Enhanced polyphosphate organism cultures in activated sludge systems. Part II: Experimental behaviour

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Abstract

In **Part I** of this series of papers, procedures were reported to develop enhanced cultures of polyphosphate organisms in modified Bardenpho and UCT systems. In this paper, from experimental observations on the steady state response of the enhanced culture systems and on batch tests in which mixed liquors drawn from these systems were subject to a variety of conditions, the compounds involved in biological excess P removal and the processes acting on these compounds are identified. A mechanistic model is proposed that explains the complex interactions between compounds and processes. Qualitatively the behaviour indicated by this model is in agreement with present understanding of the biochemistry of the biological excess P removal phenomenon.

Introduction

In **Part I** of this series of papers (Wentzel *et al.*, 1988), experimental procedures were reported whereby enhanced cultures of polyphosphate (polyP) organisms were developed in the modified Bardenpho and UCT systems. The mixed liquor in these systems were shown to comprise *Acinetobacter* spp., a polyP organism, in excess of 90 per cent. It was envisaged that, in these enhanced culture systems, the behaviour of the polyP organisms could be readily isolated because of the relatively pure assembly of these organisms. Furthermore, by developing the enhanced cultures in modified Bardenpho and UCT systems and by not positively excluding other organisms (e.g. predators), the polyP organism behaviour could be expected to conform reasonably closely to their behaviour in the normal mixed culture system.

In this paper we shall report on experimental investigations, using the enhanced cultures to identify the compounds influenced by biological excess phosphorus (P) removal; identify the processes that act on these compounds; and conceptualise a mechanistic model that qualitatively describes the kinetic and stoichiometric behaviour of the processes and compounds.

In a following paper (**Part III**) the kinetics of the processes identified as of importance in biological excess P removal and their stoichiometric effect on the compounds will be formulated and incorporated into a mathematical model.

Background

To set up an experimental protocol to achieve the objectives outlined above, one needs to have some initial conceptualisations of the processes and compounds that need to be monitored in the experiments, and the conditions under which these processes can be isolated and stimulated, i.e. some rudimentary conceptual model. As more information becomes available from the experimentation, aspects of the rudimentary model can be verified or improved on. This in turn provides guidance on further experimental tasks that need to be undertaken, so that both the model and the experimental investigation tend to evolve contemporarily. This certainly was the path followed in this study. Initial conceptualisation of the rudimentary model, and the experimental techniques used to obtain information were strongly influenced by:

- The approaches developed by Marais and Ekama (1976), Dold *et al.* (1980) and Van Haandel *et al.* (1981) in setting up a model to describe general activated sludge kinetics under aerobic, anoxic and anaerobic conditions.
- The biochemical model describing biological excess P removal by Comeau *et al.* (1985) and Wentzel *et al.* (1986).

Marais and co-workers found that description of the behaviour of activated sludge systems must include the growth (substrate utilisation) and endogenous mass loss processes. In the stoichiometry of growth, for every unit of substrate consumed, it is accepted that a constant fraction (the true specific yield) appears as new cell mass and the remainder is oxidised to generate energy for synthesis giving rise to an associated oxygen requirement. When attempting to obtain an estimate of the true specific yield, Marais and co-workers found that it cannot be determined directly from sludge production in systems operated under steady state because the active mass synthesised is subject to endogenous/maintenance/death processes. These processes not only reduce the active mass but also generate inert (endogenous) residues, both particulate and soluble, so that the observed volatile solids not only are less than that indicated by the true specific yield, but also are made up of active and endogenous particulate fractions, to give an apparent yield. Due to these endogenous effects, the apparent yield decreases as the sludge age of the system increases. If it is accepted that the endogenous residue generation is constant with respect to the active mass that disappears, then it is possible to obtain estimates of the true specific yield and endogenous mass loss by trial and error curve fitting to observed yields in a set of steady state responses over a range of sludge ages. However, Marais and Ekama (1976) found that the yield and endogenous mass loss effect act in a compensatory fashion; good 'fits' can be obtained by different pairs of true specific yield values and endogenous mass loss rates. They found it essential to obtain an independent assessment of one of the two processes. To achieve this, they established that the endogenous mass loss rate could be isolated by doing aerobic digestion batch tests on samples of mixed liquor without addition of substrate. From a semi-log plot of the oxygen utilisation rate versus time, they determined that the endogenous mass loss process conformed to first order kinetics with respect to the active mass and that the slope of the plot defines the specific endogenous mass loss rate. Applying this rate to the steady state systems over a range of sludge ages, and accepting the value for the unbiodegradable

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particulate endogenous residue fraction of the active mass measured by McCarty and Brodersen (1962) and Washington and Hetling (1965), they determined the true specific yield. In the enhanced cultures, in order to obtain information on the yield and endogenous mass loss, it was proposed to follow the approach of Marais and co-workers by:

- Running two steady state polyP organism enhanced culture systems at 10 and 20 d sludge ages respectively, to obtain apparent yield values.
- Conducting aerobic digestion batch tests on mixed liquor samples drawn from the respective steady state systems in order to determine endogenous mass loss characteristics, including stoichiometry and kinetics of the mass loss, generation of endogenous residue fractions and changes in stored polyP.

The biochemical model of Comeau *et al.* (1985), extended and modified by Wentzel *et al.* (1986) describes the behavioural patterns of the biochemical processes associated with biological excess P removal. From the biochemical model it is apparent that the approach of Marais and co-workers is not sufficient; due account must be taken of a number of additional processes and compounds that are unique to biological excess P removal. The biochemical model provides guidance on the selection of the processes and compounds and the essential requirements that make the excess P removal system work, namely an anaerobic/aerobic sequence with short-chain fatty acids present in the anaerobic stage. Also, the biochemical model can be used to develop experimental procedures because it identifies the conditions under which the short-chain fatty acids are sequestered by the polyP organisms, with associated P release, and the conditions for subsequent utilisation of the sequestered acids for growth with associated P uptake and polyP synthesis. To obtain information on the relevant processes and compounds, including kinetic information, it was proposed that a number of batch tests be undertaken using mixed liquor from the steady state enhanced culture systems:

(1) Add acetate under anaerobic conditions to observe sequestration of the acetate and associated P release.
(2) Repeat (1) above but follow with aeration to observe P uptake and oxygen utilisation rates, the latter giving a measure of stored substrate utilisation for synthesis of new cell mass and for P uptake.
(3) Repeat (2) above but, instead of aerating, add nitrate to the batch to observe denitrification behaviour.
(4) Add acetate under *aerobic* conditions to observe aerobic acetate sequestration.

Steady state response pattern

First test series

The two system configurations in which the enhanced cultures were developed are shown in Fig. 1 (a and b). Essentially the two systems conform to a 3-stage modified Bardenpho and a UCT system respectively. The enhanced culture systems were operated according to the procedures developed by Wentzel *et al.* (1988). The modified Bardenpho system was operated at 20 d sludge age and the UCT system at 10 d. Sludge age was maintained by hydraulic control, i.e. drawing off the required waste mixed liquor volume from the last aerobic reactor (Marais and Ekama, 1976). The substrate was sodium acetate, 500 mgCOD/ℓ; macro-nutrients N (35 mgN/ℓ), P (54-65 mgP/ℓ), Mg (30 mgMg/ℓ), K (149 mgK/ℓ) and Ca (15 mgCa/ℓ); micro-nutrients and growth factors as listed in Table 1. In both systems a sidestream of acid (hydrochloric) was added to each of the aerobic reactors to maintain the pH in the region ± 7,5; failure to do so resulted in a

TABLE 1
INFLUENT MICRO-NUTRIENTS ADDED PER 1 000 mgCOD SODIUM ACETATE

Chemical	mg added/1 000 mgCOD sodium acetate	
	Compound	Element
Yeast extract	50	
$FeSO_4 \cdot 7H_2O$	5,25	1,05 Fe
$ZnSO_4 \cdot 7H_2O$	1,5	0,34 Zn
$MnSO_4$	1,5	0,55 Mn
$CuSO_4 \cdot 5H_2O$	0,3	0,076 Cu
$CoCl_2 \cdot 6H_2O$	0,3	0,074 Co
$Na_2MoO_4 \cdot 2H_2O$	0,15	0,059 Mo
H_3BO_3	0,3	0,052 B
KI	0,075	0,057 I

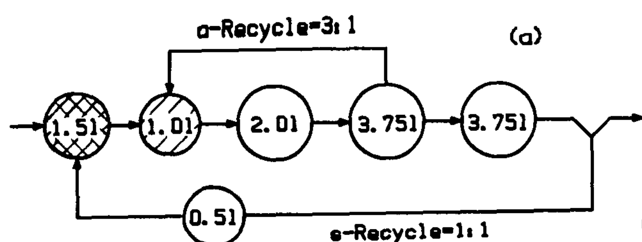

Figure 1(a)
Schematic layout of 3-stage modified Bardenpho polyP organism enhanced culture system, first test series.

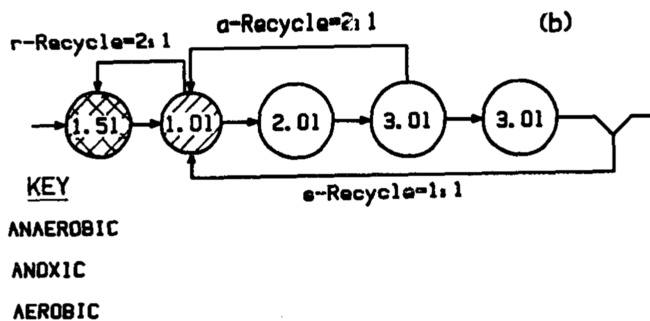

Figure 1(b)
Schematic layout of UCT polyP organism enhanced culture system.

sharp increase in the aerobic pH to values greater than 9 (as against ± 7,1 in the anaerobic zone). The necessity for acid addition is in accordance with the biochemical model of Wentzel *et al.* (1986) which predicts that the pH in the anaerobic zone would not change significantly, but would rise in the aerobic zone.

After a system had attained steady state, the system response was monitored for an extended period, about four months. The following parameters were measured: unfiltered influent and filtered effluent COD, TKN, total P and nitrate (NO_3); filtered reactor contents total P and NO_3; pH in all reactors; oxygen utilisation rates (OUR) in the aerobic reactors; total suspended solids (TSS) and volatile suspended solids (VSS) of the waste mixed liquor. Population structures of the mixed liquor samples also were analysed by the Scientific Services branch of the Johannesburg City Council using the Analytical Profile Index (API) procedure (Lötter *et al.*, 1986).

The averages of the results of all tests over the steady state periods are summarised in Table 2. Mass balances on the COD and nitrogen give recoveries of 92 and 103 per cent respectively for the modified Bardenpho system and 90 and 104 per cent respectively for the UCT system. Recoveries of greater than 90 per cent usually are indicative of acceptable mass balances (Marais and Ekama, 1976). Mass balances on P and nitrate were calculated relative to the influent flow and are shown in Table 2. From the mass of the P removed and the mass of volatile solids wasted per day, the P/VSS ratio was calculated. This ratio was checked by doing a number of direct measurements of P and volatile solids on the mixed liquor. Statistically, the two values were not significantly different. The ratios also are given in Table 2.

