increasing use of garbage grinders, the universal addition of synthetic detergents to domestic sewage, the increased use of agricultural inorganic fertilizers, and the conditioning of industrial cooling water. Thus, large quantities of mineral-enriched wastewater are discharged into relatively small geographic areas.

A paradox of modern wastewater treatment complicates the problem. As measured by the classical yardsticks of suspended solids and BOD removal, the degree of treatment has increased steadily. This has resulted in more efficient mineralization of the organic constituents of sewage. Sunlight readily penetrates the clear waters, and the minerals which have been released promote rapid and extensive growth of photosynthetic organisms, principally algae. Thus, undesirable changes in natural bodies of water, which would have required thousands of years through normal eutrophication processes, have been brought about in 5 to 10 yr by wastewater discharge (1) (2).

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The predominant opinion indicates that the high concentration of phosphorus in wastewater may be the principal factor. For example, the Wisconsin Governor's Committee Report (3) on the pollution of the Madison Lakes reached the following conclusions:

1. High concentrations of inorganic phosphorus and nitrogen are often the major cause of algal growths.
2. Sewage treatment effluent discharged into a confined body of water may contribute a considerable portion of the total fertility.
3. Lakes high in phosphorus and nitrogen usually suffer algal blooms.
4. Nitrogen can be made available to plankton by fixation from the atmosphere.
5. The removal of phosphorus from effluents should control one of the basic causes of blooms.

The research reported in this paper was directed toward the removal of phosphorus. The specific aim was to modify aeration wastewater treatment processes to produce effluents low in phosphorus.

Activated Sludge and Phosphate Uptake

Reporting adversely on the possible use of activated sludge in phosphate reduction, Lea and Nichols (4), Sawyer (5), Wuhrmann (6), and Bogan (7) based their conclusions on the unfavorable ratio of the carbon, phosphorus, and nitrogen content of sludge bacteria compared to that of raw sewage. Available carbon in sewage was

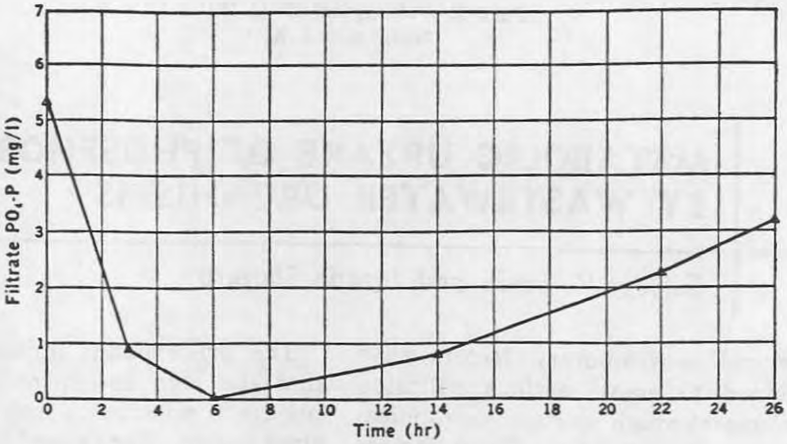

FIGURE 1.—Phosphate concentration in mixed liquor during aeration (9).

cited as being severely deficient, precluding significant phosphorus uptake.

However, some data have been reported which do not necessarily fit the carbohydrate (available carbon) limitation theory. For example, Harris (8), investigating phosphorus metabolism in zooplankton and microorganisms with the aid of P^{32}, found that the "disappearance" of 60 percent of the inorganic radiophosphorus in his experiment was caused by uptake of phosphate by the organisms present in the distilled water solution of the radiophosphorus. Furthermore, in studying phosphate fractions present in the various stages of the Baltimore sewage treatment plant, Alarcon (9) found that the dissolved phosphate content of the effluent from the activated sludge process varied widely from time to time. Since fluctuation in the rate of aeration was suspected as the cause, Alarcon took samples of mixed liquor to the laboratory for more vigorous aeration and phosphate uptake studies. The results are shown in Figure 1. Initial uptake was very dramatic and was followed by uptake at a decreasing rate which resulted in almost total removal of the dissolved phosphate by the sixth hour.

FIGURE 2.—PO_4 removal from wastewater by activated sludge (10).

Srinath *et al.* (10) conducted similar experiments in India. These studies preceded those of Alarcon, but were unknown to him. Figure 2 presents the results obtained. The resemblances to the Alarcon curves are striking.

Concurrent with the work described in this paper, Feng (11) independently reported on the use of orthophosphate concentrations in activated sludge as an index for controlling the process during operation. He also demonstrated the ability of activated sludge to remove much of the orthophosphate from solution.

Alarcon (9) and Srinath *et al.* (10) clearly demonstrated the changing rate of phosphate uptake, and, unless the sewages they used were unusually rich in carbohydrates, their work also suggested that phosphate could be taken up in excess of the limitation that would be imposed by a high C:P ratio in cellular tissue.

In addition to these suggestive results, a consideration of basic metabolic processes also supported the proposed research goal.

All living organisms depend on phosphorus for energy transfer and are believed to be fastidious to the form in which the phosphorus may be accepted from the environment. The necessary form is dissolved inorganic orthophosphate (12).

The first step in the utilization of glucose is the enzymatically induced linkage of a phosphate group to the glucose molecule to form glucose-6-phosphate. The phosphate can be accepted only from adenosine triphosphate (ATP). The fundamental importance of ATP, believed to be ubiquitous in living matter, is attributed to the high-energy bonds of the two terminal phosphate radicals. Orthophosphate cannot add directly to glucose to form glucose-6-phosphate because the reaction is endergonic, requiring approximately 3,000 cal/mole. However, the ATP molecule can donate its energy-rich terminal phosphate.

Orthophosphate *per se* plays no part in the above scheme. However, when ATP molecules are created or regen-

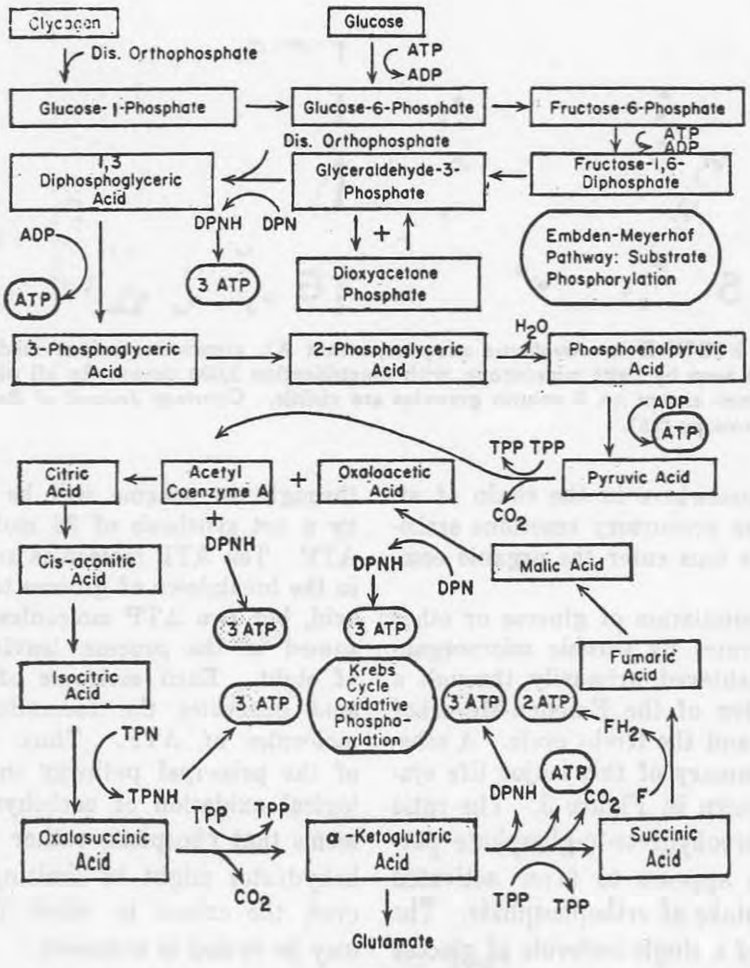

FIGURE 3.—Important aspects of phosphate metabolism in aerobic utilization of carbohydrates.

