

**BIOLOGICAL EXCESS PHOSPHORUS
REMOVAL**

**MC WENTZEL, GA EKAMA, PL DOLD,
RE LOEWENTHAL, GvR MARAIS**

WRC REPORT NO 148/1/88

UNIVERSITY OF CAPE TOWN
Department of Civil Engineering

**FINAL REPORT TO THE
WATER RESEARCH COMMISSION
ON A FOUR YEAR CONTRACT ON
RESEARCH INTO
BIOLOGICAL EXCESS PHOSPHORUS REMOVAL
(1984 – 1987)**

by

M C Wentzel, G A Ekama, P L Dold, R E Loewenthal, G v R Marais

**Research supported by the
Water Research Commission
and the Foundation for Research Development**

**Research Report No W60
Contract No K148
June 1988**

105/1/88

SYNOPSIS

The principal objective of this contract was to develop a kinetic model for biological excess phosphorus removal (BEPR) in biological nutrient removal plants, with the view to improving BEPR technology. This objective essentially required the completion of four research projects; (1) BEPR system performance, (2) substrate description, (3) modelling of BEPR and, (4) non-polyP activated sludge modelling.

1. BEPR SYSTEM PERFORMANCE

Previous research on the performance of BEPR systems, treating raw and settled municipal wastewaters, had been limited to sludge ages between 12 and 30 days and the influent COD 'standardized' at 500 mg/l. Virtually no information was available outside these ranges. In order to obtain more information on system behaviour, enquiry was directed towards behaviour at short sludge ages (3-6 days) and high and low influent COD's (1000 and 250 mg/l respectively). The results of this enquiry were most useful as it verified the behaviour inferred from the 12 to 30 day sludge age investigation, i.e. dependence of BEPR on (1) the anaerobic mass fraction, (2) influent total COD, (3) influent readily biodegradable COD (RBCOD) fraction, (4) the adverse effect of nitrate and oxygen recycled to the anaerobic reactor, (5) improved stability of systems with high influent COD, and (6) apparent low endogenous mass loss rate for the polyP organisms.

2. SUBSTRATE DESCRIPTION

The need for this project arose from the recognition of the importance of the influent COD characteristics in BEPR. Three aspects particularly were in need of attention; (1) accepting the importance of RBCOD, to seek a more expedient method for measurement of this COD fraction than the existing biological ones, (2) surmizing that the polyP organisms can utilize only short chain fatty acids (SCFA, or, VFA), to study the conversion of RBCOD to SCFA in the anaerobic reactor and, (3) accepting that BEPR is virtually proportional to the RBCOD/SCFA, to study augmentation of this fraction by acid fermentation of the slowly biodegradable COD.

With regard to (1), biological methods that had been developed are based on the response of organisms to the RBCOD fraction. However, the practical difficulties in setting up and operating these bioassay systems greatly detract from their usefulness. For this reason, research was directed to estimating the fraction by physical

separation, in this case by ultrafiltration, because it was believed that this COD fraction would consist only of small molecules. This approach proved successful, and practical, so that designers now can form reasonable estimates of this fraction by using this simple separation method.

With regard to (2), it was established that the conversion of RBCOD to SCFA is a first order reaction with respect to RBCOD and the active mass of non-polyP heterotrophs. This indicated, and was verified, that two or more anaerobic reactors in series give a higher efficiency of conversion than a single reactor with the same total anaerobic sludge mass. With this kinetic model conversion it is now possible to determine with reasonable certainty the anaerobic mass and the anaerobic reactor configuration to obtain optimal conversion of RBCOD in any situation, within the practical constraints.

With regard to (3), work on full scale systems had shown that SCFA can be generated from the slowly biodegradable COD by acid fermentation of the underflow from the primary settling tank. This task was set up in order to develop a model by means of which the mass of SCFA generated can be estimated. From batch and semi-continuous series reactor systems the kinetic behaviour was identified; that the SCFA generation is a first order reaction with respect to a fraction of the influent primary settling tank underflow sludge concentration; that anaerobic batch or reactor retention time should not exceed 9 days; that from kinetic considerations batch reactors are but slightly superior to single completely mixed reactors for the same residence times up to about 6 days.

3. MODELLING OF BEPR

This project formed the core of the whole investigation, and took up the major part of the research effort. The main development in the understanding of BEPR can be summarized as follows:

- (1) A biochemical model for BEPR was developed describing the pathways and their regulation. This biochemical model was verified over a range of different situations. The model explains most of the observations relating to BEPR in the nutrient removal systems.
- (2) Enhanced cultures of polyP organisms were successfully developed in the modified Bardenpho and UCT systems. By conducting anaerobic, anoxic and aerobic batch tests on sludge harvested from the enhanced culture

systems, the processes and compounds associated with BEPR could be delineated.

- (3) With the processes and compounds defined, a mathematical model was developed in which the kinetics of the process rate and its stoichiometry could be quantitatively expressed. This model appears to describe the BEPR remarkably closely for the batch tests on sludge harvested from the enhanced culture systems.

The tasks above in essence complete the basic enquiry into the biochemical mechanisms and kinetics of BEPR. However a number of tasks related to the application of the basic information still are to be completed, viz:

- (1) Application of BEPR model to simulate the enhanced culture systems under constant flow and load conditions.
- (2) Integration of BEPR model into the general activated sludge model for mixed cultures.
- (3) Test and refine the integrated model under constant and cyclic flow and load conditions.
- (4) Develop a simplified steady state BEPR model for design purposes.

4. NON-POLYP ACTIVATED SLUDGE MODELLING

An investigation was undertaken to substantiate the bisubstrate hypothesis for modelling the activated sludge system by (1) monitoring the response of systems to the feeding of selected substrates apparently characteristic of the bisubstrate fractions, and (2) checking in which measure the general activated sludge model, based on the bisubstrate hypothesis, simulates the observed response of the systems. The artificial substrates selected were glucose and starch, representative of readily biodegradable and slowly biodegradable fractions respectively. The conclusion formed from this investigation is that the observed response of the glucose, starch and glucose/starch substrates are closely simulated by the bisubstrate model thereby strongly supporting the bisubstrate hypothesis.

The IAWPRC Task Group on modelling biological wastewater treatment systems accepted in a large measure the activated sludge model developed by the UCT group;

some changes were made and the model incorporating these changes has become known as the IAWPRC activated sludge model. The aspects found unacceptable by the IAWPRC group were; (1) that enmeshment/adsorption is a time dependent process and, (2) that slowly biodegradable COD is modified on the organism (by enzymes attached to the cytoplasmic membrane) and then directly utilized by the same organism. Instead they proposed that; (1) enmeshment is immediate and, (2) organisms release enzymes to the bulk liquid which then solubilize the slowly biodegradable COD to RBCOD which becomes available in competition to *all* the organisms. These two versions of the general model will need to be investigated to determine which is the better.

TABLE OF CONTENTS

	Page
SYNOPSIS	i
TABLE OF CONTENTS	v
ACKNOWLEDGEMENTS	vii
PAPERS, REPORTS AND OTHER CONTRIBUTIONS DURING CONTRACT PERIOD (JANUARY 1984 TO DECEMBER 1987)	ix
CHAPTER 1 : INTRODUCTION	1. 1
CHAPTER 2 : DEVELOPMENT OF BIOLOGICAL EXCESS PHOSPHORUS REMOVAL MODEL (PROJECT 1)	2. 1
1. BIOCHEMICAL MODEL	2. 1
2. KINETIC BEHAVIOUR	2. 2
2.1 Development of enhanced culture of polyP organisms	2. 3
2.2 Process and compound identification	2. 5
2.3 Kinetic model	2.18
CHAPTER 3 : BIOLOGICAL EXCESS PHOSPHORUS REMOVAL SYSTEM PERFORMANCE (PROJECT 2)	3. 1
1. PERFORMANCE AT HIGH AND LOW INFLUENT COD STRENGTH	3. 1
1.1 Performance at 1000 mg/l influent COD	3. 3
1.2 Performance at 250 mg/l influent COD	3. 4
1.3 Discussion	3. 5
2. PERFORMANCE AT SHORT SLUDGE AGES	3. 6
2.1 Experimental investigations	3. 7
2.2 Simulation studies	3.12
2.3 Conclusions	3.13
CHAPTER 4 : SUBSTRATE IDENTIFICATION, UTILIZATION AND GENERATION (PROJECT 3)	4. 1
1. MEASUREMENT OF READILY BIODEGRADABLE COD IN WASTEWATER	4. 1
1.1 Aerobic and anoxic batch tests	4. 1
1.2 Ultrafiltration	4. 2
2. READILY BIODEGRADABLE COD UPTAKE IN ANAEROBIC ZONE	4. 4

3.	ACID FERMENTATION OF PRIMARY SEWAGE SLUDGE	4. 8
3.1	Batch tests	4. 8
3.2	Semi continuous series reactor tests	4. 9
CHAPTER 5 :	NON-POLYP ACTIVATED SLUDGE MODEL (PROJECT 4)	5. 1
1.	VERIFICATION OF BISUBSTRATE HYPOTHESIS	5. 1
2.	IAWPRC MODELLING TASK GROUP	5. 3
CHAPTER 6 :	CONCLUSIONS AND RECOMMENDATIONS	6. 1
1.	BEPR SYSTEM PERFORMANCE	6. 1
2.	SUBSTRATE DESCRIPTION	6. 1
3.	MODELLING OF BEPR	6. 2
4.	NON-POLY ACTIVATED SLUDGE MODELLING	6. 4
REFERENCES		R.1

ACKNOWLEDGEMENTS

The writers wish to express their gratitude to the following persons for their contribution to the contract research work reported here.

- Mr Taliep Lakay – Laboratory Assistant, for his invaluable help in running the experimental units and laboratory.
- Mrs Heather Bain – Clerical Assistant, for so cheerfully typing and retyping papers and reports and attending to the accounts.
- Mr Ray Bevertton – Technical Assistant, for so ably constructing, maintaining and servicing the equipment in the laboratory.

The efforts of these three persons were not only that of support, they were vital participants in the research team.

- A special word of appreciation to Dr Laurraine Lötter, Principal Professional Officer at the City Health Department of Johannesburg, for her cooperation, and collaboration on the biochemical aspects of biological excess P removal.
- A special word of thanks to Dr Herman Wiechers and Mr John McGlashan, Senior Advisers of the Water Research Commission and Chairmen of the Steering Committee for the project, for their support, positive critical comment and guidance of the research work.
- A word of thanks to the City Health Department, in particular Mr Dave Osborn, Chief Scientific Officer, and Mr Harold Nicholls (Chief Professional Officer), and the City Engineer's Departments, in particular Mr Tony Pitman, Acting Assistant City Engineer, Sewerage and Water Reclamation, all of the Johannesburg City Council, for their willing collaboration.

Acknowledgement is due to the members of the Steering Committee of the project who guided the research work during the four year period:

- | | |
|---------------------|--|
| - Dr H N S Wiechers | - Water Research Commission
(Chairman 1984 to 1986) |
| - Mr J McGlashan | - Water Research Commission
(Chairman 1987) |

- | | |
|----------------------|--|
| - Mr W H J Hattingh | - Water Research Commission |
| - Mr A R Pitman | - Johannesburg City Engineer's Department |
| - Mr H G J Beekman | - Cape Town City Engineer's Department |
| - Mr I R Morrison | - Cape Town Scientific Services Branch (alternate) |
| - Mr W van der Merwe | - Department of Water Affairs |
| - Mr J v R Stander | - Department of Water Affairs (alternate) |
| - Mr P W Weideman | - Water Research Commission
(Committee Secretary) |

PAPERS, REPORTS AND OTHER CONTRIBUTIONS
DURING CONTRACT PERIOD
(JANUARY 1984 TO DECEMBER 1987)

I BOOKS

1. Ekama G A and G v R Marais (1984). Contribution of seven of the twelve chapters in Theory, Design and Operation of Nutrient Removal Activated Sludge Processes. Published by the Water Research Commission of South Africa.
2. Henze M, C P L Grady, W Gujer, G v R Marais and T Matsuo (1987). Activated sludge model No.1. International Task Group on modelling biological wastewater treatment systems. IAWPRC Scientific and Technical Report series, Pergamon Press, Oxford.

II PAPERS

1. Systems Control

1. Dold P L, H O Buhr and G v R marais (1984). An equalization control strategy for activated sludge process control. Presented at 12th IAWPRC Conference, Amsterdam. Wat.Sci.Tech, 17, 221-234.234.
2. Dold P L, G A Ekama and G v R Marais (1985). pH control and cost savings in aerobic digestion. Presented at IAWPRC Specialized Seminar on wastewater systems control, Houston, April 1985. In Advances in Water Pollution Control: Instrumentation and Control of Water and Wastewater and Transport Systems. (Ed. R A R Drake), pp 375-382. Pergamon Press, Oxford.
3. Randall W and P L Dold (1985). Data transmission in treatment plant computer control systems. Presented at IAWPRC Specialized Seminar on wastewater systems control, Houston, April 1985. In Advances in Water Pollution Control: Instrumentation and Control of Water and Wastewater Transport Systems. (Ed. R A R Drake), pp 85-90. Pergamon Press, Oxford.
4. Dold P L and G v R Marais (1987). Survey of activated sludge process control. Proceedings IWPC (SA Branch) biennial Conference, Port Elizabeth, May 1987.

2. Anoxic-aerobic activated sludge kinetics

5. Dold P L and G v R Marais (1984). Aspects of modelling and design of the activated sludge processes. Presented at 2nd meeting of the IAWPRC Task Group on modelling of biological waste treatment. Tokyo, March 1984.

6. Dold P L and G v R Marais (1986). Evaluation of the general activated sludge model proposed by the IAWPRC Task Group. Presented at the IAWPRC Specialized Seminar on Modelling of Biological Wastewater Treatment, Copenhagen, Aug 1985. Wat.Sci.Tech, 18, 63-89.
7. Ekama G A, P L Dold and G v R Marais (1986). Procedures for determining influent COD fractions and the maximum specific growth rate of heterotrophs in activated sludge systems. Presented at the IAWPRC Specialized Seminar on Modelling of Biological Wastewater Treatment, Copenhagen, Aug. 1985. Wat.Sci.Tech, 18, 91-114.
8. Warner A P C, G A Ekama and G v R Marais (1986). The activated sludge process Part 4 - Application of the general kinetic model to anoxic-aerobic digestion of waste activated sludge. Water Research, 20, 8, 943-958.
9. Dold P L and G v R Marais (1987). Benefits of including unaerated zones in nitrifying activated sludge plants. Presented at 13th IAWPRC Conference, Rio de Janeiro, Aug. 1986. Wat.Sci.Tech, 19, 195-207.
10. Henze M, C P L Grady, W Gujer, G v R Marais and T Matsuo (1987). A general model for single sludge wastewater treatment systems. Water Research, 21, 5, 505-515.
11. Dold P L, W K Bagg, G A Ekama and G v R Marais (1985). Comparison of measurement methods for readily biodegradable COD fraction in municipal wastewater. Presented at IWPC biennial Conference, Durban, May 1985.

3. Biological phosphorus removal

12. Wentzel M C, P L Dold, G A Ekama and G v R Marais (1985). Kinetics of biological phosphorus release. Wat.Sci.Tech, 17, 57-71.
13. Ekama G A and G v R Marais (1985). Biological excess phosphorus removal in the activated sludge process in South Africa, gwfwasser/abwasser, H.5, 241-249. (in German: Zusätzliche biologische Phosphorelimination beim Belebungsverfahren-Erfahrungen in Suidafrika).
14. Marais G v R (1987). Future of biological removal of phosphorus from wastewater. Plenary address paper. Proceedings 12th Federal Convention, Australian Water and Wastewater Association, Adelaide, K18-K27.
15. Marais G v R and P L Dold (1987). Modelling of activated sludge systems for nutrient removal. Invited paper, Proceedings 12th Federal Convention, Australian Water and Wastewater Association, Adelaide, K32-44.

16. Lindrea K C, M P Peters and G A Ekama (1987). An evaluation of biological nitrogen and phosphorus removal at pilot scale for Bendigo unsettled sewage. Proceedings 12th Federal Convention, Australian Water and Wastewater Association, Adelaide, K138-K145.
17. Rabinowitz B, I Siebritz, G A Ekama and G v R Marais (1987). Phosphorus removal in activated sludge systems in(with) chemical addition. Proceedings IWPC biennial Conference, Port Elizabeth, May 1987.
18. Wentzel M C, P L Dold, R E Loewenthal, G A Ekama and G v R Marais (1987). Experiments towards establishing the kinetics of biological excess P removal. Presented at IAWPRC Specialized Conference on enhanced biological phosphate removal, Rome, September 1987. Advances in Water Pollution Control, 79-97, Pergamon press, Oxford.
19. Marais G v R and Dold P L (1987). Biological removal of carbon, nitrogen and phosphorus in single sludge systems. Invited paper, Seminar on Advances in biological treatment, Rome, November 1985, Istituto di Recherche sulle Acque/Consiglio Nazionale delle Ricerche.
20. Wentzel M C, L H Lötter, R E Loewenthal and G v R Marais (1986). Metabolic behaviour of *Acinetobacter* spp. in enhanced biological phosphorus removal - a biochemical model. Water SA, 12, 4, 209-224.
21. Lötter L H, M C Wentzel, R E Loewenthal, G A Ekama and G v R Marais (1986). A study of selected characteristics of *Acinetobacter* spp. isolated from activated sludge in anaerobic-anoxic-aerobic and aerobic systems. Water SA, 12, 4, 203-208.

III REPORTS

- W 50 Ekama G A and G v R Marais (1984) 'Preliminary Investigation for the Optimization of the Cape Flats Sewage Purification Works'
- W 51 Mellish F C S and G v R Marais (1984) 'An Investigation into an integrated Physical Chemical Biological Wastewater Treatment Process'
- W 52 Ekama G A (1984) Final report to the Water Research Commission on a four year contract on research into 'The optimization of the modified activated sludge process for nutrient removal (1980-1983)'
- W 56 Bagg W K, P L Dold and G v R Marais (1986) 'Verification of the bisubstrate concept in the activated sludge process'
- W 57 Dold P L, W K Bagg and G v R Marais (1986) 'Measurement of the readily biodegradable COD fraction in municipal wastewater by ultrafiltration'
- W 58 Burke R, P L Dold and G v R Marais (1986) 'Biological excess P removal in short sludge age activated sludge systems'

- W 59 Wentzel M C, G A Ekama, P L Dold, R E Loewenthal and G v R Marais (1988) 'Biological excess phosphorus removal in activated sludge systems'
- W 60 Wentzel M C, G A Ekama, P L Dold, R E Loewenthal and G v R Marais (1988) 'Final report to the Water Research Commission on a four year contract on research into biological excess phosphorus removal (1984-1987)'

CHAPTER ONE

INTRODUCTION

At the completion of the previous research contract in 1983,¹ kinetic modelling of carbon (COD) removal, nitrification and denitrification had been developed to a definitive level. The model was demonstrated to simulate accurately COD removal, nitrification and denitrification behaviour of all the known activated sludge systems, viz. completely mixed single and series reactor systems incorporating anaerobic, anoxic and aerobic reactors with any imposed recycles between the reactors, aerated lagoons and contact-stabilization under constant and cyclic flow and load conditions, for sludge ages ranging from 2,5 to 72 days, and anoxic-aerobic digestion of waste activated sludge for temperatures from 14 to 24° C, at laboratory, pilot or full scale.

With regard to P removal, at the time this contract commenced, the achievements in this area were not to the same level attained in COD removal, nitrification and denitrification - no kinetic model of biological excess phosphorus (P) removal (BEPR) had been developed. However, from a practical point of view, research up to 1983 had attained a considerable measure of success, viz:

- (1) Identification of the prerequisites to accomplish biological excess P removal in activated sludge systems, namely; presence of readily biodegradable COD in the influent; anaerobic/aerobic sequencing with the influent discharging to the anaerobic zone; protection of the anaerobic zone from nitrate entry.
- (2) Development of a number of activated sludge systems that incorporate the prerequisites for biological excess P removal, e.g. modified Bardenpho, UCT, AO/Phoredox systems.
- (3) Development of an empirical model to provide rough estimates of the concentration of P that could be removed by the system.
- (4) Development of guidelines whereby the most appropriate of the P removal

¹Final report to the Water Research Commission on a four year contract on research on the optimization of the modified activated sludge process for nutrient removal (1980-1983). Compiled by G A Ekama, Research Report W52, Dept of Civil Eng., Univ of Cape Town.

systems could be selected and the selected system could be designed and operated as effectively as the situation allowed.

In the empirical model, P removal was formulated in terms of some of the system parameters, such as anaerobic mass fraction, available readily biodegradable COD and active mass concentration. Organisms directly implicated in biological excess P removal did not feature and the P removal phenomenon was not linked to any basic biological or biochemical behaviour. As a consequence designs based on the model had a measure of reliability only within the range of conditions in which the model had been developed; indeed there was a measure of uncertainty even with designs within this range because the basic mechanisms underlying the behaviour were not understood.

To overcome these limitations, it became apparent that the research emphasis should change from the empirical to a more fundamental microbiological-biochemical one. In the 'empirical' approach the emphasis had been to study P removal in the system and to identify the conditions in the system that promote excess P removal; in the 'microbiological-biochemical' approach the emphasis would change to identifying and studying the microorganisms that mediate excess P removal and relating the characteristics and actions of these microorganisms to the response in the systems. Consequently, the principal objective of the research in this contract was to develop a microbiologically-biochemically based kinetic model for BEPR for incorporation in the general activated sludge kinetic model. In pursuance of this objective a number of projects were identified, viz:

- (1) Development of BEPR model in terms of polyP organism behaviour.
- (2) Performance of BEPR systems treating municipal waste waters over an extended range of influent COD concentrations and sludge ages.
- (3) Physical-chemical methods of measuring the readily biodegradable COD fraction in the influent.
- (4) Improvements to the general activated sludge kinetic model and integration of BEPR model in the general model.

Within this structure, as the investigation progressed new tasks were identified as being necessary to accomplish the main objective. To assist the reader in obtaining

an overall view of the work done, a flow diagram showing the tasks and their interactions is shown in Fig 1.1.

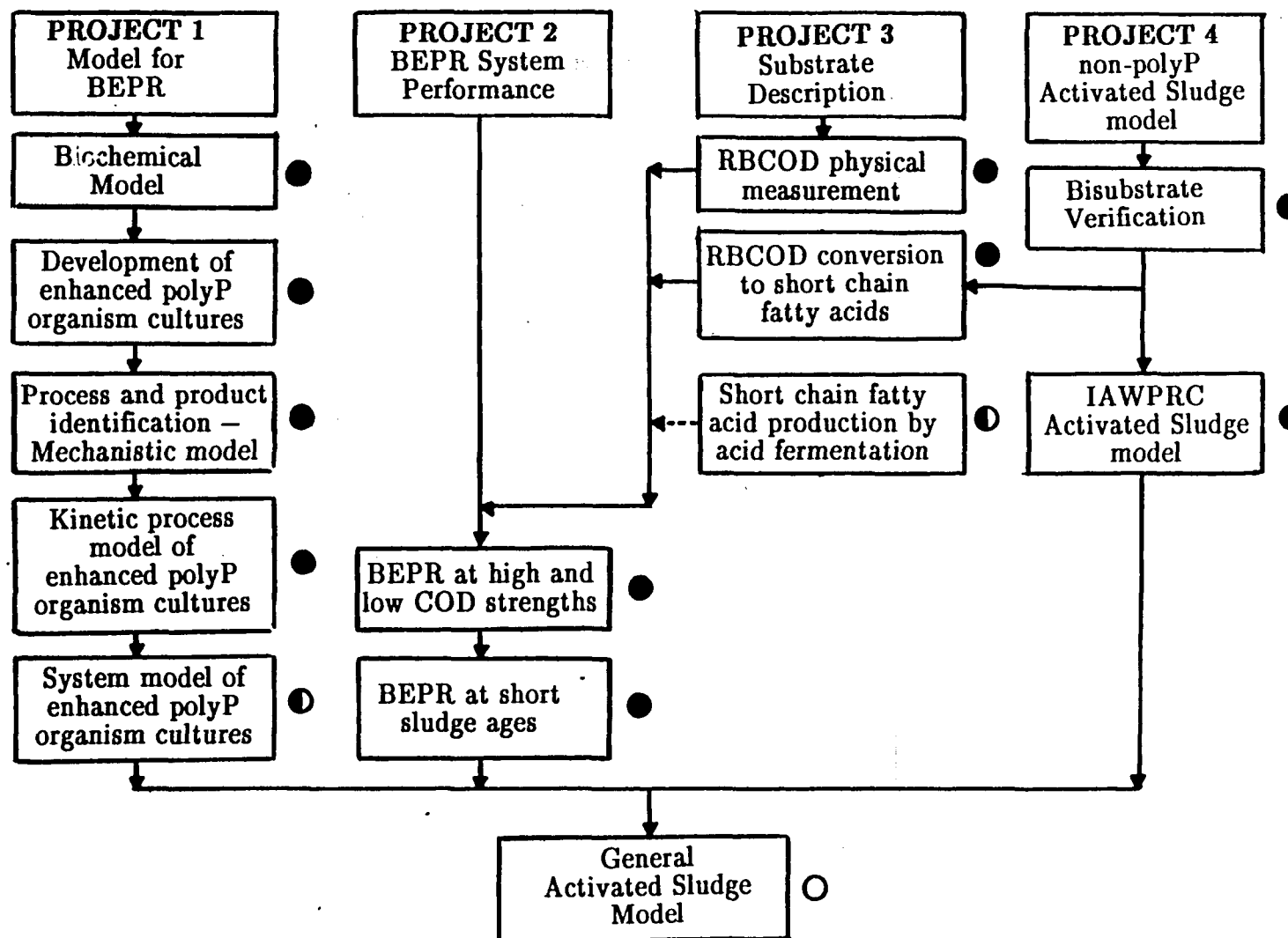


Fig 1.1: Flow diagram showing the research projects, their tasks that required completion and the interaction between these tasks.

