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INTERACTIONS BETWEEN PHOSPHATE, NITRATE AND ORGANIC SUBSTRATE IN BIOLOGICAL NUTRIENT REMOVAL PROCESSES

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ABSTRACT

The response of activated sludge following exposure to various organic compounds is decribed. Batch systems simulating the various stages of biological nutrient removal processes were used to study the dependence of phosphate release, enhanced phosphate uptake and denitrification on the nature and level of organic substrate, and the presence or absence of nitrate. The phenomenon of phosphate release is shown to be controlled primarily by the nature of the substrate rather than the creation of an anaerobic state. Certain short-chain fatty acids or their salts, such as acetate and propionate, induce phosphate release even under anoxic or aerobic conditions but with compounds such as ethanol and glucose release occurs only after the onset of anaerobiosis. Given the necessary conditions, the time course of phosphate concentration in initially anoxic mixtures of phosphate-rich sludge and short-chain fatty acids is shown to proceed in three consecutive stages, comprising primary release, anoxic uptake and secondary release respectively. It is concluded that phosphate uptake and release occur simultaneously in the presence of fatty acids, which also render the best overall phosphate removal during aeration.

KEYWORDS

Activated sludge; anaerobiosis; biological phosphate removal; nitrate; organic substrate; phosphate release.

INTRODUCTION

The presence of readily biodegradable compounds is widely acknowledged as being favourable to the achievement of enhanced biological phosphate removal in modified activated sludge plants. Various devices have been employed in full-scale applications to ensure a supply of desirable compounds which would impart extended phosphate accumulation capability to the biomass. Among these are the intermittent operation of aerators near the inlet end of an extended aeration plant to promote volatile fatty acid production in sludge settled to the floor (Venter *et al.*, 1978) and the addition of *whole* acid fermented primary sludge to the anaerobic zone (Osborn and Nicholls, 1978). More recent developments concern the external generation of soluble carbon compounds by leaching these from either acid digested sludge or sludge fermented in primary sedimentation tanks or thickeners (Pitman *et al.*, 1983; Barnard, 1984; Oldham, 1985), followed by their introduction to the process via the supernatant or settled liquor.

The pronounced improvement in biological phosphate removal, associated with the introduction of liquors containing a variety of desirable but undefined biodegradable organic compounds to modified activated sludge plants, has left an increased need of investigating the function of specific substances more closely. While the literature contains frequent references to the

response of selected organisms with respect to phosphate removal when exposed to well-defined organic substances, such observations are usually imbedded in studies aimed at other objectives. Most typically the published results concern *pure* cultures grown in batch or continuous culture on well-defined media containing the substrate of interest, such as those of Deinema *et al.* (1985). The combined response of *heterogeneous* populations usually has been investigated in relation to biomass acclimated to synthetic wastewater, such as in the studies by Fukase *et al.* (1982). Limited studies concerning the response of *sewage-grown* biomass to selected organic substrates have been reported by Rensink (1981), Potgieter and Evans (1983), Arvin and Kristensen (1985), Malnou *et al.* (1984), Meganck *et al.* (1985) and Wentzel *et al.* (1985).

This paper is concerned with the response of activated sludge when exposed under batch conditions to selected organic substances in an environment approximating the sequence of events commonly occurring in practice, namely the anoxic/anaerobic period following admixture of well-nitrified mixed liquor and influent wastewater, and the subsequent aerobic phase. The study described was aimed at determining collective response characteristics of phosphate accumulating sludges and to assess the capability of selected organic substances to induce improved uptake capacity. The rationale for this approach is, amongst others, based on the premise that if enhanced phosphate uptake can be ascribed to the presence or formation of a particular organic compound in the process, then biomass with a track record of excess phosphate accumulation at full-scale should respond favourably upon exposure to that substance during shortterm tests in a controlled system.

MATERIALS AND METHODS

Batch Tests

The response of a given biomass with respect to phosphate uptake and release, nitrification and denitrification was determined in batch tests conducted in two phases. Preparation of the sludge consisted of collecting approximately 20 ℓ of mixed liquor from the aerobic stage of the particular plant being studied, just before its entering the clarifier, and adding a sodium nitrate solution to an effective concentration of 20 mg/ℓ (as N) to prevent phosphate release during transportation. Filtered samples taken at the time of collection and after arrival were analysed to verify that phosphate had not passed to the liquid phase. The mixed liquor was subsequently aerated overnight (total period approximately 15 h) to ensure complete utilization of available substrate, following which the sludge was separated from the supernatant by centrifugation at 5 000 g for 3 min. The pellet was collected by washing with small aliquots of a tap water medium to which had been added sodium nitrate, orthophosphoric acid, ammonium chloride and sodium bicarbonate to target concentrations of 10 mg/ℓ each for nitrate (as N), phosphate (as P) and ammonia (as N), and a total alkalinity of 200 mg/ℓ (as $CaCO_3$). In cases which called for variable nitrate concentrations, sodium nitrate was added separately. Following screening through a sieve with 1.5 mm square openings to remove coarse inorganic particles the concentrated sludge mass was suspended in 18 ℓ of the tap water medium, which was then flushed for about 15 minutes with nitrogen gas introduced through porous glass diffusers. Test volumes consisting of 1.5 ℓ aliquots of this suspension were then dispensed and introduced into stoppered, magnetically stirred reactors. These had a total capacity of 2 ℓ and were equipped with a sampling port and facilities for maintaining a continuously renewed nitrogen blanket above the liquid medium. The desired quantities of the organic substances being studied were dispensed from stock solutions, then adjusted to a standard volume of 75 mℓ using distilled water, and introduced to the reactor at time zero. The anoxic/anaerobic phase was maintained for 22 to 24 h, subsequent to which air was introduced for an additional 25 to 30 h . During both phases the concentration of ammonia, nitrate, orthophosphate and the organic substances involved were determined at discrete time intervals, using filtered samples.