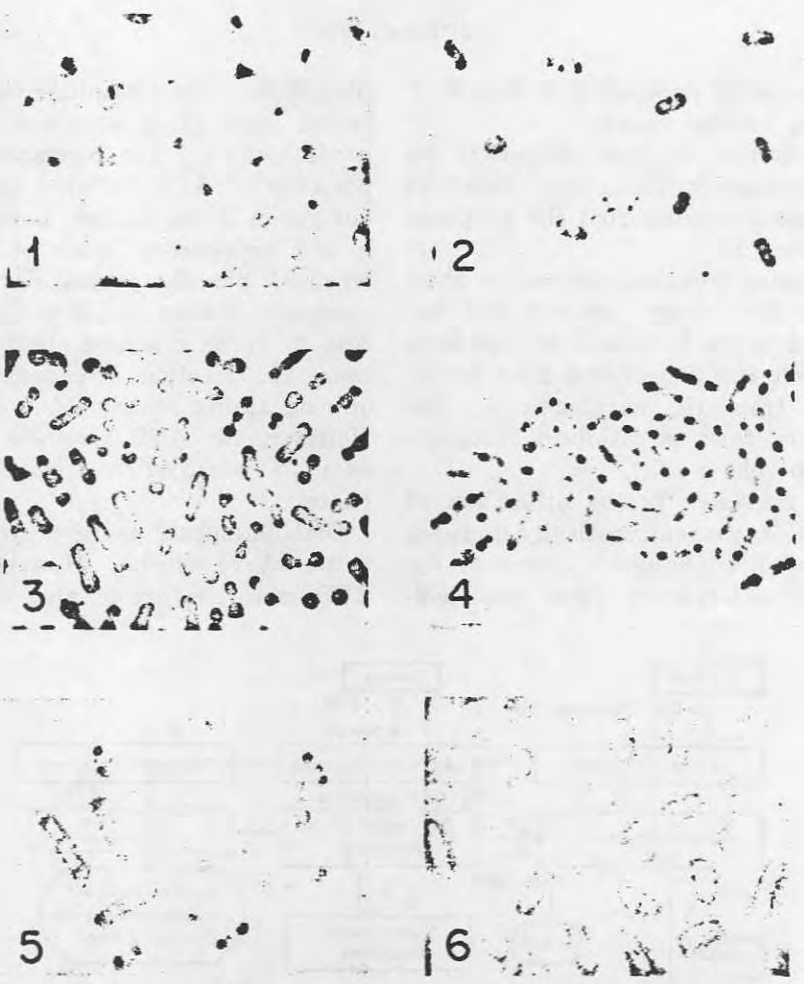

FIGURE 4.—*Aerobacter aerogenes*, strain A3, grown in various media as seen by light microscope, with magnification 3,000 times. In all pictures except no. 6 volutin granules are visible. Courtesy *Journal of Bacteriology* (15).

erated, somewhere in the chain of attending or precursory reactions orthophosphate ions enter the organic complex.

The assimilation of glucose or other carbohydrates by aerobic microorganisms is achieved primarily through a combination of the Embden-Meyerhof pathway and the Krebs cycle. A schematic summary of this major life system is shown in Figure 3. The ratio of the carbohydrate-to-phosphate participation appears to favor activated sludge uptake of orthophosphate. The journey of a single molecule of glucose through the scheme will be attended by a net synthesis of 38 molecules of ATP. Ten ATP molecules are formed in the breakdown of glucose to pyruvic acid, but two ATP molecules are consumed in the process, leaving a net of eight. Each molecule of pyruvic acid generates the formation of 15 molecules of ATP. Thus, in terms of the principal pathway in the biological oxidation of carbohydrates, it seems that phosphate rather than carbohydrates might be limiting. However, the extent to which phosphate may be cycled is unknown.

Finally, there is evidence that bacteria store phosphate. Winkler (13) stated that, since the earliest days of bacteriology, metachromatic granula have been observed in cells. These granula, sometimes called "volutin," have been found to contain considerable stored phosphate in the form of metaphosphate. They occur in fungi, algae, and bacteria. Electron microscopy (14) revealed that they are dense bodies. The rapid development of the phosphate-rich granules in *Aerobacter aerogenes* has been studied by Smith *et al.* (15) (Figure 4). If phosphorus can be stored, the C:P ratio could be variable and would not be considered too formidable a theoretical barrier to the objective of this study.

Laboratory Studies

The experimental phase of this work (16) was performed primarily at the District of Columbia Sewage Treatment Plant Research Laboratory, using sewage from the plant. This is a "high-rate" aeration plant providing approximately two hours of aeration.

Equipment and General Procedures

Unless otherwise noted, the following "standard" procedures and conditions prevailed:

1. Mixed liquor was prepared from plant raw sewage and return sludge.
2. Phosphate uptake experiments were conducted in 2-l flasks immersed in a 25°C water bath.
3. Air was applied through medium-porosity fritted glass diffusers at the rate of 40 ml (barometric pressure)/sec/1,500 ml mixed liquor.
4. Orthophosphate uptake by sludge organisms was determined by measurement of the orthophosphate concentration remaining in mixed liquor aliquots after membrane (0.45 μ) filtration.
5. Phosphate analyses were conducted by slight modification of the stannous chloride procedure described in Standard Methods for the Examination of Water and Wastewater (17), but are herein reported as mg/l phosphate phosphorus (PO_4-P).

Experimental Procedures and Results

Phosphate Uptake with and without Added Carbohydrate

In a typical experiment designed to follow the fate of dissolved phosphate in aerating mixed liquor with and without carbohydrate enrichment, 2 flasks, each containing 1,500 ml of fresh mixed liquor, were prepared. One flask received no additives while the other received 1.5 mM/l sodium succinate plus 0.5 mM/l glucose which had been found to enhance orthophosphate uptake. Samples were taken periodically during the aeration period and were filtered, and the orthophosphate concentration in the filtrate was determined as an index of uptake by the microorganisms.

In the flask containing no additives, maximum orthophosphate uptake of 63 percent was achieved after 8 hr of aeration (Figure 5). In only 4 hr, the mixed liquor containing the glucose and succinate took up 90 percent of the orthophosphate. A characteristic pattern of orthophosphate secretion subsequent to maximum uptake was evident in each flask.

This experiment showed that the addition of a carbon source does promote the uptake of phosphorus. However, it also showed that uptake could occur in the absence of added carbohydrate.

Active Uptake Fraction of Sewage

A simple, direct experiment proved that the active fraction causing orthophosphate uptake in mixed liquor is the sludge. This experiment also confirmed that orthophosphate uptake could occur in the absence of added substrate. Orthophosphate uptake ac-

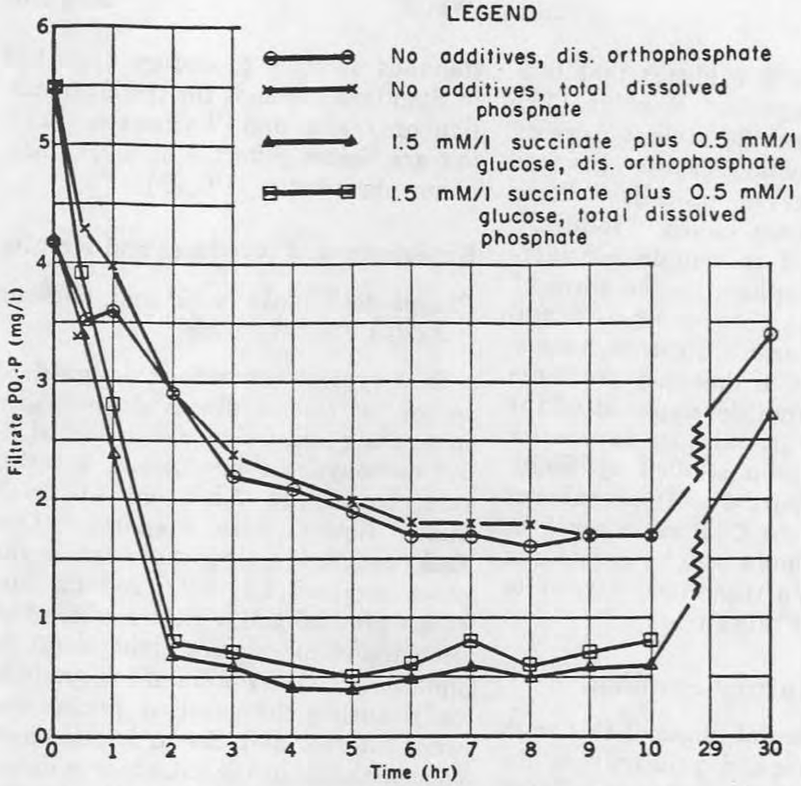

FIGURE 5.—Phosphate uptake by activated sludge.

tivity was sought in 100 percent return sludge. Two flasks containing 1,500 ml of 100 percent return sludge were placed in the water bath. One was aerated continuously and the other was stoppered and not aerated. Portions of mixed liquor were withdrawn from each flask hourly for orthophosphate analysis of the filtrate.

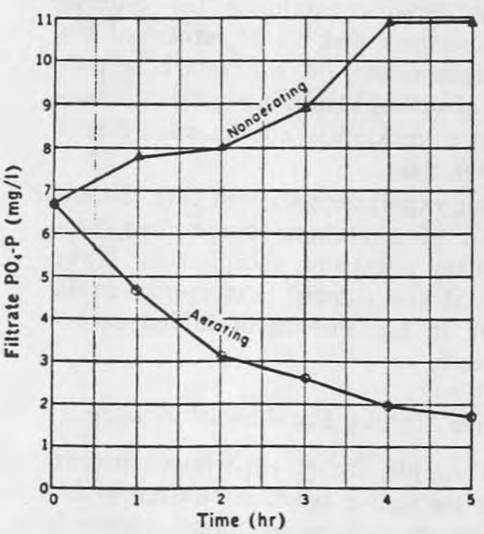

FIGURE 6.—Orthophosphate uptake by activated sludge, with 100 percent return sludge.

