The effect of acetate and other short-chain carbon compounds on the kinetics of biological nutrient removal*

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Abstract

The response of activated sludge with respect to phosphate release, phosphate uptake and denitrification following exposure to various organic compounds is covered. Batch systems simulating the anoxic-anaerobic and aerobic phases of biological wastewater treatment systems were used to highlight the interactions between phosphate, nitrate and substrate in nutrient removal processes.

Introduction

Enhanced biological phosphate removal from domestic wastewaters in full-scale activated sludge plants is currently perceived to hinge upon the provision of alternate stages in which the activated sludge is subjected to anaerobic and aerobic environments respectively. A characteristic feature of such plants is that phosphate, after being released from the biomass in the anaerobic stage, is re-incorporated in cell mass during aeration, together with part or all of the influent phosphate.

Although there are many successful applications of enhanced phosphate removal the role of particular phosphate-accumulating organisms, the mechanisms involved and indeed the function of the anaerobic stage itself, remain unclear. It has been suggested that the anaerobic state causes a stress (Nicholls and Osborn, 1979) which sets off the same enzymatic reactions as certain nutrient medium imbalances, eventually resulting in the production of polyphosphate granules. Authors such as Fuhs and Chen (1975), have advanced the theory that biological phosphate removal is due to a single bacterial group, *Acinetobacter-Moraxella-Mima*. The anaerobic stage is thought to play the role of a fermentation zone where acidogenic bacteria transform organic matter into volatile fatty acids. These fatty acids may be used by the phosphate removing bacteria and stored in the form of poly-β-hydroxybutyrate (PHB) (Nicholls, 1978; Deinema, 1980). The energy required for this storage would be supplied by the hydrolysis of the polyphosphate reserves (Rensink, 1981), which explains the release of phosphorus into the extracellular medium during the anaerobic phase. During aerobiosis, PHB could be metabolised normally and the energy liberated thereby partially used to reconstitute the polyphosphate reserves.

The favourable effect of readily bio-assimilable compounds in phosphate removing plants has been demonstrated by several investigators, who worked at full-scale and employed various devices to ensure a supply of desirable carbon compounds to the biomass. These included 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, 1984), 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 for 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 embedded 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.* (1984). 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), Malnou *et al.* (1984), Meganck *et al.* (1984) and Wentzel *et al.* (1984).

The complex and variable nature of most municipal wastewaters, the heterogeneous composition of the biomass found in activated sludge plants and the wide range of process conditions under which phosphate removal needs to be achieved, necessitate the performance of extensive tests before the effect associated with a particular organic substrate can be quantified. This paper is concerned with the interaction between activated sludge and various organic compounds in pure form. The discussion is focussed on sludge obtained from the Goudkoppies plant in Johannesburg, although samples from several other plants have been analysed in similar fashion. The results described are those associated with a specific level of substrate addition. In essence, the subset of investigations described here was aimed at uncovering the behaviour of a biomass when exposed to an environment approximating the sequence of conditions commonly occurring in practice, namely, the anoxic-anaerobic period following contact between well-nitrified mixed liquor and influent substrate, and the subsequent aerobic phase.

