Influence of nitrates on biological phosphorus removal from wastewater

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Abstract

For biological phosphorus removal two conditions are necessary, firstly, an anaerobic phase which produces some acids with short carbonaceous chains and, secondly, a biomass which contains poly-phosphate microorganisms. This biomass is induced by fermentation products but it alone is not sufficient for biological phosphorus removal.

This study shows that the release of phosphate depends on two factors which can act separately or simultaneously: the concentration of the organic substrate and the nitrates. The permissible nitrates in the return sludge recycle depend on the influent COD.

Introduction

In recent years much attention has been focused on biological phosphorus removal and many researchers, among them Barnard (1976), Nicholls (1979), Rensink *et al.* (1981), and Siebritz *et al.* (1983) have proposed different process configurations to obtain enhanced phosphate removal in biological treatment plants. These evolved out of the need to develop processes for the biological removal of both phosphorus and nitrogen compounds from wastewater. To achieve this dual objective, Barnard (1976) introduced the Phoredox system with three non-aerated zones: an anaerobic compartment for phosphate release and two anoxic (a state in which nitrates are present but no oxygen) compartments for denitrification. All authors who used this process observed that when denitrification was not complete, a major factor adversely affecting phosphorus removal was an excessive amount of nitrates or dissolved oxygen in the anaerobic basin. The nitrates introduced in the anaerobic basin with the return sludge when denitrification is incomplete, prevents the formation of an appropriate anaerobic condition for phosphorus release necessary for subsequent phosphorus uptake in the aeration basin. For the successful and consistent removal of phosphorus, Barnard (1982) for example found that the nitrate concentration in the effluent should be well below 5 mg/ℓ as N.

The purpose of the present study is in observing the behaviour of orthophosphates under aerated or non-aerated conditions in the presence of nitrates during batch experiments, the effect of nitrate concentrations (40 mg/ℓ as N) in the influent of a laboratory unit prior to phosphate removal and the evolution of phosphorus assimilation after stopping nitrate addition. To do so, in addition to classical chemical analysis, Phosphorus Nuclear Magnetic Resonance was used to observe the changes in the different phosphorus compounds with time, without disturbing the living system.

Materials and methods

Continuous laboratory pilot plant

On the basis of the diagram proposed by Barnard (1976) a process for phosphorus removal was developed. This process (Figure 1) had just one compartment in which aerobic and anaerobic conditions were obtained by altering aerated periods and non-aerated periods sequentially in time according to the cycle presented in Figure 2.

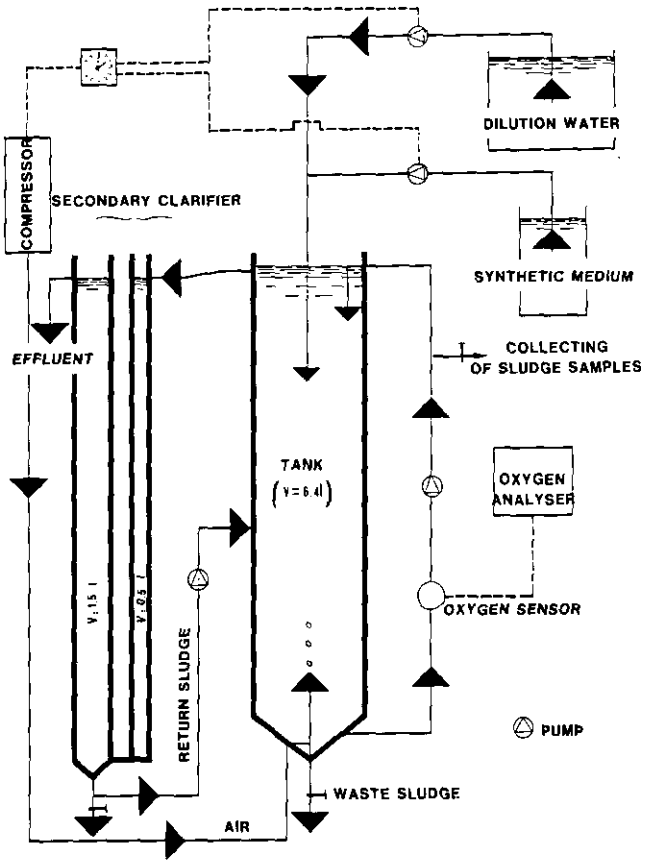

Figure 1
Schematic diagram of the laboratory pilot plant.