Aerated 100 percent return sludge showed much greater dissolved orthophosphate uptake (Figure 6) than had been achieved in any prior experiments in the course of this research program. During the 5-hr aeration period, 5.2 mg/l of orthophosphate were incorporated by the suspended solids of the return sludge.

Effect of Solids Concentration

The effect of suspended solids concentration on orthophosphate uptake by mixed liquor was investigated. Flasks of mixed liquor were prepared containing 0. 5, 10, 20, and 30 percent return sludge.

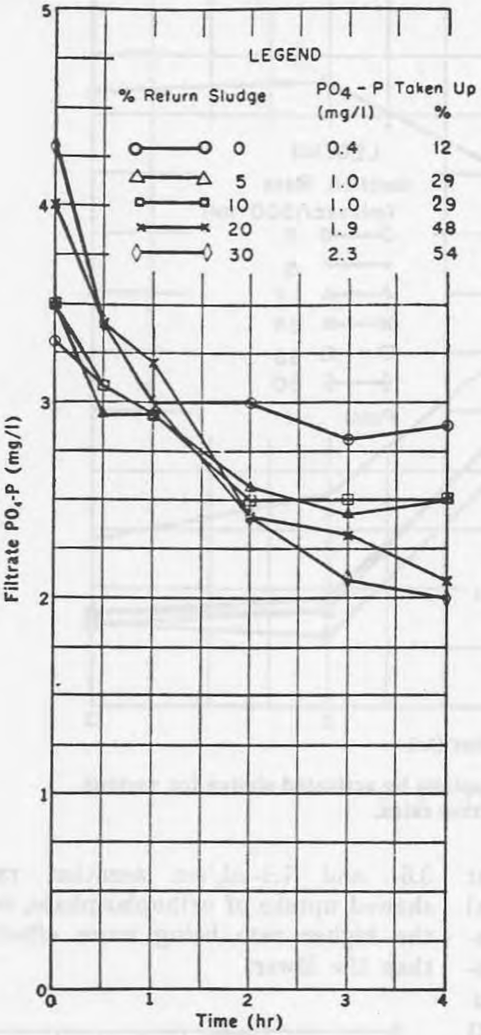

FIGURE 7.—Orthophosphate uptake by activated sludge for various percentages of return sludge.

Figure 7 reveals that as little as 5 percent return sludge increases uptake 100 percent over that of raw sewage.

Dissolved Orthophosphate Uptake as Function of Aeration Rate

Neither Alarcon (9) nor Srinath *et al.* (10), in stating that aeration of mixed liquor resulted in the uptake of orthophosphate, reported the method of aeration or quantity of air supplied. Reference to Figure 3 shows that most of the production of ATP, and presumably most of the uptake of orthophosphate, attends oxidative metabolism in the Krebs cycle.* Thus, it seemed possible that the rate of aeration would be an important factor in orthophosphate uptake.

A series of experiments was undertaken to measure dissolved orthophosphate uptake as a function of aeration rate. In the early experiments of this nature, glucose and succinate were added to the mixed liquor to amplify differences produced by various aeration rates. In one such experiment, 6 flasks of 30 percent return sludge in raw sewage were prepared. To each flask was added 1.5 mM/l sodium succinate plus 0.5 mM/l glucose. The following aeration rates were applied to 1,500-ml portions of mixed liquors: 0, 1, 5, 17, 24, 35, and 50 ml/sec. During aeration, dissolved oxygen determinations were made in each flask by means of the silver-lead oxygen electrode, according to the method described by Mancy *et al.* (18).

The dissolved orthophosphate uptake accomplished by the suspended solids in the mixed liquor can be ascertained from Figure 8. The substantial leakage of orthophosphate from the suspended solids when mixed liquor is not aerated was demonstrated. An aeration rate of 5 ml/sec resulted in uptake of 70 percent of the dissolved orthophosphate in 3 hr. Advancing the rate to 17 ml/sec increased the uptake to 80 percent. Further increase in aeration rate had relatively little effect. At the higher aeration rates, maximum uptake was achieved within two hours. At the five ml/sec rate, uptake would have continued after three hours.

Experiments next were performed to determine if the same effect could be achieved with unadulterated mixed

* The production of ATP in the Krebs cycle is referred to as oxidative phosphorylation as opposed to substrate phosphorylation in the Embden-Meyerhof pathway.

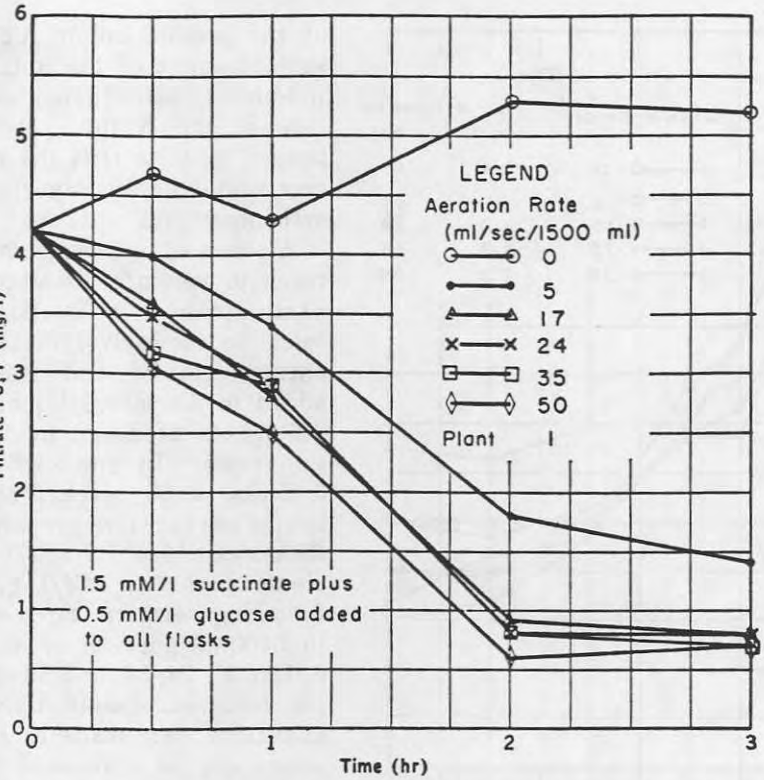

FIGURE 8.—Orthophosphate uptake by activated sludge for various aeration rates.

liquor. In view of the findings that increased aeration rates had marginal effects beyond a point somewhere between 5 and 17 ml/sec, the aeration-rate range was narrowed. The Washington, D. C., Sewage Treatment Plant aeration rate of approximately 1 ml/sec/1,500 ml was included. One further aspect was explored. Okun (19) found increased BOD reduction when oxygen was supplied to mixed liquor as the pure gas rather than in air. Seeking a similar effect on orthophosphate uptake, the new series of experiments included bubbling with pure oxygen.

The results obtained with 30 percent return sludge in raw sewage are shown in Figure 9. Aeration at the plant rate resulted in secretion of orthophosphate during the first two hours of the experiment. This phenomenon also occurs in the plant itself. The 3.6- and 7.1-ml/sec aeration rates showed uptake of orthophosphate, with the higher rate being more effective than the lower.

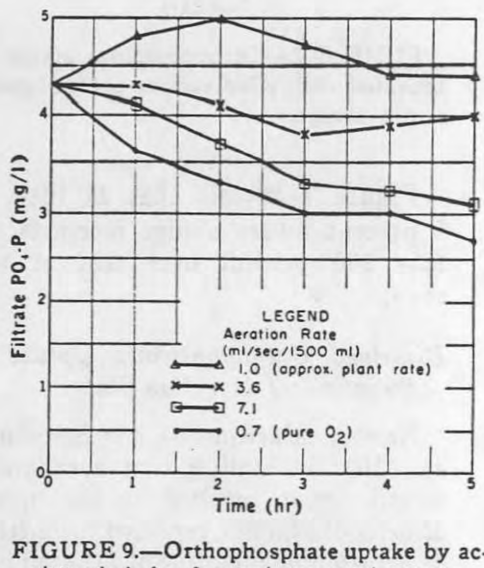

FIGURE 9.—Orthophosphate uptake by activated sludge for various aeration rates.