TABLE 2
STEADY STATE RESPONSE OF THE 20 DAY SLUDGE AGE 3-STAGE MODIFIED BARDENPHO AND 10 DAY SLUDGE AGE UCT ENHANCED CULTURE SYSTEMS

3-Stage Bardenpho system

Parameter	Value	Parameter	Value
Substrate	Acetate	VSS/TSS (mgVSS/mgTSS)	0,48
Sludge age (d)	20	ΔVSS/influent COD (mgVSS/mgCOD)	0,24
Flow rate (ℓ/d)	15	P removal (mgP/ℓ)	49,7
% *Acinetobacter* spp.*	90	P/VSS (mgP/mgVSS)	0,38
VSS (mgVSS/ℓ)	2998	ΔP/influent COD (mgP/mgCOD)	0,09
TSS (mgTSS/ℓ)	6181		

	Influent	Reactor 1	Reactor 2	Reactor 3	Reactor 4	Reactor 5	Effluent	Underflow aeration
COD (mgCOD/ℓ)	544	–	–	–	–	–	62	–
TKN (mgN/ℓ)	35,2	–	–	–	–	–	2,7	–
PO_4 (mgP/ℓ)	53,6	145,8	76,8	49,1	23,4	5,6	3,9	22,2
NO_3 (mgN/ℓ)	0	1,0	7,4	11,4	12,0	11,5	11,5	10,3
OUR (mgO/ℓ.h)	–	–	–	38,5	17,8	9,5	–	14,0
PO_4 change (mgP/ℓ influent)**	–	+ 215,7	+ 22,4	– 138,6	– 128,6	– 39,1	–	+ 18,4
NO_3 change(mgN/ℓ influent)**	–	– 8,2	– 1,3	+ 20,0	+ 3,3	– 1,1	–	– 1,2
pH	–	7,19	7,33	7,47	7,59	7,65	–	7,56

UCT system

Parameter	Value	Parameter	Value
Substrate	Acetate	VSS/TSS (mgVSS/mgTSS)	0,46
Sludge age (d)	10	ΔVSS/influent COD (mgVSS/mgCOD)	0,30
Flow rate (ℓ/d)	15	P removal (mgP/ℓ)	60,9
% *Acinetobacter* spp.*	90	P/VSS (mgP/mgVSS)	0,38
VSS (mgVSS/ℓ)	2397	ΔP/influent COD (mgP/mgCOD)	0,11
TSS (mgTSS/ℓ)	5224		

	Influent	Reactor 1	Reactor 2	Reactor 3	Reactor 4	Reactor 5	Effluent
COD (mgCOD/ℓ)	543	–	–	–	–	–	65
TKN (mgN/ℓ)	36,0	–	–	–	–	–	2,7
PO_4 (mgP/ℓ)	63,7	157,5	86,3	48,2	12,7	4,8	2,9
NO_3 (mgN/ℓ)	0	0,5	2,9	7,1	8,0	7,0	7,0
OUR (mgO/ℓ.h)	–	–	–	39,5	17,5	9,0	–
PO_4 change (mgP/ℓ influent)**	–	+ 236,2	+ 17,0	– 152,6	– 141,7	– 19,7	–
NO_3 change (mgN/ℓ influent)**	–	– 4,4	– 7,0	+ 16,9	+ 3,6	– 2,0	–
pH	–	7,18	7,27	7,30	7,59	7,61	–

* % of organisms cultured aerobically determined to be *Acinetobacter* spp. using API.
** + indicates P release/nitrification, – indicates P uptake/denitrification.

From Table 2, taking the UCT system as an example, the most striking features in the system response are:

- The specificity of the population structure; more than 90 per cent of the organisms cultured aerobically were identified to be *Acinetobacter* spp. using the API procedure.
- The extremely high phosphorus content of the sludge in the aerobic reactor, 0,38 mgP/mgVSS. This gives rise to a VSS/TSS ratio of 0,46 mgVSS/mgTSS, as against the usual 0,75 to 0,85 in activated sludge systems.
- The magnitudes of the P release, uptake and removals; 253 mgP/ℓ release (anaerobic + anoxic reactors), 314 mgP/ℓ uptake (aerobic reactor) giving a net removal of 61 mgP/ℓ (concentration with respect to influent flow).
- The high filtered effluent COD concentration. All the substrate acetate was removed in the anaerobic and anoxic zones and an apparently unbiodegradable COD was generated in the system, giving a net effluent COD concentration of 65 mgCOD/ℓ. This effluent value is considerably higher than that expected for an acetate feed to a normal activated sludge system, 15 to 20 mgCOD/ℓ. The most likely reason is the high specificity of the organism assembly – by-products generated cannot be used by the specific organism mass of the enhanced culture, whereas in a mixed culture these by-products are likely to serve as a substrate source for other species.
- The low nitrate (NO_3) removal, about 11 mgN/ℓ. Despite the fact that the influent was nearly totally readily biodegradable, and high concentrations of internally stored acetate (as PHB) were present in the anoxic zone, NO_3 removal was poor indicating that only a small fraction of the *Acinetobacter* spp. could use NO_3 as an electron acceptor. This is supported by the observation that usually no P was taken up in the anoxic reactors of the enhanced culture systems. Batch tests with nitrate as electron acceptor verified this behaviour (see under *BATCH TESTS*).
- Utilisation of NO_3 as a nitrogen source for growth. Nitrification was virtually complete in the first aerobic reactor; in the third aerobic reactor the NO_3 concentration decreased, indicating a utilisation of NO_3 as a nitrogen source for synthesis; this was confirmed by batch tests (see under *BATCH TESTS*).

Second test series

The first test series were conducted at sludge ages of 20 and 10 d using the 3-stage modified Bardenpho and UCT systems respectively. A second series of tests then were inaugurated with the objectives to investigate the effects of sludge age and influent COD on the enhanced culture steady state response pattern. Both the systems in the first series gave results consistent with each other. Hence, for reasons of convenience, the system selected for the second series was the 3-stage modified Bardenpho. In the first test series the modified Bardenpho system was operated only at 20 d sludge age. For the second series the system was operated at 10 and 7,5 d sludge age. The influent COD was reduced from the 500 mgCOD/ℓ acetate in the first series to 350 mgCOD/ℓ acetate in the second series; the added mineral nutrients were decreased proportionally. From the first series of tests and the associated batch tests it was apparent that the anaerobic mass fraction of the 20 d sludge age 3-stage modified Bardenpho system (12 per cent) would be too small to enable all the acetate to be sequestered at shorter sludge ages and accordingly the anaerobic mass fraction was increased to 31 per cent. This mass fraction is in excess of that theoretically required for complete sequestration but experience indicated that a larger anaerobic mass fraction than the theoretical was necessary to ensure that no acetate 'leaked' through the anaerobic zone in event of operator error or system malfunction (Wentzel *et al.*, 1988). The anaerobic mass fraction was divided between two reactors in series. This has the advantage that it allows the rate of acetate uptake to be determined experimentally in the first reactor. The layout of the system is shown in Fig. 1(c).

The systems were operated at steady state for 3 to 4 sludge ages using the same operational and monitoring procedures as in the first series.

The average results over the steady state periods are summarised in Table 3. Mass balances on the COD and nitrogen give recoveries of 91 and 90 per cent respectively for the 10 d sludge age system, and 93 and 95 per cent respectively for the 7,5 d sludge age system; these percentage recoveries, as mentioned earlier, indicate that the results are acceptable. Mass balances on P and nitrate were calculated relative to the influent flow, see Table 3. Mass balances on the P removed by the systems, and on the mixed liquor wasted per day, were used to determine the P/VSS ratios. These ratios also are given in Table 3.

The results obtained in the second series are consistent with those of the first series, viz. low VSS/TSS and high P/VSS ratios, large magnitudes of the P release, uptake and removal, high effluent CODs, poor denitrification and utilisation of nitrate as a nitrogen source for synthesis.

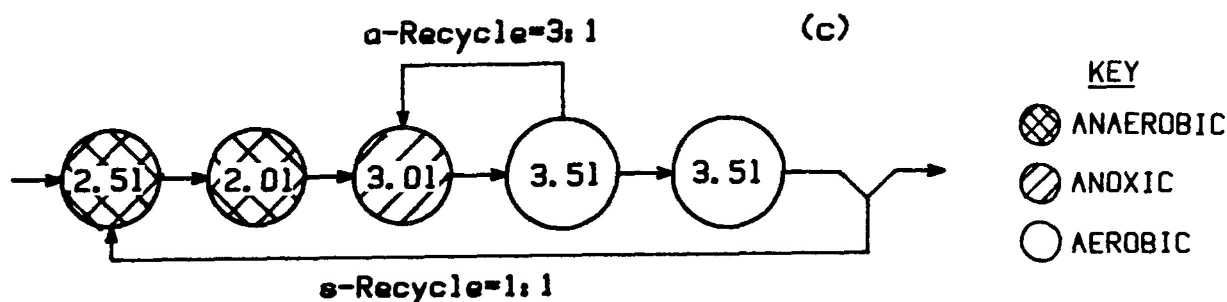

Figure 1(c)
Schematic layout of 3-stage modified Bardenpho polyP organism enhanced culture system, second test series.

TABLE 3
STEADY STATE RESPONSE OF THE 10 AND 7,5 DAY SLUDGE AGE 3-STAGE MODIFIED BARDENPHO ENHANCED CULTURE SYSTEMS

3-Stage Bardenpho system

Parameter	Value	Parameter	Value
Substrate	Acetate	VSS/TSS (mgVSS/mgTSS)	0,46
Sludge age (d)	10	ΔVSS/influent COD (mgVSS/mgCOD)	0,27
Flow rate (ℓ/d)	15	P removal (mgP/ℓ)	38,6
		P/VSS (mgP/mgVSS)	0,34
VSS (mgVSS/ℓ)	1167	ΔP/influent COD (mgP/mgCOD)	0,09
TSS (mgTSS/ℓ)	2547		

	Influent	Reactor 1	Reactor 2	Reactor 3	Reactor 4	Reactor 5	Effluent
COD (mgCOD/ℓ)	417	–	–	–	–	–	62
TKN (mgN/ℓ)	26,9	–	–	–	–	–	2,5
PO_4 (mgP/ℓ)	43,8	115,8	134,0	72,9	38,5	7,6	5,2
NO_3 (mgN/ℓ)	0	0,3	0,5	2,4	4,0	3,7	3,7
OUR (mgO/ℓ.h)	–	–	–	–	23,0	8,9	–
PO_4 change (mgP/ℓ influent)**	–	+ 181,0	+ 37,9	– 18,9	– 172	– 66,5	–
NO_3 change (mgN/ℓ influent)**	–	– 3,2	+ 0,5	– 1,06	+ 8,0	– 0,56	–
pH	–	7,09	7,10	7,40	7,71	7,70	–

Parameter	Value	Parameter	Value
Substrate	Acetate	VSS/TSS (mgVSS/mgTSS)	0,45
Sludge age (d)	7,5	ΔVSS/influent COD (mgVSS/mgCOD)	0,33
Flow rate (ℓ/d)	15	P removal (mgP/ℓ)	42,6
		P/VSS (mgP/mgVSS)	0,32
VSS (mgVSS/ℓ)	1036	ΔP/influent COD (mgP/mgCOD)	0,10
TSS (mgTSS/ℓ)	2283		

	Influent	Reactor 1	Reactor 2	Reactor 3	Reactor 4	Reactor 5	Effluent
COD (mgCOD/ℓ)	410	–	–	–	–	–	53
TKN (mgN/ℓ)	26,3	–	–	–	–	–	2,5
PO_4 (mgP/ℓ)	46,0	102,1	132,1	72,6	38,2	8,2	3,4
NO_3 (mgN/ℓ)	0	0,3	0,6	2,2	3,5	3,3	3,3
OUR (mgO/ℓ.h)	–	–	–	–	20,4	7,6	–
PO_4 change (mgP/ℓ influent)**	–	+ 154,9	+ 60,0	– 15,8	– 172,2	– 69,6	–
NO_3 change (mgN/ℓ influent)**	–	– 2,7	+ 0,5	– 0,6	+ 6,4	– 0,3	–
pH	–	7,18	7,18	7,45	7,74	7,74	–

* % of organisms cultured aerobically determined to be *Acinetobacter* spp. using API.
** + indicates P release/nitrification, – indicates P uptake/denitrification.

Batch tests

To obtain further information on the different polyP organism processes, e.g. acetate uptake, P release and uptake, growth rates, endogenous mass loss, etc., series of batch tests were undertaken using mixed liquor from the steady state enhanced culture systems. As discussed earlier, five types of batch tests were undertaken: (1) aerobic digestion of the mixed liquor; (2) response with addition of acetate under anaerobic conditions; with either (3) aerobic; or (4) anoxic response thereafter; and (5) response with acetate addition under aerobic conditions. In addition, data were accumulated from these batch tests to investigate (6) cation release and uptake.