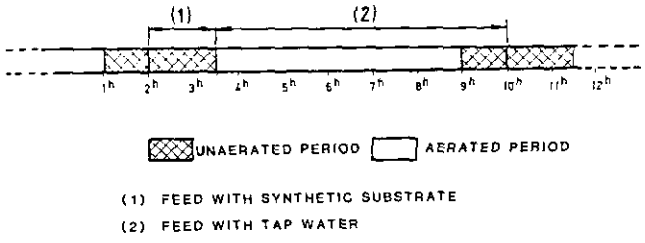

Figure 2
Feed, non-aeration and aeration period during a full cycle.

The cycles were calculated to conform to the mean retention time in a real system. The retention time of the unaerated/anoxic period was 2,5 h, chosen on the basis of experiments by Barnard (1976).

The unit was inoculated with activated sludge from Colombes, a wastewater treatment plant in the suburb of Paris and acclimatized to a synthetic feed substrate. The latter was obtained by diluting a meat extract in tap water, the characteristics of which are given in Table 1.

TABLE 1
CHARACTERISTICS OF THE SYNTHETIC SUBSTRATE USED AS FEED FOR CONTINUOUS LABORATORY PILOT PLANT AND FOR LABORATORY BATCH EXPERIMENTS

Water	19,5 %
Inorganic constituents	21,5 %
P_2O_5	7,0 %
K_2O	9,0 %
NaCl	3,5 %
not measured	2,0 %
Total nitrogen	9,0 %
Extracted matter other than nitrogen	16,5 %
Albumose, amino acids, peptone	19,0 %

When steady state was attained in the unit (unit A) (for process response see Table 2) a second unit (unit B) was started up, using the waste sludge from unit A. The two units were operating identically, in particular the aeration and feed cycles were identical in both systems. The only difference between the units was that nitrate was added to the synthetic wastewater feeding unit B. Nitrate was added to maintain the concentration in the unaerated phase at about 20 mg/ℓ (as N).

TABLE 2
PROCESS OPERATING PARAMETERS FOR CONTINUOUS UNITS

Total COD influent (mg/ℓ)	890 ± 109 (15)
Total COD effluent (mg/ℓ)	89 ± 11 (15)
BOD/COD ratio influent	0,7
TKN/COD ratio influent	0,07
PO4 influent (mg P/ℓ)	57 ± 10 (15)
PO4 effluent (mg P/ℓ)	15 ± 5 (15)
MLSS (g/ℓ)	4,8 ± 0,6
Total phosphorus in the sludge (mg P/g MLSS)	110
Sludge age (days)	10 – 13
Temperature (°C)	18

Results reported as mean ± SD (Standard deviation)
Sample size: n = 15

The following parameters were measured using the analytical methods of Standard Methods (1975): pH, mixed liquid suspended solids (MLSS), mixed liquid volatile suspended solids (MLVSS), chemical oxygen demand (COD), Kjeldahl nitrogen (TKN), nitrate nitrogen, orthophosphates and total phosphorus.

Laboratory batch experiments

Laboratory batch experiments were run to obtain experimental data that provide additional information on the effects of nitrates on the phosphorus metabolism. For these batch studies, sludge was abstracted from the continuous unit.

Experimental procedure

Six batch reactors of one liter capacity each, were filled with 300 ml of sludge and 100 ml of synthetic wastewater (the same wastewater as fed to the continuous units – meat extract). One of these received 100 ml of distilled water, the others received 100 ml distilled water containing sodium nitrate, to obtain final nitrate concentrations of 5, 10, 15, 25 and 50 mg/ℓ (as N).

Four sets of 6 batch tests were run, each set with different feed COD concentrations. In the four sets COD was added to give final concentrations in the batch reactors of 200, 250, 300 and 400 mg/ℓ. The soluble phosphorus in the 100 ml feed added was 80 mg P/ℓ, consequently, the soluble phosphorus concentration in the batch reactor feed was about 16 mg P/ℓ.

The mixed liquor was kept for 2,5 h in an unaerated state followed by 5,5 h of aeration. Samples were taken from the batch reactor at 0,5 h or hourly intervals and centrifuged at 3 000 g for 15 min. The supernatant liquid was collected for phosphate and nitrate analysis. The phosphate and nitrate results are shown plotted in Figures 3 and 4 respectively.

