Enhanced polyphosphate organism cultures in activated sludge systems – Part 1: Enhanced culture development

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Abstract

In UCT and Bardenpho systems, by starting with 100 per cent municipal waste water as influent, incrementally decreasing the waste-water fraction and increasing the acetate fraction, an enhanced culture of polyphosphate (polyP) organisms was developed. Aerobic cultures, using the API procedure, indicated greater than 90 per cent of the microorganisms as *Acinetobacter* spp. As the acetate fraction increased, macro and micro-nutrients and yeast extract addition was necessary to maintain polyP organism growth. Of the macro-nutrients, magnesium, potassium and calcium had to be available in adequate concentrations for P uptake – magnesium and potassium form the principal counter-ions for stabilising the polyP chain and, to a lesser extent, calcium; calcium was probably involved in other functions. Acid addition was necessary in the aerobic zone to maintain the pH near neutrality. At sludge ages of 10 and 20 d with 100 per cent acetate feed (500 mgCOD/ℓ) removals of about 60 and 50 mgP/ℓ respectively were attained, P/VSS (mgP/mgVSS) of about 0,38 and VSS/TSS (mgVSS/mgTSS) of 0,46 in the aerobic zone. This enhanced culture was used to obtain information on the stoichiometry and kinetics of the growth of polyP organisms in nutrient removal systems.

Introduction

In biological excess phosphorus (P) removal activated sludge systems i.e. modified Bardenpho and Phoredox (Barnard, 1976) and UCT (Siebritz *et al.*, 1980), the removal of P is mediated by organisms which have the propensity to store P as polyphosphate (polyP) granules called volutins (Buchan, 1981). Generically, such organisms may be termed polyP organisms. Up to the present only semi-empirical methods have been available to predict the biological excess P removal (Siebritz *et al.*, 1983; Wentzel *et al.*, 1985). For greater surety in prediction, it is necessary to develop a quantitative model describing the stoichiometry and kinetics of the various processes connected with biological excess P removal. There are a number of approaches to obtain this information.

The first approach is to hypothesise on the behavioural characteristics of the polyP organisms, incorporate these in a model, and by simulation obtain theoretical responses that can be compared with those observed, in this manner to assess in what measure the model is relevant. This approach has been used by Wentzel *et al.* (1985). They investigated the release of P by polyP organisms in anaerobic plug flow and batch reactors.

They established that (1) with municipal wastewater:

- the rate of release of P was governed by the rate of conversion of readily biodegradable COD (RBCOD) (see Dold *et al.*, 1980, for description of this COD fraction) to short-chain fatty acids;
- the release of P conformed to a first order type of reaction (with respect to RBCOD) at a relatively slow rate; and
- the mass of P released appeared to be proportional to the mass of RBCOD converted.

and (2) with acetate addition in anaerobic batch tests:

- the release of P conformed to a zero order type of reaction (with respect to acetate) at a relatively fast rate; and
- the total mass of P released was proportional to the mass of acetate taken up.

Under subsequent aerobic conditions they found that:

- the mass of P taken up was proportional to the mass of P released in the anaerobic reactor; and
- the mass of P taken up was only weakly dependent on the sludge age of the system; this insensitivity, they concluded, implied that the polyP organisms have a very low endogenous mass loss rate.

The response data on P release with municipal waste water allowed formulation of a kinetic relationship for the release in terms of the readily biodegradable COD (RBCOD) concentration and the normal heterotrophic (non-polyP) organism concentrations in the anaerobic zone. This formulation could be established without quantitative knowledge of the mass of polyP organisms in the system because the rate of conversion of RBCOD to short-chain fatty acids by the non-polyP organisms (a slow reaction) governed the rate of release of P by the polyP organisms (a fast reaction). With regard to the uptake of P in the aerobic reactors, the simulation approach proved unproductive; the uptake kinetics could not be formulated without explicitly incorporating the growth and death characteristics of the polyP organisms. From the experimental data it was not possible to isolate these characteristics of the polyP organisms, for reason that the response of the other heterotrophic organisms either obscured or swamped out that of the polyP organisms. Consequenty this approach is limited in the information it can provide. For an improved understanding of the biological excess P removal phenomenon different approaches need to be considered.

A second approach is to obtain information on the growth and death characteristics of the polyP organisms from pure culture studies. The organism group widely implicated in biological excess P removal is the genus *Acinetobacter*. Numerous pure culture studies have been conducted to ascertain the growth and death characteristics of strains belonging to this genus (Abbott, 1973; Abbott *et al.*, 1973; Abbott *et al.*, 1974; Ensley and Finnerty, 1980; Du Preez, 1980; Du Preez *et al.*, 1981 and van Groenestijn and Deinema, 1985). These studies were all conducted using pure cultures of specific *Acinetobacter* strains or species grown in aerobic chemostats, with very short sludge ages (up to one day) and with acetate or ethanol as influent substrate,

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TABLE 1
GROWTH AND DEATH CHARACTERISTICS OF VARIOUS *ACINETOBACTER* STRAINS (SPECIES) MEASURED IN PURE CULTURES GROWN IN AEROBIC CHEMOSTATS

Research group	Organism	Substrate	Yield (gcells/gCOD)	Max. spec. growth rate (/d)	Spec. end. mass* loss rate (/d)
Abbott *et al.* (1973, 1974)	*Acinetobacter calcoaceticus*	Ethanol	0,38	17	2
Ensley and Finnerty (1980)	*Acinetobacter* spp.	Acetate	–	14	2
Du Preez *et al.* (1981)	*Acinetobacter calcoaceticus*	Ethanol, acetate and ethanol/ acetate mixture	0,43	4-30	1-5
Van Groenestijn and Deinema (1985)	*Acinotobacter* strain A102	Acetate	0,40	17	1

* Specific endogenous mass loss rate constant calculated by us (from data supplied in the respective papers) using the Monod-Herbert approach (synthesis-endogenous respiration) instead of the Monod-Pirt approach (synthesis-maintenance energy) used in the papers.

but at high COD concentrations. The results of the studies show remarkable similarity, summarised in Table 1.

Referring to Table 1, the stoichiometric yield coefficients for acetate (0,43 gcells/gCOD) and for ethanol (0,40 gcells/gCOD) compare favourably with values reported for 'normal' activated sludge organisms fed municipal sewage (0,45 gVSS/gCOD) (Marais and Ekama, 1976). However, the respective specific kinetic rate constants for growth and decay differ significantly from those reported for 'normal' sludges in activated sludge systems: The maximum specific growth rate constant ($\hat{\mu}$) for *Acinetobacter*, with ethanol and with acetate, is extremely high, 4 to 30/d, compared with that for 'normal' activated sludge, $\hat{\mu}$ = 2 to 4/d observed by Ekama, Dold and Marais (1986). Similarly the specific endogenous mass loss rate constant also is exceptionally large, 1 to 5/d, compared with 0,24/d found by Marais and Ekama (1976). The high specific endogenous mass loss rate of the *Acinetobacter* in particular, is directly in contradiction to the conclusions of Wentzel *et al.* (1985) referred to earlier, i.e. that the rate appears to be very much *smaller* than that for the normal heterotrophs. Furthermore, on incorporating these high growth and endogenous mass loss rates into a kinetic model, it was found impossible to relate the response observed by Wentzel *et al.* (1985) on excess P removal activated sludge systems with that predicted. These differences in the responses of the pure culture and activated sludge systems, indicate that either two different polyP organism types were present, or the same organism type was present, but developed divergent behavioural characteristics when exposed to the two different sets of environmental conditions – the *Acinetobacter* pure cultures were grown aerobically at short sludge ages, whereas Wentzel *et al.* (1985) developed these organisms in mixed cultures in anaerobic/anoxic/aerobic activated sludge systems at long sludge ages. The influence of the environmental conditions on the same species could have been checked by growing the selected polyP species in pure culture in a system of interlinked chemostats simulating the same configuration as that of the Bardenpho or UCT excess P removal plants and comparing the response with that observed in the aerobic pure culture studies. Such an investigation, however, would have required specialised apparatus and techniques which were not available. To overcome this, an alternative approach was devised, as described below.

The third approach is to grow an *enhanced* culture of polyP organisms. By enhanced culture is meant: the development of a polyP organism culture by selecting a substrate and set of environmental conditions that favour polyP organisms so that they become the dominant primary organisms and their behaviour dominates the culture response. Growth of competing normal heterotrophic primary organisms will be curtailed naturally but not terminated, neither will predation by higher organisms and other interactive effects be positively excluded. Also, a strain (or strains) of polyP organism will be selected naturally and may differ from that selected artificially and grown in pure culture in a chemostat system.