Organic Compounds

The substances selected for comparison were glucose, typical bacterial end products resulting from glucose fermentation (acetate, propionate, butyrate, lactate, formate, ethanol and 2,3 butanediol), methanol and two intermediates of the tricarboxylic acid cycle (citrate and succinate) which may in turn be transformed to acetate and other end products. Where free organic acids or their salts were used in the preparation of stock solutions these were neutralized before use to pH values in the range 6.5 to 7.5. A fixed quantity of each compound, equivalent to a theoretical COD of 200 mg/ℓ, was added to each batch reactor except when the substrate concentration itself was the primary variable.

Analytical Methods

Orthophosphate, nitrate/nitrite and ammonia were determined colorimetrically using an Auto-Analyzer. Glucose was analysed according to the method described by Dubois *et al.* (1956). Following filtration through a Millex-HV 0.45 µm filter the remaining compounds were determined using a Water's Associates High Performance Liquid Chromatograph equipped with, amongst others, refraction index and variable wavelength detectors. With 0.02 M aqueous sulphuric acid as mobile phase, acetate, lactate, formate, ethanol, methanol and 2,3 butanediol were determined using an Aminex HPX-87H column and a refraction index detector. Butyrate, propionate, succinate and citrate were separated on a Whatman Partisil 5 ODS-3 RAC column and determined at 214 nm. These columns were used in conjunction with an Aminex HPX-85H pre-column and a Whatman guard column containing pellicular ODS respectively.

RESULTS AND DISCUSSION

Influence of Substrate on Phosphate Release

Admixture of biomass and substrate in a batch system as described above simulates conditions at the head of the anaerobic stage in processes such as the modified Bardenpho®. At that point a mixture of settled sludge and nitrate present in the underflow is brought into contact with influent substrate.

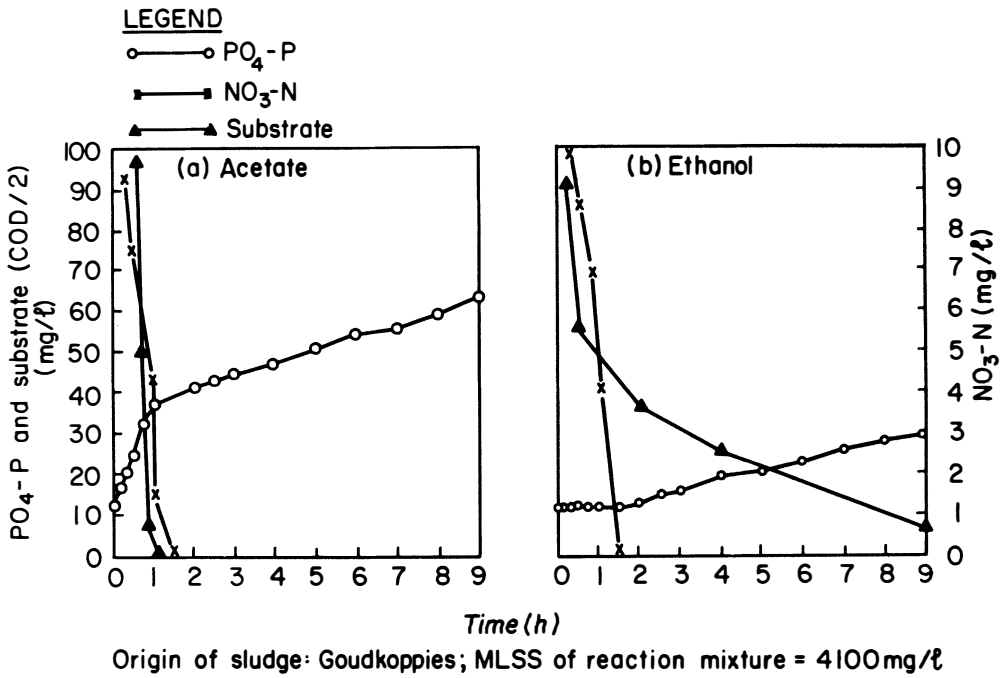

Fig. 1. Dependence of phosphate release under anoxic/anaerobic conditions on the nature of the organic substrate.

