On a two year study on the enhancement of biological phosphate removal by altering process feed composition

(PLANT AND LABORATORY STUDIES)

By DW Osborn LH Lotter AR Pitman & HA Nicholls

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JOHANNESBURG CITY COUNCIL

CITY HEALTH AND CITY ENGINEER'S

DEPARTMENTS

REPORT TO THE WATER RESEARCH COMMISSION ON A TWO YEAR STUDY ON THE ENHANCEMENT OF BIOLOGICAL PHOSPHATE REMOVAL BY ALTERING PROCESS FEED COMPOSITION (PLANT AND LABORATORY STUDIES)

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REPORT BY THE JOHANNESBURG CITY COUNCIL ON A TWO YEAR STUDY ON THE ENHANCEMENT OF BIOLOGICAL PHOSPHATE REMOVAL BY ALTERING PROCESS FEED COMPOSITION (PLANT AND LABORATORY STUDIES)

EXECUTIVE SUMMARY

1 PROJECT AIMS

During the two year extension of the Contract, the aim of the research work was to :-

- improve elutriation of readily biodegradable compounds from primary sedimentation tanks to achieve optimum use of this process
- minimise the mass of nitrate entering the anaerobic zone
- monitor oxygen utilisation rates (OUR) and oxidation reduction potentials (ORP) in the plant to optimise power consumption
- develop less labour intensive and more accurate methods of determining volatile fatty acid levels
- confirm postulates contained in the biochemical model of biological phosphorus removal experimentally.
- consolidate on-line effluent monitoring
- incorporate new design features in plant modifications.

2 RESEARCH WORK

A programme of airlancing of the sludge in the primary sedimentation tanks was undertaken in an attempt to improve elutriation of readily biodegradable COD. At the same time the effect of passing the return sludge through an anoxic zone was evaluated.

Oxygen utilisation rates in the aerobic zone and oxidation reduction potential of the anaerobic zone were monitored continuously. A number of methods for the determination of volatile fatty acids were evaluated.

Microbiological evaluation of the activated sludge process was continued. This included microscopic evaluation of the floc-forming and filamentous organisms, evaluation of isolation media and identification techniques for population studies.

Metabolic control mechanisms and enzyme behaviour were studied in Acinetobacter spp.

3 CONCLUSIONS

Operational Aspects

Experience at the Johannesburg Northern Works which makes use of both the five and three-stage Bardenpho Process, has shown that the continuous and reliable removal of phosphates to the required standard of 1 mg o-P/ℓ, is critically dependent on having an adequate supply of readily biodegradable COD present in the influent to the anaerobic zone.

Attempts to correlate the oxidation reduction potential in the anaerobic zone and influent VFA levels with effluent phosphate levels, were unsuccessful. This is probably as a result of the numerous factors affecting the process in full-scale plants.

Augmentation of the concentration of readily biodegradable COD naturally present in the influent sewage, was achieved by the fermentation of primary sludge in the sedimentation tanks, and subsequent recycle of sludge for the elutriation of soluble organics. Improved reliability of biological phosphate removal was noted.

Further enhancement of performance was achieved by periodic airlancing to prevent the conversion of fermentation products to methane, under anaerobic conditions.

The lowest effluent phosphate levels were achieved by storing sludge for 24h, recycling for 24h and storing for a further 24h. During storage periods, the sludge was aerated for 30 seconds three times a day.

Full optimisation of this procedure will only be possible when a method for monitoring readily biodegradable COD on-line has been developed.

Performance Monitoring

Considerable experience has been gained with on-line monitors measuring the concentration of phosphates, nitrates, ammonia and alkalinity in effluents. Data from this monitoring network was initially collected in a computer-based system, using programs developed in-house. This proved to be inflexible, requiring new programs to be written for any operational changes, and to produce results in a graphical form more understandable to works staff. This system was augmented with data loggers using commercially available programs. Once again, hourly average data was collected and printed out for the information of operational staff. Data captured on the logger's cassette was transferred weekly to a computer for storage and representation in graphical form.

Because of the limitations described, it is proposed to install a further supervisory system consisting of a number of Programmable Logic Controllers (PLC's), which can be accessed at frequent intervals by a computer that can plot and display data immediately. This computer should also desirably have spreadsheet capabilities which will assist in further in-depth analysis of the process. Another important use of the supervisory system is that in future, it can be coupled to a computer simulaton of the activated sludge process now under development.

On-line monitoring systems can only be justified in the case of large works and should be designed, installed and maintained by staff who are fully conversant with this field.

Design Aspects

Experience gained during the execution of this Contract has highlighted aspects to be borne in mind in the design of biological phosphorus removal plants. These include :-

- feed sewage characteristics
- primary sedimentation designed for fermentation of raw sludge
- load balancing
- unaerated mass fraction should not exceed 0,45
- placement of a second anoxic zone in front of the anaerobic zone has been successful in preventing nitrate in return sludge from entering the anaerobic zone.
- a tapered aeration system with more aeration capacity available at the inlet rather than the outlet of the aerobic zone
- standby chemical dosing systems should be designed to remove at least 50% of the incoming phosphorus load using the most dilute precipitant chemical available.

Microbiological Aspects

Sludge bulking, which was observed at the Northern Works, but not at the diffused air plants at the Bushkoppie Works, could be controlled by maintaining oxygen levels between 1 - 2 mg/ℓ.

While microscopic evaluation provides a rapid diagnostic tool to determine the general health of the plant, it was not found to be suitable as an absolute operating parameter.

Improvements were made to methods used for isolating and identifying bacteria in population studies.

A phosphate releasing capacity test was developed. This simple, inexpensive technique, shows potential in distinguishing between biologically and chemically immobilised phosphate and may be useful in monitoring activated sludge plants combining biological and chemical phosphate removal.

Development and Refinement of Techniques

Methods to determine volatile fatty acids were improved and methods for the determination of polysaccharides were developed.

Fundamental Studies on Chemical and Biological Phosphate Removal

Investigations into *in situ* phosphate precipitation indicated that separate precipitation mechanisms exist for different cations, and that precipitation does not occur in nutrient removal plants without addition of chemicals.

The additional effort and cost involved in the chemical analysis of extracellular polysaccharide compared to microscopic evaluation is not warranted, as the former technique does not provide a more sensitive indicator of plant performance than the latter.

Dehydrogenase activity is relatively easy to determine and its potential use in controlling aeration should be investigated.

A method for the analysis of alkaline phosphatase activity was also developed.

Experimental Evidence Supporting the Biochemical Model

The main hypothesis contained in the model, namely, that the process is controlled by intracellular metabolite levels rather than genetic selection, was found to hold. The studies also showed that Acinetobacter spp, as an example of a polyphosphate bacterium, have the ability to absorb substrate under one unfavourable situation (i.e. anaerobic), and to use the stored substrate as an energy source under a second unfavourable situation (i.e. lack of external carbon), to absorb other essential nutrients like phosphate. The importance of adequate aeration and minimisation of nitrate feedback to the anaerobic zone were explained during this study.

4 RECOMMENDATIONS

In addressing the specific research areas required by the Contract, researchers identified a number of ancillary processes which impinge on biological phosphate removal. In order to integrate biological phosphate removal into a total wastewater treatment protocol, various research needs into these ancillary processes were identified.

Certain research areas addressed during the Contract also require further investigation. These recommendations are intended to encompass both the research needs in biological phosphorus removal and ancillary processes.

Ancillary Processes

Combined chemical and biological phosphate removal in the activated sludge process

In order to comply with the statutory effluent standard, the biological phosphate removal process often requires supplementation with chemical addition. Optimisation of these two processes in combination is essential to ensure the most cost-effective utilisation of both. Different chemicals and dosing protocols should be investigated.

Sludge handling including dewatering, final disposal and metal extraction without loss of phosphorus from sludge

Sludge from nutrient removal plants contains large amounts of biologically immobilised phosphorus. Sludge handling can cause the release of some of this phosphorus to the liquid phase, thus requiring retreatment of the latter. Optimisation of sludge handling and disposal with minimum loss of phosphorus is essential.

Recycling sludge in the form of compost is an attractive proposition, but the metal content limits its application to agricultural land. Recovery of the heavy metals from the sludge without loss of

nutrients, would greatly enhance the attractiveness of the composting option.

Utilisation and role of particulate COD in phosphorus and nitrogen removal and its role in sludge bulking

Particulate COD contains a biodegradable component which must be utilised during the process. No research has been carried out into the optimal use of this carbon source, or its role in various processes prevailing in an activated sludge system.

Removal of non-biodegradable COD

The removal of non-biodegradable COD has, like the utilisation of particulate COD, received little or no attention. Effluent COD levels in excess of the effluent standard, have been observed to arise as result of high levels of non-biodegradable COD in the influent. Methods to reduce effluent levels to within the standard should be investigated.

Sludge bulking in nutrient removal plants

Sludge bulking is a problem experienced in a number of nutrient removal plants. While this problem may be prevented by correct design of new plants, operational protocols are required for bulking control in existing plants. Further research to consolidate design features and operational aspects is required. Alternate methods of solids separation should be investigated.

Further Research Emanating from the Contract

In addition to the abovementioned ancillary avenues which should be investigated, the following areas emanating from this Contract, deserve attention in the future :-

Updating of the biochemical and mathematical models

Current biochemical and mathematical models developed by the University of Cape Town and the City of Johannesburg, should be continually updated in the light of new findings, in order to retain these models at the forefront of research in this area.

The theoretical data generated by these models should be linked to operational aspects and then consolidated into a cohesive whole.

Consolidation of on-line monitoring protocols

Considerable progress has been made with on-line monitoring of activated sludge plants and the handling of data from the monitors. Protocols for their routine use need to be drawn up.

Monitoring effects of innovative design features on process efficiency

A number of design ideas have been generated during the research undertaken during this Contract. In order to evaluate their usefulness, their effect on process efficiency should be monitored.

Consolidation of biological monitoring procedures

Various monitoring techniques have been used during this Contract. Attempts should be made to consolidate and rationalise their use.

Handling phosphate removal plant failures

In the long sludge age plants operated in South Africa, phosphorus can be immobilised over a period of up to twenty days in the sludge.

Plant failures could result in disruption of the environment to such an extent, that the immobilised phosphate is released to the aqueous phase. Strategies should be developed to deal with such an eventuality. These should include protocols for restarting the plant.

Cost effectiveness of feed process alteration versus chemical addition for phosphorus removal

A preliminary paper study revealed that optimisation of biological phosphate removal by altering the process feed composition, is cost effective in comparison to chemical addition. However, additional factors such as possible reduced gas production, the effect of chemical addition on the salinity of the rivers, and the effect of sludge handling, should be investigated.

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Mr J E McGlashan (Chairman)	Water Research Commission
Mr G Offringa	Water Research Commission (Final Meeting only)
Mr J Goodman	Johannesburg City Council
Mr D W Osborn	Johannesburg City Council
Prof GvR Marais/Dr G Ekama	University of Cape Town
Mr A Gerber	Division of Water Technology (CSIR)
Prof P L Steyn	University of Pretoria

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ABBREVIATIONS

SRT	Sludge retention time
TP	Total phosphorus
COD	Chemical oxygen demand
S_{bs}	Readily biodegradable COD
SVI	Sludge volume index
DSVI	Dilute sludge volume index

CHAPTER ONE

INTRODUCTION

1.1 Background

Research into and development of biological nutrient removal from sewage in South Africa over the last twelve years, with much of the work being sponsored by the Water Research Commission, has resulted in significant advances in understanding the process and its successful application in practice. This knowledge has been transferred to user agencies such as consulting engineers and local authorities by way of presentations at local and international conferences, working group discussions and a comprehensive information document on the theory, design and operation of nutrient removal activated sludge processes (Water Research Commission, 1984).

The factors responsible for and controlling phosphate removal from sewage have been identified and to a limited extent quantified. The more important are the composition of the sewage, the bacterial species which remove the phosphate and the process reaction conditions, the first named being the most crucial. Parameters of importance are the quantity of short chain organic compounds, such as acetic acid, present in sewage as well as the relative proportions of carbon to nitrogen and phosphorus.

Mathematical models relating phosphate removal to the above parameters have been developed and found to give conservative estimates of the phosphate removal experienced in practice.

Preliminary investigations by a number of organisations in South Africa have identified a number of ways in which the composition of feed sewage may be altered to make it more amenable to biological phosphate removal. These techniques are all based on supplementing the feed to the biological reactor with additional readily biodegradable soluble organic matter, preferably short chain organic compounds, and include:

- Addition of industrial effluents with high organic matter content to the domestic sewage feed.
- Returning primary sludge removed from raw sewage to the biological reactor instead of anaerobically digesting and stabilizing it.
- Altering the anaerobic digestion process to proceed only up to the acid formation stage and discharging the resultant acids (i.e. short chain organics) to the head of the works.
- Operating primary sedimentation tanks to induce acid formation within the sludge layer and after elutriation discharging the resultant acids to the biological reactor.
- Expanding the anaerobic zone in biological nutrient removal processes to generate the required short chain organics within the process.

During the initial period of this contract (1984-1986) excellent progress was made in evaluating various methods of increasing the readily biodegradable COD content of sewage (Osborn et al., 1986).

The generation of volatile fatty acids in primary sedimentation tanks and recycling of the primary sludge was demonstrated as a successful means of acquiring the desired feed characteristics. The use of high rate digesters for the same purpose, while not in itself capable of generating the required amount of acid was shown to be useful as a back-up facility during times of low COD load. On-line monitoring was successfully used to evaluate the plant response to the addition of volatile fatty acids.

Preliminary results obtained from on-line monitoring of oxygen utilization rates as a means of more efficient plant control also showed promise.

In any biological system, an in depth understanding of the fundamental mechanisms involved enhances the possibility of ultimate control of the process. A number of significant observations in this area contributed to the overall understanding of the biological phosphorus removal process, and allowed the postulation of a biochemical model for the process in collaboration with the University of Cape Town.

The results of this initial work were published in 1986 (Osborn et al, 1986). During the course of these investigations a number of areas requiring further research were identified, namely :

- Methods to improve elutriation of readily biodegradable compounds from primary sedimentation tanks to achieve optimum use of this process.

- Methods to minimize the mass of nitrate entering the anaerobic zone.

- Methods for efficient monitoring of oxygen utilization rates (OUR) and oxidation/reduction potentials (ORP) in the plant to optimize power consumption.

- Less labour intensive and more accurate methods of determining volatile fatty acid levels.

- Experimental confirmation of postulates contained in the biochemical model of biological phosphorus removal.

1.2 Elutriation of volatile fatty acids from primary sedimentation tanks

In the acid fermentation of primary sludge, volatile fatty acids are produced within a sludge layer having a fairly high solids concentration. A practical problem exists in regard to the extraction of a large proportion of these acids from the sludge for addition to the activated sludge process, while retaining most of the settleable solids in the extracted sludge material. A way of doing this is to make use of the concept of primary sludge recycle. Considerable experience has been obtained at Northern Works with the procedure where the results indicate that the removal of suspended solids across the primary sedimentation tanks during primary sludge recycle periods is poor. Thus there is a need to investigate better elutriation techniques to reduce the suspended solids load on the activated sludge process. The continuous flow mode of primary sedimentation tank operation, was altered to a batch operation, where four

tanks were desludged and cleaned out in rotation. The complete desludging of the tanks should prevent loss of volatile acids by conversion to methane. It was intended to optimize mechanical operation of the thickeners to make best use of the extraction capacity of the equipment, thus promoting better partition of fatty acids from the solid to liquid phase. However, this was not possible due to lack of thickening equipment.

1.3 Methods to minimize the mass of nitrate entering the anaerobic zone

Excessive nitrate recycle via the return sludge to the anaerobic zone is a major reason for the failure of the biological nutrient removal process. A way of obviating this problem is to ensure good denitrification in the main process, which is not always easily achieved. An alternative method of ensuring that the return sludge contains minimal nitrates is to pass it through an anoxic zone before it enters the anaerobic zone.

Recent studies at Northern Works have shown that by passing return sludge on its own through a compartmentalised anoxic zone can denitrify it completely thus ensuring a nitrate free sludge feed to the anaerobic zone. In addition considerable phosphate release occurs towards the end of this anoxic zone. This release, in the absence of any added substrate, was investigated.

Operation of one module over a limited period in this manner has produced very good results. Phosphorus removal is consistent and stable and there are indications that this module can tolerate periods of low feed COD levels far better than its neighbouring module.

The effect of feed COD levels on phosphorus removal at different return nitrate levels was studied. The manipulation of feed COD by generation of S_{bs}, and its combined effect on phosphorus removal was also studied at different nitrate levels.

The rate of phosphorus uptake in relation to plant operational parameters like dissolved oxygen (DO) was also studied in order to make maximum use of aeration time.

1.4 Monitoring OUR and ORP in the plant

On-line monitoring of oxygen utilization rate (OUR) in the aerobic zone and oxidation reduction potential (ORP) in the anaerobic zone is the first step in optimizing OUR in the plant. An electronic data management and automatic control system for the above was developed and installed and evaluated on an extended time basis. The equipment has the potential to effect considerable power savings.

The ultimate aim of this work is to investigate the feasibility of feedback control of aerators. In other words activation and deactivation of aerators according to the oxygen demand. In addition attempts were made to develop operational strategies based on OUR and ORP measurements. For instance the sudden draining of a balance tank resulting in a large plug of COD to the plant can be instantly detected by an OUR measurement and a computer generated warning can be transmitted to the operator.

Time sharing techniques to utilize on-line monitors in all zones was investigated.

1.5 Determination of volatile fatty acids

Techniques for the determination of volatile fatty acids (VFA) currently in use are either too insensitive to detect the levels required or too labour intensive to allow sufficient samples to be analyzed.

Preliminary work on a high pressure liquid chromatograph indicates that this technique will be superior to gas chromatography in the research field. The further development of this technique was not pursued due to the high cost and requirement for skilled analysts. A simple test for VFA for plant operators remains elusive. The possibility of using specific enzyme assays, and other colorimetric techniques was investigated.

1.6 Biochemical studies

In a collaborative effort with the University of Cape Town a biochemical model was postulated to describe the biological removal of phosphorus.

(Wentzel et al., 1986). Metabolic studies were undertaken in an attempt to prove certain aspects of the biochemical model. The results of this research are reported in a separate document (Lotter, 1989)

The characterization of the phosphorus species present in activated sludge and the role of each in phosphorus removal was investigated.

1.7 Transfer of Data from The City Council of Johannesburg (JCC) to the Computing Centre for Water Research (CCWR)

A meeting was held to discuss the work which needed to be undertaken in order that data could be transferred from the JCC mini computer at Cydna to the CCWR computer at Pietermaritzburg. Software requirements were identified as :

- A program to write the required data to magnetic tape at Cydna
- A query program to enable the data to be searched on the CCWR computer.

Additionally a conversion process was required to overcome an incompatibility problem with the magnetic tape format.

The first program has been written and implemented at Cydna whilst an existing program was supplied to CCWR, where it was modified to run on the CCWR computer.

The incompatibility problem can most easily be solved by running the Cydna tapes on a computer on the Durban campus of the University of Natal. A second tape is then produced in the correct format for the CCWR computer.

A trial run has been undertaken and was completely successful. Information about sample points, determinands and the operation of the query, program have been supplied to CCWR for incorporation in their newsletter.

It has been decided that until the level of demand for this data has been established, only wastewater effluent samples and river samples will be included in the data bank. Should the demand warrant it, the range of sample points can be increased. The query program has at this stage been

kept as simple as possible, it, too can be enhanced if required.

1.8 General

In each of the following chapters, particular aspects of this research programme are discussed in detail. Due to the specific nature of the experimental work, these chapters have been structured in such a way as to allow each to stand alone. Chapter 8 however attempts to place all the findings in perspective.

1.9 References

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Lötter, L H (1989). Enhancement of biological phosphate removal by altering process feed composition. (Metabolic Control Mechanisms) WRC Report No 137/3/89.

Wentzel, M C, Lötter, L H, Loewenthal, R E and Marais, G v R (1986). Metabolic behaviour of Acinetobacter spp in enhanced biological phosphorus removal : A biochemical model. Water SA 12, 209 - 224.

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CHAPTER TWO

ON-LINE MONITORING : DATA PROCESSING

H A NICHOLLS AND C S STEVENS

2.1 INTRODUCTION

A number of on-line monitoring systems were examined at the Johannesburg Northern Works, to determine the reliability and stability of nutrient removal activated sludge plants. The first system, developed by the Johannesburg Electricity Department, was computer controlled and specifically designed to meet the requirements at the Northern Works. At a later stage, a data logger was connected in parallel with this system. Unfortunately, both these systems had severe shortcomings, with the result that a third system is currently being investigated. The advantages and disadvantages of all three systems, together with some comments regarding on-line monitoring in general, are presented.

2.2 COMPUTER BASED SYSTEM USING PROGRAMS DEVELOPED IN-HOUSE

2.2.1 System Description

The details of this system have already been discussed by Osborn et al., (1986), and will only be summarised below :-

The system was controlled by an Apple micro-computer which was sited in the Administration Building approximately 1,5 km from the activated sludge plants (Fig 2.1).

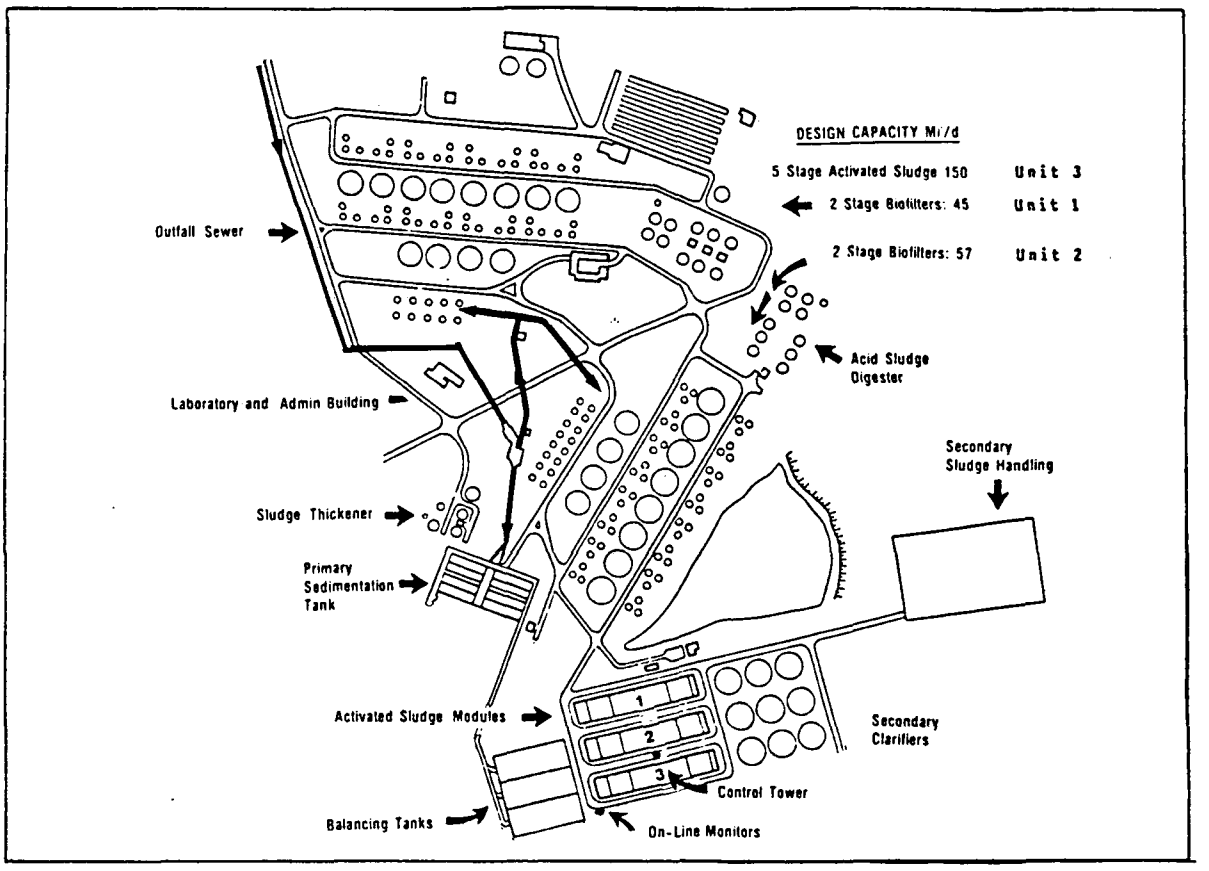

Figure 2.1 : Layout of the Johannesburg Northern Works

Data was transmitted from the computer to the outstation which was sited in the control tower, via an overhead wire system connected to modems at either end. Connection of the outstation to the monitors, was via a cable system housed in water piping to minimise damage from lightning. The parameters monitored on Module 3 of Unit 3 were as follows :-

- Phosphate in the final effluent
- Redox potential at a depth of 2 m in the middle of the anaerobic reactor
- Respiration rates at the start, middle and end of the main aeration basin.

Phosphate and nitrate levels in the effluent from Module 2 were also monitored.

The development of the computer programs to control the outstation, log the data from the monitors and control the respirometers, proved to be a long and tedious task. Approximately 1 000 hours of programming were required to develop this system. Maintenance became a major problem, for the overhead cables were often struck by lightning, causing damage to both the computer and outstation hardware. This problem was finally overcome by installing a squirrel wire above the cable and earthing the latter at roughly 200 metre intervals.

2.2.2 **Major Drawbacks To This System**

- All data collected was either stored on disk or printed out as received. Plotting facilities were not easily available since this required additional specialised programs to be written for every graph. A solution to this problem would have been to transfer the data to an IBM-compatible computer, and to then use a commercially available program for plotting purposes, but unfortunately, this facility was not available.

- Since all the programs were developed in-house, any change of staff required a new learning period before any minor or major changes could be made to the program.

- In general, in-house programs are not user-friendly and any change in operational staff required a learning period of two to three weeks.

- The display on the computer screen in the administration block was very basic, depicting only the latest concentrations. Whilst this information was of interest to the operator, it would have been considerably more useful if it had been possible to display the last twenty results, so that trends might be identified. To include trends in the program would have required major additions.

2.2.3 Presentation of Data

A printout of the data collected from the Apple computer (Table 2.1), was manually entered onto a spread sheet using an IBM computer located at a different laboratory some 35 km away. A plot of this data was then sent to the operational staff a few days later for information purposes only. A typical example of these plots is given in Figures 2.2 and 2.3.

TABLE 2.1 : TYPICAL EXAMPLE OF THE DATA PRINTED OUT BY MICRO-COMPUTER

Wed 28 May	our (0)	cc (0)	do (0)	our (1)	cc (1)	do (1)	our (2)	cc (2)	do (2)
11:53:54	42,8	0,998	1,3	14,9	0,998	2,3	12,5	0,974	1,4
12:17:20	42,2	0,998	1,5	14,8	0,997	2,1	10,2	0,986	1,3
12:40:46	45,4	0,999	1,5	15,1	0,998	2,1	12,3	0,962	1,3
13:04:12	39,9	0,999	1,5	15,1	0,997	2,0	12,2	0,963	1,2
13:27:38	50,9	0,998	1,5	15,5	0,996	1,8	12,2	0,979	1,2
13:51:04	53,5	0,999	1,4	15,7	0,997	1,6	10,4	0,980	1,1
14:14:30	56,6	0,999	1,4	15,5	0,997	1,4	8,5	0,970	0,8
14:37:56	57,0	0,998	1,3	15,7	0,997	1,3	11,7	0,979	0,7
15:01:22	56,5	0,999	1,3	15,6	0,996	1,2	10,3	0,981	0,6
15:24:48	56,6	0,999	1,4	16,6	0,997	1,0	8,9	0,976	0,6
15:48:14	57,1	0,999	1,4	16,3	0,997	1,2	11,6	0,984	0,3
16:11:40	56,3	0,999	1,3	21,3	0,998	1,1	11,1	0,982	0,4
16:35:06	56,1	0,999	1,4	22,4	0,998	1,2	11,2	0,991	0,4
16:58:32	58,0	0,999	1,3	26,4	0,998	1,3	10,2	0,973	0,3
17:21:58	56,7	0,999	1,2	27,4	0,999	1,1	9,8	0,991	0,3
17:45:24	57,4	1,000	1,2	27,2	0,998	1,0	10,2	0,983	0,3

KEY : **our = oxygen utilisation rate mg O/ℓ**
cc = correlation coefficient
do = dissolved oxygen mg O/ℓ

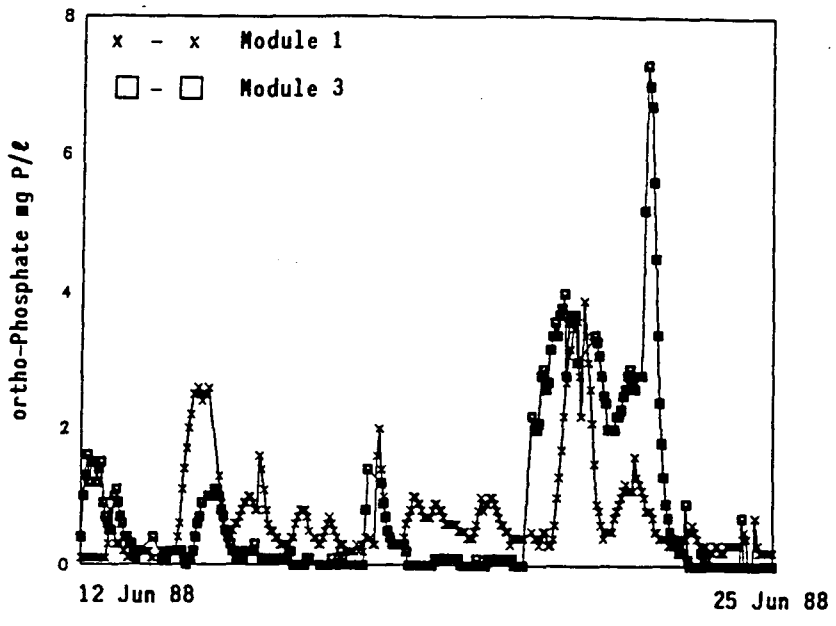

Figure 2.2 : Effluent phosphate concentrations : Modules 1 and 3

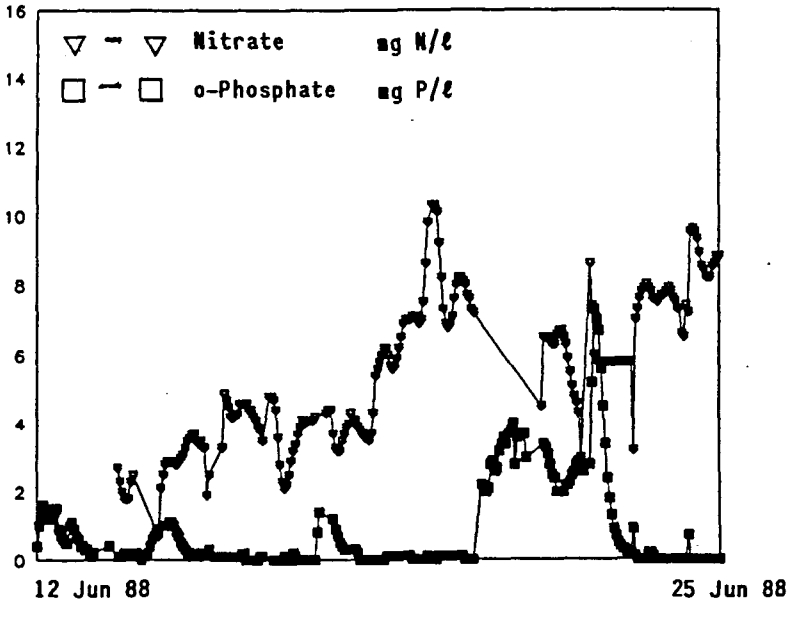

Figure 2.3 : Effluent nitrate and phosphate concentrations : Module 1

In general therefore, although this system gathered information on-line, because of the limited data processing facilities in the system itself, the full potential of on-line monitoring could not be exploited. For this reason, it was decided to connect a data logger in parallel with the computer based system.

2.3 THE USE OF DATA LOGGER SYSTEMS

The data logger used was capable of handling 30 analogue inputs which was sufficiently adequate to permit collection of data from monitors situated on the activated modules of Unit 3, and the two-stage biological filtration system of Unit 1 (Figure 2.1). Iron sulphate was being added to the latter unit to chemically remove phosphate, but resulted in an acidic effluent. Lime was therefore added as a neutralising agent. On-line measurement and recording of the alkalinity of this chemically treated water was essential if corrosion of plant was to be avoided. As all the required cables had already been installed, it only took about 10 days to have the total system connected and running.

The logger was programmed to collect readings every minute from all the monitors except the respirometers, and to average these figures every hour for retention in the logger memory and display in the administration block. Once a week, the data stored on the logger's tape cassette was transferred to a mini-computer which was used for bulk storage, and the preparation of graphical presentations. These plot programs were written in-house for another research project, and were reasonably adaptable to this application as well. Changes to permit, for example, a continuous plot of data collected over a period of two months were not easily made and would have required extensive reprogramming. The existing system permitting the collection and plotting of data on a weekly basis was therefore adopted.

Each week the Works Manager would be given a set of graphs depicting the effluent quality from both the activated sludge plants and the filter plant. For operational purposes he would have to use the

hourly printed values discussed above. Examples of both the plots and the printouts are given in Figure 2.4 and Table 2.2 respectively.

TABLE 2.2 : EXAMPLE OF HOURLY PRINTOUT FROM DATA LOGGER

```
04 RPT1  1111  054:12:00      ALK 094.42>N1  P04 007.03UN1
POP 003.23M02  N03 003.40M02  RED 425.64M03  POS 000.75M03
PH  008.98UN1  ORP 388.54M02  /

04 RPT1  1111  054:13:00      ALK 098.76>N1  P04 006.95UN1
POP 003.13M02  N03 003.45M02  RED 327.00M03  POS 000.69M03
PH  009.06UN1  ORP 405.36M02  /

04 RPT1  1111  054:14:00      ALK 098.43>N1  P04 007.10UN1
POP 002.99M02  N03 003.52M02  RED 376.65M03  POS 000.60M03
PH  008.96UN1  ORP 396.08M02  /

04 RPT1  1111  054:15:00      ALK 098.38>N1  P04 007.02UN1
POP 002.84M02  N03 003.55M02  RED 381.42M03  POS 000.55M03
PH  009.01UN1  ORP 402.09M02  /

04 RPT1  1111  054:16:00      ALK 100.18>N1  P04 006.65UN1
POP 002.57M02  N03 003.61M02  RED 383.25M03  POS 000.49M03
PH  008.90UN1  ORP 408.03M02  /

04 RPT1  1111  054:17:00      ALK 102.67>N1  P04 005.86UN1
POP 002.23M02  N03 003.72M02  RED 428.74M03  POS 000.43M03
PH  008.55UN1  ORP 406.44M02  /

04 RPT1  1111  054:18:00      ALK 106.46>N1  P04 005.07UN1
POP 002.00M02  N03 003.89M02  RED 472.95M03  POS 000.44M03
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04 RPT1  1111  054:19:00      ALK 109.41>N1  P04 004.52UN1
POP 001.81M02  N03 004.06M02  RED 379.54M03  POS 000.48M03
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04 RPT1  1111  054:20:00      ALK 114.54>N1  P04 004.66UN1
POP 001.63M02  N03 004.04M02  RED 386.25M03  POS 000.34M03
PH  007.39UN1  ORP 399.02M02  /

04 RPT1  1111  054:21:00      ALK 117.98>N1  P04 004.49UN1
POP 001.54M02  N03 004.08M02  RED 385.69M03  POS 000.31M03
PH  007.22UN1  ORP 393.51M02  /

04 RPT1  1111  054:22:00      ALK 118.51>N1  P04 004.41UN1
POP 001.53M02  N03 004.08M02  RED 385.85M03  POS 000.29M03
PH  007.41UN1  ORP 391.50M02  /
```

KEY : RPT1 = Average of data collected over 1 h

059:02:00 = Julian day:Hour:00

Chemical Parameters

Alk	Alkalinity
NO_3	Nitrate as mg N/ℓ
pH	Hydrogen ion concentration
POP, PO_4, POS	Phosphate as o-P mg/ℓ
RED	Oxidation reduction potential -ve mV

Plant

UN1	Unit 1 - Biological filters
M02	Module 2 of Unit 3
M03	Module 3 Activated sludge

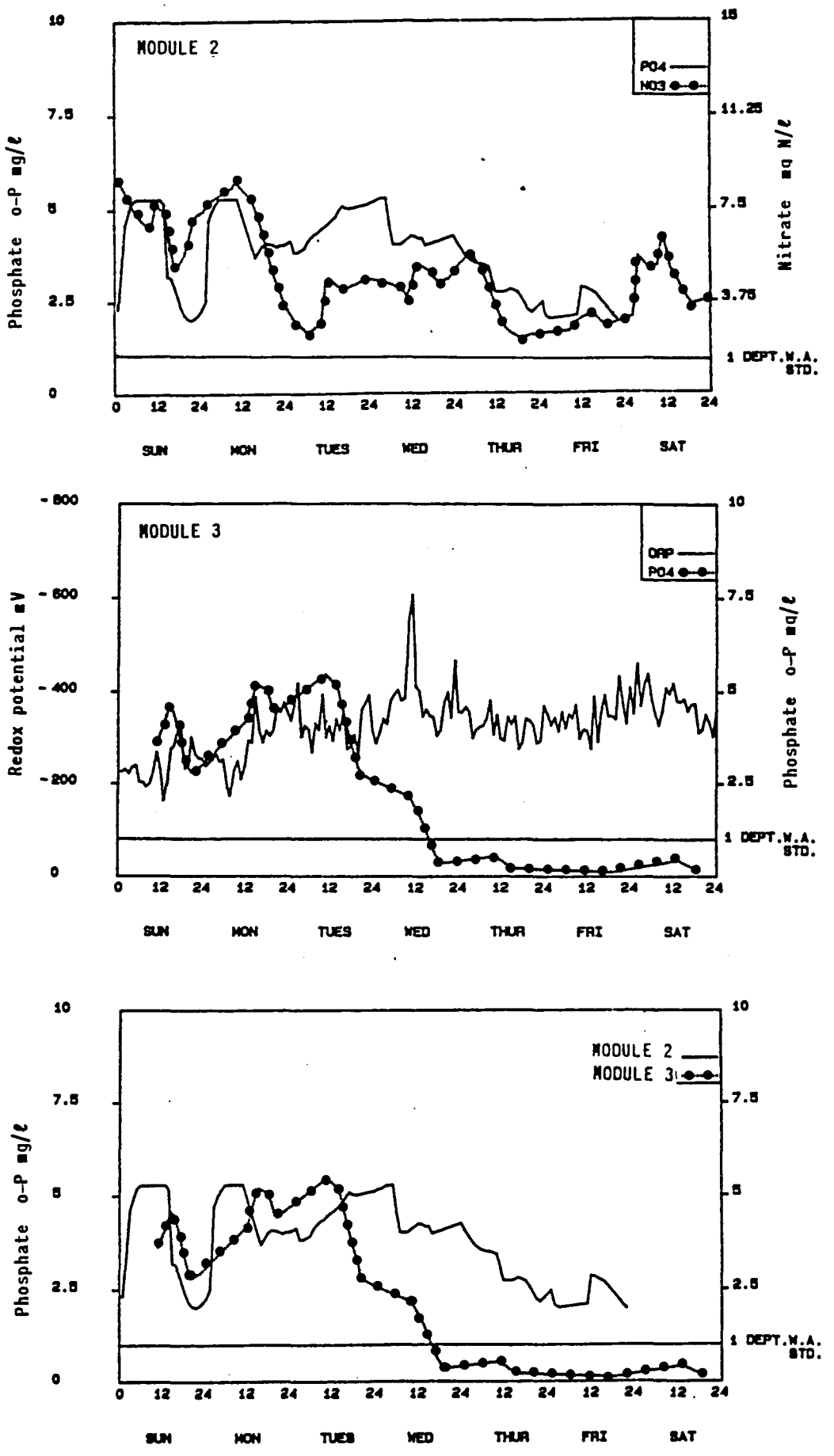


Figure 2.4 : Examples of graphs constructed from data captured by Logger 10-16 April 1988

The disadvantages of the system included inadequate facilities to control the respirometers, and furthermore, it was unable to present data collected in graphical form, to permit the determination of trends.

SUPERVISORY COMPUTER CONTROLLED SYSTEM

Because of the limitations of the two systems described above, it is proposed that they be phased out and replaced by a commercially available supervisory package, when modifications are carried out to the Northern Works in the near future. It is envisaged that a number of Programmable Logic Controllers (PLC's) will be placed at strategic points on the plant and will gather information from all the monitors, flow meters, pumps, aerators, etc., and store in their individual memories. This data will be accessed at selected fixed time intervals of, say, every five minutes, by an IBM-compatible computer located near the Manager's office.

The main advantage of this method is that all the data can be plotted and displayed instantaneously. In addition, alarms can be set to warn to operator of changing conditions. Furthermore, the data collected can be transferred to a spreadsheet for further in-depth evaluations of the process. This is a most useful and powerful feature of this system, and if utilised to its full extent, should result in an improvement in plant operation.

Another important use of the supervisory control system is that it can be coupled to a computer simulation of the activated sludge process, which will enable the operator to predict when the plant effluent is unlikely to comply with the effluent quality standard. Verification of the accuracy of this simulation model under full-scale plant operating conditions, is the subject of another research investigation currently in progress.

The design and installation of this type of control system requires the services of professional system control engineers, who can

exploit the use of PLC's to their full extent. On account of the high degree of professionalism required, these systems are considerably more expensive than data loggers. Nevertheless, when all the benefits of on-line monitors are made available to operators through the supervisory system, both the operator's understanding of the processes, as well as the plant performance, would improve. This in turn, will reduce operational costs and in a very short while, offset the costs of the supervisory system.

2.5 RESPONSE OF THE WORKS OPERATIONAL STAFF TO ON-LINE MONITORING

Until graphs were produced depicting the performance of the activated sludge plant, the works operational staff showed little interest, and in fact, were rather sceptical about the entire monitoring programme. Once the results were depicted graphically however, works operation took on a new dimension; it became a dynamic process. The daily peak effluent phosphate concentrations were noticeable, whereas previously, they were mistakenly thought to be non-existent. Furthermore, the rapidity with which these peaks increased and decreased was a surprise to all. These observations led to many questions being asked, which in turn led to more discussion, and in the end, improved plant operation.

Probably the most important aspect of on-line monitoring is that firstly, it requires a group of dedicated technicians to keep the system up and running, and secondly, informed operators who are prepared to make full use of all the data generated. In other words, it is essential to have the commitment of all staff to on-line monitoring, without which, no system will be successful, no matter how good it is!

Three levels of expertise are required :-

- technicians who can maintain the monitors from a chemical and mechanical point of view

- electronic technicians who can maintain the electronic

equipment on both the computers, monitors and PLC's etc

- programmers who can make changes to the software if and when required.

With regard to chemical and mechanical maintenance technicians, it is essential to have such a technician on site. From the experience gained during this project, the sensing devices were by far the most troublesome part of the entire monitoring and control system. Daily attention was necessary even if it amounted to only five minutes.

Contracts can be entered into with suitable companies to provide the other maintenance skills required , on a call-out basis. Probably, the ideal situation is to employ the writers of the PLC software to maintain the electronic equipment. Furthermore, if a plant is not in easy reach of help, on-line monitoring should not be considered.

2.6 COST BENEFITS OF ON-LINE MONITORING

Since the ideal monitoring and control system has not yet been installed at Northern Works, no actual cost can be given. However, without on-line monitoring, large excesses of chemicals would have been added to remove phosphate, if action had been taken only on the results obtained from 24 hour composite samples. Also, the extent of corrosion problems resulting from this chemical addition would only have become noticable once the damage had been done. Even with the primitive on-line systems in use at the Northern Works, it has been possible to keep both chemical addition and corrosion to a minimum.