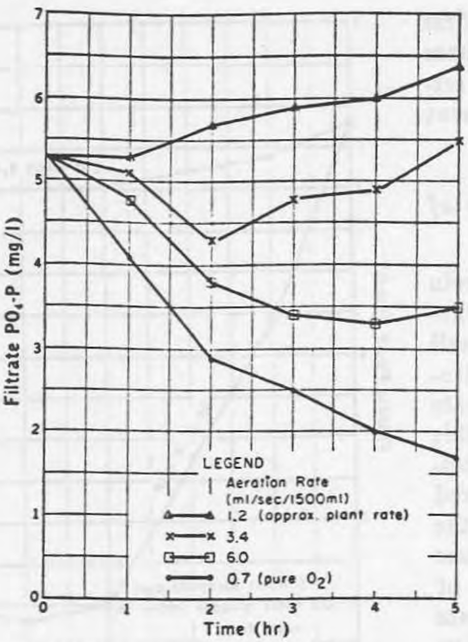

FIGURE 10.—Orthophosphate uptake by 100 percent return activated sludge for various aeration rates.

The mixed liquor supplied with pure oxygen took up a great deal more orthophosphate than did the mixed liquor supplied with an equal quantity of oxygen-in-air. The pure oxygen quickly saturated the mixed liquor to such an extent that the dissolved oxygen meter went off scale. The equal quantity of oxygen-in-air never produced dissolved oxygen concentrations approaching this level.

The effect of aeration rate on dissolved orthophosphate uptake by 100 percent return sludge, the active phosphate-uptake fraction of mixed liquor, next was explored.

Figure 10 shows that relatively small changes in aeration rate evoked pronounced differences in orthophosphate uptake by the sludge. The 1.2-ml/sec aeration rate, roughly corresponding to that of the plant, was inadequate to prevent orthophosphate leakage.

The 0.7-ml oxygen/sec and the 3.4-ml air/sec bubbling rates supplied equal quantities of oxygen. The difference in orthophosphate uptake between these two flasks is even more dramatic than it was in the case of 30 percent return sludge in raw sewage.

Of considerable significance is another consistently observed phenomenon seen very clearly in this family of curves. The valleys are longer and the troughs deeper in the direction of increased aeration. Thus, it is the rate of application of air that determines the amount of phosphate ultimately absorbed.

In conjunction with the aeration studies, experiments were conducted to determine the effect of denying air to the following preparations: raw sewage, 30 percent return sludge in raw sewage, 100 percent return sludge, and freshly settled sludge.

In all flasks the available oxygen was consumed quickly, and the dissolved orthophosphate content in the filtrate increased as shown in Figure 11. The rate of secretion varied in accordance with the suspended solids content of each flask, confirming the high phosphate-metabolic activity in the sludge organisms. The fact that the freshly settled sludge began to

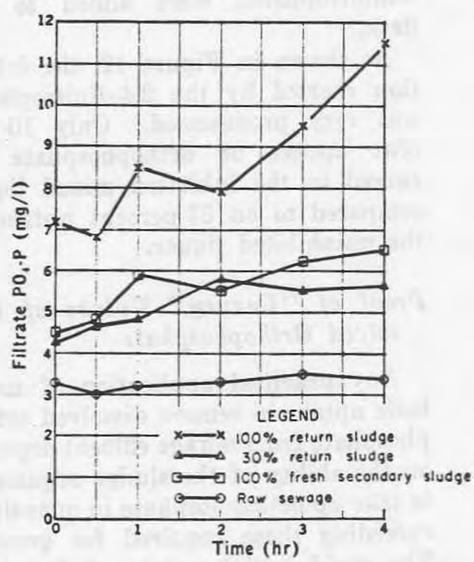

FIGURE 11.—Orthophosphate leakage from activated sludge as a function of time for various types of sludge and wastewater, during oxygen depletion.

secrete orthophosphate within the first 30 min is proof that orthophosphate leakage occurs during secondary settling in activated sludge treatment plants.

Confirmation of Biological Nature of Uptake

Oxidative phosphorylation, herein attributed to effect most of the orthophosphate uptake achieved, is well known to be inhibited by 2,4-dinitrophenol (20). Conversely, substrate phosphorylation is not affected by this substance (20). Thus, with the addition of 2,4-dinitrophenol to mixed liquor, the biological nature of the orthophosphate uptake should become evident. The amount of inhibition of orthophosphate uptake should indicate the relative importance of oxidative phosphorylation and substrate phosphorylation in orthophosphate uptake.

Two flasks were prepared with 30 percent return sludge in raw sewage. Again, for purposes of amplification of effect, 1.5 mM/l sodium succinate plus 0.5 mM/l glucose were added to each flask. In addition, 2.0 mM/l of 2, 4-dinitrophenol were added to one flask.

As shown in Figure 12, the inhibition exerted by the 2,4-dinitrophenol was very pronounced. Only 10-percent uptake of orthophosphate occurred in the inhibited mixed liquor compared to an 87-percent uptake in the uninhibited liquor.

Proof of "Luxury" Uptake of Dissolved Orthophosphate

Any practical application of metabolic uptake to remove dissolved orthophosphate from sewage effluent depends on the ability of the sludge organisms to take up orthophosphate in quantities exceeding those required for growth. The rapid initial uptake of dissolved orthophosphate demonstrated by activated sludge mixed liquor indicated that such "luxury" uptake does occur.

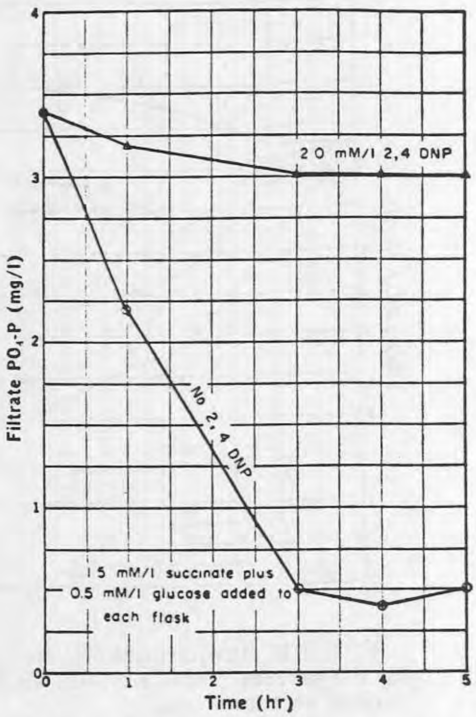

FIGURE 12.—Orthophosphate uptake by activated sludge showing effect of uncoupling oxidative phosphorylation with 2,4-dinitrophenol.

To test this, a quantity of 30 percent return sludge in raw sewage was prepared. The mixed liquor was treated in a Waring blender. One-ml aliquots were removed by pipette for total bacteria determinations. Sodium succinate and glucose then were added to the mixed liquor, which was aerated and incubated in the normal fashion. Dissolved orthophosphate and total bacterial determinations were made periodically. The results, shown in Figure 13, confirm luxury uptake of orthophosphate by mixed liquor suspended solids. Seventy percent of the dissolved orthophosphate in the mixed liquor was taken up by the solids. The bacteriological results show that this uptake was not associated with growth. Indeed, it took place in spite of an 80-percent decline in the number of viable bacteria as they adjusted to the new environment.

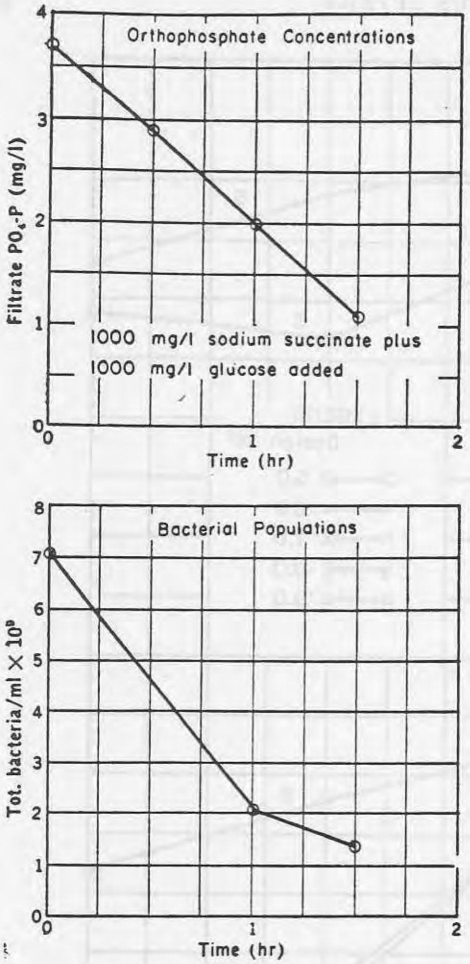

FIGURE 13.—Bacteriological demonstration of luxury uptake of orthophosphate.

Determination of Total Phosphate in Dried Sludge Solids before and after Uptake

An experiment was conducted to determine the change in total phosphate content of the sludge after uptake of orthophosphate. The total phosphorus found in the dried solids removed from freshly prepared 30 percent return sludge in raw sewage was 20,200 ppm on a weight per weight basis. This is in keeping with the one- to three-percent phosphorus content in dried protoplasm generally reported (12) (21). The 30-percent return sludge mixed liquor then was aerated for 3 hr and a similar determination was made. The dried suspended solids total phosphate phorphorus content had risen to 24,500 ppm. The orthophosphate content in the mixed liquor had fallen correspondingly from 3.6 to 0.9 mg/l, a 75-percent reduction.

