The Significance of an Anaerobic Zone for the Biological Removal of Phosphate from Wastewaters

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Abstract

Two laboratory, denitrifying, activated sludge units were used to study phosphate removal by employing an additional, anaerobic phase in one of the units. The unit with an anaerobic zone showed markedly higher phosphate removal, although phosphate accumulating bacteria were traced in both sludges. No relationship could be determined between phosphate release in the anaerobic zone and subsequent phosphate accumulation in the aerobic zone. The permissible nitrate concentration in the inflow to the anaerobic zone was also estimated.

Introduction

It would seem superfluous to elaborate on the importance of developing processes for the biological removal of compounds of phosphorus and nitrogen from wastewaters. The need for effective phosphate removal is often related to the call for less eutrophication of the impoundments into which this treated waste is disposed (Toerien, 1977). In South Africa, about 66 per cent of the impoundments are growth limited by phosphate. Nitrogen is usually the controlling factor in hyper-eutrophic impoundments and there seems to be a switch from phosphate to nitrogen as impoundments become more eutrophic. The need for phosphate removal could be superseded in time by the need for nitrogen removal.

In the denitrifying, activated sludge process, the sewage effluent passes through an anoxic zone*, followed by an aeration zone and a clarifier. The sludge, together with some of the effluent, is recycled to the anoxic zone and, in some plants, a fair volume from the aerobic zone is recycled to the anoxic zone. The nitrate formed in the aerobic zone is converted to nitrogen or nitrous oxide (N_2O) in the anoxic zone, leading to total nitrogen removal. Removal of phosphate was also sometimes observed (Barnard, 1976).

Barnard (1976), reviewing the conditions under which biological phosphate removal had taken place, postulated the apparent need for an anaerobic stage. McLaren and Wood (1976) also stressed the importance of including an anaerobic stage in denitrifying, activated sludge units. This anaerobic zone had to be seen as a requirement opposed to that of a mere anoxic stage.

The following theories have been advanced on the function of the anaerobic zone:

1. The production of certain low molecular weight fermentation products to serve as substrates for specialized bacteria able to accumulate large amounts of phosphate, such as certain *Acinetobacter* spp. (Fuhs and Chen, 1975).

2. The release of phosphate from the sludge into the supernatant liquor as soon as aerobic oxidative metabolism can no longer take place. This is followed by consumption of the phosphate in luxury amounts, owing to over-compensation when the accumulation of energy-rich phosphorus compounds becomes possible in the aerobic zone (Barnard, 1975).

3. The chemical theory of DeBoice and Thomas (1975), which is not considered in detail in this paper. It should

*In this paper 'anoxic' denotes a state in which free oxygen is absent or present in negligible amounts, while nitrate is available in quantities sufficient to allow significant denitrification. 'Anaerobic' implies the complete or virtual absence of oxygen, but a certain (as yet unspecified) inflow of nitrate/nitrite can be tolerated, dependent on the inflow of chemical oxygen demand (COD).

also be noted that chemical precipitation of a calcium phosphate mineral can take place under anaerobic, anoxic or aerobic conditions provided the pH $>$ 7,0 (Hoffmann and Marais, 1977).

It has been reported that phosphate removal could be optimized at particular phosphate to COD ratios (Carberry and Tenny, 1973) and an optimal anoxic retention time of 30 min in the absence of an anaerobic stage (Martin and Marais, 1975).

However, both McLaren and Wood (1976) and Barnard (1976) stressed the importance of a period of anaerobiosis to induce phosphate release, followed by a period of sludge aeration to promote enhanced phosphate uptake (i.e. a removal rate exceeding 50 per cent). Barnard (1976) also mentioned that nitrate in the inflow to the anaerobic zone adversely affected phosphate removal.

The need to strip phosphate from the sludge of the activated sludge process was stressed by Levin *et al.* (1975), who proposed that the stripped phosphate be recovered by chemical means.

In view of these controversial postulates and observations on the necessity of an anaerobic zone, the work reported below was carried out primarily to prove experimentally whether an additional anaerobic zone, as opposed to an anoxic zone only, is required for enhanced phosphate removal in the denitrifying, activated sludge process. A further objective was to establish the relationship, if any, between phosphate release in the anaerobic zone and its subsequent uptake in the aerobic zone of the process.