2.7 CONCLUSIONS

- The on-line monitoring discussed in this paper is only applicable to plants with effluents which have a significant impact on our rivers and dams; in other words, only large plants.

- There are no shortcuts which can be taken when installing a reliable on-line control and monitoring system. It is simply not worth the time and effort to install a system which meets half the requirements.

- For on-line monitoring to be successful it must have the desired impact on the operator.

- Only specialists in control and monitoring should design and commission on-line monitoring systems.

- It is essential to have a dedicated staff to run and maintain the system.

2.8 REFERENCES

D W, OSBORN., L H, LöTTER., A R , PITMAN., and H A, NICHOLLS. (1986). Enhancement of Biological Phosphate Removal by Altering Process Feed Composition. Report to the Water Research Commission, P O Box 824, Pretoria 0001, Republic of South Africa. WRC Report No 137/1/86.

CHAPTER THREE

PLANT CONTROL STRATEGIES : MODIFICATION OF INFLUENT SEWAGE CHARACTERISTICS

H A NICHOLLS, C S STEVENS AND S DEACON

3.1 INTRODUCTION

To achieve a consistent effluent orthophosphate concentration of less than 1 mg P/ℓ relying solely on biological phosphate removal, has been difficult to accomplish at the Johannesburg Northern Works (Nicholls et al., 1987). The main reason for this unsatisfactory phosphate removal was an inadequate supply of readily biodegradable COD or volatile fatty acids (VFA) in the feed to the activated sludge process. In order to improve this situation, volatile fatty acids were either added from an external source, or generated within the primary sedimentation tanks (Osborn et al., 1986). In both of the above procedures however, methane fermentation ultimately commenced with a concomitant loss of fatty acids (Osborn and Nicholls, 1985). To overcome this problem, air lancing of sludge which accumulated on the floor of the primary sedimentation tank, was introduced. This paper discussed the effects of this air lancing on biological phosphate removal in the activated sludge process.

Two main issues were of concern :

- would intermittent aeration prevent methane formation and result in an increase in the readily biodegradable COD in the feed to the activated sludge process ?

- would air lancing increase the concentration of suspended solids in the feed to the process and improve phosphate removal ?

Both of these issues would also influence the mass of nitrogen removed in the activated sludge plant, which in turn, would also influence the mass of phosphate removed.

3.2 HISTORICAL PERFORMANCE OF THE PRIMARY SEDIMENTATION TANKS

Nicholls et al., (1987) have described a number of procedures for operating the primary sedimentation tanks, where the sludge was allowed to accumulate and ferment on the floor of the tank, and after a period of time, the volatile acids so produced, were elutriated from the sludge by recycling it back to the inlet of the sedimentation tanks. A summary of these results together with operational data under design conditions is given in Table 3.1.

TABLE 3.1
PERFORMANCE OF THE PRIMARY SEDIMENTATION TANKS

Test	Operated as designed		Sludge recycled with no SRT control		Sludge recycled with SRT control	
	Influent	Effluent	Influent	Effluent	Influent	Effluent
COD (mg/ℓ)	580	440	600	580	520	460
TKN (mg N/ℓ)	41	39	47	50	50	49
Total P (mg P/ℓ)	11	11	14	15	13	13
Susp solids (mg/ℓ)	240	120	320	240	260	180
Volatile fatty acids (as acetic acid)(mg/ℓ)	-	-	15	40	43	48
Readily biodegradable COD (mg/ℓ)	-	-	-	-	94	120
Final effluent from activated sludge plant (mg o-P/ℓ)	4,7		3,7		2,9	
Test duration			1/1/84 - 14/5/85		1/7/86 - 1/10/87	
No of samples tested			150	150	300	300

NOTE : SRT = Sludge retention time

3.3 PERFORMANCE OF PRIMARY SEDIMENTATION TANKS WITH INTERMITTENT AIR LANCING

The primary sedimentation tanks at the Northern Works are rectangular and flat-bottomed (Figure 3.1). Sludge is scraped into a hopper at the inlet end from where it is removed. In order to prevent methane production in the hopper, air lances were installed and operated for 30 seconds at periods ranging from 3 to 12 times per day during the storage portion of the operational cycle. Three operational procedures were investigated, as depicted in Figure 3.2.

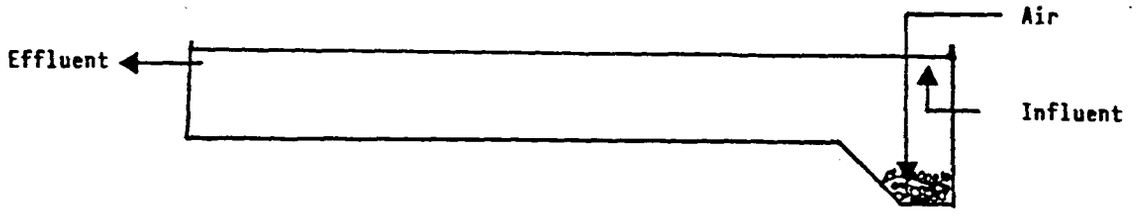


Figure 3.1 : Side elevation of the primary sedimentation tanks at Northern Works

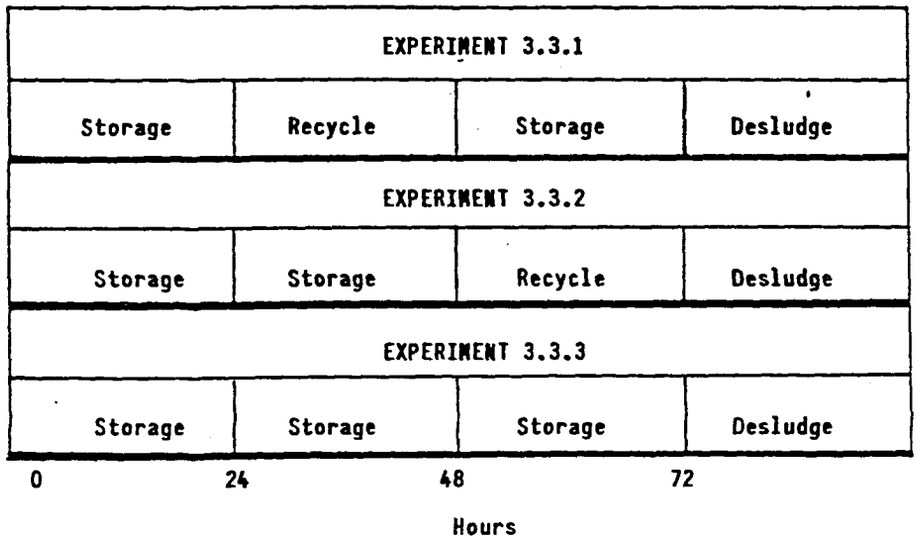


Figure 3.2 : The operation procedure of the primary sedimentation tanks for three experiments

3.3.1 Sludge Stored and Air Lanced Every Alternate Day

The primary sedimentation tanks were operated as follows :

At the start of the cycle all the tanks were desludged completely, after which sludge was allowed to accumulate for 24 hours. During the following 24 hours, sludge was recycled back to the inlet of the sedimentation tank. This was followed by another 24 hour storage period. At this stage all the tanks were desludged and the cycle repeated. Various air lancing cycles ranging from 3; 4 and 6 times per day, were experimented with during all sludge storage periods.

TABLE 3.2 : PERFORMANCE OF PRIMARY SEDIMENTATION TANKS WITH SLUDGE STORED AND AERATED INTERMITTENTLY ON ALTERNATE DAYS

No aeration periods/d	3		4		6	
Test	Feed	Effluent	Feed	Effluent	Feed	Effluent
COD (mg/ℓ)	490	480	560	490	460	470
TKN (mg N/ℓ)	40	45	42	43	40	43
Total P (mg P/ℓ)	13	13	14	14	12	13
Susp solids (mg/ℓ)	260	200	320	150	260	200
Volatile fatty acids (as acetic acid)(mg/ℓ)	44	62	60	58	32	48
Readily biodegradable COD (mg/ℓ)	91	150	150	140	76	130
Upward velocity (m/h)	0,9		0,8		0,8	
Effluent P from activated sludge plant (mg o-P/ℓ)	0,9		1,5		1,3	
Test duration	26/11/87	27/12/87	10/1/88	14/1/88	17/1/88	14/2/88
No of samples tested	19	19	5	5	60	60

3.3.2 Sludge Stored and Aerated Intermittently for Two Consecutive Days

The mode of operation during these tests was as follows :

At the start of the cycle all the sedimentation tanks were desludged. For the next two days the sludge was stored and aerated for 30 seconds every 2 hours. Thereafter, the sludge was recycled

back to the inlet of the sedimentation tank for another 24 hours. The tanks were desludged completely and the cycle repeated. The results of this test are given in Table 3.3.

TABLE 3.3 : PERFORMANCE OF PRIMARY SEDIMENTATION TANKS WITH SLUDGE STORED AND AERATED INTERMITTENTLY FOR 48 HOURS

No aeration periods/d	12	
	Influent	Effluent
COD (mg/ℓ)	530	470
TKN (mg N/ℓ)	45	47
Total P (mg P/ℓ)	13	14
Susp solids (mg/ℓ)	290	180
Volatile fatty acids (as acetic acid)(mg/ℓ)	34	52
Readily biodegradable COD (mg/ℓ)	84	120
Upward velocity (m/h)	0,9	
Effluent P from activated sludge plant (mg o-P/ℓ)	3,6	
Test duration	14/4/88	26/5/88
No of samples tested	27	

3.3.3 Sludge Stored and Aerated for Three Consecutive Days

Initially, all the sedimentation tanks were desludged. Thereafter, sludge was stored for three consecutive days, during which time it was aerated every six hours for 30 seconds. The sedimentation tanks were then desludged and the cycle repeated. It should be noted that no sludge was recycled during these tests. The results are given in Table 3.4.

TABLE 3.4 : PERFORMANCE OF PRIMARY SEDIMENTATION TANKS WITH SLUDGE STORED AND AERATED INTERMITTENTLY FOR 3 CONSECUTIVE DAYS

No aeration periods/d	4	
	Influent	Effluent
COD (mg/ℓ)	710	530
TKN (mg N/ℓ)		
Total P (mg P/ℓ)		
Susp solids (mg/ℓ)	430	230
Volatile fatty acids (as acetic acid)(mg/ℓ)	33	45
Readily biodegradable COD (mg/ℓ)	94	134
Upward velocity (m/h)		
Effluent P from activated sludge plant (mg o-P/ℓ)	2,1	
Test duration	27/5/88	14/7/88
No of samples tested	33	33

During the period December 1987 to August 1988, the effluent from the primary sedimentation tanks flowed into three activated sludge modules, one of which was operated in the Johannesburg process mode the and the other two, the Bardenpho process.

While the results obtained during the experimental period showed that storage and air lancing every alternate day to be the most useful operational mode, subsequent plant observations showed the 48 hour storage regime to be the most beneficial for enhanced biological phosphate removal.

3.4 FACTORS INFLUENCING THE PERFORMANCE OF BIOLOGICAL PHOSPHATE REMOVAL PLANTS

3.4.1 The Role of Substrate

A relationship has been established between readily biodegradable COD in the feed and the phosphorus removal efficiency of an activated sludge plant (WRD, 1984). During this study attempts

were made to establish this relationship for full-scale plants.

While alteration of the feed characteristics by primary sludge recycle (fermentation has proved to have a favourable effect on biological phosphate removal) (see Figure 3.3), an attempt was made to establish a more direct relationship.

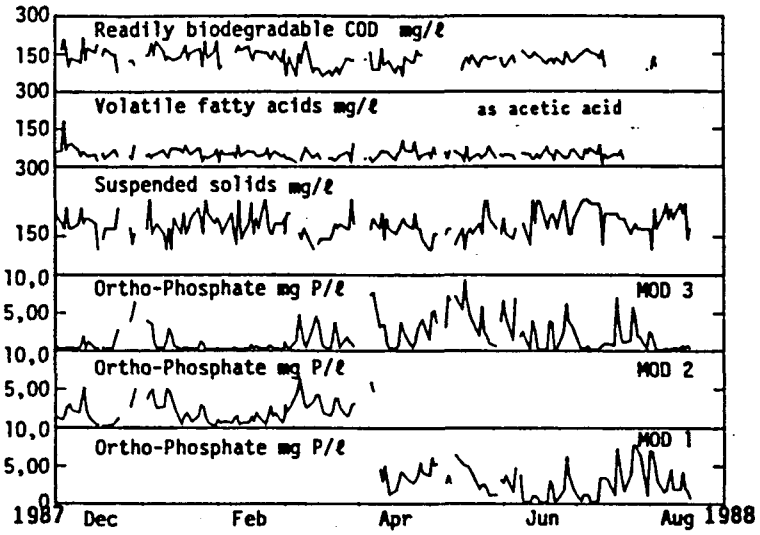


Figure 3.3 : Feed and effluent characteristics of the Northern Works activated sludge plants

Effluent phosphate levels were monitored on-line during this study, thus providing the operator and researcher with an immediate opportunity to gauge response to alterations in operational procedures.

Comparison of different plant modules is also facilitated by this technique (see Figures 3.4 and 3.5).

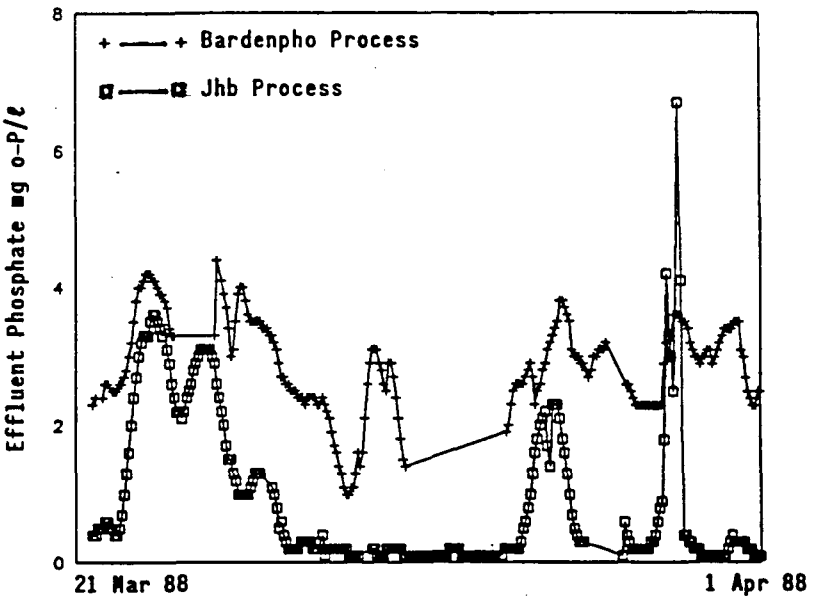


Figure 3.4 : Typical effluent phosphate concentrations produced by similar plants operating on the Johannesburg and Bardenpho processes

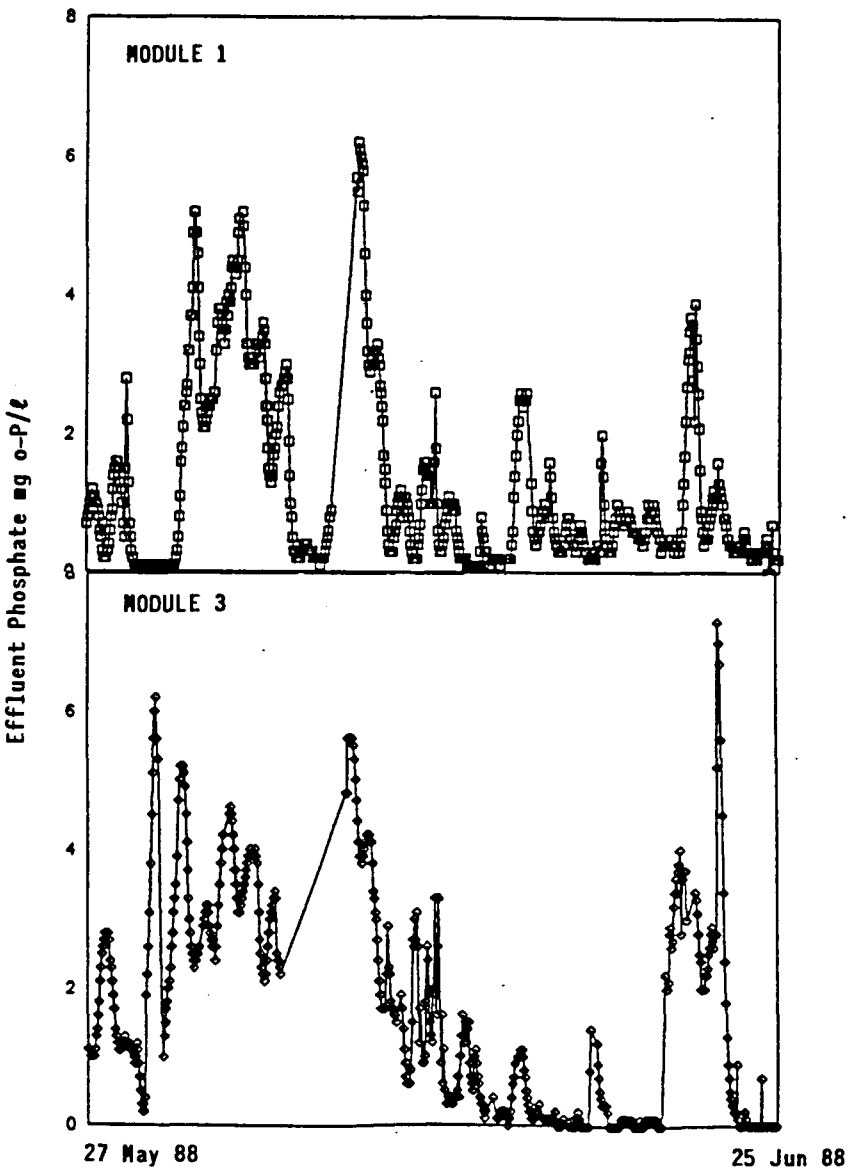
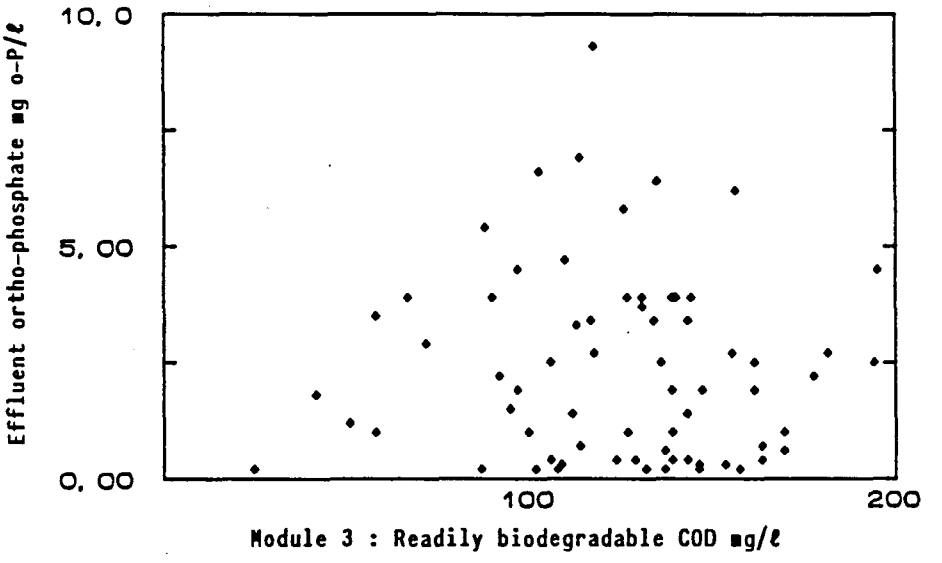


Figure 3.5 : Typical effluent phosphate concentrations produced by two similar plants operating on the Bardenpho Process

As can be seen from Figure 3.4 the Johannesburg process produced consistently lower effluent phosphate levels than the Bardenpho process. This is probably due to the prevention of nitrate return to the anaerobic zone, which is accomplished by the Johannesburg configuration.

Modules 1 and 3 performed similarly, which is not unexpected in view of their identical configurations. The modules are fed from separate balancing tanks so their slight differences in feed characteristics do occur. The modules are operated on the same basis. Readily biodegradable COD and volatile fatty acid concentrations in the feed were measured and plotted against the effluent phosphate concentrations (see Figures 3.6 and 3.7).



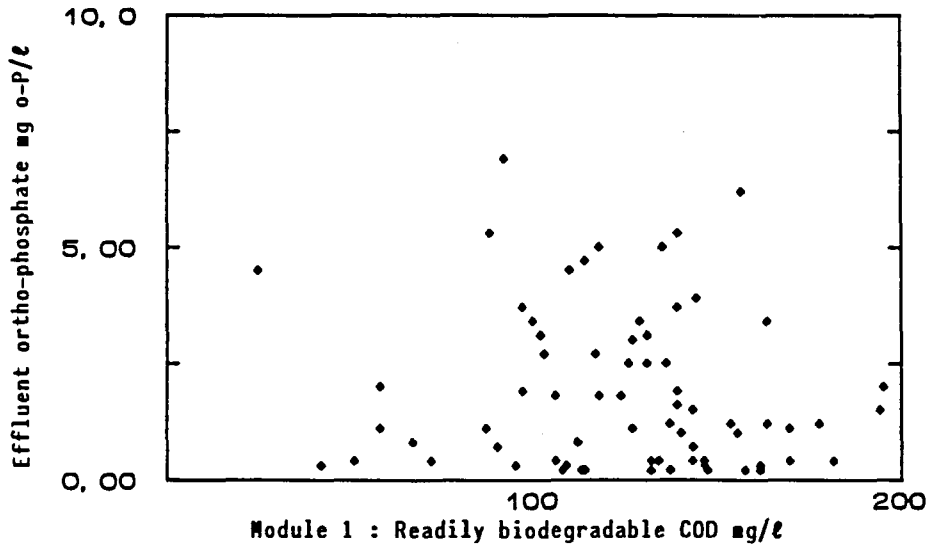


Figure 3.6 : Relationship between feed readily biodegradable COD and effluent phosphate concentrations

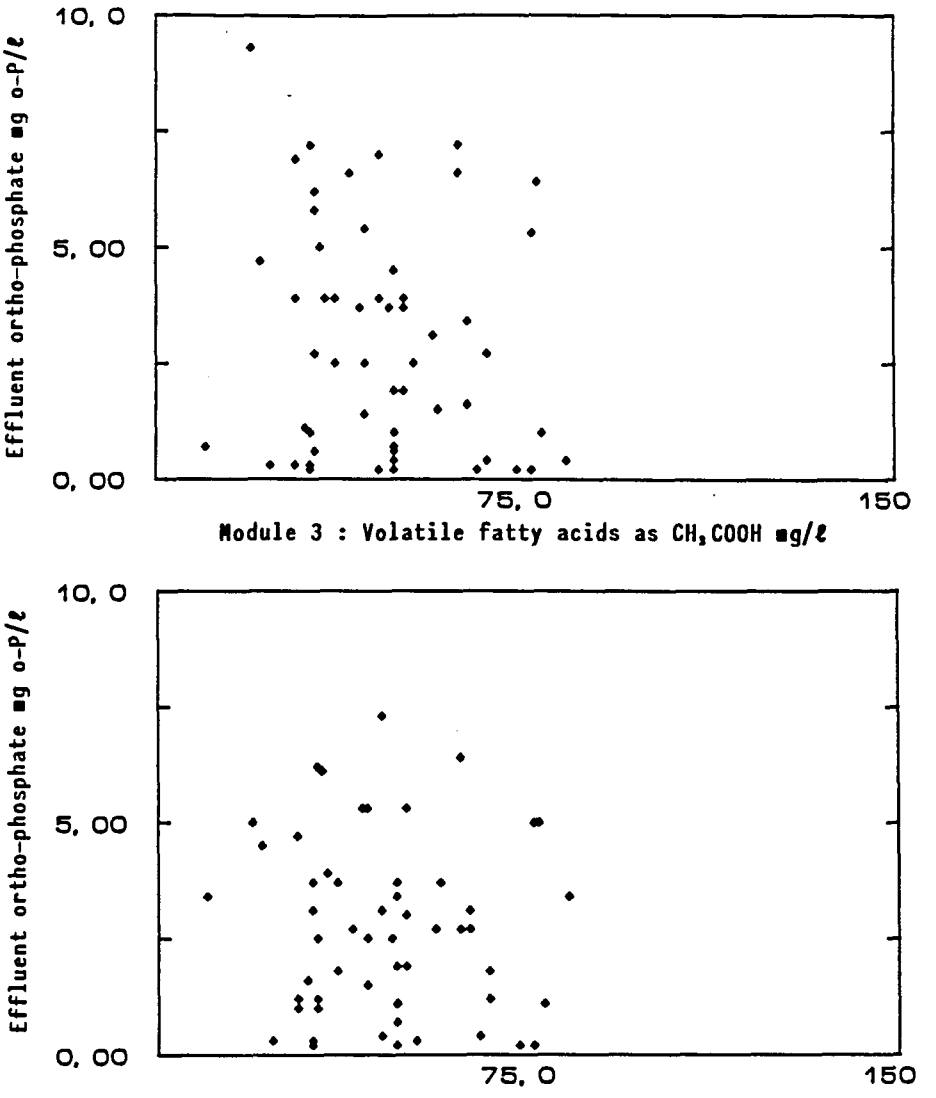


Figure 3.7 : Relationship between feed volatile acid concentrations and effluent phosphate concentrations

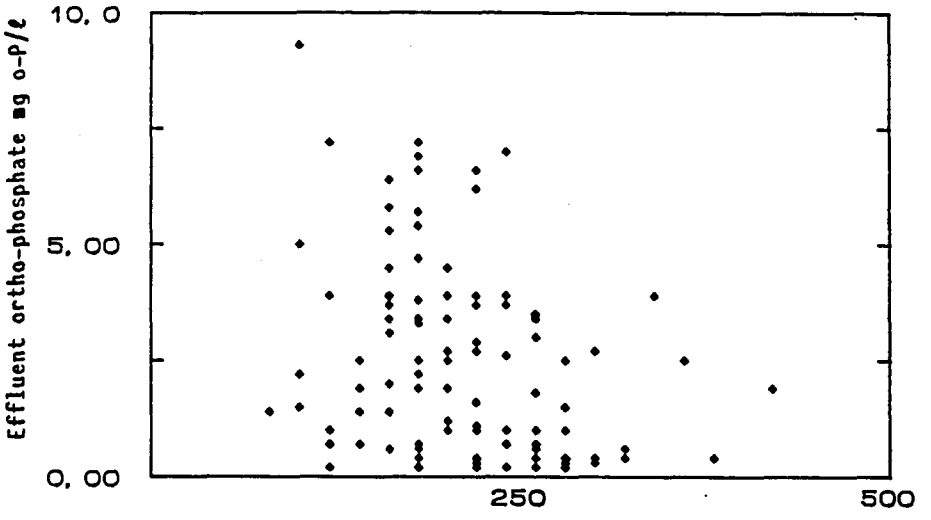
The lack of a direct relationship between these parameters is probably not unexpected due to the problems of scale.

It is virtually impossible to control a number of variables on a full-scale plant in the same way that it can be done on laboratory pilot plants.

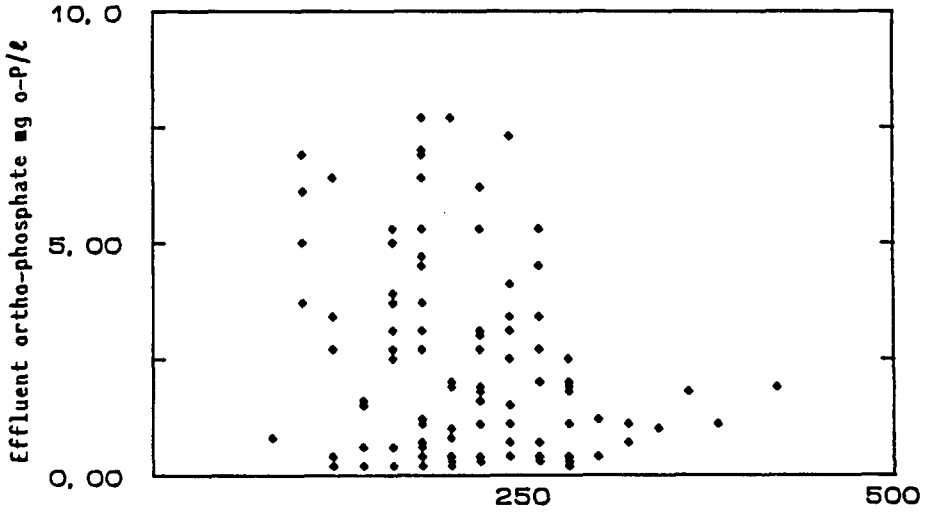
Suffice it to see that the favourable effect of the right feed characteristics on effluent phosphate levels, which is predicted by laboratory-scale work, was borne out at full-scale during this study.

3.4.2 The Role of Suspended Solids in the Feed

The air lancing of the primary sedimentation tanks caused a rise in the feed suspended solids. It was therefore decided to establish whether the improved phosphate removal could be directly related to the feed solids. Figure 3.8 reveals the absence of any correlation.



Module 3 : Influent suspended solids



Module 1 : Influent suspended solids

Figure 3.8 : Relationship between suspended solids in the feed and the effluent phosphate concentrations

3.5 EVALUATION OF ON-LINE MONITORING DATA

3.5.1 The Role of Redox Potential in the Anaerobic Reactor

A redox meter was positioned in the centre of the anaerobic reactor in a Bardenpho-type process module. The aim was to establish whether a minimum redox potential must be reached in the anaerobic reactor if consistently low effluent phosphate concentrations were to be achieved. From the results plotted (Figure 3.9), there appears to be no relationship.

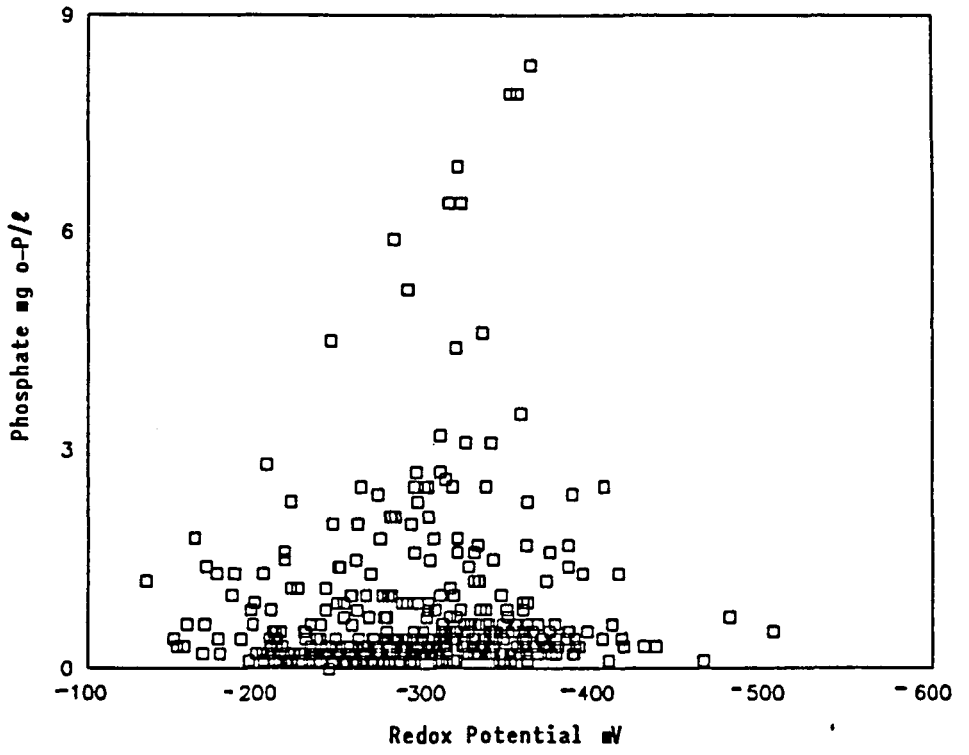


Figure 3.9 : Relationship between redox potential and effluent phosphate concentration

3.5.2 Effluent Phosphate Concentrations

A Tuesday peak was usually evident, which could either be caused by a lower readily biodegradable COD in the feed on a Sunday, or by a higher total phosphate in the feed on a Monday. Since no on-line facilities were available to measure these parameters, no conclusions could be drawn. At present, methods to monitor both these parameters on-line are being investigated.

On occasions, the effluent ortho-phosphate was below 1 mg o-P/ℓ throughout the week, while the very next week it exceeded the standard for a number of days (see Figure 3.10). If the on-line effluent nitrate data is examined, there is very little difference between the two weeks. One explanation for the difference in the phosphate removal is that the sewage feed characteristics are different, which again points to the need to monitor the readily biodegradable COD on-line.

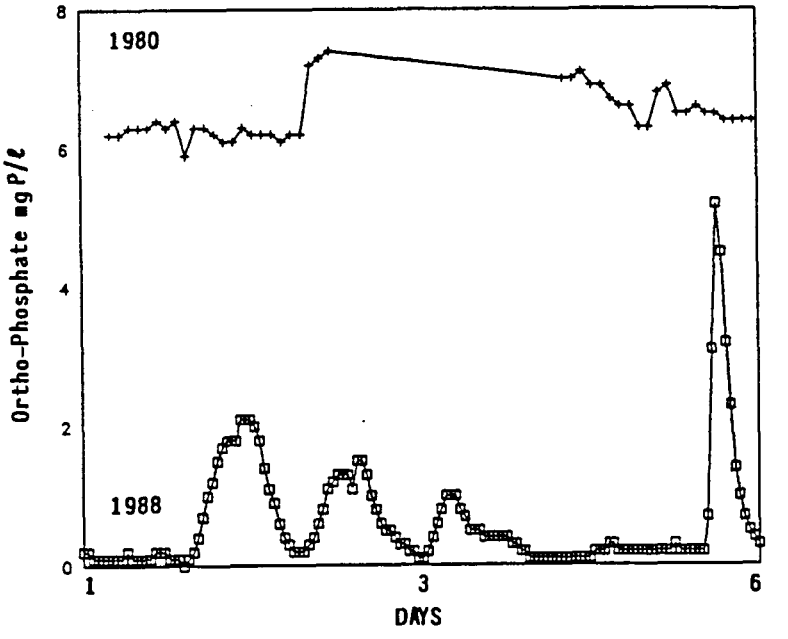


Figure 3.10 : Effluent phosphate concentration over a 6 day period in 1980 and 1988

3.6 CONCLUSIONS

- Intermittent aeration by airlancing increases the level of suspended solids in the plant feed. The latter however, does not appear to have an influence on phosphate removal.

- The increased levels of readily biodegradable COD in the plant feed promotes improved phosphate removal.

- On-line monitoring of redox potential in the anaerobic zone does not appear to be a useful parameter.

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CHAPTER FOUR

DESIGN CONSIDERATIONS

A R PITMAN

4.1 INTRODUCTION

The Johannesburg City Council has operated biological nutrient removal activated sludge plants since 1973 (Nicholls et al., 1986; Nicholls et al., 1987; Osborn and Nicholls, 1985; Osborn et al., 1986; Pitman et al., 1983; Venter et al., 1978). These are either specifically designed for biological nitrogen and phosphorus removal or have been adapted to achieve this. The plants range in size from 27 Mℓ/d to 150 Mℓ/d and were either designed as extended aeration or Phoredox/ Bardenpho processes. Initial experiences showed that the removal of nitrogen and phosphorus did not occur as readily as, for example COD removal, so that considerable research on large scale plants and process modifications were necessary to be in a position to achieve statutory effluent standards particularly the 1 mg P/ℓ o-phosphate limit. Results of research into the enhancement of biological phosphate removal carried out over the past 10 years have allowed engineers to identify a number of aspects which must be considered in the design of nutrient removal activated sludge plants.

In this chapter practical solutions to design problems experienced during the research are proposed. This chapter is not intended to serve as a complete process design guide and readers are referred to WRC 1984 for complementary information.

4.2 FACTORS AFFECTING DESIGN

4.2.1 Feed Sewage Characteristics

The quality of the sewage fed to nutrient removal activated sludge plants is an important consideration in design (Pitman et al.,

1987). The TKN:COD ratio of the feed can influence the degree of nitrogen removal. Sewage with a high ratio will not produce good denitrification. Although the discharge of effluents with a low nitrate content is not required in most areas of the R S A, the achievement of denitrification is an important prerequisite for good phosphorus removal.

Experience has also shown that when the TP:COD ratio in the feed sewage increases much above 0,020, complete biological removal of phosphorus can be difficult and simultaneous chemical precipitation is necessary to meet the Standard.

An important sewage characteristic which has an overriding effect on nutrient removal is the readily biodegradable COD (S_{bs}) or volatile fatty acid (VFA) content. When the S_{bs} or VFA content is high, biological phosphorus removal is easy. When this is low, special care must be taken with the process design. This would include facilities to increase the S_{bs} content of the feed, larger anaerobic zones in the activated sludge process and precautions to minimise nitrate and molecular oxygen entering the anaerobic zone. The high rate acid fermentation of raw sewage solids can provide a ready means of increasing the S_{bs} content of sewage. An important decision to be made is whether to feed raw or settled sewage to the reactor. Although settling can adversely affect the TP:COD and TKN:COD ratios the feeding of settled sewage direct to the activated sludge process could be inadvisable for large installations. The negative effects on the above ratios caused by the removal of primary solids can usually be compensated for by increasing the S_{bs} content of the sewage (see below). However smaller installations can usually be built on the extended aeration principle by feeding raw sewage into the reactor.

4.2.2 **Primary Sedimentation**

Unless the incoming sewage has favourable characteristics for complete biological P removal, primary sedimentation is virtually a necessity in order to retain raw sludge solids so that they can be

fermented to form additional S_{bs}. Even with extended aeration processes, primary sedimentation might be necessary. The design of these tanks must be such that sludge can be accumulated in them for extended periods i.e. 2 - 10 days. Adequate storage capacity must be provided and the mechanical equipment must be robust enough to operate on thick sludge layers. Facilities must be available to obtain the maximum S_{bs} generation while minimising any losses that may occur due to the fermentation of VFA to methane.

In this respect a multiplicity of tanks is preferred over a single tank as this makes control over the fermentation easier. Once the S_{bs} has been generated it can be added to the process either by directly feeding the fermented sludge to the activated sludge reactor (i.e. pseudo extended aeration)(see Figure 4.1) or by elutriating the S_{bs} out of the sludge (Figure 4.3). The latter is achieved either by recirculating sludge to the sewage inlet or by using elutriation thickeners. These would be raw sludge gravity thickeners with elutriation facilities. An alternative to using primary sedimentation tanks for the fermentation of sludge is to use a separate high rate acid digester (Figure 4.2).

4.2.3 **Load balancing**

Balancing the diurnal load on the process can improve and facilitate operation and reduce capital costs. However it is important to ensure that the balancing tank is properly mixed to obviate settlement of solids and that proper outlet control systems are provided. The latter should ensure that the tank does not run dry for extended periods as this can adversely affect the downstream nutrient removal process. An additional advantage of a balancing tank is that it could be used to increase the S_{bs} content of the sewage by encouraging acid fermentation in it (Figure 4.4). However in the design of such a tank it is important to minimise aeration of the sewage i.e. air bubble mixing must not be used. In designing balancing tanks, cognizance should be taken of these factors and some method of control should be incorporated. A number of researchers (inter alia Dold et al., 1982) have made recommendations in this regard.

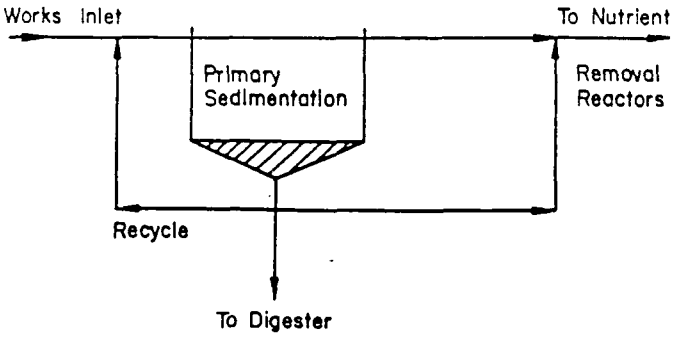

Figure 4.1 : Primary Sludge Accumulation and Recycle

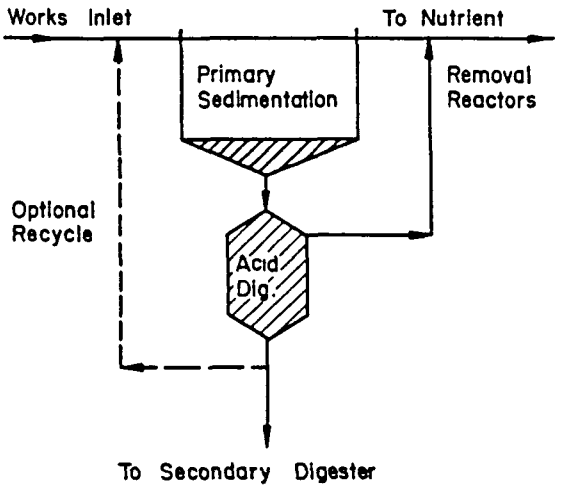

Figure 4.2 : High Rate Acid Digestion

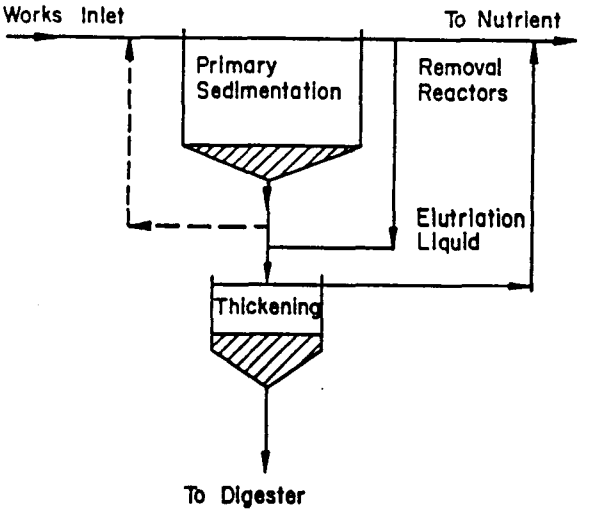

Figure 4.3 : Primary Sludge Accumulation and Elutriation Thickening

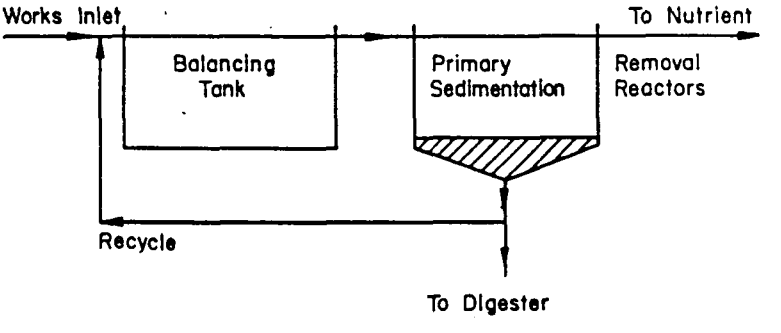

Figure 4.4 : Use of Balancing Tank as High Rate Digester

4.2.4 Reactor size and configuration

The size of the activated sludge reactor depends on the feed sewage characteristics, the design sludge age (R_s) and the mixed liquor suspended solids (X_t) concentration. Except for special circumstances design X_t values should be in the range 3000 - 4500 mg/ℓ. In addition the design aerobic sludge age should ensure complete nitrification under the worst winter conditions. In calculating R_s it should be borne in mind that it is inadvisable to have the unaerated mass fraction greater than 0,45. This limitation will then affect the sizes of anaerobic and anoxic zones.

Unless the plant is to operate on the extended aeration, long sludge age principle, the minimum sludge age to ensure nitrification under all conditions should be designed for. The minimum R_s required can be found using the UCT model (WRC 1984).