Effect of pH on Orthophosphate Uptake

To test the effect of pH on orthophosphate uptake, portions of aerating mixed liquor were maintained at or near the following pH units: 5.0, 6.0, 7.0. 8.0, 9.0. A pattern of dissolved orthophosphate uptake as a function of pH was established, as seen in Figure 14. At the design pH's 5.0 and 6.0, rapid and drastic secretion of orthophosphate by the sludge organisms took place. Conversely, at design pH's 7.0 and 8.0, strong uptake of orthophosphate occurred. At design pH 9.0, significant diminution in orthophosphate uptake was observed. This curve demonstrates that the pH effect is biological and not chemical. If the uptake had been the result of chemical precipitation with dissolved calcium present in the sewage, the amount removed would have increased rather than decreased at pH 9.0 (22).

Nonidentity of pH and Aeration Factors

Increased orthophosphate uptake had been associated with increased aeration and the pH range 7.0–8.0. The direct effect of increased aeration might have been merely to make the pH alkaline by blowing out the dissolved carbon dioxide. If this were the case, an identity would exist between the pH and the aeration-rate effects.

To determine whether these two variables were independent, 4 flasks of 30 percent return sludge in raw sewage mixed liquor were adjusted to pH 8.0, placed in the bath, and aerated continuously at respective rates of 5.6, 10.3, 19.4, and 40.0 ml/sec/1,500 ml.

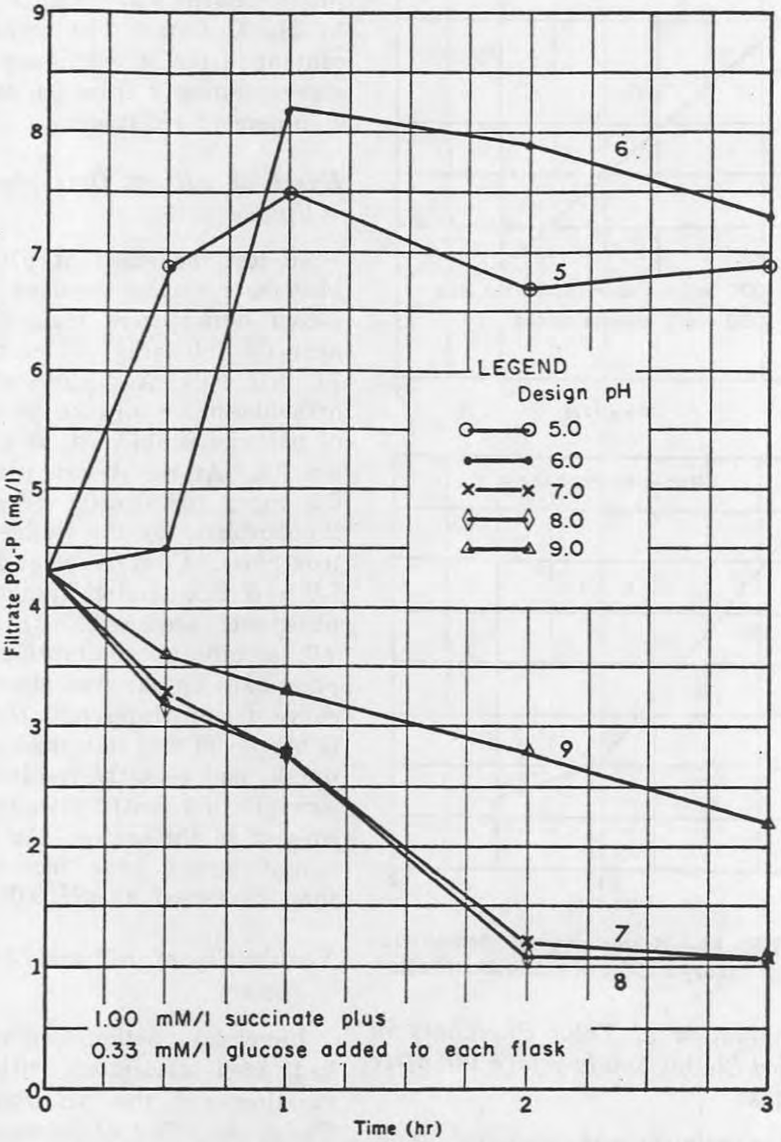

FIGURE 14.—Orthophosphate uptake by activated sludge for various pH's in range 5–9.

At half-hour intervals the pH of each mixed liquor was determined and readjusted to 8.0 ± 0.1.

The results are plotted in Figure 15. It is evident that aeration rate is an independent variable affecting orthophosphate uptake. This experiment also confirmed the earlier finding that aeration in excess of 17 ml/sec/1,500 ml became marginal with respect to orthophosphate uptake.

Phosphate Stripping of Activated Sludge Organisms

Having demonstrated luxury uptake of orthophosphate by sludge organisms and the great dependence of that uptake on pH, a means that might induce greater uptake became evident: if the phosphate reservoir of the cell could be depleted, increased uptake of orthophosphate might follow. The pH experiments had demonstrated that

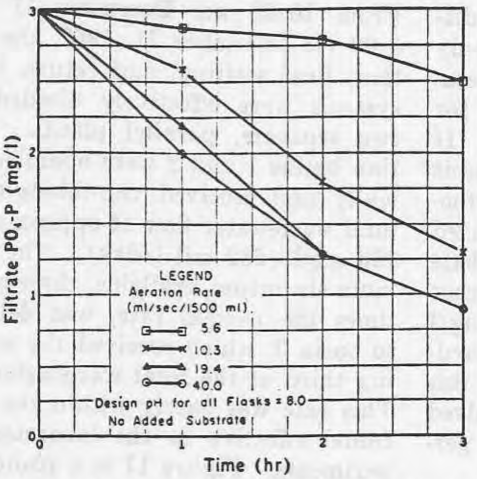

FIGURE 15.—Orthophosphate uptake by activated sludge for various aeration rates at constant pH.

acid environments caused the sludge organisms to lose phosphate. Accordingly, three portions of mixed liquor were prepared in the following manner:

Preparation of Phosphate-Stripped Sludge.—A 1,500-ml portion of 100 percent return sludge was acidified to pH 5.5 with 1.0 *N* hydrochloric acid to induce secretion of phosphate. This sludge was permitted to stand and its pH was maintained at 5.5. At the end of one hour, one liter of the clarified supernatant was decanted and replaced with one liter of stored tap water (containing orthophosphate in trace amount only). Hydrochloric acid again was added to adjust and maintain the pH at 5.5 while the reconstituted sludge was permitted to settle another hour. At the end of the second hour, one liter of the supernatant again was decanted and replaced with tap water. Two drops of 1 *N* sodium hydroxide brought the pH of the sludge back to 6.8, the same as the initial pH of the 100 percent return sludge.

Concentration of Normal Return Sludge.—While this preparation was under way, 450 ml of return sludge were centrifuged at approximately 1,700 g and the supernatant was discarded.

Preparation of Mixed Liquors.—Three flasks of 30 percent return sludge in raw sewage then were prepared. The first consisted of 450 ml of the phosphate-stripped return sludge plus 1,050 ml of fresh raw sewage. The centrifugant from the 450 ml of return sludge was added to 1,050 ml of raw sewage to constitute the second preparation. Finally, 1,500 ml of normally constituted 30 percent return sludge in raw sewage was prepared.

Experiment and Results.—The three flasks of mixed liquor then were placed in the water bath and aerated at the normal rate. Aliquots from each were withdrawn hourly for four hours and filtered, and the filtrates were analyzed for orthophosphate.

The results of the experiment are plotted in Figure 16. Starting with a dissolved orthophosphate content of 2.9 mg/l, the normal mixed liquor showed a 48-percent reduction for a total uptake of 1.4 mg/l. The mixed liquor made with centrifuged sludge depleted its initial 2.8 mg/l of dissolved orthophosphate by only 21 percent for a total uptake of 0.6 mg/l. Because of the phosphate stripping,

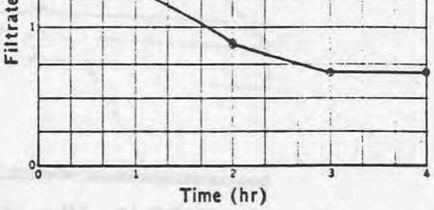

FIGURE 16.—Orthophosphate uptake by activated sludge, showing the effect of phosphate stripping of return sludge.

the mixed liquor made with the stripped sludge had an initial dissolved orthophosphate content of only 1.8 mg/l. In the course of the aeration, this was reduced 61 percent for an absolute uptake of 1.1 mg/l. If the reduction is considered on the basis of the initial 2.9-mg/l dissolved orthophosphate content of the normal mixed liquor, the net effect of the phosphate stripping was to achieve a 76-percent reduction by the removal of 2.2 mg/l of orthophosphate from the mixed-liquor filtrate. A repetition of this experiment achieved a net dissolved orthophosphate reduction of 80 percent.