Nuclear magnetic resonance

In two additional batch tests, batch reactors were inoculated with 300 ml of sludge and 300 ml of synthetic substrate (the same wastewater as fed to the continuous units – meat extract). The only difference between these batch runs was that in the feed to the one reactor, 40 mg N/ℓ of nitrate (as N) was added. These batch reactors were kept in an unaerated state for 3 h followed by an aerated period of 7 h. Mixed liquor samples were taken from the batch reactors at the end of the unaerated and aerated periods. Nuclear magnetic resonance analyses of these samples were carried out according to the ^{31}P-NMR technique as described by Florentz *et al.* (1984). The results are shown in Figure 6.

Results and discussion

Figure 3 shows the effect of nitrate on phosphate release and uptake at various initial substrate concentrations. When the initial COD was low and nitrate concentration high, the phosphorus uptake occurred during the unaerated phase.

For the same substrate concentration, initial uptake of phosphate decreased when the initial nitrate concentration increased. The presence of nitrate effected the observed phosphate release. This might be due to the anaerobic state formed as a result of the substrate strength. The nitrate oxygen was masked by the organic substrate presence and was not available: the uptake took place when the major substrate was adsorbed, this portion of substrate might be called readily biodegradable substrate (Siebritz *et al.*, 1983). Another feasible explanation is that the rate of release was higher than the rate of uptake at the beginning of the experiment. After some time, the difference between these rates dropped to zero and then became negative.

Figure 4 illustrates nitrate removal during the anoxic period due to denitrification and the nitrate formation during the aeration phase, which was the result of the ammonia oxidation.

As illustrated in Figure 5, the rate of phosphate release observed is a function of the substrate concentration (the greater the substrate concentration, the later the phosphate uptake appears for a constant nitrate concentration). From figures 3 and 5 it follows that the perturbation caused by the nitrates during the anaerobic state on the phosphate release, is dependent on the substrate strength and nitrate concentration.