The response patterns observed during the period immediately after admixture of substrate and biomass with a prior history of excess phosphate removal allow the substances studied to be divided into three classes:

- The first consists of the lower fatty acids (formic, acetic and propionic), or their salts, each of which proved capable of inducing phosphate release from sludge under both anaerobic and anoxic conditions. Figure 1(a) illustrates the time course of release typically observed for these compounds during the anoxic phase, which started with an initial nitrate concentration of 10 mg/ℓ . Practically identical response curves were

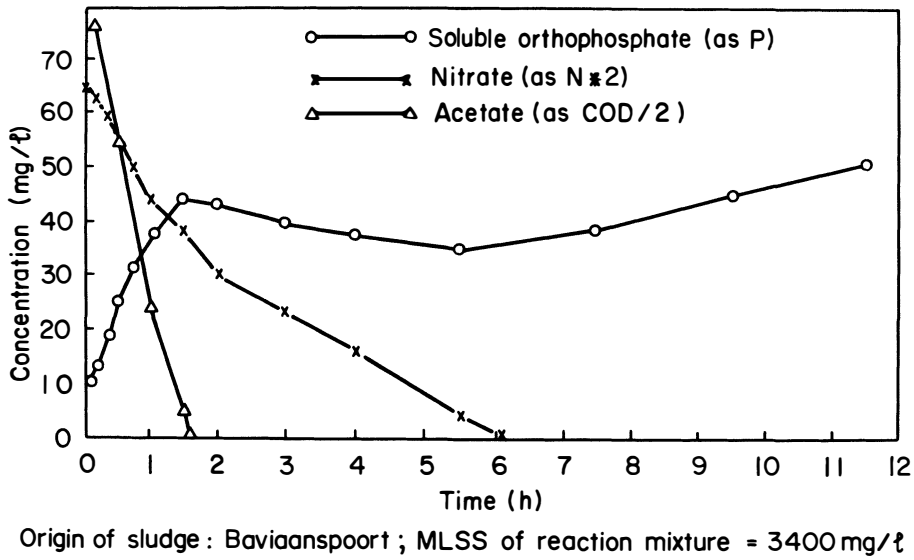

Fig. 2. Illustration of sequential periods exhibiting net phosphate release and uptake under anoxic conditions, followed by secondary release under anaerobiosis.

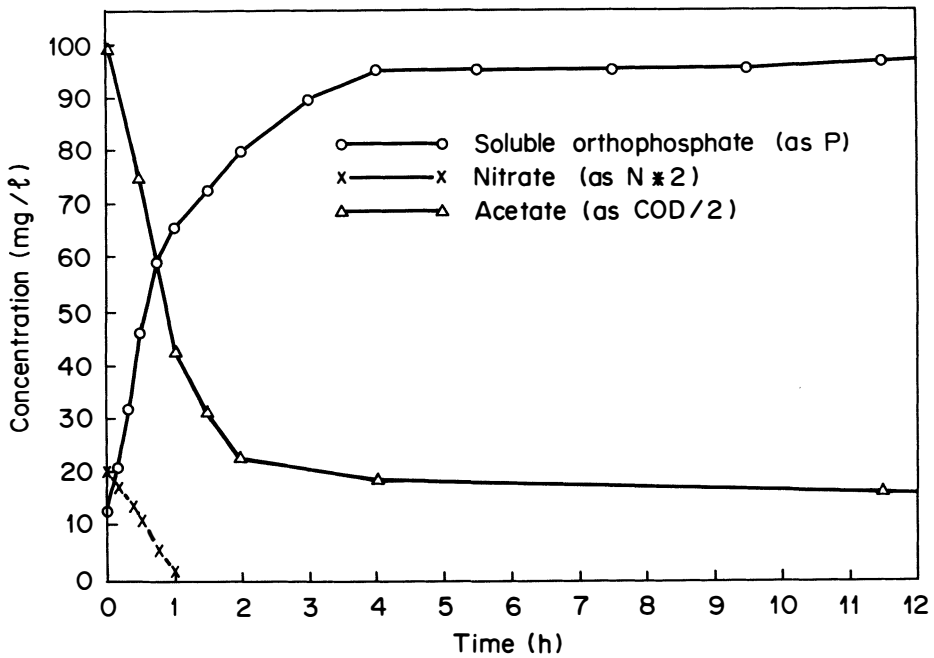

Fig. 3. Illustration of phosphate release when nitrate is consumed before complete utilization of substrate.

observed under aerobic conditions, leading to the conclusion that these compounds will consistently trigger release from phosphate-laden sludge even under conditions of

complete oxygen saturation in the bulk mixed liquor. Phosphate release commenced immediately, at a relatively high rate which remained practically linear with time up to the point where either all substrate was utilized, or the biomass was depleted of the phosphorus-containing reserves normally participating in the reaction.

- The second group comprises those compounds which, in this study, invariably failed to induce phosphate release unless strict anaerobiosis prevailed. The release pattern shown for ethanol in Figure 1(b) is typical and of the compounds investigated; this property is shared by citrate, ethanol, methanol, butanediol and glucose for all the phosphate removing plants included in the study. Substrate was removed fairly rapidly during the anoxic phase but phosphate release commenced only once nitrate had reached negligible concentrations and then at rates which were markedly slower than those observed during the early time period with compounds of group one. The results suggest that transformation of compounds in group two has to occur before phosphate release is triggered. Anaerobiosis provides a suitable environment for such reactions to take place.

- The third group consists of substances which respond similarly to those in either group 1 or group 2. They proved capable of inducing phosphate release under anoxic conditions in sludge from some plants but not in others. Of the substances studied this property was shared by butyrate, lactate and succinate. Phosphate release, once started, occurred at rates comparable to those associated with compounds of group one.