CHAPTER TWO

DEVELOPMENT OF BIOLOGICAL EXCESS PHOSPHORUS REMOVAL MODEL (PROJECT 1)

This project developed into two main tasks:

- (1) Development of a biochemical model of polyP organism behaviour.
- (2) Elucidation of the kinetic behaviour of the polyP organism mass. This task itself developed into a number of sub-tasks:
 - (i) Development of an enhanced culture of polyP organisms.
 - (ii) Identification of the processes and the compounds formed or utilized in the enhanced polyP organism culture.
 - (iii) Mathematical formulation of kinetic behaviour of the processes in enhanced polyP organism cultures.
 - (iv) Modelling of the response of enhanced polyP organism culture systems.

1. BIOCHEMICAL MODEL

Investigations into the biochemical behaviour of polyP organisms were conducted to (1) determine the characteristics that give rise to P release and uptake and (2) determine the biochemical mechanisms whereby the release and uptake are mediated.

Acinetobacter strains were isolated from both anaerobic/anoxic/aerobic systems that exhibit excess P removal and completely aerobic systems that do not exhibit excess P removal. In the strains isolated differences were looked for in carbon utilization, carbon storage as PHB and phosphorus storage as polyP. Despite the apparent different selective pressures created in the two systems no significant differences between the *Acinetobacter* strains isolated from the systems were found. A number of strains isolated from the systems could utilize glucose aerobically as substrate; such utilization most likely was via the Entner–Duodoroff pathway, a pathway which operates only with oxygen and/or nitrate present. Also, *Acinetobacter* strains isolated from both systems had the propensity to accumulate polyphosphate (polyP) and polyhydroxybutyrate (PHB) under aerobic culture conditions, with acetate and with glucose as substrate. From this study it was concluded that imposing

conditions conducive to excess phosphorus removal in a system (by anaerobic/aerobic sequencing) does not appear to stimulate new *Acinetobacter* strains, but rather to stimulate the polyphosphate and polyhydroxybutyrate accumulating propensities inherent in strains already present (Lötter, Wentzel, Loewenthal, Ekama and Marais, 1986).

From the information collected, including that on the kinetic behaviour in enhanced cultures described below, a biochemical model was developed describing (1) the pathways whereby *Acinetobacter* spp. accumulate and degrade polyP and PHB and (2) the regulation of these pathways. The regulation appears to be principally via the ATP/ADP and NADH/NAD ratios. These ratios are affected by the intracellular (e.g. PHB) and/or extracellular (e.g. acetate) substrates and the presence or absence of external electron acceptors (O_2 , NO_3) (aerobic, anoxic or anaerobic). Identification of the regulatory systems allows prediction of the response of these organisms under a variety of imposed conditions and explains the observed behaviour of biological excess phosphorus removal as observed in plants and other situations. For example, the aerobic batch test for determining the readily biodegradable COD fraction, using mixed liquor samples from excess P removal plants always have given results smaller than those obtained from the short sludge age square wave tests (WRC 1984). The biochemical model predicts that this will be so because readily biodegradable COD storage, as PHB, can take place, to a degree, under aerobic conditions and *not only* under anaerobic conditions as is widely believed. As a consequence the conditions in the tests for measuring the readily biodegradable COD can be set down more precisely (Wentzel, Lötter, Loewenthal and Marais, 1986).

2. KINETIC BEHAVIOUR

There are principally two approaches to obtain information on the kinetic behaviour of the polyP organisms. The first approach is to hypothesize on the behaviour of the polyP organisms, incorporate this in a model, and, by simulation, obtain theoretically responses that conform with those observed. This approach was used to determine the kinetics of P release with municipal wastewater as influent (Wentzel, Dold, Ekama and Marais, 1985). In developing the kinetics of P *release*, quantification of the polyP organism mass is not necessary, because the rate limiting step is mediated by the non-polyP organisms. (This aspect is discussed again in Chapter 4, section 2).

With regard to developing kinetics for the *uptake* of P, the simulation approach

proved unproductive — the uptake kinetics could not be formulated without explicitly incorporating the growth and death characteristics of the polyP organisms. From the experimental data on 'normal' mixed culture activated sludge systems it was not possible to isolate these characteristics of the polyP organisms, because the response of the other heterotrophic organism either obscured or swamped out that of the polyP organisms for all the measurable parameters such as oxygen utilization rate, VSS etc., except P. Consequently the simulation approach was limited in the information it can provide on P uptake. For an improved understanding, of P uptake specifically and the excess P removal phenomena in general, a different approach needed to be taken.

The alternative approach was to develop a system in which the polyP organism behaviour dominates the system response. This would enable the processes making up the P removal phenomenon to be identified, and the stoichiometry and kinetics of the processes to be determined. There are two approaches to this: The organisms can be grown in (1) pure or (2) enhanced cultures. Analysis of the data in the literature on pure culture studies of *Acinetobacter* spp. (e.g. Abbott, Laskin and McCoy, 1973, Du Preez 1980) indicated that the responses of these organisms in pure cultures were very different from the responses observed in activated sludge systems. Instead, it was proposed that an *enhanced* culture may be more relevant and supply the required information. By enhanced culture is meant: Development of a polyP organism culture, by selecting a substrate and set of environmental conditions that favour polyP organisms, to the extent that these become the dominant primary organisms and dominate the culture response. Growth of competing normal heterotrophic organisms will be curtailed naturally but not deliberately terminated, neither will predation by higher organisms and other interactive effects be positively excluded. Also, a strain (or strains) of polyP organism will be naturally selected that may differ from that artificially selected and grown in pure cultures.

2.1 Development of enhanced culture of polyP organisms

From the biochemical model sets of conditions that give rise to excess P removal processes were identified. One such set is to subject the organism mass to an anaerobic/aerobic sequence with short chain fatty acids fed to the anaerobic phase; these conditions are present in the Phoredox, modified Bardenpho and UCT systems, with acetate as influent. By starting with 100 percent municipal wastewater as influent, incrementally decreasing the wastewater fraction and increasing the acetate fraction, enhanced cultures of polyP organisms were developed (Wentzel, Loewenthal, Ekama and Marais, 1988a).

The most striking features of the enhanced cultures are:

- (1) The specificity of the population structure; 90 percent of the organisms cultured aerobically were identified to be *Acinetobacter* spp. using the API procedure.
- (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, as against the usual 0,75–0,85 in activated sludge systems.
- (3) The magnitudes of the P release, uptake and removals. As an example, in the UCT system [10 day sludge age, influent COD (acetate) 500 mg/l] there was 253 mgP/l release (anaerobic + anoxic reactors), 314 mgP/l uptake (aerobic reactor) giving a net removal of 61 mgP/l.
- (4) The high filtered effluent COD concentration. All the substrate acetate was removed in the anaerobic and anoxic zones but a high, apparently unbiodegradable, COD was generated in the system, giving a net soluble effluent COD concentration of 65 mgCOD/l. This value is considerably higher than that expected for acetate feed to a normal aerobic activated sludge system, 15–20 mgCOD/l. The most likely reason is the high specificity of the organism assembly. Byproducts generated cannot be used by the specific organism mass of the enhanced culture, whereas in a mixed culture these are likely to serve as a substrate source for other species.
- (5) The low nitrate (NO₃) removal, about 11 mgN/l. Despite the fact that the influent was nearly totally readily biodegradable, and a high concentration of stored acetate (as PHB) was present in the anoxic zone, nitrate (NO₃) removal was poor, indicating that only a small fraction of the *Acinetobacter* spp. could use NO₃ as an electron acceptor. This is supported by the observation that in the enhanced culture systems usually no P was taken up in the anoxic reactors.
- (6) Utilization of NO₃ as a nitrogen source for growth. Nitrification was virtually complete in the first aerobic reactor; in the third aerobic reactor the NO₃ concentration decreased, indicating a utilization of NO₃ as a nitrogen source for synthesis.

2.2 Process and compound identification

In order to obtain information on the different polyP organism processes, e.g. acetate uptake, P release and uptake, growth rates, endogenous mass loss, etc. a series of batch tests was undertaken using mixed liquor from the enhanced culture systems. Five types of tests were undertaken; (1) aerobic digestion of the mixed liquor, (2) response with addition of acetate under anaerobic conditions, with (3) aerobic response thereafter, (4) anoxic response thereafter and (5) response with acetate addition under aerobic conditions. Some results obtained in these batch tests are shown in Figs 2.1 to 2.10.

Figs 2.1a,b,c:

Oxygen utilization rate (OUR) (2.1a), total soluble phosphate and nitrate concentrations (2.1b) and $\log(\text{OUR})$ versus time profiles measured in an aerobic batch digestion test on sludge harvested from the aerobic reactor of the enhanced polyP organism culture system.

Figs 2.2a,b,c:

Oxygen utilization rate (OUR) (2.2a), total soluble phosphate and nitrate concentrations (2.2b) and soluble COD and TKN concentration versus time profiles measured in an aerobic batch digestion test on sludge harvested from the aerobic reactor of the enhanced polyP organism culture system. Fig 2c shows the increase in soluble COD during the course of endogenous mass loss.

Fig 2.3:

Statistical plot of endogenous mass loss rates (b_G) measured in a number of aerobic batch digestion tests on the enhanced polyP organism culture. Note the mean rate is only 0,043/d. This low value is in contrast to that noted on mixed culture systems receiving sewage and suggests that the polyP organism mass is not subject to predation and is therefore largely independent of organism interaction.

Figs 2.4a,b,c:

Total soluble phosphate (2.4a,b,c), acetate (2.4a,b), TKN and nitrate (2.4c) 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 (0,11 mgCOD acetate/mgVSS) (2.4a) and medium (0,265 mgCOD acetate/mgVSS) (2.4b) and high (0,90 mgCOD acetate/mgVSS) (2.4c) acetate additions. Note two-phase P release (2.4b,c) and acetate uptake (2.4b) behaviour at medium (2.4b) and high (2.4c) acetate addition. Note also that the acetate uptake rate is zero order with respect to acetate.

Fig 2.5:

Statistical plot of first phase acetate uptake rates measured in anaerobic batch tests on the enhanced polyP culture. The mean acetate uptake rate is 6,7 mgHAc/mgVSS/d.

Fig 2.6:

Statistical plot of the stoichiometric ratio between P released and acetate taken up in the first phase of acetate uptake. The mean value is 1,1 mmol P/mmol HAc.

Fig 2.7a,b,c:

Total soluble phosphate and carbonaceous oxygen utilization rate (excluding nitrification) profiles versus time upon aeration under aerobic batch conditions following anaerobic addition of acetate at a dosage of 0,207 mgCOD HAc/mgVSS (medium) (2.7a), 0,363 mgCOD HAc/mgVSS (medium) (2.7b) when there is sufficient phosphate for uptake, and at a dosage of 0,22 mgCOD HAc/mgVSS (medium) (2.7c) when there is insufficient phosphate for uptake on the sludge harvested from the aerobic reactor of the enhanced polyP organism culture system. Note the constant high initial OUR at the greatest anaerobic acetate dosage (2.7b) and hence at the greatest internally stored PHB level, indicating PHB utilization follows a saturation type (like Monod) kinetics. Note also the sudden decrease in OUR when P becomes limiting (2.7c).

Fig 2.8a,b,c:

Carbonaceous oxygen utilization rate (OUR) (2.8a), soluble phosphate, nitrate and TKN concentration (2.8b) profiles versus time in an aerobic batch test following anaerobic acetate addition of 0,125 mgCOD acetate/mgVSS on sludge harvested from the aerobic reactor of the enhanced polyP organism culture. At low anaerobic acetate addition ($< 0,14$ mgCOD HAc/mgVSS) and sufficient P for uptake during subsequent aerobic conditions, the P uptake rate is first order as indicated by the log (P concentration-P minimum) versus time plot (2.8c) (P minimum = 48,3 mgP/l).

Fig 2.9a,b:

Total soluble phosphorus and COD concentrations (2.9a) and nitrate and nitrite concentrations (2.9b) measured under anoxic batch conditions after nitrate addition to sludge harvested from the anaerobic reactor of the enhanced culture system. The low denitrification indicates that the enhanced culture denitrified poorly.

Fig 2.10a,b:

Total soluble phosphate and nitrate concentrations (2.10a) and soluble COD and acetate concentrations and total oxygen consumption rate (OUR) profiles (2.10b) measured under aerobic batch conditions with aerobic addition of acetate at a dosage of 0,324 mgCOD HAc/mgVSS (medium) on sludge harvested from the aerobic reactor of the enhanced culture system. Note that P release takes place aerobically while acetate is present.

From the experimental studies on the enhanced cultures, (1) the compounds affected by biological excess P removal and (2) the processes that act on these compounds were identified, and (3) a mechanistic model was conceptualized that qualitatively describes the behaviour of the processes and compounds. A total of 12 compounds, associated with 13 processes, were identified as being directly involved with biological excess P removal. The processes that act on the compounds were grouped into three broad categories, (1) sequestration of acetate, (2) growth and (3) endogenous mass loss. A detailed conceptual mechanistic model of the processes, and their stoichiometric interaction with the compounds, was developed. (Wentzel, Dold, Loewenthal, Ekama and Marais, 1987 and Wentzel, Ekama, Loewenthal, Dold and Marais, 1988b).

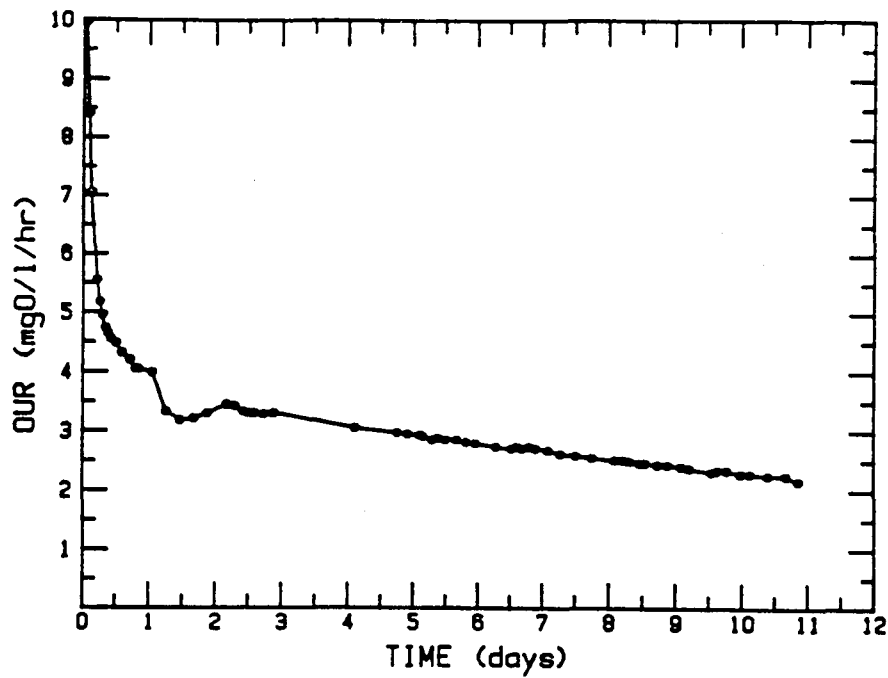


Fig 2.1a: Oxygen utilization rate (OUR) response versus time profile in an aerobic batch digestion test on mixed liquor harvested from the enhanced polyP organism culture system. MLVSS concentration = 2400 mgVSS/L

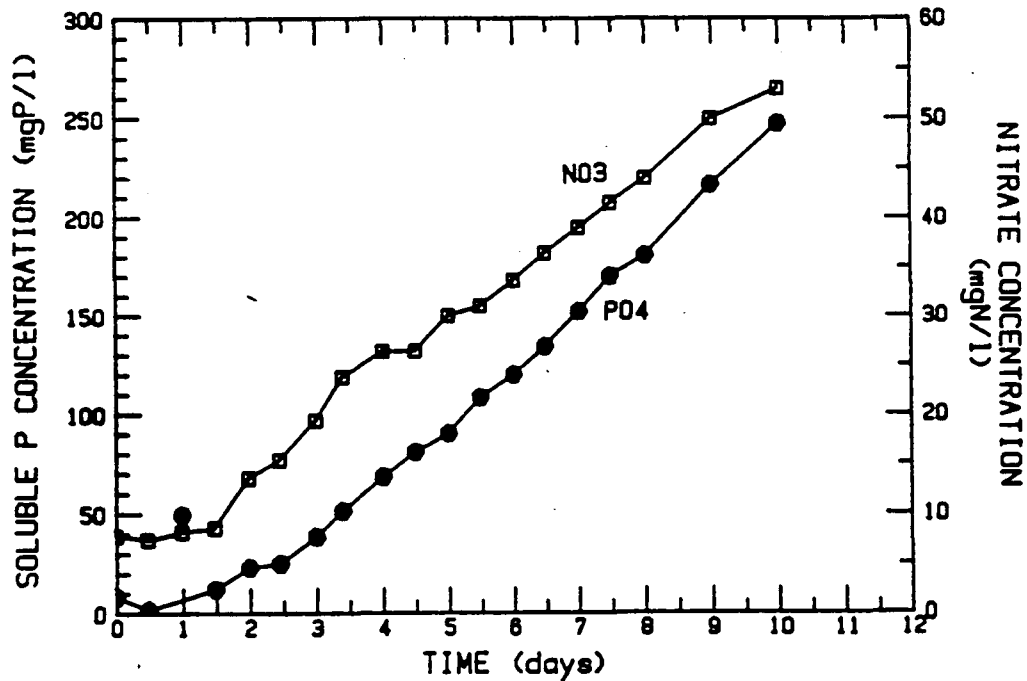


Fig 2.1b: Total soluble phosphate ($\text{PO}_4\text{-P}$) and nitrate ($\text{NO}_3\text{-N}$) concentration versus time profile for the aerobic batch digestion test in Fig 2.1a.

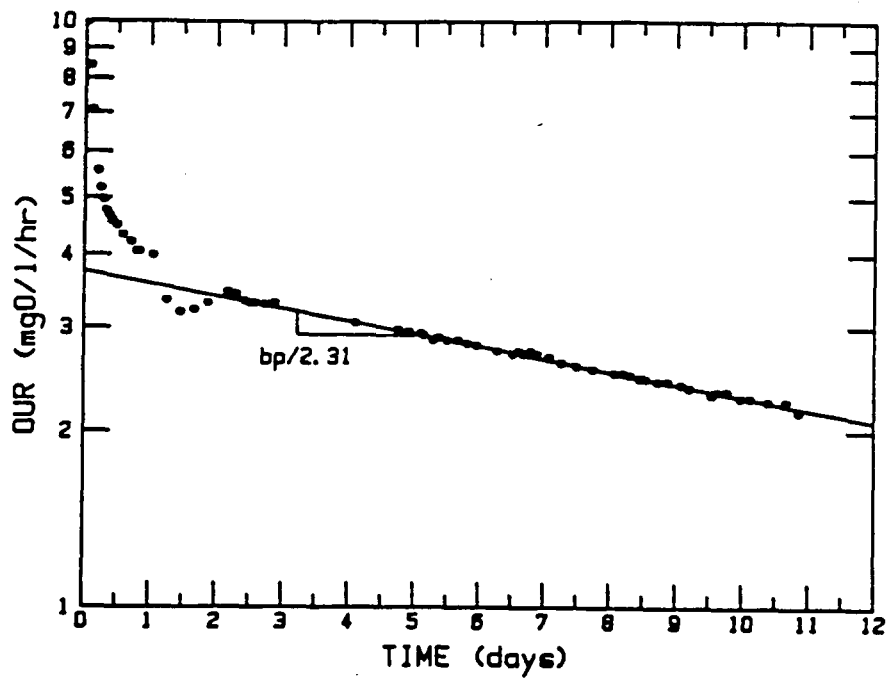


Fig 2.1c: Semi-log plot of the OUR versus time data shown in Fig 2.1c.

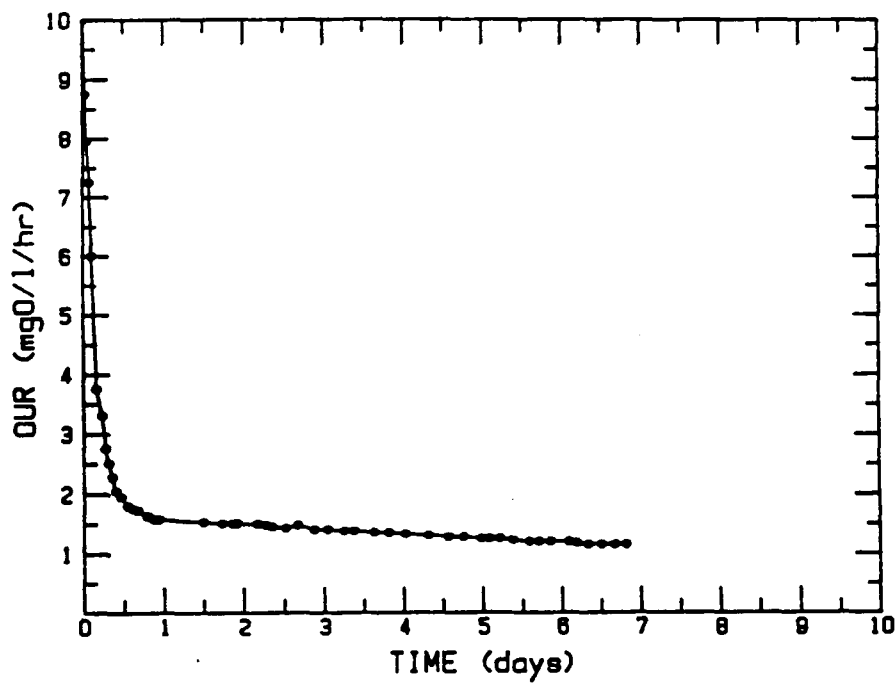


Fig 2.2a: Oxygen utilization rate (OUR) response versus time profile in an aerobic batch digestion test on mixed liquor harvested from the enhanced polyP organisms culture system. MLVSS concentration 1096 mgVSS/l .

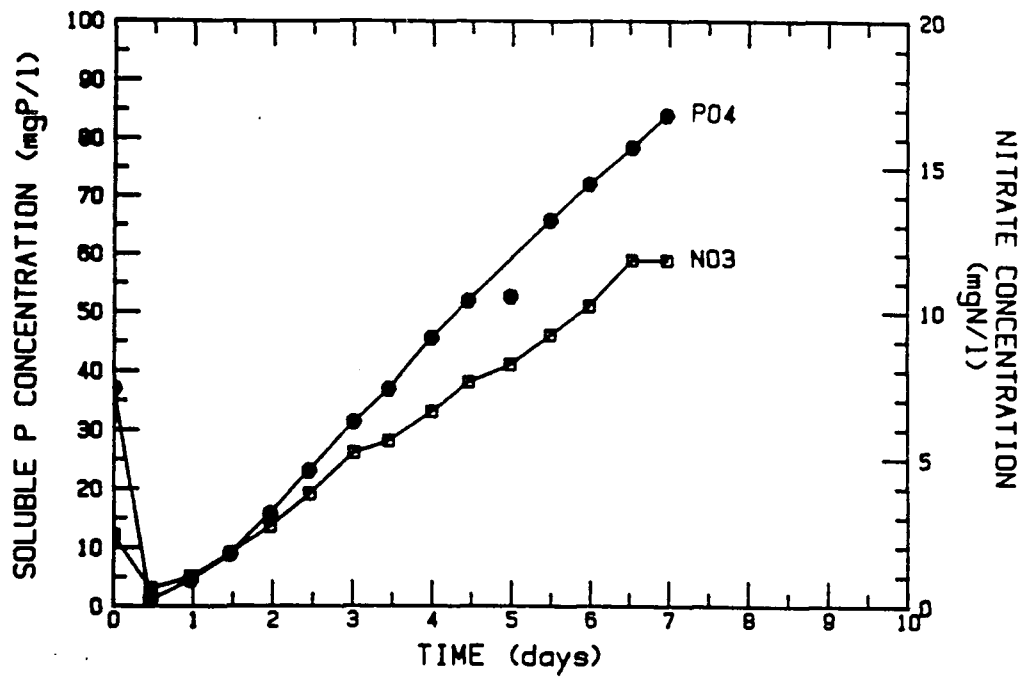


Fig 2.2b: Total soluble phosphate (PO₄-P) and nitrate (NO₃-N) concentration versus time profile for the aerobic batch digestion test in Fig 2.2a.

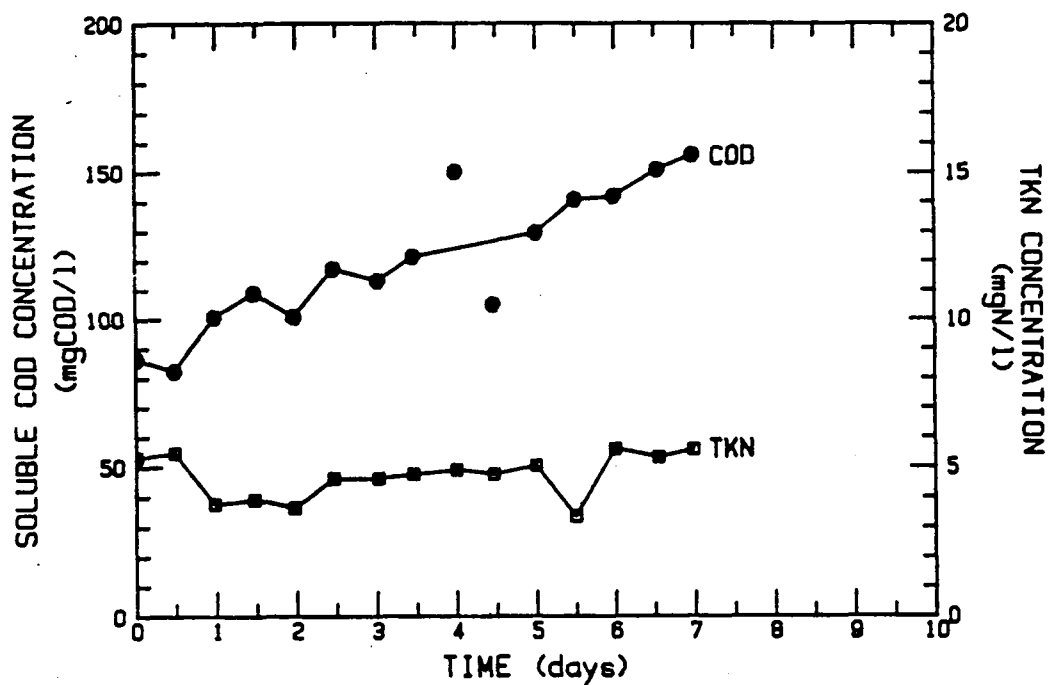


Fig 2.2c: Soluble COD and TKN concentration versus time profiles for the aerobic batch digestion test shown in Fig 2.2a.