To identify the conditions likely to produce an enhanced culture of polyP organisms, *Acinetobacter* spp. were accepted as organisms typical of the polyP group. *Acinetobacter* spp. have been shown to be present abundantly in activated sludge systems; up to 60 per cent of the organisms isolated from completely aerobic activated sludge systems treating municipal waste water, and cultured aerobically, have been found to belong to this genus (Lötter *et al.*, 1988). Yet, in aerobic activated sludge systems, these organisms have exhibited no biological excess P uptake. Lötter *et al.* (1986) concluded that these organisms have the propensity to exhibit biological excess P uptake, but this is invoked only with the appropriate substrate and environmental conditions. Wentzel *et al.* (1986) put forward a biochemical model in terms of which sets of conditions that give rise to biological excess P removal processes can be identified. One set is to subject the organism mass to an anaerobic/aerobic sequence with short chain fatty acids fed to the anaerobic phase, conditions present in the Phoredox, modified Bardenpho and UCT systems. PolyP accumulating organisms sequester the short chain fatty acids (e.g. acetate) in the anaerobic phase for their exclusive use in the subsequent aerobic phase. Hence, with acetate as the sole substrate fed to the anaerobic phase of an anaerobic/aerobic sequence, the acetate would be available only to the polyP organisms, providing a situation favourable for these organisms and enabling them to become dominant. Furthermore, according to the biochemical model of Wentzel *et al.* (1986), these conditions would stimulate the processes involved in biological excess P removal.

Based on the considerations above, an investigation was initiated to determine if an enhanced culture of polyP organisms could be developed. This paper describes our endeavours to achieve such an enhanced culture. In a later paper of this series the kinetics of growth and endogenous mass loss of these organisms, and the kinetics of P release and uptake under different environmental conditions, will be discussed in detail; for a preliminary assessment see Wentzel *et al.* (1987).

System development

When investigations into the development of an enhanced

culture of polyP accumulating organisms commenced in February 1984, little information was available in the literature on the protocol for such an investigation. As a consequence, initial experimentation was almost totally of a trial and error nature. It may be useful to other investigators, therefore, to relate briefly the evolution of the procedures, the dead ends and the byways encountered, to obtain an enhanced culture.

Phoredox system 1

Experiments were initiated using an anaerobic/aerobic (Phoredox) configuration operated at 10 d sludge age. The system set-up is shown in Fig. 1. As a control, a single completely mixed aerobic activated sludge reactor was set up, having the same volume and sludge age, and receiving the same substrate and volume of influent feed per day.

To eliminate confounding effects of nitrification and denitrification, nitrification was suppressed by periodic addition of thiourea to both systems as follows: Whenever the effluent TKN commenced to decrease, 20 mg thiourea per litre mixed liquor was added to the respective reactors.

To start up, both systems were inoculated with mixed liquor from a laboratory scale UCT system operated at 20 d sludge age on unsettled municipal waste water. To acclimatise the organism mass to acetate, the systems were fed with a mixture of 500 mgCOD/ℓ unsettled municipal waste water from Mitchell's Plain, Cape Town, plus 500 mgCOD/ℓ sodium acetate i.e. 1 000 mgCOD/ℓ. To counter the possibility of deficiency in mineral nutrients, additional inorganic *macro*-nutrients were added according to the recipes of Abbott (1973) and du Preez (1980) as set out in Table 2. In their nutrient recipes the magnesium concentrations were much lower than those given by Fuhs and Chen (1975). However, Fuhs and Chen stated that magnesium was unlikely to be a limiting nutrient as it acted as cofactor in biological reactions and hence was required only in low concentrations. Thus the magnesium and other macro-nutrient concentrations given by Abbott (1973) and Du Preez (1980) were used as a starting point. The *micro*-nutrients suggested by Du Preez were not added as it was considered that these would be adequatly supplied by the waste-water fraction and the tap water used in dilution. The characteristics of the unsettled waste-water fraction were approximately as shown in Table 3 (a) and those of the tap dilution water approximately as shown in Table 3 (b).

TABLE 2
INORGANIC MACRO-NUTRIENTS ADDED PER 1 000 mgCOD SODIUM ACETATE TO INFLUENT CONSISTING OF A MIXTURE OF UNSETTLED MUNICIPAL WASTE WATER AND SODIUM ACETATE (ADAPTED FROM DU PREEZ, 1980).

Chemical	mg added/1 000 mgCOD sodium acetate		
	Compound	Element	
$NH_4Cℓ$	162,2	42,4	N
$MgSO_4.7H_2O$	40,0	3,9	Mg
$CaCℓ_2.2H_2O$	5,34	1,45	Ca
$MnSO_4.4H_2O$	5,34	1,32	Mn
$FeSO_4.7H_2O$	0,197	0,0396	Fe
KH_2PO_4	52,66	62,91	K
K_2HPO_4	106,66	31,01	P

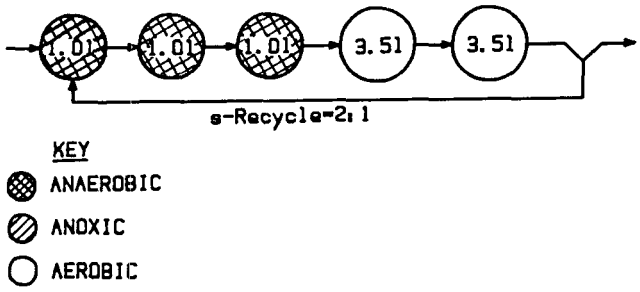


Figure 1
Schematic layout of Phoredox (A/O) system; reactor volumes in litres, sludge age 10 d and influent flow rate 7,5ℓ/d.

TABLE 3(a)
CHARACTERISTICS OF UNSETTLED MUNICIPAL WASTE WATER

Component	mg/1 000 mg COD	
Total COD (S_{ti})	1 000	COD
Readily biodegradable COD (S_{bsi})	≈ 200	COD
Unbiodegradable soluble COD	≈ 100	COD
Unbiodegradable particulate COD	≈ 80	COD
TKN	≈ 100	N
P	≈ 18	P
Mg	≈ 7,00	Mg
Ca	≈ 38	Ca
Mn	≈ 0,12	Mn
Fe	≈ 0,80	Fe
Zn	≈ 0,30	Zn
Cu	≈ 0,12	Cu
Co	≈ 0,02	Co
Mo	≈ 0,06	Mo
Al	≈ 1,80	Al
Ni	≈ 0,04	Ni

TABLE 3(b)
SELECTED CHARACTERISTICS OF TAP WATER

Component	Approximate concentration (mg/ℓ)
Mg	2,4
Ca	20,0
Mn	0,02
Fe	0,1
Zn	0,15
Cu	0,04
Co	NIL
Mo	NIL
Al	1,7
Ni	NIL

The systems were operated for two weeks, whereupon both had approximately the same sludge concentrations. The mixed liquor in the Phoredox system settled well, but that in the aerobic system bulked excessively. For the aerobic system the steady state P removal was around 0,005 mgP/mg influent COD which conforms reasonably to the normal P requirements of heterotrophs at 10 d sludge age. For the Phoredox system the steady state average P removal was 15 mgP/ℓ (i.e. 0,015 mgP/mg influent COD) and the average P release was 60 mgP/ℓ influent (0,06 mgP/mg influent COD). This removal was *less* than that obtained on a UCT system operated at 20 d sludge age with 1 000 mgCOD/ℓ unsettled municipal waste water (removal 20 mgP/ℓ i.e. 0,02 mgP/mg

Table 4
INFLUENT INORGANIC NUTRIENT CONCENTRATIONS ADDED TO INFLUENT OF 1 000 mgCOD SODIUM ACETATE, ADAPTED FROM DU PREEZ (1980)

Chemical	mg added/1 000 mgCOD sodium acetate	
	Compound	Element
NH_4Cl	153,0	40,0 N
$MgSO_4.7H_2O$	200,0	19,5 Mg
$FeSO_4.7H_2O$	0,7	0,14 Fe
$CaCl_2.2H_2O$	20,0	5,44 Ca
KH_2PO_4	98,75	118,0 K
K_2HPO_4	200,0	58,14 P

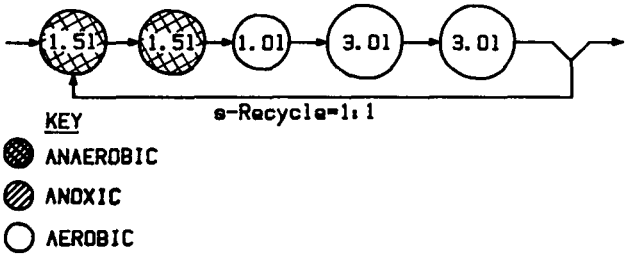


Figure 2
Schematic layout of Phoredox (A/O) system: reactor volumes in litres, sludge age 20 d and influent flow rate 15ℓ/d.

influent COD) even though the substrate fraction favourable to the polyP organisms was three times higher for the Phoredox (500 mgCOD/ℓ acetate plus 100 mgCOD/ℓ RBCOD) than for the UCT system (200 mgCOD/ℓ RBCOD). No assignable cause for this lower P removal could be discovered, except possibly a deficiency in the inorganic nutrients. In an endeavour to gain further information on the nutrient requirements, the influent was changed to pure acetate at 1 000 mgCOD/ℓ and, using the nutrient recipe of Du Preez (1980) as a basis, fractional nutrient concentrations relative to the acetate were increased to four times those suggested by Du Preez (1980). It was presumed that the concentrations reduced the likelihood that the macro-nutrients possibly could be the limiting factor.