Special attention was also given to the presence of phosphate-removing bacteria in the sludge, particularly to certain *Acinetobacter* spp. (Fuhs and Chen, 1975) and similar types.

Materials and Methods

Two Perspex, laboratory scale, denitrifying, activated sludge units, each with a total volume of 5*l*, were operated simultaneously at a constant temperature of 20°C. The units were inoculated with the same sludge taken from a pilot, activated sludge plant and fed with pasteurized (20 min at 75°C), settled, domestic sewage throughout the whole of the investigation. A layer of plastic balls was placed on the surface of the anoxic and anaerobic basins, to minimize the access of air.

The reactors were designed in such a way that the percentage retention time spent in the presence or absence of free oxygen was identical in both systems.

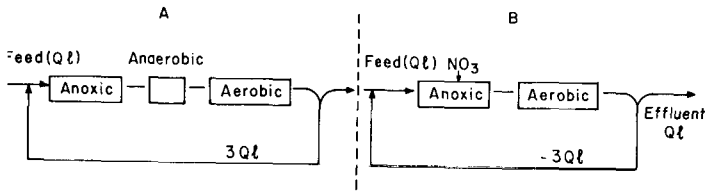
The only difference between the units was the division of the anoxic zone in unit B into an anoxic and anaerobic section in unit A (Table 1). As the anoxic basin of unit B was expected to accomplish complete denitrification of the recycled mixed liquor, it was necessary to add nitrate to the primary basin of unit B in order to prevent the development of anaerobic conditions owing to the longer anoxic retention time. Nitrate was added to maintain the concentration in the anoxic zone at about 2 mg l^{-1} (as N).

After the first period (1) of 23 days, the aeration rate had to be reduced for a period of 25 days, owing to air compressor malfunction. In addition, during this period, the COD of the influent sometimes exceeded the 500 mg l^{-1} specified in Table 1. Both units were subsequently operated for about four weeks (period 2). The performance levels matched those for period 1, before the two sludges were interchanged (period 3).

Performance of the units was assessed by regular chemical analysis of the various zones of each system. Nitrate and orthophosphate were measured on an AutoAnalyzer, using the analytical methods of the National Institute for Water Research (1974). Chemical oxygen demand, Kjeldahl nitrogen, mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) were determined manually *(Standard methods,* 1971).

On two occasions, the sludge from each unit was serially diluted and plated on nutrient agar. Twenty isolates were obtained on each occasion and plated on nutrient agar. Ten isolates from sludge A[1] were also obtained on the enrichment medium for phosphate-accumulating bacteria of Fuhs and Chen (1975) and with 15 g l^{-1} agar added. All isolates were

TABLE 1
OPERATIONAL CHARACTERISTICS OF EXPERIMENTAL UNITS



Unit A		Unit B
3,3	Total actual retention time (h)	3,3
33,3	Percentage anoxic	55,5
22,2	Percentage anaerobic	0,0
44,5	Percentage aerobic	44,5
2000	Mixed Liquor Volatile Suspended Solids (MLVSS) (mg l^{-1})	2000
15	Sludge age (d)	15
500	Influent COD (mg l^{-1})	500
3	Recycle/feed ratio	3
A[1]	Sludge designation	B[1]

TABLE 2
SUMMARY OF AVERAGE PHOSPHATE AND NITRATE CONCENTRATION AND PHOSPHATE REMOVAL RELATED TO COD REMOVAL

Conc. in mg l^{-1}	Period	1		2		3	
	Test	A	B	A	B	A	B
Orthophosphate as P in influent		9,1	9,1	9,1	9,1	9,3	9,3
Organic Phosphate as P in influent		3,4	3,0	3,6	2,9	3,2	3,0
Orthophosphate as P in aerobic basin effluent		2,5	7,1	1,4	6,6	3,0	8,0
Orthophosphate as P in clarifier effluent		3,8	7,2	2,3	6,8	4,2	8,2
Orthophosphate as P in aerobic basin influent		12,5	9,3	7,0	7,7	7,5	8,0
Nitrate in final effluent		8,3	10,9	8,8	12,7	8,5	20,0
$\Delta P/\Delta COD$		0,021	0,011	0,0235	0,011	0,021	0,0096

