JOHANNESBURG CITY COUNCIL

CITY HEALTH AND CITY ENGINEERS

DEPARTMENTS

REPORT TO THE
WATER RESEARCH COMMISSION
ON A THREE YEAR STUDY ON THE
ENHANCEMENT OF BIOLOGICAL PHOSPHATE
REMOVAL BY ALTERING PROCESS FEED COMPOSITION

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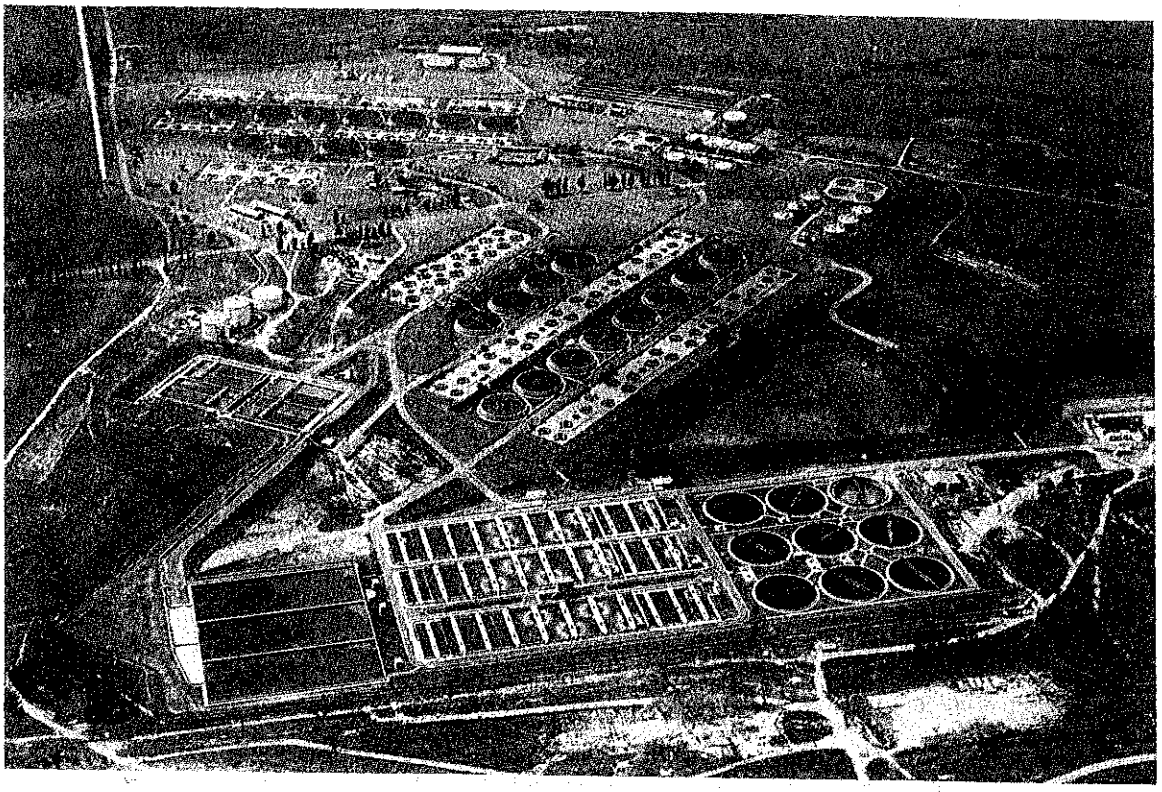
JOHANNESBURG NORTHERN WORKS



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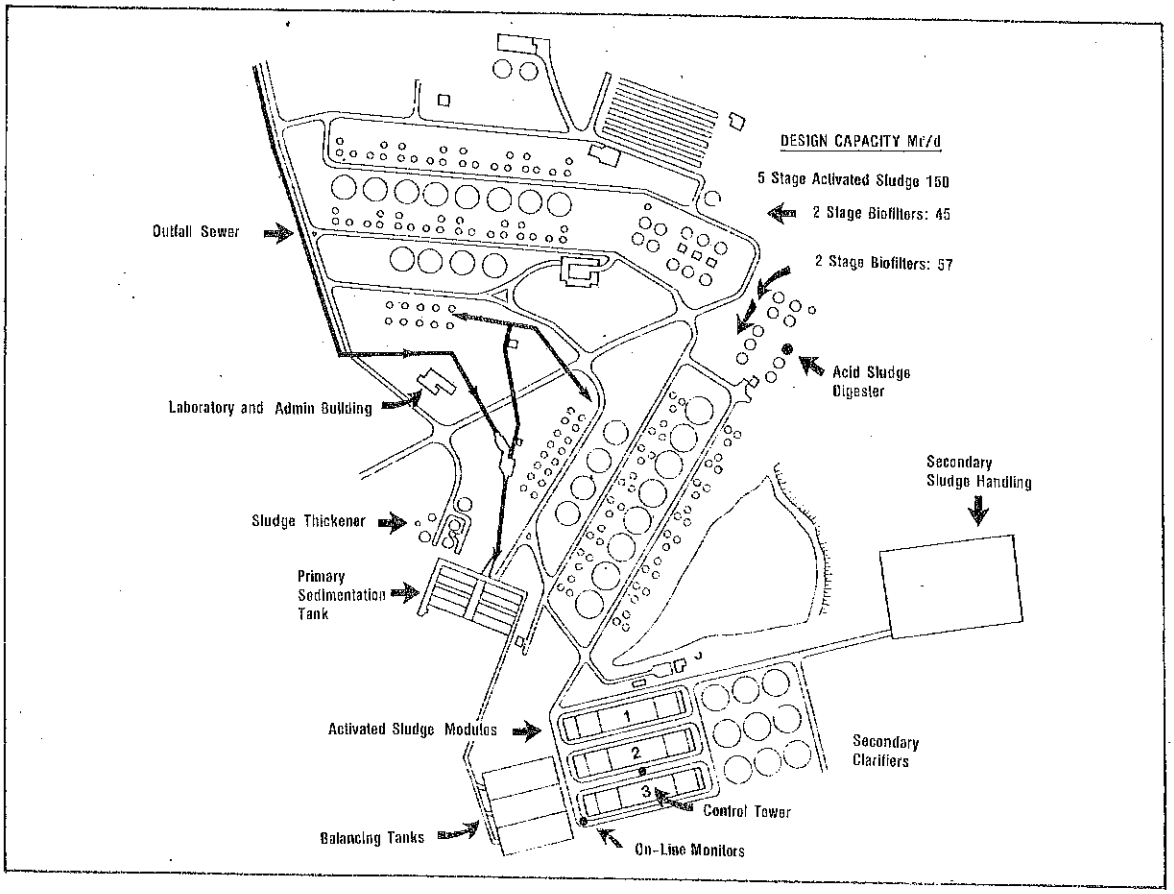

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LIST OF ABBREVIATIONS

ADP	Adenosine diphosphate
AEC	Adenylate energy charge
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
DSVI	Diluted sludge volume index
MLSS	Mixed liquor suspended solids
NAD	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide (reduced form)
o-P	Soluble orthophosphate expressed as phosphorus
PHB	Polyhydroxybutyrate
SVI	Sludge volume index
TKN	Total kjeldahl nitrogen
TP	Total phosphorus
VFA	Volatile fatty acids
VSS	Volatile suspended solids

REPORT BY THE JOHANNESBURG CITY COUNCIL ON ENHANCEMENT OF BIOLOGICAL PHOSPHATE REMOVAL FROM SEWAGE BY ALTERING PROCESS FEED COMPOSITION

EXECUTIVE SUMMARY

Background

Biological phosphate removal plants in South Africa have not always given reliable and satisfactory performance. Whilst this situation applies at the Johannesburg Northern Works, an example of a works treating a relatively weak sewage, it does not apply to the virtually identical 150 Mℓ/d Johannesburg Goudkoppies Plant, which has given consistently reliable results over a number of years. The purpose of the research reported upon herein, was to investigate the reasons therefor and to carry out modifications which would improve plant performance. During the investigational period, compliance with the statutory phosphate effluent standard was relaxed by the Department of Water Affairs.

Modification of Influent Sewage Characteristics

Research at the University of Cape Town (UCT), the National Institute for Water Research (NIWR) and elsewhere, has highlighted the dependence of phosphate removal mechanisms on the presence of certain minimum concentrations of some readily biodegradable materials including, inter alia, volatile fatty acids (VFA). These were produced at the Northern Works by the fermentation of primary sludge, either in the primary sedimentation tanks (PST), or off-line in a separate high rate "acid" digester. In both cases a sludge retention time of 3 to 4 days proved optimal.

Total sludge removal from the fermentation area at periodic intervals was necessary, to prevent the unwanted proliferation of methane forming bacteria. The mass of volatile fatty acids generated in the PST's was far greater than that in the off-line digester, and hence it is the recommended method. Elutriation of the VFA's from the sludge was achieved by the recycle of sludge to the influent sewage stream. This resulted in a

concomitant large increase in the density of the primary sludge, however, the volume of digester gas produced dropped significantly. Significant improvements in plant phosphate removal were noted but the imposition of a higher load on the bioreactor was observed. Further optimisation of this technique, using sludge thickeners, still has to be tried. The proliferation of filamentous organisms possibly as a result of the presence of VFA's is a feature that requires further investigation.

Optimal Use of Readily Biodegradable COD (S_{bs}) of Sewage

Research experience in this project identified the need for ensuring that all readily biodegradable material present in the sewage, or subsequently deliberately generated, should desirably be available to promote the growth of phosphate removing bacteria. To minimise the use of S_{bs} by denitrifiers, one five-stage 50 Mℓ/d module was structurally altered to eliminate the second anoxic zone, and to recreate it upstream of the anaerobic zone. Denitrification of the returned activated sludge was achieved by utilising the breakdown products produced by endogenous respiration of the sludge, thus leaving the S_{bs} available in the feed sewage, which was admitted to the anaerobic zone for the use of phosphate accumulating bacteria.

This mode of operation resulted in a significant improvement in reliability and level of phosphorus removal, but requires to be further proven under wet weather conditions and associated weaker sewages. Furthermore, it is a significant departure from the conventional Bardenpho process and may provide an answer to plants experiencing difficulty in maintaining a high level of phosphate removal.

Performance Monitoring

Two commercial on-line monitors for nitrogen and phosphate measurement were installed, and operational problems and costs identified. Capital costs per instrument ranged from R29 000 to R31 000 (1984). Total monthly running costs for measuring ammonia, nitrate and phosphate continuously, were R756, R535 and R271 respectively.

Measurements of oxygen utilisation rates have highlighted an unexpected

differential demand for oxygen in the main aerobic basin. Further investigations are required to evaluate this type of instrumentation for plant control purposes. A computer-based system for controlling instrumentation and collecting and storing data, has been installed. Software includes provision for automatic plant control which still has to be evaluated.

Process Modelling

The UCT model has been modified to permit its adaptation to an electronic spread sheet format, controlled by a microcomputer. In this form it is capable of use by non-computer trained staff. It has been used routinely to determine the optimal solution to plant operational problems, including identification of a need to increase S_{bs} content. With very little alteration, it is envisaged that this facility could be used for operator training in identifying solutions to problems which could arise during plant operation.

Operational and Design Aspects

The generation of volatile fatty acids by accumulation and recycle of raw sludge, necessitates designers giving special consideration to the adequacy and strength of scraper mechanisms. High sludge densities generated by this technique may require special facilities for removal, including direct connection of desludging pipes to extraction pumps.

The advantages of endogenous denitrification of secondary clarifier underflow have already been cited, but it would appear that plant performance can be further enhanced by providing for plug flow through compartmentalised anaerobic and anoxic zones. Provision of an unhindered surface path from the reactors to the final clarifiers for scum removal is essential.

Maintenance of adequate disssolved oxygen levels in the aerobic zone, appears to control the proliferation of filamentous organisms. Three-stage plants as opposed to the more conventional five-stage plants, may offer advantages in this regard, as the sludge is constantly exposed to aerobic conditions after leaving the anoxic zone.

Operational control of multiple point sludge withdrawal systems installed in secondary clarifiers, is facilitated if the operator can actually view the quality of sludge being withdrawn. Air entrainment in the underflow return system should be minimised, to prevent flotation effects and the formation of scum in the main bioreactor. Such scum or froth may be stabilised by polymers produced by the bacteria.

Designers are urged to pay special attention to the final handling and disposal of phosphate rich sludges, since they tend to leach phosphate on standing and may be more difficult to dewater and dry.

Financial Aspects

At the Northern Works, biological filter plants using chemical addition for phosphate removal and nutrient removal activated sludge plants, exist side by side. For various reasons, a cost comparison of these two systems was abandoned in favour of a desk study in which a four-stage Bardenpho process was used as a base, and to which a number of phosphate removal options were added.

The introduction of an anaerobic zone with sewage character modification to a standard four-stage denitrifying Bardenpho process, was shown to involve an additional cost of 1,6 c/kℓ. Alternatively, chemical phosphate removal in a four-stage plant will involve an additional cost of 2,47 c/kℓ. Retro-fitting of chemical dosing equipment to a two-stage biological filter plant, was calculated to add 7,62 c/kℓ to treatment costs. It would therefore appear that biological removal of phosphate is the more cost effective option.

Microbiological Studies

Severe bulking sludge problems have been experienced, particularly during the winter months, with Microthrix parvicella being identified as the causative organism. Bacterial population studies indicated a dominance of Acinetobacter in aerobic zones, with Aeromonas punctata predominating in the anaerobic zone. Sludge fermenting in PST's was found to contain large numbers of the latter species, but in "acid" digesters, Klebsiella oxytoca predominated. Degradation of the typical Acinetobacter clusters was noted

as a precursor to plant failure in regard to phosphate removal.

Biochemical Studies

Studies of this type were undertaken to provide an insight into intracellular activity, particularly in regard to substrate uptake and conversion. Monitoring of two bacterial storage products, viz polyphosphate and polyhydroxybutyrate, revealed the dependence of the metabolic reactions involved on process conditions such as nitrate levels in the anaerobic zone and sewage VFA level.

Investigations into the intracellular metabolism of Acinetobacter spp have allowed the University of Cape Town researchers, in collaboration with the Johannesburg staff, to propose a biochemical model for phosphate removal in the activated sludge process. This makes a significant contribution to the understanding of the mechanism of enhanced phosphate removal, and allows the UCT mathematical model for the process to be refined.

Future Research

During the period of this contract, a number of areas have been revealed that require further investigation to arrive at conclusive results. In regard to plant scale studies, it is believed that further experience over at least one year, should be obtained in regard to the generation of volatile fatty acids in the PST's, and the more effective use of the readily biodegradable substrate produced, by removing nitrates present in the underflow from the secondary clarifiers by endogenous denitrification.

Technology Transfer

During the Contract, a series of 30 papers were prepared for publication or presentation at scientific meetings or symposia. Two laboratory training seminars, each of two-day duration were organised, at which a total of 12 delegates received instruction into the application of fluorescent antibody techniques for the identification of bacteria. Six papers were presented at a conference held in Johannesburg on 30 October 1986, describing the work carried out under this Contract.

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Dr H N S Wiechers (Chairman)	Water Research Commission
Mr J Goodman	Johannesburg City Council
Mr D W Osborn	Johannesburg City Council
Mr J E McGlashan	Water Research Commission
Prof P L Steyn	University of Pretoria
Prof GvR Marais/Dr G Ekama	University of Cape Town
*Mr A Gerber	National Institute of Water Research (CSIR).

* Invited to the last meeting of the Committee only.

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CHAPTER ONE

Introduction

1.1 BACKGROUND

Research into biological nutrient removal from sewage has resulted in significant advances over the last ten years. Various factors have been identified as essential in the regulation of phosphate removal from sewage. The three most important of these are :-

- Composition of sewage to be treated
- Nature of phosphate removing bacteria
- Process reaction conditions.

1.1.1 Sewage Composition

Successful sewage treatment depends on the maintenance of the correct biomass in the process. This biomass must be in such a state that all process performance requirements are met. The type of biomass required to remove most of the pollutants from sewage can be establisned and maintained, using the substrate material available in the incoming sewage, it being only necessary to provide the process conditions for growth of desired biomass. However, process conditions are often not optimal for biological nutrient removal processes.

Although a high degree of denitrification can be achieved by providing the correct process conditions (feeding of nitrified mixed liquor to anoxic zones), rapid and complete nitrogen removal can only be assured if the process is fed with an adequate supply of readily biodegradable substrate

(S_{bs}) (Siebritz et al.,1983). More stringent requirements apply to the biological phosphorus removal process. In this process, most, if not all, of the phosphorus is removed by its excess accumulation in a specified biomass fraction of which Acinetobacter spp usually make up the majority species. Acinetobacter spp are obligate aerobes requiring a fairly restricted spectrum of substrates for their proliferation in the activated sludge process. These substrates include the volatile fatty acids (VFA), for example, acetic, propionic, butyric, and valeric, which are the main products of high rate anaerobic fermentation. Depending on circumstances, these short chain organic fermentation products can make up a fair proportion of the readily biodegradable substrate fed to the process.

Experience in the last few years has shown that, depending on their origin and history, sewages can contain varying quantities of the substrates required for biological nutrient removal. However, even if such substrates are present, competition for them can occur in the process. This is particularly so in the case of Acinetobacter spp where other bacteria such as denitrifiers can assimilate a large proportion of the volatile fatty acids available, thus inhibiting the growth of these phosphorus accumulating bacteria.

Current thinking on biological phosphorus removal is that the success of this process depends on providing enough of the correct short chain organic or volatile fatty acids substrates to the process and creating the environment where phosphorus accumulating bacteria can assimilate these substrates in a favourably uncompetitive environment. The latter condition can be met by providing the correct process layout, but if the desired substrates are not present in the sewage arriving at a plant, alteration of the sewage characteristics will be required.

1.1.2 Phosphate Removing Bacteria

While preliminary work has identified Acinetobacter spp (Buchan,1983) as playing the primary role in phosphate removal, the relationship between this bacterial species and others present in the activated sludge system has yet to be investigated.

1.1.3 Process Reaction Conditions

Monitoring of plants which successfully remove phosphate to below the required limit of 1,0 mg o-P/ℓ, has shown that a number of operational parameters require special attention in the achievement of this objective. For example, excess nitrate in the sludge returned to the anaerobic zone has a deleterious effect on phosphate removal. Insufficient aeration reduces the efficiency of removal, and the growth of filamentous organisms appears to be enhanced in phosphorus removal plants, causing problems with final clarification.

The Johannesburg City Council entered into a contract with the Water Research Commission, to undertake research into the areas outlined above.

Experimentation at full scale inevitably leads to fluctuations in plant performance, rendering it impossible to comply with the effluent standard at all times. To enable the research to continue, the Department of Water Affairs allowed a relaxation in the phosphate effluent standard for the duration of the Contract.

1.2 RESEARCH PROGRAMME

1.2.1 Sewage Composition

There are basically two sources of the substrates considered desirable for biological nutrient removal, suitable industrial effluents and the high rate anaerobic fermentation products (short chain organic compounds). Certain industries (e.g. brewing and fermentation) can discharge effluents containing high concentrations of short chain organic substances which can greatly enhance the S_{bs} content of sewage and hence, its potential for the removal of nutrients. In particular, industries such as the oil from coal industry, produce relatively large quantities of liquid effluents containing high concentrations of volatile fatty acids. Such wastes are ideal substrates for Acinetobacter spp and their discharge into sewers feeding biological nutrient removal plants should be welcomed, provided aeration capacity exists to handle the additional organic load and the non-biodegradable fraction in the final treated effluent is not excessive.

In most cases, suitable industrial wastes are not available and other methods have to be sought to provide the necessary substrates. At present, high rate anaerobic fermentation seems to be the only viable alternative to specific industrial wastes.

Fresh domestic sewage contains relatively small quantities of these fermentation products, but if it is retained under anaerobic conditions, acid fermentation will readily occur. The first place this can occur is in the sewerage system. Sewers with a low gradient, long retention period or involving pumping, tend to go anaerobic, particularly in warm weather. This VFA generation in sewers could explain the chance occurrence of biological phosphorus removal in certain plants not designed for this purpose.

It is felt however, that one cannot rely on in-sewer fermentation as a source of substrate for biological phosphorus removal bacteria, as modern sewers are being designed with relatively steep gradients and aerated conditions, to obviate corrosion and odour problems. In most cases therefore, consideration has to be given to the generation of necessary substrates on the site of the sewage works.

One of the main objectives of this research programme was to evaluate the various methods of increasing the readily biodegradable substrate concentrations in influent sewage.

High rate anaerobic fermentation of organic compounds in raw sewage appears to be a viable method of generating substrates necessary for biological nutrient removal (Venter et al.,1978). In general, anaerobic fermentation is slower than aerobic metabolism, taking of the order of days, instead of hours. Thus, it would be uneconomical to consider anaerobic fermentation of the whole sewage flow. The use of sewage sludges, both primary and secondary, was therefore considered.

1.2.1.1 Fermentation of raw sludge in primary sedimentation tanks

When suspended organic solids are removed from sewage in primary sedimentation tanks, they readily undergo anaerobic fermentation in the

accumulated sludge layer in the bottom of these tanks. The effect of various sludge residence times in these tanks was investigated. The same applies to an even greater extent to primary sludge in thickeners, and provides a ready method of increasing the S_{bs} and volatile fatty acid content of sewage.

1.2.1.2 Fermentation of raw sludge in a separate high rate digester

On some installations it might not be possible to carry out fermentation in sedimentation tanks or thickeners, i.e. the sedimentation tanks could have inadequate facilities to handle larger volumes of sludge and the plant may not have thickeners. The use of a separate anaerobic digester was therefore also considered. Further advantages could flow out of the use of an acid digester, in that process conditions such as retention time, mixing temperature, etc can be manipulated better than in a sedimentation tank.

1.2.1.3 Supplementary acid production in activated sludge reactors

A further source of fermentation products could be activated sludge where the fermentable substrate would be the biomass itself or adsorbed colloidal and other suspended solids. Early successes in the studies of the biological nutrient removal process were reported by Barnard(1974) and Johannesburg researchers (Nicholls,1975 and Venter et al.,1978), have indicated that considerable success could be obtained by allowing activated sludge to undergo fermentation.

In reviewing his initial studies, Barnard(1974) felt that it was the anaerobic fermentation of activated sludge in a leaking compartment adjacent to the main reactor, which provided a source of VFA for phosphorus removal. In studies at Alexandra Works (Nicholls,1975) and Olifantsvlei Works (Venter et al.,1978), successful phosphorus removal was obtained by switching off a number of surface aerators at the inlet of the extended aeration activated sludge process. These aerators were kept off for considerable periods (up to 22 to 24 h/d,), during which time activated sludge and raw sewage solids settled to the floor of the basin and underwent high rate fermentation. Measurements showed that in excess of

3000 mg/ℓ VFA (as acetic acid) could be generated in the sludge layer on the floor of the reactor. When the stationary aerators were operated for a brief period of about 2 hours, settled sludge was resuspended and the VFA made available to aerobic phosphorus accumulating organisms. When operated in this mode, the plant produced an effluent containing less than 1 mg o-P/ℓ for considerable periods.

1.2.2 Methods of Adding Fermentation Products to the Process

Once fermentation products have been produced, they have to be made available to phosphorus accumulating bacteria in quantities sufficient to satisfy their maximum demand. In this regard, competition from other aerobic and denitrifying organisms for the available substrate should be avoided by introducing the fermentation products into an anaerobic zone which has a minimal input of oxygen and nitrate.

In order to achieve this objective, various methods of adding fermentation products to the process were considered.

1.2.2.1 Addition of whole fermented raw sludge

In this option, fermented primary sludge either from the bottom of sedimentation tanks or from an acid digester was added back into the feed to the anaerobic zone of the five-stage Bardenpho process, as in Figure 1.1(a). Ideally, the quantity of "acid" sludge added should match the requirements for phosphorus removal and if there is any excess, it should pass to conventional methane producing digesters. On smaller plants where the extended aeration principle of aerobically treating raw sewage is used, all of the fermented sludge would be returned to the activated sludge process. The use of primary sedimentation tanks ahead of the so-called extended aeration process might sound strange, but if the substrates required for biological nutrient removal are not available in the feed sewage, or cannot be generated in the activated sludge reactor, their use might provide the only means of obtaining effective biological phosphorus removal.

1.2.2.2 Addition of fermented raw sludge to sewage inlet of primary sedimentation tanks

On many plants, particularly the larger ones, it will not be economical to return all the acid sludge from a digester to the activated sludge process, especially considering the additional power and tank volume required to handle all the sludge solids. In such cases it is necessary to elutriate the volatile fatty acids and other substrates out of the acid sludge. The method which was considered was to feed the "acid" sludge into the inlet to the primary sedimentation tanks where it comes into contact with a large volume of sewage for elutriation and the redundant solids are settled out for removal to a conventional digester - see Figure 1.1(b).

An example of this application is that the required volume of acid sludge is discharged continually or semi-batchwise from the acid digester to the sedimentation tank feed, and a settled sewage rich in necessary substrates, flows to the anaerobic zone of the nutrient removal process - see Figure 1.1(c). If the acid digester is the bottom of the sedimentation tank, one has the situation of primary sludge recycle.

1.2.2.3 Recycling of primary sludge

In this option sludge was accumulated in the bottom of primary sedimentation tanks and allowed to go anaerobic, and a portion of the acid sludge was recycled to the tank inlet. This recycle allows for the elutriation of the fermentation products into the main sewage flow and increases the sludge retention period in the sedimentation tanks. The degree of recycling depends on the quantity of fermentation products required by the process.

1.3 EVALUATION OF PLANT RESPONSE

In order to evaluate the effect of the addition of more readily biodegrdable substrate on the activated sludge plant, intensive monitoring of the process was required. In this respect, normal routine process parameters like COD, nitrate and phosphate had to be determined.

1.3.1 On-line Monitoring

In order to accurately monitor rapid changes which could occur in the process, it was decided to use on-line monitors to continuously monitor effluent phosphate, nitrate and ammonia levels. Furthermore, oxygen utilisation rates were monitored on a semi-batch basis at half hour intervals, at three points in the aeration basin.

1.3.2 Mathematical Modelling

The University of Cape Town's general activated sludge model was adapted for use on a microcomputer, using a spread sheet, to render the model more accessible to plant management staff.

1.3.3 Microbiological Investigations

In order to fully evaluate the effect of operational changes, it was necessary to investigate alterations in microbiological population structure and bacterial behaviour, during different modes of plant operation. To this end, a number of analytical techniques had to be developed or refined.

1.3.4 Biochemical Studies

The achievement of the objectives in this programme required the determination of parameters not normally considered to be part of routine sewage analysis. Here again, techniques had to be developed or refined. In addition, certain fundamental studies were carried out in an attempt to gain greater insight into the mechanisms of phosphorus removal. These included studies into some basic aspects of bacterial metabolism and biochemical parameters.

1.4 FINANCIAL ASPECTS

During the last decade of intensive investigation into enhanced biological phosphorus removal, no critical analysis of the cost of this process has been made, particularly in comparison to the relatively simple solution

provided by chemical precipitation. It was therefore considered expedient to address this issue during this study. An attempt was made within the prevailing accounting constraints, to identify the major cost differences between various treatment processes.

1.5 GENERAL

In each of the following Chapters, particular aspects of the research programme are discussed in detail. Largely due to the very specific nature of some of the experimental work, these Chapters have been structured in such a way as to allow each to stand alone. Chapter Ten however, attempts to place all the findings in perspective.

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CHAPTER TWO

Full scale studies in Johannesburg

2.1 INTRODUCTION

The methods of improving the characteristics of a sewage for biological phosphorus removal described in Chapter 1, were studied at full scale in Johannesburg, mainly at the Northern Sewage Works. As many of the concepts involved were new, the ideal facilities did not exist, so that existing plant had to be adapted to try and suit requirements. Often the difficulties of doing this hampered the interpretation of the data obtained. In addition, two other factors which contributed to this difficulty, were the need to produce a final effluent which satisfied the regulatory authorities as well as mechanical breakdowns and other plant or equipment deficiencies.

2.2 NUTRIENT REMOVAL UNDER OPTIMAL CONDITIONS

In contrast to the Northern Works, the five-stage Goudkoppies Works in Johannesburg is an example of a plant which receives a sewage containing more than adequate quantities of the necessary short chain organic fermentation products, such as volatile fatty acids (VFA). This plant, which is based on the five-stage Bardenpho layout is fed by two sewers, the so-called Main Sewer, which is a high gradient tunnel sewer draining the southern central city area and the Relief Sewer, which is a very low gradient (1 in 3 000) contour sewer. Sewage in the Main Sewer arrives at the plant in a fairly fresh state, while the contents of the Relief Sewer

are highly septic and contain large quantities of high strength effluents from a yeast factory.

During the course of these investigations, the characteristics of the sewage feeding Goudkoppies were examined on a number of occasions, as techniques for doing the required tests were evolved. First estimates of the readily biodegradable COD (S_{bs}) content of the Relief Sewer as determined by the denitrification test (Stern and Marais, 1974), are shown in Table 2.1. As can be seen, the Relief Sewer contains a very high strength sewage, which has a large concentration of S_{bs} (approximately 340 mg/ℓ), the highest of all sewages in Johannesburg. Later examination of the volatile fatty acid content of the sewages revealed the results shown in Table 2.2. Here we observe that the Main Sewer has a fairly low concentration of volatile fatty acid, bearing out its aerobic state. However, the Relief Sewer contains high concentrations of volatile fatty acid which results in the combined feed to the nutrient removal modules containing over 200 mg/ℓ volatile fatty acids (as acetic acid). This concentration appeared to be too high to be completely taken up by the biomass in the anaerobic zone, as 45 mg/ℓ volatile fatty acid still remained in solution in the effluent from this zone. The results confirm that significant fermentation takes place in this sewer which renders the sewage highly suitable for biological nutrient removal.

These results were followed up at a later stage by further tests for the readily biodegradable COD content of the feed sewage at Goudkoppies, using ultra- filtration (see Chapter 5 for details). The results obtained are depicted in Table 2.3, where it can be seen that a high concentration of S_{bs} (approximately 240 mg/ℓ) was found again with the strong implication that most of the S_{bs} was present as volatile fatty acid.

TABLE 2.1 : MEAN VALUES FOR THE READILY BIODEGRADABLE COD* CONTENT OF THE RELIEF SEWER FEEDING THE GOUDKOPPIES WORKS

Sample	Total COD (mg/ℓ	Readily biodegradable COD (mg/ℓ)	Readily biodegradable COD fraction
Relief sewer when yeast waste present	2 130	360	0,17
Relief sewer when yeast waste not present	950	290	0,31
Average Relief Sewer contents	1 740	340	0,20

(* As determined by the denitrification method)

TABLE 2.2 : VOLATILE FATTY ACID CONCENTRATION OF SEWAGES AND ANAEROBIC ZONE AT THE GOUDKOPPIES WORKS

Sample	** Total COD (mg/ℓ)	VFA as acetic acid (mg/ℓ)	VFA/COD ratio
Main sewer	180	24	0,13
Relief sewer with yeast waste	1 630	400	0,24
Relief sewer without yeast waste	980	230	0,23
Feed to activated sludge units *	760	220	0,29
Anaerobic zone	-	45	-

* Mixture of Main Sewer and Relief Sewer after primary sedimentation and balancing

** Results obtained from a single 24 hour composite sample.

TABLE 2.3 : MEAN VALUES FOR THE READILY BIODEGRADABLE COD* CONTENT OF THE SETTLED SEWAGE AT THE GOUDKOPPIES WORKS

	Total COD (mg/ℓ)	0,45µ filtered COD (mg/ℓ)
Feed to reactors	600	306
Final effluent	75	68
Net amount (S_{bs})	-	238
S_{bs} fraction of Total COD	-	0,40

(* Ultrafiltration)

The suitability of the sewage arriving at the Goudkoppies Works for biological nutrient removal is borne out by the results obtained during extensive monitoring. Table 2.4 shows a statistical analysis of the results obtained from about 1 000 four-hourly samples taken of the effluent from one module.

TABLE 2.4 : EFFECT OF SEPTIC SEWAGE ADDITION ON EFFLUENT QUALITY GOUDKOPPIES WORKS

	Septic Sewage Added			
	T-p	o-P	N/NH_4	N/NO_3
Arithmetic mean *	9,66	0,36	0,39	1,60
Standard deviation *	0,42	0,35	0,97	2,16
Geometric mean *	0,58	0,27	0,01	0,54

	COD	BOD	TKN	T-P
Influent to reactor	600	260	38	7,3

(* 1 000 samples taken at 4 h intervals. Results in mg/ℓ)

A further statistical evaluation of the results obtained on about 330 two-hourly composite samples is shown in Table 2.5, with the relevant histograms for Module 1 being depicted in Fig 2.1. Modules 2 and 3 returned similar performance data. As can be seen, the Goudkoppies plant can produce consistent nutrient removal with the 1 mg P/ℓ orthophosphate effluent limit being met for 95 to 97 % of the time and these conditions should be emulated on other plants.

The septic sewer conditions and the yeast industry effluent however, the conditions at Goudkoppies probably represent a special case. Nevertheless, the "acid" fermentation taking place in the Relief Sewer could be replicated by the fermentation of primary sludge on other works.

TABLE 2.5
STATISTICAL EVALUATION OF EFFLUENT QUALITY FOR YEAR 1985

GOUDKOPPIES Sample	Total P as P	ortho-P as P	TKN as N	Ammonia as N	Nitrate as N	COD	Suspended solids
	(Results expressed as mg/ℓ)						
Effluent from Module 1							
Arithmetic mean	0,98	0,34	4,34	1,40	2.40	101,34	16,04
Standard deviation	1,40	0,41	2,59	1,71	2,66	42,24	30,52
Geometric mean	0,77	0,25	3,96	0,41	1,19	95,63	11,16
Effluent from Module 2							
Arithmetic mean	1,16	0,39	3,98	0,86	2,77	100,89	17,07
Standard deviation	1,82	0,74	2,92	1,25	2,93	43.56	34,99
Geometric mean	0,79	0,23	3,52	0,24	1,51	94,81	11,32
Effluent from Module 3							
Arithmetic mean	1,77	0,35	5,53	1,19	2,00	117,31	35,93
Standard deviation	3,35	0,57	7,29	1,90	2,54	94,68	91,05
Geometric mean	1,00	0,25	4,27	0,35	0,86	104,78	16,91
Feed sewage to modules							
Arithmetic mean	9,28	6,39	47,93	34,41	-	698,61	-
Standard deviation	1,60	1,47	9,43	5,39	-	224,91	-
Geometric mean	8,68	6,22	47,01	34,00	-	666,72	-

Note : Data has been derived from 335 daily composite samples taken during the calender year 1985

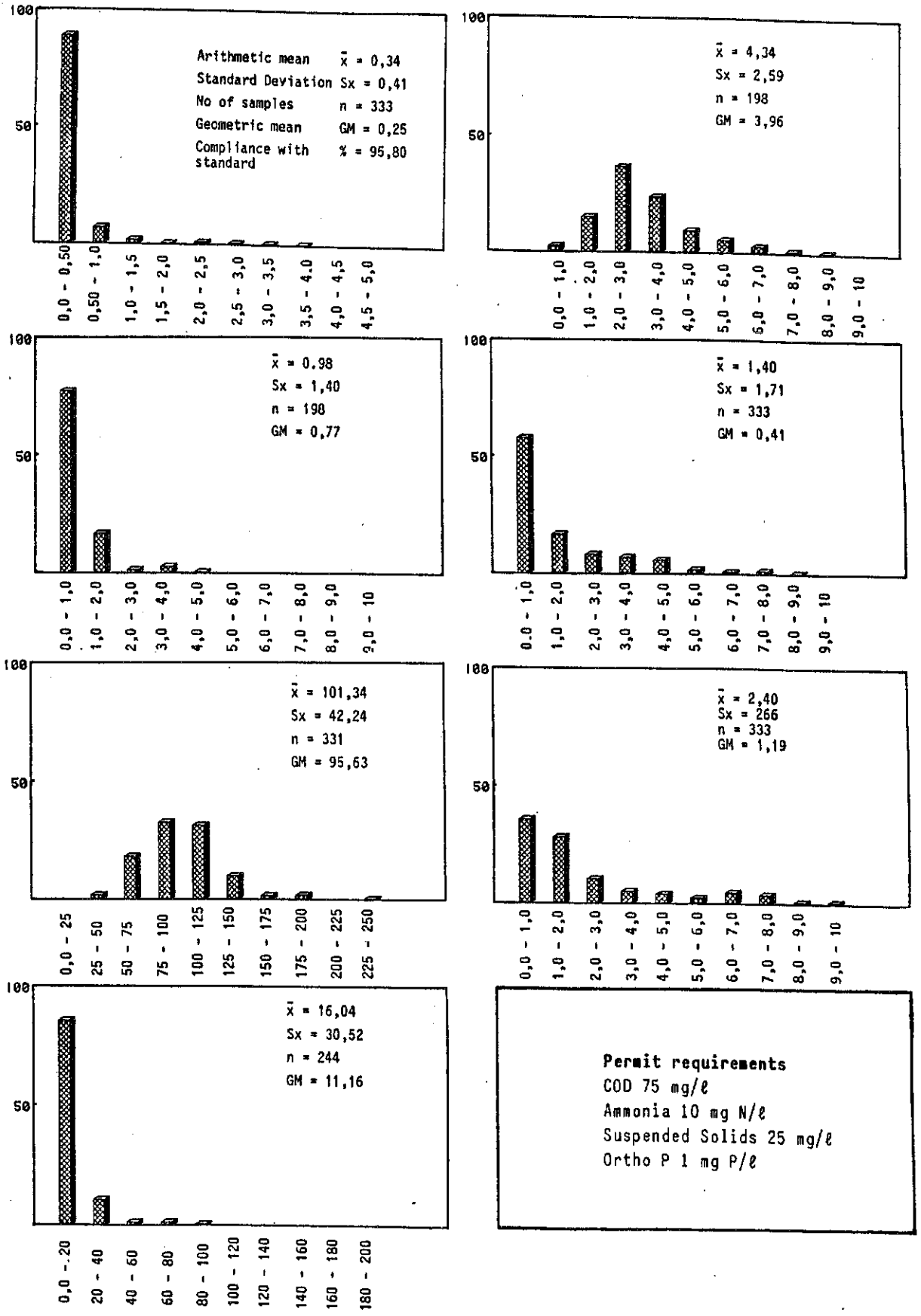


Figure 2.1 : Goudkoppies Module 1. Percentage of samples in different concentration ranges and permit requirements.

2.3 AUGMENTATION OF FATTY ACID CONTENT OF INFLUENT SEWAGE

2.3.1 Fermentation of Activated sludge in Main Reactor

Barnard (1984) has indicated that the fermentation of activated sludge could provide a volatile fatty acids substrate for biological phosphorus removal. In addition, Venter et al. (1978) showed that when the aerators at the inlet end of an extended aeration plant were switched off, the sludge settled to the floor of the reactor and readily underwent fermentation to produce volatile fatty acids.

This concept was studied further under this present contract. The aim of the experiment was to determine if fermentation could be encouraged in the anaerobic zone of the five-stage Bardenpho process by firstly, increasing the retention time in this zone by passing the majority of the feed to the first anoxic zone and secondly, by causing the activated sludge to settle on the floor of the anaerobic zone.

The following operating conditions were studied :-

(i) All the feed sewage was bypassed around the anaerobic zone and both mixers in this zone were switched off, allowing activated sludge to accumulate on the floor.

(ii) The same as in (i), except that about 25 % of the feed sewage was fed to the anaerobic zone and the remainder bypassed to the first anoxic zone.

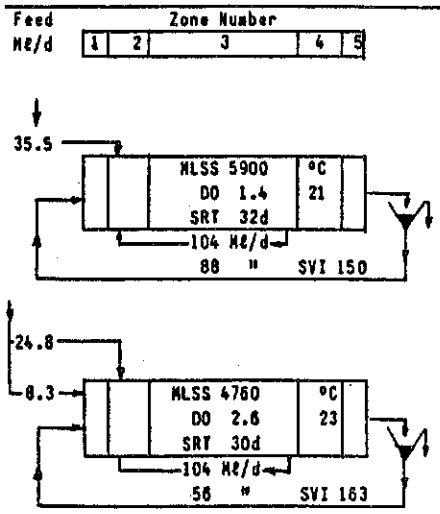
Mean steady state results obtained at the Northern Works during these operating conditions are shown in Table 2.6. It should be noted that the volatile content of the mixed liquor suspended solids in all the tests conducted at Northern Works, was 72 %.

As can be seen, nitrogen removal was good and although a considerable mass of phosphorus was removed, the phosphate standard of 1 mg o-P/ℓ could not be met. However, the fact that such quantities of phosphorus were removed, indicated that suitable conditions prevailed in the anaerobic zone

despite it receiving none or only limited, amounts of feed which contained all of the readily biodegradable COD fed to the process. Also, the readily biodegradable COD levels measured by the denitrification method (see Chapter 5), in the sludge layer on the floor of the anaerobic zone were relatively low, namely, 66 and 110 mg/ℓ.

TABLE 2.6

EFFECT OF VARIOUS OPERATIONAL MODIFICATIONS TO THE THE PERFORMANCE OF THE JOHANNESBURG NORTHERN WORKS



Sample Point	COD Total	COD S_{bs}	VFA as Acetic	Nitrogen TKN	Nitrogen NH_4	Nitrogen NO_x	Phosphorus Total	Phosphorus ortho
Feed to anoxic zone								
Influent ex								
. Balancing tank	555			54	31,6	-	15,6	10,1
Zone : Anaerobic 1		114	55		3,3	0,1		9,8
. Anoxic 2					5,4	0,5		7,8
. Aerobic 3					2,5	2,1		4,4
. Anoxic 4					2,5	0,5		7,5
Final effluent 5	71				1,9	0,2		3,7
Split Feed - Anaerobic Zone								
Influent ex								
. Balancing tank	530	68		45	30,2	-	15,3	10,9
Zone : Anaerobic		66			2,7	0,3		12,7
. Anoxic 1					4,7	0,2		10,3
. Aerobic 1					1,3	3,0		4,4
. Anoxic 2					1,4	1,2		6,9
Final effluent	67				1,4	1,8		3,7

Results expressed as mg/ℓ, where applicable

This was especially so, considering that this concentration still had to be diluted with the bulk of the zone contents which had undetectable concentrations of readily biodegradable COD (S_{bs}). By comparison, Venter et al.,(1978) found up to 1500 mg/ℓ volatile fatty acids in the sludge layer during similar experiments at the Olifantsvlei extended aeration plant. Possible explanations for the low S_{bs} levels found are :-

- the S_{bs} was used almost as quickly as it was formed
- the S_{bs} was not a suitable substrate for denitrifiers and hence could not be detected via the denitrification method.
- as not all of the feed entered the anaerobic zone, limited substrate was available for fermentation into S_{bs}.

The last one provides the most feasible explanation, especially if it is noted that the Olifantsvlei results were obtained with an unsettled sewage

feed passing straight into the inlet of the process, where aerators were turned off. Nevertheless, it is interesting to note that large masses of phosphorus were accumulated within the sludge despite there being very little S_{bs} present in the anaerobic zone.

2.3.2 Fermentation of Raw Sludge in a Separate High Rate Digester

The primary sludge settled out from sewage in sedimentation tanks, readily undergoes "acid" fermentation. The analysis of many primary sludges has shown that they can contain between 1000 and 3000 mg/ℓ total volatile fatty acids (as acetic acid) at a MLSS of 3 - 5 %, showing that some fermentation has already taken place in the primary tanks.

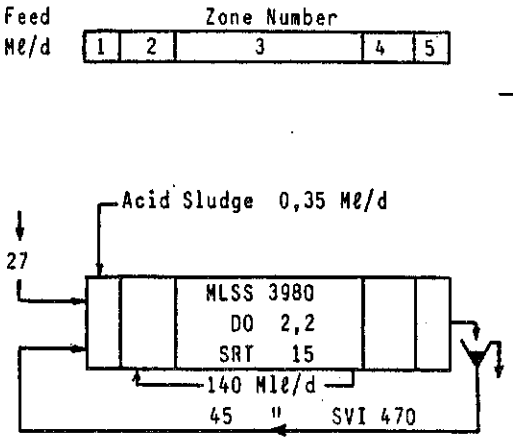
The use of primary sludge to improve nutrient removal was studied under the previous Water Research Commission contract with Johannesburg (Pitman, 1982). In order to increase the COD load on the five-stage Northern Works plant, primary sludge was fed back into the anaerobic zone. (This was conventional primary sludge containing about 2 % total solids and had not been deliberately stored in the primary sedimentation tanks). A considerable increase in both nitrogen and phosphorus removal was noted. This result pointed to the need to consider the fermentation of primary sludge as a source of volatile fatty acids for biological nutrient removal processes with a separate high rate digester providing a possibility for optimising volatile fatty acids production.

Workers in Johannesburg first experimented with high rate digestion in the early phosphorus removal studies at Olifantsvlei (Venter et al.,1978). Considerable success was obtained when sludge from an overloaded anaerobic digester with a mean retention period of around 8 days was added batchwise to an extended aeration plant.

In investigations under the present Water Research Commission contract, high rate digestion was studied at the Northern Works. At first a 2000 m^3 digester was operated on a semi-batch basis, with a mean retention period of 6 days. Acid supernatant liquor from this digester was added directly to the anaerobic zone of a five-stage Bardenpho module, over a period of only 4 h per day.

The results obtained are shown in Table 2.7 and Figure 2.2.

TABLE 2.7
PERFORMANCE OF THE JOHANNESBURG NORTHERN WORKS
WITH ADDITION OF ACID DIGESTER SUPERNATANT



Sample Point	COD	Nitrogen			Phosphorus	
		TKN	NH_4	NO_3	Total	ortho
Influent	360	38			11	7,7
Acid Sludge	26000	1180			500	
Mixture	700	53			17,5	
Zone :						
Anaerobic 1			8,1	0,1		10,5
Anoxic 2			4,9	0,8		6,6
Aerobic 3			2,0	4,0		2,9
Anoxic 4						
Final Effl			1,6	2,8		2,2

Results expressed as mg/ℓ, where applicable

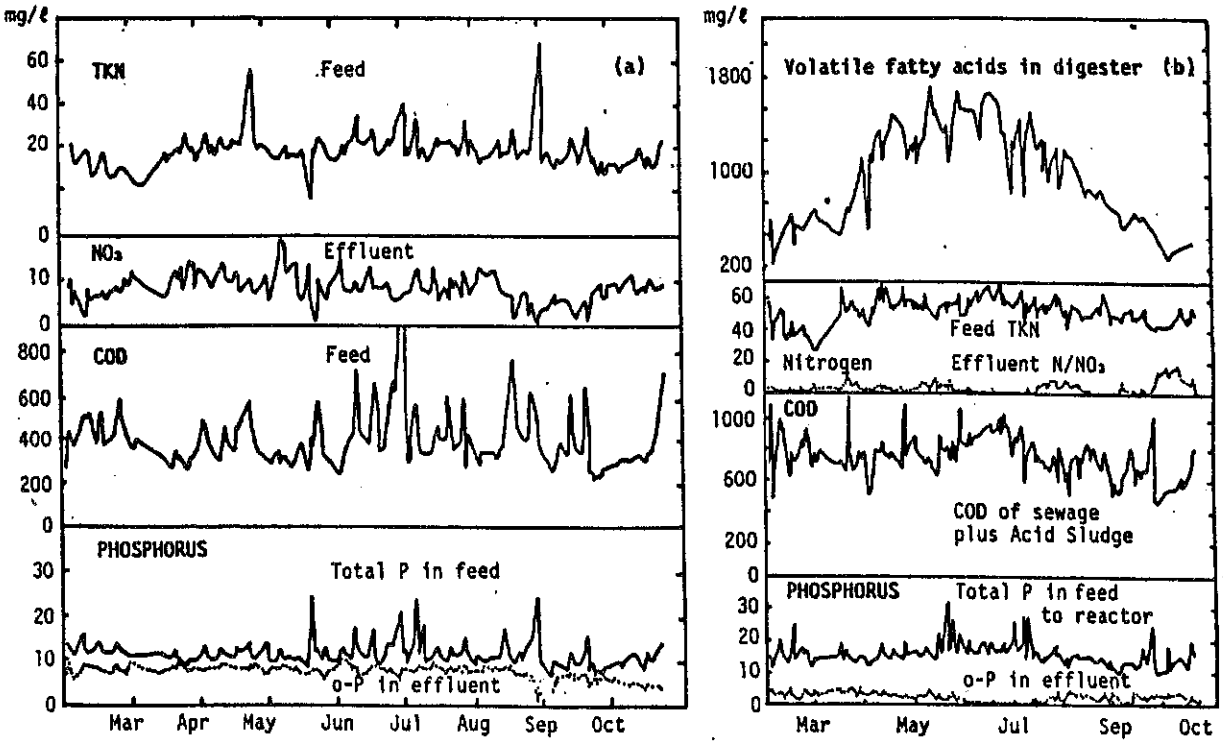


Figure 2.2 : Acid Sludge Supernatant Addition to the Northern Works

(a) Prior to acid sludge supernatant addition
(b) After acid sludge supernatant addition

The production of adequate quantities of volatile fatty acids in the digester was not as easy as at first thought, as it took some time for the concentration in the supernatant liquor to reach high levels. The highest levels (~ 1800 mg/ℓ as acetic acid), were reached in the winter months, whereafter, the volatile fatty acids production rate reduced again so that by October it became obvious that it would not be possible to maintain the digester in the acid phase and the experiment was abandoned. The warmer weather may have encouraged the growth of methane fermenters and sufficient sludge was not available to reduce the retention to more realistic values (about 2 days) for the summer conditions. In addition, the semi-batch nature of the operation did not promote the wash-out of methane fermenters from the system. Similar results were also reported by researchers at the University of the Orange Free State (Toerien et al.,1983).

Nitrogen removal improved due to the increased COD load, in particular, the increased input of S_{bs}, while phosphorus removal improved dramatically, due to the low nitrate levels in the return sludge and the improved volatile fatty acids input to the anaerobic zone. In fact, the lowest phosphate values measured in the Northern Works effluent to date, were observed when the volatile fatty acids content of the digester liquor was at its highest levels. This work has been described in greater detail by Osborn (1985) and Nicholls et al. (1986).

After plant modifications to improve draw-off and pumping, the experiments with acid digestion were continued. Here two 2000 m^3 anaerobic digesters were used to produce acid supernatant liquor for addition to the test module. The digesters were operated alternately on a fill and draw cycle, being filled with a daily batch of primary sludge over a period of 3 to 4 days and mixed after each daily feed for 4 to 6 hours. Acid supernatant liquor was fed to the anaerobic zone of the test module in daily batches (pumped over about 8 hours) for the next 2 to 3 days. The digesters were then emptied via the underflow and the cycle repeated. It should be noted that the primary sludge used as a feed to the digesters came from a nearby trickling filter plant and not the primary sedimentation tanks on the plants, which , as mentioned earlier, were being used to deliberately accumulate sludge so that it could undergo acid fermentation.

Table 2.8 shows some typical results obtained with these digesters.

TABLE 2.8
PRODUCTION OF VFA IN HIGH RATE DIGESTER
NORTHERN WORKS

Samples	Total VFA as acetic acid (mg/ℓ)
Feed sludge from primary sedimentation tanks on biofilter plant	1 080
Supernatant liquor feed to Bardenpho module	1 390
Sludge removed from bottom of acid digester and fed to secondary digesters	2 450

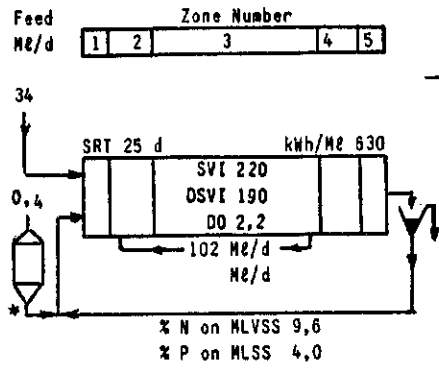
The results show that there was a considerable concentration of volatile fatty acids in the feed to the acid digester. This was due to the 12 to 18 h retention period of the sludge in the primary sedimentation tanks from the biofilter plant. The acid digestion process increased the quantity of acids produced by about 100 % but due to poor elutriation, the concentration of volatile fatty acids in the supernatant liquor was not significantly higher than that in the feed sludge. Unfortunately, the greater mass of volatile fatty acids was lost via the digester underflows. This pointed to the need to find better methods for the elutriation of volatile fatty acids products from fermented sludge solids. The acids produced in the acid digester consisted mainly of almost equal amounts of acetic and propionic acids.

Supernatant liquor from the acid digester was added to the return sludge line of one of the test modules. This module thus received volatile fatty acids from two sources; the primary sedimentation/balancing tanks and the acid liquor. It is estimated that the acid liquor accounted for about 0,5 t/d of volatile fatty acids and the feed to the reactor from the balancing tank accounted for 1,5 t/d.

The performance of this module was fairly good (see Table 2.9), with phosphorus being removed from an inlet value of 15 mg P/ℓ to an effluent orthophosphate of 1,3 mg P/ℓ. However, it was not possible to meet the 1

mg P/ℓ phosphate standard for extended periods. Nitrogen and COD removals were also good.

TABLE 2.9
THE ADDITION OF ACID SLUDGE TO THE ANAEROBIC REACTOR
NORTHERN WORKS



Sample Point	COD	VFA as acetic	Nitrogen TKN	Nitrogen NH_4	Nitrogen NO_3	Phosphorus Total	Phosphorus ortho	Susp solids
Influent	880	76	56			15		
Zone :								
Anaerobic				8,9	0,2		12	
Anoxic 1				5,8	0,6		9,3	
Aerobic 2				1,5	5,3		2,6	
Anoxic 1				5,2	1,7		16	
Aerobic 2				1,6	3,0		2,0	4200
Final Effl	87			1,1	2,9		1,3	37

Note : 1. Results expressed as mg/ℓ, where applicable.
2. Experimental period 20/5/85 - 20/6/86.
3. Number of occassions on which the plant was tested : 18.

The results of these experiments showed that the fermentation products generated during the high rate digestion of primary sludge, can greatly assist the biological nutrient removal. Whether a separate high rate digestion tank will provide the optimum results has not been proven directly, as the digesters used in these tests did not increase the volatile fatty acids production to the extent that the use of such digesters is warranted. This is because the sludge fed to the digesters already contained considerable amounts of volatile fatty acids which had been generated in the primary sedimentation tanks. Consequently, optimisation of the fermentation in primary sedimentation tanks may obviate the need for a separate high rate digester.

2.3.3 Fermentation of Raw Sludge in Primary Sedimentation Tanks

Extensive experiments were conducted at Northern Works to try and alter the characteristics of the feed to the biological nutrient removal plants by encouraging the formation of fermentation products in the primary

sedimentation tanks (PST).

Prior to these experiments, these tanks were desludged fairly frequently, and the sludge withdrawn had a total solids of about 2 % and a pH of above 6. For this study, desludging schedules were altered to allow sludge to accumulate on the bottom of the rectangular PST's where it underwent anaerobic fermentation. The sludge withdrawn now had a solids concentration in the range of 4 to 7 % and the pH dropped to about 5,5. The sludge residence period in these tanks was consequently considerably increased, but it was not possible to obtain any estimate of exact hold-up times.

Considerable quantities of volatile fatty acids were produced in the PST's with the thickened underflow sludge containing up to 3000 mg/ℓ (total as acetic acid) and the typical operating range being 1000 - 2000 mg/ℓ (total as acetic acid) with the actual value depending on sludge retention period and solids concentration. Analysis showed that in contrast to the "acid" digester, the acids produced comprised 70 % acetic acid and 28 % propionic acid (see Figure 2.3).

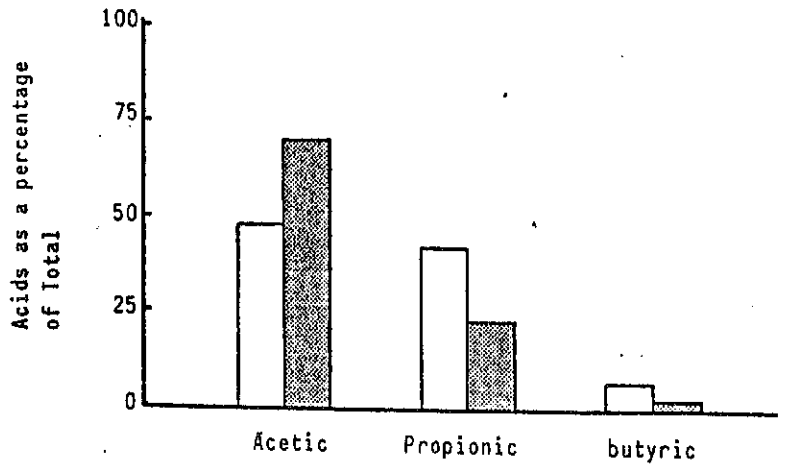


Figure 2.3 : Typical Acid Profiles in an Acid Digester ☐ and Primary Clarifier ▒

These volatile fatty acids were released into the feed to the activated sludge modules, via two basic mechanisms. In the first, about half of the fermented primary sludge withdrawn was recycled back to the inlet of the primary tanks thus allowing the fermentation products to be elutriated into the bulk liquid. An example of the effect of this procedure on the volatile fatty acids content of the sewage is shown in Table 2.10.

These results show a significant generation and release of volatile fatty acids into the reactor feeds. It is interesting to note that a further generation of volatile fatty acids occurred in the balancing tank that was not regularly drained of accumulated solids. The second mechanism of releasing volatile fatty acids into the bulk sewage was discovered when the PST recycle was stopped. When this happened, there was no significant decrease in the volatile fatty acids content of the resultant settled sewage (i.e. the concentration was still of the order of 60 mg/ℓ as acetic acid).

TABLE 2.10 : VFA PRODUCTION WHEN RAW SLUDGE WAS ACCUMULATED IN PRIMARY SEDIMENTATION TANKS AND RECYCLED TO INFLUENT OF TANKS

Sample	Total VFA concentration as acetic acid (mg/ℓ)
Outfall sewer	12
Sedimentation tank inlet	21
Sedimentation tank outlet	46
Effluent from balancing tank* that was drained daily	49
Effluent from balancing tank that was regularly drained	62

(* Refer to Fig 1.1 for layout of plant)

Visual observation of the PST's showed that an active fermentation process was occurring in these units, with significant gas evolution and flotation of solids due to gas buoyancy. In fact there was concern that some of the acids generated were being lost to methane fermentation. It was however,

concluded that diffusion and convection processes were continuing to ensure the elutriation of the VFA into the liquid phase. These findings appeared to question the need to recycle the sludge to release the VFA (see Tables 2.11 and 2.14). It is interesting to note that fermentation in the primaries could produce up to 4 t/d of total VFA (as acetic acid).

Various operational problems were experienced during these experiments, mainly due to the fact that the PST's were not designed for excess sludge accumulation. For example, the thickened sludge was more difficult to remove and pump while the scrapers tended to have difficulty in moving the sludge across the floor of the tank, and at times, were inclined to ride over the accumulated sludge layer.

Various experimental runs were carried out during this period, the results of which are described below :-

2.3.3.1 Initial studies

In this preliminary study, primary sludge was accumulated in the tanks and approximately 50 % of the sludge volume withdrawn, was recycled back to the inlet of the PST 's. The balance was wasted to digesters. The average performance of the PST's is shown in Table 2.11, with the annual means for the previous 2 years for comparison purposes.

TABLE 2.11 : PRIMARY SEDIMENTATION TANK PERFORMANCE DURING FIRST PERIOD OF SLUDGE ACCUMULATION WITH RECYCLE

		Outfall Sewer Averages			Sedimentation tank inlet Averages			Sedimentation tank outlet Averages		
		Test	1983	1982	Test	1983	1982	Test	1983	1982
Sewage										
COD	(mg/ℓ)	630	680	570	810	-	-	590	560	500
TKN	"	57	51	38	69	-	-	60	50	40
Total P	"	14	14	11	18	-	-	16	14	13
Suspended solids	"	360	270	260	490	-	-	230	170	160
Sludge										
Total solids	(%)							4,6	5,2*	2,1

* Sludge was accumulated but not completely discharged daily

As can be seen, the COD and suspended solids removal during this test period were significantly lower, resulting in a fairly strong sewage entering the plant. The performance of these modules for two consecutive operating periods is shown in Tables 2.12 and 2.13 and Figures 2.4 and 2.5.