4.2.4.1 Anaerobic zone

This zone must be large enough to ensure good P removal at all times. Research on biological P removal has not reached the stage where the optimum size of this zone can be calculated from the usual input conditions. Indications are that if the sewage fed to the process has good characteristics, the zone could have a short retention period (i.e. 0,5 - 1,0h, based on feed sewage flow rate). If feed characteristics are not optimum, retention periods of up to 6h can be considered. However bearing in mind the need to restrict the unaerated mass fraction to less than 0,45 anaerobic retention periods cannot be extended too far without affecting anoxic retention periods and hence denitrification. The anaerobic zone should preferably be in the semi-plug-flow configuration.

4.2.4.2 Anoxic zones

Anoxic zones are necessary to meet any effluent total N limits and to minimise nitrate feedback to the anaerobic zone which can affect P removal. Unless the feed sewage has a high S_{bs} content

all nitrate feedback to the anaerobic zone should be virtually eliminated. The major source of nitrate input to the anaerobic zone is the sludge recycle from the final clarifiers. In the conventional Phoredox/Bardenpho process layout (Figure 4.5) it is usually difficult to control this source unless the feed sewage TKN:COD ratio is such as to achieve virtually complete mainstream denitrification at all times. In this respect the UCT (Figure 4.6), JHB (Figure 4.7) and MUCT (Figure 4.8) layouts are superior in that they pass the return sludge through an anoxic zone, thus creating a barrier to nitrate feedback to the anaerobic zone. This effectively preserves all the S_{bs} in the feed for use by excess phosphate accumulating bacteria as it is not preferentially consumed by denitrifiers.

In the JHB process the sludge recycled from the clarifier underflow passes on its own through a separate anoxic zone. This enhances the nitrogen removal in this stream as the denitrification achieved in the clarifier is usually insignificant. Denitrification in this zone occurs by endogenous respiration and the nitrate reduction achieved depends on the endogenous respiration rate, degree of sludge thickening achieved in the clarifiers and the retention period. Experience in Johannesburg has shown that up to 10 mg N/ℓ nitrate can be removed from the return sludge in such a zone. Retention periods can vary between 0,5 and 2,0h in this zone.

As far as the mainstream primary and secondary anoxic zones are concerned the reader is referred to the UCT model (WRC 1984) for information on their process design. However the use of the secondary anoxic zone should be carefully considered. Experience in Johannesburg has shown that at best this zone will remove a maximum of 2,0 - 3,0 mg N/ℓ so that is is recommended that the mainstream secondary anoxic zone only be used in cases where raw sewage is fed to the reactor or very low effluent total N values are required. When treating settled sewage nitrogen removal in such a zone would be very small. If this zone is to be used it should be positioned (if possible) in an area of the reactor where nitrification is virtually complete but the sludge respiration rate is still relatively high.

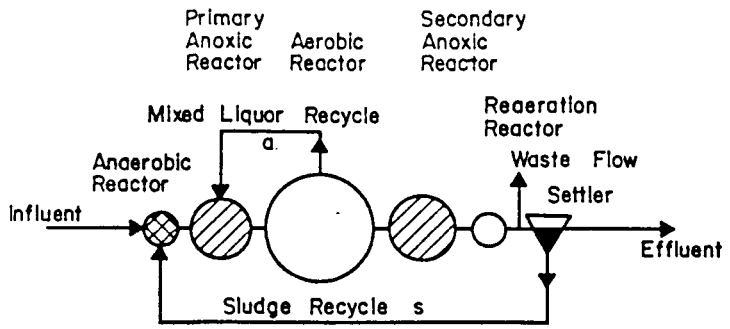

Figure 4.5 : The Phoredox Process for Biological Nitrogen and Phosphorus Removal, also Called the Modified Bardenpho Process.

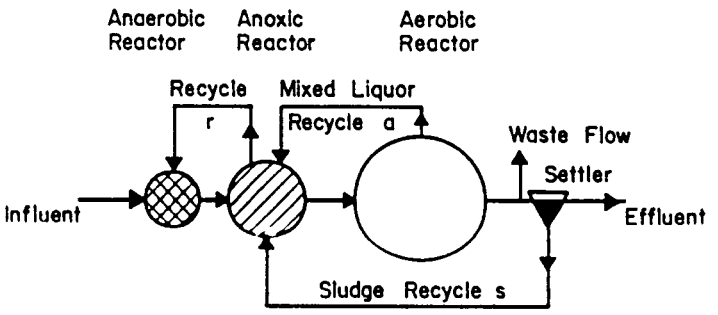

Figure 4.6 : The UCT Process for Biological Nitrogen and Phosphorus Removal.

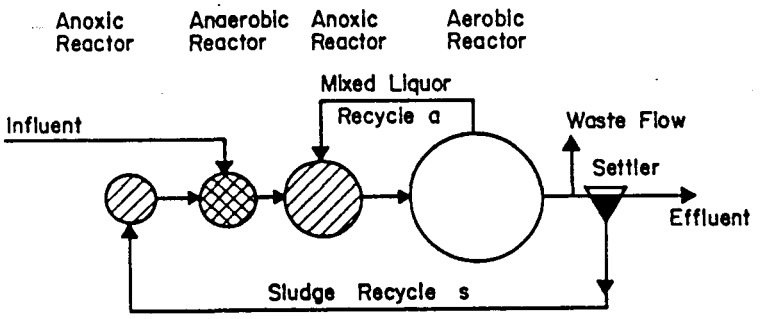

Figure 4.7 : The JHB Process for Biological Nitrogen and Phosphorus Removal.

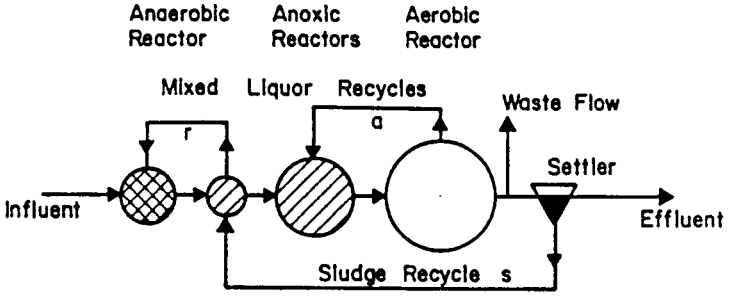

Figure 4.8 : The Modified UCT Process for Biological Nitrogen and Phosphorus Removal.

Mixed liquor recycle to the first anoxic zone should be flexibly designed to give recycle ratios varying between 2 and 6 to 1 (based on inlet flow). Excessive oxygen entrainment into this recycle should be avoided.

4.2.5 Aeration

The design of aerobic zones and aeration equipment has been found to be more important in nutrient removal plants than was at first thought. Adequate aeration is necessary to ensure rapid phosphate uptake by bacteria and efficient nitrification. It is important to adequately satisfy the respiration needs of the biomass in all parts of the aeration zone at all times.

Experience in Johannesburg has shown that the rectangular aerobic zones in nutrient removal plants are not as completely mixed as was at first thought, despite the large volume of mixed liquor being recycled through them. Semi-plug flow conditions can exist in these zones leading to a distinct respiration rate profile along their length. Such zones should therefore have a tapered aeration system with more aeration capacity available at the inlet than the outlet of the zone.

An important decision to be made is the choice of aeration system (i.e. surface aeration or diffused air). There are many pros and cons to each system. An important advantage of surface aerators is their lower capital cost while diffused air is theoretically more economical on power. Johannesburg City Council has both systems operating in nutrient removal plants and Table 1 shows mean yearly power consumptions based on COD load removed. These results are based on full scale field experience and reflect some of the inadequacies which can occur in the installation and operation of such systems. As can be seen the Bushkoppie diffused air plant has an efficiency in the middle of the range.

TABLE 4.1 : AVERAGE PROCESS POWER EFFICIENCIES AND SETTLING CHARACTERISTICS OF ACTIVATED SLUDGES IN JOHANNESBURG

Works	Process Type	Aeration System	Power Used kWh/kg COD removed	SVI mℓ/g	DSVI mℓ/g
Northern	Phoredox/Bardenpho	Surface	1,36	160	115
Goudkoppies	Phoredox/Bardenpho	Surface	0,92	136	110
Bushkoppies	Phoredox/Bardenpho	Diffused air	0,66	53	50
Alexandra	Extended Aeration	Surface	0,58	85	70
Olifants-vlei	Extended Aeration	Surface	0,53	84	73

Provided the correct gearboxes are used, surface aerators give a reliable, robust aeration system for these plants. The number and size of the aerators should be chosen so that significant areas having low D O levels or large anoxic zones will not occur when aerators are turned off to save power during periods of low incoming load. For example, if a total installed aerator power of 220 kW is necessary it would be better to use 8 x 30 kW units than 2 x 110 kW units.

Experience in Johannesburg has shown that the capital maintenance costs of diffused air systems are higher than was originally thought, particularly if the claimed better power-use efficiencies are to be experienced. Also the latter efficiencies under field conditions are not as high as expected. In addition these systems appear to be rather more sensitive to inadequacies and defects in the quality of fabrication materials, and the quality of installation maintenance and process control.

As can also be seen in Table 1 a possible advantage of the diffused air process is that it produces very good settling activated sludges. Experience at Bushkoppie has shown that the DSVI value of the sludge has never exceeded 70 mℓ/g while at Northern Works and Goudkoppies values of up to 200 mℓ/g have been experienced. However

there are strong indications from the Johannesburg experience that if diffused air is to be used in the R S A very strict quality control is necessary over fabrication and installation of mechanical equipment.

4.2.6 Final Clarifiers

These should be designed for the worst conditions that might be encountered. For example in surface aerator plants the worst conditions might be storm flow conditions and a diluted sludge volume index, DSVI of 200 mℓ/g.

It is recommended that the solids flux theory, in particular the approach described by Ekama and Marais (1985) be used in the design of these tanks. In addition circular conical bottom scraped units should be used with a minimum side water depth of 3,0 m.

Recycle pumps for return sludge should be designed to provide recycle ratios (based on inlet flow conditions) between 0,5 and 1,5:1. In addition sludge withdrawal and pumping facilities should be able to handle the higher viscosities caused by sludges containing up to 20 g/ℓ suspended solids.

4.2.7 Sludge Bulking

Sludge bulking has been a problem which has beset many biological nutrient removal plants in the R S A (Pitman 1984; Blackbeard et al., 1986; Blackbeard et al., 1988) Experience has shown that the growth of activated sludges with high DSVI values can be minimised by some or all of the following;

- Ensure that aeration system is designed such that D O levels can never drop below 0,5 mg/ℓ at any place in the aeration zones.
- Do not attempt to obtain significant denitrification in aerobic zones i.e. do not extend the primary anoxic zone into the main aerobic zone.
- Ensure semi-plug flow conditions exist in the reactor.

- Prevent the growth of scum forming organisms such as Microthrix parvicella.
- Do not increase the unaerated fraction of the process beyond 0,5.
- Give preference to diffused air aeration systems.

4.2.8 Nuisance scums

In addition to bulking sludge, nuisance scums have also plagued many plants particularly in the winter months. These scums have caused aesthetic problems and on escaping from the clarifiers can contaminate the final effluent. In addition if allowed to proliferate can exacerbate sludge bulking problems. The main organisms responsible for the growth of scums have been identified as Microthrix parvicella and Nocardia. The former is the predominant scum forming organism on Johannesburg's plants. A method of preventing such growths is to ensure that the surface floating solids or scum layers in the process do not have a longer retention period than the total solids retention period in the process i.e. the hold up or accumulation of solids behind baffles should be avoided.

In this respect the compartmentalisation of the reactor which occurs in nutrient removal plants can present problems. In addition the reseeding of the process by scum forming organisms which occurs when scum removed from clarifiers is recycled back to the inlet of the plant should be avoided.

4.2.9 Sludge wasting

Although activated sludge wasting via mixed liquor removal has become fairly commonplace, problems can occur in the practical implementation at full scale. Wasting should be accurately metered and controlled. Removal via a hand operated valve and flume, V-notch or magnetic meter can present problems so that frequent adjustments would be necessary to ensure the correct quantity is removed each day. On large installations full automatic control of sludge removal should be considered. As well as wasting mixed

liquor, provision should also be made to remove underflow sludge as a standby facility to reduce the sludge inventory quickly in an emergency. In addition the design should be such that the flow of waste sludge is not impeded for any length of time. Waste sludge handling facilities should have an almost 100 % availability or standby facilities should be provided.

4.2.10 Standby chemical addition

Although these processes should be designed to achieve the maximum biological removal of phosphorus, standby chemical addition facilities are frequently required by the Department of Water Affairs and should be provided. This is particularly so if the feed characteristics are not favourable. Conditions can occur during the year when process efficiency declines and temporary addition of chemicals can quickly rectify phosphorus removal.

Dosing systems should be designed to remove at least 50% of the incoming phosphorus load using the most dilute precipitant chemical available on the market. Provision should be available to add chemicals to various points in the process.

4.2.11 Monitoring and Control

Because of their increased sophistication, nutrient removal plants require better monitoring and control than conventional sewage treatment processes. Also the unreliability and lack of dedication to duty, of labour has made automatic control far more attractive in recent years, particularly since with the advent of Programmable Logic Controllers (PLC's) and Personal Computers (PC's). Areas requiring control would be :

- Flowrates of the feed, waste activated sludge, recycle liquor and return sludge.
- Dissolved oxygen levels in aerobic zones.
- Standby chemical addition.
- Desludging of primary sedimentation tanks and thickeners.

4.2.12 Sludge treatment and handling

Phosphorus rich sludges need to be carefully handled after withdrawal from the main process to ensure that phosphate release into solution does not occur, or if it does, that it is reprecipitated back onto the sludge. All recycled liquors removed during sludge handling must either have a low phosphorus content or the activated sludge process must have the capacity to handle the additional load imposed.

Plants in Johannesburg were designed so that primary sludge is thickened by gravity and waste activated sludge is thickened in dissolved air flotation (DAF) units. Recent experiments (Pitman et al., 1987) have shown that if the activated sludge process is operated to give an SVI of under 100 mg/ℓ and effluent nitrate concentrates below 10 mg N/ℓ, gravity thickening of waste activated sludge gives comparable results to DAF units, also release of phosphate into the overflow is minimal. Waste sludge from nutrient removal activated sludge plants is unstable and unless dewatered and dried quickly can cause odour problems. Stabilisation via digestion or composting should be considered. Both anaerobic and aerobic digestion can cause the solubilisation of sludge bound phosphorus. If necessary this phosphate should be precipitated using ferric salts or lime. The Johannesburg Municipality is at present designing and building plants to precondition digested sludge, prior to dewatering. These will incorporate aeration to strip CO_2 and ammonia and reduce alkalinity; followed by lime addition to complete the phosphate precipitation.

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CHAPTER FIVE
MICROBIOLOGY

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5.1 INTRODUCTION

Since 1975, when Fuhs and Chen postulated their microbiological basis for biological phosphate removal, namely, that the organisms responsible were capable of accumulating both polyphosphate and polyhydroxybutyrate, researchers have investigated these characteristics in activated sludge.

Buchan (1981), used electron microscopy combined with energy dispersive analysis of X-rays (EDAX) to examine the nature of the phosphorus accumulated in sludges from plants exhibiting enhanced phosphorus removal. Large phosphorus accumulations were located in identical structures in the sludges examined. The phosphorus was located in large electron-dense bodies within large bacterial cells, which were characteristically grouped in clusters. The phosphate released into the mixed liquor during anaerobiosis was observed to originate from the intracellular electron-dense inclusions. A combination of light and electron microscopy was used by Murphy and Lötter (1986), to study the effect of different carbon sources on polyphosphate synthesis and degradation. These studies were carried out with a pure culture of Acinetobacter calcoaceticus isolated from activated sludge samples. The phosphate release on addition of acetic acid under anaerobic conditions, which has been reported by a number of researchers (including Buchan, 1981, and Wentzel et al., 1985) was also observed in this study, and confirmed Buchan's observation that the phosphate originated from the electron-dense bodies. Examined under electron microscopy, these electron dense bodies were observed to coincide with the metachromatically stained inclusions seen under light microscopy (Murphy and Lötter, 1986).

In an attempt to develop a rapid method for evaluating the potential of an activated sludge biomass to release phosphate, Healey and Kerdachi (1987), determined the phosphate released on addition of sodium acetate. In a sludge where phosphate was being removed chemically, very little phosphate was released in this manner, thus confirming the biological mechanism responsible for phosphate removal.

In contrast to the dynamics of polyphosphate, polyhydroxybutyrate, a carbon storage material, is synthesized during anaerobic conditions and utilised under aerobic conditions, as observed under light microscopy by Hart and Melmed (1982), and chemically by Lötter (1987). Wentzel et al. (1986), hypothesized that the absorption of substrate and its accumulation as polyhydroxybutyrate is fundamental to the success of the biological phosphate removal process. The carbon stored in this way is utilised under aerobic conditions where extracellular biodegradable carbon is depleted. The energy derived from the utilisation of the stored carbon is used for phosphate uptake and polyphosphate synthesis.

The role of extracellular polymers in bioflocculation has been recognised for some time (Forster, 1971; Kiff, 1978; Sheintuch et al., 1986). In the case of biological phosphate removal it is possible that these polymers have an additional role to play. In view of the bacterial cell clustering, which has been reported in plants exhibiting enhanced phosphate removal (Buchan, 1981; Hart and Melmed, 1982; Osborn et al., 1986), it is probable that the polyphosphate accumulating organisms ensure the formation of clusters by secreting a biopolymer.

In addition to the phosphate removing bacteria, the so-called filamentous bacteria, which have the ability to bridge sludge flocs causing the sludge to settle poorly, may also be present. These bacteria may cause severe operational problems, i.e. bulking, if allowed to proliferate unchecked in an activated sludge system.

Sludge bulking is a world-wide problem and Blackbeard and Ekama (1984), in a survey of 111 South African activated sludge plants, showed that 78 % experienced either bulking or foaming or both, in various degrees of severity. Methods to detect filament increase early, before their rapid

and profuse growth has already created a bulking problem, are clearly essential. Sezgin et al. (1978); Palm et al. (1980) and Lee et al. (1982), all used the Sezgin et al. (1978) method for measuring the total extended filament length (TEFL) to assess the extent of bulking in activated sludge. Because the TEFL measurement is a very time-consuming and tedious method, they sought correlations between TEFL and various simpler measures of activated sludge settling, such as SVI and zone settling velocity. Lee et al. (1983) found that the Diluted Sludge Volume Index (DSVI), gave the best correlation and least scatter when compared with the TEFL, and defined a bulking sludge as having a DSVI greater than 150 mg/ℓ.

In this Chapter, results of the microscopic evaluation of both beneficial and non-beneficial micro-organisms will be discussed. The phosphate releasing capacity of activated sludge was also evaluated.

Many workers have been using GCY agar (Pike et al., 1972), for the isolation of bacteria from activated sludge. The suitability of this medium for the selection of actively metabolising bacteria from activated sludge, has subsequently been questioned, since this is a particularly nutrient rich medium which supports the growth of faster growing bacteria that are passing through the system, but actually play a minor role in the plant.

The aim of this experiment was to compare the recovery of bacteria on four different media. Plate counts on each of these media was then compared with total viable microscopic counts on the same sample. The bacteria isolated on the two media with the highest yield, were then identified and the results compared. The number of nitrifying organisms in the sample was also determined.

5.2 MATERIALS AND METHODS

5.2.1 Filamentous Organisms

Methodology for the rapid identification of the filamentous organisms responsible for sludge bulking have been developed by a

number of researchers, inter alia, Eikelboom (1975); Eikelboom and van Buijsen (1981); Lee et al. (1982, 1983) and Jenkins et al. (1984). During this study the filamentous bacteria were identified using the key developed by Eikelboom and van Buijsen (1981) and Jenkins et al (1984).

The filamentous organisms present in three and five-stage Bardenpho units at the Northern Works were examined. Later in the study, the Bushkoppie plant was also examined for the presence of filamentous organisms.

The subjective scoring of filament abundance (SFA) was carried out according to Jenkins et al. (1984) as shown in Table 5.1 :-

TABLE 5.1
SUBJECTIVE SCORING OF FILAMENT ABUNDANCE

Numerical Value*	Abundance	Explanation
0	None	
1	Few	Filaments present, but only observed in an occasional floc
2	Some	Filaments commonly observed, but not present in all flocs
3	Common	Filaments observed in all flocs, but at low density (e.g. 1 - 5 filaments per floc)
4	Very common	Filaments observed in all flocs at medium density (e.g. 5 - 20 per floc)
5	Abundant	Filaments observed in all flocs at high density (e.g. 20 per floc)
6	Excessive	Filaments present in all flocs -appears more filaments than floc and/or filaments growing in high abundance in bulk solution

Note * : This scale from 0 to 6 represents a 100 - 1000 fold of total extended filament length

5.2.2 Phosphate Removing Bacteria

Polyphosphate staining. Slides of polyphosphate accumulating Acinetobacter were stained with methylene blue (Fuhs and Chen, 1975 and Neisser staining techniques (Society of American Bacteriologists, 1957), in order to compare their suitability for this study. Prior to methylene blue staining the slides were fixed with ethanol. Slides were then stained in methylene blue (1% in water) for 5 minutes then decolourised for 5 seconds with 1% sulphuric acid. Polyphosphate appears purple against a blue background. The Neisser stain consists of two solutions. The slide is first stained with a solution containing methylene blue and crystal violet, then after washing, with an aqueous solution of Bismarck brown, polyphosphate appears dark purple against a light brown background.

Polyhydroxybutyrate staining. Slides were stained with a 1% solution of Sudan Black in ethylene glycol, followed by washing in water and staining with a 1% solution of safranin (Gurr, 1973). The specificity of Sudan Black for intracellular lipophilic material was confirmed by extracting intracellular material with chloroform overnight, then comparing Sudan Black staining before and after extraction.

Polysaccharide staining. Slides were stained with Alcian blue for 5 minutes, then washed and flooded briefly with carbol fuchsin (McKinney and Weichlin, 1953). Bacterial polysaccharide stains blue in contrast to the red colour of the cellular material. India ink was also used to demonstrate the presence of polysaccharide.

Mixed liquor samples were taken on a weekly basis and heat-fixed on microscope slides, prior to staining.

5.2.3 Evaluation of Different Media for the Isolation of Bacteria from Activated Sludge

Activated sludge samples were taken from the aerobic zone of the

Goudkoppies sewage works. GCY medium (Pike et al, 1973) and Fuhs and Chen medium (Fuhs and Chen, 1975) were diluted to a final COD of 353 and TKN of 23. Sterilised balance tank effluent (BTE) and settled sewage (SS) with similar COD and TKN values were used undiluted with Difco No 2 agar added at 12 g/ℓ. The activated sludge sample was declumped and diluted as previously described (Lötter and Murphy, 1985), and 0,1 mℓ plated out on each medium. The plates were incubated at 21 °C and the colonies counted after 3; 5 and 7 days. A medium containing containing sodium nitrite was used for the isolation of nitrifying bacteria able to grow on nitrite. 0,1 mℓ of each dilution was plated out as before, and then incubated aerobically at 21 °C for 5 days. The isolates obtained on diluted Fuhs and Chen (DF&C) and diluted GCY (DGCY), were identified using fluorescent antibody and API techniques as previously described by Lötter and Murphy (1985). Polyphosphate accumulation by each isolate was examined using the Neisser stain (Society of American Bacteriologists, 1957).

The dilute GCY medium was compared to the BTE medium in a subsequent test. The effect of replenishing the medium with fresh nutrients daily was investigated by treating the mixed liquor sample in the usual way, then filtering the suspension through 0,45µ filter and placing the filter on BTE agar. The experiment was carried out in duplicate. One membrane was allowed to incubate at 20 °C for 5 days, while the second was placed on fresh BTE agar every day for 5 days. After 5 days, colonies were individually plated onto GCY agar and incubated at 35°C for 2 days. Colonies were then identified.

5.2.4 Comparison of Identification Techniques

During this study a new test kit for the identification of Gram negative bacteria became available. The Microbact (1988) identification kit, as it is called, compared favourably in price with the API system then in use. It was therefore decided to compare the two systems.

Twenty colonies isolated from an activated sludge plant were identified using both systems. The nitrate reduction test of both systems was compared to the standard Nitrate Agar Slant test (Difco, 1957). The oxidase test was done separately.

5.2.5 Phosphate Releasing Capacity of Activated Sludge

Mixed liquor samples from the aerobic zones of the activated sludge plants at Northern Works and Goudkoppies, were taken and divided in two. One portion was centrifuged for 10 minutes at 6 500 g. The supernatant was filtered through a Whatman 541 filter and analysed for orthophosphate. Acetic acid to a concentration of 1000 mg/ℓ was added to the second portion, which was then allowed to stand overnight without aeration. The mixed liquor was then centrifuged and the supernatant treated as described.

The phosphate released was expressed as a percentage of the soluble phosphate present before acetate addition. The sludge samples were microscopically examined for polyphosphate inclusions prior and subsequent to acetate addition.

5.3 RESULTS AND DISCUSSIONS

5.3.1 Filamentous Bacteria

The results of the survey carried out during 1986 and 1987 are shown in Tables 5.2 and 5.3. The correlation between the presence of the filamentous organisms and various operating parameters are shown in Figures 5.1 and 5.2.

TABLE 5.2
FILAMENTOUS ORGANISMS IN NORTHERN WORKS MODULE 2

Date	Observations	SFA	Remarks
1986			
7/1 - 26/2	Filaments 0041 and 0092 dominant - no bridging. Filaments only in the floc	2	No bulking
16/4 - 15/6	Types 0041 and 0092 dominant - some bridging present	3	No bulking
15/6 - 30/8	Microthrix parvicella increasing		
	Microthrix parvicella dominant	4	Bulking
30/8 - 20/11	Microthrix parvicella dominant - bridging present	6	Severe bulking
Heavy rain & heat 28/11/86			
1987			
1/12/86 - 16/1	0092 dominant - little bridging	3	No bulking
28/1 - 24/3	0041 dominant - no bridging		No bulking
30/3 - 7/9	0803 dominant - no bridging	2	No bulking
14/9 - 7/10	0803 and 0041 dominant - no bridging	2	No bulking
1988			
16/10/87 - 21/3	0803 dominant - no bridging	2	No bulking

TABLE 5.3
FILAMENTOUS ORGANISMS IN NORTHERN WORKS MODULE 3
(FIVE-STAGE BARDENPHO)

Date	Observations	SFA	Remarks
1986			
1/3 - 31/5	Types 0041 and 0092 dominant - some bridging present	2	No bulking
1/6 - 30/6	0041 and 0092 dominant - Microthrix parvicella increasing	4	Bulking
7/7 - 7/1/87	0041 dominant - bridging present	4	Bulking
1987			
16/1 - 9/2	1701 dominant - little bridging	3	No bulking
18/2 - 24/3	0041 dominant - little bridging	3	No bulking
30/3 - 13/7	0803 dominant - Microthrix parvicella increasing some bridging	3	No bulking
27/7 - 16/10	Microthrix parvicella dominant - bridging present	4	Bulking
1988			
11/12/87 - 21/3	0803 dominant - little bridging	3	No bulking

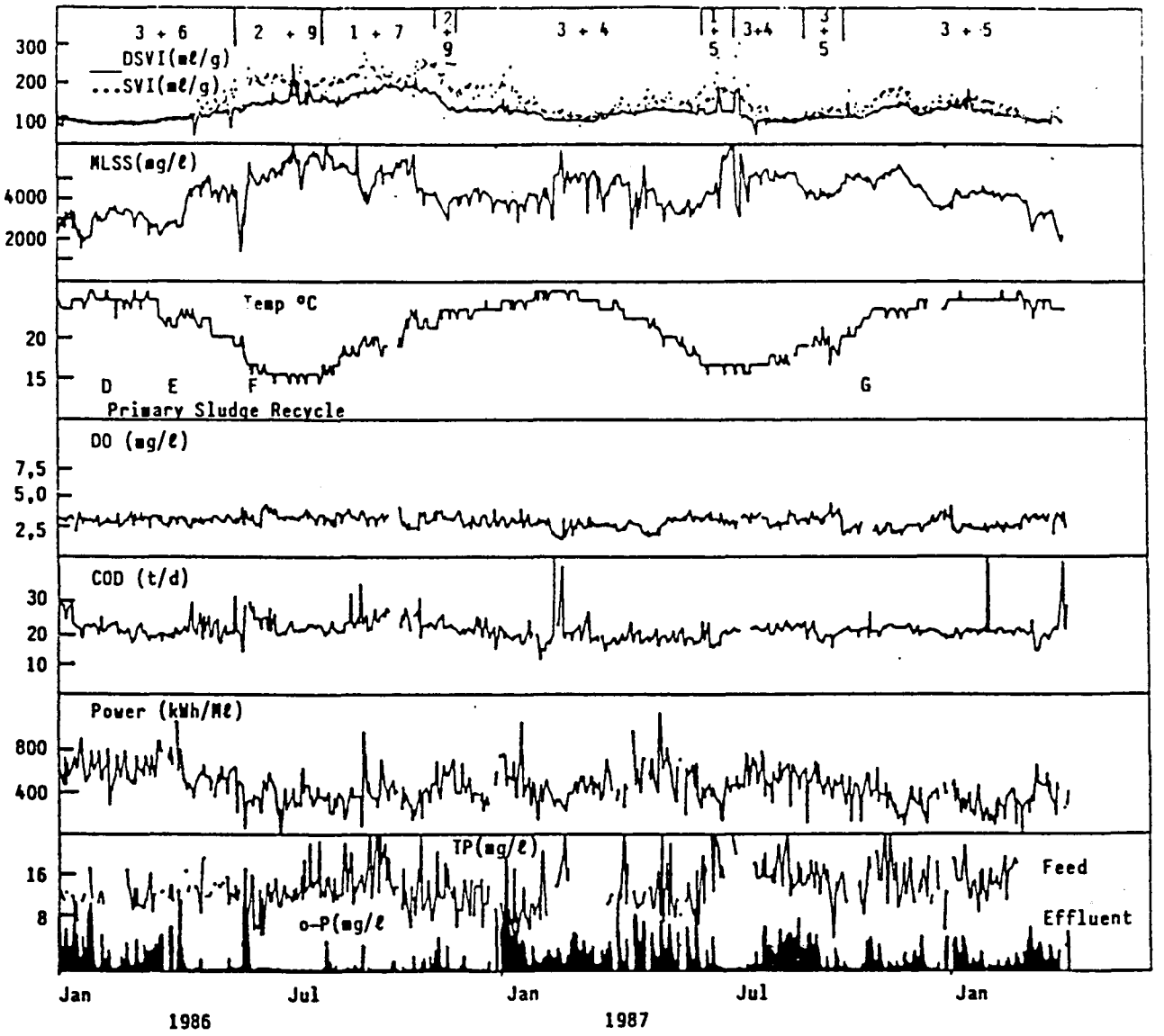

Figure 5.1 : Interrelationship between microbiological observations and plant data for Northern Works Module 2

Key : Microbiological Data

1	Severe bulking and extensive bridging
2	Filaments increasing, some bridging
3	Little or no bridging
4	Filament type 0041 dominant
5	Filament type 0803 dominant
6	Filament types 0041 and 0092 dominant
7	M. parvicella dominant
8	Filament type 1701 dominant
9	Filament type 0092 dominant

Plant Data

A	MLSS recycle point changed
B	Anoxic zone on RAS. Feed to second half of anaerobic zone
C	Blockage on second clarifier siphons
D	No control on detention time
E	Zero recycle
F	Sludge retention time 4 days
G	Sludge stored and aerated intermittently on alternate days

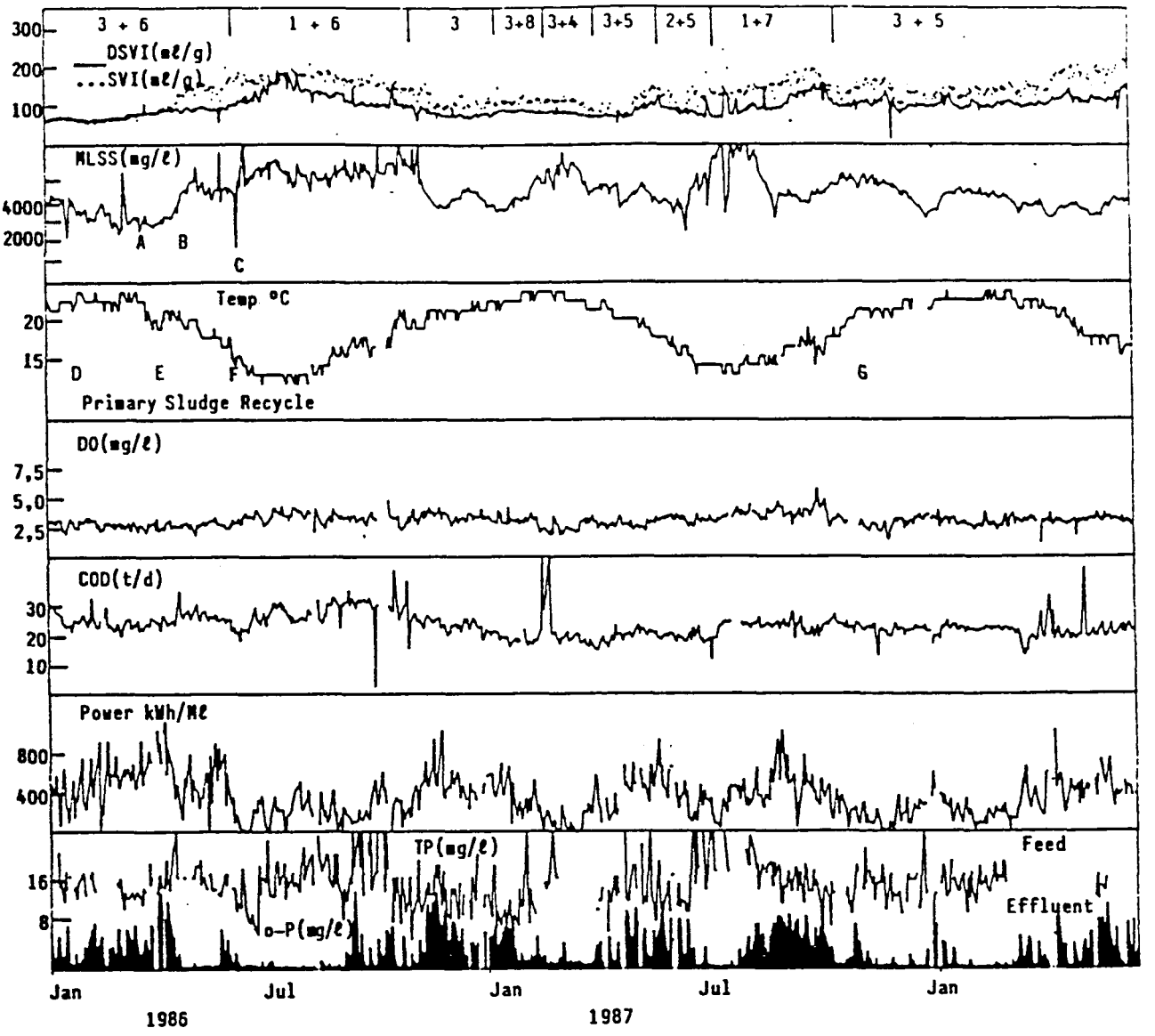

Figure 5.2 : Interrelationship between microbiological observations and plant data for Northern Works Module 3

Key : Microbiological Data

1	Severe bulking and extensive bridging
2	Filaments increasing, some bridging
3	Little or no bridging
4	Filament type 0041 dominant
5	Filament type 0803 dominant
6	Filament types 0041 and 0092 dominant
7	M. parvicella dominant
8	Filament type 1701 dominant
9	Filament type 0092 dominant

Plant Data

A	MLSS recycle point changed
B	Anoxic zone on RAS. Feed to second half of anaerobic zone
C	Blockage on second clarifier siphons
D	No control on detention time
E	Zero recycle
F	Sludge retention time 4 days
G	Sludge stored and aerated intermittently on alternate days

In Module 2, bulking was experienced between April and December 1986. In January 1986, primary sludge recycling commenced. The detention time of sludge was stabilised at four days during April 1986, at which time the SVI values started to increase. Bulking continued until January 1987, when the satisfactory effluent o-phosphate levels which had been achieved during the bulking period, started to deteriorate. Phosphate removal remained poor during the greater part of 1987, and the filamentous population changed from Microthrix parvicella domination to filaments 0041 and 0803 being the most abundant. During the same period that bulking was experienced in Module 2, SVI values in Module 3 started to increase. Bulking continued for the same period as Module 2, again coincident with satisfactory phosphate removal.

In contrast to Module 2, Microthrix parvicella was not the dominant organism. Filaments 0041 and 0092 dominated the population, which altered to 1701, 0041 then 0803, after bulking had ceased. During July 1987, Microthrix parvicella again made its appearance and SVI values rose from 100 to above 150 mℓ/g by the end of September. Phosphate removal improved dramatically during October 1987, with no concomitant increase in SVI values. Microthrix parvicella and filaments 0041 and 0092 were observed to be the most abundant filaments in South African nutrient removal plants in a survey by Blackbeard et al., (1986).

Previous experience at both the Northern and Goudkoppies Works has shown that SVI's can be radically reduced from 300 - 400 down to about 100 mℓ/g even under winter conditions, by increasing the number of aerators in operation (Melmed et al., 1986). Even better results (SVI's 70 - 80 mg/ℓ) have been achieved at the Bushkoppie Works, which operates on a three-stage Bardenpho process using diffused air for aeration. In this case, the sludge is kept totally aerobic from the time it leaves the initial and only, anoxic zone, until it reaches the final clarifiers.

Nostocoida limicola II dominated the population of Bushkoppie Module 1 for some time, later being replaced by filaments 0041 and 0803. The numbers of these organisms did not rise to levels sufficient to cause bulking during this survey. Module 3, which was only commissioned during 1987, showed a similar pattern (see Figures 5.3 and 5.4).

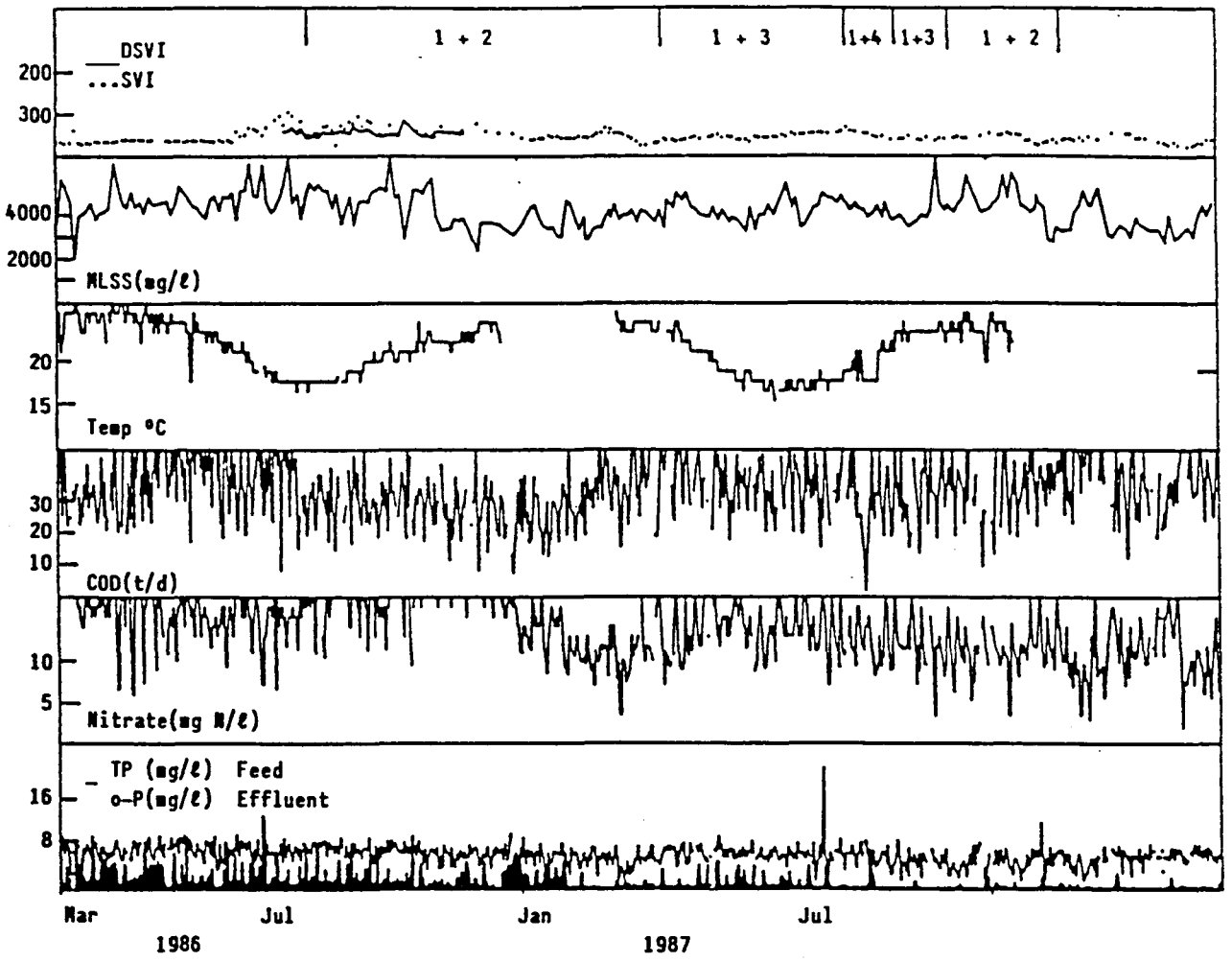

Figure 5.3 : Interrelationships between microbiological observations and plant data for Bushkoppie Module 1

Key : Microbiological Data

1 No bridging

2 Nostocoida limicola II dominant

3 Filament type 0041 dominant

4 Filament type 0803 dominant

5 Filament type 1701 dominant

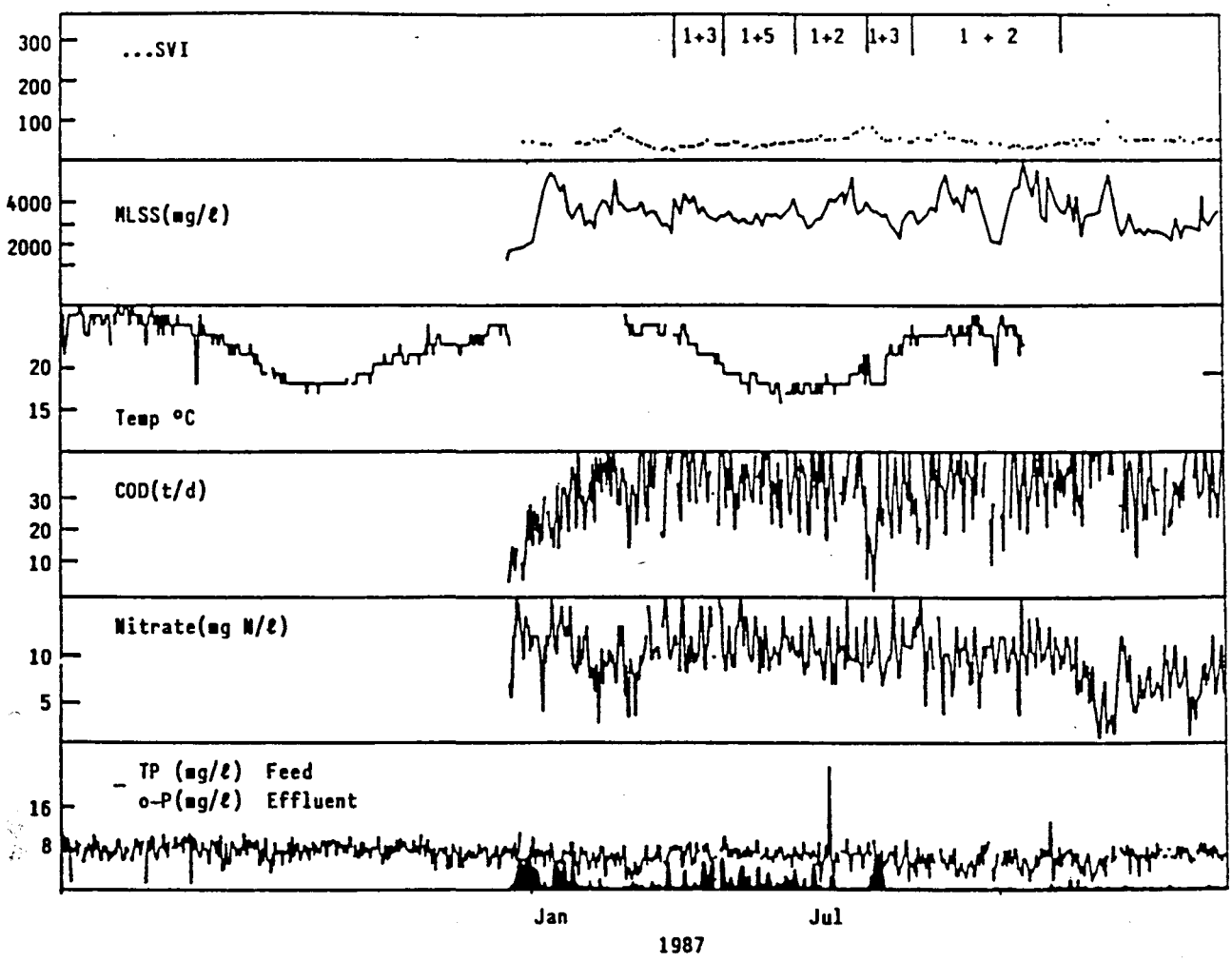

Figure 5.4 : Interrelationshops between microbiological observations and plant data for Bushkoppie Module 3

Key : Microbiological Data

1 No bridging

2 Nostocoida limicola 11 dominant

3 Filament type 0041 dominant

4 Filament type 0803 dominant

5 Filament type 1701 dominant

5.3.2 Phosphate Removing Bacteria

Staining Techniques

The comparison between the two staining techniques is shown in Figures 5.5 and 5.6. The Neisser stain appeared to provide a clearer indication of polyphosphate presence and was used throughout this study.