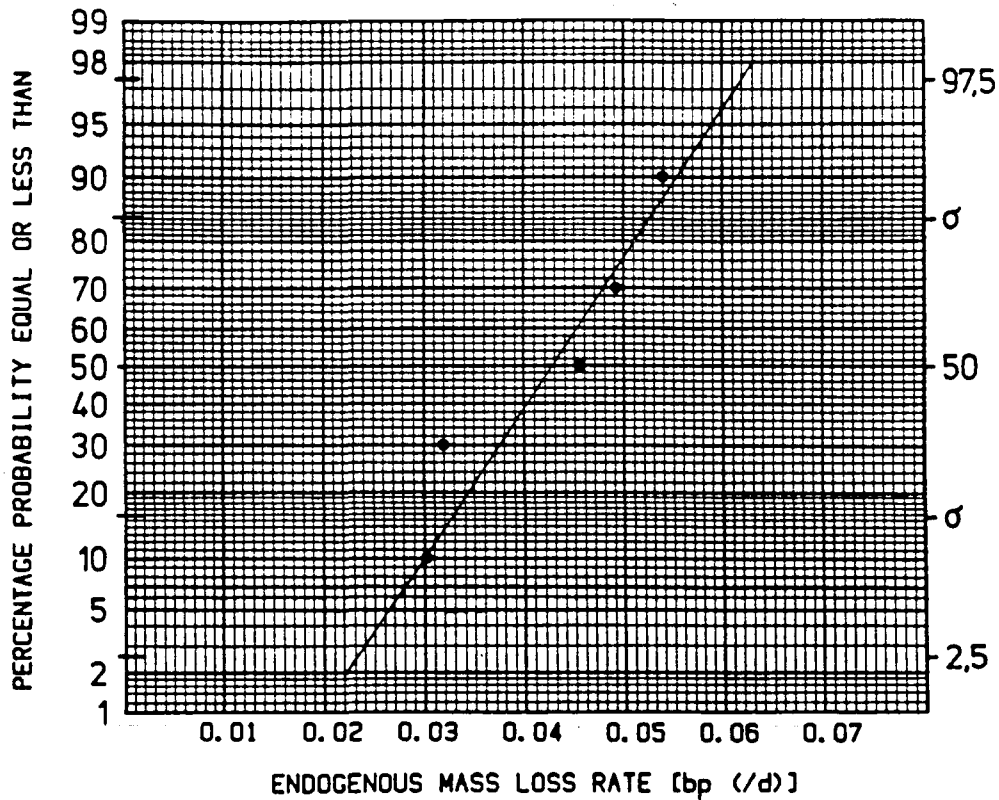


Fig 2.3: Statistical probability plot of 5 endogenous respiration rates measured in 5 aerobic batch digestion tests on the enhanced polyP organism culture. The mean rate is 0,043/d.

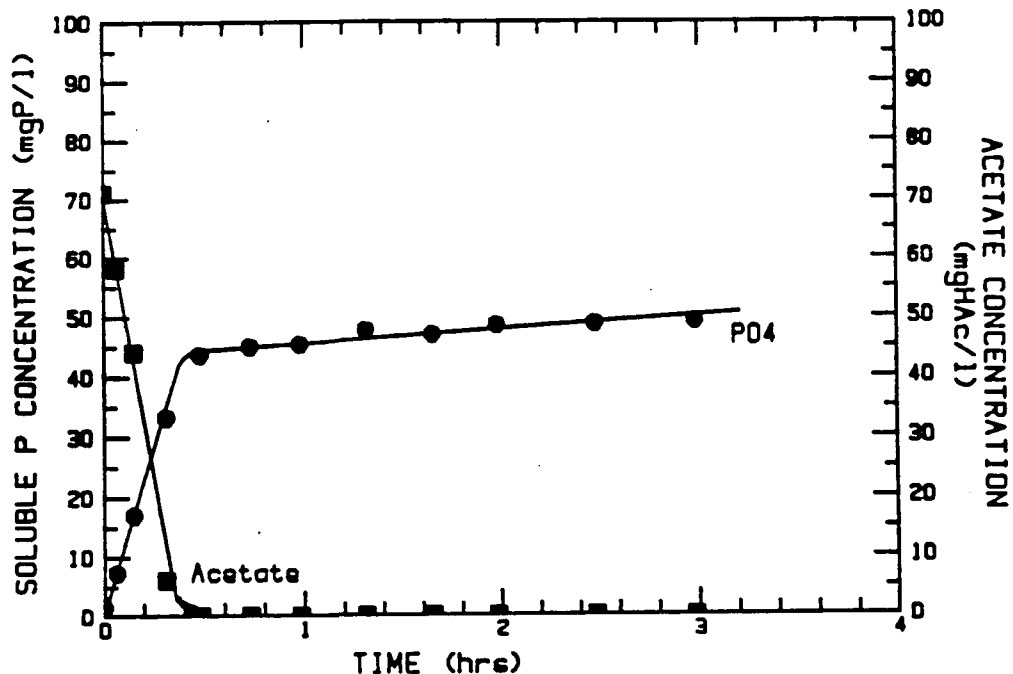
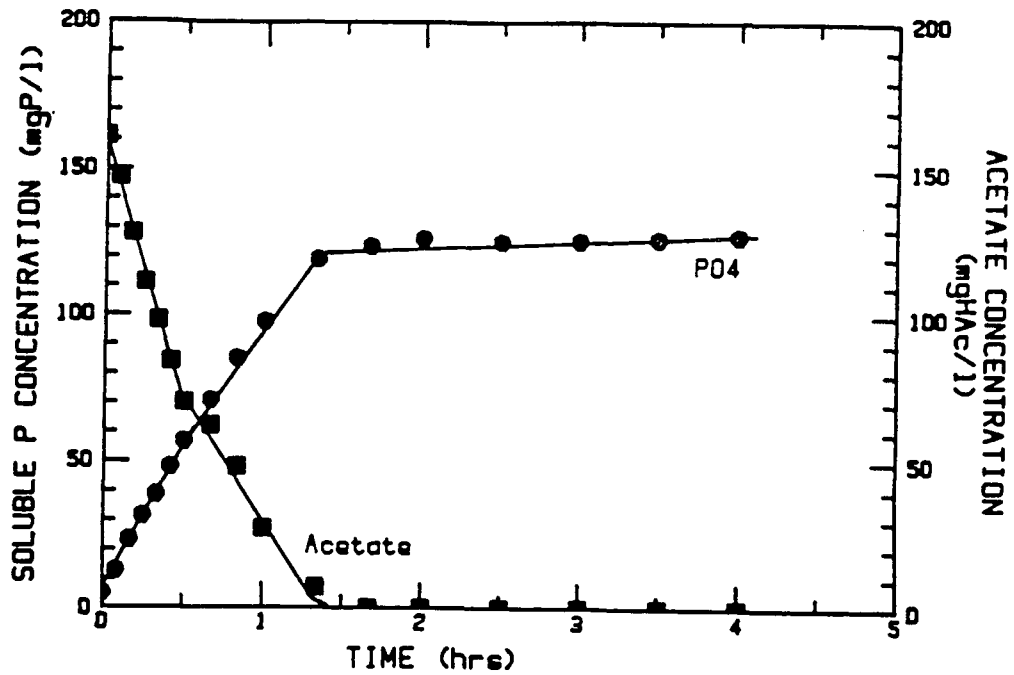
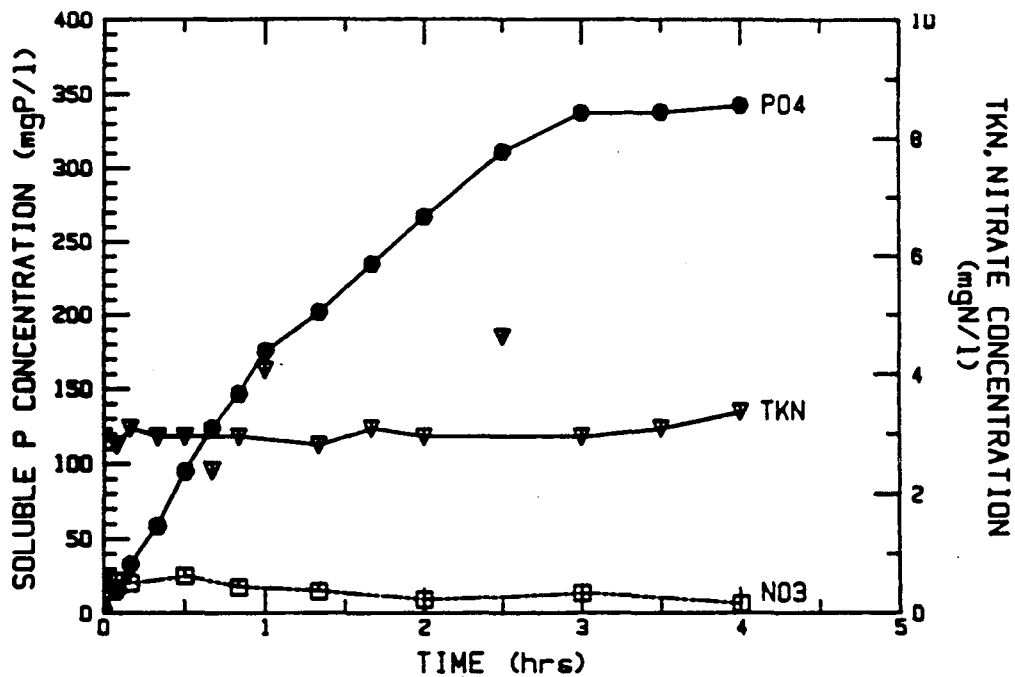


Fig 2.4a: Total soluble phosphate and acetate concentrations versus time profiles measured on an anaerobic batch test on enhanced polyP organism culture sludge at a low acetate loading rate of 0,11 mgCOD acetate/mgVSS. MLVSS = 684 mgVSS/l

**Fig 2.4b:**

Total soluble phosphate and acetate concentrations versus time profiles measured on an anaerobic batch test on enhanced polyP organism culture sludge at a medium acetate loading rate of 0,265 mgCOD acetate/mgVSS. MLVSS = 651 mgVSS/L

**Fig 2.4c:**

Total soluble phosphate, TKN and nitrate concentration versus time profiles measured in an anaerobic batch on enhanced polyP organism culture sludge at a high acetate loading rate of 0,90 mgCOD acetate/mgVSS.

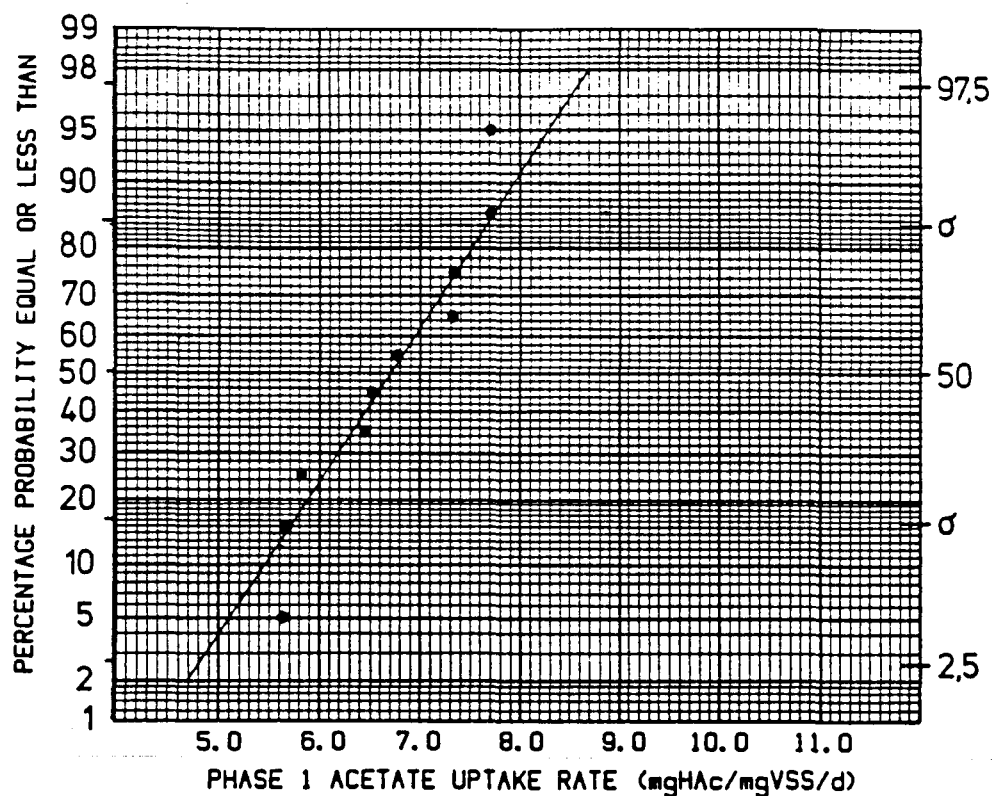


Fig 2.5: Statistical probability plot of the first phase of acetate uptake measured in a number of anaerobic batch tests on the enhanced polyP organism culture sludge.

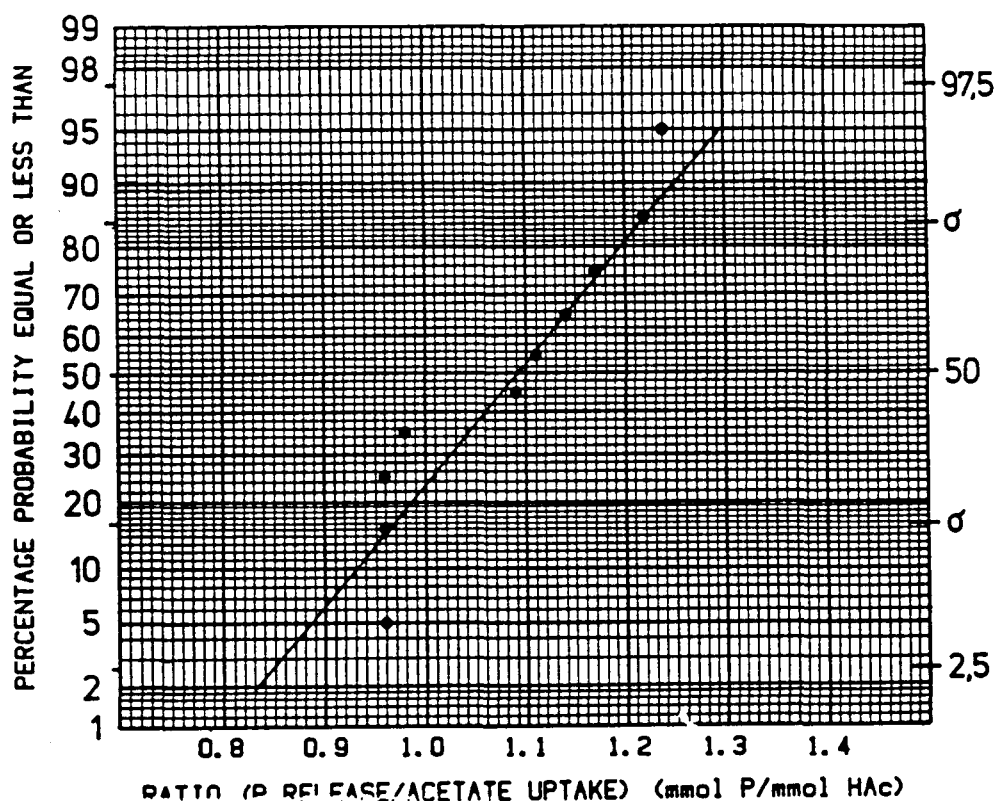


Fig 2.6: Statistical plot of the stoichiometric ratio between P released and acetate taken up in the first phase of acetate uptake for the anaerobic batch test data in Fig 2.5.

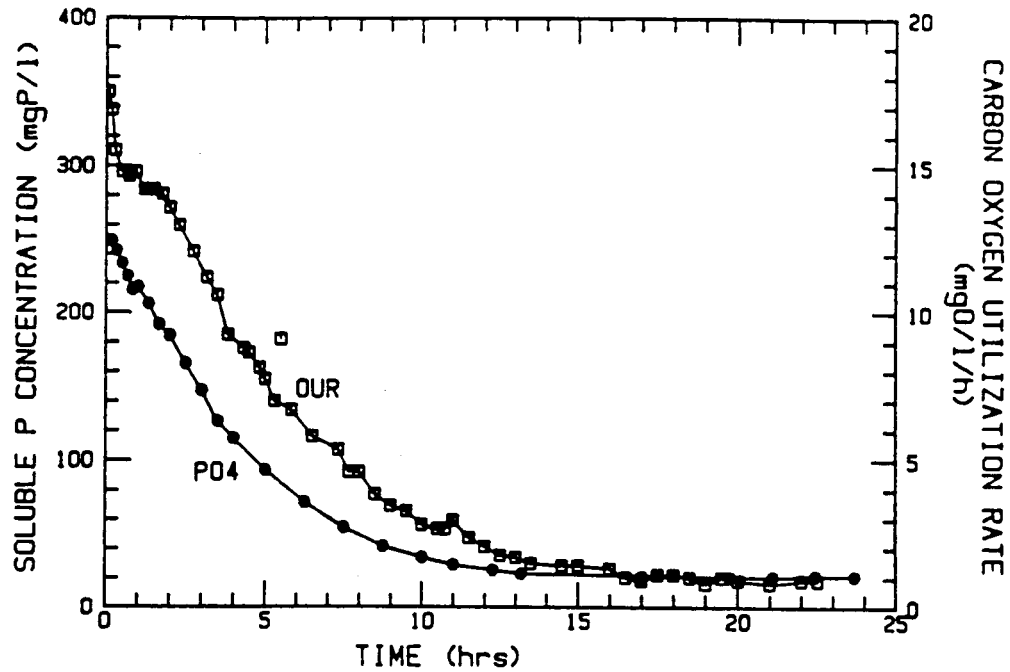


Fig 2.7a:

Total soluble phosphate ($\text{PO}_4\text{-P}$) and carbonaceous oxygen utilization rate (OUR_j) versus time profiles in an aerobic batch test on enhanced polyP organism culture sludge following anaerobic acetate addition at an acetate loading rate of 0,207 mgCOD acetate/mgVSS. The OUR decreases precipitously when the P concentration reduces to zero (MLVSS concentration = 1041 mgVSS/l).

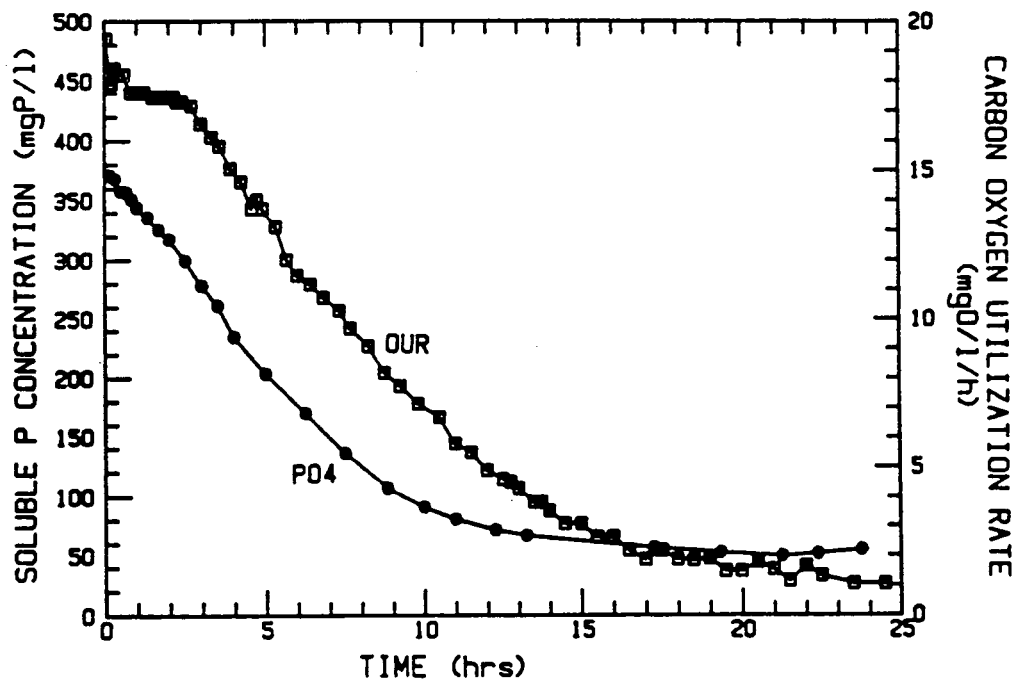


Fig 2.7b:

Total soluble phosphate ($\text{PO}_4\text{-P}$) and carbonaceous oxygen utilization rate (OUR_j) versus time profiles in an aerobic batch test on enhanced polyP organism culture sludge following anaerobic acetate addition at an acetate loading rate of 0,363 mgCOD acetate/mgVSS. The OUR decreases precipitously when the P concentration reduces to zero (MLVSS concentration = 1100 mgVSS/l).

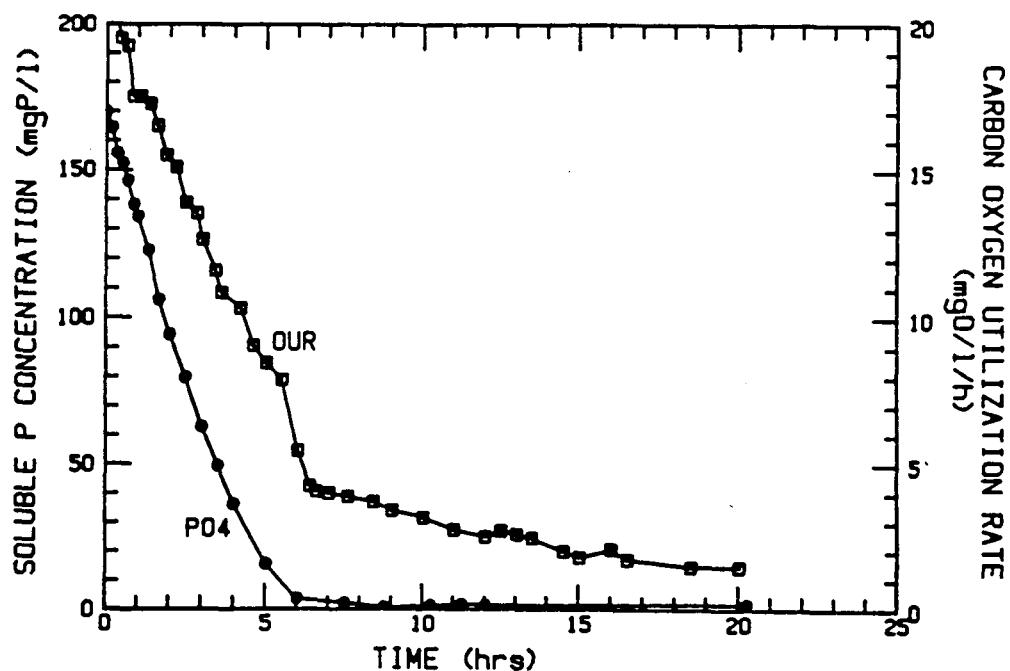


Fig 2.7c:

Total soluble phosphate ($\text{PO}_4\text{-P}$) and carbonaceous oxygen utilization rate (OUR_j) versus time profiles in an aerobic batch test on enhanced polyP organism culture sludge following anaerobic acetate addition at an acetate loading rate of 0,22 mgCOD acetate/mgVSS. The OUR decreases precipitously when the P concentration reduces to zero (MLVSS concentration = 1226 mgVSS/l).

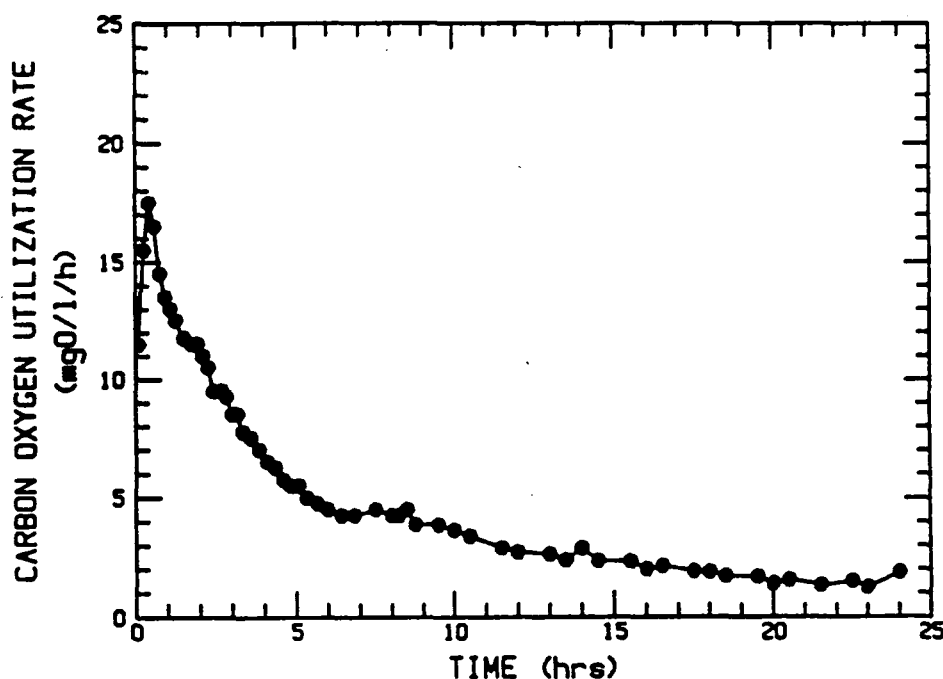


Fig 2.8a:

Carbonaceous oxygen utilization rate (OUR) versus time profile in an aerobic batch test on enhanced polyP organism culture sludge following anaerobic acetate addition at an acetate loading rate of 0,125 mgCOD acetate/mgVSS (MLVSS concentration = 1143 mgVSS/l).