TABLE 3
SUMMARY OF pH VALUES IN EXPERIMENTAL UNITS

	A		B	
Sampling point	Average	Range	Average	Range
Influent	7,37	7,22 to 7,80	7,37	7,22 to 7,80
Anoxic stage	7,52	7,33 to 7,70	7,75	7,60 to 8,20
Anaerobic stage	7,50	7,36 to 7,70	—	—
Effluent	7,39	7,34 to 7,70	7,60	7,43 to 7,94

TABLE 4
PHOSPHATE REMOVAL PERCENTAGES IN LABORATORY UNITS A AND B DURING PERIODS 1 TO 3

	Period No.	1		2		3	
	Duration of experimental run	23 days		26 days		22 days	
	Plant	A	B	A	B	A	B
	Sludge Designation	A^1	B^1	A^1	B^1	A^1	B^1
	Phosphate in feed (mg l^{-1})*	9,1	9,1	9,1	9,1	9,3	9,3
From	To	Percentage phosphate removal					
Aerobic basin effluent	Aerobic basin influent*	80	24	80	15	60	1
Aerobic basin effluent	Plant influent*	73	22	85	27	68	14
Clarifier effluent	Plant influent*	58	21	75	25	55	12
Aerobic basin effluent	Plant influent**	80	41	89	45	76	35
Clarifier effluent	Plant influent**	68	41	81	44	66	33

* Orthophosphate (as P), determined on filtered samples
**Total phosphates (as P), determined on unfiltered samples

examined for phosphate-accumulating properties in an aerated liquid medium specially designed to supply all the required nutrients (Fuhs and Chen, 1975). Phosphate accumulation was measured after 5 to 10 days.

Results

The average phosphate removal percentages, pH values, nitrate concentrations, and the bacteriological results for periods 1 to 3 are presented in Tables 2 to 5. Daily results for period 1 are detailed in Figures 1 and 2.

Discussion

The superior phosphate removal ability of the unit with an anaerobic stage is illustrated in Table 2. Unit A removed 80 per cent of the phosphate entering the aerobic basin during each of the first two periods. In contrast, the aerobic basin of unit B removed only about 20 per cent of the phosphate. The latter, being less than 50 per cent, does not constitute enhanced phosphate removal.

Since the pH in unit B was invariably higher than that of unit A (Table 3), following the theory of DeBoice and Thomas (1975) the phosphate removal should have been higher in unit B than in unit A, and this is undoubtedly not the case. Hoffmann and Marais (1977) found that relatively small increases of the pH (7,39 to 7,60) will cause only an insignificant fixation of phosphate as calcium phosphate. For marked fixation of phosphate of this type the pH has to rise above 8, while at pH values $>$ 7,00 calcium phosphate dissolves. The pH barely fluctuated between the basins of a single unit while phosphate was being released and accumulated. Thus it appears that the change in pH value cannot be regarded as a significant factor in the mechanism of phosphate release and accumulation.

TABLE 5
SUMMARY OF BACTERIAL ISOLATES FROM SLUDGES A[1] AND B[1] GIVING A POSITIVE REACTION FOR PHOSPHATE ACCUMULATION IN SPECIAL LIQUID MEDIUM

Period	Sludge	Medium	Isolate No.
1	A[1]	Nutrient agar	3, 7, 8, 16, 18
	A[1]	Fuhs and Chen (1975) agar	3, 5, 7, 8, 10
	B[1]	Nutrient agar	4, 11, 12, 14, 17
3	A[1]	Nutrient agar	2, 3, 5, 7, 10, 11, 18
	B[1]	Nutrient agar	3, 5, 7, 17