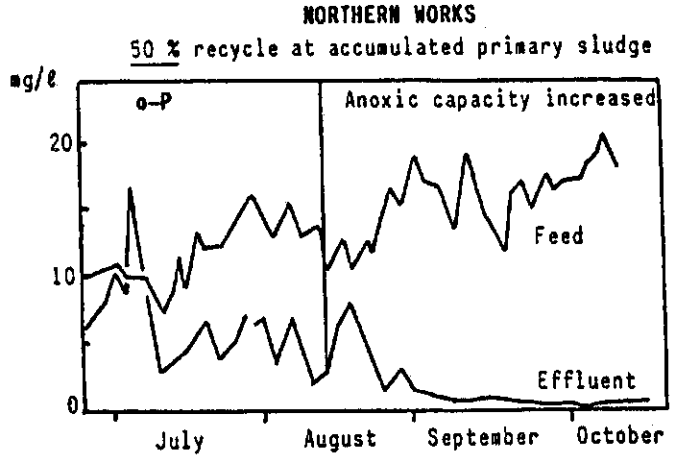


Figure 2.4 : o-P removal (with increased anoxic capacity)

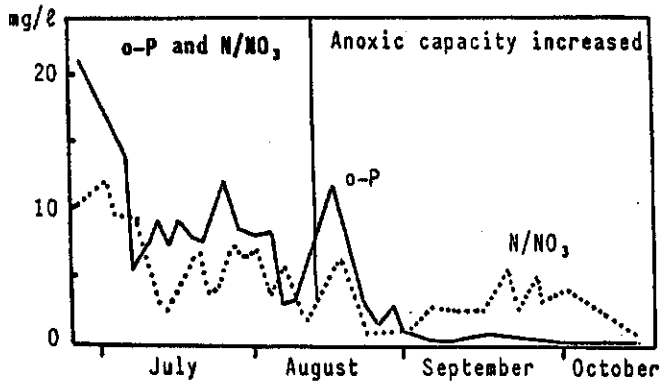
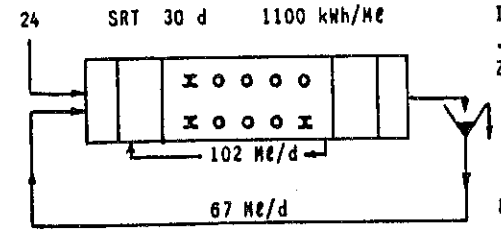


Figure 2.5 : Effluent o-P and N/NO, with two aerators off next to primary anoxic zone
7 other large aerators operating

The initial response to the new operating strategy was not very favourable, as phosphorus removal was not good. This can mainly be ascribed to the high effluent nitrate levels (10 mg N/ℓ), resulting in excessive nitrate feedback to the anaerobic zone. This is especially so when one considers the sludge recycle ratio of 1,7:1. The latter was necessary to limit solids carryover from the final clarifiers, as the activated sludge had poor settling properties.

TABLE 2.12
PLANT PERFORMANCE WITH PRIMARY SLUDGE ACCUMULATION AND RECYCLE

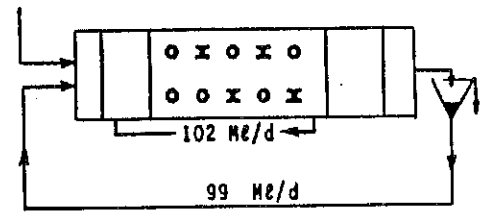


Sample Point	COD	Nitrogen TKN	Nitrogen NH_4	Nitrogen NO_3	Phosphorus Total	Phosphorus ortho	Susp solids
Influent ex							
. Balancing tank	590	60			16		230
Zone : Anaerobic 1			7,4	0,4		11	
. Anoxic 2			3,6	1,2		4,8	
. Aerobic 3			0,9	4,7		0,8	
. Anoxic 4			*3,4	1,4		* 11	
. Aerobic 5		470	0,8	3,5	280	0,9	4800
Final effluent	88		1,0	5,7		3,5	38

** 14 % N on MLVSS
5,8 % P on MLSS
DO 3,1
SVI 180
DSVI 130
O aerator on X aerator off

Note : 1. Results expressed as mg/ℓ, where applicable.
2. * High result could be due to scum on surface of reactor.
3. ** This result is abnormally high.
4. Experimental period 26/8/84 - 10/10/84.
5. Number of occassions on which the plant was tested : 18.
6. The high power consumption was due to deliberate high oxygen input to reduce SVI.

TABLE 2.13
PLANT PERFORMANCE WITH PRIMARY SLUDGE ACCUMULATION RECYCLE AND INCREASED ANOXIC CONDITIONS



Sample Point	COD	Nitrogen TKN	Nitrogen NH_4	Nitrogen NO_3	Phosphorus Total	Phosphorus ortho	Susp solids
Influent ex							
. Balancing tank	590	60			16		230
Zone : Anaerobic 1			9,9	0,2		12	
. Anoxic 2			4,6	0,9		7,0	
. Aerobic 3			1,8	4,5		2,3	
. Anoxic 4			2,0	2,3		8,7	
. Aerobic 5		470	1,4	3,5	180	1,6	5700
Final effluent	84		1,5	4,5		2,5	35

11 % N on MLVSS
4,9 % P on MLSS
DO 2,8
SVI 160
DSVI 130
O aerator on X aerator

Note : 1. Results expressed as mg/ℓ, where applicable.
2. Experimental period 26/8/84 - 10/10/84.
3. Number of occassions on which the plant was tested : 60.

To improve the situation, the size of the primary anoxic zone was increased by switching off the first two aerators at the inlet of the main aerobic zone. As can be seen in Figs 2.3 and 2.4, the effluent (and consequently, return sludge) nitrate levels dropped significantly and phosphorus removal improved a with the effluent containing an average orthophosphate concentration of 1,0 mg o-P/ℓ.

During this period, as with much of the operating life of the Northern Works plant, it was difficult to obtain proper hydraulic control of the sludge age in the test modules. This was because of metering problems and hold-ups in the sludge treatment and disposal systems. For this reason the sludge age was estimated via the MLSS concentration, using the UCT Model. Nevertheless, this sludge age gave a poor phosphorus mass balance, i.e. approximately 74 %. When the sludge age was 25 days, a 100 % recovery of phosphorus could be obtained. This highlights the difficulty of obtaining meaningful mass balances on full-scale plants.

Because more than one parameter varied, it is difficult to draw any exact conclusions as to the effects of primary sludge recycling, except for the fact that high nitrate feedback to the anaerobic zone could negate the beneficial effects of improvements in sewage characteristics brought about by primary sludge storage and recycle.

2.3.3.2 Sludge accumulation with no recycle

In this second run it was decided to examine the performance of the five-stage Bardenpho process when the sludge was accumulated in the primaries, with no primary sludge recycle. Table 2.14 shows the performance of the primary sedimentation tanks during this period.

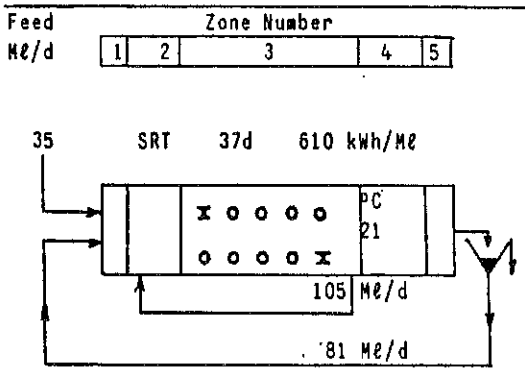
Settled sewage was fed to Module 1 and Module 2 of the Northern Works plant and these modules were operated with aerator patterns which had been shown by previous experience to give good denitrification (i.e. two aerators at the inlet of the main aerobic zone were kept off to increase the size of the first anoxic zone).

As can be seen, the COD and suspended solids removals were again low, with the efficiency of solids removal being far less than in the case of normal primary sedimentation tanks. Thus a relatively high load was maintained on the test modules and there was no major change in the quality of the feed sewage when compared with the previous period when the primary sludge was recycled.

TABLE 2.14 : PRIMARY SEDIMENTATION TANK PERFORMANCE DURING SLUDGE ACCUMULATION BUT NO RECYCLE

		Outfall sewer	Sedimentation tank inlet	Sedimentation tank outlet
Sewage				
COD	(mg/ℓ)	650	710	580
TKN	"	47	51	49
Total P	"	15	18	16
Suspended solids	"	330	380	250
Sludge				
Total solids	(%)			4,4

TABLE 2.15
PLANT PERFORMANCE WITH NO PRIMARY SLUDGE ACCUMULATED IN PRIMARY SEDIMENTATION TANK : MODULE 1 NORTHERN WORKS



Sample Point	COD	Nitrogen TKN	Nitrogen NH_4	Nitrogen NO_3	Phosphorus Total	Phosphorus ortho	Susp solids
Influent ex . Balancing tank	580	49			16		
Zone : Anaerobic 1			11	0,3		14	
. Anoxic 2			4,8	0,5		9,2	
. Aerobic 3			1,6	3,4		1,8	5000
. Anoxic 4			2,4	0,9		12	
. Aerobic 5		330	1,1	1,6	270	1,4	
Final effluent	58		1,2	1,6		1,0	19

Note : 1. Results expressed as mg/ℓ, where applicable.
2. Experimental period 14/10/84 - 27/12/84.
3. Number of occassions on which the plant was tested : 60.

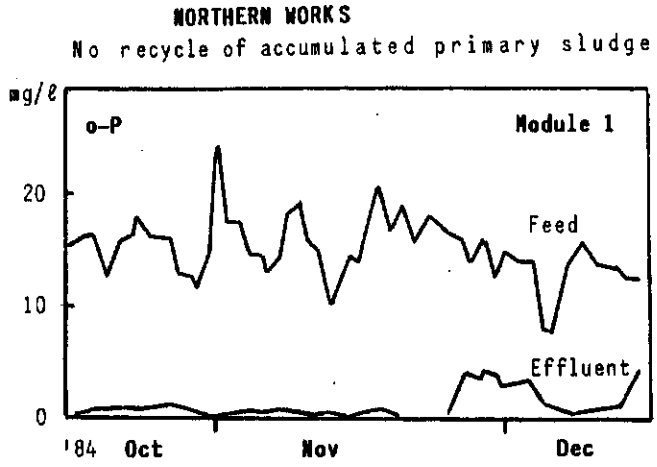


Figure 2.6: o-P removal with 1 aerator off next to primary anoxic zone. 8 other large aerators operating

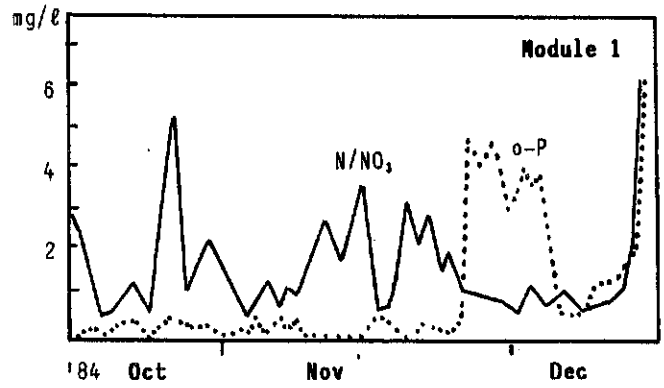


Figure 2.7 : Effluent o-P and N/NO_3 with one aerator off next to primary anoxic zone 8 other large aerators operating

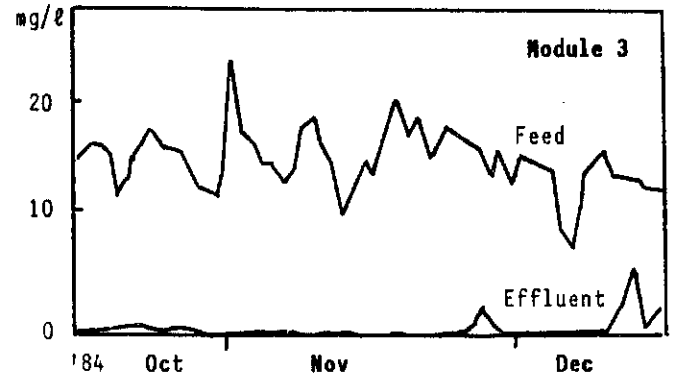


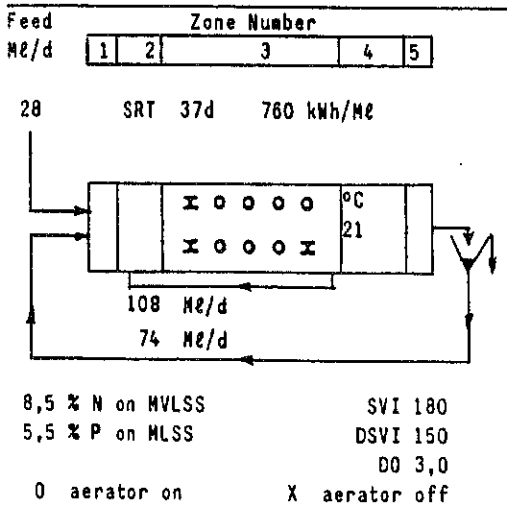
Figure 2.8 : Effluent o-P and N/NO_3 with 2 aerators off next to primary anoxic zone 7 other large aerators operating

Module 1 was operated with only one aerator closest to the primary anoxic zone switched off. Eight of the other aerators were operated, except in December when, because of the reduced load, seven were operative. The average performance of this module is depicted in Table 2.15 which shows that nitrogen and phosphorus removals were high. Figures 2.6 and 2.7 show that the effluent orthophosphate levels were below 1 mg P/ℓ for a fairly long period with a deterioration in phosphorus removal occurring towards December. Effluent nitrate levels remained fairly low, so that excess nitrate feedback to the anaerobic zone was not a problem and high release was maintained.

Module 3 was operated with the first two aerators off and seven of the other aerators in operation. The results obtained were very similar to Module 1 (see Table 2.16). In fact, as shown in Figure 2.8, this was one of the best runs experienced at the Northern Works.

Overall conclusions of this run were that good phosphorus removal was achieved because the feed sewage contained sufficient S_{bs} or volatile fatty acids to induce adequate release in the anaerobic zone. Because the return sludge nitrate levels were low, there was minimal wastage of incoming S_{bs} due to the denitrification of the return sludge.

TABLE 2.16
PLANT PERFORMANCE WITH SLUDGE ACCUMULATION
BUT NO RECYCLE : MODULE 3 NORTHERN WORKS



Sample Point	COD	Nitrogen TKN	Nitrogen NH_4	Nitrogen NO_3	Phosphorus Total	Phosphorus ortho	Susp solids
Influent ex							
. Balancing tank	580	49			16		
Zone : Anaerobic 1			7,9	0,4		12	
. Anoxic 2			4,6	0,5		5,3	
. Aerobic 3			1,4	3,2		0,7	
. Anoxic 4			3,5	0,8		15	
. Aerobic 5		300	1,2	1,6	270	0,8	4900
Final effluent	47		1,2	1,4		0,5	28

Note : 1. Results expressed as mg/ℓ, where applicable.
2. Experimental period 14/10/84 - 27/12/84.
3. Number of occassions on which the plant was tested : 60.

Other interesting observations to emerge during this period were that although aerators at the inlet of the aerobic zone were switched off to promote denitrification, aeration in the early stages of the aerobic zone was important to promote phosphate uptake and that DO levels in this region should not be less than 2 mg/ℓ. There would appear to be an unfortunate conflict of interests here, which could only be overcome by an alteration of the primary anoxic zone. The mass of phosphate released in the anaerobic zone was approximately 2,5 times the mass of phosphate entering with the feed. This was later shown to be an important criterion for the success of phosphorus removal at the Northern Works.

Again, phosphorus balances were low with only approximately 50 % of the phosphorus being accounted for. Uncertainty about the volumes of sludge wasted from the modules, was responsible for this.

2.3.3.3 Primary sludge accumulation plus recycle

In this third test, all the operating conditions were kept the same as the second, except that the primary sludge recycle was started up again with 50 % of the underflow being recycled.

The average performance of the PST's is shown in Table 2.17 and of the plant in Table 2.18 :-

TABLE 2.17 : SEDIMENTATION TANK PERFORMANCE DURING SECOND PERIOD OF ACCUMULATION IN PRIMARY SEDIMENTATION TANKS WITH RECYCLE

		Outfall sewer	Sedimentation tank inlet	Sedimentation tank outlet
Sewage				
COD	(mg/ℓ)	560	690	570
TKN	"	45	49	47
Total P	"	14	15	15
Suspended solids	"	290	390	240
VFA	"	15	22	40
Sludge				
Total solids	(%)			4,7

Again, these results were similar to those obtained before, except this time, volatile fatty acids figures were available. These show a significant increase in concentrations but this concentration was not high, compared with the Goudkoppies plant, a value of 140 mg/ℓ as reported in Section 2.2.

As shown in Table 2.18 the nitrogen removal in the test modules continued to be satisfactory, but phosphorus removal was disappointing despite the fact that the same level of phosphate release occurred in the anaerobic zones. If the effluent phosphate levels from both modules are plotted on the same graph (see Figure 2.9) one can see that with a few exceptions, they follow a very similar pattern. This would indicate that the feed quality was having the major influence on performance and that the volatile fatty acids concentrations in the feed were inadequate to ensure phosphorus removal to 1 mg P/ℓ.

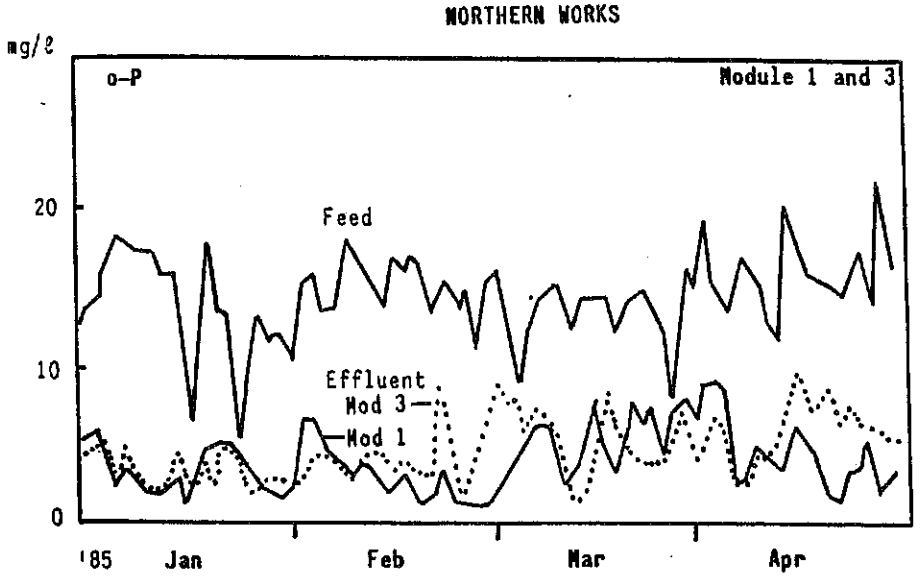


Figure 2.9 : o-P removal with 50 % of primary sludge being recycled

Another factor which may have had an effect on the results was the oxygen input to the modules. As can be seen in Figure 2.10, there was a steady decrease in the mean DO level in the main aerobic zone of Module 3. This could have prejudiced the uptake of phosphate in this zone.

NORTHERN WORKS

50 % recycle of accumulated primary sludge

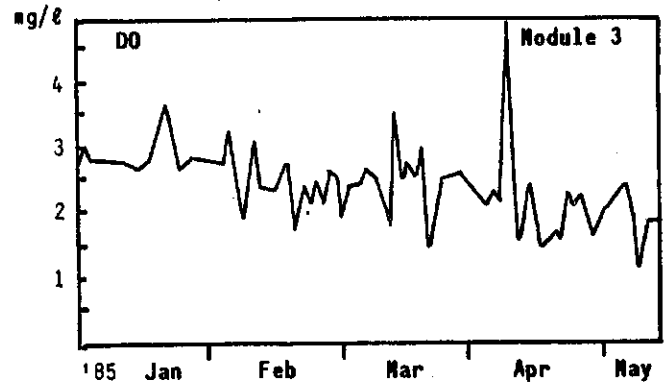
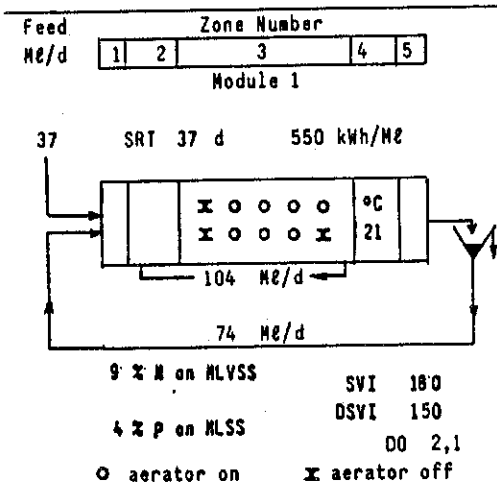


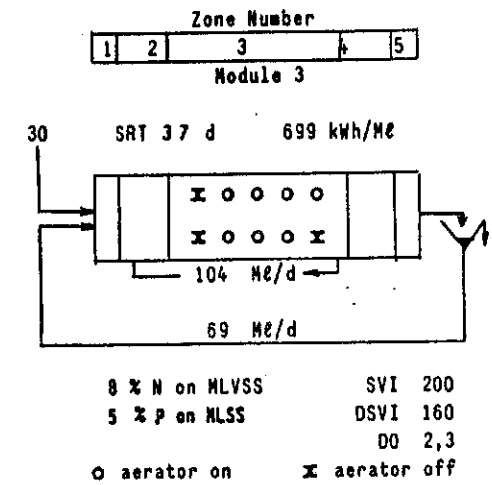
Figure 2.10 : Mean dissolved oxygen levels in main aerobic zone

TABLE 2.18

PERFORMANCE OF NORTHERN WORKS MODULES 1 AND 3 WITH PRIMARY SLUDGE RECYCLE BUT NO CONTROL ON SOLIDS RETENTION TIME



Sample Point	COD	VFA	Nitrogen TKN	NH_4	NO_3	Phosphorus Total	ortho	Susp solids
Influent ex . Balancing tank	570	45	47			15	10	240
Zone : Anaerobic 1				9,5	0,2		14	
. Anoxic 2				4,4	0,3		11	
. Aerobic 3			280	1,2	3,4	200	5,5	4500
. Anoxic 4				1,8	1,0		12	
. Aerobic 5				1,8	1,5		4,1	
Final effluent	47			0,8	1,7		3,7	10



Influent ex . Balancing tank	570	52	47			15	10	240
Zone : Anaerobic 1				6,3	0,3		12	
. Anoxic 2				4,6	0,7		9,8	
. Aerobic 3			240	1,4	3,4	190	5,8	4200
. Anoxic 4				2,9	1,1		12	
. Aerobic 5				1,0	1,5		4,8	
Final effluent	52			0,8	1,7		4,2	18

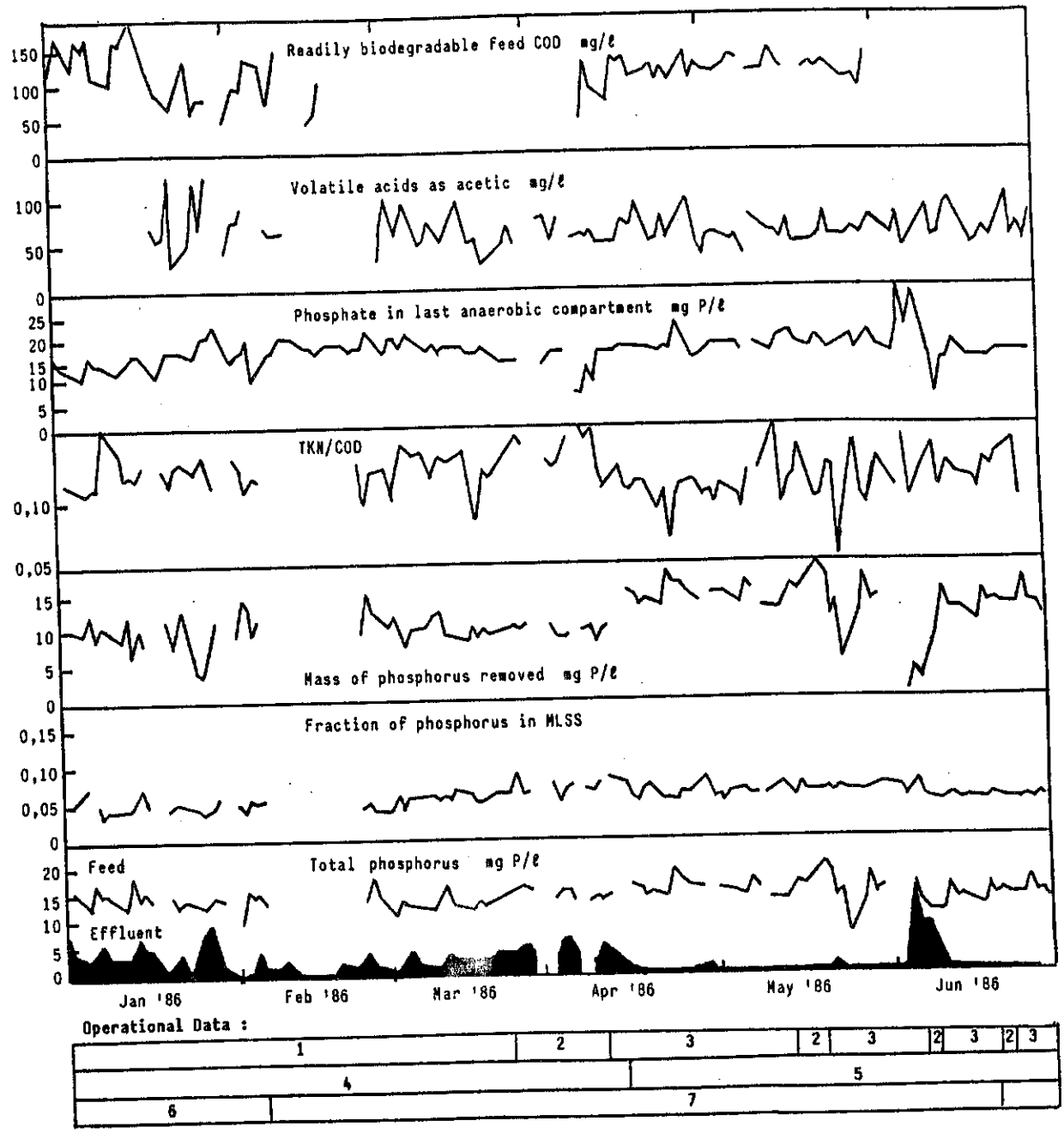
Note : 1. Results expressed as mg/ℓ, where applicable.
2. Experimental period 1/1/84 -14/5/85.
3. Number of occassions on which the plant was tested : 90.

It is interesting to note that results from Goudkoppies show a definite relationship between oxygen input to the aerobic zones and phosphate uptake. If the feed quality is suitable and there is an adequate phosphate release in the anaerobic zone, the only factor which can result in poor phosphorus removal at Goudkoppies is inadequate aeration in the aerobic zones. Therefore, it is possible that for this third run at Northern Works, there was inadequate aeration to ensure good phosphate uptake at all times.

2.3.3.4 Sludge accumulation with recycle and control of solids retention time

In all the preceding experiments where substrate was solubilised in the PST, phosphorus removal was satisfactory for periods ranging from a few weeks to a few months, after which, either the feed sewage characteristics changed or the effluent nitrate concentration increased. The net result was that the effluent phosphate concentration increased. In order to improve the reliability of the phosphate removal, it was decided to investigate the effect of controlling the retention time of solids in the PST.

From experience gained in the operation of acid digesters discussed in 2.3.1, it was known that the volatile acids produced could easily be lost, if methane fermentation were allowed to occur. This same situation could develop in a PST provided with sludge recycle facilities where, as a result of the recycle, solids retention time would increase to such an extent that a population of methane fermenters would develop, with a concomitant drop in the volatile acid concentration, which could be detrimental to phosphate removal. One way of preventing the growth of the methane fermenters, is to periodically stop the recycle and dispose of all the sludge to anaerobic digesters.

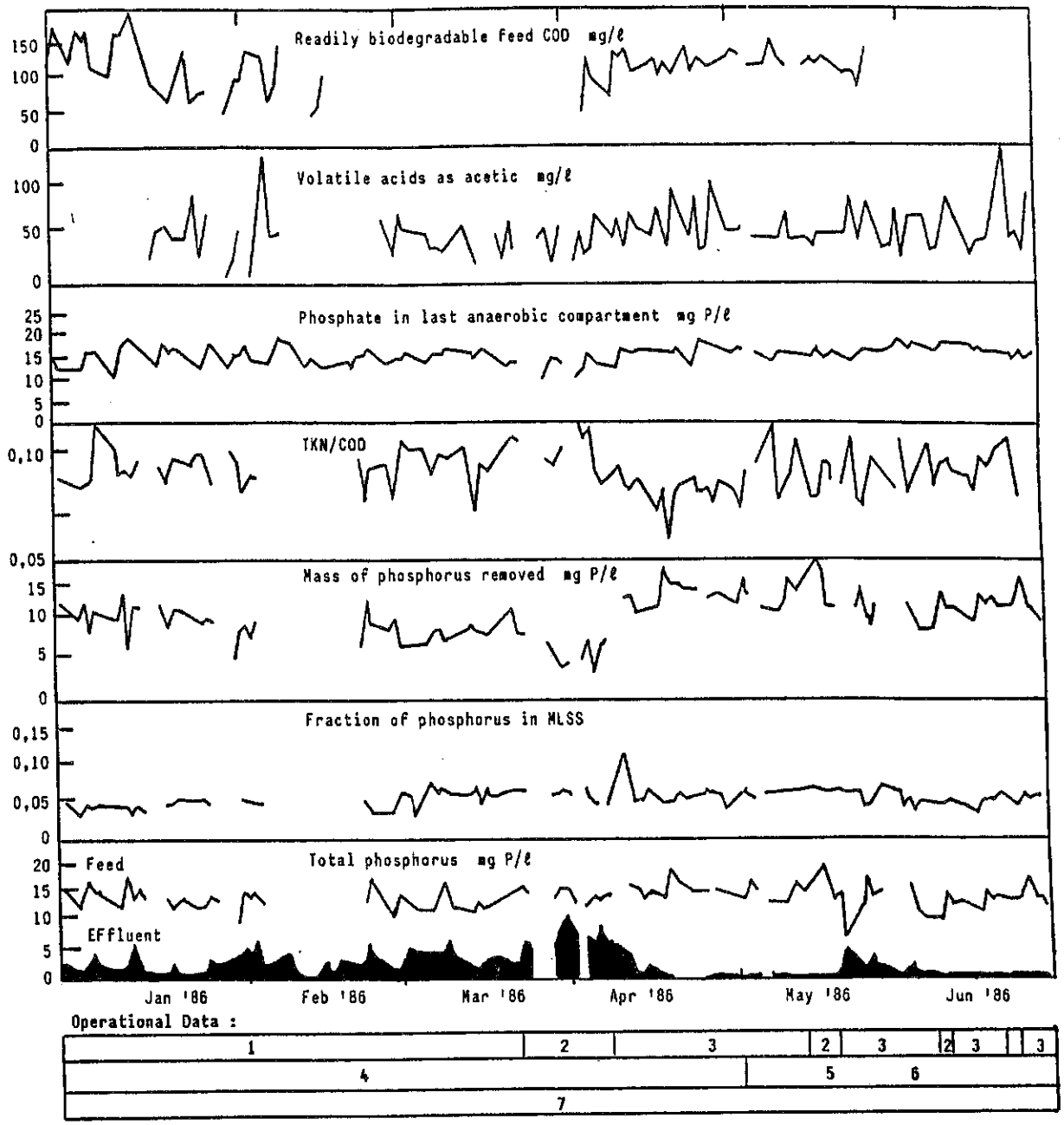


Key

1 Primary sludge recycle 1:1. All tanks desludged partially each day
2 No primary sludge recycle. All tanks desludged completely
3 Primary sludge recycle 1:1. Each tank desludged completely every 4 days
4 All feed to first anaerobic compartment
5 All feed to last anaerobic compartment
6 Three stage process
7 Five stage process

Figure 2.11 : **Parameters affecting phosphorus removal**

Module 2 - Performance with primary sludge recycle and controlled primary sludge retention times

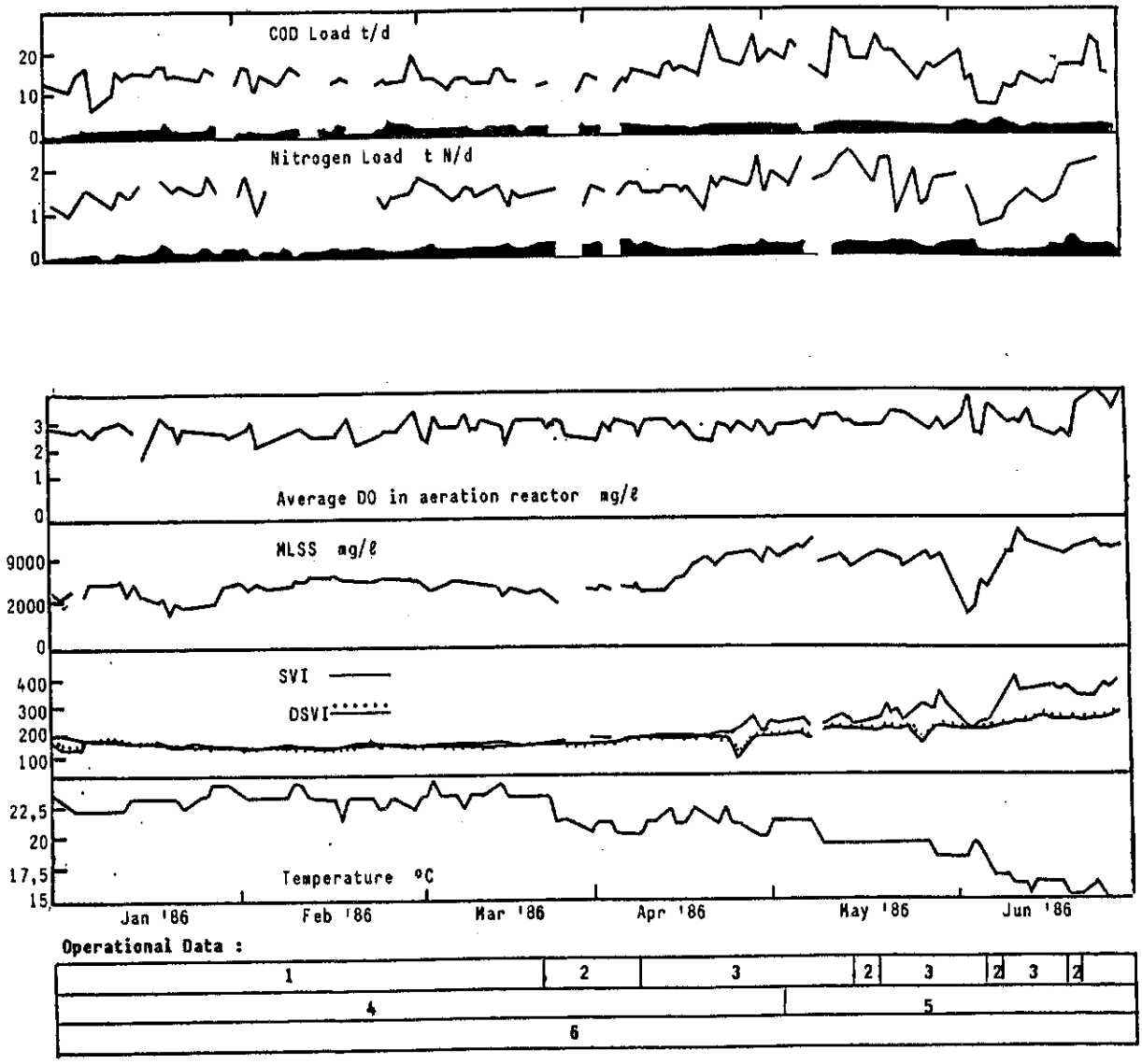


Key

1 Primary sludge recycle 1:1. All tanks desludged partially each day
2 No primary sludge recycle. All tanks desludged completely
3 Primary sludge recycle 1:1. Each tank desludged completely every 4 days
4 One MLSS recycle pump in operation
5 Two MLSS recycle pumps in operation
6 Level of weir dropped to minimum
7 Five stage process. All feed to start of anaerobic reactor

Figure 2.12 : **Parameters affecting phosphorus removal**

Module 3 - Performance with primary sludge recycle and controlled primary sludge retention times

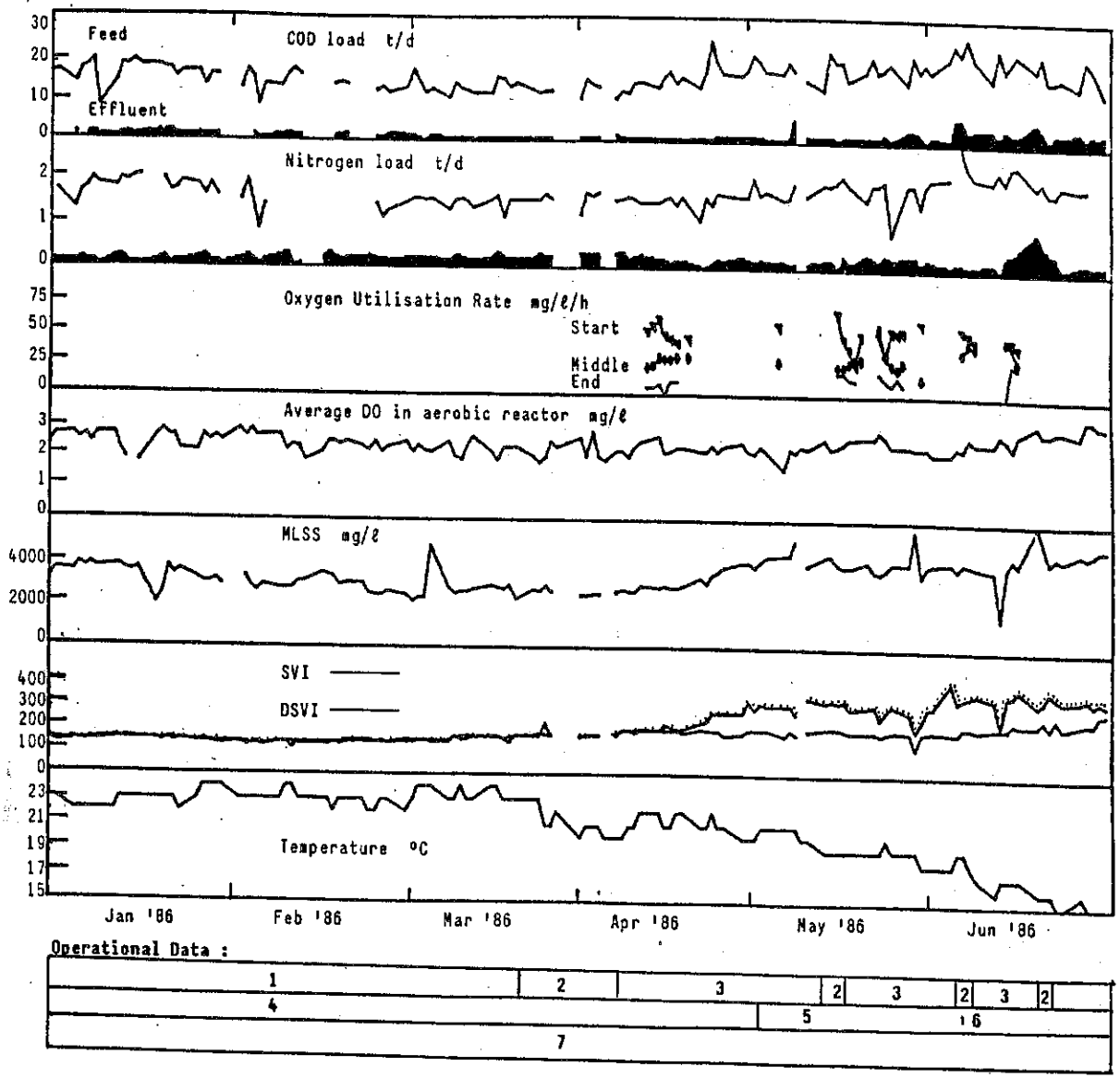


Key

1 Primary sludge recycle 1:1. All tanks desludged partially each day
2 No primary sludge recycle. All tanks desludged completely
3 Primary sludge recycle 1:1. Each tank desludged completely every 4 days
4 One MLSS recycle pump in operation
5 Two MLSS recycle pumps in operation
6 Five stage process

Figure 2.13 : **Parameter affecting oxygen demand and SVI**

Module 2 - Performance with primary sludge recycle and controlled primary sludge retention times



Key

1 Primary sludge recycle. All tanks desludged partially each day
2 No primary sludge recycle. All tanks desludged completely
3 Primary sludge recycle 1:1. Each tank desludged completely every 4 days
5 Two MLSS recycle pumps in operation
6 Level of weir dropped to minimum
7 Five stage process

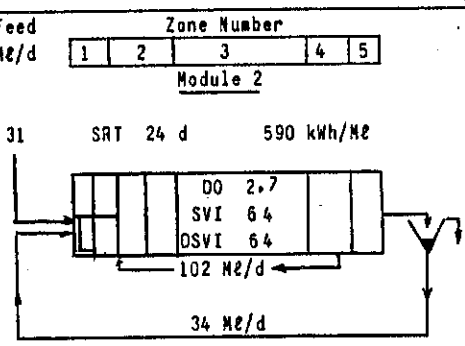
Figure 2.14 : **Parameters affecting oxygen demand and SVI**

Module 3 - Performance with primary sludge recycle and controlled primary sludge retention times

An experiment was accordingly designed, where primary sludge recycle was practised for approximately two and half months, after which the tanks were emptied of all sludge. The appropriate plant performance data in presented in Figures 2.11 to 2.14, and the average results reflected in Table 2.19.

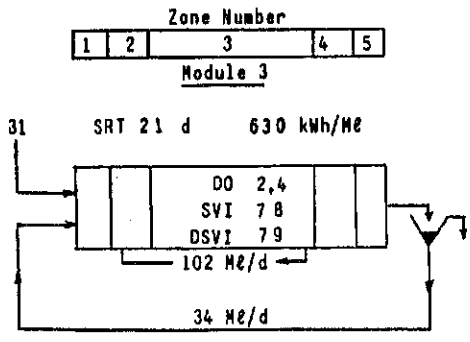
From Figures 2.11 and 2.12 it would appear that with exceptions, the effluent phosphate concentrations from Modules 2 and 3 follow the same pattern, which would indicate that the character of the feed is the over-riding control factor. At the start of this test period the colour of the settled sewage was grey, whereas towards the end it became black, similar in colour to digester supernatant liquor.

TABLE 2.19
PERFORMANCE OF MODULES 2 AND 3 WITH PRIMARY SLUDGE RECYCLE BUT NO CONTROL ON SOLIDS RETENTION TIME



Sample Point	COD	VFA	Nitrogen TKN	Nitrogen NH_4	Nitrogen NO_3	Phosphorus Total	Phosphorus ortho	Susp solids
Influent ex . Balancing tank	470	67	54			14		190
Zone : Anaerobic 1				12	0,1		16	
. Anoxic 2				4,4	1,0		9,3	
. Aerobic 3				0,8	6,6		4,0	
. Anoxic 4				0,9	6,1		3,5	
. Aerobic 5	3400		190	0,8	6,5	130	3,3	2700
Final effluent	34			0,7	7,1		3,2	8

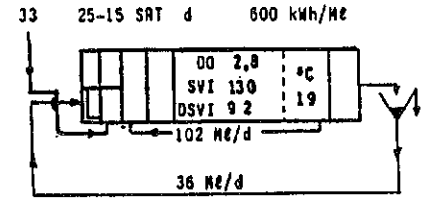
Note :Module operated as a five-stage Unit by switching off aerators in second anoxic zone



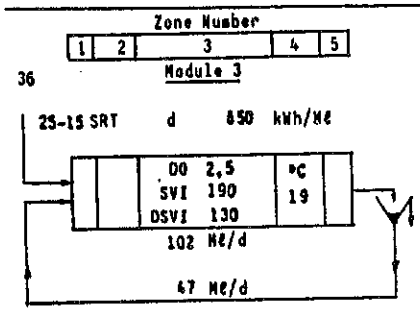
Sample Point	COD	VFA	Nitrogen TKN	Nitrogen NH_4	Nitrogen NO_3	Phosphorus Total	Phosphorus ortho	Susp solids
Influent ex . Balancing tank	470	67	54			14		190
Zone : Anaerobic 1				10	0,2		14	
. Anoxic 2				4,8	2,6		8,4	
. Aerobic 3				0,8	6,6		3,9	
. Anoxic 4				0,7	5,2		3,7	
. Aerobic 5	3400			0,6	5,1		3,5	3000
Final effluent	40			0,7	5,4		3,0	13

Note : 1. Results expressed as mg/ℓ, where applicable.
2. Experimental period 1/1/86 - 23/3/86.
3. Number of occassions on which the plant was tested : 57.

TABLE 2.20
PERFORMANCE OF NORTHERN WORKS MODULES 2 AND 3 WITH PRIMARY SLUDGE RECYCLE AND CONTROLLED SOLIDS RETENTION TIME



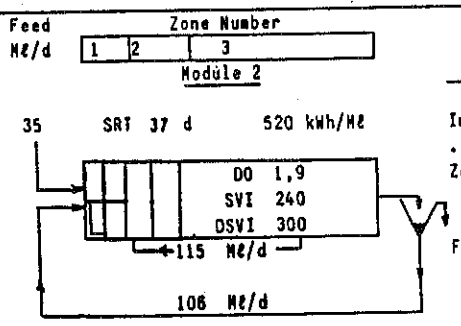
Sample Point	COD	VFA	Nitrogen TKN	Nitrogen NH_4	Nitrogen NO_3	Phosphorus Total	Phosphorus ortho	Susp solids
Influent ex								
. Balancing tank	500	61	53			15		200
Zone : Anaerobic 1				12	0,1		17	
. Anoxic 2				5,8	0,3		9,4	
. Aerobic 3				1,8	5,8		1,6	
. Anoxic 4				1,7	4,9		1,7	
. Aerobic 5	4200			1,5	5,8		1,5	3800
Final effluent	38			1,9	5,3		1,3	14



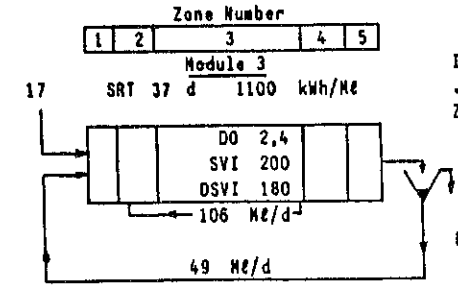
Sample Point	COD	VFA	Nitrogen TKN	Nitrogen NH_4	Nitrogen NO_3	Phosphorus Total	Phosphorus ortho	Susp solids
Influent ex								
. Balancing tank	500	61	53			15		200
Zone : Anaerobic 1				12	0,1		15	
. Anoxic 2				6,9	1,6		7,5	
. Aerobic 3				3,0	6,6		2,2	
. Anoxic 4				3,4	4,3		4,7	
. Aerobic 5	4200			2,8	4,2		1,5	4000
Final effluent	58			2,4	4,3		1,2	38

Note : 1. Results expressed as mg/ℓ, where applicable.
2. Experimental period 9/4/86 - 26/6/86.
3. Number of occassions on which the plant was tested : 57.

TABLE 2.21
PERFORMANCE OF NORTHERN WORKS MODULES 2 AND 3 PRIOR TO SWITCHING OFF AERATORS AND FEED WITH SLUDGE ACCUMULATION IN PRIMARY SEDIMENTATION TANK BUT NO RECYCLE



Sample Point	COD	Nitrogen TKN	Nitrogen NH_4	Nitrogen NO_3	Phosphorus Total	Phosphorus ortho	Susp solids
Influent ex							
. Balancing tank	460				8,8		
Zone : Anaerobic 1			9,3	0,2		12	
. Anoxic 2			4,5	0,7		8,9	
. Aerobic 3			0,6	2,1		3,8	4100
Final effluent	44		1,2	3,7		3,2	14



Sample Point	COD	Nitrogen TKN	Nitrogen NH_4	Nitrogen NO_3	Phosphorus Total	Phosphorus ortho	Susp solids
Influent ex							
. Balancing tank	560		36		8,7		21
Zone : Anaerobic 1			6,2	0,1		14	
. Anoxic 2			3,3	2,0		11	
. Aerobic 3			0,6	5,5		7,1	
. Anoxic 4			0,7	3,9		8,8	
. Aerobic 5			0,6	3,8		6,6	4800
Final effluent	49		0,6	3,8		6,2	

Note : 1. Results expressed as mg/ℓ, where applicable.
2. No results available for TKN and Total Phosphorus.
3. High power consumption on Module 3 was due to deliberate high oxygen input to reduce SVI.

This suggests that methane fermentation had occurred, but unfortunately because of insufficient data, this is not noticeable in Figures 2.11 and 2.12. In order to prevent loss of volatile fatty acids's by conversion to methane it was obviously necessary to desludge the PST's more frequently and in the next series of experiments, this was done every 12 days. The daily performance data is presented in Figures 2.11 to 2.14 and the average results are reflected in Table 2.20.

Effluent phosphate concentration dropped dramatically to below 1 mg P/ℓ and except for one or two occasions, remained at this level. Furthermore, it was interesting to note that the colour of primary sludge remained grey. From the work of Siebritz et al. (1983) one would have expected an increase in the readily biodegradable COD and volatile acid concentrations, as well as a larger release of phosphorus in the anaerobic reactor. From Figures 2.11 and 2.12 no marked change in any of the above parameters is evident. However, it should be borne in mind that only small increases of 10 - 20 mg/ℓ COD would be required to ensure low effluent phosphate concentrations, and since the scatter of the data was so large, these small changes were difficult to detect.

It was concluded from these experiments, that it was essential to control the retention time of solids in PST's if reliable phosphate removal was to be achieved.

2.4 EFFECT OF SWITCHING OFF ALL AERATORS FOR AN EXTENDED PERIOD

Experience in Johannesburg at the Alexandra Plant and elsewhere (Nicholls, 1977), has shown that switching off all the aerators and mixers in a nutrient removal plant for an extended period, can sometimes induce an improvement in phosphorus removal. It was decided to investigate this type of operational procedure at the Northern Works.

Period before switch-off

Table 2.21 shows the performance of the two modules during the period before switch-off. As can be seen, Module 2 (the three-stage Phoredox) gave the best phosphorus removal, which was partly due to the three-stage

layout and the fact that it received the greater flow. Both modules had poor settling sludges, necessitating high return sludge recycle rates and a greatly reduced feed rate to Module 3, as this module had a final clarifier out of commission for repairs.

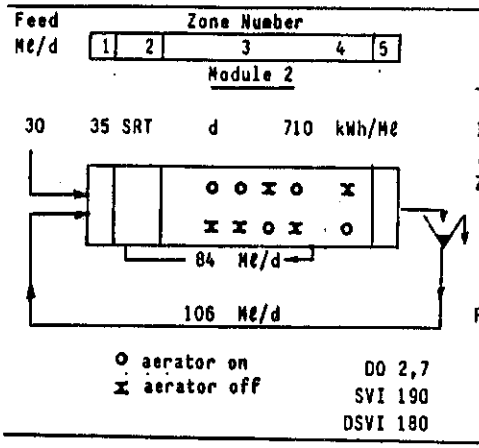
Both modules were switched-off for period of approximately 60 hours to try and induce an improvement in phosphorus removal. On restarting the mechanical plant, there was the usual phosphate peak in the effluents (up to 20 mg P/ℓ) whereafter the modules settled down to a period of improved performance.

Period after switch-off

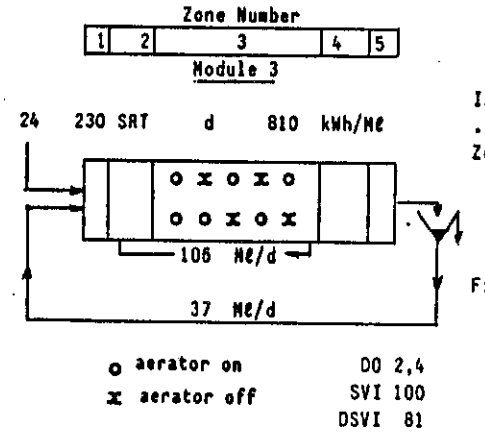
Once the modules had stabilised after the off periods, monitoring runs were carried out to assess the improved performance. This operating period was characterised by both modules having poor settling sludges, although extra aerators were operated to reduce SVI values. Return sludge recycle rates were kept high and both modules had extensive thick scum layers covering the unaerated zones. During both the period before and after switch-off, sludge was accumulated in the primaries but not recycled. The mean results obtained on Modules 2 and 3 are shown in Table 2.22. As can be seen, there is an improvement in phosphorus removal which is depicted more clearly in Figure 2.15.

After the off period, the effluent phosphate values dropped dramatically and were below 1 mg P/ℓ for extended periods. This good performance continued until heavy rains occurred in early November, whereafter, as Figure 2.15 shows, phosphate removal deteriorated and did not improve for a considerable period. The rains did not cause an increase in effluent nitrate levels, although it is interesting to note that after the rains the effluent nitrate and phosphate values followed very similar patterns. This suggests that prior to the rains, there was sufficient S_{bs} in the feed sewage, whereas after the rains there was less S_{bs}, with the result that the effluent and consequently, the return sludge nitrate levels now became important.

TABLE 2.22
PERFORMANCE OF NORTHERN WORKS MODULES 2 AND 3 AFTER SWITCHING AERATORS AND FEED FOR 30 HOURS



Sample Point	COD	Nitrogen TKN	Nitrogen NH_4	Nitrogen NO_3	Phosphorus Total	Phosphorus ortho	Susp solids
Influent ex							
. Balancing tank	530						210
Zone : Anaerobic 1			8,7	0,2	16		
. Anoxic 2			4,0	0,5	10		
. Aerobic 3							
. Anoxic 4							
. Aerobic 5			0,7	5,3	2,3		4800
Final effluent	46		0,8	4,4	1,8		14



Sample Point	COD	Nitrogen TKN	Nitrogen NH_4	Nitrogen NO_3	Phosphorus Total	Phosphorus ortho	Susp solids
Influent ex							
. Balancing tank	520				13		220
Zone : Anaerobic 1			8,7	0,3	13		
. Anoxic 2			4,1	1,6	7,3		
. Aerobic 3			0,7	6,2	2,2		
. Anoxic 4			0,8	3,9	3,9		
. Aerobic 5			0,7	4,0	2,3		4200
Final effluent	46		0,8	4,4	1,8		17

Note : 1. Results expressed as mg/ℓ, where applicable.
2. No results available for TKN and Total Phosphorus.
3. High power consumption was due to deliberate high oxygen input to reduce SVI.

As can be seen in Figure 2.15, Module 3 also showed an improvement in phosphorus removal, with the rains causing the performance to deteriorate in November. In this case however, good phosphorus removal returned within a week, unlike Module 2, where the deterioration was more prolonged.

2.5 COMPARISON BETWEEN THREE AND FIVE STAGE PHOREDOX MODULES AT NORTHERN WORKS

The Northern Works nutrient removal plant was built in the form of three parallel five-stage modules with surface aerators. Initial operating experience with this plant (Pitman, 1982) showed that limited denitrification occurred in the second anoxic zone and there were

NORTHERN WORKS

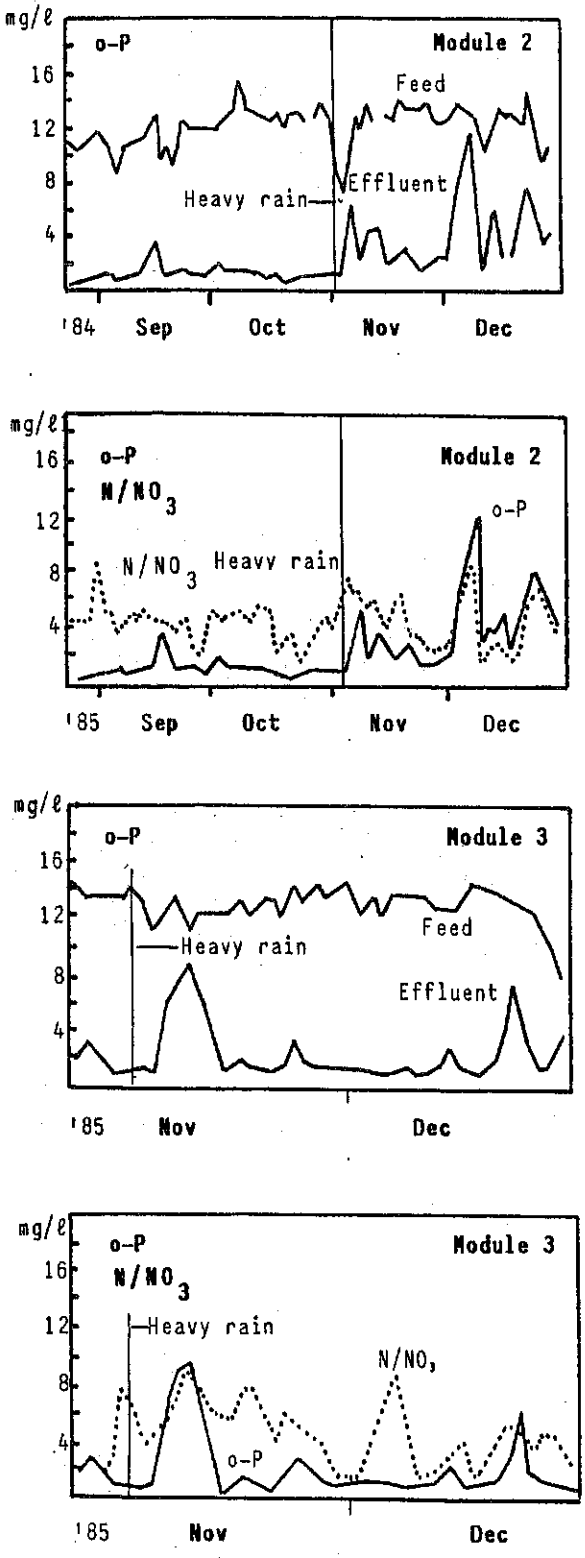


Figure 2.15 : Plant Performance

After aerators and feed were switched off for 60 h

indications that the anaerobic zone was too small. Modifications were therefore made to Module 2, in which the anaerobic basin was doubled in size and divided into five compartments in series, with hydraulic residence times of 10; 20; 30; 30 and 30 minutes respectively, based on designed flow plus return activated sludge, with the first compartment being in the form of an anoxic selector. Furthermore, the first anoxic zone was split into two compartments, whilst the second anoxic zone was eliminated.

Figure 2.16 shows a plan view of this module after the changes were made.

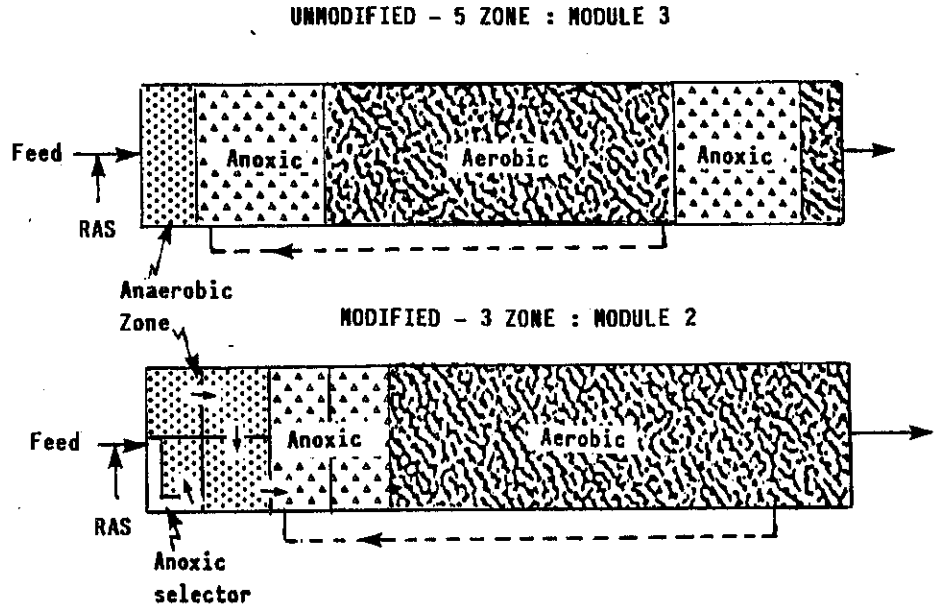


Figure 2.16 : Conversion of Northern Works 5 Zone module to a 3 Zone module

In this study, a side-by-side comparison was made between the modified module and a neighbouring five- stage module. For these experiments the flows to the two modules and the main return sludge and mixed liquor recycles, were kept much the same. The feed to the modules came from rectangular primary sedimentation tanks in which sludge was accumulated, but not recycled. Despite there being no recycle, there is a significant improvement in the S_{bs} value of the feed sewage as is shown in Table 2.23.