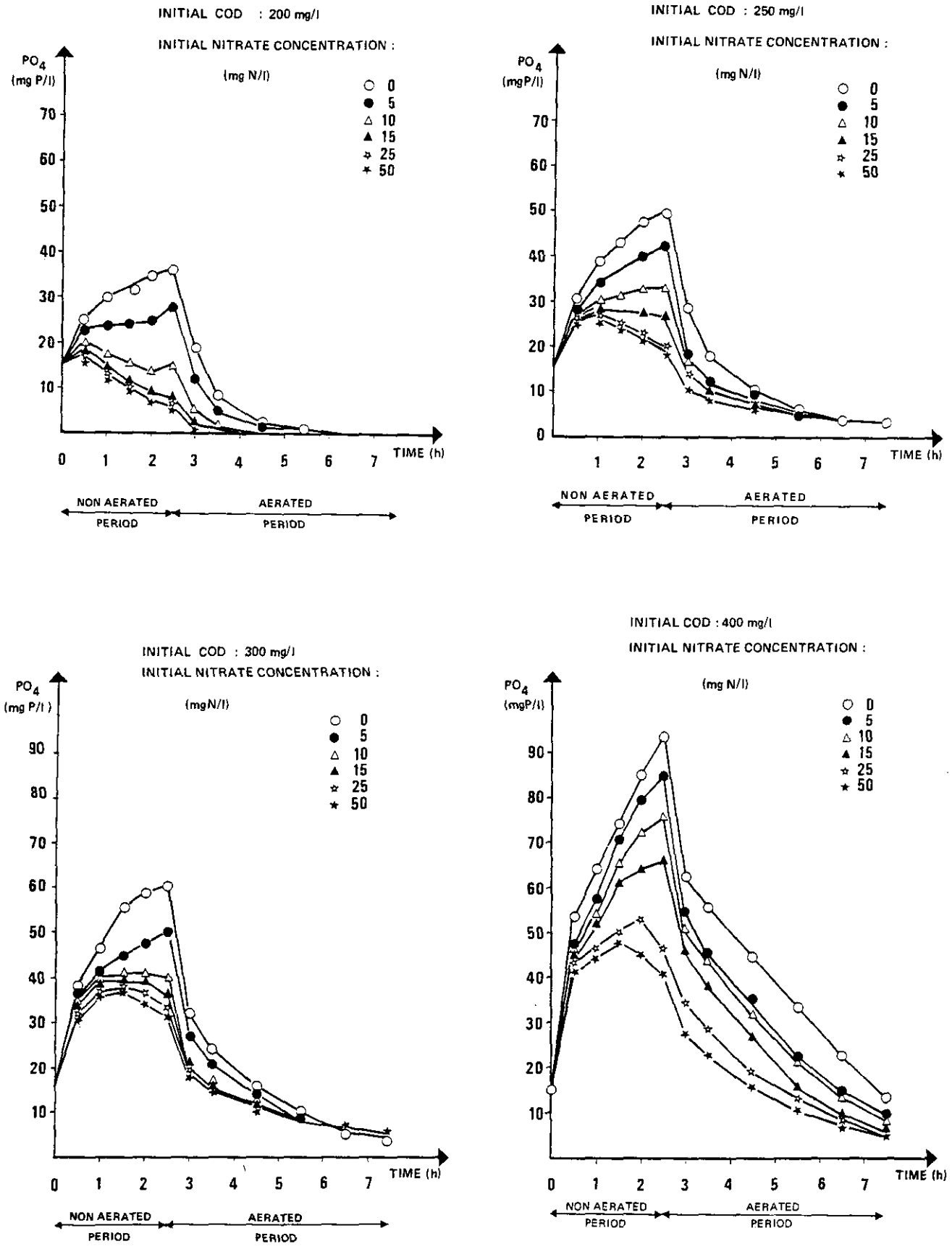

Figure 3
Effects of nitrates on phosphorus release and uptake at various initial substrate concentrations in sequentially unaerated (2,5 h) and aerated (5,5 h) batch tests.