The augmented nutrient concentrations are given in Table 4. Micro-nutrients were not added because the elements specified by Du Preez (1980) appeared to be adequately supplied in the tap water (Table 3 (b)). Immediately the new substrate was fed to the Phoredox system, the release in the anaerobic zone commenced to increase, so also the removal. An unexpected feature noted was that the pH in the aerobic reactor increased sharply relative to that in the anaerobic reactor, 8,7 as against 7,2. To counter this, 0,3 mℓ concentrated HCl per litre influent was added to the influent batch. This controlled the pH in the aerobic reactors to about 8,4, but the pH in the anaerobic reactor now fell to about 6,0. To avoid the reduction in pH in the anaerobic reactor, the HCl was drip fed to the aerobic reactor. By doing this, the pH was maintained at ± 7,4 in the aerobic and ± 7,1 in the anaerobic reactors. Maintaining the pH between 7,0 and 7,5 minimised the possibility of calcium phosphate precipitation. This method of maintaining the pH was used subsequently in all the systems operated. (The loss of protons (H^+) in the aerobic zone of biological excess P removal systems has been explained biochemically by Wentzel *et al.*, 1986).

Within about 10 d of operating the Phoredox system in the fashion described above, the release had increased to 130 mgP/ℓ influent (0,13 mgP/mg influent COD), and the removal to 20 mgP/ℓ (0,02 mgP/mg influent COD). However, the sludge settleability in the Phoredox system now deteriorated, to such an extent that the settler overflowed and the system had to be abandoned. Microscopic examination of the sludge showed that the bulking was due to the massive formation of extracellular polysaccharide slimes. Such slime formation often is due to some nutrient deficiency. Warskow and Juni (1972) had observed that, in pure culture studies, one strain of *Acinetobacter* failed to grow in an acetate-mineral medium unless a trace of yeast extract was added. Accordingly in the next series of experiments yeast extract was added to the influent.

Phoredox system 2

A Phoredox system again was set up, as shown in Fig. 2. It was hypothesised that perhaps the sludge age had been too low and this was set to 20 d. Again thiourea was added to inhibit nitrification. The total influent COD was set to 500 mgCOD/ℓ to obtain a more direct comparison with other excess P removal systems operated in the laboratory on unsettled municipal waste water. The acetate fraction in the influent was increased incrementally, keeping the total COD constant at 500 mg/ℓ i.e. if, for example, 100 mg/ℓ COD acetate was included, the sewage fraction was reduced to 400 mgCOD/ℓ. Yeast extract was added in the proportion, 1 mg extract for every 100 mgCOD of sodium acetate. With each increment in acetate the system was run until apparent steady state behaviour was attained. The acetate fraction COD concentrations were 50, 100 and 200 mg/ℓ plus yeast extract. In order that the onset of nutrient deficiency could be determined, no inorganic macro or micro-nutrients were added to the influent (except for $NH_4Cℓ$ and K_2HPO_4 to give desired N and P influent concentrations respectively). An aerobic control unit again was set up receiving the same influent and operated at the same sludge age as the anaerobic/aerobic unit.

In the Phoredox system, the P removal achieved with the increased fractions of acetate in the influent is shown in Fig. 3. For all the acetate fractions the P removal hardly changed and averaged at about 11 mgP/ℓ (i.e. 0,022 mgP/mg influent COD). In the aerobic unit P removal remained at about 0,004 mgP/mg influent COD. Settleability in the anaerobic/aerobic unit now was excellent with no slime development, indicating that the yeast extract served some essential function in the system. The aerobic system continued to bulk, but the bulking now was due to filamentous organisms, not slime formation.

In seeking a reason for the insensitivity of P removal to the acetate feed, it was hypothesised that the acetate may not be a good carbon source for metabolic synthesis and, accordingly, an influent consisting of 250 mgCOD/ℓ sewage, 125 mgCOD/ℓ acetate and 125 mgCOD/ℓ lactate was tried for both systems – P removal remained at approximately 10 mgP/ℓ (i.e. 0,02 mgP/mg influent COD) for the anaerobic/aerobic unit and 0,004 mgP/mg influent COD for the aerobic control. The acetate substrate was then abandoned and an influent of 250 mgCOD/ℓ lactate and 250 mgCOD/ℓ sewage was tried. The Phoredox system's release declined slightly, from 60 mgP/ℓ influent (0,12 mgP/mg influent COD) to 50 mgP/ℓ influent (0,10 mgP/mg in-

fluent COD), but the removal remained at 10 mgP/ℓ (0,02 mgP/mg influent COD). Evidently lactate addition conferred no advantage on the system. The influent feed was then changed to 250 mgCOD/ℓ sewage and 250 mgCOD/ℓ acetate, but the removal continued to remain at about 10 mgP/ℓ (0,02 mgP/mg influent COD). The aerobic control unit continued to remove 0,005 mgP/mg influent COD.

During these investigations it was noticed that every time thiourea was added to the system, the removal of P in both the anaerobic/aerobic and the aerobic units showed a decline. It was decided therefore to reduce the frequency of thiourea addition, but this did not improve the mean removal. It was thereupon decided to abandon thiourea addition and to reduce the NO_3^- by introducing a primary anoxic reactor with an a-recycle from the last aerobic zone to the primary anoxic reactor (i.e. setting up a laboratory-scale 3-stage Bardenpho system) (Fig. 4).

Bardenpho system

For this system, the influent was maintained at 250 mgCOD/ℓ acetate and 250 mg/ℓ sewage. The release fluctuated around 100 mgP/ℓ, but the average removal continued to remain at 10 mgP/ℓ (0,02 mgP/mg influent COD).

At this juncture an analysis of the organisms present in the mixed liquor from the Bardenpho system was undertaken by the scientific and chemical services branch of the City of Johannesburg using the Analytical Profile Index (API) procedure (Analytlab Products, 1977). This showed that, of the organisms cultured aerobically, 90 per cent were *Acinetobacter* spp. (for detailed analysis see Lötter *et al.*, 1988). It seemed clear, therefore, that there was not a nutrient deficiency insofar as growth of this species was concerned. This, together with the remarkable constancy of the P removal at about 10 mgP/ℓ (0,02 mgP/mg influent COD), irrespective of the magnitude of the release or the type of substrate added, eventually forced the conclusion that there might be a deficiency in some chemical that affected the P uptake and excess P removal. Biochemically, the counter-ion for ATP stabilisation is Mg^{2+} and it was hypothesised that, for the stabilisation of the polyP chains, the counter-ion similarly was Mg^{2+}. Accordingly, with influent remaining at 250 mgCOD/ℓ acetate and 250 mgCOD/ℓ sewage, the influent was augmented by 264 mg $MgCl_2.6H_2O$/ℓ (30 mgMg^{2+}/ℓ). Immediately the removal increased from 10 to 18 mgP/ℓ and the release from 100 to 120 mgP/ℓ influent. To confirm that the Mg^{2+} was the prime deficient nutrient the Mg^{2+} was omitted – this caused a precipitous drop in the P removal to 12 mgP/ℓ. It was concluded therefore that the 250 mgCOD/ℓ acetate-250 mgCOD/ℓ sewage mixture contained inadequate Mg^{2+}.

An examination on an ionic charge basis indicated that for every two P removed two negative charges had to be neutralised; if the charges were neutralised by magnesium, one Mg^{2+} would be required as a counter-ion for every two P removed. Accordingly, using this ratio, Mg^{2+} was added to the influent sufficient for a P removal of 30 mgP/ℓ. A steady state was attained with P removal of 25 mgP/ℓ (0,05 mgP/mg influent COD) and with P release of 110 to 130 mgP/ℓ influent (0,24 mgP/mg influent COD). This steady state was readily maintained.

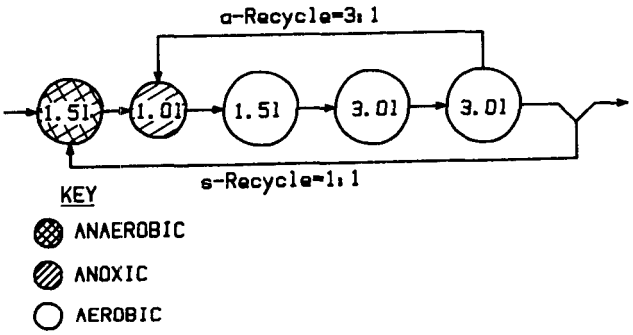


Figure 4
Schematic layout of 3-stage Bardenpho system; reactor volumes in litres, sludge age 20 d and influent flow rate 15ℓ/d.