The comparison of cells stained with Sudan Black, before and after chloroform extraction is shown in Figures 5.7 and 5.8.

Occurrence of Polysaccharide

Staining with India ink was found to be satisfactory when large amounts of polysaccharide were present (see Figure 5.9). However, in the absence of significant amounts of polysaccharide, the stain provided very little information. Alcian blue on the other hand, provided more detailed information on the occurrence of the polysaccharide and cell morphology. Polysaccharide appeared in extended aeration plants only in association with filaments (see Figure 5.10). In the three-stage Bardenpho plants, polysaccharide production clearly occurred in the aerobic zone as evidenced by the large amounts of polysaccharide associated with cell clusters (see Figure 5.11). The Alcian blue staining procedure allows the cell clusters to be observed. However, the characteristic blue staining due to polysaccharide is not as evident in the anaerobic zone (see Figure 5.12). Polysaccharide encapsulation of the cell clusters was much more apparent in the Goudkoppies plant than the two Northern Works modules. The more pronounced clustering in the Goudkoppies plant is probably as a result of the abundant polysaccharide.

The relationship between polysaccharide levels in the Northern Works plants and effluent phosphate and influent S_{bs} are shown in Figures 5.13 and 5.14.

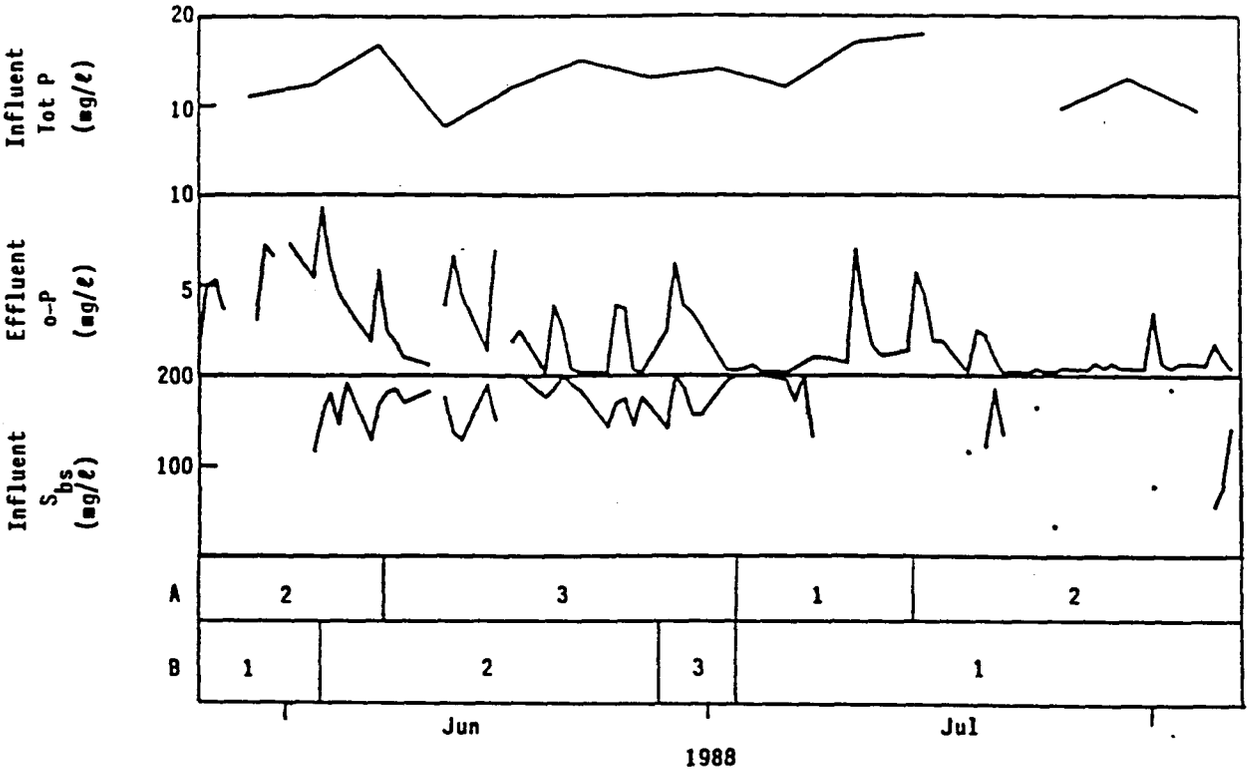

Figure 5.13 : Relationship between S_{bs}, effluent phosphate and polysaccharide in the anaerobic zone (A) and the aerobic zone (B) in Northern Works Module 3

KEY :

Anaerobic Zone

1: 0 - 10% cells surrounded by polysaccharide
2: 11 - 25% cells surrounded by polysaccharide
3: 25% cells surrounded by polysaccharide

Aerobic Zone

1: 0 - 25% cells surrounded by polysaccharide
2: 26 - 50% cells surrounded by
3: 50% cells surrounded by polysaccharide

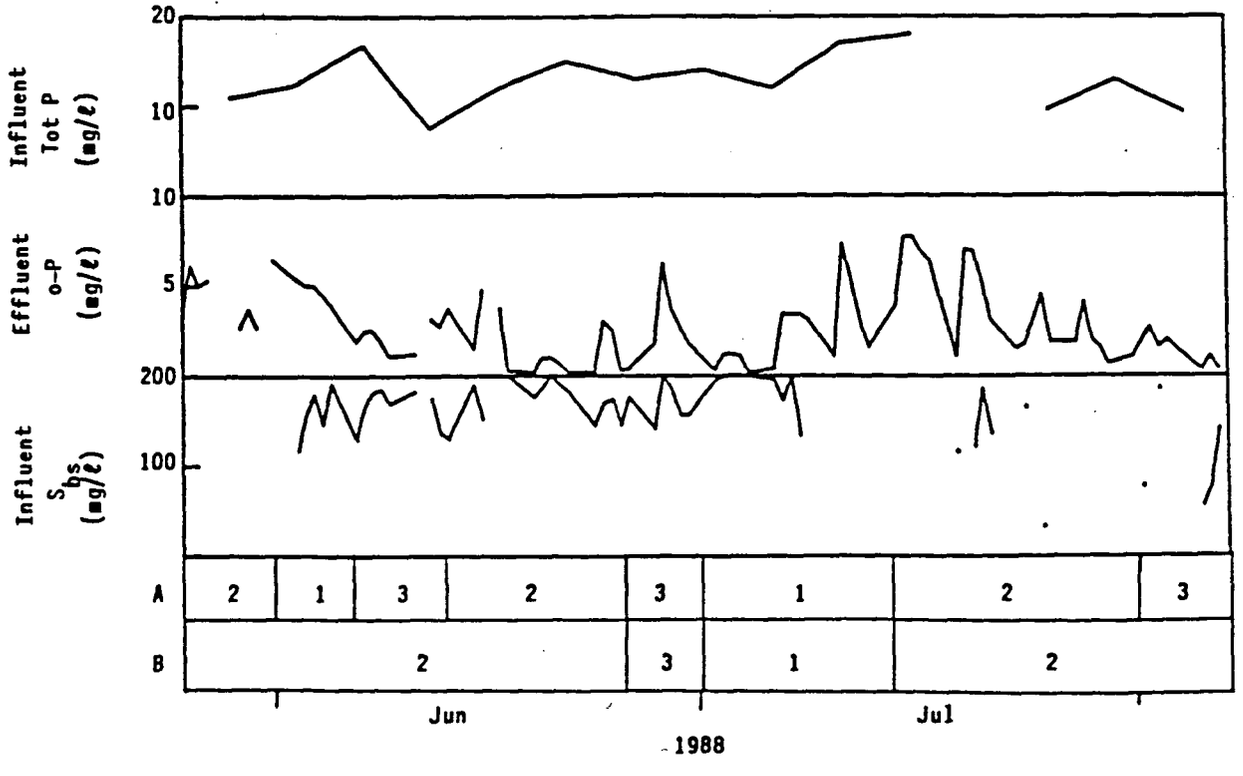

Figure 5.14 : Relationship between S_{bs}, effluent phosphate and polysaccharide in the anaerobic zone (A) and the aerobic zone (B) in Northern Works Module 1

Key as for Figure 5.13

Polyhydroxybutyrate Metabolism

Polyhydroxybutyrate (PHB) accumulation takes place in the anaerobic zone as demonstrated by the abundant inclusions observed in anaerobic zone clusters, compared to the aerobic zone. Polyhydroxybutyrate inclusions were only observed in filaments in the aerobic zone during this study. Typical examples of polyhydroxybutyrate inclusions are shown in Figures 5.15 and 5.16.

The accumulation of polyhydroxybutyrate in the filaments under aerobic conditions, in contrast to the behaviour of the poly P organisms, is not unexpected. The filamentous bacteria do not actively metabolise substrate under anaerobic conditions and are unable to absorb their preferred substrates, namely, long chain fatty acids, which can be converted to polyhydroxybutyrate. The presence of these inclusions in the aerobic zone filaments would inflate the results obtained by chemical analysis of PHB in the sludge, thus complicating the interpretation of the results. In this case, the value of microscopic evaluation is evident.

Polyhydroxybutyrate metabolism in the anaerobic and aerobic zones, was evaluated by expressing the number of cells containing inclusions in the anaerobic zone as a percentage of the total cells, and comparing this value to that obtained in the aerobic zone to estimate the amount of PHB utilised under aerobic conditions. Figure 5.17 shows the effect of PHB metabolism on the phosphate levels for the Goudkoppies plant, from October 1986 to April 1987.

Until March this plant received sewage containing high levels (greater than 200 mg/ℓ) of volatile fatty acids. The periods of satisfactory phosphate removal (effluent less than 1 mg P/ℓ) were characterised by large cells occurring in well-defined clusters. More than 75 % of the cells contained PHB inclusions and at least half the stored PHB was utilised in the aerobic zone. (Figures 5.18 and 5.19)

Figure 5.5 : Polyphosphate containing Acinetobacter cells stained with methylene blue

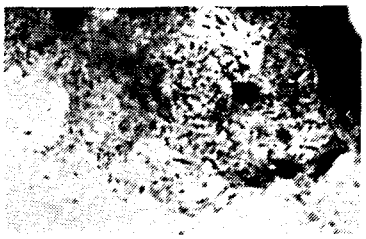

Figure 5.6 : Polyphosphate containing Acinetobacter cells stained with Neisser stain

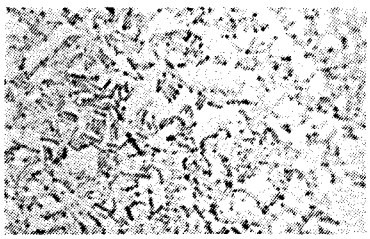

Figure 5.7 : Acinetobacter cells containing polyhydroxybutyrate stained with Sudan Black

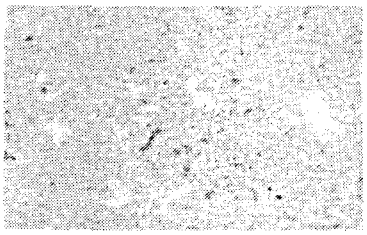

Figure 5.8 : Cells shown in Fig 5.7 after extraction with chloroform and staining with Sudan black

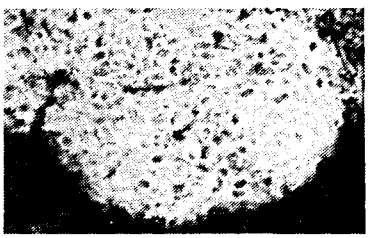

Figure 5.9 : Large cluster of cells surrounded by polysaccharide stained with India Ink, in the aerobic zone of the Goudkoppies plant

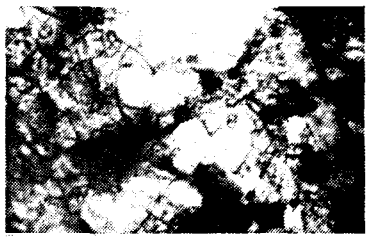

Figure 5.10 : Polysaccharide containing filaments in an extended aeration plant

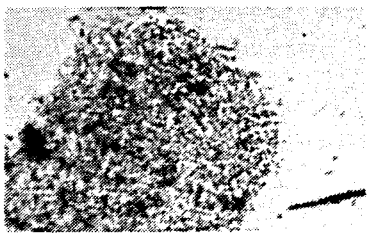

Figure 5.11 : Cell cluster with some polysaccharide in the anaerobic zone of the Goudkoppies plant

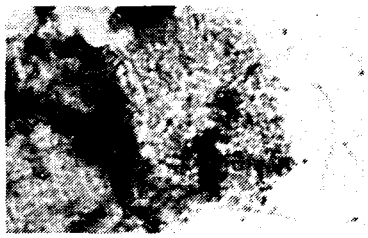

Figure 5.12 : Polysaccharide encapsulated cluster in the aerobic zone of the Goudkoppies plant

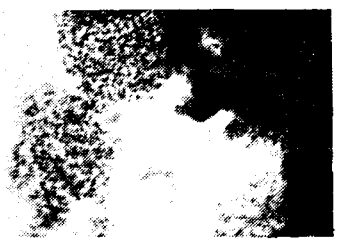

Figure 5.15 : Polyhydroxybutyrate inclusion in poly P cells in the anaerobic zone of Goudkoppies. Note the absence of inclusions in the filament

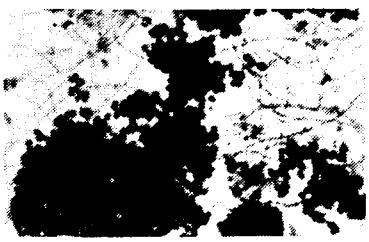

Figure 5.16 : Polyhydroxybutyrate inclusions in poly P cells in the anaerobic zone of Northern Works Module 2 during a period of satisfactory phosphate removal

Periods of unsatisfactory removal, particularly the very poor performance exhibited in March and April, were characterised by poor storage in the anaerobic zone. While utilisation in the aerobic zone remained fairly satisfactory, the poor anaerobic storage appeared to adversely affect the utilisation.

From April 1987 until the present, this plant has maintained phosphorus removal at below 1 mg o-P/ℓ. The microscopic evaluation reflected this situation.

Figure 5.20 depicts the correlation between influent volatile fatty acid levels, effluent phosphate levels and PHB metabolism for Northern Works Module 3, which is operated in essentially the same way as Goudkoppies. A significant difference is the influent volatile fatty acid level, which seldom rises above 100 mg/ℓ. The presence of volatile fatty acids in the influent is however, not in itself sufficient to ensure satisfactory PHB synthesis under anaerobic conditions, as shown by the poor synthesis observed until March. PHB synthesis improved in March and continued into April, but did not coincide with satisfactory effluent phosphate levels in the latter month. Utilisation of PHB in this plant did not at any stage reach the high levels observed in Goudkoppies. In contrast to the consistency of microbiological evaluations in this plant, the three-stage Northern Works Module 2 showed greater fluctuations in PHB utilisation and synthesis (see Figure 5.21).

As for Module 3 the influent volatile fatty acid levels very seldom exceeded 100 mg/ℓ. Again, no direct correlation between volatile fatty acid levels and PHB synthesis was observed, although satisfactory effluent phosphate levels coincided with good PHB synthesis under aerobic conditions. This plant did, on a few occasions, achieve the high PHB synthesis and utilisation levels observed in the Goudkoppies plant.

While microscopic evaluation of the plant cannot be used to predict failure, the technique has value as a diagnostic tool. Poor synthesis of PHB in the anaerobic zone can be caused by insufficient volatile acids, nitrate entering the zone (Lötter, 1987), or possibly, low abundance of poly P bacteria.

A rapid microbiological evaluation demonstrating poor PHB synthesis could indicate additional parameters to be tested. Poor utilisation in the aerobic zone is most likely to be caused by insufficient aeration. Low oxygen levels cause a build-up of NADH which is a powerful inhibitor of PHB degradation (Lötter and Dubery, 1987).

More recent results showing the relationship between influent S_{bs}, effluent phosphate and PHB metabolism are given in Figures 5.22 and 5.23.

Polyphosphate Metabolism

The accumulation of polyphosphate in the aerobic zone and its degradation under anaerobic conditions, has been reported by a number of researchers (including Buchan, 1981; Murphy and Lötter, 1986). The large, well-defined polyphosphate containing clusters typical of satisfactory phosphate removal, were observed during this study (see Figures 5.24 and 5.25).

Disintegration of the clusters and significant reduction in cell size characterises periods of unsatisfactory phosphate removal. During unsatisfactory phosphate removal, the oval coccoid type cells, typical of phosphate removing activated sludge, continue to dominate the Goudkoppies biomass (see Figure 5.26), while the clearly defined biomass structure is no longer present in Northern Works Module 3 (see Figure 5.27).

Poly P cells are visible in an amorphous non-polyphosphate containing biomass. The large empty cells, normally visible in an anaerobic zone during satisfactory phosphate removal, are shown in Figure 5.28. Figure 5.29 shows clusters of empty and polyphosphate

filled cells in the anaerobic zone of Goudkoppies, when phosphate removal had started to fail.

Similar to the way in which the polyhydroxybutyrate results were expressed, the number of cells containing polyphosphate inclusions in the aerobic zone were expressed as a percentage of the total number of cells. The polyphosphate degradation was expressed as the percentage reduction in the number of cells containing polyphosphate.

Large clusters with more than 75% of the cells containing polyphosphate, dominated the biomass during periods of satisfactory phosphate removal. Anaerobic phosphate release was also high. Phosphate release was practically undetectable at the end of March into April. Polyphosphate storage was also low during this period (see Figure 5.30).

Poor phosphate release appears to coincide with poor polyhydroxybutyrate synthesis, as can be seen by comparing Figures 5.30 to 5.32 with Figures 5.17, 5.20 and 5.21, thus lending credence to the hypothesis of Wentzel et al. (1986), who state that phosphate is released under anaerobic conditions, to reinstate the proton motive force dissipated by the uptake of acetate.

Very few large cell clusters were observed in Northern Works Module 3 during the first part of this study and very little phosphate release was observed (Figure 5.31). These observations were indicative of a non-phosphate removing biomass, as evidenced by the high effluent phosphate levels. Polyphosphate storage improved during the second period of the study, but never attained the levels observed in Goudkoppies. Phosphate release also improved and effluent phosphate levels decreased.

Northern Works Module 2 showed essentially the same pattern of polyphosphate metabolic behaviour relative to effluent phosphate levels (Figure 5.32). As with polyhydroxybutyrate microscopic evaluation, polyphosphate assessment cannot be used predictively, but can be used in conjunction with polyhydroxybutyrate as a diagnostic tool.

Figure 5.18 : Poly P cells empty of polyhydroxybutyrate in the aerobic zone of Northern Works Module 2 during a period of satisfactory phosphate removal

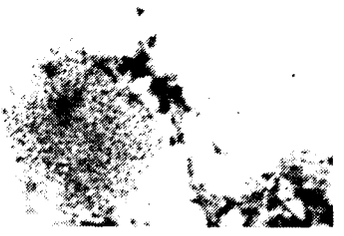

Figure 5.19 : Reduction of polyhydroxy-butyrate inclusions in the aerobic zone of Goudkoppies. Filaments containing polyhydroxybutyrate

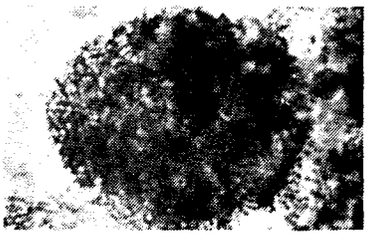

Figure 5.24 : Large poly P cluster containing polyphosphate in the Goudkoppies aerobic zone

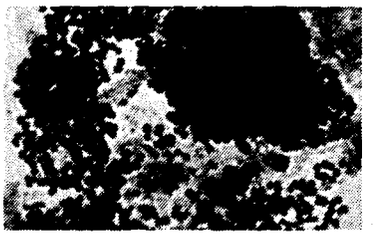

Figure 5.25 : Large poly P cluster containing polyphosphate in Northern Works Module 2 aerobic zone

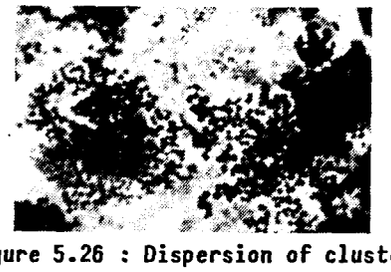

Figure 5.26 : Dispersion of cluster and reduction in size of polyphosphate containing cells. Goudkoppies aerobic zone

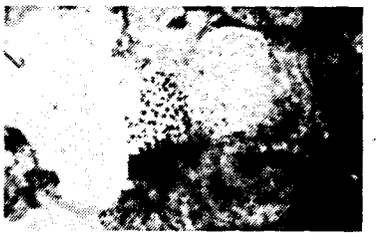

Figure 5.27 : Small polyphosphate containing cells in small groups in amorphous biomass. Northern Works Module 3 aerobic zone

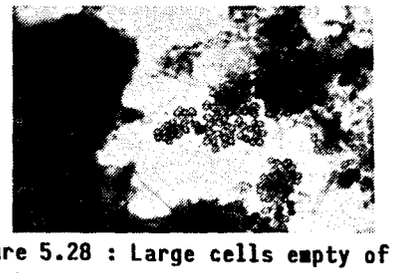

Figure 5.28 : Large cells empty of polyphosphate. Goudkoppies anaerobic zone

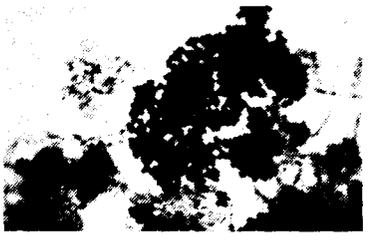

Figure 5.29 : Large empty and poly-phosphate containing cells. Goudkoppies anaerobic zone

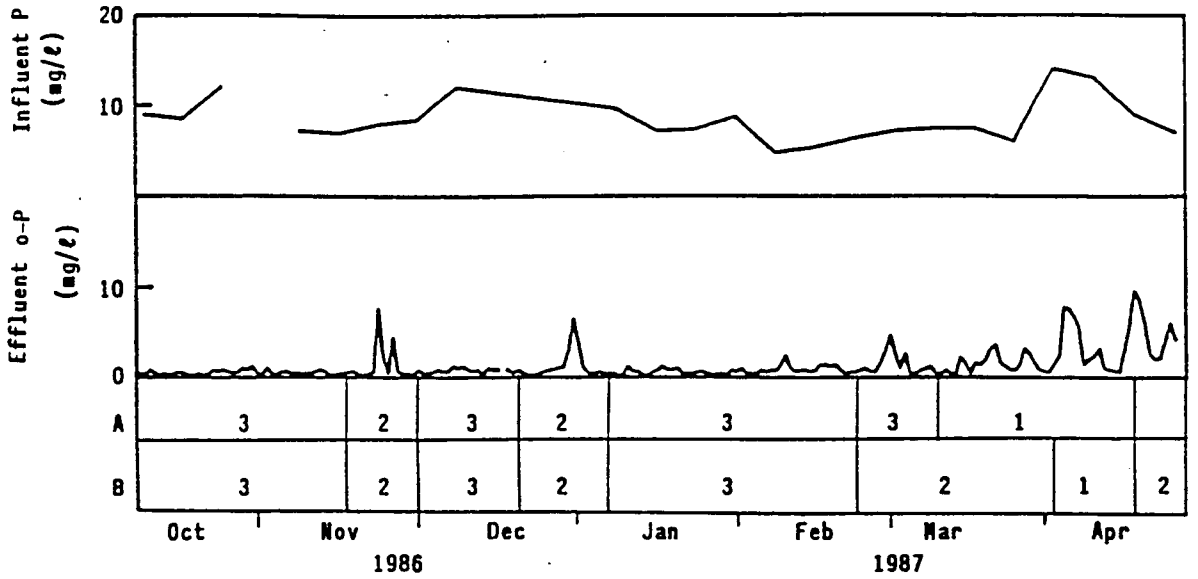

Figure 5.17 : Correlation between polyhydroxybutyrate synthesis (A) in the anaerobic zone and utilisation (B) in the aerobic zone and effluent phosphate levels - Goudkoppies

KEY :

Polyhydroxybutyrate Storage		Polyhydroxybutyrate Utilisation	
1	25% cells contain PHB	1	25% reduction in number of cells containing PHB
2	25-75% cells contain PHB	2	25-50% reduction in number of cells containing PHB
3	75% cells contain PHB	3	50% reduction in cells containing PHB

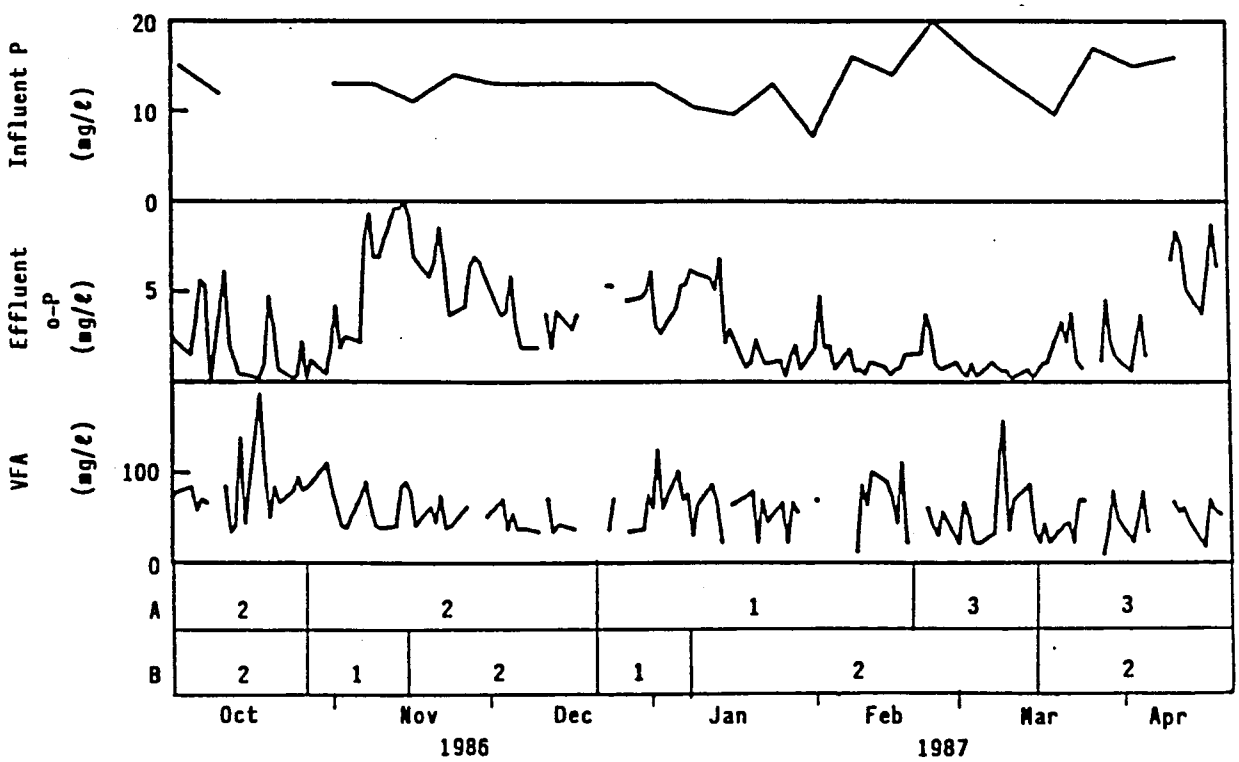

Figure 5.20 : Correlation between polyhydroxybutyrate synthesis (A) in the anaerobic zone and utilisation (B) in the aerobic zone and volatile fatty acids (VFA) levels in the influent and effluent phosphate levels - Northern Works - Module 3

KEY : as for Figure 5.17

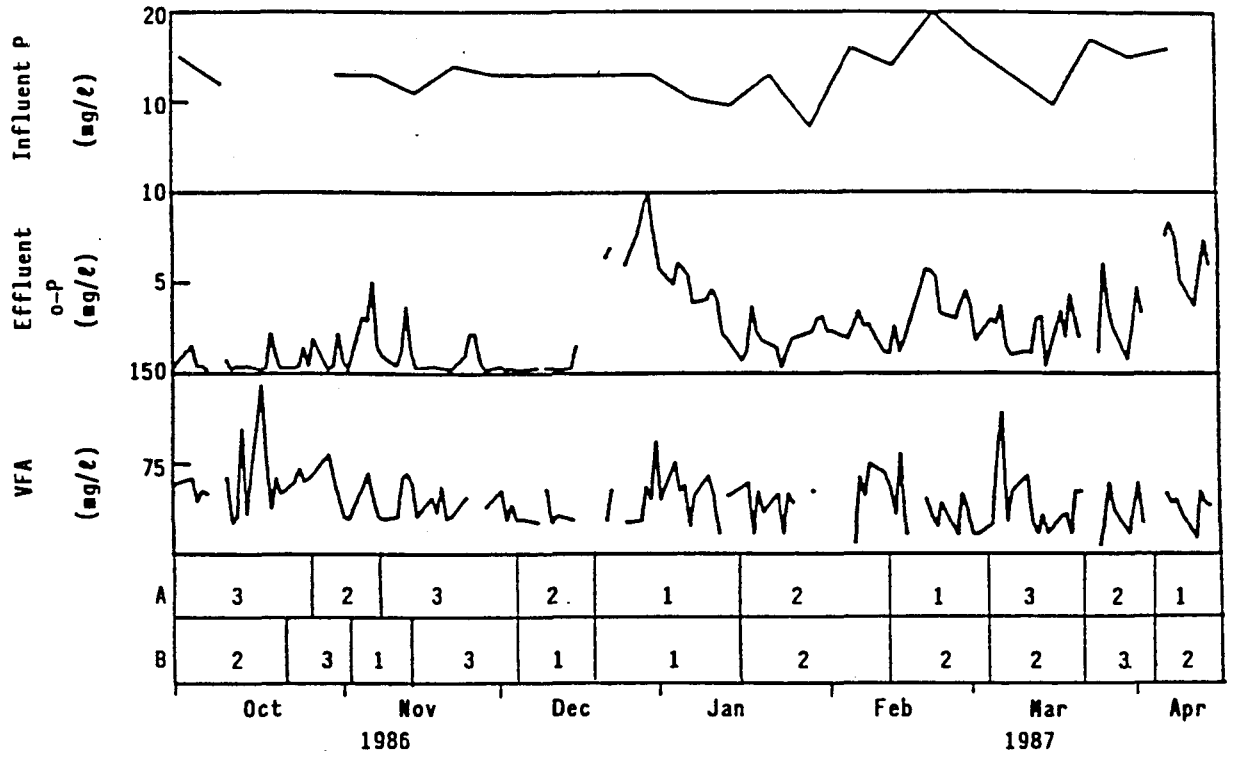

Figure 5.21 : Correlation between polyhydroxybutyrate synthesis (A) in the anaerobic zone and utilisation (B) in the anaerobic zone and volatile fatty acids (VFA) levels in the influent and effluent phosphate levels - Northern Works Module 1

KEY: As for Figure 5.17

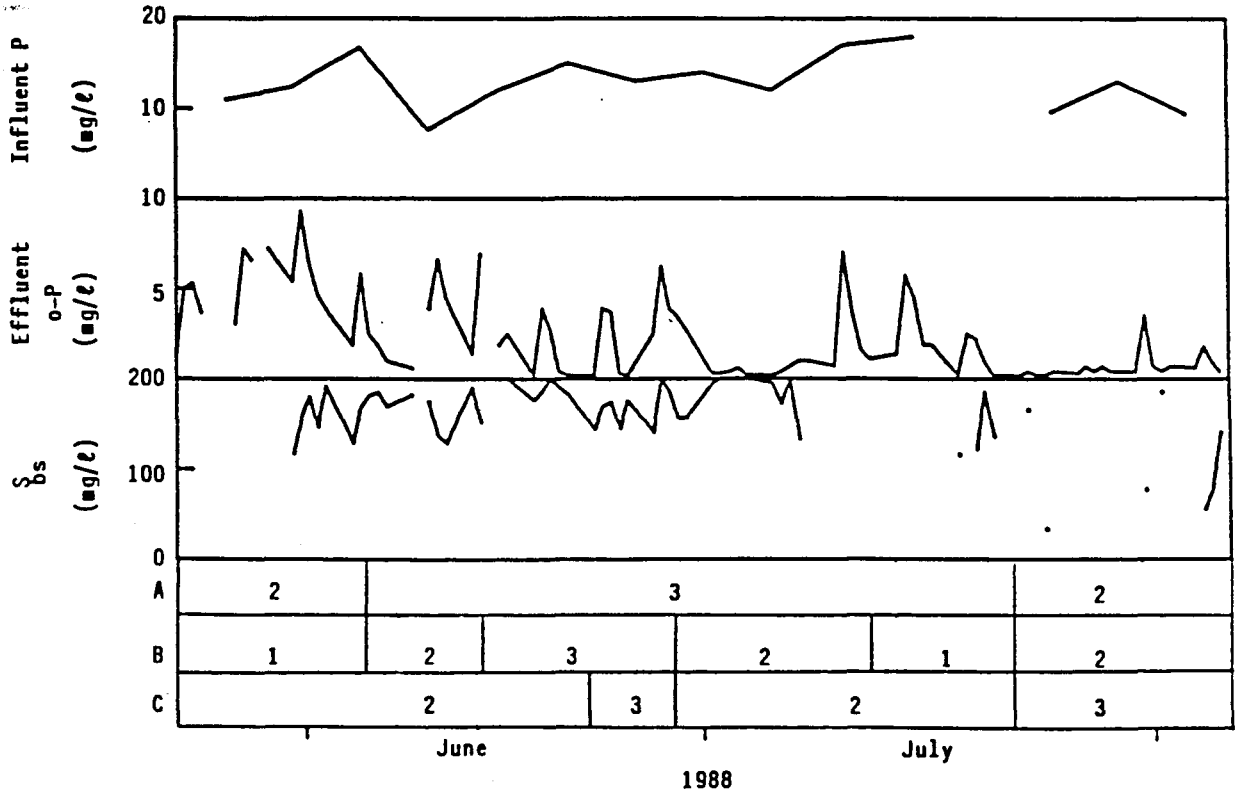

Figure 5.22 : Relationship between S_{bs}, effluent phosphate and PHB accumulation in the anaerobic zone (A); PHB utilisation in the anoxic zone (B) and PHB utilisation in the aerobic zone (C) in Northern Works Module 3

KEY: As for Figure 5.17

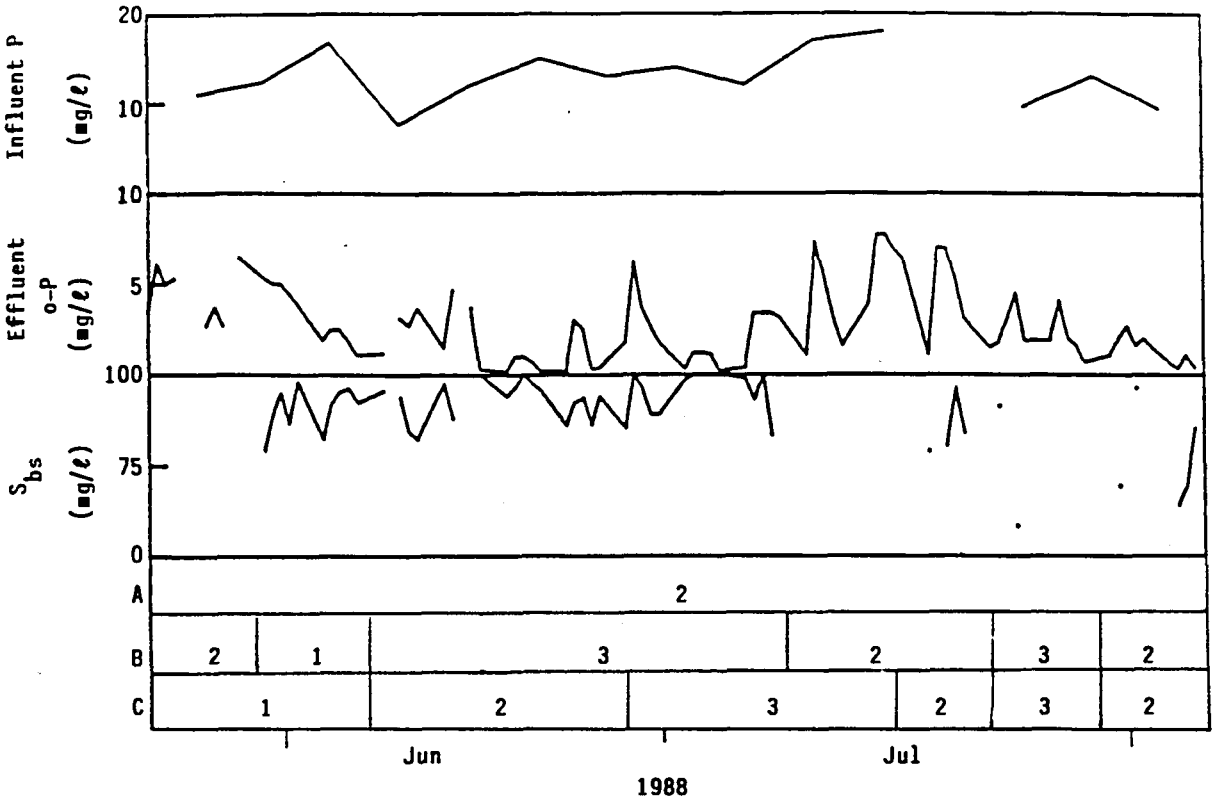

Figure 5.23 : Relationship between S_{bs}, effluent phosphate and PHB accumulation in the anaerobic zone (A); PHB utilisation in the anoxic zone (B) and PHB utilisation in the aerobic zone (C) in Northern Works Module 1

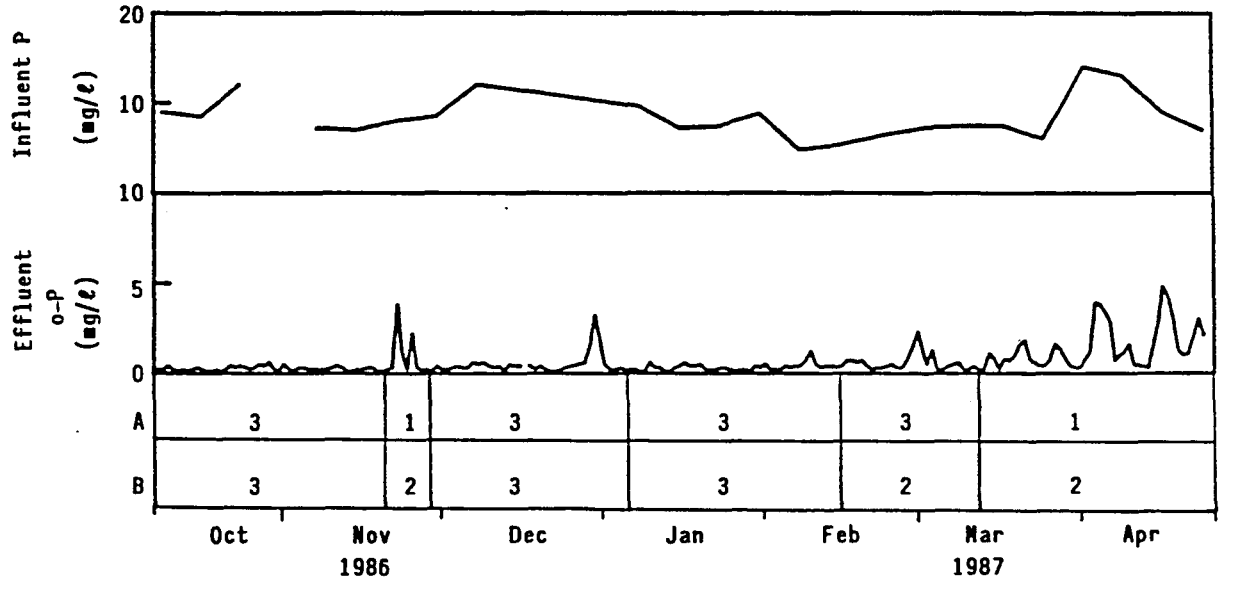

Figure 5.30 : The effect of phosphate release (A) in the anaerobic zone and polyphosphate storage (B) in the aerobic zone on effluent phosphate levels - Goudkoppies

KEY :

Phosphate Release

1 10% reduction in number of cells containing polyphosphate
2 10-25% reduction in number of cells containing polyphosphate
3 25% reduction in number of cells containing polyphosphate

Polyphosphate Storage

1 25% cells contain polyphosphate
2 25-75% cells contain polyphosphate
3 75% cells contain polyphosphate

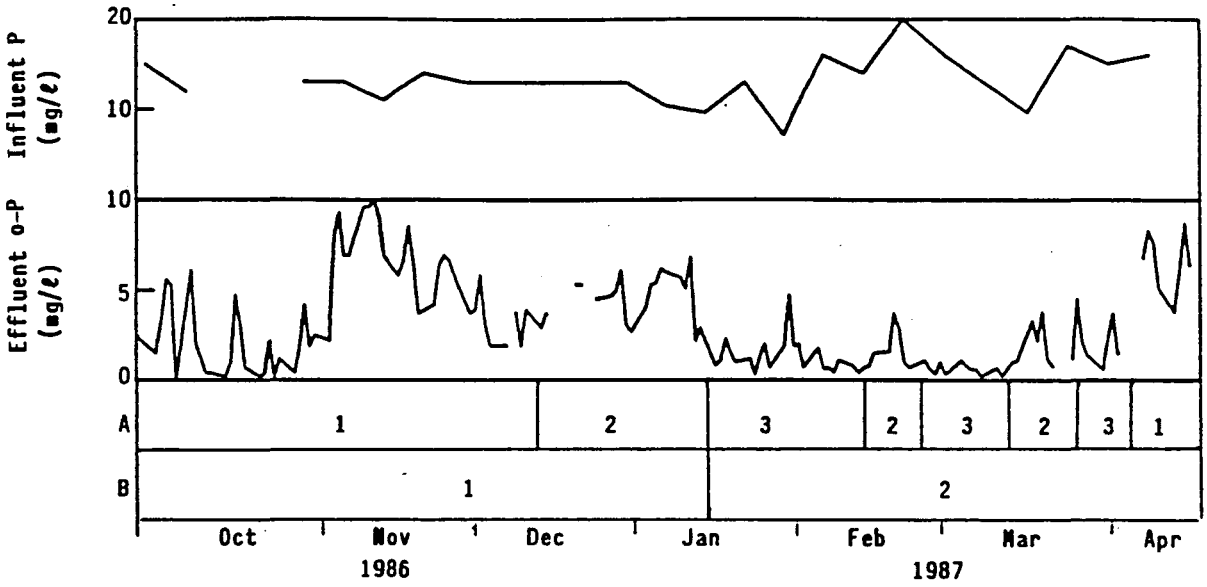

Figure 5.31 : The effect of phosphate release (A) in the anaerobic zone and polyphosphate storage (B) in the aerobic zone on effluent phosphate levels - Northern Works Module 3

KEY : As for Figure 5.30

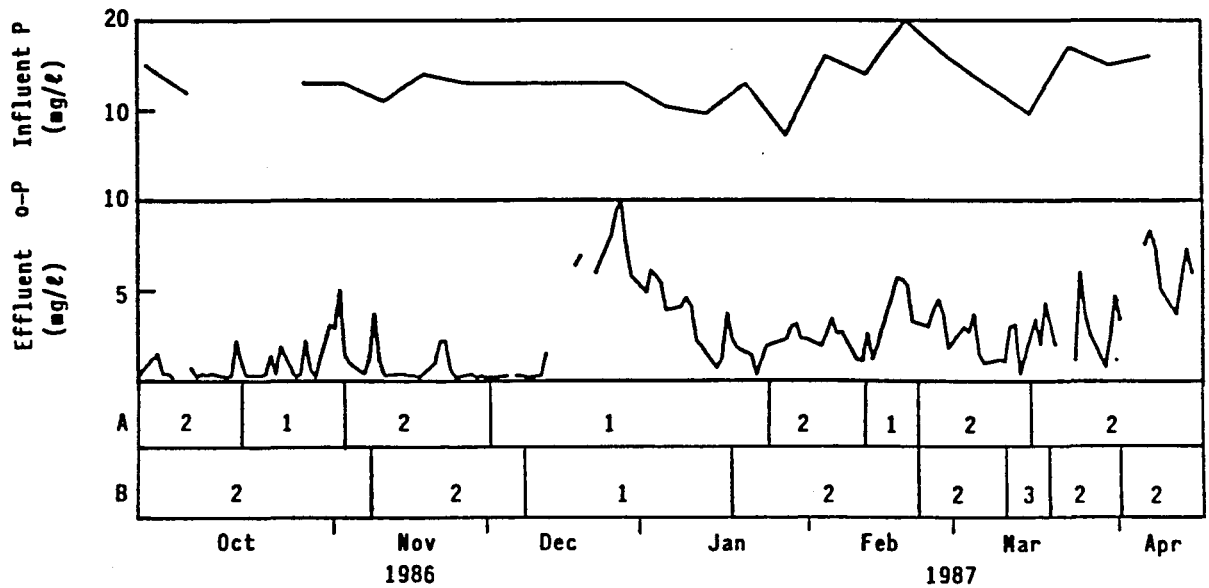

Figure 5.32 : The effect of phosphate release (A) in the anaerobic zone and polyphosphate storage (B) in the aerobic zone on effluent phosphate levels - Northern Works Module 2

KEY : As for Figure 5.30

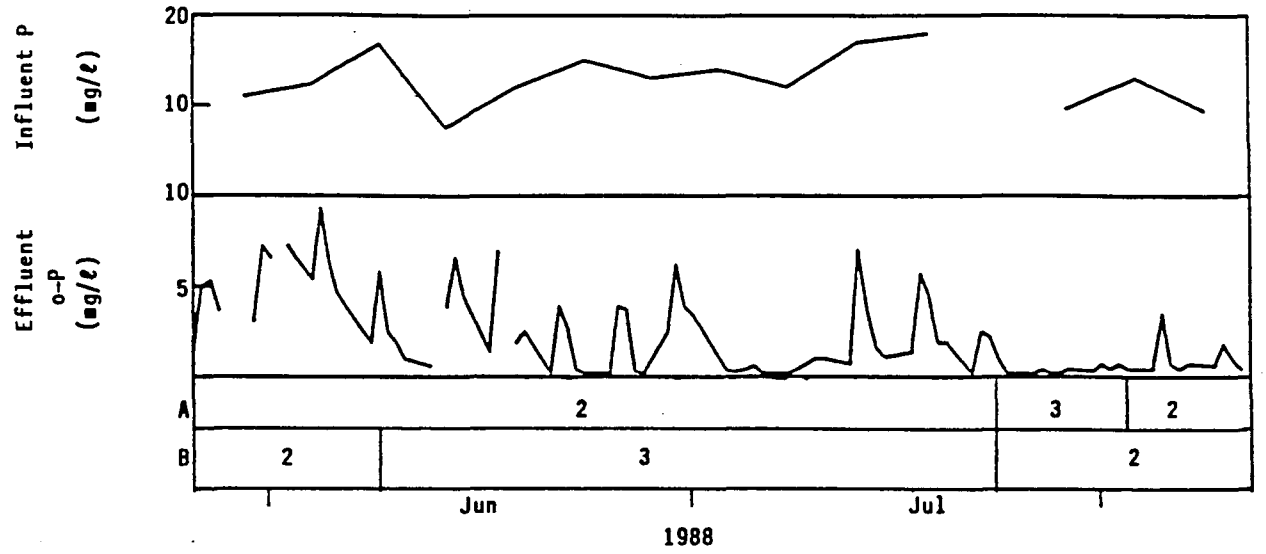

Figure 5.33 : The effect of phosphate release (A) in the anaerobic zone and polyphosphate storage (B) in the aerobic zone on efluent phosphate levels - Northern Works Module 1.