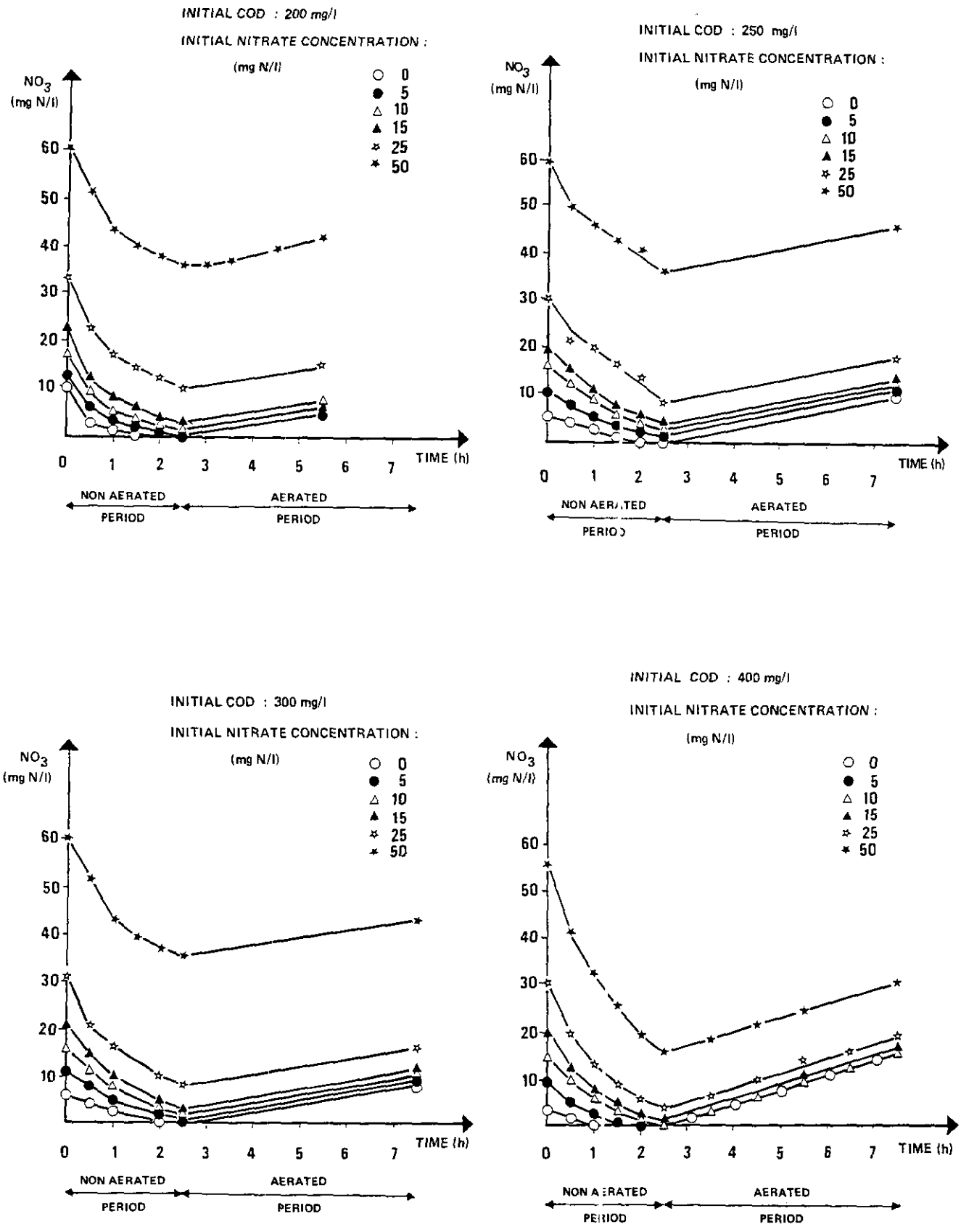

Figure 4
Denitrification and nitrification at various initial substrate concentrations.

These results are in contradiction with the theories of some other authors. Osborn and Nicholls (1978) showed that phosphate release begins only when nitrate is non-existent. McLaren and Wood (1976) observed a phosphate release when the nitrate concentration reaches 1 mg/ℓ as N, for nitrate concentration above 1 mg/ℓ, phosphate uptake occurs. This may be due to the low COD in the feed used by these authors, but unfortunately no indication of the COD concentration was given.

From the above results it is concluded that a major problem for the phosphate removal process is the nitrate concentration in the return activated sludge which affects the anaerobic state. It must be reduced to a low level, but as Davelaar *et al.* (1978) mentioned, the permissible nitrate concentration is related to the concentration of organic material expressed as COD. Consequently, recycled nitrates have a negligible effect if the influent COD is sufficiently high.

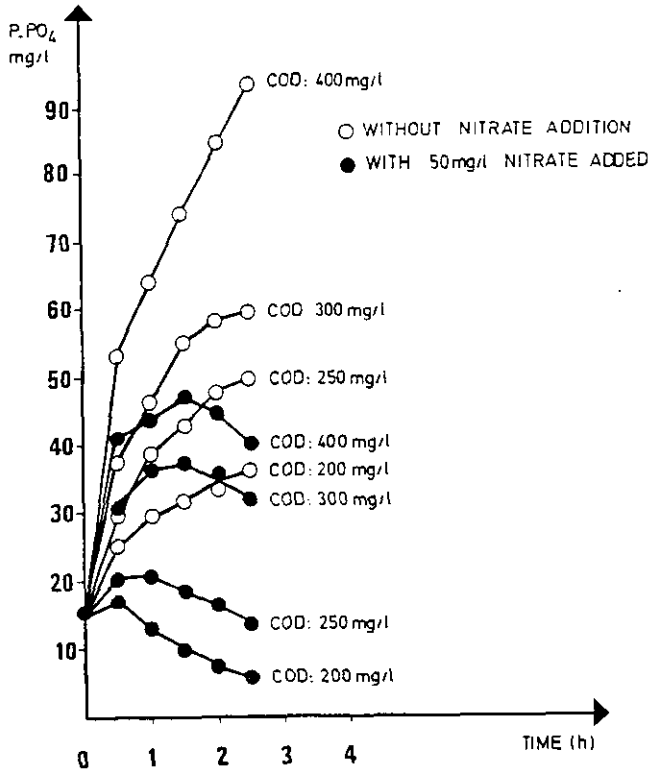

Figure 5
Effects of nitrates (50 mg N/ℓ) during an unaerated period at various initial substrate concentrations.

When the feed COD is low, the microorganisms in the sludge use nitrates during the anoxic period in order to maintain the normal functioning of their cell metabolism. This is illustrated by the ^{31}P-NMR experiments (figure 6). When the NMR spectra for reaction with or without nitrate in feed are compared after a 3 h non-aerated period, it is clear that the anaerobic state is disturbed by the polyphosphate presence. Figure 6 shows the biological character of phosphate removal.