(1) Aerobic digestion

The objective of this series of batch tests was to obtain information on endogenous mass loss behaviour. The procedure followed for these tests was that developed by Marais and Ekama (1976): Mixed liquor was drawn from the last aerobic reactor of the enhanced culture systems and placed in a continuously stirred batch reactor, without addition of substrate. At the start of the test mixed liquor samples were drawn from the batch for VSS and TSS determinations. The dissolved oxygen (DO) in the batch was monitored continuously by means of a Yellow Springs oxygen meter connected to a strip recorder. An electronic controller turned on aeration when the DO dropped below 5 mgO/ℓ, and turned off aeration when it rose above 7,5 mgO/ℓ. Floating plastic balls were used to cover the surface of the mixed liquor to minimise DO transfer at the air/liquid interface. The rate of DO decrease during the air off period gave the oxygen utilisation rate (OUR). The pH in the batch also was monitored continually and controlled to remain in the region 7,4 to 7,6 by addition of hydrochloric acid or sodium bicarbonate when necessary. At regular intervals, mixed liquor samples were drawn from the batch reactor and filtered immediately. The filtrate was analysed

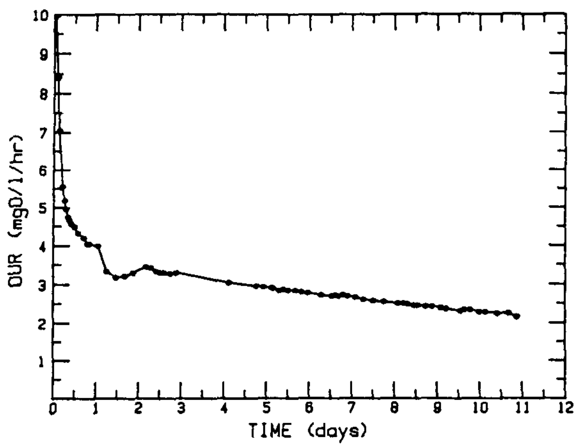

Figure 2(a)
Oxygen utilisation rate (OUR) response with time in a batch aerobic digestion of mixed liquor drawn from the enhanced culture system (VSS = 2 400 mgVSS/ℓ).

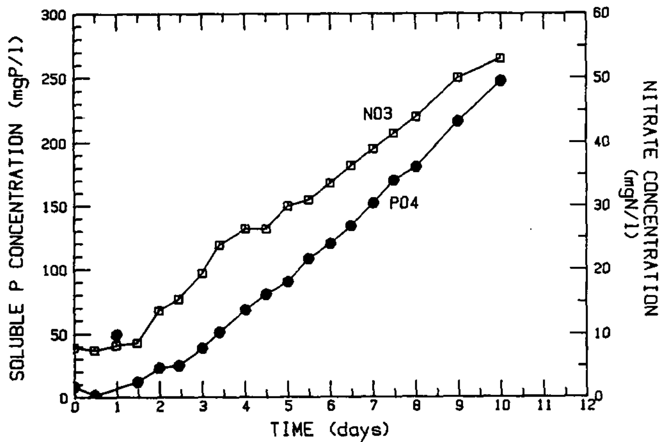

Figure 2(b)
Total soluble phosphate (PO_4) and nitrate (NO_3) concentration-time profiles for the batch aerobic digestion in Fig. 2(a).

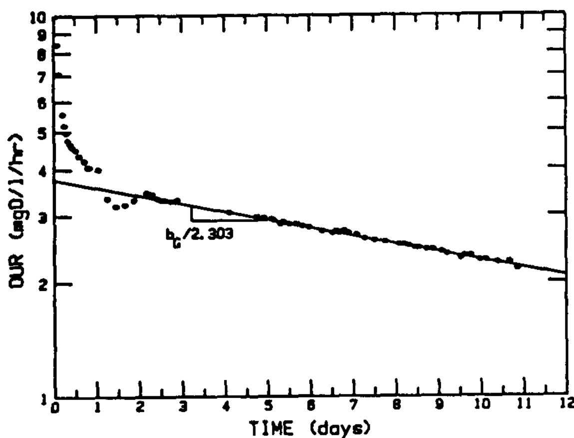

Figure 3
Semilog plot of oxygen utilisation rate (OUR) versus time for the data in Fig. 2(a).

for total P and NO_3 concentrations.

A number of batch tests were run for periods of up to 10 d; it was found that after about 10 d a qualitative change occurred in the endogenous mass loss behaviour in the batch tests, manifested by an increase in the OUR. Most probably, this was due to the development of predatory and other primary organisms in the batch. Typical response time relationships obtained are shown in Fig. 2 (a and b). The following features merit comment:

- The OUR in Fig. 2(a) is shown plotted log OUR versus time in Fig. 3. During the initial stages stored PHB still is available giving rise to a high OUR, but reduces thereafter to that associated with endogenous mass loss only. In the true endogenous phase (> 2 d) the OUR follows a first order rate with respect to the active VSS concentration. The slope of the log OUR defines the specific endogenous mass loss rate constant (b_G), i.e. b_G = 2,303.slope (log OUR). (This slope is independent of nitrification, Marais and Ekama, 1976). The b_G values from a number of such tests are shown plotted on normal probability paper in Fig. 4 giving a mean value for b_G of 0,04/d and standard deviation of the mean of ± 0,004. This mean value is very low compared with 0,24/d found for normal activated sludge (Marais and Ekama, 1976). This would indicate that very little or no species interaction, such as predation, is present; with "normal" activated sludge, species interaction has been implicated as the principal cause for the high "endogenous" rate constant observed (Dold *et al.*, 1980).
- P and nitrate concentration (Fig. 2b) both show an initial decrease, associated with stored PHB utilisation, followed by an increase, associated with endogenous mass loss. If the P release is related directly to the volatile mass loss (i.e. lysis of the P content of the mass lost) then the P concentration changes will be stoichiometrically related to the volatile mass loss, so also the nitrate (NO_3). To check this, plots were made of the concentrations of P and NO_3 versus the cumulative oxygen utilised in the digestion tests (i.e. the integral under the OUR curve), in Fig. 5. Both plots appear to

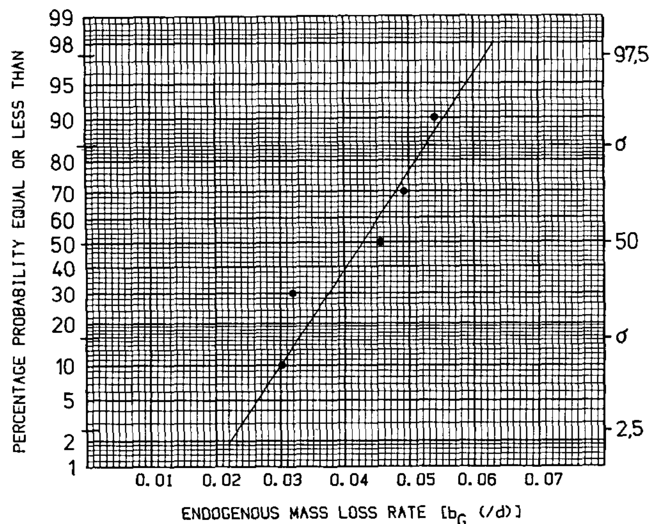

Figure 4
Statistical plot of endogenous mass loss rates (b_G) obtained from a number of aerobic digestion batch tests.

be linear in the endogenous region, thereby indicating that the P released and NO_3 generated are stoichiometrically related to the endogenous mass loss. This conclusion is true irrespective of whether all the volatile mass lost is oxidised, i.e. endogenous residue is zero, or only a fraction is oxidised with a remaining fraction being unbiodegradable, provided the fraction remains constant with time. Similarly b_G also is independent of the fraction of the endogenous mass lost that is oxidised for energy, again provided the fraction remains constant. Thus, b_G can be obtained directly from experimental observation, but the *fraction* of the endogenous mass loss that is oxidised for energy must be determined by curve fitting the theoretical OUR against the OUR observed in the digestion test, taking due account of nitrification.

Subsequently, when the kinetic model was developed and attempts were made to fit the predicted behaviour in the batch to that observed, it was found necessary to include the generation of a soluble unbiodegradable COD during the endogenous process to obtain consistency between predicted and observed data. To check whether such generation of COD did in fact occur, the aerobic digestion batch tests described above were repeated, monitoring the filtered COD over the test period. Typical results from such a test are shown plotted in Fig. 6 (a, b and c). Referring to Fig. 6 (a and b) the OUR, P and NO_3 concentration profiles conform to those in Fig. 2 (a and b). With regard to the filtered COD concentration profile (Fig. 6(c)), the COD increases with time during the course of the batch test. This verifies that, in addition to the usual generation of a particulate endogenous residue during endogenous mass loss, there is a generation of soluble endogenous 'residue' (COD). Most likely soluble residue generation is due to the high specificity of the organism population as by-products generated during endogenous mass loss processes cannot be utilised by the specific organism mass of the enhanced culture; in normal mixed culture systems, these by-products very likely would serve as a substrate for other species (This soluble unbiodegradable COD generation already had been noted earlier in the steady state systems).

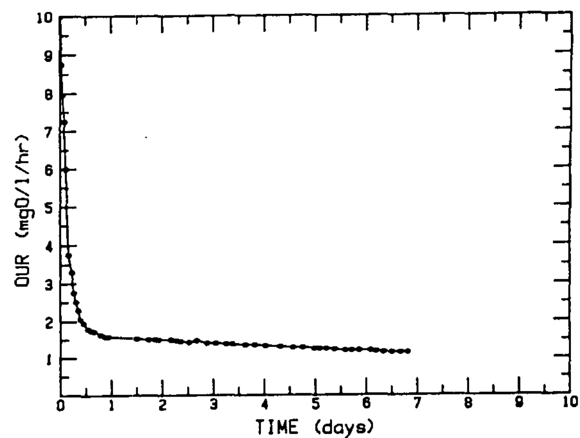

Figure 6(a)
Oxygen utilisation rate (OUR) response with time in a batch aerobic digestion of mixed liquor drawn from the enhanced culture system (VSS = 1 096 mgVSS/ℓ).

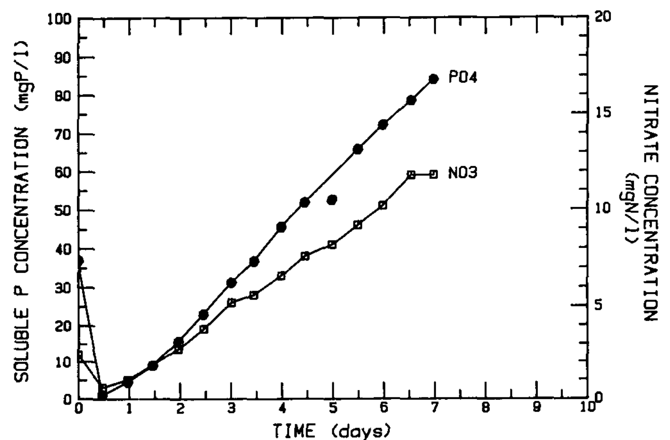

Figure 6(b)
Total soluble phosphate (PO_4) and nitrate (NO_3) concentration-time profiles for the batch aerobic digestion in Fig. 6(a).

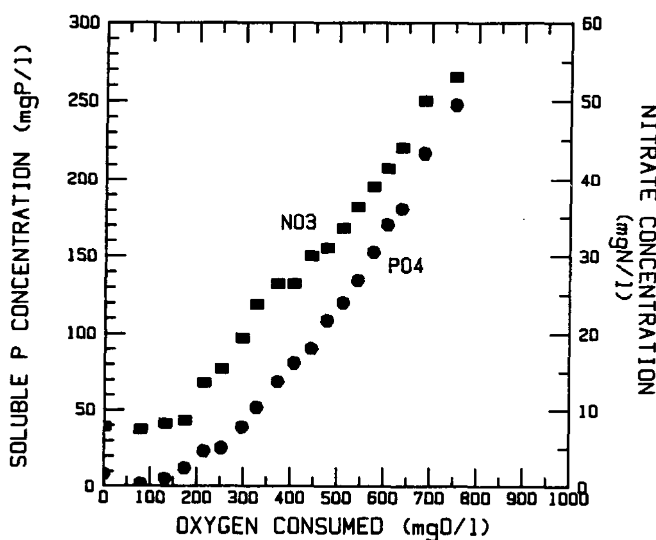

Figure 5
Plot of total soluble phosphate (PO_4) and nitrate (NO_3) concentrations (from Fig. 2(b)) versus cumulative oxygen consumed (from Fig. 2(a)), in the batch aerobic digestion in Fig. 2(a and b).