The results lead to the conclusion that the phosphate release phenomenon is primarily dependent on the nature of the feed rather than the anaerobic state as such. This statement is in agreement with observations reported by Malnou *et al.* (1984) and Hascoet and Florentz (1985). Furthermore, the results suggest that the time course of phosphate concentration in the presence of nitrate and substrate is a multivariate function, the shape of which depends, amongst others, on the nature of the substrate and the relative amounts of substrate and nitrate in the mixture. If the fact that phosphate uptake can occur under anoxic conditions (Gerber and Winter, 1985) is considered, it follows that the time course of phosphate concentration at the head of the anaerobic stage may take one of several forms. With substrates of group one, such as acetate and propionate, phosphate release and uptake can occur *simultaneously*. Under these conditions the observed shape of the phosphate concentration versus time curve is the result of these two reactions and is, amongst others, dependent on the amount and nature of the substrate as well as the relative quantities of substrate and nitrate. The response curves shown in Figure 2 represent results typically observed with compounds capable of releasing phosphate under initially anoxic (or aerobic) conditions. The early time period is dominated by rapid net phosphate release, which may gradually taper off to a period of net uptake, provided nitrate is still present and provided either substrate or phosphate reserves participating in the release reaction have reached zero levels. If complete denitrification is achieved subsequently, phosphate release once again manifests itself during the anaerobic period that follows provided, of course, that the biomass still contains phosphorus compounds susceptible to release. The response patterns in Figure 3, on the other hand, represent the situation when denitrification is complete before all substrate is utilized, and while the sludge still has the capacity to release phosphate. In this case *net* uptake does not occur during the anoxic/anaerobic phase.

For substrates other than those in group one, the onset of phosphate release following contact between sludge and fresh substrate is critically dependent on denitrification rate, seeing that those compounds are incapable of triggering release under anoxic conditions. The relative denitrification rates associated with the individual compounds varied considerably across different plants but substances such as acetate, propionate, butyrate and lactate consistently produced results near the upper end of the range, as illustrated in Figure 4 for particular cases. Substrates such as glucose, methanol and citrate, on the other hand, usually yielded values near the bottom end of the range. Low denitrification rates near 0.5 mg N/(g MLSS.h) imply that, under an initial nitrate level of 10 mg/ℓ (as N) and an MLSS concentration of 4 000 mg/ℓ, delays of about 5 h may occur before phosphate release is observed. Given sufficient time, however, phosphate was found to be released to similar eventual levels, irrespective of the nature of the substrate.

Phosphate Uptake and Nature of the Substrate

Each of the organic compounds studied was associated with phosphate uptake during the aerobic period following anoxis/anaerobiosis, as shown in Figure 5. A practically linear

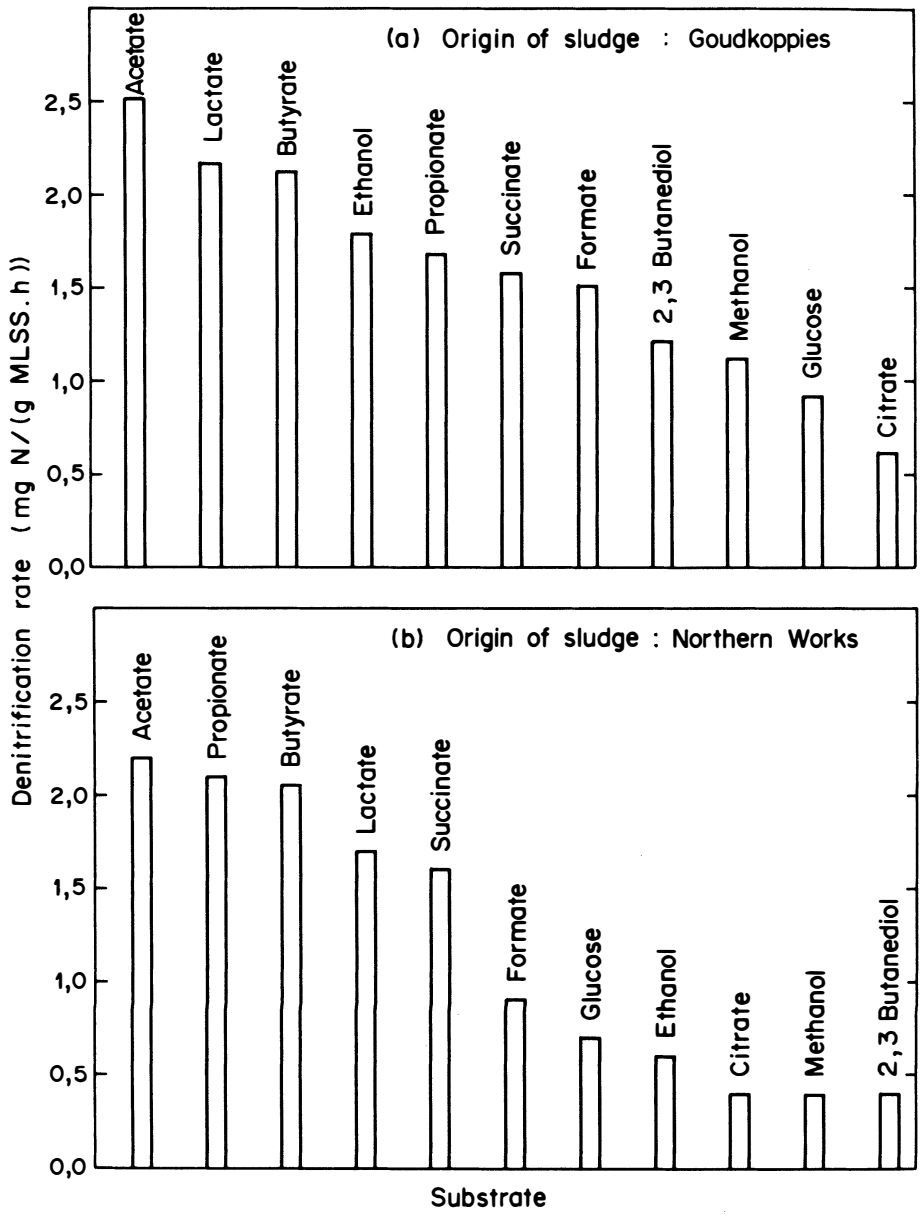

Fig. 4. Dependence of denitrification rate on nature of the substrate.

