DENITRIFICATION KINETICS IN BIOLOGICAL NITROGEN AND PHOSPHORUS REMOVAL ACTIVATED SLUDGE SYSTEMS

by

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A thesis submitted in partial fulfilment of the
requirements for the degree of Master of Science in
the Faculty of Engineering, University of Cape Town.

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July 1989

DECLARATION BY CANDIDATE

I, JOHN ANDREW CLAYTON, hereby declare that this thesis is my own work and that it has not been submitted for a degree at another university.

Signed by candidate
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25 July 1989

SYNOPSIS

In order to size the anoxic reactors in nutrient (N and P) removal activated sludge plants, it is essential to know the denitrification kinetics that are operative in such systems. To date, denitrification kinetics have been accurately defined only for systems that remove N alone; little research has been performed on denitrification in N and P removal plants.

Activated sludge systems that remove N only, usually include a primary anoxic reactor (one that precedes the aerobic reactor and receives the influent and underflow streams). In this reactor, denitrification occurs in two phases: the first is made up of a fast rate $[K_1 = 0,720 \ mgN/(mgAVSS \cdot d)]$ due to utilization of influent readily biodegradable COD (RBCOD), plus a slower background rate $[K_2 = 0,101 \ mgN/(mgAVSS \cdot d)]$ due to utilization of some of the influent particulate biodegradable COD (PBCOD); the second phase of denitrification starts after all the RBCOD has been consumed — it takes place at the background K_2 rate and continues for the remainder of the reactor retention time. Denitrification after the aerobic reactor takes place at $K_3 = 0,072 \ mgN/(mgAVSS \cdot d)$ and is due to products of cell death and lysis.

Activated sludge systems that remove N and P have an anaerobic and a primary anoxic reactor ahead of the main aeration reactor; the influent feed is discharged to the anaerobic reactor. In the anaerobic reactor, polyphosphate-accumulating organisms such as *Acinetobacter* spp. take up and store the influent RBCOD with the result that the substrate composition entering the primary anoxic reactor is no longer the same as that of the influent feed. In spite of this, experience on laboratory and full-scale N removal plants has shown that the inclusion of an anaerobic reactor (i.e. converting to an N and P removal system) does not significantly affect the degree of N removal. To explain why this is so, an investigation into the denitrification kinetics in N and P removal systems was initiated.

Along the length of a plug flow primary first anoxic reactor in a modified UCT (MUCT) system for N and P removal, nitrate, nitrite, phosphate and soluble COD concentrations were measured, allowing twenty-nine concentration profiles for these parameters to be constructed.

(i) About two-thirds of the nitrate profiles showed denitrification to occur in two phases, i.e. a fast followed by a slow rate, the other one-third showed a single slow phase of denitrification. The fast rate was $K_1' = 0,610$ mgN/(mgAVSS.d), about 85% of the fast rate in the absence of an anaerobic reactor [cf. $K_1 = 0,720$ mgN/(mgAVSS·d)]. The K_1' rate, in all likelihood, is a consequence of RBCOD leakage through the anaerobic zone; the slight reduction in magnitude is probably due to the RBCOD that leaks through the anaerobic reactor not being as readily biodegradable as that which is utilized in the latter zone.

(ii) The second rate of the two-phase profiles and the only rate in the single-phase profiles were found to be equal; this rate was $K_2' = 0,240$ mgN/(mgAVSS·d), over twice the second rate in a primary anoxic reactor without a preceding anaerobic zone [cf. $K_2 = 0,101$ mgN/(mgAVSS.d)].

(iii) In all the profiles measured, there was very little change in the phosphate concentration (a slight uptake) and virtually no change in soluble COD.

To confirm the above observations, nineteen anoxic batch tests were performed on sludge harvested from the MUCT/UCT unit. This method simulated anoxic plug flow conditions. Tests were conducted on blends of anaerobic and aerobic reactor sludge (to simulate the conditions in the plug flow first anoxic reactor) as well as on separate batches of anaerobic and aerobic reactor sludge.

(i) The batch tests on the sludge blends (those simulating conditions in the first anoxic reactor) and the tests on pure anaerobic reactor sludge showed a single denitrification rate, the mean of which was $K_2' = 0,185$ mgN/(mgAVSS·d). This rate is not significantly different (at a 95% confidence level) to the average second denitrification rate obtained in the first anoxic plug flow reactor [$K_2' = 0,240$ mgN/(mgAVSS·d)] hence the data was pooled, yielding a mean K_2' denitrification rate of 0,224 mgN/(mgAVSS·d).

(ii) All the profiles with unblended aerobic reactor sludge (corresponding to secondary denitrification) yielded slower rates; the mean was $K_3' = 0,100$ mgN/(mgAVSS·d) which is statistically significantly higher (at a 95% confidence level) than the secondary denitrification rate in N removal

systems $[K_3 = 0,072 \text{ mgN}/(\text{mgAVSS}\cdot\text{d})]$.

(iii)   The phosphate and soluble COD profiles were similar to those measured in the plug flow reactor, i.e. both parameters showed very little change.

From the plug flow and batch experiments, the denitrification kinetics in the nutrient removal MUCT/UCT system were established (it is suggested that these kinetics are characteristic of nutrient removal systems in general):

(i)   Denitrification in the primary anoxic reactor takes place at a single rate of magnitude $K_2' = 0,224 \text{ mgN}/(\text{mgAVSS}\cdot\text{d})$. The initial fast rate measured in the plug flow reactor is excluded because it did not occur in all the plug flow profiles and appeared in none of the batch tests. Additionally, it was of widely varying duration and is therefore not reliable.

(ii)   Secondary denitrification takes place at a single rate of magnitude $K_3' = 0,100 \text{ mgN}/(\text{mgAVSS}\cdot\text{d})$.

These kinetics are clearly different to the two-phase behaviour in N removal systems [cf. $K_1 = 0,720 \text{ mgN}/(\text{mgAVSS}\cdot\text{d})$ and $K_2 = 0,101 \text{ mgN}/(\text{mgAVSS}\cdot\text{d})$]. However, it seems that the loss of denitrification by the absence of the first phase in the MUCT/UCT nutrient removal system is more than compensated for by the faster 'second' phase: in this investigation, at high TKN/COD ratios (> 0,10) the effluent nitrate was lower than that predicted by the 'old' two-phase kinetics for N removal systems by about 8% (expressed relative to the influent TKN), i.e. the anoxic zone appeared to possess a higher denitrification potential than a similar-sized anoxic zone in an equivalent N removal system (viz. an MLE system). Effluent nitrate predictions with the formulated 'new' K_2' kinetics were more accurate; they only overestimated the effluent nitrate by about 3%. At lower TKN/COD ratios (< 0,10) both forms of kinetics gave equal predictions resulting in effluent nitrate concentrations that were about 3% too high (relative to the influent TKN).

To explain the high K_2' denitrification rate observed in the primary anoxic reactor of the MUCT system (relative to the K_2 rate in N removal systems), two alternative hypotheses were advanced:

(1)   The polyP organisms present in these systems are able to denitrify, thereby

adding to the denitrification by the facultative heterotrophs. Since the observed COD levels remained constant, the substrate for this would have to be the internally-stored PHB. The relatively constant P levels may be explained by there not being sufficient energy for polyphosphate accumulation when PHB is oxidized with nitrate.

(2)   The influent PBCOD undergoes some kind of modification in the anaerobic zone, making it more readily biodegradable, thus leading to an increased denitrification rate. The polyP organisms are not implicated in this hypothesis.

To evaluate these two hypotheses, a number of batch tests were performed.

(1)   Batch tests with PHB measurements

(i)   PHB concentration observations in MUCT sludge under anoxic batch test conditions.

(ii)   PHB concentration observations in enhanced polyP organism culture sludge under anoxic batch test conditions.

From these two tests it was concluded that PHB is not utilized under anoxic conditions and that the polyP organisms do not contribute to the high K_2' denitrification rate.

(2)   Batch tests without direct PHB measurements

(i)   Comparison of the denitrification rates of two sludges that differ in polyP organism PHB concentration.

(ii)   Examining the effect of primary anoxic conditions on the P uptake capability of a sludge that is subsequently aerated.

From these tests it was also concluded that the polyP organisms do not utilize PHB under anoxic conditions and therefore do not contribute to the fast K_2' rate in the primary anoxic zone of the MUCT/UCT system. The final set of tests was conducted to evaluate the alternative hypothesis, viz. the hypothesis that PBCOD

was being modified in the anaerobic reactor.

(3)   Batch tests with sewage addition

In this set of tests, the denitrification rate of aerobic reactor MUCT sludge was measured in the presence of PBCOD that had *not* passed through an anaerobic zone, i.e. sewage was added. This rate was compared to that which occurred in the presence of PBCOD that *had* passed through the anaerobic reactor, i.e. it was compared to the denitrification rate in the anoxic zone of the MUCT system (measured earlier in the investigation). The rates were found to be equal, i.e. it appears that the anaerobic reactor does not modify the influent PBCOD to a more readily biodegradable form. Consequently, the second hypothesis was also rejected.

Since experimental evidence resulted in both the proposed hypotheses being rejected, the cause for the increased 'second' rate of denitrification in the MUCT/UCT system can only be speculated upon. It is possible that the anaerobic reactor conditions the sludge in some way, simply enabling it to denitrify faster, i.e. the PBCOD hydrolysis rate is increased. This obviously requires further research. Regardless of the reason, the observation that the single K_2' rate is faster relative to the K_2 rate in N removal systems will be accepted and incorporated in the revision of the design procedure for nutrient removal plants.

ACKNOWLEDGEMENTS

I wish to express my gratitude to the following persons:

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Symbol	Description
a	Mixed liquor recycle ratio from aerobic to anoxic reactors
ADP	Adenosine diphosphate
API	Analytical Profile Index
ATP	Adenosine triphosphate
AVSS	Active volatile suspended solids
BEPR	Biological excess phosphorus removal
b_h	Specific endogenous mass loss rate for heterotrophs (/d)
COD	Chemical oxygen demand (mgCOD/ℓ)
D_p	Denitrification potential of the primary anoxic reactor (mgNO$_3$-N/ℓ influent)
D_{p3}	Denitrification potential of the secondary anoxic reactor (mgNO$_3$-N/ℓ influent)
D_{pp}	Denitrification potential of the process (mgNO$_3$-N/ℓ influent)
DO	Dissolved oxygen (mgO/ℓ)
DSVI	Diluted Sludge Volume Index (mℓ/g)
f	Endogenous residue fraction in the steady state model of Marais and Ekama (1976) (mgVSS/mgVSS)
f_{av}	Active fraction of the volatile suspended solids (mgAVSS/mgVSS)
f_{bs}	Readily biodegradable COD fraction with respect to the biodegradable COD (mgCOD/mgCOD)
f_{cv}	COD to VSS ratio of the sludge mass (mgCOD/mgVSS)
f_n	Nitrogen fraction of the sludge (mgN/mgVSS)
f_{up}	Unbiodegradable particulate fraction of the influent COD (mgCOD/mgCOD)

f_{us}	Unbiodegradable soluble fraction of the influent COD (mgCOD/mgCOD)
F/M	Food to microorganism ratio (mgCOD/mgVSS)
K_1	Specific denitrification rate (due to RBCOD) present for the first phase of denitrification in the primary anoxic reactor of N removal systems [mgNO$_3$-N/(mgAVSS.d)]
K_2	'Background' specific denitrification rate (due to PBCOD) manifested during the second phase of denitrification in the primary anoxic reactor of N removal systems [mgNO$_3$-N/(mgAVSS.d)]
K_3	Specific denitrification rate in the secondary anoxic reactor of N removal systems [mgNO$_3$-N/(mgAVSS.d)]
K_1'	First specific denitrification rate occasionally found in the plug flow primary anoxic reactor of the MUCT/UCT nutrient removal system [mgNO$_3$-N/(mgAVSS.d)]
K_2'', K_2'	The single accepted specific denitrification rate in the primary anoxic zone of the MUCT/UCT nutrient removal system [mgNO$_3$-N/(mgAVSS.d)]. Superscripts '' and ' refer to the rate before and after correction for nitrite accumulation respectively
K_3'', K_3'	The specific denitrification rate in the secondary anoxic reactors of nutrient removal systems [mgNO$_3$-N/(mgAVSS.d)] (before and after nitrite accumulation)
K_{NO_2}	The specific nitrite accumulation rate [mgNO$_2$-N/(mgAVSS.d)]
MLSS or MLTSS	Mixed liquor total suspended solids (mgTSS/ℓ)
MLVSS or VSS	Mixed liquor volatile suspended solids (mgVSS/ℓ)
MO_c	Carbonaceous oxygen demand (mgO/d)
MO_d	Equivalent oxygen demand of denitrification (mgO/d)
MO_n	Nitrification oxygen demand (mgO/d)
MS_{NO}	Mass of nitrate generated from nitrification (mgNO$_3$-N/d)
MS_{NOd}	Mass of nitrate denitrified (mgNO$_3$-N/d)
MS_{NOe}	Mass of nitrate in effluent (mgNO$_3$-N/d)

MS_{Nte}	Mass of TKN in effluent (mgN/d)
MS_{Nti}	Mass of TKN in influent (mgN/d)
MS_{te}	Mass of COD in effluent (mgCOD/d)
MS_{ti}	Mass of COD in influent (mgCOD/d)
MUCT	Modified University of Cape Town system for N and P removal
MX_N	Mass of nitrogen in waste sludge (mgN/d)
MX_{Svw}	Mass of COD in waste sludge (mgO/d)
MX_v	Mass of volatile suspended solids (mgVSS)
$M\Delta X_v$	Mass of sludge wasted per day (mgVSS/d)
N_c	Nitrification capacity (mgN/ℓ influent)
N_n	Nitrate concentration (mgN/ℓ)
N_{ne} or S_{NOe}	Nitrate concentration in effluent (mgN/ℓ)
N_s	Nitrogen concentration required for sludge production (mgN/ℓ)
N_t, N_{te} or S_{Nte}	TKN concentration in effluent (mgN/ℓ)
N_{ti} or S_{Nti}	TKN concentration in influent (mgN/ℓ)
NAD	Nicotinamide adenine dinucleotide (oxidized form)
NADH	Nicotinamide adenine dinucleotide (reduced form)
O_a	Oxygen concentration in mixed liquor a-recycle from aerobic to anoxic reactors (mgO/ℓ)
O_c or OUR	Carbonaceous oxygen demand (mgO/ℓ/d)
O_s	Oxygen concentration in the sludge underflow s-recycle (mgO/ℓ)
ΔP	Change in P concentration $-$ ive = uptake; $+$ ve = release (mgP/ℓ)
PBCOD	Particulate biodegradable COD

PHB	Poly-β-hydroxybutyrate
polyP	Polyphosphate
Q	Daily influent wastewater flow rate (ℓ/d)
r	Mixed liquor recycle ratio from anoxic to anaerobic reactors
RBCOD	Readily biodegradable COD
R_s	System sludge age (d)
s	Sludge underflow recycle ratio with respect to the feed flow rate
S_{bi}	Influent biodegradable COD concentration (mgCOD/ℓ)
S_{bsi}	Influent readily biodegradable COD concentration (mgCOD/ℓ)
SCFA	Short chain fatty acids
$S_{NO\ aer}$ or S_{NO_4}	Nitrate concentration in the aerobic reactor of the MUCT/UCT system (mgNO$_3$-N/ℓ)
$S_{NO\ anaer}$ or S_{NO_1}	Nitrate concentration in the anaerobic reactor of the MUCT/UCT system (NO$_3$-N/ℓ)
$S_{NO\ anox_1}$ or S_{NO_2}	Nitrate concentration in the first anoxic reactor of the MUCT/UCT system (mgNO$_3$-N/ℓ)
$S_{NO\ anox_2}$ or S_{NO_3}	Nitrate concentration in the second anoxic reactor of the MUCT/UCT system (mgNO$_3$-N/ℓ)
S_{NOe} or N_{ne}	Nitrate concentration in the effluent of the MUCT/UCT system (mgNO$_3$-N/ℓ)
S_{Nte}, N_t or N_{te}	Effluent TKN concentration (mgN/ℓ)
S_{Nti} or N_{ti}	Influent TKN concentration (mgN/ℓ)
S_{te}	Unfiltered effluent COD concentration (mgCOD/ℓ)
S_{ti}	Total unfiltered influent wastewater COD concentration (mgCOD/ℓ)
SVI	Sludge Volume Index (mℓ/g)
TKN	Total Kjeldahl nitrogen

UCT	University of Cape Town
V_a	Aerobic reactor volume (ℓ)
V_p	Process volume (ℓ)
X or X_v	Volatile suspended solids concentration of the sludge ($mgVSS/\ell$)
Y_h	Heterotrophic yield coefficient ($mgVSS/mgCOD$)
α	Fraction of nitrate removed by first rapid phase of denitrification in N removal plants.

CHAPTER 1

INTRODUCTION

1.1 Denitrification kinetics in nitrogen removal systems

Denitrification – the process whereby nitrate is biologically reduced to nitrogen gas – is an essential part of nitrogen removal in activated sludge systems and occurs under anoxic conditions (nitrate present, oxygen absent). In the design of the anoxic zones in biological nitrogen removal activated sludge systems, it is necessary to know the denitrification kinetics in order to estimate the degree of nitrogen removal that can be obtained. To date, the denitrification kinetics have been delineated accurately for systems that remove nitrogen only, i.e. for the Modified Ludzack-Ettinger (MLE) and 4-stage Bardenpho systems (see Figures 1.1a and 1.1b). In the MLE system, there is only a primary anoxic reactor located upstream of the main aeration reactor and it receives the influent sewage and the underflow recycle (the s-recycle) from the settling tank. The 4-stage Bardenpho system has both primary and secondary anoxic reactors, the latter being a denitrification reactor after the main aeration reactor. In both systems the mixed liquor first comes into contact with the influent sewage in the primary anoxic zone.

The denitrification kinetics that have been established for the two systems described above are shown graphically in Fig 1.2 (Stern and Marais, 1974 and van Haandel *et al.*, 1981). In the primary anoxic reactor, shown in Fig 1.2 to be a plug flow reactor, denitrification takes place in two phases – an initial phase of rapid nitrate reduction followed by a second slower phase. The overall rate during the first phase is made up of two separate rates: K_1 [due to the utilization of readily biodegradable COD (RBCOD)] plus the background K_2 [due to the utilization of particulate biodegradable COD (PBCOD)]. The second phase of denitrification is made up of only the background K_2 rate; in the secondary anoxic reactor (also shown as a plug flow reactor in Fig 1.2) there is only a single rate of denitrification (K_3) which is slower than the second rate in the primary anoxic reactor (K_2). The energy source for this form of denitrification is derived from particulate biodegradable COD generated by organism death and cell lysis. The kinetics described above are well understood and have formed the basis for the development of successful design procedures for these systems (WRC, 1984).
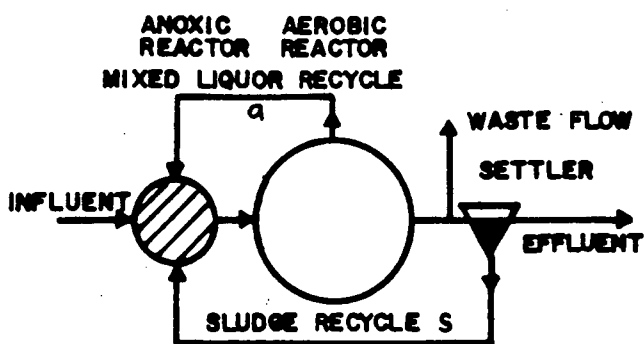
Fig 1.1(a): The Modified Ludzack-Ettinger (MLE) system.
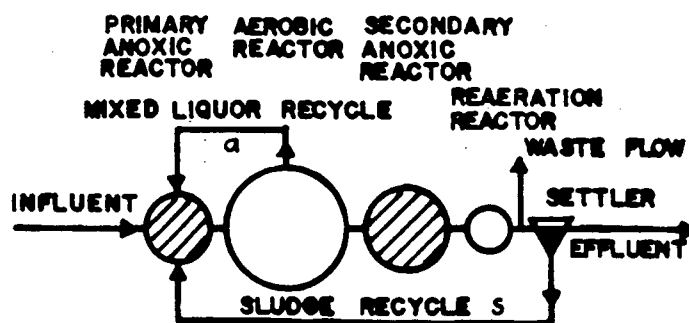
Fig 1.1(b): The 4-stage Bardenpho system.
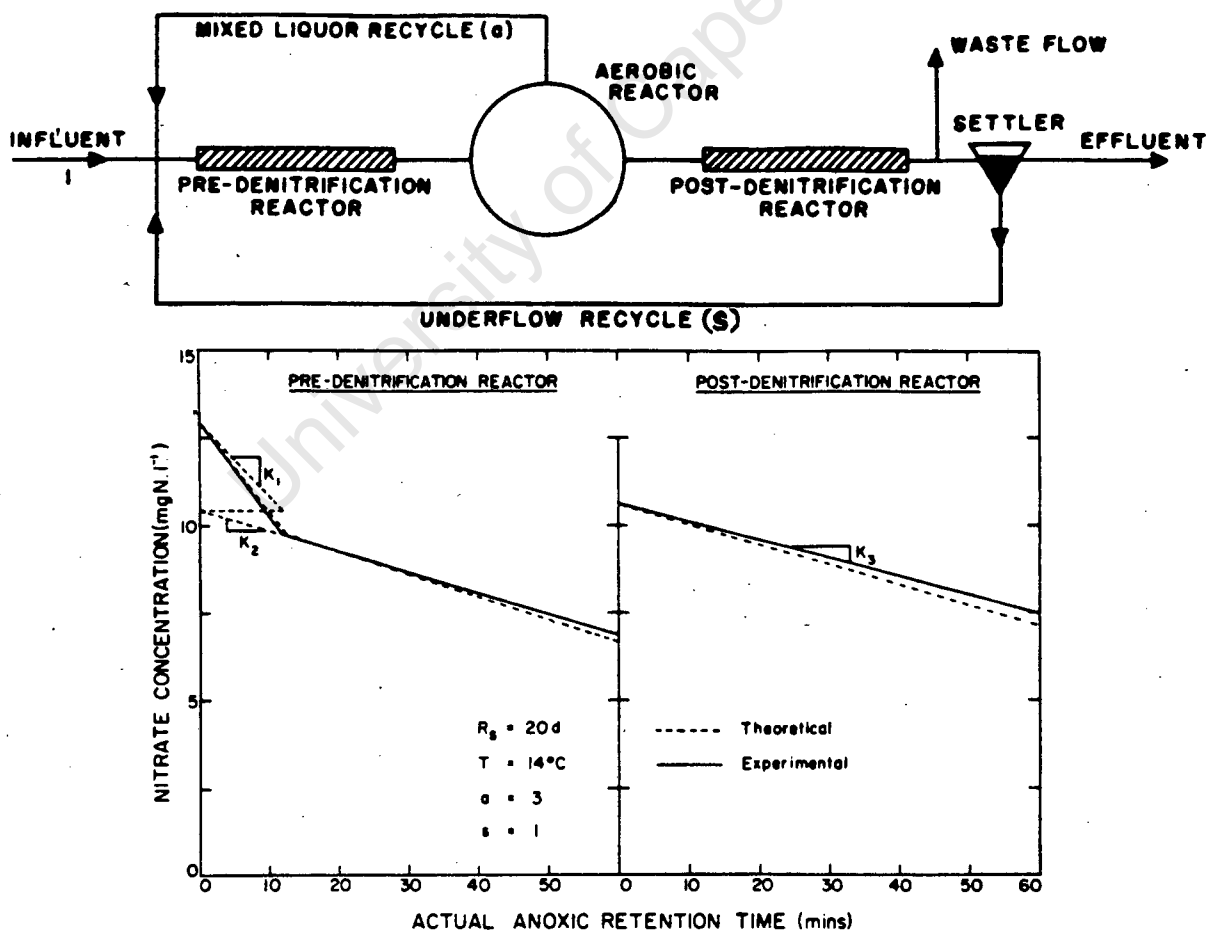
Fig 1.2: Top — Experimental denitrification system showing primary (pre-denitrification) and secondary (post-denitrification) anoxic plug flow reactors. Bottom — Comparison of nitrate concentration profiles predicted by the kinetic model with those observed under constant flow and load conditions at 20 days sludge age and 14° C.

1.2 Incorporation of biological phosphorus removal into nitrogen removal systems

To accommodate biological P removal, the system configurations for nitrogen removal were modified: an anaerobic reactor was installed upstream of the primary anoxic reactor, with the anaerobic reactor now receiving the influent sewage flow. A number of different biological N and P removal configurations have been developed. All of these function in accordance with the same biological mechanisms, and differ only in the origin of the sludge recycle stream to the anaerobic reactor. The main criterion governing the choice of the origin of the sludge recycle to the anaerobic zone is that the nitrate concentration entering the anaerobic reactor needs to be as low as possible – nitrate entering the anaerobic zone adversely affects excess biological P removal. Three types of N and P removal systems (also referred to as "nutrient removal systems") have been developed: the 3- and 5-stage Bardenpho systems (Figs 1.3a and 1.3b) in which the sludge recycle is the settling tank underflow, the UCT/Modified UCT (MUCT) systems (Figs 1.3c and 1.3d) in which the sludge recycle is a mixed liquor recycle from the primary anoxic reactor, and the Johannesburg (JHB) system (Fig 1.3e) in which the sludge recycle is from a denitrification reactor in the underflow recycle stream.

The rôle of the anaerobic zone in biological excess P removal (BEPR) is twofold: (1) to allow conversion of the influent readily biodegradable COD to short chain fatty acids (SCFA) via acidogenesis by facultative/aerobic organisms[1], (Wentzel et al., 1985) and (2) to provide an environment for polyphosphate-accumulating organisms such as Acinetobacter spp. to sequester the SCFA and store these internally as polyhydroxybutyrate (PHB). The energy for (2) originates from the cleavage of polyP chains within the Acinetobacter spp.; associated with this is a release of P to the surrounding liquid (Wentzel et al., 1986). This process of RBCOD conversion and SCFA sequestration for PHB formation depletes virtually the entire quantity of influent RBCOD, leaving only a small fraction in the anaerobic reactor effluent.

In the aerobic zone, the stored PHB is used by the polyP organisms to replenish their polyP chains and for new organism growth, resulting in a P uptake. Due to the growth of new polyP organisms, the amount of P taken up in the aerobic zone exceeds the P released in the anaerobic zone by about 14%. Consequently, systems

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[1]"Facultative" refers to organisms that are able to grow under a variety of conditions; facultative aerobic bacteria can grow in aerobic (presence of dissolved oxygen and nitrate), anoxic (absence of dissolved oxygen but presence of nitrate) and anaerobic (absence of dissolved oxygen and nitrate) zones.
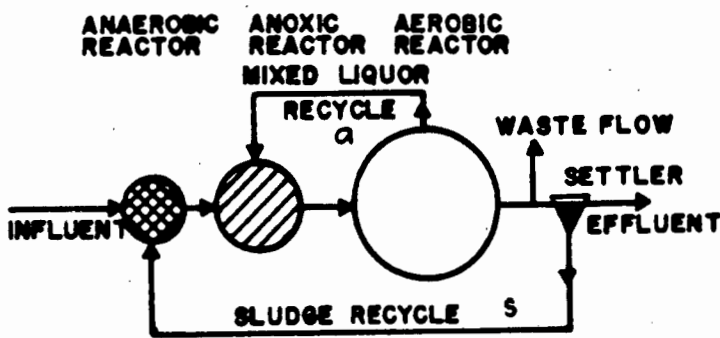
Fig 1.3(a):   The 3-stage Bardenpho system.
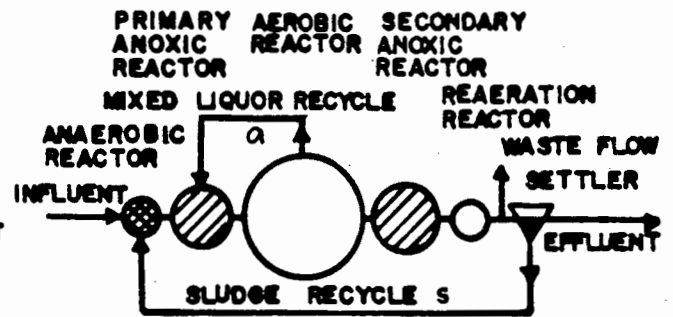
Fig 1.3(b):   The 5-stage Bardenpho system.
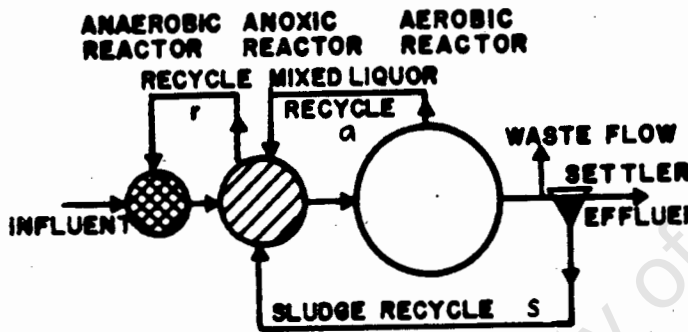
Fig 1.3(c):   The UCT system.
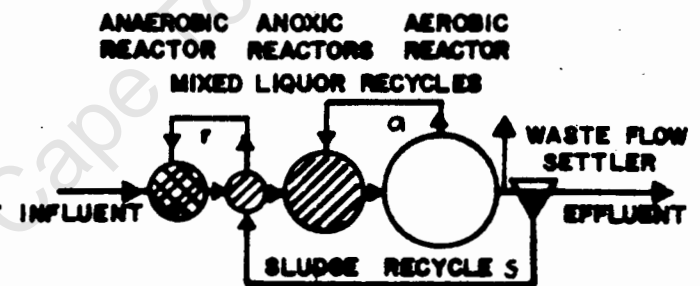
Fig 1.3(d):   The Modified UCT (MUCT) system.
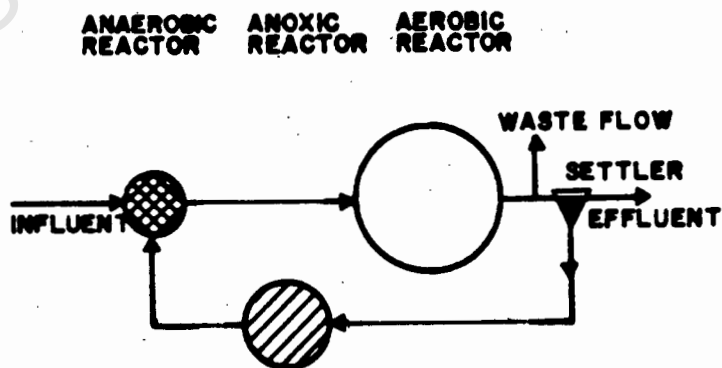
Fig 1.3(e):   The Johannesburg (JHB) system.

that achieve BEPR essentially stimulate the growth of the polyP organisms with very high P contents in the aerobic zone. The presence of polyP organisms increases the overall P content of the total VSS in the aerobic reactor from the usual 2,5 to 3% to around 8% (for normal domestic sewages with a readily biodegradable fraction of 20%). Because of the high P content of the VSS, a significant mass of P is removed from the wastewater through the daily sludge wastage from the aerobic zone.

1.3   The problem and research objective

From the behaviour of the facultative aerobic and polyP organisms outlined above, most (70 − 90%) of the RBCOD is converted to SCFA and is removed from the liquid phase in the anaerobic reactor. The remaining biodegradable COD in the effluent from the anaerobic reactor is the small amount of RBCOD not removed plus the particulate biodegradable fraction of the influent sewage. The question that arises is: What is the effect of the uptake of RBCOD in the anaerobic reactor by polyP organisms on the denitrification kinetics in the primary anoxic reactor?

In terms of the denitrification kinetics for N removal systems it would appear that the removal of RBCOD in the anaerobic reactor should result in the virtual elimination of the initial rapid rate of denitrification in the primary anoxic reactor leaving only the second slow denitrification rate. This seems to suggest that the N removal ability of nutrient removal systems should be significantly lower than that of N removal systems. However, observations on laboratory and full-scale nutrient removal systems have indicated that the introduction of an anaerobic zone and the presence of excess biological P removal that this zone stimulates, does *not* significantly affect the degree of nitrogen removal (Nicholls, 1982). Clearly, the denitrification behaviour in nutrient removal systems is more complex than meets the eye and a closer study of the behaviour is required.

The question of the influence of biological P removal on denitrification was recognized early in the development of the biological N and P removal technology. However, because empirical observations showed that the extent of denitrification appeared unaffected by BEPR, it was accepted that in the short term the established denitrification kinetics for N removal systems would apply for design of anoxic zones in N and P removal systems (as in WRC, 1984) until the uncertainties outlined above were solved. The aim of this experimental investigation was therefore set to gain insight into the denitrification kinetics in the primary anoxic reactor in nutrient

removal systems.

1.4.   Scope of work

The first part of the investigation was concerned with establishing the denitrification behaviour in the primary anoxic reactor of an MUCT/UCT system (a nutrient removal configuration). This led to the formulation of two hypotheses to account for the observed behaviour. The final part of the investigation was to collect evidence to evaluate these two hypotheses.

1.5.   Overview of thesis

In Chapter 2, a literature survey is presented reviewing the denitrification theory developed for N removal systems and the biochemical model for excess P removal; the implications of biological P removal on denitrification in an N and P removal system are also discussed. Chapter 3 outlines the experimental programme adopted for the research; this involved operating a plug flow primary anoxic reactor in an MUCT/UCT system and conducting batch tests to confirm the observed denitrification behaviour in the plug flow reactor. In Chapter 4, the plug flow and batch test experiments and their results are described. In Chapter 5, two hypotheses to explain the observed denitrification are presented and in Chapter 6, the tests that were conducted to evaluate the hypotheses are described. Chapter 7 summarizes the major conclusions and gives recommendations regarding the design of anoxic zones in N and P removal systems. Aspects that require further research in this area of work are also discussed.

CHAPTER 2

DEVELOPMENT OF BIOLOGICAL N AND P REMOVAL
THEORY: A LITERATURE REVIEW

To form a framework in which to interpret the research described in this thesis, it is necessary to highlight the main features of the theories for biological nitrogen and phosphorus removal in the single sludge activated sludge system. In this chapter, the theories of biological N and P removal (as they are understood at present) are reviewed. Application of these theories to design is also discussed, and the possible influence of P removal on denitrification is examined in terms of the theories.

2.1   Development of denitrification theory

2.1.1   The process of denitrification

Biological nitrogen removal in activated sludge systems is achieved via two mechanisms: (i) incorporation of nitrogen into the sludge mass and (ii) dissimilative reduction of nitrate to nitrogen gas. Of these two mechanisms, the latter, commonly known as denitrification, removes the major part of the influent nitrogen load – approximately 70 to 80% (van Haandel et al., 1981).

The ability of the bacterial mass in activated sludge systems to denitrify has been established in numerous cases (Christensen and Harremöes, 1977a). Denitrification is biologically mediated by organisms that are part of a group known as 'facultative heterotrophic aerobes'.[1] Redox reactions take place in which sewage is the electron donor and nitrate or nitrite the electron acceptor. The nitrogen, in the form of dissolved nitrate or nitrite, is reduced to nitrogen gas which escapes to the atmosphere:

Sewage: Organic Compound $\rightarrow$ Organisms $+ e^- + CO_2 + H_2O$ (2.1)

Nitrate: $e^- + \frac{1}{5} NO_3^- + \frac{6}{5} H^+ \rightarrow \frac{1}{10} N_2 + \frac{3}{5} H_2O$ (2.2)

Nitrite: $e^- + \frac{1}{3} NO_2^- + \frac{4}{5} H^+ \rightarrow \frac{1}{6} N_2 + H_2O$ (2.3)
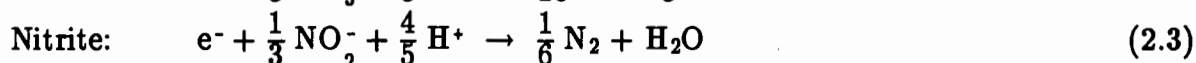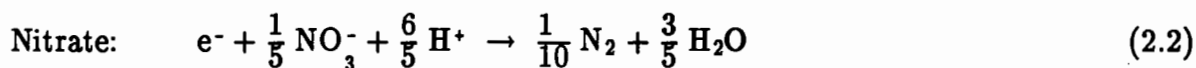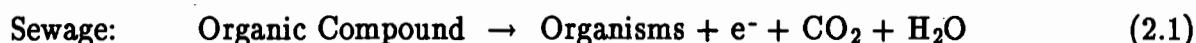
The nitrogen in the influent sewage is predominantly in the free and saline ammonia

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[1]'facultative aerobes' : these organisms can operate under anaerobic, anoxic or aerobic conditions; 'heterotrophic' : they use organic matter as an energy source.

form (NH_4^+); very little exists as oxidized nitrate or nitrite. The oxidized forms are synthesized from the ammonia through nitrification, a biological process carried out under aerobic conditions by nitrifying organisms ('obligate autotrophic aerobes'),[2] prior to denitrification. Consequently, to obtain biological N removal by denitrification, nitrification under aerobic conditions must first take place. However, denitrification per se is severely inhibited by the presence of dissolved oxygen (Chong and Morris, 1962), and for this reason a special denitrification zone (separated from the aerobic zone), is provided. This zone is unaerated and nitrate is discharged to it to create conditions that are termed 'anoxic' (nitrate but no oxygen). The positioning of the anoxic zone in relation to the aerobic zone gives rise to a number of different configurations for N removal.

2.1.2 Process configurations

The different process configurations that have been developed for biological N removal basically fall into two categories: (i) Systems in which denitrification takes place utilizing biodegradable material in the influent, i.e. the sewage is the electron donor and (ii) systems in which denitrification utilizes self-generated biodegradable material, i.e. the electron donor is provided by the biological sludge.

(i)   Influent energy source: Two systems that utilize influent biodegradable material as an energy source for denitrification are shown in Figs 2.1a and 2.1b. They are the Ludzack-Ettinger (Ludzack and Ettinger, 1962) and Modified Ludzack-Ettinger (Barnard, 1973) systems respectively. In both systems the influent sewage is fed directly to the anoxic zone. Being upstream of the aerobic reactor, the anoxic zone in these configurations is known as the primary anoxic zone. In the Ludzack-Ettinger configuration the nitrate formed through nitrification in the aerobic zone is made available for denitrification in the anoxic zone through the partial mixing of the two zones. The Modified Ludzack-Ettinger (MLE) configuration has totally separate aerobic and anoxic reactors, nitrate being recycled to the anoxic zone via a mixed liquor stream from the aerobic reactor and the underflow stream from the clarifier. This latter design gives a much improved and more uniform denitrification performance than the Ludzack-Ettinger system. Unfortunately, neither process can attain a system effluent containing no nitrate since the overflow from the clarifier is in effect the aerobic reactor

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[2] 'obligate aerobes' : these organisms can only use oxygen as an electron acceptor; 'autotrophic' : they use inorganic matter as an energy source.
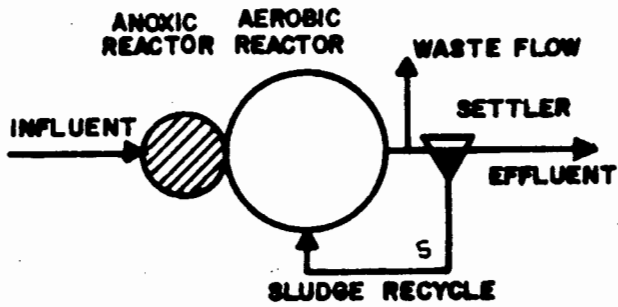
Fig 2.1(a): The Ludzack-Ettinger system.
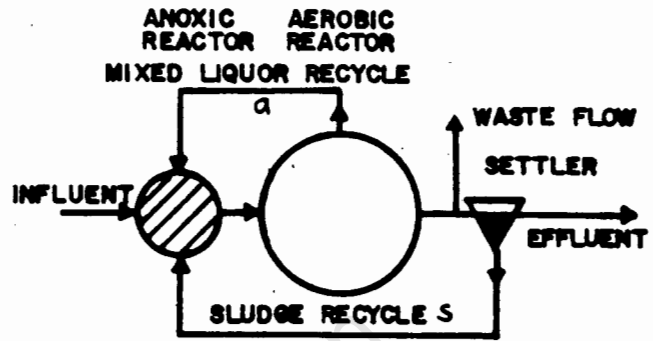
Fig 2.1(b): The Modified Ludzack-Ettinger (MLE)system
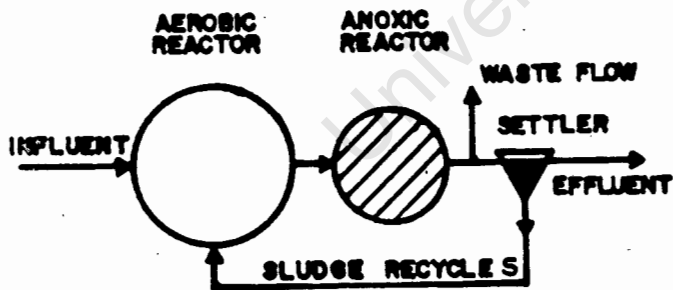
Fig 2.1(c): The Wuhrmann system.
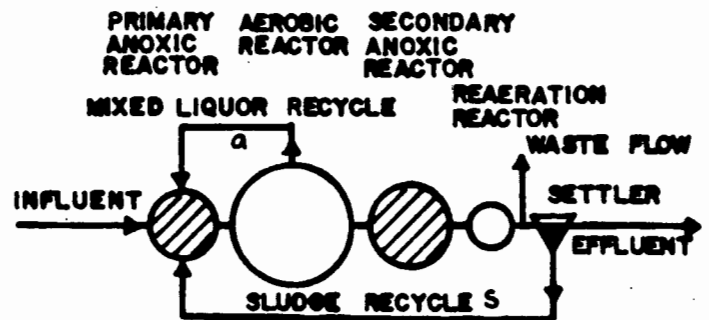
Fig 2.1(d): The 4-stage Bardenpho system.

effluent which has a high nitrate concentration.

(ii) Internally-generated energy source: The Wuhrmann system (Wuhrmann, 1964) which is based on denitrification using biodegradable material generated by the sludge itself, is shown in Fig 2.1c. The anoxic reactor is downstream from the aerobic reactor (in this instance the anoxic reactor is termed a secondary anoxic reactor) and the only material remaining for denitrification arises from products of cell death and lysis. Unlike the Ludzack-Ettinger and MLE systems described earlier, the Wuhrmann configuration has the potential of reducing the nitrate concentration in the system effluent to zero since the anoxic reactor follows the aerobic reactor, the source of the nitrate. However, because the denitrification rate using products of organism death and lysis is lower than that using influent material, a large anoxic mass fraction is needed to obtain appreciable denitrification. Large anoxic mass fractions can only be achieved at the expense of the aerobic sludge mass fraction; making the aerobic mass fraction too small can lead to loss of nitrification, the prerequisite for denitrification. The Wuhrmann system is therefore more limited in the amount of denitrification it can produce.

To attain good denitrification together with the possibility of a zero effluent nitrate concentration, Barnard (1973) combined the MLE and Wuhrmann configurations as shown in Fig 2.1d; this system has become known as the 4-stage Bardenpho system.

Over a 10 year period (1974 to 1984), the kinetics of biological denitrification in primary and secondary anoxic reactors (like those in the MLE and Wuhrmann systems respectively) were investigated. The outcome of this research is briefly reviewed below.

2.1.3   The empirical denitrification model

Carlson (1971) and Christensen and Harremöes (1977b) proposed that denitrification by activated sludge mixed liquor may be described by

$$dN_n/dt = KX \qquad (2.4)$$

where

dN_n/dt = denitrification rate [mgN nitrate/(ℓ·unit time)]

N_n = nitrate concentration (mgN/ℓ)

t = time in hours or days

X = volatile solids concentration (mgVSS/ℓ)

K = specific denitrification constant [mgN/(mgVSS·time)].

This indicates the denitrification rate to be independent of nitrate concentration, i.e. the rate is zero order relative to the nitrate concentration and depends only on the volatile suspended solids concentration.

To examine the validity of Eq 2.4, Marais and co-workers (Stern and Marais, 1974; Wilson and Marais, 1976; Marsden and Marais, 1976) undertook an extensive investigation into the denitrification kinetics in primary and secondary anoxic reactors of N removal systems under constant load and flow conditions. This was achieved by measuring nitrate concentration versus retention time profiles in plug flow primary and secondary anoxic reactors (see Fig 2.2a). Typical nitrate concentration-retention time profiles observed for the primary and secondary anoxic reactors are shown in Figs 2.2b and 2.2c respectively. The primary anoxic reactor denitrification was characterized by a two-phase behaviour: an initial sharp decline in nitrate concentration with time, followed by a second slower rate that continued until the end of the reactor. The secondary anoxic reactor showed a single rate of denitrification, slower than the second rate in the primary anoxic reactor.

Both rates in the primary anoxic reactor and the single rate in the secondary anoxic reactor were observed to be zero order with respect to nitrate concentration in agreement with Eq 2.4. However, examination of the data over a range of sludge ages (10-20 days) indicated that the denitrification rates were not proportional to the volatile solids concentration of the sludge as implied by Eq 2.4, but rather the *active* mass concentration as calculated by the steady state model of Marais and Ekama (1976) – on the basis of volatile solids concentration, the specific denitrification rate [mgNO$_3$-N/(mgVSS·d)] decreased as sludge age increased; in contrast, when the specific rate was defined in terms of the *active mass concentration*, [mgNO$_3$-N/(mgAVSS·d)], the rate was found constant with sludge age.

From the slope of the nitrate-time profiles, accepting that the initial measured rate in the primary anoxic reactor is the sum of an initial rapid rate and a continuous background slower rate (for reasons, see section 2.1.4 below), average denitrification
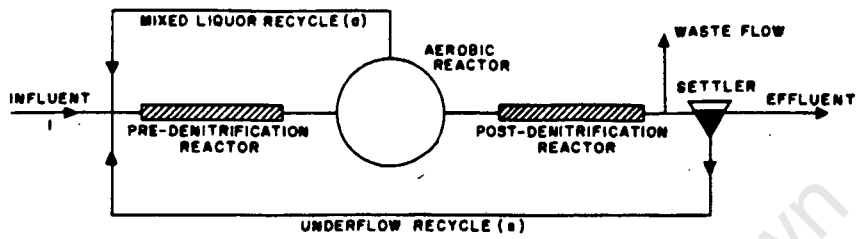
Fig 2.2(a): *Experimental system for examining the denitrification kinetics in the primary and secondary anoxic reactors of N removal systems.*

(b) Pre-denitrification   (c) Post-denitrification
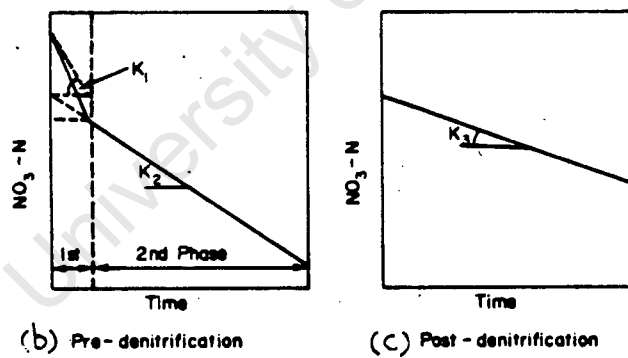
Fig 2.2(b): *Typical nitrate concentration—retention time profiles in anoxic plug flow reactors.*

rates and their temperature sensitivity were determined. The two rates in the primary anoxic and the single rate in the secondary anoxic were designated K_1, K_2 and K_3 respectively and the following average values were found:

$$K_{1_T} = 0,720 \ (1.20)^{(T-20)} \ [mgNO_3\text{-}N/(mgAVSS \cdot d)] \tag{2.5}$$

$$K_{2_T} = 0,101 \ (1,08)^{(T-20)} \ [mgNO_3\text{-}N/(mgAVSS \cdot d)] \text{ for } T \geq 13°C \tag{2.6}$$

$$K_{3_T} = 0,072 \ (1,03)^{(T-20)} \ [(mgNO_3\text{-}N/(mgAVSS \cdot d)] \tag{2.7}$$

In evaluating the three denitrification rates, it was found that they are: (1) Independent of mixing regime – in experiments where the plug flow reactors were replaced with completely mixed ones, the extent of denitrification remained unchanged; this verified that the three denitrification rates were zero order with respect to nitrate concentration; (2) Independent of the mixed liquor a-recycle ratio provided the nitrate concentration does not reduce to zero; (3) Independent of sludge age (as discussed above). Furthermore, it was found that the concentration of nitrate (with respect to the influent) removed by the first rate (K_1) is proportional to the influent COD concentration; the constant of proportionality (α) was found to be a function of the influent readily biodegradable COD (RBCOD) fraction (f_{bs}) and the stoichiometric mass of COD utilized per unit mass NO_3-N denitrified (8,6), i.e. $\alpha = f_{bs}/8,6$ (van Haandel *et al.*, 1981).

As a consequence of the above findings, it was possible to develop simple expressions for the denitrification potential of primary and secondary anoxic zones based on the K_1, K_2 and K_3 denitrification rates and the steady state activated sludge model of Marais and Ekama (1976). Before describing these expressions, it is instructive to first review the integration of the empirical denitrification kinetics into the general aerobic model for simulating cyclic flow and load conditions; this serves as a verification of the empirical denitrification kinetics discussed above.