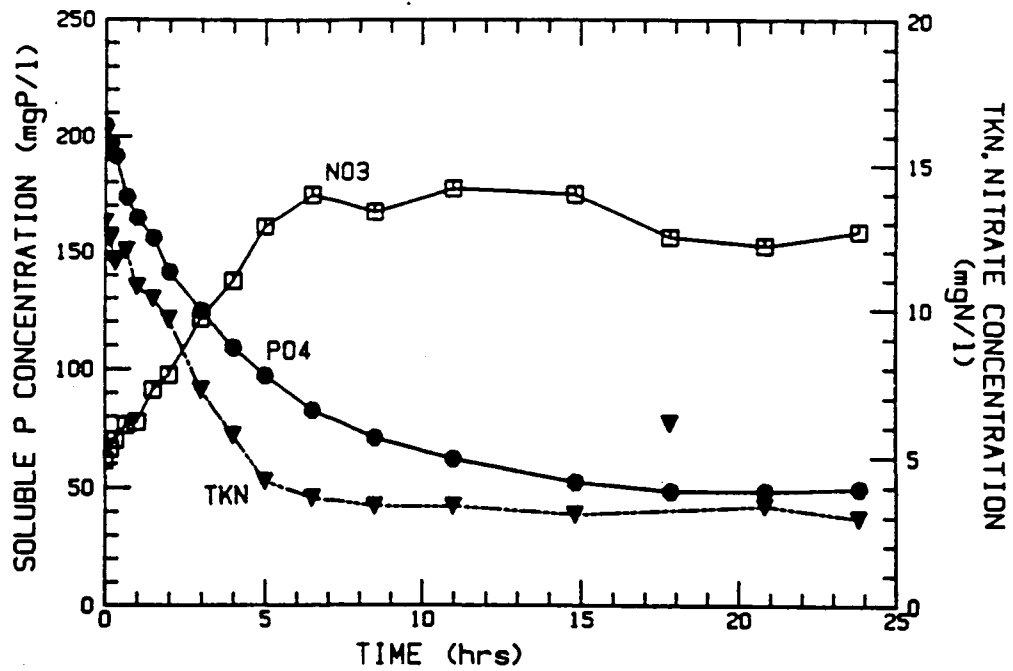


Fig 2.8b: Total soluble phosphate ($\text{PO}_4\text{-P}$), nitrate ($\text{NO}_3\text{-N}$) and TKN (TKN-N) concentration versus time profiles for the aerobic batch test in Fig 2.8a.

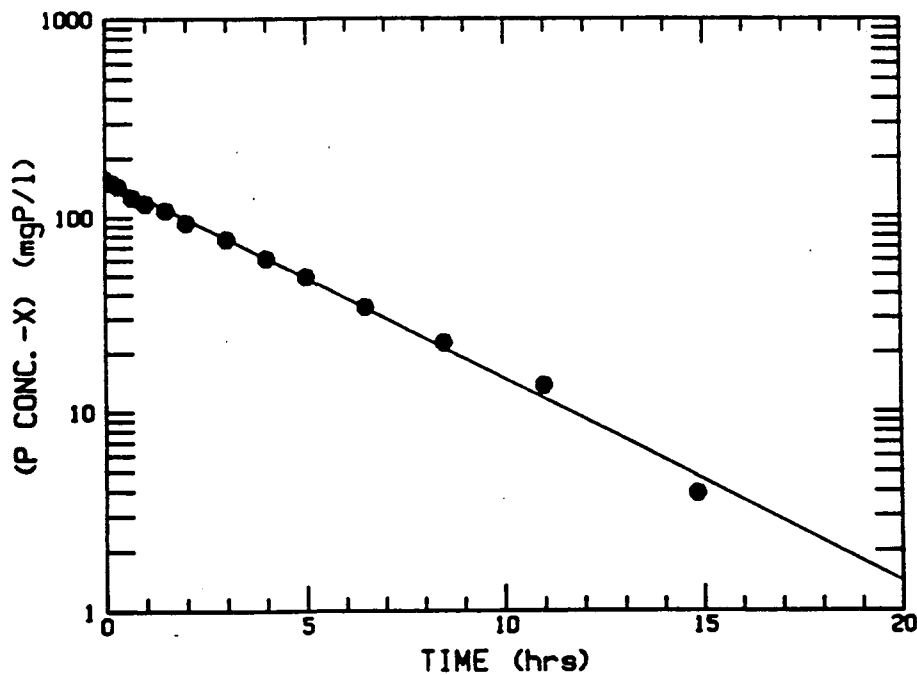


Fig 2.8c: Semi-log plot of total soluble phosphate ($\text{PO}_4\text{-P}$) concentration versus time for the aerobic batch test data in Fig 2.8b showing that the P uptake rate is first order with respect to P.

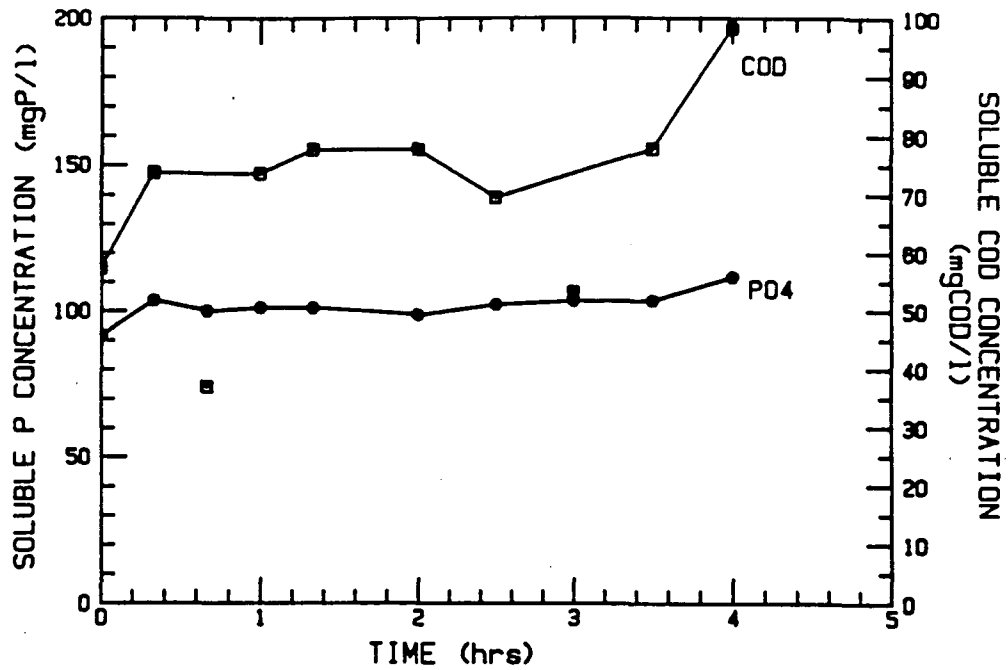


Fig 2.9a:

Total soluble phosphate ($\text{PO}_4\text{-P}$) and COD concentrations observed in an anoxic batch test on sludge harvested from the anaerobic reactor of the enhanced polyP organism culture system.

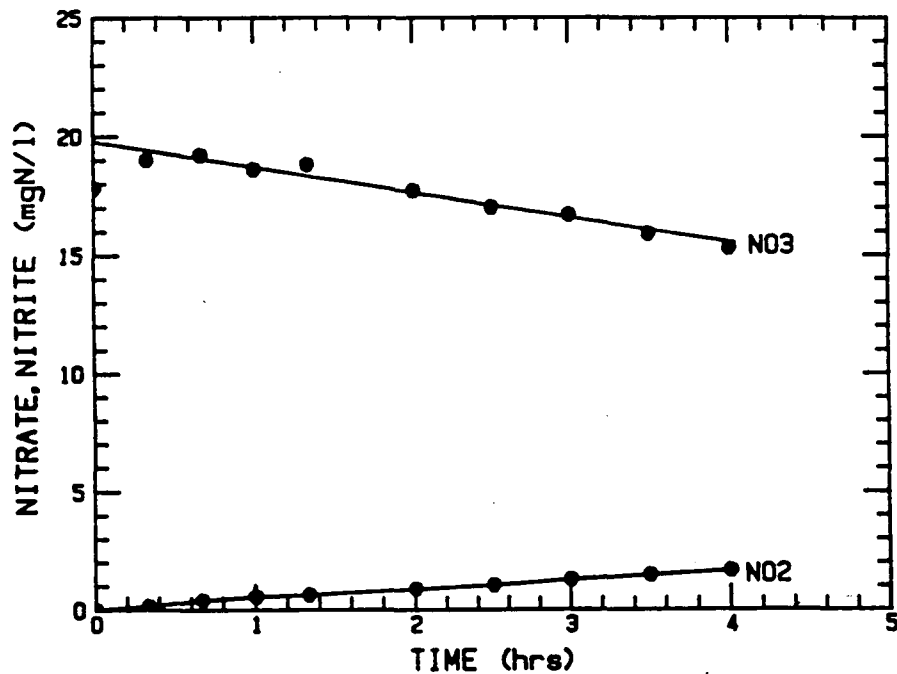


Fig 2.9b:

Nitrate ($\text{NO}_3\text{-N}$) and nitrite ($\text{NO}_2\text{-N}$) concentration versus time profiles for the anoxic batch test data in Fig 2.9a showing that the denitrification rate is low.

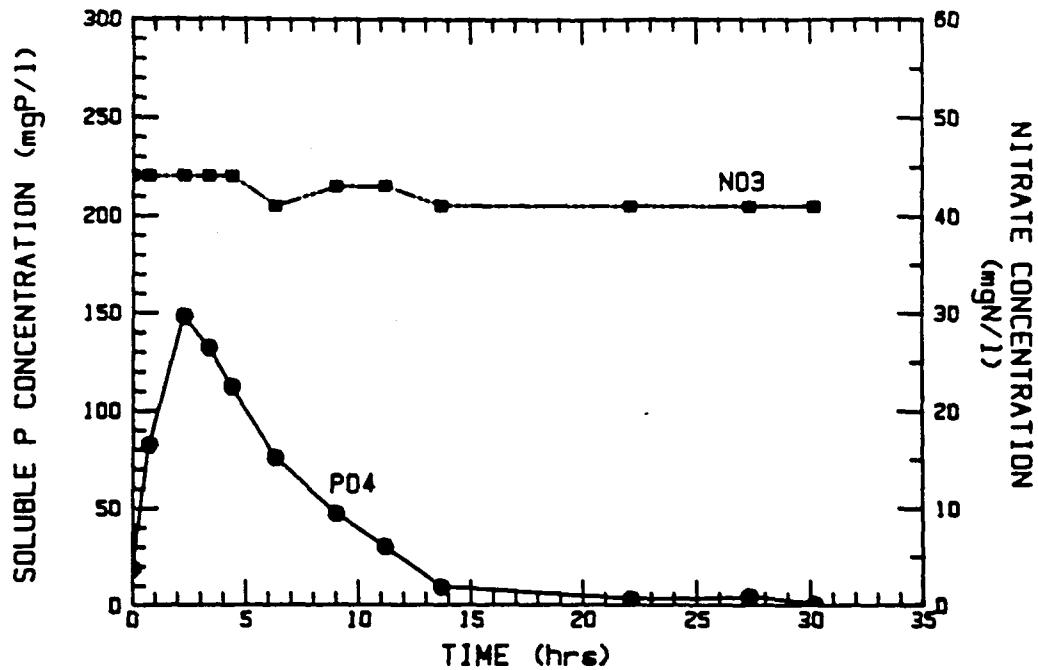


Fig 2.10a:

Total soluble phosphate ($\text{PO}_4\text{-P}$) and nitrate ($\text{NO}_3\text{-N}$) concentrations versus time profiles measured in an aerobic batch test on enhanced polyP organism culture sludge following *aerobic* acetate addition at an acetate loading rate of 0,324 mgCOD acetate/mgVSS. MLVSS concentration = 1080 mgVSS/l.

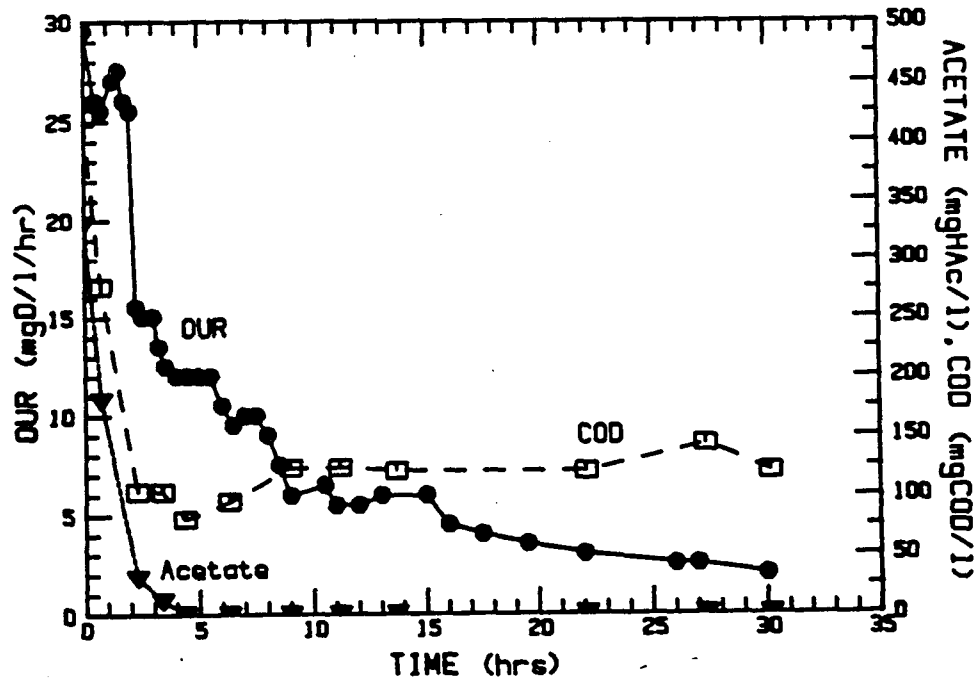


Fig 2.10b:

Oxygen utilization rate (OUR) and soluble COD and acetate concentration versus time profiles for the batch test in Fig 2.10a.

2.3 Kinetic model

The response of the enhanced cultures in the continuous steady state systems and in aerobic, anoxic and anaerobic batch tests, illustrated in Figs 2.1 to 2.10, formed the basis for formulating the kinetics of biological excess P removal and setting up a mathematical model describing this.

All the relevant process rate and stoichiometric information pertaining to the model is conveniently written in a matrix form. Such a matrix for the enhanced polyP organism culture is given in Table 2.1. The processes are listed in the rows and the compounds in the columns. At the relevant intersection points of the columns on the rows, the stoichiometric effect of a process (row) on one or more compounds (columns) is written, and the kinetic rate for the process is written down the right of the matrix. For example, uptake of acetate (row 13) increases the stored acetate, PHB, (column 5) by an amount equal to the reduction in the bulk solution acetate (column 8); hence row 13, columns 5 and 8 contain +1 and -1 respectively. The acetate uptake stimulates P release which reduces the internally stored polyP in proportion to the concentration of acetate taken up; the stoichiometric ratio being 0,50 mgP/mgCOD HAc (denoted $f_{P,rel1}$). Hence for 1 unit of acetate taken up, $f_{P,rel1}$ units of polyP are released which reduces the internally stored polyP (column 6) by $f_{P,rel1}$ and increases the bulk solution P (column 12) $f_{P,rel1}$. Consequently row 13, columns 6 and 12 contain $-f_{P,rel1}$ and $+f_{P,rel1}$ respectively. The kinetic rate for this reaction is given on the right of the matrix. Details of the matrix representation of the enhanced polyP organism culture model are given by Wentzel, Dold, Ekama and Marais, 1988c).

The calibration of the model required that the stoichiometric and kinetic constants be quantified. Essentially, there are three ways whereby this can be done: (1) From a test in which the constant is isolated and thus is directly determined. (2) From a test in which the constant is completely dominant compared to the effects of other constants and thus also is directly determined. (3) By "curve fitting", using ranges of system and batch operating conditions; this approach can be applied only if most of the other constants have been evaluated. The values determined for the various kinetic and stoichiometric constants by the three methods are listed in Tables 2.2, 2.3 and 2.4. For details of their determination see Wentzel *et al.* (1988c).

In model verification, the acceptability of the model is enhanced if, on applying the model in a range of situations, one finds consistency between observations and

Table 2.1: Process kinetics and stoichiometry for biological excess P removal.

COMPOUND	1	2	3	4	5	6	7	8	9	10	11	12	13	14	PROCESS RATE, μ_j
PROCESS	1	2	3	4	5	6	7	8	9	10	11	12	13	14	μ_j
1 Aerobic growth of $Z_{B,C}$ on S_{phb} with NH_3															$\mu_1 \left(\frac{S_{phb}/Z_{B,C}}{(K_{a1}) + S_{phb}/Z_{B,C}} \right) \left(\frac{NH_3}{(K_{N1}) + NH_3} \right) \left(\frac{P}{(K_{P1}) + P} \right) Z_{B,C}$
2 Aerobic growth of $Z_{B,C}$ on S_{phb} with NO_3															$\mu_2 \left(\frac{S_{phb}/Z_{B,C}}{(K_{a2}) + S_{phb}/Z_{B,C}} \right) \left(\frac{NH_3}{(K_{N2}) + NH_3} \right) \left(\frac{P}{(K_{P2}) + P} \right) Z_{B,C}$
3 J_1 if P_a limited															$\mu_3 \left(\frac{S_{phb}/Z_{B,C}}{(K_{a3}) + S_{phb}/Z_{B,C}} \right) \left(\frac{NH_3}{(K_{N3}) + NH_3} \right) \left(\frac{P}{(K_{P3}) + P} \right) Z_{B,C}$
4 J_2 if P_a limited															$\mu_4 \left(\frac{S_{phb}/Z_{B,C}}{(K_{a4}) + S_{phb}/Z_{B,C}} \right) \left(\frac{NH_3}{(K_{N4}) + NH_3} \right) \left(\frac{P}{(K_{P4}) + P} \right) Z_{B,C}$
5 Aerobic decay of $Z_{B,C}$															$\mu_5 \left(\frac{S_{phb}/Z_{B,C}}{(K_{a5}) + S_{phb}/Z_{B,C}} \right) \left(\frac{NH_3}{(K_{N5}) + NH_3} \right) \left(\frac{P}{(K_{P5}) + P} \right) Z_{B,C}$
6 Lysis of Polyp for J_5															$\mu_6 \left(\frac{S_{phb}/Z_{B,C}}{(K_{a6}) + S_{phb}/Z_{B,C}} \right) \left(\frac{NH_3}{(K_{N6}) + NH_3} \right) \left(\frac{P}{(K_{P6}) + P} \right) Z_{B,C}$
7 Lysis of S_{phb} for J_5															$\mu_7 \left(\frac{S_{phb}/Z_{B,C}}{(K_{a7}) + S_{phb}/Z_{B,C}} \right) \left(\frac{NH_3}{(K_{N7}) + NH_3} \right) \left(\frac{P}{(K_{P7}) + P} \right) Z_{B,C}$
8 Anaerobic decay of $Z_{B,C}$															$\mu_8 \left(\frac{S_{phb}/Z_{B,C}}{(K_{a8}) + S_{phb}/Z_{B,C}} \right) \left(\frac{NH_3}{(K_{N8}) + NH_3} \right) \left(\frac{P}{(K_{P8}) + P} \right) Z_{B,C}$
9 Lysis of Polyp for J_8															$\mu_9 \left(\frac{S_{phb}/Z_{B,C}}{(K_{a9}) + S_{phb}/Z_{B,C}} \right) \left(\frac{NH_3}{(K_{N9}) + NH_3} \right) \left(\frac{P}{(K_{P9}) + P} \right) Z_{B,C}$
10 Lysis for S_{phb} for J_8															$\mu_{10} \left(\frac{S_{phb}/Z_{B,C}}{(K_{a10}) + S_{phb}/Z_{B,C}} \right) \left(\frac{NH_3}{(K_{N10}) + NH_3} \right) \left(\frac{P}{(K_{P10}) + P} \right) Z_{B,C}$
11 Anaerobic cleavage of Polyp for maintenance															$\mu_{11} \left(\frac{S_{phb}/Z_{B,C}}{(K_{a11}) + S_{phb}/Z_{B,C}} \right) \left(\frac{NH_3}{(K_{N11}) + NH_3} \right) \left(\frac{P}{(K_{P11}) + P} \right) Z_{B,C}$
12 Conversion $S_{B,C}$ to $S_{B,A}$															$\mu_{12} \left(\frac{S_{phb}/Z_{B,C}}{(K_{a12}) + S_{phb}/Z_{B,C}} \right) \left(\frac{NH_3}{(K_{N12}) + NH_3} \right) \left(\frac{P}{(K_{P12}) + P} \right) Z_{B,C}$
13 Sequestration of $S_{B,A}$															$\mu_{13} \left(\frac{S_{phb}/Z_{B,C}}{(K_{a13}) + S_{phb}/Z_{B,C}} \right) \left(\frac{NH_3}{(K_{N13}) + NH_3} \right) \left(\frac{P}{(K_{P13}) + P} \right) Z_{B,C}$
14 Aerobic growth of Autotrophs															$\mu_{14} \left(\frac{S_{phb}/Z_{B,C}}{(K_{a14}) + S_{phb}/Z_{B,C}} \right) \left(\frac{NH_3}{(K_{N14}) + NH_3} \right) \left(\frac{P}{(K_{P14}) + P} \right) Z_{B,C}$
15 Decay of Autotrophs															$\mu_{15} \left(\frac{S_{phb}/Z_{B,C}}{(K_{a15}) + S_{phb}/Z_{B,C}} \right) \left(\frac{NH_3}{(K_{N15}) + NH_3} \right) \left(\frac{P}{(K_{P15}) + P} \right) Z_{B,C}$
$*A = -f_{ZB,C,P} - f_{P,upt}/Y_C$ $*B = -f_{ZB,C,P} - f_{P,upt}/Y_C$ $*C = f_{ZB,C,N} - f_{ZP,C} f_{ZEG,N} - f_{ZB,C} f_{ZEG,N}$ $*D = f_{ZB,C} f_{ZEG,N}$ $*E = f_{ZB,C,P} - f_{ZP,C} f_{ZEG,P}$ $*F = -(1-f_{ZP,C} - f_{ZB,C})$ $*G = f_{ZB,C,N} - (f_{ZP,C} f_{ZEG,N} + f_{ZB,C} f_{ZEG,N})$ $*H = f_{ZB,C} f_{ZEG,N}$															
Biological active Autotrophic mass - $M(COD)L^{-3}$															
Biological active polyP mass - $M(COD)L^{-3}$															
Endogenous mass - $M(COD)L^{-3}$															
Emmeshed slowly biodegradable substrate - $M(COD)L^{-3}$															
Stored PHB - $M(COD)L^{-3}$															
Stored polyP - $M(P)L^{-3}$															
Readily biodegradable "complex" substrate - $M(COD)L^{-3}$															
Readily biodegradable "acetate" substrate - $M(COD)L^{-3}$															
Ammonia nitrogen - $M(N)L^{-3}$															
Degradable soluble organic nitrogen - $M(N)L^{-3}$															
Nitrate nitrogen - $M(N)L^{-3}$															
Soluble phosphate - $M(P)L^{-3}$															
Unbiodegradable soluble substrate - $M(COD)L^{-3}$															
Oxygen-negative COD - $M(COD)L^{-3}$															
$*I = f_{ZB,C,P} (1-f_{EP,C})$ $*J = \frac{4.57 - Y_A}{Y_A}$ $*K = f_{ZB,A,N} - f_{EP,A} f_{ZEA,N}$ $*L = f_{ZB,A,P} - f_{EP,A} f_{ZEA,P}$															

Table 2.2: Description and values of stoichiometric constants in the matrix (Table 2.1).

Constant		Range of Values	Units
Symbol	Description		
A - POLYP ORGANISMS			
Y_G	Yield	0,639	mgCOD volatile mass/mgCOD
$f_{P,upt}$	Ratio P uptake/COD stored substrate utilized	0,9-1,1	mgP/mgCOD
$f_{ZBG,N}$	Nitrogen content of active mass	0,07	mgN/mgCOD active mass
$f_{ZEG,N}$	Nitrogen content of endogenous mass	0,07	mgP/mgCOD active mass
$f_{EsG,N}$	Nitrogen content of soluble unbiodegradable COD	0,07	mgN/mgCOD
$f_{ZBG,P}$	Phosphorus content of active mass (excluding polyP)	0,021	mgP/mgCOD active mass
$f_{ZEG,P}$	Phosphorus content of endogenous mass	0,021	mgP/mgCOD endogenous mass
$f_{Ep,G}$	Fraction of active mass that remains as particulate unbiodegradable residue	0,25	mgCOD endogenous mass/mgCOD active mass
$f_{Es,G}$	Fraction of active mass that remains as soluble unbiodegradable residue	0,20	mgCOD/mgCOD active mass
$f_{P,rel1}$	Ratio P release/acetate uptake for phase 1 sequestration	0,48-0,55	mgP/mgCOD
$f_{P,rel2}$	Ratio P release/acetate uptake for phase 2 sequestration	0,8-1,0	mgP/mgCOD
PSwitch	Changes acetate sequestration from phase 1 to phase 2, i.e. $f_{P,rel1}$ to $f_{P,rel2}$ and K_{p1} to K_{p2}	0,32	mgP/mgCOD active mass
f_{cv}	Ratio COD/VSS	1,42	mgCOD/mgVSS
B - AUTOTROPHS (NITRIFIERS)			
Y_A	Yield	0,15	mgCOD volatile mass/mgCOD
$f_{ZBA,N}$	Nitrogen content of active mass	0,068	mgN/mgCOD active mass
$f_{ZEA,N}$	Nitrogen content of endogenous mass	0,068	mgN/mgCOD endogenous mass
$f_{ZBA,P}$	Phosphorus content of active mass	0,02	mgP/mgCOD active mass
$f_{ZEA,P}$	Phosphorus content of endogenous mass	0,02	mgP/mgCOD endogenous mass
$f_{Ep,A}$	Fraction of active mass that remains as particulate unbiodegradable residue	0,08	mgCOD endogenous mass/mgCOD active mass

Table 2.3: Description and values of kinetic constants in the matrix (Table 2.1).