A very significant nitrate concentration in the initial stage did not permit the anaerobic state to establish and prevented phosphorus release. This observation is confirmed by the data from the continuous units (Figure 7a). The curve represents the amount of phosphate release and uptake during a cycle. It is obtained from the difference between the estimated concentration in the reactor arising from the phosphate addition in the feed and

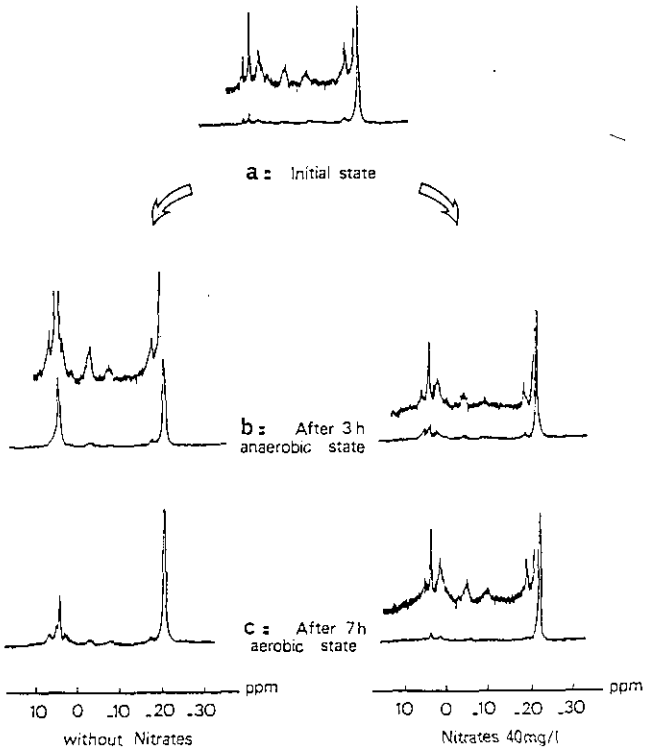

Figure 6
Evolution of ^{31}P-NMR spectra of activated sludge during an anoxic phase in the presence of nitrates (162 MHz).

the measured concentration at different times of a given cycle. A positive difference means an uptake and a negative difference a release.

For one cycle the estimated concentration can be calculated as follows:

- When the reactor feed was tap water (period 2 – figure 2), the phenomenon was a series of dilution and

$$P\,(t + \Delta t) = \frac{Pt \times Vr}{Vr \times VEau}$$

Pt: phosphorus concentration at t time
Vr: Reactor volume
VEau: Water volume received during one period

- When the reactor was fed with synthetic substrate (period 1 – figure 2), the phosphorus concentration was

$$P\,(t + \Delta t) = \frac{(Pt \times Vr) + (Pf \times Vf)}{Vr + Vf}$$

Pf: phosphate concentration in the feed
Vf: feed volume received during one period

Phosphate release was not observed for unit B and the uptake occurred during the unaerated period.

Table 3 gives the average of the different parameters measured for two months following the steady state in the two continuous units. Unit A removed a greater amount of phosphate than unit B. The latter, which does not have an anaerobic period, removed 70% of the phosphate. This might be due to the fermentation products of the feed. Indeed, during storage of the