2.1.4 Integration of empirical denitrification kinetics into the general aerobic model

The denitrification expressions developed for constant flow and load conditions are essentially empirical; except for the linkage of all the denitrification rates to the

active mass concentration and the K_1 rate also to the RBCOD fraction, there is no evident connection with the biological processes for COD removal. To find the relationship, attention was directed to the aerobic kinetic model.

In this description of the integration, a working knowledge of the aerobic general kinetic activated sludge model of Dold *et al.* (1980) is required, both in terms of its substrate definition and its description of substrate utilization. In essence, the model's substrate definition is based on a bi-substrate concept — the influent biodegradable COD is divided into two fractions, i.e. (1) a readily biodegradable soluble (RBCOD) fraction and (2) a slowly biodegradable particulate (PBCOD) fraction. With regard to substrate utilization, the RBCOD is *absorbed* and utilized directly for synthesis by the organisms at a relatively high specific rate; in contrast the PBCOD is *adsorbed* onto the organism mass and *stored* there — enzymes attached to the organism's surface *hydrolyze* the stored COD to simpler compounds which are then *absorbed* by the organisms. The overall specific rate is relatively slow and is controlled by the rate-limiting hydrolysis step. Details are given by Dold *et al.* (1980).

To incorporate the denitrification kinetics into the aerobic model, it was hypothesized that the two-phase behaviour in the primary anoxic reactor arose from the response of the active organisms to the bi-substrate nature of the influent. The hypothesis was checked by simulating *aerobic* primary and secondary plug flow reactors and comparing the predicted nitrate profiles [obtained from the dissolved oxygen (DO) profile divided by the stoichiometric electron acceptor equivalence between oxygen and nitrate, i.e. 2,86 mgO/mgNO$_3$-N] with the observed nitrate profiles. The simulated and observed nitrate profiles are strikingly similar (Fig 2.3); the two-phase behaviour in the primary anoxic reactor and single-phase behaviour in the secondary anoxic reactor are clearly reproduced in the aerobic simulations except that the simulated slopes corresponding to the K_2 and K_3 rates differ from the observed anoxic slopes. The similarity was so clearly evident that the bi-substrate hypothesis was accepted as a basis for modelling denitrification; further modifications would be made as required to form a more general anoxic-aerobic dynamic model. In terms of the model, it was hypothesized that in the primary anoxic reactor, the first phase of denitrification is made up of two rates — the initial fast rate (K_1), due to the RBCOD, plus the second background rate (K_2), due to influent PBCOD[3]; when all the RBCOD has been utilized, the K_2 rate continues

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[3]The model of Dold *et al.* (1980) suggests that the RBCOD absorption/utilization and PBCOD hydrolysis/utilization are reactions that take place simultaneously.
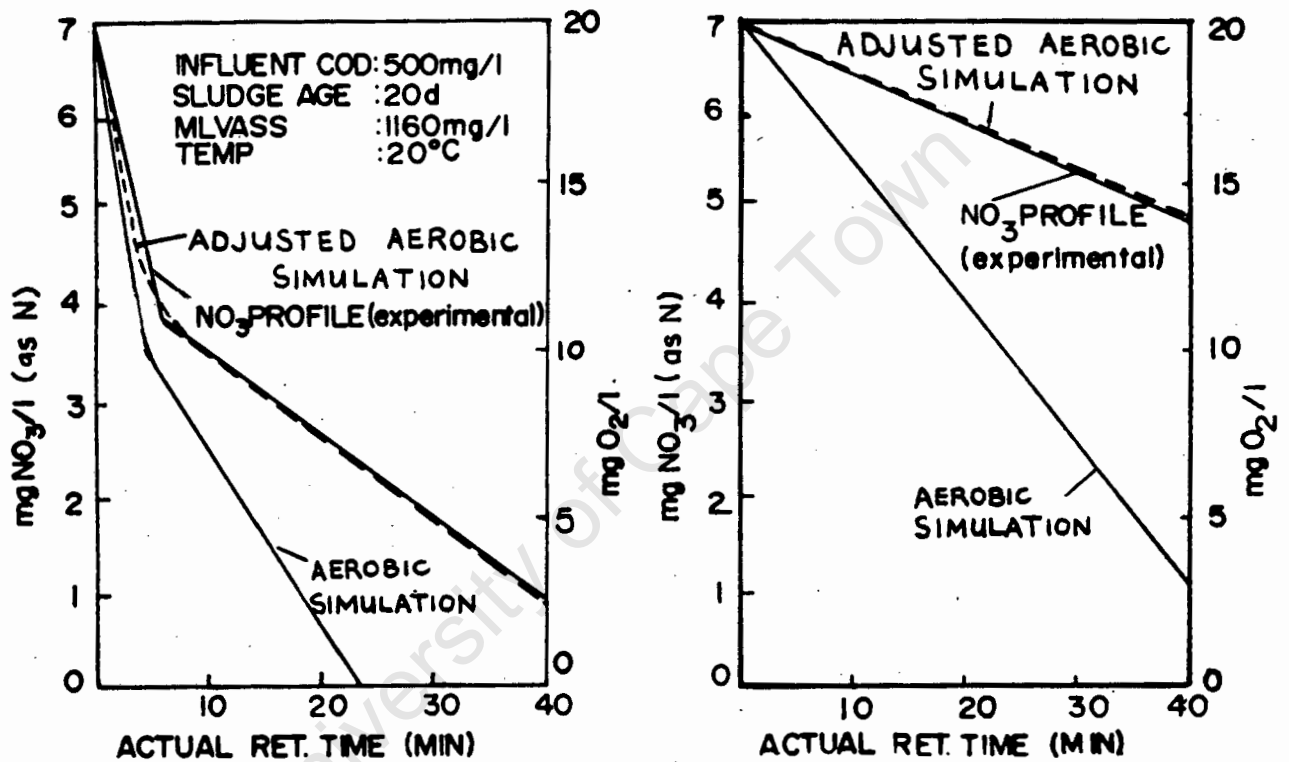
<u>*Fig 2.3*</u>:   *Experimental and simulated nitrate profiles in primary and secondary plug flow anoxic reactors (left and right respectively). The simulations were performed with the aerobic kinetic model, converting the resulting DO profiles to nitrate profiles (1mg NO_3-N $\equiv$ 2,86 mgO_2). Note the improved predictions when the hydrolysis—utilization rate of PBCOD under anoxic conditions is adjusted to 38% of its aerobic value.*

alone until the end of the reactor. For the secondary anoxic reactor it was suggested that the continuous K_3 rate is due to utilization of particulate biodegradable COD from cell death and lysis (this material is less biodegradable than the influent PBCOD and K_3 is hence slower than K_2).

The first modification made to the model was to correctly predict the K_2 and K_3 denitrification rates. This could be achieved by assuming that the hydrolysis-utilization rate of stored COD under anoxic conditions is reduced to 38% of its value under aerobic conditions (see Fig 2.3). The reason for this reduction is uncertain; it could be that only about 40% of the active organism population that grows in single sludge systems is facultative (able to utilize nitrate or oxygen as an electron acceptor), or all is facultative but the hydrolysis-utilization rate of stored COD indeed is reduced to 38% under anoxic conditions; from a modelling point of view, the actual reason is immaterial.

Another major problem that arose was one associated with endogenous mass loss/respiration kinetics[4] in the model. In order to make energy available for endogenous respiration, an electron acceptor (oxygen or nitrate), must be present. In anoxic zones, sometimes the nitrate concentration is reduced to zero, i.e. the conditions become anaerobic, in which event endogenous mass loss should cease. Modelling this situation by cessation of endogenous mass loss could not be justified in terms of biological behaviour and experimental data. The problem was resolved by abandoning the endogenous mass loss/respiration approach and replacing it with a 'death-regeneration' one. In this approach, an attempt is made to separate the reactions which possibly take place during the 'death phase' of organisms; a fraction of the active mass dies, lyses biodegradable COD back to the liquid phase, and leaves the balance as unbiodegradable endogenous residue. The lysed biodegradable COD adds to the PBCOD in the liquid and passes through the same processes of enmeshment, adsorption, storage, hydrolysis and synthesis to new organism mass as the influent PBCOD. With this approach, endogenous respiration does not exist and oxygen is consumed only for synthesis of new organism mass from either influent or lysed COD. The regenerated active mass is less than the mass that dies, due to the energy loss in the synthesis process.

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[4]Endogenous mass loss/respiration is a concept that is used to model the observation that daily a fraction of the active organism dies, or is lost, and the energy this releases is oxidized for cell maintenance.

For aerobic and anoxic conditions (nitrate concentration > 0) there is little to choose between the two approaches; by appropriate selection of constants, the two approaches lead to the same quantitative results; indeed the two approaches are linearly related (Dold *et al.*, 1980). However, when conditions become anaerobic (no oxygen or nitrate), the advantages of the death-regeneration approach become evident; the hydrolysis-utilization reactions cease, but organism death with its associated lysis of biodegradable COD continues, resulting in a build-up of biodegradable material (particulate). This accumulation can be considerable, so that when the mixed liquor enters the aerobic zone where oxygen is available, aerobic degradation of the accumulated biodegradable COD causes a high OUR; this high OUR is in accordance with observation (Ekama *et al.*, 1979) and is higher than that obtained if accumulation of biodegradable COD through lysis did not take place. In this respect, the death-regeneration approach is a convenient, simple and reliable framework in which to model the kinetics of COD degradation in anaerobic-anoxic-aerobic single sludge systems.[5]

The last modification required was a minor one and concerned nitrification: Growth of nitrifiers takes place only under aerobic conditions, but endogenous mass loss occurs throughout the system.

The modified model satisfactorily simulated anaerobic-anoxic-aerobic behaviour of COD removal, nitrification and denitrification in multi-reactor systems at temperatures ranging between $14°C$ and $22°C$ and sludge ages longer than 4 days under constant and cyclic flow and load conditions (van Haandel *et al.*, 1981). As examples, the measured and predicted responses are shown for (1) a 5 tank in-series $40 \ m^3/d$ pilot plant at 18 days sludge age under constant flow and load (Fig 2.4) and (2) a single reactor system at 6 days sludge age under constant flow and load conditions with alternating anoxic (5h) and aerobic (19h) conditions (Fig 2.5).

With the general model calibrated, reasons for the constancy of K_1, K_2 and K_3 under constant flow and load conditions were sought. With regard to K_1, which arises from utilization of RBCOD, the rate of utilization remains constant right down to low RBCOD concentrations. This is due to the low half saturation coefficient in the

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[5]'single sludge systems' refers to those systems in which the same sludge mass is utilized for COD and nitrogen removal (and possibly P removal as well). By contrast, multiple sludge systems have separate reactors with settlers in series, each system performing a different function of either COD removal, nitrification and/or denitrification, etc.
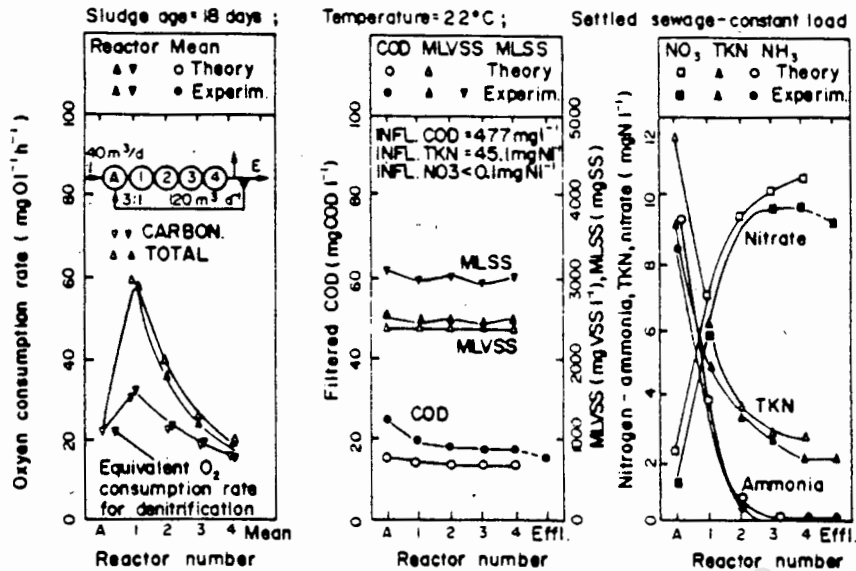
Fig 2.4: Comparison of the response profiles theoretically predicted with those experimentally observed in a 5 in-series reactor pilot plant (40 m³/d), of which the first reactor is anoxic, under constant flow and load conditions at 18 days sludge age, pH 7,8 and 22° C.
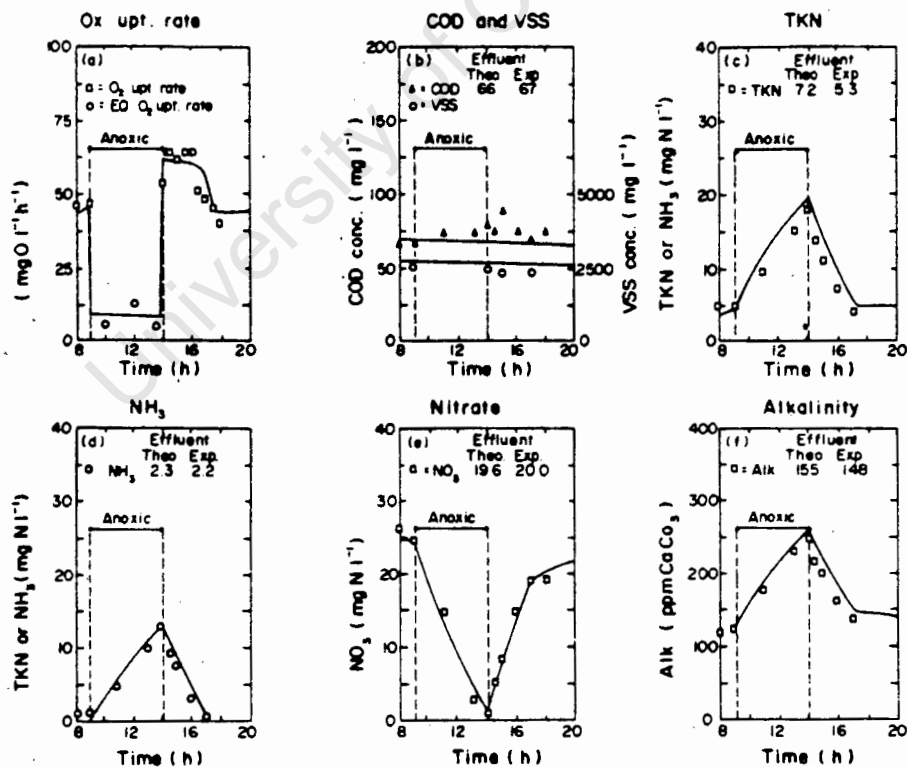
Fig 2.5: Comparison of the response profiles theoretically predicted with those experimentally observed in a single reactor alternating anoxic (5h)/aerobic (19h) under constant flow and load conditions at 6 days sludge age, pH 7,0 and 20° C.

Monod equation for utilization of RBCOD. With regard to K_2 and K_3, the level of stored COD changes relatively little through the anoxic reactor due to the reduced hydrolysis rate. The reason for the K_2 being higher than K_3 arises from different levels of stored COD; in the primary anoxic reactor the level is high because PBCOD for storage originates from the influent and organism death, whereas in the secondary anoxic, the level is low because PBCOD originates only from death. It was concluded that the K_1, K_2 and K_3 denitrification "constants" have no fundamental kinetic significance; their constancy is the result of a combination of kinetic reactions which fortuitously, show little variation with sludge age in the range 10 to 30 days (Fig 2.6). Temperature does affect the K constants but once the kinetic constants are adjusted for temperature, again the K rates show little variation at different sludge ages (Fig 2.6). The experimentally measured and theoretically predicted K values between 12°C and 22°C are shown in Fig 2.7 (van Haandel *et al.*, 1981).

It can be concluded both from experimental observation and theoretical kinetic points of view, that acceptance of constant values for K_2 and K_3 at any temperature is acceptable for design. It will be seen below that this in fact is done to estimate the denitrification potential under constant flow and load conditions. With regard to K_1, this rate can change significantly because the RBCOD utilization rate can change appreciably depending on the mixing regime in the anoxic (or aerobic) reactor (see Ekama *et al.*, 1986 and Still *et al.*, 1986). However, its variation does not affect design significantly because normally, primary anoxic reactors are sufficiently large to allow complete utilization of RBCOD even when the utilization is low.

2.1.5 Design of denitrification systems

The general anaerobic-anoxic-aerobic model described above requires the system configuration, load conditions and system parameters to be completely defined before the system response can be simulated. Consequently, the model is not useful for design because it does not provide system parameters for optimum nitrogen removal except by a very tedious repeated trial simulation of selected configurations and system parameters. To reduce the number of trials, the empirical denitrification kinetics for constant flow and load conditions were taken a step further by developing a system design and optimization procedure. This procedure, which is briefly described below, allows direct calculation of the system configuration and operational parameters to achieve maximum removal of nitrogen in a single sludge
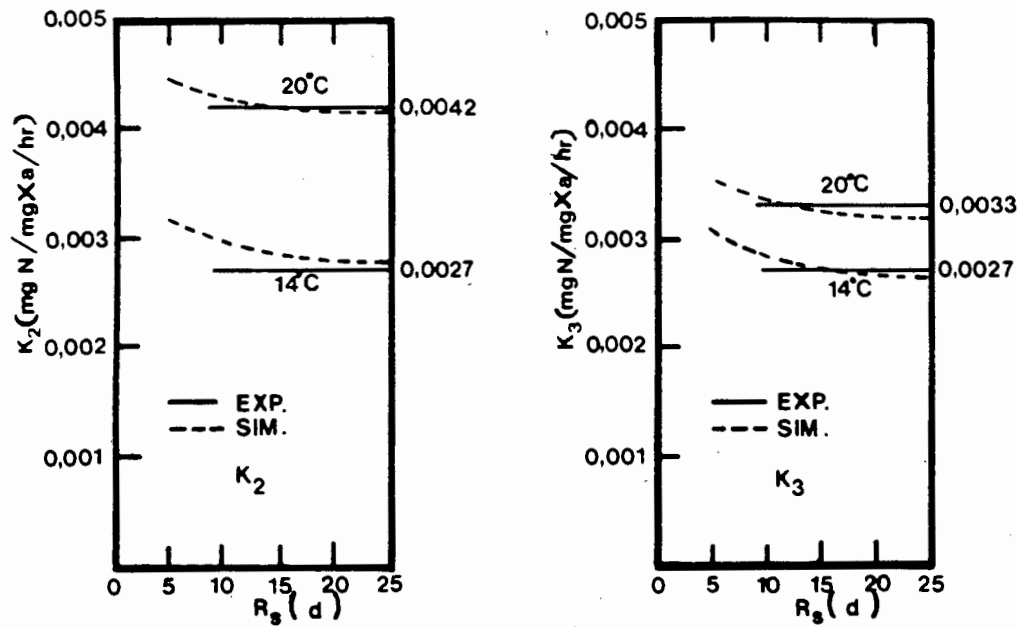
Fig 2.6: _Theoretically predicted and experimentally observed second primary anoxic and secondary anoxic denitrification rates (K_2 and K_3 respectively) versus sludge age at 14° C and 20° C. For design purposes the K_2 and K_3 rates can be accepted as constant with sludge age for constant flow and load conditions._
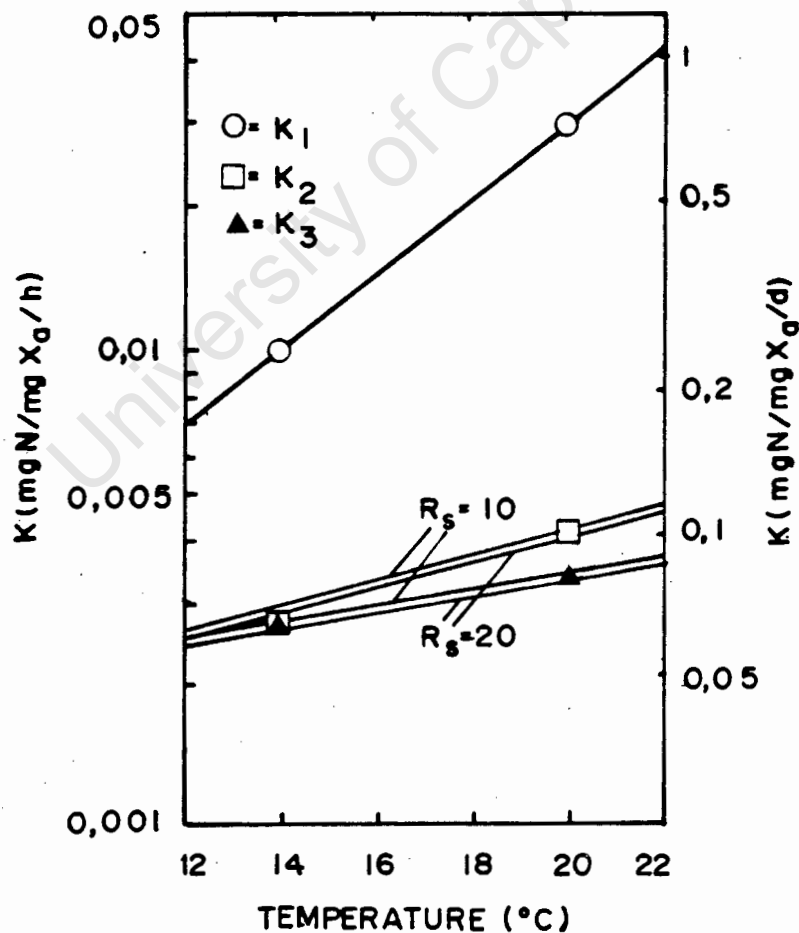
Fig 2.7: _Theoretically predicted and experimentally observed denitrification rates K_1, K_2 and K_3 versus temperature at 10 and 20 days sludge age showing the sensitivity of the denitrification rates to temperature._

nitrification-denitrification system, within the constraints of the system itself and the sewage characteristics.

With the aid of the K_1, K_2 and K_3 denitrification rates and the steady state activated sludge model of Marais and Ekama (1976), van Haandel *et al.* (1982), Ekama *et al.* (1983) and WRC (1984) developed simple expressions for the denitrification *potential* of primary and secondary anoxic reactors, i.e. the concentration of nitrate (with respect to the influent) that a reactor can denitrify:

For the primary anoxic reactor:

$$D_{p1} = S_{bi} \{ \alpha + K_2 f_{x1} Y_h R_s / (1 + b_{hT} R_s) \}$$
(2.8)

where

D_{p1} = denitrification potential of the primary anoxic reactor (mgN/ℓ influent)
S_{bi} = biodegradable COD concentration of the influent (mgCOD/ℓ)
α = fraction of nitrate removed by the initial rapid phase of denitrification
  = $f_{bs} (1 - f_{cv} Y_h)/2,86$
f_{bs} = readily biodegradable fraction of the influent biodegradable COD
f_{cv} = COD to VSS ratio of volatile sludge mass
  = 1,48 mgCOD/mgVSS
Y_h = heterotrophic organism yield coefficient
  = 0,45 mgVSS/mgCOD
f_{x1} = primary anoxic sludge mass fraction
R_s = sludge age (d)
b_{hT} = endogenous mass loss rate for heterotrophic organisms at T°C
  = $0,24 (1,029)^{(T-20)}$ (/d)

For the secondary anoxic reactor:

$$D_{p3} = S_{bi} f_{x3} K_3 Y_h R_s / (1 + b_{hT} R_s)$$
(2.9)

where

D_{p3} = denitrification potential of the secondary anoxic reactor (mgN/ℓ influent)

f_{x3} = anoxic sludge mass fraction of the secondary anoxic reactor.

From these two equations it can be seen that the denitrification potential of primary or secondary anoxic reactors is a function of the influent biodegradable COD concentration (S_{bi}) and the size of the anoxic zone (f_{x1} or f_{x3}) and for the primary anoxic reactor, additionally the readily biodegradable COD fraction (f_{bs}). In practice, where primary anoxic reactors usually have mass fractions that vary between 20 and 30%, the influent RBCOD fraction (f_{bs}) contributes about 50% to the denitrification potential.

The denitrification potential parameter is extremely useful in design because together with the nitrification capacity, it is relatively simple to calculate (1) the optimum mixed liquor recycle ratio, (2) whether or not complete denitrification can be achieved, and if not (3) the effluent nitrate concentration for various N removal systems such as the 4-stage Bardenpho or the modified Ludzack-Ettinger configurations. Details of this design procedure are given by Ekama *et al.* (1983), in WRC (1984) and in the general anaerobic-anoxic-aerobic model by van Haandel *et al.* (1982).

The design/optimization procedure allows one to determine the optimum system configuration and operation parameters for maximum nitrogen removal, subject to the constraints of the sewage characteristics. Although the procedure was developed for constant flow and load conditions, simulations with the general anaerobic-anoxic-aerobic model have shown that its solutions are reliable also for cyclic flow and load conditions.

2.2   Excess biological phosphorus removal

In 1976, Barnard noted that enhanced biological P removal is induced if at some point in the activated sludge process the mixed liquor is maintained under anaerobic conditions such that phosphorus is released from the organism mass. This condition was achieved efficiently by placing an anaerobic reactor upstream of the primary anoxic reactor of the 4-stage Bardenpho or MLE systems (Fig 2.8a and 2.8b). These configurations have become known as the modified 5-stage or 3-stage Bardenpho systems respectively.

The 12 years of research aimed at modelling biological excess P removal (BEPR) following this development basically falls into two periods: (1) Empirical steady

PRIMARY AEROBIC SECONDARY
ANOXIC REACTOR ANOXIC
REACTOR REACTOR

MIXED LIQUOR RECYCLE   REAERATION
                           REACTOR
ANAEROBIC   WASTE FLOW
REACTOR   SETTLER
INFLUENT   EFFLUENT

SLUDGE RECYCLE S
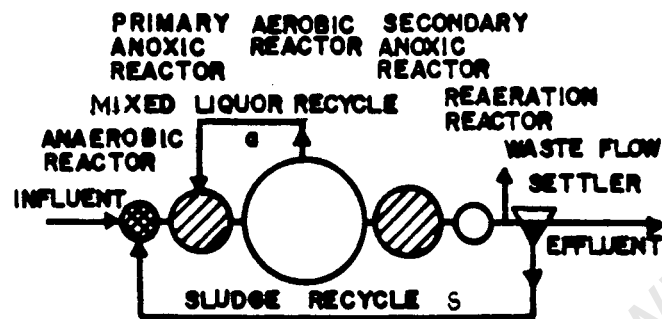
Fig 2.8(a):   *The 5-stage Bardenpho system for N and P removal.*

ANAEROBIC ANOXIC   AEROBIC
REACTOR REACTOR   REACTOR

RECYCLE MIXED LIQUOR
          RECYCLE
                              WASTE FLOW
                                 SETTLER
INFLUENT   EFFLUENT

SLUDGE RECYCLE S
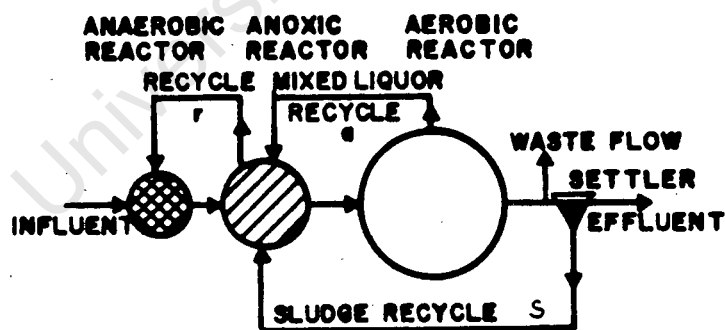
Fig 2.8(b):   *The 3-stage Bardenpho system for N and P removal.*

state model development (1976-1983) and (2) kinetic model development (1983-1988). The first period arose out of an urgent need in South Africa to develop design criteria for BEPR; this form of P removal was preferred compared to chemical precipitation because it is more economical and does not contribute to mineralization (build up of chlorides and sulphates) of receiving streams and impoundments. In a large measure, the objective of developing design criteria to satisfy the short term need was met by 1983. Since 1983, research focused on kinetic modelling to improve understanding and refine the design criteria to satisfy the long term need. The major objective of this second period was to develop a kinetic model for integration into the general model for COD and N removal described above. The achievements of the two periods of research are outlined briefly below.

2.2.1 Empirical model development

The main difficulty encountered during the pre-1983 period was the lack of an acceptable mechanistic explanation of BEPR: although a number of mechanisms had been advanced, observations on different system configurations and operational procedures did not support the mechanisms unambiguously. Initial research into BEPR produced information of a parametric nature and this formed the basis for understanding the process. By 1983, a large measure of success had been achieved (Nicholls, 1975; McLaren and Wood, 1976; Osborn and Nicholls, 1978; Simpkins and McLaren, 1978; Nicholls and Osborn, 1979; Siebritz *et al.*, 1981, 1983; Pitman, 1982; Nicholls *et al.*, 1982; Barnard, 1983a, 1983b; Ekama *et al.*, 1983), culminating with the publication of a design guide for nutrient removal systems (WRC, 1984). The most important findings were:

(1) The prerequisites to obtain BEPR were identified, i.e. the presence of RBCOD in the influent; sequential anaerobic/aerobic conditions with the influent sewage discharged to the anaerobic zone; P release in the anaerobic zone and protection of the anaerobic zone from nitrate (and oxygen) entry.

(2) P release in the anaerobic zone is essential for P uptake in the subsequent aerobic zone, to give a net P removal in the system. The P release/uptake is the result of polyphosphate accumulating organisms (also known as 'polyP organisms') such as *Acinetobacter* spp.: In the anaerobic zone, the polyP organisms sequester substrate (in the form of short chain fatty acids or SCFA) and store these as poly-β-hydroxybutyrate (PHB), with polyphosphate (polyP) cleavage for energy supply and hence P release; in

the aerobic zone, the PHB is utilized for growth and polyP synthesis and hence P uptake. The excess uptake arises from the growth of new polyP organisms. The P removed from the wastewater is ultimately disposed of through daily wastage of a portion of P-rich aerobic reactor sludge. Any oxygen or nitrate entering the anaerobic zone reduces the substrate necessary for polyP organism metabolism and hence reduces the P removal.

(3)   A number of systems were developed which incorporate the prerequisites for BEPR, i.e. 3- and 5-stage Bardenpho, UCT and modified UCT (MUCT), and Johannesburg (JHB) systems.

(4)   An empirical model was developed that provided approximate estimates of the concentration of P that could be removed in a system.

(5)   Design criteria were set whereby the most appropriate system for BEPR could be selected, and the selected system designed and operated as effectively as the particular situation allowed.

In the empirical model (Siebritz et al., 1983), the P removal was formulated in terms of the parameters that were found to influence it – the RBCOD concentration in the anaerobic zone available for BEPR and the fractional mass of sludge in the anaerobic zone. With these parameters, the P content of the active organisms could be empirically calculated. This, together with the P content of the inert fractions of the VSS, enabled the mass of P removed with the waste sludge to be determined. The P removal in mgP/ℓ influent was found by converting the mass of P in the waste sludge to an equivalent influent concentration. A comparison of the calculated and measured P removal is shown in Fig 2.9.

The organisms associated with BEPR, i.e. Acinetobacter spp., together with their basic biological and biochemical behaviour, were not directly implicated in the empirical model. As a consequence, designs based on the parametric approach had a measure of reliability only within the range of conditions in which the empirical model was developed; indeed there was a measure of uncertainty even for designs within the range because the basic mechanisms underlying the behaviour were not understood. Clearly, the need existed for a more fundamentally-based model. Accordingly, an investigation was commenced which focused on the P release and uptake behaviour. This investigation marked the beginning of the second period into
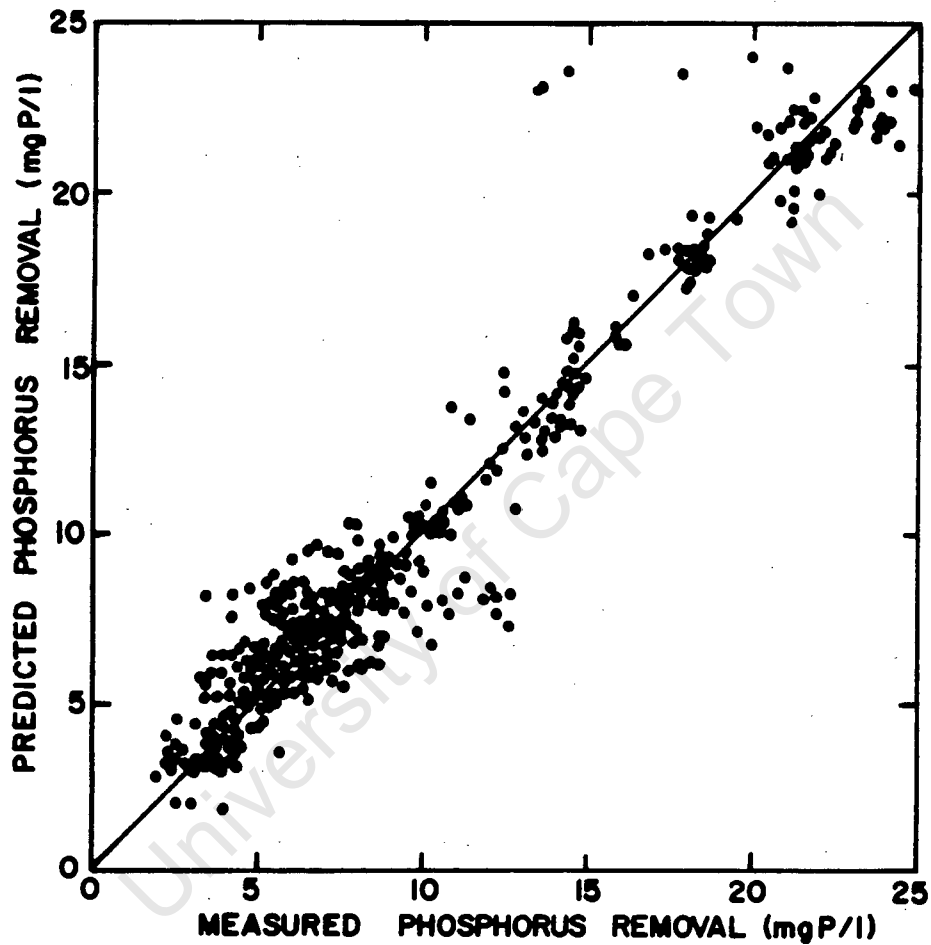
Fig 2.9:   *Comparison of predicted biological excess phosphorus removal by the empirical (parametric) model with that observed under different operating conditions: influent total COD 250-800 mgCOD/l and RBCOD 70-220 mgCOD/l; sludge age 13 and 25 days, temperature 12° C to 20° C and anaerobic mass fraction 0,05 to 0,20.*

BEPR research.

2.2.2   Kinetic model development

From the investigation into the kinetics of P release (Wentzel *et al.*, 1985) it was found that in the anaerobic zone:

(1)   RBCOD is converted to short chain fatty acids (SCFA) by non-polyP facultative aerobes,

(2)   the SCFA are taken up by the polyP organisms at a high rate, with associated P release,

(3)   the uptake rate of SCFA is governed by the rate of SCFA production from RBCOD,

(4)   the rate of P release can be modelled as a first order reaction with respect to the active non-polyP heterotrophic mass and the RBCOD concentration in the anaerobic zone,

and in the aerobic zone:

(5)   P uptake is linearly related to P release,

(6)   Sludge age has minor effect on the magnitude of P uptake, for a constant P release.

It appeared from the investigation that whereas P release and its kinetics could be modelled without direct consideration of the polyP organisms, P uptake and its kinetics could not be modelled without explicit incorporation of the polyP organism mass.   This conclusion pointed to the need to enquire into the occurrence of the polyP organisms and their characteristics.

The empirical information built up during the pre-1983 period, indicated that *Acinetobacter* spp. are the most likely organisms mediating BEPR. A study of the prevalence of this organism indicated that they are present in significant concentrations in both anaerobic/anoxic/aerobic systems that exhibit BEPR and completely aerobic systems that do not exhibit BEPR. To obtain more information

on these organisms, *Acinetobacter* spp. were isolated from anaerobic/anoxic/aerobic and completely aerobic systems. From a study of their glucose and acetate utilization, carbon storage (as PHB) and phosphorus storage (as polyP), it was concluded that imposing conditions conducive to BEPR in a system (by anaerobic/aerobic sequencing) does not select new *Acinetobacter* strains, but rather stimulates the polyP and PHB accumulating propensities inherent in strains already present (Lötter *et al.*, 1986). This raised questions regarding the biochemical mechanisms whereby polyP and PHB are synthesized and degraded.

In the literature, a number of biochemical models had been proposed to explain polyP and PHB metabolism in biological excess P removal. Of these, the model of Comeau *et al.*, (1986) although only conceptual and limited in the situations it could explain, showed the most promise. This model contained two new proposals: (1) Under anaerobic conditions the tricarboxylic acid (Krebs) cycle is operative, and (2) the proton motive force (pmf) must be maintained (although generally accepted in biochemistry, not considered in the previous models).

From a study of the biochemical literature, a more general model was developed by Wentzel *et al.* (1986) for the polyP organism *Acinetobacter* spp. It incorporates the two proposals of Comeau *et al.* (1986) and quantitatively delineates the various biochemical pathways in the BEPR phenomenon. Two key parameters were identified that appear to regulate the pathways: the ATP/ADP and the NADH/NAD ratios. Having identified the regulatory parameters, the model could be extended to situations other than anaerobic/aerobic BEPR systems. It was found that the regulatory parameters are influenced by substrate concentration — intracellular or extracellular (PHB and, say, acetate respectively), and the presence or absence of external electron acceptors (aerobic, anoxic or anaerobic conditions). In terms of the ATP/ADP and NADH/NAD ratios, detailed explanations could be given of the behaviour of *Acinetobacter* spp. over a wide range of conditions.

Having resolved the biochemical aspects, attention was focused on the kinetic modelling of P uptake. Unlike P release, which is controlled by the RBCOD modifications mediated by the non-polyP heterotrophs, P uptake is controlled almost entirely by the polyP organisms. It was not possible to obtain metabolic and kinetic information on the polyP organisms from 'normal' mixed cultures receiving sewage, because the response of the polyP organisms in these systems is largely obscured by the action of the non-polyP heterotrophs. To obtain kinetic information on P uptake

required cultures in which the polyP organisms are dominant. Although *Acinetobacter* spp. could be isolated in pure cultures, data in the literature indicated that the response of *Acinetobacter* spp. in pure cultures was different to that inferred from BEPR in activated sludge systems. Consequently, instead of pure cultures, enhanced cultures (cultures in which the polyP organisms are the dominant primary ones) were developed by feeding acetate to the anaerobic zone of an anaerobic-anoxic-aerobic system: By starting with 100 percent municipal wastewater as influent to 'normal' activated sludge UCT and Bardenpho systems, then incrementally decreasing the wastewater fraction while increasing the acetate fraction, the polyP organisms became dominant.[6] Following this procedure, four enhanced cultures were developed: one in a UCT system at 10 days sludge age and three in modified Bardenpho systems at 20, 10 and 7,5 days sludge ages respectively (Wentzel *et al.*, 1987; 1988a). The behaviour of the enhanced culture was continually monitored to check that it remained qualitatively (but not quantitatively) similar to that in 'normal' mixed cultures exhibiting BEPR.

Some striking features of the enhanced cultures are:

(1)   The specificity of the population structure: 90 percent of the organisms cultures aerobically were identified to be *Acinetobacter* spp. using the API procedure (Analytlab Products, 1977).

(2)   The extremely high phosphorus content of the sludge in the aerobic reactor, 0,38 mgP/mgVSS: as a consequence, the VSS/TSS ratio is 0,46 mgVSS/mgTSS in the aerobic reactor, compared to the usual 0,75-0,85 in activated sludge systems.[7]

(3)   The magnitudes of the P release, uptake and removals: 250 mgP/ℓ release (anaerobic + anoxic reactors), 310 mgP/ℓ uptake (aerobic reactor) giving a net removal of 60 mgP/ℓ (concentrations with respect to influent flow) for 500 mgCOD/ℓ acetate feed.

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[6]As the acetate fraction increased, macro- and micro-nutrients and yeast extract addition became necessary to maintain polyP organism growth.

[7]The polyphosphate chains in the polyP organisms are counterbalanced with cations, mainly Mg^{2+} (Wentzel *et al.*, 1988a). When a sludge sample containing a significant fraction of polyP organisms exhibiting BEPR is dried for a TSS and VSS test, the polyphosphates and cations precipitate and add to the inorganic (ash) content of the sample. Consequently with BEPR, the TSS increases causing the VSS/TSS ratio to decrease.

To obtain information on the different polyP organism processes, e.g. acetate uptake, P release and uptake, growth rates, endogenous respiration, etc. a series of batch tests was undertaken using mixed liquor from the enhanced culture systems, viz. (1) aerobic digestion of the mixed liquor, (2) response with addition of acetate under anaerobic conditions, with (3) aerobic response thereafter and (4) response with acetate addition under aerobic conditions (Wentzel et al., 1987, 1988b). Some results obtained are shown in Figs 2.10 and 2.11.

The polyP organism behaviour observed in the enhanced culture systems and in the aerobic, anoxic and anaerobic batch tests, was used to identify and formulate the kinetics of the biological processes making up the BEPR phenomenon. In the interests of brevity, it is not possible to describe the details that entered into the development and calibration of the model; these have been published by Wentzel et al. (1987, 1988c). For our purposes, it is sufficient to show that the predicted behaviour of various compounds compare well with those observed in the different batch tests. This can be seen in Figs 2.10 and 2.11 in which the experimental responses (symbols) shown are theoretically simulated (lines): the predicted behaviour can be seen to conform remarkably well to the data. The model also has been applied to simulate the continuous-feed multi-reactor enhanced culture systems; in these also, the predicted behaviour conforms very well with that observed.

With regard to kinetic modelling of biological excess phosphorus removal, the model developed so far is specific to anaerobic/aerobic behaviour of enhanced cultures, receiving acetate only as substrate; it does not include anoxic behaviour principally because the enhanced cultures showed minimal denitrification response and it was therefore not possible to quantify such behaviour. The possibility of denitrification by the polyP organisms was, in fact, one of the facets considered in the research described in this thesis. Incorporation of the enhanced culture model into the general kinetic model will require modelling the interactions between the non-polyP and polyP organisms. This is not expected to present serious difficulties: the polyP organisms seem not to be prone to predation and the polyP and non-polyP cultures appear to have little interaction — by and large they can be modelled independently. The only significant interaction is in the anaerobic zone: with municipal sewages, most of the short chain fatty acids for the polyP organisms are generated by the conversion of RBCOD by non-polyP organisms. This interaction is already included in the enhanced culture model. Refinement of the model, and its integration with the general model, are aspects that are receiving attention at present. Some
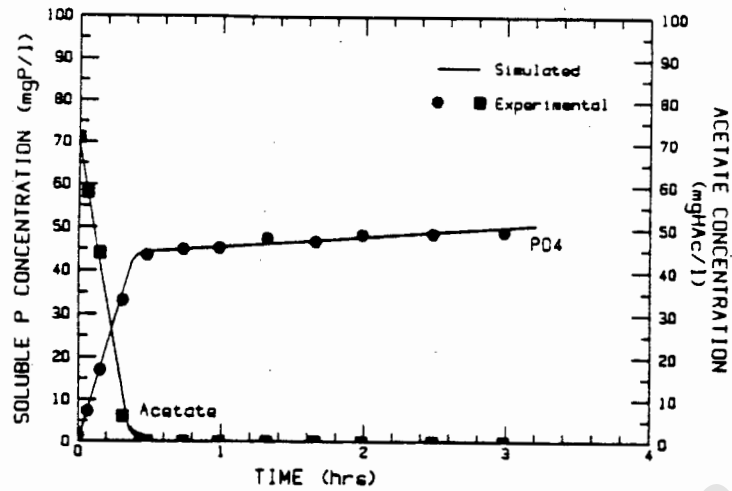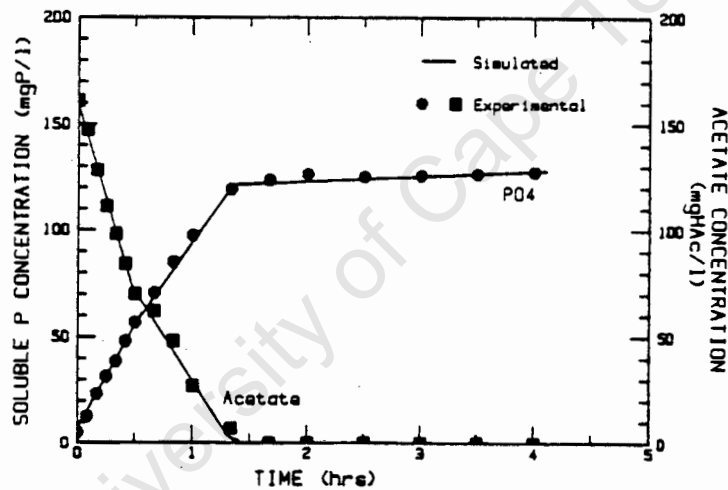
<u>**Fig 2.10**</u>:   *Total soluble phosphate and acetate concentration profiles with time, measured in anaerobic batch tests on sludge harvested from the aerobic reactor of the enhanced polyP organism culture system at low and medium acetate conditions [0,11 mgCOD acetate/mgVSS in Fig 2.10(a) and 0,265 mgCOD acetate/mgVSS in Fig 2.10(b) respectively]. Note the two-phase P release and acetate uptake at medium acetate conditions [Fig 2.10(b)]. The acetate uptake rate is zero order with respect to acetate.*
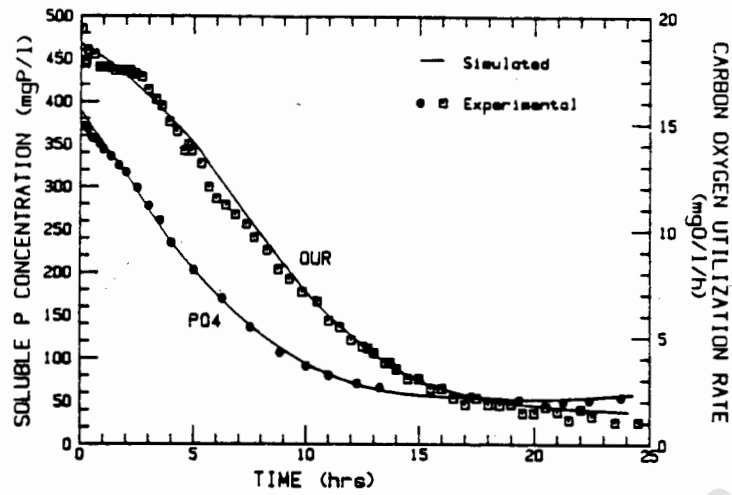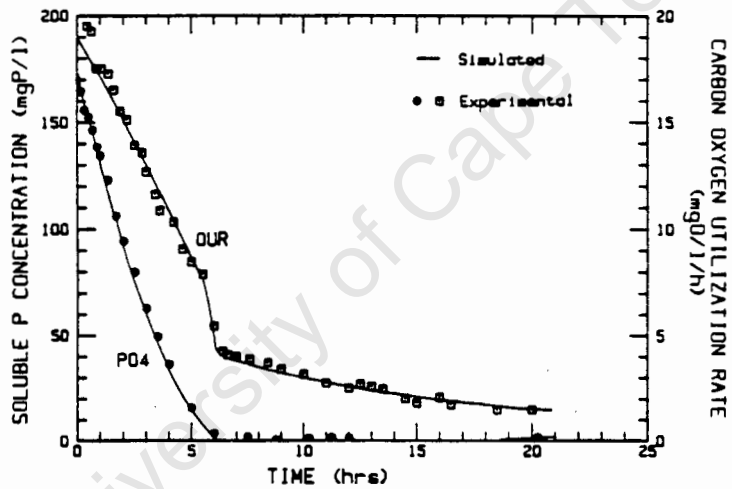
Fig 2.11:   *Total soluble phosphate and carbonaceous oxygen utilization rate (excluding nitrification) profiles versus time of enhanced culture sludge under aerobic conditions. The sludge in Fig 2.11(a) received medium acetate addition (0,363 mgCOD acetate/mgVSS): there is sufficient phosphate for uptake. The sludge in Fig 2.11(b) received a low acetate dose (0,22 mgCOD acetate/mgVSS): there is insufficient phosphate for uptake. The sludge that received the higher acetate dose [Fig 2.11(a)] resulting in a higher stored PHB level, had a constant high initial OUR — this indicates PHB utilization has saturation type (like Monod) kinetics. Note in Fig 2.11(b) the sudden decrease in OUR when P becomes limiting.*

modifications to the general model may arise out of the integration.

2.3   Biological nitrogen and phosphorus removal

With the aid of the theories of biological nitrogen and phosphorus removal presented above, it is instructive at this stage to briefly describe the sequence of reactions that effect these removals in a nutrient removal system as the mixed liquor passes through the various zones, i.e. anaerobic, anoxic and aerobic.

2.3.1   Anaerobic zone

The first stage is to subject the mixed liquor to anaerobic conditions in the presence of a high concentration of readily biodegradable COD (RBCOD) or short chain fatty acids (SCFA). This allows the facultative aerobes to acidify the RBCOD to SCFA and the polyP organisms to take up the SCFA with a concomitant internal PHB storage and release of P. Anaerobic conditions are maintained by avoiding, as much as is possible, the ingress of dissolved oxygen or nitrate to the anaerobic reactor; the high RBCOD/SCFA concentrations are obtained by discharging the influent sewage to the anaerobic reactor and the mixed liquor is introduced via a mixed liquor recycle. To avoid nitrate ingress, this recycle should contain only very low concentrations of nitrate (< 2 mgN/ℓ). Consequently, the mixed liquor stream should be very well denitrified.