TABLE 2.23 : READILY BIODEGRADABLE COD CONTENT OF INFLUENT SEWAGE DURING COMPARISON OF 3 vs 5 STAGE PHOREDOX PROCESSES

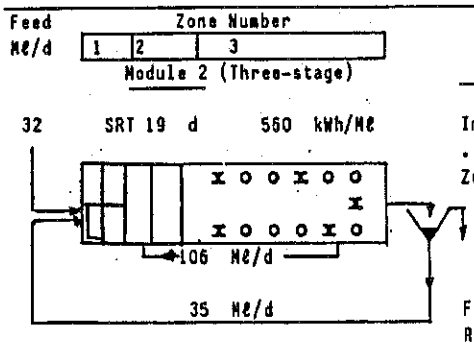
Sample	S_{bs}* (mg/ℓ)
Raw sewage	73
Inlet to primary sedimentation tank	87
Effluent from primary sedimentation tanks	104

(* As estimated from the 0,45μ microfiltration technique)

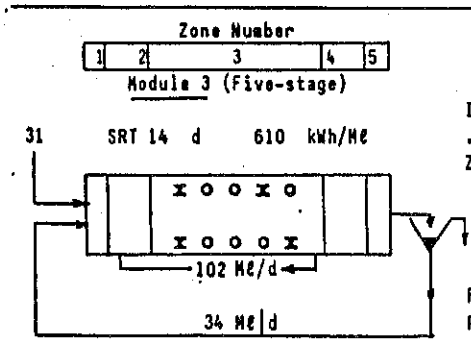
This improvement in S_{bs} concentration was probably caused by the diffusion and convection of fermentation products from the accumulated sludge layers in the sedimentation tanks.

The performance of the two modules during this comparative period is shown in Tables 2.24.

TABLE 2.24
PERFORMANCE OF THREE-STAGE VERSUS FIVE-STAGE BARDENPHO PROCESS



Sample Point	COD	VFA	Nitrogen TKN	Nitrogen NH_4	Nitrogen NO_3	Phosphorus Total	Phosphorus ortho	Susp solids
Influent ex								
. Balancing tank	430	64						170
Zone : Anaerobic 1				13	0,1		17	
. Anoxic 2				4,7	0,9		9,5	
. Aerobic 3				0,9	6,3		2,8	2800
Final effluent	32			0,8	6,8		2,2	10
Return sludge					4,8			5400



Sample Point	COD	VFA	Nitrogen TKN	Nitrogen NH_4	Nitrogen NO_3	Phosphorus Total	Phosphorus ortho	Susp solids
Influent ex								
. Balancing tank	430	53						170
Zone : Anaerobic 1				11	0,2		14	
. Anoxic 2				4,8	3,0		8,5	
. Aerobic 3				0,8	7,1		4,5	
. Anoxic 4				0,7	5,9		4,2	
. Aerobic 5				0,7	5,8		3,9	2800
Final effluent	37			0,7	6,1		3,5	
Return sludge					4,8			5300

Note : 1. Results expressed as mg/ℓ, where applicable.

As can be seen, both modules achieved similar degrees of nitrification and denitrification. The five-stage module displayed limited denitrification in the second anoxic zone, while the nitrate levels measured in the first anoxic zone indicated that the denitrification capacity in the zone had been exceeded. The three-stage unit had very similar nitrate levels, indicating that the separate second anoxic zone was not really necessary and that the effect of the zone could possibly be created by switching off aerators towards the end of the aerobic zone. In addition, nitrate concentrations at the outlet of the first anoxic zone were significantly lower than equivalent results for the five-stage module, possibly indicating that the compartmentalisation of this zone provided increased denitrification capacity.

Reference to Table 2.24 will show that the three-stage module displayed a slightly better phosphorus removal than the five-stage module, due to the better phosphorus release in the anaerobic zone. The enlarged compartmentalised anaerobic zone probably contributed to this. The modules had very similar sludge settling properties (see Figures 8.2 and 8.3 in Chapter 8), with the three-stage unit displaying slightly lower SVI values than the five-stage module. The fact that there were no major differences in nutrient removal between the modules, and that the 1 mg o- P/ℓ phosphate standard could only be met on few occasions during the test period would point to other factors, in particular, the feed quality having a larger influence on nutrient removal at Northern Works.

2.6 DENITRIFICATION OF RETURN ACTIVATED SLUDGE

Examination of performance data derived from the Johannesburg Bushkoppie Works showed that providing SVI of the mixed liquor was low, MLSS concentrations could be doubled or trebled in the secondary clarifiers. Under these conditions, considerable denitrification was found to occur, resulting in a loss of 5 to 7 mg N/ℓ as nitrate. These observations were further quantified by carrying out laboratory scale denitrification tests on return activated sludge obtained from both the Goudkoppies and Bushkoppie Works. The test samples were artificially spiked with known amounts of nitrate and the endogenous denitrification rates monitored over a period of time, as reflected in Figure 2.17.

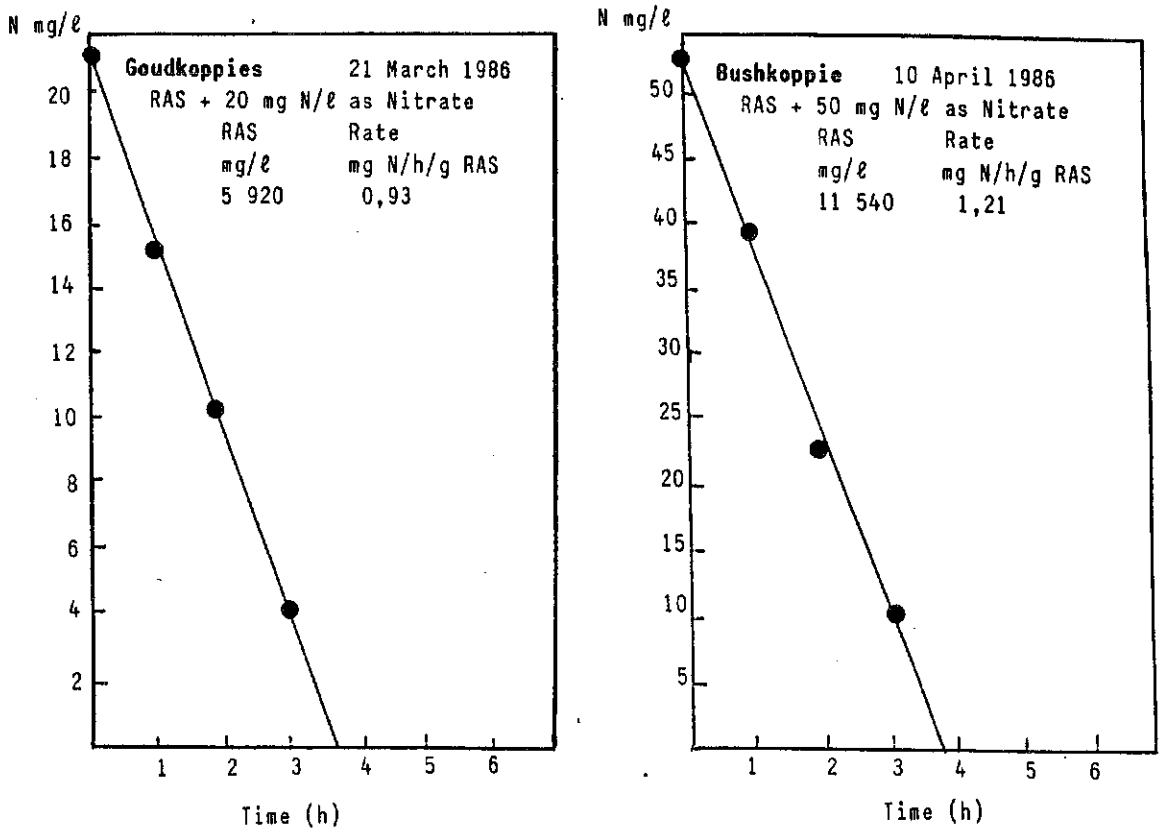


Figure 2.17 : Endogenous denitrification potential from the Goudkoppies and Bushkoppie Works

It will be noted that the denitrification rates associated with the Bushkoppie sludge are higher than those for the Goudkoppies sludge. This is due to raw sludge from the PST's having been added to the influent sewage entering the biological reactor at Bushkoppie. At Goudkoppies, all the incoming sewage was subjected to primary sedimentation.

Removal of nitrates from the return activated sludge before entering the anaerobic zone, would prevent readily assimilable COD in the influent sewage from being used up for denitrification purposes and make it available for phosphorus accumulating bacteria. As this concept appeared to have considerable merit, it was decided to apply these principles to the operation of the newly modified Module 2 at the Northern Works. Figure 2.16 shows the alteration of this module to three-stage operation and Figure 2.18 shows how it was possible to direct the feed to this unit into the lower half of the enlarged anaerobic zone. This situation was not ideal, as the contact time of the return activated sludge with influent sewage was reduced to a minimum, but this was dictated by the availability of existing pipework. On the other hand, endogenous denitrification facilities, although too large, were provided ahead of the anaerobic zone.

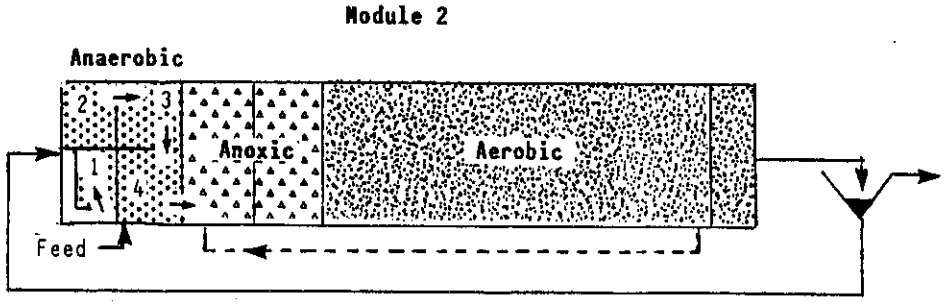
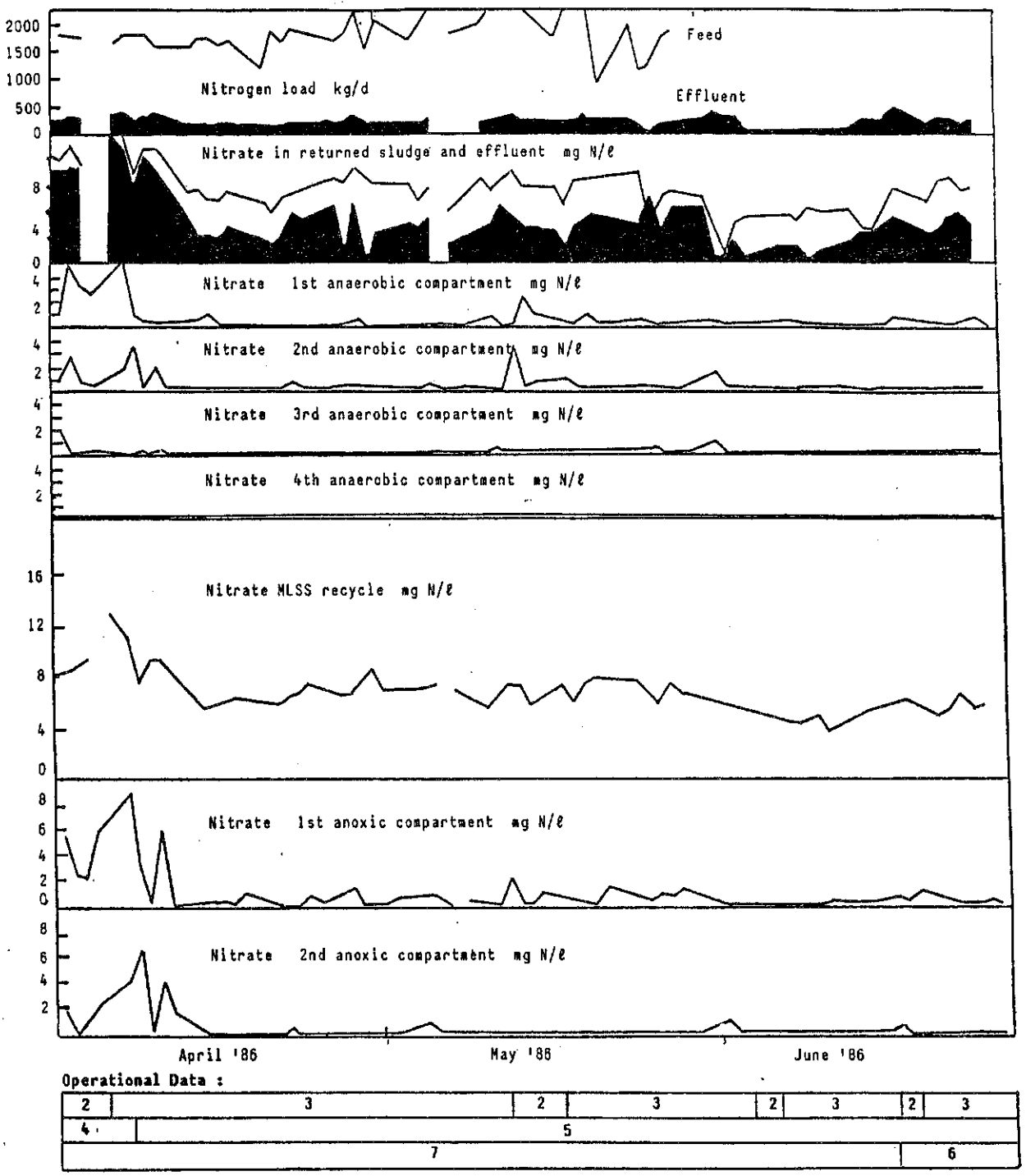


Figure 2.18 : Further Modifications to Module 2 to Provide endogenous denitrification of returned activated sludge

Plant performance during this mode of operation is reflected in Figures 2.19 and 2.20. From Figure 2.19, it will be noted that denitrification of return activated sludge is virtually complete by the end of anaerobic Compartment 1, thus ensuring that virtually all the readily asssimilable COD in the influent feed is available for uptake by phosphorus accumulating bacteria in Compartment 4. In regard to Figure 2.20 it will be noted that phosphorus release occurred in Compartments 2 and 3 in the absence of an externally supplied substrate. This phenomenon has also been noticed on occasions, in the second anoxic zone at the Northern Works and has also been reported by Barnard (1985). Reasons for this release are hypothesized by Wentzel et al. (1986).

Reference to Figures 2.19 and 2.20 show that the performance of the modified Module 2 and unmodified Module 3, were very similar. This would



Key :

2 No primary recycle.
All tanks desludged completely

3 Primary sludge recycle 1:1.
Each tank desludged every 4 d

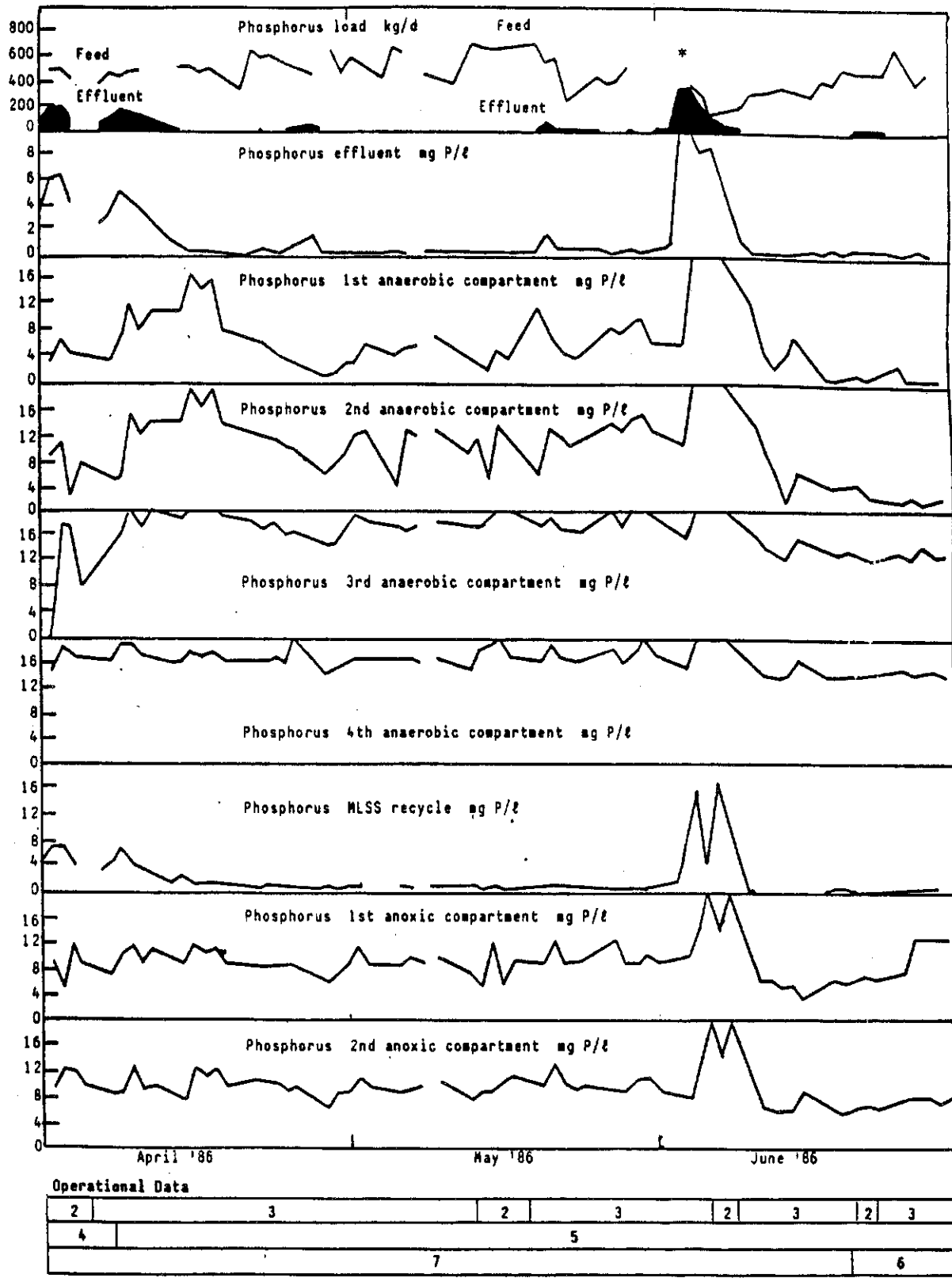
4 All feed to first anaerobic compartment

5 All feed to last anaerobic compartment

6 Three stage process

7 Five stage process

Figure 2.19 : Module 2 – Nitrate removal in compartmentalised anaerobic and anoxic reactors



Key

* Power failure

2 No primary sludge recycle.
All tanks desludged completely

3 Primary sludge recycle 1:1.
Each tank desludged every 4 d

4 All feed to first anaerobic compartment

5 All feed to last anaerobic compartment

6 Three stage process

7 Five stage process

Figure 2.20 : Module 2 - phosphate release in compartmentalised anaerobic and anoxic reactors

imply that the presence of relatively low concentrations of nitrate in the RAS is not a major problem, provided there was an adequate supply of S_{bs} available in the influent sewage.

Operational staff at the Northern Works have gained the impression that the modifications to Module 2 have conferred a greater stability to the unit. This will be tested to the full during the forthcoming summer rain period, when weaker sewages will be available for treatment. At the Bushkoppie Works the problem of nitrates in return activated sludge is far more severe and it is proposed to repeat this experiment after structural modifications have been made to this plant in the latter half of 1986.

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CHAPTER THREE

On-line monitoring of plants

3.1 INTRODUCTION

Under normal routine operating conditions, the influent to the plant and the effluent produced from each module, would be sampled at hourly intervals with samples being composited at the end of the day, and analysed for the eight parameters indicated in many of the tables presented in Chapter 2. In the event of problems being experienced, each zone of the different modules would be individually investigated. All the data generated at the Northern works is stored in a computer, which facilitates the generation of reports or graphs for the works management, depicting trends or abnormalities in works performance.

As the main objective of the investigations carried out at Northern Works, was to change the influent sewage characteristics with a view to improving the reliability of nutrient removal, it was essential to have a comprehensive record of phosphate, nitrate and ammonia concentrations in the effluent. To this end, a Bran and Lubbe on-line phosphate analyser and a Technicon ammonia and nitrate analyser were installed. Two different instrument suppliers were used simply to gain experience of different on-line designs.

Johannesburg has frequently used the University of Cape Town (UCT) model (WRC, 1984), to carry out mass balances across the plant and to predict the effect of any proposed process modifications. This model incorporates factors based on oxygen utilisation rates (OUR) and it was therefore

decided that it was essential to also measure this parameter. Howell et al., (1984) and Sollfrank and Guijer (1985), have demonstrated that this parameter can also be used successfully for control purposes. OUR was measured in the main aerobic basin of Module 3 of the Northern Works, using three large respirometers linked by a personal computer for control and data logging.

3.2 DESCRIPTION OF MONITORS

3.2.1 Bran and Lubbe Phosphate Monitor

Effluent was pumped continuously through a cartridge filter (pore size 25 µm) to an overflow chamber within the monitor. Accumulation of solids in this chamber necessitated the replacement of the filter supplied, with one having a 1µm pore size.

The monitor operates in a batch mode, taking a new sample every 12 minutes. Appropriate volumes of sample and reagents are added by means of a number of cam-controlled peristaltic pumps, to a reaction vessel where the characteristic molybdenum blue complex is developed. The intensity of the colour formed is proportional to the concentration of orthophosphate and is measured by means of a photo-electric cell.

3.2.2 Technicon Ammonia and Nitrate Monitors

In these instruments provision is made for an electronically-controlled system to purge the feed lines and pre-instrument filter with air, prior to drawing in a sample for analysis. The principle of operation of these monitors is similar to the laboratory Autoanalysers, where a single peristaltic pump controls the flow of sample and required analytical reagents through a number of narrow bore plastic tubes of different but known diameter. After a suitable reaction time the colours developed are measured photo-electrically and recorded. Ammonia is determined by the Bertholdt reaction which involves the use of sodium phenoxide, followed by sodium hypochlorite, to produce a green colour which is thought to be closely related to indophenol. A solution of potasium tartrate (Rochelle Salt) is added to the sample stream to prevent the precipitation as

hydroxides of any heavy metals which may have been present in the sample. Nitrate is estimated by first reducing it to nitrite in a copper cadmium column. The nitrite formed is then reacted with sulphanilamide under acid conditions to form a diazo compound, which is then coupled with N-1-naphthyl-ethylenediamine dichloride to form a reddish-purple azo dye for colorimetric estimation.

The monitors have individual dedicated electronic systems which monitor the flow of the sample through the system, process the data and provide a hard copy record.

3.2.3 Respirometers

The industrial respirometers used were of the same design as described by Nicholls (1982) and diagramatically depicted in Figure 3.1 :-

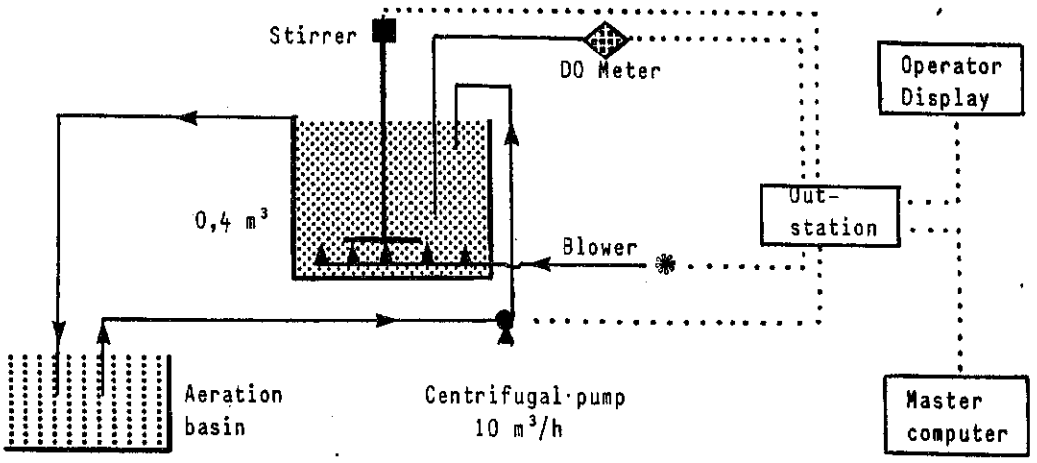

Figure 3.1 : Respirometer and Control System
.... Electrical connections

Three units were installed on the primary aerobic zone of Module 3 in positions indicated in Figure 3.2. The operating cycle of each individual monitor was as follows :-

First, the pump was energised by the computer to pass mixed liquor from the aeration tank through the respirometer for a period of ten minutes, which ensured that the contents of the respirometer were replaced four times.

Next, the pump was switched off and an initial reading of dissolved oxygen taken. In practice, this reading was found to be 0,2 - 0,3 mg/ℓ lower than the DO measured at the corresponding point in the aeration tank.

In order to measure the OUR, the DO level of the respirometer contents was increased to 4 to 5 mg O_2/ℓ by switching on the blower and bubbling air through the liquid for about two minutes. Care had to be exercised here as excessive aeration would have resulted in some of the readily biodegradable substrate being utilised before the OUR could be measured. After turning the blowers off and allowing a 15 second period for the DO to stabilise, a series of DO measurements were taken at 5 second intervals, over a period of five minutes. From these readings the computer calculated the OUR and correlation factors and stored this information in an appropriate file on disk. During this entire procedure, the contents of the respirometer were gently mixed in such a manner as to minimise the ingress of oxygen through the surface. This entire cycle was repeated on all three respirometers at approximately 25 minute intervals.

3.3 SAMPLE COLLECTION AND INSTRUMENT HOUSING

Local site conditions necessitated the monitoring instruments being housed next to the anaerobic zone of Module 3, i.e. some 500 to 700 m from the final clarifiers (Figure 3.2).

Effluent from the final clarifiers was pumped to the monitoring station by means of a submersible pump via a smooth-walled polyethylene pipe to a small settling tank, which acted as a trap for any solids accidentally carried over with the clarifier effluent. Volumes far in excess of the requirements of the monitors, were delivered to the pre-monitor clarifier to ensure that liquid residence time in the pipeline was less than five minutes, and to minimise the growth of biological film on the pipe. Tests showed that there was no change in the chemical characteristics of the effluent. Once a week, as a precautionary measure, the pipeline was flushed out with a concentrated hypochlorite solution. Settled sludge and excess effluent from the pre-monitor settling tank, were returned to the anaerobic zone of the main plant. Further conditioning of the effluent was achieved by filtration through a 25 μm stainless steel filter.

3.4 OPERATIONAL EXPERIENCE AND COST ASPECTS

Teething problems were experienced with the commissioning of some of the instruments. Three months elapsed before the nitrate monitor reached a state of satisfactory operation. The main problem was blockages, which were attributed to a reaction between the wetting agent used to facilitate reagent flow, and the ammonium chloride solution. This problem diminished considerably when use of the wetting agent was discontinued. Other blockages were traced to very fine suspended material entering with the reagents. These problems were solved by doubling the size of tne pump tubes and reactor coils, with the obvious disadvantage of increasing the cost through greater usage of chemicals.

The ammonia monitor took nine months to commission, largely due to a faulty pump and recorder. Minor blockage problems were also experienced and as with the nitrate monitor, solved by increasing the size of the pump tubes and reactor coils.

In order to ensure near 100 % reliability, pump tubes on these instruments had to be replaced every two weeks, which added considerably to the maintenance costs. After these changes were effected, very little further difficulty was experienced.

The phosphate analyser was delivered with large sized pump tubes i.e. about three times the size of those in the nitrogen analysers and operated reliably from the day it was installed.

None of these monitors could be left unattended for any length of time. They were checked daily and often some minor attention was required for leaking tubes and adjustment of bubble pattern. More detailed servicing was carried out on the Technicon Analyser once every two weeks, involving replenishing reagents and replacing pump tubes. Manual calibrations were also carried out using standard solutions. Such a service, including the making up of reagents, would take an experienced person about 8 hours.

The Bran and Lubbe phosphate monitor was serviced every 4 months. Replacement of tubes took only a few minutes, but cleaning of glassware and

making up of reagents still occupied the time of a technician for a period of 8 hours.

The stainless steel filters interposed between the monitors and the external settling tank, required manual washing and backwashing with tap water every 8 hours but to date have not required acid washing. Cartridge filters were washed daily and replaced on a monthly basis.

In practice it was found necessary to employ a Laboratory Technician to carry out the above servicing programme. When it was necessary to seek assistance from the suppliers of these instruments, their representatives responded promptly to service calls.

With regard to the respirometers, it was found necessary to spend about 5 minutes per respirometer per day cleaning the DO probes with water and restandardising the DO meter. The meters were originally mounted on the respirometer and were subject to some degree of vibration, which in turn, caused an occasional breakage of a galvonometer spring. This fault was corrected by mounting the instrument in a weatherproof box alongside the respirometer.

The capital and estimated running costs associated with the nitrate, ammonia and phosphate monitors are given in Table 3.1 :-

TABLE 3.1
CAPITAL AND OPERATIONAL COST OF MONITORS

	TECHNICON		BRAN AND LUBBE
	Ammonia	Nitrate	Phosphate
	R	R	R
Purchase price (1984)			
Monitor and filtration system	31 308	31 308	28 890
Running Costs (1986) (Rand/month)			
Chemicals	235	27	46
Pump tubes	106	93	52
Labour : skilled	380	380	98
: unskilled	35	35	75
Total running cost/month*	756	535	271

*Excludes any provision for worn components, which was not necessary during the two-year period of operation reported upon. It should also be noted that the price of the pump tubes, which were imported, was very dependent on the exchange rate at the time of purchase.

3.5 DATA COLLECTION SYSTEM

In view of the large number of operating commands which have to be given to the respirometers, and the vast amount of data which is subsequently generated, it was decided to install a micro-computer (in this case, an Apple), to carry out these tasks. It also has sufficient capacity to store data generated by the ammonia, nitrate and phosphorus monitors, and facilitated its manipulation into a variety of formats suitable for use by operating, laboratory and management staff. It was envisaged (at some later date) that this system should also be compatible with, and capable of integration with a far more comprehensive plant data collection, monitoring and control system.

The Telecommunications Branch of the Council's Electricity Department were commissioned to design the whole system, and to integrate into this plan

the necessary equipment and hardware to carry out the Council's commitments to the Water Research Commission. Due to shortages of suitably trained staff and monetary restrictions, this project, including the small section involving the on-line monitors, took longer than expected to complete. In designing this system, cognisance had to be taken of the high humidity and spray content of the environment in the immediate vicinity of the activated sludge plant. As the works are situated in an area of extremely high thunderstorm activity, precautions had to be taken to protect vulnerable electronic equipment from damage due to lightning induced voltage surges. In these circumstances the best practical option appeared to be to place the systems master station in the administration buildings, located 1,5 km from the actual plant and to site a micro-processor-based outstation in the control tower near the plant (see Figure 3.2 for schematic layout).

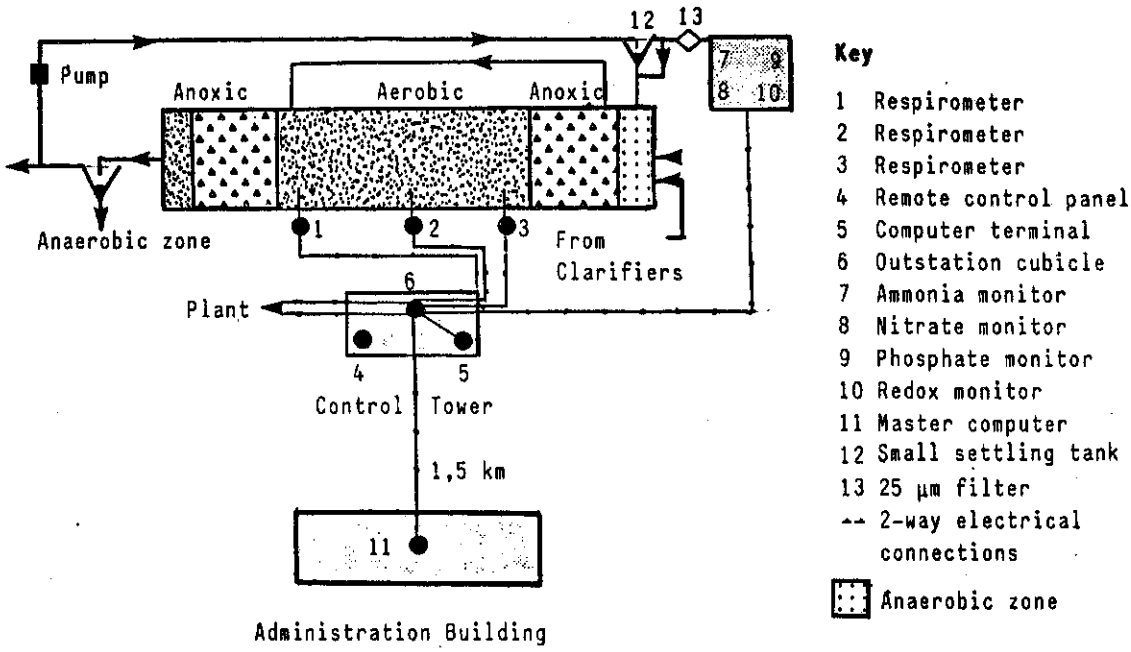

Figure 3.2 : Schematic Layout of Sampling Point, Monitoring and Remote Control Systems

The master station comprises the Apple computer that runs an executive program which requests information from, and gives control instructions to, the outstation, in order to control the monitors or plant. It provides a current status display, logs important plant parameters for example, on disk and allows for the running of other utility tasks. The software is written in a high level Pascal language with many of the routines and procedures being provided as library files, thus enhancing modularity and flexibility. One of the advantages of Pascal is the almost self-documenting nature of the language, especially when care has been taken in choosing variable names.

System restrictions made it necessary for the routines for communication with the outstation to be written in Assembler language. These, and a number of other routines are transparent to the user. The programs and procedures are written in such a manner that the keyboard is alive at all times. Many of the procedures are incorporated as library units (Unit Sewerage, Unit Respirometer and Unit Equipment), which are called by the executive program "Sewermain". The system also has automatic start-up facilities, enabling it to re-enter the program into the micro-computer after a power failure.

At the request of the master station, the outstation will perform one of the following functions :-

(a) clear (turn off) all control relays
(b) perform a digital control
(c) read digital input data
(d) read analogue input data
(e) read totaliser values
(f) return error status to the master station.

In the case of (c), the current state of all the digital inputs is recorded. As far as (d) is concerned, the current values of the selected block of analogue inputs is recorded, while in the case of (e), the current count value of all totaliser inputs is recorded.

Two types of control are provided, viz continuous and momentary. The continuous control uses a single relay which, when closed, turns the plant item on and when open, turns it off, for example, the respirometers, pumps and blowers. The momentary control makes use of two relays, one which turns the plant item on and the other switches it off. The appropriate relay closes for approximately 0,5 seconds and then automatically opens, implying that the plant has its own mechanism for latching on.

This system makes provision for possible future control of plant motors on aerators or mixers, should it be decided to automate plant control. Equipment operated by a momentary control will be unaffected by an outstation failure, whereas that operated by a continuous control, would be switched off. A modem designed and constructed by the Electricity Department allows the computer to communicate over a two-wire line with the outstation.

The outstation was assembled from 19 inch printed circuit cards and racks, manufactured by Telkort (Pty) Ltd and mounted in a cabinet specifically designed for operation in an environment detrimental to electrical equipment. It is of the type extensively used in the Electricity Department's telecontrol system and is micro-processor controlled. The number of inputs or control outputs is easily expanded by the addition of plug-in cards. Status inputs (on/off or digital inputs) from the plant are connected to optical isolators, while analogue signals are afforded protection by voltage limiting diodes, on an analogue protection card. Controls are performed by contacts of output relays. Provision is also made for up to six totaliser (pulsing) inputs. Input from on-line monitors, which are sited about 200 m from the outstation, are conveyed in a single line, making use of a multiplexor.

All the outstation functions are controlled by an assembler programme which resides in EPROM (Erasable Programmable Read Only Memory) on the micro-processor card. This programme scans the digital, analogue and totaliser inputs on command from the master station and also looks after the communication protocols. A terminal is provided to display the output of the computer to works operators. Considerable capacity for expansion is available, and in the future, consideration could possibly be given to

automating a system bringing samples from the various zones in the activated sludge plant to the on-line analysers, for analysis on a time-sharing basis.

3.6 RESULTS AND DISCUSSION

3.6.1 Phosphate, Ammonia and Nitrate Analysers

Whilst the monitors are expensive to purchase and to operate, they have the very obvious advantage of providing a continuous record of plant performance. Plant operators have benefited greatly by having information readily available on plant performance. By scrutinising the data throughout the day, they obtained a far greater appreciation for the process. If, for example, the effluent nitrate was seen to be increasing, plant changes could be made and the success or otherwise, was immediately visible to the operator. In fact, they became so dependent on the instruments that they found difficulty in operating the plant when these units were off-line.

Once the manufacturers had been able to correct initial teething problems and the Council had been able to make available the part-time services of a Laboratory Technicial, to assist in the day to day running of the instruments, very little difficulty was experienced with the maintenance of this equipment. During the investigational period the electronic side of the monitors remained trouble-free. More cost effective use of this instrumentation could possibly be made by making provision for the contents of individual zones to be analysed. Early warning of process problems would then be rapidly available to plant operators. A prerequisite for this extended facility would be that some device would have to be installed to remove activated sludge solids from the liquid to be analysed.

3.6.2 Respirometers

The basic objective of installing respirometers was to obtain meaningful values of oxygen utilisation but, since these were based on the measurement of DO, it was also possible to gain an additional benefit by recording the DO content of the liquor initially pumped from the aerobic reactor to the

respirometer. Whilst this would not represent the absolute value in the reactor, it would provide adequate guidance to the operator and show the effect of any plant changes. Its recorded value would furthermore permit the works management staff to judge the effectiveness of the power used on the plant. Moreover, it was believed that this procedure would overcome previous difficulties experienced on the Johannesburg nutrient removing activated sludge plants in measuring and recording DO levels with commercially available instrumentation.

Regarding the measurement of OUR, initial experiments had shown that there was a dramatic drop over a short distance, at the head of the aeration basin (see Figure 3.3). In order to obtain a representative figure for the entire primary aerobic zone, it was decided to install three respirometers (Figure 3.2) and average the individual results.

To verify that this average figure was acceptable, it was inserted into the UCT model (WRC 198 4), to see whether reasonable COD and TKN mass balances could be obtained. Such an exercise is reflected in Table 3.2, from which it was concluded, bearing in mind the errors that could be introduced in sampling and flow metering, that the calculated mass balances were acceptable.

TABLE 3.2

NITROGEN AND COD MASS BALANCES ACROSS AN ACTIVATED SLUDGE PLANT AT NORTHERN WORKS

Nitrogen Balance		COD Balance	
	mg N/ℓ		mg COD/ℓ
TKN influent	39	Influent	240
TKN effluent	2,1	Effluent	46
Nitrate in effluent	17	Oxygen demand (OUR)	128
Total nitrogen in waste sludge	2,9	COD in waste sludge	43
Nitrogen loss due to denitrification	16,8		
Total nitrogen recovered	38,8	Total COD recovered	217
% recovery	99	% recovery	90

An example of the data produced by the respirometers is reflected in Table 3.3. The time intervals between readings is about 25 minutes. All data is stored on disk and can be recalled for review by the operating staff.

This information has been reproduced in graphical form as depicted in Figure 3.3 and from which the following can be observed :-

(a) The oxygen utilisation rate at the beginning of the primary aeration zone was on average for the day in question, 5,3 times that at the end of the aeration basin.

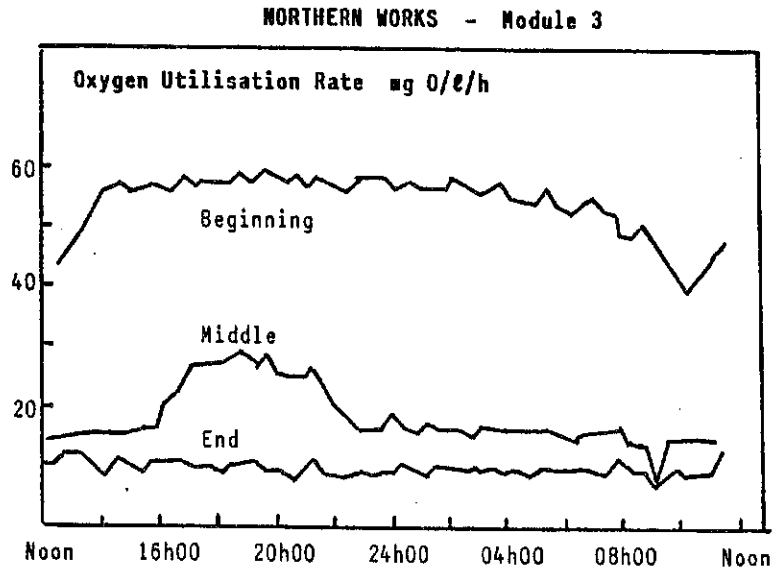

Figure 3.3 : Oxygen Utilisation Rates at Different Points in Primary Aeration Basin

(b) The maximum OUR appears to be sustained for a period of about 14 hours, compared to a sustained 3 hour peak for sewage entering the works (10h00 - 13h00). Whilst this indicates that considerable attenuation of oxygen demand requirements has taken place, it also suggests that introduction of balancing tank controls may be cost effective. Such a strategy would be particularly effective, if it proved possible to postpone the maximum demand for energy, into a time period when lower off peak tariffs were applicable for electricity supply.

(c) The pronounced transient OUR peak exhibited in the middle of the primary anoxic zone, may be due to the oxygen demand exhibited by

the sudden release of sludge accumulated in the balancing tank. It may be hypothesized that the addition al quantity of particulate matter present in the influent sewage, is initially adsorbed on to the activated sludge and solubilised in the aerobic zone, thus creating an additional demand for oxygen. An alternative possibility is that the extra oxygen demand is being created by nitrifying bacteria.

(d) Table 3.3 reveals the presence of a considerable oxygen gradient in the primary aerobic zone. Dissolved oxygen levels average 1,35 mg/ℓ at the beginning of the basin, 1,40 mg/ℓ in the middle and 0,67 mg/ℓ at the end, dropping at times to 0,2 - 0,3 mg/ℓ. Aerators at the end of this zone are often switched off to lower the carry-over of dissolved oxygen into the second anoxic zone (see photograph at the front of this document). Low oxygen conditions are thereby created in certain areas, which are conducive to the growth of filamentous organisms and the production of a bulking sludge (see Section 8.2). A high OUR at the end of the primary aeration zone would likewise indicate the presence of unoxidised particulate matter on the floc. Eikelboom (1983) considers this to be undesirable, as potential adsorption sites on the returned sludge from the secondary clarifiers are not available for the removal of soluble organic pollutants from the influent sewage. Such compounds are then able to pass through the primary aerobic zone where, in dilute concentration, they preferentially accelerate the growth of filamentous organisms. When non-operational aerators are switched on in the aerobic zone, there is an immediate increase in OUR, due to the resuspended sludge which has been lying under anaerobic conditions, beneath the stationary aerators.

The respirometric monitors described, can provide a wealth of most useful information to a knowledgable works management. For example, the data collected can be used to reliably pinpoint plant problems, malfunctioning of equipment and faulty operational procedures. Knowledge of OUR is a prerequisite for the use of the UCT model (WRC, 198) (see Chapter 7) for calculating mass balances. With slight modifications the respirometers can also be used to measure maximum specific growth rates of heterotrophs,

TABLE 3.3
TYPICAL DATA GENERATED BY THE RESPIROMETERS AT NORTHERN WORKS

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	1,5	14,9	0,993	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	12,1	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
18:08:50	57,2	0,999	1,4	27,9	0,998	0,9	9,0	0,985	0,3
18:32:16	57,5	0,999	1,3	28,6	0,997	0,9	10,3	0,983	0,3
18:55:42	58,7	0,999	1,3	29,1	0,996	1,0	10,3	0,988	0,3
10:19:08	57,1	0,998	1,3	27,5	0,997	0,9	11,6	0,976	0,3
19:42:34	59,1	0,999	1,2	28,9	0,997	1,0	9,0	0,967	0,3
20:06:00	58,5	0,999	1,2	24,9	0,998	1,1	9,5	0,981	0,4
20:29:26	57,8	0,999	1,2	25,2	0,997	1,0	8,0	0,963	0,2
20:52:52	58,4	0,999	1,2	24,9	0,998	0,8	8,9	0,969	0,3
21:16:18	57,3	0,999	1,1	26,8	0,997	1,2	11,9	0,953	0,3
21:39:44	58,7	1,000	1,1	22,5	0,996	1,2	9,9	0,981	0,3
22:03:10	57,5	0,999	1,2	19,5	0,995	1,0	9,4	0,964	0,3
22:26:36	56,4	0,998	1,3	17,5	0,996	0,9	8,7	0,963	0,3
22:50:02	57,6	0,998	1,2	16,4	0,991	1,1	9,2	0,986	0,4
23:13:28	58,2	0,999	1,2	16,5	0,993	1,1	8,7	0,950	0,3
23:36:54	58,2	0,999	1,3	16,6	0,994	0,9	9,6	0,970	0,5
00:00:20	58,0	0,999	1,2	19,4	0,996	1,2	9,1	0,963	0,2
00:23:46	56,7	0,999	1,2	16,3	0,993	1,2	10,4	0,965	0,3
00:47:12	57,8	0,999	1,3	15,8	0,991	1,1	9,7	0,964	0,3
01:10:38	56,3	0,999	1,2	17,0	0,996	1,2	9,0	0,968	0,3
01:34:04	56,7	0,998	1,4	16,4	0,996	1,2	10,1	0,956	0,3
01:57:30	56,5	0,999	1,2	16,4	0,997	1,0	10,2	0,973	0,6
02:20:56	58,4	0,999	1,3	16,4	0,996	1,1	9,8	0,973	0,3
02:44:22	56,7	0,998	1,2	15,7	0,995	1,4	10,0	0,965	0,5
03:07:48	56,0	0,998	1,4	16,5	0,997	1,1	9,8	0,984	0,4
03:31:14	56,3	0,998	1,2	16,7	0,996	1,1	10,4	0,984	0,4
03:54:40	56,8	0,999	1,2	16,0	0,996	1,1	9,3	0,983	0,5
04:18:06	55,9	0,999	1,1	16,4	0,997	1,2	9,4	0,967	0,5
04:41:32	55,1	0,998	1,3	16,2	0,996	1,4	8,2	0,978	0,5
05:04:58	54,8	0,998	1,4	16,1	0,997	1,2	9,1	0,966	0,6
05:28:24	56,7	0,999	1,2	16,6	0,997	1,3	10,0	0,968	0,6
05:51:50	53,7	0,998	1,3	15,6	0,997	1,4	9,8	0,987	0,6
06:15:16	52,2	0,999	1,3	14,7	0,998	1,3	10,6	0,986	0,6
07:02:08	55,1	0,999	4,4	15,8	0,999	2,3	9,4	0,988	1,2
07:25:34	53,2	0,999	1,4	16,1	0,998	1,6	8,6	0,981	0,7
07:49:00	52,1	0,999	1,5	16,0	0,995	1,4	12,0	0,978	0,8
07:59:34	49,9	0,999	1,5	14,2	0,990	1,9	11,2	0,978	1,2
08:23:00	48,6	0,999	1,5	14,1	0,992	1,7	10,8	0,964	1,2
08:46:26	50,8	0,999	1,5	14,1	0,997	1,9	10,0	0,987	1,3
09:09:52	49,2	0,958	1,6	6,5	0,891	1,8	6,0	0,965	1,2
09:33:18	45,5	0,999	1,6	14,8	0,998	2,1	8,3	0,965	1,3
09:56:44	43,9	0,999	1,7	14,6	0,997	1,8	10,3	0,972	1,5
10:20:10	39,2	0,997	1,6	14,8	0,997	2,5	8,8	0,971	1,4
10:43:36	41,1	0,997	1,6	14,5	0,996	2,1	8,9	0,973	1,3
11:07:02	45,0	0,999	,15	14,8	0,998	2,5	8,8	0,970	1,4
11:30:28	46,5	0,998	1,5	14,8	0,996	2,4	13,0	0,967	1,5

NOTE (a) our(0); our(1); our (2) represent oxygen utilization rates at the beginning, middle and end of primary aeration basin.

(b) cc = correlation coefficient.

(c) do = approximate dissolved oxygen concentration in mg/ℓ in primary aeration basin at same points as in (a).

a parameter which has a bearing on the control of filamentous organisms (Ekama et al., 1985).

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CHAPTER FOUR

Microbiological studies

4.1 INTRODUCTION

In activated sludge the relative numbers of different bacterial species are mainly determined by their growth rate and the availability of suitable food. The dominant organisms in terms of efficient sewage purification are not necessarily those occurring in the largest numbers, but those that make the greatest contribution to the necessary metabolic activity.

The dominant bacteria will be those capable of most effectively utilising the sewage and forming flocs, to ensure their retention in the system. The nature of bacteria in the plant is determined chiefly by the composition of the sewage and process configuration.

Considering these two characteristics, it is clear that the anaerobic, anoxic and aerobic zones in an activated sludge plant, will stimulate the dominance of different bacteria. For example, the anaerobic zone environment will favour the proliferation of bacteria capable of fermentation, i.e. non-oxidative metabolism. A number of bacteria capable of this type of metabolism are also capable of oxidative metabolism, i.e. facultative bacteria.

The transition from fermentative metabolism to oxidative metabolism does not occur rapidly. Consequently, facultative bacteria entering the aerobic zone will be at a distinct competitive disadvantage against obligate aerobes for available substrate, thus limiting their proliferation

under these conditions.

The production by facultative anaerobes of fermentation products which are eminently suitable for denitrifying and phosphorus accumulating bacteria, illustrates the mutual advantages available to bacteria in an activated sludge system.

The discussion above has been confined to bacteria beneficial to sewage purification. There are however, bacteria which may cause severe operational problems if allowed to proliferate unchecked in an activated sludge system. These are the so-called filamentous bacteria, which have the ability to bridge sludge flocs causing the sludge to settle poorly. This so-called sludge bulking and scum formation, are problem areas, which are of similar importance as sewage purification by bacteria.

The various bacterial groups encountered in multistage biological nutrient removing activated sludge systems will be discussed below :-

4.2 METHODOLOGY

In order to study the various bacteria present in nutrient removing activated sludge, a number of microbiological techniques had to be refined and new ones had to be developed.

4.2.1 Microscopic Investigations

Slides were prepared by thoroughly mixing the sample and taking one drop from a Pasteur pipette onto a glass microscope slide, and allowing it to dry. Slides were stained for polyphosphate accumulation by the Methylene Blue method described by Fuhs and Chen(1975) and the Neisser stain (Society of American Bacteriologists,1957). Polyhydroxybutyrate accumulation was visualised by staining with Sudan Black (Gurr, 1973), or Nile Blue (Ostle and Holt, 1982). For phase contrast microscopy one drop of sample was placed on a microscope slide with a loop or Pasteur pipette. A cover slip was then placed on the drop and pressed down gently.

4.2.2 Bacterial Identification

Filamentous bacteria were identified by using the key developed by Eikelboom and van Buijsen(1981).

Bacterial population studies were carried out by isolating organisms from activated sludge mixed liquor and subjecting the isolates to identification by the API 20E technique, combined with a fluorescent antibody technique (Cloete et al.,1985). The procedures used are described in detail by Lötter and Murphy(1985). Aeromonas spp were distinguished from each other by using the butanediol dehydrogenase test (Schubert, 1974).

4.3 NUTRIENT REMOVING ORGANISMS

Population studies on activated sludge plants have been undertaken by a number of researchers (Brodisch and Joyner, 1983; Buchan, 1983; Cloete et al.,1983). However, these studies were confined to the aerobic phase of the process. Later research was extended to include the study of anaerobic and anoxic zones (Lötter and Murphy, 1985). The identification of bacterial species is time-consuming and not suitable for routine plant monitoring.

Polyphosphate and polyhydroxybutyrate accumulation in activated sludge has been demonstrated microscopically (Fuhs and Chen, 1975; Buchan, 1981; Hart and Melmed, 1982). Microscopic evaluation of activated sludge plants mignt therefore provide a satisfactory monitoring technique for P removal efficiency. A number of studies were undertaken to investigate this possibility.

Studies on the bacterial population structures of activated sludge plants revealed the presence of Aeromonas hydrophila in the anaerobic zone (Lötter and Murphy,1985). In other population studies, Aeromonas punctata was found to be the dominant Aeromonas species present (Brodisch,1985).

The API 20E test cannot distinguish between these two Aeromonas species (Analytlab Products,1977). It was therefore decided to use another test capable of distinguishing between the two.

Butanediol dehydrogenase activity was used to distinguish Aeromonas hydrophila from Aeromonas punctata, the species of Aeromonas previously found in nutrient removal activated sludge plants (Brodisch, 1985; Lötter and Murphy, 1985).

4.3.1 Population Studies

The aerobic zone of the five stage Bardenpho plant at Northern Works (Module 3), was sampled on three occasions when the phosphate content of the sludge of the plant differed considerably (see Tables 4.1 and 4.2).

TABLE 4.1
CHEMICAL COMPOSITION OF BALANCE TANK EFFLUENT (MODULE 3)
TO PLANT AT TIME OF AEROBIC ZONE SAMPLING

Parameter	Sample 1	Sample 2	Sample 3
COD as oxygen	490	410	580
Kjeldahl N as N	42	46	60
Ammonia as N	31	31	40
Total phosphorus as P	12	12	12
Orthophosphate as P	7,2	7,2	7,1

TABLE 4.2
CHEMICAL COMPOSITION OF FINAL EFFLUENT
AT TIME OF AEROBIC ZONE SAMPLING

Parameter	Sample 1	Sample 2	Sample 3
COD as oxygen	56	55	55
Total nitrogen as N	9,5	7,8	6,7
Ammonia as N	Nil	Nil	Nil
Nitrate as N	8,6	4,3	6,6
Total phosphorus as P	7,5	9,6	8,3
Orthophosphate as P	6,8	8,7	8,2
Phosphorus removal mg P/g VSS	1,29	1,73	0,90

The three bacterial population distributions for these three states of phosphate removal, are shown in Figure 4.1.

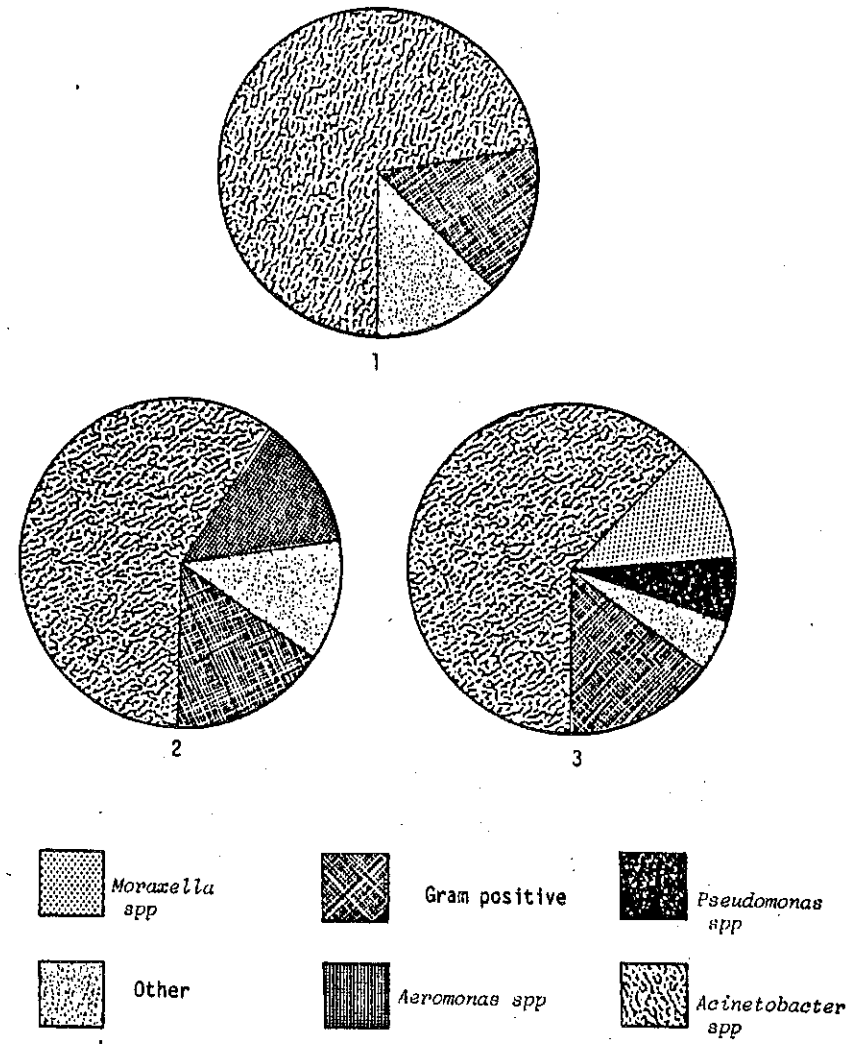

Figure 4.1 : Heterotrophic bacteria present in the primary aerobic zone of a five stage Bardenpho plant at different levels of phosphorus removal. (1) 1,29 mg P/g VSS; (2) 1,73 mg P/g VSS; (3) 0,90 mg P/g VSS.

The numbers are expressed as a percentage of the total number of colonies that grew. Bacterial species which represented less than 5 % of the population have been grouped together under "other".

In addition to the bacteria shown in Figure 4.1, the aerobic zone samples contained Flavobacterium spp, Bordetella bronchiseptica, Citrobacter spp, Shigella spp, Pasteurella spp and Yersinia enterocolitica.

The dominance of Acinetobacter spp in aerobic zone samples is in agreement with the results of other workers (Buchan, 1983; Kerdachi and Roberts, 1983), who undertook studies on full-scale plants. The dissimilarity with the results of Brodisch and Joyner (1982) who reported Pseudomonas spp as the most dominant bacterium, can possibly be attributed to the fact that their work was on pilot plants and that a scale effect occurred.

The complete dominance of the obligate aerobe Acinetobacter spp over facultative anaerobes in this zone is probably due to the relatively slow transition from fermentative to oxidative metabolism, which the latter have to undergo to compete in the aerobic environment.

The anaerobic and anoxic zones of this plant were sampled on two separate occasions of different phosphorus removal capacity (see Tables 4.3 and 4.4).

TABLE 4.3
CHEMICAL COMPOSITION OF FEED (BALANCE TANK EFFLUENT) TO PLANT AT THE TIME OF ANOXIC AND ANAEROBIC ZONE SAMPLING

Parameter	Sample 1	Sample 2
COD as oxygen	620	930
Kjeldahl as N	56	48
Ammonia as N	35	27
Total phosphorus as P	18	13
Orthophosphate as P	9,9	6,6
pH	7,0	7,1

TABLE 4.4
CHEMICAL COMPOSITION OF FINAL EFFLUENT AT TIME OF ANOXIC AND ANAEROBIC ZONE SAMPLING

Parameter	Sample 1	Sample 2
COD as oxygen	65	170
Total nitrogen as N	7,7	9,6
Ammonia as N	Nil	Nil
Nitrate as N	6,7	0,5
Total phosphorus as P	3,3	6,9
Orthophosphate as P	Nil	Nil

The bacterial population distributions for these two levels of phosphate removal are shown in Figures 4.2 and 4.3.

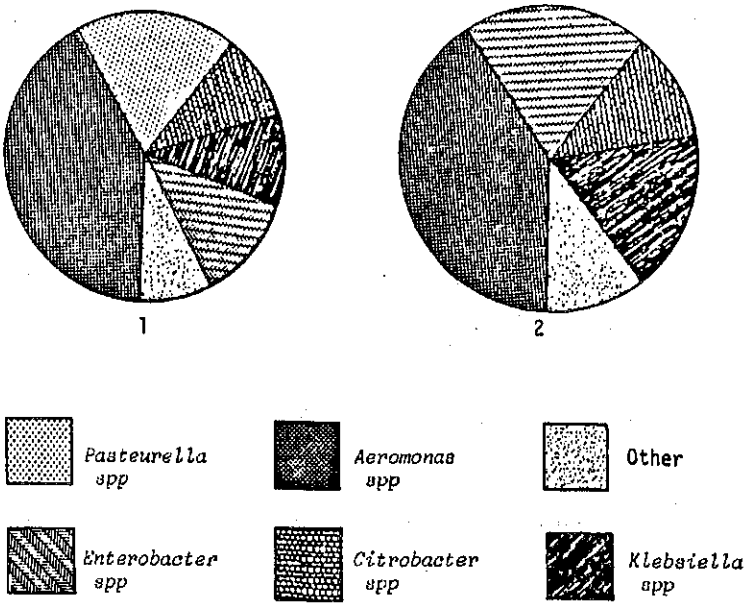

Figure 4.2 : Relative proportions of heterotrophic bacteria in the anaerobic zone of the five-stage Bardenpho process. (Anaerobic incubation).