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

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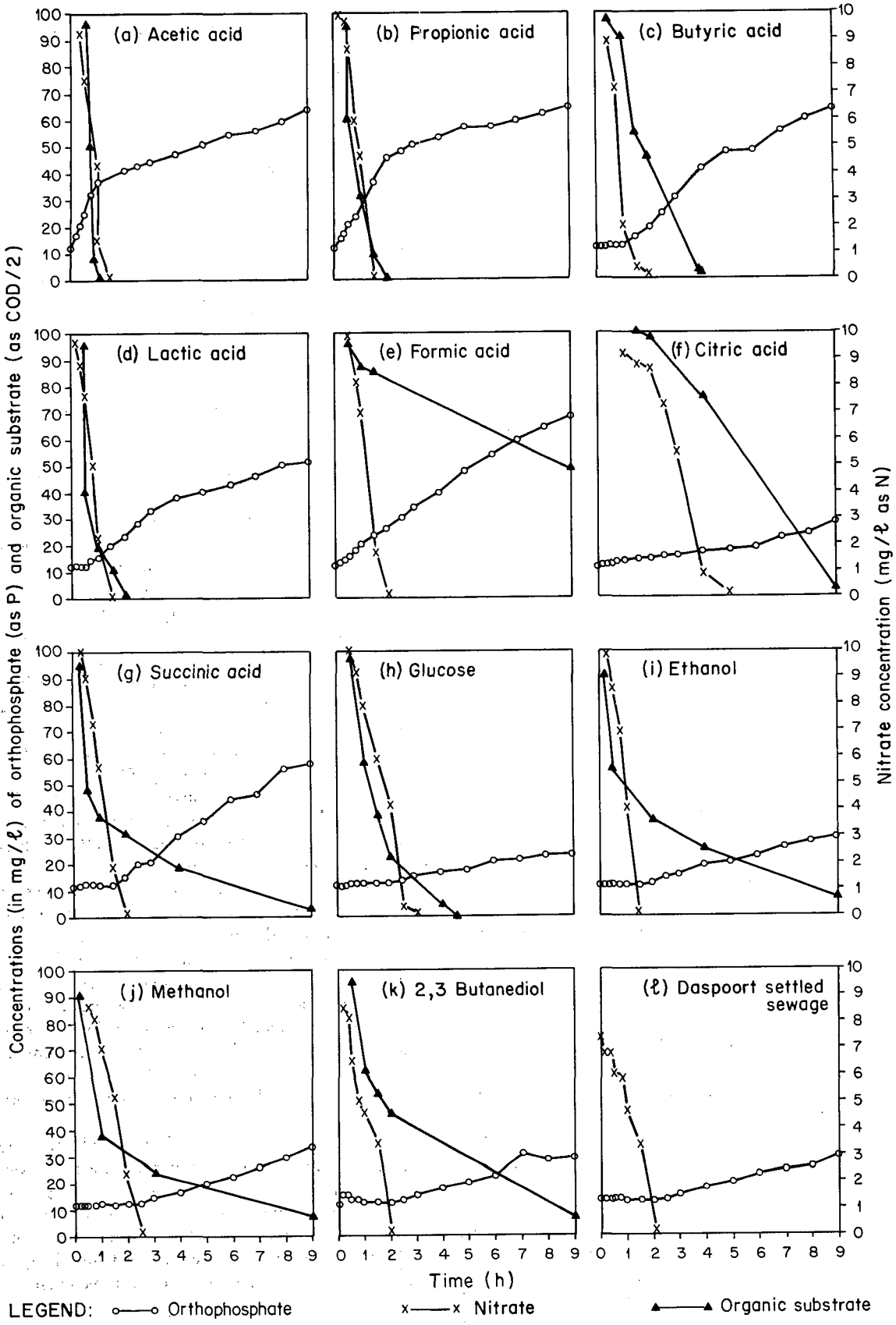

Figure 1
Temporal variation of orthophosphate and nitrate concentrations under anoxic-anaerobic conditions, in response to the addition of various substrates to Goudkoppies mixed liquor (MLSS concentration of reaction mixture = 4097 mg/ℓ)

consisted of collecting approximately 20 ℓ of mixed liquor from the aerobic stage of the particular plant being studied, just before it enters the clarifier, and adding a sodium nitrate solution to an effective concentration of 20 mg/ℓ (as N) to prevent phosphate release during transport to the NIWR laboratories. 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 $CaCO_3$). 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 min 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, adjusted to a standard volume of 75 mℓ using distilled water, and then introduced to the reactor. The anoxic-anaerobic condition was maintained for 22 to 23 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 endproducts 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.

Analytical methods

Analytical methods were generally as published in Standard Methods (1981), with orthophosphate, nitrate/nitrite and ammonia determined colorimetrically using an Auto-Analyser. 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.