Most municipal sewages have only a very low concentration of SCFA (< 50 mg/ℓ) and the bulk of the SCFA's are formed via acidogenesis of RBCOD to SCFA by facultative aerobic organisms in the anaerobic reactor. Owing to the relatively short mixed liquor retention time in the anaerobic reactor, only a small amount of particulate biodegradable COD (PBCOD) is hydrolyzed and acidified. Consequently, the RBCOD in the sewage (usually about 20 to 30% of the total COD) forms the main supply of SCFA and hence substrate for the polyP organisms.

The formation of SCFA by acidogenesis is a much slower reaction than the uptake of SCFA by polyP organisms. Therefore, the acidogenesis rate governs the rate of SCFA uptake, PHB formation and P release. Furthermore, acidogenesis of RBCOD is rarely complete in the anaerobic reactor and a fraction of the influent RBCOD (10–30%, depending on the total COD concentration) passes through to the subsequent primary anoxic reactor (Wentzel *et al.*, 1985).

It is important that nitrate and oxygen are positively excluded from the anaerobic

zone because these compounds adversely affect the mass of SCFA sequestered by the polyP organisms: with nitrate or oxygen present, the facultative aerobic organisms are able to oxidize the RBCOD via the Krebs cycle, with the result that SCFA are not generated. The reduction in SCFA generation is in proportion to the amount of nitrate and oxygen available. Under these conditions, the polyP organisms are able to store less PHB hence BEPR is effectively reduced. Normally, the dissolved oxygen in the recycle flow to the anaerobic reactor is low; however, the nitrate concentration in the recycle to the latter reactor can be considerable in nitrification/denitrification plants with high TKN/COD ratios ($> 0,08$). For this reason, several N and P removal plant configurations have been developed that avoid nitrate discharge to the anaerobic reactor. These systems were mentioned in Chapter 1: the 5-stage Bardenpho, the UCT/Modified UCT (MUCT) systems and the Johannesburg system.

The effluent from the anaerobic reactor passes to the primary anoxic reactor.

2.3.2   The primary anoxic zone

In the primary anoxic reactor, dissolved oxygen is absent. Consequently, denitrification takes place, i.e. nitrate is reduced to nitrogen gas via the biological reactions (Eqs 2.1 to 2.3).

The substrate for the denitrification is the biodegradable COD that passes out of the anaerobic reactor. From the reactions in the anaerobic reactor, this is principally the PBCOD and the residual RBCOD not acidified. One might surmise that denitrification with these two substrates is the same as that in the primary anoxic reactor receiving sewage, i.e. an initial rapid rate stimulated by the utilization of RBCOD and a second continuous slower rate stimulated by the utilization of PBCOD; the extent of denitrification by the initial rapid rate should in this instance be significantly less because the RBCOD concentration leaving the anaerobic reactor has been drastically reduced compared to that in the influent. Because the RBCOD contributes about 50% to the denitrification attainable in a primary anoxic reactor, it would therefore appear that denitrification in the primary anoxic reactor with a preceding anaerobic reactor should be considerably reduced compared to that in a primary anoxic reactor without a preceding anaerobic reactor.

The above conclusion would be valid if the polyP organisms do not contribute to denitrification: the RBCOD/SCFA converted to PHB in the anaerobic reactor

would pass through the primary anoxic zone and only be utilized in the aerobic reactor for P uptake, polyP synthesis and polyP organism growth. If the polyP organisms in the system *are* able to denitrify, then *not all* the RBCOD/SCFA taken up in anaerobic reactor would be lost for denitrification — instead of facultative aerobic organisms using the RBCOD directly, the polyP organisms might be able to denitrify utilizing some of the internally-stored PHB which they obtained from the RBCOD in the anaerobic reactor.

From the above it can be seen that an important question to be answered is: Can polyP organisms in an anaerobic-anoxic-aerobic activated sludge system denitrify? Various researchers have reported that under anoxic conditions, phosphorus uptake can occur (Simpkins and McLaren, 1978 and Hascoët *et al.*, 1985). In making similar observations, Comeau *et al.* (1985) also noted that under anoxic conditions the PHB levels of the sludge decrease with time. The above evidence suggests that some of the polyP organism population may be capable of nitrate reduction, i.e. denitrification. This is supported by the work of Lötter (1985) which demonstrated that approximately 50 percent of *Acinetobacter* spp. strains isolated are capable of nitrate reduction. Recently Lötter *et al.* (1986) found that of the *Acinetobacter* spp. able to reduce nitrate, the majority could only do so as far as nitrite, while only a minority could reduce nitrate (and nitrite) to nitrogen gas. Apart from these observations, the net denitrification behaviour of a mixed culture in the primary anoxic reactor of an N and P removal system has received little attention.

In modelling the behaviour of the *Acinetobacter* spp. under anoxic conditions, Wentzel *et al.* (1986) considered all three groups of *Acinetobacter* : (i) those unable to utilize nitrate as an external electron acceptor, (ii) those able to reduce nitrate to nitrite only, and (iii) those able to reduce nitrate to nitrogen gas. In the primary anoxic reactor, Wentzel *et al.* (1986) proposed that the first group, unable to reduce nitrate, would recognize the environment as anaerobic and release P. Groups (ii) and (iii) would reduce nitrate to differing degrees, but take up P at a slow rate. In addition to the action of the polyP organisms, the non-polyP organisms also reduce nitrate to nitrogen gas. The expected overall behaviour in the primary anoxic reactor is a combination of the behaviour of the three *Acinetobacter* spp. groups and the facultative heterotrophic group: Wentzel *et al.* (1986) speculated that the overall behaviour is likely to be a rapid decrease in nitrate, an associated decrease in PHB and either a slow increase or decrease in P concentration depending on the relative fractions of *Acinetobacter* spp. present.

The effluent from the primary anoxic zone passes into the aerobic reactor. The assumed biochemical activity in the latter reactor is described next.

2.3.3 The aerobic zone

In the aerobic zone, the PBCOD remaining in the effluent from the primary anoxic reactor is utilized by the heterotrophic organisms, oxygen serving as the electron acceptor. Also, the obligate aerobic nitrifiers convert free and saline ammonia to nitrate. Generally, the aerobic sludge mass fraction (50 to 60% of the total sludge mass) and sludge age (20–30 d) of nutrient removal systems are such that utilization of PBCOD and nitrification are complete. The only PBCOD that remains is generated by the sludge mass itself via organism death and cell lysis; additional ammonia also becomes available from ammonification of the organic N that is released in cell lysis.

With regard to the polyP organisms, those that are obligate aerobes (those able to utilize only oxygen as an electron acceptor) utilize their internally-stored PHB to resynthesize polyP chains, taking P up from the liquid; simultaneously they grow new polyP organisms which also synthesize polyP; it is the uptake of P by these new cells that effects the higher P uptake relative to the P release. The polyP organisms that are facultative (those that can grow under both anoxic and aerobic conditions) should act the same as the obligate aerobic ones, except they would already have utilized some of the stored PHB for denitrification in the primary anoxic zone. The fraction of stored PHB that is utilized by the facultative polyP organisms in the anoxic and aerobic zones is unknown.

For systems that have a secondary anoxic zone, i.e. the 5-stage Bardenpho configuration, the mixed liquor passes from the aerobic zone to the secondary anoxic zone.

2.3.4 The secondary anoxic zone

In the secondary anoxic reactor, the only substrate available to facultative heterotrophic organisms is PBCOD generated by the sludge itself via organism death and cell lysis. This PBCOD subsequently undergoes the usual hydrolysis and utilization steps. However, under these conditions, the organism death and cell lysis is the rate limiting step. The relatively slow organism death rate causes the denitrification rate K_3 (due to the hydrolysis and utilization of the lysed material) to be slow, considerably slower (about 2/3rds, depending on the temperature) than that

due to the hydrolysis rate of the influent PBCOD in the primary anoxic reactor (K_2).

The organism death and cell lysis causes a slow continuous release of organic N which is readily converted to ammonia. Therefore, in the secondary anoxic reactor there is a slow continuous increase in ammonia concentration, at a rate of about 1/3rd of the nitrate reduction (denitrification).

Considering the polyP organisms, the stored PHB is likely to have been nearly or completely utilized in the aerobic reactor and P uptake and polyP synthesis is likely to be complete. As for the primary anoxic reactor, Wentzel *et al.* (1986) considered the activity of all three groups of *Acinetobacter* spp. to predict the overall polyP organism behaviour in the secondary anoxic reactor. Due to the low levels of internally stored PHB and influent biodegradable material, it is probable that all three groups of *Acinetobacter* will behave as if under endogenous conditions: Group (i) (those unable to reduce nitrate) will respond as under anaerobic endogenous conditions whilst groups (ii) and (iii) (those able to reduce nitrate) will behave as if under aerobic endogenous conditions. In terms of the model, Wentzel *et al.* (1986) speculated that a slow rate of P release would be observed due to endogenous polyP organism death of group (i) and polyP cleavage for cell maintenance by groups (ii) and (iii). However, if adequate PHB (and nitrate) is initially present, polyP storage by groups (ii) and (iii) will probably continue as in the aerobic zone and at first exceed the P release by group (i); later when the stored PHB is low, the situation will change to a net P release.

2.4 The effect of BEPR on denitrification in N and P removal plants

From the above description of the reactions involved in denitrification and BEPR, it is clear that in biological N and P removal plants (1) the anaerobic reactor alters the composition of the influent stream before it reaches the primary anoxic reactor and (2) there is the possibility that the polyP organisms present in such a system partake in the nitrate reduction in the primary anoxic zone. Both these effects therefore may considerably alter the form of the denitrification kinetics and therefore the extent of denitrification in the primary anoxic reactor of N and P removal plants relative to plants removing N only. It seems unlikely that the polyP organisms should influence denitrification in the secondary anoxic reactor because of their low PHB levels.

Although from theoretical considerations it appears that the extent of denitrification in the primary anoxic reactor of nutrient removal systems should be different to that in N removal systems, observations on laboratory and full-scale nutrient removal systems have indicated that he introduction of an anaerobic zone and the BEPR that this stimulates, does not significantly affect the degree of nitrogen removal (Nicholls, 1982). Because of this latter fact, in the past, design of the anoxic zones in N and P removal plants followed the same procedure as for N removal plants (WRC, 1984). However, now that more attention is being given to the modelling of BEPR in nutrient removal systems, knowledge of the possible interactions between BEPR and nitrogen removal has become increasingly important. Consequently, a detailed investigation into the denitrification kinetics in N and P removal plants was implemented.

CHAPTER 3

OVERVIEW OF EXPERIMENTAL INVESTIGATION

The experimental programme was divided up into three sequential parts:

PART 1: establishing the denitrification behaviour in the primary anoxic zone of a laboratory Modified UCT (MUCT) nutrient removal (N and P) system,

PART 2: formulating hypotheses to account for the observed behaviour, and

PART 3: performing specific tests to provide experimental evidence to establish which of the proposed hypotheses best explains the observed behaviour.

A brief description of each part is given below so that the rationale of the investigation can be appreciated before details of the results are discussed.

PART 1: ESTABLISHING THE DENITRIFICATION BEHAVIOUR IN THE PRIMARY ANOXIC ZONE OF A MODIFIED UCT NUTRIENT REMOVAL SYSTEM

The denitrification kinetics in systems with primary anoxic zones *without* preceding anaerobic reactors (i.e. N removal systems) were reviewed in Chapter 2 and it was concluded that the kinetics are well established and understood. In contrast, the denitrification kinetics in primary anoxic zones with preceding anaerobic reactors (i.e. in N and P removal systems) have not been well understood and it was accepted up until the present that they were the same as those in N removal systems. The reason for this is that observations on laboratory and full scale N and P removal plants indicated the N removal as predicted by the established kinetics of N removal plants was not reduced (or increased) significantly by incorporation of an anaerobic reactor to stimulate biological excess P removal (BEPR).

As a starting point of the investigation, the denitrification kinetics in the primary anoxic zone of an MUCT system for N and P removal (Fig 3.1) were examined. This was done by replacing the first completely mixed anoxic reactor of the system with a plug flow reactor (Fig 3.2). Details of the experimental set-up are discussed in Appendix A and the manner in which the system was operated and tested are
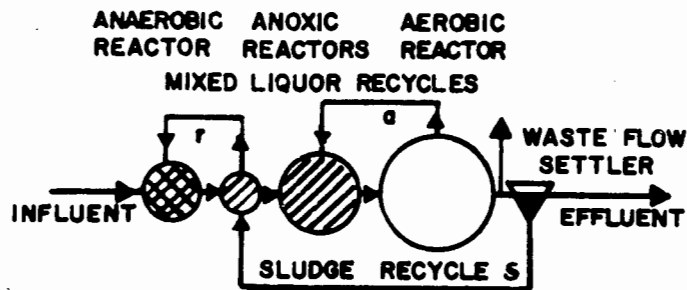
Fig 3.1: The MUCT nutrient removal system with completely mixed reactors. Note that the anoxic zone (a primary anoxic zone) is made up of two reactors — a first anoxic reactor followed by a slightly larger second anoxic reactor.
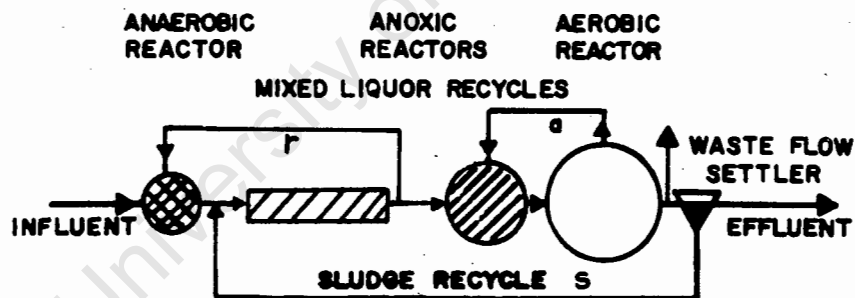
Fig 3.2: The MUCT nutrient removal system with its completely mixed first anoxic reactor replaced by a plug flow reactor. The denitrification kinetics were studied by measuring nitrate concentration versus retention time profiles along the length of the plug flow reactor.

given in Appendix B. By extracting samples along the length of the plug flow reactor and measuring *inter alia* nitrate concentrations, the rate of denitrification could be determined. This procedure was adopted because it was shown to be reliable in the previous denitrification studies in systems without anaerobic reactors (Stern and Marais, 1974; Wilson and Marais, 1976 and Marsden and Marais, 1976). In this investigation, additional parameters such as phosphate and soluble COD ($<0,45\mu m$) concentrations were measured.

Two considerations led to the selection of the MUCT configuration as the N and P removal system in which to study the effect of BEPR on denitrification: (1) This configuration has the merit that it avoids nitrate discharge to the anaerobic reactor (nitrate entering the anaerobic reactor adversely affects BEPR), even when denitrification is not complete; the MUCT configuration therefore may be operated close to its optimum P removal performance so that the full effect of P removal on denitrification can be observed. (2) By manipulation of the mixed liquor recycle from the aerobic to the second anoxic reactor (the a-recycle; see Fig 3.1 and 3.2), the nitrate concentration in the system effluent and hence that recycled to the first anoxic plug flow reactor via the s-recycle could be controlled. In this manner, optimal use could be made of the length of the plug flow reactor for measuring denitrification profiles.

During this part of the investigation, in which a number of nitrate, phosphate and soluble COD ($<0,45\ \mu m$) concentration profiles were measured, it became evident that the above two objectives were conflicting. In order to derive the full benefit of the complete length of the plug flow reactor to measure denitrification profiles, the nitrate concentration in the effluent of the plug flow reactor had to be maintained between 2 and 3 mgN/ℓ. Because the r-recycle to the anaerobic reactor was taken from the end of the plug flow reactor (see Fig 3.2), this nitrate concentration was recycled back to the anaerobic reactor via the r-recycle and had a detrimental effect on biological P removal. To minimize this effect, the MUCT system was converted to a UCT system (see Fig 3.3), the mixed liquor a-recycle was removed, and the r-recycle to the anaerobic reactor was taken from the effluent of the *second* anoxic reactor instead of the first anoxic reactor. In this mode of operation, the only nitrate entering the first anoxic zone was that recycled via the underflow recycle (the s-recycle). The first and second anoxic reactors remained plug flow and completely mixed respectively. It was anticipated that even though the removal of the a-recycle would increase the nitrate concentration discharged to the plug flow first anoxic
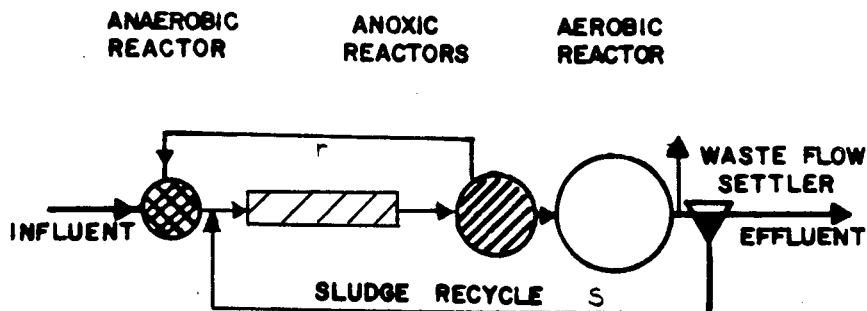
Fig 3.3: *The UCT nutrient removal system with a plug flow first anoxic reactor and no a-recycle. This configuration obviated the recycle of high nitrate concentrations (present at the end of the plug flow reactor) back to the anaerobic reactor. P removal was hence improved. Further denitrification profiles were measured in the plug flow reactor of this system.*
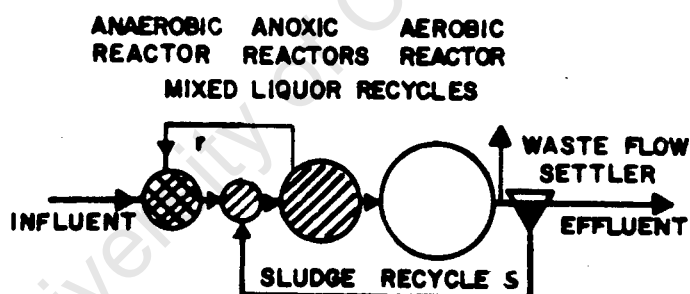
Fig 3.4: *The UCT nutrient removal system with a completely mixed first anoxic reactor and no a-recycle. The denitrification kinetics were studied further with anoxic batch tests on sludge taken from the parent UCT/MUCT system.*

reactor (via the s-recycle), yielding a nitrate concentration in the effluent of this reactor greater than 2 to 3 mgN/ℓ, the second anoxic reactor would be sufficiently large to completely denitrify this residual nitrate, so that a zero nitrate concentration would be discharged to the anaerobic reactor (via the r-recycle). Experimental data confirmed that the conversion did not affect the denitrification kinetics in the plug flow reactor.

With the MUCT and UCT systems, 29 denitrification profiles were measured in the plug flow first anoxic reactor. The profiles showed a significantly different denitrification behaviour to that observed in N removal systems (without anaerobic reactors). However, several technical problems were experienced in both the plug flow reactor operation and sampling (see Chapter 4 and Appendix B for details) which cast some doubt on the accuracy of the observed denitrification behaviour. It was therefore decided to check the observed plug flow denitrification profiles by conducting complementary denitrification tests in satellite anoxic batch reactors containing sludge abstracted from the UCT system. This method eliminated the technical problems encountered with the plug flow reactor. Since the plug flow reactor was of no further use, it was replaced with an equivalent volume completely mixed reactor (Fig 3.4). This change was not expected to affect the denitrification kinetics since earlier experiments with N removal systems showed that denitrification is zero order with respect to nitrate concentration, i.e. independent of the anoxic mixing regime.

In the batch tests, sludge from the anaerobic and aerobic reactors was blended in the same proportions as would happen at the entrance of the plug flow reactor, i.e. according to the system recycles. Denitrification behaviour was examined in the batch tests by taking samples at various time intervals and measuring the nitrate, phosphate and soluble COD (<0,45 μm) concentrations - this allowed the nitrate, phosphate and soluble COD concentration versus time profiles to be constructed. Details of the experimental equipment and testing procedures are given in Appendix C. Four anoxic batch tests were performed with the system operating in the UCT mode; the system was then converted back to the MUCT mode (Fig 3.1) and a further fifteen batch tests were conducted.

The batch reactor profiles were found to be the same as those observed in the plug flow reactor. Consequently, both sets of data could be accepted with confidence that they accurately reflect the denitrification behaviour in the MUCT/UCT system.

From the plug flow and batch tests, the denitrification kinetics were formulated to give a quantitative description for the denitrification behaviour in nutrient the MUCT/UCT nutrient removal system. These kinetics were compared to those in systems removing N alone and were found to be significantly different. It is suggested that the kinetics established for the MUCT/UCT system are probably also valid for other nutrient removal configurations.

This part of the investigation was concluded with a quantitative check of the formulated denitrification kinetics – the kinetics were employed to predict the overall nitrogen removal performance of the MUCT/UCT system as observed during the entire investigation. A satisfactory close prediction between the calculated and observed nitrogen removal was obtained – a closer prediction than by the N removal denitrification kinetics.

PART 2: DEVELOPMENT OF HYPOTHESES TO ACCOUNT FOR THE OBSERVED DENITRIFICATION IN THE MUCT/UCT SYSTEM

Part 1 of the investigation established that the denitrification kinetics observed in the MUCT/UCT system were different to those observed in N removal systems. In the second part of the investigation, possible explanations for the different observed behaviour were sought. Two hypotheses were formulated that appeared to explain the observations. These hypotheses were based not only on the rate of nitrate reduction, but also on the other parameters measured in the anoxic plug flow and batch tests, such as the phosphorus and soluble COD concentrations. The hypotheses and their implications are described in detail in Chapter 5.

PART 3: EXPERIMENTS TO EVALUATE THE HYPOTHESES

In the third and final part of the investigation, attempts were made to find evidence that would indicate which of the two proposed hypotheses was the correct one to account for the observed denitrification behaviour in the MUCT/UCT system. Using sludge from certain reactors in the MUCT/UCT system, batch tests were conducted under various conditions in order to highlight different aspects of the denitrification and biological excess P removal behaviour. The design of each batch experiment depended to a degree on the outcome of the previous one; the objectives and experimental conditions are discussed in detail together with the results of each test in Chapter 6. By adopting this flexible approach and building on the results of the previous tests, an acceptable explanation for the observed denitrification behaviour in the MUCT/UCT nutrient removal system was obtained.

CHAPTER 4

EXPERIMENTAL INVESTIGATION PART 1 : ESTABLISHMENT OF DENITRIFICATION BEHAVIOUR IN THE ANOXIC ZONE OF AN MUCT/UCT NUTRIENT REMOVAL SYSTEM

Part 1 of the experimental investigation comprises 4 sections, each of which was briefly discussed in Chapter 3, viz:

(1)   establishment of denitrification behaviour in a continuous plug flow first anoxic reactor of an MUCT/UCT nutrient removal system,

(2)   confirmation of plug flow results with supplementary batch tests,

(3)   delineation of denitrification kinetics from the plug flow and batch test data and a comparison of the denitrification kinetics with those in N removal systems, and

(4)   verification of the denitrification kinetics.

The results obtained in each of these 4 sections are discussed in detail below.

4.1   Establishment of denitrification behaviour in plug flow reactor
4.1.1   Operation and day-to-day performance of the laboratory activated sludge system with the plug flow reactor

(i)   The MUCT mode of operation (day 1 to 198)

It was mentioned in Chapter 3 that Part 1 of the investigation started by replacing the first completely mixed anoxic reactor of an MUCT system (Fig 4.1a) with a plug flow reactor (Fig 4.1b). Denitrification profiles were then measured in the latter reactor. The MUCT system was selected as the appropriate system in which to study the effect of BEPR on denitrification because it (i) may be operated close to its optimum P removal performance, even when denitrification is not complete (nitrate is excluded from the anaerobic zone) and (ii) the nitrate load on the first anoxic reactor can be easily controlled by manipulation of the a-recycle. The initial design features and operating parameters for this system configuration appear in
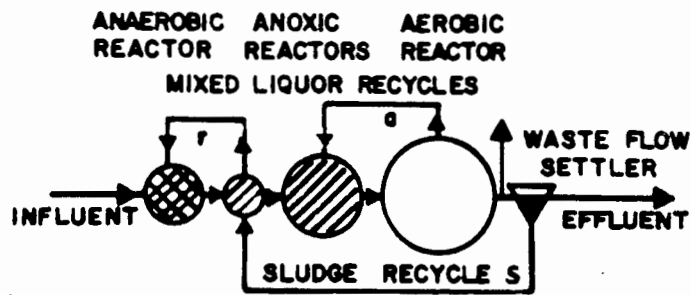
<u>*Fig 4.1(a)*</u>:   *The MUCT configuration, chosen as the nutrient removal system in which to study the effect of BEPR on denitrification.*
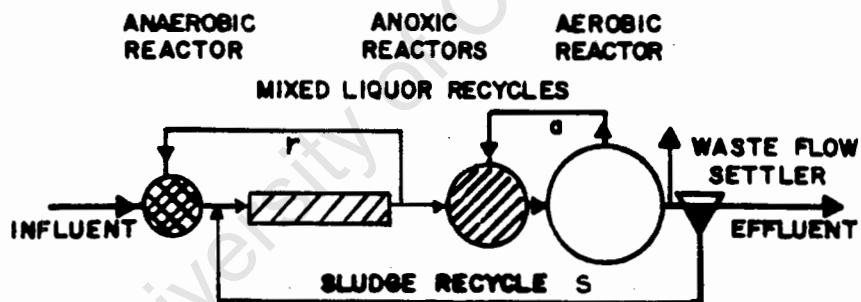
<u>*Fig 4.1(b)*</u>:   *The first anoxic reactor of the MUCT system was replaced with a plug flow reactor so that nitrate concentration versus retention time profiles could be measured.*

Table 4.1. Details of the experimental apparatus and testing procedures appear in Appendices A and B respectively.

The system was operated in the MUCT mode with a plug flow first anoxic reactor for the first 198 days of the investigation. During this time, the performance of the MUCT system was monitored by making certain daily measurements, i.e. overall COD removal (Fig 4.2) and nitrogen removal (Fig 4.3), effluent nitrate and phosphate concentrations from each reactor (Figs 4.4 and 4.5 respectively), system P removal, i.e. influent P minus effluent P concentrations (Fig 4.6), MLTSS and VSS concentrations (Fig 4.7), DSVI (Fig 4.8) and oxygen utilization rate (OUR) in the aerobic zone (Fig 4.9).

Table 4.2 gives a summary of all the operational changes that were made while the plug flow reactor was in operation. During the MUCT mode of operation, the following changes were made to the system. (1) On day 58 the influent TKN concentration was increased from around 80 mgN/ℓ to 135 mgN/ℓ (see Fig 4.3) by daily additions of $NH_4C\ell$ to the sewage feed. This was done to obtain more nitrification and hence recycle more nitrate to the plug flow reactor via the underflow recycle. The aim was to allow denitrification to take place for most of the length of the plug flow reactor so that good nitrate reduction versus time profiles could be measured. (2) In an attempt to control bulking[1] (which was a problem for the major part of this investigation – see Fig 4.8), on day 79 the a-recycle was changed from 1:1 to 3:1 (relative to the influent flow) to decrease the hydraulic retention time in the second anoxic zone – it was thought that this would improve the settleability of the sludge (Siebritz et al., 1983). This measure was not successful in controlling the bulking; it was thought that the increase in a-recycle had resulted in an increase in the discharge of dissolved oxygen (DO) to the second anoxic reactor – a situation that was thought to promote the growth of certain filamentous organisms (Blackbeard et al., 1988). (3) To lower the DO discharge to the latter reactor, a 1ℓ anoxic zone was installed on the a-recycle on day 140. (In order to keep the same total sludge mass in the system, the aerobic reactor volume was decreased from 9ℓ to 8ℓ). Unfortunately, this measure also was unsuccessful in controlling bulking – the DSVI actually increased further (see Fig 4.8). In view of this, on day 183 the a-recycle was changed back to 1:1 and the 1ℓ zone on the latter stream was

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[1] "bulking" describes a situation of poor sludge settleability. It limits the rate at which the sludge may be separated from the final effluent in the settling tank. Generally, the system may be said to have reached a bulking point when the DSVI exceeds 150 mℓ/g (Blackbeard et al., 1988).

Table 4.1: Initial design and operating parameters for the laboratory-scale MUCT system with plug flow 1st anoxic reactor.

Parameter	Value
Sludge age	18,5 d
Influent: Mitchell's Plain raw sewage	
Flow	10 ℓ/d
COD concentration	1000 mg/ℓ
Readily biodegradable COD concentration	240 mg/ℓ
TKN concentration	80-100 mgN/ℓ*
Total P concentration	32 mgP/ℓ**
Reactor volumes; mass fractions	
Anaerobic	6ℓ : 0,16
1st Anoxic	2,5ℓ : 0,13
2nd Anoxic	4,0ℓ : 0,22
Aerobic	9ℓ : 0,49
Unaerated mass fraction	0,51
Recycles	
Underflow (s-recycle)	1:1 10ℓ/d
Mixed liquor - Aerobic to 2nd anoxic (a-recycle)	1:1 10ℓ/d
Mixed liquor - 1st Anoxic to Anaerobic (r-recycle)	1:1 10ℓ/d
VSS concentration	2450 mg/ℓ
MLSS concentration	3200 mg/ℓ
Mass MLSS in system	59,2 g

* The TKN/COD ratio varied between sewage batches.

** The influent was supplemented with 17 mgP/ℓ orthophosphate to raise the concentration to about 32 mgP/ℓ. This ensured that the system was never underloaded with respect to its P removal capability.

Table 4.2: Operational changes made to the laboratory system with the plug flow first anoxic reactor (Day 1 to 311).

Day No.	Change	Reason
1	Install plug flow 1st anoxic reactor in MUCT system; a-recycle 1:1 r-recycle 1:1, s-recycle 1:1	—
58	Influent TKN boosted with NH_4Cl to approx. 135 mgN/l	To ensure sufficient NO_3 present for denit. profiles
79	Change a-recycle from 1:1 to 3:1	To decrease hydraulic retention time in 2nd anoxic reactor and so improve settleability
140	Install 1l anoxic zone on a-recycle; reduce aerobic reactor volume from 9l to 8l	To lower DO discharge to 2nd anoxic reactor and so improve settleability
183	Change a-recycle from 3:1 to 1:1; remove anoxic zone on a-recycle; increase aerobic reactor volume from 8l to 9l	The previous two measures were unsuccessful in improving settleability
199	Change to UCT configuration; remove a-recycle, reduce NH_4Cl supplement to feed	To reduce NO_3 recycled to the anaerobic zone and so increase P removal
	Increase s-recycle from 1:1 to 2:1	To improve clarifier operation
214	Install 1l anoxic zone after P.F. reactor; reduce aerobic reactor from 9l to 8l	To increase amount of denitrification and reduce NO_3 recycled to anaerobic zone
224	Increase s-recycle from 2:1 to 3:1	To further improve clarifier operation
238	Remove 1l anoxic zone after P.F. reactor; increase aerobic reactor from 8l to 9l	This zone was found to be superfluous
312	Replace P.F. reactor with completely mixed reactor; NH_4Cl supplementation to feed stopped	Further denitrification profiles to be measured with batch tests

INFLUENT & EFFLUENT COD
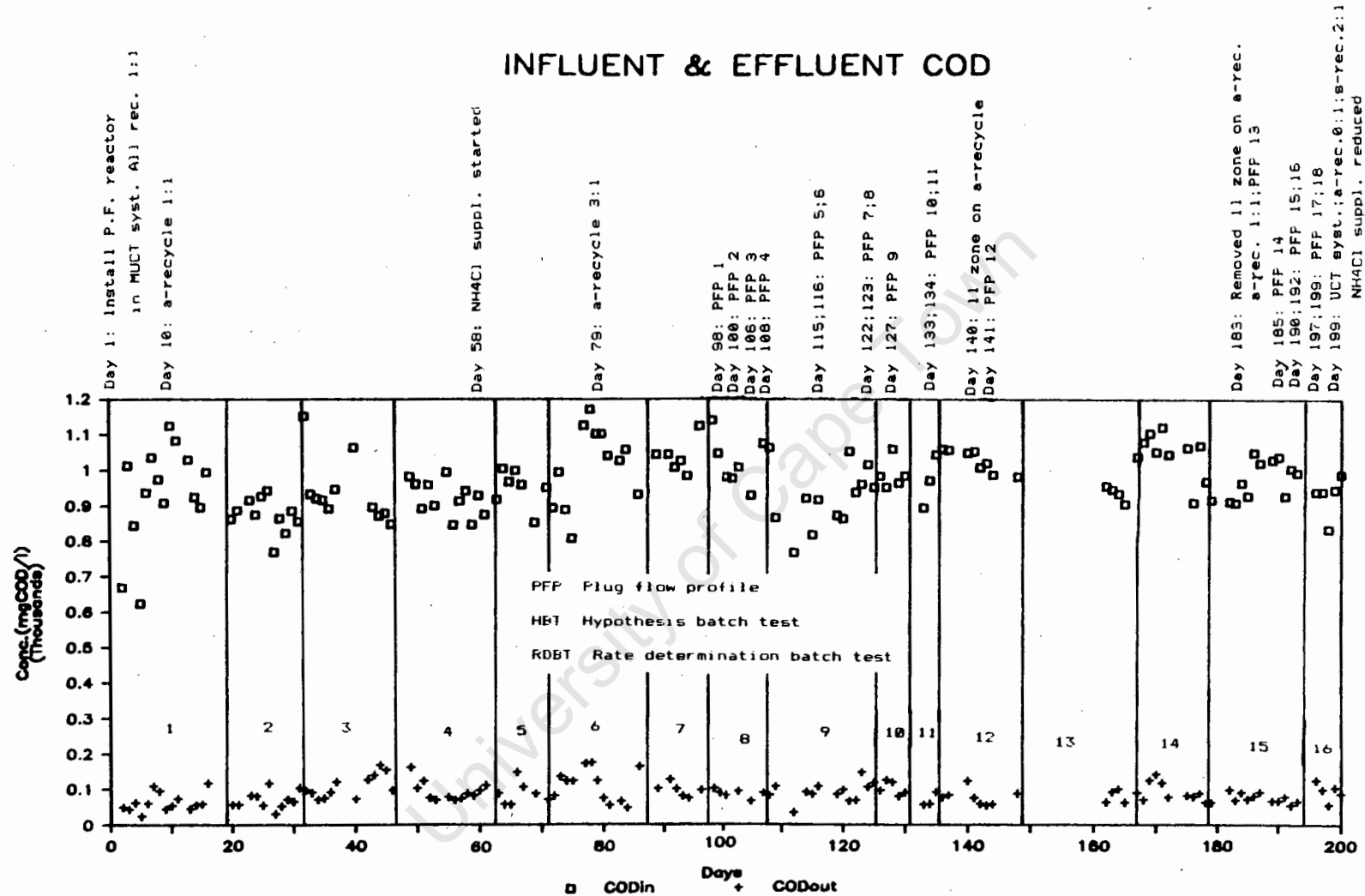
Fig 4.2: *The daily COD removal performance of the MUCT system for day 1 to 200. Included on this figure are the operational changes that were made during this period as well as the time of each new sewage batch (the vertical lines). The days of the plug flow reactor denitrification profiles (section 4.1) are also shown.*
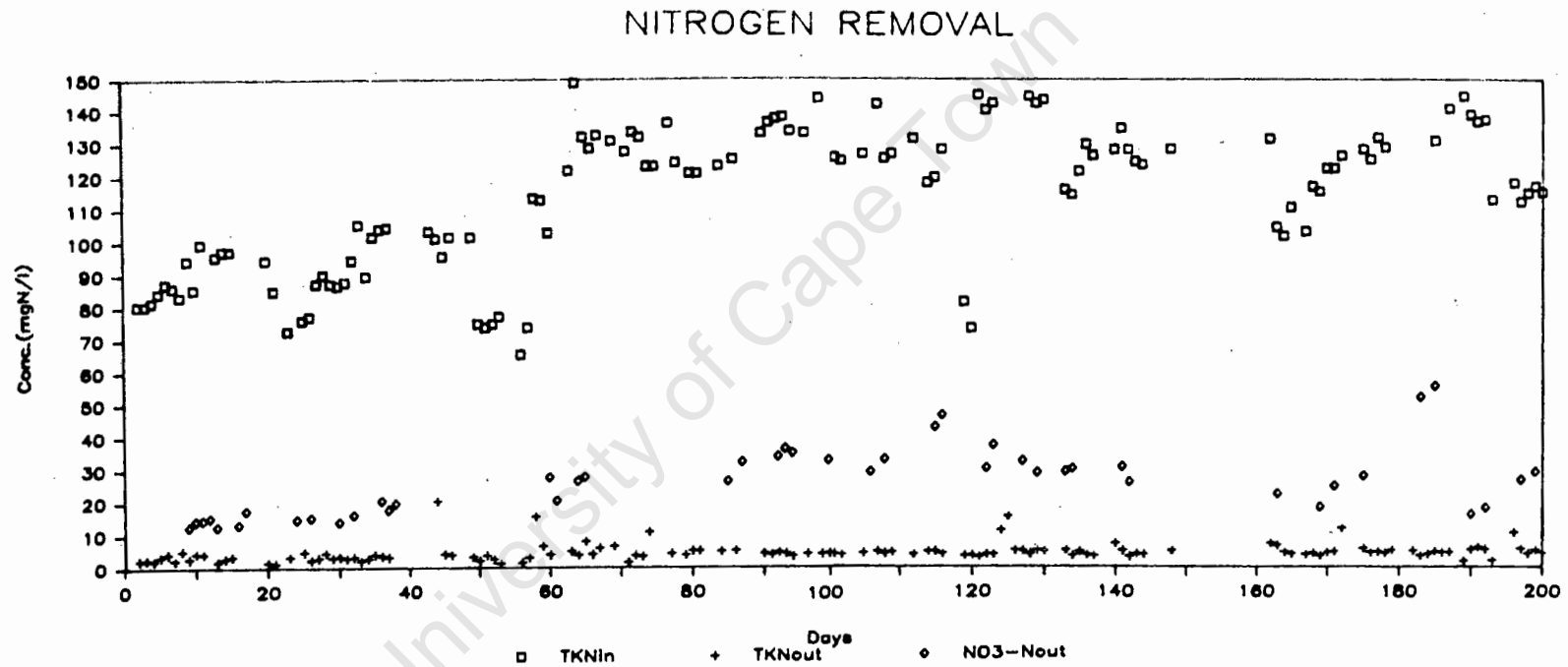
NITROGEN REMOVAL

Fig 4.3:   *The daily nitrogen removal performance of the MUCT system for day 1 to 200. The net amount of nitrogen removed (mgN/ℓ influent) by the system on any day may be estimated by subtracting the effluent TKN and nitrate concentrations from the influent*   *TKN concentration (as mgN/ℓ). All three latter concentrations are of interest and therefore appear separately. Note that on day 58, NH₄Cℓ supplementation of the feed was commenced to increase the amount of nitrification occurring. The influent TKN increased accordingly.*

NO3—N LEVELS IN EACH REACTOR

4.8
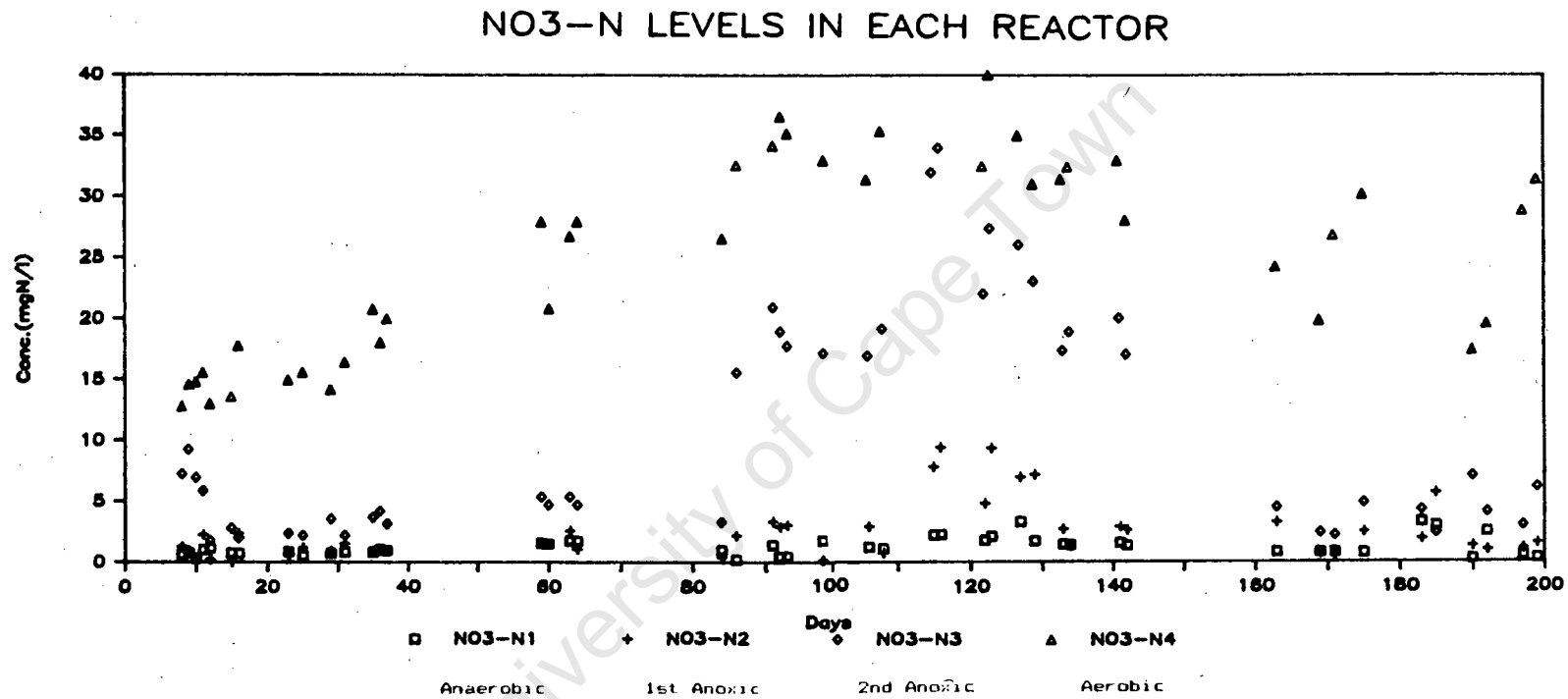
Fig 4.4: The nitrate concentration (as mgN/l) in each reactor of the MUCT system for day 1 to 200. Note the relatively high effluent nitrate concentrations in the effluent of the 1st anoxic plug flow reactor (as much as 8 to 9 mgN/l around day 120).
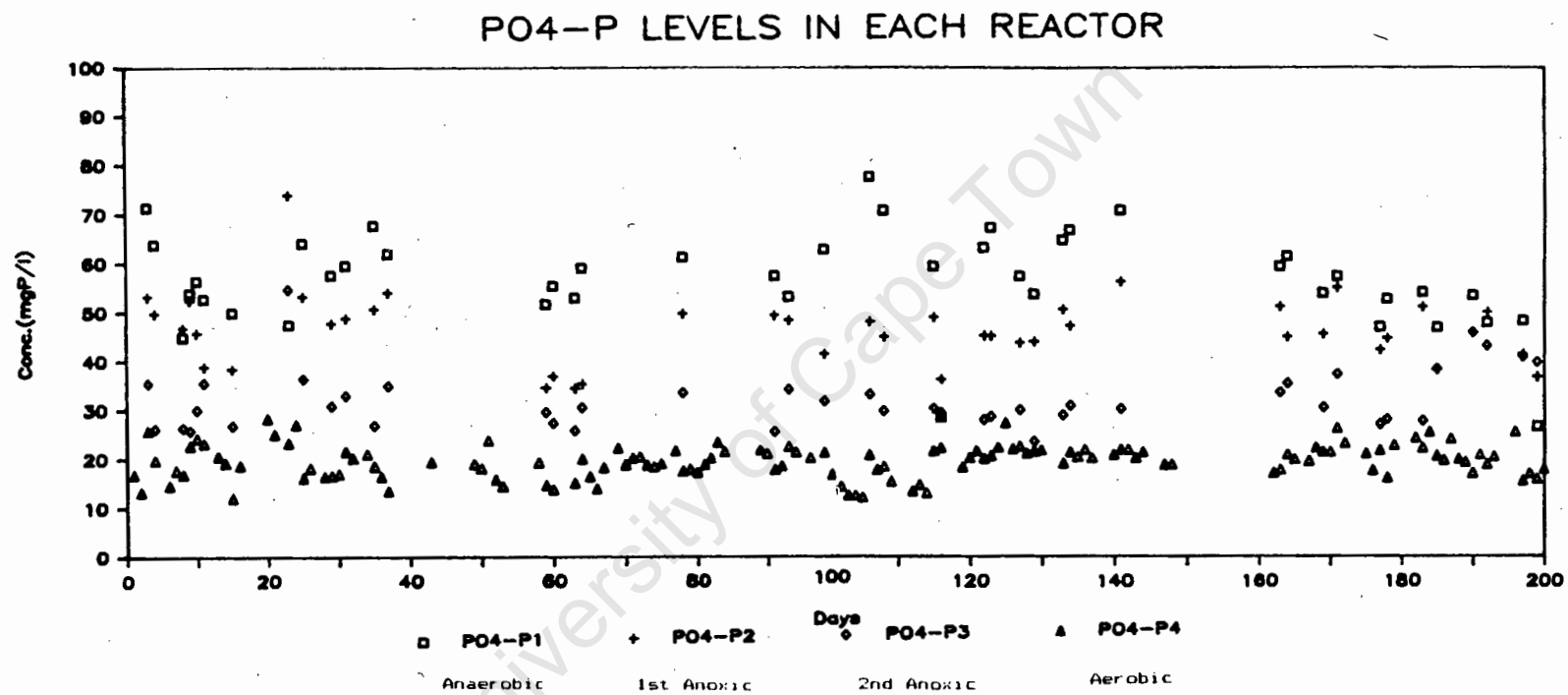
Fig 4.5: The phosphate concentration (as mgP/ℓ) in each reactor of the MUCT system for day 1 to 200.