Plant-Scale Studies

In order to ascertain whether or not the laboratory results had some validity for plant operation, it was necessary to apply some aspects of the laboratory findings on a plant scale.

Experimental Conditions

Through the cooperation of the District of Columbia Department of Sanitary Engineering, the District Sewage Treatment Plant was made available. From 10:00 AM December 17 until 5:00 PM December 21, 1962, the aeration, final settling, and return sludge systems were effectively divided into two separate, parallel plants. Aeration basins 1 and 2 were operated normally and received two-thirds of the total wastewater flow of approximately 180 mgd (680 mil l/day). The maximum air output available, three to four times the normal rate, was delivered to basin 3, which received the remaining third of the total wastewater flow. This rate was barely within the range found effective in the laboratory experiments. Figure 17 is a photograph of the aeration basins taken during the experiment.

Results of Experiment

Dissolved Orthophosphate

The influent and effluent dissolved orthophosphate concentrations in filtered mixed liquor samples from each aeration system are given in Figure 18. Figure 18a compares influent dissolved orthophosphate with effluent dissolved

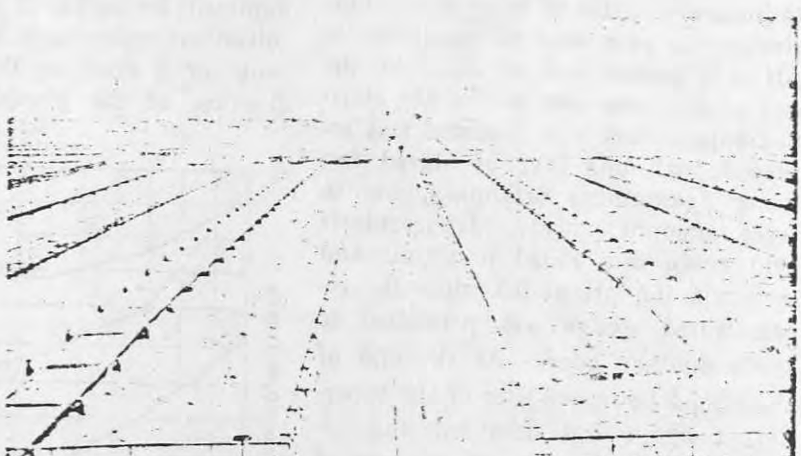

FIGURE 17.—View of aeration basins of District of Columbia Sewage Treatment Plant during increased aeration experiment, Dec. 17–21, 1962. Basins at left were aerated at 3 to 4 times the rate of those to the right.

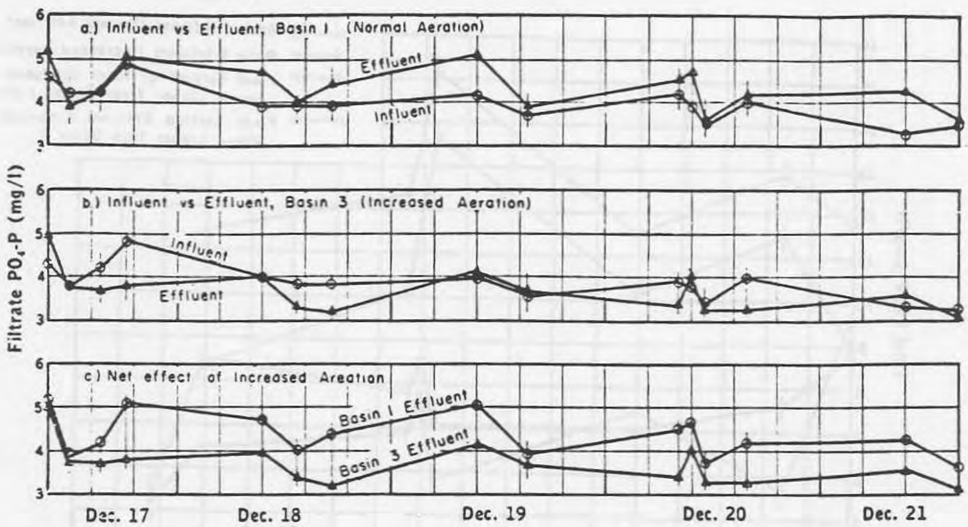

FIGURE 18.—Results of plant-scale increased-aeration experiment, showing orthophosphate changes through the aeration basins.

orthophosphate in aeration basin 1. The dissolved orthophosphate content of the mixed liquor increased during the normal aeration process.

Influent and effluent dissolved orthophosphate data for basin 3 are presented in Figure 18b. The effect of the increased aeration in promoting orthophosphate uptake is apparent. Figure 19a presents the percent reductions achieved, with the average percentage being close to the 9-percent reduction shown by the laboratory experiment in which the aeration was 3.6 times the normal plant rate and in which 30 percent return sludge, as opposed to the plant's 10 percent, was used.

One significant result of the experiment was the fact that the system never equilibrated; that is, the uptake of dissolved orthophosphate in basin 3 did not end because of phosphate satiation of the sludge organisms that were being recycled.

Dissolved orthophosphate reduction has been discussed above in absolute terms, based on the initial concentration in the aeration basin influent.

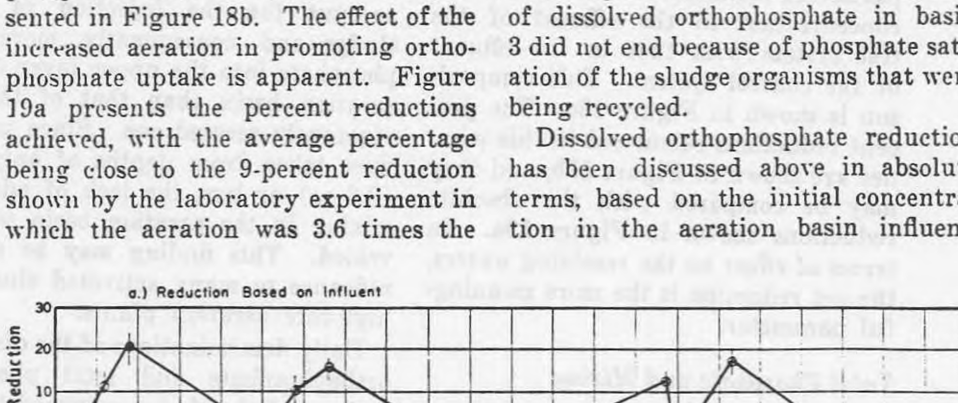

FIGURE 19.—Results of plant-scale increased-aeration experiment, showing percent dissolved orthophosphate reductions through aeration basins.

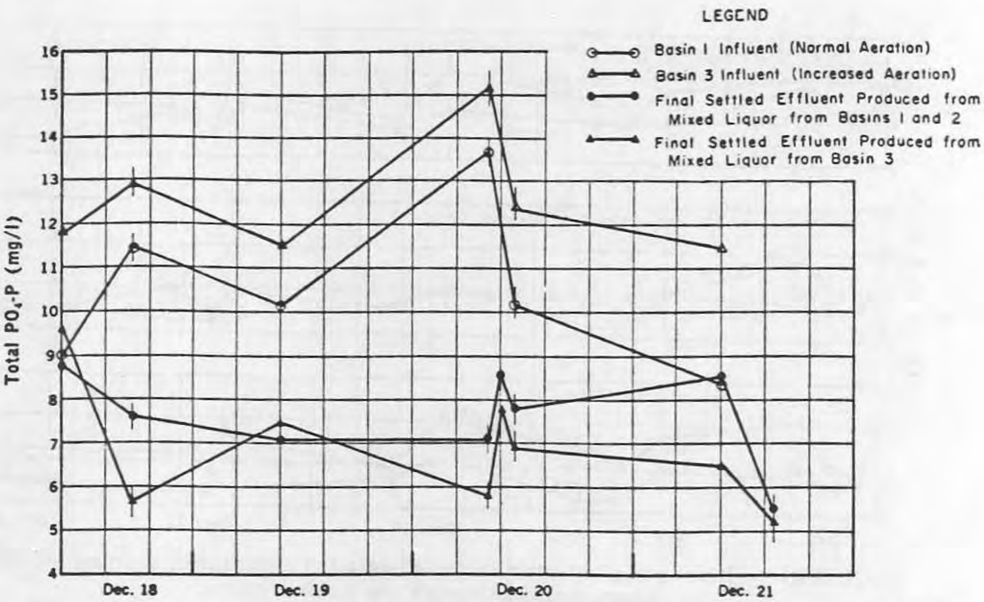

FIGURE 20.—Results of plant-scale increased-aeration experiment, showing effect of aeration and final settling on total phosphate content.