Symbol	Constant Description	Range of Values	Units
A - POLYP ORGANISMS			
μ_{G1}	Maximum specific growth rate with no soluble P limit	0,9-1,1	/d
μ_{G2}	Maximum specific growth rate with soluble P limit	0,42	/d
K_{sG1}	Growth rate half saturation coefficient with no soluble P limit	0,18	mgCOD/l
K_{sG2}	Growth rate half saturation coefficient with soluble P limit	0,18	mgCOD/l
b_G	specific endogenous mass loss rate	0,03-0,04	/d
b_{pp}	specific polyP cleavage rate for anaerobic "maintenance" energy generation	0,03	/d
K_c	Specific rate for conversion of "complex" readily biodegradable COD to short chain acids	0,04	mgCOD/mgCOD active mass/d
K_{p1}	Specific rate of acetate uptake for first phase sequestration	6,0	mgCOD/mgCOD active mass/d
K_{p2}	Specific rate of acetate uptake for second phase sequestration	2,6	mgCOD/mgCOD active mass/d
B - AUTOTROPHS (NITRIFIERS)			
μ_A	Maximum specific growth rate	$\pm 0,35$	/d
K_{NH}	Growth rate half saturation coefficient	1,0	mgN/l
b_A	Specific decay rate	0,04	/d

Table 2.4: Switching functions used in the matrix (Table 2.1).

Abbreviation	Formulation	Value of half saturation coefficient (K)
Air on	$\frac{O}{K_{OH} + O}$	$K_{OH} = 0,002 \text{ (mgO/l)}$
Air off	$\frac{K_{OH}}{K_{OH} + O}$	
NH ₃ limit	$\frac{N_{h3}}{K_{NA} + N_{h3}}$	$K_{NA} = 0,05 \text{ (mgN/l)}$
NO ₃ limit	$\frac{N_{o3}}{K_{NO} + N_{o3}}$	$K_{NO} = 1,0 \text{ (mgN/l)}$
P limit	$\frac{P}{K_{LP} + P}$	$K_{LP} = 0,1 \text{ (mgP/l)}$
PolyP limit	$\frac{P_{polyP}}{K_{xp} + P_{polyP}}$	$K_{xp} = 1,0 \text{ (mgP/l)}$
Ac limit	$\frac{S_{bs,a}}{K_{SSEQ} + S_{bs,a}}$	$K_{SSEQ} = 1,0 \text{ (mgCOD/l)}$

predictions. In the enhanced culture model the predicted behaviour of various compounds compared remarkably well with those observed in the different batch tests. This can be seen in Figs 2.11a,b and c, 2.12a and b and 2.13a, b and c in which the experimental data shown in Figs 2.2a, b and c, 2.4a and b and 2.7a, b and c are simulated. Even though the same set of tests was used for evaluating the constants as and verifying the model, the closeness with which the predictions conform to the observations over the wide range of conditions in the tests constitutes powerful evidence for the acceptability of the model. When considering the complexity of biological excess P removal behaviour the behaviour predicted by the model can be seen to conform remarkably well to the observed data.

The model developed is specific to anaerobic/aerobic behaviour of enhanced cultures, receiving acetate only as substrate; it does not include anoxic behaviour principally for reason that the enhanced cultures showed minimal denitrification response and it was not possible therefore to quantify denitrification behaviour. In the absence of experimental results, empirical denitrification behaviour will have to be incorporated in the model. Incorporation of the enhanced culture model in mixed culture behaviour receiving municipal sewage will require modelling the interactions between the non-polyP and polyP organisms. This is not expected to present serious difficulties; the polyP and non-polyP cultures appear to have little interaction so that 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 readily biodegradable COD by non-polyP organisms. This interaction is already included in the enhanced culture model.

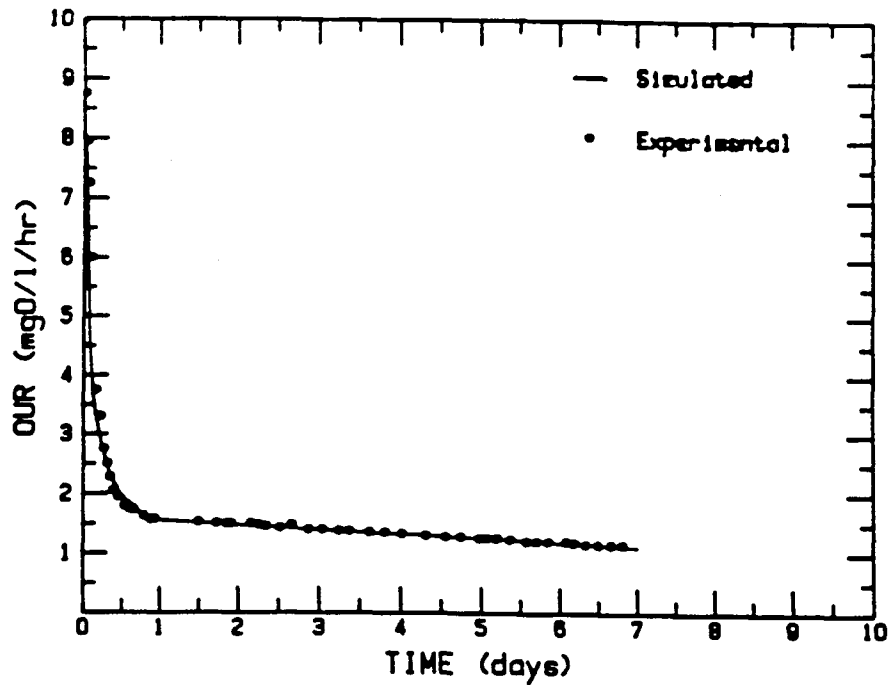


Fig 2.11a: Experimentally observed and theoretically simulated oxygen utilization rate (OUR) versus time profiles for the aerobic batch digestion test data shown in Fig 2.2a.

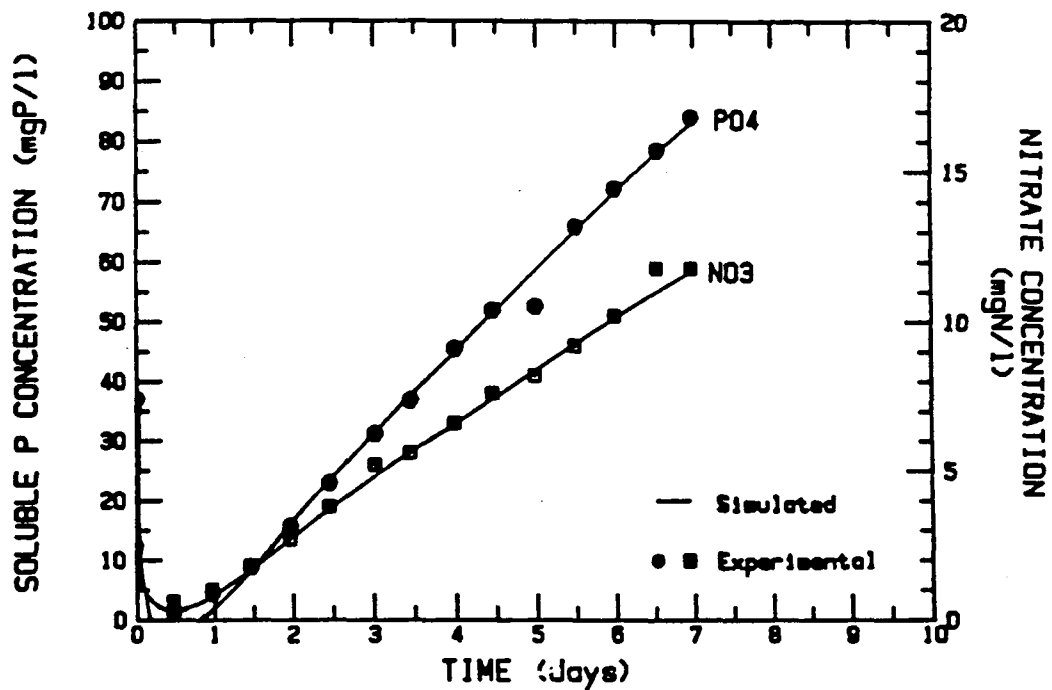


Fig 2.11b: Experimentally observed and theoretically simulated total soluble phosphate ($\text{PO}_4\text{-P}$) and nitrate ($\text{NO}_3\text{-N}$) concentration profiles for the aerobic batch digestion test data shown in Fig 2.2b.

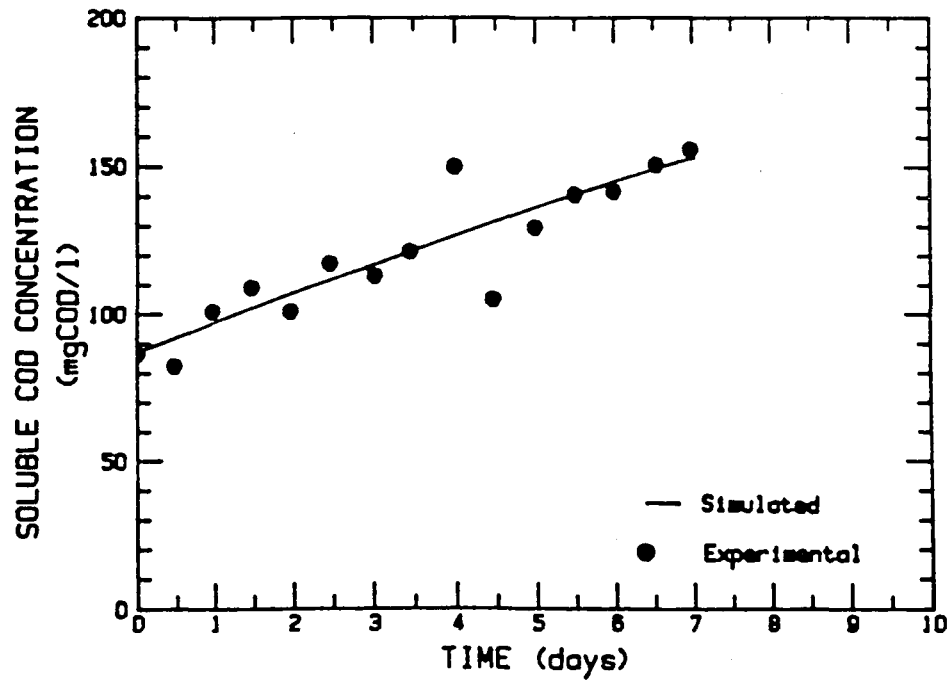


Fig 2.11c: Experimentally observed and theoretically simulated filtered COD concentration versus time profiles for the aerobic batch digestion test data shown in Fig 2.2c.

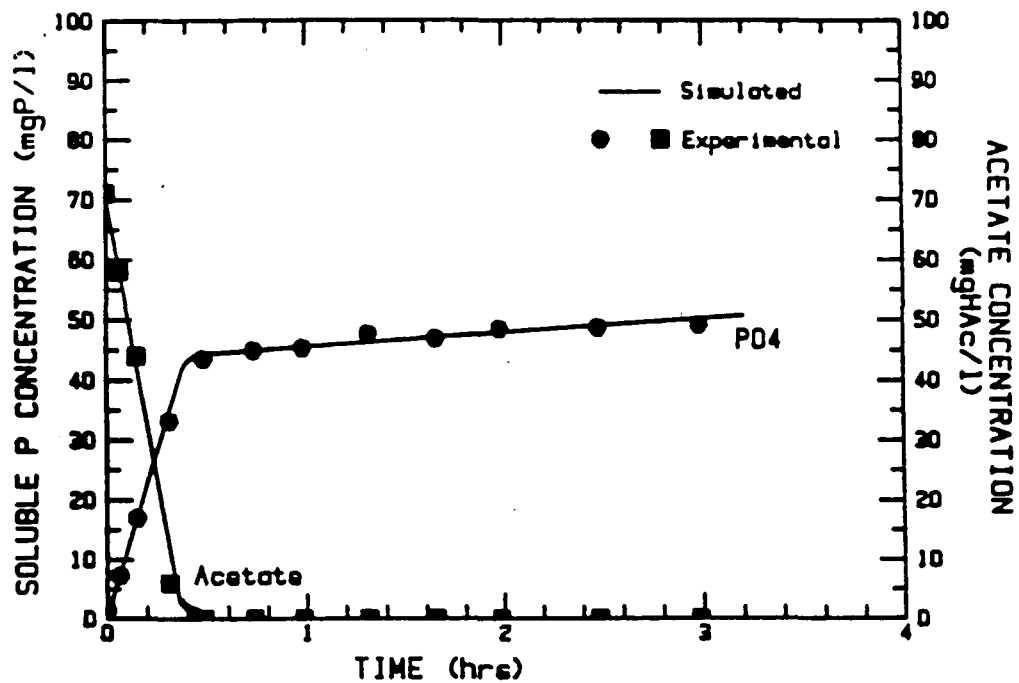


Fig 2.12a: Experimentally observed and theoretically simulated total soluble phosphorus ($\text{PO}_4\text{-P}$) and acetate concentration versus time profiles for the anaerobic batch test data shown in Fig 2.4a.

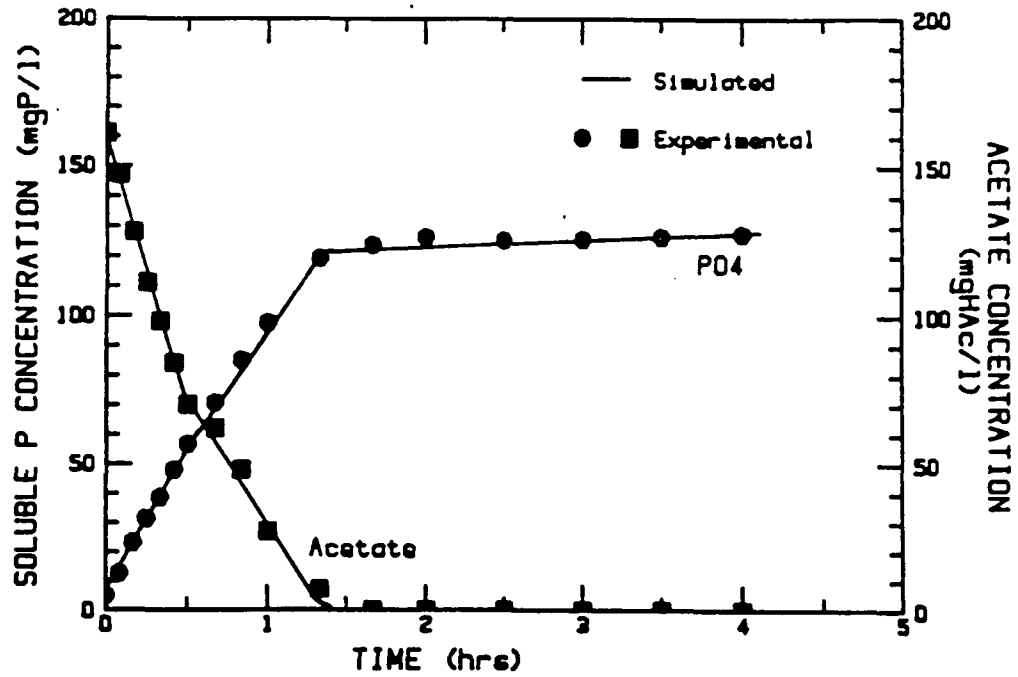


Fig 2.12b: Experimentally observed and theoretically simulated total soluble phosphorus ($\text{PO}_4\text{-P}$) and acetate concentration versus time profiles for the anaerobic batch test data shown in Fig 2.4b.

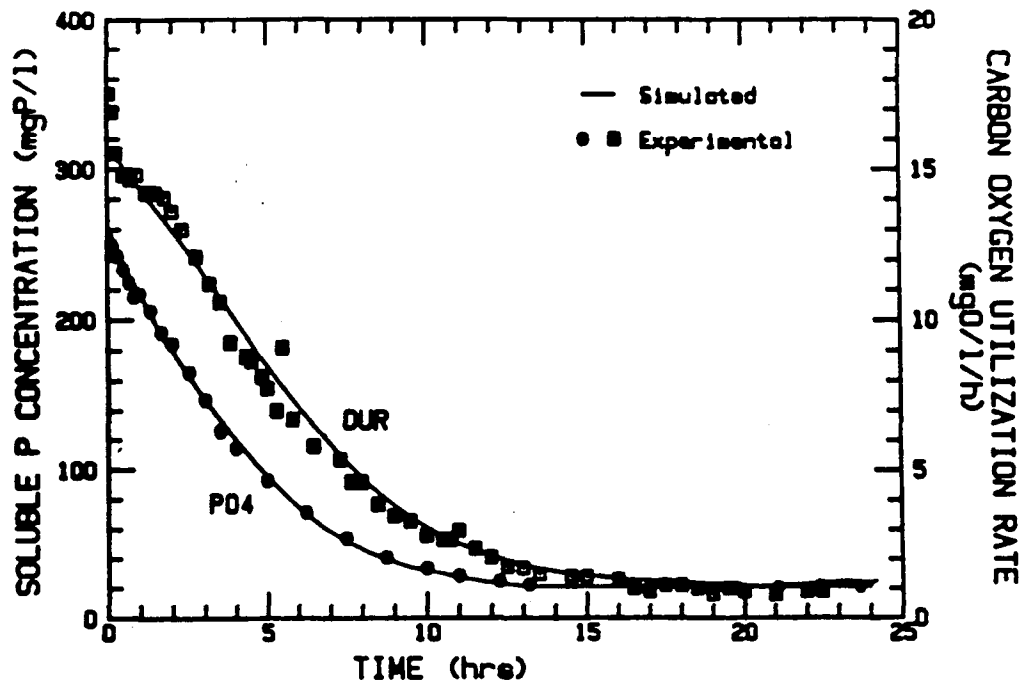


Fig 2.13a: Experimentally observed and theoretically simulated carbonaceous oxygen utilization rate (OUR) and total soluble phosphate ($\text{PO}_4\text{-P}$) concentration for the aerobic batch test data shown in Fig 2.7a.

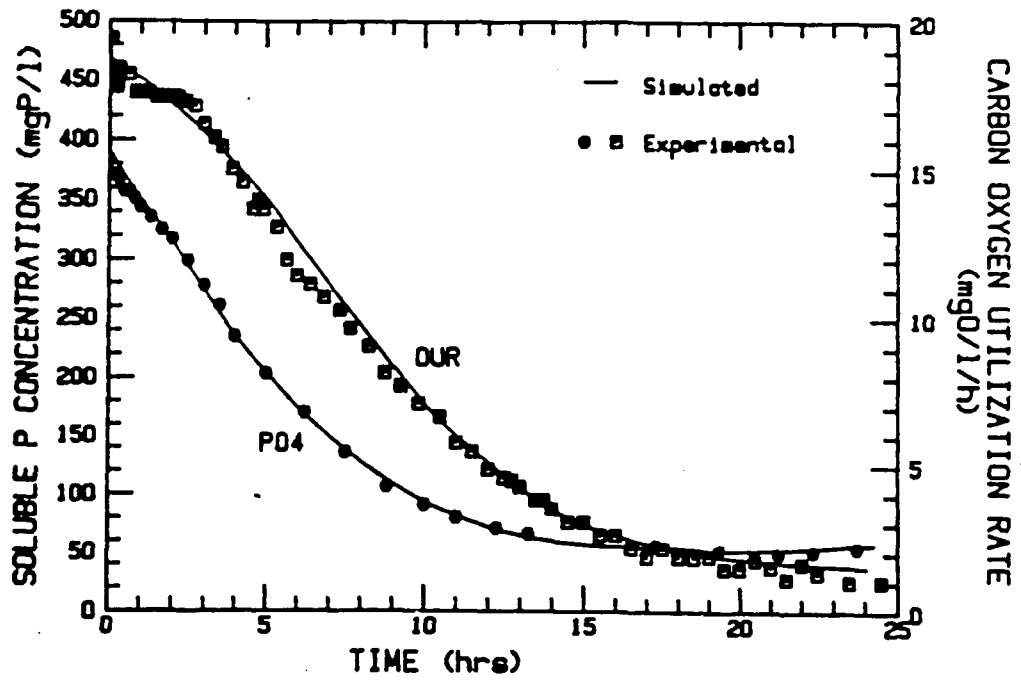


Fig 2.13b: Experimentally observed and theoretically simulated carbonaceous oxygen utilization rate (OUR) and total soluble phosphate (PO₄-P) concentration for the aerobic batch test data shown in Fig 2.7b.

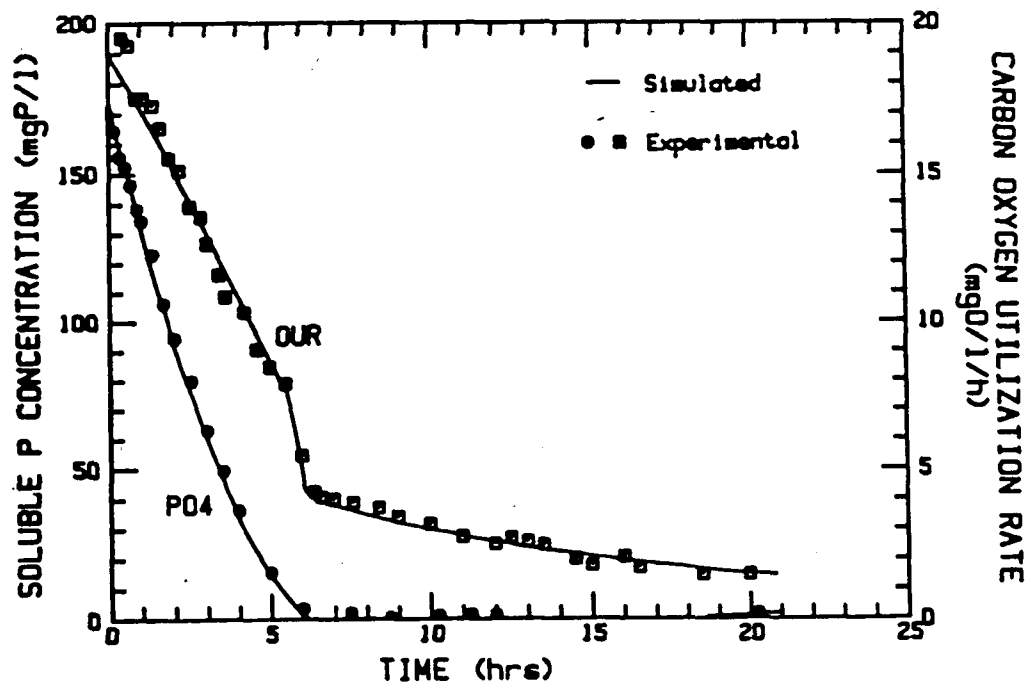


Fig 2.13c: Experimentally observed and theoretically simulated carbonaceous oxygen utilization rate (OUR) and total soluble phosphate (PO₄-P) concentration for the aerobic batch test data shown in Fig 2.7c.

CHAPTER THREE

BIOLOGICAL EXCESS PHOSPHORUS REMOVAL SYSTEM PERFORMANCE (PROJECT 2)

1. PERFORMANCE AT HIGH AND LOW INFLUENT COD STRENGTHS

From the enquiry into the kinetics of P release in the anaerobic reactor (Wentzel *et al.*, 1985) it appeared that for the same sewage, the P removal per influent COD would be lower/higher for influent COD strengths lower/higher than 500mg/l. Also on full scale plants, it was noticed that with the onset of the drought in 1983, which resulted in higher sewage strengths (from around 500 mg/l to 1000 mg/l), biological nutrient removal in general and BEPR in particular was considerably better per mg influent COD and easier to achieve (performance more stable and less sensitive to upsets). Accordingly, to investigate this aspect further, two laboratory scale nutrient removal systems were run at influent COD strengths of 1000 and 250 mg/l respectively.

Experiments were conducted on modified UCT type systems because with these systems, the P removal performance is separated from the nitrogen removal performance. Two systems were set up. The sludge age was set at 20 days, and operating temperature 20° C. The anaerobic zone was 6l, 1st anoxic 2,5l, 2nd anoxic 3,5l and aerobic 10l giving an anaerobic mass fraction of 0,16, unaerated mass fraction of 0,48. The mixed liquor recycles were (1) aerobic to 2nd anoxic (a-recycle) 2:1, (2) 1st anoxic to anaerobic (r-recycle) 1:1 and (3) underflow (s-recycle) 1:1. One system was fed 1 000 mg/l COD sewage strength, the other 250 mg/l. The same sewage and the same mass of COD per day was fed to both systems: The high strength system received 15l/d at 1000 mgCOD/l and the low strength system received 60 l/d at 250 mgCOD/l; for the low strength system, the 1 000 mg/l COD feed was diluted 4 times with tap water to give 250 mg/l COD. The 1 000 mgCOD/l system was operated for about 9 months, i.e. 13 sludge ages, and the 250 mgCOD/l system for more than 5 months, i.e. 8 sludge ages. The results, discussed below, are based on the average performance over these periods. Performance data i.e. effluent COD, TKN and nitrate concentrations and P removal in mgP/l are given for a 4½ month part of the 9 month period in Fig 3.1 for the 1000 mgCOD/l system.