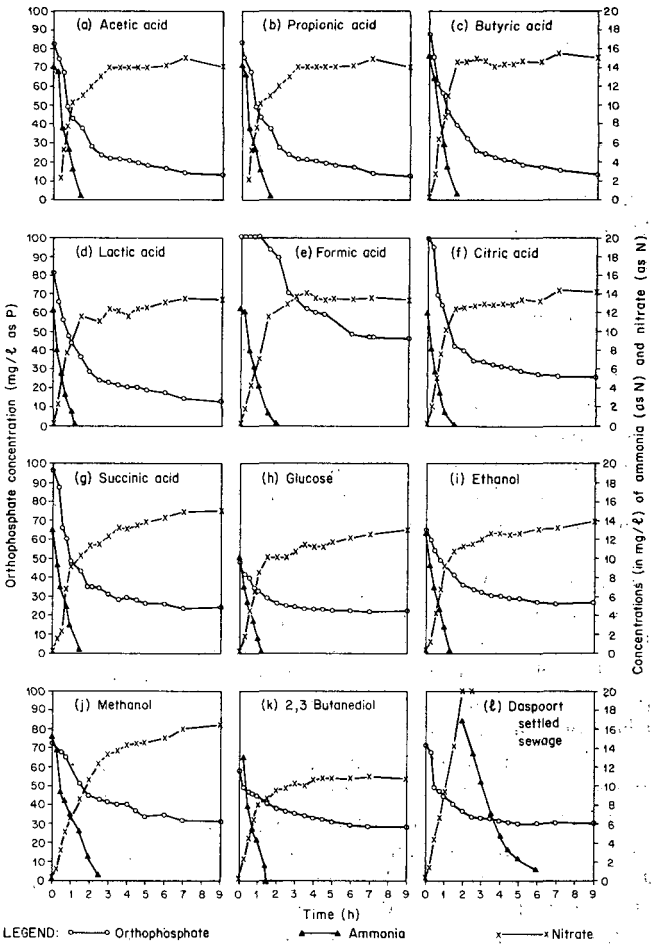

Figure 2
Temporal variation of orthophosphate, nitrate and ammonia concentrations under aerobic conditions, after the exposure of Goudkoppies mixed liquor to various substrates under anoxic-anaerobic conditions (MLSS concentration of reaction mixture = 4097 mg/ℓ)

Results and discussion

Temporal concentration profiles of orthophosphate, nitrate and the various organic compounds during the anoxic-anaerobic phase are given in Figure 1 and the corresponding results for the aerobic phase in Figure 2.

The results indicate that formate, acetate and propionate each induces effective phosphate release, *even in the presence of nitrate*. (Fig. 1(a), (b) and (e)). This observation has been found to hold for sludge from each of seven different plants examined so far, provided the plant exhibits at least a marginal degree of enhanced phosphate removal. Both acetate and propionate disappeared relatively quickly from the liquid phase, the point of zero concentration coinciding with a pronounced reduction in phosphate release rate. The slope of the latter part of the release curve did not suddenly diminish to zero but tapered off gradually over a period of some 15 h, the eventual phosphate concentration being about 85 mg/ℓ (refer values at zero time on Fig. 2). In contrast, formic acid was utilized much more slowly and resulted in a

linear release rate over the first seven hours of the anoxic-anaerobic phase. The rate subsequently tapered off to zero at an eventual phosphate concentration of about 100 mg/ℓ, the highest concentration induced by any of the substances investigated.

Phosphate release was effectively *prevented* during the anoxic phase for all the organic substrates studied other than the three mentioned above, which is in line with practical experience. Substrate was removed from the liquid phase but significant phosphate release commenced only once nitrate had been reduced to negligible concentrations. This proved true for sludge from all other plants investigated so far, except for butyric and lactic acid, which did result in some phosphate release during the anoxic phase in two instances (results not presented in this paper), albeit at a significantly lower rate than during anaerobiosis. Generally speaking, butyrate, lactate and succinate resulted in phosphate release rates comparable to or better than obtained with formate, irrespective of whether release occurred under anoxic or anaerobic conditions. The remaining substances (citrate, glucose, ethanol, methanol and 2,3 butanediol) resulted in relatively much lower phosphate release rates, with such release occurring only during the anaerobic phase. A similar conclusion holds for Daspoort settled sewage, which was known to support excellent phosphate release and uptake in continuous-flow experimental activated sludge systems operated in the NIWR laboratories at the time.