relationship between the overall mass of phosphate respectively released and taken up per unit volume of reaction mixture was found to hold for the various substrates except formate. This compound proved particularly successful in stimulating phosphate release but led to relatively large phosphate residuals after aeration, even though substantial amounts were indeed removed.

The results in Figure 5 show that *net* phosphate uptake in the batch systems described was difficult to achieve. Furthermore, net removal was uncorrelated with prior release levels. Of the compounds investigated, only acetate, propionate, butyrate and lactate proved capable of reducing the released mass of phosphate back to original levels, but even in these cases

disappointingly little of the amount present initially was removed. It appears that there is some critical factor associated with batch systems as operated here which renders the sludge incapable of reducing phosphate to the near-zero concentrations obtained in continuous-flow configurations.

Phosphate release and uptake under variable nitrate concentrations

Figure 6 illustrates phosphate release and uptake patterns observed when dosing acetate to phosphate-laden sludge initially containing nitrate varying between 0 and 93 mg/ℓ (as N). The corresponding decline in nitrate concentrations during the anoxic/anaerobic period is illustrated in Figure 7. Practically identical response curves have been observed for propionate and the other compounds (such as butyrate) which respond similarly to group 1 substances in particular cases.

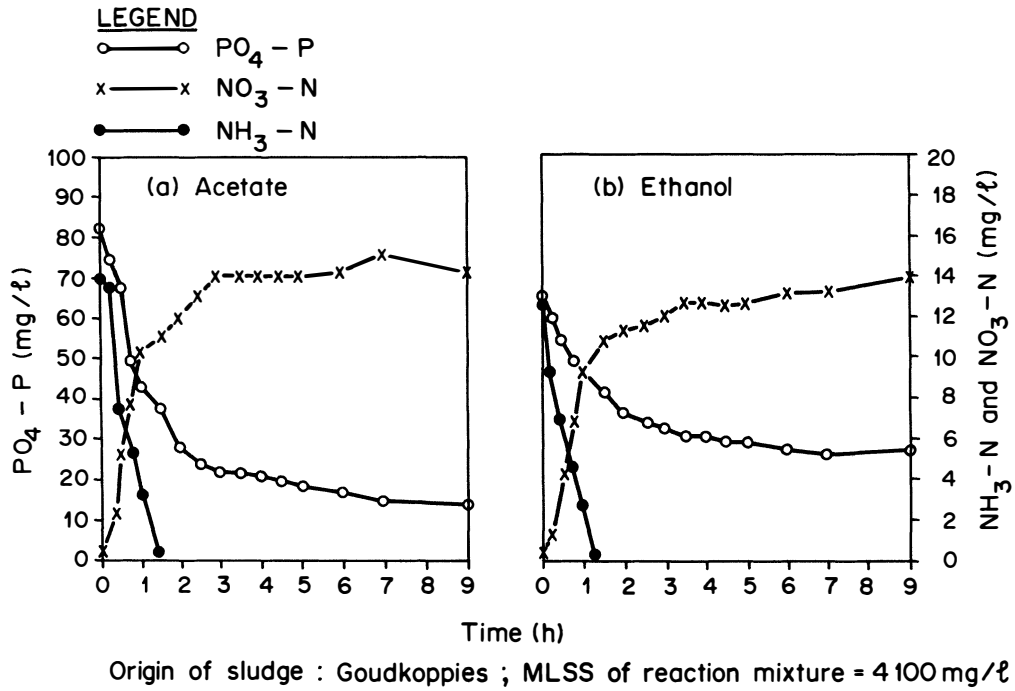

Fig. 5. Dependence of aerobic phosphate uptake on the nature of the organic substrate introduced during anoxis/anaerobiosis.

The results, which are similar for all plants examined, clearly illustrate the inability of nitrate to prevent phosphate release in the presence of certain substrates. The amount of phosphate released is shown to decrease with increasing nitrate levels but as suggested above, this is considered due to phosphate uptake occurring simultaneously with release, rather than to *suppression* of release. The collection of response curves is governed by a lower envelope which represents, at a given moment, the maximum amount by which net phosphate release can be diminished as a result of nitrate in the medium. That segment of the response pattern associated with secondary release departs from this limiting curve at time instants corresponding to the achievement of zero nitrate levels in the liquid medium (refer Fig. 7). The succession of initial release, uptake during the remaining anoxic period and secondary release during the subsequent anaerobic phase can be distinguished clearly for initial nitrate levels above 21 mg/ℓ . At lower initial concentrations nitrate was completely consumed within the phase of rapid phosphate release and hence the secondary release phenomenon did not occur. The ultimate phosphate concentrations after 24 h of anoxis/anaerobiosis