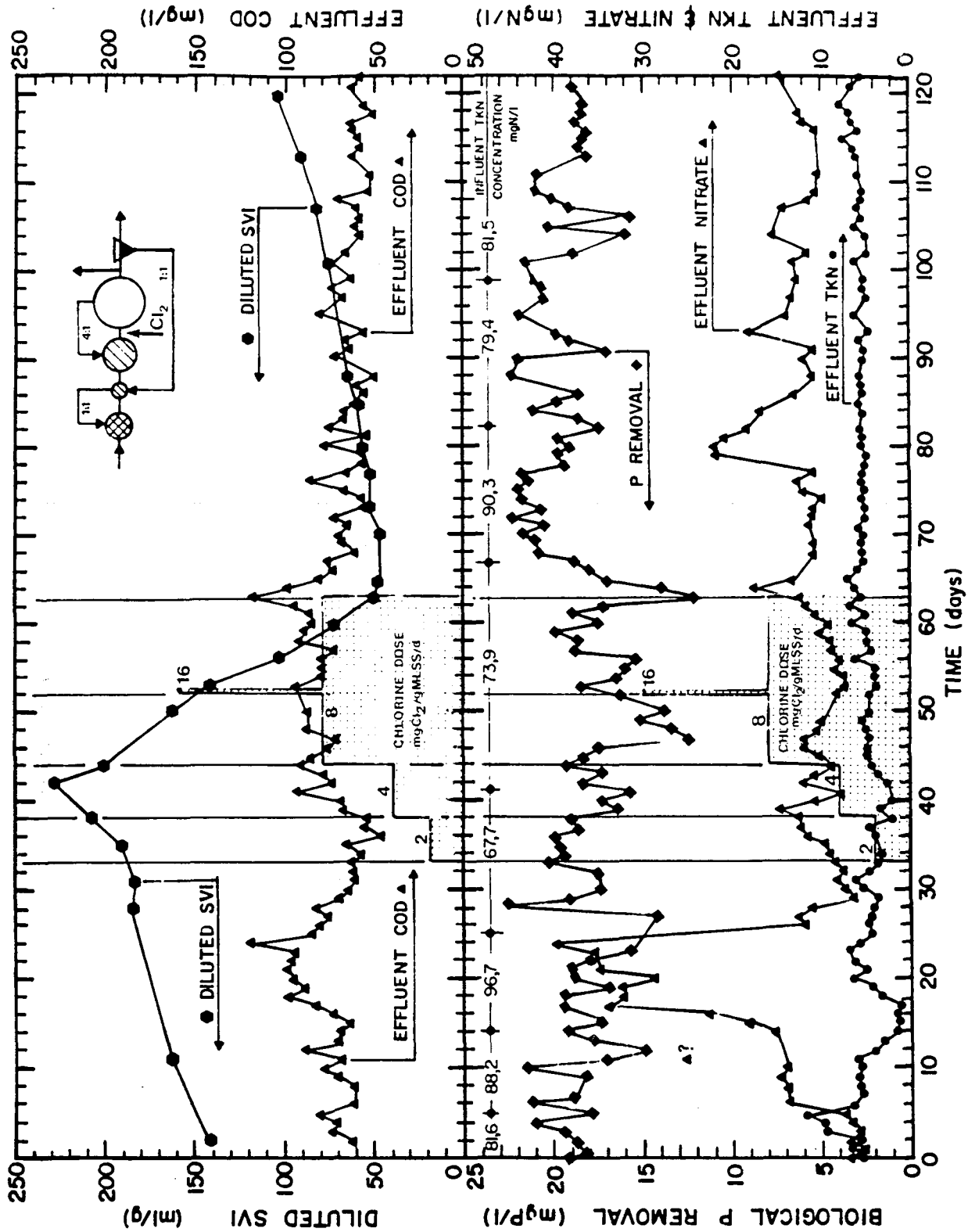


Fig 3.1: Performance data [unfiltered effluent COD and DSVI, top; effluent nitrate and TKN (unfiltered) concentration and P removal, bottom] of the modified UCT system at 20 days sludge age, 20° C treating 1000 mgCOD/l raw sewage for a period of 6 sludge ages.

1.1 Performance at 1 000 mg/l influent COD

The average P removal was about 22 mgP/l i.e. 0,022 mgP/mgCOD. The measured readily biodegradable COD (RBCOD) fraction was about 22 percent with respect to total COD. Calculated P removal according to Wentzel *et al.* (1985) is 21 mg/l, that is, the predicted and observed P removals are closely equal. Nitrification was complete at all times with the effluent TKN concentration varying between 4 and 6 mgN/l. Denitrification also was good; the effluent nitrate concentration ranged between 7 and 30 mgN/l. This variation was due to a different influent TKN concentration between batches of sewage, from 85 to 120 mgN/l; high effluent nitrate concentrations were associated with high influent TKN concentrations and *vice versa*. Despite the range of nitrate concentrations in the effluent, P removal was not affected; in the UCT/MUCT systems the anaerobic reactor usually is completely protected from the adverse effect of nitrate entering the anaerobic reactor.

Chlorination and bulking: From the commencement of the investigation, the sludge settleability progressively deteriorated until after about 2 months, a severe bulking problem developed with the DSVI above 230 ml/g (see Fig 3.1). Bulking was caused by the low F/M filaments 0092, 0041 and *M.parvicella*, three of the filaments common in full scale nutrient removal plants (see Blackbeard, Gabb, Ekama and Marais, 1988). The sludge was chlorinated to improve the settleability and to check the effects of this on nutrient removal (for details see Lakay, Wentzel, Ekama and Marais, 1988). Following a lead-in period at chlorine overall mass dose rates of 2 and 4 mgCl₂/(gMLSS.d), dosing at 8 mgCl₂/(gMLSS.d) continued for 19d, during which time the DSVI decreased from 230 to 48 ml/g. Dosing was terminated when the system manifested overdosing symptoms e.g. high effluent turbidity. During dosing, COD removal was essentially unchanged except when overdosing became apparent at the end of the dosing period, nitrification was unaffected and complete and denitrification was only marginally affected.

Chlorination and P removal: The effect of chlorination on P removal was as follows: On increasing the chlorine dose P removal was temporarily reduced but recovered fully in the following 3 to 5d, the duration of the recovery depending on the magnitude of the dose increase (Fig 3.1). However, after 16d of chlorination at 8 mgCl₂/(gMLSS.d) when the DSVI had declined to less than 50 ml/g, P removal began to decline precipitously. On day 19, when chlorination was terminated, P removal had declined to 12 mgP/l. After cessation of chlorination the P removal recovered to its normal value of 21 mgP/l within 5d (for details see Lakay *et al.*, 1988). In practical situations where it is mandatory to set a target DSVI at say 100

or 120 mg/l, conditions of overdosing would not occur, in which event chlorination is unlikely to affect nitrification, denitrification and biological P removal. After the P removal re-established itself at around 21 mgP/l it remained at this level for the remaining 5 months of the investigation, with the exception of one period of toxicity during which the P removal was below 20 mgP/l for 3 weeks (see 1985 Steering Committee report for details).

1.2 Performance at 250 mg/l influent COD

Average P removal (ΔP) was 3,8 mgP/l. This gives a $\Delta P/COD$ of 0,015 for 250 mgCOD/l influent. According to Wentzel *et al.* (1985) the $\Delta P/COD$ values should have been 0,017 mgP/mgCOD, that is, the removal observed was slightly less than that predicted. The lower value observed probably arises from the higher flows (influent and recycle flows 4 times higher) through the plant (for the same mass COD load) when low COD strengths are treated. These higher flows transport greater masses of dissolved oxygen to the anaerobic reactor per unit influent COD; this causes a loss of some RBCOD because the oxygen will serve as electron acceptor and less RBCOD will be available for uptake by polyP organisms in the anaerobic reactor. When this loss of RBCOD was taken into account in the theoretical prediction, the predicted and observed P removals corresponded closely.

With regard to the lower value $\Delta P/COD$ for the lower influent COD concentration the theory of Wentzel *et al.* (1985) provides the following explanation: The greater the influent COD concentration, the greater the proportion of readily biodegradable COD converted to volatile fatty acids which are taken up by polyP organisms. In the two investigations above the predicted conversion at 1 000 mgCOD/l this is over 90%, at 250 mgCOD/l, only about 70%. For the same RBCOD fractions, the $\Delta P/COD$ accordingly will be higher for the high influent COD's.

Nitrification was complete at all times with the effluent TKN varying between 2 and 3 mgN/l. Effluent nitrate concentration ranged between 5 and 20 mgN/l. This variation was due principally to variations in influent TKN concentration in different sewage batches; the TKN varied between 20 and 30 mgN/l; high effluent nitrate concentrations coincided with high influent TKN sewage batches. Despite the range in effluent nitrate concentration, the nitrate recycled to the anaerobic zone always was maintained at less than 1 mgN/l except for the highest TKN batches when the effluent nitrate was around 20 mgN/l; over these periods the nitrate in the recycle to the anaerobic reactor increased to about 3 mgN/l and caused a reduction in P

removal to about 2,5 mgP/ℓ, from about 4,0 mgP/ℓ when there was no nitrate recycled.

In terms of the empirical theory of Wentzel *et al.* (1985), to improve the P removal, the anaerobic zone should be enlarged as this would improve the degree of conversion of RBCOD. Accordingly, to improve the P removal at the low influent strength, the anaerobic reactor was increased from 6ℓ to 10ℓ giving an increase in anaerobic mass fraction from 0,16 to 0,24. All other parameters remained unchanged. The data recorded over a 6 week period showed that P removal increased from a previous average of 3,8 mgP/ℓ to about 4,6 mgP/ℓ i.e. $\Delta P/COD = 0,018$ a value in accordance with that predicted for the enlarged anaerobic zone.

For the system receiving the low influent COD, the sludge settleability was, on average, quite poor and ranged between 120 and 180 mL/g DSVI with filamentous organisms similar to those normally identified in nutrient removal systems i.e. 0092, 0041, *M.parvicella*, 0675 and 0914. The sludge of this system was not chlorinated because the clarifier was sufficiently large to deal with the high hydraulic load (4 times higher than that in the 1 000 mgCOD/ℓ system) and poor settleability. For the high and low COD strength systems, the volume of the reactors was the same because the mass of COD treated daily and sludge age were the same; however, to obtain the same clarifier overflow rate, the surface area needs to be 4 times greater for the low COD strength system than that for the high COD strength system, because the influent flow is 4 times greater. Consequently, in practice for the same mass COD load per day the higher hydraulic loads of weak sewages would result in much larger clarifier surface areas per mass unit of COD treated than for strong sewage.

1.3 Discussion

From the results obtained on the two investigations, it was concluded that both denitrification and biological P removal efficiencies (mgN or P removed/mgCOD influent) are adversely affected by low COD strengths, confirming under laboratory conditions the observations made at full scale and the theoretical predictions. With denitrification, the reduction appears to be caused principally by dissolved oxygen in the recycle flows. With excess P removal the reduction appears to be a consequence of two effects (1) the kinetics of P release in the anaerobic reactor and (2) the concentration of dissolved oxygen in the influent flow and in the underflow recycle in the modified Bardenpho system. The magnitudes of these effects differ significantly between high and low influent COD concentrations.

With a high influent COD (1000 mg/l) P removal was 22 mg/l (0,022 mgP/mg influent COD) with a variation of less than 1 mg/l (5%) over a week for a specific batch of sewage; the system maintained this stability without undue attention to operation.

With a low influent COD (250 mg/l) P removal was 3,8 mgP/l (0,015 mgP/mg influent COD) with a variation of 1 mg/l (25%) for a specific batch of sewage; operation was erratic, the oxygen concentration had to be maintained at a fixed value (of 1,5 mgO/l) within close limits.

The marked reduction in mean removal per influent COD at the lower influent COD arises from the kinetics of conversion of readily biodegradable COD to short chain fatty acids in the anaerobic reactor; the degree of conversion, for the same anaerobic mass fraction, declining with a decline of the influent COD (Wentzel *et al.*, 1985).

The higher instability at the lower influent COD arises from an increased sensitivity of the system to the concentration of oxygen in the recycles and influent flows. For example, for the same recycle ratio, for say 1 mg increase in the oxygen concentration in the recycle to the anoxic zone, the larger volume of flow per COD in the low 250 mgCOD/l strength system will transport 4 times as much oxygen than the high 1000 mgCOD/l strength system. Thus in the low COD system denitrification will be reduced, which in turn adversely effects the P release in the anaerobic reactor. In general in plants treating low COD strengths of 300 mg/l or less the anaerobic mass fraction should be higher than for high influent strengths; very careful control needs to be exercised on the oxygenation rate if reasonably stable results are to be obtained, and, special precautions should be incorporated in the design to limit oxygen entrainment at pumps, overflow weirs, mixers and areas of turbulence. The higher the influent COD strength the lower the level of process supervision needs to be and the easier it becomes to attain the biological N and P removal potential of the sewage and system.

2. PERFORMANCE AT SHORT SLUDGE AGES

In the original agreement of this contract, an evaluation was to be made of the feasibility of the Phostrip P removal system, or a suitable modification of it, for implementation in South Africa. From an enquiry into this system, focusing mainly on the design and operation of the stripping tank in a system that must produce an effluent low in COD, ammonia, nitrate and phosphorus concentrations, it was concluded that the system had little potential for application in South Africa. After

consultation with the advisors of the Water Research Commission, it was concluded that an experimental investigation into high rate (short sludge) biological excess P removal (BEPR) systems such as Phoredox, 3-stage Bardenpho, UCT/modified UCT and Johannesburg systems, would be more fruitful. The area of application for high rate excess P removal systems was envisaged to be in upgrading trickling filter plants where a nitrate effluent standard does not have to be met. The envisaged treatment scheme was an initial high rate non-nitrifying excess P removal activated sludge system followed by further organic material (COD) removal and nitrification on the existing trickling filter. Accordingly the Phostrip project was replaced by an experimental evaluation of short sludge age (< 6 days) BEPR activated sludge systems (Burke, Dold and Marais, 1986).

The objectives in this task were (1) to test, at laboratory-scale, the behaviour of short sludge age biological excess P removal systems, (2) to assess the response of the systems under non-nitrifying and nitrifying conditions, (3) to determine the optimal system configuration(s) and operational parameters, and (4) to check the settling characteristics of the mixed liquor produced in the systems. A secondary objective was to use the observed response data to test the predictive qualities of both the general IAWPRC activated sludge model of Dold and Marais (1985) and the semi-empirical biological excess P removal model of Wentzel *et al*, (1985).

2.1 Experimental investigations

A number of laboratory scale anaerobic/aerobic and anaerobic/anoxic/aerobic systems were operated with different sludge ages, all less than 6 days, different anaerobic, anoxic and aerobic mass fractions, mixed liquor recycle ratios and COD loading rates, all operated at 20°C and receiving Mitchell's Plain raw sewage feed at 500 mgCOD/ℓ strength. Mitchell's Plain raw sewage has a RBCOD fraction of about 22% with respect to the total COD and a TKN/COD ratio of about 0,1 mgN/mgCOD.

Phoredox system: Almost all of the limited literature available on biological excess P removal at short sludge ages (< 6 days) deals with the non-nitrifying Phoredox (or A/O) system. Accordingly, this system was selected as the starting configuration, Fig 3.2a. The system was operated at a 4 day sludge age with a 50 percent unaerated mass fraction, a 1:1 underflow recycle ratio and influent COD of 500 mg/ℓ. It was assumed that the short aerobic sludge age of 2 days would prevent nitrification.

The system provided good excess P removal i.e. 15 mgP/l ($\Delta P/COD = 0,03$ mgP/mgCOD), excellent settleability (DSVI - 70 ml/g) and adequate COD removal (approximately 90 percent) but, against expectations, the system nitrified partially. To eliminate nitrification, the system sludge age was reduced to 3 days while leaving all other process parameters unchanged. The system was operated at a 3 day sludge age on two separate occasions. On the first occasion, the nitrification slowly decreased to about 2 mgN/l and very good P removal was attained 15 mgP/l ($\Delta P/COD = 0,03$ mgP/mgCOD). On the second occasion, the system nitrified partially and produced a lower P removal 10 mgP/l ($\Delta P/COD = 0,02$ mgP/mgCOD). The reduction in P removal was due to with the detrimental effect of nitrate discharge to the anaerobic reactor. The results also indicated that it is possible to obtain nitrification at system sludge ages as low as 3 days with a 50 percent unaerated mass fraction, i.e. with an aerobic sludge age of 1,5 days.

At 3 days system sludge age, both Phoredox systems produced turbid effluents with high COD concentrations (about 15 percent of the COD in the influent). Furthermore the settleability of the sludges was poor (DSVI - 150 ml/g) due to the presence of *Sphaerotilus natans*.¹

3-Stage modified Bardenpho system: As a consequence of the inability to prevent nitrification by lowering the aerobic sludge age to 1,5 days, it was decided to accept nitrification and to study systems, such as the 3-stage Bardenpho and UCT systems, that provide a measure of control over the nitrate concentration in the recycles to the anaerobic reactor. However, to retain the short sludge age character, an upper limit of 6 days was imposed on the sludge age of the systems. The first system tested was the 3-stage Bardenpho system, Fig 3.2b, with a sludge age of 6 days, an anaerobic mass fraction of 25 percent, an anoxic mass fraction of 25 percent and an underflow recycle ratio of 1:1. The a-recycle ratio was initially set at 2:1 but, when the nitrate concentration in the anoxic reactor was measured to be zero, the a-recycle ratio was increased to 4:1; a positive nitrate concentration leaving the anoxic reactor then indicated that the system was operating at its full denitrification

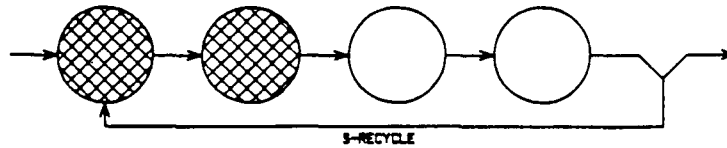
¹Gabb *et al.* (1988) have shown that the growth of *S.natans* on laboratory systems arises from their growth on the wetted walls of the influent feed lines with the result that the system is continually seeded with these filamentous organisms. From their own and published research they show that anaerobic reactors in enhanced P removal systems, anoxic reactors receiving sufficient nitrate, or aerobic selectors act as selectors against *S.natans*. Consequently it is unlikely that *S.natans* will cause bulking in full scale high rate Phoredox systems because in full scale systems the seeding effect is at least 2 orders of magnitude lower than in laboratory scale systems due to the lower surface area/volume ratio.

potential, the effluent nitrate was 13 mgN/l. The system nitrified completely but the denitrification fluctuated, thereby causing a variation in the nitrate concentration in the underflow to the anaerobic reactor; concomitantly, the excess P removal was variable; the mean removal was about 8 mgP/l ($\Delta P/COD = 0,016$ mgP/mgCOD) which is significantly less than that achieved in the non- or partially nitrifying 3 and 4 day sludge age Phoredox systems. The system produced a high COD removal (± 92 percent), a good settling sludge (± 100 ml/g) and a clear effluent.

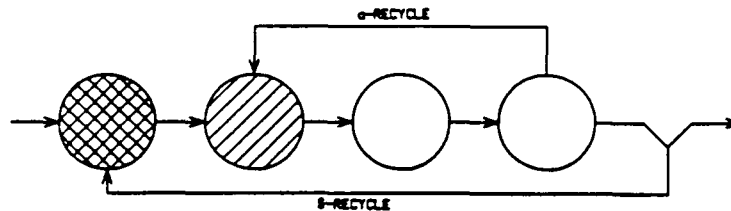
UCT system: A UCT system, Fig 3.2c, was operated with a 6 day sludge age, a 14 percent anaerobic mass fraction, a 29 percent anoxic mass fraction, an underflow recycle ratio of 1:1 and an r-recycle ratio of 1:1. The a-recycle ratio was initially set at 2:1 but later reduced to 1:1 when it was found that the anoxic reactor was overloaded with nitrate. The system nitrified completely throughout its period of operation. With an a-recycle ratio of 2:1, about 4 mgN/l of nitrate was present in the r-recycle to the anaerobic zone and the system P removal was about 7 mgP/l ($\Delta P/COD = 0,014$ mgP/mgCOD). On reducing the a-recycle ratio to 1:1, the nitrate in the r-recycle stream decreased to near zero and the excess P removal increased steadily until, by the time the experiment was terminated, the P removal was about 14 mgP/l ($\Delta P/COD = 0,028$ mgP/mgCOD). This removal was achieved despite the high effluent nitrate concentration of about 10,5 mgN/l. The sludge settleability was generally good (DSVI - 110 ml/g) and the COD removal was consistently high (± 93 percent).

The results from the 6 day sludge age study on the 3-stage modified Bardenpho and UCT systems indicate that: In the UCT system, by appropriate selection of mixed liquor recycle ratios, the systems can remove about 0,028 mgP/mg influent COD from wastewaters with TKN/COD ratios of 0,1 mgN/mgCOD and higher. In contrast, the 3-stage Bardenpho system can remove only about 0,016 mgP/mg influent COD. The reason for the higher efficiency of the UCT system is its ability to selectively remove nitrate from the recycle to the anaerobic reactor; in the 3-stage Bardenpho system, the nitrate concentration in the underflow recycle is the same as that in the aerobic reactor. The sludge settleability and COD removal for both the 3-stage Bardenpho and UCT systems were good.

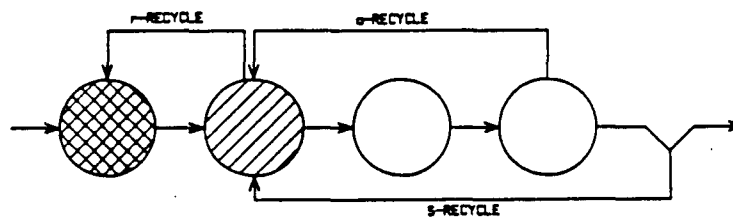
Johannesburg system: The third system operated to minimize the nitrate in the recycle to the anaerobic zone was the Johannesburg system, Fig 3.2d. This system was initially operated at a 3 day system sludge age with a 24 percent anaerobic mass

**Fig 3.2a:**

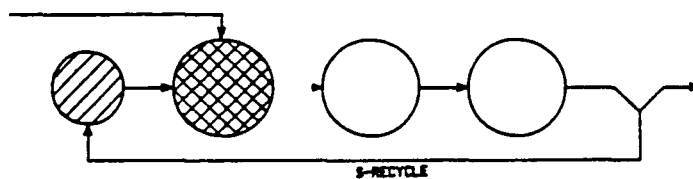
Diagrammatic representation of the 2 stage Phoredox (anaerobic-aerobic) system operated at 3 to 4 days sludge age.

**Fig 3.2b:**

Diagrammatic representation of the 3 stage Bardenpho (anaerobic-anoxic-aerobic) system operated at 6 days sludge age.

**Fig 3.2c:**

Diagrammatic representation of the UCT system (anaerobic-anoxic-aerobic) system operated at 6 days sludge age.

**Fig 3.2d:**

Diagrammatic representation of the Johannesburg (anoxic-anaerobic-aerobic) system operated at 6 days sludge age.

fraction, 24 percent anoxic mass fraction and a 1:1 underflow recycle ratio. The system nitrified partially and the anoxic reactor successfully removed all the nitrate from the underflow recycle stream. An excess P removal of between 8 and 9 mgP/l ($\Delta P/COD = 0,016$ to $0,018$ mgP/mgCOD) was consistently attained. This removal was lower than that attained in the 3 day sludge age Phoredox system possibly due to the smaller anaerobic mass fraction in the Johannesburg system compared to the other system. The COD effluent quality was very similar to that of the other 3 day sludge age systems (i.e. 85% COD removal).

The Johannesburg system was also operated at a 5 day sludge age with a 24 percent anaerobic mass fraction and a 36 percent anoxic mass fraction. The underflow recycle ratio was initially set at 0,5:1 and, in this mode of operation, the nitrate leaving the underflow anoxic reactor proved to be zero and the excess P removal was about 13 mgP/l ($\Delta P/COD = 0,026$ mgP/mgCOD). The underflow recycle ratio was later raised to 1:1; this caused the nitrate concentration in the underflow anoxic reactor to vary between 1 and 5 mgN/l and the excess P removal to decrease to about 7 mgP/l ($\Delta P/COD = 0,014$ mgP/mgCOD). The settleability of the 5 day sludge age Johannesburg system was relatively poor with a mean DSVI of approximately 130 ml/g with the filamentous organism *S.natans* being the cause.

The experimental results indicate that, by adopting the Johannesburg configuration, the anaerobic reactor can be protected (by the anoxic reactor) from nitrate in the underflow recycle stream for wastewaters with TKN/COD ratios at least as high as 0,1 mgN/mgCOD for underflow recycle ratios of about 0,5 to 0,7. In proposing a Johannesburg system, it is important to ensure that the anoxic reactor in the underflow either is sufficiently large, or, for a selected anoxic mass fraction the recycle ratio can be reduced sufficiently for complete denitrification in the underflow anoxic reactor. The second option will be dependent on the settleability of the sludge. In full scale plants settleability can be expected to be good (probably DSVI < 100 ml/g); *S.natans* is unlikely to proliferate in the full scale system, Gabb *et al.* (1988).

In all the short sludge age systems operated, good nitrogen recoveries of about 97 percent were obtained but the COD recoveries were very poor, between 70 and 87 percent. Four factors serially or cumulatively could have led to the low COD recoveries:

- (1) The effluent COD sample was always filtered and therefore any particulate material carried over in the effluent was not accounted for.
- (2) Hydraulic control of sludge age was by 'batch' removal of sludge twice a day. At short sludge ages batch removal introduces uncertainty as to the exact sludge age and hence, in the mass balance.
- (3) In the anaerobic reactor COD may have been lost as hydrogen gas due to acidogenesis.
- (4) Slime accumulated rapidly around the mixing impellers in the aerobic reactors of the systems. This slime, a polysaccharide, was not accounted for in the mass balance.

In general, experience in the UCT laboratory has indicated lower COD recoveries in anaerobic/anoxic/aerobic systems than in aerobic systems. This suggests that COD loss due to hydrogen formation, and other anaerobic reactions that may be taking place in the unaerated zone, are responsible for the low COD recoveries. Further research in this direction is required.

2.2 Simulation studies

The responses of the various experimental systems were simulated using the IAWPRC general activated sludge model (see Dold and Marais, 1986). Certain changes had to be made to the input parameters of the model in order to accurately simulate the observed results. The changes were as follows:

- (1) To account for the high effluent COD values obtained from the short sludge age systems, it was necessary to increase the value of the unbiodegradable soluble COD fraction of the influent wastewater.
- (2) To account for the low experimental COD recoveries, it was assumed that the entire COD loss was due to acidogenesis and the values of the total COD and the readily biodegradable COD in the influent had to be adjusted accordingly.
- (3) It was assumed that there was conversion of slowly biodegradable COD to readily biodegradable COD in the anaerobic zone.