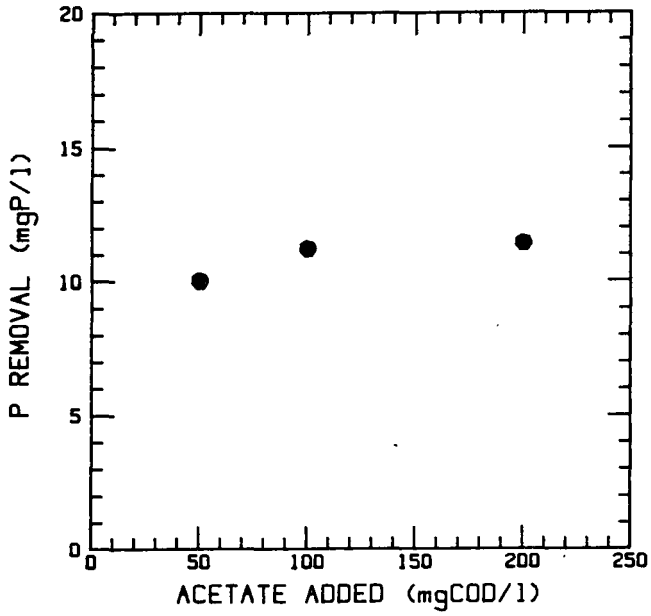


Figure 3
Phosphorus (P) removal versus acetate added to the influent to Phoredox system (Fig. 2) when there is a deficiency of magnesium. Total COD is 500 mg/ℓ.

Once the importance of magnesium was established, the acetate fraction was increased to 300 mgCOD/ℓ; the sewage fraction correspondingly decreased to 200 mgCOD/ℓ; the magnesium was increased proportionally, also the yeast extract, but no other nutrient was augmeted. However, no apparent change was observed in the magnitude of P release and P removal, from that obtained with lower acetate concentration. Moreover, the system response now was unstable, exhibiting cyclic fluctuations. It was hypothesised that such fluctuations could be the result of some further nutrient limitation. The element calcium (Ca^{2+}) functions as a co-factor in numerous enzyme reactions in biological systems and also has been implicated as another cation stabilising the polyP chain (Buchan, 1981). In consequence, it was decided to ensure adequate calcium by increasing calcium in the influent by 10 mgCa^{2+}/ℓ. Immediately the P release, uptake and removal exhibited an increase; P removal stabilised at about 30 mgP/ℓ (i.e. 0,06 mgP/mg influent COD) and release at 160 to 180 mgP/ℓ influent (i.e. ≈ 0,34 mgP/mg influent COD). Evidently the Ca^{2+} ion was an essential macro-nutrient in polyP metabolism.

At the beginning of June 1985, and at intervals thereafter, the acetate fraction was progressively increased to 350, 400 and

500 mgCOD/ℓ, the sewage fraction being decreased correspondingly to 150, 100 and zero mgCOD/ℓ. The macro-nutrients, Mg^{2+} and Ca^{2+}, and the yeast extract were increased proportionally to the acetate in accordance with the proportions set out in Table 5. P was added to the influent in sufficient concentrations so as to ensure that the effluent always contained P. The P was added as K_2HPO_4. With this addition K did not become limiting (on a molar basis two K were added for every P). However, batch tests conducted on sludge samples drawn from the enchanced cultures indicated that K release and uptake was linearly associated with P release and uptake, 0,3 moles K/mole P. Similarly, in the batch tests, Mg release and uptake was linearly associated with P release and uptake, 0,26 moles Mg/mole P. In contrast, very little Ca release and uptake was associated with the P release and uptake, 0,05 moles Ca/mole P. Nitrogen was added as ammonia to maintain an influent TKN/COD ratio of about 0,06 mgN/mgCOD. When the acetate fraction increased to 400 mgCOD/ℓ and above, the possibility of inadequacy of micro-nutrients was raised. In pure culture studies Du Preez (1980) described a micro-nutrient solution for growth of *Acinetobacter*, a polyP organism. As a *precautionary* measure, a similar micro-nutrient solution was added to the influent to supply elements essential to biological function. The concentrations of micro-nutrients added to the influent, adapted from Du Preez (1980), are given in Table 5. The data obtained, on P release and uptake (and hence P removal) versus acetate added in this series of tests, are shown plotted in Fig. 5 – the responses are near linear.

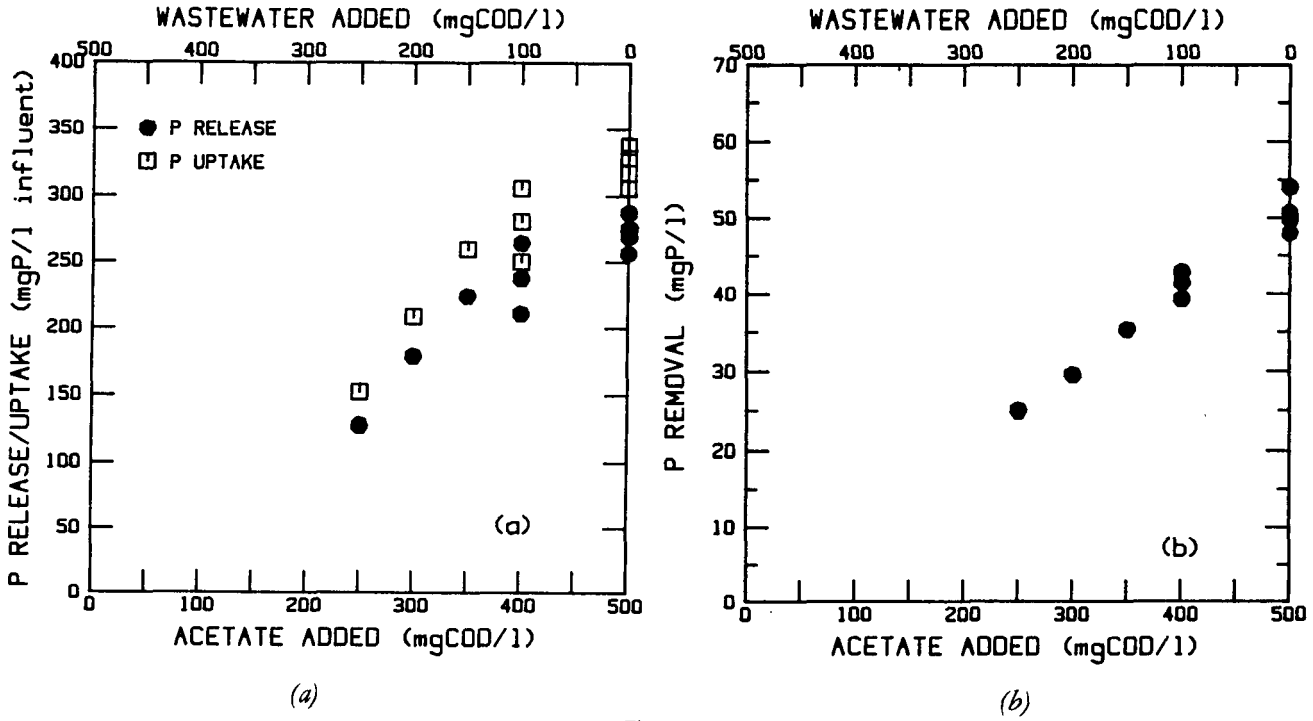
After the system with acetate as the sole carbon and energy source had been operating stably for about a month, the sludge again was analysed using the Analytical Profile Index (API) method. The analysis indicated that, of the organisms cultured aerobically, virtually 100 per cent were *Acinetobacter* spp. (Lötter *et al.*, 1988). An enhanced culture of the polyP organisms had been achieved.

TABLE 5
INFLUENT MACRO AND MICRO-NUTRIENT ADDED PER 1 000 mgCOD SODIUM ACETATE TO THE INFLUENT, ADAPTED FROM DU PREEZ (1980)

Chemical	mg added/1 000 mgCOD sodium acetate	
	Compound	Element
	Macro-nutrients	
$MgCl_2.6H_2O$	468	56 Mg
$CaCl_2.2H_2O$	117	32 Ca
Yeast extract	10	
	Micro-nutrients	
$FeSO_4.7H_2O$	5,25	1,05 Fe
$ZnSO_4.7H_2O$	1,5	0,34 Zn
$MnSO_4$	1,5	0,55 Mn
$CuSO_4.5H_2O$	0,3	0,076 Cu
$CoCl_2.6H_2O$	0,3	0,074 Co
$Na_2MoO_4.2H_2O$	0,15	0,059 Mo
H_3BO_3	0,3	0,052 B
KI	0,075	0,057 I

UCT system

Concomitant with the Bardenpho system, a UCT system was set up for development of another enhanced culture of polyP organisms in May 1985. A UCT system was selected as it reduces the nitrate recycled to the anaerobic reactor. Furthermore, a comparison of UCT system behaviour with that of the Bardenpho system would be possible. To obtain some information on the effect of sludge age on polyP organism behaviour, it was decided to operate the UCT system at a sludge age of 10 d, as opposed to the Bardenpho system sludge age of 20 d. The layout of the UCT system is given in Fig. 6. Following the success of operation of the Bardenpho system with a mixture of 250 mgCOD/ℓ acetate, 250



(a) *(b)*

Figure 5
Total soluble phosphorus (P) (a) release and uptake, and (b) removal in 3-stage Bardenpho system (Figs. 4 and 8) with increasing concentration of acetate in a total influent COD of 500 mg/ℓ (waste water + acetate).