KEY : As for Figure 5.30

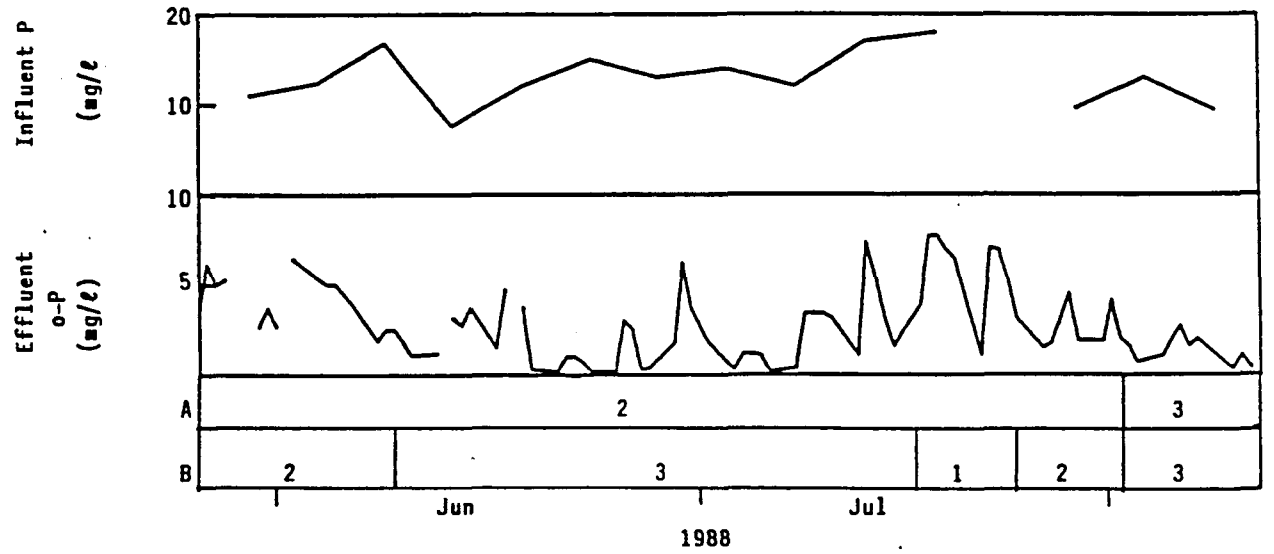

Figure 5.34 : The effect of phosphate release (A) in the anaerobic zone and polyphosphate storage (B) in the aerobic zone on effluent phosphate levels - Northern Works Module 3

KEY : As for Figure 5.30

More recent microscopic evaluation revealed the same trends (see Figures 5.33 and 5.34).

Bushkoppie Works Start-up

Microscopic examination under low magnification (125x) revealed that the bacterial flora were initially restricted to a few clumps sparsely distributed within the sewage. A high rate of cell proliferation resulted in much larger clumps and bridging by filaments appeared within a week.

Within two weeks the typical activated sludge floc had formed. The structure of the floc was studied in more detail at a higher magnification (1250x). Very little difference was observed between the three zones in the first week, although small polyphosphate containing cells started to develop around the edges of the flocs. After two weeks, distinction between zones became possible.

The cells in the anaerobic zone remained small and contained little polyphosphate. The anoxic zone cells contained larger granules. This trend continued into the aerobic zone where the cells and granules were larger and more cells contained polyphosphate. Within a month, clusters of cells were becoming well-defined in all zones. Subsequently, all three zones contained large cells appearing in well-defined clusters with the storage of polyphosphate in the aerobic zone resulting in cells three times larger than those in the anaerobic zone. During this period the effluent phosphate level had decreased significantly, and towards the end of September had decreased to below 1 mg o-P/ℓ. Figure 5.35 depicts the effect of the polyphosphate behaviour on effluent phosphate levels. In October however, the average cell size in all the zones suddenly decreased dramatically, a phenomenon which coincided with a period of poor phosphate removal.

Phosphate release in the anaerobic zone and uptake in the aerobic zone still occurred, but the size of the cells was not sufficient to reduce phosphate levels to below 1 mg o-P/ℓ.

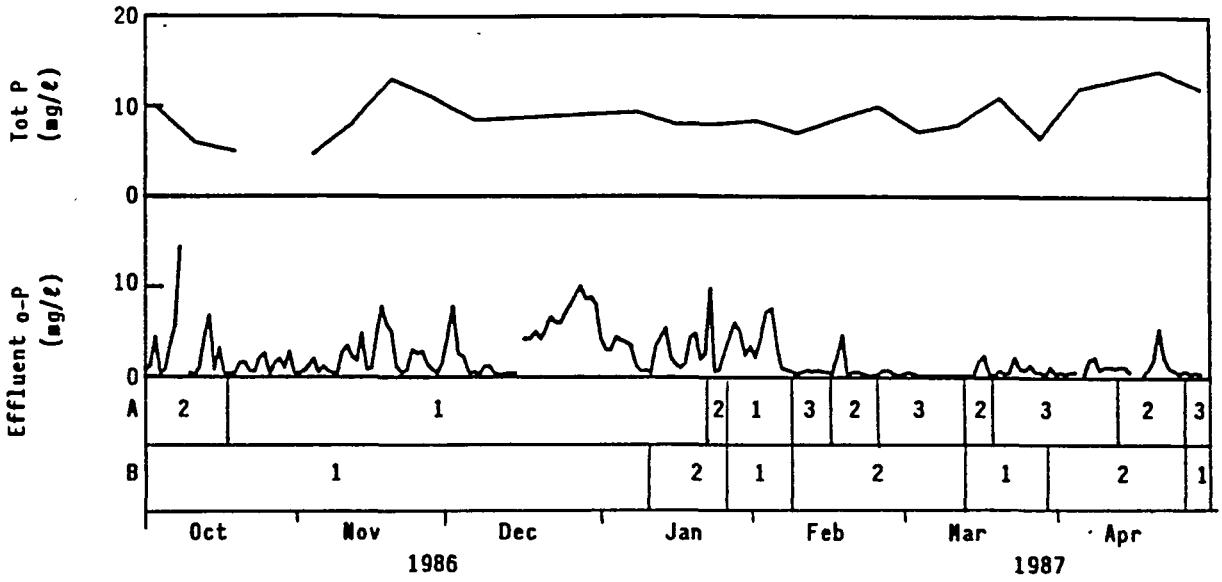

Figure 5.35 : The effect of phosphate release (A) in the anaerobic zone and polyphosphate storage (B) in the aerobic zone and effluent o-phosphate levels-Bushkoppie Module 1; influent total P

KEY : As for Figure 5.30

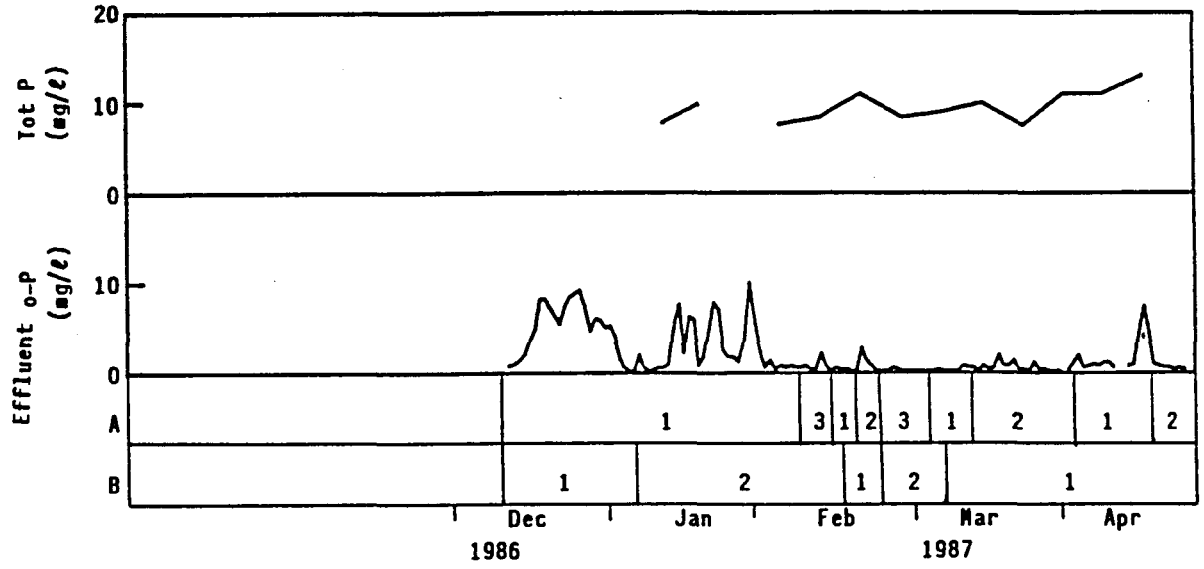

Figure 5.36 : The effect of phosphate release (A) in the anaerobic zone and polyphosphate storage (B) in the aerobic zone and effluent o-P levels-Bushkoppie Module 3; influent total P

KEY : As for Figure 5.30

No explanation for this dramatic change and the subsequent return to efficient operation in November, was apparent.

The Bushkoppie plant was subsequently monitored from October 1986. Figure 5,36 depicts the effect of polyphosphate behaviour on the effluent phosphate levels. The plant's performance remained poor until February 1987, when the sewage with high volatile acid levels was diverted from Goudkoppies. Phosphate release improved dramatically and significant amounts of polyphosphate were stored, causing the effluent phosphate level to decrease to below 1 mg o-P/ℓ.

In December 1986, a second module of Bushkoppie was commissioned using waste activated sludge from the first module as inoculum. The diversion of sewage from Goudkoppies affected this module in the same way as Module 3, and satisfactory phosphate removal was achieved (see Figure 5.36). Both these modules have continued to maintain an effluent phosphate level of less than 1 mg o-P/ℓ.

5.3.4 Evaluation of Different Media for the Isolation of Bacteria from Activated Sludge

The plate counts obtained on the different media after incubation for 3; 5 and 7 days are given in Tables 5.4; 5.5 and 5.6.

TABLE 5.4
PLATE COUNTS OBTAINED ON THE FOUR MEDIA AT DIFFERENT DILUTIONS AFTER THREE DAYS OF INCUBATION AT 21 °C

Medium	Dilution			
	-3	-4	-5	-6
DGCY	>500	42	3	0
	>500	42	2	0
DF&C	>500	23	0	0
	>500	12	0	0
BTE	>500	9	0	0
	260	20	0	0
SS	22	2	0	0
	130	2	0	0

The dilute GCY medium gave the highest counts. GCY agar has previously been recommended as the medium giving the highest plate counts for activated sludge samples (Pike et al., 1972). The total relatively small increase in counts for the lowest dilution on this medium, after an additional 2 to 4 days incubation, indicates substrate depletion at this concentration.

TABLE 5.5
PLATE COUNTS OBTAINED ON THE FOUR MEDIA AT DIFFERENT DILUTIONS AFTER FIVE DAYS OF INCUBATION AT 21 °C

Medium	Dilution			
	-3	-4	-5	-6
DGCY	> 500	> 500	19	1
	> 500	> 500	10	3
DF&C	> 500	325	2	0
	> 500	390	2	0
BTE	> 500	260	2	0
	> 500	195	2	0
SS	260	2	0	0
	130	2	0	0

TABLE 5.6
PLATE COUNT OBTAINED ON THE FOUR MEDIA AT DIFFERENT DILUTIONS AFTER SEVEN DAYS OF INCUBATION AT 21 °C

Medium	Dilution			
	-3	-4	-5	-6
DGCY	> 500	> 500	19	1
	> 500	> 500	11	3
DF&C	> 500	> 500	2	0
	> 500	> 500	8	0
BTE	> 500	260	3	0
	> 500	230	2	0
SS	260	2	0	0
	130	2	0	0

The lower figures obtained for the lowest dilution on balance tank effluent after the longer incubation period, appear anomalous.

Balance tank effluent counts differed only slightly from the counts obtained with dilute Fuhs and Chen medium. Settled sewage appeared to be a very poor medium for the isolation of colonies (see Table 5.7).

TABLE 5.7
VIABLE PLATE COUNTS ON DIFFERENT MEDIA AFTER FIVE DAYS

Medium	Viable Plate Count CFU/mℓ
DGCY	$1,5 \times 10^{6}_{5}$
DF&C	$3,6 \times 10^{5}_{5}$
BTE	$2,3 \times 10^{5}_{4}$
SS	$2,0 \times 10^{4}$

The mixed liquor sample was also examined microscopically for viable bacteria using the Acridine Orange technique. $2,03 \times 10^{8}$ bacterial cells were counted with a viability of 98,8 %. These results indicate that none of the media investigated isolated the total bacteria present in the sample.

In an attempt to approach this total, nitrifying bacteria were isolated (see Table 5.8).

TABLE 5.8
VIABLE COUNTS OF NITRIFYING BACTERIA

Medium containing	Viable Plate Count CFU/mℓ
Ammonium	$6,0 \times 10^{4}_{5}$
Nitrite	$1,2 \times 10^{5}_{5}$
Total	$1,8 \times 10^{5}$

The addition of these bacteria to the highest total in Table 5.7 still does not approach the Acridine Orange total. The composition of the two synthetic isolation media did not influence the population diversity significantly (see Tables 5.9 and 5.10).

TABLE 5.9
POPULATION COMPOSITION ON DILUTE GCY MEDIUM

Bacterium	Percentage of total
Acinetobacter calcoaceticus var lwoffi	30
Achromobacter spp	10
Aeromonas spp	7,1
Bordetella/Alcaligenes	7,1
CDV VE2	8,5
Pseudomonas	37

TABLE 5.10
POPULATION COMPOSITION ON DILUTE FUHS AND CHEN MEDIUM

Bacterium	Percentage of total
Acinetobacter calcoaceticus var lwoffi	36
Achromobacter spp	8,2
Aeromonas hydrophila	5,5
Bordetella/Alcaligenes	5,5
CDV VE2	6,9
Pseudomonas	37
Gram positive	1,4

TABLE 5.11
POPULATION COMPOSITION ON DILUTE GCY AND BTE MEDIUM

Bacterium	Percentage of Total			
	GCY (25x dilute) agar		BTE agar	
Gram positive (discarded)	4,55		6,38	
No growth	4,55		4,26	
Gram negative :				
Moraxella spp	27,27		25,5	
Alcaligenes faecalis	18,18		25,5	
Aeromonas hydrophila	4,55		14,89	
Flavobacterium spp	13,63		8,51	
Pseudomonas spp :				
Ps.fluorescens	2,27		4,26	
Ps.diminuta	4,55	9,09	2,14	6
Ps.cepacia	2,27		-	
Vibrio spp :	6,81		-	
V.parahaemolyticus	-	9,08	2,14	6,42
V.alginolyticus	2,27		2,14	
V.cholera	-		2,14	
Acinetobacter calcoaceticus var lwoffi	-		2,14	
Actinobacillus spp	4,55		-	
Pasteurella spp	4,55		-	
	100,0		100,0	

The population compositions with dilute GCY and BTE medium, are shown in Table 5.11. The total plate counts were 4,4 x 10^9 CFU/mℓ and 4,7 x 10^9 CFU/mℓ respectively.

Individual colonies were replated onto dilute media but did not flourish and organisms such as Pasteurella spp and Acinetobacter spp normally observed on GCY were not detected in high numbers in this study. Examination of Table 5.11 reveals that significant differences in population composition are obtained with the two media. The propensity for polyphosphate accumulation was also not affected by the synthetic isolation medium (see Tables 5.12 and 5.13).

TABLE 5.12
POLYPHOSPHATE ACCUMULATION BY ISOLATES ON FUHS AND CHEN MEDIUM

Bacterium	Percentage accumulating polyphosphate
Acinetobacter calcoaceticus var lwoffi	54
Achromobacter spp	Nil
Moraxella spp	3
Bordetella/Alcaligenes	6
Pasteurella spp	Nil
Pseudomonas spp	37
Gram positive	Nil

TABLE 5.13
POLYPHOSPHATE ACCUMULATION BY ISOLATES ON DILUTE GCY MEDIUM

Bacterium	Percentage accumulating polyphosphate
Acinetobacter calcoaceticus var lwoffi	48
Achromobacter spp	Nil
Aeromonas hydrophila	8
Bordetella/Alcaligenes	12
CDV VE2	Nil
Pseudomonas	32

The total plate counts obtained with constant nutrients for five days and daily nutrient replenishment are given in Table 5.14.

TABLE 5.14
TOTAL PLATE COUNTS WITH NUTRIENT REPLENISHED AND UNREPLENISHED MEDIA

Medium	Total Plate Count CFU/mℓ	
	1	2
Replenished daily	$4{,}40 \times 10^9$	$4{,}15 \times 10^9$
Unreplenished	$4{,}65 \times 10^9$	$4{,}45 \times 10^9$

The lower counts obtained with replenished media could be as a result of growth being retarded, while bacteria adapted to the fresh medium.

The population compositions for these two procedures are given in Table 5.15.

TABLE 5.15
COMPARISON OF BACTERIAL POPULATIONS WHEN (A) NUTRIENTS REMAIN CONSTANT FOR 5 DAYS AND (B) NUTRIENTS ARE FRESHLY REPLACED DAILY FOR 5 DAYS

Bacterium	Percentage of Total	
	A	B
Gram positive (discarded)	10,47	11,88
No growth after inoculation	0	1,00
Gram positive :		
Acinetobacter spp	3,86	5,47
Alcaligenes spp	23,05	19,29
Actinobacillus spp	2,44	3,47
Aeromonas hydrophila	8,48	13,42
Flavobacterium spp	12,64	5,47
Moraxella spp	16,72	17,29
Pasteurella spp	7,18	9,47
Pseudomonas spp	11,10	9,24
Vibrio spp	4,06	4,00
	100,0	100,0

As can be seen from Table 5.15, certain bacteria are favoured by daily nutrient replenishment, while others are inhibited.

5.3.5 Comparison of Identification Techniques

The identification of the twenty bacterial colonies obtained with the two systems is shown in Table 5.16.

TABLE 5.16
COMPARISON OF API IDENTIFICATION TECHNIQUES AND MICROBACT IDENTIFICATION SYSTEM

Sample No	API Identification	Microbact Identification
3	Pseudomonas spp	Ps fluorescens
5	Alcaligenes spp	A faecalis
6	A lwoffi	A lwoffi
12	A lwoffi	A lwoffi
13	Enterobacter agglomerans	E agglomerans
15	Pseudomonas cepacia	Ps cepacia
18	Aeromonas hydrophila	A hydrophila
19	Klebsiella oxytoca	K oxytoca
26*	Cedecia spp	Pseudomonas spp
27	Flavobacterium spp	Flavobacterium spp
28	Moraxella spp	Moraxella spp
29	Alcaligenes spp	A faecalis
20	A lwoffi	A lwoffi
34	Pasteurella spp	P haemolytica
35	Xanthomonas maltophilia	Xanthomonas maltophilia
37	Ps fluorescens	Ps fluorescens
44	Pasteurella spp	P haemolytica
45	Klebsiella spp	K oxytoca
57	Ps cepacia	Ps cepacia
58	Moraxella spp	Moraxella spp

The comparison of the nitrate reduction tests is given in Table 5.17.

Ninety five percent of the samples shown positive by the standard nitrate agar test were found positive by the Microbact system, in contrast to only 80 % for the API test.

From Table 5.16 it is clear that the API and the Microbact identification systems generally provide the same identification. It can also be noted that the Microbact system seems to be more

species specific in certain cases. The anomaly with sample 26* is more than likely due to experimental error such as the inoculation of the systems using a mixed or contaminated culture.

TABLE 5.17
COMPARISON OF NITRATE REDUCTION USING API, MICROBACT AND NITRATE AGAR SLANTS

Sample No	Nitrate Agar	API	Microbact
3	+	+	+
5	-	-	-
6	-	-	-
12	-	+	-
13	+	+	+
15	+	+	+
18	+	-	+
19	+	+	+
26	+	+	+
27	-	+	-
28	-	-	-
29	-	-	-
30	+	+	+
34	+	+	+
35	+	+	-
37	+	-	+
44	+	+	+
45	+	+	+
57	+	+	+
58	-	-	-

Key : + = positive
- = negative for nitrate reduction

Table 5.17 shows that the Microbact nitrate reduction test is more reliable than the API nitrate reduction test. The nitrate reduction test does not always affect the identification procedure, but when interested in these properties, this would be a problem.

The oxidase test on the other hand, does not affect the identification of the organism. The API oxidase test has been shown to be unreliable and since the Microbact system does not cater for the oxidase test, this test must be done separately, thus giving a more reliable result.

The Microbact system has 24 tests (excluding the nitrate reduction test, which is done in the ONPG well), in contrast to the 20 tests of the API system. The Microbact system appears to be more reliable and more species specific than the API test. In view of the lower cost, this system is now used routinely.

The dilute GCY gave the highest viable plate count. GCY agar has previously been recommended as the medium giving the highest plate counts for activated sludge samples (Pike et al., 1972). The total count determined microscopically by the Acridine Orange method was $1,7 \times 10^8$.

5.3.6 Phosphate Releasing Capacity of Activated Sludge

Significant differences in the average phosphate released by the sludge from the three plants were observed (see Table 5.18).

TABLE 5.18
AVERAGE PHOSPHATE RELEASE TEST VALUES

Plant	Phosphate release (%)
Northern Works Module 1	97
Northern Works Module 3	70
Goudkoppies	121

As has been previously observed, complete release of intracellular polyphosphate could not be achieved (Lötter, 1985). The amount of phosphate released does not appear to be solely dependent on the amount of polyphosphate in the floc. See Figures 5.37 to 5.39).

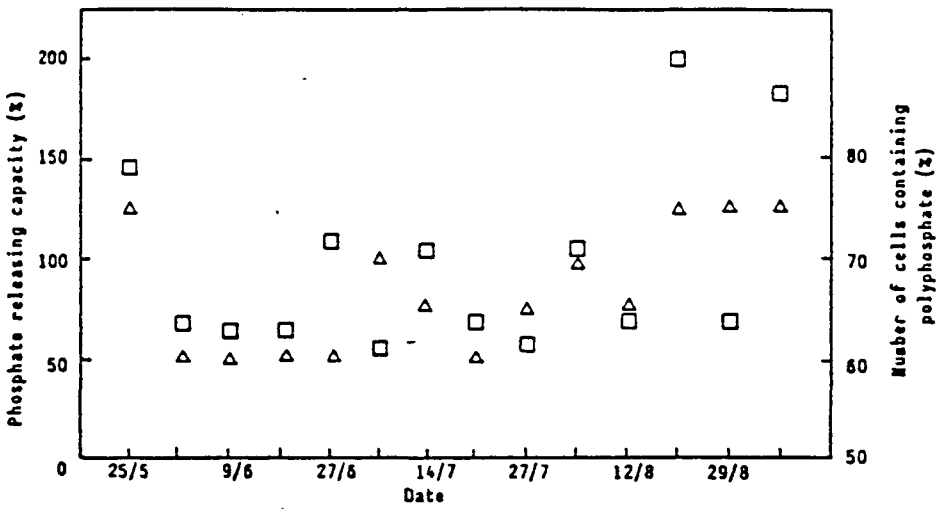

Figure 5.37 : Effect of phosphate releasing capacity on intracellular polyphosphate : Northern Works Module 1

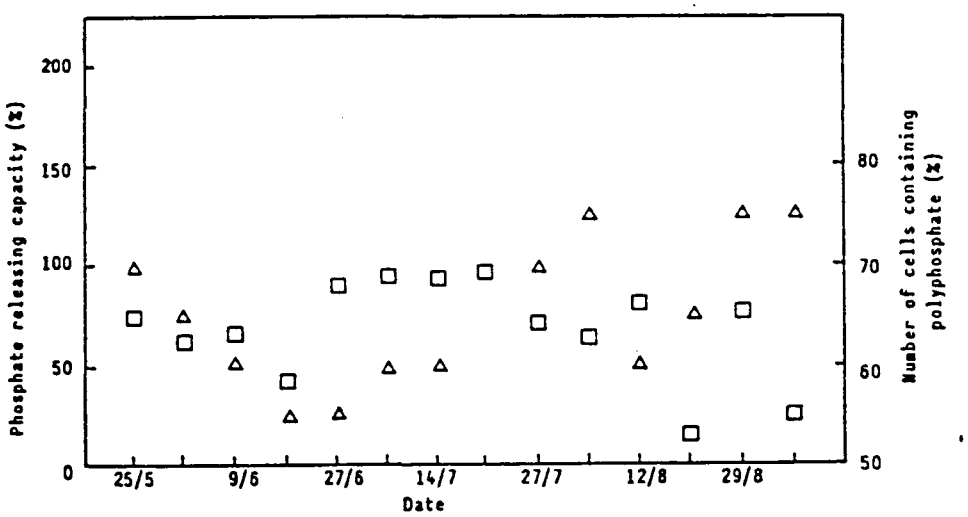

Figure 5.38 : Effect of phosphate releasing capacity : □ ; on intracellular polyphosphate : △ ; Northern Works Module 3

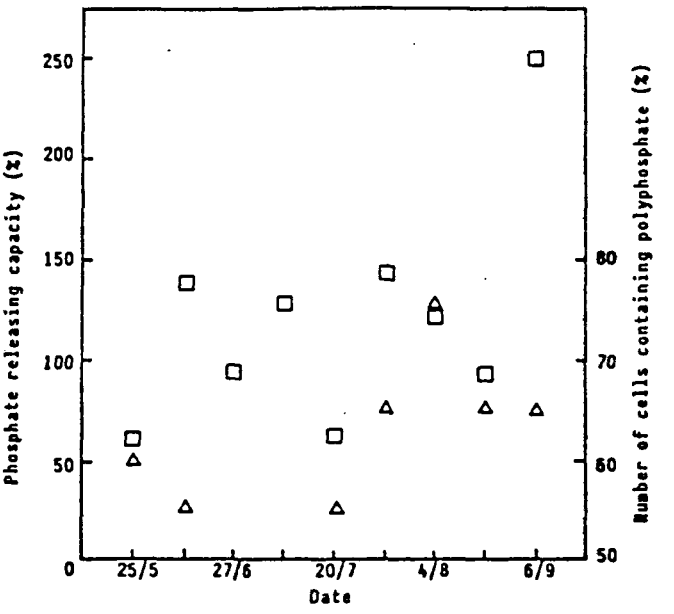

Figure 5.39 : Effect of phosphate releasing capacity: □ ; and intracellular polyphosphate: △ ; Goudkoppies

Phosphate release has been shown to occur as a result of substrate uptake under anaerobic conditions (Wentzel et al., 1985; Wentzel et al., 1986). The different release abilities of the sludge may therefore be linked to their substrate absorption capacity.

During the period of study, Goudkoppies effluent phosphate levels were maintained at zero and both Northern Works modules performed poorly, while no direct correlation between effluent phosphate levels and phosphate releasing capacity could be observed (see Figures 5.40 and 5.41).

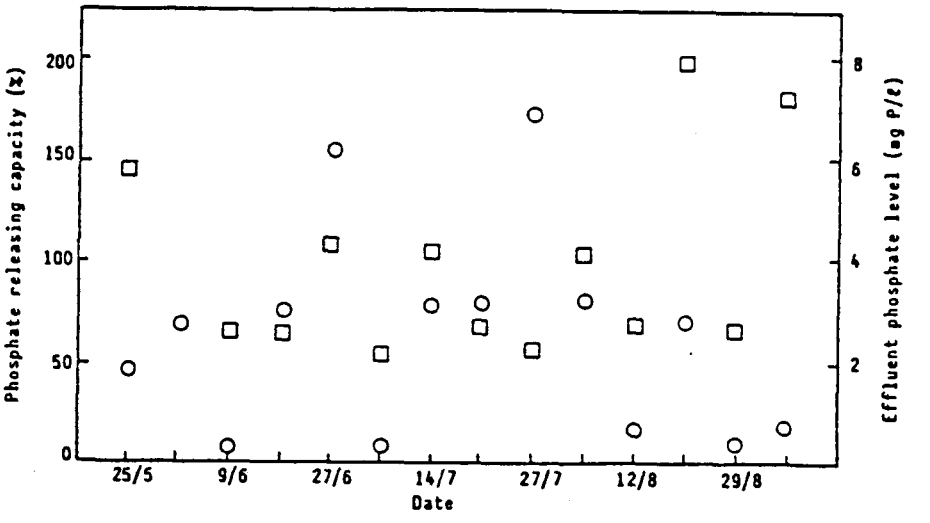

Figure 5.40 : Effect of phosphate releasing capacity: ; on effluent phosphate levels: ; Northern Works Module 1

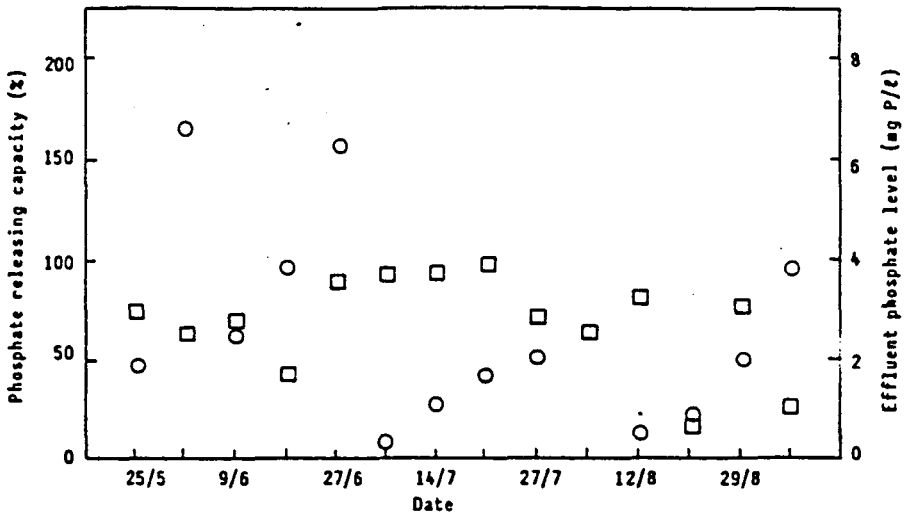

Figure 5.41 : Effect of phosphate releasing capacity: ; on effluent phosphate levels: ; Northern Works Module 3

The higher average phosphate releasing capacity observed for the efficient phosphorus removal plant at Goudkoppies, provides a stimulus for further investigation.

Wentzel et al. 1985, observed a linear relationship between anaerobic phosphorus release and aerobic uptake. Further manipulation of the data could well refine this test into a simple useful parameter.

5.4 CONCLUSIONS

5.4.1 Filamentous Organisms

Sludge bulking observed at the Johannesburg Northern Works plant is caused by Microthrix parvicella. Results have shown that bulking can be controlled in nutrient removal plants, by supplying adequate aeration (i.e. DO levels above 2 mg/ℓ).

To date, bulking problems have not been experienced at the diffused air plants at the Bushkoppie Works which continually maintain a higher DO than the Northern Works. In addition, different types of filaments were observed in this plant, compared to Northern Works.

5.4.2 Phosphate Removing Bacteria

The effect of microbial metabolism on effluent phosphate levels can be readily monitored by microscopic evaluation. While microscopic evaluation provides a rapid diagnostic tool for the general health of the plant, it is not suitable as an absolute operating parameter.

5.4.3 Comparison of Different Media for the Isolation of Bacteria from Activated Sludge

The results show that different media give different population compositions, as expected. It is therefore not possible to compare the population studies of various researchers, when different media have been used. The technique currently being used is beneficial in the study of the effects of operational changes on bacterial population.

5.4.4 Comparison of Identification Techniques

The Microbact identification system was shown to be more species specific and cost effective than the API system, and is now used routinely with a separate oxidase test.

5.4.5 Phosphate Releasing Capacity of Activated Sludge

These preliminary results have indicated that the phosphate releasng capacity test has potential as an easy, inexpensive technique, to predict deterioration in phosphorus removal.

Further work is required to ensure that this initial promise can be fulfilled in practice. This could provide a useful tool in supplementing biological removal with chemical addition.

5.5 REFERENCES

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CHAPTER SIX

DEVELOPMENT AND REFINEMENT OF ANALYTICAL TECHNIQUES

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6.1 THE DETERMINATION OF VOLATILE FATTY ACIDS

6.1.1 Introduction

The importance of the readily biodegradable COD present in sewage and in particular, the volatile fatty acid level, has been clearly demonstrated (Osborn et al., 1986).

A number of methods have been described for the determination of volatile fatty acids. A colorimetric method (Montgomery et al., 1962) has been widely used for routine sludge samples, while more sophisticated chromatographic techniques have been used in research work.

Gas chromatography was successfully used in evaluating volatile acid production in primary sedimentation tanks and digesters (Osborn et al., 1986). Preliminary studies showed that high pressure liquid chromatography also had potential for the analysis of different volatile acids (Osborn et al., 1986).

While chromatographic methods provide reliable results and allow individual acids to be quantified, these techniques are not available to average routine laboratories. For routine plant performance data, the total volatile acid level should be sufficient. A method that would allow the routine determination of volatile fatty acids, was required.

6.1.2 Materials and Methods

6.1.2.1 Colorimetric method

The colorimetric method described by Montgomery et al. (1962) was modified by the addition of acetic acid to samples at a concentration of 100 mg/ℓ. A calibration curve was constructed

in the range 0 - 1000 mg/ℓ acetic acid. The method was evaluated using raw sewage, settled sewage and balance tank effluent from the Johannesburg Northern Works.

6.1.2.2 Enzymatic assay

Gas chromatographic analysis revealed that acetic acid constituted 65 - 80 % of the volatile acids produced during fermentation in primary settling tanks. It was therefore decided that determination of this acid alone would provide a good measure of the total volatile fatty acids.

In an attempt to obtain a method which could be adopted for automatic analysis, acetic acid was determined by an enzymatic assay (Boehringer-Mannheim, 1986). The assay method is based on the following principle :

In the presence of the enzyme acetyl-CoA synthetase (ACS) with cofactors adenosine-5'-triphosphate (ATP) and Co-enzyme A (CoA) Acetic acid (acetate) is converted to acetyl-CoA (1).

(1) $\text{Acetate} + \text{ATP} + \text{CoA} \xrightleftharpoons{\text{ACS}} \text{Acetyl-CoA} + \text{AMP} + \text{pyrophosphate}$

Acetyl-CoA reacts with oxaloacetate to form citrate in the presence of citrate synthase (CS).

(2) $\text{Acetyl-CoA} + \text{oxaloacetate} + H_2O \xrightleftharpoons{\text{CS}} \text{citrate} + \text{CoA}$

The oxaloacetate required for reaction (2) is formed from malate and nicotinamide-adenine dinucleotide (NAD) in the presence of malate dehydrogenase (MDH) (3). In this reaction NAD is reduced to NADH.

(3) $\text{Malate} + NAD^+ \xrightleftharpoons{\text{MDH}} \text{oxaloacetate} + \text{NADH} + H^+$

The determination is based on the formation of NADH measured by the increase in absorbance at 340 nm.

A Boehringer-Mannheim test kit (Cat no 148261) for the determination of acetic acid in foodstuffs was used for the assay.

The method was evaluated using balance tank effluent samples from the Johannesburg Northern Works.

6.1.2.3 High pressure liquid chromatography

Samples were adjusted to pH 2 suitably diluted and filtered through a 0,45 µm filter into vials for chromatographic analysis using UV detection at 210 nm. A Brownlee Polypore H 10 micron column was used with a mobile phase 0,01N H_2SO_4.

6.1.3 **Results and discussion**

6.2.3.1 Colorimetric method

The addition of acetic acid at a concentration of 500 mg/ℓ to samples containing between 10 - 50 mg/ℓ total volatile acids allowed the low levels to be determined. See Figure 6.1 for calibration curve.

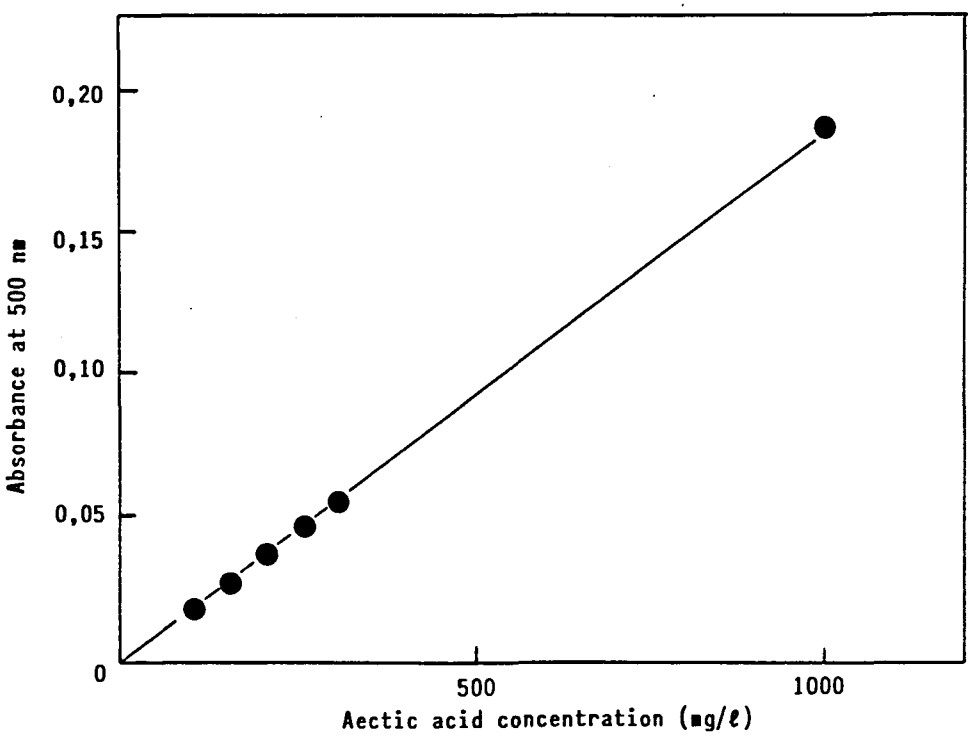

Figure 6.1 : Calibration curve for colorimetric determination of volatile acids.

Recoveries using this method were in excess of 80%.