When the above factors were taken into account, the IAWPRC model on the whole simulated the observed response of short sludge age systems reasonably closely. However, the modifications are open to question and merit further investigation if a solution to the poor COD recoveries is to be found.

2.3 Conclusions

This study gives rise to the following conclusions with regard to selection of short sludge age P removal systems:

- (1) Biological excess P removal systems operated at 3 days system sludge age and 50 percent unaerated mass fractions
 - (i) will give poor COD reduction and turbid effluents
 - (ii) if the systems do not nitrify, will give high specific removals of phosphorus, up to 0,03 mgP/mg influent COD for 'normal' municipal waste flows,
 - (iii) if the systems nitrify, the Phoredox and A/O systems will give sharply reduced P removals
 - (iv) will give good settling sludges.

As it does not seem possible to guarantee non-nitrification even at 1,5 days aerobic sludge age it is recommended that the Phoredox and A/O systems are not considered as viable short sludge age P removal systems.

- (2) Biological excess P removal systems operated at 6 days sludge age with 50% unaerated mass fractions
 - (i) will give good settling sludges
 - (ii) will give good COD removal > 90%
 - (iii) are likely to nitrify
 - (iv) with nitrification the UCT system provides the best security for consistently high specific P removals (0,028 mgP/mgCOD) with

underflow recycle ratios of 1:1 and TKN/COD ratios of 0,12 mgN/mgCOD

- (v) with nitrification the Johannesburg system can produce a nitrate-free underflow recycle to the anaerobic zone for recycle ratios of 1:1 with $\text{TKN/COD} = 0,07$ or recycle ratios of 0,6:1 with $\text{TKN/COD} = 0,1$ to give specific P removals of 0,028 mgP/mgCOD.
- (vi) with nitrification the modified 3-stage Bardenpho cannot reduce the nitrate in the underflow recycle to the anaerobic zone to zero; this causes that the specific P removal is significantly reduced, to as low as 0,014 mgP/mgCOD. In consequence this system is not recommended.
- (vii) sludge ages for these short sludge age systems should not be less than about 5 days to preserve system stability and give good quality COD reduction.

CHAPTER FOUR

SUBSTRATE IDENTIFICATION, UTILIZATION AND GENERATION (PROJECT 3)

1. MEASUREMENT OF READILY BIODEGRADABLE COD IN WASTEWATER

The importance of the influent readily biodegradable COD (RBCOD) concentration for biological excess phosphorus removal was firmly established in the previous contract (Ekama, 1984). The method for determining this COD fraction was biological, in a 2,5 day sludge age single completely mixed aerobic reactor under square wave daily cyclic loading. The RBCOD fraction was estimated from the change in oxygen utilization rate when the feed period terminated. This method of estimation, however, is cumbersome as it requires the operation of a laboratory scale activated sludge unit. Accordingly simpler methods of investigation were sought.

Three simpler alternatives were devised i.e. (1) aerobic and (2) anoxic batch tests and (3) microfiltration. A brief evaluation of these three is given below.

1.1 Aerobic and anoxic batch tests

The method and details of these two tests have been reported by Ekama, Dold and Marais, (1986). With both methods, reproducible results in close conformity with the square wave tests can be obtained. These tests have the advantage that the maximum specific growth rate of the heterotrophs, nitrification and denitrification rates also can be determined provided the appropriate experimental conditions are imposed and the data properly analyzed. The test procedures *per se* are not difficult but require careful attention and considerable experience and appreciation of activated sludge and associated measurement techniques to obtain reliable and reproducible results. Biological sludges have an inherent variability in response and a reliable result is obtained only by repeating the tests a number of times and averaging the results. Of great importance is that the source sludge must be from systems that do *not* give biological excess P removal. Tests done with sludges from biological P removal systems give very low (only 2/3 to 3/4 of the expected value) results *because* the short chain organic acid component of the RBCOD is taken up by polyP accumulating (e.g. *Acinetobacter*) organisms under *aerobic* conditions, as predicted in the biochemical model of biological excess P removal, see Wentzel *et al.* (1986) and Chapter 2. These aspects detract from the biological batch methods of

measurement of RBCOD. To obviate the complications of the biological methods, RBCOD measurement by physical separation was investigated.

1.2 Ultrafiltration

Practical difficulties in estimating the readily biodegradable COD fraction by the biological methods prompted enquiry into the feasibility of a determination based on ultrafiltration. The hypothesis was that the readily biodegradable COD must consist of molecules of small mass that can pass through the cytoplasmic membrane without first requiring extracellular breakdown. Accordingly a series of ultrafiltration tests were undertaken to test the hypothesis and, if successful, develop a detailed testing procedure. It was established that the method indeed has potential provided the following testing procedure is followed:

- (1) Remove as much *particulate material* in the influent sewage sample by centrifuging at 3 000 to 4 000g for 30 minutes.
- (2) Prefilter *effluent* and *centrifuged influent* samples once through filter paper (Whatmans No.1) and twice through glass micro fibre filter membranes (Whatmans GF/C).
- (3) Filter samples through 0,45 μ m filter membrane.
- (4) After 0,45 μ m filtration, the samples are ready for ultrafiltration through 100 000 or 10 000 molecular mass cut-off filter membranes.

A detailed description of the method is given by Dold, Bagg and Marais (1986).

Specific conclusions from this investigation are as follows:

- (1) An appreciable fraction of the 0,45 μ m filtered COD in municipal wastewaters consists of organic material which is not degraded under normal operating conditions in the activated sludge process. From molecular mass distribution (MMD) studies it would appear that almost all of the 0,45 μ m filtered influent COD material of molecular mass greater than approximately 1 000 is "unbiodegradable" – the influent and effluent MMD profiles essentially are identical in this region. This can be seen in Fig 4.1 which shows the MMD of the dissolved organic carbon (DOC) in the influent and effluent from a full scale activated sludge plant treating municipal

wastewater. From Fig 4.1 it can be seen that the major portion of the influent 0,45 μm filtered COD in a municipal wastewater is material of low molecular mass (< 200). The portion of the 0,45 μm filtered COD (in a relative sense, equivalent to DOC) which is removed in the treatment process is almost entirely in this low molecular mass fraction; this observation supports the supposition that the readily biodegradable COD (RBCOD) comprises small molecules (MMD < 200).

- (2) The very close similarity between influent and effluent 0,45 μm filtered MMD profiles, except for the RBCOD, indicates that the microbial byproduct formation contribution (via cell lysis) to the effluent unbiodegradable 0,45 μm filtered COD is minimal. This conclusion appears reasonable for sludge ages longer than 2 days for then the microbial ecosystem appears to be "fully" developed with the result that byproducts formed by some organisms are utilized as substrate by other organisms, consequently, the unbiodegraded soluble byproducts do not become significant in the effluent. At sludge ages shorter than 2 days, the ecosystem is dominated by the faster growing class of organisms and some of the byproducts are not utilizable, the unbiodegraded soluble byproducts become significant thereby increasing the "unbiodegradable" soluble effluent COD.
- (3) From (1) and (2) above an accurate estimate of the RBCOD concentration in a municipal wastewater can be obtained by ultrafiltration. Filtration of the influent will isolate both the soluble readily biodegradable and soluble unbiodegradable COD ($S_{bsi} + S_{usi}$) while filtration of the effluent from a long sludge age activated sludge system receiving the wastewater will provide a close estimate of the soluble unbiodegradable COD (S_{usi}) only. The RBCOD is therefore found by difference, i.e.

$$\text{RBCOD} = \text{Influent ultrafiltrate COD} - \text{Effluent ultrafiltrate COD}.$$

This implies that the soluble unbiodegradable COD of the influent unbiodegradable soluble COD, S_{usi} , can be approximated closely by assuming it to be equal to the effluent soluble COD, S_{use} .

- (4) There is a large degree of flexibility in selection of the suitable nominal molecular mass cut-off for the ultrafiltration membrane. A cut-off of 1 000, 10 000 or 100 000 seem to be equally suitable: Unbiodegradable MMD up to

100 000 molecular mass cut-off membranes were tested extensively and found to give values for the RBCOD that correlated well with those determined by the square-wave biological method (Fig 4.2).

- (5) A prerequisite for obtaining reliable data with the ultrafiltration method is correct sample treatment prior to ultrafiltration, to remove high molecular mass humic compounds that cause filter blinding. Pretreatment is by centrifuging influent samples at 3 000 to 4 000g and filtering both the centrifuged influent and prefiltered (through filter paper) effluent samples through glass microfibre filters, followed by 0,45 μm membrane filtration.
- (6) If ultrafiltration is omitted, the filtrates from the 0,45 μm stage will yield an estimate proportional to the influent RBCOD, with a proportionality factor of about 0,8 for the two sewages tested (Fig 4.3). This simplified technique may be useful for obtaining approximate estimates where the facilities for ultrafiltration are not available, provided the proportionality factor is checked on a number of other sewages.

Physical separation by ultrafiltration now has been shown to provide accurate estimates of the readily biodegradable influent COD concentration. The particular advantage of this method is its simplicity and the speed of determination when compared to the bioassay methods.

The method has not been tested exhaustively; wastewaters from only two plants have been analyzed so that there is a need to conduct tests on wastewaters from a wide range of locations to check the conclusions of this study. Despite these shortcomings it is *recommended* that the ultrafiltration method is adopted as measure for the RBCOD.

2. READILY BIODEGRADABLE COD UPTAKE IN ANAEROBIC ZONE

As noted earlier, the importance of the readily biodegradable COD (RBCOD) concentration for biological excess P removal was firmly established in the previous contract. However, although this link between RBCOD and P removal was recognized, the P removal was formulated only empirically in terms of the RBCOD, amongst other parameters. Therefore, it was required that a more fundamental understanding and formulation of the link be developed. Accordingly, an extensive investigation into P release and removal with regard to the RBCOD was inaugurated. In anaerobic batch tests the P release behaviour was found to be

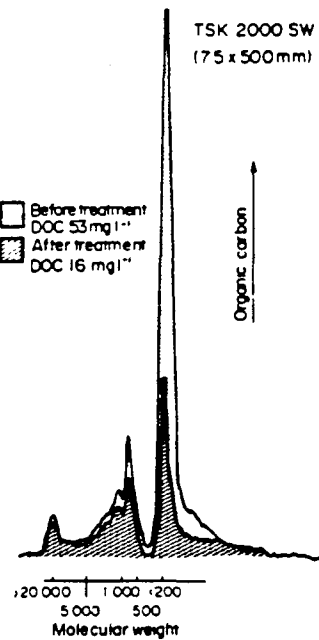


Fig 4.1: Molecular mass distribution of the dissolved organic carbon (DOC) in the influent (before treatment) and effluent (after treatment) from a full scale activated sludge plant treating municipal sewage (after Gloor *et al.*, 1981).

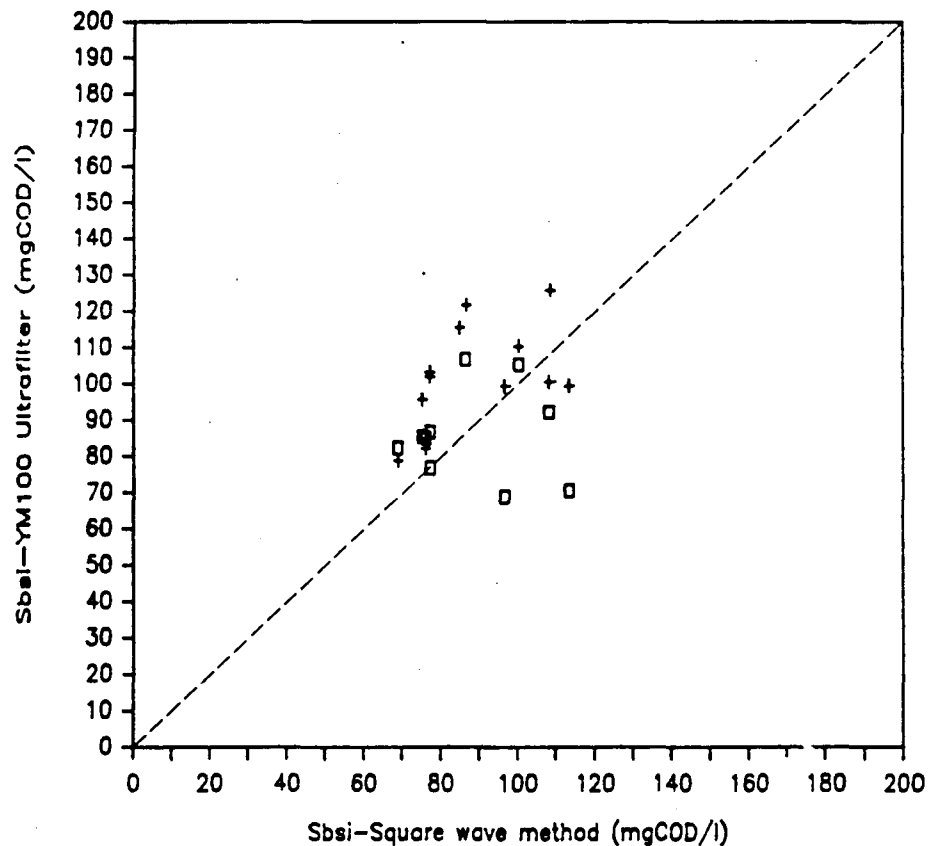


Fig 4.2: Comparison of the 100 000 molecular mass cut-off ultrafiltered COD and the biological square wave short sludge age methods for measuring the readily biodegradable COD in municipal sewage.

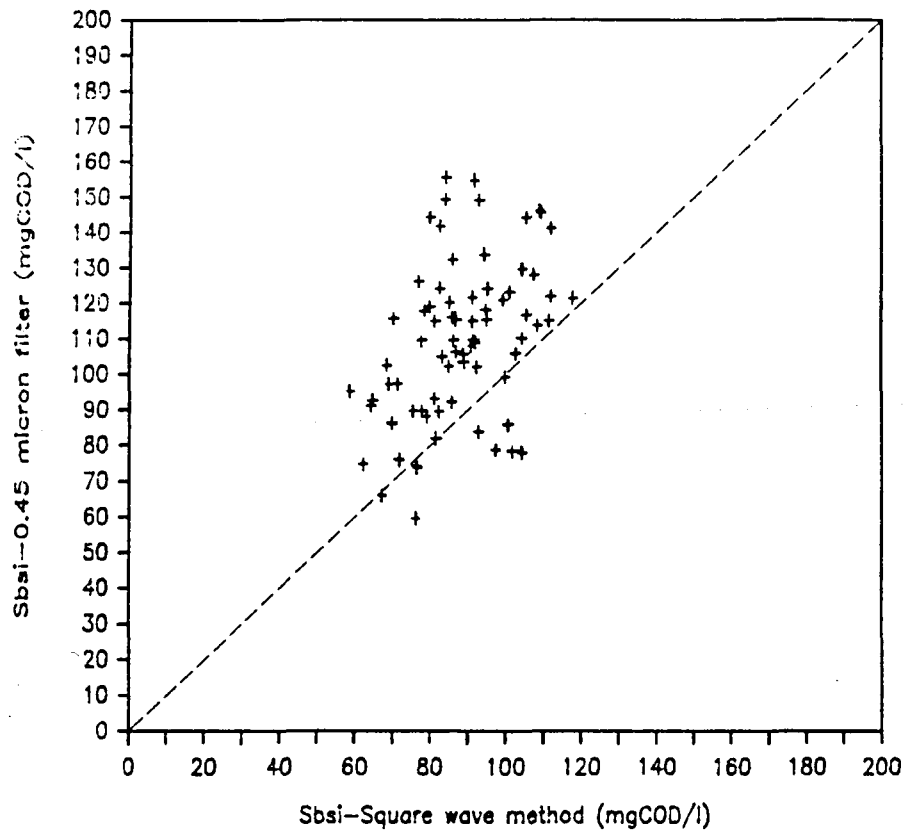


Fig 4.3: Comparison of the 0,45 μ m filtered COD and the biological square wave short sludge age methods for measuring the readily biodegradable COD in municipal sewage.

different with acetate and with unsettled municipal wastewater as substrate: With acetate a rapid linear increase in P concentration was observed, with wastewater P concentration increased but at a rate that declined continually. This difference in behaviour was hypothesized to be due to the presence of an intermediate step in the sequestration process with wastewater as influent – conversion of the RBCOD to acetate (or similar short chain fatty acids) by non-polyP heterotrophs possessing the Embden-Meyerhof pathway, in this way to make acetate available to the polyP organisms for sequestration. From the strong correlation between P removal and the RBCOD of the influent it can be inferred that only the RBCOD fraction of the wastewater can be converted to short chain fatty acids. However, in the batch tests with wastewater as substrate no short chain fatty acids could be detected. From this it was concluded that the rate of conversion by the non-polyP organisms is much slower than the rate of sequestration by the polyP organisms, in which event the rate of conversion controlled the rate of sequestration and associated P release. Consequently the kinetics of P release, with sewage as substrate, could be modelled in terms of the rate of conversion of readily biodegradable COD by the heterotrophic non-polyP mass, a first order reaction with respect to the active heterotrophic organism mass and the concentration of readily biodegradable COD, see Wentzel, Dold, Ekama and Marais (1985).

This model implied that as the anaerobic mass is increased so the mass of readily biodegradable COD converted will do likewise, but at a declining rate. Furthermore, being a first order type reaction, the anaerobic mass necessary to convert any selected fraction of the readily biodegradable COD can be reduced by having plug flow or in-series-reactors instead of a single anaerobic reactor.

Using the UCT system, the P release conversion model was tested over a wide range of anaerobic mass fractions, in single, series and plug flow anaerobic reactors at different system sludge ages. Very good correlation between predicted and observed results were obtained.

With regard to the subsequent P uptake, experimental data showed the magnitude of the P uptake to be strongly linked to the magnitude of P release, or equivalently to the magnitude of S_{bs} converted, in the anaerobic reactor.

The model was applied to the data obtained in the previous contract, in which single reactors were used with fixed anaerobic mass fractions. This analysis showed that the empirical formulation (that P removal or P release was proportional to S_{bs} -25)

was approximately valid for the restricted range of anaerobic mass fractions, sludge ages and RBCOD fractions investigated. However, with the model a general solution for determining P release, the magnitude of S_{bs} converted, etc. is now available over extended ranges of anaerobic mass fractions, influent RBCOD fractions, total influent COD and sludge ages.

The P release with wastewater as substrate could be modelled without implicating the polyP organisms *per se*, because the rate of P release was controlled by the rate of conversion of RBCOD.

3 ACID FERMENTATION OF PRIMARY SEWAGE SLUDGE

The biochemical model, described earlier, predicts that P removal is dependent on the short chain (or volatile) fatty acids supplied to the polyP organisms in the anaerobic reactor of the UCT and modified Bardenpho systems. Experiments on full scale plants at Johannesburg had shown that if the underflow from the primary settling tanks is subject to acid fermentation appreciable quantities of volatile fatty acids (VFA) can be generated. These, when fed to the anaerobic zone, increase the P removal potential of systems. However, little quantitative information was available on the mass of VFA that could be generated by acid fermentation. Accordingly, research into this aspect was undertaken.

Acid fermentation of primary (raw) sewage sludge was studied at laboratory scale under (3.1) batch and (3.2) semi-continuous series reactor conditions at 20°C.

3.1 Batch tests

The objectives of the batch tests were to enquire into onset of acid fermentation, proportion of VSS solubilized, proportion of acids in solubilized COD and the types of acids formed.

From eight successful batch tests each of 9 to 19 days duration, at initial VSS concentrations ranging from 3 000 to 40 000 mgVSS/L, the following conclusions were drawn:

- (1) There is a variable lag phase before acid production commences ranging from zero to 7 days.
- (2) The lag phase showed no consistency and did not appear to affect the

maximum attainable VFA concentration of the sludge or depend on the VSS concentration.

- (3) Neglecting the lag period, peak acids concentration is achieved 6 to 9 days after commencement of fermentation; VFA generation appeared to follow a first order type reaction.
- (4) The maximum potential yield of VFA's is approximately 0,125 mgVFA (as COD) per mg initial VSS (as COD) at 6 to 9 days. At 3 to 4 days the yield is approximately 0,075 mgVFA (as COD) per mg initial VSS (as COD).
- (5) The total VFA generated is 60% to 90% of the 0,45 μm filtered COD generated. Proportions of VFA produced are acetic:propionic:butyric:valeric: 1:1:0,08:0,07.
- (6) Of the total acids generated 40 to 50% is acetate, 40 to 60% propionate, other higher acids such as butyrate and valeric together make up about 0 to 20%.
- (7) The lowest pH in the batch tests coincides with the peak acid concentration and ranges between 5,0 and 5,5. There appears to be no correlation between the initial VSS concentration and minimum pH.
- (8) As the batch test progresses, after centrifugation and Whatman No.1 filter paper pre-filtration, the filtrate becomes increasingly more difficult to filter through 0,45 μm filter membranes. This possibly is due to long chain fatty acid production which blinds the 0,45 μm filter membranes.

3.2 Semi-continuous series reactor tests

The objective of these tests was to enquire into acid fermentation behaviour under continuous operating conditions, as it would be implemented at full scale.

The experimental system comprised 3 to 4 reactors in series each with 1 day retention time. At start, all the reactors were filled with primary sludge. Thereafter twice daily, half the volume of the last (3rd) reactor was wasted and replaced with an equal volume from the 2nd reactor. The second reactor was filled with half the volume of the first reactor, and the first reactor was fed with primary sludge feed. The system was operated for 5 to 6 days to reach steady state whereafter the VSS,

COD of the VSS, 0,45 μm filtered COD and VFA were measured daily on each reactor before feeding. The operating temperature was 20° C.

From 4 successful tests, each operated for about 20 days after steady state was achieved, having influent VSS concentrations ranging between 37 000 to 57 000 mgVSS/L, the following conclusions can be drawn:

- (1) No lag period was observed, i.e. VFA production commenced immediately.
- (2) The yield of VFA (as COD) ranged from 0,06 to 0,10 mgVFA (as COD) per mg of initial VSS (as COD) i.e. between 6 to 10 percent of the VSS is solubilized to VFA (as COD).
- (3) About 50 to 75% of the 0,45 μm filtered COD is VFA.
- (4) Proportions of VFA generated, acetic to propionic to butyric to valeric = 1:1:0,3:0,1.
- (5) Of the total VFA generated, 43% was acetic, 41% propionic and 16% butyric and valeric.
- (6) The pH was never lower than 5,2 for the duration of the experiment.
- (7) The 0,45 μm filtered COD vs time plots approximated to those of the batch tests and appeared also to conform to a 1st order type reaction.

From the specific rate constant for the 1st order reaction, it would appear that a series reactor system confers little advantage over a single reactor system with retention time equal to the sum of the retention times of the reactors in the series.

CHAPTER FIVE

NON-POLYP ACTIVATED SLUDGE MODEL

As stated in the introduction, a general activated sludge model for non-polyP behaviour had been developed under the previous contracts (Dold, Ekama and Marais, 1980; van Haandel, Ekama and Marais, 1981). A basic assumption in this model is that the influent biodegradable COD can be subdivided into two sharply different fractions, readily biodegradable and slowly biodegradable. This division was established from the oxygen utilization rate response and denitrification response when wastewater was added to mixed liquor under aerobic and anoxic conditions respectively. It was desirable to check if a wastewater could be constituted of these two substrate fractions.

1. VERIFICATION OF BISUBSTRATE HYPOTHESIS

This investigation was undertaken to substantiate the bisubstrate hypothesis for modelling the activated sludge process by (1) monitoring the response of systems to the feeding of selected substrates apparently characteristic of the bisubstrate fractions, and (2) checking in what measure the general activated sludge model, based on the bisubstrate hypothesis, simulates the observed bisubstrates (Bagg, Dold and Marais, 1986). The artificial substrates selected were glucose and starch, representative of readily biodegradable and slowly biodegradable substrate fractions respectively.

Aerobic activated sludge systems were run under constant flow and square wave cyclic flow conditions with glucose only, starch only and glucose/starch mixtures. From the constant flow response the specific yield values were determined and the reliability of the data checked by doing mass balances on the COD. From the cyclic flow response, the specific rate constants for growth and solubilization respectively were determined by simulation using the general model and specifying the concentrations of readily and slowly biodegradable fractions equal to the stoichiometric equivalent concentrations of glucose and starch in the feed. The constants thus determined were compared with the "standard" constants for municipal wastewaters. With a purely readily biodegradable substrate (glucose) it was necessary to increase only the maximum specific growth rate constant for heterotrophs, μ_H from 2,50 to 3,0/day and the half saturation coefficient, K_S , from 5,0 to 10,0 mgCOD/l; the specific yield constant, Y_H remained as before, i.e. $Y_H =$

0,666 mg cell COD yield/mgCOD utilized. For a purely particulate slowly biodegradable substrate (maize starch) and a mixture of glucose and maize starch, the growth rate constant, μ_H , remained at 2,5/d, the solubilization rate for particulate substrate, K_H , had to be reduced from 2,20 to 1,80 mgCOD/mg cell COD/day and the specific yield, Y_H , reduced from 0,666 to 0,592 mg cell COD yield/mgCOD utilized. With these constants reasonably good simulated fits to the observed data were obtained. Compared to the standard constants the deviations of the constants for these specific substrates are small and very likely are due to the specificity of the substrates – it is to be expected that both the readily and slowly biodegradable COD fractions would be influenced in some degree by the chemical structure or the organic material in each fraction.

Examples of the experimental data obtained are given in Figs 5.1 to 5.4:

In Fig 5.1 the experimental and simulated diurnal carbonaceous oxygen utilization rate (COUR i.e. excluding nitrification) response to an 8h square wave feed pattern with glucose as substrate (8h feed followed by 16h no feed) is shown; at feed commencement the COUR rapidly increases from an endogenous respiration level and thereafter remains constant; at feed termination, the COUR decreases precipitously back to the endogenous respiration level again. This type of response indicates that the glucose is utilized as fast as it is discharged to the system.