P REMOVAL/LITRE INFLUENT
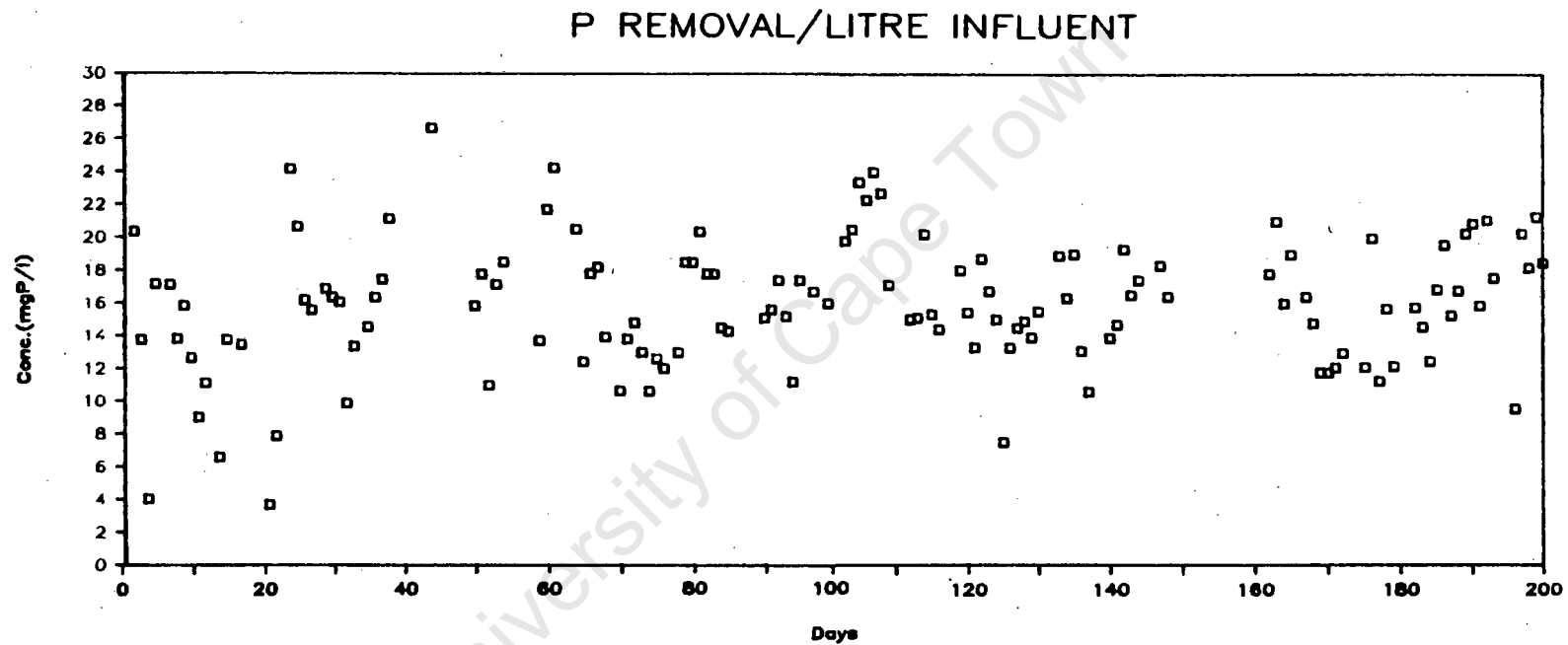
Fig 4.6:   *The P removal (per litre of influent) given by the MUCT system for day 1 to 200.   The removal was generally erratic and below 20 mgP/ℓ, the expected amount for this system (Wentzel et al., 1985).*

MLTSS & MLVSS CONCENTRATIONS
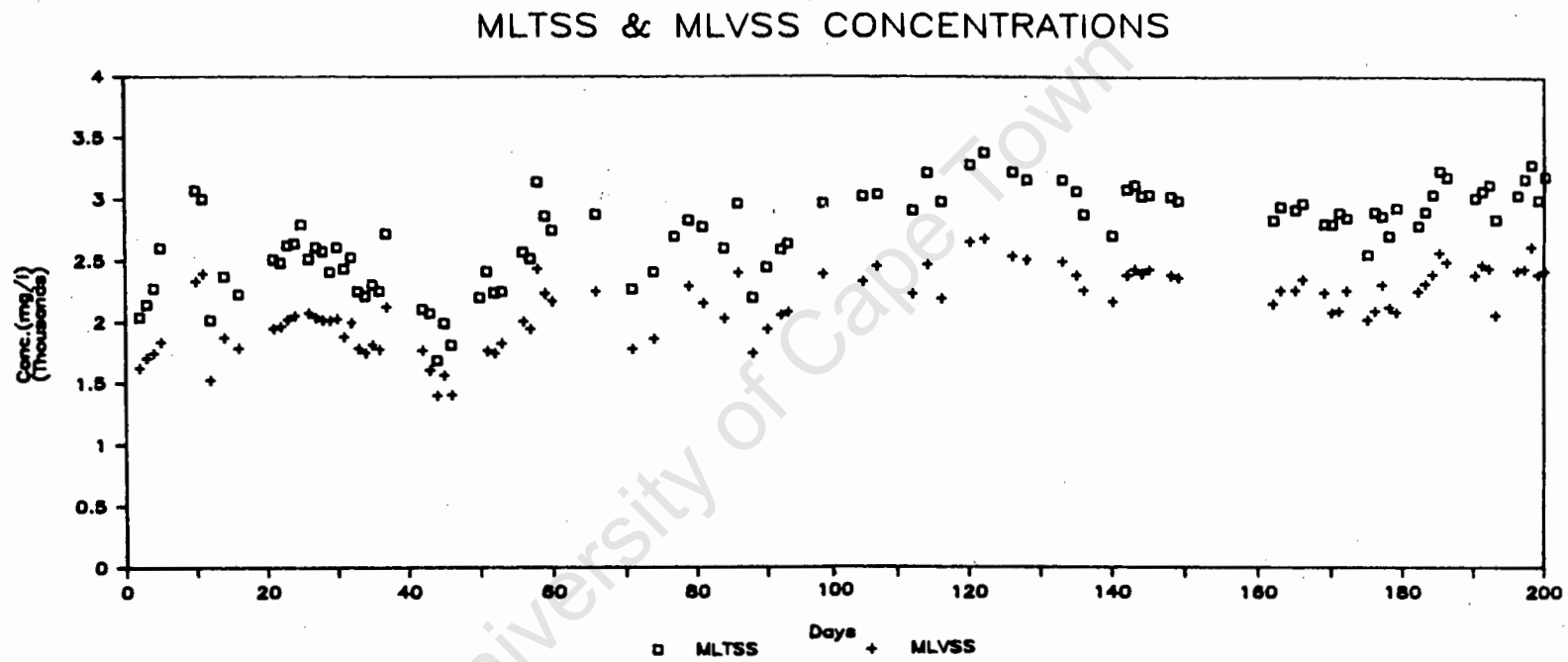
Fig 4.7:   The MLTSS and MLVSS concentrations in the MUCT system for day 1 to 200.

DSVI

DSVI (ml/g)

Days
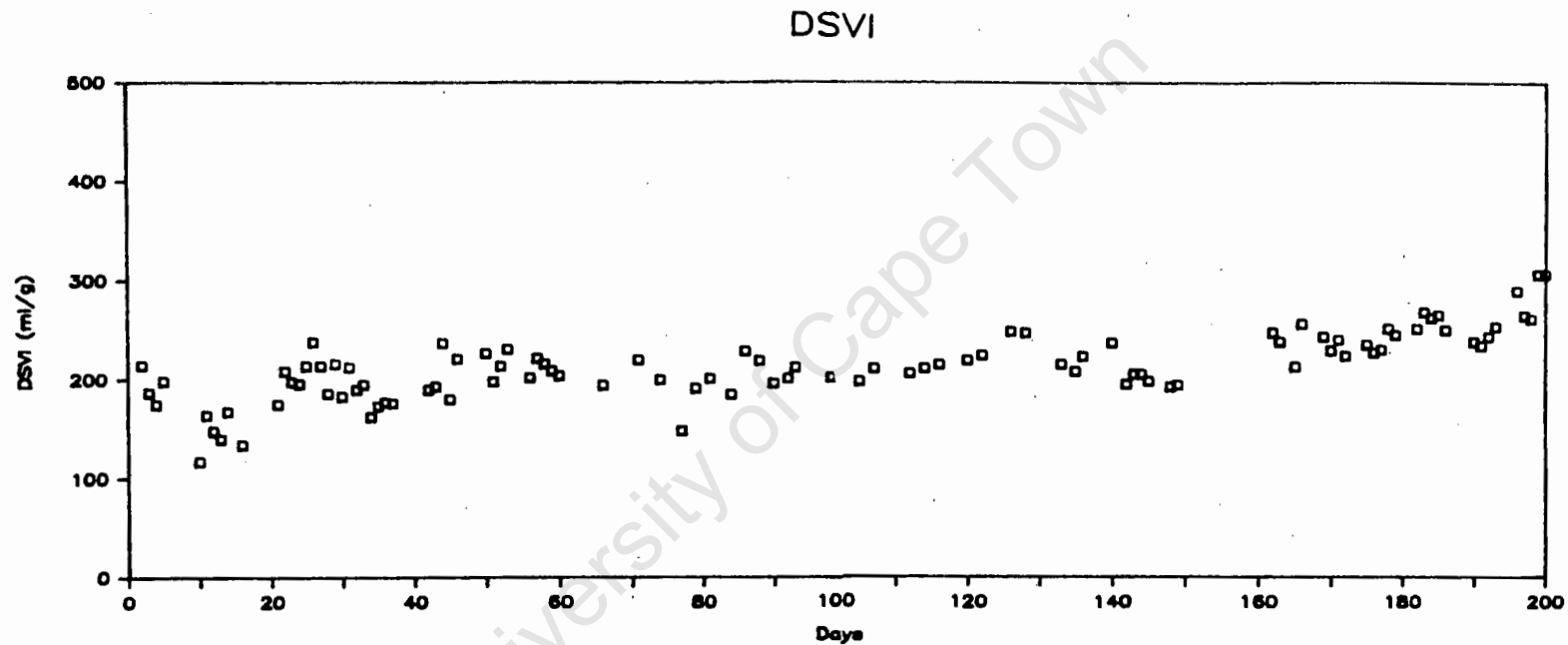
Fig 4.8:   *The DSVI of the MUCT system sludge for day 1 to 200.   The DSVI remained constant around 200 ml/g for most of this period, but started to increase from about day 180.*

OXYGEN UTILIZATION RATE

4.13
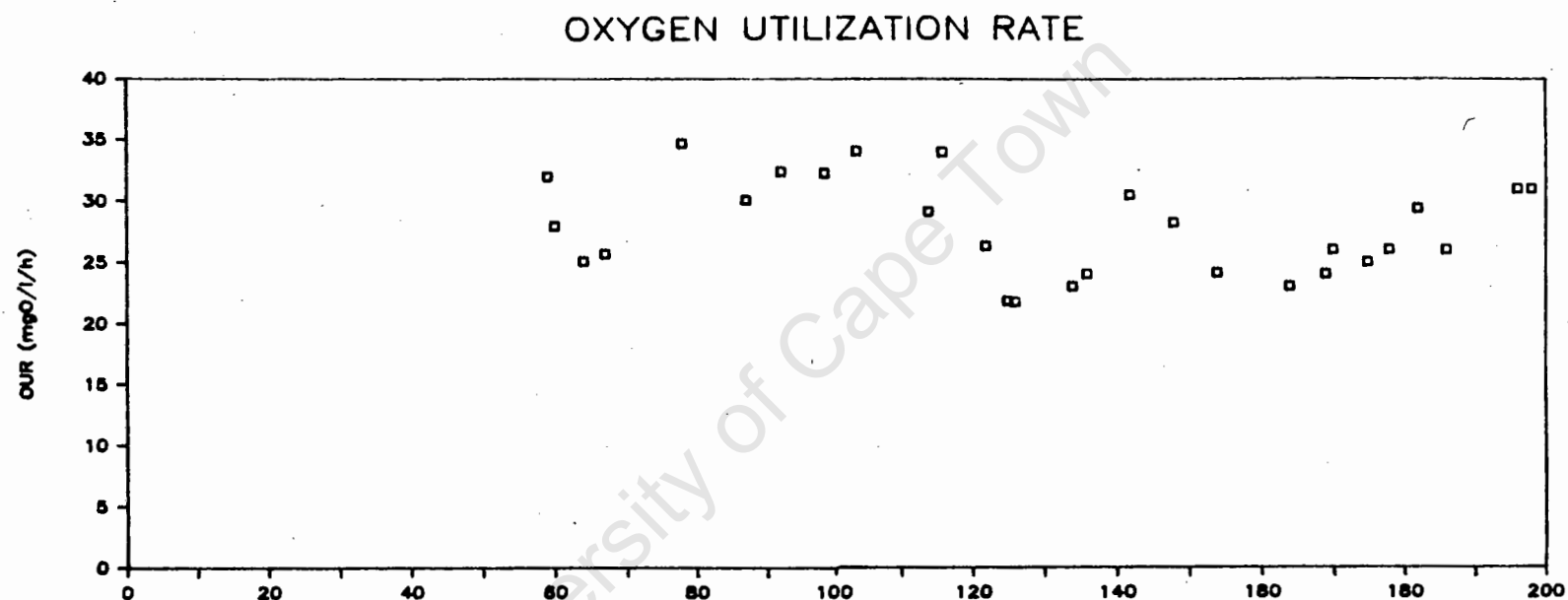
Fig 4.9:   *The oxygen utilization rate of the MUCT system sludge measured in the aerobic reactor for day 1 to 200.*

removed.[2] The aerobic reactor volume was changed back to 9ℓ.

During the time that the system was operated in the MUCT mode (day 1 to 198), it can be seen from Figs 4.2 and 4.3 that the COD and nitrogen removal were good. The P removal during this period was erratic and generally below 20 mg/ℓ (Fig 4.6). The expected P removal for the system was about 20 mg/ℓ (Wentzel *et al.*, 1985), i.e. the system was removing less P than expected. The probable reason for the poor P removal was that nitrate was being recycled to the anaerobic reactor via the r-recycle – high nitrate concentrations existed at the end of the first anoxic plug flow reactor (the source of the r-recycle), sometimes as much as 9 mgN/ℓ (see Fig 4.4). These high nitrate concentrations arose from the attempts to load the plug flow reactor to its fullest potential in order to measure denitrification profiles.

Over this first 198 day period, during which the system was operated in a MUCT mode, 17 denitrification profiles were measured in the plug flow reactor.

(ii)   The UCT mode of operation (day 199 to 311)

In the investigation, optimal P removal was desired so that the maximum effect of BEPR on denitrification could be observed. To improve the P removal performance, on day 199 the laboratory system was altered to operate in the UCT mode with no a-recycle (Fig 4.10). The UCT mode was brought into operation by taking the recycle to the anaerobic reactor (r-recycle) from effluent of the *second* anoxic reactor (instead of from the effluent of the first) and eliminating the a-recycle. These measures were taken to keep the nitrate concentration in the r-recycle as low as possible. The UCT mode of operation was maintained with no a-recycle for the remainder of the time that the plug flow reactor was operational in the system, i.e. from day 199 to day 311. Other changes that were made on day 199 were: (1) Ammonium chloride supplementation of the feed was reduced to lessen the amount of nitrification occurring and (2) the underflow s-recycle was set at 2:1 to improve clarifier operation because bulking was still a problem. The sludge age, sewage characteristics and reactor volumes remained unchanged (see Table 4.1).

The day-to-day performance of the UCT system (from 199 to 311) is given in Figs 4.11 to 4.18, as follows: COD removal (Fig 4.11), nitrogen removal (Fig 4.12), effluent nitrate and phosphate concentrations from each reactor (Figs 4.13 and 4.14

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[2]Details regarding the sludge settleability and filmentous organism growth in the system during the investigation are given in Appendix J.

ANAEROBIC   ANOXIC   AEROBIC
REACTOR   REACTORS   REACTOR
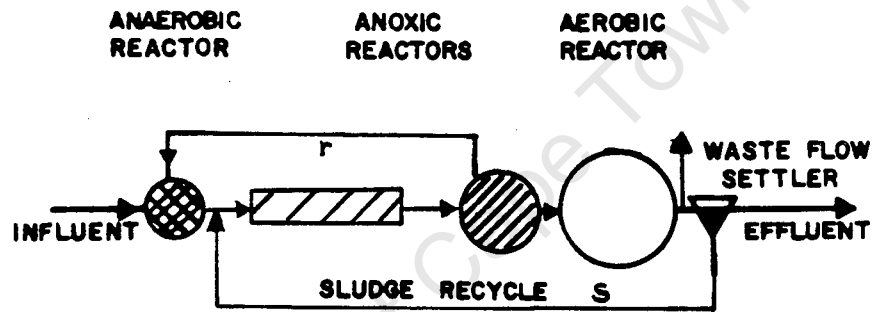
Fig 4.10:   On day 199 the MUCT system with its 1st anoxic plug flow reactor, was
converted to operate in the UCT mode with no a-recycle. This is
shown above. This configuration prevented the recycle of the high
nitrate concentrations (present at the end of the 1st anoxic plug flow
reactor) back to the anaerobic reactor. P removal was hence improved.
The plug flow reactor was retained so that further denitrification
profiles could be measured.

INFLUENT & EFFLUENT COD
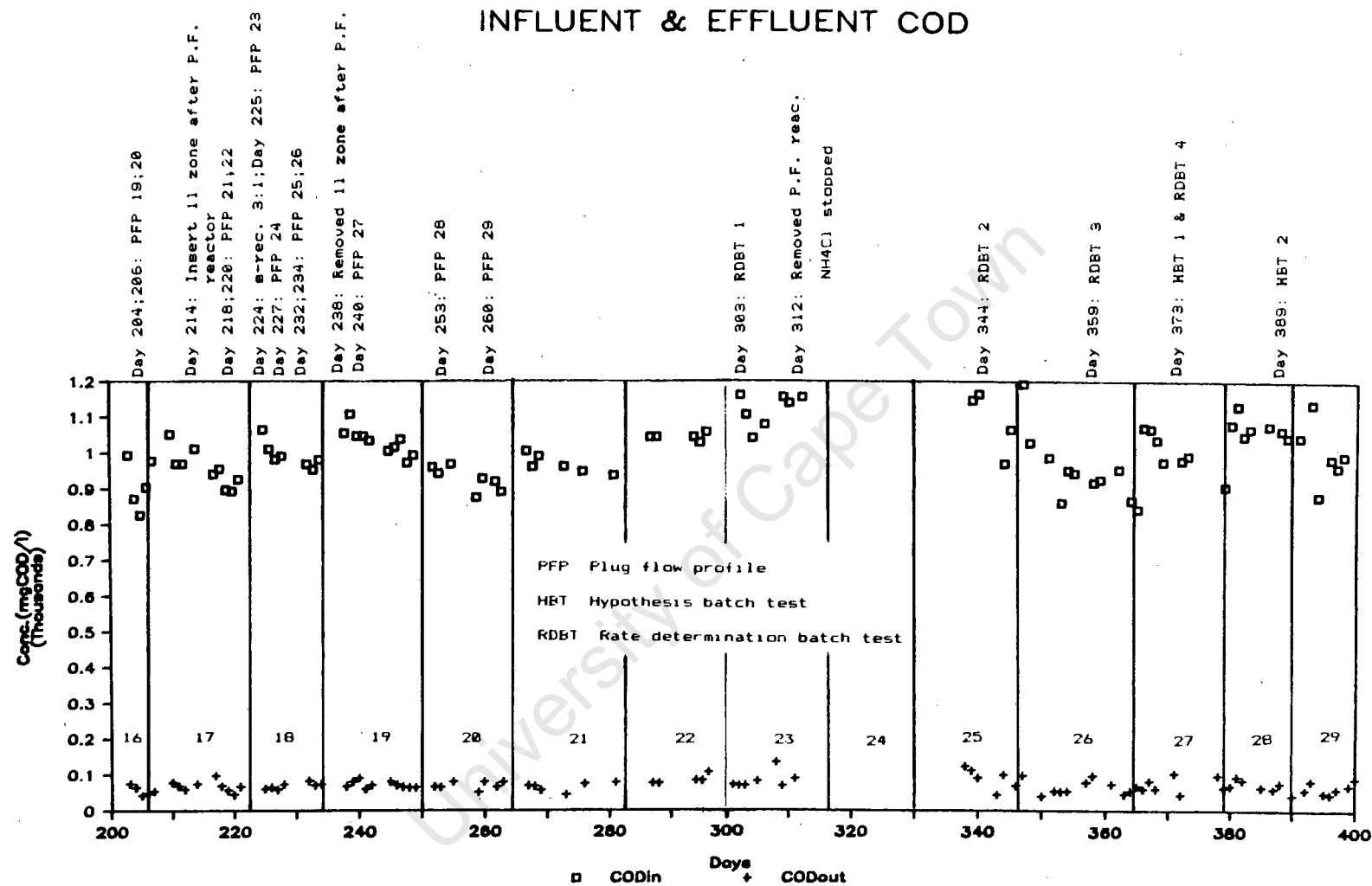
Fig 4.11: The daily COD removal performance of the UCT system for day 200 to 400. The operational changes, new sewage batches, and days for the plug flow profiles (section 4.1), rate determination batch tests (section 4.2) and hypothesis batch tests (Chapter 6) are included.
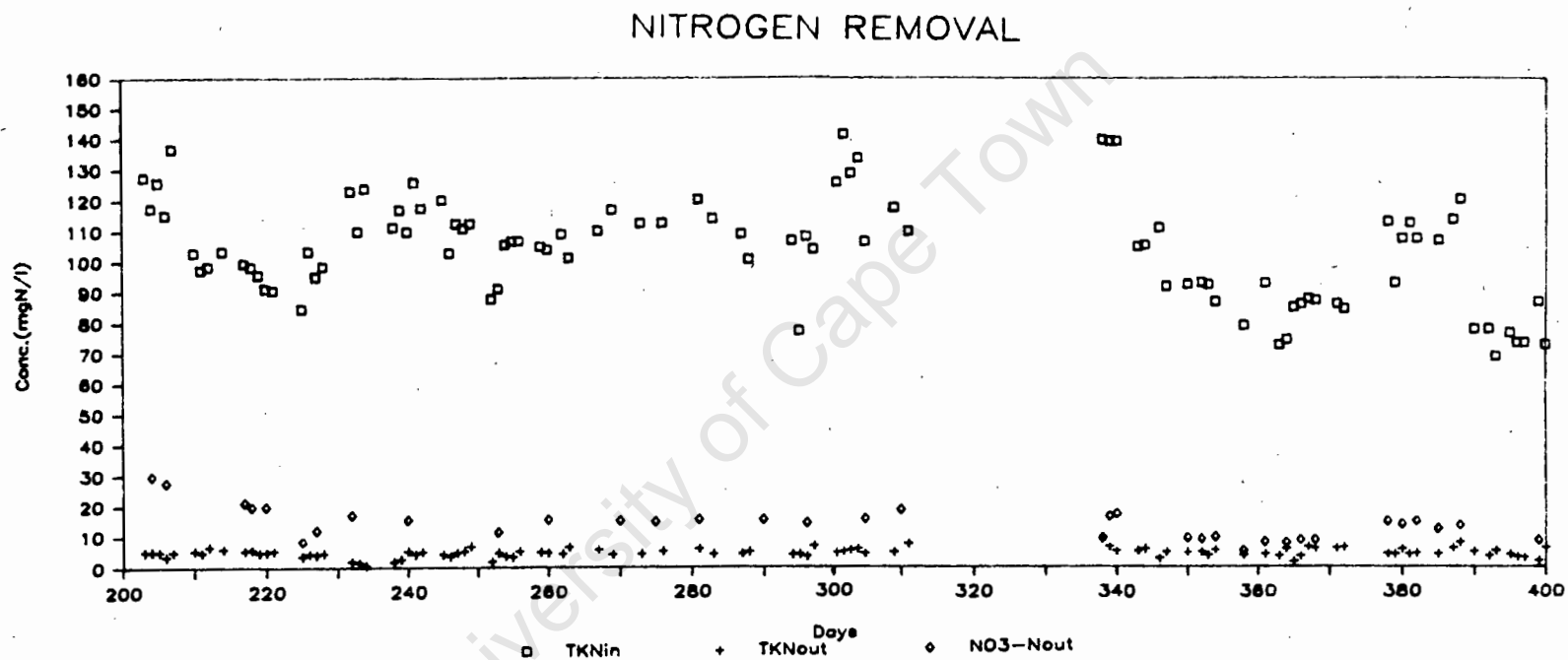
NITROGEN REMOVAL

Fig 4.12: The daily nitrogen removal performance of the UCT system for day 200 to 400. For day 200 to 311 the influent TKN was around 110 mgN/ℓ, lower than that prior to day 199. This was due to NH_4Cl supplementation of the feed being reduced on day 199. On day 312 the NH_4Cl supplementation was stopped completely and the influent TKN dropped to about 85 mgN/ℓ.
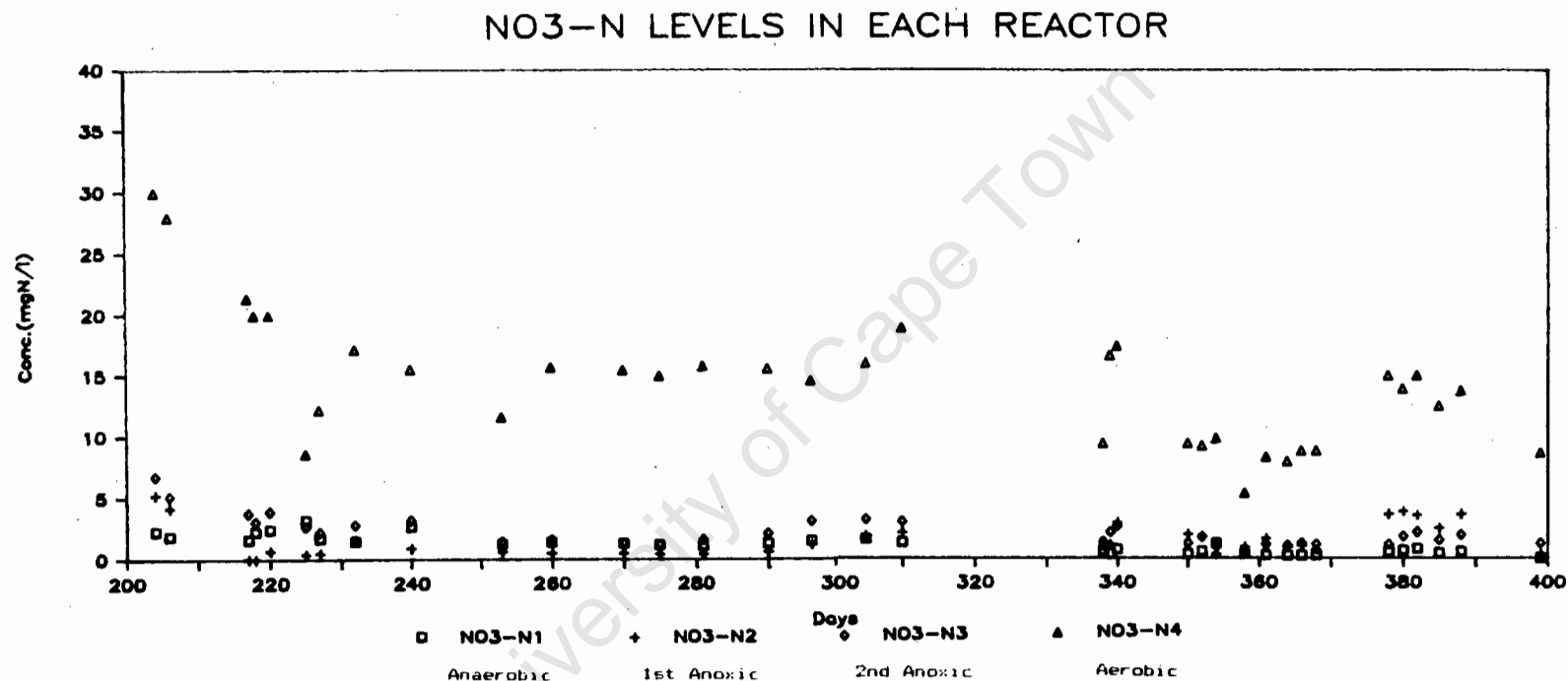
Fig 4.13: *The nitrate concentrations (as mgN/ℓ) in each reactor of the UCT system for day 200 to 400. With the $NH_4Cℓ$ supplementation to the sewage feed having been reduced on day 199 and totally stopped on day 312, the nitrate concentrations are generally lower than in the period prior to day 199.*

PO4-P LEVELS IN EACH REACTOR
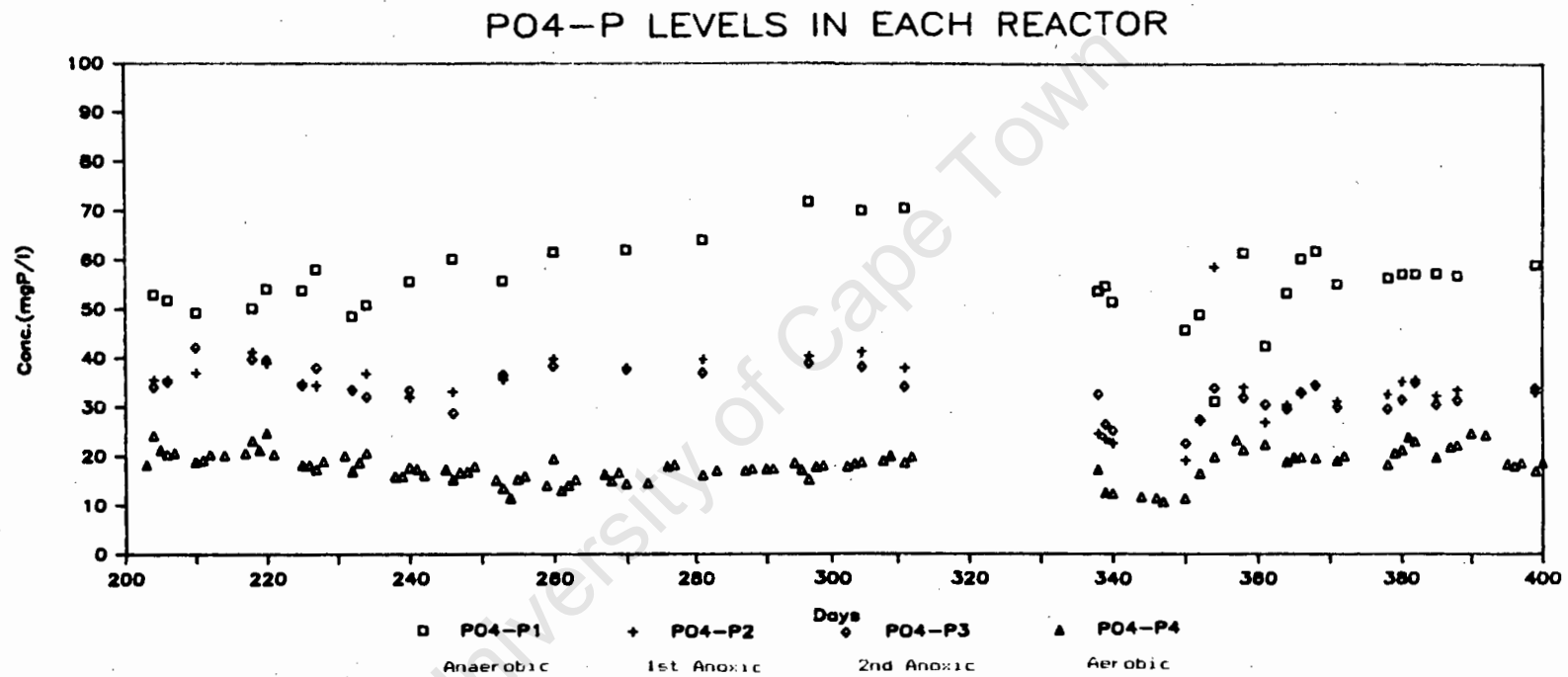
Fig 4.14: The phosphate concentrations (as mgP/ℓ) in each reactor of the UCT system for day 200 to 400.

P REMOVAL/LITRE INFLUENT
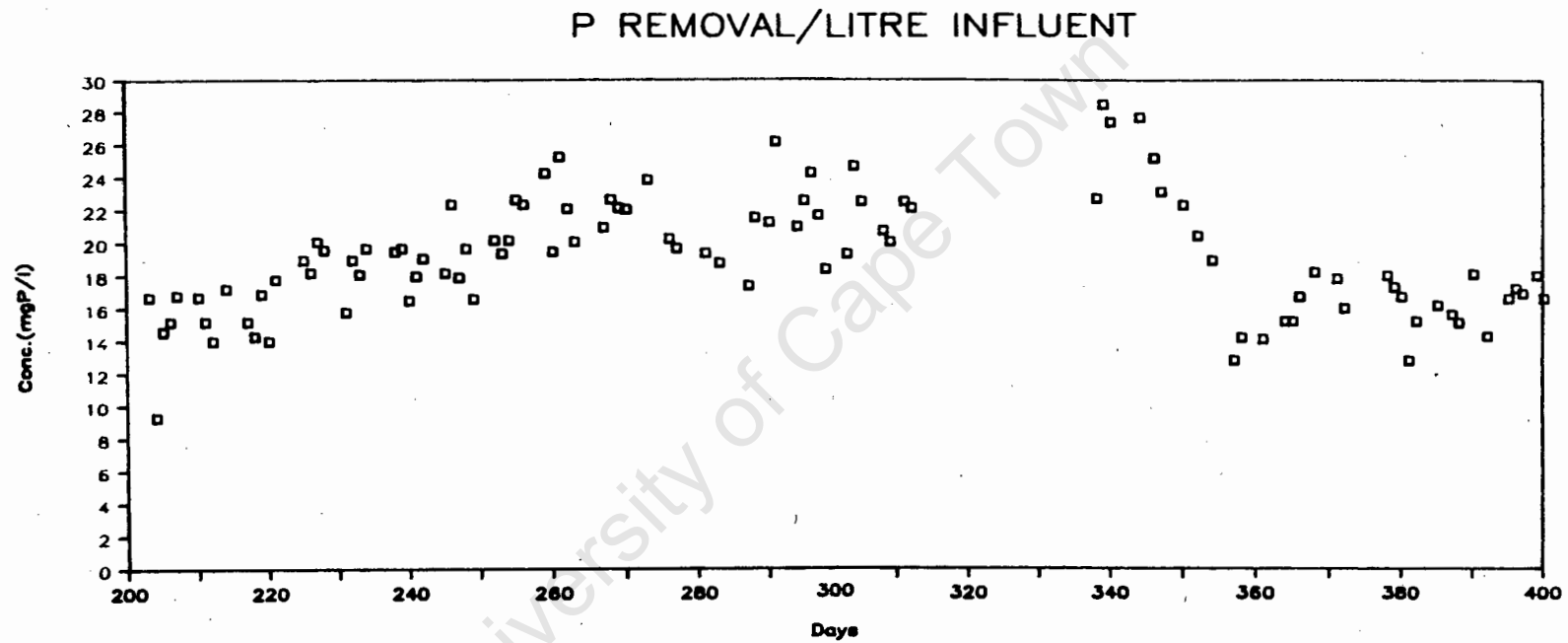
Fig 4.15: The P removal (per litre of influent) given by the UCT system for day 200 to 400. Note that for day 200 to 347 the P removal was far less erratic than that prior to day 200, and reached values of over 20 mgP/ℓ. The sewage batch fed between days 347 and 363 (No.26) seems to have contained toxins that inhibited BEPR somewhat : there was a sharp decline in P removal to 13 mgP/ℓ, which recovered to 17 mgP/ℓ within 3 days.

MLTSS & MLVSS CONCENTRATIONS
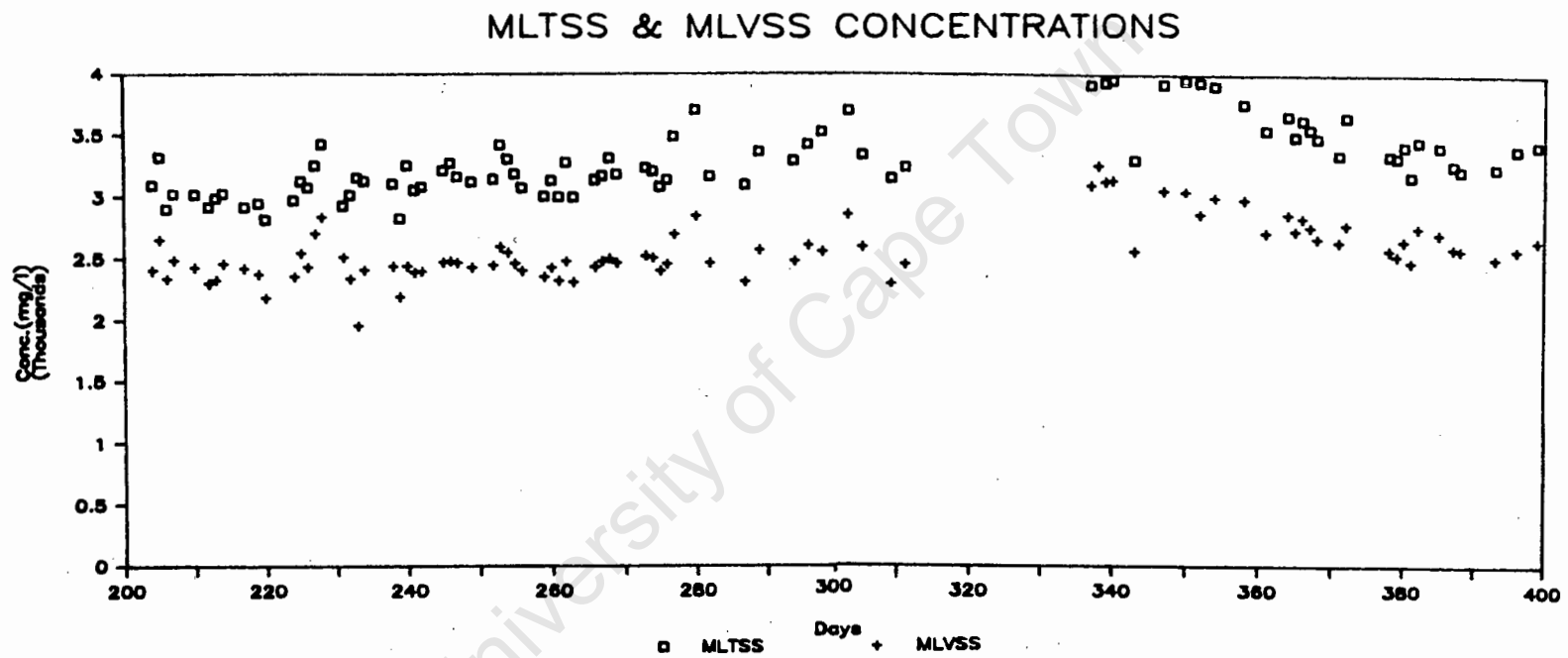
Fig 4.16: The MLTSS and MLVSS concentrations in the UCT system for day 200 to 400.
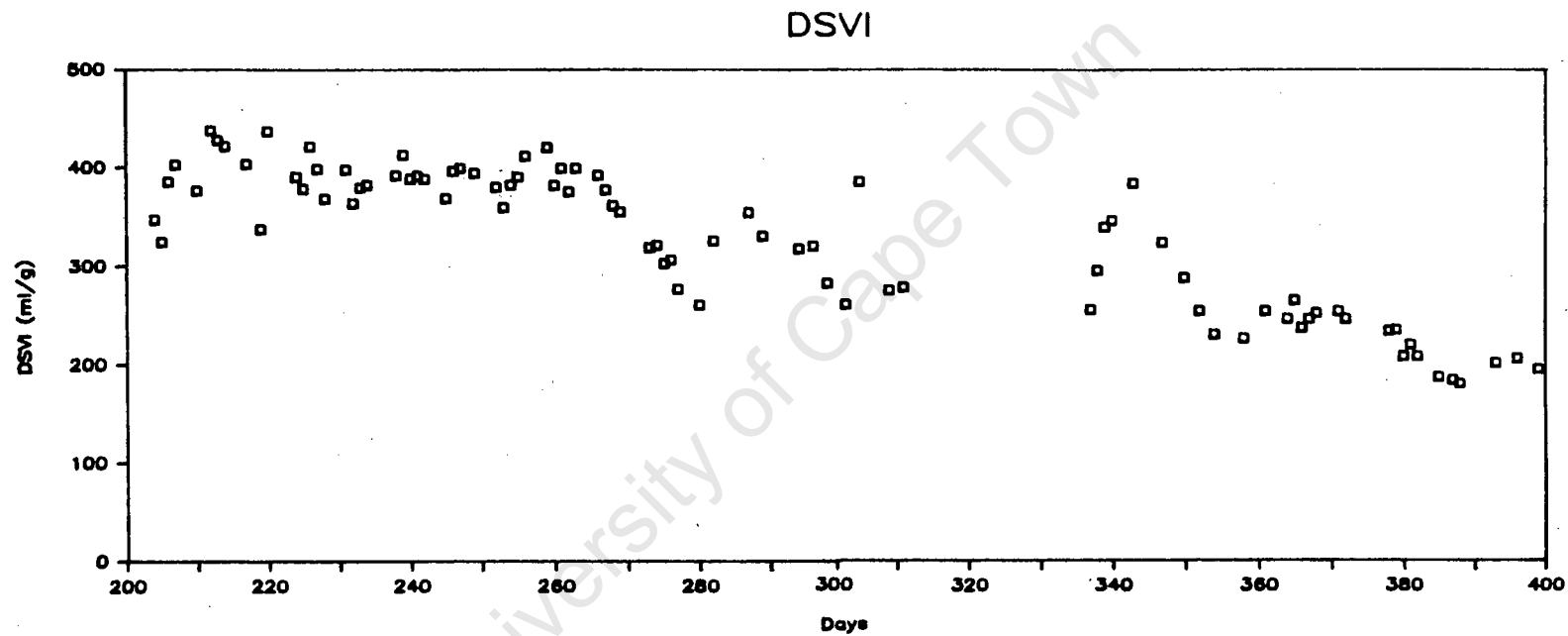
DSVI

Fig 4.17: *The DSVI of the UCT system sludge for day 200 to 400. The DSVI was around 400 ml/g from day 200 to 265, after which it showed a gradual decreasing trend to 200 ml/g by day 400.*

OXYGEN UTILIZATION RATE
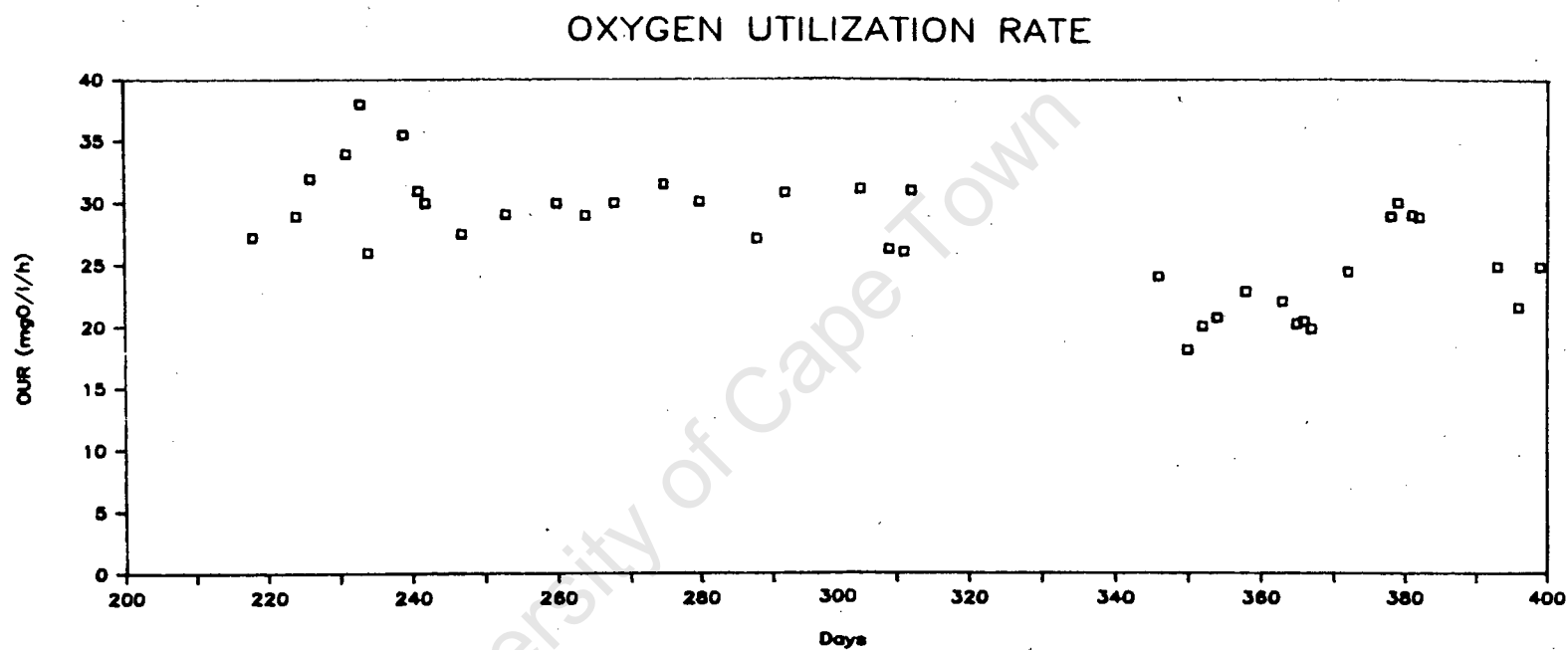
Fig 4.18: The oxygen utilization rate of the UCT system sludge measured in the aerobic reactor for day 200 to 400.

respectively), system P removal, i.e. influent minus effluent P concentrations (Fig 4.15), MLTSS and MLVSS concentrations (Fig 4.16), DSVI (Fig 4.17) and OUR (Fig 4.18) in the aerobic reactor.

Two changes were made to the system during the UCT mode period (see Table 4.2): (1) On day 214 a 1ℓ anoxic zone was inserted between the plug flow reactor and second anoxic reactor to further reduce the nitrate concentration being recycled to the anaerobic reactor (from the second anoxic reactor). However, once the system stabilized this was found superfluous and it was removed on day 238. (2) The underflow (the s-recycle) ratio was increased from 2:1 to 3:1 on day 224, since at this point the DSVI had reached 400 mℓ/g (Fig 4.17).

Over the period that the system was operating in the UCT mode, good COD and nitrogen removal were again obtained (see Figs 4.11 and 4.12). As expected, the P removal also became less erratic and improved to close to 20 mgP/ℓ (see Fig 4.15).

With the system operated in the UCT mode, 12 denitrification profiles were measured in the plug flow reactor.

4.1.2 Observed denitrification behaviour in the plug flow anoxic reactor

To place the plug flow reactor profiles in context with the overall MUCT/UCT system performance, the days on which the profiles were measured are marked on the overall system response graphs in Fig 4.2 and Fig 4.11. Over the first 198 days while the system was operated in the MUCT mode 17 profiles were measured. For the next 113 days (day 199–311) while the system was operated in a UCT mode, 12 profiles were measured. The profile results from both periods showed little difference and typical nitrate and nitrite concentration versus time profiles are given in Figs 4.19 and 4.20; typical phosphate and soluble ($<0,45\mu$m) COD concentration-time profiles are shown in Fig 4.21. The nitrate, nitrite, phosphate and soluble COD data for all 29 profiles is listed in Appendix D.

Examining the nitrate concentration profiles, of the 29 measured, 20 showed a two-phase denitrification behaviour — an initial rapid rate of nitrate disappearance followed by a second slower one; the other 9 profiles consisted of only one rate; Figs 4.19 and 4.20 show examples of profiles with two and one denitrification rate(s) respectively.

MUCT TWO-PHASE DENITRIFICATION
NITRATE & NITRITE

$K_1'' + K_2''$

K_2''

K_{NO_2}

Conc.(mg/l)

Time(min)

□ NO3–N   + NO2–N
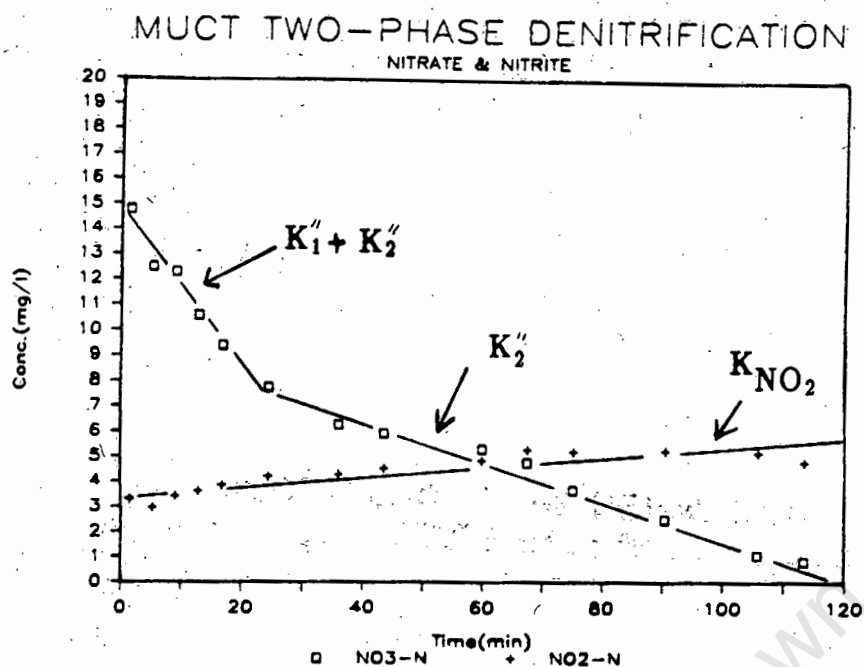
Fig 4.19: *A typical example of the two-phase denitrification profiles obtained in the plug flow first anoxic reactor of the MUCT/UCT system. Note that although the nitrate reduction occurs in two phases, the nitrite accumulation rate is continuous.*

MUCT ONE-PHASE DENITRIFICATION
NITRATE & NITRITE

K_2''

K_{NO_2}

Conc.(mg/l)

Time(min)

□ NO3–N   + NO2–N
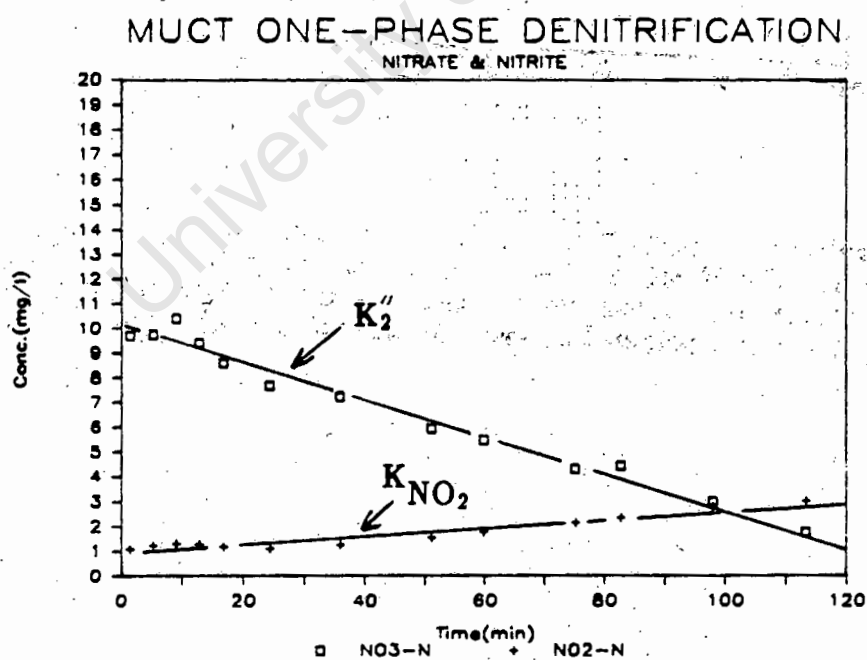
Fig 4.20: *A typical example of the single-phase denitrification profiles obtained in the plug flow first anoxic reactor of the MUCT/UCT system.*

In all the profiles, irrespective of whether there was one or two phases of denitrification, (i) P uptake was very slow (Fig 4.21), (ii) the nitrite concentration increase in most cases was small (Figs 4.19 and 4.20) and (iii) $<0,45\mu$m COD concentration remained unchanged (Fig 4.21).