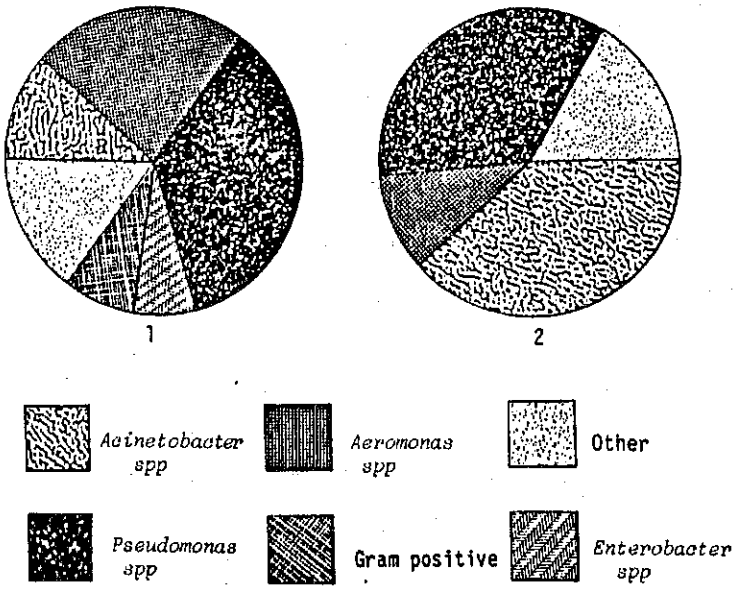

Figure 4.3 : Relative proportions of heterotrophic bacteria in the primary anoxic zone of the five-stage Bardenpho process. (Anaerobic incubation with nitrate in the medium).

As expected, facultative anaerobes dominate the anaerobic zone. The importance of Aeromonas punctata as an acid producer has previously been noted by Brodisch (1985). The presence of these bacteria in this zone would supplement the suitable substrate available for Acinetobacter spp.

As shown in Figure 4.3, the denitrifying Pseudomonas spp constitute about one-third of the anoxic zone population. The remainder of the populations however, differ substantially between the two zones and might account for the difference in denitrification ability of the plant.

The comparison between the zones, achieved by this study, demonstrate the effect of the three different zone environments on metabolic behaviour.

The anaerobic zone, which is rich in readily biodegradable substrate and devoid of oxygen, encourages non-oxidative metabolism, i.e. facultative anaerobes will dominate this zone in terms of metabolic activity. On the same basis, the anoxic zone will allow oxidative metabolism with nitrate as the terminal electron acceptor to dominate.

4.3.2 Microscopic Evaluation

Samples were taken daily from all five zones of the five-stage Northern works plant (Module 3), over an eight week period. Slides of activated sludge samples were stained for the presence of polyphosphate inclusions. A system of values from 0 - 10 was adopted for assessing the level of phosphorus accumulation as polyphosphate. The orthophosphate in the supernatant was also determined.

Acinetobacter calcoaceticus was seen as single cells, chain formations and clusters, ranging in size from a few cells to aggregates containing an estimated many thousands. When phosphate uptake was good, the cells tended to be large and swollen, i.e. from the normal 1,5 µ x 2,0 µ to 3,5 µ x 5,0 µ. Streptococcal forms were very much enlarged and the clusters, some of which became very large, were dense and compact. A fuzzy outline was sometimes associated with the very full clusters, suggesting the possible presence of extracellular polymer. Figures 4.4 to 4.7 show these characteristics.

When the plant is not removing excess phosphate the individual Acinetobacter are small (1,5 x 2,0 µ or even less), there are many chain forms, clusters are small to medium in size and do not have a dense compact appearance. The polyphosphate granules, where present, are small and

usually, the number of Acinetobacter appears to be much reduced (see Figures 4.8 to 4.11). The results of the microscopic assessment and orthophosphate analyses of the various zone samples, are shown graphically in Figure 4.12.

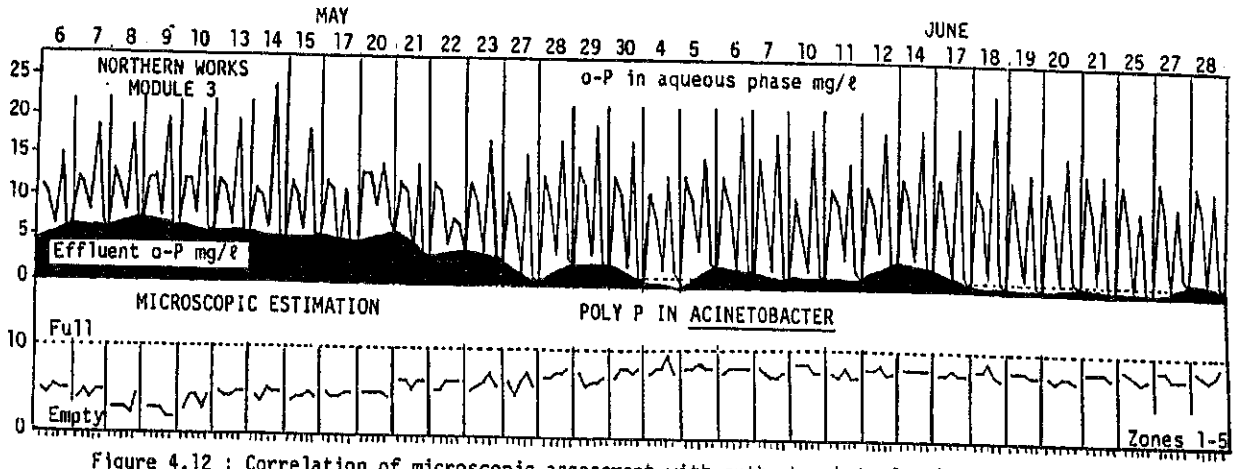

Figure 4.12 : Correlation of microscopic assessment with orthophosphate levels in the various zones of Northern Works Module 3

Note : The areas between each pair of vertical lines reflects conditions from left to right in the anaerobic, anoxic, aerobic, anoxic, aerobic zones and final effluent with the position of individual zones shown in the lower horizontal axis

From the graph it may be seen that the microscopic evaluation closely follows the general trend of removal or failure to remove phosphate, as shown by effluent phosphate concentration. However, there do not appear to be changes in microscopic appearance concomitant with P uptake and release in the various zones. In particular, it would be expected that the release in the anaerobic zone, and the massive release shown chemically to take place in the anoxic zone, would be reflected microscopically as the presence of a larger number of empty clusters, than would be seen in the other zones. Wentzel et al. (1986) have in fact shown that only a fraction of the P that can be released by the mixed liquor in the presence of excess acetic acid, is actually released in the system (about 20 %).

It may be postulated that there is a certain inertia in the emptying of the volutin granules so that it would take time for the microscopic picture to change. Also, there is most likely a small amount of phosphate uptake and release at all times, so that a certain number of phosphate containing cells and clusters of cells will always be seen.

When plant performance has been satisfactory and there is a sudden fall-off of removal, there are changes in the morphology of the Acinetobacter. Firstly, the clusters of which the majority were large, dense and compact, become 'disrupted' with the cells of the clusters being dispersed and reduced in size. Many more single cells appear. This can be described as an open, or scattered, look. These conditions are illustrated in Figures 4.13 to 4.16. The numbers of Acinetobacter can be assumed to be decreasing, because the large clusters break up and it is likely that there is washout of the single cells and very small clusters, and since conditions are not favourable to proliferation of Acinetobacter, their numbers are reduced.

While this work has shown that microscopic examination of mixed liquor samples is not sensitive enough to provide early warning signals of plant failure or recovery, the observations can be useful in plant operation. For example, microscopic evaluation of polyphosphate accumulation by Acinetobacter can be correlated with the results of chemical analyses, to show whether a plant is removing well or not and the overall rating is a good indication of plant performance.

The formation of larger, tighter, denser colonies of Acinetobacter, enlarged chain forms, swollen individual cells and sometimes, a fuzzy outline to the clusters, when the plant is beginning to pick-up after a period of poor removal, seems to herald an improvement in polyphosphate accumulation. Cluster break-up with the manifestation of the 'disrupted' look, smaller cells, smaller clusters and empty clusters, indicates the failure of polyphosphate removal.

A typical Acinetobacter cell cluster as observed in wet preparation, is shown in Figure 4.17.

4.4 FERMENTATION ORGANISMS

In order to gain greater insight into the production of volatile fatty acids (VFA) by fermentation, it is essential to identify the responsible organisms. Bacterial population studies have been used to explain the specific behaviour of anaerobic digestion (Cookson and Burbank, 1965;

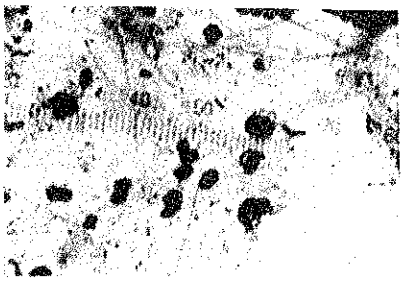

Figure 4.4
Single Acinetobacter cells showing the enlarged streptoccal form, typical of good phosphate uptake.

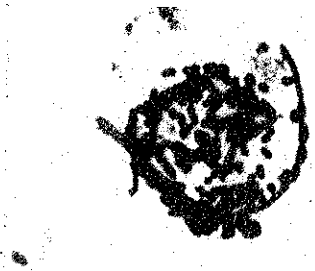

Figure 4.5
Acinetobacter cells in chain and loose cluster formation during a period of good phosphorus removal.

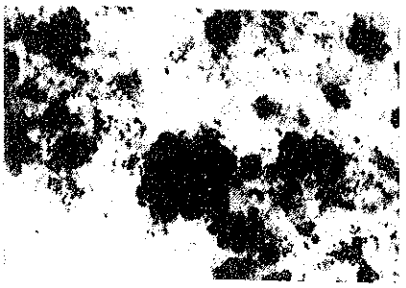

Figure 4.6
Acinetobacter cells in dense compact clusters during a period of good phosphorus removal.

Figure 4.7
Fuzzy outline associated with full clusters.

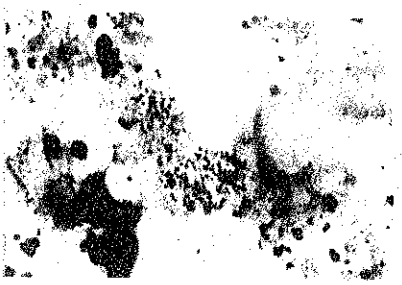

Figure 4.8
Single Acinetobacter cells showing the reduced size typical of poor phosphate uptake.

Figure 4.9
Acinetobacter cells containing small polyphosphate granules during a period of poor phosphorus removal.

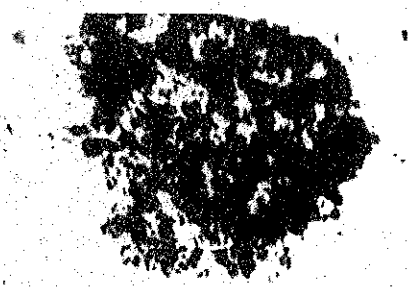

Figure 4.10
A loose cluster of Acinetobacter cells typical of poor phosphorus removal.

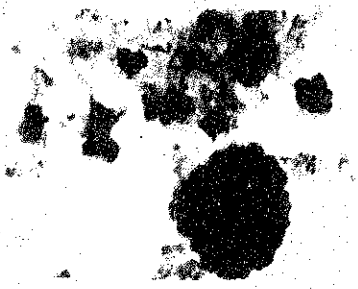

Figure 4.11
A small dense cluster of Acinetobacter cells typical of poor phosphorus removal.

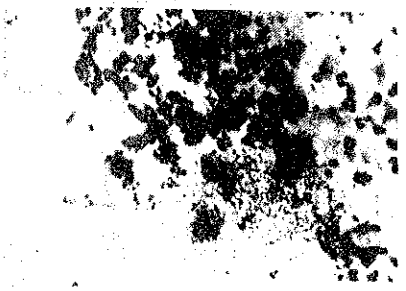

Figure 4.13
Dispersion of cell clusters and reduction in size during sudden onset of poor phosphorus removal.

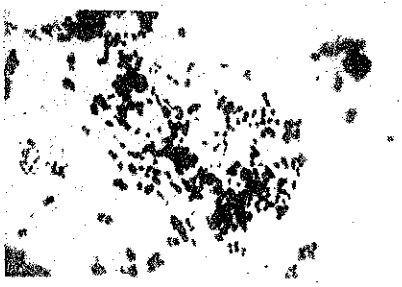

Figure 4.14
Most Acinetobacter cells appear singly during the period described in Figure 4.13.

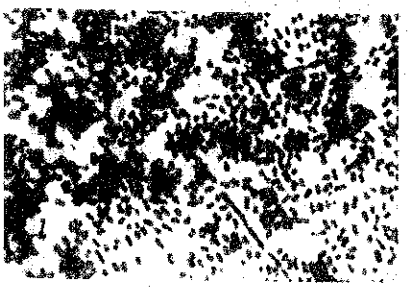

Figure 4.15
A total lack of clustering is observed during this phase of poor removal.

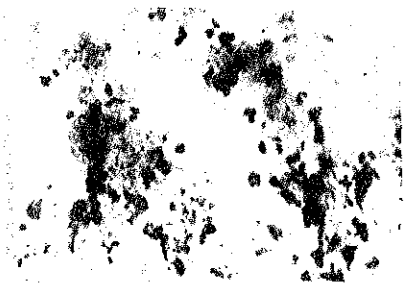

Figure 4.16
The Acinetobacter cells decline in number as well as size.

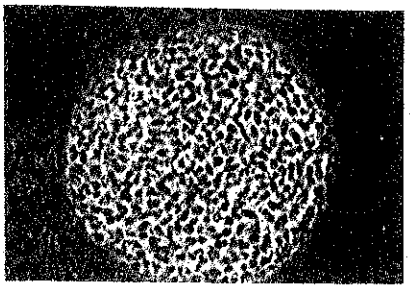

Figure 4.17
Wet preparation of Acinetobacter cell cluster in activated sludge.

Toerien, 1967, Toerien et al.,1967). This research however, has concentrated on the conversion of carbohydrates to methane and not on the formation of volatile fatty acids, which is, in fact, an intermediate step in this process.

Two groups of bacteria have been implicated in this process, namely, the acid forming bacteria which ferment glucose to produce a mixture of acetic, propionic and butyric acids and the acetogenic bacteria, which convert propionic and butyric acid to acetic acid (Mosey, 1983).

The bacteria capable of growth under anaerobic conditions were identified where possible. The number of colonies is expressed as a percentage of those that were incubated. Any species comprising less than 5 % of the total are grouped together under "other".

The bacterial population structures of the feed to a high rate acid digester with a four day retention time and in the digester, were investigated (see Figure 4.18).

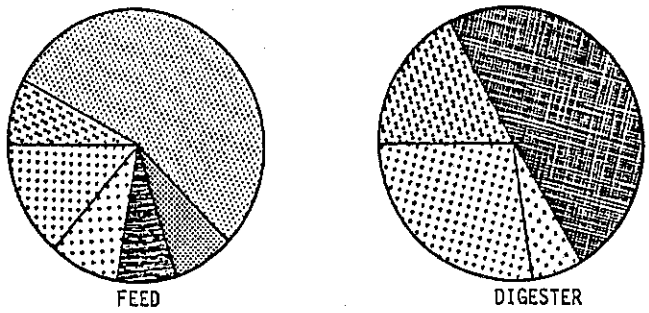

Figure 4.18 : Relative proportions of heterotrophic bacteria in a high rate acid digester and its feed.

KEY :

Unidentified
Other
Klebsiella spp
Aeromonas hydrophila
Enterobacter spp
Pseudomonas spp
Aeromonas punctata

The environment inside the digester clearly promotes a dramatic population shift from one which comprises Aeromonas punctata as the dominant facultative anaerobe to one in which Klebsiella oxytoca assumes the dominant facultative role. The isolation and identification of obligate anaerobes in this type of sample is clearly essential for a complete population study.

The population of another primary sedimentation tank upstream of an activated sludge plant is shown in Figure 4.19.

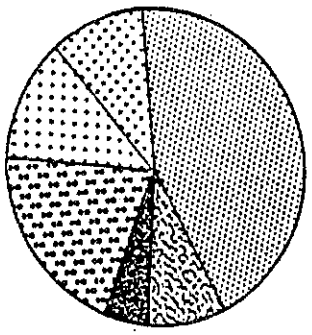

Figure 4.19 : Relative proportions of heterotrophic bacteria in the sludge of a primary sedimentation tank.

Key as for Figure 4.18 except for *Escherichia coli*

The results indicate the presence of a number of obligate anaerobes as observed in the anaerobic digester. The facultative population is however, considerably different with Aeromonas punctata the dominant organism.

The presence of Klebsiella oxytoca and Aeromonas spp in digesters with short retention times, has been reported previously (Halukinen et al., 1985).

The difference in population in the primary sedimentation tank is probably due to differences in feed composition. The similarity between the population of the feed to the digester and the primary sedimentation tank is not unexpected, as the feed to the digester originates from the primary sedimentation tank of biological filter units. Brodisch (1985) referred to the importance of Aeromonas punctata as a producer of acetic acid. While Aeromonas spp are known to ferment glucose to acetic, propionic and

butyric acids (Schubert, 1974), 2,3-butanediol is the main product of glucose fermentation by Klebsiella spp (Orskov,1974).

The difference in acid production profiles between the two processes has already been discussed in Chapter 2 and can probably be attributed to these population differences.

Further research will be required if the optimisation of acid production by the main bacterial species observed here is to be achieved.

4.5 FILAMENTOUS ORGANISMS

4.5.1 Scum Formation

The formation of scum on the surface of activated sludge process reactors has been observed on numerous plants throughout the world. If this scum is retained within the plant, it does not cause any serious problems, although it may result in the entire surface being covered, thus reducing the area open to the atmosphere, which could result in less efficient oxygen transfer if surface aerators are used. Effluent quality will obviously suffer when scum passes over the final clarifier weirs.

Jenkins et al. (1984a) have examined activated sludge derived scums and shown them to contain predominantly, either Nocardia or Microthrix parvicella. These filamentous bacteria have the ability to bridge floc particles and it is furthermore hypothesised, that they have cell walls that allow bubble attachment, which, when significant enough, may ultimately cause flotation of floc to form a scum. Eikelboom (1982) maintains that these filamentous bacteria generally contain large quantities of lipids, which decrease their density in relation to water and cause them to rise to the surface of the reactor. Eikelboom (1977) and Jenkins et al. (1984), have shown that the microbial composition of scums on activated sludge plants, can be reasonably predicted where the food to micro-organism loading on the plant is low.

During the observation period, the feed:micro-organism (F/M) ratio was 0,06 under normal operation. The appearance of the scum on the plant varied

from a light, frothy texture (Figure 4.20), to a heavy, mucoid mass (Figure 4.21). The colour ranged from cream to brown, becoming black and crusty on standing. It occurred as discreet islands, or as a blanket covering the entire surface. In the aerobic zone it formed a thin layer of foam in quiescent areas, where the aerators were switched off. The scum under investigation had been present in fairly large amounts throughout the previous drought-stricken summer.

Stereo microscopic examination revealed the following :

- Beneath a dark, dried-out surface film, the scum was found to contain a myriad of tiny bubbles coated with sludge, giving an overall honeycomb appearance. The walls of these bubbles were gelatinous in nature, and when pierced, the skin would fold back upon itself and a stable, hollowed out aperture would be formed, there being no flow of material back into the cavity thus formed. This phenomenon was particularly noticeable when the scum had thickened.

- The fragile, light bubbles in the froth from the aerobic zone were easily broken and the contents were very fluid.

- The clusters of bubbles from the surface of the final clarifiers, which resembled a white, net-like stucture (Figures 4.22 and 4.23), were found to be difficult to puncture.

Routine samples of activated sludge taken from the plant for microbiological analysis were seen to contain large quantities of gas which had lifted the entire solids content to the surface of the jar. This gas was identified as air.

Using the key developed by Eikelboom and van Buijsen (1981) the filaments present in the reactors and the scum were identified. The results are shown in Table 4.5 :-

TABLE 4.5
FILAMENTOUS BACTERIAL POPULATION AT NORTHERN WORKS

Filament	Quantity
*0803	Abundant
0041	Few
0675	Scanty
0092	Few
Nocardia	Scanty
Microthrix parvicella	Scanty

*The occurrence of this organism as a dominant filament is unusual, and it has not been observed as such in other plants in this country (Ekama 1986).

According to Jenkins et al.,(1984a) these filaments are all indicative of a low food to micro-organism (F/M) ratio, which was the case at the time of sampling. However, the small amount of Nocardia and Microthrix parvicella, is unexpected in view of Eikelboom's suggestion that one of these two filaments normally dominates in scum formation (Eikelboom and van Buijsen, 1981). In contrast, at the Northern Works there appeared to be no difference in the relative numbers of filamentous organisms present in the bulk of the liquid and scum, thus indicating that no selective concentration was taking place in the foam.

The same situation however, did not apply to the Acinetobacter population. Slides stained by Gram's method (Cruikshank 1960) showed these aerobic bacteria to be more swollen and clustered (Figure 4.25) in the scum, while in the MLSS they appeared concave and occured, not in clusters, but as single rods.

Subsequent to the observations described above, raw sludge recycling was introduced on the primary clarifiers and after a few weeks, a dramatic change in the microbial composition of the scum layer was noted, as it then consisted almost entirely of Microthrix parvicella. Obviously the chemical characteristics of the influent sewage also play an important role

in the preferential selection for certain filamentous bacteria.

Biosurfactant production by Nocardia erythropolis has been noted by Margaritis et al. (1979), and Sar and Rosenberg (1983) have reported on the extracellular production of bio-emulsifiers by sixteen different strains of Acinetobacter calcoaceticus. It is suggested that such substances may well be produced in the scum layers of nutrient removing plants thus imparting tremendous strength and stability to entrained air bubbles.

Under semi-plug flow conditions, the concentration of syndets will obviously be higher at the influent end of the reactor, and is more likely to emulsify any bubble stabilisers present, giving rise to a structurally weaker scum. Towards the end of the aerobic zones however, bacteria such as Acinetobacter, which are commonly found in nutrient removing plants, may have produced polymers for film stabilisation and at the same time, the available detergents will have been degraded. Under these conditions, stable and strong bubble formation could be expected and is indeed found.

Heavy showers of rain have been found to rapidly break up scum layers and it may well be that floating solids and entrapped air, are forced below the surface where detergents have a better opportunity of sequestering the bubble stabiliser, thus contributing to its collapse.

4.5.2 Sludge Bulking

A major problem in the operation of the activated sludge process is the growth of filamentous organisms,which leads to sludge bulking. As a result of its lower settling rate and much lower compaction rate, the separation of bulking sludge during the secondary clarification step becomes very difficult.

The prevention of sludge bulking has largely consisted of the use of chemicals such as chlorine to suppress the growth of the causative organisms.

Methodology for the rapid identification of filamentous organisms

responsible for sludge bulking have been developed by a number of researchers, inter alia, Eikelboom (1975);Lee et al. (1982) and Jenkins et al. (1984b).

A study was undertaken to evaluate the nature and degree of sludge bulking at the Northern Works Plant and to identify the dominant causative organisms. The filamentous organisms present in the three and five-stage modules at the Northern Works were monitored over a period of 12 months. The three-stage module incorporated an anaerobic selector for the period of the study. The results of the study are summarised in Tables 4.7 and 4.8.

The scoring of filament abundance (SFA) was undertaken according to Jenkins et al. (1984b) (see Table 4.6).

TABLE 4.6
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)

The bridging between flocs is shown in Figures 4.26 to 4.29.

Microthrix parvicella entwines the Acinetobacter cell clusters, forming a

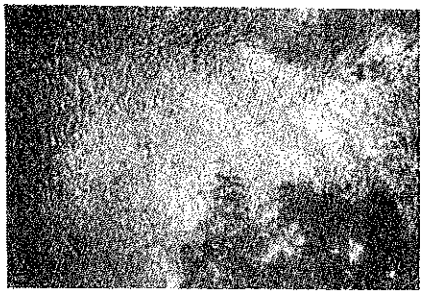

Figure 4.20
Light frothy scum.

Figure 4.21
Heavy mucoid scum.

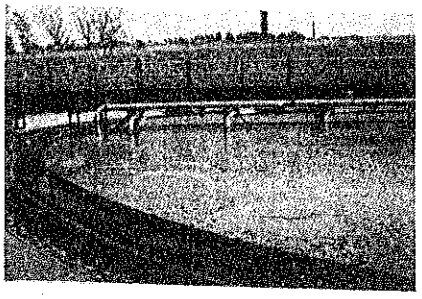

Figure 4.22
Net-like scum structure on surface of secondary clarifiers.

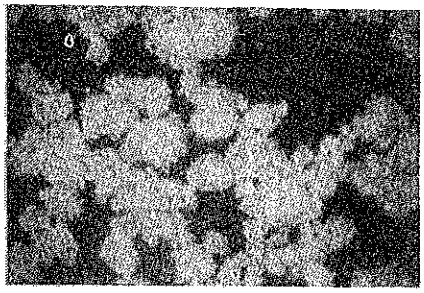

Figure 4.23
Close-up of net-like structure of scum shown in Figure 4.22.

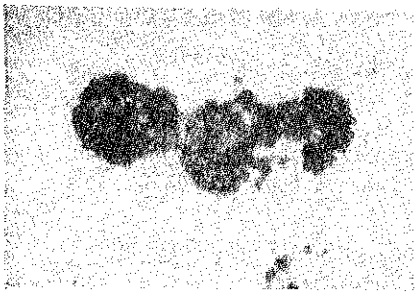

Figure 4.24
Acinetobacter cells present in scum in anaerobic zone.

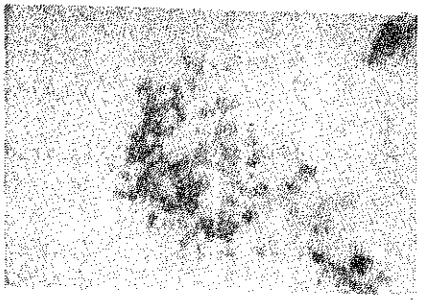

Figure 4.25
Acinetobacter cells in mixed liquor of anaerobic zone at the same time as Figure 4.23 was taken.

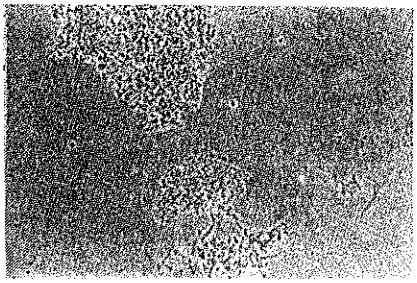

Figure 4.26
Wet preparation of activated sludge; very few filaments.

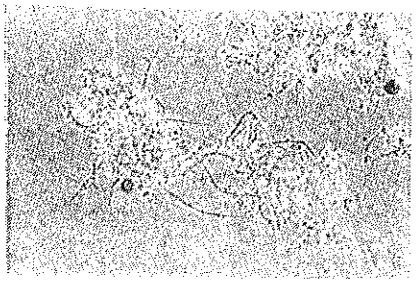

Figure 4.27
Filaments in all flocs; medium density and bridging between flocs.

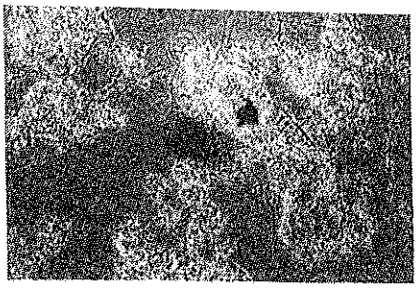

Figure 4.28
Abundant filaments; bridging between flocs.

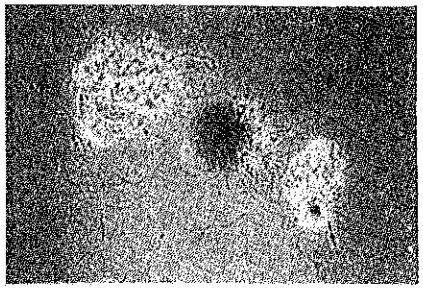

Figure 4.29
Bridging between flocs starting to break up.

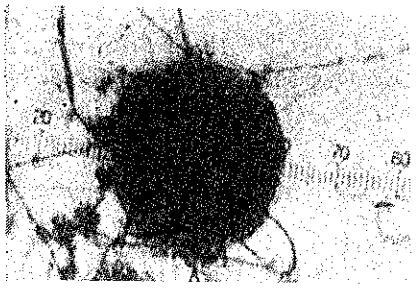

Figure 4.30
Dense cluster of Acinetobacter surrounded by filaments.

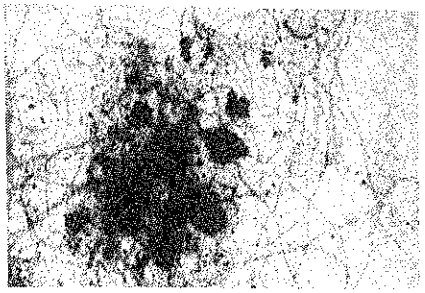

Figure 4.31
Abundant Microthrix parvicella intertwined with Acinetobacter cells, Methylene blue stain.

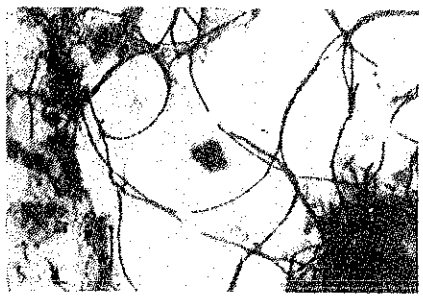

Figure 4.32
Microthrix parvicella breaking up. Gram stain.

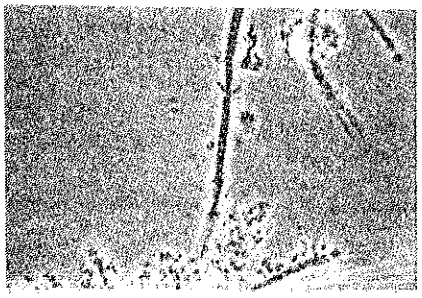

Figure 4.33
Wet prepparation of filament 0041. Phase contrast microscopy

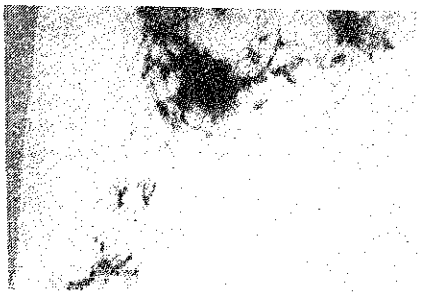

Figure 4.34
Wet preparation of filament 0092. Phase contrast microscopy

TABLE 4.7
FILAMENTOUS ORGANISMS IN THE NORTHERN WORKS MODULE 2
(THREE STAGE PHOREDOX WITH ANAEROBIC SELECTOR)

Date	Observations	SFA	Remarks
1985.05.13 to 1985.09.10	Microthrix parvicella dominant - bridging between floc	4	Severe bulking
1985.09.11 to 1985.10.23	Microthrix parvicella dominant - some bridging present	3	Severe bulking
1985.12.20	Microthrix parvicella dominant - no bridging	2	Slight bulking
1986.01.07 to 1986.04.15	Filaments mainly in floc - no bridging dominant filaments 0041 and 0092	1	No bulking
1986.04.16 to	Some bridging present - Microthrix parvicella increasing 0041 and 0092 dominant	2	No bulking

TABLE 4.8
FILAMENTOUS ORGANISMS IN THE NORTHERN WORKS (MODULE 3)
(FIVE STAGE PHOREDOX)

Date	Observations	SFA	Remarks
1985.05.15 to 1985.08.30	Bridging between floc - Microthrix parvicella dominant	4	Bulking
1985.09.01 to 1985.10.27	No bridging between floc - Microthrix parvicella dominant	2	Slight bulking
1985.11.1 to 1986.02.28	No bridging - 0041 and 0092 dominant	1	no bulking
1986.03.01 to 1986.05.30	Some bridging present - 0041 and 0092 dominant	2	No bulking

dense mat of Acinetobacter flocs in a mass of filaments (see Figures 4.30 and 4.31). Figure 4.32 depicts the breaking up of Microthrix parvicella. Filaments 0041 and 0092, which were also observed in the Northern Works Plant are shown in Figures 4.33 and 4.34.

According to a survey carried out on South African activated sludge plants, the filamentous organisms most dominant in mixed liquor samples, were Type 0041, Type 0092, Type 0675, Type 0194 and Microthrix parvicella (Blackbeard and Ekama,1985; Ekama,1986). This is in contrast to surveys undertaken in the USA (Jenkins et al.,1984) and Europe (Eikelboom,1977). Different types of filaments have been associated with different operational problems (Jenkins et al.,1984). The filaments observed at the Northern Works are indicative of a low F/M condition, as is the case with most South African plants, which are operated at long sludge ages (Blackbeard and Ekama, 1985).

While specific reasons for the growth of filamentous organisms under low F/M conditions are poorly understood (Jenkins et al.,1984), the severe low F/M bulking observed in systems incorporating an anaerobic reactor has enjoyed special attention during this investigation. In these systems very little S_{bs} (10 mg/ℓ) will be available for filamentous growth in the anaerobic zone, due to the sequestration of S_{bs} by floc formers in the anaerobic zone (Wentzel et al.,1985).

An explanation for bulking in this type of system is provided by the IAWPRC hydrolysis hypothesis (Dold and Marais, 1985). According to this hypothesis, particulate biodegradable COD is hydrolysed to readily biodegradable COD (S_{bs}) in the bulk liquid. Eighty to ninety percent of this hydrolysis occurs in the aerobic zone. Due to the slow rate of hydrolysis compared to S_{bs} uptake, the S_{bs} concentration remains low in the aerobic zone, giving the filaments a growth advantage over floc forms, thus causing bulking to occur (Ekama and Marais,1985).

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CHAPTER FIVE

Biochemical studies

5.1 INTRODUCTION

While the operational aspects of an activated sludge system are of paramount importance in the evaluation of any plant, the nature of the sewage and the biological nature of the process cannot be overlooked.

Any biological system can be broadly studied under two disciplines, namely, microbiology and biochemistry. As the microbiology has already been discussed in Chapter 4, only the biochemistry will be discussed further here.

The study of the biochemistry of a biological system provides insight into intracellular activity. Generally speaking, the processes of interest are substrate uptake and conversion. In the study of substrate uptake, the nature of the substrate requires definition. Some aspects of sewage composition are discussed in relation to the process.

The conversion products derived from nutrient uptake (polyphosphate and polyhydroxybutyrate) and which are of particular interest, are also considered. Intracellular activity is dependent on a source of energy. In this instance, the energy rich compound adenosine triphosphate (ATP) provides the required energy. The levels of this compound in the system are discussed, as is the enzymatic activity involved in certain fermentation processes under anaerobic conditions.

5.1.1 Fermentation Enzymes

The incorporation of an anaerobic zone has been shown to enhance the phosphorus removal capability of a number of activated sludge plants (Barnard, 1976; Davelaar et al.,1978). The presence of nitrates in this zone however, negates the beneficial effect (Osborn and Nicholls,1978). The precise function of this zone remains uncertain (Fuhs and Chen,1975; Nicholls and Osborn,1979; Buchan,1982).

Some workers have postulated that the anaerobic zone provides a stress environment for aerobic bacteria, which stimulates enhanced phosphorus removal in subsequent aerobic zones (Nicholls and Osborn, 1979), while others consider the anaerobic zone as a manufacturing zone for substrate, particularly suitable for the proliferation of phosphorus removing bacteria in the aerobic zone (Fuhs and Chen,1975; Deinema et al.,1980; Buchan, 1982; Brodisch,1985). Acinetobacter spp has been identified as the predominant aerobic organism responsible for phosphorus removal (Fuhs and Chen,1975; Buchan,1983; Lötter,1985). Studies with strains of these bacteria isolated from Johannesburg sewage purification works have shown that enhanced growth and phosphorus accumulation occurred when the isolates were grown on carbon sources which were also products of bacterial fermentation (Lötter,1985).

Anaerobic zone enzymatic studies were undertaken to determine whether a correlation exists between the activity of enzymes involved in the production of fermentation products and enhanced biological phosphorus removal.

5.1.2 ATP Levels

The determination of viable sewage biomass is an important parameter in assessing the efficiency of activated sludge sewage treatment. The use of cellular ATP content as a measure of sludge viability has been used by a number of researchers (Weddle and Jenkins,1971; Levin et al.,1975; Nelson and Lawrence,1980).

A number of workers have reported the determination of ATP in biological samples by using the so-called luciferin-luciferase reaction, where the oxidation of luciferin is catalysed by luciferase in the presence of ATP. One photon of light is emitted for every molecule of ATP. The measurement of the emitted light allows the ATP concentration to be determined (Cole et al.,1976; Kucnerowicz and Verstraete,1979; Nelson and Lawrence,1980). A commercial test kit (Aquatox Kit, Weil Organisation (Pty) Ltd) is available for the determination of ATP, using the luciferin-luciferase technique. It was decided to investigate the use of this kit in the determination of ATP in activated sludge.

The adenylate energy charge (AEC) which provides an indication of the metabolic condition of a biological system, has previously been determined in activated sludge systems, and while the results were not useful in predicting specific behaviour, trends in plant stability correlated with the AEC value in pilot plant studies (Potgieter, 1981).

The aim of this study was to develop a technique for determining ATP in activated sludge samples and then to compare the ATP levels with the performance of the plant and to determine AEC levels in a full-scale plant.

5.1.3 Polyphosphate Accumulation

Microscopic examination of sludge samples from aerobic zones of phosphorus removing plants, has revealed the presence of large numbers of cells containing polyphosphate inclusions (Fuhs and Chen, 1975; Buchan, 1981). Anaerobiosis caused the disappearance of these inclusions with a concomitant increase in the soluble orthophosphate level in the mixed liquor (Buchan,1981). Polyphosphate inclusions occur in a variety of bacteria (Harold,1966; Dawes and Senior,1973; Jones and Chambers,1975). There is still uncertainty as to the role of this storage product in bacterial metabolism. The detection of enzymes which catalyse the direct transfer of phosphate residues, without the participation of adenosine triphosphate (ATP) (Kulaev et al.,1961; Harold,1966), lends support to the hypothesis that these compounds act as phosphagens (Muhammed et al.,1959, Suzuki et al.,1972).

A number of researchers however, favour a role for polyphosphate in the regulation of cellular orthophosphate levels (Harold,1962; Kulaev,1973; Nesmeyanova et al.,1974). Regulation of glycolysis through control of the ATP/ADP level by polyphosphates is also an hypothesis supported by the observations of a number of researchers (Kulaev,1973; Nesmeyanova et al., 1974). In an attempt to learn more about the role of this compound in enhanced phosphorus removal by the activated sludge process, the distribution of phosphorus compounds in sludges under aerobic and anaerobic conditions was investigated.

5.1.4 Polyhydroxybutyrate Accumulation

Polyhydroxybutyrate is a reserve of carbon and energy which accumulates in a variety of micro-organisms, generally under conditions of nutrient imbalance (Dawes and Senior,1973). The accumulation of polyhydroxybutyrate by an Acinetobacter calcoaceticus var lwoffi strain isolated from an activated sludge plant, was first demonstrated by Fuhs and Chen in 1975. Subsequent investigations indicated that a number of Acinetobacter strains were capable of carbon storage by this route (Lawson and Tonhazy,1980; Lötter et al.,1986a). The metabolism of polyhydroxybutyrate by Acinetobacter spp in the activated sludge process has been described in detail by Wentzel et al.,(1986).

Microscopic examination of activated sludge for the presence of polyhydroxybutyrate has revealed an increase in the anaerobic zone and subsequent depletion on aeration (Hart and Melmed,1982; Lötter et al., 1986b). While the metabolism of polyhydroxybutyrate has not been studied in the presence of nitrate, the deleterious effect on nitrate of anaerobic zone behaviour has been well documented (Barnard,1976; Osborn and Nicholls, 1978; Siebritz et al.,1983).

The aim of this study was to investigate the synthesis and utilisation (degradation) of polyhydroxybutyrate in the anaerobic, anoxic and aerobic zones of three of Johannesburg's activated sludge plants with different process configurations.

5.1.5 Sewage Composition

Studies on the effect of different carbon substrates on tne phosphorus removal of an activated sludge biomass have demonstrated the substrate specific nature of this phenomenon (Gerber et al.,1986). The beneficial effect of readily biodegradable carbon compounds on biological phosphorus removal has been demonstrated by a number of researchers (Osborn and Nicholls,1978; Pitman et al.,1983; Siebritz et al., 1983; Barnard,1984).

The nature of the biodegradable portion of sewage entering an activated sludge plant will clearly have an effect on the biological phosphorus removal efficiency. Methods for improving the sewage composition for phosphorus removal were studied (see Chapter 2) and analytical techniques were required to monitor the efficiency of these procedures. The readily biodegradable fraction of the sewage in general terms and in particular, volatile fatty acids had to be monitored.

The effect of substrate on sludge bulking, a problem sometimes encountered in phosphorus removing activated sludge plants has been studied in some detail by Slijkhuis(1983). In particular, the stimulatory effect of long chain fatty acids on the growth of certain filamentous organisms was demonstrated.

The analysis of readily biodegradable COD (S_{bs}), volatile fatty acids and long chain fatty acids was therefore undertaken. The method used for the routine determination of volatile fatty acids in activated sludge (Nicholls et al.,1986)has two shortfalls; it is not intended for use below 50 mg/ℓ and it does not distinguish between the different acids (Montgomery et al., 1962). The importance of specific acids in phosphorus removal has been clearly demonstrated (Wentzel et al.,1985, Murphy and Lötter,1986). It was therefore considered essential to determine the different acids separately.

The most useful technique for this purpose is gas chromatography (Packett and McCune,1965; Mahadevan and Stenroos,1967; Ottenstein and Bartley, 1971; Di Corcia and Samperi,1974).

The aim of this investigation was to develop a technique suitable for the accurate quantification of volatile fatty acids in various sewage samples. To this end, various gas chromatographic techniques were investigated. A satisfactory method was developed and used routinely for some time. However, the technique was tedious and in attempting to reduce the manpower requirements of the procedure, attention was given to less tedious technique, i.e. high pressure liquid chromatography, which is described below.

5.2 METHODOLOGY

5.2.1 Fermentation Enzymes

Mixed liquor samples were drawn from the anaerobic zones of Johannesburg's Northern and Goudkoppies Works activated sludge plants.

The enzymes were extracted by ultrasonic disintegration in a Tris buffer (Kotze,1981). The temperature of the sample was kept below 10 °C by immersing a stainless steel cooling coil into the suspension during ultrasonication. The cold homogenate was centrifuged at 35 000 x g at 4 °C for 30 minutes. The cell-free supernatant was freeze dried. The freeze-dried sample was resuspended in one-tenth the original volume of distilled water and dialysed against 0,1M Tris buffer overnight. The solution inside the dialysis bag was centrifuged at 35 000 x g at 4 °C and the supernatant retained for enzymatic assays. The activities of β-HO-butyrate dehydrogenase and alcohol dehydrogenase were determined by measuring the amount of $NADH_2$ produced during the formation of acetoacetate and acetaldehyde respectively (Williamson and Mellanby,1974; Kersters and de Ley,1966). Malate dehydrogenase and lactate dehydrogenase were determined by measuring the utilisation of $NADH_2$ in the formation of malate and lactate respectively (Bergmeyer and Bernt,1974; Schwartz and Bodansky, 1966).

The phosphotransacetylase activity was determined by measuring the amount of acetyl-SCoA formed from acetyl phosphate (Klotsch, 1969). The enzyme activity was also determined in the presence of nitrate, by adding nitrate to the assay mixture to a final concentration of 10 mg/ℓ (as N).

5.2.2 ATP Levels

Mixed liquor samples were taken from the aerobic zone of the Northern Works for refinement of the technique used for ATP determination. The samples were extracted as soon as possible after sampling.

ATP was extracted from the sludge by a bacterial cell ATP releasing agent (Lumac Cat No 9228) in an ice bath for 30 minutes. The resulting mixture was centrifuged at 500 x g for 5 minutes and the supernatants retained for ATP analysis. The ATP content of the supernatant was determined using an ATP Test Kit (Lumac Cat No 9240).

The optimum extraction time was determined and the effect of sample storage on the ATP concentration was investigated. Once optimisation of the technique had been accomplished, samples from the anaerobic, anoxic and aerobic zones of the Northern and Goudkoppies Works, were taken for ATP determination.

Mixed liquors from three zones of the Goudkoppies Plant were also taken for the determination of the adenylate energy charge (AEC).

Samples of Acinetobacter cell suspensions grown aerobically in acetate and succinate and subjected to anaerobiosis were also taken, to investigate ATP determination in this micro-organism. The Lumac AEC test kit (Lumac Cat No 4642) was used for the determination. Adenylate phosphates were extracted from samples as described for ATP extraction from sludge.

5.2.3 Polyphosphate Accumulation

Mixed liquor samples were drawn from the aerobic zones of the Johannesburg Northern and Goudkoppies sewage purification plants and from the reactors at the Johannesburg Olifantsvlei and Alexandra sewage purification works.

Analyses (described below) were carried out immediately. The remainder of the samples were then allowed to become anaerobic at room temperature. After 72 hours anaerobiosis the samples were analysed again.

A 40 mℓ aliquot of the well-shaken sample was centrifuged at 10 000 g for ten minutes. The residue was washed with saline solution and the resulting suspension recentrifuged. Orthophosphate was determined in the combined supernatants. Fractionation of the phosphorus compounds in the sludge pellet was accomplished by Harold's procedure (Harold,1960). The pellet was shaken with 40 mℓ cold 0,5M perchloric acid, centrifuged and the extraction repeated. The extracts were pooled. The residue was then extracted with 40 mℓ ethanol for 30 minutes. After centrifugation, a 40 mℓ ethanol:ether (3:1) mixture was added to the residue. The mixture was boiled for one minute and then centrifuged. These two extracts were pooled. The residue was then extracted twice with 40 mℓ portions of hot 0,5M perchloric acid at 70 °C. The extracts were pooled.

An aliquot of the cold perchloric acid extract was taken for an orthophosphate and total phosphorus determination. Approximately 500 mg phosphate-free charcoal was then added to the extract and the suspension shaken well. Total phosphorus and polyphosphate determinations were carried out on the filtrate.

The orthophosphate result provides the cellular orthophosphate level and the difference between the total phosphorus before and after charcoal treatment, the nucleotide phosphorus level. Total phosphorus was determined on the ethanol extract to provide the lipid phosphorus level. The hot perchloric acid extract was subjected to total phosphorus determination; charcoal treatment and subsequent phosphorus determination as described above. The difference in total phosphorus before and after charcoal treatment provides the nucleic acid phosphorus level.

The polyphosphate in this fraction is defined as acid insoluble polyphosphate, while that in the cold extract is known as acid soluble polyphosphate.

5.2.4 Polyhydroxybutyrate Accumulation

Mixed liquor samples were drawn from the anaerobic, anoxic primary and secondary aerobic zones of the Northern Works and Goudkoppies plants and analysed for polyhydroxybutyrate by the method of Braunegg et al.,(1978), with slight modifications. Fifty millilitres of the well-shaken sample was freeze-dried. The freeze-dried sample was resuspended in 10 mℓ acidic methanol (3,0 mℓ H_2SO in 97 mℓ methanol) and 10 mℓ chloroform, in a sealed test tube. The samples were placed in an oven at 100 °C for three hours. After cooling, 10 mℓ water was added and the samples shaken for 10 minutes. The chloroform layer was filtered through phase separating paper (Whatman PS1) and the resultant 3-HO methyl ester subjected to gas chromatographic analysis on a 1,0 m 3 % SP 1000 column at 100 °C, with a nitrogen flow rate of 35 mℓ/min.

5.2.5 Sewage Composition

5.2.5.1 Volatile fatty acids (VFA)

Samples from a balancing tank, primary sedimentation tank and digester were used to refine the gas chromatographic method for VFA determination. The pH of samples was raised to 12,0 immediately after sampling.

The samples were treated in a variety of ways, as described below. Balancing tank effluent samples were acidified and analysed directly by gas chromatography or extracted with ether and the ether extract subjected to gas chromatography.

Digester and sedimentation tank samples were extracted with diethyl ether after acidification or steam-distilled and the distillate extracted with ether, before gas chromatographic analysis.

Acidified samples were also steam-distilled and the distillates titrated with 0,1N sodium nydroxide, then evaporated to dryness at 60°C under vacuum. Dichloroacetic acid and acetone were added to the residue (Horwitz,1980) and the solutions subjected to gas chromatographic analysis.

Aqueous samples were injected onto a 2,0 m Chromosorb 101 column at 150 °C (Mahadevan and Stenroos,1967) or a 1,5 m 10 % SP 1200/1 % H_3PO_4 column at 150 °C (Ottenstein and Bartley,1971).

Diethyl ether and acetone/dichloroacetic acid extracts were chromatographed on a 10 m 530µ Carbowax 20M column with temperature programming from 80 °C to 150 °C, which was a modification of the technique of Packett and McCune (1965).

Aqueous solutions of volatile fatty acids were also analysed by HPLC in an attempt to expedite analyses.

5.2.5.2 Long chain fatty acids

Samples of mixed liquor were extracted with chloroform and the chloroform extract filtered through phase separating paper (Whatman PS1). The extract was evaporated to dryness under vacuum. The fat was then saponified by treatment with methanolic sodium hydroxide and the resulting soaps converted to methyl esters of the fatty acids by reaction with a boron trifluoride-methanol complex.

The methyl esters were extracted into heptane and subjected to gas chromatographic analysis on a 25m 5 % phenyl methyl silicone column of 0,32 mm diameter, using helium as a carrier gas.

5.2.5.3 Readily biodegradable COD

Due to its biological nature the determination of this parameter is most readily carried out by bioassay. In fact, three bioassay methods are available, namely :-

- continuous aerobic method (Ekama and Marais,1984)
- batch denitrification method (Stern and Marais,1974)
- batch aerobic method (Ekama and Marais,1984)

These were evaluated to determine the most appropriate for routine monitoring purposes.

Using the continuous aerobic method, S_{bs} values were determined twice a day on different samples of raw sewage, settled sewage and flow balancing tank effluent, at the Johannesburg Northern Works. To test the suitability of the denitrification method, recovery studies were conducted on two substrates; sodium acetate, which is readily biodegradable and starch, which is slowly biodegradable. The batch aerobic method was evaluated using sodium acetate.

The batch denitrification method was subsequently evaluated on the Goudkoppies plant, in an attempt to assess the effect of yeast effluent on the S_{bs} concentration.

Snap samples of sewage were taken from the sewer when yeast waste was present and also when it was absent. Five hundred mℓ of each sample was mixed with 2 ℓ return sludge and 1,5 ℓ dilution water, giving a final reaction volume of 4 ℓ. During the test, the mixture was stirred at a very slow rate to avoid oxygen introduction from the atmosphere but sufficient to maintain the sludge in suspension. Between 50 and 100 mg nitrate (as N) was added to each reaction volume and the denitrification rate monitored by taking small samples at various time intervals.

Similar experiments were carried out in an aspirator bottle, introducing nitrogen gas continuously into the mixture by means of a sintered-glass bubbler. The dilution water was air-stripped with nitrogen gas for 60 minutes prior to use. Samples were taken from the bottom of the aspirator. Large samples (approximately 100 mℓ), were taken to eliminate the possibility of a "dead volume" in the tap. Only 20 mℓ of this sample was filtered (immediately), the remainder being poured back into the reaction mixture. On a number of samples the length of the experiment was increased to obtain complete denitrification.

The problems experienced with the practical implementation of the above techniques, led researchers at the University Cape Town to consider alternative, less tedious techniques.

A method based on the determination of the COD of the filtrate through a 0,45 µm filter was suggested. Correlation with the bioassay techniques

has demonstrated the necessity for a correction factor of 0,8 (Bagg, 1986).

Therefore

S_{bs} = 0,8 (COD 0,45 µm filtrate influent - COD 0,45 µm filtrate effluent).

This method was used routinely during this investigation to determine S_{bs} values. Evaluation of this method with pure substrates was also undertaken.

5.2.6 General

In the studies described above, the volatile suspended solids content of the samples was determined by igniting at 550 °C, the residue retained on a glass fibre filter and dried at 105 °C (American Public Health Association,1981). Total phosphorus determinations were carried out by the digestion procedure of Jirka (Jirka et al.,1976), followed by the orthophosphate determination by the molybdenum blue method (Canelli and Mitchell,1975). Polyphosphate was determined by hydrolysis in 1M hydrochloric acid at 70 °C for 15 minutes, followed by the orthophosphate determination.

5.3 RESULTS AND DISCUSSION

5.3.1 Fermentation Enzymes

Preliminary experiments were carried out to determine optimum sonication times and levels. Negligible enzymatic activity was observed without sonication, confirming the intracellular location of the enzymes. Malate dehydrogenase activity was determined on samples sonicated at 120 W and 600 W. Even 90 minutes sonication at 120 W failed to release the maximum amount of enzyme. Sonication at 600 W however, produced satisfactory recovery of the enzyme.

Activities of all the enzymes in the study were determined after various periods of sonication. Fourteen minutes was chosen as an optimum period. Freeze drying of the extract had no significant effect on enzyme activity. All enzyme activities were expressed per gram of total volatile suspended solids. Alcohol dehydrogenase activities were negligible in the preliminary experiments and assays were subsequently discontinued.

The enzymatic activities in four different plants are given in Table 5.1.

TABLE 5.1
ENZYMATIC ACTIVITY OF MIXED LIQUOR FROM ACTIVATED SLUDGE PLANTS

Plant	Phosphorus removal mg P/g VSS	Enzyme activity µmole/g VSS			
		LDH	MDH	HO-BDH	PTA
Goudkoppie	2,81	0,61	68	1,0	1,4
Goudkoppie	2,56	3,0	220	0,63	0,58
Goudkoppie	2,78	0,53	438	1,3	1,3
Northern Works	2,45	0,13	6,6	0,05	0,06

LDH	Lactic dehydrogenase
MDH	Malate dehydrogenase
HO-BDH	β-hydroxybutyrate dehydrogenase
PTA	Phosphotrahsacetylase

Lactate and malate dehydrogenase activities showed very poor correlation with phosphorus removal. These determinations were not continued beyond the preliminary experiments. β-HO-butyrate dehydrogenase and phosphotransacetylase activity was determined routinely on the two plants over a twelve-month period. The results are shown in Figures 5.1 to 5.4. The relationships between phosphorus removal and the two enzyme activities are very similar, nevertheless the activities in the two plants differ considerably.

A satisfactory method for the determination of lactate, malate and β -HO-butyrate denydrogenase and phosphotransacetylase activities in activated sludge samples has been developed. While a number of workers have used enzymatic activities in aerobic zones as an indication of activated sludge performance (Toerien and Kotze,1967; Teuber and Brodisch,1977), no enzymatic activities have previously been reported for the anaerobic zone.

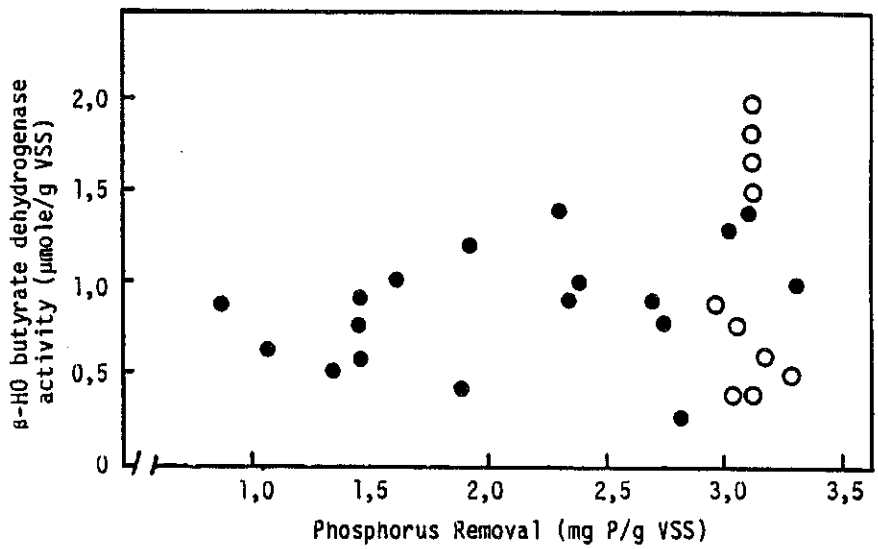

Figure 5.1 : Variation in phosphorus removal at Northern Works with different β-HO butyrate dehydrogenase activities
● No primary sludge recycle
○ Primary sludge recycle

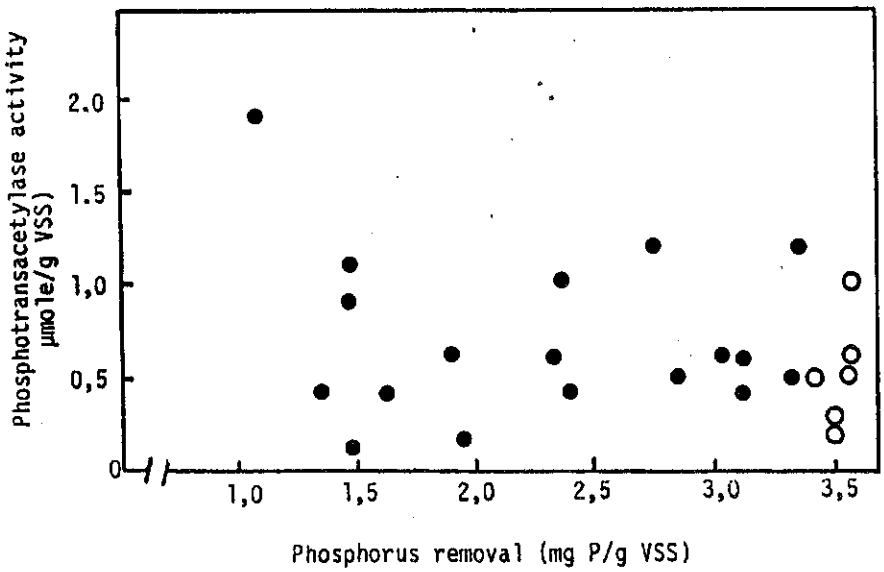

Figure 5.2 : Variation in phosphorus removal at Northern Works with different phosphotransacetylase activities
● No primary sludge recycle
○ Primary sludge recycle

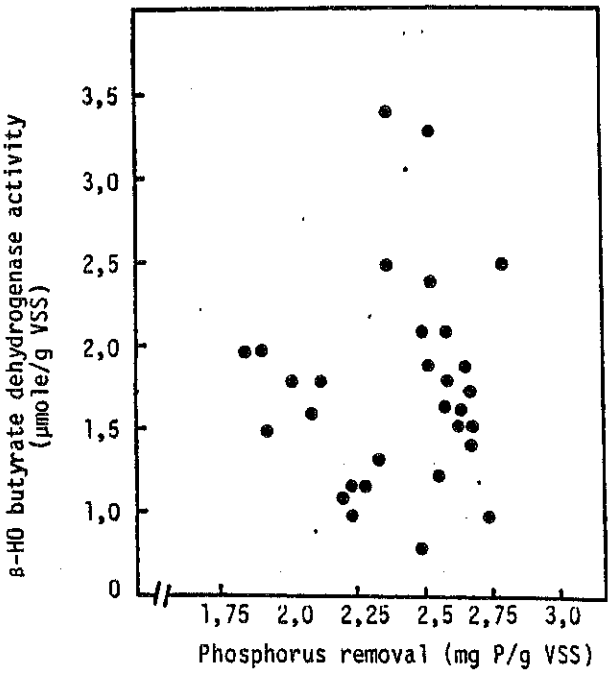

Figure 5.3 : Variation of phosphorus removal at Goudkoppies with different β-HO butyrate dehydrogenase activities

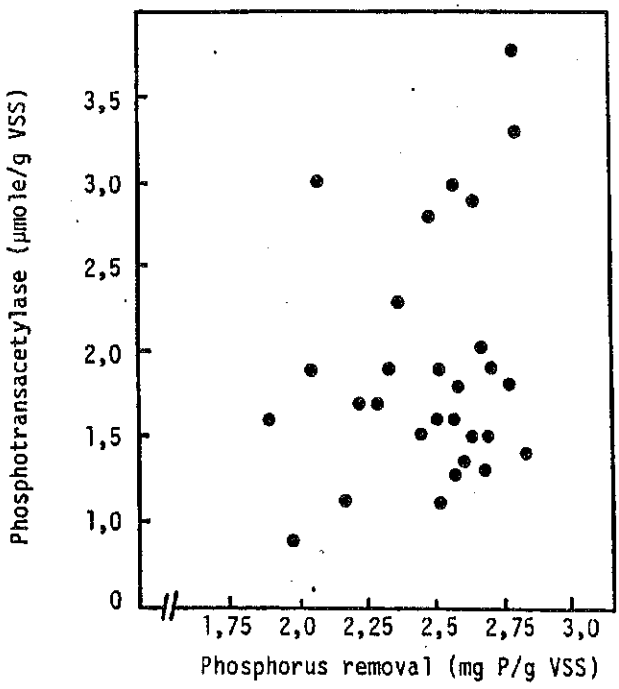

Figure 5.4 : Variation of phosphorus removal at Goudkoppies with different phosphotransacetylase dehydrogenase activities

The Northern Works plant appeared more sensitive to changes in its anaerobic zone, as reflected in variations in enzymatic activity, than the Goudkoppies plant. The latter plant receives an influent containing high concentrations of low molecular weight compounds in contrast to the Northern Works plant, which, for the greater part of the monitoring period relied solely on *in situ* generation of suitable substrate for *Acinetobacter spp*.