TABLE 6.1 : VOLATILE FATTY ACID CONCENTRATIONS AT NORTHERN WORKS USING THE COLORIMETRIC ASSAY

Date	Sample	Volatile acid concentration as acetic acid mg/ℓ
11/4	RPS	291
	BE_1	77
	BE_3	69
12/4	RPS	637
	BE_1	86
	BE_3	76
13/4	RPS	427
	BE_1	82
	BE_3	90
14/4	RPS	404
	BE_1	109
	BE_3	85
17/4	RPS	377
	BE_1	78
	BE_3	74
18/4	RPS	429
	BE_1	72
	BE_3	81
19/4	RPS	300
	BE_1	83
	BE_3	78

RPS : Return primary sludge
BE_1 : Balance tank effluent, module 1
BE_3 : Balance tank effluent, module 3

Typical results obtained with this method are given in Table 6.1. The method was considered suitable for routine use provided the analyst worked accurately (See Appendix 6.1 for complete method).

6.1.3.2 Enzymatic assay

The enzymatic assay proved satisfactory in terms of standard deviation, recovery and specificity. (See Tables 6.2 and 6.3)

TABLE 6.2 : REPLICATE ANALYSIS OF ACETIC ACID IN BALANCE TANK EFFLUENT USING THE ENZYMATIC ASSAY

Sample	Acetic acid mg/ℓ
1	26,9
2	26,7
3	26,8
4	26,7
5	28,8
6	28,2
7	29,0
8	27,7
9	26,9
10	29,2
Mean	27,7
Std Dev	1,03

TABLE 6.3 : RECOVERY OF ACETIC AND PROPIONIC ACID FROM SPIKED BALANCE TANK EFFLUENT (BTE) USING THE ENZYMATIC ASSAY

Sample	Percentage recovery (%)	
BTE + 100 mg/ℓ acetic acid	acetic	68,5
BTE + 100 mg/ℓ propionic acid	propionic	NIL
BTE + 50 mg/ℓ acetic + 50 mg/ℓ propionic acid	acetic	67,8
	propionic	NIL

The results show that this method is extremely reproducible and specific for acetic acid.

Results obtained with this method are given in Table 6.4.

TABLE 6.4 : RANGE OF RESULTS FOR ACETIC ACID DETERMINATION USING THE ENZYMATIC ASSAY

Sample	Acetic acid (mg/ℓ)
N3BE2 (balance tank effluent)	10,8 - 45,5
N3BE3 (balance tank effluent)	14,9 - 45,2
NOS (raw sewage)	3,4 - 11,0

While the method is extremely useful in a laboratory with access to UV absorbance spectrophotometry, it requires fairly skilled analysts and was not considered useful for routine plant use. The use of a UV detector for automatic analysis is feasible but costly. The results of this evaluation have shown that an enzymatic assay for the determination of acetic acid in sewage is available.

6.1.3.3 High pressure liquid chromatography (HPLC)

Preliminary investigations revealed that only acetic and propionic acid could be detected at the 5mg/ℓ level. Due to the high proportion of these two acids in the samples, (80 - 95 % of the volatile acids) further work was restricted to these two compounds. A typical chromatogram is shown in Figure 6.2.

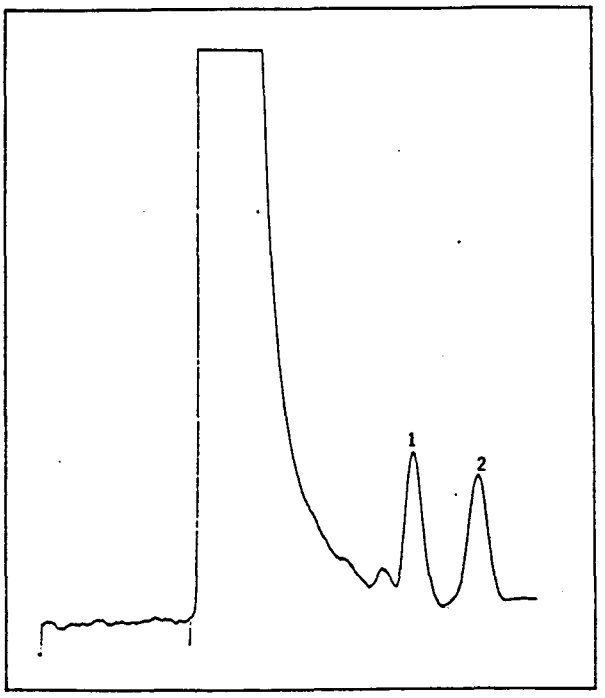

Figure 6.2 : High pressure liquid chromatogram of acetic (1) and propionic acid (2) at 5 mg/ℓ.

Integration of the peak area proved unsatisfactory and peak heights were used. See Figure 6.3 for calibration curve.

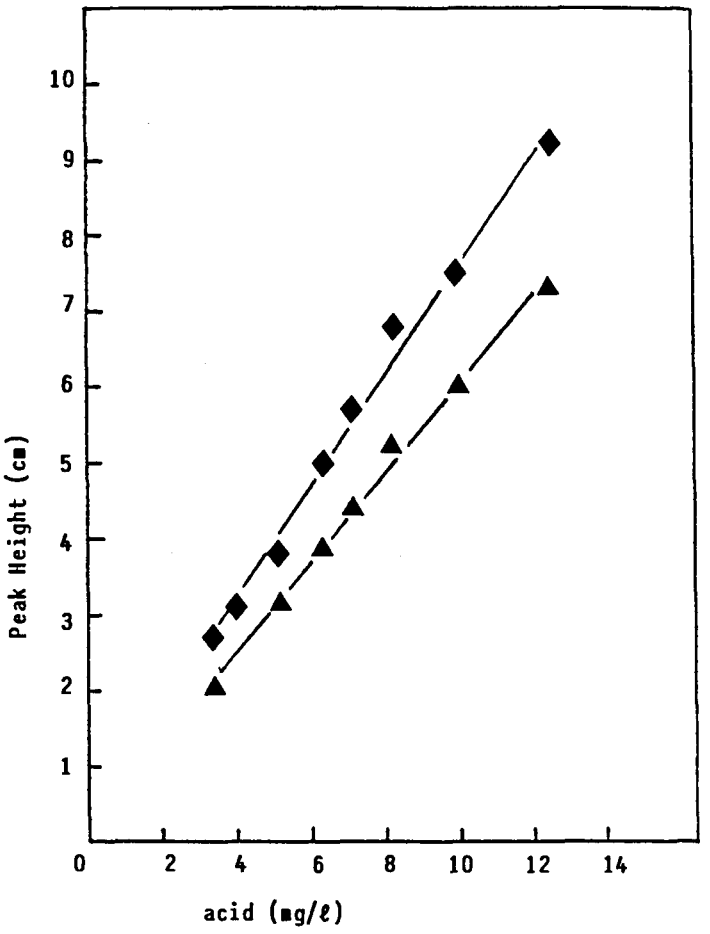

Figure 6.3 : Calibration curve for acetic ◆—◆ ; propionic ▲—▲ acids.

The reproducibility of the method is reflected in Table 6.5.

TABLE 6.5 : REPLICATE ANALYSES OF ACETIC AND PROPIONIC ACID USING THE HPLC METHOD

Sample	Acetic acid (mg/ℓ)	Propionic acid (mg/ℓ)
1	5,5	5,7
2	6,1	5,7
3	4,6	5,5
4	6,0	6,4
5	4,7	5,0
6	5,7	6,3
7	4,8	5,2
8	5,8	5,5
9	6,2	5,3
10	4,8	5,2
Mean	5,4	5,6
Std Dev	0,64	0,46

Balance tank effluent was spiked with two different concentrations of acetic and propionic acid to assess recovery of the method. The results are given in Table 6.6.

TABLE 6.6 : RECOVERY OF ACETIC AND PROPIONIC ACID IN BALANCE TANK EFFLUENT (BTE) USING THE HPLC METHOD

Sample	Percentage recovery (%)	
	Acetic acid	Propionic acid
BTE + 2,86 mg/ℓ acetic acid	100	-
BTE + 2,86 mg/ℓ propionic acid	-	59
BTE + 5,72 mg/ℓ acetic acid	93	-

Results for Northern Works are given in Table 6.7.

6.2 DETERMINATION OF ORTHOPHOSPHATE

Orthophosphate levels in sewage samples are determined by continuous analysis, using the acid molybdate blue method. The linear range of this method is 1 - 10 mg/ℓ P. Concentrations below 1 mg/ℓ were reported as nil. It was considered necessary to investigate the feasibility of extending the range of the method, in order to accurately determine phosphate levels between 0 - 1 mg/ℓ P. It was found to be possible to determine levels of phosphate between 0 - 1 mg/ℓ P by changing the configuration of the Auto Analyzer (See Figure 6.4 for calibration curve).

It was however impossible to extend the range of either method to include both high and low values. In order to run both analyses routinely separate instruments are required.

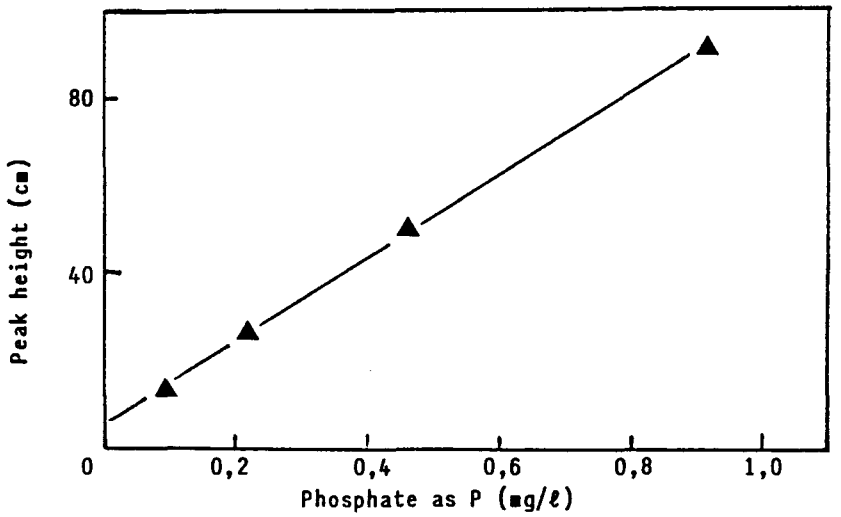

Figure 6.4 : Calibration curve for phosphate concentration.

TABLE 6.7 : VOLATILE FATTY ACID CONCENTRATIONS AT NORTHERN WORKS USING THE HPLC METHOD

Date	RPS Acetic acid	RPS Propionic acid	BE_1 Acetic acid	BE_1 Propionic acid	BE_3 Acetic acid	BE_3 Propionic acid	S_{bs} TE
20/4	170	90	31	11	35	11	-
21/4	189	106	29	11	31	13	-
24/4	259	192	30	12	25	10	-
25/4	234	208	22	8	28	12	-
26/4	142	98	29	11	28	13	-
27/4	155	82	49	13	35	10	-
28/4	205	139	25	4	28	8	-
01/5	142	135	26	NIL	10	8	-
02/5	221	184	25	8	21	NIL	-
04/5	170	102	41	10	158	6	-
08/5	69	33	39	5	87	8	-
09/5	-	-	102	NIL	213	12	-
10/5	379	184	126	9	96	8	-
12/5	442	122	58	9	126	6	-
15/5	145	94	26	8	32	15	88
16/5	170	94	23	6	21	NIL	120
17/5	404	653	18	8	16	8	140
18/5	445	445	25	9	22	19	110
19/5	161	110	27	11	25	10	140
22/5	331	343	15	7	17	10	97
23/5	149	155	26	11	32	13	130
24/5	-	-	24	12	23	9	140
25/5	398	485	32	24	28	16	140
26/5	316	306	28	13	25	12	130
31/5	-	-	23	11	17	12	130
02/6	388	310	92	10	22	7	-
05/6	139	90	14	9	16	9	97
06/6	-	-	22	6	20	9	140
09/6	161	126	25	13	26	11	110
14/6	382	281	21	12	55	10	160
15/6	-	-	29	16	27	17	160
16/6	-	-	20	9	15	11	140
19/6	221	196	28	22	26	24	140
20/6	88	67	19	NIL	26	10	110
21/6	-	-	25	11	-	-	130
22/6	221	201	544	11	36	10	110
23/6	196	173	23	14	32	18	130
26/6	-	-	21	15	-	-	110
27/6	133	134	16	8	30	16	160
28/6	126	97	14	8	17	10	140
29/6	136	99	17	14	11	10	120
30/6	-	-	13	10	29	10	120

RPS : Return primary sludge
BE_1 : Balance tank effluent, module 1
BE_3 : Balance tank effluent, module 3

6.3 DETERMINATION OF POLYSACCHARIDES IN ACTIVATED SLUDGE

6.3.1 Introduction

Morphological biochemical studies of floc-forming bacteria indicate that exocellular polymers are responsible for the flocculating growth habits of these bacteria (Friedman et al., 1969).

Bacterial aggregation is generally ascribed to exocellular polysaccharides, although polyamino acids could also play a functional role in this respect. These compounds are excreted from the surface of the cell under different physiological conditions. The availability of certain substrates and relative nutrient concentrations also influences the composition and concentration of exocellular polymers (Harris and Mitchell, 1973).

The role of extracellular polymers in bioflocculation has been recognised for some time (Forster, 1971; Kiff, 1978; Sheintuch et al., 1986). In the case of biological phosphate removal it is possible that these polymers have an additional role to play. In view of the bacterial cell clustering, which has been reported in plants exhibiting enhanced phosphate removal (Buchan, 1981; Hart and Melmed, 1982; Osborn et al., 1986), it is possible that the polyphosphate accumulating organisms ensure the formation of clusters by secreting a biopolymer.

Microscopic evaluation of polysaccharide formation in activated sludge plants indicates a correlation between abundance of polysaccharide and cluster formation (see Chapter Five). In order to investigate this phenomenon in greater depth it was decided to attempt quantitation of polysaccharide levels.

6.3.2 Materials and Methods

6.3.2.1 Technique development

Mixed liquor samples were drawn from the aerobic zone of an activated sludge plant to evaluate published methods. The steam extraction procedure of Brown and Lester (1980) was evaluated by determining hexoses, and protein on the extract before and after steaming.

Hexoses were determined by the anthrone method which involved formation of a colour complex which absorbs at 620 nm (Plummer, 1978). Protein was determined by the dye-binding method of Bradford (1976) using Biorad protein reagent.

Pentoses were determined as described by Borrow and Jefferys (1956). The pentose sugar was converted to furfural by hydrochloric acid. The furfural was then measured colorimetrically after treatment with aniline acetate.

Hexosamines were determined by the reaction of deaminated hexosamine with indole and hydrochloric acid, after deacetylation by hydrolysis (Glick, 1955).

All samples were hydrolyzed prior to sugar determination.

6.3.3 Results and discussion

The calibration curve for hexose determination is given in Figure 6.5. The technique was easy to use and the calibration curve satisfactory. Protein determinations were successfully carried out using the Bradford (1976) method. See Figure 6.6 for calibration curve. The standard deviation for these two techniques when applied to mixed liquor samples was satisfactory (See Tables 6.9 and 6.10).

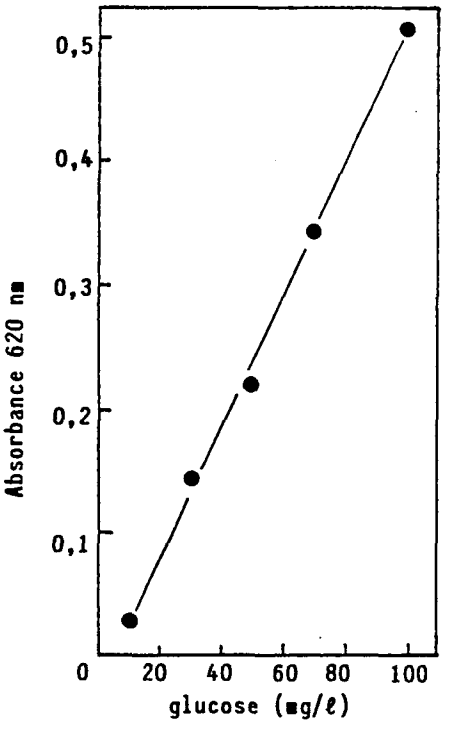

Figure 6.5 : Calibration of hexose concentration.

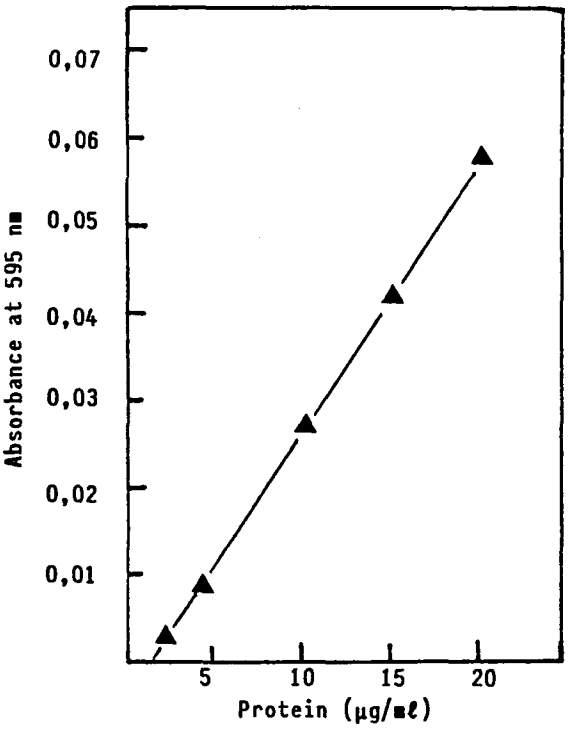

Figure 6.6 : Calibration curve for protein determination.

TABLE 6.9 : REPRODUCIBILITY OF HEXOSE DETERMINATION

Sample No	Hexose concentration mg/ℓ
1	0,050
2	0,043
3	0,055
4	0,058
5	0,048
6	0,044
7	0,047
8	0,046
9	0,049
10	0,043
Mean	0,048
Std deviation	0,0051

TABLE 6.10 : REPRODUCIBILITY OF PROTEIN DETERMINATION

Sample No	Protein concentration mg/ℓ
1	0,048
2	0,039
3	0,039
4	0,051
5	0,056
6	0,045
7	0,044
8	0,042
9	0,045
10	0,049
Mean	0,046
Std deviation	0,005

The effect of steaming time on hexose and protein concentration in supernatant fluid was assessed. The results are given in Table 6.11.

TABLE 6.11 : EFFECT OF STEAMING TIME ON PROTEIN AND HEXOSE CONCENTRATION

Steaming time (min)	Protein concentration (mg/ℓ)	Hexose concentration (mg/ℓ)
10	0,019	0,057
20	0,036	0,071
30	0,060	0,071

The protein concentration increased dramatically with increased steaming time indicating destruction of cell walls. The additional hexose extracted by this procedure could therefore have been intracellular. As the investigation was intended to determine extracellular polysaccharides only, steaming was restricted to 10 minutes in subsequent investigations.

The method for determination of pentoses was found to be unsuitable for concentrations below 0,2 mg/mℓ as ribose (See Figure 6.7 for calibration curve). The levels of pentose in the activated sludge samples were below this minimum detectable level. Concentration of the sample extracts by lyophilization however allowed the pentose levels to be determined. As in the case of hexoses, negligible amounts of free pentose was present in the mixed liquor (See Table 6.12).

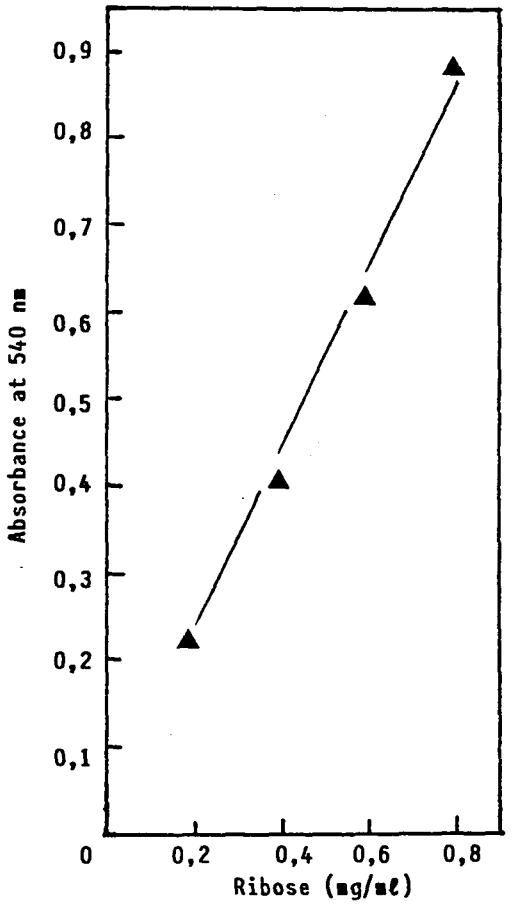

Figure 6.7 : Calibration of pentose concentration

TABLE 6.12 : PENTOSE CONCENTRATIONS IN ACTIVATED SLUDGE EXTRACTS

Sample	Pentose as mg/mℓ ribose	
	Before steaming	After steaming
NW Module 2	0,23	13
NW Module 3	0,17	12
Alexandra	0,15	13

The method is fairly tedious and coupled with its lack of sensitivity was not considered suitable for further use (See Figure 6.8 for calibration curve). The hexosamine method was found to have a minimum detectable limit of 20 µg/mℓ. The hexosamine levels in the sample were below this level, thus requiring a concentration step before the sugar analysis.

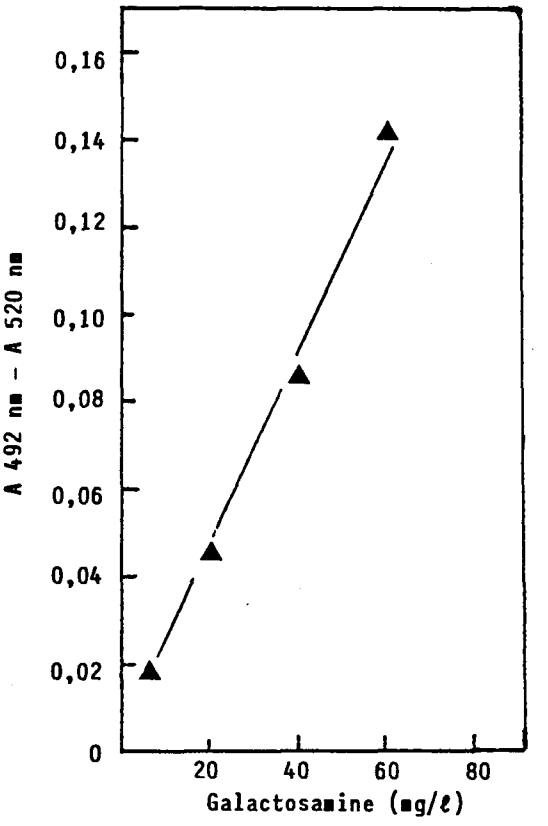

Figure 6.8 : Calibration curve for hexosamine concentration.

The difficulties experienced with these methods led to the consideration of a gas chromatographic technique for the analysis of hexoses, pentoses and hexosamines.

6.4 DETERMINATION OF POLYPHOSPHATE CHAIN LENGTH

Acinetobacter were grown in Fuhs and Chen (1975) medium under aerobic conditions. After addition of acetate, the culture was incubated overnight aerobically and the cells were harvested by centrifugation. The cell pellet was resuspended in 0,05 M Phosphate buffer, pH 7,0 and the cells were lysed by ultrasonication to extract the polyphosphate into the solution. Cell debris was removed by centrifugation.

The extract was subjected to polyacrylamide gel electrophoresis (Robinson et al., 1984). A mixture of polyphosphate standards was run concurrently with the samples. The gels were stained with 0,05 % ortho-Toluidine blue.

A similar procedure was carried out on cells harvested from mixed liquor from the sewage works. The cells were suspended in 0,1 M Tris/Borate buffer, pH 8,3, which was also the electrophoresis buffer. Electrophoresis was performed as before.

Protein precipitation with various concentrations of Trichloracetic acid was performed on the mixed liquor extracts in an attempt to enhance the polyphosphate bands. The protein precipitate was removed by centrifugation and the supernatant was dialysed and freeze dried. The residue was redissolved in buffer to one third of the original volume. An untreated aliquot of the mixed liquor extract was also freeze dried and reconstituted to one third of its original volume. Electrophoresis was performed on these samples, as previously described.

Finally protein precipitation was carried out on a mixed liquor extract using ammonium sulphate followed by hot Trichloracetic acid treatment of the resultant precipitate. Ammonium sulphate was added to the mixed liquor extract to 45% saturation. The precipitate was removed by centrifugation. The supernatant was retained and the pellet resuspended in hot 10% Trichloracetic acid. The supernatant obtained by centrifugation of the TCA

extract and the previously obtained supernatant were dialysed, freeze dried and the residues dissolved in small volumes of buffer.

The electrophoresis procedure was repeated on these samples.

Results were disappointing in that no strong discrete bands were obtained for different polyphosphate molecular masses.

6.5 CONCLUSIONS

Methods were successfully developed for the routine determination of volatile fatty acids and polysaccharides in sewage and activated sludge samples. A method for the determination of polyphosphate chain length was investigated. The method proved more difficult to use on activated sludge than anticipated and requires further work.

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DETERMINATION OF TOTAL VOLATILE FATTY ACIDS IN SEWAGE

APPARATUS

Spectrophotometer (visible range).

Centrifuge.

Water bath.

REAGENTS

Sulphuric acid 50 mℓ/100 mℓ

Add with care and constant stirring 100 ± 1 mℓ of sulphuric acid to 100 ± 1 mℓ of water and cool.

Sodium hydroxide 18 g/100 mℓ

Dissolved 90 ± 1 g of sodium hydroxide in about 400 mℓ of water. Cool and dilute to 500 mℓ with water.

Acidic ethane diol reagent

Mix 30 ± 1 mℓ of ethane diol with 4,0 ± 0,1 mℓ of 50 mℓ/100 mℓ sulphuric acid. Prepare this reagent freshly each day. The ethane diol should not make a significant contribution to the blank. Replacement of the ethane diol every month is usually necessary.

Hydroxylammonium sulphate 10 g/100 mℓ

Dissolve 10,0 ± 0,1 g of hydroxylammonium sulphate in about 80 mℓ of water and dilute with water to 100 mℓ. Store in a refrigerator. Prepare fresh solutions monthly.

Hydroxylamine reagent

Mix 20,0 ± 0,5 mℓ of 18 g/100 mℓ sodium hydroxide with 5,0 ± 0,1 mℓ of 10 g/100 mℓ hydroxylammonium sulphate. Prepare this reagent immediately before it is required.

Acidic ferric chloride reagent

Dissolve 20,0 ± 0,1 g of ferric chloride hexahydrate in about 500 mℓ of water. Add 20,0 ± 0,1 mℓ of sulphuric acid conc, and dilute with water to 1 litre and filter if necessary. Store in refrigerator. Prepare fresh solutions monthly.

Standard acetic acid solutions

Solution A (1 000 mg/ℓ)

Weigh 1,00 ± 0,01 g of glacial acetic acid in a stoppered weighing bottle and transfer quantitatively to a 1 litre calibrated flask and dilute with water to 1 litre. Prepare fresh solutions monthly.

CALIBRATION STANDARDS

To a series of 100 mℓ calibrated flasks pipette 1,0; 2,0; 3,0; 4,0;; 5,0; 6,0 of standard acetic acid solution A and dilute to the mark with water. These flasks contain respectively 10, 20, 30, 40, 50 and 60 mg/ℓ acetic acid.

PROCEDURE

Sample preparation

Centrifuge samples at about 8 500 x g and retain about 10 mℓ of supernatant. Filter samples through Whatman 1 filter paper.

ANALYTICAL METHOD

1. Pipette 0,5 mℓ of sample into a dry test tube and add 55 µℓ 1 000 mg/ℓ acetic acid soltion. Add from a microburette 1,7 mℓ of acidic ethane diol reagent and mix thoroughly.
2. Heat in a boiling water bath for 3 minutes. Immediately cool test tube in cold water.
3. Add 2,5 mℓ of the hydroxylamine reagent and mix thoroughly. Allow to stand for 1 minute.

4. Add 10,0 mℓ of acid ferric chloride reagent into a 15 mℓ volumetric flask. Quantitatively transfer the solution in the test tube to the flask. Make up to the mark with distilled water, shake vigorously and allow flask to stand with stopper removed for 30 minutes.
5. Measure the absorbance of the solution (As) as 500 nm.
6. Repeat steps 1 - 5 using 0,5 mℓ distilled water as a blank, and 0,5 mℓ of each standard solution.
7. Prepare a calibration curve using the absorbances of the standard solutions and determine the acetic acid concentration in the sample solutions.

CHAPTER SEVEN

FUNDAMENTAL STUDIES : CHEMICAL AND BIOLOGICAL PHOSPHATE REMOVAL

LAURRAINE H LÖTTER

7.1 INTRODUCTION

The hypothesis of a conceptual model of the biochemical processes responsible for the phenomenon of enhanced biological removal, has provided a foundation for more detailed research into this phenomenon. Experimental attempts to prove the validity of the model have been reported elsewhere (Lötter, 1989). In addition to these studies, a number of investigations were carried out to clarify specific aspects of plant behaviour. The results of these investigations are reported here.

7.2 CHEMICAL PRECIPITATION OF PHOSPHATE IN ACTIVATED SLUDGE WITHOUT ADDITION OF CHEMICALS

7.2.1 Introduction

Removal of phosphorus in excess of normal metabolic requirements is now a well accepted phenomenon. Although the phenomenon has been under fairly intensive study for some years, complete agreement on the mechanism of the phenomenon has not been achieved. A number of researchers claim that the process depends entirely on biological processes, while others see an important role for chemical precipitation biologically mediated or otherwise.

The transformations of phosphorus between the bulk liquid and the sludge can be schematically depicted as illustrated in Figure 7.1.

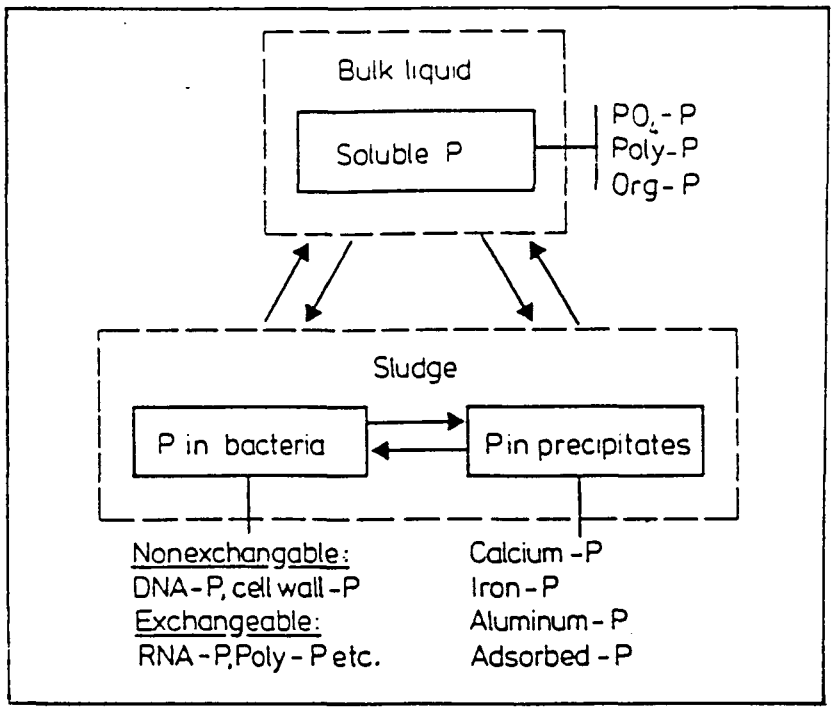

Figure 7.1 : Transformation of phosphorus between the bulk liquid and sludge (Arvin, 1985)

Chemical precipitation can be divided into bulk precipitation and biofilm precipitation. In bulk precipitation, the formation of the solid phosphate phase is governed by the composition of the bulk liquid, while the biofilm precipitation occurs within the micro-environment of a biofilm. Accelerated bulk precipitation caused by elevated phosphorus concentrations in anaerobic zones, is one form of biologically mediated calcium phosphate precipitation which has been observed (Arvin, 1983).

Phosphate removal by chemical precipitation has also been observed by Menar and Jenkins (1970).

Calcium phosphate precipitation inside denitrifying biofilms has also been observed, due to the increased pH caused by denitrification (Arvin and Kristensen, 1983). Calcium phosphate precipitation was also observed in a non-nitrifying anaerobic/aerobic activated sludge process (Lan et al., 1983).

In order to evaluate the phosphate precipitation potential of the

bulk feed to the Northern Works and Goudkoppies activated sludge plants, the precipitation of calcium, iron, aluminium, magnesium and phosphate from the bulk feed through pH variation, was investigated.

Materials and Methods

Balance tank effluent from the Northern Works and Goudkoppies plant was used. Sulphuric acid was added to reduce the pH level to below 3. The pH was then raised by addition of sodium hydroxide to the solution. pH adjustment was carried out with constant stirring. Between pH adjustments, the stirring was ceased and solutions allowed to stand for 3 minutes. Samples were taken at every pH level, filtered and analysed for phosphate, iron, aluminium, magnesium and calcium.

The experiments were repeated in the presence of iron and aluminium added to a concentration of 10 mg/ℓ each, in separate experiments.

Results and Discussion

Significant precipitation of phosphorus and calcium occurred at pH values in excess of pH 8,0 (see Figures 7.2 and 7.3). No significant precipitation of magnesium or iron was observed during these experiments. The pH range at which calcium phosphate precipitation occurs does not prevail in an activated sludge plant. It is therefore, unlikely that calcium phosphate precipitation could take place spontaneously under normal plant conditions. Addition of iron and aluminium to the samples before pH manipulation, resulted in greater precipitation of phosphate, but did not affect the amount of calcium precipitation (see Figures 7.4 and 7.5). These results signify separate precipitation mechanisms for the different cations, rather than co-precipitation reactions. The precipitation of phosphate in these experiments occurred at the same pH levels observed by Diamadopoulis and Benedek (1984).

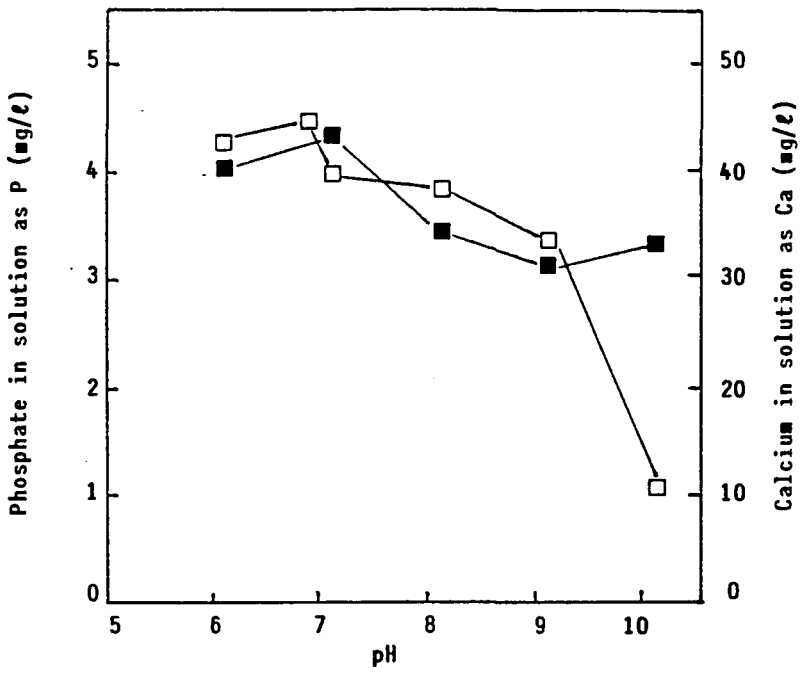

Figure 7.2 : Effect of pH on phosphate and calcium precipitation in Goudkoppies influent
■——■ calcium; □——□ phosphate

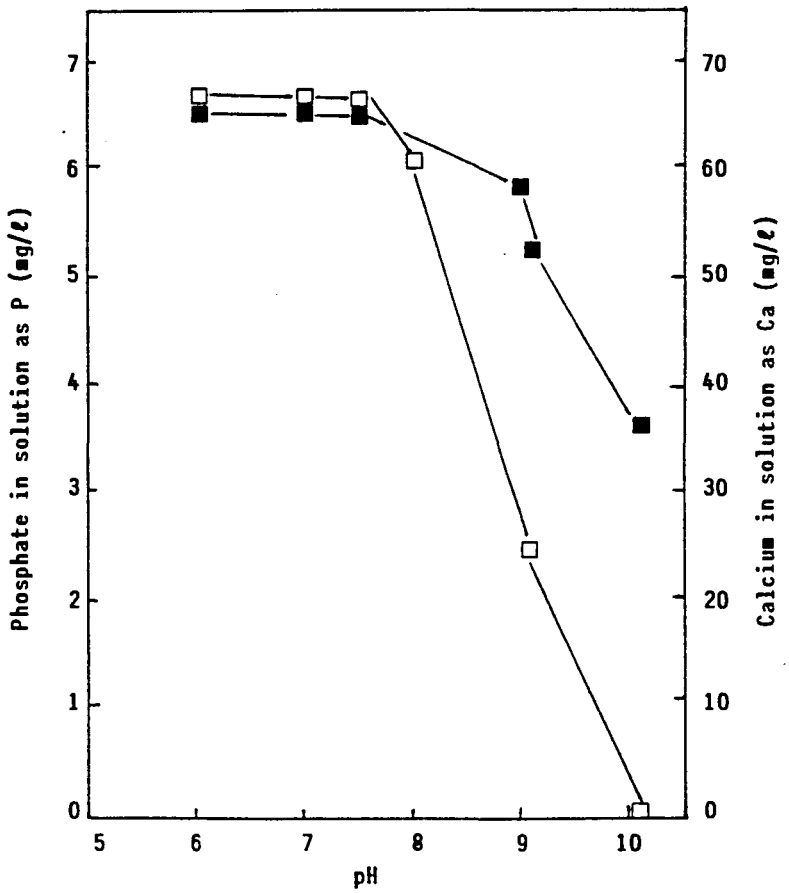

Figure 7.3 : Effect of pH on phosphate and calcium precipitation in Northern Works influent
■——■ calcium; □——□ phosphate

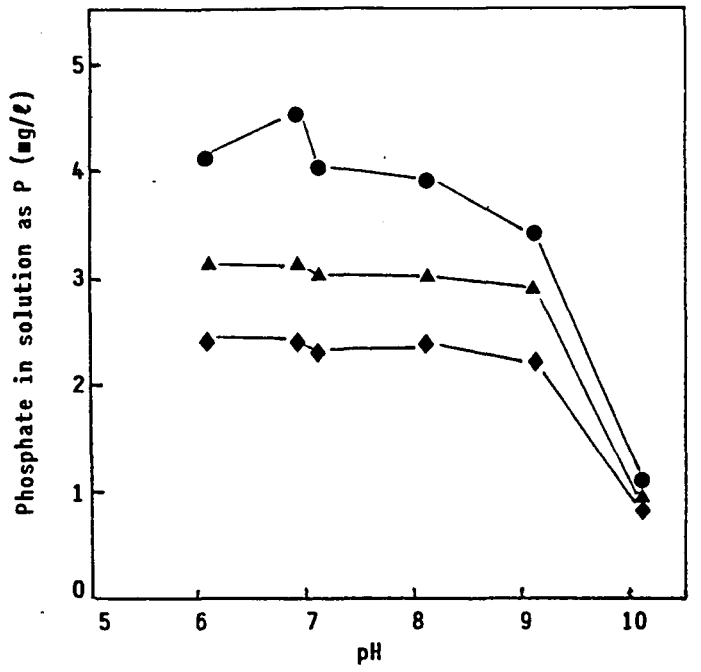

Figure 7.4 : Effect of pH on phosphate precipitation in Goudkoppies influent
●——● control;
▲——▲ in the presence of iron
♦——♦ in the presence of aluminium

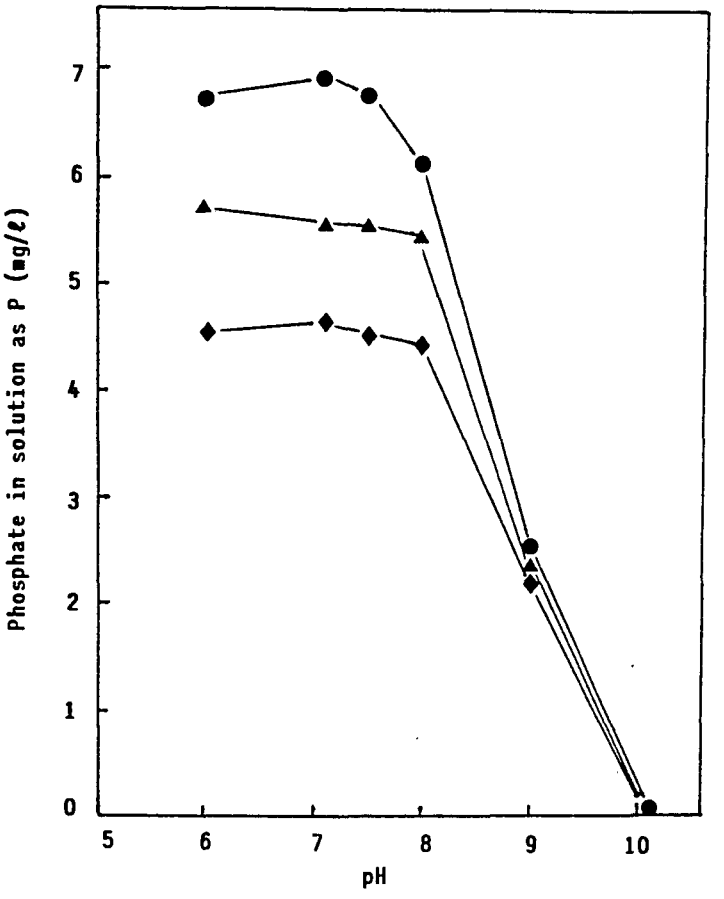

Figure 7.5 : Effect of pH on phosphate precipitation in ●——● control;
▲——▲ in the presence of iron;
♦——♦ in the presence of aluminium

7.3 THE EFFECT OF SOME NUTRIENTS ON SLUDGE BULKING

7.3.1 Introduction

In an attempt to relate sludge surface properties to its settling characteristics, electrophoretic mobility studies were undertaken. Mobility measurements carried out in the sludge liquor showed that the average mobility was directly proportional to the sludge volume index (SVI) (see Figure 7.6). The SVI in turn, was found to be a logarithmic function of the ratio of ammonia-nitrogen to soluble phosphate in the liquors (see Figure 7.7) (Forster, 1968).

Since the surface charge has been found to be directly related to sludge settling characteristics, compounds which could alter the surface charge could affect the settling characteristics.

The formation of extracellular polysaccharides has been associated with flocculation by a number of researchers (Friedman et al., 1969 and Forster, 1971). It has also been shown that as the polysaccharide content of activated sludge increases, so the settling characteristics deteriorate (Forster, 1976).

The SVI levels and nutrient levels were compared, as were SVI levels and polysaccharide levels.

7.3.2 Results and Discussion

The ammonia-nitrogen phosphate ratios did not exhibit the logarithmic relationship observed by Forster (1968), (see Figures 7.8 to 7.10).

Forster's (1968) data was taken from a plant with an average ammonia level of 18,8 over the period under discussion, while the Northern Works modules under discussion nitrify to the extent that ammonia levels seldom exceed 1 mg/ℓ as N. The metabolic processes attributed to the high SVI in Forster's work (1968), could not be relevant in the Northern Works plants due to the low ammonia levels.