4.1.3 Evaluation of nitrate profiles

(i) Separation into first and second rate components

In the denitrification kinetics for N removal systems (see Chapter 2, section 2.1.3), the initial rapid decrease in nitrate concentration was accepted to arise from two denitrification rates, viz. a fast rate (K_1) due to influent RBCOD utilization and a background slower rate (K_2) due to influent PBCOD utilization. The rationale for this is that RBCOD utilization and PBCOD hydrolysis and utilization are reactions that take place simultaneously. The slower rate of nitrate reduction (K_2) manifests itself after the RBCOD has all been depleted. The denitrification rate due to influent RBCOD (K_1) can be obtained by subtracting the background rate (K_2) from the initial rate of rapid nitrate reduction ($K_1 + K_2$). Specific denitrification rates were obtained by adjusting the observed nitrate reduction rates for the active mass fraction of the VSS and for nitrite formation.

The above approach was also adopted to evaluate the denitrification profiles measured in the MUCT/UCT system. After adjustment for active VSS, the two measured rates will be denoted K_1'' and K_2'' to distinguish them from the kinetic values K_1 and K_2 for N removal systems; after further adjustment for nitrite formation the rates will be referred to as K_1' and K_2'.

(ii) Adjustment for active mass of VSS

Stern and Marais (1974) found that the rates of denitrification [mgNO$_3$-N/(ℓd)], when divided by the MLVSS (X_v) to obtain the specific denitrification rate mgNO$_3$-N/(mgVSS.d), decreased as the sludge age increased. They concluded that this was because the activity (i.e. active fraction of the MLVSS) decreased as sludge age increased. When the denitrification rates were expressed in terms of the *active* VSS concentration X_a, i.e. mgNO$_3$-N/(mgAVSS·d), they found the resulting specific rates to be independent of sludge age.

Following this approach, the denitrification rates measured in this investigation also were reduced to specific rates with respect to active VSS. The active fraction of the VSS is a function of the system parameters such as sludge age and the sewage characteristics (see Marais and Ekama, 1976). Because the same system parameters and sewage source were used throughout the investigation, a constant value for the active fraction (f_{av}) of the VSS sludge mass was accepted.

The active VSS concentration at any time during the investigation therefore was accepted to be a constant fraction of the measured VSS (X_v) concentration, i.e.

$$X_a = f_{av}.X_v \qquad (mgAVSS/\ell) \tag{4.1}$$

where f_{av} = 0,24 mgAVSS/mgVSS for this study.

Hence the specific denitrification rate K_1'' (and K_2'') is given by

$$K_1'' \text{ (and } K_2'') = \frac{\left\{ \begin{array}{c} \text{nitrate concentration reduction} \\ \text{per unit time, } mgN/(\ell.d) \end{array} \right\}}{\{X_a, mgAVSS/\ell\}} \tag{4.2}$$

(iii)   Adjustment for nitrite formation

Basing the K_1'' and K_2'' rates on nitrate disappearance is not an accurate measure of the rates of denitrification to nitrogen gas because some nitrate is reduced only as far as nitrite. The nitrite formation rate (see Figs 4.19 and 4.20) is a measure of the quantity of nitrate reduced only to nitrite. The difference between the nitrate disappearance rate and the nitrite appearance rate is the rate of denitrification to nitrogen gas.

When nitrate is denitrified only to nitrite instead of to N_2 gas, the electron-accepting capacity of the nitrate is reduced; only 2/5 of the electrons are available, hence

$$K' \text{ (nitrate to } N_2 \text{ gas)} = K_{NO_3}'' - 3/5 \, K_{NO_2} \tag{4.3}$$

where K_{NO_2} is the rate of formation of NO_2-N

K''_{NO_3} is the rate of disappearance of NO_3-N.

Hence, the initial observed fast rate $(K''_1 + K''_2)$ corrected for nitrite formation is $(K''_1 + K''_2 - 3/5\,K_{NO_2})$ and the second slow rate (K''_2) corrected for nitrite formation is $(K''_2 - 3/5\,K_{NO_2})$.

By subtracting the adjusted second rate from the adjusted initial rate, the latter may now be split into its two components, viz:

$$K'_1 = (K''_1 + K''_2 - 3/5\,K_{NO_2}) - (K''_2 - 3/5\,K_{NO_2}) \qquad (4.4)$$
$$= K''_1$$
$$K'_2 = (K''_2 - 3/5\,K_{NO_2}) \qquad (4.5)$$

where K'_1 and K'_2 are the rates adjusted for nitrite accumulation.

From the above it can be seen that the adjustment for nitrite is not divided between the two rates: the adjusted (K'_1) and unadjusted (K''_1) initial rapid rates are equal, and the second slow rate (K''_2) is reduced by the full nitrite formation rate. This method of the nitrite adjustment is justified because the nitrite formation rate K_{NO_2} appears constant throughout the plug flow reactor length; cessation of the initial rapid rate (K''_1) does not appear to reduce the nitrite formation rate (Fig 4.19).

After adjustment for nitrite formation, the K'_1 and K'_2 rates were plotted on probability paper. The K'_1 rate data is log − normally distributed with a geometric mean value of $\bar{K}'_1 = 0{,}610 \overset{x}{\div} 1{,}48$ mgNO$_3$-N/(mgAVSS·d) (Fig 4.22) and the K'_2 data is normally distributed with a mean of $\bar{K}'_2 = 0{,}240 \pm 0{,}053$ mgNO$_3$-N/(mgAVSS·d) (Fig 4.23). Both means are quoted at the 95% confidence level.

It is common in biological systems that generally when a kinetic rate is high, the variance of the data also is high, and the error in the reading is proportional to the magnitude of the reading; when a kinetic rate is low, the variance of the data also is low and the error in the reading is independent of the magnitude of the reading. In the former case, the data will be log− normally distributed and in the latter it will be normally distributed.
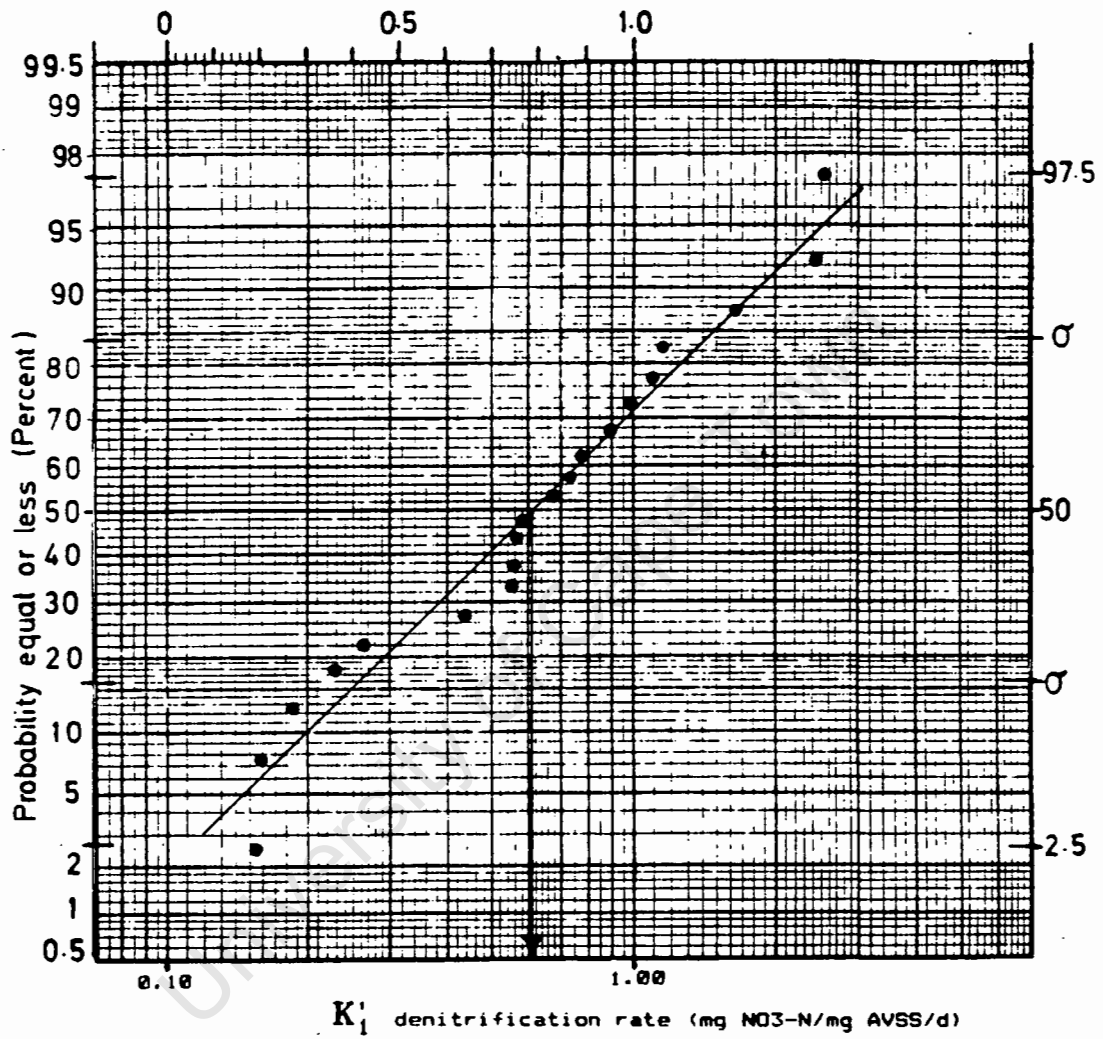
Fig 4.22:   Probability distribution of the K_1 data obtained in the plug flow first anoxic reactor of the MUCT/UCT system.
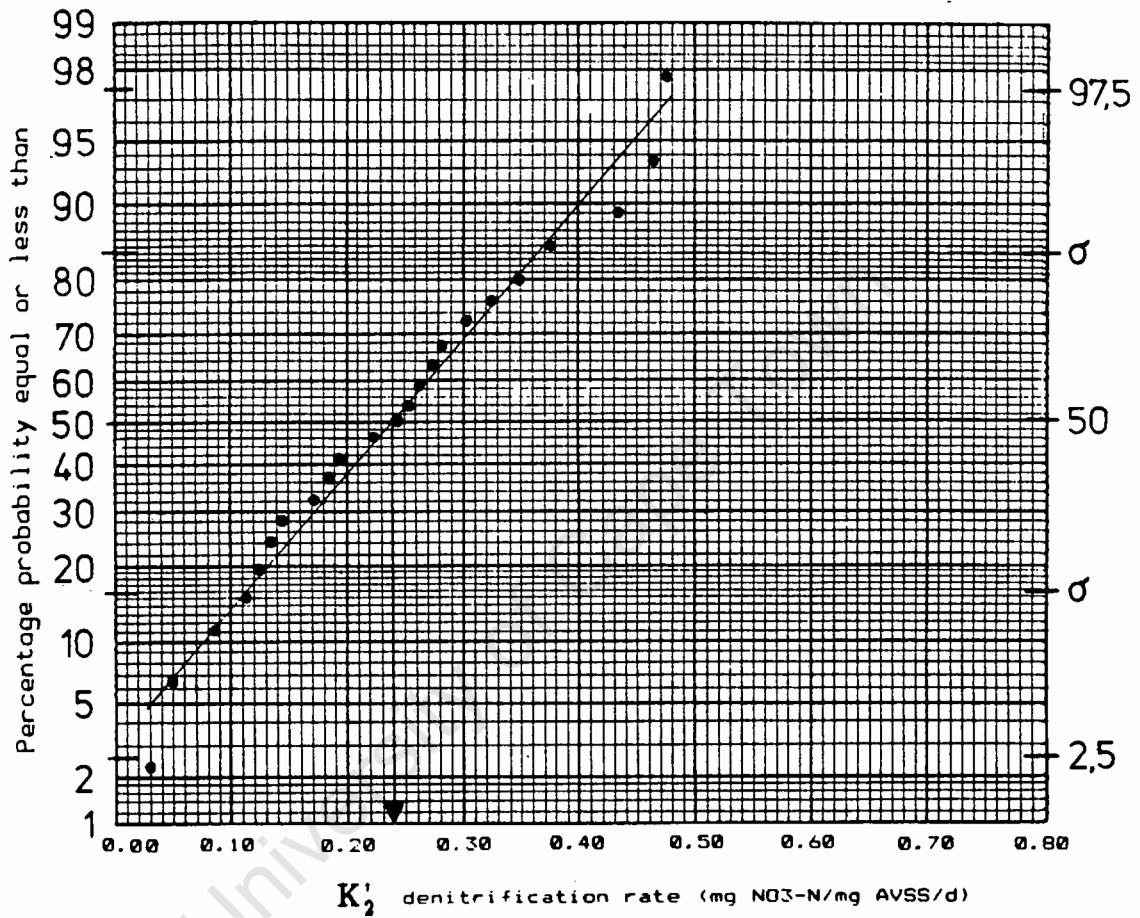
Fig 4.23: _Probability distribution of the K_2 data (adjusted for nitrite accumulation) obtained in the plug flow first anoxic reactor of the MUCT/UCT system._

4.1.4   Possible reasons for observed behaviour

Twenty (out of the total of 29) denitrification profiles showed a two-phase denitrification behaviour like that shown in Fig 4.19; the other nine had a single phase of denitrification (Fig 4.20). Examination of both the second rate (K_2') in the profiles with a two-phase behaviour and the single rate in the profiles with a single phase of denitrification indicated that statistically there was no significant difference between these sets of rates [a t-test (Walpole and Myers, 1978) showed the means of the two sets *not* to be significantly different at the 95% confidence level]. Consequently, these two sets of rates were grouped together as a single family of "second" slow rates of denitrification, the members of which are denoted K_2'. However, an explanation needed to be found why some profiles exhibited an initial rapid rate of denitrification (K_1').

The similarity of the two-phase behaviour in some of the denitrification profiles to that in the primary anoxic reactor in N removal systems provided the clue for a hypothesis for the occasional two-phase behaviour in this investigation: in those profiles with a two-phase behaviour not all the readily biodegradable COD was taken up in the anaerobic reactor — some passed through to the anoxic reactor and stimulated a short initial high denitrification rate. In those profiles with a single slow rate it is probable that all the RBCOD was utilized in the anaerobic reactor, hence the initial rapid rate was absent. This explanation accounts for the wide variation in the extent of denitrification (mgN/ℓ removed) by the initial rapid rate — the extent varied from zero (i.e. no initial rapid rate) to as high as 24 mgN/(ℓ influent) and depended on the concentration of RBCOD that passed through the anaerobic zone.

The measured average first denitrification rate of $K_1' = 0,610$ mgN/(mgAVSS·d) is relatively close to the first denitrification rate in N removal systems, i.e. $K_1 = 0,720$ mgN/(mgAVSS·d). This similarity between the rates supports the hypothesis that the initial rapid rate (K_1') in this investigation was due to RBCOD leakage from the anaerobic reactor. The fact that K_1' is slightly lower than K_1 is probably because the RBCOD that passes through the anaerobic reactor is not as readily biodegradable as that acidified to SCFA and used by the polyP organisms in the anaerobic reactor itself.

The measured average second slower denitrification rate K_2' (of all 29 profiles) is 0,240 mgN/(mgAVSS·d). This is two and a half times greater than the second rate

(K_2) in the N removal systems, i.e. $K_2 = 0,101$ mgN/(mgAVSS·d). It seemed that the presence of the anaerobic reactor stimulated a much more rapid second rate of denitrification in the subsequent anoxic zone of the MUCT/UCT system. Reasons for this were thought to be either additional denitrification by the polyP organisms (see Chapter 2, section 2.3.2) or modification of the normal PBCOD in the anaerobic zone to a more easily biodegradable form.

Before a detailed experimental examination of the reasons for the higher second rate of denitrification was commenced, it was felt important to first check the observed results in the plug flow reactor by a different method. This was principally because several technical difficulties were encountered with the plug flow reactor operation and the measurement of the denitrification profiles in it. These problems are discussed in Appendix B. It is possible that these difficulties resulted in disturbances in the plug flow reactor that caused it to deviate, sometimes considerably, from ideal plug flow behaviour. To obviate the technical difficulties of the plug flow reactor, satellite anoxic batch experiments, which simulate ideal plug flow conditions, were conducted on sludge drawn from the MUCT/UCT system. These batch test experiments are discussed next.

4.2 Anoxic batch tests using sludge from the laboratory activated sludge system

4.2.1 Operation and day-to-day performance of the laboratory activated sludge system during the period the anoxic batch tests were performed

(i) The UCT mode of operation including the plug flow reactor (day 199 to 311)

The first anoxic batch test was performed on day 303 while the system was still operating in the UCT mode including the plug flow reactor. This was the only batch test performed with UCT system sludge whilst the plug flow reactor was in operation. At this time the COD, nitrogen and phosphorus removals were all good (see Figs 4.11, 4.12 and 4.15 respectively).

(ii) The UCT mode of operation with a completely mixed first anoxic reactor (day 312 to 402)

On day 312 the plug flow first anoxic plug flow reactor was replaced by a completely mixed reactor of equal volume (2,5ℓ) - see Fig 4.24; the sludge age, sewage characteristics and other reactor volumes remained the same as in Table 4.1. Since further denitrification rate determinations were to be obtained from anoxic batch
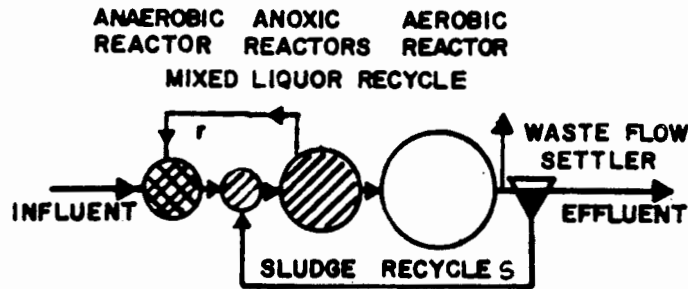
Fig 4.24: The laboratory system was changed to this UCT configuration with a completely mixed first anoxic reactor on day 312. The denitrification kinetics were studied further with the aid of anoxic batch tests on sludge taken from the parent MUCT/UCT system.
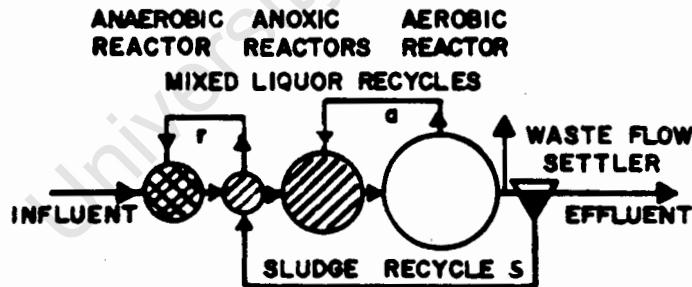
Fig 4.25: The MUCT system with a completely mixed first anoxic reactor and all its recycles set at 1:1. This configuration was in operation from day 402 until 569, during which more anoxic batch tests were performed.

tests, the plug flow reactor was of no further use.

The system was operated in the UCT mode with no a-recycle and an s-recycle of 3:1 from day 312 to day 402. During this period 3 batch tests, i.e. batch tests 2, 3 and 4 were performed. The system COD and nitrogen removals were fairly good (see Figs 4.11 and 4.12). After removing the plug flow reactor on day 312, NH_4Cl addition to the daily sewage feed was stopped and consequently the influent TKN concentration was generally low – about 85 mgN/ℓ (see Fig 4.12). Up until day 347, the P removal (Fig 4.15) was good – above 20 mgP/ℓ. However, from day 347 to 363 the P removal showed a sharp decline to about 13 mgP/ℓ. It is suspected that the sewage batch for this period (Batch 26) contained toxins that inhibited BEPR. On day 363 (the advent of sewage Batch 27), the P removal started to recover and reached about 17 mgP/ℓ within 3 days.

(iii) The MUCT mode of operation with a completely mixed first anoxic reactor
        (day 402 to 569)

Having performed batch tests on sludge from the system operating in the UCT mode, it was at this point desired to use sludge from a MUCT system to see if the mode of operation altered the denitrification capability of the sludge in any way. On day 402 the system was changed back to the MUCT mode with all recycles (a-, r- and s-) set at 1:1 (Fig 4.25). The sludge age, sewage characteristics and the volumes of the reactors remained unchanged. The system response during the period of MUCT mode operation, i.e. day 402 to 569, is shown in Figs 4.26 to 4.33. The COD and nitrogen removals remained good (see Figs 4.26 and 4.27) and the P removal improved slightly to around 19 mgP/ℓ (see Fig 4.30).

Operational changes during this last period of the research are summarized in Table 4.3. During this period of operation, as in previous periods, the measured effluent nitrate concentration from the second anoxic reactor remained above zero – about 2 to 3 mgN/ℓ (see Fig 4.28). Bulking research in the Water Research Laboratory (UCT) at this time indicated that residual nitrate in the anoxic zones of nutrient removal systems may encourage poor settleability. Since bulking was still a problem in the system in this period (Fig 4.32), it was attempted to reduce the nitrate concentration in the second anoxic reactor through reducing the a-recycle (containing nitrate from the aerobic reactor) from 1:1 to 0,5:1 on day 442. This change appeared to make little difference to the nitrate concentration in the second anoxic reactor (Fig 4.28). The DSVI actually started to increase on day 450 (Fig 4.32) from the

Table 4.3: Operational changes made to the laboratory system with the completely mixed first anoxic reactor (Day 312 to 569).

Day No.	Change	Reason
312	P.F. reactor in UCT system replaced with completely mixed reactor; NH_4Cl feed supplementation stopped	Further denitrification profiles to be measured with batch tests
402	Change from UCT configuration to MUCT; all recycles 1:1	To note whether mode of operation affected batch test results
442	Reduce a-recycle from 1:1 to 0,5:1	To reduce the nitrate concentration in the 2nd anoxic reactor in an attempt to alleviate bulking
469	Increase s-recycle from 1:1 to 2:1	To improve clarifier operation
485	Waste additional sludge to reduce the VSS from 2500 mg/l to 1900 mg/l	To test whether VSS would remain at its theoretical concentration (WRC 1984)
569	Terminate investigation	-

INFLUENT & EFFLUENT COD
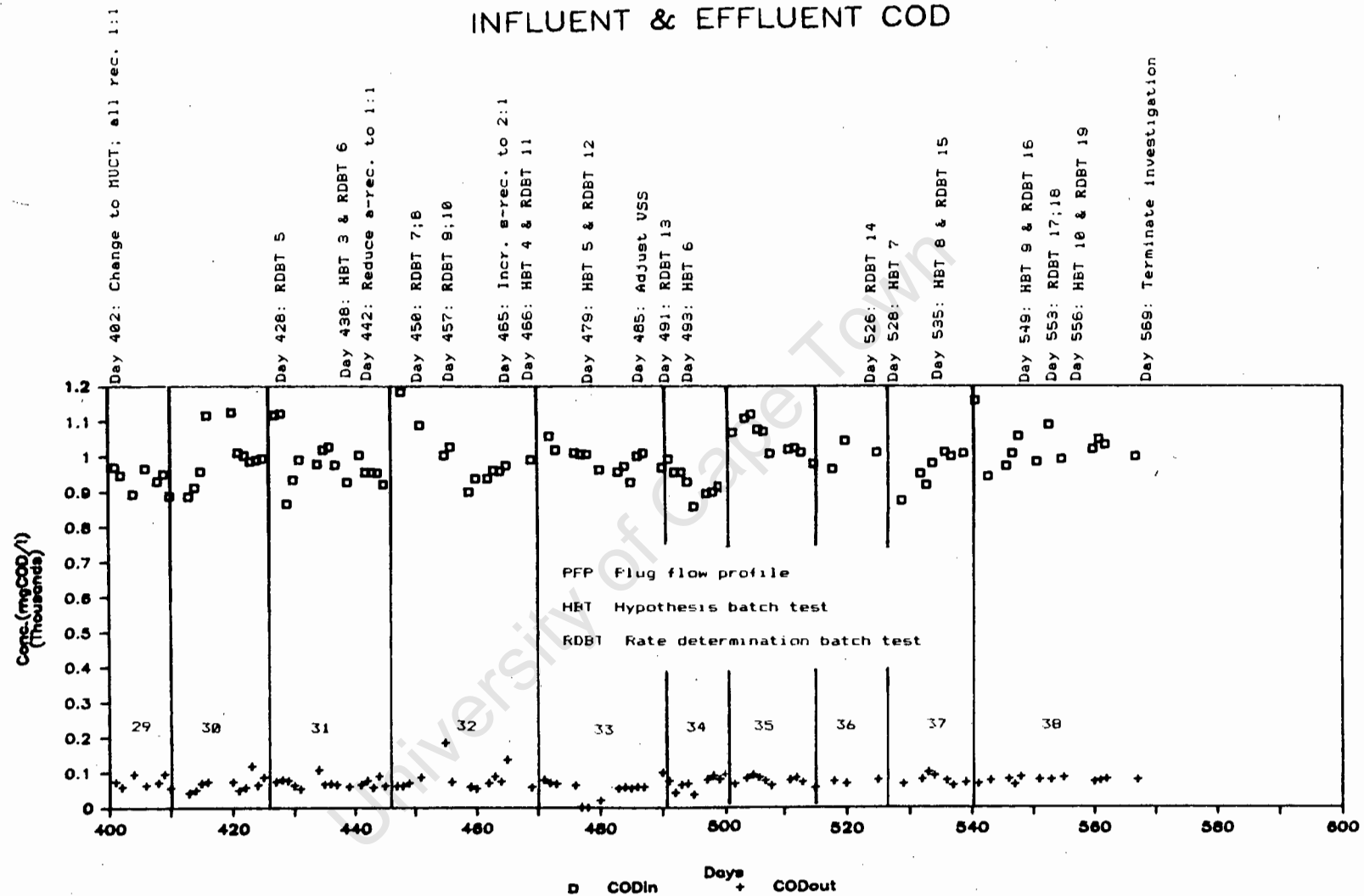
Fig 4.26: The daily COD removal performance of the MUCT system for day 400 to 569. The operational changes, new sewage batches, and days for the rate determination batch tests (section 4.2) and hypothesis batch tests (Chapter 6) are included.

NITROGEN REMOVAL

4.38
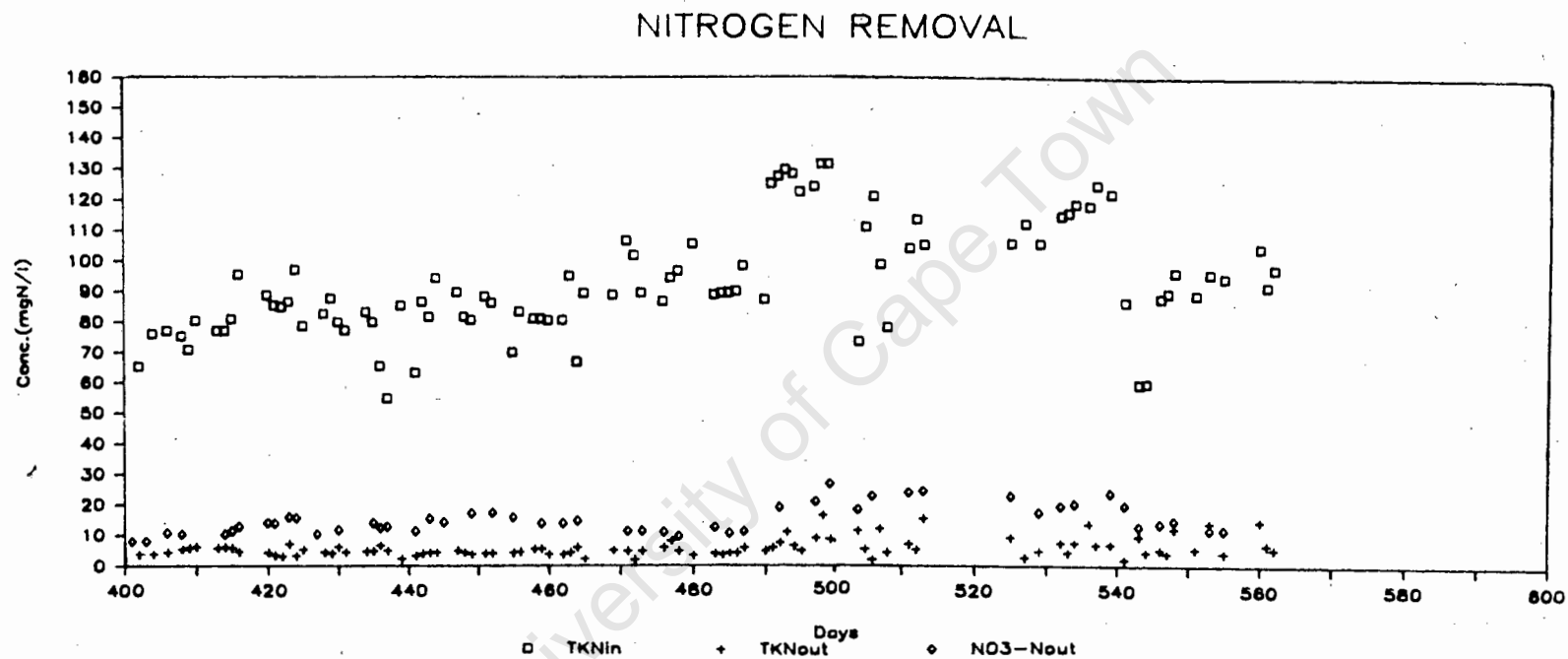
Fig 4.27:   *The daily nitrogen removal performance of the MUCT system for day 400 to 569.*

NO3-N LEVELS IN EACH REACTOR

4.39
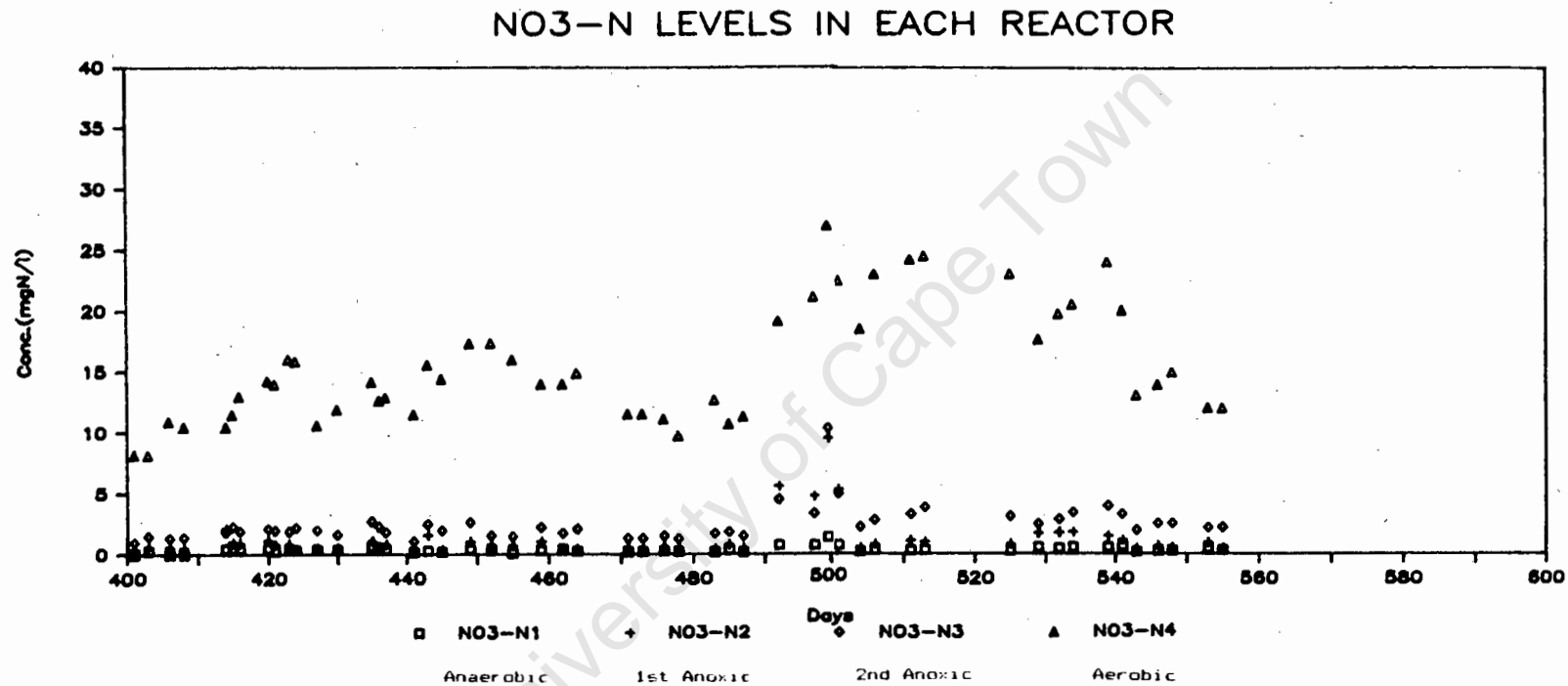
Fig 4.28: *The nitrate concentrations (as mgN/ℓ) in each reactor of the MUCT system for day 400 to 569. Note the residual nitrate in the second anoxic reactor (2 to 3 mgN/ℓ), in spite of a reduction in the a-recycle to this reactor on day 442.*

PO4-P LEVELS IN EACH REACTOR
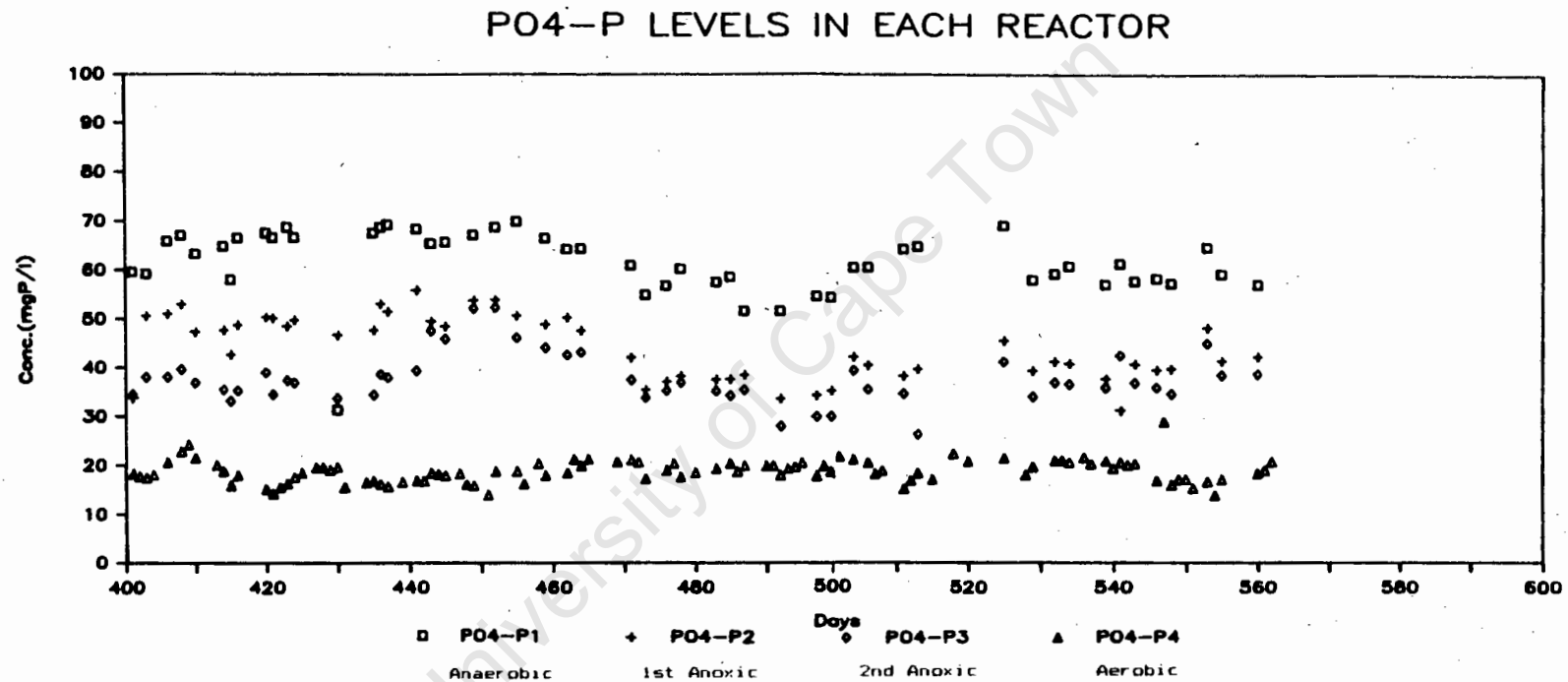
Fig 4.29: The phosphate concentrations (as mgP/ℓ) in each reactor of the MUCT system from day 400 to 569.

P REMOVAL/LITRE INFLUENT
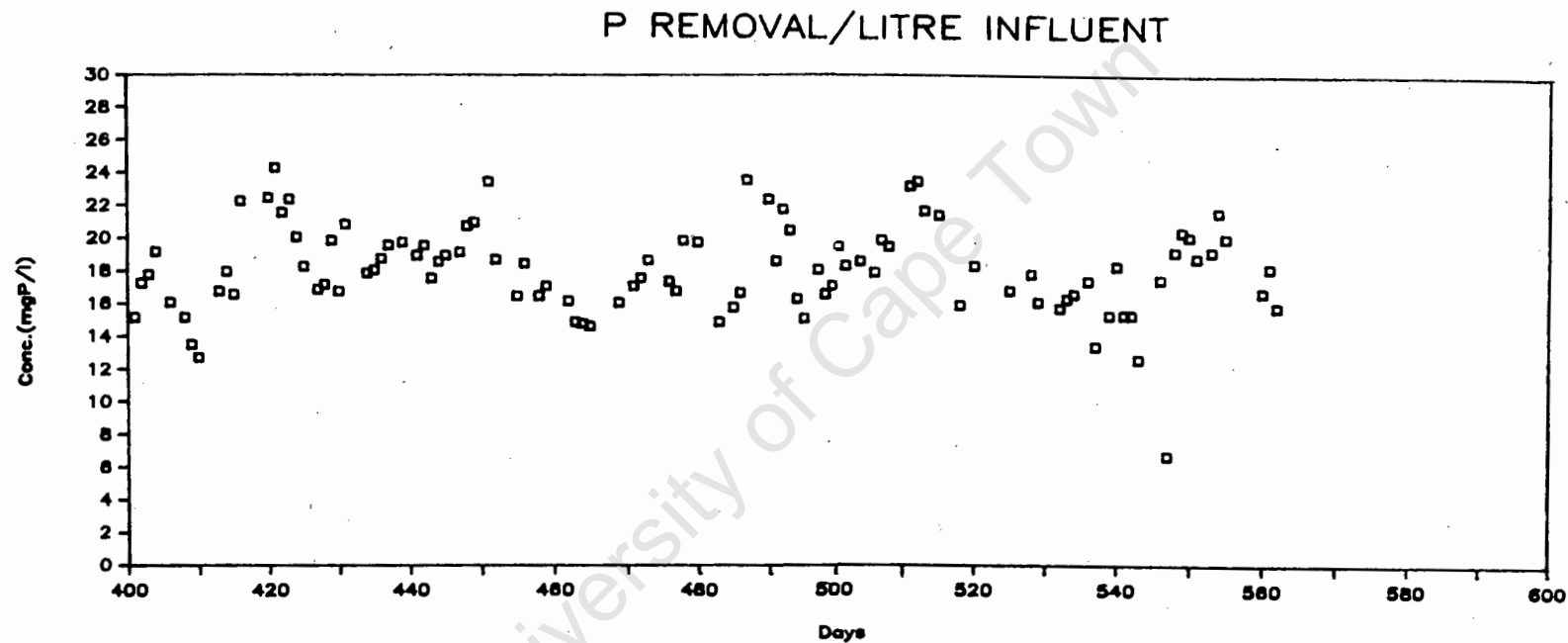
Fig 4.30: The P removal (per litre of influent) given by the MUCT system for day 400 to 569. The removal seemed to recover from the toxic batch 26 (see Fig 4.15) and reached about 19 mgP/ℓ in this period.

MLTSS & MLVSS CONCENTRATIONS
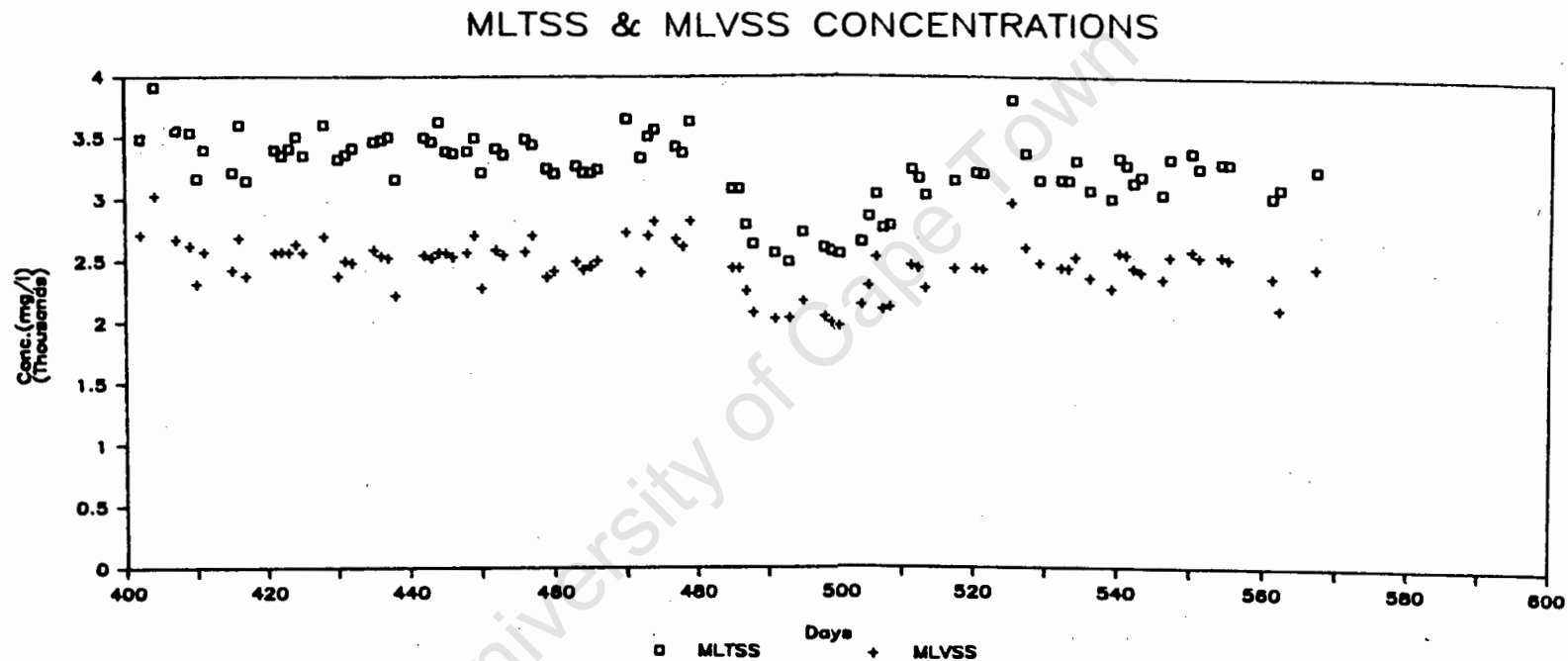
Fig 4.31: The MLTSS and MLVSS concentrations in the MUCT system for day 400 to 569. Note that on day 485 both concentrations drop sharply. The lowering of MLTSS and MLVSS was achieved by wasting an extra 4ℓ of sludge. The object was to see if the MLVSS would remain at its theoretical value for the system — 1900 mg/ℓ. This was not so: by day 525 the concentration had increased back to 2500 mg/ℓ. See text for explanation.
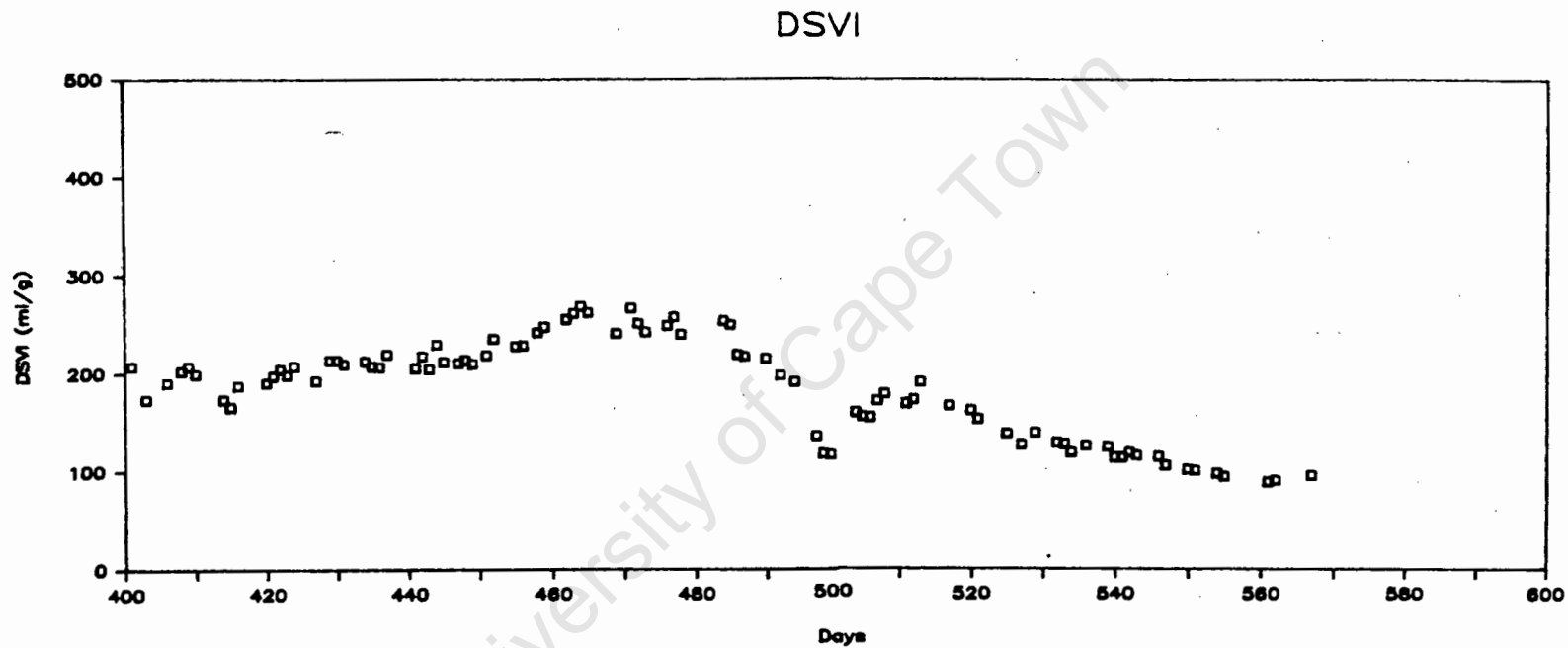
DSVI

Fig 4.32: *The DSVI of the MUCT system sludge for day 400 to 569. The DSVI remained at 200 mℓ/g until day 480, after which it showed a gradual improvement (for no apparent reason) to below 100 mℓ/g by the end of the investigation.*

OXYGEN UTILIZATION RATE

4.44
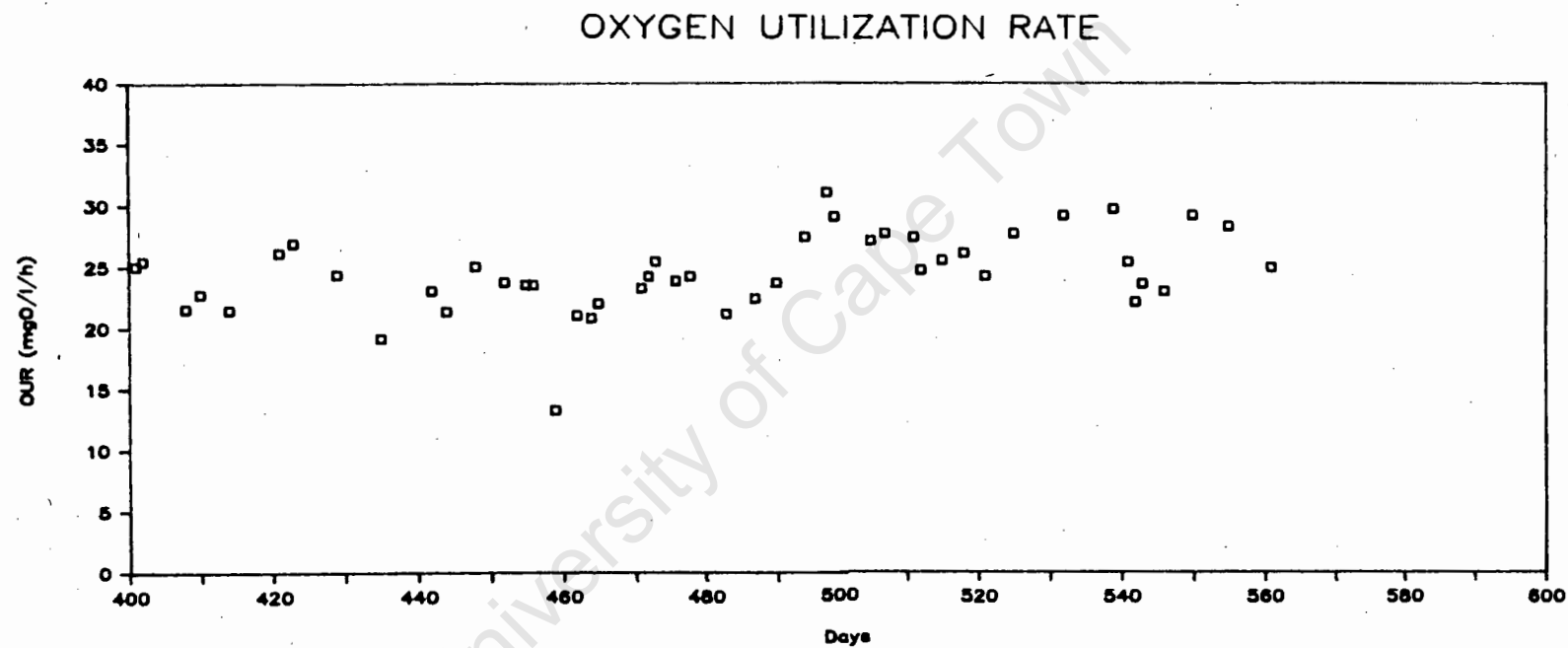
Fig 4.33: *The oxygen utilization rate of the MUCT system sludge measured in the aerobic reactor for day 400 to 569.*

level of 200 mℓ/g it had attained just prior to this period. The s-recycle was increased to 2:1 on day 465 to cope with this.