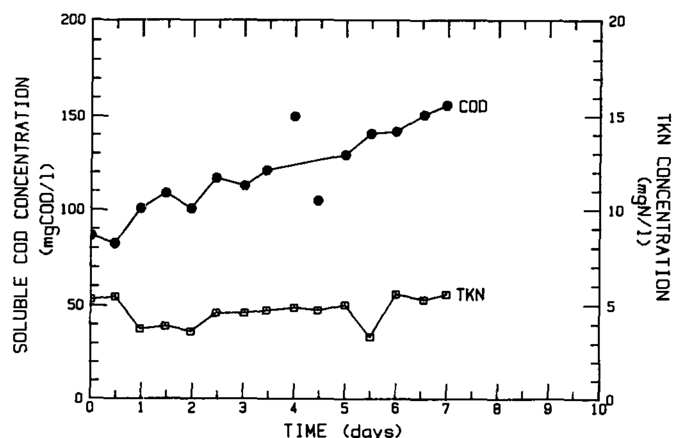

Figure 6(c)
Soluble COD and TKN concentration-time profiles for the batch aerobic digestion in Fig. 6(a).

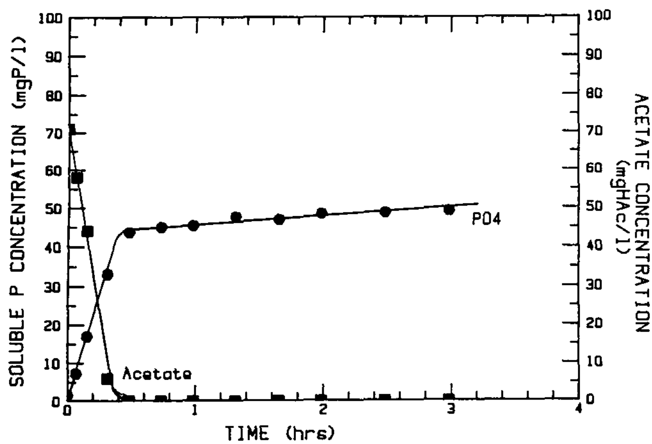

Figure 7
Total soluble phosphate (PO_4) and acetate concentration-time profiles with anaerobic addition of 0,11 mgCOD acetate/mgVSS to a mixed liquor batch drawn from the Bardenpho enhanced culture system (VSS = 684 mgVSS/ℓ).

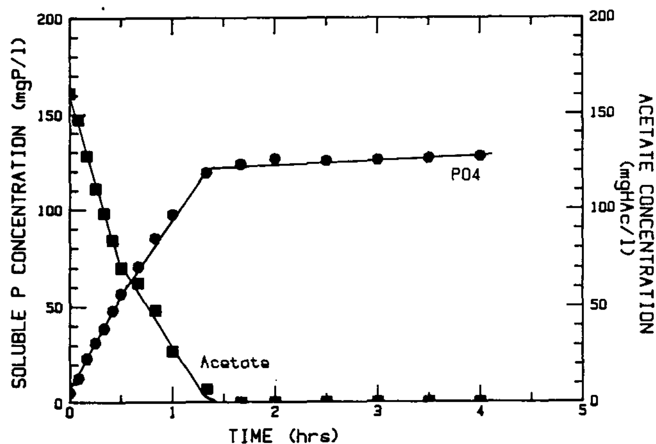

Figure 8
Total soluble phosphate (PO_4) and acetate concentration-time profiles with anaerobic addition of 0,265 mgCOD acetate/mgVSS to a mixed liquor batch drawn from the Bardenpho enhanced culture system (VSS = 651 mgVSS/ℓ).

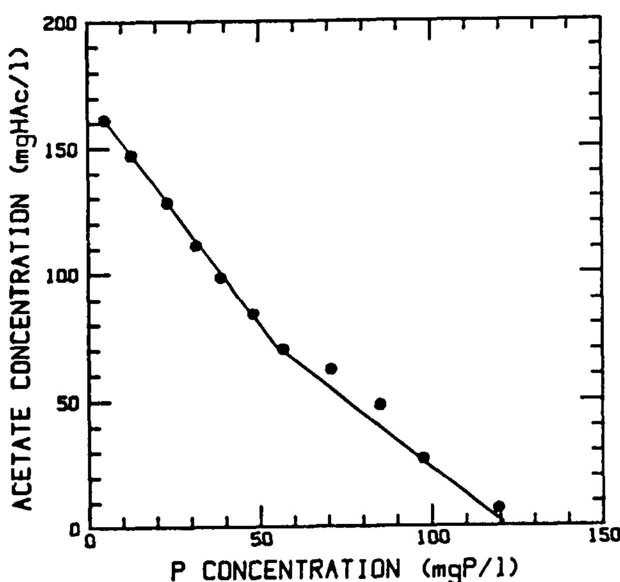

Figure 9
Plot of acetate concentration versus total soluble phosphate concentration for the time paired data in Fig. 8.

(2) Anaerobic response with acetate addition

The objective of this series of batch tests was to gain information on the kinetic behaviour of the anaerobic sequestration reactions with associated P release hypothesized in the biochemical models.

Batches of mixed liquor were drawn from the last aerobic reactor of the enhanced culture systems, diluted appropriately, so as to reduce the rates of reaction per litre batch volume for accurate measurements, and placed in covered, continuously stirred, batch reactors. Nitrogen gas was bubbled continuously through the batch mixed liquor to strip out any oxygen and to prevent oxygen ingress into the batch during the course of the experiment. The effect of nitrate (NO_3) was eliminated by keeping the batch unaerated until the nitrate was depleted before adding the acetate. The pH in the batch was continuously monitored and maintained in the region 7,4 to 7,6. At intervals samples were taken from the batch and filtered immediately. The filtrate was analysed for total P, acetate and NO_3 concentrations. (In all these tests NO_3 concentrations were zero). A series of tests was undertaken with serially increasing additions of acetate per mgVSS. The following observations were made:

(i) With relatively low acetate addition, i.e. 0,11 mgCOD acetate/mgVSS (Fig. 7), the disappearance of acetate is linear, so also the release of P. This implies that the reactions are independent of the acetate concentration and probably dependent only on the polyP organism concentration. When the acetate is depleted, P release continues, but at a greatly reduced rate (see also Wentzel *et al.*, 1985).

(ii) With relatively high acetate addition, 0,265 mgCOD acetate/mgVSS (Fig. 8), while acetate is present, both the disappearance of the acetate and the release of P show a two-phase linear relationship. The first phase conforms to that in (i) above. The rate in the second phase is slower than that in the first phase (about half) and shows a different stoichiometric ratio between P released and acetate taken up. The point in the test at which the change occurs is not easily determined from the concentration-time profiles, but can be more clearly distinguished by plotting the time paired data of acetate and P against each other (Fig. 9). From a series of such tests on a particular mixed liquor it was found that the first linear phase operated for acetate addition up to approximately 0,14 mgCOD/mgVSS. With higher additions of acetate the first phase operates until approximately 0,14 mgCOD/mgVSS acetate has been sequestered, thereafter the second phase is observed. In such instances after all the acetate has been sequestered, again the P release continues, but at the greatly reduced rate as in (i) above.

(iii) With excess acetate addition, the second phase of acetate uptake also terminates, even though acetate is still available. At this termination point it appears that all the P that can be released has been released (Fig. 10). After the termination there is only a slight further release of P with time. From these tests the fraction of P that can be released with excess acetate addition was approximately 70 per cent of the P contained in the batch sludge. This fraction probably corresponds to the low molecular weight polyP identified in the organism mass by Mino *et al.* (1984). They also identified a high molecular weight polyP but they found that this was not released. Their studies were conducted using mixed cultures so that it is not clear whether the two polyP types were present in the same organism, or in different organism species.

In the batch studies the fraction of the P content of the mixed liquor that was released (up to 70 per cent) is greatly in excess of the fraction usually released in the anaerobic reactors of modified Bardenpho and UCT enhanced culture systems. In these systems the acetate addition per mgVSS in the anaerobic reactor is so low that only about 13 per cent of the P content of the VSS is released (Tables 2 and 3), i.e. about 20 per cent of the P that *can* be released. Thus, the extreme conditions imposed in the batch tests are unlikely to be replicated in the P removal systems – generally in these systems, the P release will be such that it remains in the first linear release phase.

For the series of tests with acetate addition as in (i) to (iii) above, the rates of acetate uptake and P release, and the stoichiometric ratios between the P released and the acetate taken up for both phases of P release were determined. The values for the first phase are shown plotted on normal probability paper in Fig. 11 (a and b) respectively. From these plots and similar plots for the second phase, the mean values for the rates and the ratios were determined and are listed in Table 4. With regard to the stoichiometric ratio for the the first phase, it is 1,08 mol P released/mol acetate taken up or equivalently 0,52 mgP/mgCOD sodium acetate. This value is in close conformity with that predicted by the biochemical model of Wentzel *et al.* (1986) and also agrees with observations on P removal systems treating municipal waste water (Wentzel *et al.*, 1985).

(3) Aerobic response after P release

This series of batch tests was conducted to investigate the aerobic response following anaerobic P release. Mixed liquor samples were drawn from the last aerobic reactor of the enhanced culture systems and placed in continuously stirred batch reactors. The surface of the mixed liquor was covered, as described previously. The batch was aerated continuously for approximately 24 h to utilise any stored PHB carried over from the steady state systems. During this period OUR, total soluble P, TKN and NO_3 concentrations were monitored and the pH controlled, as described previously. After 24 h the air was switched off and nitrogen gas

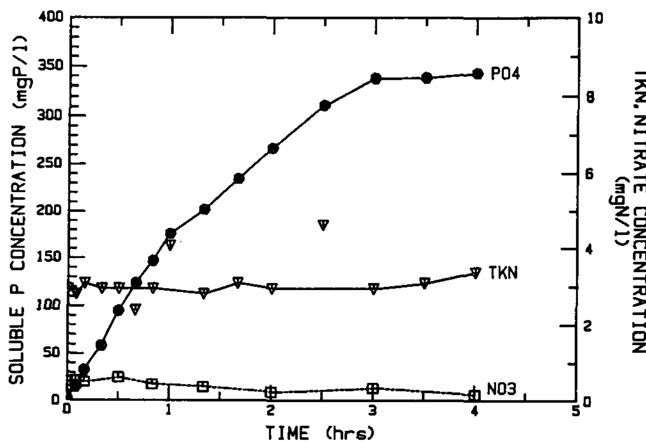

Figure 10
Experimentally observed total soluble phosphate (PO_4), TKN and nitrate (NO_3) concentration-time profiles with anaerobic addition of excess acetate (0,9 mgCOD acetate/mgVSS) to a mixed liquor batch drawn from the Bardenpho enhanced culture system.

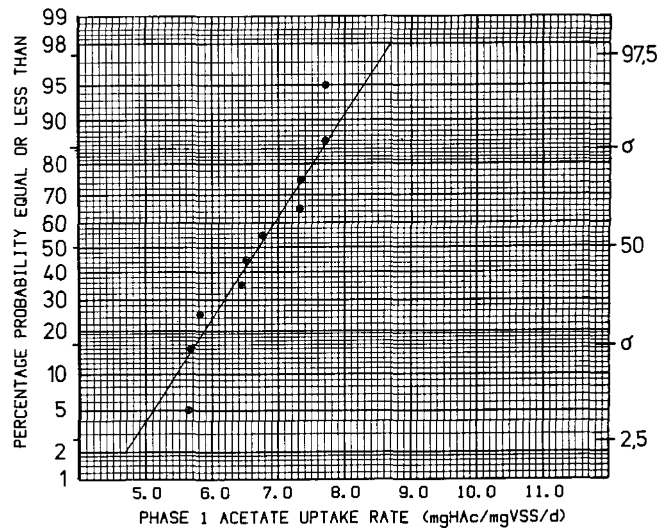

Figure 11(a)
Statistical plot of first phase rates of acetate uptake in anaerobic batch tests such as in Fig. 7.