The observation that phosphate release can occur under anoxic (or even aerobic) conditions leads to the conclusion that the release phenomenon is primarily dependent on the nature of the organic substrate implicated in the reaction 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). The time course of phosphate release under *anoxic* conditions is, however, dependent on several additional factors, amongst others the maximum amount of phosphate that can be released to the liquid medium, as well as the relative quantities of lower fatty acid and nitrate. This is illustrated in Fig. 3, which indicates a transition of phosphate release to phosphate uptake during the anoxic phase. With acetate this has been observed to occur as soon as the substrate reaches negligible levels (Fig. 3) and also when the biomass has reached the state of being fully drained of 'labile' phosphate, in the latter case irrespective of whether unutilized substrate is present or not but again only in the presence of positive nitrate concentrations. It is surmised that the release and uptake reactions actually occur simultaneously but that the latter is masked by the rapid initial release rates. Once nitrate has been reduced to negligible levels uptake stops and phosphate release mechanisms once more predominate (Fig. 3). These results indicate that one or more bacterial species capable of utilizing nitrates as a final electron acceptor may play an active role in phosphate removal.

Denitrification and ammonia utilization rates under anoxic and aerobic conditions respectively are illustrated in Fig. 2 and summarised in Table 1. Of the substrates other than settled sewage the lowest denitrification rate was observed with citrate, followed by glucose, methanol and 2,3 butanediol in that order. The highest rates were achieved with acetate, butyrate and lactate, a result which is in agreement with sludge examined from other plants. Ammonia utilization rates proved much more uniform although the results for methanol and, to a lesser extent glucose and formate, were conspicuously lower than the rest. Both denitrification and ammonia utilization proceeded linearly in the range of positive nitrate and ammonia concentrations respectively.

The time course of phosphate uptake under aerobic conditions is illustrated in Fig. 2. The results indicate virtually identical uptake patterns for acetate, propionate, butyrate and lactate, which all had phosphate concentrations in the range 80 to 90 mg/ℓ (as P) following anaerobiosis. The response of these substances is emulated by citrate and succinate but for the fact that the initial phosphate levels are higher in the latter case. The higher starting values of about 100 mg/ℓ are responsible for the uptake curves being displaced vertically and thus also for the higher residual phosphate concentrations at the end of the aerobic period. Formate induced phosphate release to levels practically identical to citrate and succinate, but fared worse than these substances with respect to uptake during the aeration phase. Uptake commenced after a lag time of about 1 h but the phosphate concentration could be reduced ultimately to only about 50 mg/ℓ. Citrate, succinate, glucose, ethanol, methanol, 2,3 butanediol and Daspoort settled sewage resulted in eventual concentrations varying between 20 and 40 mg/ℓ but all shared the inability of formate to effect complete uptake of phosphate released during the anoxic-anaerobic phase of this experiment. In contrast, each of acetate, propionate, butyrate and lactate proved capable of taking up not only the released mass of phosphate but also some of the amount present initially. The explanation for

TABLE 1
NET RATES OF DENITRIFICATION AND AMMONIA UTILIZATION UNDER ANOXIC AND AEROBIC CONDITIONS RESPECTIVELY, FOLLOWING EXPOSURE OF GOUDKOPPIES SLUDGE TO VARIOUS ORGANIC SUBSTRATES (mg N/(g MLSS.h))

Substrate	Reaction rate		Substrate	Reaction rate	
	Denitrification	Ammonia oxidation		Denitrification	Ammonia oxidation
Acetate	2,51	2,91	Succinate	1,58	2,28
Propionate	1,68	2,33	Glucose	0,92	1,99
Butyrate	2,13	2,33	Ethanol	1,79	2,38
Lactate	2,17	2,58	Methanol	1,12	1,38
Formate	1,51	1,94	2,3 Butanediol	1,22	2,24
Citrate	0,61	2,54	Settled sewage*	0,64	1,53