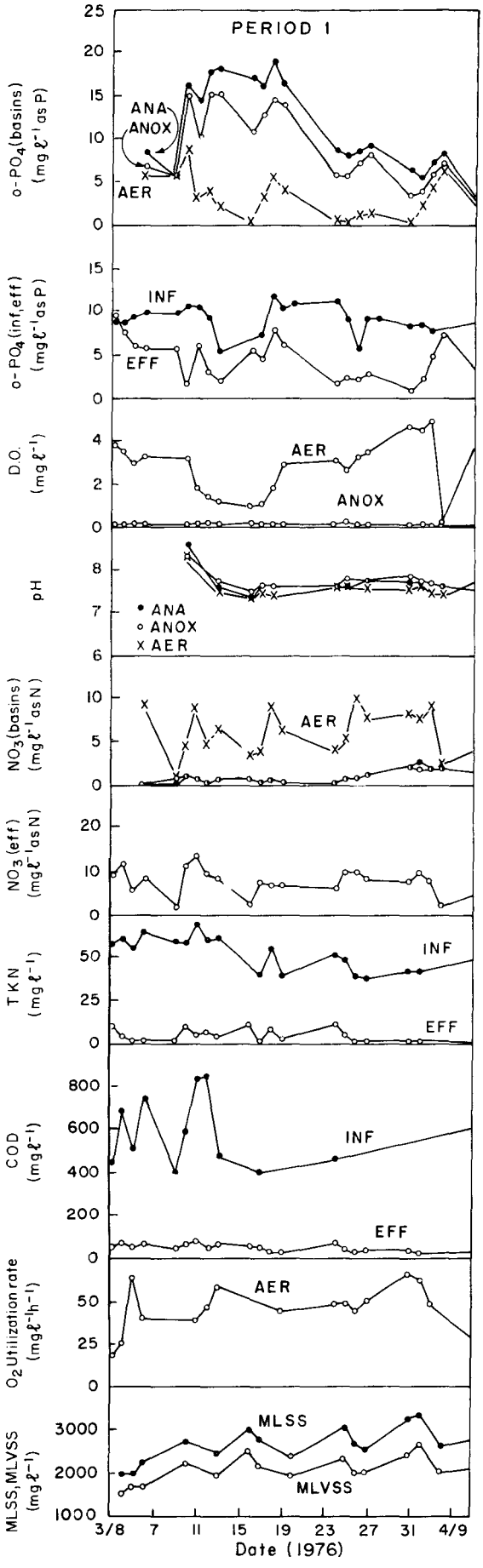


Figure 1
Removal of phosphate in unit A (anaerobic basin)

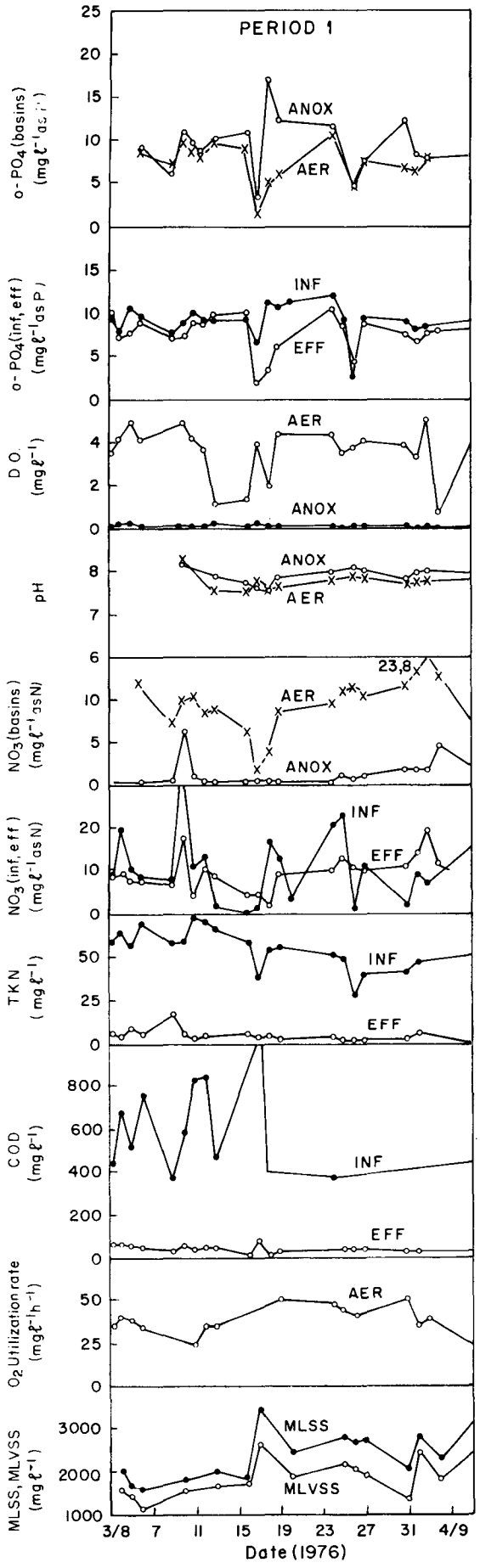


Figure 2
Performance of unit B (without anaerobic basin)