During the final part of the monitoring period the Northern Works influent was enriched by recycling sludge rich in volatile fatty acids from the primary sedimentation tank. Phosphorus removal improved and sensitivity to changes in enzymatic activity decreased.

The significance of acetate in the anaerobic zone has been shown by a number of workers (Lötter,1985; Wentzel *et al*,1985) and the sensitivity of the plant to the activity of phosphotransacetylase, which is a key enzyme in its formation is not unexpected.

It has been suggested by some researchers that carbonaceous substrate absorbed by *Acinetobacter* under anaerobic conditions is stored as polyhydroxybutyrate (Nicholls and Osborn,1979). The correlation betweeen the activity of β-HO-butyrate-dehydrogenase, which catalyses an essential step in the formation of this storage polymer and phosphorus removal, could well be due to its role in polyhydroxybutyrate formation rather than its role in the production of fermentation products. The presence of nitrate had no significant effect on lactate, malate or β-HO-butyrate dehydrogenase activity. Phosphotransacetylase activity was inhibited by 1 mg/ℓ nitrate as N (see Figure 5.5).

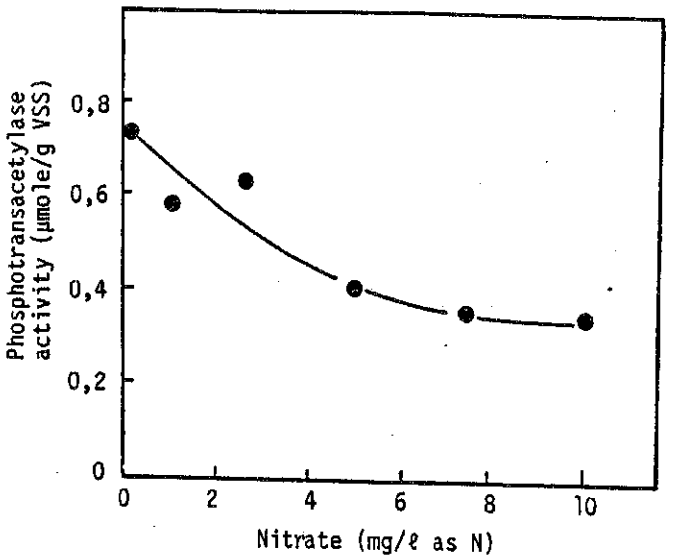

Figure 5.5 : The effect of nitrate on phosphotransacetylase activity

Some researchers posultate that this is due to the consumption of readily biodegradable COD by denitrifying bacteria (Siebritz et al.,1983), while others implicate nitrate as an inhibitor of enzymatic reactions (Buchan,1983). While inhibition of phosphotransacetylase was observed at a level of 1 mg/ℓ nitrate as N, it is unlikely that this is sufficient to inhibit the entire process. The presence of nitrates is more likely to create an environment in which some facultative anaerobes are able to metabolise substrate along oxidative pathways, rather than the fermentative pathways which give rise to the carbon sources preferred by Acinetobacter spp.

The correlation between certain enzyme activities and phosphorus removal, suggests an advantage to be gained by the presence of certain facultative anaerobes and Acinetobacter in the same plant (Brodisch,1985).

5.3.2 ATP Levels

The effect of various sample preparation methods on resultant ATP

concentrations is shown in Table 5.2.

TABLE 5.2
ATP CONCENTRATIONS AFTER VARIOUS TREATMENTS

Sample Treatment	ATP (µg/mℓ)
Sonication in releasing agent	1,1
Homogenised in releasing agent	1,9
Extraction with releasing agent in ice bath	2,2

The extraction with the releasing agent, without mechanical assistance, produces the highest ATP levels. This procedure was used throughout. The effect of extraction time on ATP concentration is shown in Figure 5.6. There is no significant difference in extraction after 30 minutes.

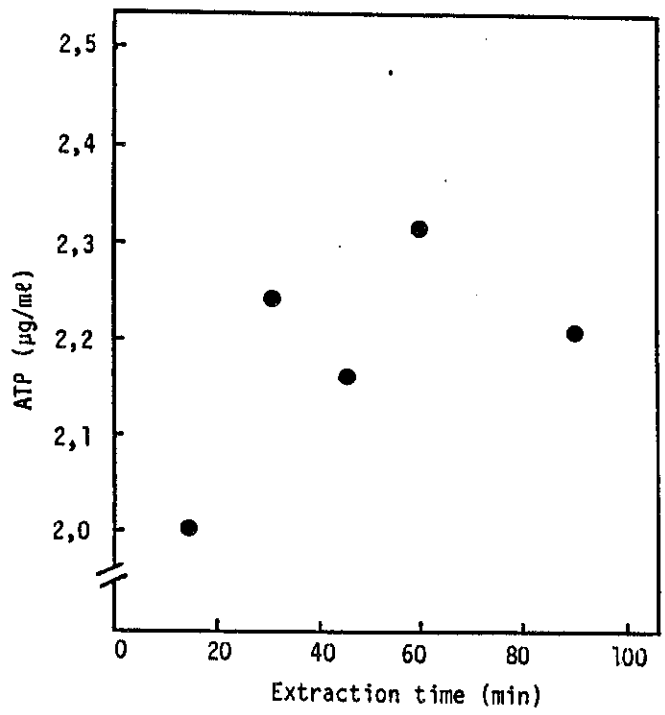

Figure 5.6 : ATP levels with different extraction times

The storage of frozen extracts was found to have no effect on ATP levels.

However, storage of the mixed liquor at 4 °C resulted in a considerable decrease in the ATP levels (see Table 5.3).

TABLE 5.3
EFFECT OF STORAGE ON ATP LEVELS

Sample	ATP (µg/mℓ)
Sample analysed immediately after extraction	2,3
Sample stored overnight at 4 °C prior to extraction	0,5
Sample aerated overnight prior to extraction	0,8
Sample extract frozen for 2 days	2,2

The standard deviation achieved with this procedure is satisfactory (see Table 5.4).

TABLE 5.4
STANDARD DEVIATIONS OBTAINED WITH ATP DETERMINATION AFTER EXTRACTION WITH RELEASING AGENT

Mean ATP (µg/mℓ)	Standard deviation
2,229	0,157
1,882	0,045
0,465	0,058

The results for the samples from the activated sludge plants were expressed as a fraction of volatile suspended solids (VSS) (see Tables 5.5 and 5.6).

TABLE 5.5
ATP LEVELS IN VARIOUS ZONES OF GOUDKOPPIE WORKS

Date	ATP (µg/g VSS) Anaerobic zone	Anoxic zone	Aerobic zone
28 February 1985	475	697	672
8 March 1985	392	566	632
18 March 1985	342	344	399
17 April 1985	169	115	244
13 May 1985	89	60	100

TABLE 5.6
ATP LEVELS IN VARIOUS ZONES OF NORTHERN WORKS

Date	ATP (µg/g VSS) Anaerobic zone	Anoxic zone	Aerobic zone
1 March 1985	560	531	672
8 March 1985	125	162	827
18 March 1985	271	384	521
17 April 1985	326	364	590
3 May 1985	143	152	225
13 May 1985	66	134	123
4 June 1985	47	54	54
12 June 1985	14	19	11
20 June 1985	13	18	35

ATP levels in activated sludge plants are an indication of viable biomass (Weddle and Jenkins,1971). The generally slight differences in ATP levels in the different zones are therefore in agreement with the results for viable counts of the different zones observed by Lötter and Murphy (1985). The values obtained for aerobic zone samples are in agreement with the results obtained by other researchers for a range of growth rates (Weddle and Jenkins,1971).

A linear relationship between specific ATP concentrations and specific substrate removal, was observed by Kucnerowicz and Verstraete(1979). This type of linear relationship was not observed in either of the two plants investigated (see Figures 5.7 and 5.8).

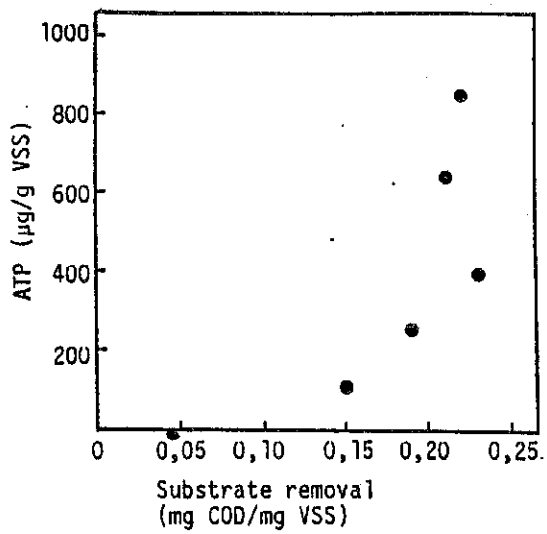

Figure 5.7 : Variation of substrate removal with ATP levels at Goudkoppies

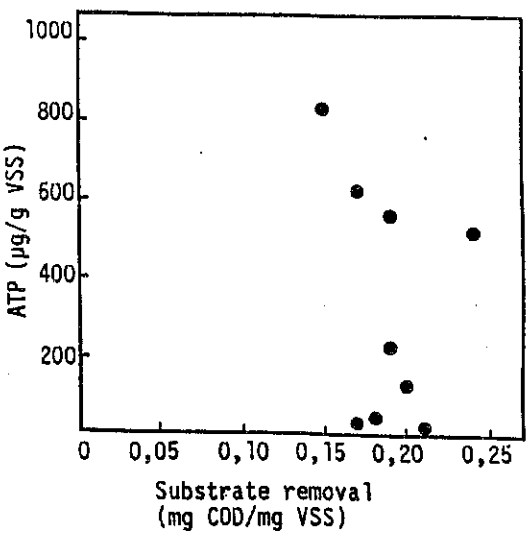

Figure 5.8 : Variation of substrate removal with ATP levels at Northern Works

The same applies to the relationship between specific phosphorus removal and specific ATP concentration (see Figures 5.9 and 5.10)

There could be a number of explanations for this. One possibility is that the consistent substrate removal achieved in both plants is independent of viable biomass, in that both plants contain an excess of viable bacteria over the minimum required to achieve removal. In the case of Goudkoppies, where a consistent level of phosphorus removal is achieved, the same applies.

FIGS 5.9 and 5.10

In contrast, the situation at Northern Works is not as stable in this respect and phosphorus removal is dependent on a minimum viable biomass being maintained. The implications of this possibility in terms of volatile acid addition, are clear. A feed containing a consistent level of volatile acids above a required minimum, is essential for the stable operation of this plant. A shift in bacterial population could also be responsible for this lack of correlation (Chiu et al.,1973). This is not considered important, as significant population shifts have not been shown to occur over periods of varying phosphorus removal (Lötter and Murphy, 1985).

The adenylate energy charge provides a measure of metabolic energy stored in the adenine nucleotide pool and is defined as the ratio between adenylate phosphates, as follows :-

$$AEC = \frac{ATP + 0{,}5\ ADP}{ATP + ADP + AMP}$$

The AEC value can vary between 0 and 1,0. The latter value being attained when all adenylate phosphate is in the ATP form, i.e. maximum energy storage level, while levels near zero indicate energy depletion (Atkinson, 1968). The AEC levels for the Goudkoppies activted sludge plant on a few occasions are given in Table 5.7.

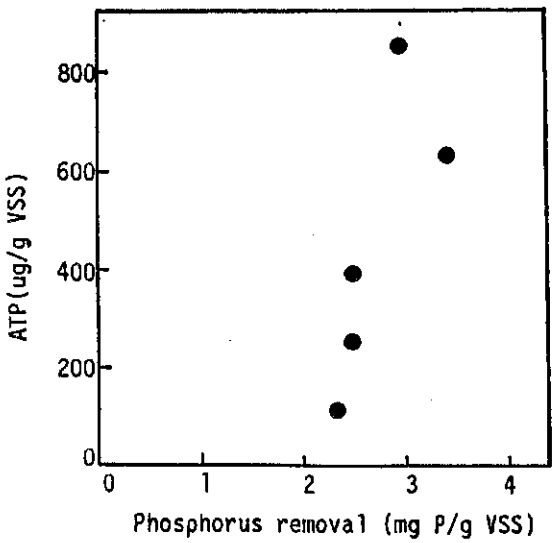

Figure 5.9 : Variation of phosphorus removal with ATP levels at Goudkoppies

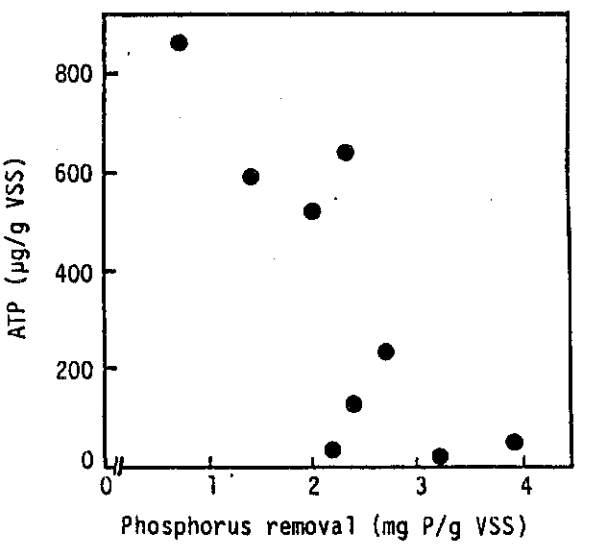

Figure 5.10 : Variation of phosphorus removal with ATP levels at Northern Works

TABLE 5.7
AEC LEVELS IN THE GOUDKOPPIES PLANT

Date	Sample	AEC
1986.03.18	Anaerobic zone	0,14
	Anoxic zone	0,22
	Aerobic zone	0,22
1986.03.26	Anaerobic zone	0,12
	Anoxic zone	0,05
	Aerobic zone	0,34
1986.04.03	Anaerobic zone	0,11
	Anoxic zone	0,08
	Aerobic zone	0,25
1986.04.21	Anaerobic zone	0,08
	Anoxic zone	0,10
	Aerobic zone	0,14

These results were considered unsatisfactory, in that values below 0,5 indicate significant loss of viability (Chapman et al.,1971). It was then decided to investigate the method in greater depth before proceeding with the determination on activated sludge samples.

The calibration curves for the three adenylate phosphates are shown in Figure 5.11.

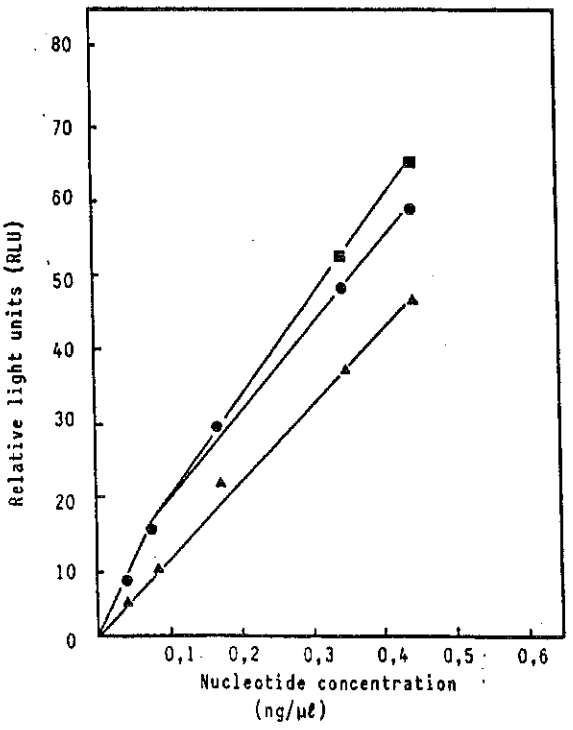

Figure 5.11: Standard curves for AMP ■-■
ADP ●-● and ATP ▲-▲

The lower values obtained for AMP are not satisfactory.

Critical evaluation of the enzymatic assay system employed in the determination, did not provide a satisfactory answer. The progress curves for the reaction showed no evidence of product inhibition (see Figures 5.12 and 5.13).

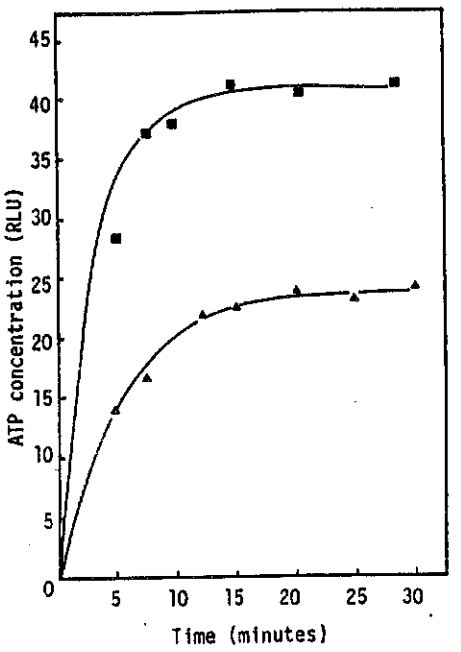

Figure 5.12: Progress curves of AMP conversion to ATP with 0,25 ng/µℓ AMP ▲ ▲ and 0,50 ng/µℓ AMP ■ ■

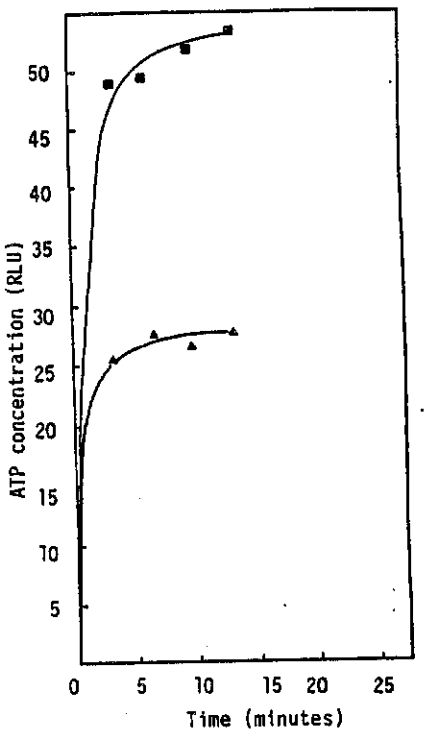

Figure 5.13: Progress curves of ADP conversion to ATP with 0,25 ng/µℓ ADP ▲ ▲ and 0,50 ng/µℓ ADP ■ ■

It was therefore decided to determine adenylate phosphate levels in bacterial suspensions. The determination of adenylate mono- and diphosphates is based on conversion of these species to ATP and subsequent quantification of the latter. The values obtained for bacterial suspensions are given in Table 5.8.

TABLE 5.8
ADENYLATE PHOSPHATE LEVELS IN BACTERIAL SUSPENSIONS

Growth Medium	ATP	ADP+AMP (ng/µℓ)	ATP+ADP+AMP
Aerated :			
succinate	0,39	1,0	0,68
acetate	0,73	1,0	0,35
Unaerated :			
succinate	0,24	0,34	0,09
acetate	0,26	0,43	0,06

The above results are unsatisfactory and the technique therefore requires further investigation.

5.3.3 Polyphosphate Accumulation

As can be expected with a biological system, the levels of phosphorus in various fractions varied over a wide range (see Tables 5.9 and 5.10).

Extracellular orthophosphate increased dramatically after anaerobiosis whereas in almost all samples a slight drop in cellular orthophosphate was observed. The same applies to nucleotide levels. Nucleic acids on the other hand, showed little decrease and in some cases, even a slight increase was observed. Fairly dramatic decreases in acid soluble and acid insoluble polyphosphate were observed. No significant correlation between any of the phosphate fractions and the phosphorus removal capacity of the plant was observed.

Comparison of the phosphorus distribution patterns in sludges from the Northern Works plant, a plug flow process, and Olifantsvlei, a single mixed reactor extended aeration plant, at different phosphorus removal capacities, are presented in Figures 5.14 and 5.15.

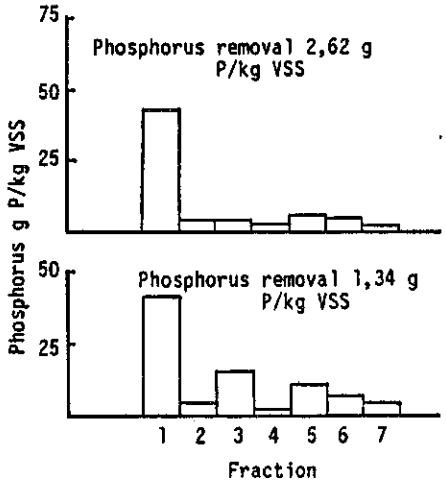

Figure 5.14: Distribution of phosphorus compounds in Olifantsvlei sludge at different phosphorus removal capacities.

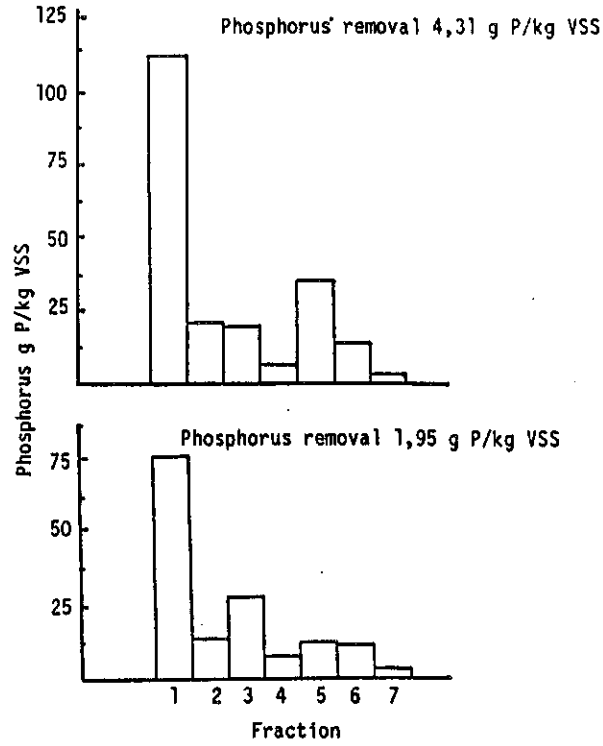

Figure 5.15: Distribution of phosphorus compounds in Northern Works sludge at different phosphorus removal capacities.

Key to fractions: 1 : Total; 2 : Cellular orthophosphate; 3 : Nucleotides;
4 : Nucleic acids; 5 : Acid soluble polyphosphate;
6 : Acid insoluble polyphosphate; 7 : Phospholipid.

While the total phosphorus levels in the two Olifantsvlei samples do not differ significantly, the distribution pattern does show slight differences. The Northern Works samples differ significantly in both their total and polyphosphate phosphorus levels.

The effect of anaerobiosis on the intracellular fractions is shown in Figure 5.16.

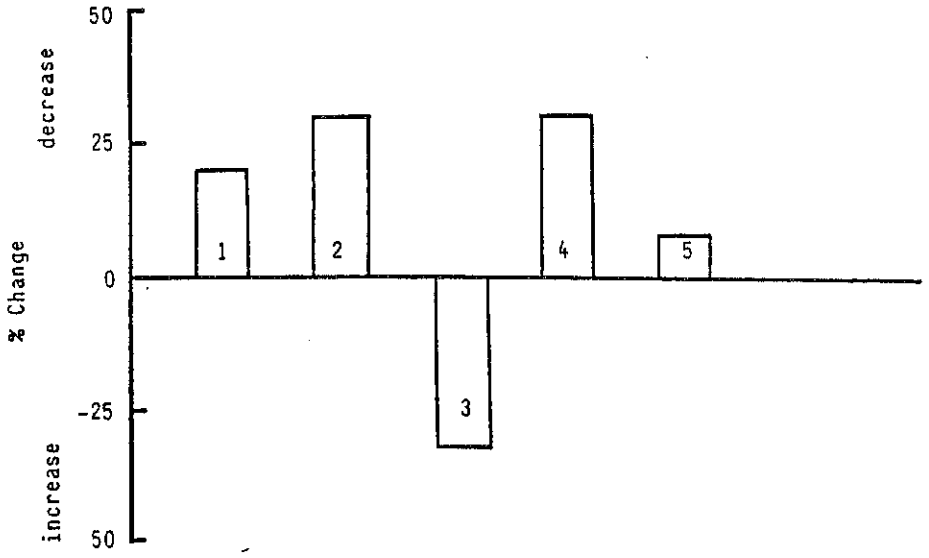

Figure 5.16 : Effect of anaerobiosis on intracellular phosphorus

1 : orthophosphate 2 : nucleotides
3 : nucleic acids 4 : acid soluble polyphosphate
5 : acid insoluble polyphosphate

The extracellular orthophosphate increased dramatically as has been observed previously by a number of researchers (Sekikawa et al, 1966; Fuhs and Chen,1975; Wentzel et al.,1985).

The decrease in the polyphosphate fraction confirms Buchan's microscopic observation that polyphosphate granules disintegrate under anaerobic conditions (Buchan,1981). These observations point to the hydrolysis of polyphosphate to orthophosphate, as the source of the increase in extracellular orthophosphate.

The lack of satisfactory correlation between biological phosphorus fractions and phosphorus removal capacity, cannot be explained at this stage. The poorly defined separation achieved by this type of fractionation procedure might, in part however, account for this lack of correlation.

5.3.4 Polyhydroxybutyrate Accumulation

Typical chromatograms obtained during the analysis of polyhydroxybutyrate are shown in Figure 5.17. As can be seen from this Figure, there are no interferences in the region of the β-hydroxybutyrate methyl ester peak. Recovery of the methyl ester was in excess of 80 %. The poly-

TABLE 5.9
DISTRIBUTION OF PHOSPHORUS COMPOUNDS IN SLUDGE FROM NORTHERN WORKS AEROBIC ZONE

Fraction	Minimum Value mg P/g VSS	Maximum Value mg P/g VSS
Cellular orthophosphate	7,9	18,0
Nucleotides	7,6	18,5
Nucleic acids	1,2	4,9
Polyphosphate (acid sol)	0,6	25,0
Polyphosphate (acid insol)	8,4	18,0
Phospholipid	0,5	1,8
TOTAL	**31**	**98**

TABLE 5.10
DISTRIBUTION OF PHOSPHORUS COMPOUNDS IN SLUDGE FROM GOUDKOPPIES AEROBIC ZONE

Fraction	Minimum Value mg P/g VSS	Maximum Value mg P/g VSS
Cellular orthophosphate	1,9	11,0
Nucleotides	7,4	15,0
Nucleic acids	1,5	5,2
Polyphosphate (acid sol)	3,3	6,5
Polyphosphate (acid insol)	3,4	24,0
Phospholipid	1,2	2,8
TOTAL	**40**	**52**

hydroxybutyrate levels, expressed as a fraction of the volatile suspended solids in various zones of the plants, are given in Table 5.11. A reduction in polyhydroxybutyrate concentration from the anaerobic zone to the aerobic zones in all three plants is evident. However, in order to evaluate the role of polyhydroxybutyrate in the process, mass balances had to be calculated.

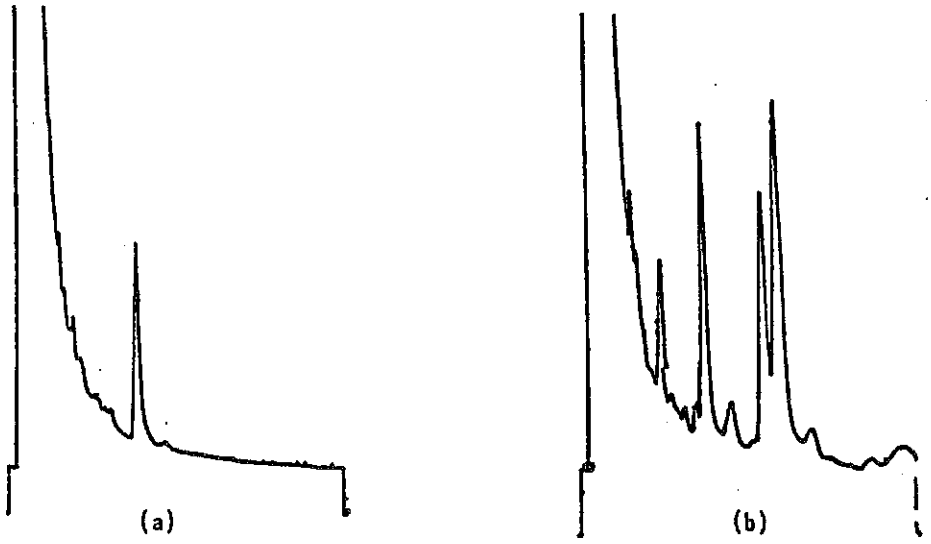

Figure 5.17 : Gas chromatographic separation of β-HO butyrate methyl ester (a) β-HO butyrate methyl ester; (b) mixed liquor containing β-HO butyrate methyl ester

Throughout the period under discussion, the Goudkoppies plant received in excess of 2 000 kg/d of volatile fatty acids and the nitrate in the return sludge remained below 1,0 mg/ℓ (as N). Under these conditions, polyhydroxybutyrate was consistently synthesized in the anaerobic zone and utilised in the anoxic and aerobic zones (Figure 5.18).

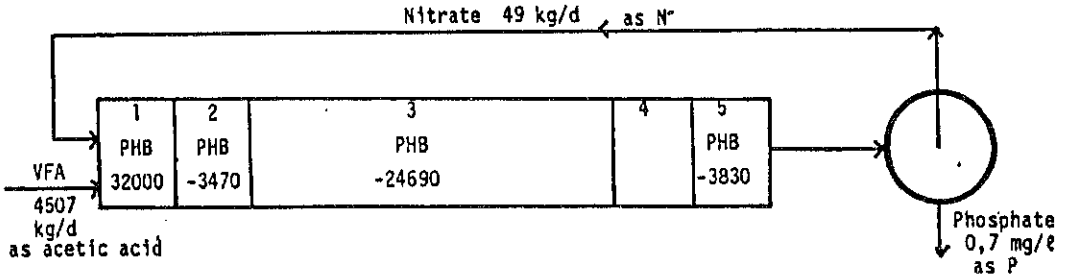

Figure 5.18 : Typical results of polyhydroxybutyrate synthesis and degradation in Goudkoppies activated sludge plant

- denotes degradation. Results in kg/d

1 anaerobic; 2 anoxic; 3 aerobic;
4 anoxic; 5 aerobic

TABLE 5.11
RANGE OF POLYHYDROXYBUTYRATE LEVELS
IN THE THREE PLANTS STUDIED

Sample Description	Polyhydroxybutyrate ug/g VSS	
	Minimum Value	Maximum Value
Goudkoppies Module 2		
Anaerobic zone	37	190
Anoxic zone	13	58
Primary aerobic zone	13	50
Secondary Aerobic zone	12	40
Northern Works Module 2		
Anaerobic zone	15	79
Anoxic zone	20	49
Primary aerobic zone	12	51
Northern Works Module 3		
Anaerobic zone	21	55
Anoxic zone	20	44
Primary aerobic zone	4	44
Secondary aerobic zone	4	42

These observations correlate well with the biochemical model postulated by Wentzel et al.,1986). The utilisation of polyhydroxybutyrate in the anoxic zone of this plant indicates a high number of Acinetobacter spp capable of nitrate reduction (Wentzel et al.,1986). The presence of these bacteria in this zone possibly makes a significant contribution to the efficient denitrification achieved by this plant.

In the Northern Works Module 2 three-stage Bardenpho plant, the volatile fatty acid feed to the plant varied between 540 and 3 420 kg/d, and the nitrate in the return sludge to the anaerobic zone, varied between 0 and 690 kg/d. See Figure 5.19 for typical results.

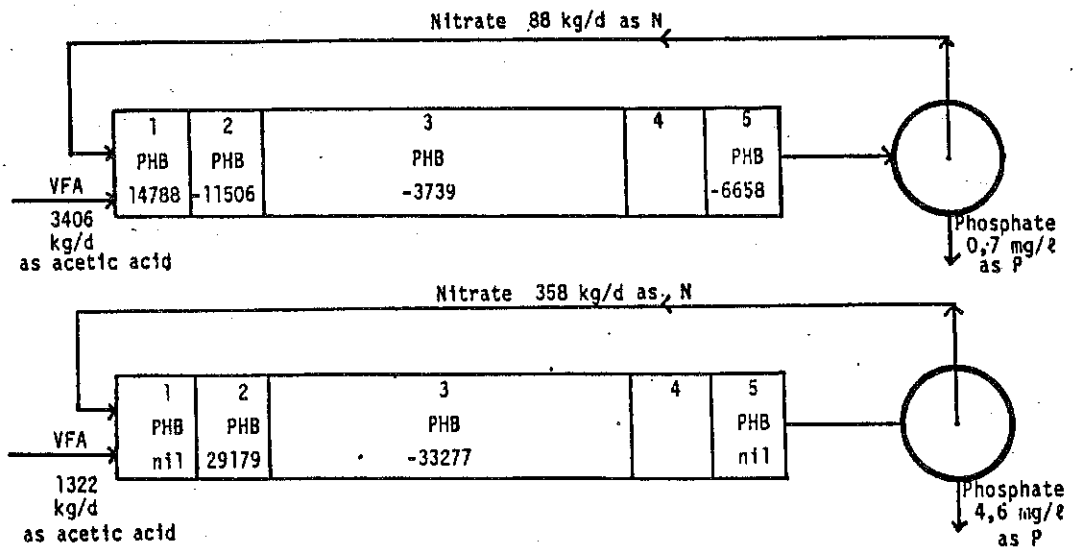

Figure 5.19 : Typical results of polyhydroxybutyrate synthesis and degradation in Northern works Module 2

- denotes degradation. Results in kg/d.
1 anaerobic; 2 anoxic; 3, 4, 5 aerobic

It is clear from these results that nitrate in the anaerobic zone and insufficient volatile fatty acids in the feed inhibits the synthesis of polyhydroxybutyrate, unless excess volatile fatty acid is present. The same typical picture is observed for Northern Works Module 3 (see Figure 5.20).

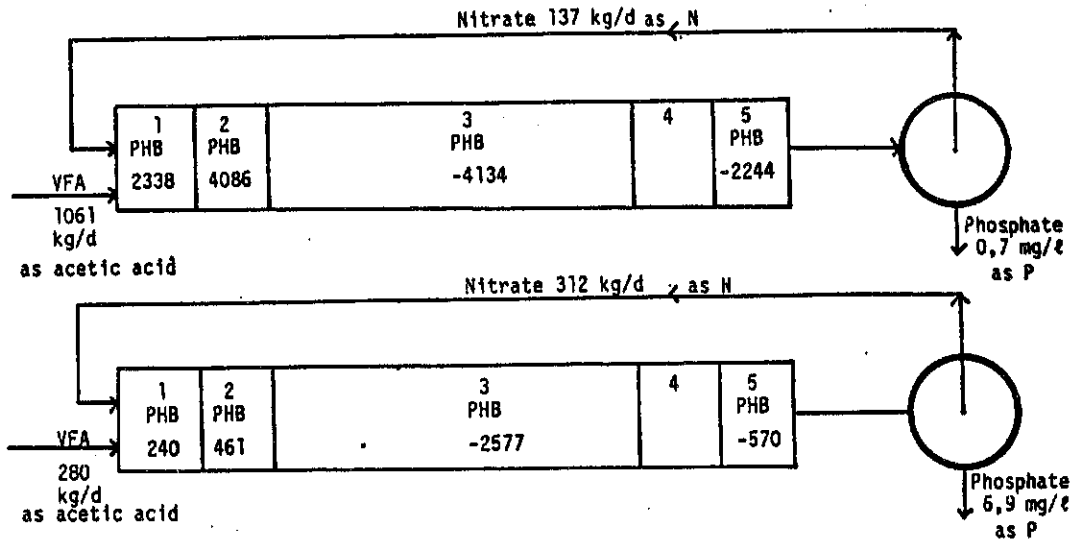

Figure 5.20 : Typical results of polyhydroxybutyrate synthesis and degradation in Northern Works Module 3

- denotes degradation. Results in kg/d.

1 anaerobic; 2 anoxic; 3 aerobic; 4 anoxic; 5 aerobic

A relatively low amount of volatile fatty acid is enough to initiate significant synthesis of polyhydroxybutyrate, as long as the nitrates are not excessively high.

No direct correlation between influent volatile fatty acid, effluent phosphorus and nitrate levels and polyhydroxybutyrate synthesis and utilisation, could be observed.

5.3.5 Sewage Composition

5.3.5.1 Volatile fatty acids

The chromatographic columns recommended for aqueous samples by column suppliers could not in fact, withstand frequent injections of aqueous

samples without deterioration. This technique was therefore not pursued. The Carbowax 20M column was subsequently used throughout. Diethyl ether extraction of the digester and sedimentation tank samples directly, was found to be impossible, due to the formation of emulsions and co-extraction of interfering substances. This method was therefore discontinued for this type of sample, in favour of diethyl ether extraction of the steam distillate.

The recovery and reproducibility of this method was not satisfactory (see Table 5.12) :-

TABLE 5.12
RECOVERY AND REPRODUCIBILITY OF ANALYSIS OF VOLATILE FATTY ACIDS USING DIETHYL ETHER EXTRACTION WITH STEAM DISTILLATION

Acid	Percentage Recovery	Mean* (mg/ℓ)	Standard Deviation
Acetic	60 -120	8,5	2,65
Propionic	37 - 60	4,4	1,06
Iso-butyric	30 - 90	3,9	2,75
n-butyric	40 - 80	5,1	1,93
Iso-valeric	40 - 90	5,3	2,45
n-valeric	40 - 90	6,1	2,13

* n = 10

The chromatograms of these samples also revealed a high background level, which probably contributed to the poor reproducibility (see Figure 5.21).

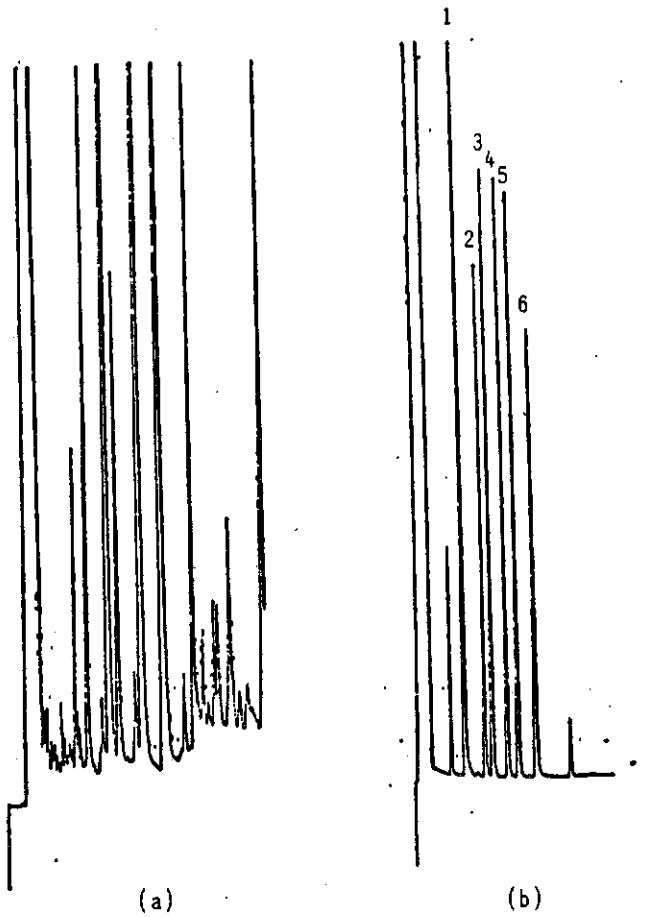

Figure 5.21: Chromatograms of steam distillates extracted with diethyl ether
(a) Digester supernatant liquor
(b) Standard containing 1:acetic acid; 2:propionic acid; 3:iso-butyric acid; 4:n-butyric acid; 5:iso-valeric acid; 6:n-valeric acid.

This method was discontinued in favour of the steam distillation with dichloroacetic acid/acetone method. The chromatograms obtained with this method were free of interfering peaks (see Figure 5.22).

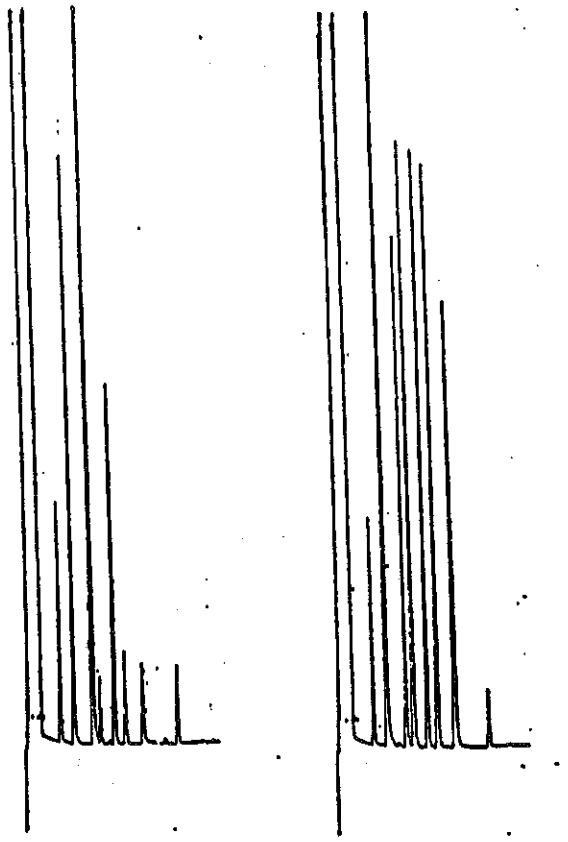

Figure 5.22: Chromatograms of samples treated by steam distillation and dichloroacetic acid/acetone
(a) Digester supernatant liquor
(b) Standard as in Figure 5.21

The recoveries and reproducibility were satisfactory (see Table 5.13).

TABLE 5.13
RECOVERY AND REPRODUCIBILITY OF ANALYSIS OF VOLATILE FATTY ACIDS USING STEAM DISTILLATION WITH DICHLOROACETIC ACID AND ACETONE

Acid	Percentage Recovery	Mean (mg/ℓ)	Standard Deviation
Acid	91 - 98	48	1,84
Propionic	92 - 100	9,7	0,42
Iso-butyric	94 - 99	9,6	0,29
n-butyric	94 - 95	9,5	0,06
Iso-valeric	92 - 99	9,5	0,38
n-valeric	89 - 96	9,3	0,36

An attempt to omit the steam distillation step for the balance tank samples resulted in the appearance of a large unknown peak (see Figure 5.23).

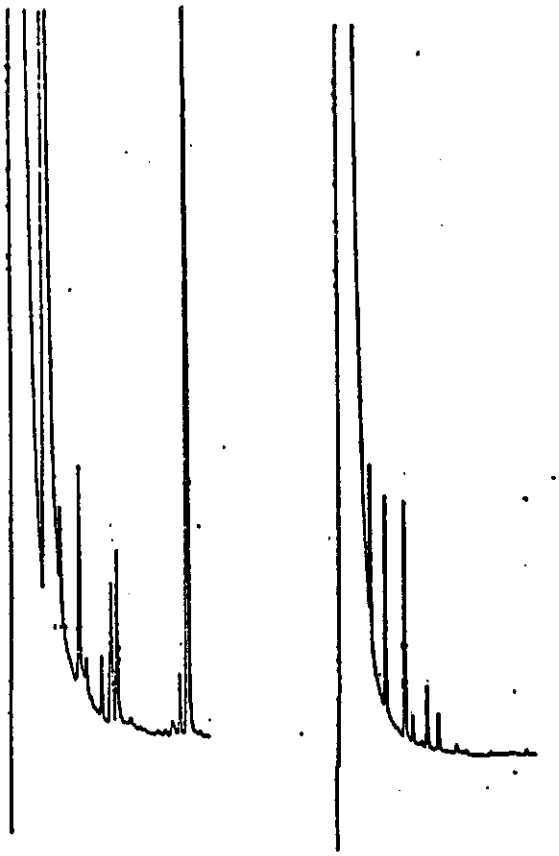

Figure 5.23: Chromatograms of balance tank effluent
(a) without steam distillation
(b) with steam distillation

Using high pressure liquid chromatography, volatile fatty acids were successfully separated on a Polypore H column (Brownlee Labs PPH-GU), using 0,01N H_2SO_4 as mobile phase and detection at 210 nm (see Figure 5.24). The minimum detection level could be decreased using a refractive index detector.

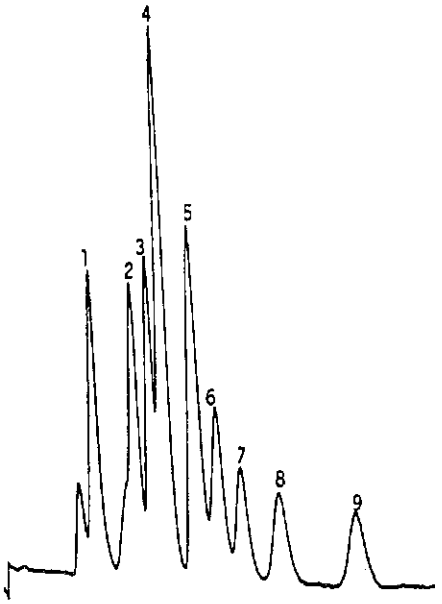

Figure 5.24: High pressure liquid chromatogram of volatile fatty acids standard solution containing 1: citric acid; 2: lactic acid; 3: formic acid; 4: acetic acid; 5: propionic acid; 6: iso-butyric acid; 7: n-butyric acid; 8: iso-valeric acid; 9: n-valeric acid

5.3.5.2 Long chain fatty acids

The technique described 5.2.5.2 resulted in satisfactory determination of the main long chain fatty acids occurring in sewage (see Figure 5.25).

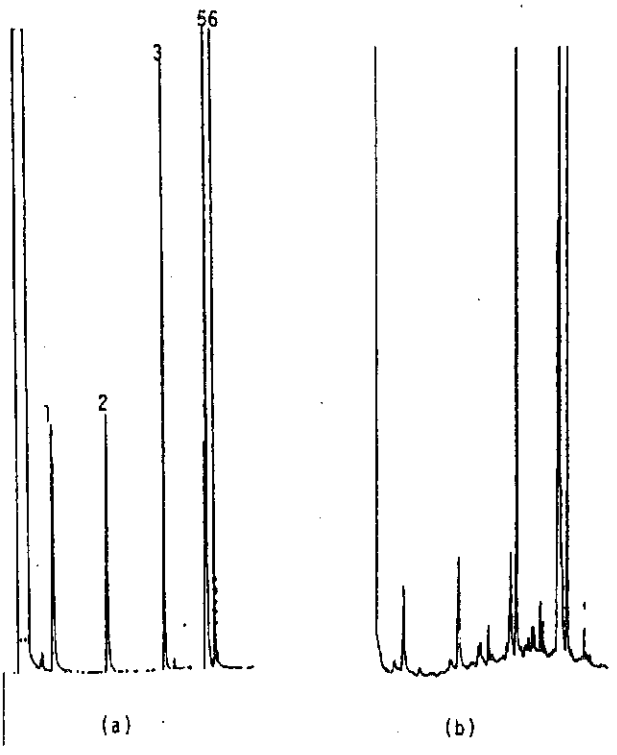

Figure 5.25: Chromatograms of long chain fatty acids (a) 100 µg/ℓ standard; 1: lauric acid; 2: myristic acid; 3: palmitic acid; 4: linoleic acid; 5: oleic acid; 6: stearic acid. (b) sewage extract.

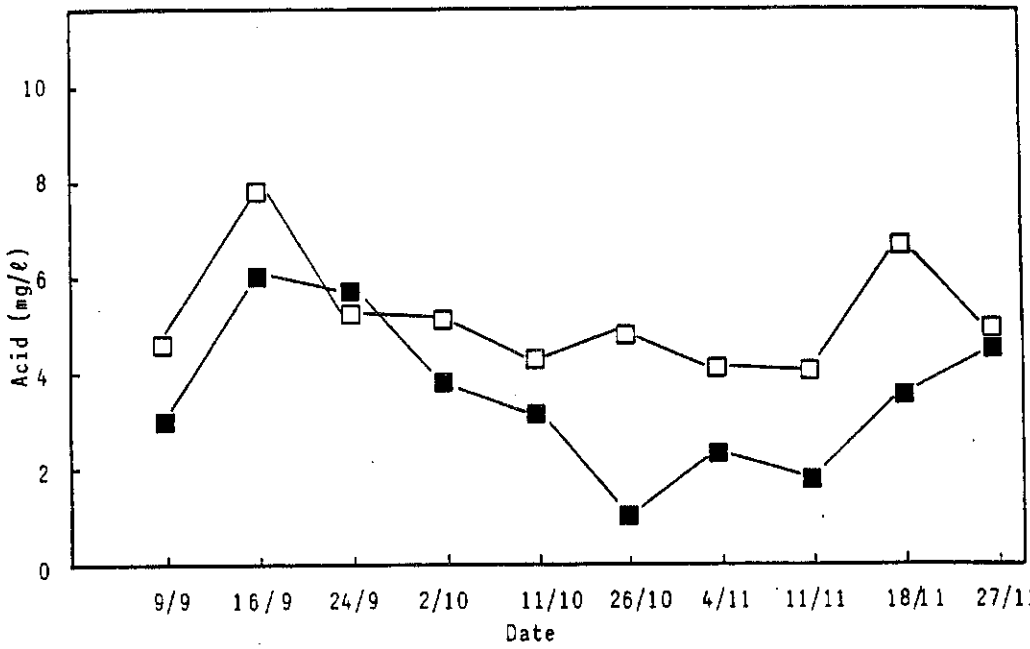

Figure 5.26: Long chain fatty acids in balance tank effluent of Northern Works Module 2. Palmitic□-□and oleic acid■-■

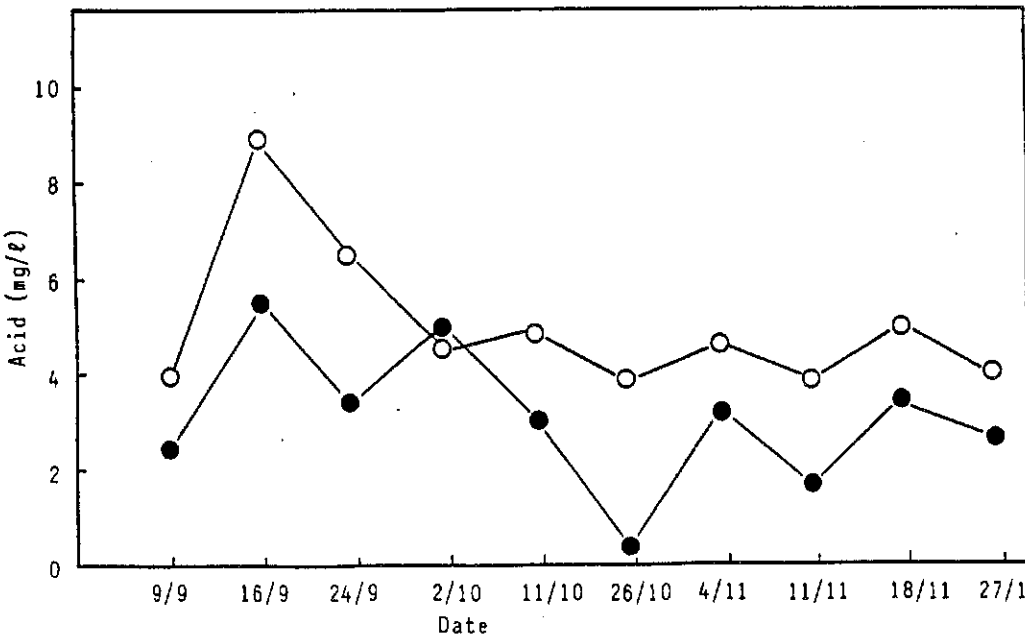

Figure 5.27: Long chain fatty acids in balance tank effluent of Northern Works Module 2 stearic○-○and linoleic acid●-●

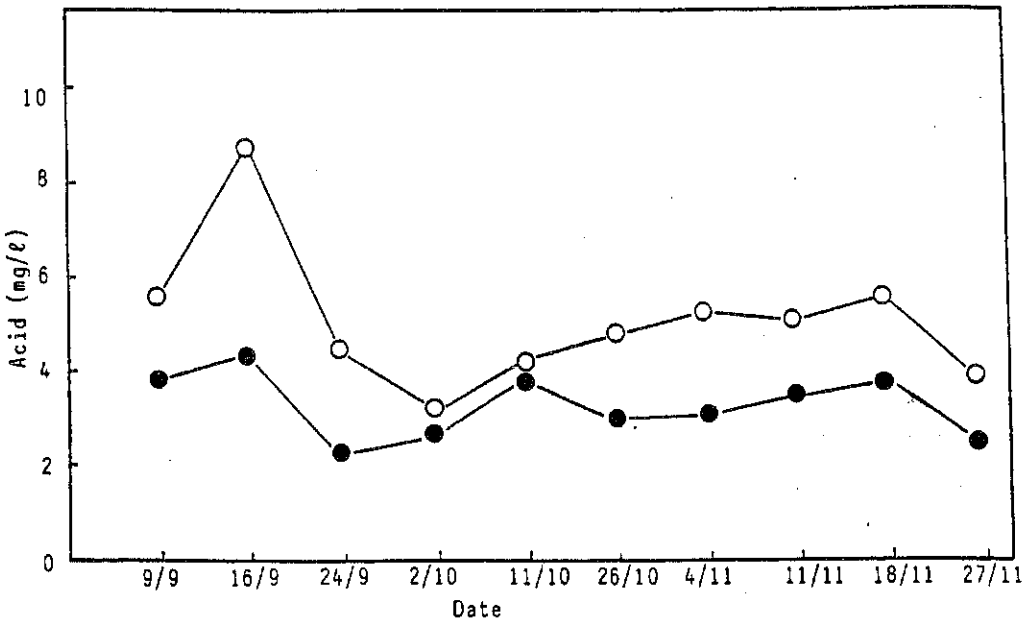

Figure 5.28: Long chain fatty acids in balance tank effluent of Northern Works Module 3 stearic○-○and linoleic acid●-●

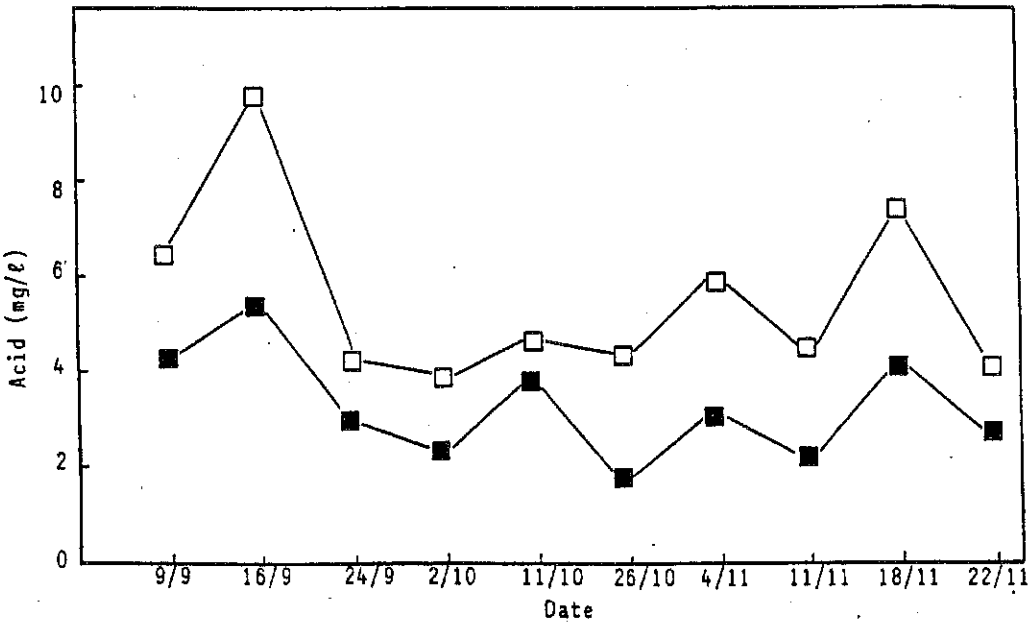

Figure 5.29: Long chain fatty acids in balance tank effluent of Northern Works Module 3 palmitic□-□and oleic acid■-■

The levels of palmitic, linoleic, oleic and stearic acid varied between less than 1 and 10 mg/ℓ in the balance tank effluents of the Northern Works plant (see Figures 5.26 to 5.29).

Attempts to correlate the acid concentration with sludge volume index (SVI) and dilute sludge volume (DSVI), were unsuccessful.

Considering the fact that oleic acid has been shown to stimulate the growth of Microthrix parvicella (Slijkhuis,1983), the most abundant filamentous organism present (see Chapter 4), this lack of direct correlation between substrate entering the anaerobic zone and filamentous growth would appear to lend support to the hypothesis of Ekama and Marais(1985), that bulking in phosphorus removal plants develops due to the low S_{bs} content produced by hydrolysis of particulate biodegradable COD in the aerobic zone.

Investigation of the partitioning of some long chain acids between the liquid and solid phase reveals very rapid uptake of these compounds in the anaerobic zone. Filamentous organisms, being aerobes (Houtmeyers,1978), could not be responsible for the rapid reduction in long chain acid levels in the liquid phase. See Figures 5.30 to 5.32 for typical results.