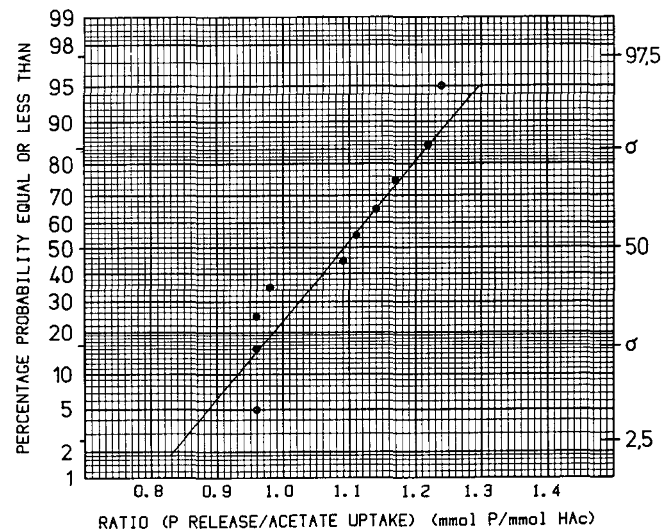

Figure 11(b)
Statistical plot of first phase stoichiometric ratio between P release and acetate uptake.

TABLE 4
RATE OF ACETATE UPTAKE, P RELEASE AND THE RATIO P RELEASED/ACETATE UPTAKE, FROM ANAEROBIC BATCH TESTS ON ENHANCED CULTURE SLUDGES

Rate of acetate uptake (mgCOD*/mgVSS.d)		Rate of P release (mgP/mgVSS.d)		Ratio P release/ acetate uptake (mgP/mgCOD*)**	
Phase 1	Phase 2	Phase 1	Phase 2	Phase 1	Phase 2
7,17	3,11	3,73	2,74	0,52	0,88

* To convert to mgHAc divide mgCOD by 1,07
** To convert to mmol P divide mgP by 30,97, to mmol acetate divide mgCOD by (60.1,07)

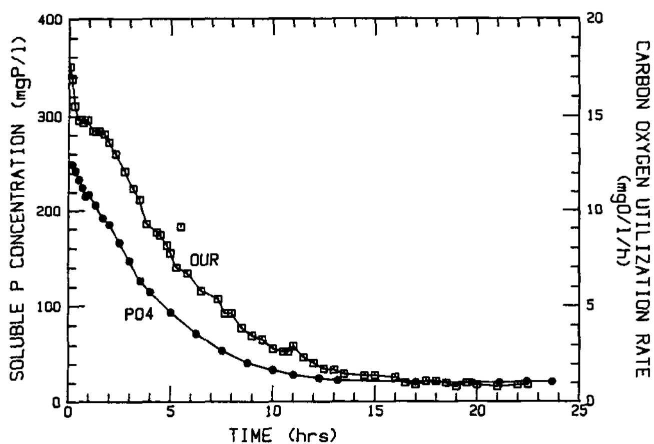

Figure 12
Total soluble phosphate concentrations (PO_4) and carbonaceous oxygen utilisation rate (OUR) on aeration following anaerobic acetate addition of 0,207 mgCOD acetate/mgVSS to mixed liquor batch drawn from the Bardenpho enhanced culture system (VSS = 1 041 mgVSS/ℓ).

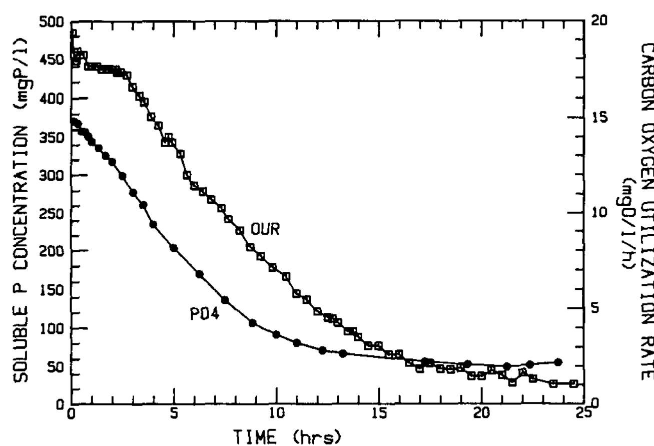

Figure 13
Total soluble phosphate concentrations (PO_4) and carbonaceous oxygen utilisation rate (OUR) on aeration following anaerobic acetate addition of 0,363 mgCOD acetate/mgVSS to mixed liquor batch drawn from the Bardenpho enhanced culture system (VSS = 1 100 mgVSS/ℓ).

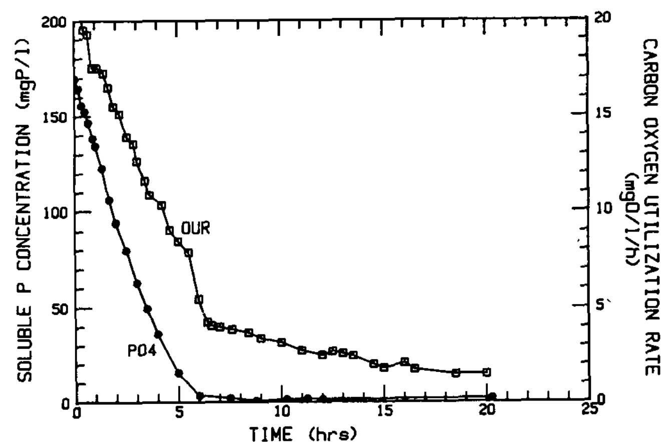

Figure 14
Total soluble phosphate concentrations (PO_4) and carbonaceous oxygen utilisation rate (OUR) on aeration following anaerobic acetate addition of 0,22 mgCOD acetate/mgVSS to mixed liquor batch drawn from the Bardenpho enhanced culture system. The PO_4 concentration falls to zero during the course of this test (VSS = 1 226 mgVSS/ℓ).

was continuously bubbled through the mixed liquor to strip off oxygen and to prevent oxygen ingression. Total soluble P, TKN and NO_3 concentrations were monitored and pH controlled as before. This state was maintained until the NO_3 concentration reduced to zero, whereupon a selected mass of standard substrate and nutrients (sodium acetate, yeast extract, K_2HPO_4, $MgCl_2.6H_2O$, and NH_4Cl, Wentzel *et al.*, 1988) were added. The batch was maintained in an anaerobic state by bubbling nitrogen gas through the mixed liquor. Total soluble P, TKN, NO_3 and acetate concentrations were monitored and pH controlled as before. The acetate concentration reduced to zero during this time. (This period corresponds to that described in (2) above, i.e. anaerobic response with acetate addition.) After the acetate reduced to zero the air was switched on and the OUR, total soluble P, TKN and NO_3 concentrations were monitored and pH controlled as before.

In this series of batch tests the focus of interest was on the final aeration period. Accordingly, the relevant responses observed for two typical tests are shown plotted in Figs. 12 and 13 for aeration following anaerobic P release with acetate addition of 0,207 and 0,363 mgCOD/mgVSS respectively. To eliminate the confounding effects of OUR for nitrification, this OUR was estimated from the increase in NO_3 in the test and subtracted from the observed OUR to give the OUR for carbonaceous material oxidation only. It is this carbonaceous OUR that is shown in the Figures.

Three features in the response curves are to be noted:

(i) The OUR curves indicate a saturation type reaction. With the lower addition of acetate to the anaerobic zone, the OUR shows a declining magnitude from the start of aeration (Fig. 12) whereas with higher addition of acetate, the OUR exhibits an initial plateau and thereafter declines (Fig. 13). This behaviour implicates a Monod type of relationship between the specific substrate utilisation (or growth) rate and the substrate concentration stored internally (as PHB) per unit of volatile active mass (polyP organisms).

(ii) The change in P concentration appears to be linked to substrate utilisation: Initially (Fig. 13), the P concentration drops linearly with time (<5 h) thereafter the change in P concentration exhibits a first order type of reaction with respect to itself. This behaviour conforms to that expected of the substrate concentration in the reactions governed by a Monod type of relationship. Since internally stored acetate (as PHB) forms the substrate and not the P, an assumption that links PHB utilisation directly with P uptake for polyP formation seems reasonable.

(iii) When the OUR levels off, the P concentration commences to increase, indicating the start of the endogenous digestion phase.

A further aerobic batch test, following anaerobic acetate addition of 0,215 mgCOD/mgVSS (as in (2)(ii) above), was conducted in which there was not sufficient soluble P for uptake. The P and carbonaceous OUR responses are shown plotted in Fig. 14. Three features in this test are to be noted:

(i) The initial stage of the test shows the OUR decreasing from the start indicating unsaturated internally stored PHB conditions similar to Fig. 12.

(ii) After about six hours the soluble P concentration reaches zero. When the P concentration reaches zero there is a precipitous drop in the OUR.

(iii) The P concentration continues to remain close to zero, and the OUR continues to decrease but at a significantly reduced rate compared to the initial stage, but greater than that associated with endogenous mass loss.

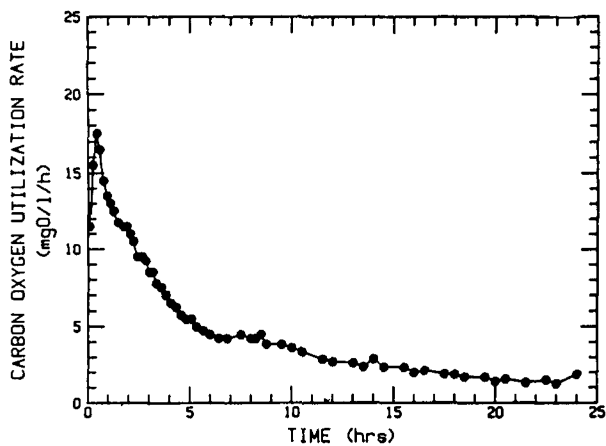

Figure 15(a)
Carbonaceous oxygen utilisation rate (OUR) on aeration following anaerobic acetate addition of 0,125 mg acetate/mgVSS to mixed liquor batch drawn from enhanced culture system. The TKN concentration falls to a steady value during the course of this test (VSS = 1 143 mgVSS/ℓ).

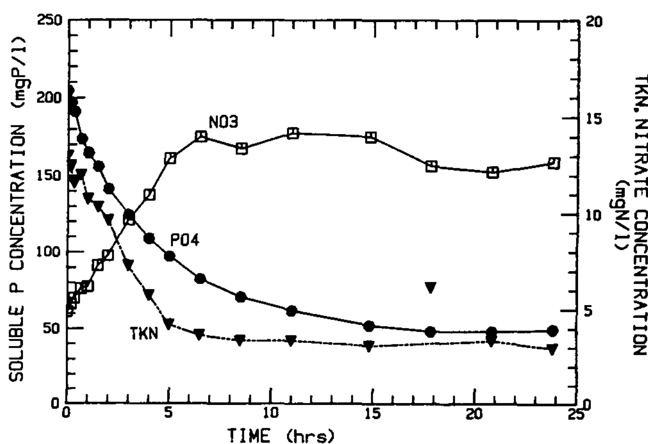

Figure 15(b)
Total soluble phosphorus (PO_4), TKN and nitrate (NO_3) concentration-time profiles for the batch test in Fig. 15(a).

The results of this batch test indicate that when P becomes limiting a qualitative change in the polyP organism behaviour takes place. It appears that stored substrate utilisation continues, but at a reduced rate and without the associated P uptake for polyP formation. Necessarily the P requirements for normal cell synthesis would be supplied from the polyP pool. This behaviour of the polyP organisms has been noted by Van Groenestijn and Deinema (1985).

A final aerobic batch test, following anaerobic acetate addition of 0,135 mgCOD/mgVSS, was conducted to investigate the utilisation of nitrate as a nitrogen (N) source for cell synthesis. From the steady state response of the enhanced culture systems, described previously, it was concluded that nitrate can serve as a N source for synthesis. However, the steady state system data provided no kinetic information as to differences that may arise when utilising nitrate as opposed to ammonia as a nitrogen source (e.g. change in growth rate). Accordingly it was proposed to investigate changes in kinetic behaviour when the nitrogen source is changed from ammonia to nitrate.