TABLE 6
CHARACTERISTICS OF AN ENHANCED CULTURE AND A 'NORMAL' MIXED CULTURE UCT SYSTEM

Parameter	Enhanced cultures		'Normal' UCT mixed culture
	Bardenpho	UCT	
Sludge age (d)	20	10	20
Influent substrate	Acetate	Acetate	Unsettled municipal waste water
Influent COD (mgCOD/ℓ)	544	543	500
Effluent COD (mgCOD/ℓ)	62,1	64,9	40
P removal-ΔP (mgP/ℓ)	49,7	60,9	10
ΔP/ΔCOD (mgP/mgCOD)	0,10	0,12	0,02
P/VSS (mgP/mgVSS)	0,38	0,38	0,15
VSS/TSS (mgVSS/mgTSS)	0,46*	0,46*	0,75-0,85

* *in final aerobic reactor*

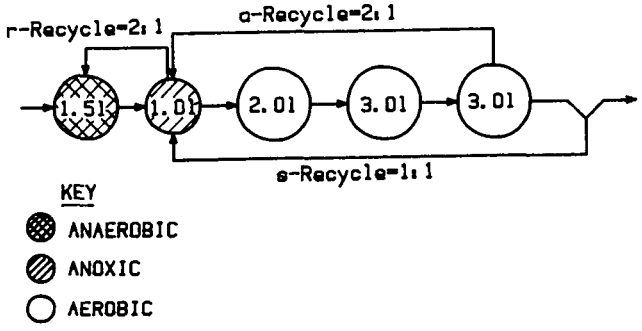


Figure 6
Schematic layout of UCT system; reactor volumes in litres, sludge age 10 d and influent flow rate 15ℓ/d.

mgCOD/ℓ sewage, yeast extract and mineral nutrients, and to acclimatise the organisms to acetate substrate, it was decided to start the UCT system with this influent mixture. Waste mixed liquor from the enhanced culture Bardenpho system, together with mixed liquor from a laboratory-scale modified UCT activated sludge system treating raw sewage, was used to inoculate the system. The acetate fraction of the influent again was increased incrementally, from 250 to 350 and finally to 500 mgCOD/ℓ, with a corresponding decrease in the sewage fraction – 250, 150 and 0 mgCOD/ℓ. Macro and micro-nutrients and yeast extract were added to the influent in the proportions set out in Table 5. As with the Bardenpho system, it was necessary to add HCl to the aerobic reactors of the UCT system, to maintain pH in the region 7,0 to 7,6; an increase in the acetate fraction of the influent necessitated a corresponding increase in the HCl added. The P removal versus time data, after the acetate concentration was increased from 350 to 500 mgCOD/ℓ, is shown in Fig. 7. This plot is instructive also in that it showed that stable P removal could be obtained for more than 100 d. The data obtained on P release and uptake (and hence removal) versus acetate added is shown plotted in Fig. 8; the responses are near linear. Again from these responses, it is clear that an enhanced culture of polyP organisms had been attained.

In both the Bardenpho and the UCT systems, some of the characteristics of the enhanced cultures were evaluated from the results during the steady state periods. These are listed in Table 6 and compared with the same parameters obtained on a UCT system with 20 d sludge age treating unsettled municipal waste water, also of 500 mgCOD/ℓ (see Table 3 (a) for characteristics of municipal waste water). The P/VSS ratios listed in Table 6 were obtained from mass balances of the average P removals and sludge wastages, and from averages of the measurement of the volatile solids. These ratios were checked by doing a number of direct measurements of P and volatile solids on the sludges. Statistically, the two values were not significantly different. Clearly, the sludges developed in the enhanced culture systems differed markedly from those found in normal activated sludge systems – the behaviour of the polyP organisms dominated in the enhanced culture systems, whereas it virtually was swamped out in the activated sludge system receiving municipal waste water as influent. This enhanced culture served as the base material for studying the *kinetic behaviour* of the polyP organisms. (Wentzel *et al.*, 1987).

System behaviour and problems

Decline in P uptake rate

On a number of occasions under steady state operation with an acetate-sewage mixture as influent, after the main problems regarding nutrients etc. had been resolved, a slow decline in P release, uptake and removal was observed. On each occasion deterioration in system response coincided with a new batch of sewage in the sewage-acetate mixture. Initially it was thought that the new batch of sewage was deficient in some essential nutrient. For this reason the influent concentrations of a number of nutrients were sequentially increased over a period of two weeks. Increasing the influent TKN, Mg^{2+}, Ca^{2+}, yeast extract and micro-nutrient concentrations had no effect on P release and removal. However, on changing to another sewage batch, P release, uptake and removal improved. During a period of decline a characteristic response was that P uptake continued to take place in the settling tank – as if the uptake rate appeared to have declined in the aerobic reactors. The relative magnitudes of P uptake in the aerobic reactors indicated that the P uptake reaction is of a first order nature, an observation verified in batch

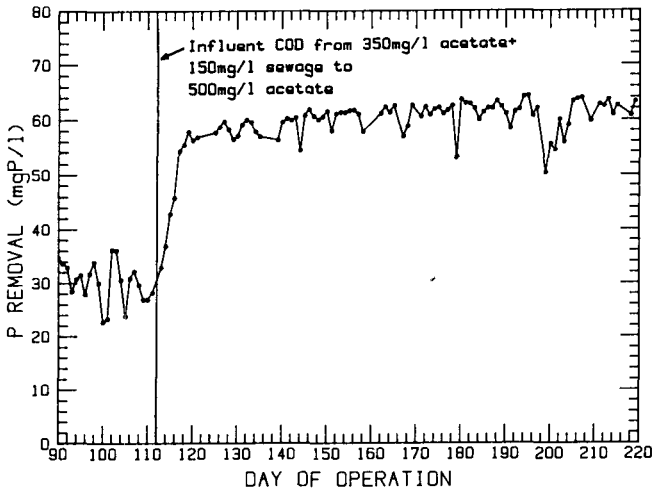


Figure 7
Total soluble phosphorus (P) removal versus time in the UCT system (Fig. 6) when the influent was changed from 350 mgCOD/ℓ acetate + 150 mgCOD/ℓ unsettled municipal sewage to 500 mgCOD/ℓ acetate on day 112.