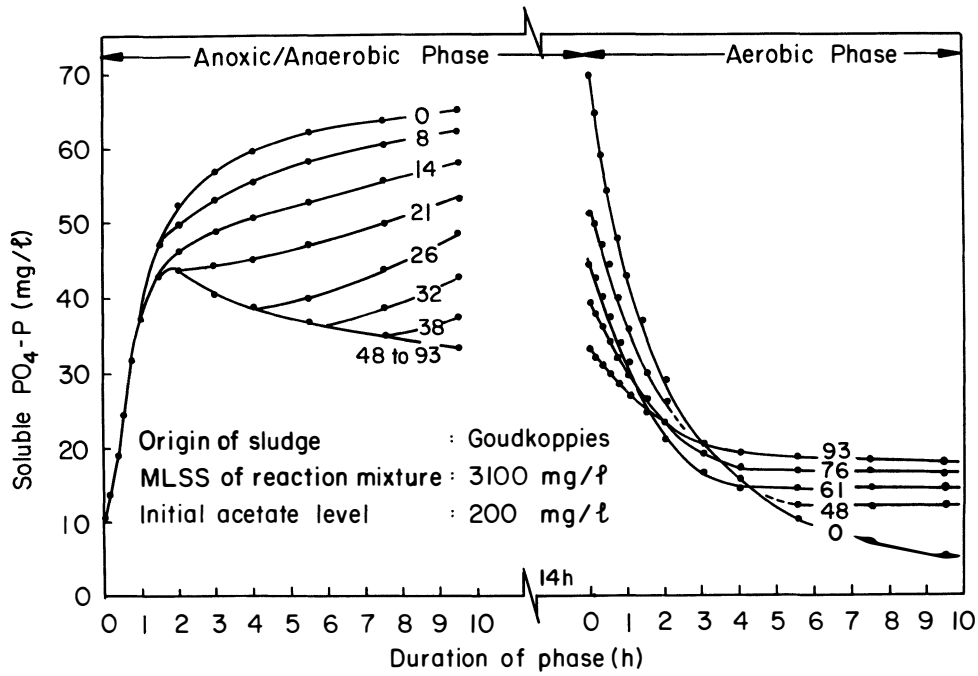

Fig. 6. Phosphate release and uptake as a function of initial nitrate concentration.

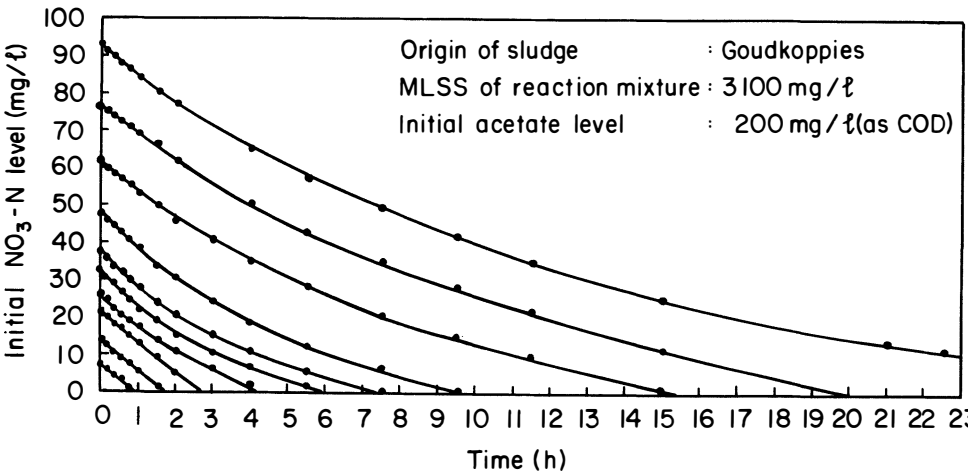

Fig. 7. Decline of nitrate concentrations during a 24 h period of anoxis/anaerobiosis, using acetate as substrate.

are presented by the initial values of the aerobic period (Fig. 6). These show that the composite envelope was fully resolved during the intervening 14 h excluded from the figure.

A maximum of approximately 83 mg/ℓ NO_3-N was removed during the 24 h anoxic/anaerobic phase (Fig. 7). The system with an initial nitrate level of 93 mg/ℓ thus remained anoxic throughout the unaerated phase. It is noteworthy that phosphate removal nevertheless occurred during the subsequent aerobic phase (Fig. 6), which suggests that periodic failure to achieve anaerobiosis does not immediately lead to comprehensive loss of excess phosphate uptake capability.

The results indicate a progressive reduction in phosphate removal capability with increasing initial nitrate concentrations, an observation which is in line with practical experience that excessive amounts of nitrate in the sludge recycle are detrimental to phosphate removal. It is noteworthy, once again, that even though the sludge proved capable of removing some 65 mg/ℓ phosphate (as P) with no nitrate present initially, the *net* removal constituted only about 5 mg/ℓ, which was achieved in the case of zero initial nitrate concentration.

Phosphate Uptake and Release Patterns under Variable Substrate Concentrations

Figure 8 illustrates phosphate release and uptake patterns when exposing phosphate-rich sludge to acetate in the range 0 to 800 mg/ℓ (as COD), initially in the presence of 10 mg/ℓ nitrate (as N). Virtually identical response curves were obtained with propionate, irrespective of the origin of the sludge. The same holds true for butyrate in those cases when it is capable of inducing phosphate release in anoxic or aerobic environments.