A batch of mixed liquor was drawn from the last aerobic reactor of the enhanced culture system and placed in a continuously stirred batch reactor. The same procedure as described above was followed with the following exceptions: Less ammonia was added with the acetate at the start of the anaerobic period; it was envisaged that the ammonia would reduce to zero during the subsequent aerobic period, while substrate still was being utilised *inter alia* for synthesis, enabling the change in kinetic behaviour to be monitored. At the start of the aeration period 5 mgN/ℓ batch volume of $NaNO_3$ was added to ensure sufficient nitrate would be present. The response profiles for the aeration period following anaerobic acetate addition of 0,135 mgCOD/mgVSS are shown plotted in Fig. 15 (a and b). The following features in the response curves are to be noted:

(i) During the initial phase of the aeration period (0 to 5,5 h), TKN is present and the OUR and P concentration profiles correspond to those in Fig. 12. The TKN concentration declines during this period due to utilisation of ammonia for nitrification and as a N source for cell synthesis. The nitrification reaction is reflected in the increase in the NO_3 concentration.

(ii) When all the available TKN has been utilised (after approximately 5,5 h), ammonia no longer is available as a N source for synthesis and the NO_3 concentration commences to decrease indicating utilisation of nitrate as a N source for synthesis. This observation verifies the steady state system observations.

(iii) When the change occurs, from ammonia to nitrate as a N source for synthesis, no observable change in the carbonaceous OUR or P concentration profiles are apparent. This was verified as follows: The P concentration decreases with time eventually to a stable value (X = 48,24 mgP/ℓ). This value is substracted from the observed P concentration during the period of change and the difference plotted log (P concentration – X) versus time (Fig. 16); the plot is linear. This would indicate the utilisation of NO_3 as opposed to ammonia as a nitrogen source does not affect the kinetic behaviour of polyP organism growth reactions.

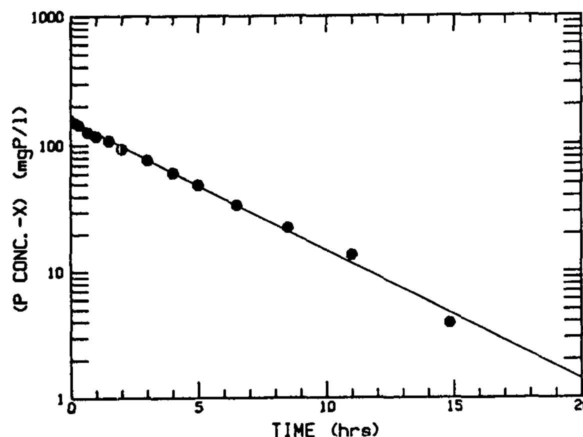

Figure 16
Semilog plot of total soluble phosphorous (PO_4) concentration versus time for the data in Fig. 15(b) (X = 48,24).

(4) Anoxic response after P release

It was concluded earlier, from data obtained on the steady state enhanced culture systems that, in these systems, denitrification by the polyP organisms is minimal. Accordingly batch tests were conducted to investigate this aspect further.

Mixed liquor was drawn from the anaerobic reactor of the enhanced culture system. Analysis of acetate in the anaerobic reactor had shown the concentration to be zero, indicating that all the acetate had been sequestered in the anaerobic zone of the system and no acetate was carried over to the batch test. The mixed liquor was placed in a sealed batch reactor and continually sparged with nitrogen gas to prevent oxygen ingression into the mixed liquor. At the start of the test samples were drawn for VSS and TSS determinations. At time zero, ± 20 mgN/ℓ as KNO_3 was added to the batch. During the course of the test total soluble P, NO_3, NO_2, COD and intracellular PHB concentrations were monitored and the pH controlled, as described previously. The relevant response curves are shown plotted in Fig. 17 (a and b). The following observations were made:

(i) The nitrate concentration slowly decreased during the course of this test, in a linear fashion, due to denitrification. The rate of denitrification was determined to be 0,043 mgN/mgVSS.d. This rate is very much lower than those measured for mixed culture activated sludge systems (Van Haandel *et al.*, 1981).

(ii) Nitrite concentration increased very slowly during the course of the batch test. Approximately 40 per cent of the nitrate was reduced to nitrite only. This is in conformity with Lötter *et al.* (1986) who showed that a number of *Acinetobacter*, a polyP organism, strains can reduce nitrate to nitrite only.

(iii) The P concentration showed minor increase during the test. This would indicate that, in the enhanced cultures, insignificant P uptake occurs with nitrate as external electron acceptor.

(iv) The intracellular PHB concentration remained approximately constant. This would indicate that PHB is not being utilised as a substrate source for denitrification.

(v) The soluble COD concentration increased slightly during the course of the test indicating accumulation of soluble unbiodegradable COD, as noted earlier.

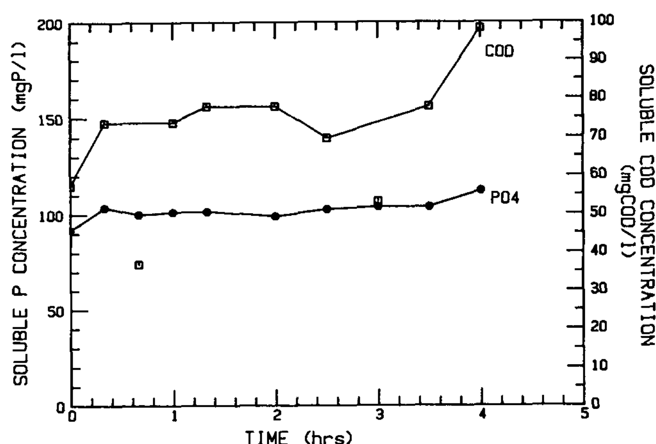

Figure 17(a)
Total soluble phosphorus (PO_4) and COD concentrations observed on addition of KNO_3 to a batch of mixed liquor drawn from the anaerobic reactor of the enhanced culture system (VSS = 594 mgVSS/ℓ).

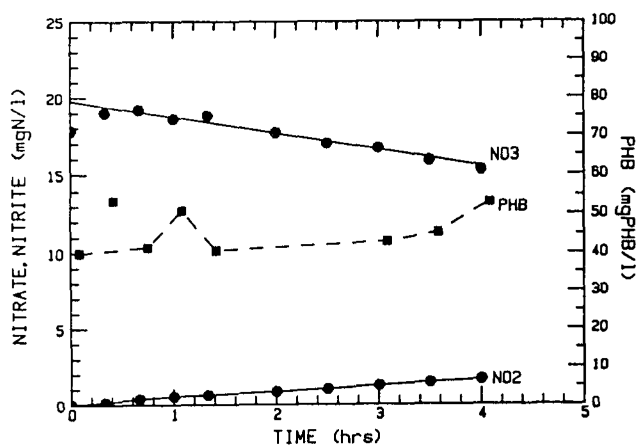

Figure 17(b)
Nitrate (NO_3), nitrite (NO_2) and intracellular PHB concentration-time profiles for the batch test in Fig. 17(a).

The data from this batch test supports the conclusion, drawn from the steady state systems, that denitrification by the polyP organisms (and hence associated P uptake potential) ***in the enhanced cultures*** is negligible, i.e. denitrification can be neglected when developing a kinetic model to describe the behaviour of such systems.

(5) Acetate addition under aerobic conditions

Mixed liquor samples were drawn from the last aerobic reactor of the enhanced culture systems and placed in batch reactors. As described previously, the surface of the batch mixed liquor was covered with floating plastic balls. The batch was aerated and OUR, total soluble P, TKN, COD and NO_3 concentrations monitored and pH controlled as before. When all the stored PHB had been depleted (judged from P concentration and OUR response i.e. after about 15 h in Figs. 12 and 13) acetate was added and aeration continued as before. The response curves following acetate addition are shown in Fig. 18 (a and b). The interesting features are:

(i) The acetate disappears at a fast rate with an associated *release* of P. This behaviour appears similar to that with acetate addition under anaerobic conditions, i.e. P release associated with acetate uptake and storage as PHB.

(ii) Initially the OUR is high and remains high until the acetate concentration becomes zero.

(iii) The P concentration increases but at a declining rate to reach a maximum concentration that appears to coincide with the complete removal of acetate. Thereafter, the P concentration declines as described in the aerobic tests in (3) above.

(iv) The OUR drops precipitously when the acetate concentration reaches zero. If it is accepted that the polyP organisms utilise only stored PHB for growth, then a precipitous OUR decrease is not expected because the stored PHB remains high at the time the acetate is depleted. The precipitous decrease in OUR therefore, would indicate that the polyP organisms also utilise acetate directly for growth under aerobic conditions, while simultaneously taking up acetate,

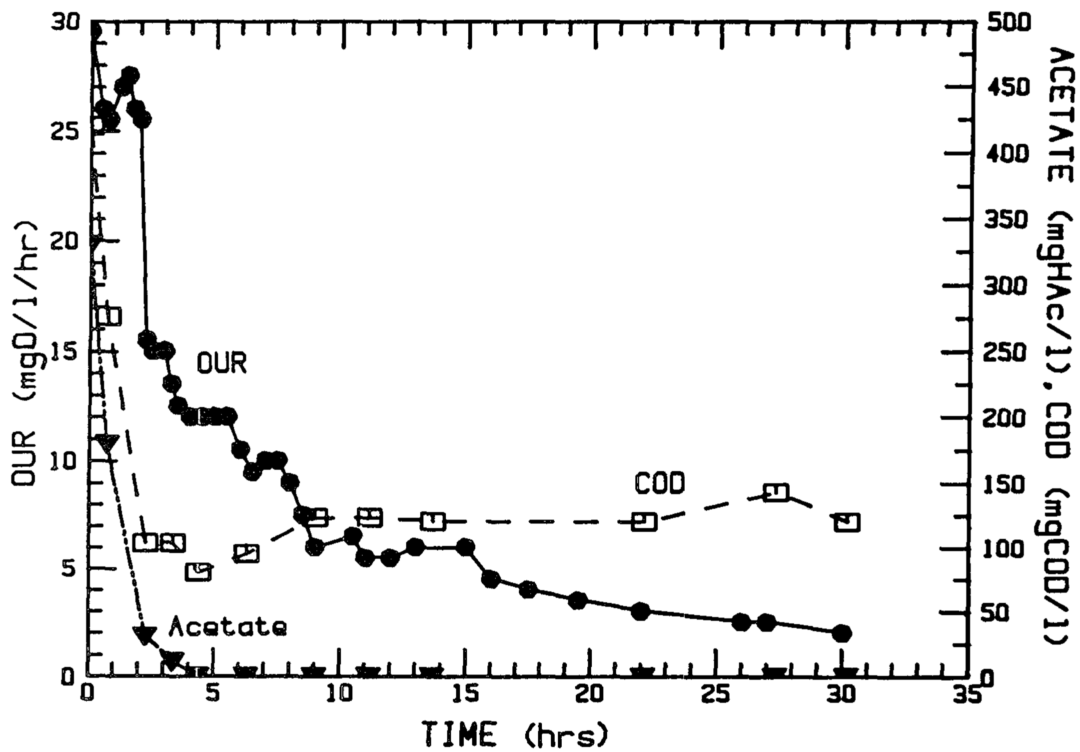

Figure 18(a)
Total oxygen utilisation rate (OUR), COD and acetate concentration-time profiles observed on aerobic acetate addition of 0,324 mgCOD acetate/mgVSS to a batch of mixed liquor drawn from the Bardenpho enhanced culture system. (VSS = 1 080 mgVSS/ℓ).