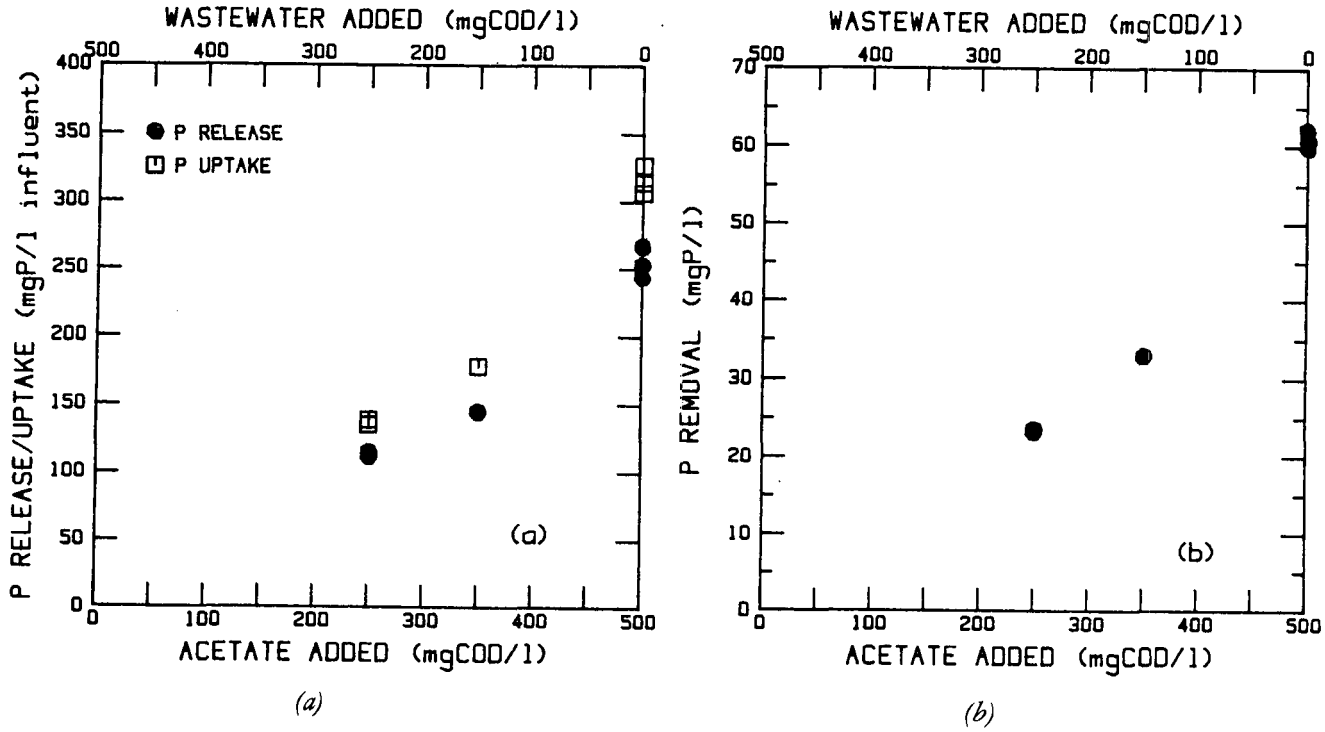


Figure 8
Total soluble phosphorus (P) (a) release and uptake, and (b)removal in UCT system (Fig. 6) with increasing concentration of acetate in a total influent COD of 500 mg/ℓ (wastewater + acetate).

tests. It was reasoned, in consequence, that uptake could be improved if the aerobic zone was converted to operate in a more plug flow fashion by recycling sludge from the second aerobic reactor to the anoxic reactor (a-recycle), and not from the last aerobic reactor (Fig. 9). Making this change to the Bardenpho system indeed did improve the uptake and removal (verified by making a similar change to the parallel UCT system). For the Bardenpho system, except for odd batches of sewage, the change caused the response of the system to be more stable. With these odd batches of sewage again it was noted that the lower removal was associated with P uptake in the settling tank; again it was concluded that the polyP organisms were not being given sufficient time to utilise all the stored PHB – it would appear that, if polyP organisms entered the anaerobic zone retaining unused PHB from the preceding aerobic phase, the metabolic controls of the organisms suffered a disturbance. Also, incomplete utilisation of PHB resulted in incomplete uptake of P. Thus, for the Bardenpho system, it was decided to aerate the underflow sludge prior to entry into the anaerobic reactor. The underflow rather than the mixed liquor was aerated because its concentration was double that of the mixed liquor and thus a much smaller size reactor was needed to obtain the desired mass fraction. A mass fraction of 9 per cent was selected, giving an actual retention time of 0,8 h. The reactor was completely mixed and aerated to maintain oxygen at about 2 to 4 mgO/ℓ.

Immediately after introducing the aeration reactor into the Bardenpho system receiving an influent mixture of 400 mgCOD/ℓ acetate and 100 mgCOD/ℓ sewage, P removal increased and stabilised, while P release decreased (from 240 to 210 mgP/ℓ influent, i.e. 0,48 to 0,42 mgP/mg influent COD). P uptake now was apparently complete because a net *release* of P of about 15 mgP/ℓ influent was observed in the re-aeration reactor. In general, provision of this extra aerobic zone very positively lent stability to the system. On introducing this reactor, there was a change in the P behavioural pattern in the anoxic reactor. Prior to underflow aeration a net P *uptake* was observed in the anoxic reactor; after underflow aeration commenced a net P *release* of about 5 to 10 mgP/ℓ influent was observed, a very small mass compared to the total release in the anaerobic reactor.

Settling behaviour

Also of interest is the settling response of the mixed liquor in both the Bardenpho and the UCT systems. Settling behaviour tended to follow P removal behaviour in a consistent fashion: A deterioration in P removal through operator error or system

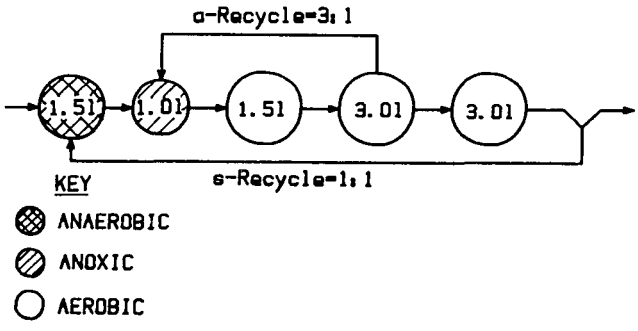


Figure 9
Schematic layout of 3-stage Bardenpho system with a-recycle from second aerobic reactor; reactor volumes in litres, sludge age 20 d and influent flow rate 15ℓ/d.

malfunctions resulted in a deterioration in the settling characteristics, indicated by an observable rise of the sludge level in the settling tank.

When settling was good, operational problems were experienced due to the rapid clumping of the sludge which caused blocking of the tubes connecting the reactors. Also, sludge in the settling tank tended to form bridges through which the liquid readily filtered. This bridge formation caused a build-up of sludge in the settler. A recycle was introduced from the underflow to the settler influent line. This prevented bridging in the sludge layer and effectively resolved the settler problem.

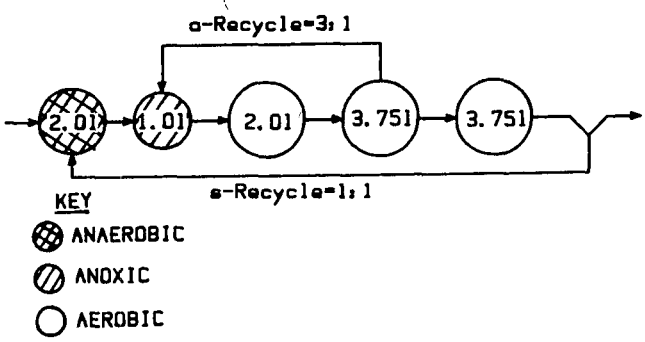


Figure 10
Schematic layout of 3-stage Bardenpho system; reactor volumes in litres, sludge age 10 d and influent flow rate 15ℓ/d.

Fluctuating decline in system response

After about six months operation of the systems at steady state with pure acetate, yeast extract and mineral nutrient influent, system P response started to exhibit fluctuations with a gradual deterioration in the P removal. Initially it was thought that this deterioration was due to a micro-nutrient deficiency – in Cape Town there are three sources for the water in the distribution system; these differ significantly in chemical quality. It was surmised that some of the source water may be deficient in micro-nutrients not provided in the added recipe. Consequently, it was decided to add to the influent as complete a micro-nutrient recipe as possible. The most extensive recipe in the literature is that of Kaiser and Hanselmann (1982). This recipe includes the elements Ni and Al in addition to those listed in Table 5. Accordingly these elements were added to the influent in the ratios suggested by Kaiser and Hanselmann. This, however, did not improve the response – P removal continued to decline, eventually to 10 mgP/ℓ influent (0,02 mgP/mgCOD), well below the expected 50 mgP/ℓ influent (0,1 mgP/mgCOD). The apparent independence of the system P performance on the influent micro-nutrient recipe led to the conclusion that some change had occurred in the structure of the bacterial population. An analysis of the sludge population using the API method indicated that only 25 per cent of the organisms cultured aerobically were *Acinetobacter* spp; the dominant primary organisms in the system were *Pseudomonas* spp. Although *Pseudomonas* spp. have been shown to accumulate P (Suresh *et al.*, 1984), this accumulation clearly is not of the same magnitude as the P accumulation exhibited by *Acinetobacter* spp.

The reasons for the gradual progress to dominance of the *Pseudomonas* spp. are not clear, but its commencement appeared to be associated with some malfunction of the system (e.g. blocking of tubes causing mixed liquor overflow) and/or its operation (e.g. breakdown of a pump). When, for example, the feed pump broke down, the practice was to feed the remaining daily feed at an augmented rate so as not to disturb the long-term steady state. Often, when this was done acetate 'leaked' through the anaerobic reactor; it is possible that the leakage initiates and promotes the growth of *Pseudomonas* spp. in the anoxic and aerobic zone, starting the process towards dominance of these organisms. Leakage of acetate also would bring about an associated reduction in the growth of polyP organisms, eventually to place the organisms at a competitive disadvantage in the anaerobic/anoxic/aerobic sequencing system. It would seem that great care must be taken not to 'overload' the polyP organisms in the anaerobic reactor to such a degree that leakage of acetate occurs from this reactor. This can be accomplished by observing two operational procedures; by limiting the acetate load increments and by enlarging the anaerobic reactor so as to accommodate any perturbation in acetate load and operating conditions.