TABLE 6
CORRELATION COEFFICIENT FOR THE RELATIONSHIP BETWEEN PHOSPHATE RELEASE IN THE ANAEROBIC BASIN AND OVERALL PHOSPHATE REMOVAL

Period	1	2	3
Correlation coefficient	−0,35	0,13	0,28
Number of observations	24	26	22

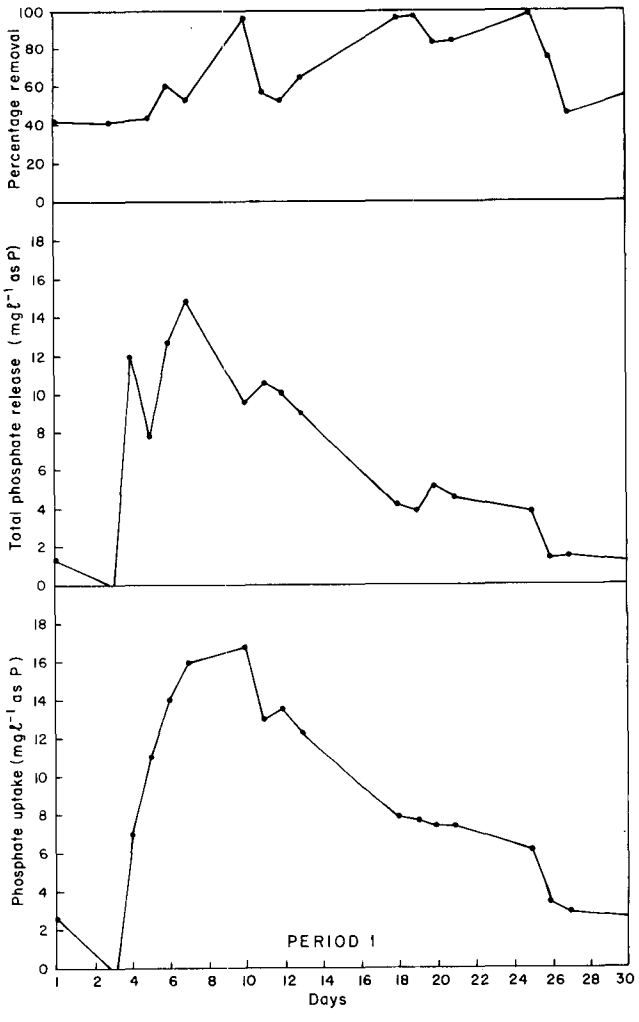


Figure 3
Time series presentation of phosphate release, uptake and total removal in unit A

The results revealed that the mode of operation, rather than the character of the sludge, was of critical importance in determining the extent of phosphate removal in the units studied. After maintaining sludge B^1 in a reactor not showing enhanced phosphate removal for a period of almost three months, phosphate removal by this sludge commenced almost immediately upon being subjected to anaerobic conditions (Table 2). If seen in conjunction with the results in Table 5, it appears reasonable to conclude that sludge B^1 had been potentially able to accomplish enhanced phosphate removal at all times, the operating conditions merely being the triggering mechanism. It is perhaps fortuitous that the necessary microflora were present in the inoculum used for this study, but the pilot plant from which the sludge was obtained was fully aerated and consequently exerted no trend towards the selection of a specialized biomass. Since the feed was pasteurized prior to feeding the units, the implication is that no major change in the composition of the microflora was necessary to accomplish phosphate accumulation.

Overall phosphate removal during period 1 was considerably lower than that during period 2. Furthermore, unit A was affected more severely during period 1 by phosphate release in the clarifier. This phenomenon is not fully understood yet but may be partially or wholly a result of dissolved oxygen concentrations in the aerator, which averaged between 1 and 2 mg l^{-1} during period 2, compared with about 3 mg l^{-1} during period 1.

Consistent phosphate release took place in the clarifier (Table 4). The actual retention time in the clarifier was 1,3 h, which was probably too long and caused local development of anaerobic conditions. Therefore some consideration should be given to sludge separation by methods such as flotation to avoid the development of anaerobic conditions, if the process of biological phosphate removal is to be optimized.