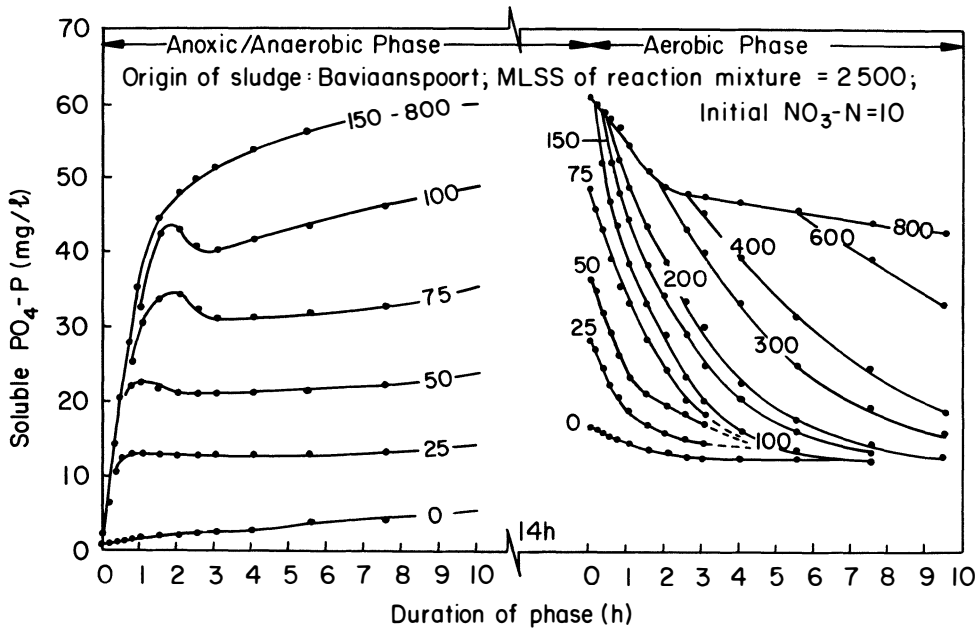

Fig. 8. Phosphate release and uptake as a function of initial acetate concentration (mg/ℓ as COD).

The results indicate that, under the specific experimental conditions, about 150 mg/ℓ acetate (as COD) was required to solubilize that component of accumulated phosphate participating in the release reaction. Higher concentrations had no further effect. The concentration quoted includes the amount required for denitrifying the nitrate present initially, the effect of which is evident from the two turning points in some of the response curves, resulting in the three distinct segments associated with net primary release, net anoxic uptake and net secondary release respectively.

The uptake patterns in Figure 8 suggest that phosphate is removed asymptotically under aerobic conditions to the same ultimate level, independent of acetate concentration. This was indeed verified by continued monitoring over an aeration period totalling 24 h (results not shown). Noteworthy is the fact that *initial* uptake rates decreased with increased acetate concentration from around 150 mg/ℓ (as COD), despite all these cases starting the uptake phase from an identical initial phosphate level. This time lag in the onset of relatively rapid uptake was particularly severe at acetate levels above 400 mg/ℓ (as COD), which suggests that excessive fatty acid levels indeed may be detrimental to the achievement of good phosphate uptake within the hydraulic residence times normally available in full-scale plants. It would appear that the time lag, *for organic compounds of group one*, is

consistently associated with cases in which free substrate remains in the liquid medium following anoxis/anaerobiosis. The amount of substrate consumed during the unaerated phase clearly is dependent on the amount of nitrate present initially and the amount of sludge-bound phosphorus capable of participating in the release reaction, but usually equalled about 150 mg/ℓ (as COD) in this study. It is considered that the delay in the onset of rapid phosphate uptake is partly, if not wholly, due to the presence of such free substrate at the beginning of aeration. It has been pointed out above that compounds of group one are capable of triggering phosphate *release* not only under anaerobiosis but also under anoxis and aeration. Phosphate *uptake*, on the other hand, occurs only under anoxic or aerobic conditions, with aerobic uptake rates significantly higher than the anoxic equivalent (Gerber and Winter, 1985). By extrapolation it follows that phosphate release and uptake occur *simultaneously* even under *aerobic* conditions as long as a free substrate of group one is present. The associated time course of phosphate concentration represents the resultant of the opposing two reactions, with net uptake rates less than those observed in the absence of free substrate. This implies that when substrates with response patterns similar to group one compounds are introduced into nutrient removal processes then, ideally, their amounts should be matched with the demand associated with denitrification and phosphate release. Also, the introduction of such compounds into the aeration stage appears to be counter-productive.

Effect of Phosphate Uptake and Release on the Concentration of Other Inorganic Components

The time course of selected cation and anion concentrations typically associated with phosphate release and uptake, under fatty acid levels not exceeding 200 mg/ℓ (as COD), is illustrated in Figure 9. The results show that calcium concentration in the liquid medium remained practically constant during both the anoxic/anaerobic and aerobic phases of the experiment. This indicates that calcium was not involved in precipitation or dissolution reactions involving phosphate. In contrast, release and uptake of phosphate were accompanied by similar response patterns for potassium, magnesium *and sulphate*.