To illustrate the instability that can develop in the system with small anaerobic mass fractions, a description of the subsequent development of an enhanced culture is instructive. A 3-stage Bardenpho system at 10 d sludge age with anaerobic mass fraction of 16 per cent, see Fig. 10, was started with mixed liquor from a laboratory-scale UCT system receiving unsettled municipal waste water. Acetate served as the sole influent substrate, with yeast extract and mineral nutrients added according to Table 5. The P removal versus time plot is shown in Fig. 11. The plot shows that when on day *12* the influent acetate concentration was increased from 250 to 350 mgCOD/ℓ, the system did not respond in a satisfactory manner; the removal decreasing to a minimum value by day *32*. However, when on day *41* the anaerobic mass fraction was increased from 16 to 32 per cent, the P removal increased in a fluctuating manner until the maximum removal was achieved, around day *78*. This highlights the importance of sufficient anaerobic mass fraction for enhanced culture development. Also, it is clear from this plot that the system can take considerable time to develop the 'mature' enhanced culture. This may not always be the case. On other occasions, see Fig. 7, the enhanced culture developed more rapidly.

Details of procedures recommended for the development of enhanced cultures of polyP organisms are given in Appendix A.

Discussion

From the experimental work outlined above it is evident that:

- An enhanced culture of polyP organisms was developed successfully by addition of acetate to the anaerobic reactor of anaerobic/aerobic sequencing activated sludge systems. When acetate served as the sole carbon and energy source, provided mineral nutrients and yeast extract also were added, almost 100 per cent of the organisms cultured aerobically were identified, using the API method, to be *Acinetobacter* spp., a polyP organism. In the enhanced cultures the behaviour of the polyP organisms dominated the system response. Furthermore, experimental response characteristics of the enhanced cultures obtained subsequently (Wentzel *et al.*, 1987) showed that the polyP organisms behaviour in the enhanced cultures approximated their behaviour in the 'normal' excess P removal activated sludge systems receiving municipal sewage. The enhanced cultures therefore served as a source for obtaining information on the stoichiometric and kinetic behaviour of polyP organisms in nutrient removal systems, such as the modified Bardenpho and UCT systems.

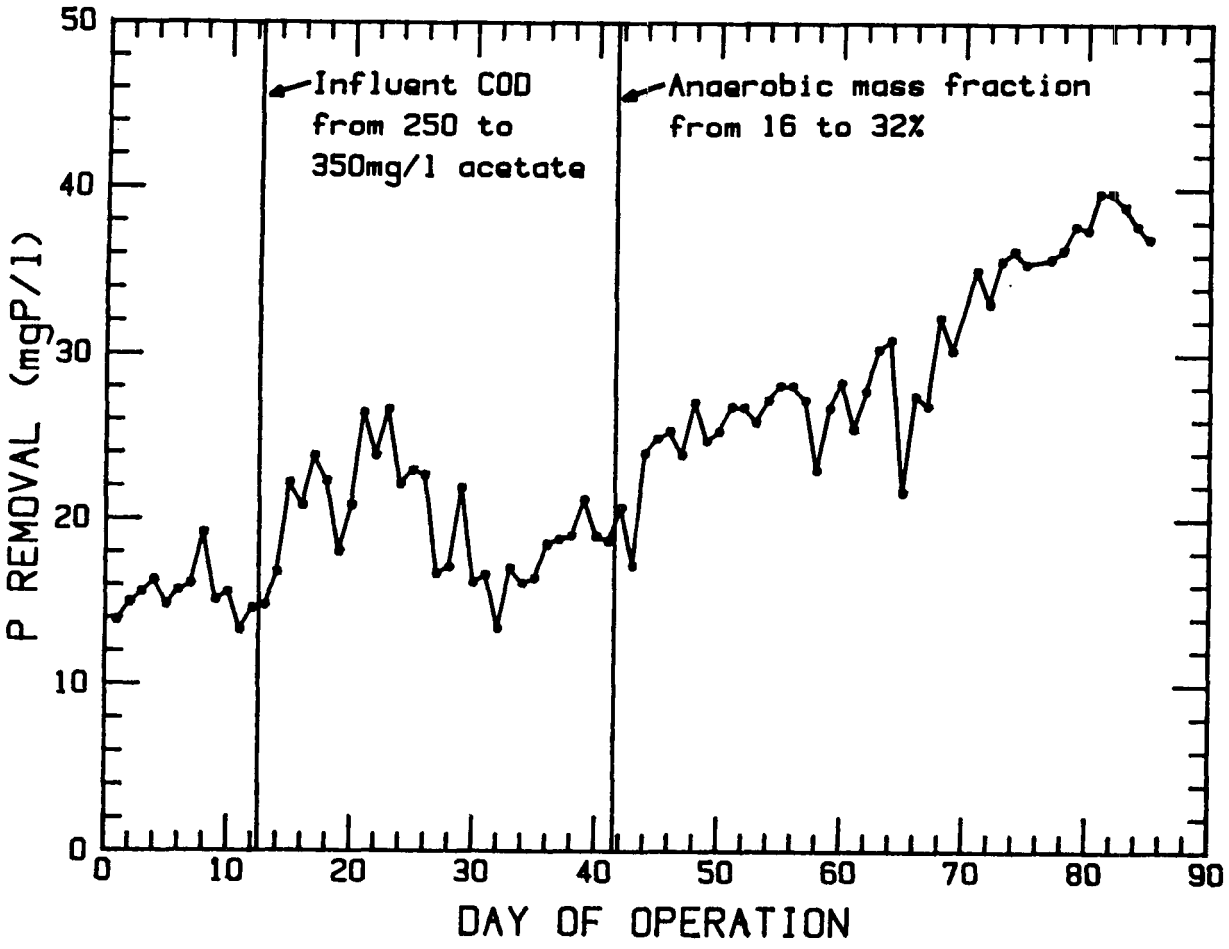


Figure 11
Total soluble phosphorus (P) removal versus time in the Bardenpho system (Fig. 10); influent changed from 250 mgCOD/ℓ acetate to 350 mgCOD/ℓ acetate on day 12 and anaerobic mass fraction increased from 16 to 32 per cent on day 41.

- Inorganic nutrients play a vital role in polyP accumulation. Potassium and magnesium are essential in biological excess P removal as these serve as counter-ions for stabilisation of the polyP chains. Calcium also has an important function in biological excess P removal, probably as a co-factor in biochemical reactions, and perhaps as another counter-ion in polyP stabilisation. These three elements, if they are present in insufficient quantities, limit excess P removal.

- Yeast extract appears to be essential in enhanced cultures of polyP organisms receiving a single substrate. The yeast extract provides growth factors essential for cell metabolism. In mixed cultures receiving multiple substrates these growth factors probably are supplied by other organism types present in the system.

- The P uptake reaction appears to be first order – improved system operation is attained by operating aerobic reactors as plug flow rather than as a completely mixed single reactor. There is also evidence that if the utilisation of PHB is not completed in the aerobic reactors it adversely affects the system P response. To overcome this problem it may prove beneficial to include an aeration reactor in the underflow recycle stream.

- The biological P release has a minor effect on anaerobic reactor pH, provided pH is close to 7,0. However, P uptake increases the pH of the mixed liquor in its passage through the series system of aerobic reactors. In enhanced culture systems, the pH has been observed to increase to above 9 which can cause collapse of the system. With enhanced culture systems it is necessary therefore to add acidity to each of the aerobic reactors to maintain the pH near about 7,5. A detailed biochemical explanation of this phenomenon has been given by Wentzel *et al.* (1986).

- In the anaerobic/aerobic system with acetate as substrate, *Pseudomonas* spp. may replace *Acinetobacter* spp. as the dominant primary organisms. All the factors giving rise to this shift in bacterial population structure are not yet clear, but certainly overloading of the organism mass in the anaerobic

reactor with acetate can induce this shift by leakage of acetate through the anaerobic reactor to the aerobic zone. *Pseudomonas* spp. do exhibit excess P removal, but at a magnitude much lower and also at P uptake rates much slower than those of *Acinetobacter* spp.

References

ABBOTT, B.J. (1973) Ethanol inhibition of a bacterium (*Acinetobacter calcoaceticus)* in a chemostat culture. *J. Gen. Microbiol.* **75** 383-389.

ABBOTT, B.J., LASKIN, A.I. and McCOY, C.J. (1973) Growth of *Acinetobacter calcoaceticus* on ethanol. *Appl. Microbiol.* **25** 787-792.

ABBOTT, B.J., LASKIN, A.I. and McCOY, C.J. (1974) Effect of growth rate and nutrient limitation on the composition and biomass yield of *Acinetobacter calcoaceticus. Appl. Microbiol.* **28** 58-63.

ANALYTLAB PRODUCTS (1977) Analytical Profile Index: Enterobacteriaceae and other gram negative bacteria.

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

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.

DOLD, P.L., EKAMA, G.A. and MARAIS, G.v.R. (1980) A general model for the activated sludge process. *Prog. Wat. Tech.* **12** 47-77.