The net dissolved orthophosphate reduction achieved by the increased rate of aeration is determined by a comparison of the dissolved orthophosphate concentration in the effluent of the test system with that in the effluent of the control system. This comparison is shown in Figure 18c. The percent reductions calculated in this manner are shown in Figure 19b, and they may be compared with the absolute reductions shown in Figure 19a. In terms of effect on the receiving waters, the net reduction is the more meaningful parameter.

Total Phosphate and Mixing

The results of the total phosphate analyses performed on samples taken from the influent and effluent ends of basins 1 and 3 are presented in Figure 20. The consistent and substantial difference found between the total phosphate concentrations of the two influent mixed liquors demands explanation. Such a difference did not exist in the case of the dissolved orthophosphate concentrations, as may be seen by comparing the influent curves of Figures 18a and 18b.

The major cause of the difference is the better mixing of the return sludge with the influent sewage in the more highly aerated basin. This would account for the injection of more sludge and, consequently, more total phosphate into the upper layer of this aeration basin than that of the less vigorously aerated one. Since samples were taken from depths of only 2 ft (0.6 m) or less, the lack of adequate mixing in the aeration basin was revealed. This finding may be of significance to many activated sludge or high-rate aeration plants.

Daily determinations of the dissolved orthophosphate and total phosphate content of the sludge returned to each aeration system are given in Table I. The trends shown are consistent with increased orthophosphate uptake by sludge organisms in the more highly aerated system. Thus, the average dissolved orthophosphate content in the sludge liquor generated from basins 1 and 2 was higher than that in the sludge found in basin 3. A greater degree of uptake by the organisms in basin 3 is responsible for this. This increased uptake by the more highly

TABLE I.—Results of Plant-Scale Increased-Aeration Experiment, Dec. 17–21, 1962

Date	Time	Phosphate Content of Return Sludges			
		Dissolved Orthophosphate (mg P/l)		Total Phosphate (mg P/l)	
		Sludge Originating in Basins 1 and 2	Sludge Originating in Basin 3	Sludge Originating in Basins 1 and 2	Sludge Originating in Basin 3
12/17	2:30 PM	—	—	47.5	58.0
12/18	2:00 PM	5.0	4.7	41.9	53.7
12/19	1:00 PM	5.6	4.7	47.5	41.9
12/20	4:30 PM	5.1	4.4	53.4	60.2
12/21	4:00 PM	5.0	5.2	69.6	62.0
Avg.		5.2	4.8	52.0	55.2

aerated sludge is confirmed by the total phosphate values.

pH

Table II shows that the pH in basin 3 was consistently higher than that in basin 1 for the period of the study. Moreover, the pH increased as the flow progressed through basin 3. The change in pH may have contributed to the increased uptake of orthophosphate. Although all pH measurements were within the favorable 7–8 range, some laboratory evidence indicated greater activity at the higher end of the range.

Dissolved Oxygen

Dissolved oxygen data taken at the influent ends, midpoints, and effluent ends of the aeration basins were very informative. These profiles are shown in Figure 21. Surprisingly, the dissolved oxygen concentrations in the influent ends of the aeration basins were almost always greater for basin 1, the one receiving less air. As previously stated, the laboratory findings showed that the rate of aeration, rather than merely the total quantity of air supplied, exercised a pronounced control over the amount of phosphate uptake. Thus, the more highly aerated organisms may have depleted the dissolved oxygen in the mixed liquor until the residual was less than that in the normally aerated mixed liquor. This same phenomenon had been observed in the laboratory-scale experiments performed in two-l flasks.

TABLE II.—Results of Plant-Scale Increased-Aeration Experiment, Dec. 17–21, 1962

Date	Location	pH	
		Basin 1	Basin 3
12/19	Influent end	7.1	7.2
	Effluent end	7.1	7.5
12/20	Influent end	7.2	7.4
	Effluent end	7.2	7.7

It can be concluded that neither aeration basin received sufficient oxygen at the influent end. The proximity of the two dissolved oxygen curves establishes this fact. If the oxygen demand in the highly aerated basin had been met, the dissolved oxygen concentrations would have been higher than those in the normally aerated basin. On the other hand, if the organisms in both basins received adequate oxygen, the dissolved oxygen in basin 3 would have exceeded that in basin 1 by virtue of the different rates of oxygen application.

The dissolved oxygen picture was radically different by the time the two mixed liquors reached the midpoints of their flows through the aeration basins. The midpoint dissolved oxygen record in Figure 21 shows that the dissolved oxygen in the normally aerated basin decreased. This occurred

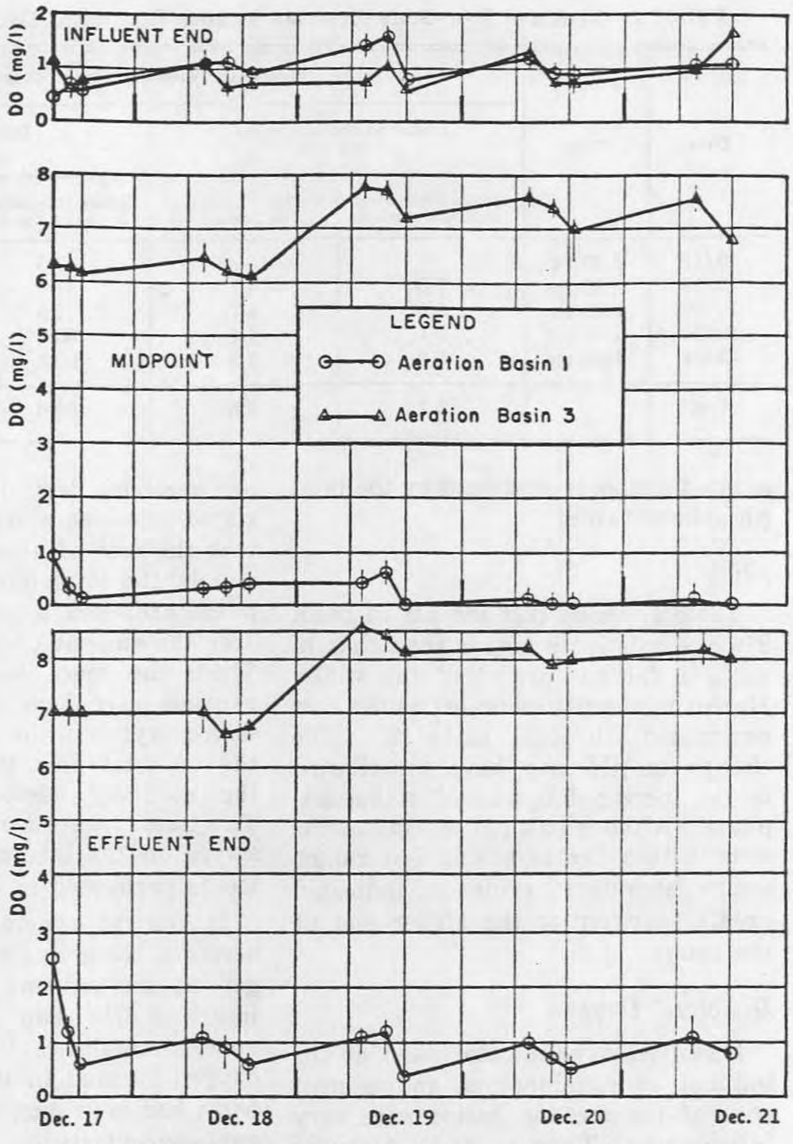

FIGURE 21.—Results of plant-scale increased-aeration experiment, showing dissolved oxygen content in aeration basins.

despite the fact that approximately one hour of aeration had been applied by the time the mixed liquor reached the midpoint. For approximately half of the experimental 5-day period, dissolved oxygen at the midpoint in this basin was absent or no higher than 0.1 mg/l. Thus, periods of anaerobiosis actually developed in the aeration basin. Under such conditions, phosphate taken up earlier undoubtedly leaked out of the activated sludge organisms.

On the other hand, the mixed liquor at the midpoint in aeration basin 3 was approximately three-quarters saturated with oxygen. This indicates that by the time the mixed liquor reached the midpoint of its flow through aeration basin 3 the organisms had completed substrate assimilation and were undergoing endogenous respiration. The dissolved oxygen data for the effluent end of aeration basin 3, also presented in Figure 21, lends support to this conclusion because the dissolved oxygen levels are similar in quantity and pattern to those at the midpoint. Thus, apparently little additional oxygen was consumed by the organisms in the latter half of the aeration period.

In the effluent end of basin 1 (Figure 21) the dissolved oxygen recovered to the influent-end level. However, the oxygen level is not sufficiently high to indicate that the organisms have completed assimilation of the available substrate. Thus, the detention period in the highly aerated basin was probably longer than it needed to be, whereas, in the normally aerated basin, the need for additional detention existed.