*Daspoort settled sewage, diluted to a COD level of 440 mg/ℓ

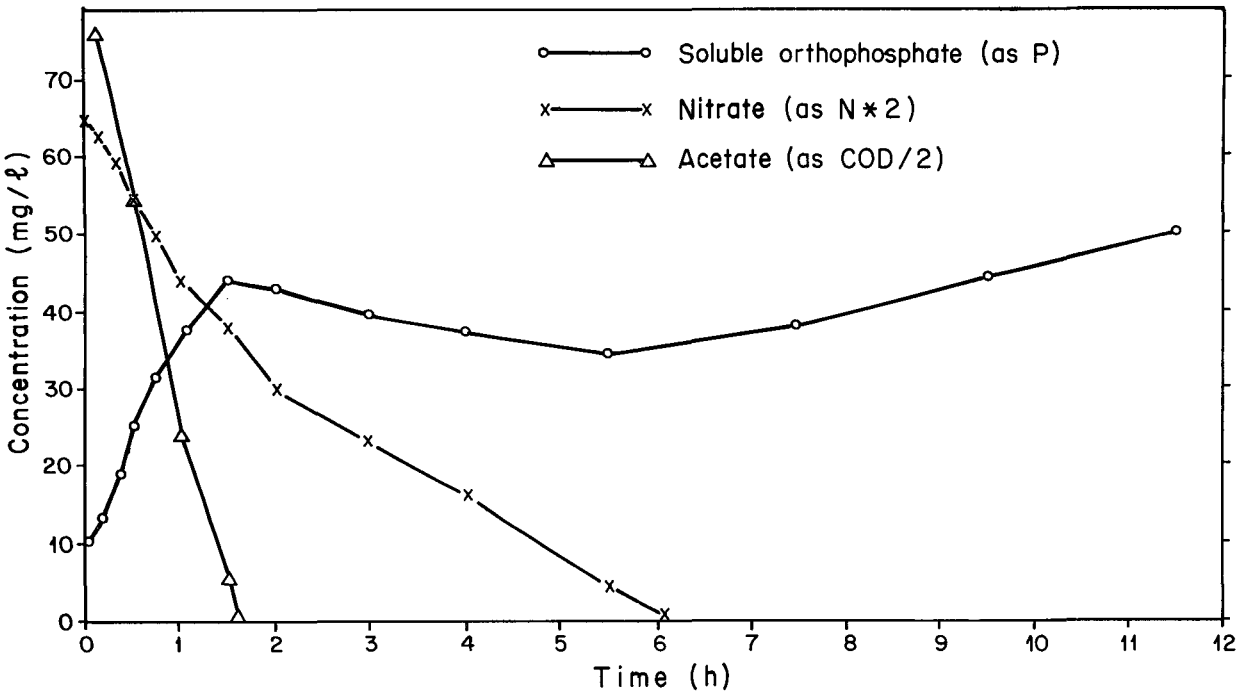

Figure 3
Illustration of sequential periods exhibiting net phosphate release and uptake under anoxic conditions, followed by further release under anaerobiosis.

this improved uptake ability has not been resolved but is probably related to different mechanisms by which the various compounds are transported across the cell wall. However, even with substances such as acetate, *net* phosphate removal was relatively low compared to the performance of continuous-flow prototypes under comparable conditions. It appears that an as yet unknown factor limits the degree of phosphate removal in batch systems such as those operated during this study.

Summary and conclusions

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

Rapid phosphate release can occur under anoxic conditions but in the study reported here this capability was confined to formate, acetate and propionate.

With substrates such as citrate, succinate, glucose, ethanol, methanol and 2,3 butanediol the release of phosphate occurs only after the onset of anaerobiosis. In the study reported here this dominance of the nitrate ion over phosphate release also holds for butyrate and lactate. It is considered that anaerobiosis provides the environment in which the stated compounds are converted to products which trigger phosphate release, rather than bringing release about by itself.

Denitrification rates associated with the pure compounds studied varied over the range 0,61 to 2,51 mg N/(g MLSS.h), with citrate and glucose at the lower end and acetate at the upper end of this domain.

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 composition and relative amounts of substrate and nitrate in the mixture. Three segments, which can be associated with net phosphate release and uptake, followed by further net release, have been distinguished with acetate as carbon source in the presence of relatively high initial nitrate concentrations. This response pattern is indicative of *simultaneous* phosphate release and uptake within the sludge mass. The observed time course of phosphate concentration is the resultant of these opposing reactions, each of which may dominate depending on the specific conditions in the reaction mixture.

The most favourable net phosphate removal from solution is obtained by the use of acetate, butyrate, propionate and lactate. Formate stimulates particularly good phosphate release but is worst in respect of overall net removal under conditions such as those pertaining to this study.

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