DU PREEZ, J.C. (1980) Growth kinetic studies of *Acinetobacter calcoaceticus* with special reference to acetate and ethanol as carbon sources. Doctoral thesis, Univ. of Orange Free State, Bloemfontein, SA.

DU PREEZ, J.C., TOERIEN, D.F. and LATEGAN, P.M. (1981) Growth parameters of *Acinetobacter calcoaceticus* on acetate and ethanol. *Eur. J. Appl. Microbiol. Ecology.* **2** 119-138.

EKAMA, G.A., DOLD, P.L. and MARAIS, G.v.R. (1986) Procedures for determining influent COD fractions and the maximum specific growth rate of heterotrophs in activated sludge systems. *Wat. Sci. Tech.* **18** 91-114.

ENSLEY, B.D. Jr. and FINNERTY, W.R. (1980) Influences of growth substrates and oxygen on the electron transport system in *Acinetobacter* spp. *J. Bacteriol.* **142** 859-868.

FUHS, G.W. and CHEN, M. (1975) Microbiological basis of phosphate removal in the activated sludge process for the treatment of wastewater. *Microbiol Ecology.* **2** 119-138.

KAISER, J.P. and HANSELMANN, K.W. (1982) Fermentative metabolism of substituted monoaromatic compounds by a bacterial community from anaerobic sediments. *Arch. Microbiol.* **133** 185-194.

LÖTTER, L.H., WENTZEL, M.C., LOEWENTHAL, R.E., EKAMA, G.A. and MARAIS, G.v.R. (1986) A study of selected characteristics of *Acinetobacter* spp. isolated from activated sludge in anaerobic/anoxic/aerobic and aerobic systems. *Water SA* **12**(4) 203-208.

LÖTTER, L.H., WENTZEL, M.C., EKAMA, G.A. and MARAIS, G.v.R. (1988) An investigation into the heterotrophic bacterial population of various activated sludge plants. (In preparation).

MARAIS, G.v.R. and EKAMA, G.A. (1976) The activated sludge process Part I – steady state behaviour. *Water SA* **2**(4) 164-200.

SIEBRITZ, I.P., EKAMA, G.A. and MARAIS, G.v.R. (1980) Excess biological phosphorus removal in the activated sludge process at warm temperate climates. *Proc. Waste Treatment and Utilisation.* Vol. **2** 233-251. Robinson, C.W., Moo-Young, M. and Farquhar, G.J. (Editors) Pergamon Press, Toronto.

SIEBRITZ, I.P., EKAMA, G.A. and MARAIS, G.v.R. (1983) A parametric model for biological excess phosphorus removal. *Wat. Sci. Tech.* **15** 127-152.

SURESH, N., WARBURG, R., TIMMERMAN, M., WELLS, J., COCCIA, M., ROBERTS, M.F. and HALVORSON, H.O. (1984) New strategies for the isolation of micro-organisms responsible for phosphate accumulation. *Proc. IAWPRC Post-conf. Seminar on Enhanced Biological Phosphorus Removal from Wastewater;* Paris, Vol. 1 132-144.

VAN GROENESTIJN, J.W. and DEINEMA, M.H. (1985) Effects of cultural conditions on phosphate accumulation and release by *Acinetobacter* strain 210A. *Proc. of Int. Conf. on Management Strategies for Phosphorus in the Environment.* Selper Ltd., London.

WARSKOW, A.L. and JUNI, E. (1972) Nutritional requirements of *Acinetobacter* strains isolated from soil, water and sewage. *J. Bacteriol.* **112**(2) 1014-1016.

WENTZEL, M.C., DOLD, P.L., EKAMA, G.A. and MARAIS, G.v.R. (1985) Kinetics of biological phosphorus release. *Wat. Sci. Tech.* **17** 57-71.

WENTZEL, M.C., DOLD, P.L., LOEWENTHAL, R.E., EKAMA., G.A. and MARAIS, G.v.R. (1987) Experiments towards establishing the kinetics of biological excess phosphorus removal. In *Biological phosphate removal from wastewaters.* Ramadori, R. (Editor)Pergmon Press, Oxford.

WENTZEL, M.C., LÖTTER, L.H., LOEWENTHAL, R.E. and MARAIS, G.v.R. (1986) Metabolic behaviour of *Acinetobacter* spp. in enhanced biological phosphorus removal – a biochemical model. *Water SA* **12**(4) 209-224.

WATER RESEARCH COMMISSION (WRC) (1984) Theory design and operation of nutrient removal activated sludge processes. Published by WRC, P.O. Box 824, Pretoria 0001.

APPENDIX A

Procedures for development of an enhanced culture of polyP organisms

The following procedures are proposed to develop an enhanced culture of polyP organisms:

- The system must incorporate anaerobic/aerobic sequencing of the mixed liquor in which the recycling of NO_3^- to the anaerobic zone is kept to a minimum. This can be accomplished by setting up a UCT or a 3-stage Bardenpho system, the latter being the simplest. Systems with sludge ages of 10 and 20 d have been successfully used to obtain enhanced cultures. An anaerobic mass fraction of 30 per cent (for P release) and an anoxic mass fraction of about 8 per cent (for nitrate reduction) are recommended. The anaerobic mass fraction is greatly in excess of that theoretically required to sequester the acetate. Lower mass fractions (down to 10 per cent) have been used successfully but experience has shown that small fractions can, on occasion, result in 'leakage' of acetate through the anaerobic reactor; leakage appears to stimulate growth of *Pseudomonas* spp. which, once established, can lead to complete deterioration of polyP organism growth.

- The aerobic zone should consist of a series of reactors, to approach plug flow, rather than a single completely mixed reactor. This will promote greater efficiency in P uptake as the uptake reaction is of a first order nature. A minimum of three aerobic reactors in series is suggested. The a-recycle to the anoxic zone preferably should be from the second aerobic reactor in the series, at around 3:1 with regard to the influent flow. With some response situations it may help to include a small aerobic reactor ($\pm$ 8 per cent mass fraction) in the underflow recycle (see relevant section in the paper).

- The system may be started using mixed liquor from a municipal activated sludge system; preferably, but not necessarily, one that is exhibiting excess P removal.

- Acetate has been found to be a satisfactory substrate. (This does not imply that other lower fatty acids would be unsuitable). On starting the system, an acetate-sewage mixture, 5:95, is suggested as influent. The system is run until all the added acetate is removed in the anaerobic zone (or until P release appears to have attained a maximum steady state value). The acetate fraction of the influent then is increased incrementally at say 5 per cent increments, the sewage fraction being correspondingly decreased. Mineral nutrients and growth factors must be added to the influent in proportion to

the acetate added, as listed in Table 5. Sufficient P needs to be added to the influent to ensure that P always is present in the effluent, roughly 0,12 mgP/mgCOD as acetate. Addition of P as K_2HPO_4 will ensure sufficient K in the influent.

- Acid must be added as a sidestream to each aerobic reactor to maintain pH ± 7,4 to exclude precipitation of P and to prevent inhibition of the polyP organism due to too high a pH.

- Settling tank design must be adequate due to the unique settling problems of the enhanced culture. Settling tank preferentially should be of the sloping type (Marais and Ekama, 1976) with a recycle of about 1:1 from the underflow to the influent flow of the settler.

- The reactors should be equipped with extra overflow pipes because the sludge tends to block the tubes connecting the reactors. Trays should be provided underneath the system to catch spillage; such trays should be plastic coated to prevent contact of the sludge with zinc and other metal coatings. Contact with these coatings can act very adversely on the system response.

- The system volumes and influent loads must be selected so that VSS is less than 2 500 mgVSS/ℓ. The following design procedure, adapted from Water Research Commission (1984) is suggested:

$$MX_v = Q.S_{ti} \left(\frac{Y.R_s}{1 + b.R_s} \right) (1 + f.b.R_s) \quad (1)$$

where Q = influent flow rate (ℓ/d)
S_{ti} = influent COD concentration (mgCOD/ℓ as acetate, propionate, butyrate, lactate)
R_s = sludge age (d)
MX_v = system volatile solids mass (mgVSS)
Y = specific yield = 0,43 (mgVSS/mgCOD)
b = specific endogenous mass loss rate = 0,04(/d) for polyP organisms
f = endogenous residue fraction = 0,25 (mgVSS/mgVSS).

$$X_v = \frac{MX_v}{V} \quad (2)$$

$$X_t = X_v/f_i \quad (3)$$

where V = volume of system for modified Bardenpho (ℓ)
X_v = volatile solids concentration (mgVSS/ℓ)
X_t = total solids concentration (mgTSS/ℓ)
f_i = VSS/TSS = 0,46 (mgVSS/mgTSS)

- Fresh influent must be made daily and the influent kept in a covered drum at 4°C. The drum and feed line must be cleaned with boiling water daily.