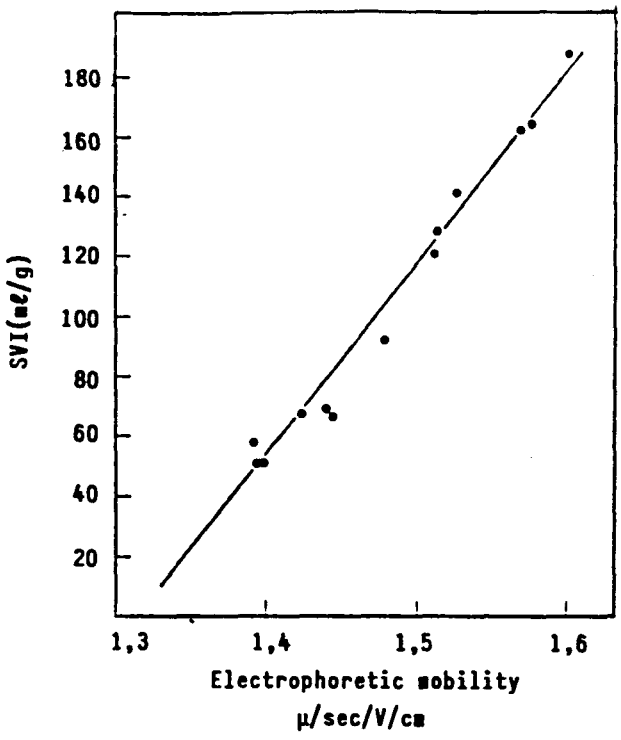

Figure 7.6: Relationship between the SVI of activated sludge and electrophoretic mobility of the particle (Forster, 1968)

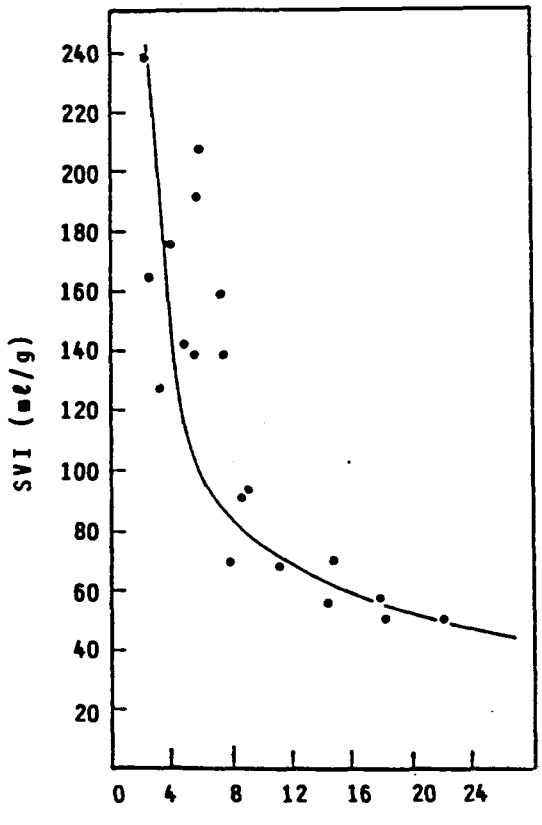

Figure 7.7: Relationship between SVI of activated sludge and $[NH_3]/[PO_4]$ of aeration tank liquor

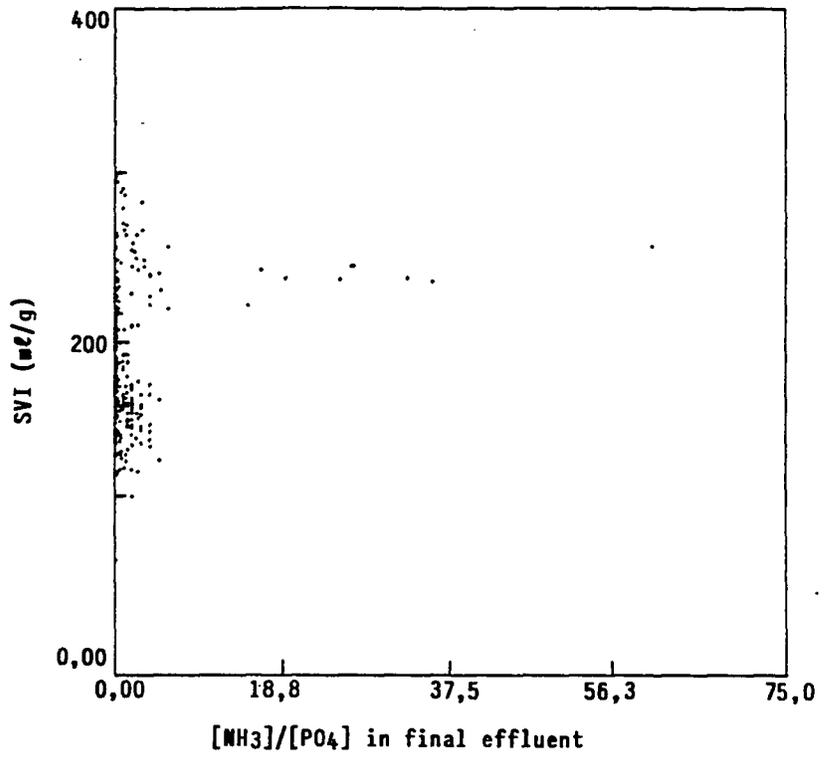

Figure 7.8: Relationship between SVI and effluent [NH3]/[PO4] for Northern Works Module 3

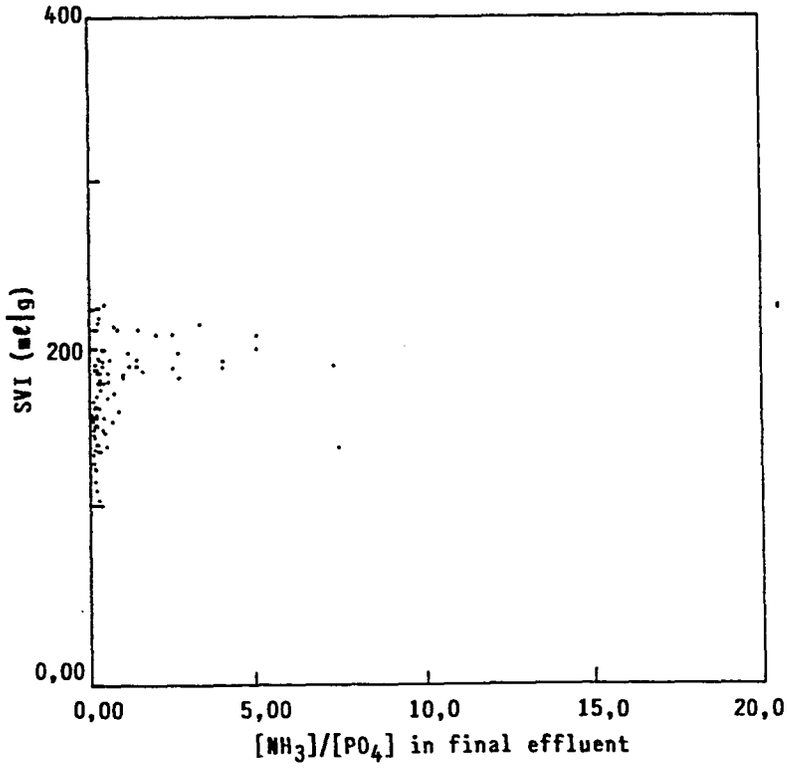

Figure 7.9 : Relationship between SVI and effluent [NH_3]/[PO_4] for Northern Works Module 1

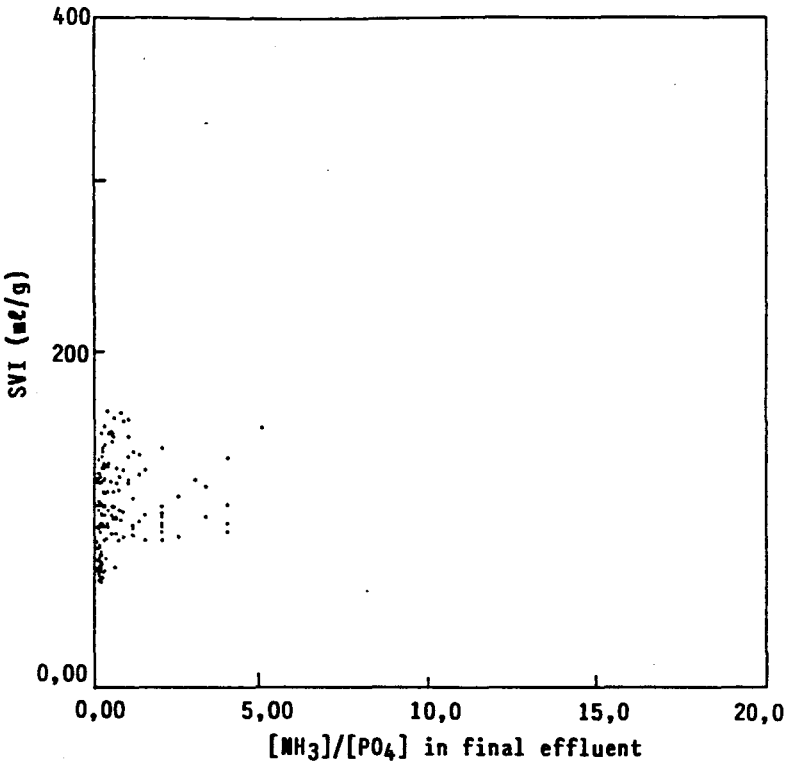

Figure 7.10 : Relationship between SVI and effluent [NH3]/[PO4] for Northern Works Module 2

7.4 EXTRACELLULAR POLYSACCHARIDES IN ACTIVATED SLUDGE

7.4.1 Introduction

The significance of extracellular polysaccharides in activated sludge was discussed in Chapter 6. The method described in the same Chapter was used to survey the extracellular polysaccharide levels in four activated sludge plants.

7.4.2 Materials and Methods

Mixed liquor samples were drawn from the anaerobic, anoxic and aerobic zones of four activated sludge plants.

Polysaccharides were extracted by the steam procedure of Brown and Lester (1980). Steaming was restricted to 10 minutes to ensure extraction of extracellular material only. After hydrolysis, various colorimetric procedures were used to determine hexoses

(Plummer, 1978), pentoses (Borrow and Jefferys, 1956) and hexose-amines (Glick, 1955).

7.4.3 Results and Discussion

Preliminary studies revealed that the level of extracellular pentoses and hexoseamines in the activated sludge were below 200 µg/ℓ respectively. The determination of these compounds was therefore not pursued. The hexose levels varied considerably between plants and between zones (see Figures 7.11 to 7.14). The average figures for the period studied are given in Table 7.1.

TABLE 7.1
AVERAGE EXTRACELLULAR HEXOSE LEVELS IN ACTIVATED SLUDGE

Plant	Hexose µg/mg VSS		
	Anaerobic Zone	Anoxic Zone	Aerobic Zone
Northern Works Module 2	29	40	34
Northern Works Module 3	26	37	26
Goudkoppies	21	25	22
Bushkoppie	27	25	32

During the survey period, Goudkoppies and Bushkoppie maintained an effluent phosphate level of below 1 mg o-P/ℓ as P. Northern Works Modules 1 and 3 achieved an effluent below 1 mg o-P/ℓ only occasionally.

The extracellular hexose values observed are considerably higher than those observed for non-nutrient removing activated sludge (Brown and Lester, 1980). The presence of extracellular slime has been shown to correlate well with satisfactory phosphorus removal (Lötter and Murphy, 1988). Quantitative analysis of the hexose did not provide a more sensitive correlation. The relatively high levels in the anoxic zones of the Northern Works compared to the other two plants, should be further investigated.

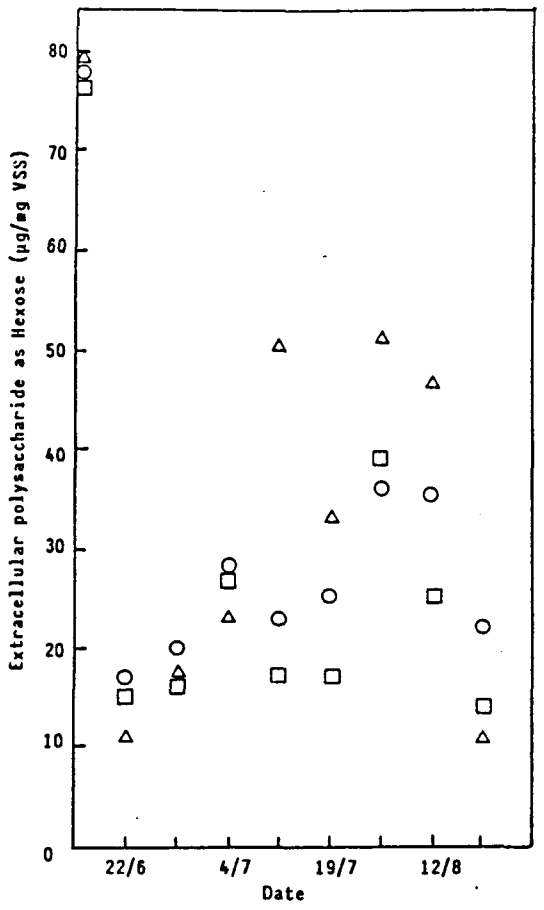

Figure 7.11: Extracellular polysaccharide levels in the anaerobic: ; anoxic: ; aerobic: zones of Northern Works Module 1

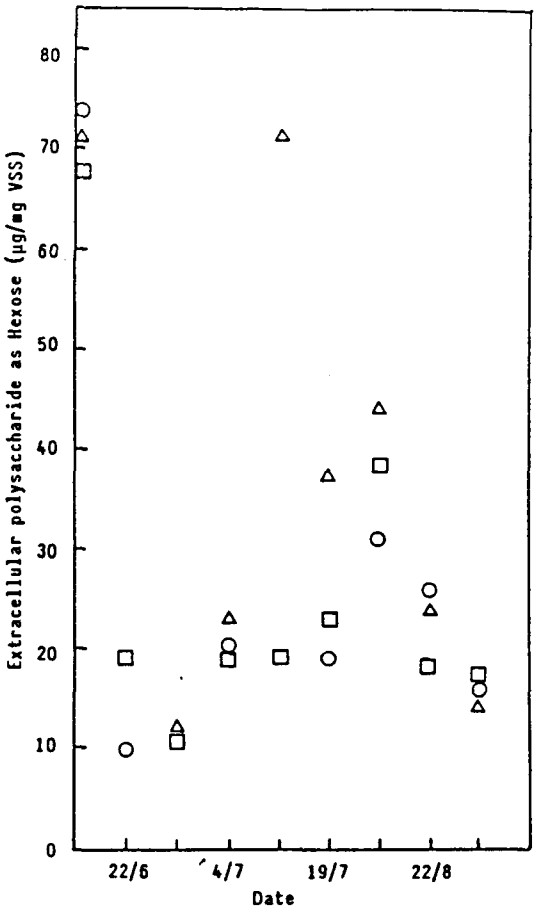

Figure 7.12: Extracellular polysaccharide levels in the anaerobic: ; anoxic: ; aerobic: zones of Northern Works Module 3

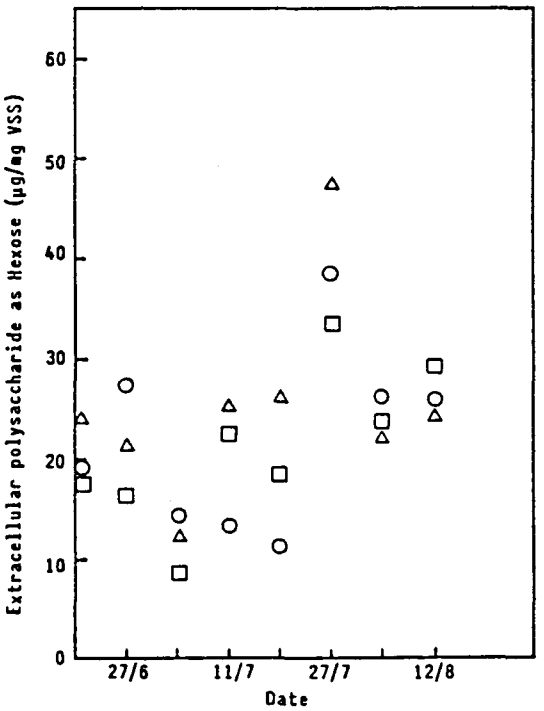

Figure 7.13: Extracellular polysaccharide levels in the anaerobic: ; anoxic: ; aerobic: zones of Goudkoppies Module 2

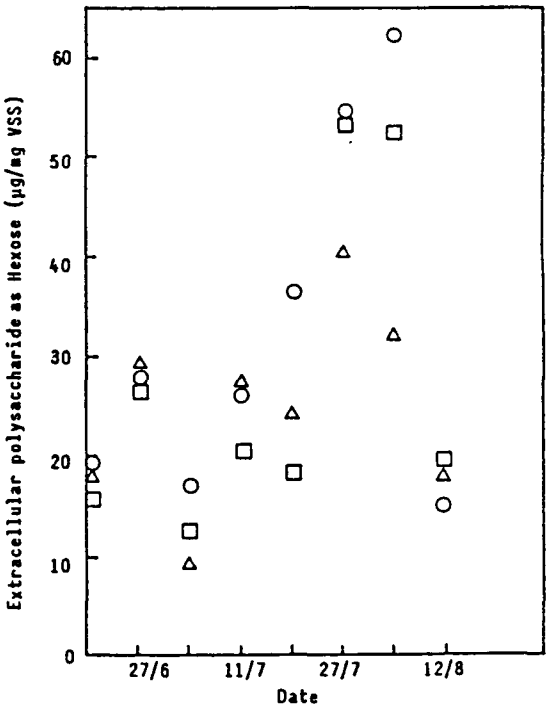

Figure 7.14: Extracellular polysaccharide levels in the anaerobic: ; anoxic: ; aerobic: zones of Bushkoppie Module 1

The polysaccharide content of activated sludge is known to increase as settling characteristics deteriorate (Forster, 1976). The polysaccharide levels were plotted against volume indices in order to test this observation for nutrient removal activated sludge (see Figures 7.15 and 7.16)

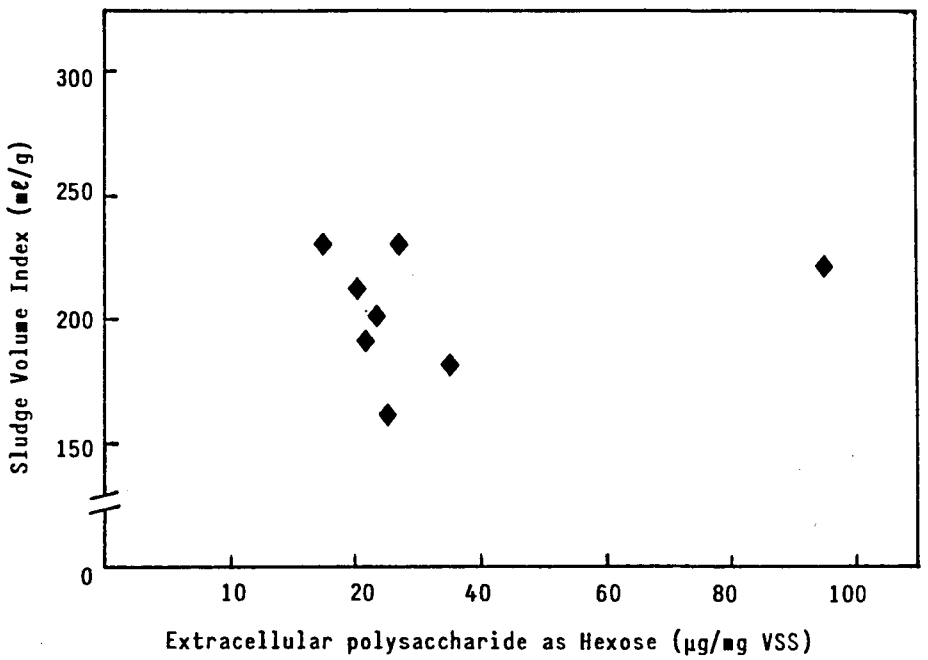

Figure 7.15 : Effect of extracellular polysaccharide levels on sludge volume indices. Northern Works Module 1.

In the case of Northern Works Module 1, the polysaccharide levels tended to remain within a narrow range which has no apparent effect on the SVI. The SVI for Northern Works Module 3 also showed no sensitivity to extracellular polysaccharide levels.

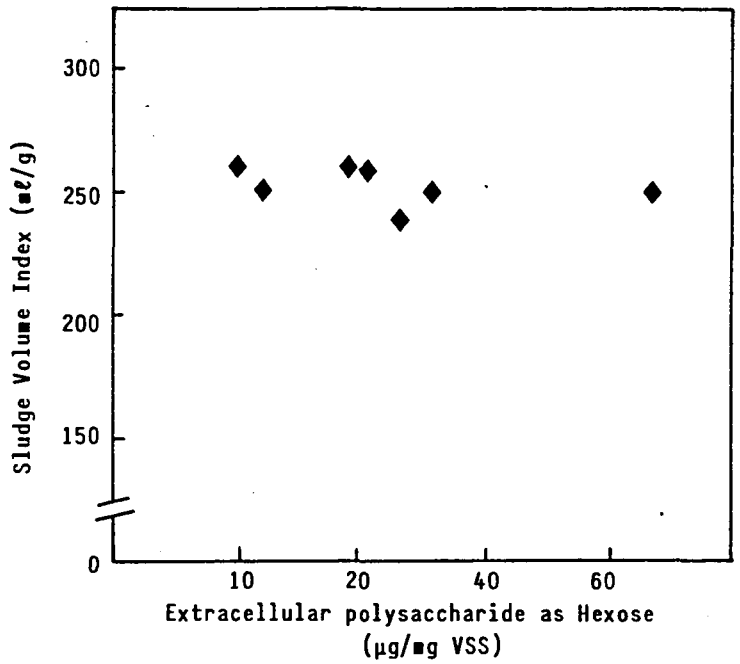

Figure 7.16 : Effect of extracellular polysaccharide on sludge volume indices. Northern Works Module 3.

7.5 PHOSPHORUS FRACTIONATION

7.5.1 Introduction

In an attempt to learn more about the role of polyphosphate in enhanced phosphorus removal, the distribution of compounds in sludge was investigated. Results did not provide good correlation between biological phosphorus fractions and phosphorus removal capacity.

The poorly defined separation achieved by the fractionation could not have accounted for this lack of correlation (Osborn et al., 1986), and it was decided to commission the Division of Water Technology of the CSIR to investigate the matter further.

7.5.2 Materials and Methods

An activated sludge sample was drawn from the primary aerobic zone of the Northern Works plant. It was cooled on ice during transit and aerated overnight at room temperature. Fractionation commenced the following morning.

The sludge was subjected to MLSS and VSS determination at 105 °C and 550 °C respectively. It was concentrated approximately six times and the degree of concentration determined by a repeat analysis for MLSS and VSS. The mixed liquor supernatant was removed by centrifugation (ca. 1500 x g, 5 minutes). Extraction of the concentrated samples was performed at 0 °C with the following solutions (in this order) : 0,9% NaCl; 0,5M PCA; dilute NaOH (pH 9 to 11); 1M NaOH. All the centrifugations were performed as above, and extracts stored at 40 °C before analysis.

Extracts and whole sludge were subjected to analyses for total P, ortho P and 7-minute P (de Haas et al., 1988a and b), RNA-P (Cerrioti, 1955) and DNA-P (Burton, 1968). Metal cations were determined on the hydrolysate produced for total P analyses, using colorimetry or atomic absorption spectrometry (American Public Health Association, 1985). Gel chromatography of Whatman No 41 filtered extracts was performed using Sephadex G-25 in a column measuring 97 cm x 1,6 cm, eluted with 0,1M KCl at 30 mℓ/h, collecting 100 fractions (2 mℓ each). The fractions were subjected to the 7-minute P assay, as above.

7.5.3 Results and Discussion

The Northern Works sample was found to contain 64,79 mg P/g VSS (44,37 mg P/g MLSS). The VSS/MLSS ratio was 0,68. Recovery of total phosphate in the extracts was 102% (less than 2% in residue), that of RNA-P 93% and DNA-P 83%. Seven-minute P determinations of whole sludge are not possible, preventing calculation of the recovery for that assay.

Figure 7.17 indicates that between 50 and 57% of the sludge total P was present as poly P. The reason for uncertainty in the figure lies with partial interferences in the ortho P, poly P and nucleic acid assays.
Firstly, the term "maximum additional ortho/poly P" appears in Figure 7.17, as a result of a small but measurable positive interference of poly P in the ortho P assay. If this interference

is at a level found to be maximal using pure reagents, these sectors of the pie may represent poly P entirely. If no interference exists, only ortho P will be represented by these sectors.

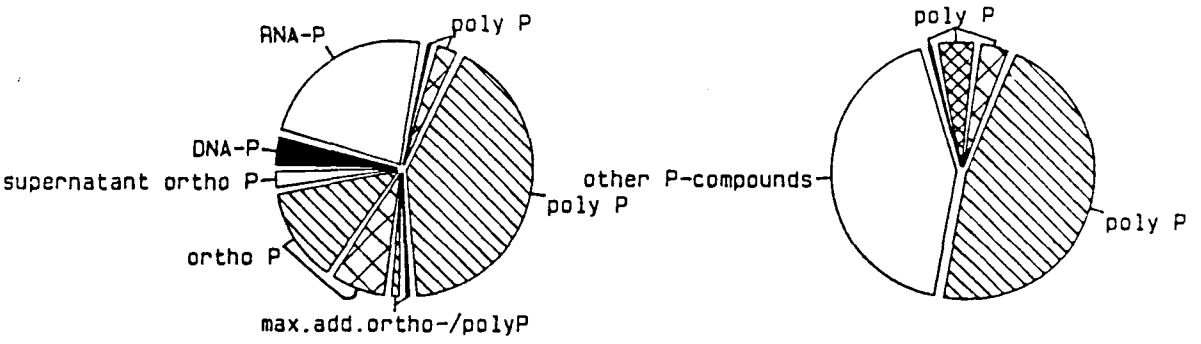

Figure 7.17 : Phosphorus fractionation of activated sludge from Northern Works

Secondly, the RNA method used has a tendency to overestimate RNA by a small but significant margin, as a result of the presence of carbohydrates originating in the bacterial cell wall matrix. For this reason, where poly P is calculated by difference from the total P, less ortho and nucleic acid P, poly P may be underestimated by a small margin.

Thirdly, the 7-minute P method does not completely exclude P originating fromm nucleic acids, meaning that poly P may be slightly overestimated in this method. Despite these interferences, the two methods for determining poly P given in Figure 7.17 agreed closely (4,5 tp 7% difference). By far the largest proportion of the poly P was acid extractable (PCA extract).

Gel chromatography (Figures 7.18 and 7.19) confirmed the presence of polyphosphate in the PCA and dilute NaOH fractions. The peak eluted at or near the void volume of the column (Fraction No 42) represents poly P, whilst that near Fraction No 80 represents ortho-P.

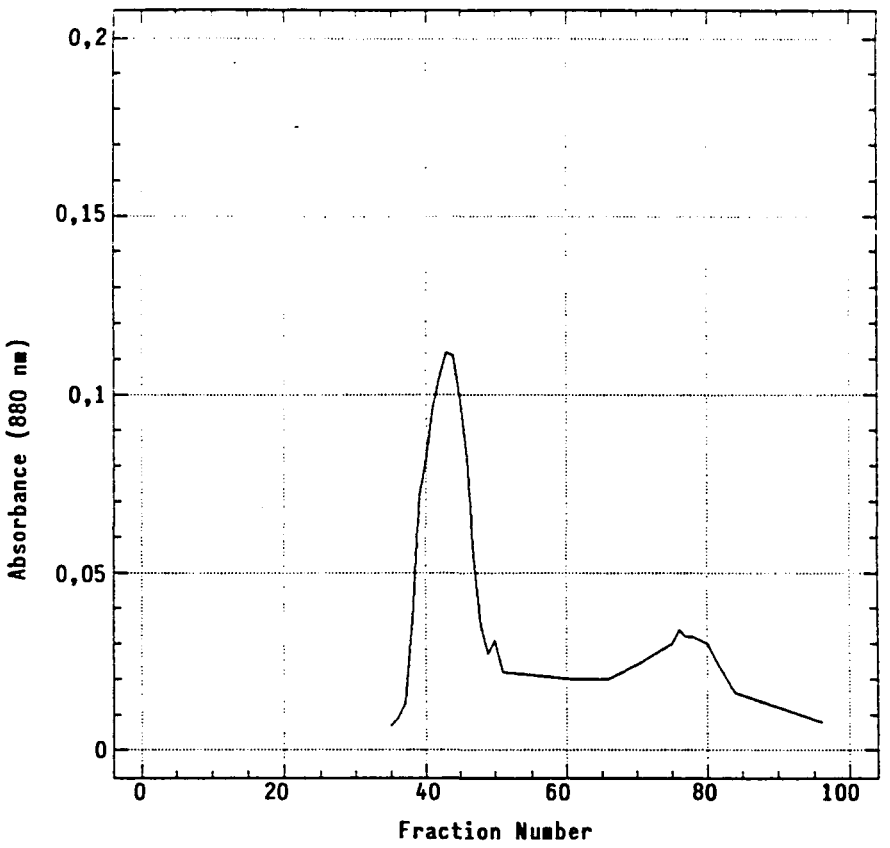

Figure 7.18 : Gel chromatography of the dilute NaOH extract

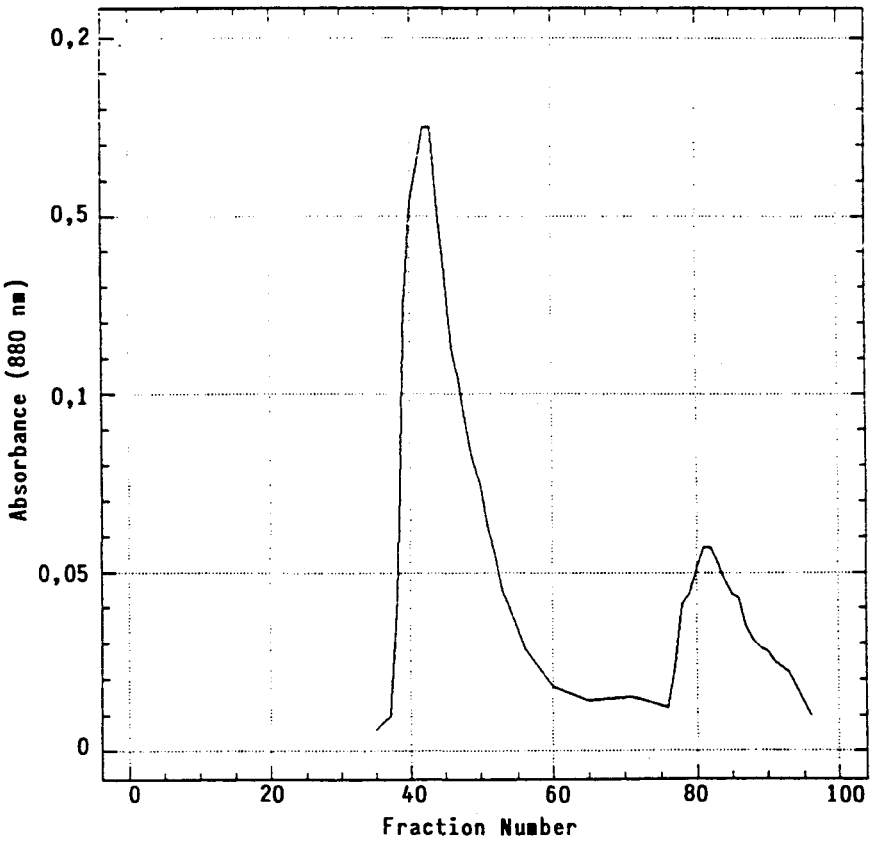

Figure 7.19 : Gel Chromatography of the PCA extract

Orthophosphate occurred in both the acid (PCA) and dilute alkali (NaOH) extracts, in the respective ratio of ca. 14% and 9% relative to the sludge total P. It is most likely that this represents the maximum contribution from chemically precipitated P in this sludge sample since the supernatant ortho P was removed separately, and the sludge pellet washed with saline (negligible P found in this extract).

From Table 7.2, it is clear that potassium, calcium and magnesium were mainly co-extracted with phosphorus (Figure 7.17). The same was true to a lesser degree for iron, more of which remained in the residue. It follows that iron indigenous to the sludge is tightly complexed and associated with phosphorus to a lesser extent than potassium, calcium and magnesium. It is however, impossible to infer the formula of the chemical P precipitate in the sludge from this data.

TABLE 7.2
METAL CATION ANALYSES FROM EXTRACTS FROM ACTIVATED SLUDGE SAMPLED AT NORTHERN WORKS, 16 MARCH 1988

Extract	K	Mg	Ca	Fe
Supernatant	7,11 (1,82)	7,11 (2,92)	7,11 (1,77)	0,15 (0,03)
0,9% NaCl	1,11 (0,28)	1,11 (0,46)	1,22 (0,55)	0,03 (0,01)
0,5M PCA	12,60 (3,22)	12,60 (5,18)	12,60 (3,14)	2,63 (0,47)
Dilute NaOH	2,37 (0,61)	1,18 (0,49)	1,18 (0,30)	0,50 (0,09)
1M NaOH	3,84 (0,98)	1,28 (0,53)	1,28 (0,32)	0,48 (0,09)
Residue	0,86 (0,22)	0,86 (0,35)	0,86 (0,35)	3,75 (0,67)
% in whole sludge (MLSS)	1,90	1,64	1,72	0,51

Figures in mg/g VSS or (10^{-4} mol/g VSS) unless otherwise stated

7.6 ENZYMATIC ASSAYS

7.6.1 Introduction

While microscopic evaluation of carbon and phosphorus accumulation

in nutrient removal activated sludge plants can be used to monitor the effects of changes in microbial metabolism on effluent phosphate levels, it is not suitable as an operational parameter (Lötter and Murphy, 1988).

The traditional technique of using chemical parameters to monitor plant performance, provides a measure of plant performance but particularly in the case of phosphate removal, is not a diagnostic tool in terms of the causes of plant failure.

A simple routine test is required which takes into account the biological nature of the process, and would therefore have some predictive diagnostic value. Biological monitoring techniques are widely used in evaluating bio-processes. Enzymatic assays have been used in activity studies on activated sludge, as have quantitative determinations of biological compounds (Weddle and Jenkins, 1971; Kucnerowicz and Verstraete, 1979; Richards et al., 1984).

Dehydrogenase activity has been widely used as an indicator of sludge viability and reaction to toxic influents (Legeron and Ben Aim, 1976; Broecker and Zahn, 1977; Awong et al., 1985; Anderson et al, 1988). No attempts have previously been made to correlate it to nutrient removal.

Phosphate acivity has also been used as an indicator of biomass activity in environments where phosphate metabolism was being studied (Reichardt, 1971; Flint and Hopton, 1977; Hassan and Pratt, 1977).

Alkaline and neutral phosphatase activity have been studied in trickling filter and activated sludge plants (Flint and Hopton, 1977; Teuber and Brodisch, 1977).

As in the case of dehydrogenase activity, phosphatase activity has not been correlated with nutrient removal.

This study addressed these issues.

7.6.2 Materials and Methods

Mixed liquor samples were drawn from various zones of a number of Bardenpho activated sludge plants in Johannesburg.

Dehydrogenase activity was determined essentially by the method of Lenhard et al, (1964). The method described by these authors used glucose as substrate. The method was modified to use -HO-butyrate as substrate. Using this method, dehydrogenase activity was determined in anaerobic, anoxic and aerobic zones of the plants.

Phosphatase activity was determined essentially by the method of Hassan and Pratt, (1977). Preliminary investigation of this method revealed that reproducible results were difficult to obtain. Rigorous testing of the method was then carried out using a commercial alkaline phosphatase preparation (Boehringer Mannheim Cat No 108154).

The effect of enzyme and substrate concentration and reaction time on enzyme activity was investigated.

Samples were then taken from the various zones of the Northern Works activated sludge plant.

7.6.3 Results and Discussion

In the case of dehydrogenase activity, sonication of the sample before addition of the reagents, allowed satisfactory reproducibility to be achieved (see Table 7.3).

TABLE 7.3
REPRODUCIBILITY OF DEHYDROGENASE ACTIVITY

Sample	Enzyme Units
1	1,10
2	1,38
3	1,06
4	1,08
5	1,16
6	1,21
7	1,17
8	1,24
9	1,27
10	1,40
Mean	1,21
Standard deviation	0,12

The enzyme activity was expressed as nM triphenyl formazan (TF) product formed per mg volatile suspended solids (VSS). The average enzyme activities for various zones of some Johannesburg plants are shown in Tables 7.4 and 7.5.

TABLE 7.4
AVERAGE DEHYDROGENASE ACTIVITY IN ACTIVATED SLUDGE WITH GLUCOSE AS SUBSTRATE

Plant	Enzyme activity nM TF/mg VSS		
	Anaerobic Zone	Anoxic Zone	Aerobic Zone
Northern Works Module 2	37	34	34
Northern Works Module 3	37	35	30
Goudkoppies	35	36	37
Bushkoppie	32	33	33

TABLE 7.5
AVERAGE DEHYDROGENASE ACTIVITY IN ACTIVATED SLUDGE WITH β-HO-BUTYRATE AS SUBSTRATE

Plant	Enzyme activity nM TF/mg VSS		
	Anaerobic Zone	Anoxic Zone	Aerobic Zone
Northern Works Module 2	35	32	31
Northern Works Module 3	33	33	34
Goudkoppies	32	34	35
Bushkoppie	30	32	30

The variation in enzyme activity for the various zones is shown in Figures 7.20 to 7.27.

No significant differences in activity were observed between zones or plants. This is in contrast to the differences observed between plants in respect of fermentation enzymes (Lötter and van der Merwe, 1987). As can be seen from Figures 7.20 to 7.27, activity varies over time. This variation could not be directly correlated to phosphorus removal. The substrate used also appears to have little effect on the activity. This could be due to the complex nature of the reaction mixture. Activity is measured directly on the sample without removal of matrix components. This is done to preserve the simplicity of the technique.

Dehydrogenase activity is a measure of oxidative behaviour and as such, has a linear correlation with Oxygen Utilisation Rate (OUR) (Awong et al., 1985; Anderson et al., 1988). It might be beneficial to investigate the use of the technique as an alternative to the more expensive OUR.

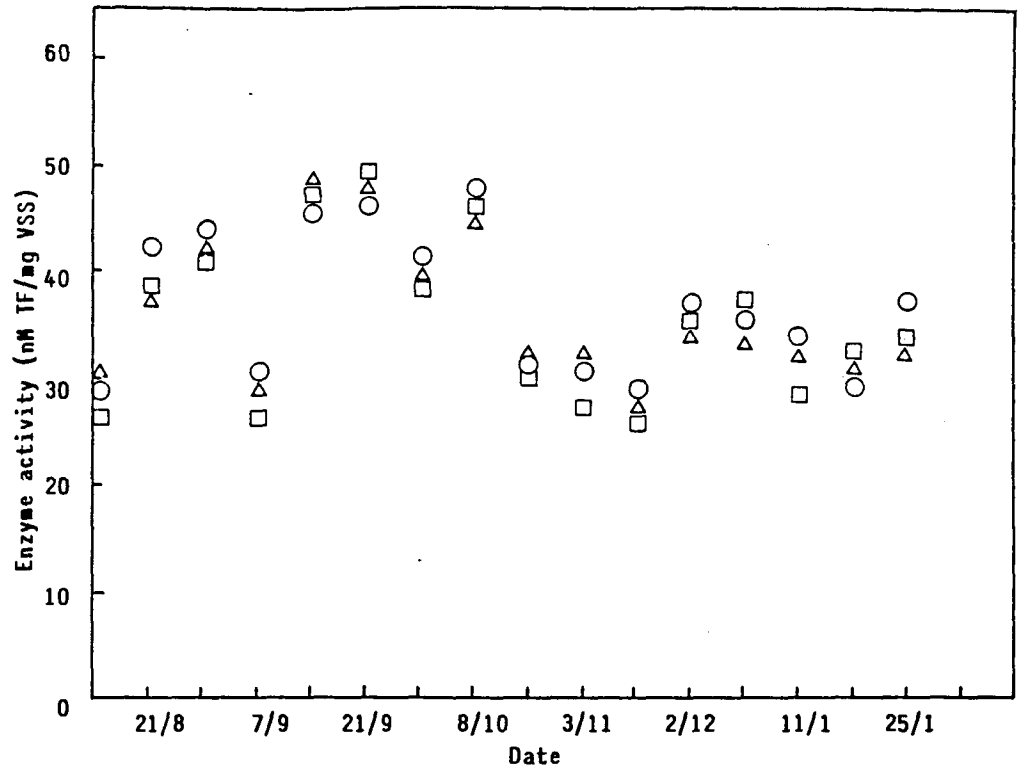

Figure 7.20 : Dehydrogenase activity in the anaerobic : □ ; anoxic : △ ; aerobic : ○ zones of Goudkoppies with glucose as substrate.

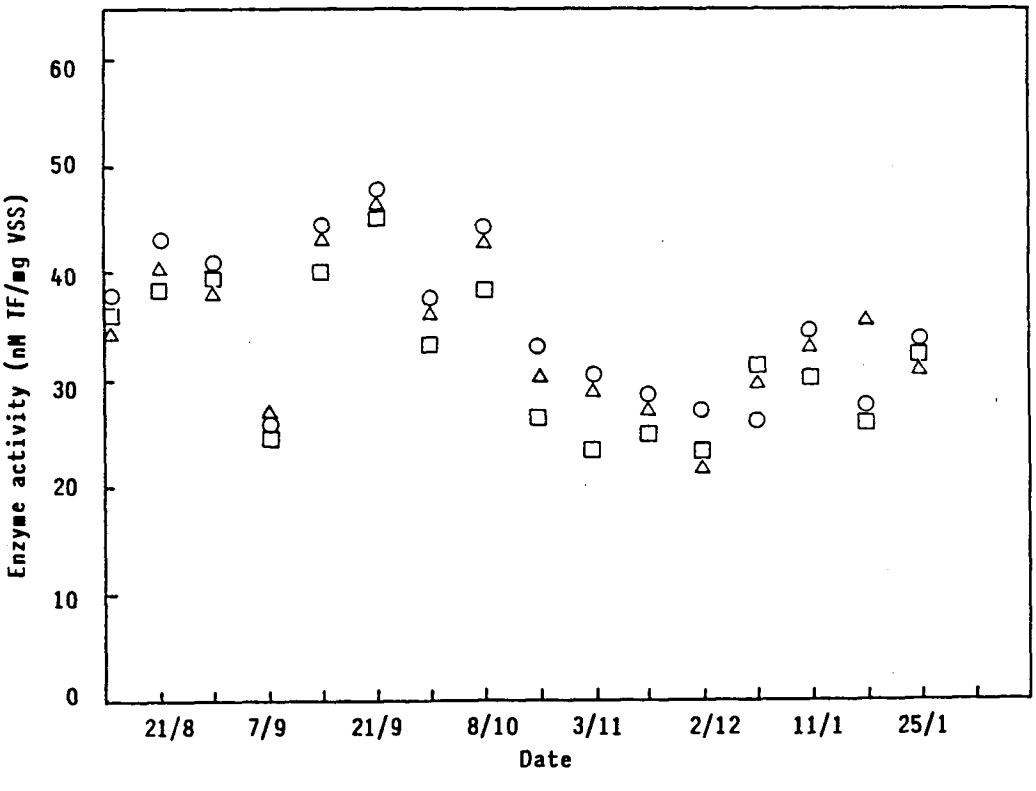

Figure 7.21 : Dehydrogenase activity in the anaerobic : □ ; anoxic : △ ; aerobic : ○ zones of Goudkoppies with β-HO-butyrate as substrate.