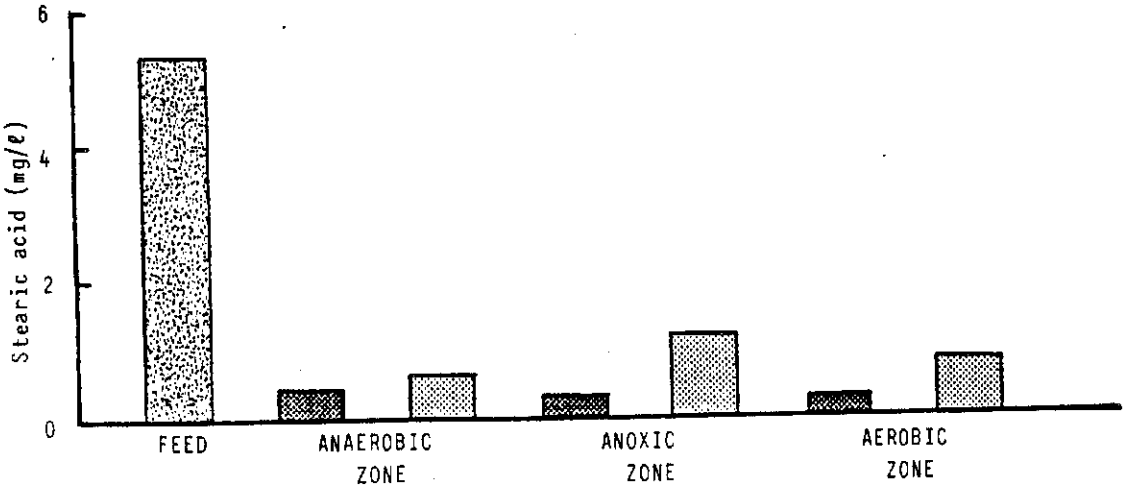

Figure 5.30 : Uptake of stearic acid in a three stage activated sludge plant showing partition between liquid ▓ and solid ▒ phases

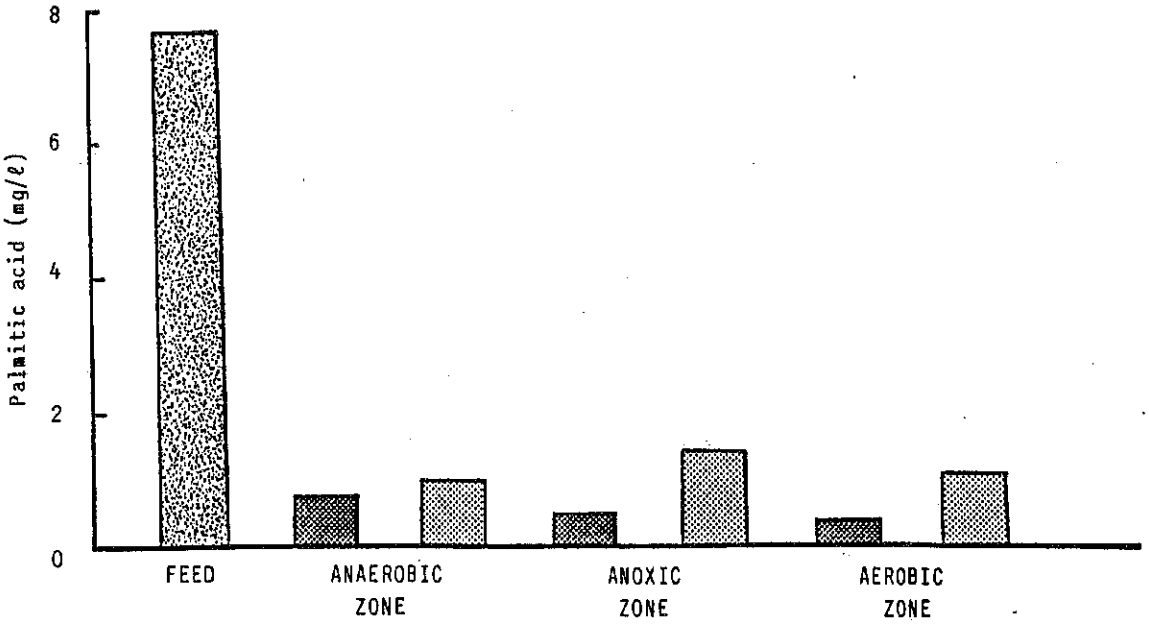

Figure 5.31 : Uptake of palmitic acid in a three stage activated sludge plant showing partition between liquid ▓ and solid phases ▒

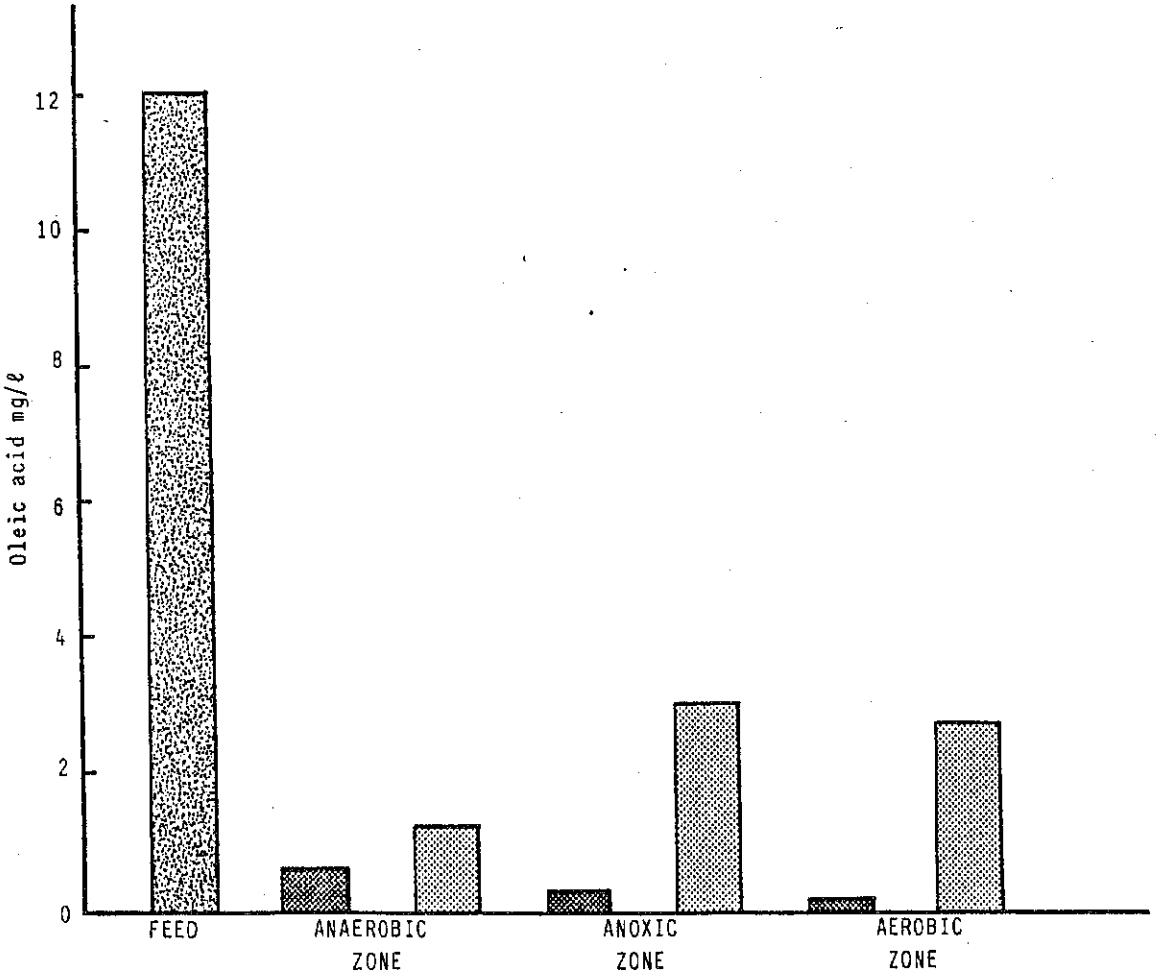

Figure 5.32 : Uptake of oleic acid in a three stage activated sludge plant, showing partition between liquid ▓ and solid phases ▒

5.3.5.3 Readily biodegradable COD

Using the continuous aerobic method, S_{bs} values were determined on the Northern Works. The variation between daily values was approximately 20 %, which was considered acceptable. However, when, in order to check the repeatability of the method, the S_{bs} was determined for 15 consecutive days, four times daily, on the same daily feed sample from Northern Works, a large scatter in the results became apparent.

The causes of this scatter were investigated and could not be pinpointed. Similar results were reported by Simpkins(1982). This contrasted with UCT workers (Dold,1984) who reported a maximum variation on this method of 20 %. In addition to these problems, this method also proved to be tedious and difficult to operate, and could not be used to determine any in situ generation of S_{bs}. For these reasons further evaluation of this method was abandoned in favour of the batch denitrification method.

Recoveries of acetate and starch were determined with this method

Results showed a very good recovery of acetate while, as expected, the starch did not exhibit any readily biodegradable component. The probable suitability of this method was thus indicated. The main disadvantage is that only those substrates which can be utilised by denitrifiers can be detected. Principal advantages are its simplicity and the fact that it can also be used to measure in situ generated fermentation products.

Typical curves obtained using the denitrification method at Goudkoppies Works, are shown in Figures 5.33 and 5.34.

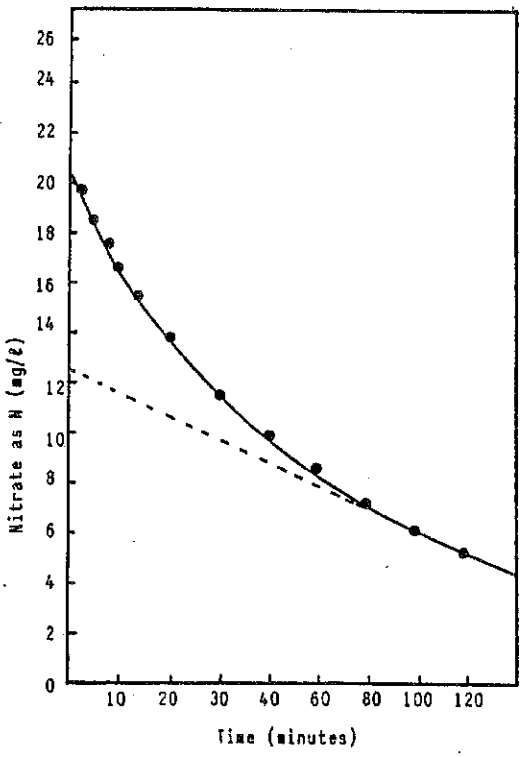

Figure 5.33 Denitrification rate without nitrogen purge.

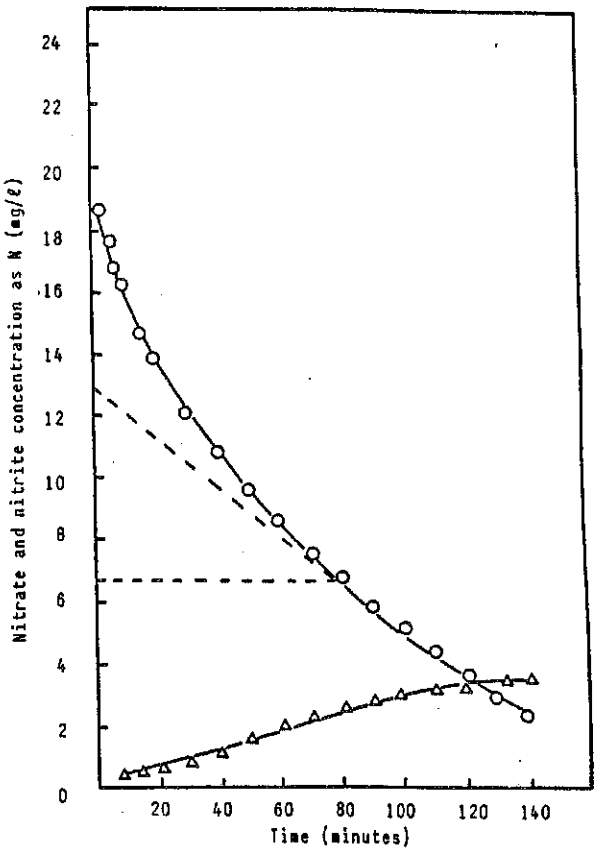

Figure 5.34 Denitrification rate curve with nitrogen purge nitrate○-○; nitrite△-△

Two definite straight rate lines normally observed using the above method, were not seen, only a gradual change or curve. This was initially thought to be due to air entrainment. However, further work showed that this was not the case. Nitrite increases each time until nitrate is depleted, whereafter it is rapidly depleted.

The presence of yeast waste in the Goudkoppies sewage did not increase the f_{bs} as expected.

The methods described above are however, extremely tedious and not easily reproducible, and the ultrafiltration method described by Bagg(1986) was implemented for routine use. The method was found to be satisfactory with a relative standard deviation of 6,3 %.

Typical results for activated sludge plant influents are given in Table 5.14.

TABLE 5.14
TYPICAL S_{bs} VALUES USING THE ULTRAFILTRATION METHOD

Sample	S_{bs}
Goudkoppies	299
Bushkoppie	248
Northern Works - Module 2	82
Northern Works - Module 3	153

Attempts to monitor the generation of S_{bs} in the primary sedimentation tanks, using this technique however, proved unreliable.

While the increase in S_{bs} is sometimes clearly distinguishable (see Table 5.15), the results are not reproducible.

TABLE 5.15
S_{bs} GENERATION IN NORTHERN WORKS PRIMARY SEDIMENTATION TANKS

Sample	S_{bs}
Outfall sewer	250
Tank influent	260
Tank effluent	290
Balancing tank effluent 2	426
Balancing tank effluent 3	338

In an attempt to isolate the problem, the effect of 0,45 µm filtration on volatile fatty acid determinations was studied (see Table 5.16 and 5.17).

TABLE 5.16
VOLATILE FATTY ACIDS ON FILTERED (F) AND UNFILTERED SAMPLES

Sample	Acetic acid (mg/ℓ)	Total VFA as acetic acid (mg/ℓ)
N BTE 2	31	43
N BTE 2 - F	34	48
N BTE 3	26	32
N BTE 3 - F	18	21
N BTE 2	30	47
N BTE 2 - F	42	62
N BTE 3	41	51
N BTE 3 - F	50	61
G BTE	47	82
G BTE - F	60	99
BIN	39	53
BIN - F	45	55

The apparent enrichment due to filtration requires explanation. Analysis of distilled water filtered through 0,45 µm filters showed no contamination by the filters.

Table 5.17 shows the loss of long chain fatty acids by 0,45 µm filtration.

TABLE 5.17
LONG CHAIN FATTY ACIDS ON FILTERED (F) AND UNFILTERED SAMPLES

Sample	Total long chain fatty acids (mg/ℓ)
N BTE 2	6,8
N BTE 2 - F	2,3
N BTE 3	4,4
N BTE 3 - F	1,9

Preliminary results of volatile fatty acid recovery in the COD determination are satisfactory - see Table 5.18. Samples of balancing tank effluent were spiked with known quantities of acetic and propionic acid.

TABLE 5.18
RECOVERY OF FATTY ACIDS DURING COD MEASUREMENT

Sample	COD	COD due to spiking
BTE	230	
BTE + 100 mg/ℓ each propionic and acetic acid	461	231
- do -	466	236
- do -	466	236
BTE + 50 mg/ℓ each propionic and acetic acid	358	128
- do -	353	123
- do -	348	118

The recovery of acetic and propionic acid at 100 mg/ℓ levels is 91 % (Theoretical COD 258), and 95 % at the 50 mg/ℓ level.

The unreliability of the method can therefore not be attributed to poor recovery of volatile fatty acids using the technique.

The lack of correlation between S_{bs} and volatile fatty acid values could perhaps be attributed to the additional biodegradable COD present in the form of non-volatile fatty acid compounds, which have not yet been identified.

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CHAPTER SIX

Bacterial metabolism

6.1 INTRODUCTION

During research into the mechanism of enhanced biological phosphate removal in activated sludge systems, interest has focussed on microbiological and biochemical phenomena. At the microbiological level the identification of the dominant bacteria responsible for phosphorus removal, have been identified (Buchan, 1983; Kerdachi and Roberts, 1983; Lötter and Murphy, 1985). These bacteria, Acinetobacter calcoaceticus, are capable of storing large amounts of phosphorus as polyphosphate (Buchan, 1983). It has however, also been shown that the presence of these bacteria is not sufficient to guarantee good phosphorus removal (Cloete et al.,1985; Lötter and Murphy, 1985). Significant numbers of Acinetobacter spp have been observed in completely aerobic systems which were not removing phosphorus (Lötter et al., 1986).

Research on the molecular level has revealed the importance of substrate composition, and in particular, the role of acetate in enhanced phosphorus removal (Fuhs and Chen, 1975; Wentzel et al., 1985). On addition of acetate to a mixed liquor sample from the aerobic zone of an activated sludge plant, phosphate is released (Lötter, 1985; Wentzel et al., 1985). The amount of phosphorus released on addition of acetate, is considerably higher than on the addition of succinate, which, unlike acetate, requires energy to enter the cell (Lötter, 1985). The phosphate released in this manner is utilised by the cell to reinstate the proton motive force, which has been dissipated by the absorption of acetic acid (Comeau et al., 1985).

The conversion of acetate to polyhydroxybutyrate under anaerobic conditions provides an endogenous substrate for energy generation under subsequent aerobic conditions where exogenous substrate has been exhausted (Wentzel et al., 1986).

In order to gain further understanding of the mechanisms involved in the above process, various metabolic reactions were studied in pure cultures of Acinetobacter calcoaceticus isolated from activated sludge plants. The production of acetic acid in the activated sludge system has also engaged the interest of researchers. Studies in this area revealed Aeromonas punctata to be the dominant bacteria in volatile fatty acid production in primary sedimentation tanks. Some characteristics of these bacteria were also studied.

6.2 THE EFFECT OF PLANT CONFIGURATION ON SELECTED CHARACTERISTICS OF ACINETOBACTER SPP

6.2.1 Aim of the Study

In anaerobic/aerobic systems, Acinetobacter spp accomplish excess P removal through the accumulation of P as polyphosphate in metachromatic granules which may occupy up to 60 % of the cell volume (Buchan, 1983). The behaviour of aerobic systems tends to indicate that the metabolic phosphorus requirements of Acinetobacter spp, are about the same as those of other heterotrophs. Comparing the behaviour of these organisms in anaerobic/aerobic and completely aerobic systems, raises the question as to whether the Acinetobacter strains in the two systems are different; whether the one group has the propensity for phosphorus accumulation as polyphosphate and the other not, or, whether all these strains have the propensity to store polyphosphate, the propensity being invoked if appropriate conditions are imposed. This question is addressed by this study.

6.2.2 Methodology

Mixed liquor samples from (1) the aerobic zone of a three-stage laboratory scale Bardenpho system exhibiting excess P removal with a twenty day sludge

age, and from (2), two completely aerobic systems with twenty day sludge age not exhibiting excess P removal, one with a selector and one without, were used for the isolation of Acinetobacter spp. The samples were treated as described by Lötter and Murphy(1985) and twenty five Acinetobacter isolates from each system were retained for further study. The nitrate reducing propensity of each isolate was tested by growth in nitrate agar and subsequent determination of nitrogen and nitrite. Polyhydroxybutyrate accumulation by the Acinetobacter isolates was determined by growth on nutrient agar, augmented with β-hydroxybutyrate (Bovre et al.,1972) followed by microscopic evaluation after Sudan Black staining, as described by Gurr(1973). Each isolate was grown in Fuhs and Chen(1975) medium. Solutions of the medium, containing only acetate or glucose at a theoretical COD of 4 000 mg/ℓ, were inoculated with 1,0 mℓ of a cell suspension with an optical density of 1,0 and incubated aerobically for five days at 20 °C.

After the incubation period, slides were made of the cell suspensions and polyphosphate accumulation was evaluated using a Neisser stain (Society of American Bacteriologists,1957). Thereafter, the cell suspensions were centrifuged in tared centrifuge tubes at 20 000 g. The supernatant was retained for the determination of COD and the mass of cells was determined. The COD was determined by the standard dichromate oxidation method (American Public Health Association,1981).

6.2.3 Result and Discussion

The nitrate reducing capacity of Acinetobacter isolates are given in Table 6.1. It is evident from this Table that a number of Acinetobacter isolates are capable of nitrate reduction. This is in agreement with the findings of previous workers on the capacity for nitrate reduction by Acinetobacter spp (Lötter,1985; van Groenestijn and Deinema,1985). Also, it is evident that little difference in the capacity for nitrate reduction, exists between isolates from the anaerobic/ anoxic/aerobic and the aerobic systems. Thus the propensity for nitrate reduction does not appear to be induced by environmental conditions, that is, the inclusion of an anoxic zone in the activated sludge system does not induce the capacity for nitrate reduction in Acinetobacter spp in the system. The majority of the

Acinetobacter isolates able to reduce nitrate, reduced the nitrate to nitrite. Only a minority of isolates reduced nitrate to nitrogen.

TABLE 6.1
PERCENTAGE OF ACINETOBACTER ISOLATES CAPABLE OF NITRATE REDUCTION

Isolate source	Nitrate reduction to nitrite (Percentage of total)	Nitrate reduction to nitrogen (Percentage of total)
Aerobic zone Bardenpho	32	6
Continuous aerobic unit	43	5
Continuous aerobic unit with selector	42	12

The microscopic evaluation of cells for polyphosphate and polyhydroxybutyrate accumulation was carried out by preparing microscopic slides from aerobic cultures of each isolate, and appropriately staining for the polyphosphate and polyhydroxybutyrate inclusions. For each isolate the slide was categorised by assigning a value of 0 - 4 to the slide, according to the number of cells containing inclusions. For each system, the number of isolates in each category was expressed as a percentage of the total number of isolates. These results are depicted graphically in Figures 6.1 to 6.3.

Referring to Figures 6.1 and 6.2, polyphosphate accumulation occurred in Acinetobacter isolates from the aerobic and from the anaerobic/anoxic/aerobic systems, on both glucose and acetate substrates. Furthermore, there is no significant difference in the proportions of occurrence. It would thus appear that the Acinetobacter isolates from the completely aerobic systems, while not accumulating polyphosphate in the system, did in fact possess the same propensity to accumulate polyphosphate under certain conditions, as isolates from the anaerobic/anoxic/aerobic system, where Acinetobacter strains did accumulate polyphosphate. Thus, the environment of the system from which the Acinetobacter was isolated, namely anaerobic/anoxic/aerobic or aerobic only, does not appear to have a significant effect in selecting out strains which have the propensity to

accumulate polyphosphate, but rather that the anaerobic/aerobic system invokes a latent propensity in the organism for poly P accumulation.

With regard to storage of polyhydroxybutyrate, all the isolates irrespective of their origin, appeared to exhibit similar propensities to accumulate polyhydroxybutyrate under the aerobic culture conditions (see Figure 6.3), even though this accumulation has not been observed in completely aerobic activated sludge systems.

From the above it would appear that the propensities to accumulate polyhydroxybutyrate and polyphosphate, are inherent characteristics of some Acinetobacter strains, regardless of the system from which they are isolated.

6.3 THE EFFECT OF ACETATE AND SUCCINATE ON POLYPHOSPHATE FORMATION AND DEGRADATION IN ACINETOBACTER SPP

6.3.1 Aim of the Study

Phosphate starvation stimulates rapid phosphate uptake on resuspension of Escherichia coli in a phosphate rich medium (Medveczky and Rosenberg, 1971) and has been shown to induce polyphosphate accumulation in Aerobacter aerogenes. The release of phosphate on addition of acetate could stimulate a condition of phosphate starvation in Acinetobacter calcoaceticus strains in the anaerobic zone of an activated sludge system. In this study an attempt was made to compare the effect of acetate and succinate treatment with phosphate starvation on polyphosphate accumulation.

6.3.2 Methodology

An Acinetobacter isolate from a five-stage Bardenpho plant which had been maintained by a weekly subculture onto GCY agar (Pike et al., 1972), and once-monthly subculture onto acetate/sewage agar (Fuhs and Chen, 1975), was used to inoculate acetate/sewage medium (Fuhs and Chen, 1975). Samples were taken for the experiments during the stationary phase. The sample treatments included the addition of acetic or succinic acid to a final

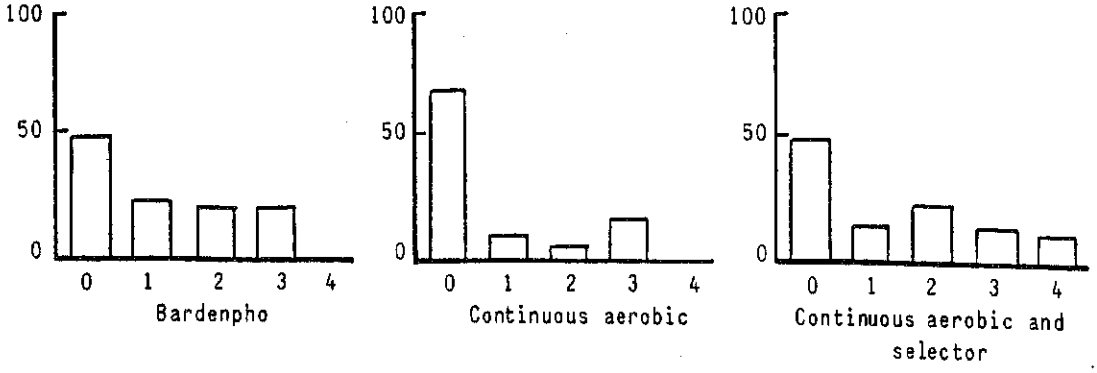

Figure 6.1 : Polyphosphate accumulation in acetate medium

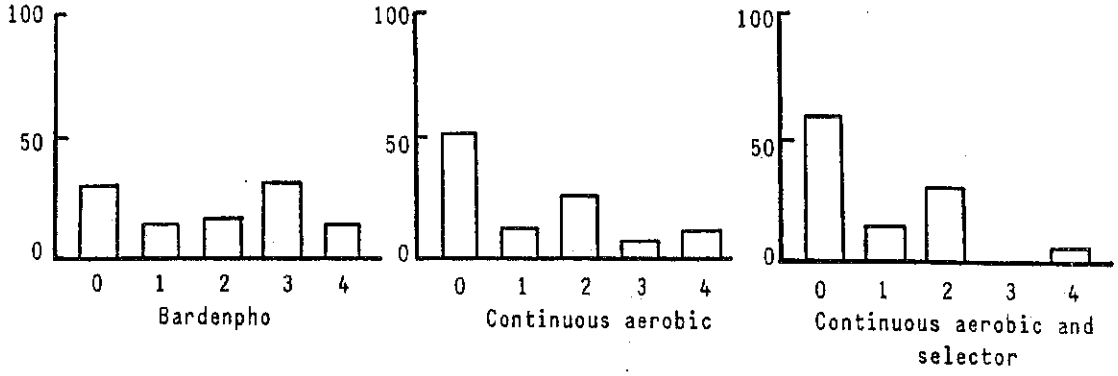

Figure 6.2 : Polyphosphate accumulation in glucose medium

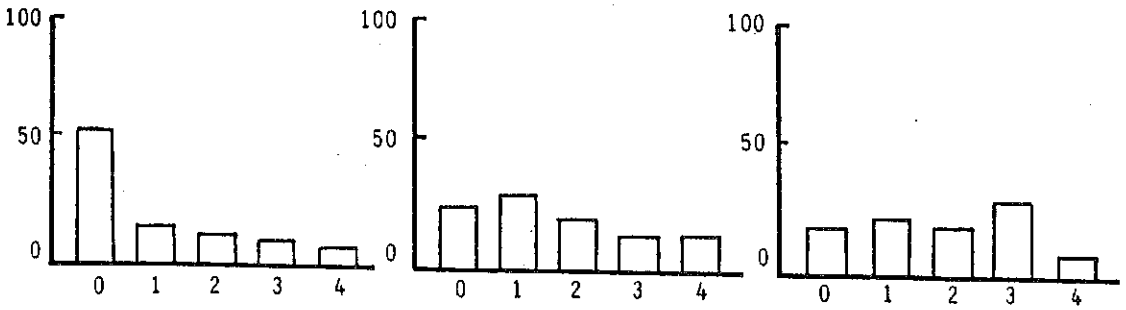

Figure 6.3 : Polyhydroxybutyrate accumulation on nutrient agar containing β-HO butyrate

Key to Figures 6.1 to 6.3 :

0 - no cells contain inclusions
1 - one quarter cells contain inclusions
2 - half cells contain inclusions
3 - three quarters cells contain inclusions
4 - all cells contain inclusions

concentration of 100 mg/ℓ or resuspension in acetate/sewage medium (Fuhs and Chen, 1975) modified by the omission of phosphate and fermented raw sewage. The samples were stirred at room temperature for the duration of the experiment. Control samples were run simultaneously and all experiments were carried out in duplicate.

Forty millilitre aliquots were taken from each sample at 40 minute intervals over the experimental period of 160 minutes. At the end of this period, each sample was centrifuged at 5 000 g for five minutes and the residue resuspended in 250 mℓ acetate/sewage medium (Fuhs and Chen, 1975), modified by the omission of fermented raw sewage. The resuspended samples were aerated at room temperature and 40 mℓ aliquots taken at 40 minute intervals for 160 minutes The phosphorus fractions in the samples were determined essentially according to the procedure proposed by Harold (1960) and described in 5.2.3.

6.3.3 Results and Discussion

The results are expressed as mg P/g cells. Phosphate starvation and acetate treatment resulted in release of phosphate to the external medium. Acetate has previously been shown to stimulate phosphorus release in activated sludge (Wentzel et al., 1985; Lötter, 1985). The similar behaviour observed here with Acinetobacter, suggests that these bacteria are the main source of phosphate release in the activated sludge. See Table 6.2 for a summary of results.

TABLE 6.2
PHOSPHATE RELEASED AND ABSORBED DURING TREATMENT

	Phosphate released	Phosphate absorbed
	mg P/g Cells	
Control	2	3
Acetate	32	43
Phosphate starvation	10	5

Succinate treatment resulted in no more release than the control. The difference in acetate and succinate behaviour is almost certainly due to their different membrane transport properties (Lötter, 1985). Again, increased phosphate uptake was observed on aeration of the treated samples. The dramatic increase stimulated by acetate is again evident. However, the effect is more marked in pure culture than in activated sludge (Murphy and Lötter, 1986), suggesting that the possible link between acetate and phosphorus metabolism is specific to Acinetobacter spp.

No significant variation in intracellular components was observed in the control and succinate treated sample culture. During phosphate starvation, a decrease in acid soluble polyphosphate and intracellular orthophosphate was observed. Very little change is observed in the acid insoluble polyphosphate. In the acetate treated sample, a decrease in all three fractions, at similar rates, was observed (see Figures 6.4 and 6.5).

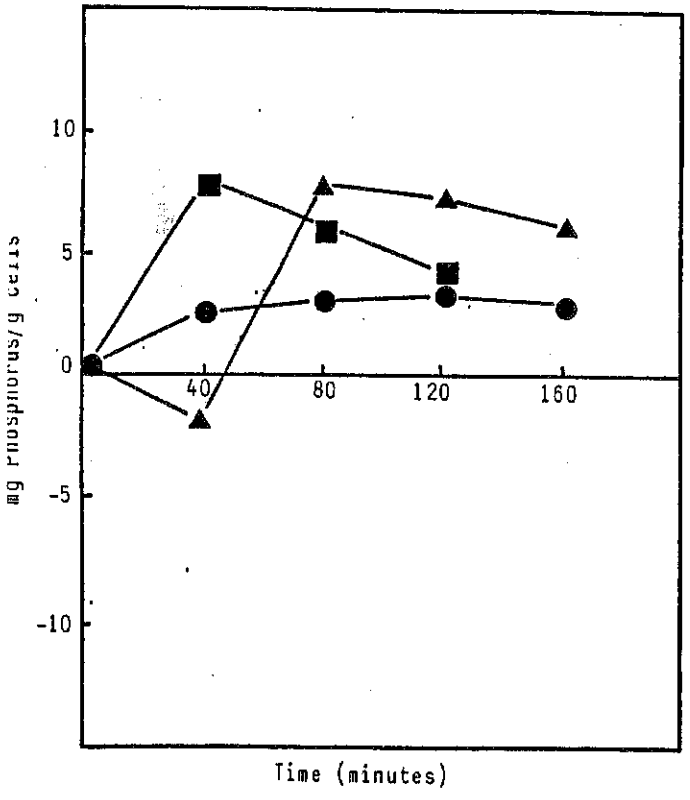

Figure 6.4 : Net variation in intracellular phosphorus fractions during aeration after suspension in phosphate free medium

Intracellular phosphate ▲—▲
Acid soluble polyphosphate ■—■
Acid insoluble polyphosphate ●—●

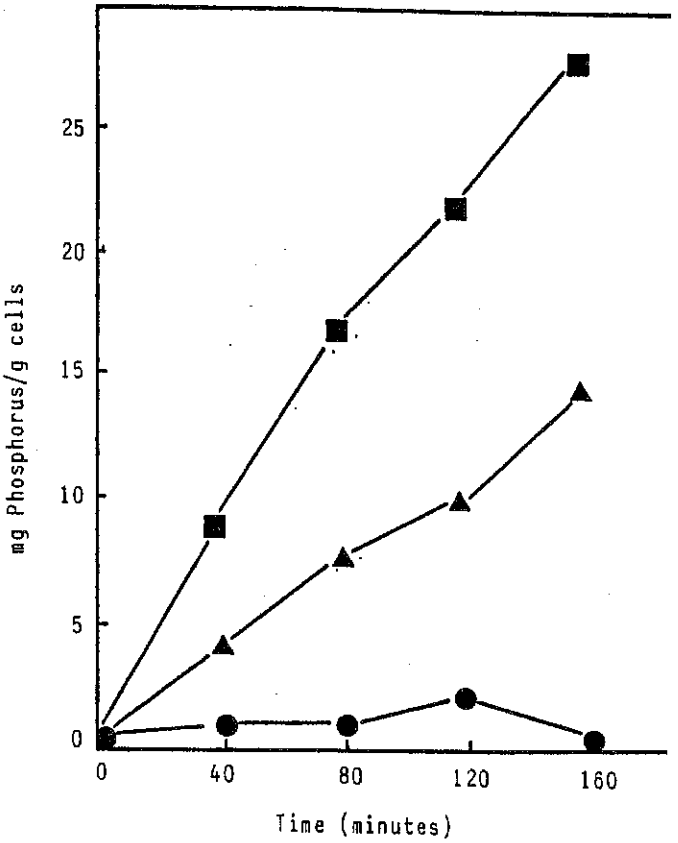

Figure 6.5 : Net variation in intracellular phosphc fractions of acetate treated cells du aeration

Intracellular polyphosphate ▲—▲
Acid soluble polyphosphate ■—■
Acid insoluble polyphosphate ●—●

It is clear from the results of this study, that the stimulation of phosphate uptake by acetate, observed by some workers (Marais and Ekama, 1982), is not solely as a result of phosphate deprivation. The significant difference between acetate treatment and phosphate starvation suggests a direct link between acetate and polyphosphate metabolism.

6.4 ACETATE UPTAKE AND METABOLISM IN ACINETOBACTER SPP

6.4.1 Aim of the Study

The addition of VFA, in particular acetic acid, has been shown to enhance biological phosphorus removal in activated sludge (see Chapter 2). In addition, enhanced polyphosphate formation was observed in an Acinetobacter strain on the addition of acetic acid under anaerobic conditions (6.3). The question of the fate of acetic acid in Acinetobacter spp under anaerobic conditions, remains to be answered.

Researchers have reported that Acinetobacter strains capable of enhanced phosphate uptake in pure culture, are also capable of storing PHB (Fuhs and Chen, 1975; Lawson and Tonhazy, 1980). The accumulation of PHB under anaerobic conditions and depletion under aerobic conditions has been observed in activated sludge (Hart and Melmed, 1982; Lötter et al., 1986). It has been postulated that acetic acid is taken up by Acinetobacter under anaerobic conditions and metabolised to PHB, which in turn, is utilised as a carbon energy source in the aerobic zone (Wentzel et al., 1986). The aim of this study was to investigate this postulate.

6.4.2 Methodology

The test bacterium, a strain of Acinetobacter calcoaceticus var lwoffi, was isolated from the aerobic zone of a five-stage Bardenpho plant exhibiting phosphorus removal. The organism was isolated and characterised as described previously (Lötter and Murphy, 1985). The isolate was maintained by weekly sub-culture onto Acinetobacter agar (Fuhs and Chen, 1975).

A five litre aerated stock culture was kept in a base medium containing : $(NH_4)_2SO_4$, 2 g/ℓ; $MgSO_4$, 0,5 g/ℓ; KH_2PO_4 , 0,25 g/ℓ; $CaCl_2.2H_2O$, 0,2 g/ℓ;

sodium acetate, 5 g/ℓ (Fuhs and Chen, 1985). Two litres of this culture were drawn off weekly and 400 mℓ fresh medium added daily to maintain the culture for subsequent experiments. The culture was boosted periodically with an overnight culture of fresh cells, to maintain an optical density at 520 nm of between 1 and 2.

Five hundred mℓ stock culture was centrifuged at 2000 g for five minutes and the pellet added to 2 000 mℓ base medium without sodium acetate, after an aliquot had been taken for blank readings. The cells were stirred for one hour to disperse the pellet and acclimatise them to the new medium. Acetate was then added to a final concentration of 5 g/ℓ. A sample was taken (t=0) and the subculture was divided into three aliquots. Aliquot 1 was purged with nitrogen and left anaerobic for three hours, after which it was aerated for 21 hours (anaerobic/aerobic). Aliquot 2 was purged with nitrogen and left anaerobic for 24 hours (anaerobic). Anaerobiosis was determined by the use of Anaerotest papers (Merck). Aliquot 3 was aerated for 24 hours. Samples were taken at 3 hourly intervals for 24 hours.

The acetic acid concentration in the cell-free medium was determined by the gas chromatographic method described in Section 5.2.5.1. The PHB content of the cells was determined by a modification of the method described in Section 5.2.4.

The cell mass was determined by measuring the optical density at 520 nm and reading the mass from a calibration curve of optical density against mass.

6.4.3 Results and Discussion

The uptake of acetic acid under anaerobic conditions is shown in Figure 6.6.

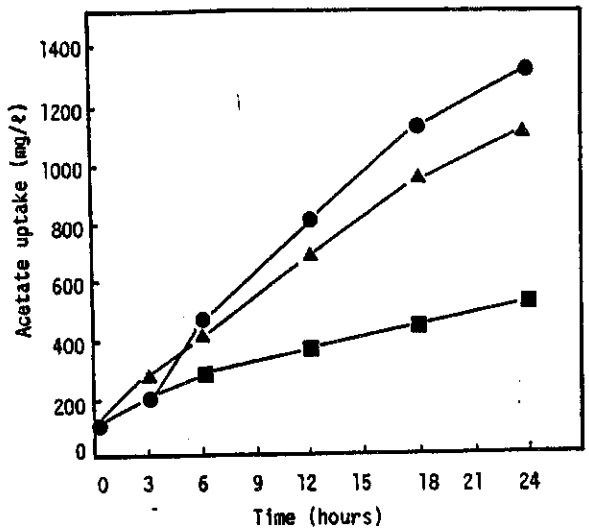

Figure 6.6 : Uptake of acetic acid by Acinetobacter cells under anaerobic ■—■ ; anaerobic/aerobic ▲—▲ and aerobic ●—● conditions

Acetic acid uptake under aerobic conditions increases linearly over the 24 hour experimental period. Under anaerobic conditions much slower uptake increases linearly to a lower maximum than observed under aerobic conditions. The initial period of anaerobiosis in the third sample appears to stimulate uptake on subsequent aeration. As Acinetobacter spp are obligate aerobes (Juni, 1978), no growth can be expected under anaerobic conditions. However, the increased uptake under aerobic conditions is at least partially due to the growth demands on exogenous carbon, as can be seen in Figure 6.7.

The downward trend of the acetic acid uptake curve in the case of the anaerobically pretreated aerobic sample indicates a growth rate in excess of the uptake rate after 18 hours. The uptake of acetic acid under anaerobic conditions is not unexpected, as previous researchers have reported the absence of an energy requirement for bacterial uptake of acetic acid (Konings et al., 1981).

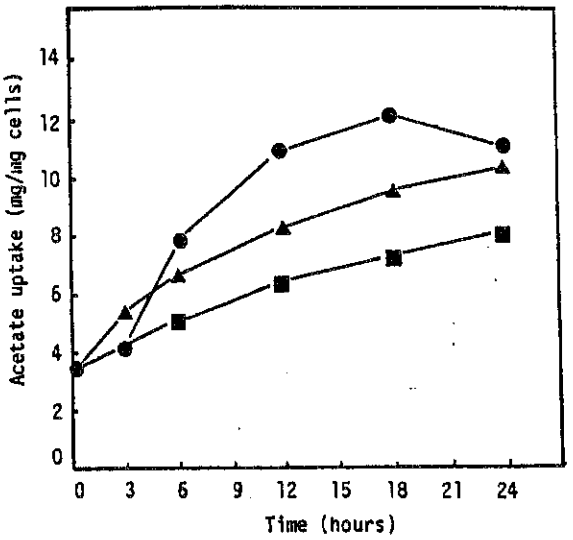

Figure 6.7 : Acetate uptake by Acinetobacter cells under anaerobic ■—■ ; anaerobic/aerobic ●—● ; and aerobic ▲—▲ conditions

The apparent stimulation of aerobic metabolism by anaerobic pretreatment in Acinetobacter spp requires further investigation. These results could be considered in the same light as the increased phosphorus metabolism observed after anaerobic pretreatment of Acinetobacter cells (Deinema et al., 1980; Murphy and Lötter 1986).

The fate of PHB under the experimental conditions is shown in Figure 6.8. In the continuously aerated sample, no significant change in the PHB level was observed, while in the anaerobically pretreated sample, a slight reduction in PHB is observed during aeration. The initial utilisation of PHB can probably be ascribed to rapid demands being made on all available carbon sources after a period of stress.

The synthesis of PHB which is observed under anaerobic conditions, confirms the postulates of Wentzel et al., (1986) who describe the the regulatory role of PHB in maintaining the ratio between the reduced and oxidised form of nicotinamide adenine dinucleotide (NADH/NAD) under anaerobic conditions.

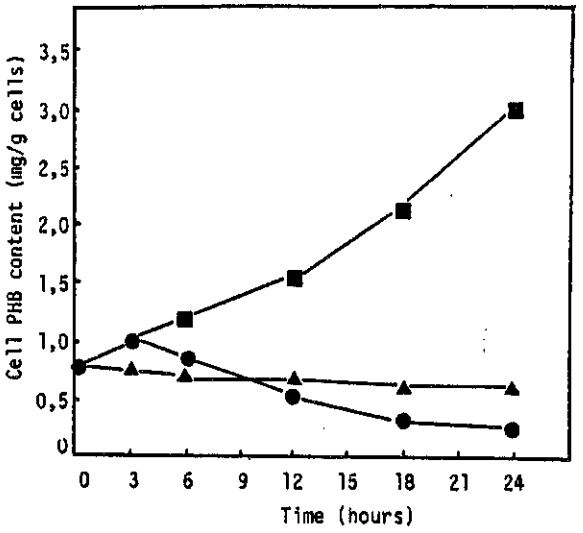

Figure 6.8 : PHB synthesis and utilisation in Acinetobacter cells under anaerobic ■—■ ; anaerobic/aerobic ▲—▲ and aerobic ●—● conditions

This study has shown that the obligate aerobe Acinetobacter is capable of acetic acid uptake and storage under anaerobic conditions. More detailed studies of acetic acid/PHB metabolism however, remain to be undertaken.

6.5 POLYPHOSPHATE KINASE ACTIVITY IN ACINETOBACTER SPP

6.5.1 Aim of the Study

Polyphosphate accumulation has been demonstrated in Acinetobacter strains, under various culture conditions (6.3.3). The synthesis of polyphosphate in bacteria is generally catalysed by a polyphosphate kinase according to the following reaction (Harold, 1966; Suzuki et al., 1972; Kulaev, 1975).

$$ATP + (PO_4)_n \xrightarrow{\text{polyphosphate kinase}} ADP + (PO_4)_{n+1}$$

The aim of this study was to investigate the effect of various culture conditions on polyphosphate kinase activity from an Acinetobacter strain.

6.5.2 Methodology

In order to simulate the anaerobic/aerobic sequencing of the activated sludge process, substrate (acetate or succinate) was added to bacterial suspensions under anaerobic conditions. Samples were taken during this period and during the subsequent aerobic period. Control samples did not

undergo an anaerobic phase.

Bacterial suspensions were subjected to ultrasonication in Tris-HCl buffer at pH 7,0 in the presence of glass beads. After ultrasonication the suspension was centrifuged at 20 000 g and the supernatant frozen. Frozen extracts retained their enzymatic activity indefinitely. The polyphosphate kinase activity was assayed by measuring the incorporation of ^{32}P from $\gamma^{32}P$-ATP into polyphosphate (Robinson et al., 1984).

6.5.3 Results and Discussion

The variation of polyphosphate kinase activity with growth rates is shown in Figure 6.9.

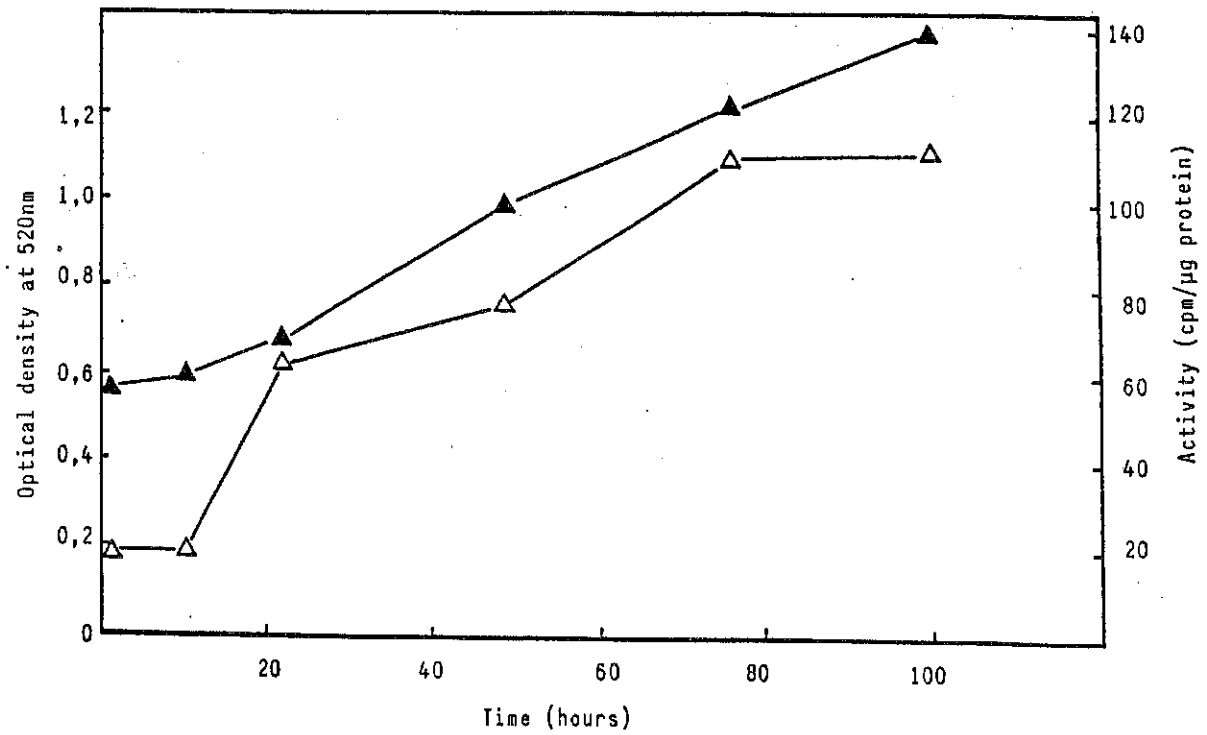

Figure 6.9 : Polyphosphate kinase activity at different stages of growth. Growth, △–△, enzyme activity, ▲–▲

Polyphosphate kinase activity was highest at the end of the growth period. This is not unexpected, as during this period of growth, the demands on ATP levels for cell synthesis are considerably reduced and the nucleotide can be channelled into polyphosphate formation.

Growth in acetate after a period of anaerobiosis stimulates enzyme activity, while the anaerobic conditions cause the succinate culture to reduce polyphosphate synthesis (see Figures 6.10 and 6.11).

An explanation for this apparent anomaly must be sought in the different uptake characteristics of the two substrates. Acinetobacter spp are obligate aerobes. Oxidative phosphorylation to produce ATP does not therefore take place under anaerobic conditions. Substrates like succinate, which are absorbed via an active transport mechanism (Ramos and Kaback, 1977) cannot be taken up under anaerobic conditions, while acetate can be transported by passive diffusion (Konings et al., 1981) and is absorbed. Acetate uptake under these conditions has in fact been observed in Acinetobacter spp (see Section 6.4).

The acetate grown cells enter the aerobic phase with sufficient carbon for metabolism, cell growth can immediately take place and ATP can be channelled to polyphosphate synthesis much more rapidly, than in the case of succinate grown cells which have to absorb carbon and metabolise it, before the luxury of polyphosphate storage can be attempted, as evidenced by the slow rise in polyphosphate kinase activity after anaerobiosis, shown in Figure 6.11.

The stimulation of polyphosphate synthesis by acetate absorption under anaerobic conditions, is not entirely due to the phosphate starvation effect experienced under these conditions (see Section 6.3).

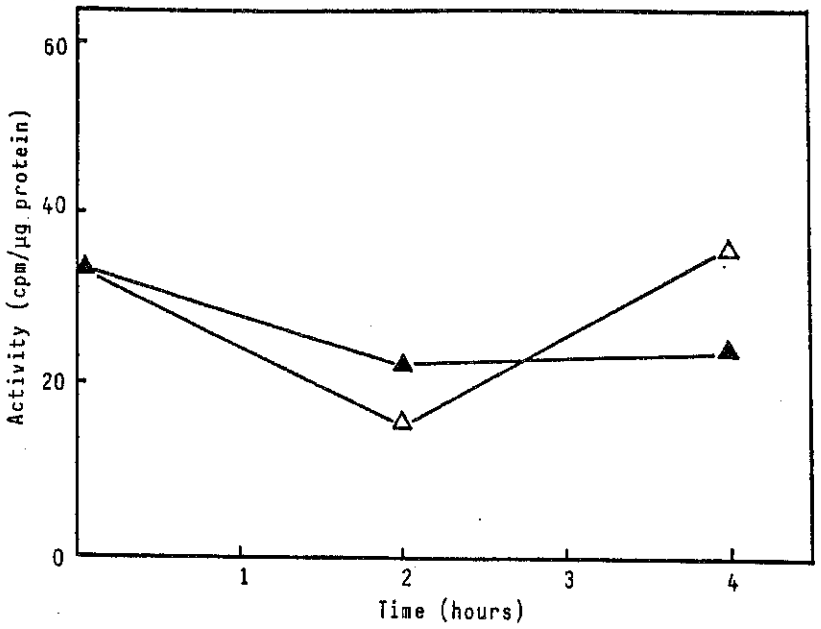

Figure 6.10: Effect of anaerobic conditions on polyphosphate kinase activity in acetate grown Acinetobacter cells△-△anaerobic treatment▲-▲aerobic control.

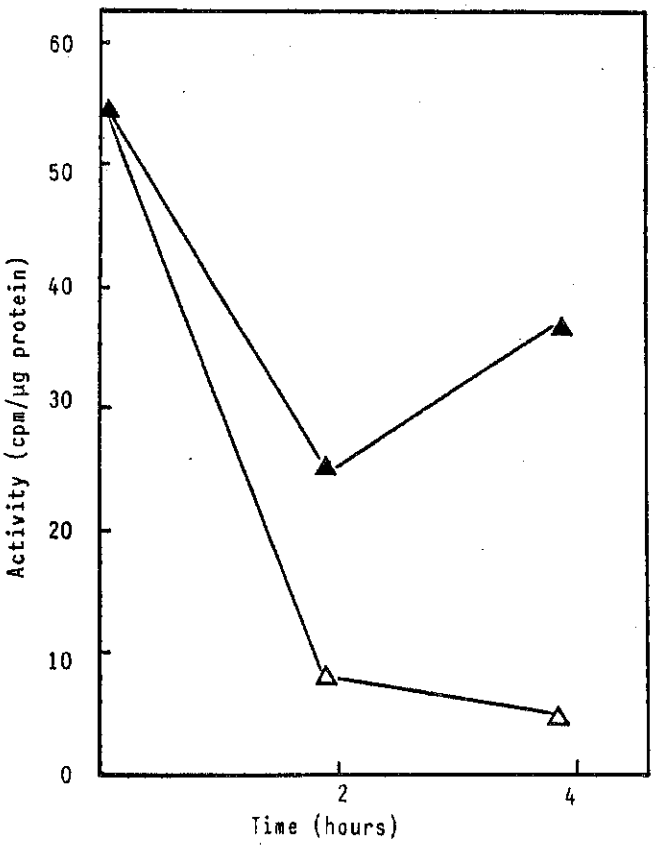

Figure 6.11: Effect of anaerobic conditions on polyphosphate kinase activity in succinate grown Acinetobacter cells △-△anaerobic treatment▲-▲aerobic control

6.6 PROTEIN PROFILES IN ACINETOBACTER SPP

6.6.1 Aim of the Study

One of the unique characteristics of a living cell is its ability to permit complex reactions to proceed rapidly without an external energy source. The principal agents which participate in these reactions are proteins called enzymes.

In general terms, enzyme catalysed reactions are dependent on the presence of the relevant enzyme and its activity. Clearly regulation of reaction velocities can be achieved in two ways, namely, one, to control the synthesis of the enzyme and two, to control the activity in some way. Understanding the regulation of enzymatic catalysis in living cells is the main key to understanding fundamental mechanisms. In this study the protein profiles of an Acinetobacter strain grown under anaerobic and aerobic conditions, were investigated in an attempt to gain insight into the regulation of enzymatic activity under these conditions.

6.6.2 Methodology

Cultures of an Acinetobacter strain isolated from the aerobic zone of the Northern Works activated sludge plant, were grown in Fuhs and Chen (1975) medium under aerobic conditions. Acetate was added to two aliquots of the stock culture. After overnight incubation, one under anaerobic and one under aerobic conditions, cells were harvested by centrifugation. Protein was extracted by ultrasonication of the cell pellet in a 0,05M phosphate buffer at pH 7,0.

The extract was subjected to polyacryladmide gel electrophoresis according to Laemmli (1970), using 12,6 % gels containing sodium dodecyl sulphate. Gels were stained with Coomassie blue before viewing.

6.6.3 Results and Discussion

Typical results of electrophoresis of the two extracts are shown in Figure 6.12. Anaerobiosis does not appear to alter the protein profile in any way.

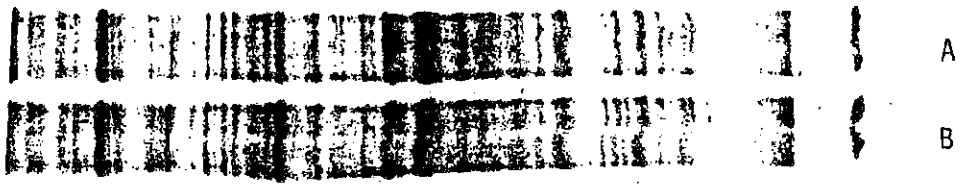

Figure 6.12: SDS PAGE Electrophoretic patterns of protein extracts from Acinetobacter cells grown aerobically (A) and anaerobically (B)

In other words, synthesis of specific proteins does not occur under these conditions. Alterations in enzymatic activity under anaerobic conditions are therefore controlled by other mechanisms. This finding supports the hypothesis of Wentzel et al. (1986), who propose an important feedback control mechanism in the form of varying NADH/NAD ratios in the control of the tricarboxylic acid cycle and polyhydroxybutyrate metabolism.

6.7 EFFECT OF CULTURE CONDITIONS ON ISOCITRATE DEHYDROGENASE ACTIVITY IN ACINETOBACTER SPP

6.7.1 Aim of the Study

The tricarboxylic acid cycle plays two fundamental roles in microbial metabolism; it provides both the precursors of many cell constituents and the energy to effect cell growth and maintenance. The continuous functioning of the cycle requires replenishment of intermediates which are drained away during growth. In the case of two carbon atom substrates, this replenishment can be a problem due to the loss of two moles of carbon dioxide during the cycle.

The operation of the glyoxylate cycle can overcome the problem by replacing the tricarboxylic acid cycle steps, which lead to the loss of 2 moles of carbon dioxide, with reactions leading to a net increase in the organic acid carbon of the system (see Figure 6.13) The enzyme, isocitrate dehydrogenase is the branch point enzyme between the glyoxylate and tricarboxylic acid cycles. The activity of this enzyme in Acinetobacter

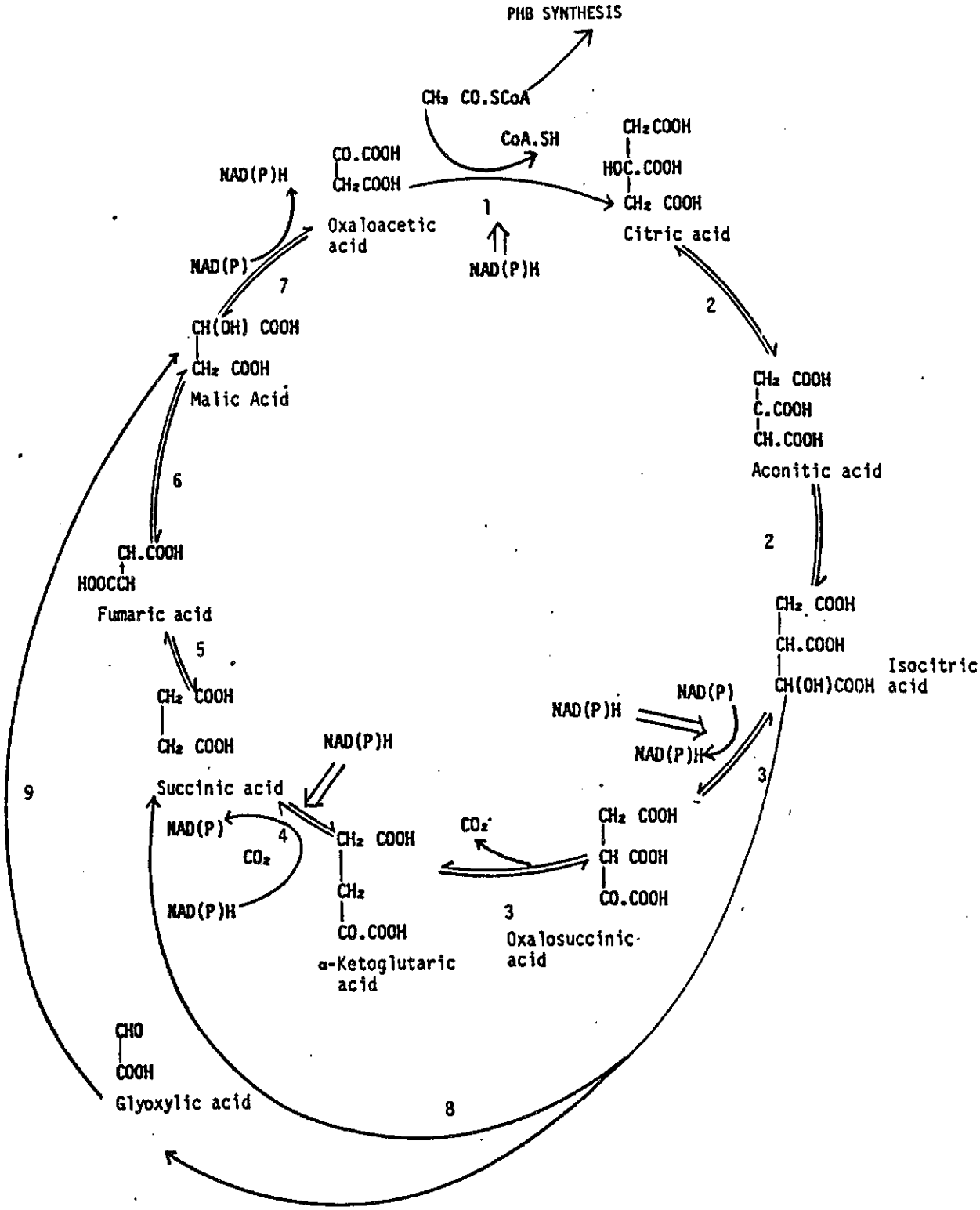

Figure 6.13 : Carbon metabolism in Acinetobacter spp

Key to enzymes :

1	Citrate synthase	2	Aconitase
3	Isocitrate dehydrogenase	4	α-ketoglutarate dehydrogenase
5	Succinate dehydrogenase	6	Fumarase
7	Malate dehydrogenase	8	Isocitrate lyase
9	Malate synthase	‡	indicates inhibition

spp was studied in an attempt to pinpoint control mechanisms in the metabolism of this organism.

6.7.2 Methodology

An Acinetobacter strain isolated from an activated sludge plant was grown in Fuhs and Chen (1975) medium without fermented raw sewage. In one case, the sodium acetate was replaced by sodium succinate. Bacterial cells were harvested by centrifugation after overnight growth in the single carbon medium.

The cell pellet was resuspended in 0,05M phosphate buffer and subjected to ultrasonication. The resulting suspension was centrifuged at 20 000 g for 10 minutes and the supernatant retained for enzymatic analysis.

The isocitrate dehydrogenase activity was determined by monitoring the production of NADPH at 340 nm (Holms and Bennett, 1971).

6.7.3 Results and Discussion

The progress curves of isocitrate dehydrogenase activity for cells grown in acetate and succinate, are given in Figures 6.14 and 6.15.

The decrease in activity with time indicates inhibition. In order to investigate this aspect further, the determination was repeated with less enzyme extract. It appears from Figures 6.16 and 6.17 that the extract contains substances inhibitory to the enzyme activity.

Further investigations revealed that using 0,2 mℓ enzyme extract for the incubation time of 2 minutes, would provide a satisfactory result (see Figure 6.18).