By day 569 (when the investigation was terminated) the DSVI had dropped to below 100 mℓ/g; the reasons for the initial bulking and subsequent improvement are not clear – further research regarding bulking is currently in progress. Details regarding settleability and filamentous organism identification for the MUCT/UCT system are given in Appendix J.

On day 485 a change was made to the system VSS. During the investigation the VSS had steadied out at about 2500 mgVSS/ℓ (see Fig 4.31); according to WRC (1984) the concentration of VSS in the system should be lower than this – about 1900 mgVSS/ℓ. It was decided to waste additional sludge on day 485 in order to bring the VSS concentration to the calculated value. The objective of this was to see if the VSS concentration would stay close to the calculated value or revert to the higher value it had attained prior to day 485. Based on previous experience with activated sludge systems in the Water Research Laboratory, it was accepted that this step would not affect the bacterial population in the system in terms of its kinetic and microbiological response. Consequently, it was anticipated that the results of the batch tests would not be affected – the results of batch tests and system performance such as COD, N and P removal confirm this. Subsequent to reducing the VSS concentration from 2500 mg/ℓ to 1900 mg/ℓ on day 485, the concentration increased back to its original value of about 2500 mgVSS/ℓ by day 525, i.e. in about 2 sludge ages (see Fig 4.31).

In investigating the causes of the high VSS concentration, the system operation and feeding procedure were carefully scrutinized. The most likely cause was traced to the sewage batch preparation, which resulted in a larger than usual unbiodegradable particulate COD fraction (f_{up}) in the sewage; this fraction becomes enmeshed in the sludge mass of the system and adds to the VSS concentration (for details, see Appendix E).

During this period of MUCT mode of operation, the final 15 batch tests (i.e. batch tests 5 to 19) were performed, all identified on the day-to-day record of influent and effluent COD concentrations (Fig 4.26).

The experimental investigation was terminated at the end of this period, on day 569.

4.2.2 Observed denitrification behaviour in anoxic batch tests

Altogether, 19 anoxic batch tests for denitrification rate determinations were performed on the system sludge, the first on day 303 and the last on day 556.. The day on which each was conducted is indicated on Figs 4.11 and 4.26.

In 5 batch tests, (i.e. batch tests 1, 2, 3, 17 and 18), sludge from the anaerobic and aerobic reactors was blended in proportion to typical recycle flows at the entrance to the first anoxic reactor in the MUCT/UCT system. Different proportions to simulate different s-recycle flows were tried (the higher the s-recycle, the greater the aerobic to anaerobic reactor sludge mass ratio). In 8 tests (i.e. batch tests 4, 6, 9, 10, 11, 12, 13 and 14) sludge from only the anaerobic reactor and in 6 tests (i.e. batch tests 5, 7, 8, 15, 16 and 19) sludge from only the aerobic reactor was tested. The reason for testing unblended anaerobic and aerobic reactor sludges was to check if the denitrification rates differed significantly from those in blends of anaerobic and aerobic sludges.

All 19 batch tests were conducted under anoxic conditions in a closed vessel and were gently mixed with a magnetic stirrer. A nitrogen atmosphere was maintained above the mixed liquor to avoid oxygen dissolution into the liquid phase. At regular intervals, samples were withdrawn and immediately filtered through Whatman's No.1 paper. Nitrate, nitrite, phosphate and soluble COD ($<0,45\mu$m) concentrations were determined on the filtrate. The general batch test apparatus and procedure are discussed more fully in Appendix C. Typical results obtained for the pure anaerobic, blended and pure aerobic sludges are shown in Figs 4.34, 4.35 and 4.36 respectively. All the batch test results are listed in Appendix F.

Irrespective of blending or not, all the batch tests showed similar denitrification behaviour. As shown in Figs 4.34 to 4.36, typical batch denitrification behaviour comprised (i) only a single phase of nitrate reduction, (ii) very little P uptake and (iii) constant soluble COD ($<0,45\mu$m) concentration. This behaviour conforms to that observed in the plug flow reactor profiles except for the following main aspect: an initial rapid rate was *not* observed in *any* of the batch tests whereas in about two-thirds of the plug flow reactor profiles an initial rapid rate did occur.

The denitrification rates were calculated from the nitrate concentration versus time profiles observed in all 19 batch tests. The denitrification rates were reduced to specific rates by dividing by the active VSS concentration in the batch test (i.e. 0,24

ANAEROBIC REACTOR SLUDGE ANOXIC
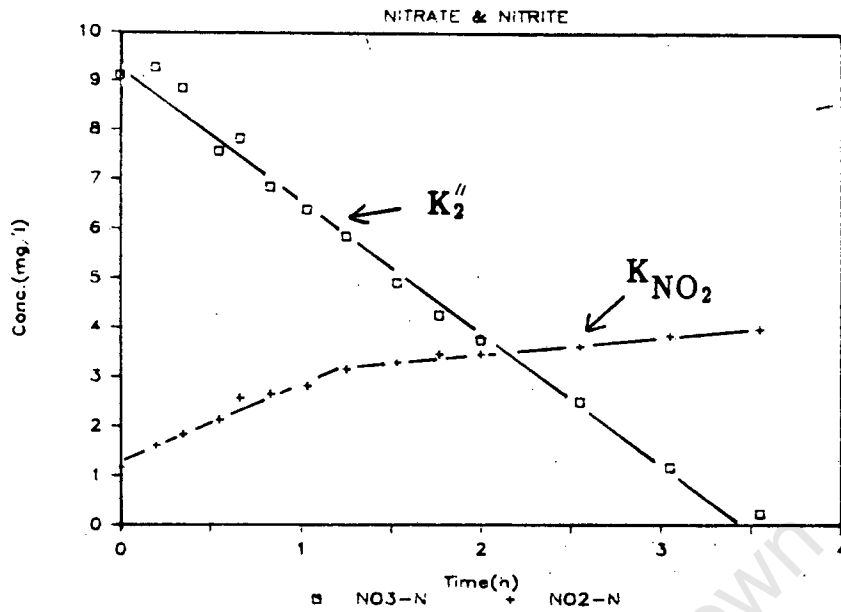
NITRATE & NITRITE

Fig 4.34(a): Typical nitrate and nitrite concentration versus time profiles for the anoxic batch tests on sludge from the anaerobic reactor of the MUCT/UCT unit. A single rate of nitrate reduction is evident.

PHOSPHATE & SOLUBLE COD
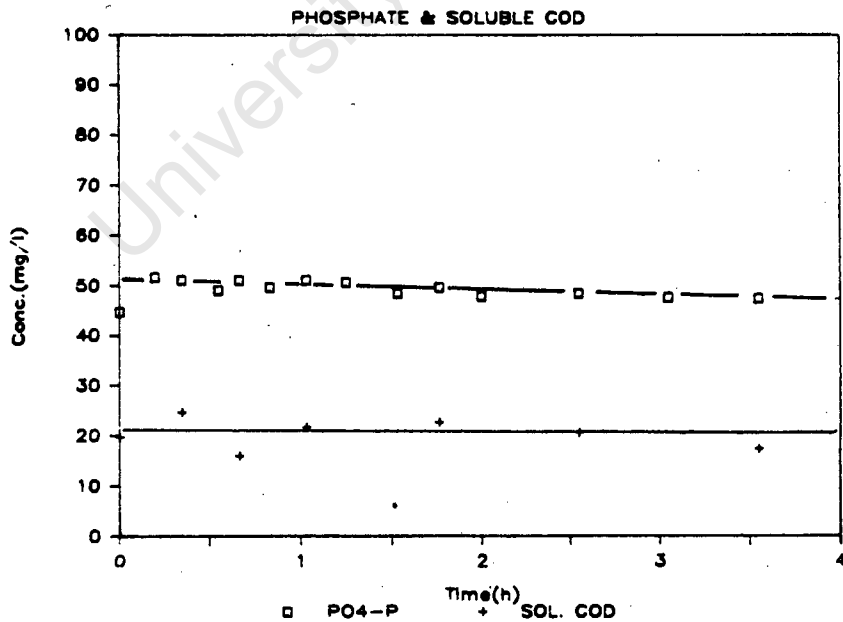
Fig 4.34(b): Typical phosphate and soluble COD ($< 0,45\mu m$) concentration versus time profiles for the anoxic batch tests on sludge from the anaerobic reactor of the MUCT/UCT unit. Note both concentrations remain essentially constant throughout the test.

SLUDGE BLEND HELD ANOXIC
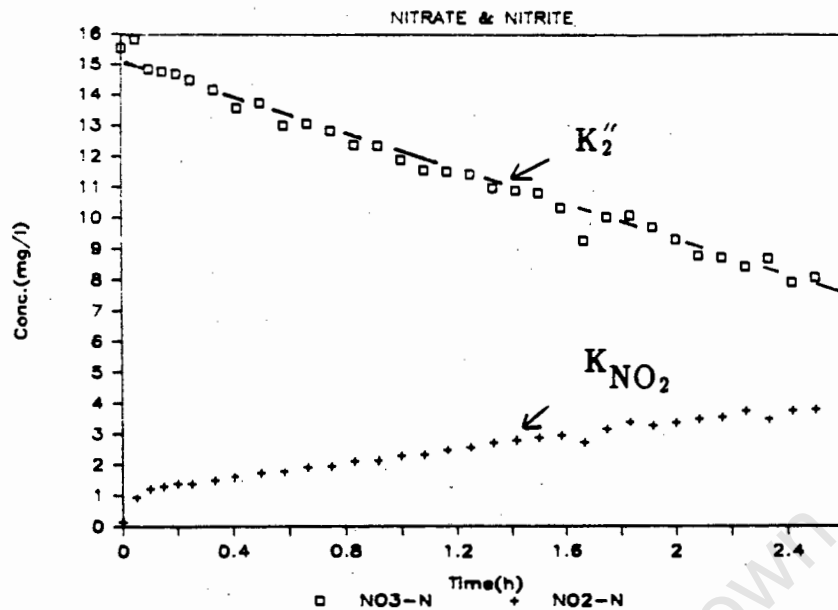
Fig 4.35(a): *Typical nitrate and nitrite concentration versus time profiles for the anoxic batch tests on blended sludge from the anaerobic and aerobic reactors of the MUCT/UCT unit. There is only one rate of denitrification. (The ratio of anaerobic to aerobic reactor sludge mass is 1:3 for this particular example).*
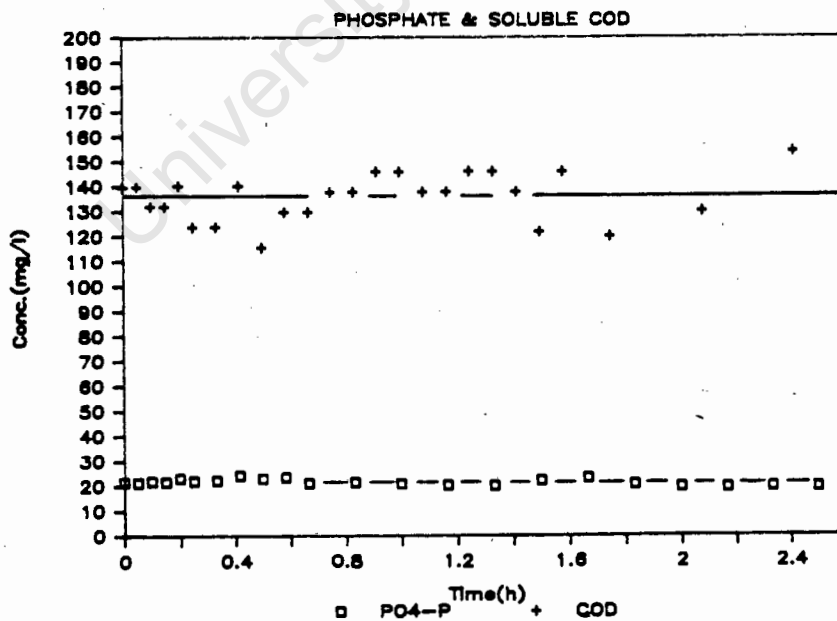
Fig 4.35(b): *Typical phosphate and soluble COD (< 0,45μm) concentration versus time profiles for the anoxic batch tests on blended sludge from the anaerobic and aerobic reactors of the MUCT/UCT unit. Both concentrations remained approximately constant over the test period.*

AEROBIC REACTOR SLUDGE ANOXIC
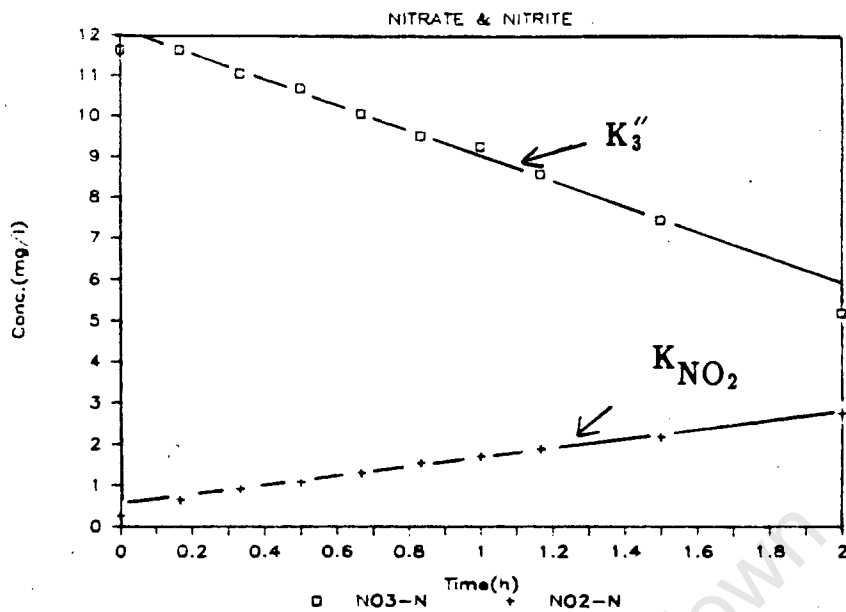
Fig 4.36(a): Typical nitrate and nitrite concentration versus time profiles for the anoxic batch tests on sludge from the aerobic reactor of the MUCT/UCT unit. As for the other batch tests, there is only a single rate of denitrification.
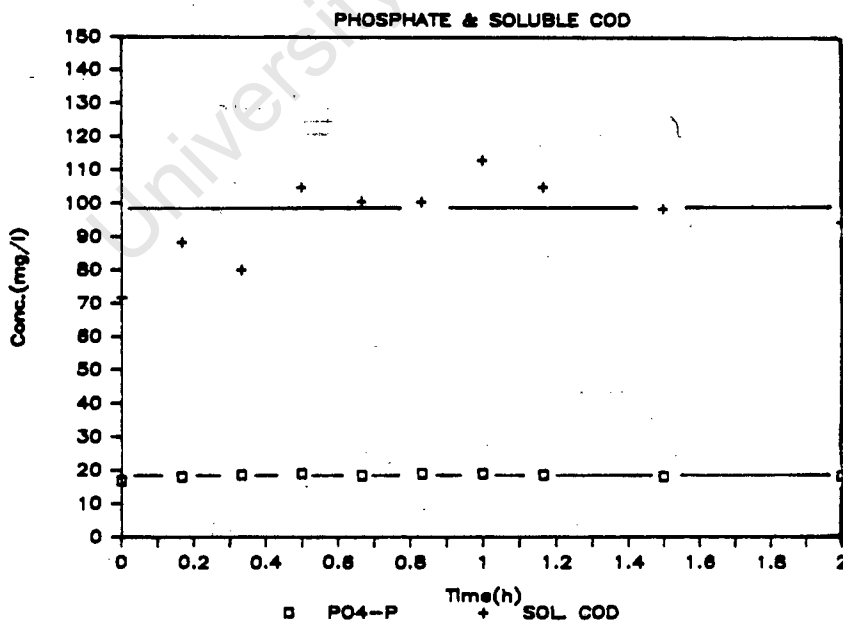
Fig 4.36(b): Typical phosphate and soluble COD (< 0,45μm) concentration versus time profiles for the anoxic batch tests on sludge from the aerobic reactor of the MUCT/UCT unit. Both parameters show little change throughout the test.

Table 4.4: Denitrification rates of anoxic batch tests on anaerobic reactor sludge only, blends of anaerobic and aerobic reactor sludge, and aerobic reactor sludge only.

Set No.	Ratio of anaerobic to aerobic reactor sludge mass	Day No.	Denitrification rate (corrected for K_{NO_2}) [mgN/(mgAVSS·d)]
1	1:0	373	0,194
	1:0	438	0,192
	1:0	457	0,176
	1:0	457	0,234
	1:0	466	0,254
	1:0	479	0,184
	1:0	491	0,163
	1:0	526	0,149
2	1:2*	553	0,173
	1:3	553	0,139
	1:3	303	0,196
	1:4	344	0,188
	1:4	359	0,157
3	0:1	428	0,100
	0:1	450	0,095
	0:1	450	0,110
	0:1	535	0,080
	0:1	549	0,134
	0:1	556	0,079

* These particular ratios were chosen to correspond to different s-recycle ratios in the system, i.e. 1:2 corresponds to an s-recycle ratio of 1:1, 1:3 corresponds to an s-recycle of 2:1 and 1:4 corresponds to an s-recycle of 3:1.

of the measured VSS concentration) and were also corrected for nitrite formation in the same way as the rates obtained from the plug flow reactor tests (see section 4.1.3). The results are listed in Table 4.4 and are set out in three groups, viz. those conducted with (1) anaerobic reactor sludge only, (2) blends of anaerobic and aerobic reactor sludges and (3) aerobic reactor sludge only. Each set is plotted on probability paper (Figures 4.37, 4.38 and 4.39). The means are 0,195 mgN/(mgAVSS·d), 0,170 mgN/(mgAVSS·d) and 0,100 mgN/(mgAVSS·d) for sets 1, 2 and 3 respectively. From these means it appears that sets 1 and 2, both of which included anaerobic reactor sludge, have approximately equal means; set 3, where only aerobic reactor sludge was tested, has a considerably lower mean. The mode of operation (i.e. UCT or MUCT) did not appear to influence the grouping.

The means of sets 1 and 2 (tests wherein anaerobic reactor sludge was present) were compared statistically with a t-test (Walpole and Myers, 1978). It was found that the means of these two sets were not different at a significance level of 0,05 (a 95% confidence interval). A t-test comparison between set 3 (aerobic reactor sludge) and sets 1 and 2 confirmed the initial impression that set 3 is different from the other two; the mean of this set of batch tests *is* different at a significance level of 0,05 to the means of both sets 1 and 2 (wherein anaerobic reactor sludge was present).

In terms of the general activated sludge model it is possible to explain (a) the difference between the means of batch tests with anaerobic reactor sludge (sets 1 and 2) and the means of the batch tests with aerobic reactor sludge only (set 3) and (b) the similarity between the means of the sets incorporating anaerobic sludge:

(a)   Sludge originating from the anaerobic reactor has contained in it stored PHB, particulate biodegradable COD (PBCOD) as well as products of cell death and lysis. In contrast, sludge originating from the aerobic reactor has contained in it only products of cell death and lysis. Under anoxic conditions, the anaerobic sludge therefore yields a higher denitrification rate because of the higher substrate content compared to the aerobic sludge; this is comparable to denitrification in N removal plants without anaerobic reactors (e.g. 4-stage Bardenpho configuration) where the second rate of denitrification in the primary anoxic reactor (K_2) is higher than the single rate in the secondary anoxic reactor (K_3) — see Chapter 2.

(b)   Considering the blended sludge batch tests (set 2), these also contain stored
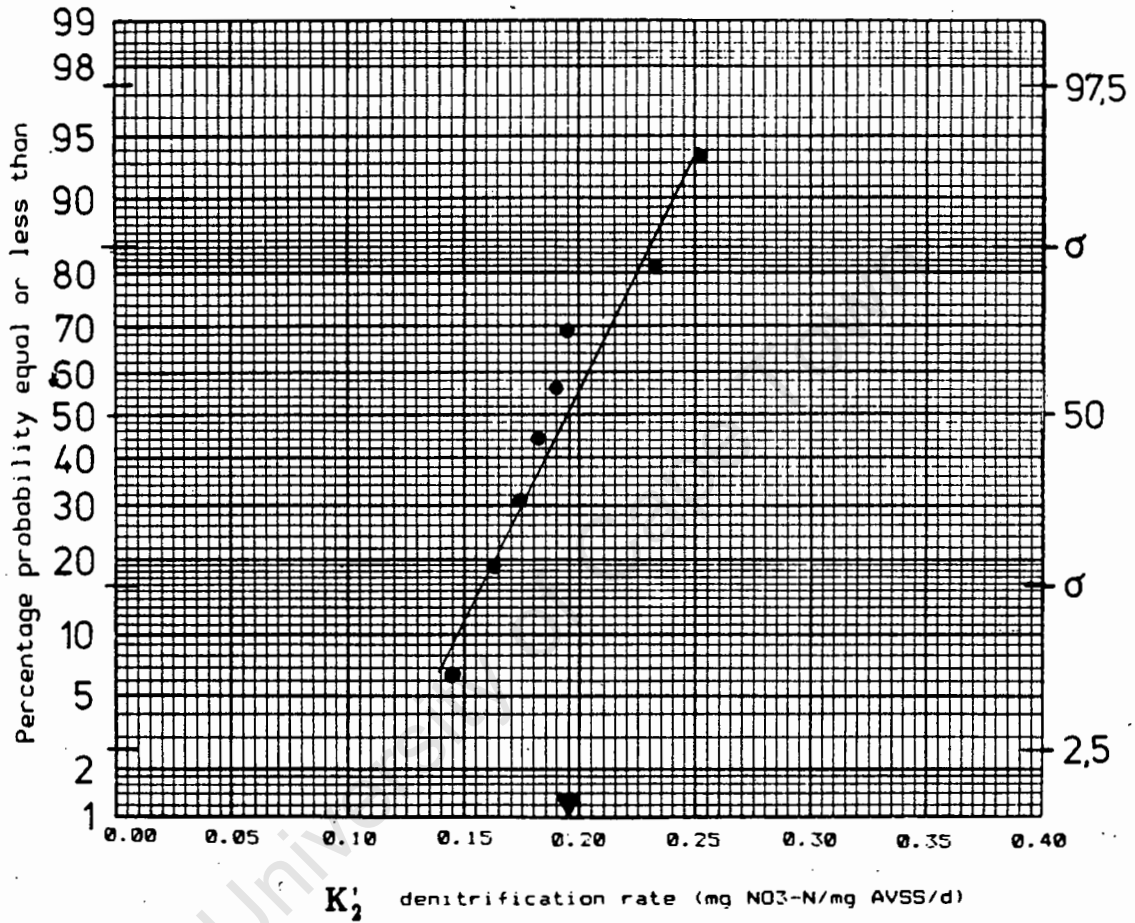
Fig 4.37: *Probability distribution of the K_2 specific denitrification rates obtained in the anoxic batch tests with anaerobic reactor sludge. The mean rate is 0,195 mgN/(mgAVSS.d).*
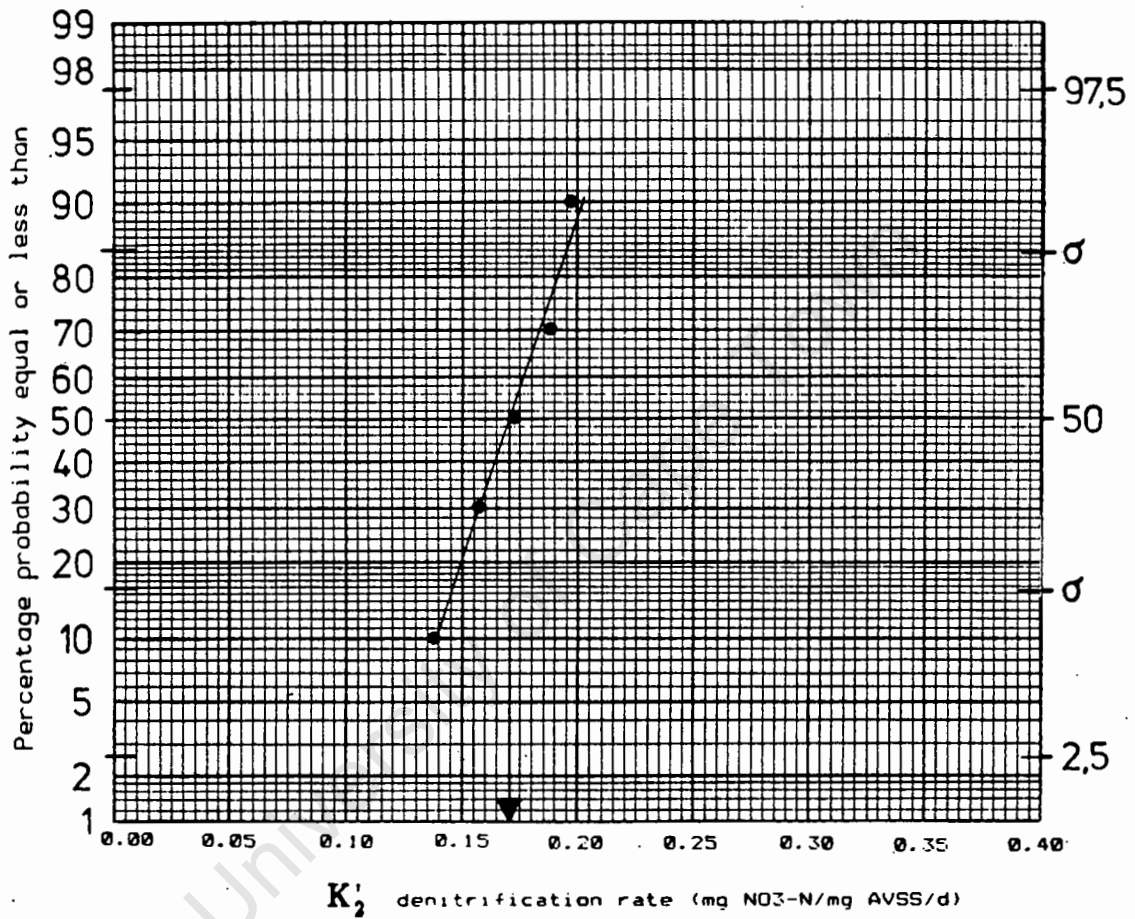
<u>**Fig 4.38**</u>:   *Probability distribution of the K_2' specific denitrification rates obtained in the anoxic batch tests with blends of anaerobic and aerobic reactor sludge. The mean rate is 0,170 mgN/(mgAVSS.d).*
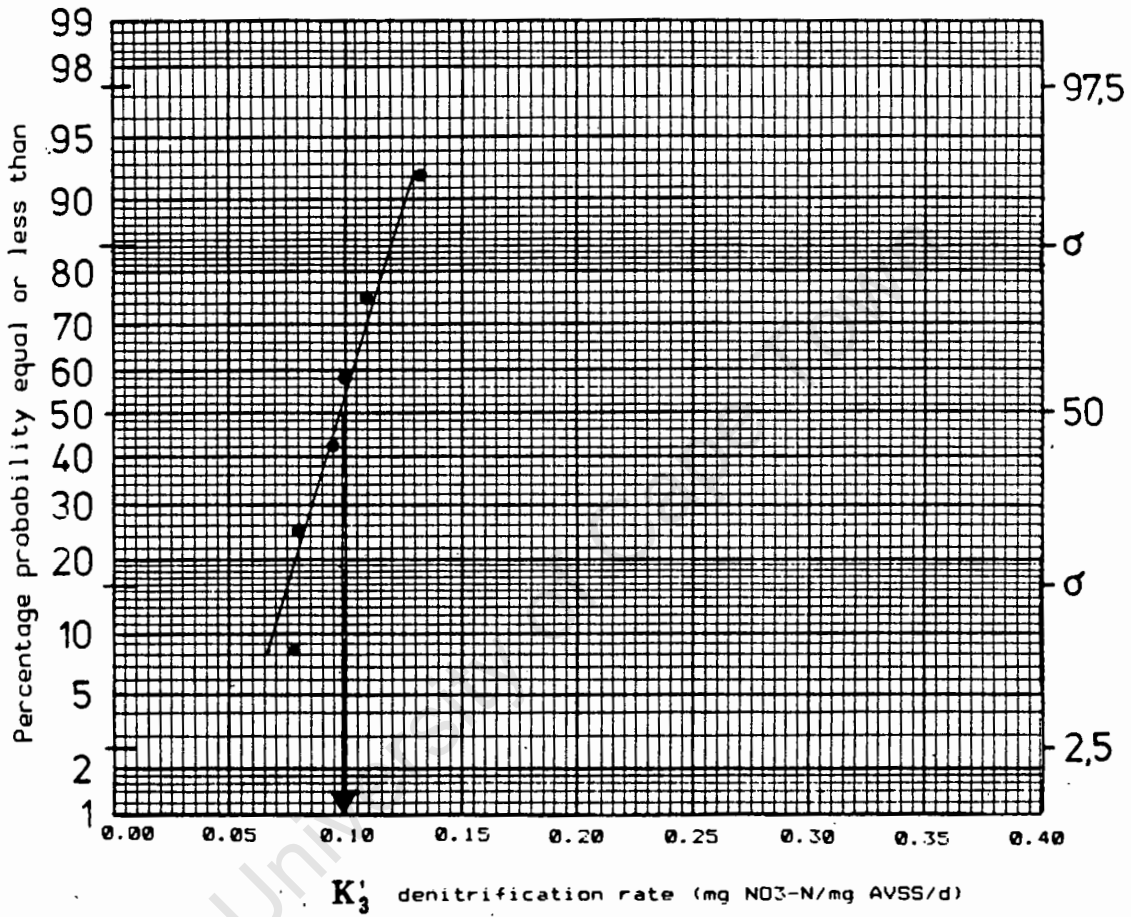
Fig 4.39: *Probability distribution of the K_3' specific denitrification rates obtained in the anoxic batch tests with aerobic reactor sludge. The mean rate is 0,100 mgN/(mgAVSS.d).*

PHB, PBCOD and products of cell death and lysis, because of the presence of anaerobic sludge in the tests. However, the mass of this substrate per mass of VSS is lower than for pure anaerobic reactor sludge because it has been 'diluted' by the aerobic reactor sludge (which only contains products of cell death and lysis). Van Haandel *et al.* (1981), found that the K_2 denitrification rate in N removal systems is virtually independent of the PBCOD/VSS mass ratio, provided the ratio is above a certain minimum. This is because the rate is governed by a surface active site type reaction which operates as a saturation mechanism. Because the anaerobic to aerobic sludge mass ratio is directly related to the PBCOD/VSS ratio, it follows that the denitrification rate of blended anaerobic and aerobic reactor sludge should be independent of the actual ratio of sludges. This was observed in this investigation -- the blended sludge batch tests all had similar denitrification rates close to those of pure anaerobic reactor sludge.

In light of the above, the denitrification rates obtained from blends of anaerobic and aerobic reactor sludges (set 2) were grouped together with the rates arising from purely anaerobic reactor sludge (set 1). Those obtained from purely aerobic reactor sludge (set 3) were kept separate.

The denitrification rates of the tests including anaerobic reactor sludge (sets 1 and 2) appear to be normally distributed (Fig. 4.40); the mean is $\bar{K}_2^{\prime} = 0,185 \pm 0,015$ mgNO$_3$-N/(mgAVSS·d). The denitrification rate arising from these two sets is denoted '$K_2^{\prime}$' because of its similarity to the $K_2^{\prime}$ rate measured in the plug flow reactor, where anaerobic reactor sludge was also present. A comparison between these two rates is given in section 4.2.3. The aerobic reactor sludge denitrification rates (set 3) are plotted out in Fig 4.39 and also appear to be normally distributed; this set has a mean of $\bar{K}_3^{\prime} = 0,100 \pm 0,021$ mgNO$_3$-N/(mgAVSS·d). The rate from this set is denoted '$K_3^{\prime}$' because of its equivalence to the K_3 rate in secondary anoxic reactors in N removal plants -- in these reactors the sludge also originates from the aerobic reactor. A comparison between $K_3^{\prime}$ and K_3 is discussed further in section 4.3. The above means are quoted at the 95% confidence level.

4.2.3 Comparison of plug flow and batch test denitrification rates
It was concluded above that the denitrification behaviour observed in the plug flow reactor and batch tests are similar except that in the plug flow reactor, an initial rapid rate was observed in about two-thirds of the profiles whereas in the batch tests

no initial rapid rate was observed. In this section, the slow rate of denitrification (K_2') observed in the plug flow reactor is compared quantitatively with the K_2' rate observed in the batch tests (the rate arising from sets 1 and 2).

The average K_2' rate from the plug flow reactor tests was calculated (in section 4.1.3) to be 0,240 ± 0,053 mgNO$_3$-N/(mgAVSS·d) and that for the anoxic batch tests was 0,185 ± 0,015 mgNO$_3$-N/(mgAVSS·d). A statistical F-test on the two data sets indicated that their standard deviations (σ) are not the same. A specific t-test formula was therefore required (Walpole and Myers, 1978) to compare the means. Applying the specific t-test formula showed that the mean rates are not different at the 0,05 level of significance (95% confidence limits). Hence, it could be assumed that the data sets originate from the same population of denitrification rates. This indicates that the technical difficulties encountered with the plug flow reactor did not significantly influence the results and accordingly, both the plug flow and batch test data sets (excluding the batch tests on purely aerobic reactor sludge) could be combined. A probability plot of the combined data sets appears in Fig 4.41: the mean denitrification rate $\bar{K}_2'$ is 0,224 ± 0,036 mgNO$_3$-N/(mgAVSS·d) at the 95% confidence interval.

From the statistical analysis it is clear that there is a good correlation between the $\bar{K}_2'$ denitrification rates obtained from the plug flow and batch test methods. However, the plug flow reactor method is less precise in that it has a greater sample standard deviation of the rate than the batch test method: the sample standard deviation of the rate with the plug flow method is 0,126 mgN/(mgAVSS·d) compared to 0,025 mgN/(mgAVSS·d) for the batch tests. This is probably because of the susceptibility of the plug flow method to error due to the operational difficulties such as sampling, hydraulic disturbances, wall growths, etc. (see Appendix B). Because of this, it is recommended that future studies into denitrification kinetics should be performed using batch tests.

The plug flow and batch test results were similar not only in the K_2' denitrification rate, but also in other aspects. The batch tests, like the plug flow reactor profiles, showed (1) very little phosphorus uptake and (2) virtually no change in soluble (<0,45μm) COD concentrations (see Figs 4.34 to 4.36). The only significant difference is in the occasional occurrence of an initial fast denitrification rate observed in the plug flow reactor, which was not observed in the batch tests. The occurrence of the initial fast rate in the plug flow reactor was interpreted as a
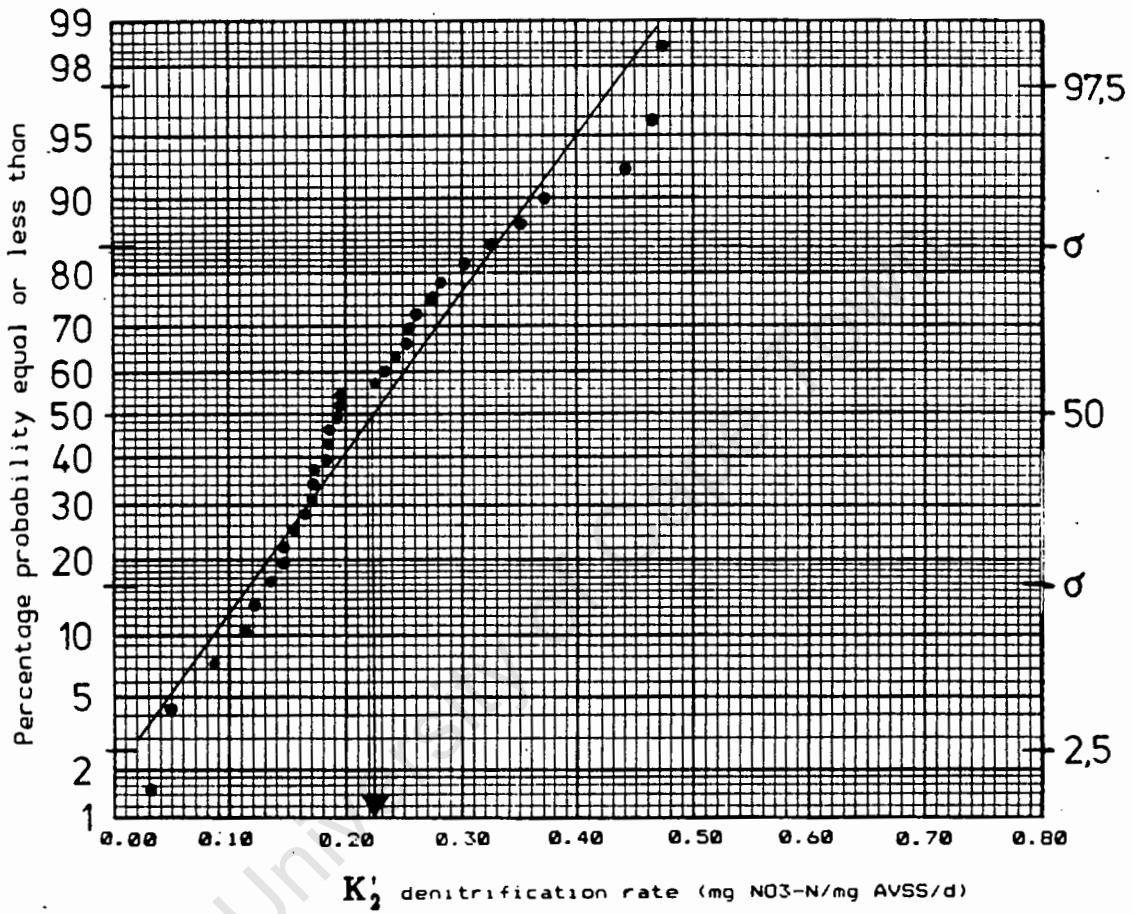
Fig 4.41: Probability distribution of the combined set of K_2' specific denitrification rates obtained in the plug flow reactor and anoxic batch tests. The mean rate is 0,224 mgN/(mgAVSS.d).

leakage of readily biodegradable COD through the anaerobic reactor. No explantion can be advanced why an initial fast rate was observed occasionally in the plug flow reactor but not in the batch tests.

In conclusion, the primary anoxic denitrification behaviour in a MUCT/UCT system was established via two methods that agreed closely, indicating that the results are acceptable for formulating the denitrification behaviour in nutrient removal systems.

4.3 Formulation of denitrification kinetics for N and P removal systems including a comparison with the kinetics for N removal systems

In describing primary denitrification (that in the primary anoxic reactor) in the MUCT/UCT system, the initial rapid rate of nitrate reduction was ignored: this rate occurred infrequently, was of a widely varying duration and took place only in the plug flow reactor. Therefore, from a design point of view, it cannot be relied upon. Consequently, it will be accepted that the denitrification kinetics in the anoxic zone following the anaerobic reactor are described by a single zero order rate of nitrate reduction: $K_2' = 0,224$ mgN/(mgAVSS·d). Secondary denitrification (denitrification *after* the aerobic reactor) also occurs at a single zero order rate: K_3' = 0,100 mgN/(mgAVSS·d). Although secondary denitrification normally does not occur in a system such as the MUCT/UCT configuration (the system does not include a secondary anoxic reactor), the K_3' rate measured in this investigation (on sludge from the aerobic reactor of the MUCT/UCT unit) will be useful for N and P removal systems such as the 5-stage Bardenpho configuration (Fig 2.8a) that do include secondary anoxic reactors. Figure 4.42 and Table 4.5 summarize and compare the denitrification kinetics of the MUCT/UCT nutrient removal system with the denitrification kinetics observed in plants removing N only.

It is probable that the kinetics established for the MUCT/UCT system also apply in other nutrient removal systems. Previous observations have shown that the inclusion of an anaerobic reactor in various laboratory and full scale N removal plants does not affect the denitrification performance. The denitrification kinetics established for the MUCT/UCT system in this investigation can explain this: in the primary anoxic zone, the absence of the fast initial rate (the absence results from the anaerobic reactor removing most of the influent RBCOD) is compensated for approximately by the high "second" rate (relative to K_2 in N removal systems). The net N removal is therefore the same.

In comparing the denitrification kinetics of the nutrient removal MUCT/UCT
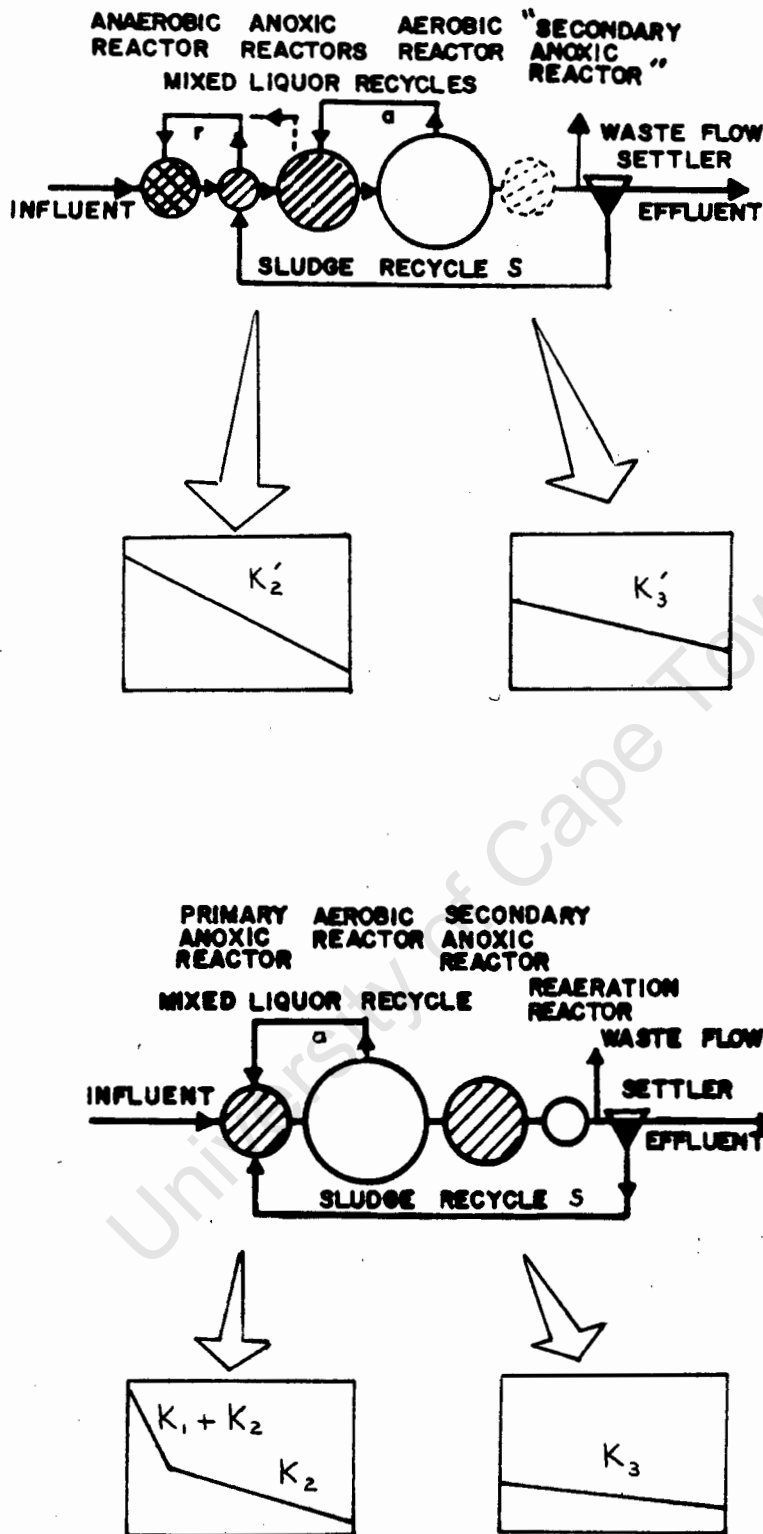
Fig 4.12: Comparison between the denitrification kinetics measured in the nutrient removal MUCT/UCT system (top) and the kinetics in N removal systems (bottom).

Table 4.5:   Comparison between the denitrification kinetics in systems with and without anaerobic reactors.

MUCT/UCT system	System without anaerobic reactor
Primary denitrification via a single rate, K_2'	Primary denitrification via two rates, K_1 and K_2
$K_2' = 0{,}224$ mgN/(mgAVSS·d)	$K_1 = 0{,}720$ mgN/(mgAVSS·d) $K_2 = 0{,}101$ mgN/(mgAVSS·d)
*Secondary denitrification via $K_3' = 0{,}100$ mgN/(mgAVSS·d)	Secondary denitrification via $K_3 = 0{,}072$ mgN/(mgAVSS·d)

*   Normally there is no secondary denitrification in the MUCT system. This K_3' rate was measured using sludge from the aerobic reactor of the MUCT system - it may be applicable to other P removal systems with secondary anoxic reactors.

system with those in N removal systems, it is noted that primary denitrification in the MUCT/UCT configuration occurs via a single rate, denoted K_2', whereas in systems without an anaerobic reactor the denitrification is described by a two-phase behaviour: an initial fast rate, $(K_1 + K_2)$, followed by a slower rate, K_2. The reason for the absence of the initial fast rate when an anaerobic reactor precedes the anoxic zone is probably because in the anaerobic zone the polyP organisms sequester practically all the influent RBCOD (see Chapter 2) leaving none for denitrification in the subsequent primary anoxic zone. This explanation led to speculation about the cause of the single denitrification rate (K_2') in the anoxic zone of the MUCT/UCT system. If virtually all the influent RBCOD is removed in the anaerobic reactor, the single denitrification rate in the subsequent anoxic reactor should be due to the remaining influent PBCOD. If this is so, then one would expect a rate of denitrification of similar magnitude to that attributable to utilization of PBCOD in N removal systems, i.e. $K_2 = 0,101$ mgN/(mgAVSS·d). However, the observed rate of denitrification is $0,224$ mgN/(mgAVSS·d), over twice the K_2 rate in N removal systems.

Two possible hypotheses for the faster rate of denitrification after an anaerobic reactor were proposed earlier, i.e. (1) additional denitrification by polyP organisms and (2) possible modification of the influent PBCOD to a more easily biodegradable form in the anaerobic reactor. These hypotheses are presented in detail in Chapter 5 and experiments to test them are described in Chapter 6.

Accepting the observed K_2' rate, the denitrification potential of the primary anoxic reactor in a nutrient removal system is similar to that in N removal systems (Eq 2.8) except there is no RBCOD denitrification, so equivalently $f_{bs} = 0$ and K_2' replaces K_2, i.e.

$$D_{p1} = S_{bi} \left(\frac{Y_h R_s}{1 + b_{hT} R_s} \right) f_{x1} K_2' \quad (\text{mgN}/\ell \text{ influent}) \qquad (4.6)$$

With regard to the secondary denitrification rate obtained from the MUCT/UCT system sludge, K_3', it seems that this rate is significantly faster than that in systems excluding an anaerobic reactor, i.e. $K_3' = 0,100$ mgN/(mgAVSS·d) compared to $K_3 = 0,072$ mgN/(mgAVSS·d) respectively; in fact, at a confidence limit of 95%, K_3' and K_3 are statistically different. The reason for the difference between K_3' and K_3 is not clear: both denitrification rates should depend only on products of cell death

and lysis and should therefore be of similar magnitude. Fortunately, for design of N and P removal systems that include secondary anoxic reactors, the accuracy of K_3' is not as important as K_2': the major part of the denitrification is achieved via the K_2' rate in the primary anoxic reactor. In these systems either K_3' or K_3 may be used to size the secondary anoxic rector.

Accepting the observed K_3' rate, the denitrification potential of the secondary anoxic reactor in a nutrient removal system is similar to that in N removal systems (Eq 2.9) except that K_3' replaces K_3, i.e.

$$D_{p3} = S_{bi} \left(\frac{Y_h R_s}{1 + b_{hT} R_s} \right) f_{x3} K_3' \quad (mgN/\ell \text{ influent}) \tag{4.7}$$

4.4 Testing the denitrification kinetics established for the MUCT/UCT system

At this stage it was decided to check the established denitrification kinetics in the primary anoxic reactor of the MUCT/UCT system (the 'new' kinetics) by using them to predict the system N removal. Accordingly, six steady state periods in the laboratory MUCT/UCT system performance were identified and the average system effluent nitrate concentrations were compared with predictions based on the average denitrification rate (K_2') observed in the plug flow and batch tests. For comparison, predictions of the system effluent nitrate concentrations were also made with the original K_1 and K_2 denitrification rates (the 'old' kinetics) developed for systems without anaerobic reactors. Another important parameter, phosphorus uptake/release in the anoxic zone of the system, was also examined and compared to the behaviour observed in the plug flow and batch tests. The steady state periods with their average experimental data and predictions appear in Table 4.6. Since the denitrification kinetics (old and new) are zero order with respect to nitrate concentration, the mixing regime does not change the denitrification potential, both the plug flow and completely mixed anoxic modes of operation were therefore considered. Steady state periods 1 and 2 are representative of the MUCT/UCT system with the plug flow first anoxic reactor and periods 3 to 6 are representative of the system with a completely mixed first anoxic reactor.