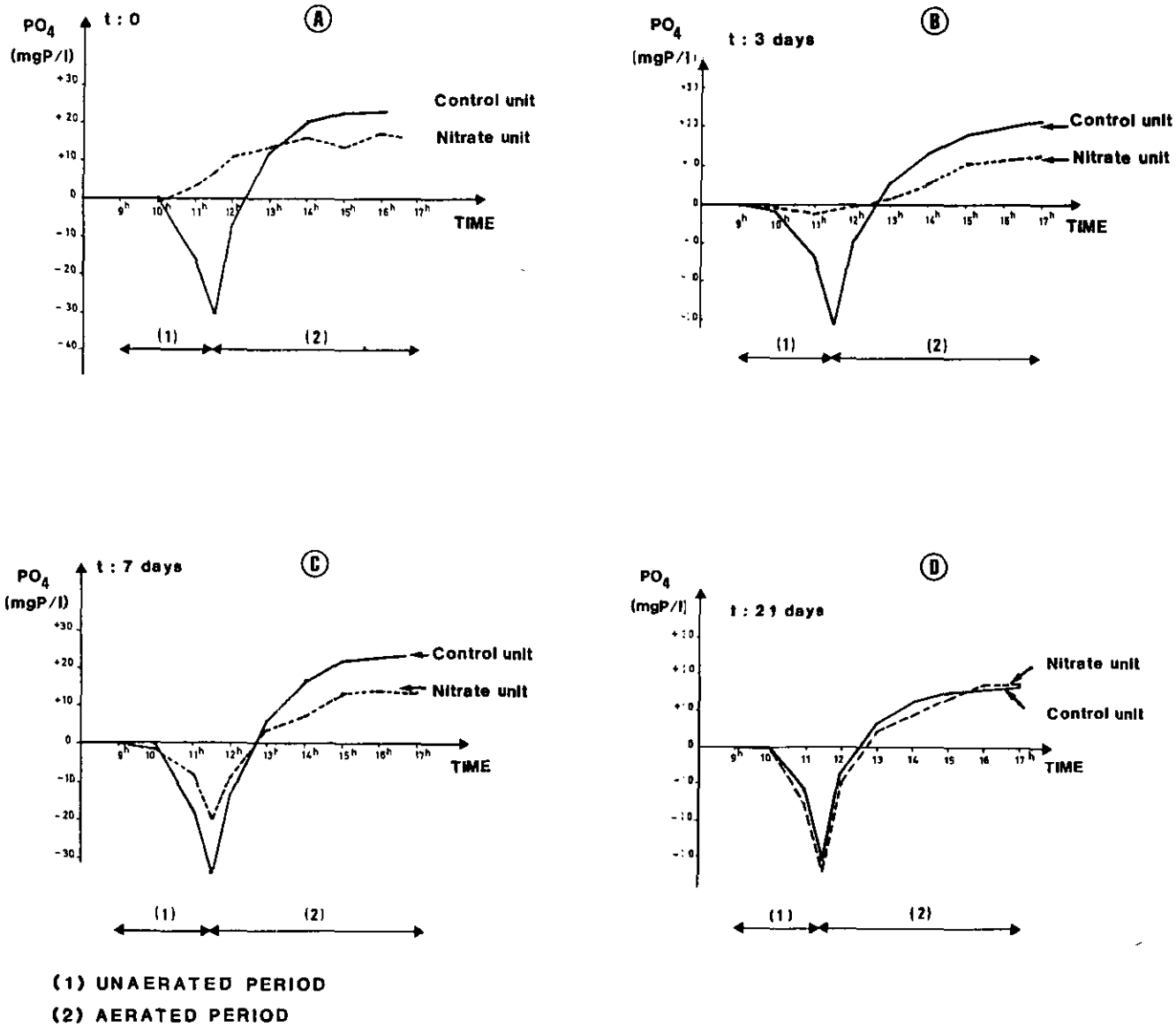

Figure 7
Phosphorus release and uptake during a transition period after stopping nitrate addition in unit B's feed.
Figure 7a Initial state
Figure 7b After 3 days
Figure 7c After 7 days
Figure 7d After 20 days

synthetic wastewater some volatile acids were formed, acetate for example, which could induce the development of Acinetobacter. The role of Acinetobacter in phosphate removal is well-known (Buchan, 1981; Deinema *et al.*, 1980) Table 4 gives the results of a gas chromatographic analysis of the substrate.

For more information and a better understanding of the

TABLE 3
OPERATIONAL CHARACTERISTICS OF UNITS AFTER FOUR MONTHS OPERATION

Parameters	Unit A Unit without nitrates	Unit B Unit with nitrates
Flow (ℓ/day)	8,2 ± 1,1	8,4 ± 0,8
Total COD influent (mg/ℓ)	897 ± 119	879 ± 138
Total COD effluent (mg/ℓ)	90 ± 16	80 ± 20
% total COD removal	90	91
PO4 influent (mg P/ℓ)	65 ± 11	62 ± 10
PO4 effluent (mg P/ℓ)	8 ± 3	19 ± 6
PO4 removal %	88	70
NO3 influent (mg N/ℓ)	0	37,2 ± 5,2
NO3 effluent (mg N/ℓ)	7,8 ± 1,1	44,5 ± 6,4
MLSS (g/ℓ)	5,0 ± 0,9	5,7 ± 0,8
% mineral MLSS	34,5	38,6
% volatile MLSS	65,5	61,4
Load (kg COD/kg MLSS/day)	0,23 ± 0,05	0,20 ± 0,04
Hydraulic retention time (d)	0,78	0,76
Ratio P/COD (mg P/mg COD)	0,070 ± 0,004	0,070 ± 0,004
Ratio ΔP/ΔCOD (mg P/mg COD)	0,069 ± 0,006	0,054 ± 0,006
pH	8,06	7,95
Sludge age (days)	10 – 15	
Recycle/feed ratio	1 : 1	
Temperature (°C)	20 – 22	

Results reported as mean + SD (Standard Deviation)
Sample size: n = 16

TABLE 4
VOLATILE ACIDS OBTAINED BY GAS CHROMATOGRAPHY OF THE SUBSTRATE (AFTER 24 h AT 22 °C)
SUBSTRATE COD = 1 g/ℓ

Nature	Concentration (mg/ℓ)
Acetic acid	164,0
Propionic acid	11,5
Butyric acid	10
Methyl 4 – valeric acid	9,7
Heptanoïc acid	5,2
Hexanoïc acid	14,0

high ability to remove phosphate by unit B, the nitrate addition in the feed was stopped. The different curves in Figure 7b and 7d show that the phosphate uptake drops progressively in the anaerobic period to zero level and that the phosphate release then increases. After twenty days the performance of the two units is similar as indicated in Figure 7d.