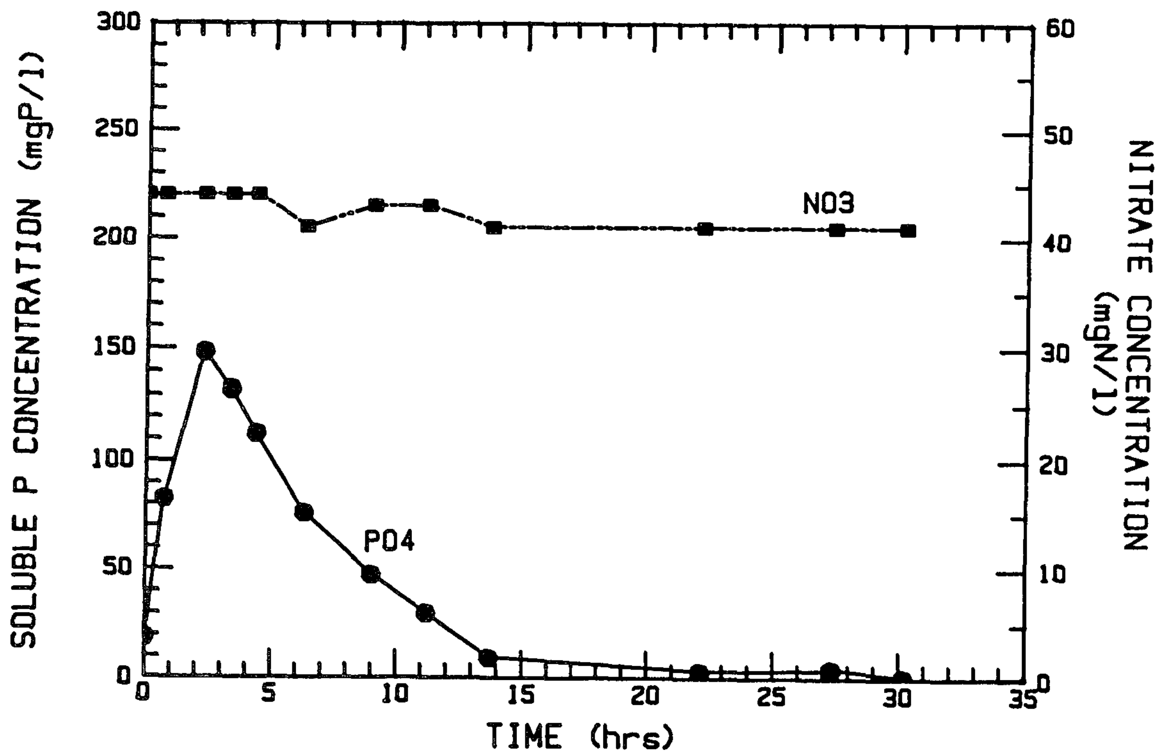

Figure 18(b)
Total soluble phosphate (PO_4) and nitrate (NO_3) concentrations observed on aerobic acetate addition of 0,324 mgCOD acetate/mgVSS to a batch of mixed liquor drawn from the Bardenpho enhanced culture system (VSS = 1 080 mgVSS/ℓ).

storing it as PHB and releasing P.

(v) Oxygen utilisation continues at a rate higher than that for endogenous respiration after the acetate concentration becomes zero. This indicates a utilisation of stored PHB as described in the aerobic tests in (3) above.

(vi) The soluble COD initially decreases, due to acetate uptake, but thereafter slowly increases. This behaviour appears to indicate that during endogenous respiration there is a generation of "unbiodegradable" soluble COD, as noted before.

This test verifies the prediction of the biochemical model of Wentzel *et al.* (1986), that acetate storage as PHB with P release can take place under aerobic conditions, i.e. an anaerobic state is not a prerequisite to obtain P release with acetate addition. This however does not imply that an aerobic system could be operated with acetate as influent to give biological excess P removal (Wentzel *et al.*, 1988)

(6) Cation release and uptake

In the development of the steady state enhanced culture systems, it was found that adequate quantities of magnesium (Mg) were essential for the successful operation of biological excess P removal systems (Wentzel *et al.*, 1988). They hypothesised that the function of Mg is to serve as the principal counter-ion stabilising the polyP chain. Consequently, the pattern of Mg release and uptake should be closely related to the P release and uptake. Accordingly, it was decided to investigate this aspect further.

For the batch tests described above in (1), (2) and (3) the samples also were analysed for the cations magnesium (Mg), calcium (Ca), potassium (K) and sodium (Na). For each batch test the cation concentration was plotted against the P concentrations. From these plots the ratio cation released/taken up per P released/taken up can be calculated. The mean values obtained from all the results are listed in Table 5 together with similar results obtained by other research groups. Referring to Table 5, the results obtained in this investigation are in agreement with those obtained by other research groups, with the exception of Na: In this study no association was found between Na and P release/uptake but Fukase *et al.* (1982) found a significant association. Very little Ca release/uptake accompanies the P release/uptake, 0,05 mol Ca/mol P. This excludes the possibility that calcium phosphate dissolution/precipitation respectively is the cause for the observed changes in P concentration. Both Mg and K are released and taken up in concert with P release and uptake. Probably, both these ions stabilise the polyP chains by acting as counter-ions. The ratio (total mol + ve released)/(mol P released) is 0,92 which conforms reasonably to the ratio of 1,0 predicted by the biochemical model of Wentzel *et al.* (1986).

TABLE 5
RATIO OF CATIONS RELEASED, OR TAKEN UP, TO P RELEASED, OR TAKEN UP

Research group	(Cation release/uptake) / (P release/uptake) (mmol cation/mmol P)				Charge balance (mmol + Ve/ mmol P)
	Mg	K	Ca	Na	
Fukase *et al.* (1982)	0,28	0,31	0,05	0,12	1,09
Arvin *et al.* (1985)	0,32	0,23	–	–	0,87
Comeau *et al.* (1985)	0,24	0,34	0,06	–	0,94
This study	0,26	0,30	0,05	0	0,92

* The charge balance is the ratio of the sum of the positive charges released or taken up to P released or taken up.

In the steady state enhanced culture systems the importance of Ca and Mg has been amply demonstrated by Wentzel *et al.* (1988). With regard to K, phosphorus was always added as K_2HPO_4, thus the importance of K could not be demonstrated in these systems.

Discussion

It is most unlikely that a model can be developed that describes a physical phenomenon completely; theoretically a complete description should include aspects down to the most fundamental level. To take an extreme example, in the description of a biosystem, for completeness DNA molecule behaviour should be included. Instead less complete models are developed, at some level of organisation (Odum, 1971); only characteristics important at that level are incorporated in the model description. Success in modelling greatly depends on the ability to identify the level of organisation and the principal or relevant characteristics controlling the phenomenon at that level. For example, we cannot directly implicate the biochemical control mechanisms of P release and uptake as described in the biochemical model of Wentzel *et al.* (1986) (such as ADP/ATP and NAD/NADH ratios) but rather implicate parameters that appear to be important at the level of interest, in this instance the mass behaviour of a population of selected microorganisms. Monod's equation is a typical example; it relates the rate of growth of an organism mass directly to substrate concentration, whereas at a lower level of organisation, the molecular level, the growth rate is controlled also by ADP/ATP and NAD/NADH ratios, amongst others.

As we have stated earlier, to set up a mathematical model that describes the stoichiometry and kinetic behaviour of the biological excess P removal phenomenon it is required to:

(1) identify the essential compounds utilised or formed;
(2) identify the processes acting on these compounds;
(3) conceptualise a qualitative mechanistic model of the behaviour; and
(4) formulate mathematically the process rates, stoichiometry and transport relationships.

We shall consider (1) to (3) only; the mathematical model will be set out in Part III of this series of papers.

(1) Compounds

There appear to be 12 essential compounds *directly* involved in biological excess P removal. Some of these compounds are directly observable whereas with others, their existence has to be inferred, either from hypothesised biochemical behaviour or from the requirement of mass balances, because the means for measuring these compounds have not been available in the laboratory, or, where the means have been available, these have not been sensitive enough to give quantitative parameters. Compounds directly observable are concentrations of acetate, soluble P, oxygen, nitrate, ammonia, readily biodegradable "complex" COD and unbiodegradable soluble COD. Compounds inferred are polyphosphate (polyP), polyhydroxybutyrate (PHB), apparently soluble unbiodegradable organic N, endogenous particulate residue and polyP organisms.

(2) Processes

The processes that act on the compounds above are identified by

observing changes in the compounds under a variety of conditions. In this manner, eventually, 13 essential processes have been identified.

(3) Conceptual model

From the experimental investigation it is evident that the processes that act on the compounds can be grouped into three broad categories:
(i) sequestration of acetate;
(ii) growth; and
(iii) endogenous mass loss.

Description of the conceptual model is facilitated if each of the three categories is developed separately.

(i) *Sequestration of acetate* Sequestration can take place under anaerobic or aerobic conditions. This is illustrated in Figs. 7, 8 and 10, and 18 (a and b) respectively.

In the anaerobic situation, the compound acetate is taken up and stored as PHB. The rate of acetate sequestration can be observed directly and appears to be zero order with respect to the acetate concentration, as observed by the linearities in the acetate — time plots (Figs. 7, 8 and 10). The mean specific rate estimates (i.e. rate/volatile solids) for the two phases of sequestration from a number of anaerobic sequestration batch tests on mixed liquor from the 20 d sludge age enhanced culture system are given in Table 4. Concomitant with acetate sequestration, stored polyP is cleaved and P released to the bulk liquid, causing the observable increase in soluble P concentration (Figs. 7, 8 and 10). There appear to be three phases of sequestration; in the first phase the mass of P released appears to be proportional to the mass of acetate taken up, see mean values in Table 4; apparently only a fraction of the polyP can be released at this first rate, approximately 30 per cent. Should acetate still be available when this polyP fraction is exhausted, a second rate of acetate uptake appears to operate; not only is this rate of acetate uptake slower than the first rate, but also the mass of P released per mass acetate stored is higher, Fig. 8 and Table 4. To this second phase also there appears to be a limit, probably the mass of polyP available. If acetate still is present at the end of the second phase, sequestration of acetate appears to cease, but a very low rate of P release still is apparent, see Fig. 10, most likely due to death of organisms, see endogenous mass loss discussion below. Should the acetate concentration become zero during either the first or the second phase of P release then both types of sequestration cease and a third phase of P release occurs; in this phase P is released without acetate uptake and storage (no acetate being available, see Figs. 7 and 8). It is hypothesised that the third phase of P release is one due to polyP cleavage for maintenance energy requirements under anaerobic conditions, see endogenous mass loss discussion below.

The second phase of P release, and thus the potential release of all the polyP, is unlikely to occur in the steady state continuous flow enhanced culture systems because the substrate loading rates per unit mass of polyP organisms are much less than the loading rate that can be imposed in a batch test; usually only the first sequestration phase will be encountered. In the system operation, second phase P release might occur only under injudiciously high acetate load increments in system startup, or under extreme cyclic loading. Normally this would also be true for mixed culture excess P removal systems.

In the aerobic situation the batch tests indicate that sequestration of acetate as described for the anaerobic situation, also takes place under aerobic conditions, Fig. 18 (a and b). However, the rates of aerobic sequestration cannot be observed directly because three phenomena take place at the same time: Acetate uptake for storage as PHB with associated P release; direct uptake and utilisation of acetate for growth without P release or uptake; and utilisation of stored PHB with associated P uptake. Normally in enhanced culture and mixed culture excess P removal systems, significant concentrations of acetate in aerobic reactors are unusual and aerobic sequestration is unlikely. From Wentzel *et al.* (1988) it would appear that in enhanced cultures, where acetate does pass into the aerobic section (due to malfunction or inappropriate operation), the specific rate of acetate uptake of the non-polyP organisms (that may be present) is higher than that for the polyP organisms, and the non-polyP organisms tend to increase their concentration. Should the acetate 'leakage' continue, a slow decline of polyP organism growth takes place, leakage of acetate increases and the enhanced culture collapses. The instability inherent in this behavioural pattern makes that it is not possible to model aerobic acetate sequestration. For modelling purposes a limitation must be imposed: it is assumed that the anaerobic phase is sufficient to sequester all the available acetate.

(ii) *Growth* Growth processes require the presence of an external electron acceptor. The external electron acceptors for polyP organism growth usually are oxygen (Juni, 1978) or nitrate (Lötter, 1985). However, in the enhanced culture systems, the conditions did not induce growth of denitrifying polyP organism species (or did only slightly). This is illustrated from the steady state results, Tables 2 and 3, and in the batch test, Fig. 17. As a consequence, no information on the behaviour of the denitrifying polyP organism species could be obtained. Hence, in this model, denitrifying by polyP organisms will not be included. (However, effort in this regard should not be abandoned because there is strong evidence that there are polyP organism species that do use nitrate, with significant growth and polyP accumulation, e.g. Comeau *et al.*, 1987).