(i) Nitrogen and COD balances

Before the predictive power of the new denitrification kinetics was tested, the accuracy of the system response data was checked by performing nitrogen and COD balances for each steady state period (see Appendix H). Nitrogen balances of only

Table 4.6: Experimental and predicted denitrification behaviour of the MUCT/UCT system during six steady state periods.

Period	Configuration and 1st anoxic mixing regime[1]		TKN/COD[2] ratio	Experimentally observed means (mgN/l)				Theoretical S_{NOe} (mgN/l)		Prediction[3] error (%)		Anoxic ΔP[4] (mgP/l inf.)	N balance (%)	COD balance (%)
				S_{NO_1}	S_{NO_2}	S_{NO_3}	S_{NOe}	K_2	K_1+K_2	K_2	K_1+K_2			
1 (Day 11-28)	MUCT r=1 a=1 s=1	P.F.	0,089	1,0 (0,0)[5]	0,5 (0,0)	2,5 (0,0)	15,0	18,1	18,1		3,9	− 4	89	99
2 (Day 199-209)	UCT r=1 a=0 s=2	P.F.	0,129	1,0 (0,0)	2,0	3,0 (0,0)	29,0	31,5	38,6	2,0	7,8	− 8	95	97,9
3 (Day 350-368)	UCT r=1 a=0 s=3	C.M.	0,080	0,8 (0,0)	1,5 (0,0)	1,0 (0,0)	9,0	13,0	13,0		5,0	+ 5	87	85
4 (Day 425-441)	MUCT r=1 a=1 s=1	C.M.	0,070	0,3 (0,0)	0,5 (0,0)	2,0 (0,0)	13,0	14,2	14,2		1,7	+ 4	99	85
5 (Day 526-538)	MUCT r=1 a=0,5 s=2	C.M.	0,118	0,5 (0,0)	1,6	3,0 (0,0)	20,5	24,6	29,2	3,5	7,5	− 1	87	100,2
6 (Day 542-555)	MUCT r=1 a=0,5 s=2	C.M.	0,090	0,3 (0,0)	0,6 (0,0)	2,0 (0,0)	14,0	17,6	17,6		4,0	− 5	88	87

[1] P.F. = plug flow; C.M. = completely mixed
[2] Average influent COD = 1000 mg/l
[3] % error = (error in S_{NOe})/TKN$_{in}$
[4] −ve = uptake, +ve = release
[5] bracketed figures were used in the material balances − see text.

4.64

60–70% could be obtained with the measured data; it was suspected that more denitrification was taking place than the readings indicated, i.e. the nitrate concentrations in the effluent from the anoxic and anaerobic reactors were possibly lower than those measured (the measured values were between 0,3 and 3 mgN/ℓ). This suspicion arose from some of the plug flow reactor denitrification profiles: in some of these profiles it happened on occasion that after the nitrate concentration had been reduced to zero, measurements showed that it started to increase again, sometimes up to 2 or 3 mgN/ℓ (see Appendix D for evidence of this). Under anoxic conditions, such a formation of nitrate is not possible and it is thought that towards the end of the denitrification period, some interference with the nitrate measurement takes place.

When the nitrogen and COD balances were re-calculated, assuming the nitrate concentrations in the anaerobic, first anoxic and second anoxic reactors to be zero (see Table 4.6), improved N balances of 85–100% were achieved. (It should be noted that the effluent nitrate concentrations of the first anoxic reactor in periods 2 and 5 were not changed from their experimental values since the actual nitrate load on the reactor was high in these periods). The fact that reducing the effluent nitrate concentrations in the first three reactors improved the N balances, supports the guess that those concentrations were lower than observed (i.e. interference with the measurement might have occurred). The new denitrification kinetics are also in agreement with this: apart from the exceptions mentioned (periods 2 and 5), the denitrification potential predicted for the anoxic (and anaerobic) reactors is greater than the nitrate load imposed on them by the recycles, i.e. the new kinetics suggest that the effluent nitrate concentrations from these reactors should have been zero.

(ii) System effluent nitrate concentration

The theoretical effluent nitrate concentrations in Table 4.6 were calculated according to the procedure set out in WRC (1984); predictions incorporating both the new and the old denitrification kinetics were made. For the new denitrification kinetics, the denitrification potential of the primary anoxic zone of the system was calculated with the aid of Eq (4.6), i.e. that based on a single fast K_2' rate with no contribution by RBCOD; for the old kinetics the denitrification potential was estimated with Eq (2.8), i.e. that based on the original K_1 and K_2 rates (observed in systems without anaerobic reactors) which have been used in the past for estimating N removal in N and P removal systems. Sample calculations are given in Appendix G.

Points to note are:

(1)   For all the steady state periods chosen, the effluent nitrate concentration is
        overpredicted by both the old and new denitrification kinetics, i.e. the
        observed effluent nitrate concentration was lower than predicted.

(2)   In steady state periods 1, 3, 4 and 6 the effluent nitrate concentrations
        predicted by both methods are identical.

(3)   For periods 2 and 5 the new kinetics predict an effluent nitrate concentration
        (i) which is lower than that predicted by the old kinetics and (ii) which is
        closer to the measured value.

The fact that both the old and new kinetics predict system effluent nitrate
concentrations that are too high shows that both sets underestimate the
denitrification potential of the primary anoxic reactors.

In the periods where the predicted effluent nitrate concentrations are identical for
both the old and the new kinetics (periods 1, 3, 4 and 6), the influent TKN/COD
ratio was low (less than 0,10). For these periods the nitrate load imposed upon the
anoxic zone was less than the theoretical denitrification potential given by both the
old and the new kinetics; the theoretical nitrate concentration in the effluent is
hence a function only of the nitrification capacity and the recycles and is
independent of the denitrification potential (see Appendix G). In these cases, both
forms of kinetics equally overpredict the effluent nitrate concentrations by between 2
and 5% (the effluent nitrate concentration error is expressed as a percentage of the
influent TKN concentration).

It is in periods 2 and 5 that the relative merits of the new kinetics become evident.
In these periods the influent TKN/COD ratios were high (> 0,10); the
denitrification potential predicted by the new kinetics is able to fully remove the
nitrate load recycled to the anoxic zone whereas the denitrification potential
predicted by the old kinetics is not sufficient to do so. Consequently, the theoretical
system effluent nitrate concentration is lower (and therefore closer to the real value)
when calculated according to the new denitrification kinetics rather than the old
kinetics. From Table 4.6, the new kinetics only overestimate the system effluent
nitrate concentration by about 3% compared to the old kinetics which overestimate

by about 8% (relative to the influent TKN concentration). It can be seen that at high TKN/COD ratios (above 0,10), where accurate estimation of the denitrification potential is required to calculate the effluent nitrate concentration, the new kinetics give a closer estimate of the system effluent nitrate concentration than the old.

(iii) Phosphorus uptake during the anoxic zone

No appreciable P uptake was observed in any of the anoxic plug flow reactor profiles or batch tests (see sections 4.1.2 and 4.2.2). This observation was checked against phosphorus concentration data measured in the MUCT/UCT system during the identified steady state periods. With the aid of a material balance for phosphorus over the complete anoxic zone (i.e. 1st and 2nd reactors) of the laboratory system, P release/uptake (release +ve; uptake −ve) was calculated for the identified steady state periods (see Table 4.6).

For some periods (periods 1, 2, 5 and 6) there was a slight uptake of P, in others (3 and 4) a slight release, but on the whole the change in P concentration through the anoxic zone was small. The uptake that did take place – at most 8 mgP/ℓ influent – is insignificant compared to the P uptake of 50-60 mgP/ℓ influent that takes place in the aerobic reactor. The P release/uptake response of the laboratory MUCT/UCT system therefore supports the observations in the batch tests and plug-flow profiles.

4.5 Conclusion

The above observations confirm that the plug flow anoxic reactor profiles and the batch test results give a reasonably accurate prediction of denitrification behaviour in the MUCT/UCT unit. The measured denitrification kinetics lead to predictions that overestimate the system effluent nitrate concentration only slightly; nevertheless, they are acceptable for describing denitrification in the MUCT/UCT configuration and at high TKN/COD ratios (> 0,10) give improved predictions compared to denitrification kinetics for N removal systems, the kinetics used for nutrient (N and P) removal systems in the past.

With the denitrification kinetics for the MUCT/UCT laboratory system established and verified, a mechanism for the behaviour was sought. Two hypotheses are presented in Chapter 5 and experiments to test them are described in Chapter 6.

CHAPTER 5

EXPERIMENTAL INVESTIGATION PART 2:
TWO HYPOTHESES FOR THE OBSERVED DENITRIFICATION
BEHAVIOUR IN THE ANOXIC ZONE OF THE MUCT/UCT SYSTEM

In Chapter 4, the denitrification behaviour in the primary anoxic zone of the N and P removal MUCT/UCT system was compared to that in the primary anoxic zone of systems removing N only. The only significant difference between the two sets of kinetics that could not be readily accounted for was the magnitude of the rate of denitrification ($K_2^!$) in the primary anoxic reactor of the MUCT/UCT system: the rate is more than twice the corresponding K_2 rate in N removal systems. In this Chapter, two hypotheses are presented to explain the cause for this high rate; experiments to verify one or other of the hypotheses are described in Chapter 6.

Each of the hypotheses for the high $K_2^!$ denitrification rate has a different fundamental basis:

Hypothesis 1: Additional denitrification by polyP organisms utilizing stored PHB;

Hypothesis 2: Modification of influent particulate biodegradable COD (PBCOD) in the anaerobic zone to a more easily degradable form.

Hypothesis 1: Additional denitrification involving polyP organisms
The first hypothesis suggests that the $K_2^!$ rate is made up of two components, i.e. (1) the usual denitrification rate by the facultative heterotrophs, like that in an N removal system plus (2) a denitrification rate by the polyphosphate-accumulating organisms utilizing stored PHB. If this hypothesis is correct, it means that (a) some polyphosphate-accumulating organisms are facultative aerobes and (b) these organisms are in a growth cycle utilizing stored PHB, but without polyphosphate accumulation in the anoxic zone.

Biochemically, statement (a) above is possible because Lötter *et al.* (1986) have identified facultative *Acinetobacter* strains. Statement (b) arises from the observation that during denitrification (i) the soluble COD concentration in the

liquid remains unchanged and (ii) the soluble phosphate concentration decreases only very slightly, much less than expected if polyphosphate accumulation was taking place. From calculations based on the measured nitrate and phosphate reductions, the amount of phosphate taken up can be accounted for approximately by the normal P requirement for organism mass synthesized with nitrate serving as the terminal electron acceptor.

Consequently, the facultative polyP organisms are behaving in a similar fashion to the other facultative organisms except the former is growing on stored PHB (accumulated in the anaerobic reactor) while the latter is growing on hydrolysis products of particulate biodegradable COD passing through the anaerobic zone. This hypothesized behaviour of the polyP organisms is not at variance with the biochemical model by Wentzel *et al.* (1986). It is possible the Adenolate energy level[1] stimulated by oxidation with nitrate is not high enough to trigger polyphosphate accumulation utilizing stored PHB, with the result that there is no appreciable uptake of phosphorus.

Hypothesis 2: Modification of influent PBCOD in the anaerobic reactor
The second hypothesis does not implicate the polyP organisms. It suggests that the high K_2' denitrification rate is a consequence of a partial solubilization of the influent PBCOD in the anaerobic reactor before it enters the anoxic zone. The theory of excess P removal accepts that facultative aerobic heterotrophs convert the 'readily biodegradable' substrate to short chain fatty acids (SCFA) for uptake by the polyP organisms (Wentzel *et al.*, 1985; Brodisch and Joyner, 1983). It is possible that these or similar organisms might alter the nature of some of the PBCOD substrate to a more easily biodegradable form. On reaching the anoxic zone (after the anaerobic reactor) the modified PBCOD would be oxidized at a faster rate compared to that in an N removal system, wherein the PBCOD is unmodified because of the absence of the anaerobic reactor.

This hypothesis does not contradict the observations that the P and soluble COD concentrations remain virtually unchanged in the anoxic zone: the slight P uptake can be accounted for by the growth requirements of the denitrifiers; the constant soluble COD concentration arises because it is the particulate and modified

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[1]The ATP/ADP ratio was one of the key parameters identified by Wentzel *et al.* (1986) to explain the behaviour of *Acinetobacter* spp. over a wide range of conditions.

particulate BCOD that is being utilized for denitrification, most of the soluble biodegradable COD having been utilized earlier in the anaerobic zone by the polyP organisms.

Although both of the hypotheses adequately explain the observed high denitrification rate in the MUCT/UCT system, they differ on one major point, i.e. the ability of the polyP organisms to denitrify. Noting this difference, it was decided to design and perform a number of different types of experiments that would indicate whether or not polyP organisms could aid denitrification significantly. These experiments and their results are described in the next Chapter.

CHAPTER 6

EXPERIMENTAL INVESTIGATION PART 3:
EVALUATION OF THE PROPOSED HYPOTHESES TO EXPLAIN THE
DENITRIFICATION KINETICS IN THE MUCT/UCT
NUTRIENT REMOVAL SYSTEM

Two hypotheses to explain the high denitrification rate (K_2') in the primary anoxic zone of the MUCT/UCT nutrient (N and P) removal system (relative to K_2 in N removal systems) were proposed and discussed in Chapter 5, viz.:

1. The polyP organisms present are able to denitrify utilizing their internally-stored PHB and so contribute to the normal denitrification by facultative organisms that utilize particulate biodegradable COD (PBCOD);

2. The polyP organisms present are unable to denitrify and the PBCOD is modified to a more easily biodegradable form in the anaerobic reactor, upstream of the primary anoxic zone; the modified PBCOD leads to a faster denitrification rate by the facultative organisms.

In this chapter, batch tests that were conducted to test the validity of each of the above hypotheses are described and discussed. The batch tests were conducted on sludge harvested from the same laboratory system that was operated for the earlier plug flow and batch test rate determination experiments.

6.1   Parent system operation and performance during the batch tests

The batch tests described in this chapter were conducted between days 373 and 556, i.e. a period that coincided approximately with the time for the rate determination batch tests described in Chapter 4. From day 373 to day 401, the system was being operated in the UCT mode and from day 402 to day 556 in the MUCT mode. The daily performance of the system is shown in Figs 4.11 to 4.18 (for day 373 to 400) and Figs 4.26 to 4.33 (for day 401 to 556); the various operational changes that were made are indicated in Figs 4.11 and 4.26 and are summarized in Table 4.3. The overall system performance and the reasons for the changes in operation during this period were discussed in Chapter 4, section 4.2.1.

Altogether, 10 batch tests were conducted to test the hypotheses. Nine of these were done on days 373, 438, 466, 479, 491, 526, 535, 549 and 556 with sludge abstracted from the MUCT/UCT system. An additional batch test was conducted on sludge harvested from an enhanced polyP organism culture system operated in the experiments by Wentzel *et al.* (1987, 1988 a,b and c). In the enhanced culture, which is fed 100% acetate as influent substrate, the polyP organisms dominate the biocenosis in the system so that more than 90% of the organisms are *Acinetobacter* spp. The P removal obtained with such a culture is 60 mgP/ℓ for 500 mg influent COD/ℓ, i.e. ΔP/COD = 0,12 mgP/mgCOD compared to 20 mgP/ℓ for 1000 mgCOD/ℓ raw sewage in the MUCT/UCT system, i.e. ΔP/COD = 0,02 mgP/mgCOD. The batch test was conducted on this sludge in order to compare the denitrification rate of the polyP organisms in the enhanced culture with that of the polyP organisms in the mixed culture MUCT/UCT system receiving sewage. Details of this enhanced culture work by Wentzel *et al.* (1987, 1988a, b and c) were reviewed in Chapter 2, section 2.2.2.

The first hypothesis implicates polyP organism denitrification utilizing internally-stored PHB. Accordingly, as an initial objective, batch tests were conducted in which PHB concentrations in the sludge mass were measured during the denitrification process.

6.2   Batch tests with PHB measurements

If polyP organisms can denitrify, their internally-stored PHB is the only substrate available to them; they are apparently unable to utilize PBCOD and the previous experiments in the plug flow and batch reactors indicated that the soluble COD (filtered to <0.45μm) in the liquid remained unchanged. In view of this, an initial objective in the batch test work was to measure PHB concentrations in the sludge mass under anoxic conditions.

6.2.1   PHB concentration observations in MUCT sludge under anoxic batch test conditions

To study whether or not PHB is utilized under anoxic conditions, anoxic batch tests were conducted on sludge abstracted from the anaerobic reactor of the laboratory system. Anaerobic reactor sludge was selected because the polyP organisms in this reactor contain the highest concentration of internally-stored PHB in the system. The batch tests were run for between 4 and 6 hours during which samples were taken and analyzed for PHB, nitrate and nitrite concentrations. General details of the

batch test procedure are given in Appendix C; this includes the specific method for PHB analysis.

Three anoxic batch tests with PHB analysis were conducted on sludge from the MUCT system. The batch tests were conducted on sludge abstracted from the system on days 438, 466 and 526 (see Fig 4.26). Due to various analytical difficulties (see Appendix C for details), reliable results for PHB concentrations were obtained from only one test, i.e. the one done on day 526. Figures 6.1a and 6.1b show the nitrate, nitrite and PHB concentration profiles measured in this anoxic batch test. For comparison, PHB concentrations of the sludge in the anaerobic, second anoxic and aerobic reactors of the MUCT unit are also plotted. Important points to note from Figs 6.1a and 6.1b are:

1. The denitrification rate K_2' is 0.149 mgN/(mgAVSS·d)

2. For the first $4\frac{1}{2}$ hours in the batch test, the PHB concentration in the sludge remained approximately constant at 12,4 mgPHB/gVSS, the same concentration as that of the sludge in the anaerobic reactor.

3. From $4\frac{1}{2}$ to 6 hours the PHB concentration decreased, but during this period the denitrification rate remained unchanged.

4. The PHB level of the sludge in the second anoxic reactor lies between that of the sludge in the anaerobic and aerobic reactors respectively.

The batch test denitrification rate is $K_2' = 0,149$ mgN/(mgAVSS·d), taking into account correction for the nitrite formation. This is slightly lower than the estimated mean for the plug flow and batch tests discussed earlier [0,224 mgN/(mgAVSS.d)], but is still significantly higher than the value of 0,101 mgN/(mgAVSS·d) observed in systems without an anaerobic reactor. This test therefore supports the earlier denitrification work.

The measured PHB levels provide evidence for the denitrification mechanism of MUCT system sludge. For the first $4\frac{1}{2}$ hours, the PHB concentration remained constant at the same value as that in the anaerobic reactor of the MUCT system. This indicates that no PHB was utilized and implies that the polyP organisms did not take part in the denitrification process for the first $4\frac{1}{2}$ hours. However, in the

ANAEROBIC REACTOR SLUDGE ANOXIC

NITRATE & NITRITE

$K_2' = 0.149$ mgN/(mgAVSS.d)

□ NO3—N   + NO2—N
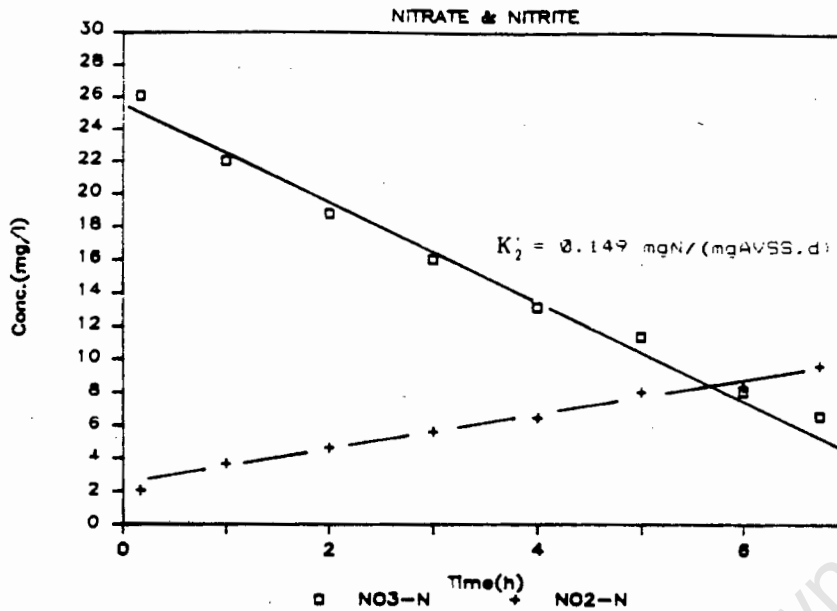
Fig 6.1(a):   *Denitrification profile of an anoxic batch test on sludge taken from the anaerobic reactor of the MUCT unit on day 526. Note the similarity to earlier anoxic batch tests (Chapter 4).*

PHB LEVELS OF SLUDGE

Anaerobic reactor

2nd Anoxic reactor
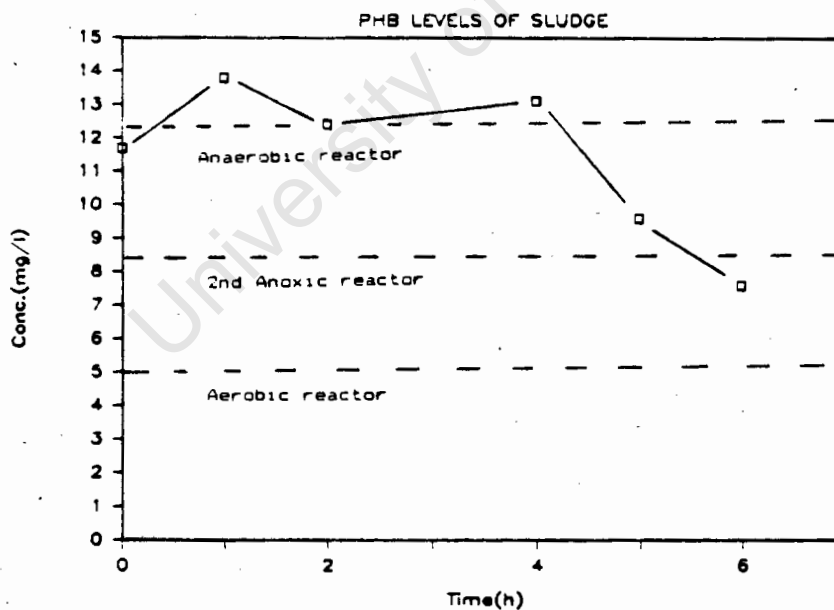
Aerobic reactor

Fig 6.1(b):   *PHB levels of the anaerobic reactor sludge throughout the batch test. Note that although there appears to be a drop in PHB concentration during the final 2 hours of the test, there is no change of denitrification rate (Fig 6.1a) over the same period, i.e. it is unlikely the polyP organisms took part in the denitrification. The PHB levels of the sludge in the various reactors (shown as dotted lines) support this (see text).*

final 2 hours of the test, the PHB concentration was considerably lower than that in the first 4 hours. This seems to indicate that in the final 2 hours, PHB *was* utilized by the polyP organisms for denitrification. However, there are two reasons to doubt this: (1) In the final part of the test period there is no increase in the denitrification rate; if PHB *was* utilized for denitrification by the polyP organisms there should have been an increase in the rate. (2) PHB was not utilized in the anoxic zone of the MUCT system; the PHB concentration in the second anoxic reactor (8,3 mgPHB/gVSS) is lower than that in the anaerobic reactor (12,4 mg PHB/gVSS), but this difference can be accounted for exactly by dilution from the underflow, r– and a– recycles.

It was concluded from this batch test that PHB was not utilized by the polyP organisms under anoxic conditions – it appeared that these organisms do not contribute to denitrification in the MUCT system sludge.

6.2.2 PHB concentration observations in an enhanced polyP organism culture sludge under anoxic batch test conditions

To examine the response of polyP organisms under anoxic conditions more closely, a batch test similar to the one discussed above was conducted on an enhanced polyP organism culture sludge. This sludge was obtained from the enhanced polyP organism culture developed and described by Wentzel *et al.* (1988a) (see Chapter 2, section 2.2.2). With the enhanced polyP organism culture sludge, the denitrification behaviour of the polyP organisms could be examined in the absence of the denitrification by 'normal' activated sludge facultative heterotrophs. In addition to this, since enhanced cultures do not positively exclude other organisms (e.g. predators), the polyP organism behaviour can be expected to simulate, reasonably closely, polyP organism behaviour in normal mixed culture systems (e.g. in the MUCT system).

Enhanced culture sludge was abstracted from the anaerobic reactor of the enhanced culture system (to obtain the highest concentration of internally-stored PHB per gVSS) and nitrate was added to create anoxic conditions. The batch test was run for about 4 hours and during this period, nitrate, nitrite, soluble COD ($<0,45\mu$m filtered), phosphate and PHB concentrations were measured regularly. The results are shown in Figs 6.2a and 6.2b.

In Figs 6.2a and 6.2b the following features of the enhanced culture batch test are

ENHANCED CULTURE SLUDGE ANOXIC
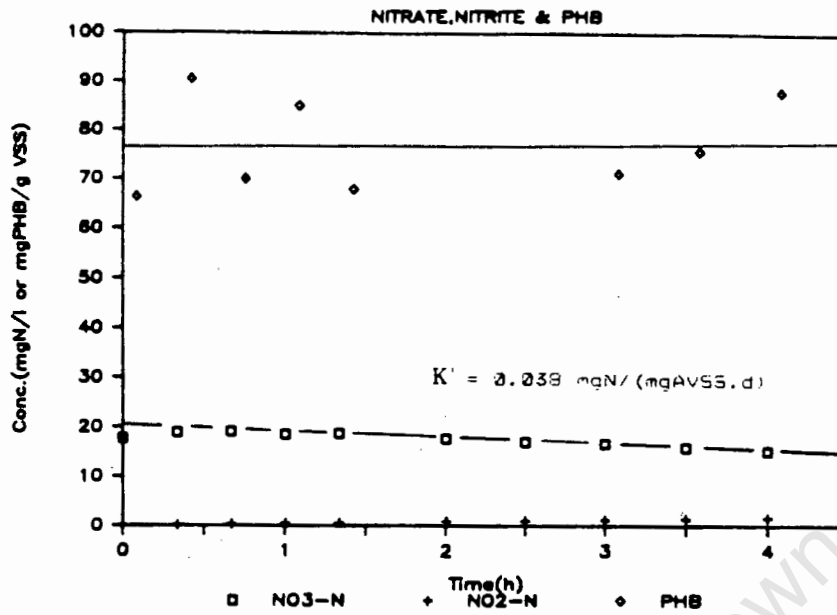
Fig 6.2(a): *Nitrate, nitrite and PHB concentration versus time profiles in an anoxic batch test on enhanced polyP organism culture sludge. There is virtually no denitrification and no appreciable change in the PHB concentration.*
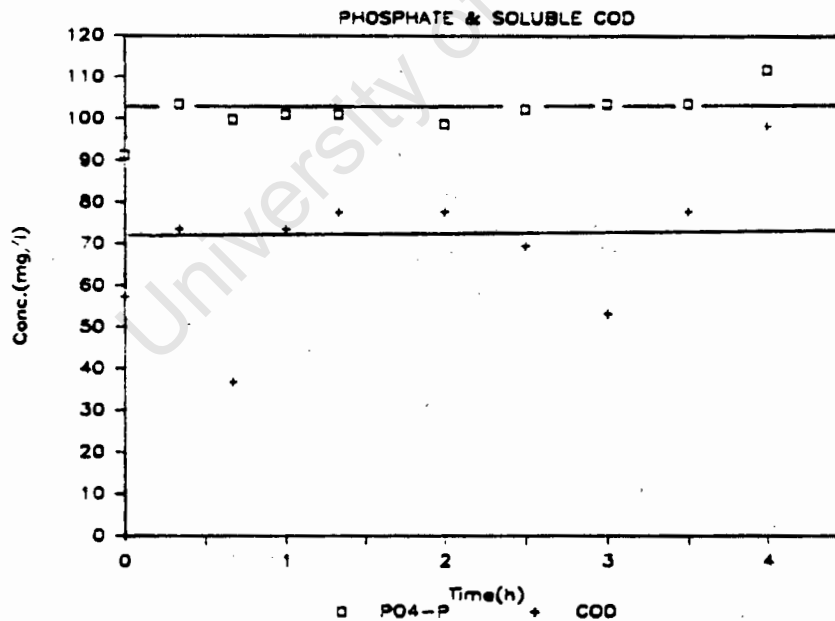
Fig 6.2(b): *Phosphate and soluble COD (< 0,45μm) concentration versus time profiles for the enhanced culture anoxic batch test. Both parameters stay relatively constant pointing to polyP organism inactivity under anoxic conditions.*

apparent:

1. The phosphate and PHB concentrations remained constant.

2. The soluble COD varied between 50 and 100 mg/ℓ, but showed neither an increasing nor decreasing trend.

3. The nitrate reduction was very low — the denitrification rate (corrected for nitrite) is 0,038 mgN/(mgAVSS·d), where the active polyP organism mass was calculated in accordance with the polyP organism model of Wentzel et al. (1989).

The soluble COD and phosphate concentration profiles conform to those observed in the Chapter 4 plug flow and batch reactors (from which the denitrification rates were established), i.e. no soluble COD utilization and phosphate uptake. As in the batch test with MUCT system sludge described in section 6.2.1, there was no PHB utilization. However, the denitrification rate was very low, i.e. 0,038 mgN/(mgAVSS·d) compared to an average of 0,224 mgN/(mgAVSS·d) for the MUCT system sludge. The low denitrification rate in a sludge which (i) has such an enormous ability to remove P and (ii) is designed to simulate polyP organism behaviour in normal mixed cultures, provides strong evidence that the polyP organisms do not contribute significantly to denitrification in the primary anoxic reactor of the MUCT/UCT system. This conclusion is in conformity with that drawn from the first batch test described above.

6.3   Batch tests without direct PHB measurement

In order to substantiate the conclusions of the batch tests in which PHB was measured directly, alternative batch test conditions were devised from which the role of polyP organism PHB in denitrification could be inferred without direct measurements of PHB.

6.3.1   Comparison of denitrification rates of two sludges that differ only in PHB content

If the increased denitrification rate is due to denitrification by the polyP organisms utilizing internally-stored PHB (hypothesis 1), then a sludge with a high PHB concentration, i.e. one that has been exposed to anaerobic PHB-accumulation conditions, should have a higher denitrification rate than a sludge with a low PHB concentration, i.e. one that has been exposed to aerobic PHB-utilization conditions.

The procedure for obtaining two sludges that differ only in their PHB content was as follows:

1. Six litres of sludge was harvested from the aerobic zone of the MUCT system on day 556. This aerobic reactor sludge has a low PHB concentration because most of the PHB has been utilized for P uptake. Also, only a low concentration of particulate biodegradable COD (PBCOD) is present in this sludge because practically all the PBCOD from the influent will have been hydrolyzed and utilized; only the low PBCOD concentration originating from organism death and lysis is present.

2. The 6 litres of sludge was divided equally between two 3 litre batch reactors, denoted A and B.

3. Both batches were held unaerated for 12 hours; during this time the nitrate and nitrite concentration in the mixed liquor was completely denitrified so that after the 12 hour period both sludges had become anaerobic. The amount of time needed for this was estimated from the nitrate concentration in the aerobic reactor sludge and the secondary anoxic denitrification rate measured in earlier batch tests (see Chapter 4, Table 4.5).

4. At $12\frac{1}{4}$ hours after the start, 64 mg/ℓ acetate was added to only one of the batches, i.e. Batch A. Batch B received a quantity of distilled water equal to the volume of acetate solution added to Batch A. A period of $2\frac{3}{4}$ hours was allowed for acetate uptake, estimated from previously observed acetate uptake rates (see Wentzel et al., 1985). The acetate added to Batch A was rapidly taken up and stimulated a concomitant P release; according to the biochemistry of excess biological P removal, PHB is formed during acetate uptake and P release (Wentzel et al., 1986). The quantity of acetate that was added was calculated from the amount of P release normally observed in the anaerobic reactor of the MUCT system. Acetate uptake was complete in 2 hours, i.e. at 14 hours after the start of the test. Batch A now had a higher PHB concentration than Batch B. It should be noted that sewage was not used to stimulate PHB formation and P release, because with sewage, PBCOD is also added. Since PBCOD utilization is invoked in the second hypothesis for the high denitrification rate, the possible influence of this COD was eliminated by adding acetate only.

5. At 15 hours after the start of the batch test, 25 mgN/ℓ nitrate was added to both Batches A and B to create anoxic conditions. Over the following 6 hours, i.e. up to 21 hours after the start of the test, samples were taken and tested for nitrate, nitrite and phosphate. The results of the whole of the 21 hour period of both Batches A and B are shown in Figs 6.3a and 6.3b. Batch test procedure and sample handling details are given in Appendix C.

Examining the results in Figs 6.3a and 6.3b, the following features are noteworthy:

1. There are three sequential phases on each batch test: a first anoxic phase (from 0 to 10,5 hours), an anaerobic phase (from 10,5 to 15 hours) and a second anoxic phase (from 15 to 21 hours).

2. During the first anoxic phase, the residual nitrate in both Batches A and B was denitrified from about 15 to 0 mgN/ℓ at a rate of 0,056 mgN/(mgAVSS·d). Neither batch showed any significant P uptake during this period.

3. During the anaerobic phase, the acetate added to Batch A was completely taken up in 2 hours and stimulated a P release of 43 mgP/ℓ; in Batch B, which did not receive acetate, a small P release of 10 mgP/ℓ was observed.

4. During the second anoxic phase, the denitrification rates in both batches were approximately the same, i.e. for A, 0,082 and for B, 0,079 mgN/(mgAVSS·d). As expected, these rates are low and are in the region of the rate for secondary denitrification, i.e. K_3 = 0,072 mgN/(mgAVSS·d) for N removal systems and K_3' = 0,100 mgN/(mgAVSS·d) for N and P removal systems — see Chapter 4, Table 4.5.

5. During the second anoxic phase, Batch A (with acetate addition) showed a considerable P uptake of 30 mgP/ℓ whereas Batch B (no acetate addition) showed a marginal P uptake of 5 mgP/ℓ.

Under anaerobic conditions, Batch A (with acetate addition) showed a much greater P release than Batch B (without acetate addition) so that it could be assumed that Batch A had a much larger stored PHB concentration than Batch B at the start of the second anoxic phase. Incidentally, the P release in Batch B in the absence of acetate uptake, called secondary release, does not have PHB storage associated with

EFFECT OF STORED HAc ON DENIT.
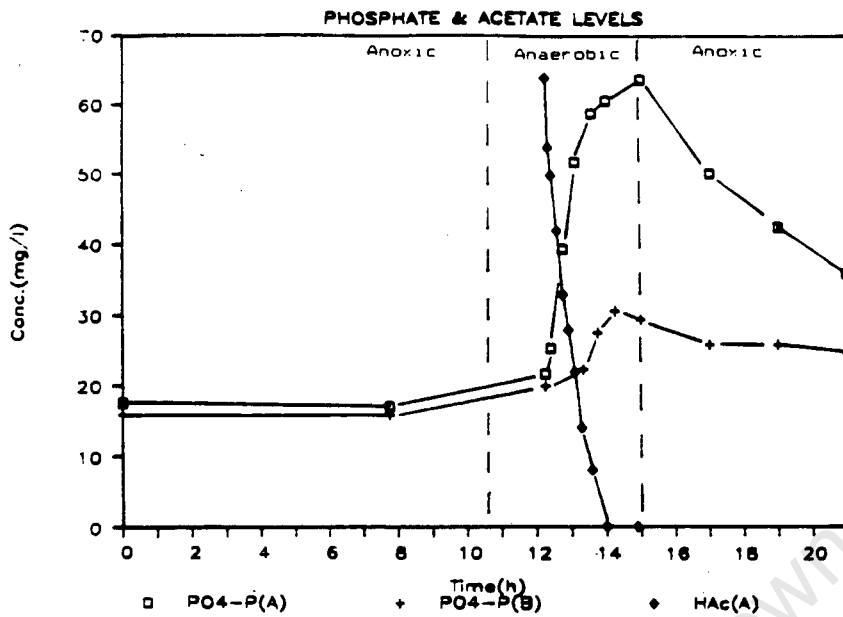
Fig 6.3(a): *Phosphate and acetate levels for the anoxic batch test comparing the denitrification rate of two sludges differing in PHB content. The acetate added to Batch A during the anaerobic period stimulated a large P release and a corresponding formation of PHB. At the start of the second anoxic period, Batch A therefore had more stored PHB than Batch B.*
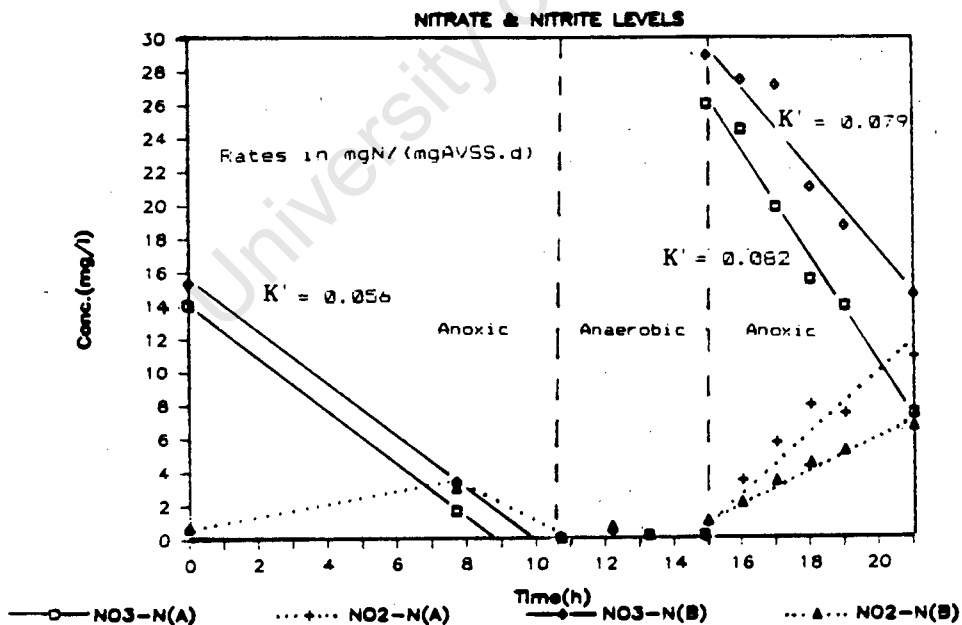
Fig 6.3(b): *Nitrate and nitrite levels in the anoxic batch test comparing the denitrification rate of two sludges differing in PHB content. Note that in the second anoxic period, Batch A (having more stored PHB) has a very similar denitrification rate (corrected for nitrite accumulation) to Batch B (having less stored PHB).*

it (Wentzel *et al.*, 1986). Despite the difference in PHB concentration, Batches A and B had the same low denitrification rate during the second anoxic phase, i.e. around 0,080 mgN/(mgAVSS·d), rates associated with secondary anoxic denitrification.

The observation that the denitrification rate for Batch B is low is expected: the sludge originated from the aerobic reactor and denitrification after the main aeration reactor is associated with only utilization of substrate from cell death and lysis, i.e. secondary anoxic denitrification. The observation that PHB-rich Batch A had a similarly low denitrification rate indicates that in this batch the denitrification also is only secondary anoxic denitrification. Had the polyP organisms in Batch A taken part in the denitrification, utilizing PHB, the denitrification rate should have been significantly faster.

From the above it appears that the PHB concentration and hence the polyP organisms did not play a role in the denitrification. This conclusion agrees with that drawn from the previous two batch tests.

Aside from this important conclusion, there are two observations that warrant further discussion: (i) The denitrification rate of both batches during the initial anoxic period was approximately 0,056 mgN/(mgAVSS·d). Since the sludge originated from the aerobic reactor of the MUCT unit, it was expected that these rates should be close to those obtained in the second anoxic period, i.e. those associated with secondary denitrification [between 0,072 and 0,100 mgN/(mgAVSS·d)]. These rates are therefore significantly lower than expected. However, both rates were calculated with only two nitrate concentration measurements; it is possible that the actual profiles were steeper than those that are drawn. An accurate rate determination can only be done with a large number of nitrate concentration samples. (ii) During the second anoxic phase, Batch A showed a considerable P uptake, whereas in earlier batch tests (and Batch B in this experiment) there was very little change in P concentration under anoxic conditions. The reason for the P uptake in Batch A in this experiment is uncertain. Although this P uptake seems to implicate the polyP organisms in some way, it does not appear to be connected to polyP organism denitrification: as pointed out above, the denitrification rate of Batch A (where there was considerable P uptake) is no different to that of Batch B (where there was only a marginal P uptake).

6.3.2 **Phosphorus uptake capability in the aerobic zone after exposure to primary denitrification conditions**

If polyP organisms utilize PHB in the primary anoxic zone, then less PHB will be available in the aerobic zone for P uptake. Consequently, a sludge with a high PHB concentration, (one from an anaerobic zone where a significant P release took place), when exposed to an anoxic period, should have less P uptake under aerobic conditions than one not exposed to an anoxic period. This was investigated by running a series of batch tests as follows:

1. Sludge was harvested from the anaerobic reactor of the MUCT system and divided into two equal batches, denoted A and B.

2. Directly after harvesting, one of the batches (Batch A) was aerated continuously; the other (Batch B) was anoxic for about 4 hours after harvesting and was then aerated continuously. The initial anoxic conditions in Batch B were created by keeping the sludge unaerated and adding about 10 mgN/ℓ to it.

3. From both batches, samples were abstracted regularly and the nitrate, nitrite and phosphate concentrations were determined. Both batches were run for a total of 24 hours.

Four of these batch test sets were conducted; the results from these are given in Appendix I. The days on which these test sets were done are 373, 479, 491 and 526 (see Figs 4.11 and 4.26). Similar results were obtained in all 4 sets. For the purposes of discussion, the results of the test done on day 373 will be considered, the results of which are shown in Figs 6.4a and 6.4b. From Figs 6.4a and 6.4b the following features are noteworthy:

1. The anoxic and aerobic periods of Batch B are clearly discernible − during the first 4 hours (anoxic), the nitrate concentration steadily decreased at a rate (corrected for nitrite accumulation) of 0,209 mgN/(mgAVSS·d). Over the same period the P concentration remained approximately constant at around 50 mgP/ℓ These observations are in conformity with all the previous observations on anoxic batch tests using sludges harvested from the anaerobic reactor.

2. When aeration commenced after a 4 hour anoxic period in Batch B, the nitrate

P UPTAKE STUDY
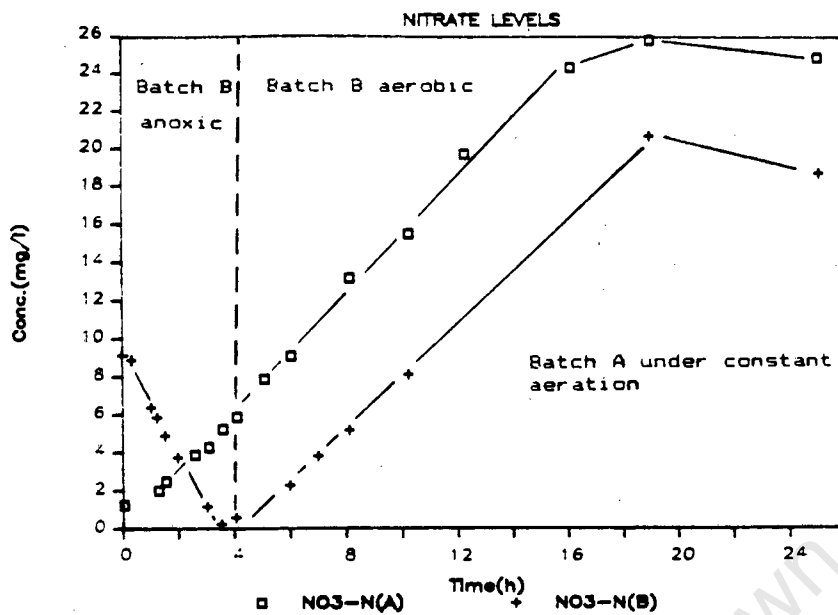
Fig 6.4(a):   Nitrate levels in a batch test designed to study the effect of an anoxic period on the P uptake capability of aerated MUCT/UCT sludge. Batch A was aerated continuously while Batch B was held anoxic for 4 hours, then aerated.
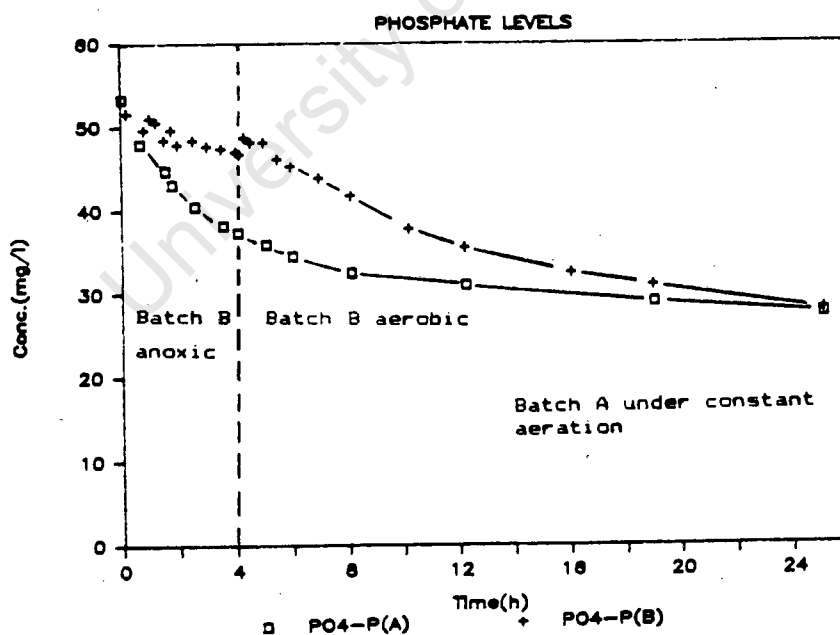
Fig 6.4(b):   Phosphate levels for the P uptake study: note that the overall P uptake exhibited by Batch A (constantly aerated) is the same as for Batch B (anoxic/aerobic).

Table 6.1: Comparison of the P uptake capability of aerated anaerobic reactor sludge with and without a preceding anoxic period.

Test No	P uptake capability during aerobic period (mgP/gVSS)	
	Aerobic period preceded by anoxic period	Batch under constant aeration
1	19,3	18,9
2	24,7	23,6
3	25,1	26,0
4	18,4	20,1
Mean	21,9	22,1

concentration increased linearly due to nitrification. The P concentration decreased from about 52 mgP/ℓ down to 27 mgP/ℓ during 20 hours of aeration.

3. When aeration commenced at the start of the test in Batch A, the nitrate concentration increased linearly due to nitrification, the nitrification rate being approximately the same as that for Batch B (i.e. the nitrate profiles are parallel). The P uptake also commenced immediately from the start of the test and the P concentration went from 52 mgP/ℓ down to the same value as Batch B, i.e. about 27 mgP/ℓ, after 24h aeration.

The results above are confirmed by the other 3 batch test sets: the P uptake capabilities in mgP/gVSS[1] for both Batches A and B observed in the 4 batch test sets are given in Table 6.1 – on average the P uptake for the anoxic/aerobic Batch B (21,9 mgP/gVSS) is virtually identical to that of the continuously-aerated Batch A (22,1 mgP/gVSS).

It can be concluded from these batch test sets that because an initial anoxic period did not reduce the P uptake capability of the sludge, no PHB was utilized during the anoxic period. Consequently, the polyP organisms appear not to have taken part in the denitrification during the initial anoxic period. This conclusion supports the conclusions of the previous batch tests conducted.

6.4 Denitrification in the presence of sewage particulate biodegradable COD which has not passed through an anaerobic zone

The batch tests described in sections 6.2 and 6.3 above focused on determining whether or not PHB was utilized by the polyP organisms to aid denitrification in the primary anoxic reactor. It was concluded from these tests that the polyP organisms did not to any significant degree utilize their internally-stored PHB and consequently did not contribute to the denitrification. The first hypothesis to explain the high primary anoxic denitrification rate therefore has to be rejected. In this section, batch test experiments are described that investigate the second hypothesis, i.e. that the influent PBCOD is modified in the anaerobic reactor to a more readily utilizable form (for details see Chapter 5).

If the anaerobic reactor does modify the influent PBCOD to a more readily utilizable

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[1]Since the VSS concentration varied slightly between Batches A and B, and between test sets, the uptake capabilities are normalised with respect to VSS concentration.

form, then there should be a difference between the K_2 rate of a sludge that is fed influent sewage directly and the K_2 rate of a sludge that receives influent sewage which has passed through an anaerobic zone. In a sense, this difference has already been noted, in that the K_2' denitrification rate observed in the MUCT/UCT system operated in this investigation was higher than the K_2 rate observed in nitrogen only removal systems (see Chapter 4, Table 4.5). However, this comparison is not sufficiently accurate to accept or reject the second hypothesis because the two rates were observed on different sludges receiving different sewages. In this section, the comparison will be made in controlled experiments on the same sludge receiving one sewage.