Using this technique, the isocitrate dehydrogenase activity of cells grown in acetate and succinate were compared. Preliminary results show that isocitrate dehydrogenase activity decreases during growth on acetate. This indicates that the glyoxylate shunt is operational in Acinetobacter spp under these conditions, similar to the observations with Escherichia coli

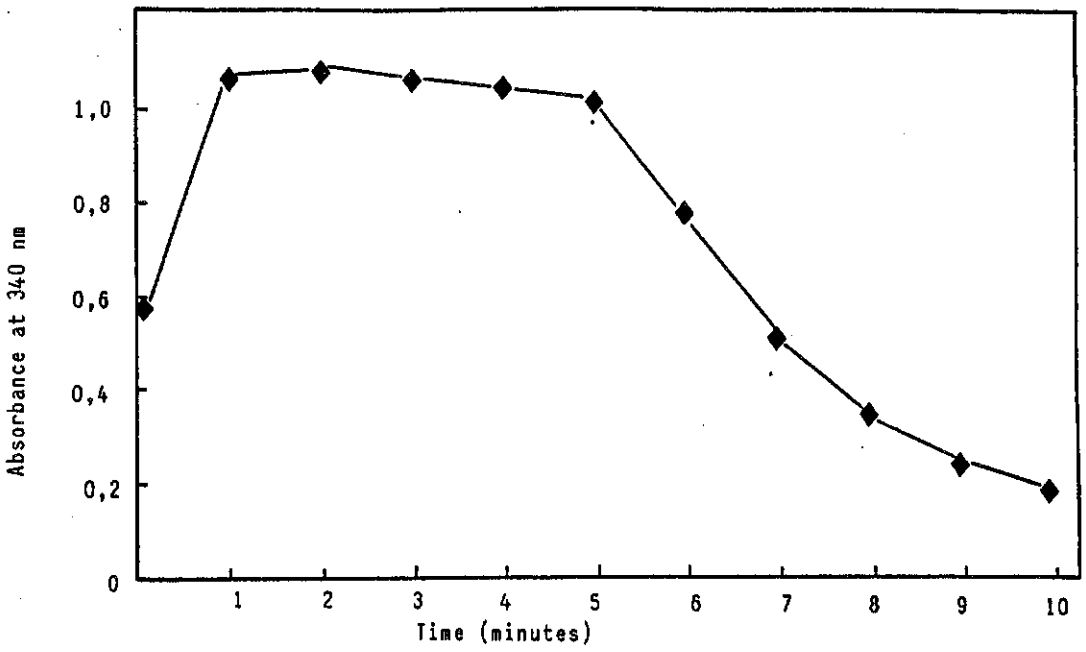

Figure 6.14: Effect of incubation time on isocitrate dehydrogenase activity in Acinetobacter cells grown in acetate medium.

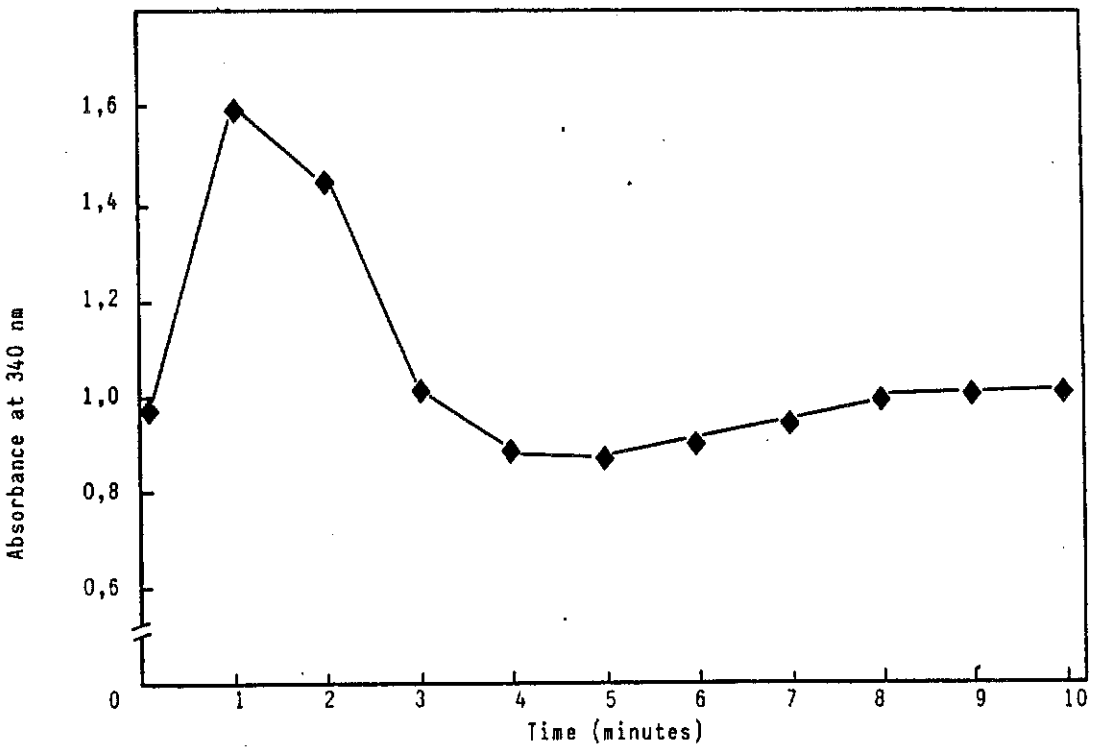

Figure 6.15: Effect of incubation time on isocitrate deydrogenase activity in Acinetobacter cells grown in succinate medium

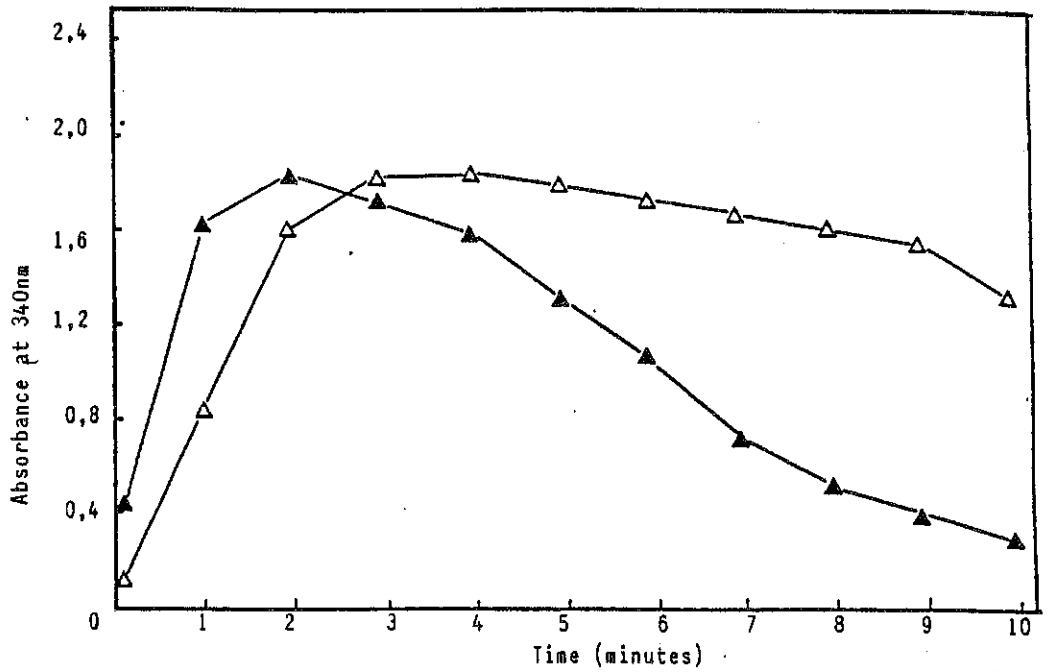

Figure 6.16: Effect of incubation time on isocitrate dehydrogenase activity in acetate grown Acinetobacter cells with 0,2 mℓ extract△-△ and 0,5 mℓ extract▲-▲

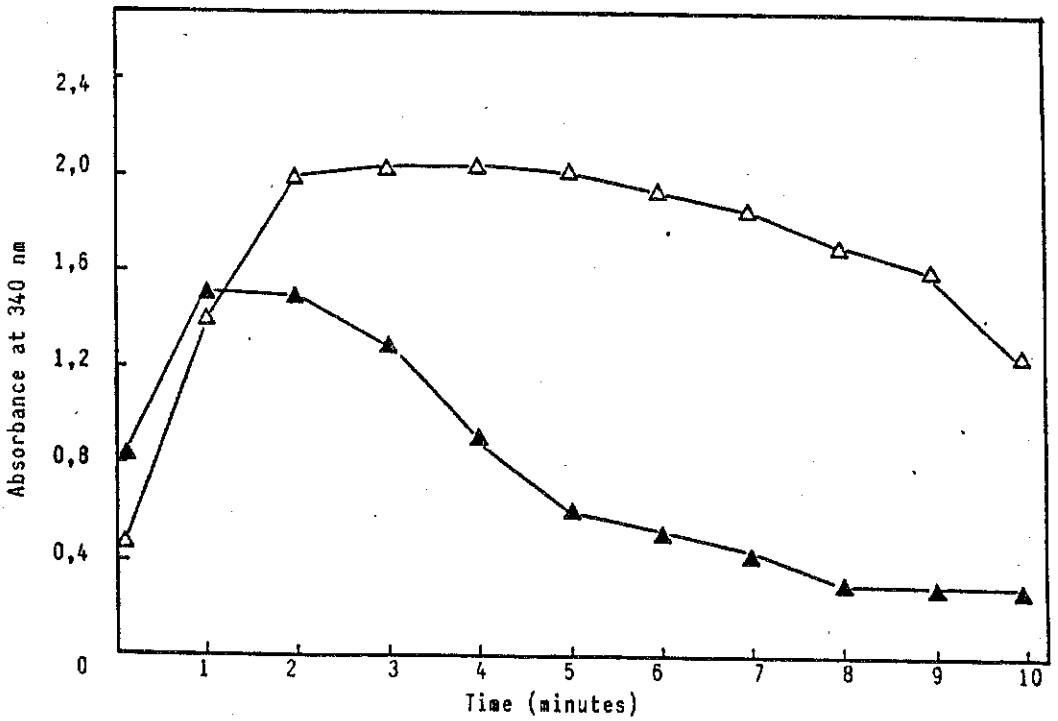

Figure 6.17: Effect of incubation time on isocitrate dehydrogenase activity in succinate grown acinetobacter cells with 0,2 mℓ extract△-△ and 0,5 mℓ extract▲-▲

(Holms and Bennett, 1971; Maloy and Nunn, 1982).

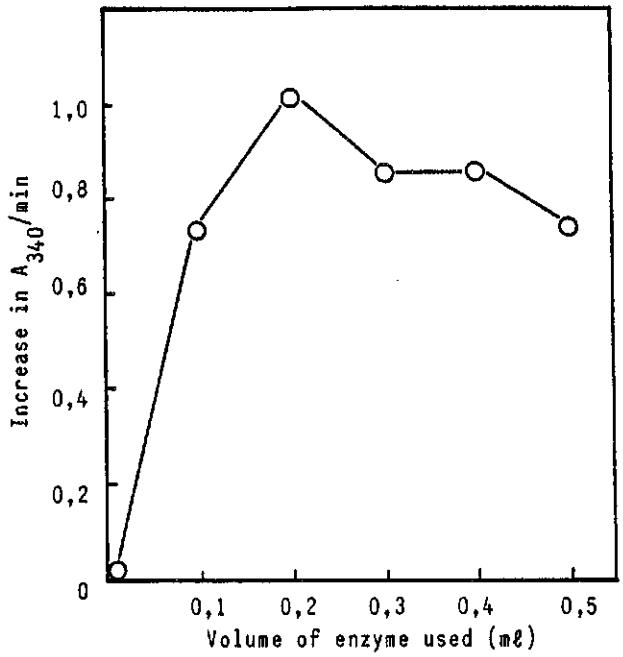

Figure 6.18: The effect of enzyme concentration on isocitrate dehydrogenase activity.

6.8 THE EFFECT OF ANAEROBIOSIS ON POLYPHOSPHATE CHAIN LENGTH IN ACINETOBACTER SPP

6.8.1 The Aim of the Study

Although the metabolism of polyphosphates has been studied in a number of bacterial species (Kulaev and Vagabov, 1983), the physiological significance of different chain length polyphosphates remains a matter of some controversy. Mino et al. (1985) after studying phosphorus turnover in activated sludge, concluded that low molecular mass polyphosphates function as an energy pool and high molecular mass polyphosphates function as phosphorus source for intracellular phosphorus requirements. Low molecular mass or acid soluble polyphosphates are assumed to have a chain length of $n > 5$ and acid insoluble, or high molecular weight polyphosphates, $n > 25$ (Kulaev and Vagabov, 1983). The problem of accurate quantification of polyphosphates of different chain length, has not been satisfactorily resolved.

Column chromatography is widely used to separate molecules of different molecular mass. In this study, fractionation of polyphosphate on Sepharose 4B was investigated (Rao et al., 1985).

6.8.2 Materials and Methods

A Sepharose 4B column (15 x 220 mm) was equilibrated with 50 mM Tris-HCl (pH 7,6), containing 4 mM EDTA and 145 mM NaCl. The column was standardised with polyphosphates of known molecular mass. Fractions were collected and polyphosphate determined by hydrolysis and subsequent automated determination of orthophosphate by the molybdate blue method (Canelli and Mitchel, 1975). Acinetobacter cell suspensions were extracted with alkaline hypochlorite (Rao et al., 1985).

6.8.3 Results and Discussion

A calibration curve of molecular mass against elution volume, was prepared by loading polyphosphates of varying molecular masses onto the column (see Figure 6.19).

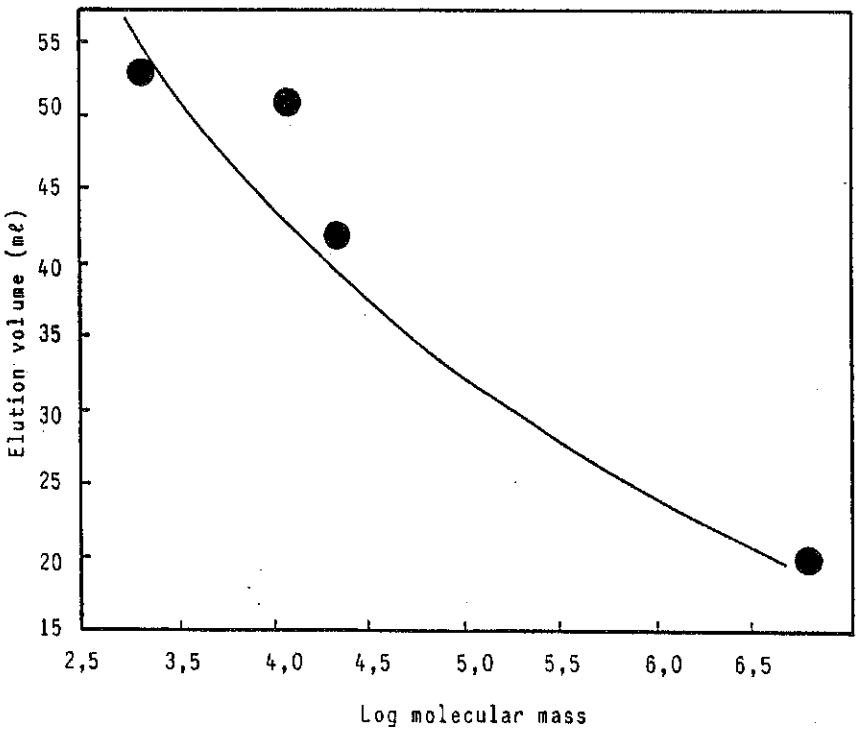

Figure 6.19: Calibration curve of elution volume vs molecular mass for sepharose 4B column

The polyphosphate chain length profiles for two cultures of Acinetobacter cells, one grown under aerobic conditions and one under anaerobic conditions, are given in Table 6.3.

These results are only preliminary and require verification before interpretation is attempted. The results however, do show that this technique shows promise in the study of polyphosphate metabolism under anaerobic and aerobic conditions.

TABLE 6.3
POLYPHOSPHATE CHAIN LENGTH PROFILES OF ACINETOBACTER SPP

Aerobic n =	Anaerobic n =
9265	6122
2798	414
93	60
22	13
16	

6.9 SOME CHARACTERISTICS OF FACULTATIVE ANAEROBES

6.9.1 Aim of the Study

Bacterial population studies on acid digesters, primary sedimentation tanks and the anaerobic zones of activated sludge plants, revealed the presence of three main species of acid producing bacteria, namely, Klebsiella oxytoca, Aeromonas punctata and Citrobacter spp (see Chapter 4). The aim of this study was to investigate the acid and gas producing characteristics of these bacteria in greater detail.

In addition, the nitrate reducing ability of the dominant facultative organisms in the anaerobic zone, namely, Aeromonas spp, was studied in an attempt to gain further insight into the deleterious effect of nitrate in this zone.

6.9.2 Methodology

Strains of Klebsiella spp, Aeromonas spp and Citrobacter spp isolated from an activated sludge plant, were used for the study. Oxidative and fermentative glucose degradation was studied, using the OF glucose medium according to Hugh and Leifson (Merck, 1984).

Tubes of this medium containing Durham tubes were inoculated in duplicate with the strains to be studied. After three days incubation, the tubes were examined for gas and acid production.

In order to identify the gas produced, tubes containing the same medium as described above, were inoculated with the bacteria and the Durham tubes were replaced with glass tubes stoppered at the upper end with a rubber septum to allow any gas produced to be withdrawn by syringe for subsequent gas chromatographic analysis.

Hydrogen and carbon dioxide were both determined using a thermal conductivity detector. Hydrogen was analysed on a molecular sieve 5A column at 60 °C with Argon as carrier, while carbon dioxide was analysed on a silica gel 60 - 80 mesh column at 110 °C, with Helium as carrier gas.

Isolates were also tested for nitrate reduction (Difco, 1953).

6.9.3 Results and Discussion

All the strains tested produced acid on utilising glucose oxidatively or fermentatively. The number of strains producing gas are shown in Figure 6.20.

The absence of gas production in Aeromonas punctata strains identifies them as being Aeromonas punctata subsp caviae (Schubert, 1974).

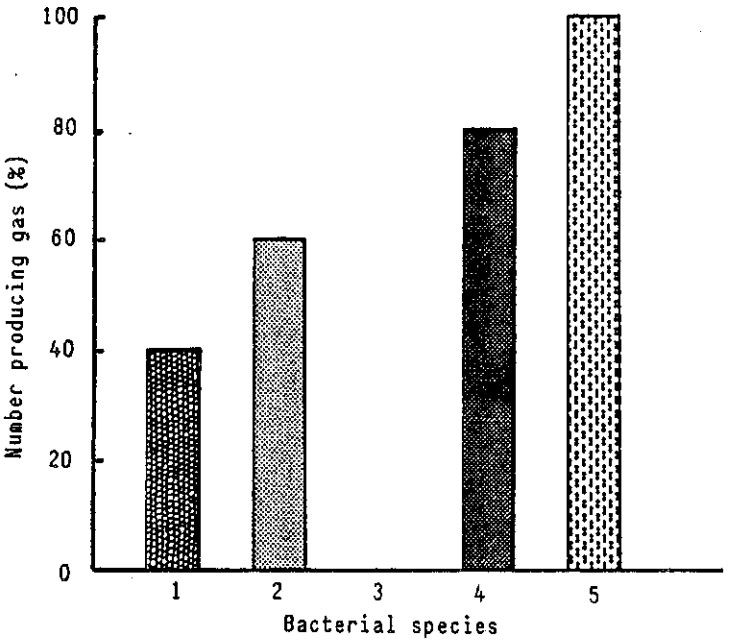

Figure 6.20: Gas production by facultative anaerobes isolated from acid sludge.
1: Klebsiella pneumoniae; 2: Klebsiella oxytoca; 3: Aeromonas punctata;
4: Aeromonas hydrophila;
5: Citrobacter spp

All the isolates tested produced both carbon dioxide and hydrogen in varying amounts (see Table 6.4). With the exception of Aeromonas punctata all the main acid producing strains produce carbon dioxide and hydrogen, which in turn, provides the substrate for the hydrogen utilising methane bacteria (Mosey, 1983).

TABLE 6.4
GAS PRODUCTION IN FACULTATIVE ANAEROBES

Isolate	Gas production %			
	Anaerobic		Aerobic	
	Carbon dioxide	Hydrogen	Carbon dioxide	Hydrogen
Klebsiella pneumoniae	40	45	48	43
Klebsiella pneumoniae	12	71	13	65
Klebsiella oxytoca	22	35	32	30
Klebsiella oxytoca	10	81	12	76
Klebsiella oxytoca	33	62	35	55
Aeromonas hydrophila	12	19	15	22
Aeromonas hydrophila	6	23	9	42
Aeromonas hydrophila	37	40	34	49
Citrobacter spp	7	2	13	18
Citrobacter spp	15	33	18	44
Citrobacter spp	7	3	6	5

The capacity of Aeromonas spp to reduce nitrate is given in Table 6.5.

TABLE 6.5
NITRATE REDUCTION IN AEROMONAS SPP

	Percentage of samples reducing nitrate to	
	Nitrite	Nitrogen
Aeromonas punctata	92	88
Aeromonas hydrophila	100	100

In the presence of nitrate in the anaerobic zone, these bacteria will utilise readily biodegradable substrate for denitrification, thus competing with Acinetobacter spp for substrate. In the absence of nitrate, Aeromonas spp would metabolise substrate via fermentative pathways, thus supplementing the supply of volatile fatty acids.

6.10 Conclusions

While the work described above cannot be considered definitive in terms of solving the mechanism of biological phosphorus removal, it has provided some foundations for future investigations.

The recognition of polyhydroxybutyrate and polyphosphate accumulation propensities as inherent characteristics of certain Acinetobacter strains, has allowed investigators to concentrate on the factors regulating these functions.

The success of the biological phosphate removal process depends on efficient metabolic switch-over under sequential anaerobic/aerobic conditions. Preliminary investigations into this crucial area have revealed that regulation of these events is not a result of differences in protein synthesis.

In view of this observation, future investigations into metabolic regulation should concentrate on feedback control mechanisms.

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CHAPTER SEVEN

Process Modelling

7.1 INTRODUCTION

The general activated sludge model developed by the University of Cape Town (UCT) has been shown to correctly predict, with remarkable accuracy, the performance of both pilot (Ekama et al.,1978; van Haandel et al.,1981) and full-scale plants Nicholls et al., 1982). Until recently the use of this model was confined to persons having access to main-frame computers. To overcome this problem, the steady state model equations were applied to a spread sheet (Visicalc) which was used in conjunction with a micro-computer. This chapter describes how this was achieved and further demonstrates how this facility can be used to optimise performance.

7.2 THE VISICALC PROGRAM

Visicalc is a commercial program for use on a number of microcomputers and in this instance, consisted of a spread sheet comprising 254 rows and 59 columns, i.e. a 254 x 59 matrix. Each point in this matrix is defined by column and row numbers. To distinguish between rows and columns, the rows have numeric values from 1 to 254 and columns have characters such as A, B, C, ...Z,AA,AB...AZ, BA, BB...BK.

Each point in this matrix can either contain a **label** or a **value.** The **labels** can be characters or figures which make up headings or titles on the spread sheet. Each matrix point can only accommodate for 9 characters, hence if more are needed, the adjacent matrix point must be used. In this

(**) Kinetics of biological phosphorus removal
(Wentzel et al., 1985).

The equations used in this paper have been given the same numbers as in the above publications, for easy cross-referencing. The character before the equation will refer to which of the abovementioned publications is being referenced, i.e. either * or ** above. The definitions of the various symbols used are detailed in Appendix 7.1.

In setting up the spread sheet, only the first three columns are used and approximately 200 rows. The first two columns (A and B) have been reserved for naming constants or variables, while in the third column the value of the corresponding constant, variables or equations are given.

The inputs into the Visicalc UCT model can be further divided into three groups :-

- Kinetic constants
- Plant and operational details
- Sewage characteristics

(1) Kinetic constants

The kinetic constants used in the model are given in Table 7.1 . Some of these, viz (b_{hT}, μ_{nmT}, k_{1T} and k_{3T}) are temperature dependent and are automatically corrected for temperature when the work sheet is calculated.

The value of % volatile solids (f_i) and the specific growth rate of nitrifiers (μ_{nm20}), should be measured (Ekama et al., 1978) since these values can vary from plant to plant.

TABLE 7.1

KINETIC CONSTANTS

Column No	A and B	C
8	**Kinetic Constants**	
9		
10	f_{cv}	1,48
11	Y_h	0,45
12	f	0,20
13	f_p	0,02
14	f_i	0,72
15	b_{h20}	0,24
16	b_{hT}	0,23
17	μ_{nm20}	0,36
18	μ_{nmT}	0,32
19	k_{120}	0,72
20	k_{1T}	0,72
21	k_{2T20}	0,1008
22	k_{2T}	0,0933
23	k_{3T20}	0,0768
24	k_{3T}	0,0746
25	f_n	0,10
26	α	0,03
27	c_{sp}	0,50
28	k_p	0,06
29	n	1

If these constants are used in any equation anywhere in the spread sheet, they will be referred to by this location e.g. the value of Y_h in Table 7.1 has a location C11 and the value of f_n a location C25. Whatever values are in these locations, will not change in sympathy with plant operating conditions. When using the UCT model to optimise the process, each operating parameter should be changed in turn to see what effect it has on the effluent quality.

(2) Plant Operational Details

The actual operating conditions at the Johannesburg Northern Works are reflected in Table 7.2 :-

TABLE 7.2

PLANT AND OPERATIONAL PARAMETERS

JOHANNESBURG NORTHERN WORKS

Row No	Column A and B	C
31	Plant details	
32		
33	Mass fractions	
34		
35	f_{xa} Anaerobic	0,08
36	f_{x1} Primary anoxic	0,16
37	f_{x3} Secondary anoxic	0,16
38	f_{xdm} Allowable	0,32
39	No. anaerobic basins	1,00
40	Flows Me/d	
41		
42	Settled sewage feed	15,00
43	Returned sludge	42,00
44	MLSS recycle	102,00
45	Waste sludge	0,78
46	Total Volume	29,18
47		
48	Recycle Ratios	
49		
50	s Sludge return	2,8
51	a MLSS Recycle	6,8
52	R_s Sludge age	37
53	Dissolved Oxygen	
54		
55	Primary aeration	2,00
56	Returned sludge	1,00
57	Total Power kWh	
58	Total kWh	2100
59	Temperature °C	19
60		

Note $f_{xdm} = f_{x1}+f_{x3}$ the value in location C38 would be +C36 + C37, the +ve sign before the first C signifies that C is a **value** and not a **label.**

(3) Sewage Characteristics

The composition of sewage received at treatment plants can vary considerably, depending on, e.g. the length of outfall sewer and the presence of industrial effluents. This means that these inputs will also vary from one plant to the next. In fact, sometimes they can vary from day to day.

Ekama et al.,(1978) developed a system whereby the available substrate in the sewage could be assessed on a uniform basis. This required measurement of the following fractions :-

- Unbiodegradable particulate COD (f_{up})
- Soluble and unbiodegradable COD (f_{us})
- Readily biodegradable COD (f_{bs})

The feed COD, TKN and total phosphorus concentrations are also required together with the nitrate concentration (N_{nr}) in the returned sludge which, if not available, can be assumed to have the same value as in the effluent. The Visicalc model overcomes this problem by predicting the nitrate in the effluent and then applying this value to N_{nr}. The Visicalc calculations can be iterated 4 to 5 times until $N_{nr} = N_{te}$. The method of iteration may vary with the type of spread sheet used. In this instance, the following expression was entered in location C75 at @ IF (C111 0, 0, C111) where C111 represents the location where the value of this effluent nitrate concentration is calculated.

The sewage characteristics entered into the spread sheet for the Johannesburg Northern Works are given in Table 7.3. These values can obviously be replaced by any measured values for specific sewages and operating conditions.

TABLE 7.3
SEWAGE CHARACTERISTICS
JOHANNESBURG NORTHERN WORKS

Row No	Column No	
	A and B	C
61	**Sewage characteristics**	
62		
63	**Feed conc m/ℓ**	
64	COD	584
65	TKN as N	46
66	Total P as P	20
67		
68	**COD Fractions**	
69	f_{bs}	0,24
70	f_{up}	0,07
71	f_{us}	0,05
72		
73	**Estimation of NO_3**	
74	N_{nr} returned	3,17
75	N_{te} returned	3,17
76		
77	**P Removal MX_{ah}/Q**	
78	1st MX_a/Q_h	True/False
79	MX_{ah}/Q Temporary	810
80		

With all the above input values the model can now be applied to predict plant performance.

7.3.1 Prediction Equations

The UCT model can be broken up into a number of sections :-

- Composition of the feed COD
- Composition of the MLSS
- Nitrification
- Denitrification
- Oxygen utilisation
- Biological phosphorus removal

These sections are not self-supporting and for this reason the order they are put into the whole work sheet is important, because sometimes it is necessary to solve one equation first and to use the data obtained in subsequent equations. It is important to note that all the input to the following equations are either given above in the sewage and plant characteristics or constants are derived from other equations.

Composition of the feed COD

The UCT group divides the feed COD into a number of different fractions, which either directly or indirectly, describe the availability of COD to micro-organisms (WRC, 1984).

These fractions are as follows :-

Concentration of unbiodegradable inert COD in feed	(S_{ui})
Concentration of unbiodegradable particulate inert COD in feed	(S_{upi})
Biodegradable COD concentration in feed	(S_{bi})
Readily biodegradable COD concentration in feed	(S_{bsi})
Readily biodegradable COD concentration in anaerobic reactor	(S_{bsa})
Readily biodegradable COD concentration available for conversion	(S_{bsi})
Readily biodegradable COD concentration in last anaerobic reactor	(S_{bs})

The values of all the above fractions can be calculated using the equations below. These are inserted in Column C adjacent to the appropriate text which is located in Column A and B in the spread sheet.

$$S_{ui} = (f_{us} + f_{up})S_{ti} \qquad * 2.5$$

$$S_{upi} = f_{up}\, S_{ti} \qquad * 2.4$$

$$S_{bi} = S_{ti}\,(1 - f_{up} - f_{us}) \qquad * 2.7$$

$$S'_{bsi} = S_{bsi} - r.\ 8{,}6.\ N_{nr}) - s\ 3{,}0 \qquad ** 2$$

$$S_{bsa} = (f_{bs}\, S_{bi} - S_{bs})/(1 + s) \qquad * 7.1b$$

$$\Delta S_{bs} = s(8{,}6\ N_{ns} + 3{,}0.0_s) + 3{,}0.0_i \qquad * 7.2b$$

$$S_{bsn} = \frac{S'_{bsi}/(1 + r)}{\left[1 + K\ \frac{f_{xa}}{N}\ \frac{MX_{ah}}{Q}/(1 + r)\right]^n} \qquad ** 9$$

Equation **2 has been modified slightly to include the dissolved oxygen concentration in the returned sludge.

To illustrate how these equations are entered into the Visicalc worksheet, consider :

$$S_{ui} = (f_{us}+f_{up})\, S_{ti} \qquad * 2.5$$

f_{us}, f_{up} and S_{ti} are in locations C71; C70 and C64 respectively, hence the equation

$$S_{ui} = (f_{us}+f_{up})\, S_{ti} = (C71+C70)\ C64$$

Sometimes the values determined by one equation are used in a second equation, e.g. :

$$S_{bsi} = f_{bs}\,(S_{bi}) \qquad * 2.8a$$

S_{bi} is determined by equation *2.7 located in C84 and f_{bs}, a variable, located in C69. Hence the expression entered in location C85 is

(C84.C69)

Table 7.4 depicts where these equations are entered into the spread sheet.

TABLE 7.4
CALCULATED COD CHARACTERISTICS DISPLAYED ON THE SPREAD SHEET

Row No	Column No	
	A and B	C
81	COD Fractions	
82	S_{ui}	29
83	S_{upi}	60
84	S_{bi}	494
85	S_{bsi},	118
86	S_{bsi}	39
87	S_{bsa}	9
88	S_{bsn}	3,6
89		

Composition of the mixed liquor suspended solids

In order to express the behaviour of the mixed liquor suspended solids (X_t) correctly, it is necessary to establish what mass of viable micro-organisms were present. Here again, the UCT group divides the X_t into a number of categories :-

- Active mass (X_a)
- Endogenous mass (X_e)
- Inert mass (X_i)
- Volatile mixed liquor (X_v)
- Total mixed liquor (X_t)
- Mass of non-poly P organisms in system (MX_{ah}/Q)

Each of the above concentrations may be described by equations which are either related to each other or to the input parameters discussed earlier.

$$X_a = \frac{S_{bi}\ Y_h R_s}{(1+b_h R_s)} \qquad * 4.10$$

$$X_e = f\ b_h\ R_s\ X_a \qquad * 4.11$$

$$X_i = \frac{f_{up}\ S_{ti}\ R_s}{f_{cv}} \qquad * 4.12$$

$$X_v = X_a + X_e + X_i \qquad * 4.13$$

$$X_t = X_v / f_i \qquad * 4.14$$

$$\frac{MX_{ah}}{Q} = \frac{[S_{bi} - (S'_{bsi} - (1+r)S_{bsN})]Y_h\ R_s}{(1+b_h\ R_s)} \qquad **10$$

Each of these equations are then inserted into the spread sheet as indicated below.

MX_{ah}/Q is estimated in conjunction with Equation **9 (S_{bsn}) by iteration and has been located in position C78 and C79.

C78 gives MX_{ah}/Q a value of zero after which it then calculates a value for
$MX_{ah/Q}$ in location (C97) which is the same value as C79.

The iteration is repeated until the value in C79 remains constant. With the present spread sheet, the values entered in C78 and C79 are @IS ERROR (C85) and @IF (C78, 0, C97). The method of iteration will depend on the type of spread sheet used.

TABLE 7.5
CALCULATED SLUDGE MASS CONCENTRATIONS
DISPLAYED ON SPREAD SHEET

Row No	Column	
	A and B	C
90	**Fraction of MLSS**	
91		
92	X_a	854
93	X_e	1475
94	X_i	1512
95	X_v	3842
96	X_t	5336
97	$\frac{MX_{ah}}{Q}$	810
98		

7.3.2 Nitrification

Provided there is sufficient oxygen available and the sludge age is above the minimum for nitrification (which is always the case in Johannesburg), then it is assumed that all available nitrogen is converted to nitrate. However, not all of the incoming TKN is available since some is used by the micro-organisms themselves for growth and some is contained in the effluent. All remaining TKN is then assumed available. This available mass is referred to as the nitrification capacity, N_c and the mass which is taken up into the sludge is denoted by N_s and are estimated by :-

$$N_s = \frac{f_n \; V \; X_v}{R_s \; Q} \qquad \text{derived from} \qquad * 4.23$$

$$N_c = N_{ti} - N_{te} - N_s \qquad * 5.29$$

N_{te} is the total nitrogen in the effluent. Under the warm weather conditions in Johannesburg, a value of 2 - 3 mg/ℓ is often obtained. Hence, for the purpose of this exercise it was assumed $N_{te} = 2{,}5$.

The nitrification capacity is a very useful parameter for its magnitude can give the operator an idea of what nitrate concentration could be expected if no denitrification occurred.

The insertion of the above equations into the spread sheet are given in Table 7.6 :-

TABLE 7.6
CALCULATION OF NITRIFICATION CAPACITY AND NITROGEN IN MLSS

Row No	Column No	
	A and B	C
99	**Nitrification**	
100		
101	N_s	10,38
102	N_c	33,34
103		

7.3.3 Denitrification

The concept of denitrification potential is described in WRC(1984) . This parameter estimates from the feed sewage characteristics and the plant operating conditions, the capacity of the anoxic reactors to remove nitrate. Again, it is a most informative parameter when compared with the actual amount of nitrogen removed in the anoxic reactor, the difference will give an indication as to how efficiently the denitrification process is working.

The equations for calculating the denitrification potential in both the primary and secondary anoxic reactors are given below :-

$$D_p = S_{bi}\,[\alpha + K_2 f_{xi}\, Y_h\, R_s/(1+b_h\, R_s)] \qquad *6.20$$

$$\alpha = f_{bs}\,(1 - f_{cv}\, Y_h)/2{,}86$$

(α = Alpha inputs in spread sheet i.e. C26)

$$D_p = S_{bi}\, f_{x3}\, K_3\, Y_h\, R_s/(1+b_{hT}\, R_s) \qquad *6.22$$

The total denitrification capacity is given by :

$$D_p = D_{p1} + D_{p3}$$

For a Bardenpho process the nitrate in the effluent can be estimated from the equation given below. This equation takes into account the dissolved oxygen (DO) concentrations in the streams entering both the anoxic and anaerobic reactors.

$$N_{ne} = \frac{\left[\frac{N_c}{a+S+1} + \frac{O_a}{2{,}86}\right]\left[a + \frac{K_{2T}}{K_{3T}}(s+1) + \frac{s\,O_s}{2{,}86}\right] - D_{pp}}{\frac{K_{2T}}{K_{3T}} + S\left[\frac{K_{2T}}{K_{3T}} - 1\right]} \qquad *6.24$$

D_{pp} is similar to equation 6.20 except that f_{xi} is replaced by f_{xdm}, i.e. anoxic mass fraction.

The above equations are inserted into the spread sheet as depicted in Table 7.7.

TABLE 7.7
ESTIMATION OF DENITRIFICATION POTENTIALS
AND NITRATE IN THE EFFLUENT

Row No	Column No	
	A and B	C
104	**Denitrification Pot**	
105		
106	D_{p_1} Primary	26,6
107	D_{p_2} Secondary	9,6
108	Total	36,2
109	D_{pp} maximum	39,4
110		
111	**Effluent NO_3**	3,17
112		

7.3.4 Oxygen Demand

This parameter is very useful to operators, because by using the model, the oxygen demand can be optimised. For example, the effects of diluting peak demands by switching in the internal MLSS recycle pumps, can be investigated.

The model considers three different oxygen demands :-

- Oxygen required for carbon access material $M(O_c)$
- Oxygen required for nitrification $M(O_n)$
- Oxygen "recovered" via denitrification $M(O_d)$

The total oxygen demand $M(O)_T$ may be expressed as follows :-

$$M(O)_T = M(O_c) + M(O_n) - M(O_d)$$

The relevant equations for each of the above parameters are given below and their inclusion in the spread sheet is given in Table 7.8.

$$M(O_c) = M\ (S_{ti})(1-f_{us}-f_{up})\left[(1-f_{cv}\ Y_h)+f_{cv}(1-f)_{bh}\frac{Y_h\ R_s}{(1+b_h\ R_s)}\right] \quad *4.15$$

$$M(O_n) = 4{,}57\ M(N_{ne}) \quad *5.39(a)$$

$$M(O_d) = 2{,}86\ (N_c - N_{nr})Q \quad *6.32$$

TABLE 7.8
ESTIMATION OF THE OXYGEN DEMAND

Row No	Column No	
	A and B	C
113	**Oxygen Demand**	
114		
115	$M(O_c)$ Carbonaceous	6016
116	$M(O_n)$ Nitrification	2385
117	$M(O_d)$ Denitrification	1294
118	$M(O)_T$ Total	7005
119		

7.3.5 Biological Phosphorus Removal

The UCT group have developed a parametric and a kinetic model for excess biological phosphorus removal. Both of these models have been included in the spread sheet.

The parametric model (Siebritz et al, 1983) requires the following values which then result in an equation which estimates the mass of phosphorus that can be removed.

S_{bi}	readily biodegradable COD in influent
S_{bsa}	readily biodegradable COD conc in anaerobic reactor
P_f	excess phosphorus removal propensity factor
	coefficient of excess phosphorus removal
P_s	phosphorus removal (mg/ℓ)

The equations describing S_{bi} and S_{ba} were considered previously (C84 and C87 repectively) and those describing P_f, and P_s are given below :-

$$P_f = (S_{bsa} - 25)\, f_{xa} \qquad *7.5$$

$$\gamma = 0{,}35 - 0{,}29 \exp(-0{,}242\, P_f) \qquad *7.7$$

$$P_s = S_{ti} \left\{ \frac{(1-f_{us}-f_{up})\, Y_h}{(1+b_{hT}\, R_s)} (\gamma + f_p\, fb_{hT}\, R_s) + f_p \frac{f_{up}}{f_{cv}} \right\} \qquad *7.6$$

The equations describing the kinetic model (Wentzel et al, 1985) are given below :-

The magnitude of phosphorus release in the nth reactor is given by ΔP_n

$$\Delta P_n = C_{sp}\, S'_{bsi} \left[\frac{1}{\left(1+K\, f_{xa}\, \frac{MX_{ah}}{N}/\frac{(1+r)}{Q}\right)^{n-1}} - \frac{1}{\left[1+Kf_{xa}\, \frac{MX_{ah}}{N}/\frac{(1+r)}{Q}\right]^{n}} \right] \qquad **11$$

The magnitude of phosphorus removal in the aerobic reactor is given by :-

$$P\ (\text{removal}) = (a'-1)P\ (\text{release}) + P\ (\text{metabolic}) \qquad **14$$

$$P\ (\text{metabolic}) = 0{,}03\ MX_v/(Q.R_s) \qquad **15$$

a' could have values ranging from 1,145 to 1,198

At this point the above two equations have not been finalised. However, should any alterations to the above equation be required, it is a simple matter to update the spread sheet. This point is emphasized in Appendix 7.2 where it will be noted that the phosphorus removal predicted is considerably less than that achieved in practice.

The format of these equations in the work sheet is given in Table 7.9.

TABLE 7.9
ESTIMATION OF THE VARIOUS PARAMETERS
ASSOCIATED WITH BIOLOGICAL PHOSPHATE REMOVAL

Row No	Column No		
	A and B		C
120	**Phosphate Removal** (Kinetic)		
121			
122	**Parametric Model**		
123			
124	S_{bi}		494
125	S_{ba}		9,6
126	P_f		0,00
127	Y		0,06
128	P_s		2,60
129			
130	**Kinetic Model**		
131			
132	ΔP_n		11,1
133	P removed		6,9
134			

At this stage all the steady state equations have been included into the spread sheet.

7.3.6 Check on the Visicalc UCT Model

To check the Visicalc UCT model, input data given in Theory, Design and Operation of Nutrient Remocal Activated Sludge Processes (WRC, 1984), was inserted into the Visicalc spread sheet. The program was iterated until N_{nr} and N_{te} (C74 and C75) had the same value and MX_{ah}/Q Temporary at site C79 remained constant. The various results were then checked against the values given in Table 7.1 of the abovementioned document. The agreement was excellent, indicating that this model was free of logic errors.

7.3.7 Incorporation of Actual Plant Performance into the Spread Sheet

The next requirement was to compare plant performance with model predictions. Up to this point in the development, only the model has been entered into the spread sheet. To improve the usefulness of the spread sheet, the actual analytical data representing the plant peformance were added. The parameters considered were soluble ammonia, nitrate and

phosphate in each reactor, as well as the effluent. Details of how they were entered into the work sheet are given in Table 7.10.

TABLE 7.10
ANALYTICAL DATA ENTERED IN THE SPREAD SHEET

Row No	Column No A and B	C
135	**Analytical Analyses**	
136		
137	**Anaerobic**	
138	Ammonia	6
139	Nitrate	0,30
140	o-P	14
141		
142	**Primary anoxic**	
143	Ammonia	3,40
144	Nitrate	2,30
145	o-P	11,00
146		
147	**Primary aeration**	
148	Ammonia	0.90
149	Nitrate	5,80
150	o-P	7,40
151		
152	**Secondary anoxic**	
153	Ammonia	1,00
154	Nitrate	3,70
155	o-P	8,00
156		

Row No	Column No A and B	C
157	**Re-aeration**	
158	Ammonia	0,80
159	Nitrate	2,10
160	o-P	6,40
161		
162	**Effluent**	
163	Total COD	48,00
164	TKN	2,00
165	Ammonia	0,80
166	Nitrate	4,00
167	Total P	7,00
168	o- P	6,20
169	Suspended Solids	25,00
170		
171	**Suspended Solids**	
172	Returned sludge	6600
173	MLSS	4900
174	MLVSS	3528
175	Total P MLSS	-
176	TKN MLSS	-
177	SVI	190
178	DSVI	170
179		

With all the analytical plant data entered, the next step was to conduct mass balances over each reactor with respect to nitrogen and phosphorus. The equations describing the mass balance were inserted in the appropriate location, as depicted in Table 7.11.

TABLE 7.11
MASS BALANCE CALCULATION FOR NITRATE, AMMONIA AND PHOSPHORUS

Row No	Column No	
	A and B	C
180	**Mass Balances**	
181		
182	**Nitrate**	
183	Anaerobic	10,06
184	Primary anoxic	16,2
185	Secondary anoxic	2.1
186		
187	**Ammonia**	
188	Anaerobic	-5,50
189	Primary anoxic	33,6
190	Primary aeration	2,6
191	Secondary anoxic	-0,10
192	Re-aeration	0,20
193		
194	**Phosphorus**	
195	Anaerobic	-15,84
196	Primary anoxic	-13,08
201	Secondary anoxic	-0,60

A negative value in Table 7.11 indicates a release of phosphorus.

7.3.8 Reporting on Plant Performance

With all the information on the model and the plant entered into the work sheet what is now required is that this information be processed into a meaningful report which could then be disseminated to the operators and managers. To achieve this objective a suitable report is formatted in a different area of the same work sheet. An example of a report, detailing where it is included in the work sheet is given in Appendix 7.2.

Relevant information is extracted automatically from Column C and inserted into the report where desired. For example, consider the actual MLSS conc in L119 - the value inserted here would be C173, which is the value originally inserted into the spread sheet. Should additional information be required in the report this can readily be added, e.g. volatile acid concentration J84 to N84.

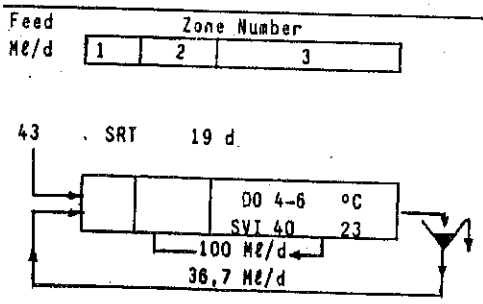
This section illustrates the power of the electronic spread sheet where data processing and report writing are handled simultaneously.

7.4 PROBLEM SOLVING USING VISICALC

7.4.1 Plant Data Used in Case Studies

Three different applications of the UCT model to solve plant problems will be discussed. Data used was obtained from the Johannesburg Bushkoppie Plant and shown into Table 7.12.

TABLE 7.12
OPERATING CONDITIONS AND AVERAGE (1 MONTH) ANALYTICAL DATA
FOR JOHANNESBURG BUSHKOPPIE PLANT



Sample Point	COD	S_{bs}	Nitrogen TKN	Nitrogen NH_4	Nitrogen NO_3	Phosphorus Total	Phosphorus ortho	Susp solids
Influent ex								
. Balancing tank	671	134	48,2			4,8		
Zone : Anaerobic 1				17	0,2		4	
Anoxic 2				9,7	2,2		9,1	
Aerobic 3				0,1	14,2		3,1	2720
Final effluent	8		1,8	0,2	14,8	3,2	2,1	40

Results expressed as mg/ℓ, where applicable

7.4.2 Evaluation of Correctness of Plant Data

Wentzel et al.,(1985) have indicated that the feed sewage characteristics play a vital role in the biological phosphorus removal process. From their work and from the feed sewage characteristics measured at Bushkoppie, good nitrogen and phosphorus removal would have been expected. In practice however, as can be seen from Table 7.12, the nutrient removal was

not acceptable, with effluent concentrations of 14 mg N/ℓ nitrate and 2 mg P/ℓ phosphate. In order to establish why the plant was not performing as expected, an in-depth evaluation of the process was conducted. All the relevant information was fed into the spread sheet and then the results calculated (see Appendix 7.3). Arising out of this assessment, the following was noted :-

- The readily biodegradable COD concentration in the feed was most favourable from a phosphorus removal point of view.

- With a TKN/COD ratio of 0,07 good nitrogen removal would be expected. This was not the case.

- The dissolved oxygen concentrations in the main aeration basin were excessively high at 4 - 6 mg/ℓ. The reason for this was that the oxygen control system installed, required that all four modules be in operation for this system to work effectively.

- The SVI and DSVI were extremely favourable.

In any evaluation of a plant the first point is always to establish that the data is correct and meaningful. Ekama et al.,(1976) have described methods where nitrogen and COD mass balances were estimated across the plant and recoveries between 90 and 110 % would be considered acceptable. All the relevant information to conduct these balances was incorporated into the work sheet and it was a matter of extracting this information. Only a nitrogen balance was possible at Bushkoppie, since there were no facilities to measure the oxygen utilisation rate of the mixed liquor, and therefore, COD balance was not possible. Details of the nitrogen balance are given in Table 7.13.

TABLE 7.13
NITROGEN MASS BALANCE ACROSS THE
JOHANNESBURG BUSHKOPPIE PLANT

	mg N/ℓ
TKN feed	48,2
TKN effluent	1,8
Nitrate effluent	14,8
Loss of N due to denitrification	35,8
Nitrogen in waste sludge	11,2
Nitrogen recovered	63,6
Percent nitrogen recovered	132

The nitrogen recovery as shown in Table 7.13 was totally unacceptable.

Since all the flow meters on this plant are checked regularly and are known to have been accurate, the only source of errors could be sampling and/or the chemical analysis.

In order to locate the error, theoretical and actual nitrogen removal in the primary anoxic reactor was checked and found to be 29,4 mg N/ℓ and 23,6 mg N/ℓ respectively.

This agreement was considered acceptable, particularly as the theoretical value did not take into account the oxygen in the recycled mixed liquor which would decrease this value. The theoretical and actual effluent nitrate concentrations were then checked and found to be 9 mg N/ℓ and 14 mg N/ℓ respectively, which indicated that more than the measured nitrogen must have been available for nitrification. Furthermore, if one considers that 12 mg N/ℓ of nitrogen were also removed in the anaerobic reactor, then the overall unaccounted nitrogen was (12 + (14-9)) = 17 mg N/ℓ. There are two sources from which additional nitrogen could come, viz. either the sludge via endogenous respiration, which was unlikely in this case, or that the value of the TKN concentration in the feed was incorrect and should be in the region of 17 + 48 = 65 mg N/ℓ.

In order to check the latter point, all the concentrations of the feed TKN and COD were averaged from the time the plant was commissioned and compared with the values under discussion, as depicted in Table 7.14.

TABLE 7.14
COMPARISON OF AVERAGE TKN AND COD CONCENTRATIONS WITH ACTUAL CONCENTRATIONS IN THE TEST PERIOD

	TKN (mg N/ℓ)	COD (mg/ℓ)	TKN/COD
Average for period under discussion	48	670	0,07
Overall average	66	930	0,08

The average value of the TKN given in Table 7.15 of 66 mg N/ℓ, was almost identical with the estimated value of the TKN of 65 mg N/ℓ. As the COD values could not be checked, in all probability, the measured value would also be too low.

Therefore, using the model and the spread sheet, it was not only possible to highlight an erroneous result, but also to suggest what the correct result might have been. To complete this investigation the corrected TKN and COD values were inserted into the model and the plant performance predicted, as reflected in Table 7.15.

TABLE 7.15
COMPARISON BETWEEN THE PREDICTED AND ACTUAL EFFLUENT NITRATE AND PHOSPHATE CONCENTRATIONS

	Actual	Predicted
Effluent nitrate (mg N/ℓ)	14,8	11,8
Effluent phosphorus (mg P/ℓ)	2,1	2,9

The agreement in Table 7.15 was within 10 %, which again indicated that the estimate of TKN and COD concentrations was reasonable.

7.4.3 Optimisation of the Bushkoppie Process

Examination of Table 7.12 shows that the effluent phosphorus concentration did not meet the 1 mg/ℓ effluent standard. From the work of Siebritz et al.,(1983) there are two changes which could improve the situation :-

- Reduce the nitrate concentration in the returned sludge stream entering the anaerobic reactor

- Increase the readily biodegradable COD concentration in the feed.

To investigate these points further, a range of nitrate concentrations in the returned sludge from 0 to 7 mg N/ℓ was entered into the spread sheet and their effect on phosphate removal calculated. The results are depicted in Figure 7.1.

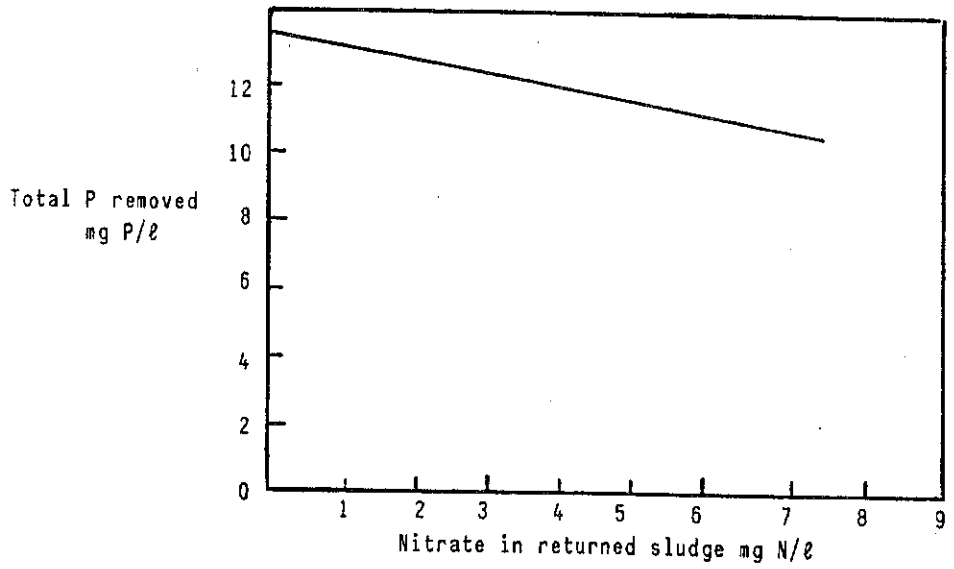


Figure 7.1 : Theoretical concentration of nitrate in the return sludge which would result in an effluent phosphorus concentration of ±1 mg P/ℓ.

From Figure 7.1 the maximum permissible nitrate concentration in the returned sludge was, theoretically, 7 mg N/ℓ. From Table 7.14 this concentration was 14 mg/ℓ, which meant that an additional 7 mg N/ℓ must be

removed via denitrification. There were two ways of achieving this removal :-

- The returned sludge could be retained in a denitrification reactor for a short period before being discharged to an anaerobic reactor (Pitman 1986).

- The reactor feed S_{bs} could be increased COD solubilisation by recycling primary sludge to the head of works (See 2.3.3).

The first suggestion requires structural modifications to the plant, while the second requires only pumping, which is far easier and cheaper to implement. In order to investigate how much COD had to be solubilised, a range of S_{bs} values was entered into the spread sheet and the effects on both nitrate and phosphate removal calculated. The results are given in Figure 7.2.

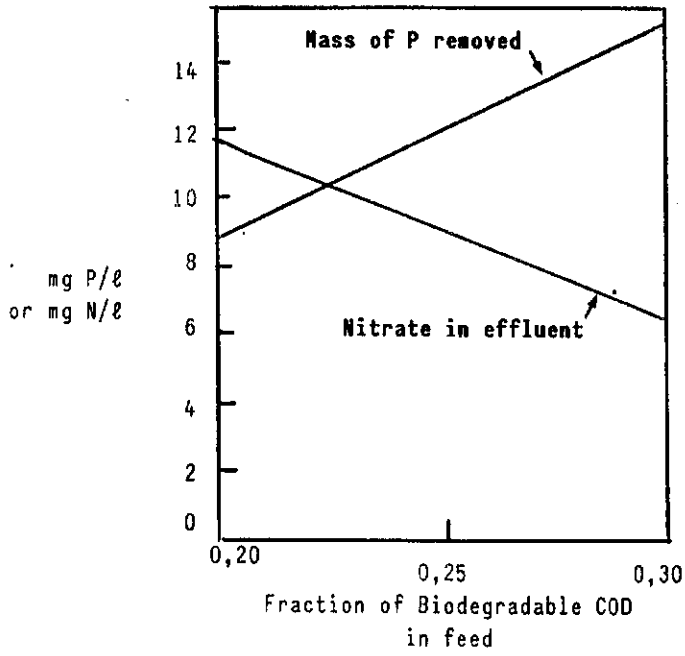


Figure 7.2 : The theoretical relationship between effluent nitrate concentration and mass of P removed at Bushkoppie at various fractions of readily biodegradable Feed COD.

As shown in Figure 7.2, it was evident that the fraction of biodegradable COD in the feed should be increased from 0,20 to 0,30 if 7 mg N/ℓ were to be denitrified and an effluent phosphorus concentration of less than 1 mg P/ℓ was to be achieved.

With the aid of the model, the works manager can test the various options available to him and also obtain an idea of how to achieve a certain objective, e.g. how much solubilisation of the feed COD was required. The net overall result would certainly be an improvement in effluent quality.

7.4.4 Comparison of a Three-Stage versus a Five-Stage Option

Where there appears to be a nitrate problem as described in 7.4.3, a five-stage process may have been more desirable, as additional nitrate would be removed in the second anoxic reactor. Performance of the plant operated in a five zone mode can easily be predicted by making use of the same spread sheet, as already described. In this instance, the mass fraction of MLSS in the second anoxic zone was increased from 0 to 0,17 i.e. f_{x3} = 0,17. The results were then calculated and are given in Appendix 7.3 and a summary is given in Table 7.16.

TABLE 7.16
A THEORETICAL COMPARISON BETWEEN A 3 AND 5 STAGE BUSHKOPPIE TYPE PLANT

	3 Stage Process	5 Stage Process
Effluent nitrate (mg N/ℓ)	11,8	1,0
Mass of phosphorus removal (mg P/ℓ)	8,9	13,2
Total oxygen demand (kg O/d)	30 200	28 990

As can be seen in Table 7.16 the five-stage process with these feed characteristics would theoretically be preferable. Furthermore, the second anoxic reactor was estimated to remove approximately 14 mg/ N/ℓ, which would account for the lower oxygen demand. In addition, the lower effluent nitrate concentration would greatly improve the mass of phosphorus which could be removed.

This again highlights the usefulness of the model and how easily investigations can be carried out by works managers.

7.5 CONCLUSIONS

The use of the electronic spread sheet technique has permitted the sophisticated UCT model to become available to wastewater plant management staff. Works management staff do not require to have any computer programming knowledge to make use of this facility, but must have a good working knowledge of the basic concepts involved.

Repetitive use of this system has resulted in greater confidence on the part of the Johannesburg works management team, the reliability of the model, and its usefulness in solving both day to day and future design problems.

The electronic spread sheet version of the UCT model provides a very useful teaching medium for the training of new staff. Coupling it with interactive videos, would make an even more effective teaching aid. Using the system, the operator can make a change to one parameter in the spread sheet, and immediately see the ripple effect that this change has on other parameters and final effluent quality. A more widespread adoption of this technology will improve the working knowledge of the process by operational staff, and result in a more effectively managed works. As further technological advances are made and more accurate equations become available for the description of various unit processes, these can easily be incorporated into an updated version of the spread sheet.