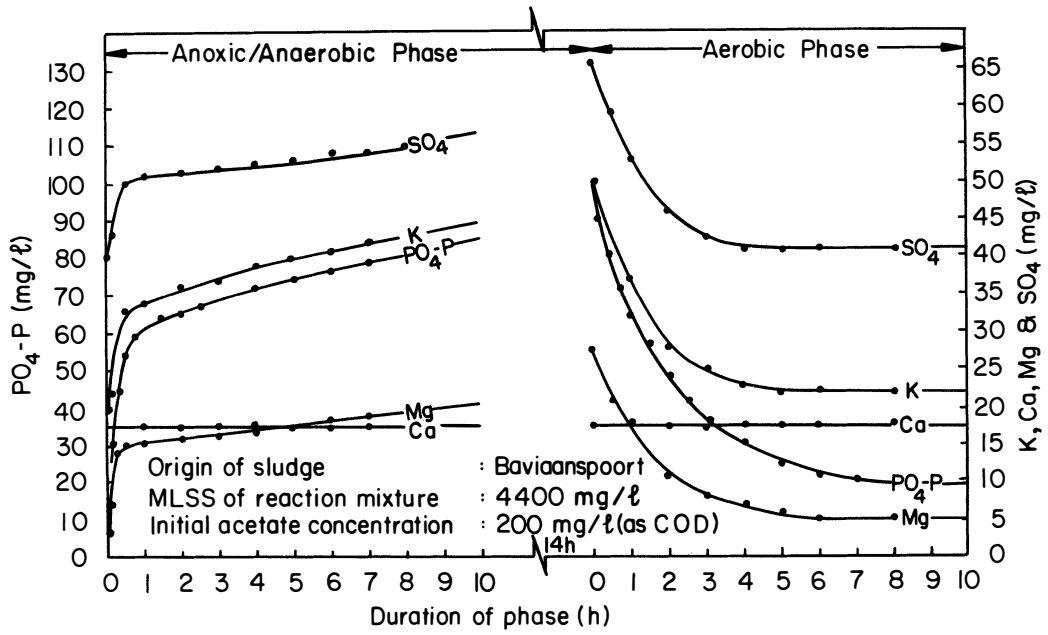

Fig. 9. Time course of selected inorganic ion concentrations accompanying phosphate release and uptake.

Electroneutrality constraints on the liquid medium dictate that phosphate release and uptake should be accompanied by molecular transport of equivalent amounts of cationic counter-ions. The exchange ratios of potassium and magnesium both varied closely around 0.25 mol/mol P. The role of sulphate, which appeared and disappeared in close association with phosphate and

in a ratio of 0.09 mol S/mol P, is yet to be resolved. Although neither of the three elements is considered to have been limiting under the conditions of this study the results show that, in general the amount available of any or all may be exhausted during aeration, an event which would probably lead to impaired phosphate uptake.

The results in Figure 9 represent initial fatty acid concentrations not exceeding 200 mg/ℓ (as COD). Higher levels were consistently accompanied by rising pH values in the range 7.5 to 9.0, which in turn were associated with reductions in calcium concentration, presumably through the precipitation of calcium compounds. These were the only conditions under which changes in calcium concentration were observed. The removal of calcium from the liquid phase was accompanied by a marked delay in the onset of rapid phosphate uptake under aeration, referred to in the previous paragraph (Fig. 8).

SUMMARY AND CONCLUSIONS

The release of phosphate from sludge acclimatized to excess phosphate removal is primarily dependent on the nature of the substrate interacting with the biomass and not the creation of an anaerobic state *per se*.

Compounds such as formate, acetate and propionate are capable of inducing release from phosphate-laden sludge under anaerobic, anoxic and aerobic conditions but with glucose, ethanol, methanol, citrate and 2,3 butanediol release is delayed until after the onset of anaerobiosis.

The time course of phosphate concentration following contact between *nitrified* mixed liquor and fresh substrate is a multivariate function, the shape of which depends, amongst others, on the nature of the substrate and the relative amounts of substrate and nitrate in the mixture. Three segments, associated with sequential periods during which net primary release, net uptake and secondary release, respectively occur have been distinguished with the carbon sources capable of releasing phosphate under anoxic conditions. The results suggest that phosphate release and uptake occur *simultaneously* upon admixture of phosphate-laden sludge and these substrates under anoxic conditions.

Increasing initial nitrate concentrations at a given level of substrate addition lead to progressively reduced phosphate release during anaerobiosis and diminished uptake under aeration. However, substantial uptake still occurs even in systems which, on singular occasions, remain anoxic prior to aeration.

The exposure of activated sludge to acetate, propionate and butyrate levels *higher* than that required for denitrification and liberation of the phosphate fraction normally participating in the release reaction, delays the onset of rapid phosphate uptake under subsequent aeration. This can be ascribed to *simultaneous* release and uptake occurring under aerobic conditions when free amounts of these substrates are present.

Uptake and release of magnesium and potassium accompanied phosphate uptake and release respectively. Exchange ratios of approximately 0.25 mol cation/mol P were observed during release and uptake. Sulphate concentrations increased and decreased likewise, with molar variations of about 0.09 mol S/mol P.

Phosphate release and uptake could not be accounted for by dissolution or precipitation of calcium phosphate compounds. Calcium concentration remained constant except under the addition of excessive amounts of substrate, which resulted in rising pH values during aeration and a concomitant reduction in calcium levels. Phosphate uptake was slowed down markedly under these conditions, especially during the initial stage.

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