Finally, it appears that the character of the sludge is important because after stopping the nitrate addition, the phosphate release did not cease immediately. Sludge B required seven days to release phosphate. This time is necessary to select microbial populations or to modify the enzymatic activities of the bacteria (Florentz and Hartemann, 1982). These observations are in accordance with the results obtained by Osborn and Nicholls (1978), who concluded that phosphorus removal is directly associated with change in the biomass induced by the anaerobic period. If it is not the case, the release should have occurred immediately after stopping the nitrates in the feed.

Conclusions

When sludge is acclimatized to phosphate removal, the introduction of nitrates during the anaerobic period changes its behaviour. This study illustrates the following points:

- Not only the concentration of nitrates being introduced in the anaerobic zone is important, but the substrate concentration expressed as COD must be taken into account. It is important to note that nitrates, even in high concentrations during the aerobic stage do not prevent good phosphorus uptake by microorganisms, although they can prevent the formation of an anaerobic phase required for the luxury uptake of phosphorus.
- The utilization of the ^{31}P-NMR shows that the polyphosphates are not used during the non-aerated period because the ATP pool can be maintained by the glycolitic metabolism.
- The continuous addition of nitrates during the non-aerated phase, modified either the microbial population or the enzymatic activities, because the release of phosphate is not immediate after stopping the nitrate addition to the feed, but takes seven days to occur. This time is necessary to acclimatize the biomass to the alternating anaerobic-aerobic phases.

References

BARNARD, J.L. (1976) A review of biological phosphorus removal in the activated sludge process. *Water SA* 2(3) 136-144.

BARNARD, J.L. (1982) The influence of nitrogen on phosphorus removal in activated sludge plants. *Water Sci. Tech.* 14 1/2 31-45.

BUCHAN, L. (1981) The location and nature of accumulated phosphorus in seven sludges from activated sludge plants which exhibited enhanced phosphorus removal. *Water SA* 7(1) 1-7.

DAVELAAR, D., DAVIES, T.R. and WIECHERS, S.G. (1978) The significance of an anaerobic zone for the biological removal of phosphate from wastewaters. *Water SA* 4(1) 54-60.

DEINEMA, M.H., HABETS, L.H.A., SCHOLTEN J., TURKSTRA, E. and WEBERS M.A.A.M. (1980) The accumulation of polyphosphate in *Acinetobacter* spp. *Microb. Letters* 9 275-279.

FLORENTZ, M. and HARTEMANN, P. (1982) Enzymatic study of activated sludge in aerobic-anaerobic run. *Environ. Tech. Letters* 3 345-350.

FLORENTZ, M., GRANGER, P. and HARTEMANN, P. (1984) Use of ^{31}P-NMR spectroscopy and electron microscopy in the study of phosphorus metabolism of micro-organisms of wastewater. *Applied Environ. Microbiol.* 47(3) 341-348.

Mc LAREN, A.R. and WOOD, R.J. (1976) Effective phosphorus removal from sewage by biological means. *Water SA* 2(1) 47-50.

NICHOLLS, H.A. (1979) Kinetics of phosphorus transformations in aerobic and anaerobic environments. *Progress Wat. Tech.* 10 Suppl. 1 89-102.

OSBORN, D.W. and NICHOLLS, H.A. (1978) Optimisation of the ac-

tivated sludge process for the biological removal of phosphorus. *Prog. Wat. Tech.* **10** 1/2 261-277.

RENSINK, J.H., DONKER, H.J.G.H. and DE VRIES, H.P. (1981) Biological phosphorus removal in domestic wastewater by the activated sludge process. 5th European Sewage and Refuse Symp. EAS. Munich - 487-502.

SIEBRITZ, I., EKAMA, G.A. and MARAIS, G.v.R. (1983) A parametric model for biologic il excess phosphorus removal. *Water Sci. Tech.* **15** 3/4. 127-152.

STANDARD METHODS for the examination of Water and Wastewater (1975) 14th Edi ion, American Public Health Association, Washington D.C.