The coefficient of correlation between phosphate release in the anaerobic basin and overall phosphate removal is given in Table 6. The results do not indicate a definite relationship between these two parameters. A time series presentation of phosphate release, uptake and total removal is given in Figure 3.

Close inspection of daily results for the experimental periods revealed that relatively poor phosphate removal often coincided with a low influent COD. During the period of highest efficiency, the ratio Δ P/ Δ COD* surpassed 0,02, the value determined by Martin and Marais (1975). Such a dependence is to be expected if phosphate accumulation is a biological growth dependent phenomenon.

It was recognized that the results reported in this paper could yield valuable information regarding the permissible concentrations of nitrate entering the anaerobic zone, an aspect which is, together with the above, of the utmost importance in drafting design criteria for biological denitrification and phosphate removal processes. These would obviously be affected by factors such as strength of the influent to the plant, degradability of the organic matter concerned, concentration of such organic material in the anaerobic zone, (expressed as mg l^{-1} COD), the volatile suspended solids (VSS) concentration in this zone and the phosphate content of the sludge.

The inflowing concentrations of nitrate entering the anaerobic stage of unit A averaged less than 1 mg l^{-1} (as N) during periods 1 and 2. During the intermediate period when operational difficulties were experienced, the concentration averaged 1,9 mg l^{-1} and it was observed that phosphate removal efficiency was reduced. It appeared that the permissible nitrate concentration was related to the concentration of organic material, expressed as COD. In other laboratory tests during periods of excellent phosphate removal (80 per cent or higher), nitrate concentrations of up to 4,5 mg l^{-1} could be tolerated to enter the anaerobic phase in the presence of about 300 mg l^{-1} COD. In a pilot plant at Daspoort, at times of excellent phosphate removal, corresponding figures for nitrate of between 1,5 and 2,0 mg l^{-1}, and 160 mg l^{-1} COD have been observed in the anaerobic phase. During the successful laboratory runs referred to in this paper, nitrate concentrations of up to about 1 mg l^{-1} did not seem to interfere in the presence of COD values of about 140 mg l^{-1} in the anaerobic phase.

Extrapolating present findings to applications in practice, it would seem that the following requirements must be met in order to ensure enhanced phosphate uptake:

1. An anaerobic zone characterized by a maximum permissible nitrate inflow.

2. Phosphate-accumulating bacteria such as *Acinetobacter phosphadevorus* (NRRL/B-8058), claimed to be essential for enhanced phosphate removal or luxury phosphate uptake by Yall *et al.* (1975).

3. A suitable COD to phosphate ratio in the influent.

Conclusions

1. The inclusion of an anaerobic zone is essential for phosphate removals in excess of 80 per cent in a biological denitrifying sewage treatment plant (Table 4). Because phosphate removal is related to the influent COD in any plant, it is to be noted that with an anaerobic zone the $\Delta P/\Delta COD \cong 0{,}021$, while without this zone $\Delta P/\Delta$ COD is only 0,011 (Table 2). The latter P removal can be attributed to the normal P requirement to convert COD into sludge.

2. The permissible nitrate concentration in the inflow to the anaerobic zone is a function of the concentration of degradable organic material and amounts to about 1 mg l^{-1} (as N) at COD levels of about 140 mg l^{-1}. Should the permissible levels be exceeded, it would be necessary to create an opportunity for the excess to be consumed, either by way of an extra basin prior to the anaerobic stage or a plug flow arrangement.

3. Significant release of phosphate was achieved in the anaerobic zone of unit A when operated with either sludge A^1 or B^1, although there did not appear to be any relationship between the amount of phosphate released and the percentage overall removal. This indicates that the release of phosphate may be a sign of the establishment of suitably anaerobic conditions, rather than an essential intermediate in the process.

4. Bacteriological examination revealed that there was no essential difference in the numbers of phosphate-accumulating bacteria present in sludges A^1 and B^1. It is apparent that both sludges had the capacity to induce enhanced phosphate removal when suitable operating conditions were provided.

5. Anaerobic conditions in the clarifier must be avoided in order to avoid phosphate release.

*Reduction in phosphate over reduction in COD concentration

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