BOD

Returning to the classical yardstick for sewage treatment plant performance, Table III presents BOD information on both systems. Five out of the six samples taken showed a significantly higher BOD reduction achieved by the increased-aeration system. For the 3 days of record, the BOD reduction showed a 21-percent improvement as the result of increased aeration.

Futuristic Sewage Treatment Plant

It is possible to visualize a future sewage treatment plant which would incorporate successfully the real and implied advantages resulting from this research program. The reader is cautioned that the primary guideline for the proposed innovations is the reduction of dissolved orthophosphate and that possible disadvantages to other objectives of sewage treatment would have to be considered carefully.

Figure 22 is a diagrammatic concept of this plant. On entering the plant, the raw wastewater would be monitored continuously for pH and adjusted to maintain the range of 7 to 8 with suitable chemicals such as hydrochloric acid, sodium hydroxide, or lime. The wastewater then would pass through conventional screening and grit-removal units. Leaving the grit chamber, it would receive return sludge in a flash mixer to insure immediate and maximum dispersal of the sludge particulates. Primary settling would be eliminated to prevent the reduction in particulate carbohydrates delivered to the aeration basin. A portion of these carbohydrates would hydrolyze and induce additional uptake of dissolved orthophosphate.

The mixed liquor from the flash mixer would be received in a two-stage oxygenation-aeration basin. Pure oxygen would be supplied at approximately 0.3 to 0.5 ml/sec/l in the first portion of the basin which would provide a short detention period of perhaps 15 min. The detention period in

TABLE III.—Results of Plant-Scale Increased-Aeration Experiment, Dec. 17–21, 1962 Effect on BOD

Date	Time	BOD of Influent to Aeration Basins (mg/l)	Final Settled Effluent				
			Originating in Basins 1 and 2		Originating in Basin 3		
			BOD (mg/l)	Reduction (%)	BOD (mg/l)	Reduction (%)	Improvement over Effluent Originating in Basins 1 and 2
12/19	10:00 AM	132	77	42	52	61	32
	3:00 PM	163	50	69	40	75	20
12/20	10:00 AM	123	50	59	32	74	36
	3:00 PM	129	49	62	41	68	16
12/21	10:00 AM	132	56	58	33	75	41
	3:00 PM	139	38	73	46	67	−21
Avg.		136	53	61	41	70	21

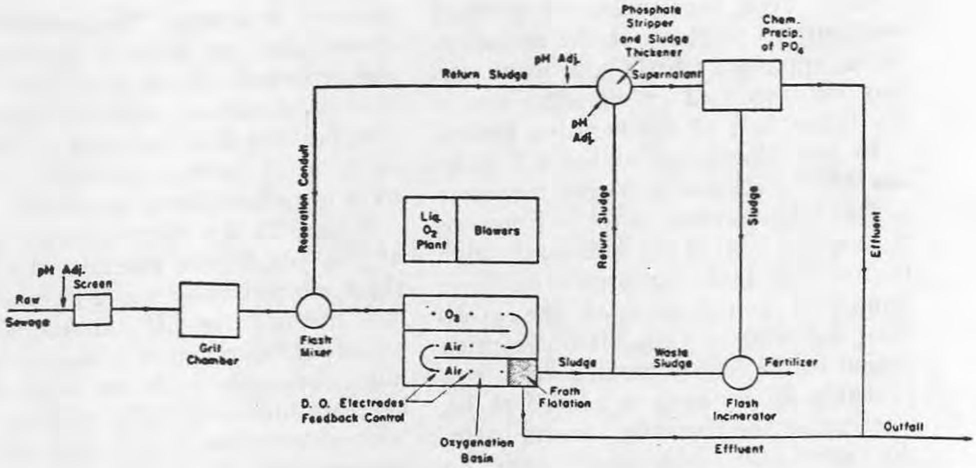

FIGURE 22.—Flow diagram of proposed wastewater treatment process for removal of phosphate in addition to usual biological treatment.

the second section of the basin would be longer. Air would be administered here at a rate between 3 and 10 ml/sec/l. Oxygen levels in both sections of the basin would be controlled by feedback mechanisms operating through a series of dissolved oxygen electrodes distributed throughout the basin. The dissolved oxygen content would be maintained at approximately 0.5 mg/l throughout the basin.

The effluent mixed liquor would flow into a froth-flotation unit where the sludge would be removed by air. The clarified waste then would be discharged to the effluent outfall, possibly after chlorination. The harvested sludge would be divided into two portions, 30 percent for return and the remainder for wasting. The wasted sludge would be flash incinerated for conversion into fertilizer with a considerably higher phosphate content than now is found in sludge.

The return sludge, in an amount equivalent to the solids in a 30-percent return sludge mixture, would be delivered to a combination phosphate stripper and sludge thickener. Here the pH would be adjusted to 5.0 to 6.0 for 10 to 20 min to induce considerable quantities of intracellular phosphate to leak out into the liquid phase of the mixed liquor. The sludge would be permitted to thicken and settle under slow mechanical stirring. The phosphate-enriched supernatant, only a small fraction of the raw sewage volume, would flow into a chemical precipitation basin where lime would be added and mixed, and the tricalcium phosphate produced would be permitted to settle. This precipitate would be carried to the flash incinerator. From the chemical basin, the phosphate-depleted effluent would join the effluent from the aeration basin for possible chlorination and subsequent discharge to the outfall. On leaving the phosphate stripper, the thickened return sludge would be adjusted to pH 7.0 to 8.0 with lime and returned to the flash mixer through a reaeration conduit in which it would be aerated vigorously enroute.

Economics

It is premature to speak of the economics associated with metabolic removal of dissolved orthophosphate or possible benefits which might result. Only the basic principles have been explored. No detailed consideration can be given yet to the engineering aspects of the process. The principal elements of that cost, however, seem to be associated with blower size and power, possibly with oxygen production, and with pH adjustment. None of these items can be estimated at this time.

Offsetting the possible cost increases are potential savings in the elimination of primary and secondary sedimentation, and possible elimination of digestion if the froth-harvested waste sludge is incinerated and its Btu content utilized directly. In addition, possible value of the sludge as fertilizer must be considered. To date, sewage sludge has been of marginal value as fertilizer largely because of the low phosphate content.

However, the benefit which would accrue to the receiving waters through the control or elimination of algal blooms is the primary consideration in phosphate removal. That such a benefit would result is supported by the recent findings of Shapiro and Ribeiro (23) in their work on the Potomac estuary.

Pollution control advantages always have been hard to evaluate financially. In Washington, D. C., however, one might estimate these savings by considering an alternative to phosphate reduction. One such alternative might be the construction of an outfall to carry the sewage effluent to the Chesapeake Bay (24). The capital costs of this project recently have been estimated at approximately $100,000,000. Annual operating costs for required pumping stations also are associated with the long-distance outfall. Thus, at least for Washington, a fairly good estimate exists of the competitive costs allowable for an economic phosphate-removal process.

Conclusions

The following principal conclusions summarize this research effort:

1. "Luxury" uptake of dissolved orthophosphate by sludge organisms, that is, uptake in the absence of growth, has been demonstrated.

2. Internal phosphorus concentrations in sludge organisms need not increase more than 25 percent above those readily achieved in these preliminary experiments to effect complete removal of dissolved orthophosphate from sewage effluent.

3. Dissolved oxygen is essential to and exerts profound control over orthophosphate uptake. The rate at which oxygen is applied greatly affects the orthophosphate-uptake capacity of the sludge organisms.

4. In wastewater treatment plant operation, dissolved inorganic orthophosphate leaks out of sewage organisms when the dissolved oxygen level of the sewage is permitted to fall. Under conditions of insufficient aeration, the leakage may occur in the aeration basin. However, even if sufficient oxygen is applied in the aeration basin, leakage will occur in secondary settling as the sludge blanket rapidly consumes available dissolved oxygen.

5. In laboratory experiments the application of pure oxygen evoked markedly better uptake of orthophosphate than did the application of equal quantities of oxygen-in-air.

6. The pH of the mixed liquor vitally affects orthophosphate uptake, with a maximum uptake occurring in the pH range 7.0–8.0.

7. A small amount of return sludge in raw sewage dramatically improves orthophosphate uptake over that obtainable in raw sewage alone. However, while increasing the percentage of return sludge further enhances uptake, the proportionality of the effect diminishes rapidly.

8. Dissolved orthophosphate uptake may be enhanced significantly by stripping phosphate from the sludge organisms through pH adjustment before returning the sludge to the aeration basin for additional uptake.

9. In laboratory experiments, dissolved orthophosphate removals occasionally as high as 80 percent were achieved by aerating mixed liquors without the addition of chemicals or substrates.

10. On a plant scale, increased aera-

tion promoted orthophosphate uptake, substantiating laboratory results.

11. The prospects for achieving major reduction of dissolved inorganic orthophosphate from sewage effluent by sludge metabolism through modification of aeration sewage treatment processes seem promising and deserve further attention.

Acknowledgments

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