In Fig 5.2, the experimental and simulated diurnal COUR response to an 18h square wave feed pattern (18h feed, 6h no feed) with starch as substrate is shown; during the feed and non-feed periods, the COUR increases and decreases only marginally with the result that the COUR remains at fairly constant elevated value. This arises because the starch is a particulate slowly biodegradable substrate requiring hydrolysis before utilization. The feed rate of starch is much greater than the utilization rate, so that a large mass of unutilized starch accumulates in the system. Because the organism mass responds to accumulated starch, which varies marginally between feed and non-feed periods, the COUR is continually high, varying only marginally over the day.

In Fig 5.3, the experimental and simulated diurnal COUR response to 12h square wave feed pattern with boiled starch as substrate is shown; at feed commencement the COUR increases relatively rapidly from endogenous respiration levels to a maximum, which is lower than that for glucose; at feed terminations, the COUR initially decreases precipitously, and thereafter decreases slowly over about 8h to the

endogenous respiration level. This type of response indicates that boiling the starch, which is a particulate slowly biodegradable substrate, converts much of it to a readily biodegradable, i.e. glucose, form. The fraction that is not converted is utilized at a rate slower than that at which it is discharged to the system and accumulates in the system during the feed period. After feed termination the accumulated starch is utilized slowly.

In Fig 5.4, the experimental and simulated diurnal COUR response to a 12h square wave feed pattern with a glucose and starch (50/50) mixture as substrate. The COUR response reflects closely the simulated response of a combination of readily and slowly biodegradable feed fractions.

Comparing the glucose-starch COUR response with that for sewage (Fig 5.5) it can be seen that similar responses are obtained: The COUR (i.e. excluding nitrification) for sewage also is a super position of the OUR for the utilization of readily and slowly biodegradable substrates: At feed termination, there is a precipitous drop in COUR; after feed termination the COUR remains high for about 2h after which it commences a steady decline; about 6h after feed termination, the COUR levels off at that associated with endogenous respiration (note the experimental OUR data in Fig 5.5 includes nitrification).

The conclusion formed from this investigation is that the observed response of the glucose, starch and glucose/starch substrates are closely simulated by the bisubstrate model thereby strongly supporting the bisubstrate hypothesis.

2. IAWPRC MODELLING TASK GROUP

In 1983 the International Association for Water Pollution Research and Control (IAWPRC) formed a task group to select and present the best kinetic model for the activated sludge process.

The members of the Group were Prof. M Henze, Technical University of Denmark (Chairman), Prof W Gujer, Swiss Federal Institute of Technology (EAWAG), Prof L Grady, Clemson University, South Carolina, Prof G Marais, University of Cape Town and Prof T Matsuo, University of Tokyo. The group met four times over the next 3 years and presented their conclusions in a special report (Henze *et al.*, 1987). Some aspects of this report are relevant to the modelling effort in this contract:

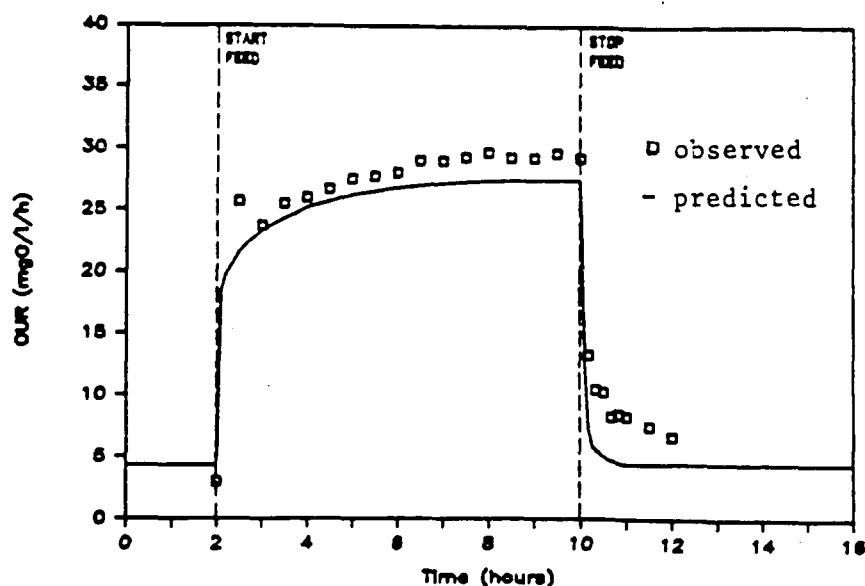


Fig 5.1: Experimentally observed and theoretically predicted carbonaceous oxygen utilization rate (OUR) in a flow through completely mixed aerobic activated sludge system at 2.5 days sludge age and 20°C under daily cyclic square wave loading conditions (7.3/4h feed-16.1/4h no feed) with glucose as substrate.

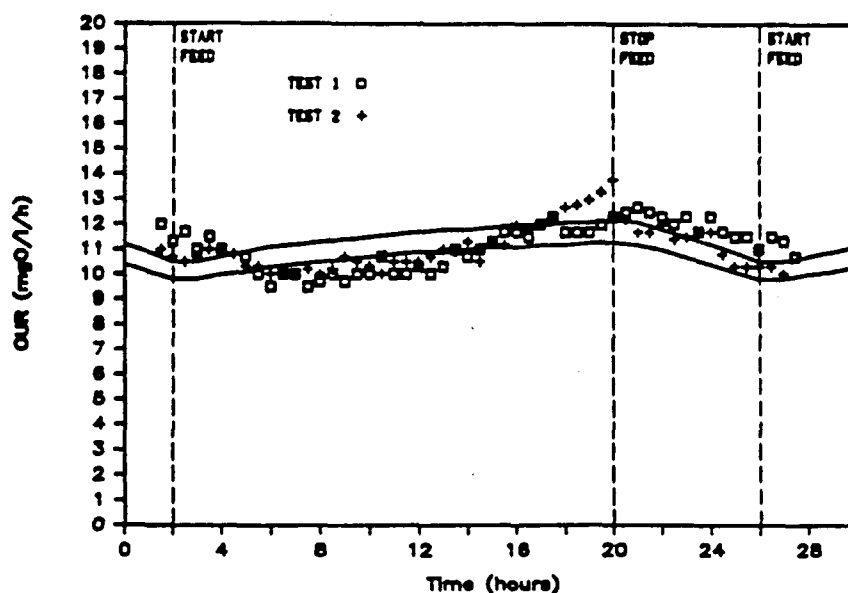


Fig 5.2: Experimentally observed and theoretically predicted carbonaceous oxygen utilization rate (OUR) in a flow through completely mixed aerobic activated sludge system at 10 days sludge age and 20°C under daily cyclic square wave loading conditions (18h feed/6h no feed) with maize starch as substrate.

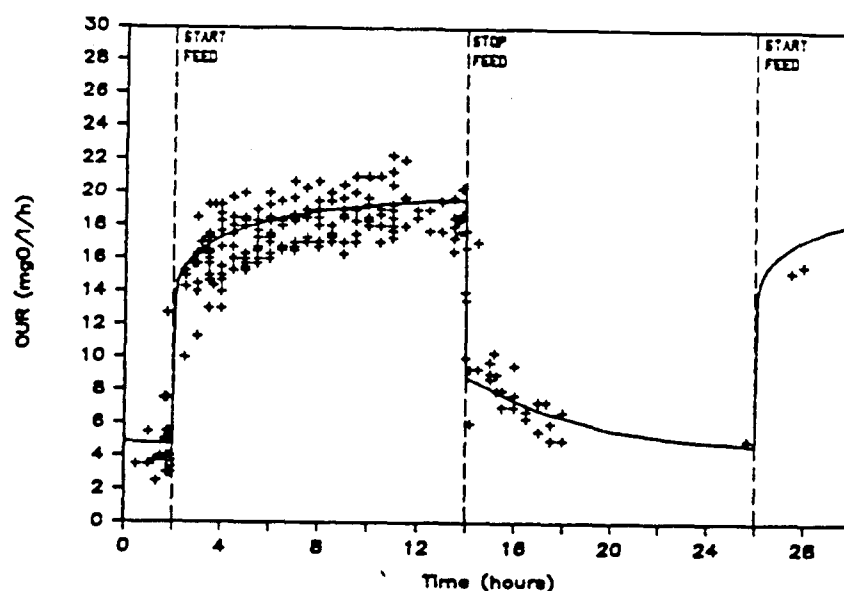


Fig 5.3: Experimentally observed and theoretically predicted carbonaceous oxygen utilization rate (OUR) in a flow through completely mixed aerobic activated sludge system at 2.5 days sludge age and 20°C under daily cyclic square wave loading conditions (12h feed/12h no feed) with boiled maize starch as substrate.

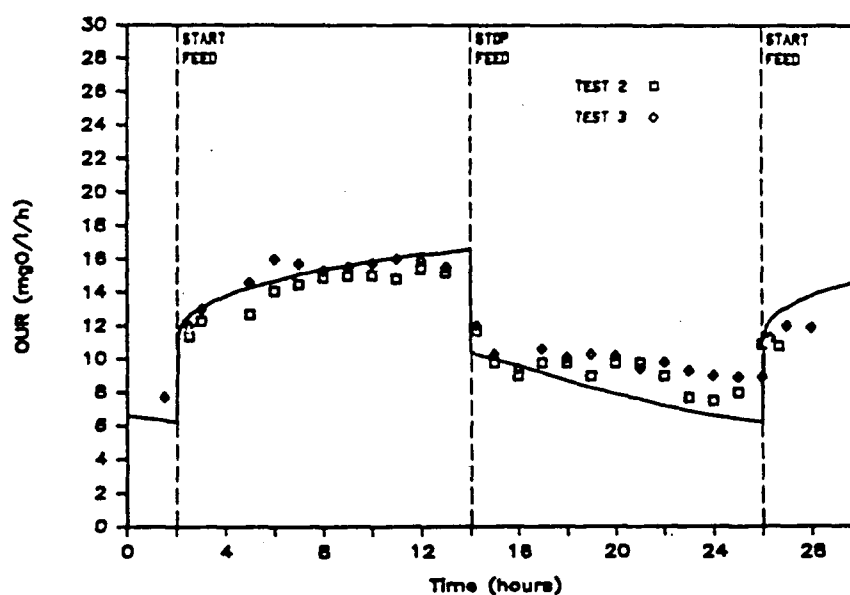


Fig 5.4: Experimentally observed and theoretically predicted carbonaceous oxygen utilization rate (OUR) in a flow through completely mixed aerobic activated sludge system at 10 days sludge age and 20°C under daily cyclic square wave loading conditions (12h feed/12h no feed) with glucose and maize starch as substrate.

- (1) The overall structure of the UCT general kinetic model for COD removal, nitrification and denitrification, with two modifications, was adopted by the Task Group. The accepted model was designated the IAWPRC general activated sludge model. It is particularly gratifying that the two main tenets in the UCT model were adopted for the IAWPRC model i.e. (i) the bisubstrate approach, in which the biodegradable influent COD is divided into readily (soluble) biodegradable and slowly biodegradable (particulate and colloidal) and (ii) the death-regeneration approach, in which cell death at a high rate takes place, releasing biodegradable COD to the bulk liquid which serves as substrate to regenerate new live mass.
- (2) A matrix presentation of kinetic models was adopted as the standard format for presenting the details of kinetic models. This form of presentation greatly facilitates ready comprehension of the processes and the compounds on which they act and provides a structure for systematic computation of system response in numerical solution procedures. The matrix method has been adopted by the UCT group for presentation of models.
- (3) Two important differences between the UCT and IAWPRC models arise with (i) adsorption and (ii) the utilization of the slowly biodegradable COD:

Adsorption: In the UCT model, adsorption is considered to proceed at a defined kinetic rate. In contrast the IAWPRC model accepts that enmeshment/adsorption is immediate and there is no need to model it kinetically.

Utilization: In the UCT model, the adsorbed slowly biodegradable COD is utilized directly by the organism mass; this occurs independently of the utilization of RBCOD. The slowly biodegradable COD is hydrolyzed on the surface of the organisms and then absorbed directly for synthesis. In contrast, in the IAWPRC model slowly biodegradable COD is hydrolyzed in the liquid to readily biodegradable COD by free extracellular enzymes and released to the bulk liquid, where it adds to the readily biodegradable COD derived from the influent. The readily biodegradable COD then is utilized by the microorganisms in competition, for synthesis of active mass. There still is a matter of controversy over between the two approaches; both give virtually identical predictions, but conceptually they differ markedly. The implications of

the two conceptualizations have not been worked out but there is little doubt that this matter needs to be resolved.

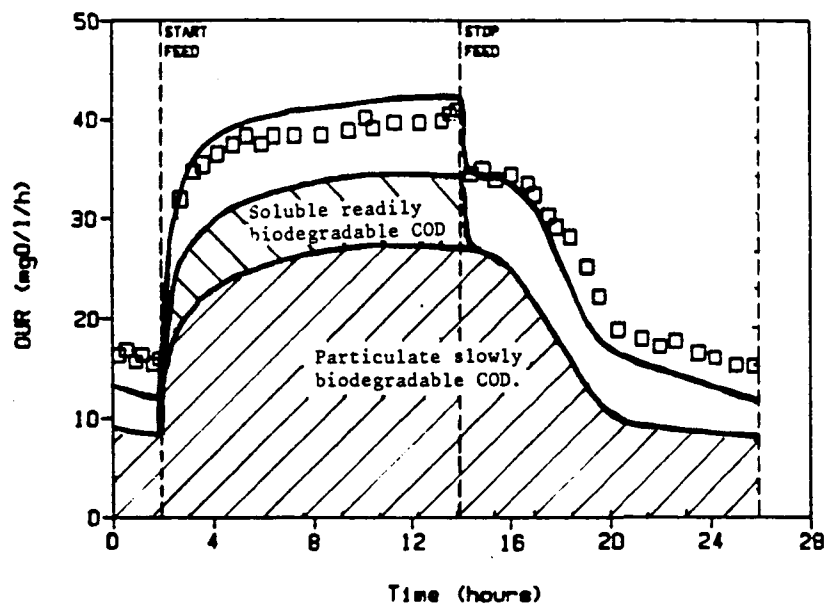


Fig 5.5: Experimentally observed total oxygen consumption rate (TOUR) and theoretically predicted TOUR and carbonaceous OUR (COUR) in a completely mixed aerobic activated sludge system at 2.5 days sludge age and 20°C under daily cyclic square wave loading conditions (12h feed/12h no feed) with municipal sewage as substrate. Note that COUR is a combination of the OUR for utilization of soluble readily biodegradable COD (RBCOD) like glucose and particulate slowly biodegradable COD (SBCOD) like starch.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

The principal objective of this contract was to develop a kinetic model for biological excess phosphorus removal (BEPR) in biological nutrient removal plants, with the view to improving BEPR technology. Referring to Fig 1.1, this objective essentially required the completion of four research projects; (1) BEPR system performance, (2) substrate description, (3) modelling of BEPR and, (4) non-polyP activated sludge modelling.

1. BEPR SYSTEM PERFORMANCE

Previous research on the performance of BEPR systems, treating raw and settled municipal wastewaters, had been limited to sludge ages between 12 and 30 days and the influent COD 'standardized' at 500 mg/l. Virtually no information was available outside these ranges. In order to obtain more information on system behaviour, enquiry was directed towards behaviour at short sludge ages (3-6 days) and high and low influent COD's (1000 and 250 mg/l respectively). The results of this enquiry were most useful as it verified the behaviour inferred from the 12 to 30 day sludge age investigation, i.e. dependence of BEPR on (1) the anaerobic mass fraction, (2) influent total COD, (3) influent readily biodegradable COD (RBCOD) fraction, (4) the adverse effect of nitrate and oxygen recycled to the anaerobic reactor, (5) improved stability of systems with high influent COD, and (6) apparent low endogenous mass loss rate for the polyP organisms.

These findings greatly assisted in identifying further research requirements. The information now available on system performance is sufficiently extensive so that there is little to be gained at the moment from further research in this area.

2. SUBSTRATE DESCRIPTION

The need for this project arose from the recognition of the importance of the influent COD characteristics in BEPR. Three aspects particularly were in need of attention; (1) accepting the importance of RBCOD, to seek a more expedient method for measurement of this COD fraction than the existing biological ones, (2) surmizing that the polyP organisms can utilize only short chain fatty acids (SCFA, or, VFA), to study the conversion of RBCOD to SCFA in the anaerobic reactor and, (3) accepting that BEPR is virtually proportional to the RBCOD/SCFA, to study

augmentation of this fraction by acid fermentation of the slowly biodegradable COD.

With regard to (1), biological methods that had been developed are based on the response of organisms to the RBCOD fraction. However, the practical difficulties in setting up and operating these bioassay systems greatly detract from their usefulness. For this reason, research was directed to estimating the fraction by physical separation, in this case by ultrafiltration, because it was believed that this COD fraction would consist only of small molecules. This approach proved successful, and practical, so that designers now can form reasonable estimates of this fraction by using this simple separation method. In essence, this work is complete, but supporting data from a number of different wastewaters still are required to check the generality of the approach.

With regard to (2), it was established that the conversion of RBCOD to SCFA is a first order reaction with respect to RBCOD and the active mass of non-polyP heterotrophs. This indicated, and was verified, that two or more anaerobic reactors in series give a higher efficiency of conversion than a single reactor with the same total anaerobic sludge mass. With this kinetic model conversion it is now possible to determine with reasonable certainty the anaerobic mass and the anaerobic reactor configuration to obtain optimal conversion of RBCOD in any situation, within the practical constraints. This work is essentially complete.

With regard to (3), work on full scale systems had shown that SCFA can be generated from the slowly biodegradable COD by acid fermentation of the underflow from the primary settling tank. This task was set up in order to develop a model by means of which the mass of SCFA generated can be estimated. From batch and semi-continuous series reactor systems the kinetic behaviour was identified; that the SCFA generation is a first order reaction with respect to a fraction of the influent primary settling tank underflow sludge concentration; that anaerobic batch or reactor retention time should not exceed 9 days; that from kinetic considerations batch reactors are but slightly superior to single completely mixed reactors for the same residence times up to about 6 days. This task still requires research at laboratory scale to establish optimal conditions, and certainly will require verification at full scale.

3. MODELLING OF BEPR

This project formed the core of the whole investigation, and took up the major part

of the research effort. The main development in the understanding of BEPR can be summarized as follows:

- (1) A biochemical model for BEPR was developed describing the pathways and their regulation. This biochemical model was verified over a range of different situations. The model explains most of the observations relating to BEPR in the nutrient removal systems.
- (2) Enhanced cultures of polyP organisms were successfully developed in the modified Bardenpho and UCT systems. By conducting anaerobic, anoxic and aerobic batch tests on sludge harvested from the enhanced culture systems, the processes and compounds associated with BEPR could be delineated.
- (3) With the processes and compounds defined, a mathematical model was developed in which the kinetics of the process rate and its stoichiometry could be quantitatively expressed. This model appears to describe the BEPR remarkably closely for the batch tests on sludge harvested from the enhanced culture systems.

The tasks above in essence complete the basic enquiry into the biochemical mechanisms and kinetics of BEPR. Insofar as it applies to BEPR in activated sludge systems, the writers are of the opinion that adequate basic information is available and, at the moment, no further research work on this aspect appears to be needed. However a number of tasks related to the application of the basic information still are to be completed, viz:

- (1) Application of BEPR model to simulate the enhanced culture systems under constant flow and load conditions.
- (2) Integration of BEPR model into the general activated sludge model for mixed cultures.
- (3) Test and refine the integrated model under constant and cyclic flow and load conditions.
- (4) Develop a simplified steady state BEPR model for design purposes.

Essentially, developments in the technology of BEPR no longer depend on a better understanding of the phenomenon itself, but on a better understanding of how to deal with problems that can develop in the operation of BEPR plants, viz: (1) control of filamentous organism growth (bulking), (2) augmentation of the readily biodegradable COD by *inter alia* acid fermentation, (3) efficient back-up systems to maintain high P removals during periods of plant malfunction, e.g. aeration breakdown, that can cause massive P discharges, and (4) procedures that limit P feedback to the biological system from the liquid discharges and supernatants generated in sludge stabilization and dewatering unit operations.

4. NON-POLYP ACTIVATED SLUDGE MODELLING

The IAWPRC Task Group on modelling biological wastewater treatment systems accepted in a large measure the activated sludge model developed by the UCT group; some changes were made and the model incorporating these changes has become known as the IAWPRC activated sludge model. The aspects found unacceptable by the IAWPRC group were; (1) that enmeshment/adsorption is a time dependent process and, (2) that slowly biodegradable COD is modified on the organism (by enzymes attached to the cytoplasmic membrane) and then directly utilized by the same organism. Instead they proposed that; (1) enmeshment is immediate and, (2) organisms release enzymes to the bulk liquid which then solubilize the slowly biodegradable COD to RBCOD which then becomes available in competition to *all* the organisms. Both modifications are not accepted by the UCT group.

Further research into the following is recommended:

- (1) Make a thorough comparison between the predictive powers of the IAWPRC and UCT models using experimental data generated at UCT. This exercise will serve also to calibrate the models accurately.
- (2) Rewrite the UCT model computer programme to make it suitable for personal computers; the programme should be interactive, and well documented for ready use by consulting engineers.
- (3) Distribute freely the computer programme. Initial indications are that this programme will be technically superior to those produced elsewhere by the other members of the IAWPRC Task Group.

REFERENCES

1. Abbot B J, A I Laskin and C J McCoy (1973). Growth of *Acinetobacter calcoaceticus* on ethanol. Appl. Microbiol., 25, 787-792.
2. Blackbeard J R, D M D Gabb, G A Ekama and G v R Marais (1988) Identification of filamentous organisms in nutrient removal activated sludge plants in South Africa. Water SA, 14(1), 29-33.
3. Bagg W K, P L Dold and G v R Marais (1986) "Verification of the bisubstrate concept in the activated sludge process" Res Rep W 56, Dept of Civil and Chemical Eng, Univ of Cape Town.
4. Burke R, P L Dold and G v R Marais (1986) "Biological excess P removal in short sludge age activated sludge systems" Res Rep W 58, Dept of Civil and Chemical Eng, Univ of Cape Town.
5. Dold P L, G A Ekama and G v R Marais (1980) A general model for the activated sludge process. Prog.Wat.Tech., 12, 47-77.
6. Dold P L, W K Bagg and G v R Marais (1986) "Measurement of the readily biodegradable COD fraction in municipal wastewater by ultrafiltration". Res Rep W 57, Dept of Civil and Chemical Eng, Univ of Cape Town.
7. Dold P L and G v R Marais (1986) Evaluation of the general activated sludge model proposed by the IAWPRC Task Group. Presented at the IAWPRC Specialized Seminar on Modelling of Biological Wastewater Treatment, Copenhagen, Aug 1985. Wat.Sci.Tech., 18, 63-89.
8. Du Preez J C (1980). Growth kinetic studies of *Acinetobacter calcoaceticus* with special reference to acetate and ethanol as carbon sources. Doctoral thesis, Univ. of Orange Free State, Bloemfontein, S.A.
9. Ekama G A (1984) Final report to the Water Research Commission on a four year contract on research on the optimization of the modified activated sludge process for nutrient removal (1980-1983). Research Report W52, Dept of Civil Eng, Univ of Cape Town.
10. Ekama G A, P L Dold and G v R Marais (1986). Procedures for determining influent COD fractions and the maximum specific growth rate of heterotrophs in activated sludge systems. Presented at the IAWPRC Specialized Seminar on Modelling of Biological Wastewater Treatment, Copenhagen, Aug. 1985. Wat.Sci.Tech., 18, 91-114.
11. Gabb D M D, Ekama G A, Jenkins D and Marais G v R (1988) Incidence of *Sphaerotilus natans* in laboratory scale activated sludge systems. Presented at 14th IAWPRC Biennial Conference, Brighton, July 1988, Wat.Sci.Tech.
12. Gloor R, H Leidner, K Wuhrman and T Fleischmann (1981). Exclusion chromatography with carbon detection. A tool for further characterisation of dissolved organic carbon. Water Research, 15(4), 457-462.
13. Henze M, C P L Grady, W Gujer, G v R Marais and T Matsuo (1987). Activated sludge model No.1. International Task Group on modelling biological wastewater treatment systems. IAWPRC Scientific and Technical Report series, Pergamon Press, Oxford.

14. Lötter L H, M C Wentzel, R E Loewenthal, G A Ekama and G v R Marais (1986). A study of selected characteristics of *Acinetobacter* spp. isolated from activated sludge in anaerobic-anoxic-aerobic and aerobic systems. Water SA, 12, 4, 203-208.
15. Lakay M T, Wentzel M C, Ekama G A and Marais G v R (1988) Bulking control with chlorination in a nutrient removal activated sludge system. Water SA, 14(1), 35-42.
16. van Haandel A C, G A Ekama and G v R Marais (1981). The activated sludge process Part 3: single sludge denitrification. Water Research, 15, 1135-1152.
17. Wentzel M C, P L Dold, G A Ekama and G v R Marais (1985). Kinetics of biological phosphorus release. Wat.Sci.Tech., 17, 57-71.
18. Wentzel M C, L H Lötter, R E Loewenthal and G v R Marais (1986). Metabolic behaviour of *Acinetobacter* spp. in enhanced biological phosphorus removal - a biochemical model. Water SA, 12, 4, 209-224.
19. Wentzel M C, P L Dold, R E Loewenthal, G A Ekama and G v R Marais (1987). Experiments towards establishing the kinetics of biological excess P removal. Presented at IAWPRC Specialized Conference on enhanced biological phosphate removal, Rome, September 1987. Advances in Water Pollution Control, 79-97, Pergamon Press, Oxford.
20. Wentzel M C, R E Loewenthal, G A Ekama and G v R Marais (1988a). Enhanced polyphosphate organism cultures in activated sludge systems - Part 1: Enhanced culture development. Water SA, 14, 2, 81-92.
21. Wentzel M C, G A Ekama, R E Loewenthal, P L Dold and G v R Marais (1988b). Enhanced polyphosphate organism cultures in activated sludge systems - Part 1: Experimental behaviour. Submitted to Water SA.
22. Wentzel M C, P L Dold, G A Ekama and G v R Marais (1988c). Enhanced polyphosphate organism cultures in activated sludge systems - Part 3: Kinetic modelling. Submitted to Water SA.
23. WRC Manual (1984) Theory, design and operation of nutrient removal activated sludge processes. Water Research Commission, Pretoria, South Africa.