The batch tests were conducted according to the following procedure:

1. Four litres of sludge at 2500 mgVSS/ℓ were taken from the aerobic reactor of the MUCT system. Aerobic reactor sludge was selected, because in this reactor the influent PBCOD concentration is a minimum.

2. The 4 ℓ of sludge was divided into two 2 ℓ batches; to one batch (Batch A), 1 ℓ of raw sewage at a COD strength of 1200 mg/ℓ was added and to the other batch (Batch B), 1 ℓ of tap water was added.

3. The conditions in both batches were held anoxic by non—aeration and adding nitrate at the start of the test.

4. At regular time intervals, samples were abstracted from both batches and analyzed for nitrate, nitrite and phosphate concentrations.

Two batch test sets of this type were conducted on days 535 and 549. The results of both test sets are shown in Figs 6.5 and 6.6. Some important aspects of the results to note are:

1. In both test sets, the batch that received sewage (Batch A) showed the expected two-phase denitrification behaviour, i.e. an initial rapid rate followed by a second slower rate, typical of primary anoxic denitrification in N removal systems; the batches that received water (Batch B) showed the expected single phase denitrification behaviour, i.e. a single slow rate of denitrification typical of secondary anoxic denitrification.

DENITRIFICATION WITH SEWAGE

Day 535

NITRATE & NITRITE

Batch A: Sewage added
Batch B: Tap water added

$K_1'' + K_2''$

K_2''

K_3''

K_{NO_2}

$K_1' = 0.191$
$K_2' = 0.230$
$K_3' = 0.087$
mgN/(mgAVSS.d)

□ NO3-N(A)   + NO2-N(A)   ♦ NO3-N(B)   ▲ NO2-N(B)
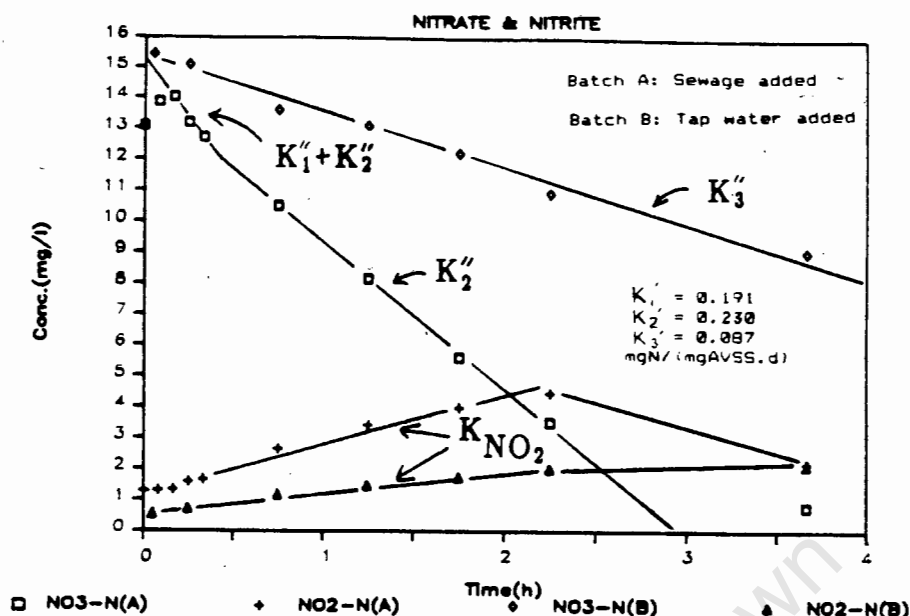
Fig 6.5(a): *Nitrate and nitrite concentration versus time profiles in an anoxic batch test to determine the response of MUCT/UCT (aerobic reactor) sludge to PBCOD that had not passed through an anaerobic reactor, i.e. sewage was added. The K_2' rate of Batch A (resulting from fresh sewage PBCOD) is very similar to the K_2' rate established for the anoxic zone of the MUCT/UCT unit (resulting from sewage PBCOD which has passed through the anaerobic reactor).*

PHOSPHATE LEVELS

□ PO4-P(A)   + PO4-P(B)
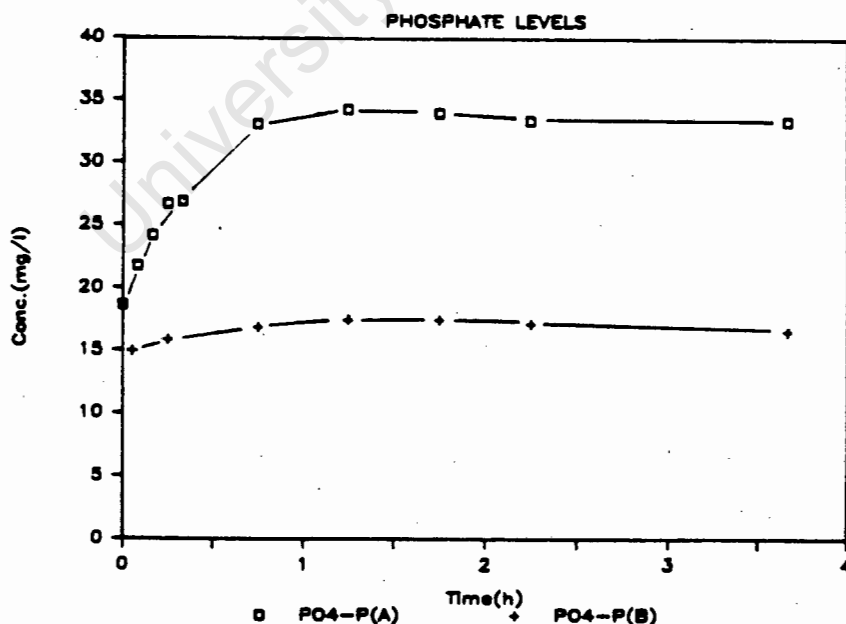
Fig 6.5(b): *Phosphate concentration versus time profiles of the two batches of aerobic reactor sludge held anoxic; Batch A (which received sewage) showed a large degree of P release during the first phase of denitrification — this resulted from the uptake and storage of RBCOD by the polyP organisms. Neither Batch A or the control showed much P uptake during the remainder of the anoxic period.*

DENITRIFICATION WITH SEWAGE

Day 549
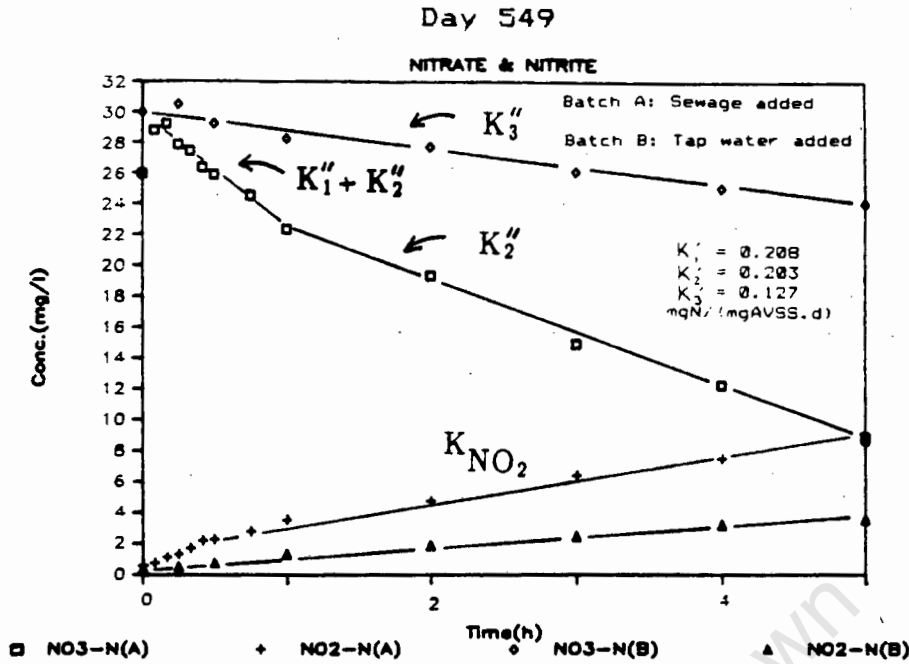
Fig 6.6(a): *Nitrate and nitrite profiles in the second anoxic batch test examining the response of MUCT/UCT sludge to sewage. The Batch A K_2 rate (resulting from fresh sewage PBCOD) is again similar to the K'_2 rate for the anoxic zone of the MUCT/UCT unit (resulting from ex-anaerobic reactor PBCOD).*
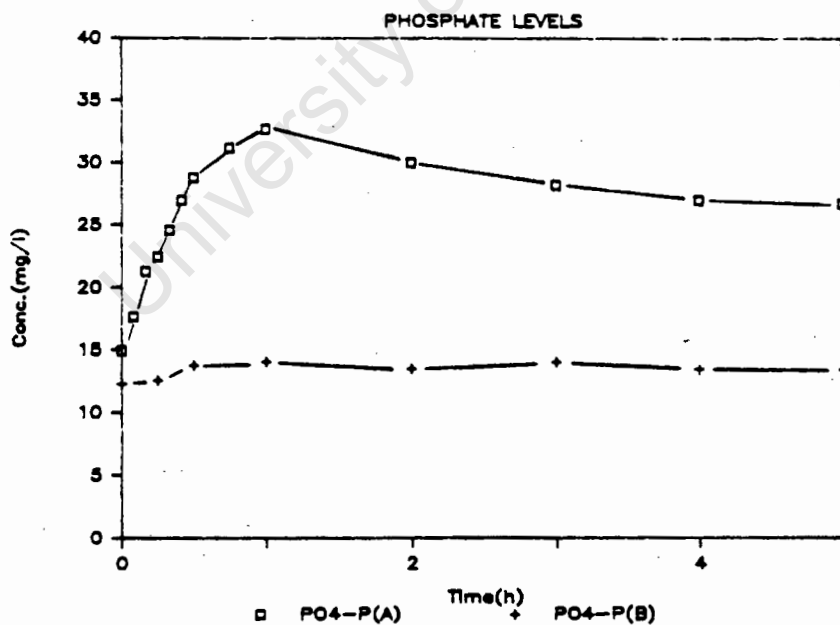
Fig 6.6(b): *Phosphate profiles in the second anoxic batch test examining the response of MUCT/UCT sludge to sewage. As in the first test, Batch A (with sewage) showed an initial phase of P release during which the polyP organisms took up PBCOD. For the rest of the anoxic period, Batch A showed a gradual P uptake, more so than in the test on Day 535.*

6.19

2. In the batches that received water (the B Batches):

    2.1   The average denitrification rate was 0,107 mgN/(mgAVSS·d) which is very similar to the average secondary anoxic denitrification rate (K_3') observed earlier in this investigation, i.e. 0,100 mg N/(mgAVSS·d) (see Chapter 4, Table 4.5).

    2.2   The P concentration remained essentially constant throughout the batch test period, an observation also in conformity with the earlier batch tests.

3. The similarity of response of the secondary anoxic denitrification behaviour of the sludge (shown by the B Batches) to that observed in the earlier batch tests described in Chapter 4, indicated that the sludge had very similar qualities compared to those in the earlier tests.

4. In the batches that received sewage (the A Batches):

    4.1   The average initial rapid denitrification rate (appropriately separated from the background second slower rate — see Chapter 4, Section 4.1.3) was 0,200 mgN/(mgAVSS·d). This rate is 3 times slower than that observed in the earlier plug flow reactor experiments on the MUCT sludge [i.e. 0,610 mgN/(mgAVSS·d)] and just over 3,5 times slower than that observed in N removal sludge, i.e. 0,720 mgN/(mgAVSS·d)[2].

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[2]Although not of central importance in this investigation, the initial denitrification rate shown by the MUCT system sludge warrants further discussion. It has been observed by Gabb et al. (1988) that exposing activated sludge to RBCOD under plug flow conditions actually stimulates a RBCOD uptake rate 2 to 3 times higher than under completely mixed conditions. Plug flow reactors have been employed to study the denitrification kinetics in both N removal systems (previously) and N and P removal systems (this investigation). In terms of the stimulation effect, the RBCOD utilization rate in N removal systems was high [K_1 = 0,720 mgN/(mgAVSS.d)] because the influent RBCOD was discharged directly to the head of the plug flow reactor; in the plug flow reactor operated in this investigation, the RBCOD utilization rate was lower [K_1' = 0,610 mgN/(mgAVSS.d)] — since only

a minor fraction of the influent RBCOD reached the plug flow reactor (most was removed in the anaerobic reactor), the stimulation effect was weak (in Chapter 4, an alternative explanation was given: the RBCOD leaking through the anaerobic reactor is not as easily biodegradable as that taken up by the polyP organisms); in the batch test receiving sewage, the RBCOD utilization rate was lower still [K_1 = 0,200 mgN/(mgAVSS.d)] — at this time the MUCT system was being operated with a completely mixed first anoxic reactor, hence there was a complete absence of the stimulation effect.

4.2.   During the initial phase of denitrification (the first 60 minutes), both tests showed a rapid P release.   Even though conditions were anoxic (as opposed to anaerobic), P release and PHB storage by polyP organisms took place due to the high short chain fatty acid (SCFA) and RBCOD concentrations in the liquid at the start of the test[3] (Wentzel et al, 1987). The internally-stored PHB acquired during this stage would not have affected the subsequent denitrification rates (K_2') — it was shown in earlier batch tests that the polyP organisms do not play a role in denitrification for the sludge under investigation.

4.3   The average second denitrification rate was 0,217 mgN/(mgAVSS·d), which is very close to the value observed in earlier experiments $[K_2' = 0,224$ mgN/(mgAVSS·d) — Chapter 4, Table 4.5], but more than 2 times higher than that observed in N removal systems $[K_2 = 0,101$ mgN/(mgAVSS·d)].

4.4   For the test performed on day 535, there was a very slight P uptake in Batch A during the second phase of denitrification — this agrees with the earlier batch tests (see Chapter 4, section 4.2.2) where the P uptake could be approximately accounted for by organism growth.

For the test on day 549, Batch A showed a more noticeable P uptake during the second phase of denitrification.   It will be recalled that similar P uptake behaviour under anoxic conditions was observed in the batch test described in section 6.3.1.   As in the latter test, the reason for the P uptake in the test on day 549 is uncertain;   if the polyP organisms *were* replenishing their polyphosphate pool during the second phase of denitrification, it is unlikely that nitrate was being used as an electron acceptor: the denitrification rate [0,203 mgN/(mgAVSS·d)] is in fact lower than that of Batch A on day 535 [0,230 mgN/(mgAVSS·d)] where

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[3]This occurs also under aerobic conditions (Wentzel *et al.*, 1987).   An important consequence of this polyP organism behaviour is that sludges that exhibit biological excess P removal (sludges from nutrient removal plants) cannot be used in anoxic or aerobic batch tests to estimate the RBCOD concentration in sewage (Ekama *et al.*, 1986).   In such tests, the initial rapid oxygen (in aerobic tests) and nitrate (in anoxic tests) utilization rates *are* correctly given, but the *concentrations* of oxygen and nitrate *utilized* during the initial rapid rates are too low to correctly give the influent RBCOD concentration, due to the uptake and internal storage of RBCOD by the polyP organisms.

there was no noticeable polyP activity (little change in P concentration). As in the batch test described in section 6.3.1, the P uptake in the test on day 549 was therefore not considered as being evidence for polyP organism denitrification.

The focal point in this set of tests is the second denitrification rate observed in the batches that received sewage. This rate is almost identical to that observed in the earlier plug flow and batch reactor tests, i.e. 0,217 compared to 0,224 mgN/(mgAVSS·d). This similarity of rate was observed despite the fact that in the earlier tests, the PBCOD of the influent passed through an anaerobic reactor, whereas in the tests under discussion, the PBCOD did not. These results therefore indicate that the second hypothesis to explain the high K_2' denitrification rate, i.e. that the anaerobic reactor modifies the PBCOD to a more easily utilizable form, also has to be rejected.

6.5   Conclusions

In this chapter, batch tests were described to evaluate the validity of two hypotheses for the high K_2' denitrification rate in the primary anoxic reactor of nutrient (N and P) removal systems (more than two times higher compared to K_2 in N removal systems). The hypotheses were:

1. The polyP organisms which effect biological excess P removal utilize some internally-stored PHB acquired in the anaerobic zone for denitrification and so aid the denitrification in the primary anoxic reactor.

2. The influent particulate biodegradable COD is modified in the anaerobic reactor to a more easily usable form, leading to a fast denitrification rate in the primary anoxic reactor.

The results of the batch tests indicated that both hypotheses have to be rejected: it was shown by direct measurement and inference that for the MUCT/UCT system sludge studied, internally-stored PHB was not utilized under anoxic conditions — as this is the only source of substrate for the polyP organisms, these organisms appear not to contribute to denitrification in this system; it was also shown that the K_2' denitrification rate of sludge in the primary anoxic reactor (after the anaerobic reactor) is equal to that of aerobic reactor sludge receiving sewage, i.e. the anaerobic zone does not appear to modify the influent PBCOD from its original state.

In the absence of alternative hypotheses at this stage, it has to be accepted that for the primary anoxic reactor, nutrient removal sludges apparently have a higher 'second' denitrification rate than the corresponding K_2 rate in nitrogen removal sludges. At present it is not known which environmental conditions present in nutrient removal systems, but absent in N removal systems, stimulate this increased 'second' denitrification rate. It is possible that the anaerobic reactor stimulates a qualitative change in the sludge mass that causes the hydrolysis of particulate biodegradable COD to be accelerated. In activated sludge there are two sources of PBCOD, i.e. that from the influent and that generated by the sludge itself through death-regeneration. If nutrient removal sludges *do* acquire an improved PBCOD hydrolysis rate, one would expect the denitrification rate in the secondary anoxic reactor also to be increased due to the accelerated hydrolysis of PBCOD arising from death-regeneration; this was in fact observed in the investigation (see Chapter 4, Table 4.5). Until greater clarity on this phenomenon is obtained, for design of primary anoxic zones in nutrient removal plants, it can be accepted with reasonable confidence that the initial rapid denitrification rate does not take place and that the second phase takes place at an accelerated rate, i.e. at about twice the rate of the second denitrification phase in N removal systems.

An aspect that needs to be given attention to produce a consistent integrated design procedure for biological N and P removal in nutrient removal plants, is the conclusion that the polyP organisms do not contribute to the denitrification. These organisms sequester for their exclusive utilization all the RBCOD of the influent; this means that less COD is available for the growth of the denitrifying facultative heterotrophic mass with the result that a lower active organism mass (active VSS) is performing the observed denitrification than that estimated from the existing design procedure (WRC, 1984). From Wentzel *et al.* (1989), growth of polyP organisms on RBCOD to achieve biological excess P removal reduces the non-polyP heterotrophic mass by about 40%. However, in the evaluation of the kinetic data in this thesis, it was assumed that all the influent biodegradable COD mass (RBCOD and PBCOD) forms non-polyP heterotrophic mass. For consistency with the observation that polyP organisms do not contribute to denitrification, the active mass used for the denitrification rate calculations needs to be reduced by about 40% to give the non-polyP heterotrophic active mass. In terms of the non-polyP organism active mass, the denitrification rates reported in this theses, are therefore about 40% higher, i.e. the observed K_2' denitrification rate in the primary anoxic reactor of a nutrient removal system is 0,224 mgNO$_3$-N/(mgAVSS·d) with respect to the total

active VSS, but is 0,314 $mgNO_3$-N/(mg non-polyP AVSS·d) with respect to the non-polyP active mass. This aspect will be dealt with in greater detail in the revised nutrient removal design manual which is being compiled at present.

CHAPTER 7

CONCLUSIONS OF THE RESEARCH AND RECOMMENDATIONS REGARDING THE DESIGN OF ANOXIC ZONES IN NUTRIENT REMOVAL PLANTS

The aim of this investigation was to establish and find reasons for the denitrification kinetics in nutrient (N and P) removal systems. To recap, the motivation behind the research was as follows:

In the past, all denitrification studies have been performed on N removal systems. In the primary anoxic reactor (receiving the influent sewage flow, upstream of the aerobic reactor), denitrification takes place according to two rates: a fast rate $[K_1 = 0,720 \text{ mgN/(mgAVSS.d)}]$ of short duration and a slower background rate $[K_2 = 0,101 \text{ mgN/(mgAVSS.d)}]$ which is present throughout the reactor. The K_1 rate utilizes all the influent readily biodegradable COD (RBCOD) while the K_2 rate utilizes some of the influent particulate biodegradable COD (PBCOD).

In nutrient removal systems, there is an anaerobic reactor which receives the influent sewage upstream of the primary anoxic reactor. The organisms responsible for P removal (*Acinetobacter* spp.) take up most of the RBCOD in this reactor so that the substrate composition entering the primary anoxic reactor is no longer the same as that of the influent feed. Because of this change in influent composition, it seemed likely that the denitrification kinetics in the primary anoxic reactor should be different to those in N removal systems. However, experimental observations have shown that the inclusion of an anaerobic reactor in an N removal system does not significantly affect the overall N removal performance. To explain the apparent anomaly, this investigation was initiated.

7.1   Denitrification behaviour in the nutrient removal MUCT/UCT system

Selecting the MUCT/UCT configuration as the nutrient removal system for the investigation, the first anoxic reactor was operated in a plug flow mode so that nitrate, nitrite, phosphate and soluble COD concentrations could be measured versus reactor retention time. Twenty-nine such profiles were constructed; these profiles were supplemented with nineteen similar concentration-time profiles obtained in anoxic batch tests aimed at simulating the plug flow reactor operation. These

experiments enabled the following major conclusions to be drawn:

(1)   Denitrification behaviour in the anoxic zone (a primary anoxic zone) of the nutrient removal MUCT/UCT configuration is characterized by a single, continuous, zero order (with respect to nitrate) rate of nitrate reduction of magnitude $K_2' = 0,224$ mgN/(mgAVSS.d) at 20°C. This behaviour is clearly different to that observed in the primary anoxic zones of N removal systems — there is no initial fast rate and the rate that does occur is over twice the slow K_2 rate.

(2)   The absence of the initial fast denitrification rate (K_1), a rate which *is* observed in systems without anaerobic reactors (N removal systems), is presumably due to the RBCOD (responsible for such a rate) being taken up by the polyP organisms in the preceding anaerobic reactor that is present. However, the measured K_2' rate is over twice that expected due to the remaining PBCOD (the K_2 rate in N removal systems) and this needed further investigation.

(3)   Although secondary denitrification (after the aerobic reactor) does not occur in the MUCT/UCT configuration, aerobic reactor sludge from this system was used to measure the latter denitrification rate since it may also apply to other P removal systems that do possess secondary anoxic zones. It was found to be $K_3' = 0,100$ mgN/(mgAVSS.d) at 20°C, slightly higher than secondary denitrification rates measured in N removal systems [cf. $K_3 = 0,072$ mgN/(mgAVSS.d)].

(4)   It is probable that the denitrification kinetics established for the nutrient removal MUCT/UCT configuration are also valid for other nutrient removal systems. It was noted in this investigation that the nitrogen removal attained did not appear to be severely affected by the presence of the anaerobic reactor — the measured effluent nitrate concentration was only slightly different (lower) compared to that predicted by the 'old' two-phase kinetics for systems without anaerobic reactors. Since it has been observed that the denitrification performance of other laboratory and full-scale N removal systems is not diminished by the inclusion of an anaerobic reactor, it is probable that the kinetics established for the MUCT/UCT nutrient removal system hold for nutrient removal systems in general. It appears

that the high single rate in the primary anoxic reactor makes up for the absence of the fast initial rate found in N removal systems.

7.2   Reasons for the K_2' denitrification rate measured in the MUCT/UCT system

Two hypotheses were formulated to explain the fast K_2' denitrification rate in the primary anoxic zone of the MUCT/UCT system.

Hypothesis 1:   The polyP organisms are able to denitrify using stored PHB, thus contributing to the nitrate reduction rate of the normal denitrifiers.

Hypothesis 2:   The PBCOD undergoes some kind of modification in the anaerobic reactor making it more easily biodegradable; in the primary anoxic reactor, the denitrification rate stimulated by this PBCOD would hence be increased.

A number of batch tests were conducted on the MUCT/UCT system sludge to evaluate these hypotheses. The following conclusions were drawn:

(1)   Experiments aimed at directly or indirectly examining the PHB levels of the activated sludge of the MUCT/UCT system during an anoxic period indicated that PHB was not being utilized by the polyP organisms. Similar results were obtained for an enhanced polyP organism culture sludge. It was therefore concluded that the polyP organisms in the MUCT/UCT system are not able to denitrify; the high K_2' denitrification rate could not be explained by additional nitrate reduction by such organisms — the first hypothesis had to be rejected.

(2)   In the final set of tests, the denitrification rate of aerobic reactor MUCT sludge was measured in the presence of PBCOD that had *not* passed through an anaerobic reactor (sewage was added). It was seen that the rate corresponding to the utilization of the PBCOD was no different to that in the primary anoxic zone of the laboratory system, where the PBCOD *had* passed through an anaerobic reactor. The conclusion from this was that the anaerobic reactor does not modify the influent PBCOD in any way, making the second hypothesis for the K_2' rate also invalid.

(3) An alternative explanation to account for the K_2' rate is that the denitrifiers in the MUCT/UCT system are simply able to hydrolyze the influent PBCOD in the anoxic zone more rapidly than in systems exhibiting the normal K_2 rate of nitrate reduction (N removal systems). Exactly what environmental conditions present in the MUCT/UCT system are responsible for this are uncertain, but it is suspected that the anaerobic reactor *per se* plays a rôle in some way, possibly acting as a selector in favour of denitrifiers capable of high PBCOD hydrolysis rates. This hypothesis was not tested directly and requires further investigation.

7.3 The design of anoxic zones in nutrient removal systems and recommendations regarding future denitrification research

(1) This investigation succeeded in establishing the denitrification kinetics for the MUCT/UCT nutrient removal system. It is likely that these kinetics also hold for other nutrient removal systems. Although the reasons for the behaviour are still not entirely clear, it is recommended that the above established kinetics are used (rather than the 'old' kinetics for N removal systems) in the design of the anoxic zones in MUCT/UCT and other nutrient removal configurations. At low TKN/COD ratios (< 0,10) there is little to choose between the two sets of kinetics — both overestimate the effluent nitrate concentration by about 3% (relative to the influent TKN concentration). However, at higher TKN/COD ratios (> 0,10) the effluent nitrate concentration prediction given by the 'new' kinetics (an overestimate of about 3%) is slightly better than that given by the 'old' kinetics (an overestimate of about 8%).

(2) The final explanation given for the high K_2' denitrification rate in the MUCT/UCT system was that the denitrifiers have a faster hydrolysis rate of influent PBCOD than in systems without anaerobic reactors (N removal systems). This explanation was not implicitly tested for and it is recommended that this receives further attention.

(3) Since it was concluded in this investigation that polyP organisms in the MUCT/UCT system do not denitrify, the specific denitrification rates reported in this thesis actually need readjustment. The specific denitrification rates in this study were calculated assuming that all of the active mass is able to denitrify; however, recent research into biological

excess P removal has shown that about 40% of the active mass consists of polyP organisms, which are unable to denitrify. The observed denitrification rates are therefore about 40% higher with respect to the non-polyP active mass, viz. the denitrification rate in the primary anoxic reactor of the MUCT/UCT system is 0,224 $mgNO_3$-N/(mgAVSS.d) with respect to the total active mass, but 0,314 $mgNO_3$-N/(mgAVSS.d) with respect to the non-polyP active mass. It is recommended that this aspect be given attention in future nutrient removal design procedures.

(4)   It is recommended that any future research performed to study denitrification behaviour in a particular system be conducted with anoxic batch tests rather than installing plug flow reactors. The latter give rise to several procedure-related problems that may affect the experimental results; the batch test method is easier to perform and accurately simulates ideal plug flow behaviour.

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APPENDIX A

EXPERIMENTAL APPARATUS MAKING UP THE MUCT/UCT SYSTEM

A.1 Introduction

A description of the apparatus making up the MUCT/UCT system used in the research is given in this appendix. Items covered are:

(i)   the typical design of a completely mixed reactor,

(ii)   a more detailed description of each reactor, including the first anoxic plug flow reactor,

(iii)   the settling tank,

(iv)   general layout,

(v)   pump and tubing, and

(vi)   feed container.

The system as a whole is shown schematically in Fig A.1. The anaerobic, second anoxic and aerobic reactors were completely mixed, the first anoxic reactor being plug flow for the first half of the research programme (Fig A.1a); for the latter half of the programme the first anoxic reactor was completely mixed (Fig A.1b). A general description of the make-up of a completely mixed reactor is now covered, after which each reactor is dealt with in more detail.

A.2 Typical reactor design

Schematic drawings of the anaerobic/anoxic and aerobic reactors are given in Fig A.2. Each reactor was made of a perspex tube with diameters and operating volumes as follows: anaerobic reactor – 150mm diameter and 6ℓ volume; first anoxic reactor (when it was completely mixed) – 140mm diameter and 2,5ℓ volume; second anoxic reactor – 140mm diameter and 4ℓ volume; aerobic reactor – 195mm diameter and 9ℓ volume. A flat perspex disc formed the base of each vessel. The inlet and outlet ports were formed by drilling into the base two 10mm diameter holes

ANAEROBIC   ANOXIC   AEROBIC
REACTOR   REACTORS   REACTOR

MIXED LIQUOR RECYCLES

WASTE FLOW
SETTLER

INFLUENT   EFFLUENT

SLUDGE RECYCLE S

---- OPTIONAL RECYCLE
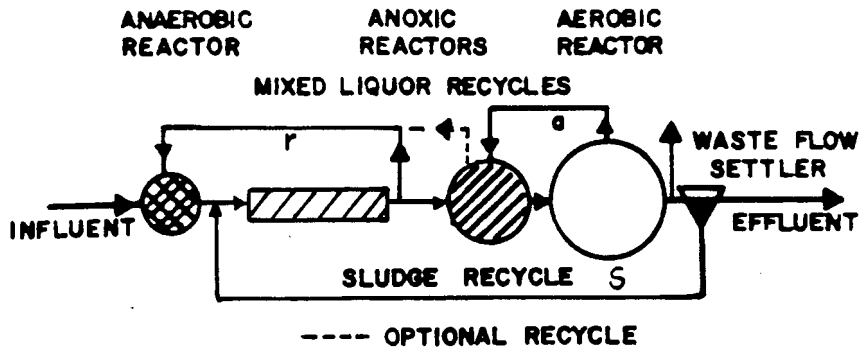
Fig A.1a: The MUCT/UCT system with a plug flow first anoxic reactor for the first half of the investigation. With the r-recycle taken from the end of the plug flow reactor, the system was in the MUCT mode; with the r-recycle taken from the second anoxic reactor, the system was in the UCT mode.

ANAEROBIC ANOXIC   AEROBIC
REACTOR   REACTORS   REACTOR
MIXED LIQUOR RECYCLES

WASTE FLOW
SETTLER

INFLUENT   EFFLUENT

SLUDGE RECYCLE S

---- OPTIONAL RECYCLE
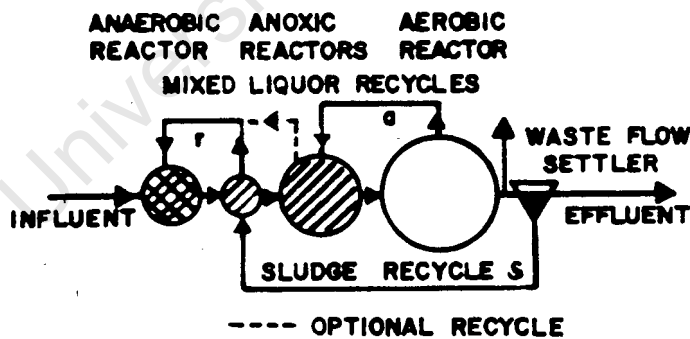
Fig A.1b: The MUCT/UCT system with a completely mixed first anoxic reactor for the second half of the investigation. Taking the r-recycle from the first anoxic reactor corresponded to the MUCT mode and taking the r-recycle from the second anoxic reactor corresponded to the UCT mode.
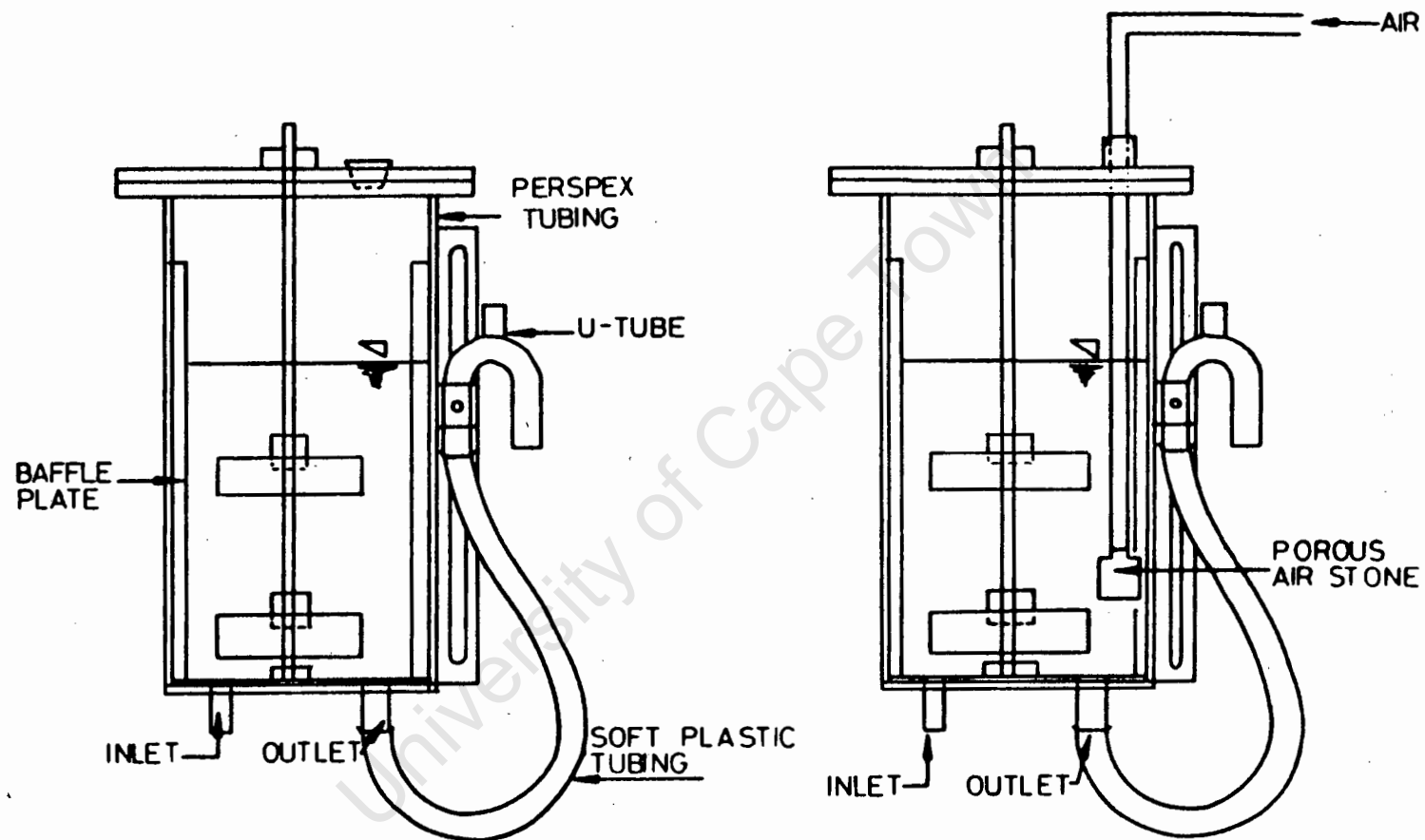
A.3

**Fig A.2**: _Details of construction of the anaerobic/anoxic and aerobic reactors._

over which short lengths of perspex tube were glued. The outlet port was connected to an inverted U—tube, the height of which was used to control the liquid volume in the reactor. Each vessel was also fitted with two vertical perspex baffles, glued opposite each other to the inside walls. These baffles improved mixing and prevented the formation of a vortex on the liquid surface.

The top of each reactor was covered with a perspex lid on which was mounted a stirrer motor. This brushless 115 V AC motor had an output of 30 rpm and drove a stainless steel shaft with perspex paddles to keep the reactor contents thoroughly mixed but without creating surface turbulence. The reactor lid had a 40mm diameter access port for cleaning, taking samples and inserting pH and oxygen probes. If the reactor was anaerobic or anoxic, when not in use the port was sealed with a rubber bung. For such reactors, a Styrofoam disc was also floated on the surface of the liquid to prevent oxygen from dissolving, the stirrer shaft passing through the disc.

A.3   Anaerobic reactor
The purpose of the anaerobic reactor was to allow P release to take place as described in Chapter 2. It received the influent flow of raw sewage to the system (10ℓ/d) as well as the r-recycle from either the first or second anoxic reactor, depending on whether the unit was being operated as a MUCT or UCT system respectively. The anaerobic reactor was operated at a volume of 6ℓ and was kept covered with black plastic to prevent light from entering — light encourages algae growth on the inside of the reactor walls.

A.4   First anoxic reactor
A.4.1   Plug flow mode
At the start of the investigation, the first anoxic reactor was plug flow. The overflow from the anaerobic reactor, together with the underflow stream, fed into the first anoxic reactor at a set rate (as opposed to being gravity fed). Since the ratio of anaerobic to aerobic reactor sludge at the entrance to the plug flow anoxic reactor was important, both streams were pumped into the reactor. The reactor itself consisted of nearly 32m of soft plastic tubing of inner diameter 10mm and with sample ports every metre. The total volume was $2,5\ell$. The tubing was coiled around a drum with a diameter of approximately 30cm (see Fig A.3). The drum had its axis along the horizontal and in order to keep the sludge in the coiled tube from settling out, the drum was rotated slowly (~0,5 rpm) by a motor that reversed the direction
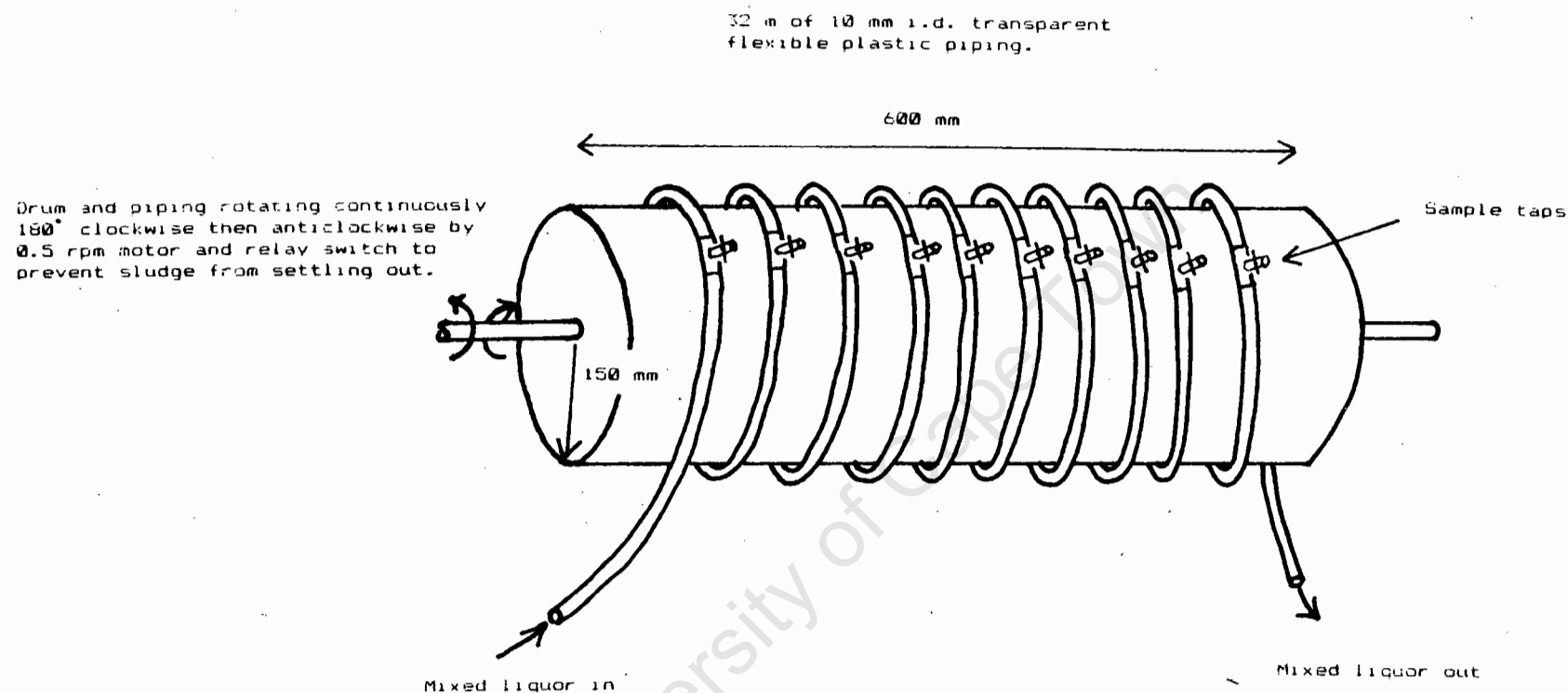
32 m of 10 mm i.d. transparent
flexible plastic piping.

600 mm

Drum and piping rotating continuously
180° clockwise then anticlockwise by
0.5 rpm motor and relay switch to
prevent sludge from settling out.

Sample taps

150 mm

Mixed liquor in

Mixed liquor out

Fig A.3: The plug flow first anoxic reactor of the MUCT/UCT system for measuring nitrate, nitrite, phosphate and soluble (< 0,45μm) COD concentration versus retention time profiles. The drum was mounted horizontally at waist-height (for easy sample-taking) and was rotated along its axis by a 0,5 rpm motor that reversed direction every 180°. This latter measure ensured that the sludge did not settle out in the coils.

of rotation every 180°. If the unit was being operated as a MUCT system, at the end of the first anoxic plug flow reactor, a recycle (the r-recycle) was taken back to the anaerobic reactor before the stream entered the second anoxic zone. For a normal UCT system, the effluent from the first anoxic reactor entered the second anoxic zone directly, the r-recycle being taken from the effluent of the latter reactor.

A.4.2 Completely mixed mode

Approximately half way through the research programme, it was decided to remove the plug flow anoxic reactor, replace it with a completely mixed reactor and conduct further denitrification studies using anoxic batch tests. The reason for this was to eliminate the problems experienced with the denitrification profiles measured in the plug flow anoxic reactor – details are given in Chapter 4 and Appendix C. The completely mixed first anoxic reactor had the same volume as the plug flow reactor (viz. 2,5ℓ) and its construction was similar to that of the other reactors described in paragraph A.2.

A.5 Second anoxic reactor

The second anoxic reactor was completely mixed and was operated at a volume of 4ℓ. It received the effluent from the first anoxic reactor as well as a recycle stream from the aerobic reactor – the a-recycle. The second anoxic reactor's purpose was to remove the nitrate load imposed on it via the a-recycle as well as any residual nitrate from the first anoxic zone. The effluent stream from the second anoxic reactor led into the aerobic reactor, described next.

A.6 Aeration reactor

Also completely mixed, this reactor was filled to 9ℓ and a constant stream of air was bubbled through it. Aeration was through a porous stone (of the type normally used in fish tanks) attached to the end of a perspex tube passing through the top cover. The airstone was positioned close to the bottom of the reactor, well out of line of the paddles. Air was supplied via a low pressure line from a large compressor, the airstream being controlled by a fine needle valve. The dissolved oxygen concentration was maintained between two and four mgO/ℓ to ensure nitrification while limiting the oxygen entering the anoxic zone via the a-recycle. The overflow from the aerobic reactor flowed into the settling tank.

A.7 Settling tank

The settling tank was constructed from 80mm diameter perspex tube, as shown in

Fig A.4. It was mounted on a wooden holder and set at an angle of 60° to the horizontal. The mixed liquor inlet and the underflow recycle outlet were located opposite each other at the bottom of the settler and were set at 90° to each other. The effluent outlet was located near the top of the settler tube.

To prevent sludge from adhering to the sides and bottom of the settler and to prevent the sludge from settling in a solid mass, two wiper blades were attached to a central shaft to sweep the sides and bottom of the tank. The central shaft was driven at 1,33 rpm by a 220V AC electric motor that was controlled by a timer, allowing the shaft to rotate one revolution every 3 minutes.

A.8   General layout

Apart from the plug flow first anoxic reactor (which was mounted horizontally in a free-standing frame at waist-height), the reactors were mounted on a vertical board, each reactor being set with its overflow point higher than that of the receiving reactor so that the reactors discharged under gravity from one to the other. The flow through the plug flow reactor was driven by the same pump as used for the recycles and the feed to the system (see section A.9). Beneath the whole system, a shallow metal tray was installed to collect and retain any sludge spilled due to blockages, split pipes or broken connections. Spilled sludge was returned to the unit. The whole system was run in a laboratory controlled at a temperature of 20° C.

A.9   Pump and tubing

The pump used for feeding the system, as well as for the recycles, was of the peristaltic variety developed by the Civil Engineering Workshop at the University of Cape Town. The pump had eight channels, each calibrated to pump 10ℓ/d. The flow rate in a channel could be adjusted by varying the tension of the pump tubing in that particular channel and once the tension was set, the flow remained constant for a considerable time. Pump tubing was replaced every two months to avoid sludge spillage through perished tubes. The 0,5 kW brushless 115V AC motor driving the peristaltic pump was extremely reliable and required little maintenance. It was connected to an on/off timer thus enabling the daily flow rates to be set very accurately.

The tubing used to connect the various reactors was of soft transparent plastic of the type used in hospitals. By using soft plastic, wall growths could easily be dislodged by periodical squeezing and tapping of the tubing. The tube lengths between
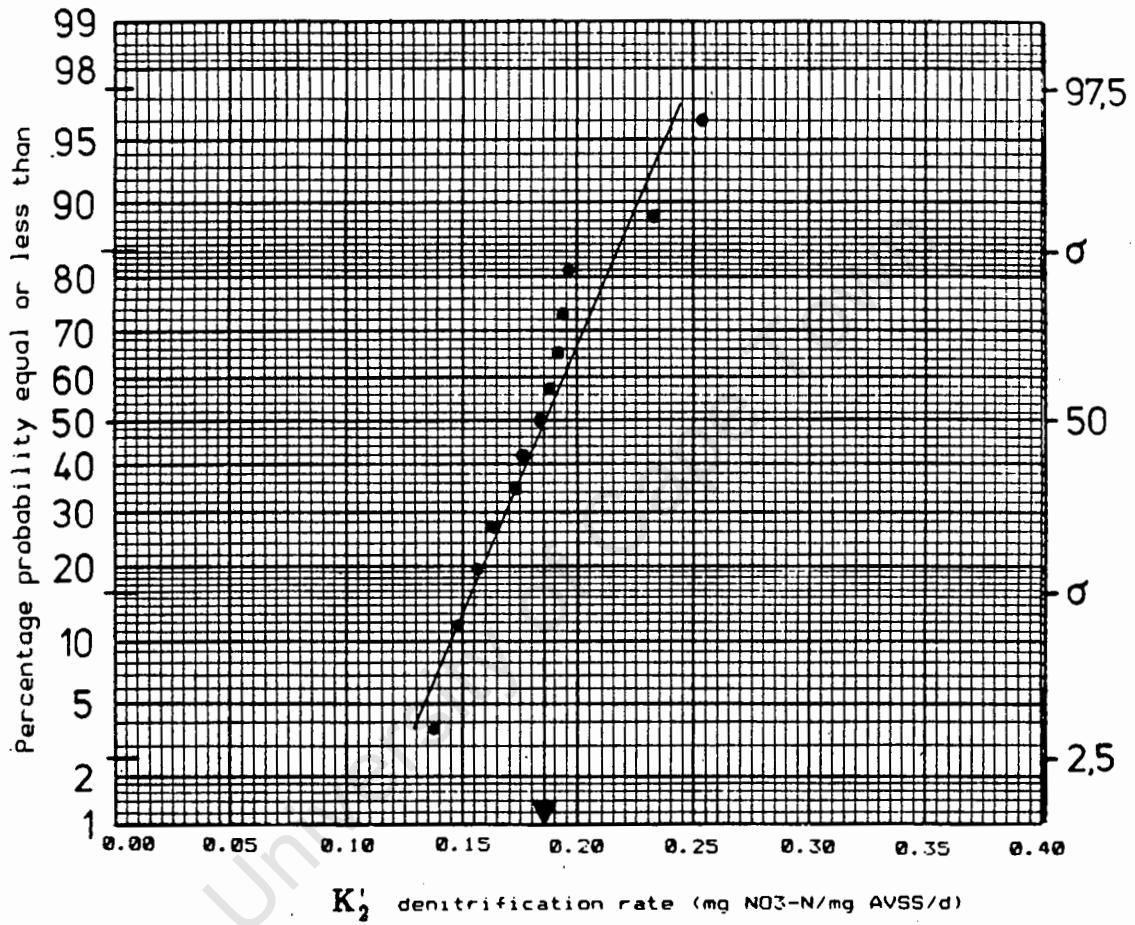
Fig 4.40:   _Probability distribution of the combined set of K_2 specific denitrification rates obtained in the anoxic batch tests with anaerobic reactor sludge only and with blends of anaerobic and aerobic reactor sludge.   The mean rate is 0,185 mgN/(mgAVSS.d)._