Growth of polyP organisms can take place utilising a number of different substrates; external, such as acetate, glucose etc., or internal, such as PHB. However, in the anaerobic/aerobic situation specific to excess P removal systems, if properly designed, all the acetate is stored as PHB in the anaerobic reactor and growth will take place in the subsequent aerobic reactor utilising the stored PHB as the only substrate source. In the aerobic reactor a fraction of the PHB is utilised to synthesize new cell material and the remainder is oxidised to provide energy for growth and for taking up and storing P as polyP volutins; the oxidation action gives rise to the observed OUR, and the P uptake to the observed decrease in P concentration (Figs. 12 and 13). In the batch tests acetate added under anaerobic conditions was completely sequestered (as PHB). Under the subsequent aerobic conditions both the OUR and P uptake appear to be closely associated; as there is no other signicant energy source this would indicate that P uptake and storage as polyP are linked to PHB utilisation.

Under aerobic conditions, should the soluble P concentration become zero, PHB utilisation continues but at a reduced rate. The PHB now appears to be utilised solely for growth because no soluble P is available for uptake and storage as polyP (Fig. 14). The P requirements for cell synthesis probably are supplied by the polyP pool.

Ammonia is used as a nitrogen source for synthesis. Should ammonia become limiting, nitrate can serve as an alternative nitrogen source (Fig. 15).

(iii) ***Endogenous mass loss*** One definition for endogenous mass loss is the loss of active mass when an organism mass is aerated with no substrate added, for example in the aerobic digestion of mixed liquor. This definition however, can be shown to be inadequate. Indeed, there exists no unambiguous definition for the term endogenous mass loss. The reason for this is that no adequate mechanistic explanation for the endogenous mass loss phenomena has evolved. Finding a mechanistic explanation has troubled research workers since Monod in (1949) ". . . the study of bacterial 'death', i.e. of the negative phases of growth, involves distinct problems and methods". This view also is reflected in the 1976 symposium on "The Survival of Vegetative Organisms" in which it was concluded "very little is known of the death and declining phase" (Trinci and Thurston, 1976).

Herbert (1958) was the first to attempt a quantitative description of endogenous mass loss. To account for the apparent decrease in the specific yield with increase of organism retention time he proposed that active mass is lost by oxidation to generate energy for cell maintenance. He modelled the rate of the loss as proportional to the active mass. McKinney and Ooten (1969) independently proposed endogenous mass loss and formulated it in the same manner as Herbert (1958), but extended Herbert's model by proposing that, with the mass loss was associated the generation of an inert particulate fraction, which they called the endogenous residue. Also, they formulated mathematically the oxygen requirements and the generation of the endogenous residue in terms of the active mass loss. The consequence of hypothesising an inert endogenous residue was that it no longer was acceptable to equate the active volatile mass to the total volatile mass. The generation of endogenous residue was confirmed by McCarty and Broderson (1962), Washington and Hetling (1965), Marais and Ekama (1976) and Warner *et al.* (1983).

Pirt (1975) proposed an alternative explanation for the phenomenon. Pirt did not recognise endogenous mass loss *per se* but accounted for the apparent decrease in specific yield with increase in sludge age, as follows: The organism requires energy to maintain essential cell function, termed "maintenance energy". Working with *pure cultures* Pirt hypothesised that, provided an *external substrate is available* to the organism mass, a fraction is oxidised to supply the energy requirements for maintenance, a fraction is oxidised to supply the energy requirements for growth and the balance is synthesised into new cell mass. The reduced yield with increase in sludge age was accounted for by the increased fraction of the input substrate needed to be utilised for maintenance energy of the greater sludge mass. Pirt did not address the problem of the mass loss observed where no external substrate was available to the organism mass in, for example, batch digestion.

Both the Pirt and Herbert/McKinney *et al.* models lead to similar behavioural patterns provided adequate external substrate is available. If no external substrate is provided, the Herbert/McKinney *et al.* model still provides a satisfactory explanation whereas the Pirt model provides no guidance; in this respect the Herbert/McKinney *et al.* model is superior.

A further "philosophical" difficulty with Pirt's approach is that, with adequate external substrate available for maintenance energy, the approach implies the organism has an indefinite life span, i.e. with external substrate available an organism does not die. With regard to the Herbert/McKinney *et al.* model, although not directly stated, the model implies that organism death does occur. In a batch digestion test with time the model predicts that the active organism mass decreases, eventually to a minute fraction of the original mass, as is observed (Marais and Ekama, 1976). If the organism does not die it implies that its mass must decrease correspondingly eventually to near zero, that is, the major fraction of the metabolic material is oxidised. This does not seem a tenable explanation for one cannot conceive of the organism decreasing below some critical mass and still remaining viable. A reasonable alternative is that a fraction of the mass must die to supply energy for maintenance of the rest. The fact that inert material is generated during digestion implies that death must have occurred leaving a remnant of unbiodegradable particulate cell material.

A deficiency in the Herbert/McKinney *et al.* model became apparent when an organism mass was subject to digestion operated under a sequence of anaerobic and aerobic periods (Warner *et al.*, 1983). Following an anaerobic period, at the beginning of the aerobic period, the oxygen demand was much higher that that measured for endogenous mass loss when the anaerobic period was not imposed. However, the total oxygen demand during the aerobic period of the anaerobic-aerobic sequence equalled the total demand in the completely aerobic digestion test for the time period spanning the anaerobic and aerobic periods. These observations cannot be accounted for in terms of the Herbert/McKinney *et al.* model which does not incorporate description of organism response under anaerobic conditions.

A model to incorporate the behavioural pattern observed above, and mixed culture behaviour, was proposed by Dold *et al.* (1980). The antecedent to this model is of interest as it will assist in understanding the extensions to the Dold *et al.* model proposed in this paper. Bhatla and Gaudy (1965), while investigating the BOD-time curve, pasteurised a fraction of the inoculum (to kill protozoa) and thereafter inoculated the pasteurised and unpasteurised inocula, respectively, into a glucose-based substrate. Oxygen consumption and the numbers of bacteria and protozoa were measured over the period of the test, approximately 8 d. The pasteurised culture, after a delay, showed rapid total oxygen uptake over the subsequent 24 h and thereafter very little additional uptake. The viable bacterial numbers increased concurrently with the oxygen demand and thereafter showed a very small rate of decline. With the unpasteurised inoculum the initial total oxygen uptake also was rapid over a period of one day; thereafter it levelled off for a short period, whereupon a secondary phase of oxygen uptake developed over the next six days, almost equal in magnitude to that during the first phase. During the first phase of oxygen uptake the bacterial numbers increased concurrently, to approach a concentration approximately equal to that with the pasteurised inoculum. However, concomitant with the second phase of oxygen demand, the bacterial numbers declined rapidly and the protozoan numbers increased. Evidently the predation of the protozoa on the bacterial population was the cause of the second phase of oxygen demand, the bacteria serving as a substrate source for growth of the protozoa so that the oxygen uptake most probably was principally to obtain energy for protozoal synthesis.

Dold *et al.* (1980) hypothesised that, in mixed cultures, predation is a significant cause of death of organisms, liberating substrate from which the remaining organisms resynthesise new cell mass. Predation, they proposed, occurs in situations where added substrate is present and where it is absent, under aerobic conditions and short periods of anaerobiosis. In the situation where no external substrate is added, e.g. in aerobic digestion, they proposed that the oxygen demand associated with endogenous mass loss arises, in fact, from the energy requirements for resynthesis of organism mass (regeneration) from the substrate liberated by predation (death). In aerobic situations where external substrate is added, Dold *et al.* (1980) proposed that the

"death-regeneration", and associated oxygen demand, also is present. Under anaerobic periods predation continues liberating substrate but, because external electron acceptors are not available, the substrate accumulates causing an initial high oxygen demand under aerobic conditions subsequently imposed. They did not address the problem of "maintenance energy" but from the Bhatla and Gaudy investigation this would be relatively small compared to the regeneration energy requirements and therefore could be "absorbed" with the regeneration energy requirements. They accepted the proposals of McKinney for endogenous residue generation but modified these proposals appropriately to fit into the "death-regeneration" mode. This model has performed excellently in describing activated sludge systems (IAWPRC Task Group, 1987).

In applying the model of Dold *et al.* (1980) to a monoculture or an enhanced culture, or a mixed culture from which predators have been removed, it is evident that predation cannot be present. In such systems necessarily maintenance energy effects must dominate. It is hypothesised that under the stress of no available external substrate, a fraction of the weaker organisms will die lysing cell contents, biodegradable substrate and unbiodegradable particulate and soluble residues, into the bulk liquid. The remaining live organisms oxidise the biodegradable substrate fraction to obtain energy for maintenance only, energy for regrowth not being available. That is, in a mono or enhanced culture, in effect the rate of death will be controlled by the maintenance energy requirements. With active predation however, as is normal in mixed cultures, substrate 'liberated' is in excess of maintenance energy requirements and allows regrowth.

The mechanisms for death-maintenance described above, relate to situations where no external substrate is added to the organism mass. However, it can be hypothesised that the same mechanisms also will be active even if an externally added substrate is present, for the following reasons. There is evidence that organisms have a limited life span so that, as the culture ages, death and lysis of a fraction of the organism population will occur naturally, irrespective of whether externally added substrate is present or not (Postgate, 1976, Trinci and Thurston, 1976). (For example, scar formation on the cell wall each time it divides, after many divisions, may weaken the organism to the extent that it splits open; Postgate, 1976). In consequence death-maintenance is hypothesised always to be present, under aerobic and anaerobic conditions, with or without substrate addition.

The death-maintenance hypothesis outlined above can be applied to the polyP organism enhanced cultures as follows:

- Under *aerobic* conditions a death rate is continuously present resulting in lysis of biodegradable and unbiodegradable material to the bulk liquid. The polyP organisms, having the Entner-Doudoroff pathway (Juni, 1978), can abstract sufficient energy for maintenance functions by oxidising the lysed biodegradable substrate. Thus the OUR under endogenous conditions (i.e. no added substrate) will be proportional to the maintenance energy requirements. The unbiodegradable lysed material cannot be utilised by the polyP organisms and appears as a particulate and soluble residue. Under the death concept the stored polyP content of the cell mass that is lost also is released to the surrounding liquid and hence the P release would be linked directly to the fraction of P per unit organism mass, and the death rate.

The explanation above is consistent with the observed behaviour. In the aerobic digestion batch tests the OUR is proportional to the active mass remaining indicated by the linearity of the semilog plot of OUR versus time (Fig. 3); the integral of the OUR with time is linear with respect to the P concentration with time (Fig. 5); the increase in P concentration is consistent with the polyP content of the lysed active mass (this effect is shown in **Part III** of this series); and there is a build-up of soluble ***apparently*** unbiodegradable COD (Fig. 6(c)).

Under anaerobic conditions it can be hypothesised that death of the weaker organisms continues as described above for aerobic conditions. However, because the Entner-Doudoroff pathway cannot operate if oxygen is absent, maintenance energy requirements must be obtained by some other mechanism. We hypothesise that this energy is obtained by cleavage of stored polyP so that the P release in a digestion batch test under anaerobic conditions should be higher than under aerobic conditions. This is indeed so as indicated by comparing the P release rates in Fig. 2(b) and Fig. 12. The substrate released by death which, under aerobic conditions, was oxidised for maintenance energy cannot be utilised under anaerobic conditions. *For the purposes of modelling,* we hypothesise that this substrate fraction accumulates as a soluble unbiodegradable material. This introduces an inconsistency in the model because the excess biodegradable material generated in the anaerobic phase could be utilised for growth in the aerobic phase. However, accommodation of this effect will greatly complicate the model because it implies polyP organism growth from extracellular substrate without polyP accumulation; as the effect seems to be relatively minor and was not observable experimentally, the stated hypothesised behaviour is accepted.

Conclusion

In this paper a mechanistic model describing the biological excess P removal phenomena has been presented. The model describes specifically the behaviour of polyP organisms in enhanced culture systems receiving acetate only as substrate. The behaviour indicated by this model is qualitatively in agreement with the biochemical model of Wentzel *et al.* (1986) and the experimental observations.

This mechanistic model forms the basis for a quantitative mathematical model, to be describd in **Part III** of this series of papers.

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