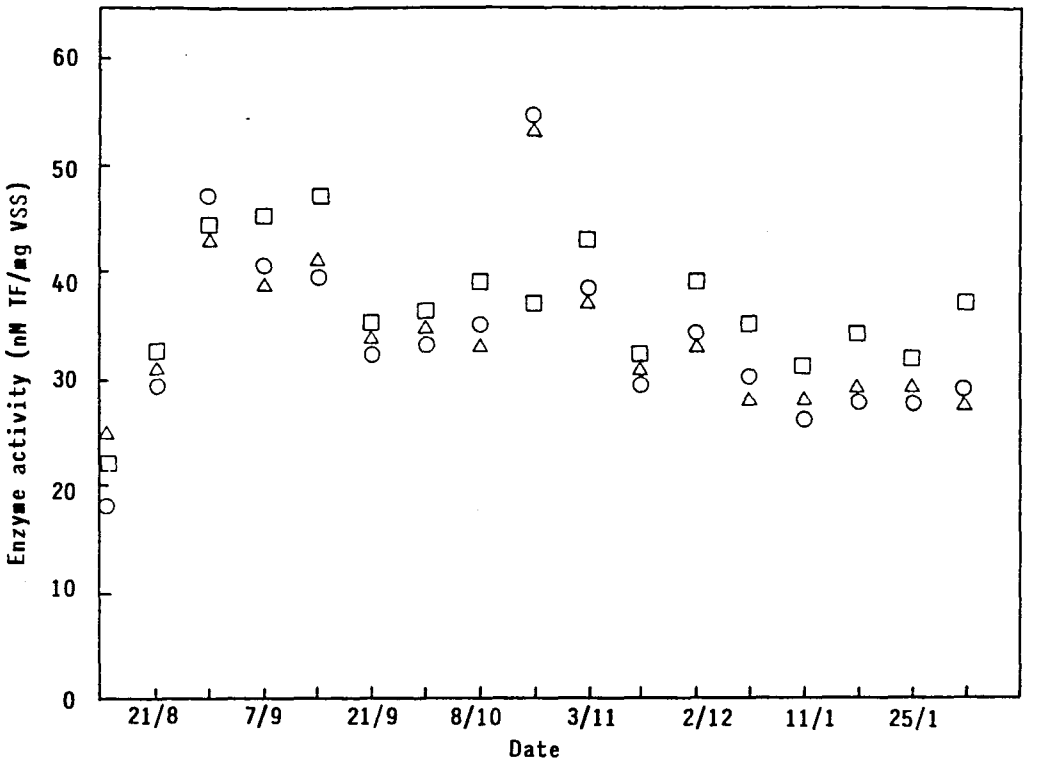

Figure 7.22 : Dehydrogenase activity in the anaerobic : □ ; anoxic : △ ; aerobic : ○ zones of Northern Works Module 2 with glucose as substrate.

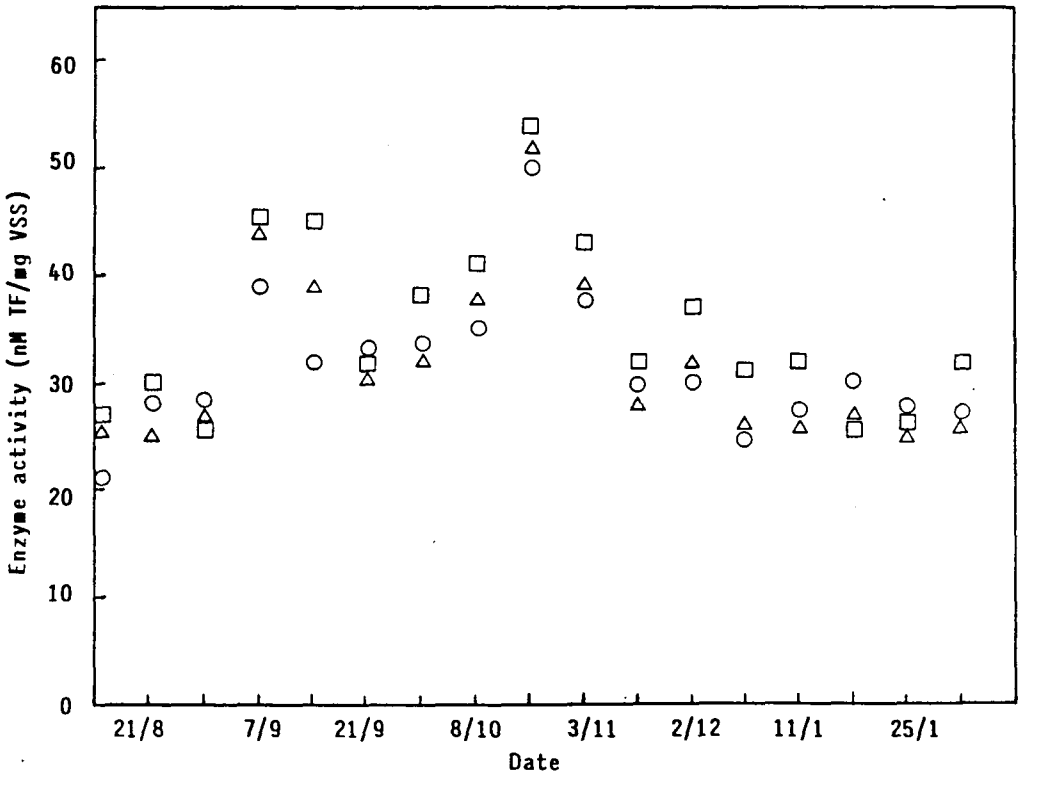

Figure 7.23 : Dehydrogenase activity in the anaerobic : □ ; anoxic : △ ; aerobic : ○ zones of Northern Works Module 2 with β-HO-butyrate as substrate.

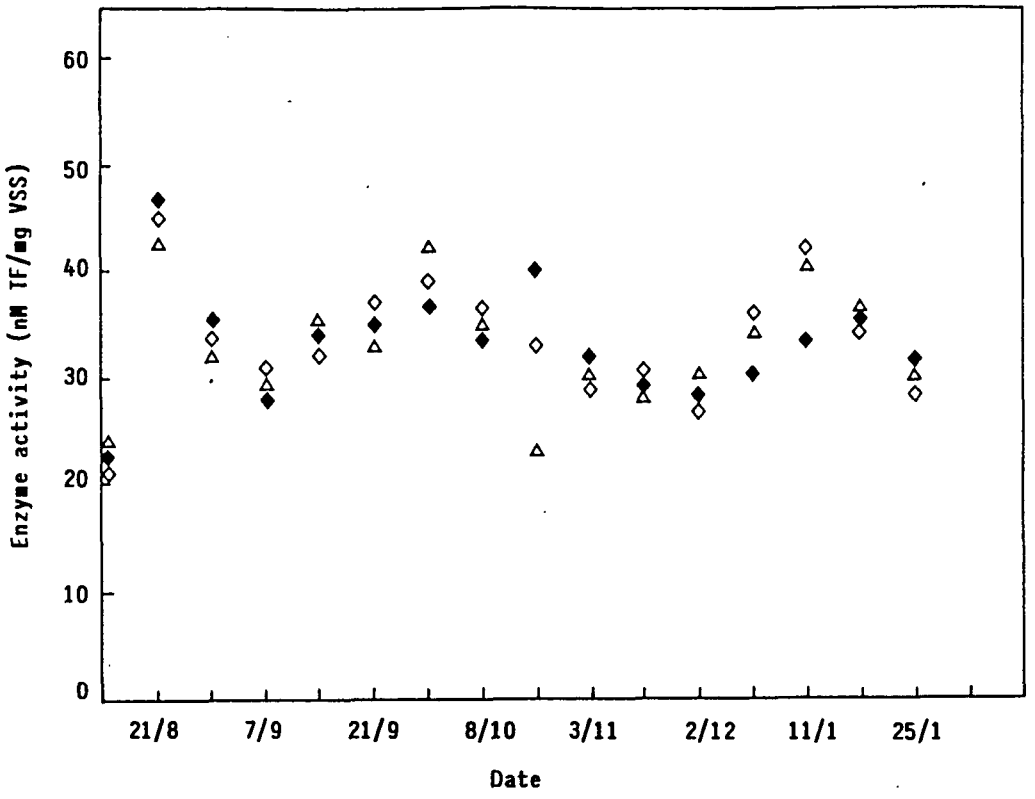

Figure 7.24 : Dehydrogenase activity in the anaerobic: ◇ ; anoxic △ ; aerobic: ◆ ; zones of Northern Works Module 2 with β-HO-butyrate as substrate

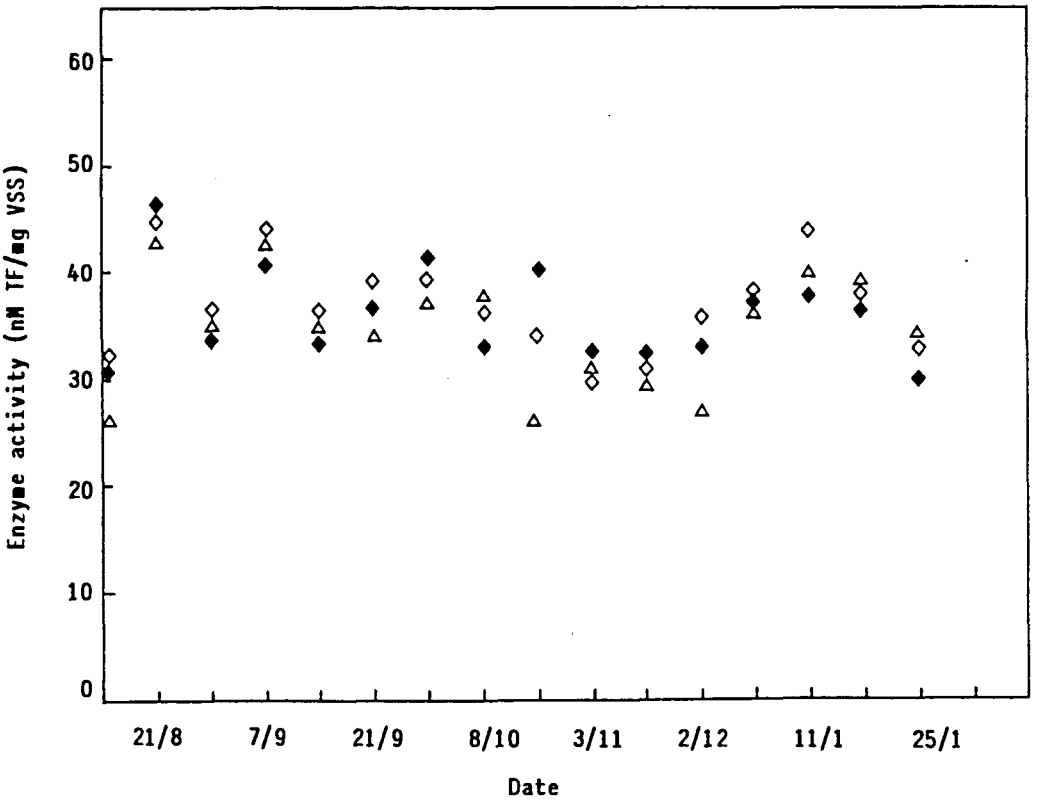

Figure 7.25 : Dehydrogenase activity in the anaerobic: ◇ ; anoxic: △ ; aerobic: ◆ ; zones of Northern Works Module 2 with glucose as substrate

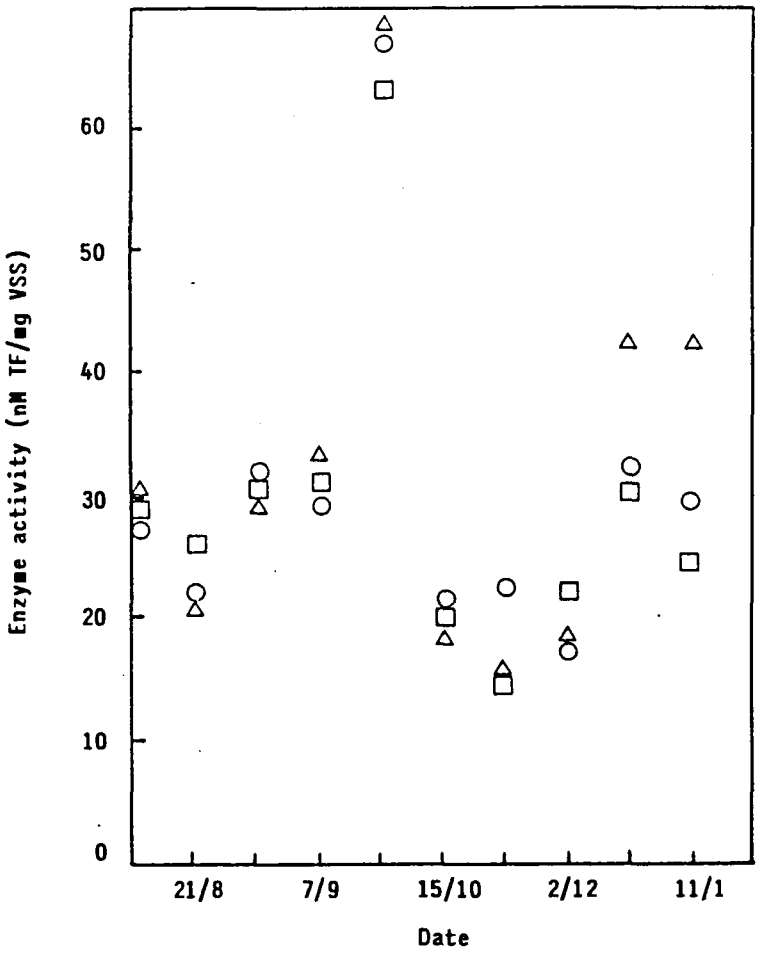

Figure 7.26 : Dehydrogenase activity in the anaerobic: ; anoxic: △ ; aerobic: ; zones of Bushkoppie with β-HO-butyrate as substrate

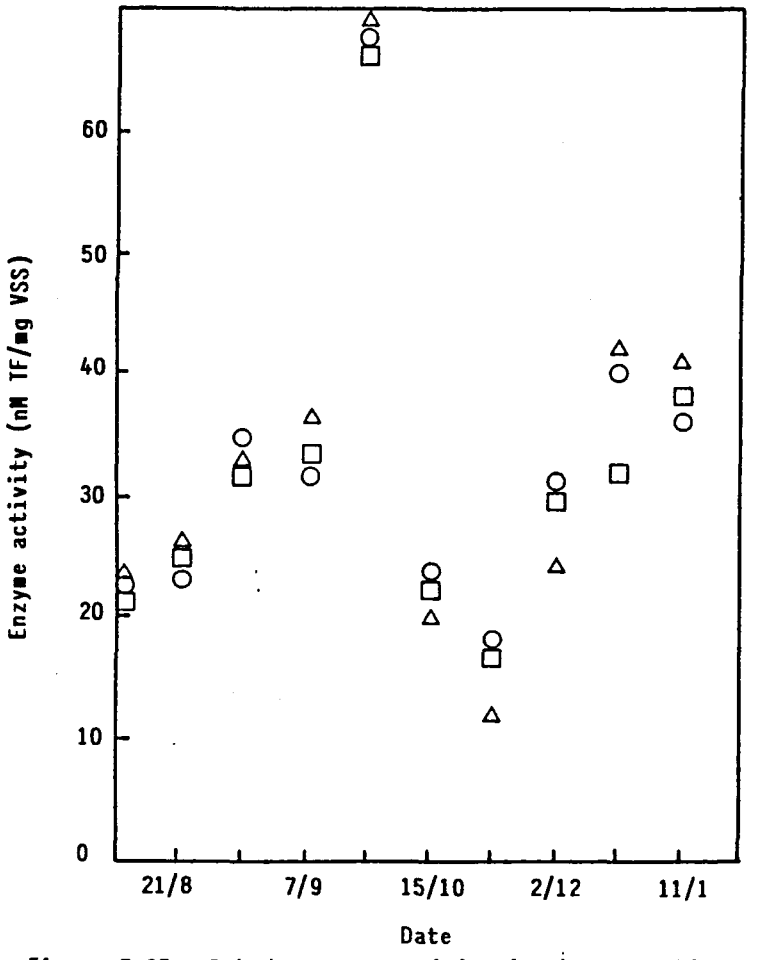

Figure 7.27 : Dehydrogenase activity in the anaerobic: ; anoxic: ; aerobic: ; zones of Bushkoppie with glucose as substrate

Alkaline phosphatase catalyses the hydrolysis of a phosphate residue from a variety of compounds. The analysis is based on the hydrolysis of p-nitrophenyl phosphate to p-nitrophenol.

Results of the preliminary investigations of the alkaline phosphatase are given in Figures 7.28 to 7.31.

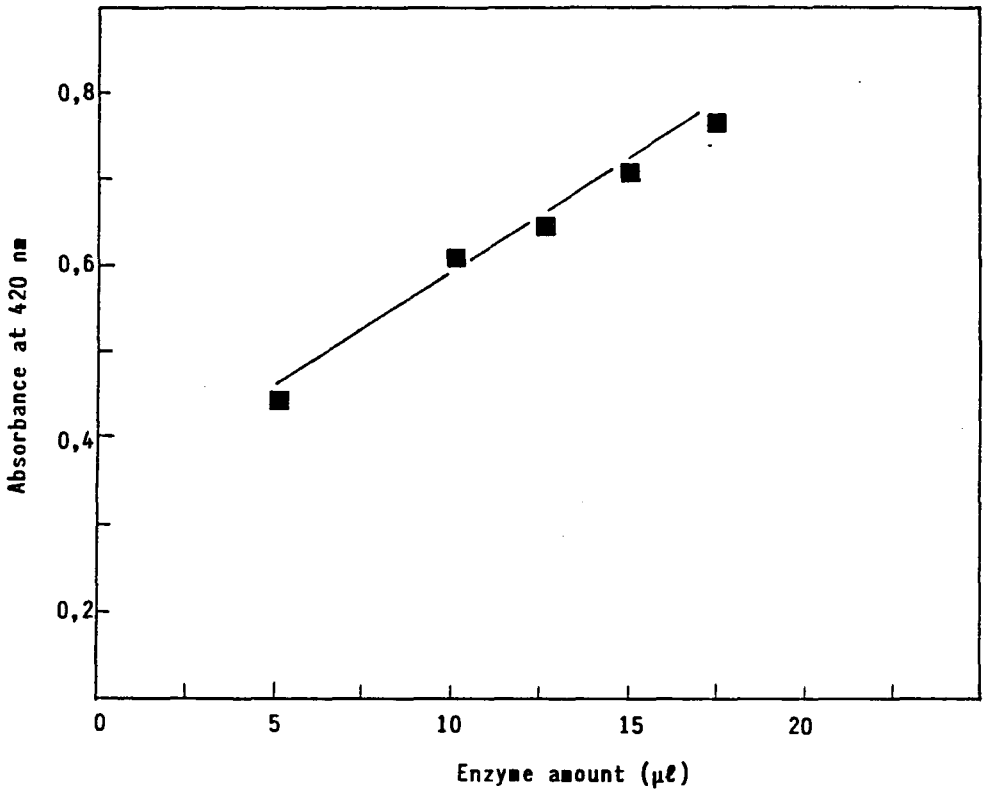

Figure 7.28 : The effect of enzyme concentration on reaction rate

Figure 7.28 shows the range of linearity of enzyme concentration in the presence of saturating substrate concentrations. Five to 20 µℓ of a diluted commercial enzyme preparation (1:20) can be used to achieve a first order reaction.

Figure 7.29 gives the effect of substrate concentration on reaction rate. As can be seen from the curve, substrate volumes above 1,0 mℓ do not result in an increase in reaction rate.

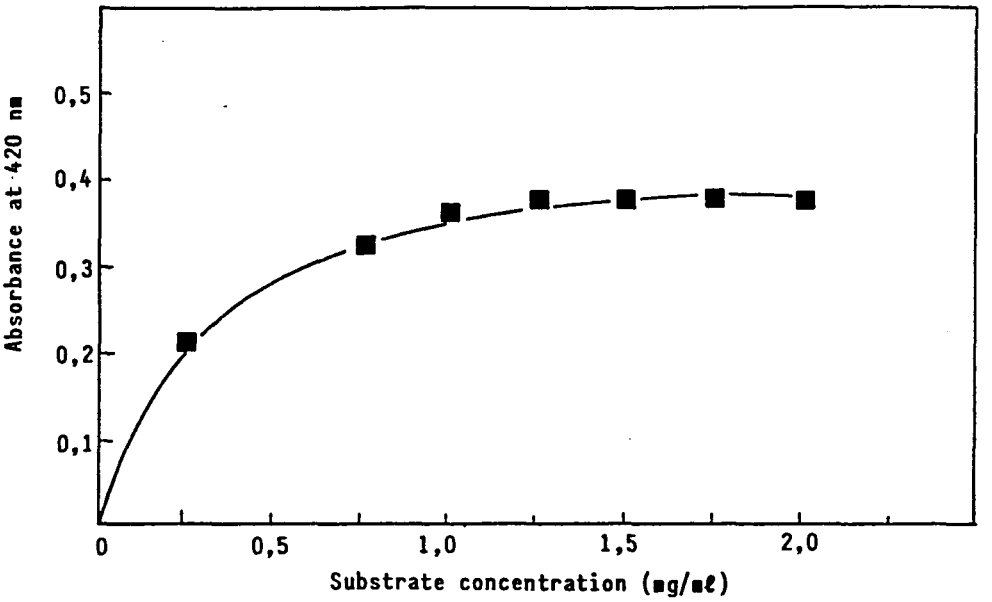

Figure 7.29 : Effect of substrate concentration on reaction rate

The data from Figure 7.29 was replotted according to Lineweaver-Burk to check the accuracy of the hyperbolic plot (see Figure 7.30). A substrate volume 0,5 mℓ was subsequently used. The effect of reaction time on reaction rate is shown in Figure 7.31. A reaction rate of 30 minutes was used in further work.

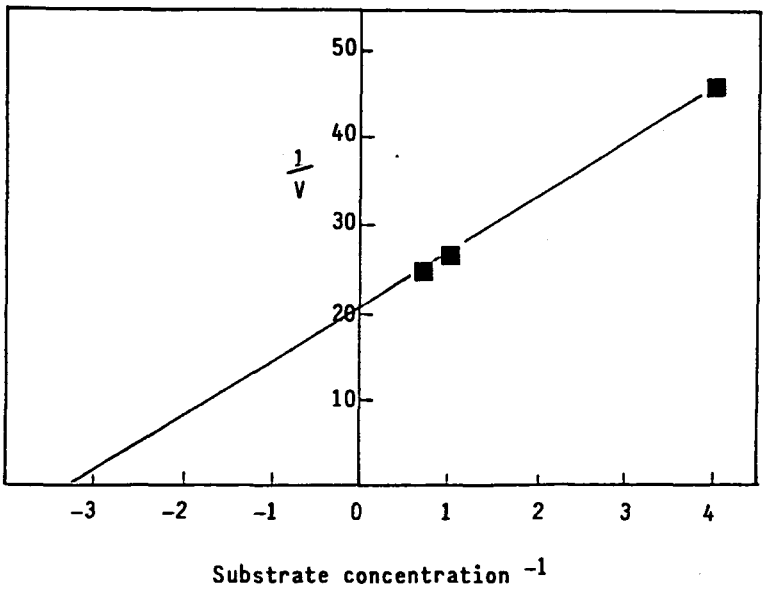

Figure 7.30 Lineweaver-Burk manipulation of data in Figure 7.29

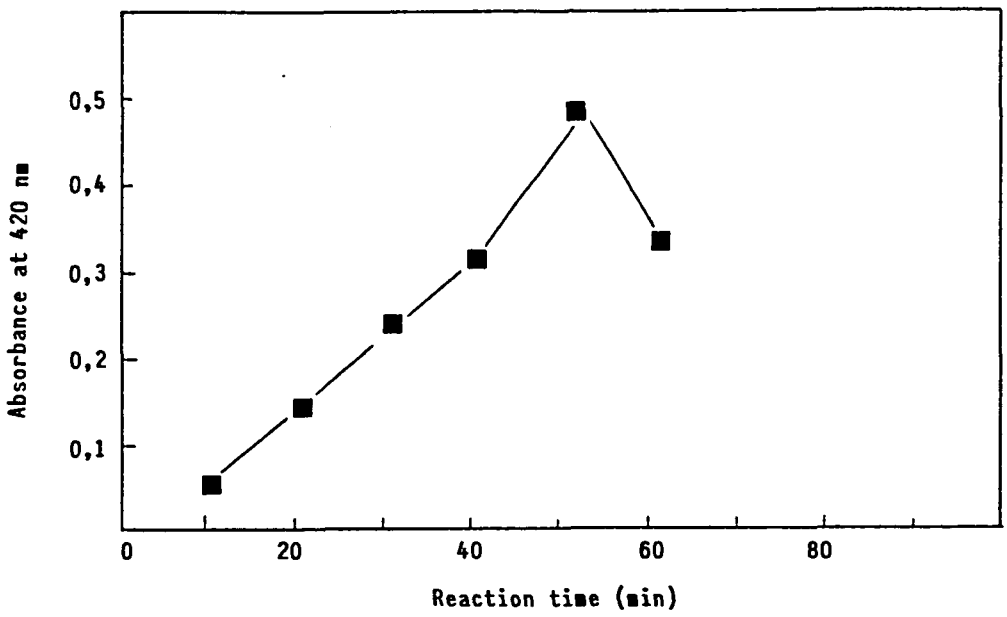

Figure 7.31 : Effect of reaction time on reaction rate

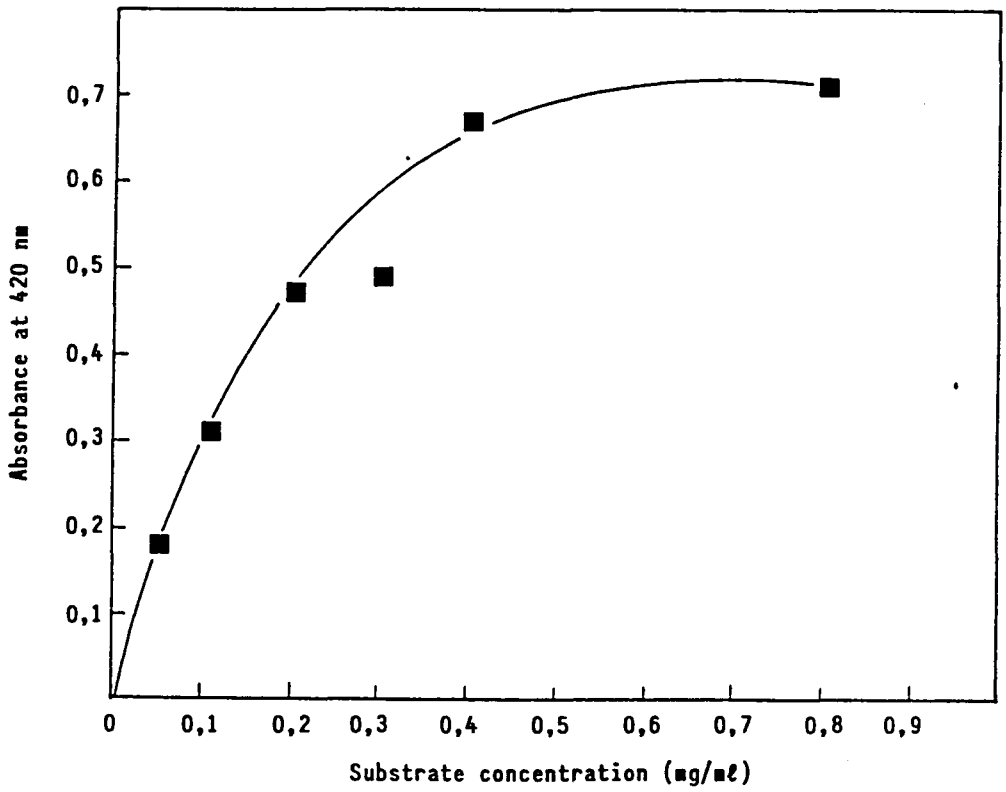

Figure 7.32 : Typical calibration curve obtained with enzymatic formation of p-nitrophenol

Difficulties were experienced with using various concentrations of p-nitrophenol to prepare a calibration curve. It was therefore decided to prepare a calibration curve using p-nitrophenyl phosphate conversion to p-nitrophenol by the commercial enzyme preparation. A typical calibration curve obtained in this way is shown in Figure 7.32. Some results of the activated sludge samples are shown in Table 7.6.

TABLE 7.6
ALKALINE PHOSPHATASE ACTIVITIES IN NORTHERN WORKS ACTIVATED SLUDGE PLANT

Zone	Enzyme activity µM substrate/g VSS
Anaerobic	2,10 - 5,71
Anoxic	2,07 - 2,20
Aerobic	1,05 - 1,93

Samples are now being routinely analysed for this parameter in an attempt to correlate it with effluent phosphate levels.

7.7 CONCLUSIONS

Some of the studies described above confirmed a number of previously held hypotheses, while others opened the door to further investigation.

Chemical precipitation as a removal mechanism in the activated sludge system has long been a controversial issue. These studies have shown that under conditions prevailing in Johannesburg plants, this is not a significant factor.

The efficient nitrification in the Johannesburg plants precludes the accumulation of ammonia which could serve as a nutrient for filamentous growth, and the correlation between nutrient levels and SVI values was not observed.

The additional effort and cost involved in the chemical analysis of extracellular polysaccharide compared to microscopic evaluation is not warranted, as the former technique does not provide a more sensitive indicator of plant performance than the latter.

A satisfactory phosphorus fractionation procedure has been developed by the Division of Water Technology, which will allow a more accurate estimation of different biological phosphorus forms. These data will be useful in refining the biochemical model of the process.

Dehydrogenase activity is relatively easy to determine and its potential use in controlling aeration should be investigated.

A method for the routine determination of alkaline phosphatase activity in activated sludge has been refined.

7.8 ACKNOWLEDGEMENTS

The work described in 7.5 of this Chapter, was carried out by David de Haas, of the Division of Water Technology of the CSIR.

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CHAPTER EIGHT
CONCLUSIONS AND RECOMMENDATIONS

8.1 INTRODUCTION

The research carried out during the period 1984 to 1986 was reported in 1986 (Osborn et al., 1986).

The further research carried out during the period 1986 to 1988 is reported in two separate reports; this one, and a second one by Lötter (1989).

In this Chapter the research carried out over the full Contract period, 1984 to 1989, is reviewed. Recommendations for future research in this and related areas, are also discussed.

8.2 PROCESS FEED COMPOSITION

These studies have confirmed that the success of biological nutrient removal is very dependent on the characteristics of the influent sewage. If the feed has an adequate concentration of readily biodegradable substances (S_{bs}), in particular, anaerobic fermentation products such as volatile fatty acids (VFA), phosphorus removal has a good chance of success. Sewage having a total COD of about 500 mg/ℓ, a VFA content of greater than 70 mg/ℓ as acetic acid and an S_{bs} of greater than 120 mg/ℓ, has been successfully treated in the Johannesburg three and five-stage Bardenpho plants, to give an effluent having very low concentrations of both orthophosphate (less than 1,0 mg o-P/ℓ) and total nitrogen (less than 5,0 mg N/ℓ).

The production of volatile fatty acids either by fermentation in the primary sludge tanks, or by the fermentation of primary sludge in high rate anaerobic digesters operated in a batch mode, is examined. In both cases it was necessary to maintain a sludge retention time (SRT) of 3 to 4 days and to ensure that the VFA's produced were not converted to methane.

High rate digesters were found to produce roughly equal quantities of acetic and propionic acid, whereas more acetic than propionic acid was produced in the primary sedimentation tanks (PST's). Furthermore the mass of total volatile fatty acids produced in the off-line digesters was far less than that produced in the PST's, where the more desirable acetic acid predominated.

Fermentation of sludge in the PST's became the routine method for VFA production. The VFA's produced by this process tend to remain adsorbed to the sludge under hydraulically static conditions and must be elutriated into the liquid phase if they are to serve any useful purpose in the activated sludge reactor.

A number of elutriation regimes incorporating recycling of the sludge back to the inlet of the sedimentation tanks, were examined. Phosphate removal was improved by this procedure. However, consistent compliance with the effluent standard was not achieved. Intermittent air lancing of the sludge in the PST's combined with recycling was found to give very good results.

During summer, the best results were obtained when sludge was accumulated for 24 hours, airlanced for 30 seconds three times during the next 24 hours, and finally stored for a further 24 hours. The effect of the lower winter temperature on the above procedure, still requires investigation. Raw sludge gravity thickeners with elutriation facilities can also be used for this purpose.

8.3 DENITRIFICATION

Experience in Johannesburg (and elsewhere), has shown that when dealing with predominantly domestic sewage containing limited amounts of naturally occurring readily biodegradable substrate, it is essential to limit the presence of nitrates in the anaerobic zone to an absolute practical minimum. This will ensure that the readily biodegradable substrate is made available to phosphorus accumulating bacteria and is not utilised by denitrifying bacteria.

To assist in achieving this objective, one 50 Mℓ/d module at the Northern Works was converted to three-stage operation by eliminating the second anoxic zone. The original anaerobic zone was enlarged however, and compartmentalised in such a way that the returned sludge could be held and denitrified under endogenous conditions (i.e. no sewage added), in the first few compartments. This modification became known as the Johannesburg Process. The successful operation of the plant under these conditions is highly dependent on having a sludge with a low SVI, thereby permitting high concentrations of returned sludge to be held in the newly created anoxic zone ahead of the bioreactor. Under such circumstances, the concentration of denitrifiers is likely to be about twice that present in conventional anoxic zones. The modification appeared to confer additional stability on the biological removal of phosphorus over an extended period.

Experience in Johannesburg has shown that up to 10 mg N/ℓ nitrate can be removed from the return sludge in such a zone at retention periods varying between 0,5 to 2,0 h.

8.4 AERATION

It has been demonstrated that aeration has a marked effect on nutrient removal processes. Aeration levels should be such to maintain DO levels of at least 2 mg/ℓ throughout the aerobic stages. This is particularly necessary at the initial entry to the aerobic zone, as phosphorus uptake is extremely rapid at this point. Excess

aeration towards the end of the aeration zone should be avoided however, as excessive nitrification will occur, and increase the denitrification requirements of the process.

Experience in Johannesburg has shown that the rectangular aerobic zones in nutrient removal plants are not as completely mixed as was first thought, despite the large volume of mixed liquor being recycled through them. Semi-plug flow conditions can exist in these zones, leading to a distinct respiration rate profile along their length. Such zones should therefore have a tapered aeration system with more aeration capacity available at the inlet, than at the outlet of the zone.

8.5 ON-LINE MONITORING

The monitoring of effluent phosphate levels on-line has proved useful in the comparison of different process configurations. These data will also be utilised to calculate chemical dosage where this is required to achieve compliance with the effluent standard.

A number of methods of collecting and processing the data from on-line analysers were investigated.

The prediction for the future is that a number of Programmable Logic Controllers (PLC's) will be placed at strategic points in the plant to collect data from monitors. The data will be accessed and processed by a central computer, which will provide the plant manager with up-to-date operational data.

8.6 MICROBIOLOGICAL STUDIES

Microthrix parvicella and filament types 0041 and 0092 were identified as the main causative organisms in scum formation and sludge bulking in the Northern Works activated sludge plant. Bulking caused by these organisms can be controlled by adequate aeration. Bulking problems have never been experienced at the diffused air plants at the Bushkoppie Works. Different types of filaments were

observed in this plant, as compared to Northern Works. Acinetobacter spp dominated the phosphorus removing organism population in the aerobic zones under the test conditions applied.

The effect of microbial metabolism on effluent phosphate levels can be readily monitored by microscopic evaluation. While microscopic evaluation provides a useful tool for the general health of the plant, it is not suitable as an absolute operating parameter.

Comparison of different media for the isolation of activated sludge show that these give varied population compositions, as is expected. It is therefore, not possible to compare the population studies of some researchers when different media have been used. The technique currently being used is beneficial in the study of the effects of operational changes on bacterial population.

A new identification system, namely, the Microbact Identification System, was shown to be more species specific and cost effective than the API system, and is now used routinely with a separate oxidase test.

These preliminary results have indicated that the phosphate releasing capacity test has potential as an easy, inexpensive technique, to predict deterioration in phophorus removal.

Further work is required to ensure that this method can be utilised successfully in practice. This could provide a useful tool in supplementing biological removal with chemical addition.

8.7 BACTERIAL METABOLISM

A number of hypotheses incorporated in the current biochemical model of enhanced biological phosphate removal from activated sludge, were evaluated experimentally during this study. The main hypothesis, namely, that the process is controlled by intracellular metabolite levels rather than genetic selection, was found to hold.

The studies also showed that Acinetobacter spp, as an example of a polyphosphate bacterium, possessed strategies to absorb substrate under one unfavourable situation (i.e. anaerobic), and to use the stored substrate as an energy source under a second unfavourable situation (i.e. lack of external carbon), to absorb other essential nutrients like phosphate. The necessity for adequate aeration was also emphasized by these studies.

8.8 DEVELOPMENT AND REFINEMENT OF TECHNIQUES

During the execution of the Contract, methods for the routine monitoring of plant response parameters such as volatile fatty acids and S_{bs} had to be refined. While these two parameters were successfully determined during the Contract period, some further refinements are required.

In attempts to gain further insight into the mechanism of enhanced biological phosphate removal, techniques were modified for the analysis of activated sludge mixed liquor. Subsequently, poly-hydroxybutyrate was successfully determined in mixed liquor.

Attempts to determine activated sludge energy levels by analysis for ATP were unsuccessful.

Methods were successfully developed for the routine determination of volatile fatty acids and polysaccharides in sewage and activated sludge samples. A method for the determination of polyphosphate chain length was investigated, but which proved more difficult to use on activated sludge than anticipated, and requires further work.

8.9 FUNDAMENTAL STUDIES : CHEMICAL AND BIOLOGICAL PHOSPHATE REMOVAL

In situ chemical precipitation as a removal mechanism in the activated sludge system has long been a controversial issue. These studies have shown that under conditions prevailing in Johannesburg plants, this is not a significant factor.

The efficient nitrification in the Johannesburg plants precludes the accumulation of ammonia which could serve as a nutrient for filamentous growth, and the correlation between nutrient levels and SVI values was not observed.

The additional effort and cost involved in the chemical analysis of extracellular polysaccharide compared to microscopic evaluation, is not warranted, as the former technique does not provide a more sensitive indicator of plant performance than the latter.

A satisfactory phosphorus fractionation procedure has been developed by the Division of Water Technology of the CSIR, which will allow a more accurate estimation of different biological phosphorus forms. These data will be useful in refining the biochemical model of the process.

Dehydrogenase activity is relatively easy to determine and its potential use in controlling aeration could be investigated.

A method for the routine determination of alkaline phosphatase activity in activated sludge has been developed.

8.10 FINANCIAL ASPECTS

A 50 000 Mℓ/d four-stage Bardenpho process with primary sedimentation and flow balancing, was used as a base for a paper study, to which a number of phosphate removal options were added . The cost of each option was then calculated, assuming the same unit electricity and chemical costs.

The introduction of an anaerobic zone and sewage character modification involves an additional cost of 1,60 c/kℓ, while the phosphate removal by chemical addition would incur an additional cost of 2,47 c/kℓ.

In contrast to the above, the retrofitting of chemical dosing equipment to a two-stage biological filter plant, would involve an additional 7,62 c/kℓ.

Although these figures may serve as a useful guide in decision making, they cannot be seen in isolation. Each individual situation must be evaluated on its own merits, taking other relevant factors into account. For example, the scrapping of a biological filter unit which has not been fully amortised, may not be an economical proposition.

8.11 MATHEMATICAL MODELLING

Until recently, the use of the general activated sludge model developed by the University of Cape Town, was confined to people with access to a mainframe computer. During this study, the model equations have been successfully applied to a spread sheet, used in conjunction with a micro-computer.

The use of this technique has permitted the sophisticated UCT model to become available to wastewater plant management staff, enabling them to observe the effect of a change in one parameter on other parameters, including effluent quality. This allows staff to make operational decisions with far greater confidence.

8.12 RECOMMENDATIONS FOR FUTURE WORK

In addressing the specific research areas required by the Contract, researchers identified a number of ancillary processes which impinge on biological phosphate removal. In order to integrate biological phosphate removal into a total wastewater treatment protocol, various research needs into these ancillary processes were identified.

Certain research areas addressed during the Contract also require further investigation. These recommendations are intended to encompass both the research needs in biological phosphorus removal and ancillary processes.

8.12.1 Ancillary Processes

Combined chemical and biological phosphate removal in the activated sludge process

In order to comply with the statutory effluent standard, the biological phosphate removal process often requires supplementation with chemical addition. Optimisation of these two processes in combination is essential to ensure the most cost-effective utilisation of both. Different chemicals and dosing protocols should be investigated.

Sludge handling including dewatering, final disposal and metal extraction without loss of phosphorus from sludge

Sludge from nutrient removal plants contains large amounts of biologically immobilised phosphorus. Sludge handling can cause the release of some of this phosphorus to the liquid phase, thus requiring retreatment of the latter. Optimisation of sludge handling and disposal with minimum loss of phosphorus is essential.

Recycling sludge in the form of compost is an attractive proposition, but the metal content limits its application to agricultural land. Recovery of the heavy metals from the sludge without loss of nutrients, would greatly enhance the attractiveness of the composting option.

Utilisation and role of particulate COD in phosphorus and nitrogen removal and its role in sludge bulking

Particulate COD contains a biodegradable component which must be utilised during the process. No research has been carried out into the optimal use of this carbon source, or its role in various processes prevailing in an activated sludge system.

Removal of non-biodegradable COD

The removal of non-biodegradable COD has, like the utilisation of particulate COD, received little or no attention. Effluent COD levels in excess of the effluent standard, have been observed to arise as result of high levels of non-biodegradable COD in the influent. Methods to reduce effluent levels to within the standard should be investigated.

Sludge bulking in nutrient removal plants

Sludge bulking is a problem experienced in a number of nutrient removal plants. While this problem may be prevented by correct design of new plants, operational protocols are required for bulking control in existing plants. Further research to consolidate design features and operational aspects is required. Alternate methods of solids separation should be investigated.

8.12.2 Further Research Emanating from the Contract

In addition to the abovementioned ancillary avenues which should be investigated, the following areas emanating from this Contract, deserve attention in the future :-

Updating of the biochemical and mathematical models

Current biochemical and mathematical models developed by the University of Cape Town and the City of Johannesburg, should be continually updated in the light of new findings, in order to retain these models at the forefront of research in this area.

The theoretical data generated by these models should be linked to operational aspects and then consolidated into a cohesive whole.

Consolidation of on-line monitoring protocols

Considerable progress has been made with on-line monitoring of

activated sludge plants and the handling of data from the monitors. Protocols for their routine use need to be drawn up.

Monitoring effects of innovating design features on process efficiency

A number of design ideas have been generated during the research undertaken during this Contract. In order to evaluate their usefulness, their effect on process efficiency should be monitored.

Consolidation of biological monitoring procedures

Various monitoring techniques have been used during this Contract. Attempts should be made to consolidate and rationalise their use.

Handling phosphate removal plant failures

In the long sludge age plants operated in South Africa, phosphorus can be immobilised over a period of up to twenty days in the sludge.

Plant failures could result in disruption of the environment to such an extent, that the immobilised phosphate is released to the aqueous phase. Strategies should be developed to deal with such an eventuality. These should include protocols for restarting the plant.

Cost effectiveness of feed process alteration versus chemical addition for phosphorus removal

A preliminary paper study revealed that optimisation of biological phosphate removal by altering the process feed composition, is cost effective in comparison to chemical addition. However, additional factors such as possible reduced gas production, the effect of chemical addition on the salinity of the rivers, and the effect of sludge handling, should be investigated.

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CHAPTER NINE

PUBLICATIONS

During the course of this contract, Johannesburg City Council Staff have presented results at international and local conferences and a number of papers have been published. A list, covering the full contract period from 1984 to 1988, appears below for easy reference.

BARNARD J L and NICHOLLS H A. (1989) Case Studies in N and P removal. Paper to be presented at the Seminar on nutrient removal. Mount Grace Hotel Magaliesberg.

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