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LIST OF SYMBOLS

a	mixed liquor recycle ratio from the aerobic to the anoxic reactors. Subscript o denotes optimum
b_{hT}	Endogenous mass loss rate for heterotrophic organisms at T°C(/d) = b_{h20} $(1,029)^{(T-20)}$
b_{h20}	The rate at 20 °C = 0,24/d
C_{sp}	Stiochiometric ratio ($\Delta P: \Delta S_{bs}$) = 0,5 mg (PO_4 -P)/mg COD converted
D_p	Denitrification potential (mg N/ℓ influent)
D_{p1}, D_{p3}	Subscripts 1 and 3 refer respectively to the primary and secondary anoxic zone
D_{pp}	Denitrification potential of the process when the maximum anoxic sludge mass fraction is all in the form of a primary anoxic reactor
f	Unbiodegradable fraction of active mass = 0,20 mg VSS/mg VSS
f_{bs}	Readily biodegradable COD fraction of the influent with respect to the biodegradable COD concentration
f_{cv}	COD to VSS ratio of the volatile sludge mass = 1,48 mg COD/mg VSS
f_i	MLVSS to MLSS concentration ratio of the mixed liquor
f_n	Nitrogen fraction of the MLVSS (mg N/mg VSS) 0,10 mg N/mg VSS
f_p	Phosphorus fraction of the inert MLVSS and endogenous residue MLVSS = 0,015 mg P/mg VSS
f_u	Unbiodegradable COD fractions in the influent (mg COD/mg COD). Additional subscripts p and s refer respectively to particulate and soluble fractions
f_x*	General parameter for sludge mass fractions *Additional subscripts a, b, d, 1,3 and m refer respectively to anaerobic, total aerobic, total anoxic, primary anoxic, secondary anoxic and maximum unaerated allowable sludge mass fraction
f_{xdm}	Additional subscripts t and m following the d subscript refer respectively to the total and maximum allowable sludge mass fractions
K	General parameter for denitrification rate (mg NO_3/mg VASS/d)
K_1, K_2, K_3	Subscripts 1 and 2 refer respectively to the 1st and 2nd rates in the primary anoxic and 3 to the rate in the secondary anoxic. Additional subscripts T and 20 refer to T °C and 20 °C respectively
K_p	First order rate constant in phosphate removal (/d)
M	Prefix denoting mass as opposed to concentration of a variable
MX_{ah}	Mass of non-poly P organisms in system (mg VASS)
N	General parameter denoting nitrogen concentration (mg N/ℓ).
n	Number of anaerobic reactors of equal volume in series
N_a, N_n	Subscripts a, n, o, t and u, refer respectively to ammonia, nitrate, biodegradable organic nitrogen, total TKN and soluble unbiodegradable organic nitrogen concentrations

N_o,N_t	Additional subscripts e, i, r, s and a refer respectively to the concentrations in the effluent, influent, r-, s- and a-recycle flows
N_c	Nitrification capacity (mg N/ℓ influent)
N_s	Nitrogen required for sludge production (mg N/ℓ influent)
O	General parameter for oxygen
O_c,O_n O_d,O_t	Subscripts c, n, d and t refer respectively to the oxygen demands for carbonaceous material degradation, nitrification, that recovered by denitrification and total oxygen demand
O_i,O_a ,O_s	Subscripts i, a and s refer respectively to the dissolved concentrations in the influent a- and s-recycles
P_f	Excess P removal propensity factor (mg COD/ℓ)
ΔP_n	Phosphorus release in nth reactor per litre influent flow (mg P/ℓ)
P_s	Phosphorus in daily sludge wastage per ℓ influent flow (mg P/ℓ) i.e. the phosphorus removal from the wastewater
P_t	Total phosphorus concentration (mg P/ℓ) Additional subscripts i and e refer respectively to influent and effluent
Q	Daily mean influent flow rate (ℓ/d)
R_s	Sludge age (d)
r or s	Returned sludge ratio based on influent flow
S	General parameter denoting COD concentration
S_b,S_u, S_t	Subscripts b, u and t refer respectively to biodegradable, unbiodegradable and total COD concentrations
S_{bs},S_{up} S_{bi},S_{bsi}	Additional subscripts i, e, s and p refer respectively to concentrations in the influent and effluent, and readily biodegradable and particulate COD
S'_{bsi}	Readily biodegradable COD available for conversion per litre influent (mg COD/ℓ)
S_{bsa}	Readily biodegradable COD concentration in the anaerobic reactor
S_{bSN}	Readily biodegradable COD concentration leaving the last anaerobic reactor (mg COD/ℓ)
S_{COD}	Substrate concentration with respect to COD
T	Temperature °C
v	Volume of waste sludge abstracted from process reactor per day
V	General parameter denoting volume. Subscripts p and r refer respectively to the total process and reactor
X	General parameter denoting sludge mass concentration. Subscripts a, e, i, v, t and n refer respectively to active, endogenous, inert, volatile, total and nitrifier sludge concentrations. Additional subscripts f and i, and a, d and b refer respectively to concentrations in effluent and influent and those in the anaerobic, anoxic and aerobic reactors
Y_h	Heterotrophic organism yield coefficient = 0,45 mg VSS/mg COD
α	Denitrification attributable to the readily biodegradable COD (mg NO_3-N/mg biodegradable influent COD)
γ	Coefficient of excess phosphorus removal (mg P/mg VASS) i.e. the proportion of phosphorus in the active mass

Δ	Prefix denoting the change in the parameter following.
μ_n	Specific growth rate of nitrifiers (/d). Subscript m denotes the maximum rate Additional subscripts T and 20 refer respectively to the rate at T °C and 20 °C
2,86	Oxygen equivalent of nitrate i.e. 2,86 mg oxygen can accept as many electrons as 1 mg NO_3-N nitrate
8,6	mg mass of COD utilised per mg NO_3-N nitrate denitrified
4,57	mg mass of oxygen required for nitrifying 1 mg N nitrate

APPENDIX 7.2

EXAMPLE OF A REPORT WHICH WAS ENTERED INTO THE VISICALC WORK SHEET

Row No	Column Number J	K	L	M	N	O	P
10							
11	PHOSPHATE REMOVAL STUDIES AT THE NORTHERN WORKS						
12	**********						
13							
14							
15	JULY 1985						
16	*********						
17							
18	The experiments were conducted on a five-stage Bardenpho Plant						
19							
20							
21							
22	Plant operating conditions during the test period						
23	**********						
24							
25							
26	All balancing tank effluent fed to the anaerobic reactor						
27	No primary sludge recycle						
28	Balancing tank not emptied each day						
29							
30	Liquid retention times (h)		Actual	Nominal			
31							
32	Anaerobic reactor		1,27	3,57			
33							
34	Primary anoxic reactor		0,69	7,32			
35							
36	Primary aerobic reactor		2,31	24,48			
37							
38	Secondary anoxic reactor		1,93	7,32			
39							
40	Re-aeration reactor		1,05	4,00			
41							
42	Overall			46,69			
43							
44	Solids retention time			37			
45							
46	Returned sludge ratio to anaerobic reactor			2,8			
47							
48	Returned sludge to anoxic reactor			0,00			
49							
50	MLSS recycle ratio			6,8			
51							
52	Dissolved oxygen conc (mg/ℓ) in primary aeration reactor						
53							
54	Bridge 1	1,4					
55	Bridge 2	1,4					
56	Bridge 3	1,6					
57	Bridge 4	1,8					
58	Bridge 5	1,8					
59	Average	1,60					
60	Power used per cubic meter treated kWh/m³			459			
61							
62	MLSS conc	4900					
63							
64	Temperature (°C)	19					
65							
66							
67	Influent feed conditions						
68	**********						
69							
70							
71	Average concentration (mg/ℓ)						
72							
73	COD	584					
74	TKN as N	46					
75	Total P as P	20					
76	Ortho P as P	10,00					
77	TKN/COD ratio	0,08					
78							

APPENDIX 7.2 (cont)

Row No	J	K	L	M	N	O	P
79	Anaerobic reactor:conditions in anaerobic reactor						
80	**********						
81							
82	Solids (mg/ℓ)				4200		
83	Readily biodegradable COD (mg/ℓ)				105		
84	Volatile acids (mg CH_3 COOH/ℓ)				75		
85							
86	Phosphate removal or release (mg P/ℓ)						
87	**********						
88	(-ve value indicated release)						
89							
90	Anaerobic			-15,88			
91	Primary anoxic			-13,1			
92	Primary aeration			3,6			
93	Re-aeration			0,6			
94	Overall			11,70	13,8		
95							
96	Nitrate removal (mg N/ℓ)						
97	**********						
98							
99	Anaerobic			10,1			
100	Primary anoxic			16,2			
101	Diluted SVI			2,1			
102							
103	Settling Properties						
104	**********						
105	SVI			150			
106	DSVI			100			
107							
108	Comparison with UCT Model						
109	**********						
110							
111	Constants used						
112							
113	f_{bs}			0,24			
114	f^{up}			0,09			
115	f_{us}			0,05			
116							
117	Test		Actual value	Predicted value			
118	==========						
119	MLSS (mg/ℓ)		4900	5337			
120	----------						
121	Nitrate removal						
122	(mg N/ℓ)						
123							
124							
125	Anaerobic & anoxic		26,26	26,64	(Anoxic only)		
126							
127	Secondary anoxic		2,10	9,57			
128	----------						
129	Effluent nitrate						
130	(mg/ℓ)		4,00	3,17			
131	----------						
132	Effluent phosphate		15,84	11,1			
133	(mg P/ℓ)						
134	Release in anaerobic						
135	Overall removal		13,80	7,18			
136	==========						
137							
138	Performance of the process						
139	**********						
140	Test (mg/ℓ)		Feed	Effluent			
141	==========						
142							
143	COD		440,00	77,00			
144	TKN as N		44,0				
145	Nitrate as N		0,00	1,20			
146	Total P as P		14,00				
147	Ortho P as P		0,00	2,30			
148	Suspended solids			17,00			
149							
150	==========						

JOHANNESBURG BUSHKOPPIE PLANT
PREDICTION OF PLANT PERFORMANCE USING THE
UCT MODEL AND A VISICALC SPREAD SHEET

Column No	Column A and B	C	D
8	**Kinetic Constants**		
9			
10	f_{cv}	1,48	
11	Y_h	0,45	
12	f	0,20	
13	f_p	0,02	
14	f_i	0,72	
15	b_{h20}	0,24	
16	b_{hT}	0,26	
17	μ_{nm20}	0,36	
18	μ_{nmT}	0,51	
19	k_{120}	0,72	
20	k_{1T}	0,72	
21	k_{2T20}	0,101	
22	k_{2T}	0,127	
23	k_{3T20}	0,072	
24	k_{3T}	0,078	
25	f_n	0,10	
26	α	0,03	
27	c_{sp}	0,50	
28	k_p	0,06	
29	n	3	
30			
31	**Plant Details**		
32			
33	Mass Fractions		
34			
35	f_{xa} Anaerobic	0,09	
36	f_{x1} Primary Anoxic	0,17	
37	f_{x3} 2nd Anoxic	0,00	
38	f_{xdm} Allowable	0,17	
39	No. Anaerobic basins	3	
40	**Flows Mℓ/d**		
41			
42	Settled sewage feed	43,3	
43	Returned sludge	36,7	
44	MLSS recycle	100,00	
45	Waste sludge	1,80	
46	**Total Volume**	34,20	
47			

48	**Recycle Ratios**	
49		
50	s sludge return	0,85
51	a MLSS recycle	2,30
52	R_s Sludge age	19
53	**Dissolved Oxygen**	
54		
55	Primary aeration	2,00
56	Return sludge	1,00
57	**Total Power kWh**	
58	Total kWh	2100
59	**Temperature °C**	23
60		
61	**Sewage Characteristics**	
62		
63	**Feed conc m/ℓ**	
64	COD	671
65	TKN as N	48,2
66	Total P as P	11,8
67		
68	**COD Fractions**	
69	f_{bs}	0,20
70	f_{up}	0,09
71	f_{us}	0,07
72		
73	**Estimation of NO_3**	
74	N_{nr} returned	9,07
75	N_{te}	9,07
76		
77	**P Removal MX_{ah}/Q**	
78	1st MX_a/Q_h	True/False
79	MX_a/Q Temp	730
80		
81	**COD Fractions**	
82	S_{ui}	40
83	S_{upi}	89
84	S_{bi}	541
85	S_{bsi},	108
86	S_{bsi}	39
87	S_{bsa}	21
88	S_{bsn}	4
90	**Fraction of MLSS**	
91		

92	X_a	775
93	X_e	770
94	X_i	1147
95	X_v	2693
96	X_t	3741
97	$\frac{MX_{ah}}{Q}$	730
98		
99	**Nitrification**	
100		
101	N_s	11,20
102	N_c	35,17
103		
104	**Denitrification Pot**	
105		
106	D_{p1} Primary	29,4
107	D_{p3} Secondary	0,00
108	Total	29,42
109	D_{pp} maximum	29,42
110		
111	**Effluent NO_3**	9,07
112		
113	**Oxygen Demand**	
114		
115	$M(O_c)$ Carbonaceous	18226
116	$M(O_n)$ Nitrification	6960
117	$M(O_d)$ Denitrification	3232
118	$M(O_T)$	21953
119		
120	**Phosphate Removal (Kinetic)**	
121		
122	**Parametric Model**	
123		
124	S_{bi}	541
125	S_{ba}	21,5
126	P_{xf}	0,00
127	Y	0,06
128	ΔP_s	3,96
129		
130	**Kinetic Model**	
131		
132	ΔP_n	15,6
133	P removed	7,5
134		

APPENDIX 7.4

JOHANNESBURG BUSHKOPPIE PLANT
THEORETICAL COMPARISON OF A 3 AND 5 STAGE PHOREDOX PROCESS
USING THE UCT MODEL AND A VISICALC SPREAD SHEET

		5 Stage	3 Stage
	Column		
Column No	A and B	C	D
8	**Kinetic Constants**		
9			
10	f_{cv}	1,48	1,48
11	Y_h	0,45	0,45
12	f	0,20	0,20
13	f_p	0,02	0,20
14	f_i	0,72	0,72
15	b_{h20}	0,24	0,24
16	b_{hT}	0,26	0,26
17	μ_{nm20}	0,36	0,36
18	μ_{nmT}	0,51	0,51
19	k_{120}	0,72	0,72
20	k_{1T}	0,72	0,72
21	k_{2T20}	0,101	0,101
22	k_{2T}	0,1272	0,1272
23	k_{3T20}	0,072	0,072
24	k_{3T}	0,078	0,078
25	f_n	0,10	0,10
26	α	0,03	0,03
27	c_{sp}	0,50	0,50
28	k_p	0,06	0,60
29	n	3	3
30			
31	**Plant Details**		
32			
33	Mass Fractions		
34			
35	f_{xa} Anaerobic	0,09	0,09
36	f_{x1} Primary Anoxic	0,17	0,17
37	f_{x3} 2nd Anoxic	0,17	0,00
38	f_{xdm} Allowable	0,34	0,17
39	No. Anaerobic basins	3	3
40	**Flows Mℓ/d**		
41			
42	Settled sewage feed	43,3	43,3
43	Returned sludge	36,7	36,7
44	MLSS recycle	100	100
45	Waste sludge	1,8	1,8
46	**Total Volume**	34,2	34,2

Recycle Ratios			
	s sludge return	0,85	0,85
	a MLSS recycle	2,3	2,3
	R_s Sludge age	19	19
Dissolved Oxygen			
	Primary aeration	2,0	2,0
	Return sludge	1,00	1,0
Total Power kWh			
	Total kWh	2100	2100
Temperature °C		23	23
Sewage Characteristics			
Feed conc m/ℓ			
	COD	930	930
	TKN as N	66	66
	Total P as P	11,8	11,8
COD Fractions			
	f_{bs}	0,20	0,20
	f_{up}	0,13	0,13
	f_{us}	0,06	0,06
Estimation of NO_3			
	N_{nr} returned	0,99	11,8
	N_{te} returned	0,99	11,8
P Removal MX_{ah}/Q			
	1st MX_a/Q_h	True/False	
	MX_a/Q Temp	904	999
COD Fractions			
	S_{ui}	55,8	55,8
	S_{upi}	123	123
	S_{bi}	750	750
	S_{bsi}	150	150
	S_{bsi}	139	60
	S_{bsa}	75	33
	S_{bsn}	11,4	4,3
Fraction of MLSS			

92	X_a	1074	1074
93	X_e	1068	1068
94	X_i	1590	1590
95	X_v	3733	3733
96	X_t	5185	5185
97	$\frac{MX_{ah}}{Q}$	904	999
98			
99	**Nitrification**		
100			
101	N_s	15,2	15,2
102	N_c	48,7	48,7
103			
104	**Denitrification Pot**		
105			
106	D_{p1} Primary	40,8	40,8
107	D_{p3} Secondary	14,3	0
108	Total	55,1	40,8
109	D_{pp} maximum	0,99	11,80
110			
111	**Effluent NO_3**	64,02	40,77
112			
113	**Oxygen Demand**		
114			
115	$M(O_c)$ Carbonaceous	25261	25261
116	$M(O_n)$ Nitrification	9627	9617
117	$M(O_d)$ Denitrification	5902	4563
118	$M(O_T)$	28985	30324
119			
120	**Phosphate Removal (Kinetic)**		
121			
122	**Parametric Model**		
123			
124	S_{bi}	750	750
125	S_{ba}	75,9	33,3
126	P_{xf}	4,58	0,75
127	Y	0,25	0,11
128	ΔP_s	16,4	8,2
129			
130	**Kinetic Model**		
131			
132	ΔP_n	59,4	26,5
133	P removed	13,22	8,94
134			

CHAPTER EIGHT

Operational and design aspects

8.1 INTRODUCTION

The successful operation of nutrient removing activated sludge plants is very dependent on good operation, coupled with good design. Without the necessary facilities even the best operator will be placed at a severe disadvantage.

The experiments carried out on the Northern Works have highlighted a number of areas where the original design concepts could be improved. However, before examining each unit process in detail, it is considered expedient to first draw attention to the operational problems which can be caused by sludge bulking, the ultimate cure for which, may affect a number of unit processes downstream of the primary settling tanks (PST).

8.2. SLUDGE BULKING

Sludge bulking is a worldwide problem and Blackbeard and Ekama(1984), in a survey of 111 South African activated sludge plants, have shown that some 78 % experienced either bulking or foaming (or both), in various degrees of severity. Bulking is caused by the excessive growth of filamentous organisms and Lee et al. (1983) have found that the Diluted Sludge Volume Index (DSVI) gave the best correlation and least scatter, when compared with Total Extended Filament Length (TEFL), (Figure 8.1) and defined a bulking sludge as having DSVI >150 mg/ℓ.

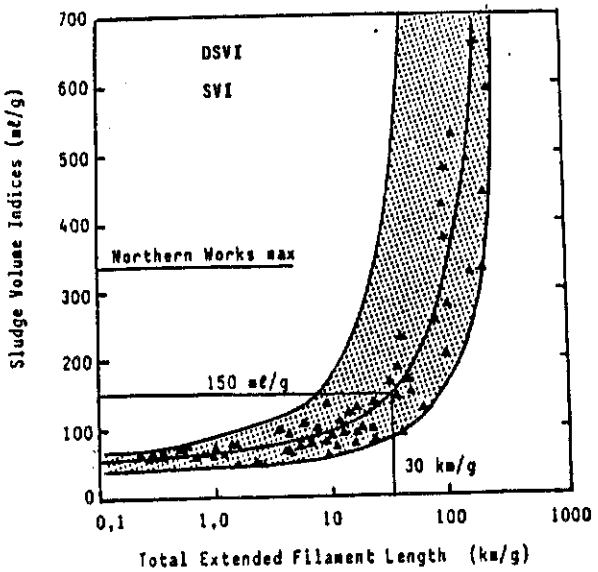


Figure 8.1 : Relationship between TEFL and DSVI as derived by Lee et al, (1983) and also showing the corresponding range of SVI as presented by Ekama et al, (1985)

The operation of two modules of the Northern Works activated sludge plant for an 18 month period is reflected in Figures 8.2 and 8.3, from which it will be noted that sludge bulking conditions were present for long periods of time. Some of the problems which have been incurred because of this condition are described below.

Sludge age control on this plant was achieved by withdrawing known volumes of MLSS from the second aerobic basin. However, as the mass of sludge withdrawn per unit volume slowly deteriorated, a point was ultimately reached where the full capacity of the withdrawal pipe was used and it was no longer possible to prevent the MLSS in the system from rising. This situation could only be rectified by making alterations to withdraw sludge via the secondary clarifier underflow pipe.

High SVI's also caused the solids content of the air flotation thickener to drop significantly, which caused a drop in efficiency of the belt presses, necessitating further units having to be used to handle the increased volume of more dilute sludge. Reduction of the MLSS to more realistic levels was therefore a lengthy procedure. Under these conditions sludge age control proved to be difficult and a situation was finally reached

where rising concentrations of ammonia in the effluent reflected an apparent loss of nitrifiers from the system.

Increased carryover of solids from the secondary clarifiers when the DSVI reached 150 mℓ/g, necessitated reducing the load on modules designed for 50 Mℓ/d to 35 Mℓ/d. Furthermore, the increased volume of secondary clarifier underflow required to prevent solids carryover, resulted in additional loads of nitrate being returned to the anaerobic zone. Retention times in both the anaerobic and primary anoxic zones were decreased, which detrimentally affected biological phosphorus removal.

Three other effects of high SVI's at the Northern Works may be mentioned, viz higher consumption of power to sustain higher levels of MLSS in the aeration basins and an increase in blockages experienced with the filters associated with the on-line monitors. Entrainment of air by the filamentous MLSS often occurs and results in the formation of unsightly surface scums (Hart,1985) (see 4.5.1). Low SVI's on the other hand, may give rise to such a dense concentration of sludge in the secondary clarifiers, that siphons are difficult to start again if they fail.

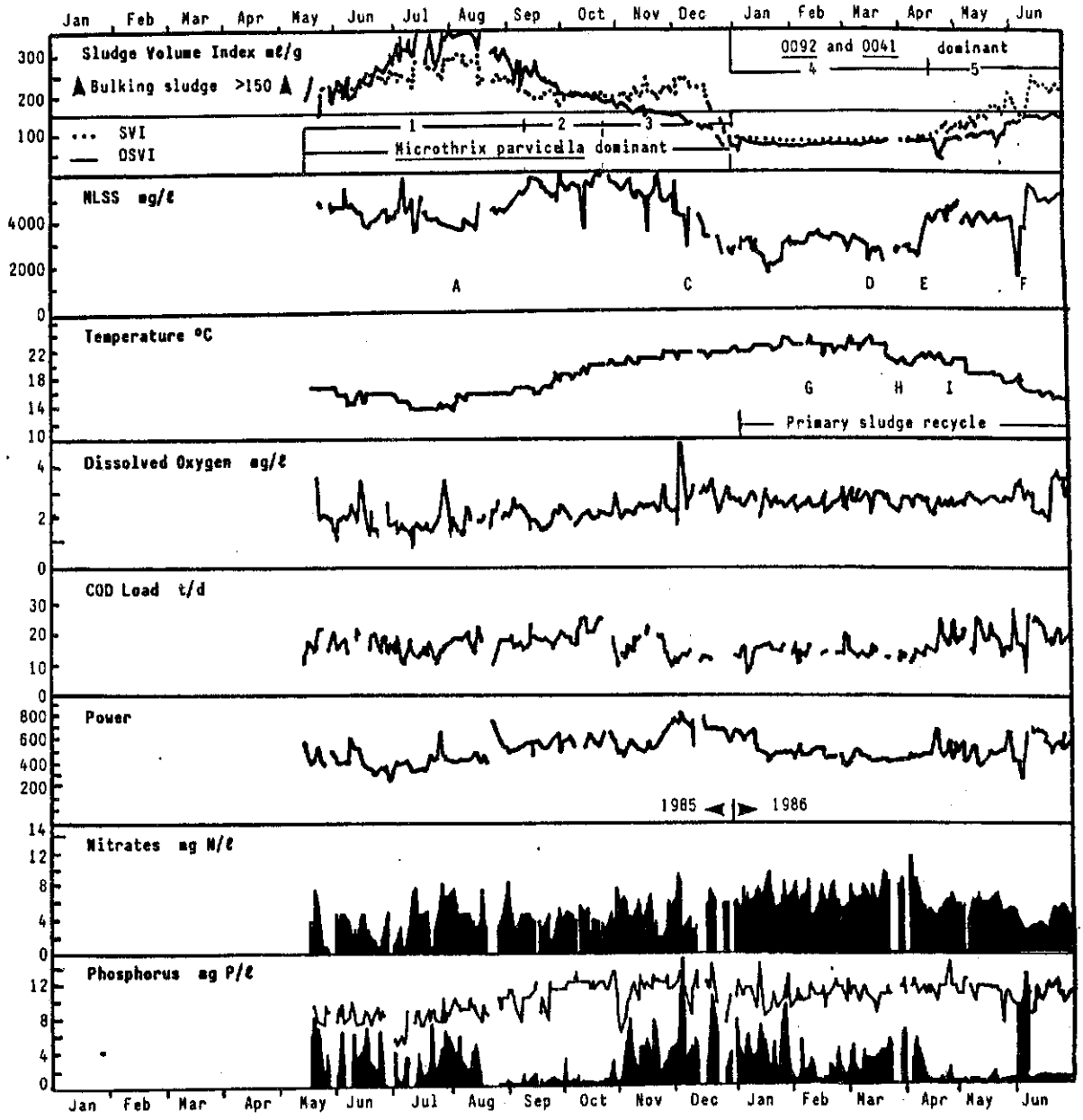
From the above it will be realised that the presence of bulking sludge can create major operational problems. Their solution rests in the hands of researchers to identify the precise mechanism whereby filamentous organisms gain a selective growth advantage over floc forming bacteria, and for the designer to provide the necessary process and plant configuration to implement these findings. Reference to Figures 8.2. and 8.3. will show that the bulking problems experienced at the Northern Works, were largely due to the presence of Microthrix parvicella and occurred mainly in the winter. During the warmer summer months, filaments Type 0092 and 0041 (Eikelboom and van Buijsen,1981) were present but did not adversely affect plant operation (see also 4.5.2).

Some of the approaches considered for controlling Microthrix parvicella at the Northern Works, and some of the techniques actually tried, are described below.

NORTHERN WORKS : MODULE 2 : THREE-STAGE PHOREDOX

Microbiological Data

1 Severe bulking and extensive bridging
2 Filaments decreasing, some bridging
3 No bridging
4 No bridging, 0091 and 0042 present
5 Some bridging. Microthrix parvicella increasing



Plant Data

A 12 t Ferric chloride added 16 Aug '85
B Aerators/mixers off for 60 h 24 Aug '85
C Scum removal path created 12 Dec '85
D MLSS recycle point changed
E Anoxic zone on RAS. Feed to 2nd half of of anaerobic zone
F Blockage on 2nd clarifier syphons
G No control on detention time
H Zero recycle
I Sludge retention time 4 d

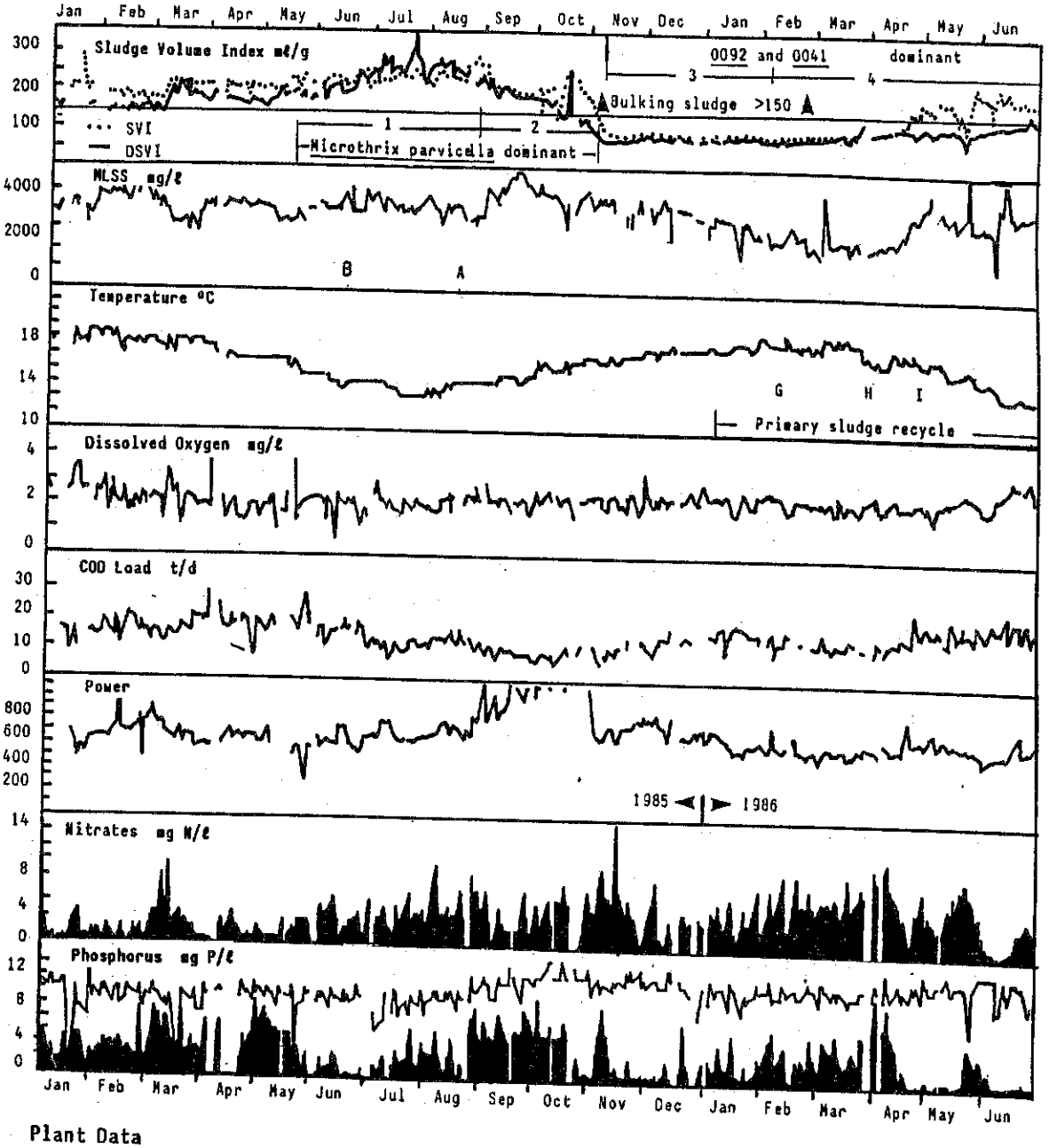
Note : Largely operated on a 5 stage process by creating artificial second anoxic zone

Figure 8.2 : Interrelationship of microbiological observations and other plant operational parameters: Module 2.

NORTHERN WORKS : MODULE 3 : 5 STAGE PHOREDOX

Microbiological Data

1 Severe bulking with bridging
2 Bridging decreasing
3 Few filaments - no bridging
4 Filaments increasing - some bridging



Plant Data

A 12 t Ferric chloride added 16 Aug '85
B Aerators/mixers off for 60 h 19 June '85
G No control on sludge retention time
H Zero recycle
I Sludge retention time 4 d

Figure 8.3: Interrelationship of microbiological observations and other plant operational parameters : Module 3.

Chlorination

Jenkins et al. (1985) have reported considerable success with the control of bulking sludge in the USA, by carefully controlled addition of chlorine. This technique has been successfully applied to some works in South Africa, but its use at the Northern Works was not possible, as chlorination equipment with sufficient capacity was not available on site. Furthermore, with the local cost of chlorine being about R1/kg Cl_2 (1985), the cost of treating a 50 Mℓ/d module for a period of two weeks would be R6 000.

Shock dosing with ferric chloride

There are obvious advantages to be gained if the light bulking sludge can be made heavier, to permit greater compaction in the secondary clarifier. A number of flocculating agents can be used for this purpose, including polymers and cheaper inorganic salts such as the conventional ferric salts and alum. Rensink et al. (1979) have shown that the continuous addition of 25 - 50 mg/ℓ of iron to the influent sewage can effectively control bulking and at the same time, remove phosphorus. This procedure would obviously defeat the plant design objective to remove phosphorus biologically. However, it was felt that temporary relief might be obtained if a single, heavy dose of ferric chloride was added to the activated sludge tanks. The additional weighting effect of adsorbed iron hydroxide might have reasonably been expected to last, with diminishing effect, for one sludge age or about 3 weeks.

On 16 August 1985, 12 t of ferric chloride was added to the anaerobic zone of Module 2 over a period of several hours and a similar procedure adopted for Module 3. No detrimental effects were noted on the biomass, however, the secondary clarifier effluent quality improved dramatically for a few days, but thereafter the original problem re-appeared. Continuous addition of iron salts for a limited period thereafter was not considered, as Wagner(1981) claimed that iron salts were ineffective against Microthrix parvicella.

Enhanced anaerobiosis

As described in Section 2.4, the aerators and mixers in Module 2 were shut down on 24 August 1985 and remained inoperative for a period of about 60 h. The main purpose of this experiment was to trigger enhanced phosphorus removal in which objective it was particularly successful (Figure 8.2). A similar exercise was carried out on Module 3, also with good results regarding phosphorus removal (Figure 8.3).

Enhanced aeration

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 (Figure 8.4). This has the effect of both increasing the dissolved oxygen levels and reducing oxygen deficient areas to a minimum. Preventative action must be taken at an early stage when the appearance of filamentous organisms is first noted.

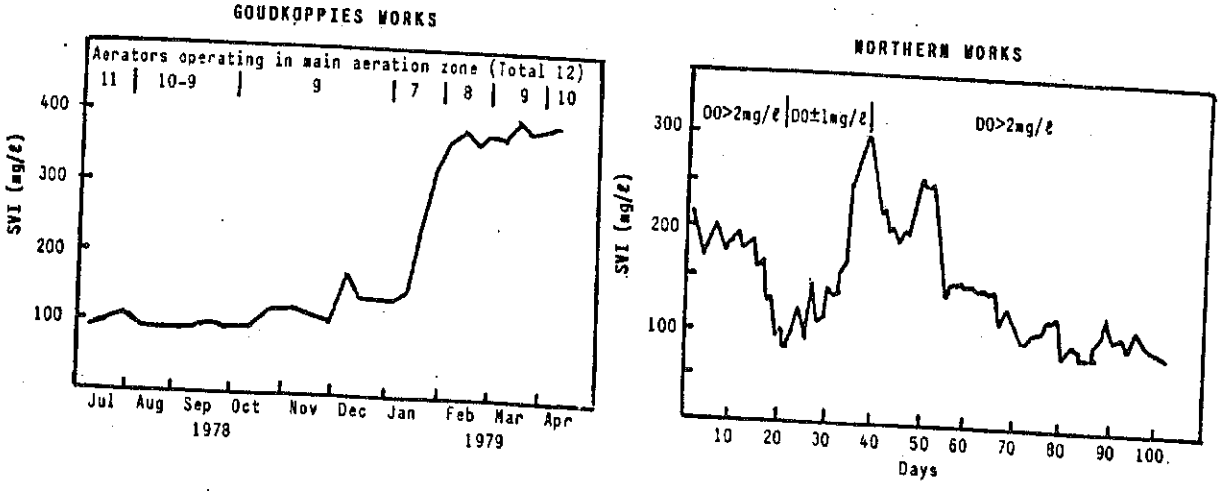


Figure 8.4 : The effect on increased aeration on the SVI at the Northern and Goudkoppies Works

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 anoxic zone until it reaches the final clarifiers. From these observations it would seem that the growth of filamentous organisms is promoted by the quasi-aerobic conditions created by turning surface aerators off. These findings give support to Eikelboom's(1983) postulation that all adsorbed COD present on the floc must be oxidised before the sludge is returned to the process via the clarifier underflow. Potential adsorption sites are thereby made available for the immediate uptake of readily assimilable organic material which therefore cannot gain entry to the aerobic zone where, in very dilute concentrations, it can promote the selective growth of filamentous organisms.

Two potential solutions to the bulking sludge problem at the Northern Works therefore suggest themselves, viz improvement of oxygen distribution through the aerobic zone and modification of the plant to three-zone operation with its relevant improved oxygen regime.

Module 2 at the Northern Works was converted to three-stage operation mainly to improve phosphorus removal as described in 2.5 by eliminating the second anoxic zone and simultaneously almost doubling the size of the anaerobic and anoxic zones. Regrettably however, it could not be run for any length of time to replicate the Bushkoppie conditions. Existing bulking conditions necessitated decreasing the volume of sewage treated by this module. To avoid over-aeration and over-nitrification, which in turn would detrimentally affect biological phosphorus removal, certain surface aerators had to remain switched off to create secondary anoxic conditions. With the return of summer in the second half of 1985, SVI's returned to reasonable values which were maintained during the winter of 1986, it is hoped to increase the load to this module during the latter half of 1986 and obtain a fairer comparison between three and five-stage processes.

Oxygen input to both Module 2 and 3 was increased in November 1985, by increasing the number of aerators in operation. Although the dissolved oxygen levels only increased marginally, the DSVI values in both units

continued to drop. This may have been due to the MLSS being exposed to aerobic conditions for a longer period of time, or due to the lowering of the mass of activated sludge in the system as a result of deliberately increased sludge withdrawal rates.

In June 1986, Module 3 was operated with all aerators in service but with the level of the control weir lowered, thus decreasing the depth of immersion of the aerators. Oxygen concentration levels were thereby maintained, but the power input per unit volume decreased. A concomitant rise of 40 to 50 % in OUR at the end of the primary aerobic basin was noted indicating the presence of unoxidised adsorbed material. The ultimate effect that this will have on SVI remains to be seen.

Reduction of MLSS

Reference to Figures 8.2 and 8.3 will show that SVI values dropped dramatically in sympathy with reduced MLSS levels, whilst DSVI did not show the same sensitivity to MLSS concentrations. For an in-depth review of the merits and demerits of these two parameters, the reader is referred to Ekama et al. (1985). While SVI and DSVI were not coincident in the presence of Microthrix parvicella, they have similar values when Microthrix parvicella is absent, and filaments Type 0092 and 0041 (Eikelboom and Van Buijsen,1981) are less abundant. It is of interest to note however, that with the onset of winter in 1986, SVI values started to exceed the DSVI values. This observation coincided with the reappearance of Microthrix parvicella and the point of deviation of these two indices may well prove to be the operator's first indication of the appearance of filamentous bacteria.

Scum removal

Hart(1985) has shown that the foam on the Northern Works activated sludge units can contain similar filamentous bacteria to those found in the main body of the tank. If such scum is therefore allowed to be mechanically trapped on the surface of various zones, it can continuously re-inoculate the MLSS. Pretorius and Laubscher(1986) have suggested that an expedient method of removal is to interpose a selective flotation unit just ahead of

the secondary clarifiers. Instead of adopting this solution, some sections of the zone dividing baffles protruding above the water level were removed to provide a continuous flow path for any floating material to the secondary clarifiers where it was removed in scum traps. This was carried out on 12 December 1985, and the majority of scum virtually disappeared overnight. As the SVI's were low at this time, it cannot be claimed that this modification had any beneficial effect on this index.

Introduction of selectors

Chudoba et al. (1973); Rensink(1974); Jenkins et al. (1985) have proposed the introduction of a small aerobic or anoxic reactor ahead of the main biological reactor as a means of giving floc forming micro-organisms a growth advantage over the filamentous types. Eikelboom(1983) however, has imposed a further condition that the returned sludge should not have unoxidised COD occupying adsorption sites on the sludge.

Advantage was taken of the modification to Module 2 to a three zone process to incorporate an anoxic selector into the influent section of the anaerobic zone, as depicted in Figure 2.15. This small plug flow unit (nominal residence time ≈10 minutes) received influent settled sewage and returned sludge, with its associated nitrates, hence the use of the term anoxic as opposed to anaerobic.

After completion of the modifications, Module 2 had to be commissioned with an inoculum of sludge containing Microthrix parvicella. However, a comparison of Figure 8.2. with Figure 8.3 will reveal that the introduction of the selector did not result in the elimination of Microthrix parvicella from Module 2, but it did add to the compartmentalisation of the anaerobic zone and this appeared to decrease the variability in effluent quality.

8.3 INTERACTION OF OPERATION AND DESIGN

The publication "Theory, Design and Operation of Nutrient Removal Activated Sludge Processes" (WRC, 1984) provides an in-depth study of this topic and the comments that follow should therefore be read in conjunction with this document.

8.3.1 Primary Sedimentation

Operational experience at the Northern Works has shown that the accumulation of sludge in the primary sedimentation tanks with the provision to recycle it to the head of works to elutriate the VFA's produced, has a very beneficial and stabilising effect on biological phosphorus removal. The addition of a recycle facility is relatively inexpensive in relation to the derived benefits, and its inclusion as a design feature should be considered essential.

In the case of small plants, primary sedimentation facilities may be omitted as a means of reducing overall costs, but in future, serious consideration may have to be given to the inclusion of these units as a potential means of modifying the characteristics of the incoming sewage. Without the correct composition, biological phosphate removal will not be to an efficient phosphate level of less than 1 mg/ℓ and phosphate precipitating chemicals will have to be added.

Accumulation and recycling of primary sludge is not without its problems. Considerable thickening can take place and sludge containing up to 7 % solids has been experienced. Withdrawal pipework and pump design must be adequate to meet this situation. Sludge scraper mechanisms must also be of adequate strength and design to handle greater volumes of accumulated raw sludge. In rectangular tanks, scrapers may lift and ride over the sludge layer, which will then remain in the tank and turn anaerobic. In the case of circular tanks, the scraper drive wheels have been known to lift-off the tank wall when the scraper attempts to move a thick layer of sludge.

From an operational point of view, if sludge is left too long in a clarifier, anaerobiosis is likely to set in and tank contents will turn black, with concomitant liberation of methane gas. This implies the destruction of the VFA which are required for enhanced biological phosphorus removal. This should be avoided at all costs and the solution appears to be to run the primary clarifiers as semi-batch sludge fermenters, with a sludge retention time of 3 to 4 days. If multiple tanks are available, say 4, then the operating cycle would be such that

every day the sludge in **one** tank would be removed to anaerobic digesters, whilst that in the remaining three, would be recycled to a point upstream of the primary sedimentation tanks. Depending on local circumstances and the facilities available, operating strategies can be varied, the main aim being to increase the concentration of anaerobic fermentation products to as high as necessary.

Alternatives to the use of primary sedimentation tanks, are to conduct high rate fermentation in primary sludge thickeners, or in a separate high rate digester. The latter option is particularly attractive for the generation and storage of acid fermentation products to be fed into the system when the incoming sewage is particularly deficient in these materials, for example, holiday weekends such as Christmas, New Year and Easter. Again, it would be important to retain the correct sludge retention time of 3 to 4 days, using semi-batch operation and elutriating the fermented sludge by recycle back into the main sewage flow, or the inlet of thickeners. It should be noted however, that the actual mass of VFA available in the supernatant from high rate digesters is far lower than that generated in primary sedimentation tanks.

Designers must take note of the fact that if organic substances are solubilised by fermentation in either primary sedimentation tanks or high rate digesters, less sludge will be available for the production of sludge gas in conventional mesophilic digesters. Nonetheless, if as much as 30 % of the influent sewage solids are converted to organic acids, there should be ample sludge left over to provide heating for conventional digesters.

In conveying the settled sewage to the anaerobic reactor, the designer should ensure that flumes,weirs, head loss devices and other potentially aerating equipment such as Archimedian screw pumps, are kept to an absolute minimum. This is to limit the bacterial degradation or stripping of VFA's before they reach the point where they are required. An example of such a design approach was reported by Barnard(1984), in designing the Kelowna Works in British Columbia, Canada, installed a special device in the PST effluent channel to ensure that the water in the channel was automatically adjusted to be maintained just below the level of the overflow sill on the PSTs, thus ensuring minimum drop of liquid and diminishing air entrainment.

When primary sedimentation tanks are not included in a plant design, the operator may have to resort to fermenting activated sludge within the main reactor, by switching off aerators and/or stirrers and allowing the sludge to settle and undergo fermentation on the reactor floor. Elutriation can be achieved by switching on the mechanical plant at predetermined intervals.

8.3.2 The Anaerobic Zone

The success of the biological removal process is dependent on the exposure of activated sludge to anaerobic conditions, that is, both oxygen and nitrate must be absent. Under these conditions, phosphorus is released by the sludge into solution, to concentrations above the feed sewage. Wentzel et al. (1985) have shown this to be an important phenomenon, as they were able to demonstrate that the magnitude of biological excess P uptake was strongly linked to the magnitude of P release in the anaerobic zone. If the feed contains 10 mg P/ℓ, then a typical level in the anaerobic zone would be 15 mg P/ℓ. Furthermore it is vital to the success of biological phosphorus removal that readily biodegradable substances be present in this zone for uptake by phosphorus accumulating bacteria, of which Acinetobacter spp is usually the dominant species. If such substances are not present in sufficient quantity, their concentration must be enhanced by one of the methods illustrated in Figure 1.1 (Chapter 1).

It is imperative to take every care to ensure that where supplies of readily assimilable substrate (S_{bs}) are limited, every endeavour is made to prevent the ingress of oxygen, which may be present as air entrained with the feed, or of recycled nitrate. For every 1 mg N/ℓ as nitrate 8,6 mg S_{bs}/ℓ are removed and for every 1 mg O/ℓ introduced 3 mg S_{bs}/ℓ are removed. The University of Cape Town (UCT) process (WRC, 1984) has been introduced to safeguard the anaerobic zone from the adverse effects of nitrates, but involves the introduction of an additional recycle.

Pitman(1986) has suggested an alternative to the UCT process for minimising the effect of nitrates in the anaerobic zone. He noted that with the very low SVI sludges prevailing at the Bushkoppie Works, excellent compaction of sludge took place in the secondary clarifiers and that significant

denitrification was also achieved. The rate of denitrification was ascertained from laboratory experiments and found to coincide with the endogenous rate reported in the Water Research Commission Monograph on biological nutrient removal WRC(1984).

This technique was tested in practice by diverting the sewage feed from Module 2 to the second half of the anaerobic zone.

Very preliminary but apparently favourable results achieved by this modification are more fully described in 2 6. Success is very dependent on being able to concentrate a large mass of returned sludge solids in the compartmentalised first half of the modified anaerobic zone. Application of this process is therefore critically dependent on the absence of bulking sludge, i.e. one having low DSVI characteristics.

From a designer's point of view, the provision of facilities to denitrify the return sludge, possibly in the secondary clarifier or in a special endogenous denitrification reactor, with the elimination of the space-consuming second anoxic zone, warrants serious consideration. A disadvantage of this proposal is that higher concentrations of nitrate are likely to be present in the effluent. Such facilities would ensure that the vitally necessary, but often in short supply, readily biodegradable component is not wasted on denitrification, but is diverted to where it will be effectively used by phosphorus accumulating bacteria. Such a design should also permit biological phosphorus removal to be more reliably achieved with lower strength sewages, than is currently the case. Plug flow conditions appear to further enhance reliability and phosphorus removal. However, care must be taken to ensure that surface scum can easily pass through any baffles that may be installed.

8.3.3 Denitrification Zones

Because of the profound effect that nitrates can have on phosphate removal, it is very important to ensure good denitrification in the process. Although the necessary provision must be made in the process design for denitrification, the quality of the sewage can also greatly influence the results obtained.

Primary anoxic reactors, in the presence of an adequate supply of S_{bs}, make use of the rapid denitrification kinetics described in WRC(1984). Secondary anoxic reactors basically rely on the slower, endogenous rates of denitrification which may be speeded up somewhat, if adsorbed particulate biodegradable material is either present or perhaps deliberately added. In normal circumstances it is doubtful if this zone would remove more than 3 mg N/ℓ as nitrate, but in some circumstances where readily biodegradable COD is not in adequate supply, 3 mg N/ℓ as NO_3 could use up 3 x 8,6 or 25 mg COD which may be critical for phosphorus removal.

Where surface aerators are used the designer should be cognizant of their powerful pumping action and capability of pushing unwanted highly aerated water into the anoxic zones. Design procedures to minimise this effect are essential. Barnard et al. (1985) have claimed that the compartmentalised design of the Kelowna Works in Canada, has assisted in the denitrification of weak sewages and it would seem that the limited experience gained with Module 2 at the Northern Works, supports this claim.

Mixed liquor recycle ratios in nutrient removing activated sludge plants are generally in the range 2:1 to 4:1, although higher recycle ratios can be used for wastewaters having very high S_{bs} values due to the presence of industrial waste. Ideally, the mixed liquor recycle ratio should be adjusted to a value where nitrates are just passing out of the first anoxic zone, e.g. 1 mg N/ℓ. In practice, due to changing conditions, this ideal cannot be met and the ratio is set at some fixed value near the optimum and is not frequently readjusted.

The introduction of anoxic zones results in a saving of power and a recovery of alkalinity. Effluents from nutrient removing activated sludge plants, can therefore be usefully blended with biological filter effluent, to which alkalinity destroying acidic metal salts have been added to precipitate phosphates. The secondary release of phosphorus in secondary anoxic zones as reported by Barnard (1984), would be prevented by replacing this zone with denitrification facilities on the returned sludge line.

8.3.4 Aerobic Zone

Proper control of aeration is important, as under-aeration can seriously inhibit nitrification and phosphate uptake in aerobic zones, while over-aeration can be wasteful and produce unnecessary endogenous nitrification.

Experience has shown that if DO levels are maintained between 2 - 3 mg/ℓ throughout the aerobic zones, good performance can be expected. Another criterion is that aeration intensity should be such that all the ammonia is oxidised in the first quarter of the aerobic zone. If the ammonia levels at this point exceed 2 mg N/ℓ, then the possibility exists that there will be insufficient remaining aeration capacity to ensure good phosphorus uptake. A third criterion could be to ensure that the OUR should be below 15 mg O/ℓ/h at the end of the aeration tank.

The on-line monitors described in 3.2.3 have shown that despite the dilution effect of the mixed liquor recycle, a definite oxygen demand profile exists, which suggests that designers should consider providing tapered aeration facilities in the basin. This effect is achieved in practice at the Northern works, by switching aerators "on" or "off" as required, to maintain the DO level. As already pointed out in Section 8.2, it is believed that this mode of operation promotes the formation of quasi- aerobic areas which are conducive to the growth of filamentous bacteria.

When recycling of sludge is practised on the primary sedimentation tanks, an additional soluble and particulate COD load will be carried forward to the biological reactor. Provision must therefore be made for the extra oxygen demand created thereby, and also for an increase in the MLSS. The additional degree of solubilisation required in the primary sedimentation tanks, will depend on how much nitrate is entering the anaerobic zone. If this concentration is high, say up to 10 mg N/ℓ, then a considerable mass of influent COD will be used up for denitrification. Under these circumstances, it is probably safer to have some 150 mg/ℓ of S_{bs} in the influent to the anaerobic zone, i.e. about 100 mg/ℓ above that present in the influent sewage. Carryover of suspended solids during periods of

recycling on the primary sedimentation tanks, may be as high as double under non-recycle conditions. If sludge age control is by means of wastage from the aerobic zone, then adequate provision must be made to withdraw the additional mass of MLSS.

Plug flow conditions improve phosphorus uptake potential, but the designer should be conscious of the increased demand for oxygen at the head of the aerobic zone. Increasing the mixed liquor recycle rate could be expected to flatten the demand curve somewhat, but may detrimentally affect the operation of the primary anoxic zone.

8.4 FINAL CLARIFIERS

The Northern Works activated sludge plant is provided with flat bottomed, circular, centrally fed clarifiers in which sludge is removed by siphons carried on a twin-arm rotating bridge. This system was adopted in the belief that accumulated sludge would be rapidly removed before anaerobiosis could set in and release phosphorus into the effluent.

A number of operational problems have been experienced with this system, of which designers should take note. Underflow recycle ratios should be kept as low as possible to minimise nitrate feedback to the anaerobic zone. However, the settling properties of the sludge and the clarifier design usually dictate how low this ratio can be set. Typical operating ranges are 0,5 to 1,0. The design of the siphons used was such that certain minimum flows were required to prevent sludge settlement and blockage of the siphon pipes. This made it operationally difficult to reach the lower recycle range. Furthermore, the discharge was at no stage visible and it was therefore not possible to see whether clear effluent or sludge was being sucked up individual pipes. In retrospect, and taking into account experience at other Johannesburg activated sludge plants, it is believed that scraped tanks may have provided more satisfactory results.

Provision of waste sludge withdrawal facilities from the underflow recycle system is necessary to augment sludge age control facilities that may be sited on the aeration tanks. Designers should also provide adequate scum removal systems. Turbulence on the return sludge system should be avoided

to prevent entrained air from being discharged to the anaerobic zone, where it will permit unwanted biological reduction of the readily biodegradable matter present in the influent sewage and possibly lead to scum formation.

For a more detailed consideration of the design of final clarifiers, see Ekama and Marais(1986).

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CHAPTER NINE

Financial Aspects

9.1 INTRODUCTION

The purpose of this chapter is to attempt to identify the major cost differences between various treatment processes in order to facilitate decisions regarding process selection. It was the intention to quantify the actual costs for the various P-removal options, from the cost analysis printouts provided by a computer based accounting system.

This proved to be impractical because the programme did not permit retrieval of informantion in a form suitable for this purpose.

Moreover the cost figures were influenced to a large degree, by vastly differing capital charges due to the source of funding and time of construction of the various installations being compared. Hence it was decided to compare only cost differences for the various options under consideration.

The costs given have been estimated for a plant capable of treating 50 000 m^3/d situated on the Witwatersrand. Caution should be exercised when using these costs for plants of different capacities located in other parts of the country. In particular the cost of chemicals depends heavily on transport costs. Thus the further the plant from the chemical factory, the higher the cost of transport and the total cost of the chemical, and the more favourable is the non-chemical option.

9.2 FACTORS TO BE CONSIDERED IN ECONOMIC EVALUATIONS

9.2.1 Timing of Cash Flows

In comparing the economic advantages of biological phosphate removal as opposed to chemical phosphate removal the essential economic comparison is the cost of high initial capital investment and low operating costs of biological phosphate removal against low initial costs and high operating costs of chemical phosphate removal.

The method of analysis requires careful consideration if the true costs are to be determined. The true unit cost of treatment greatly depends on the initial capacity of the plant, initial sewage flow and the growth rate in sewage flow. Most sewage treatment plants are designed to provide for some growth in flow and there is a time lag before the plant operates at its design capacity. This concept is illustrated in Figure 9.1.

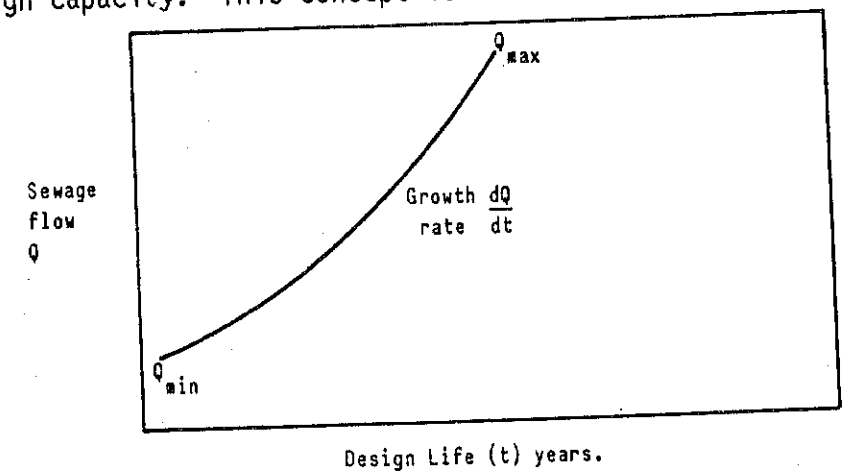

Figure 9.1 : Rate of growth of flow to a typical sewage works.
Q_{min} = Initial flow.
Q_{max} = Plant capacity.

In processes with high initial capital costs this capital investment is initially under-utilized although the full cost must be paid for in the form of interest and redemption of the capital. In processes with high operating costs resulting from addition of chemicals to the process, the cost is directly related to the sewage flow and will increase as sewage flows increase. Thus the essential difference between the cost of the two processes is the timing of the cash flows and relative costs should be evaluated using a technique such as the Discounted Cash Flow Method

(Horngren, 1977) which incorporates the consideration of cash flows over the economic life of the project.

This method of evaluation is beyond the scope of this chapter.

9.2.2 Cost of Capital or Interest Rate

In order to perform the economic analysis an appropriate interest rate must be chosen. Quoted interest rates contain an element which anticipates future inflation while for purposes of analysis the real interest rate must be used (i.e. a rate in which inflation has been discounted). In a study carried out in the USA the long term historical real rate of return of various financial instruments was found to be as follows:-

Instrument	Real return (%)
Treasury bills	0
Treasury bonds	1
Corporate bonds	1,4
Common stocks	6,1

If the same conditions are assumed to hold in South Africa, the interest rate on Treasury bills can be taken as a surrogate for the long-term inflation rate and the real interest rate should not exceed about 6%. In the case of municipal stocks the real interest rate is likely to be considerably lower.

In the following analyses an interest rate of 6% has been used.

9.3 COST OF REMOVING PHOSPHORUS VIA DIFFERENT PROCESS CONFIGURATIONS

9.3.1 Basic Assumptions

The cost of electricity based on the Johannesburg tariff ruling on 1 July 1986.

Scale 1: Service charge per day 45,00c
Energy charge/KWh 2,71c
Demand charge/kVA/day 44,28c

The cost of chemicals is based on:-

Fe^{3+} = R1,267/kg (incl. GST).
Lime = R125,66/t (incl. GST).
Polyelectrolyte a) as a settlement aid R5,00/kg
b) for sludge dewatering R7,00/kg

The major cost differences resulting from the implementation of a number of process modifications to a base process will be considered in the following sections.

Case 1:

50 000 m^3/d 4 stage Bardenpho process with primary sedimentation and flow balancing.

This is taken as the base case from which cost differences will be estimated. Denitrification is included to restore some of the alkalinity lost in the nitrification process so that addition of an alkali is not required when metal salts are used to precipitate orthophosphate.

Case 2:

50 000 m^3/d 4 stage Bardenpho process with primary sedimentation, flow balancing and chemical addition ($FeCl_3$) for phosphate removal.

Case 3:

50 000 m^3/d 5 stage Bardenpho process with primary sedimentation and flow balancing.

This case assumes that the characteristics of the influent sewage are suitable for biological orthophosphate removal.

Case 4:

50 000 m^3/d Bardenpho process with primary sedimentation flow balancing and facilities to modify influent sewage characteristics.

Case 5:

50 000 m^3/d 5 stage Bardenpho process without primary sedimentation, with facilities to modify influent sewage characteristics.

Case 6:

50 000 m^3/d 2 stage biological filtration plant with chemical addition.

These cases are discussed in detail below.

9.3.2 Case 2:
50 000 m^3/d 4 stage Bardenpho process with primary sedimentation, flow balancing and chemical addition.

Phosphate Removal

Flow	50 000 m^3/d
Orthophosphate concentration	10 mg P/ℓ
Mass of phosphorus to be removed = 0,010 x 50 000	500 kg/d
Mass of Fe^{3+} required at 1,8:1 mass ratio = 1,8 x 500	900 kg/d
Annual cost of Fe^{3+} = 900 x 365 X 1,267	R416 200

Capital Costs

Chemical dosing equipment complete	R100 000

Operating Costs

Interest and redemption on capital (6% p.a.)	R 10 300
Electricity	R 1 000
Maintenance : 3% of R100 000	R 3 000

Chemicals	
Total annual operating costs	R416 200
	R430 500
ADDITIONAL UNIT COST OF CHEMICAL ADDITION:	2.36c/kl
Sludge Disposal	
Gravity Thickening	c/kℓ
See Appendix 9.1	0,173
Belt Presses	
See Appendix 9.2	
Cost of sludge disposal R318,43/day	0,637
TOTAL UNIT COST OF WASTE ACTIVATED SLUDGE DISPOSAL	0,810
Additional unit cost of chemical sludge disposal (based on 15% increase in sludge mass resulting from chemical addition)	0,109
TOTAL ADDITIONAL COST	2,47 c/kℓ

9.3.3 Case 3:

50 000 m³/d 5 stage Bardenpho process with primary sedimentation and flow balancing.

This involves the addition of an anaerobic zone to the 4 stage Bardenpho configuration.

Flow	50 000 m³/d
Volume of anaerobic zone with a nominal 1 hour retention time	2 083 m³
Energy density for mixing	10 W/m³
Power required for mixing (say)	20 kW

Cost of Anaerobic Zone

Capital Costs

Anaerobic zone including baffling	R180 000
Mechanical mixing equipment	R 30 000
	R210 000

Operating Costs

Interest and redemption on capital (6% p.a.)	R 18 300
Electricity	R 8 300
Maintenance : 1% of R210 000	R 2 100
Total Operating Costs	R 28 700

ADDITIONAL UNIT COST OF ANAEROBIC ZONE 0,157 c/kℓ

Sludge Disposal

It is assumed that the sludge from this plant configuration has an SVI of 150 mℓ/g; considerably poorer than that for the plant described in Case 1. The high SVI will result in additional cost being incurred in the disposal of sludge. (See Appendix 9.2). The cost premium incurred is estimated at R44,50/t dry sludge (DS).

Additional cost of this process is 6 440 x R44,50	R286,58/d
Additional unit cost of sludge dewatering	0,573 c/kℓ
TOTAL ADDITIONAL UNIT COST	0,730 c/kℓ

9.3.4 Case 4:
50 000 m^3/d 5 stage Bardenpho process with primary sedimentation, flow balancing and facilities to modify influent sewage characteristics.

Four methods are available to amend the characteristics of the sewage entering the biological reactor:

- the addition of primary sludge to the reactor, as and when required,
- the addition of supernatant liquor from an anaerobic digester operating in the acid phase,
- the recirculation of primary sludge through the primary sedimentation tanks to generate and elutriate fatty acids,
- elutriation of fatty acids from primary sludge via the supernatant liquor from sludge thickeners.

The latter two options are preferred as:

- they have proven more easy to control,
- some control is possible over the amount of additional readily biodegradable COD added,
- less particulate COD is added to the reactors requiring less electricity for aeration and producing less sludge.

For the purposes of this exercise only the case of primary sludge recirculation will be considered. The costs associated with elutriation via thickeners should be very similar to primary sludge recirculation.

Capital Costs

If pumps of sufficient size are available, the capital costs associated with the process are small involving only the alteration of existing pipework and possibly the laying of additional pipework.

The costs of primary sedimentation facilities are given in Case 5.

Effect on Operating Costs

Indications from plant monitoring at Northern Works supported by work at the University of Cape Town show that about 10 mg COD/ℓ of readily biodegradable COD (S_{bs}) is required to remove 1 mg o-P/ℓ.

For a fresh domestic sewage with an effluent orthophosphate concentration of 10 mg P/ℓ about 100 mg COD/ℓ of readily biodegradable COD would be required in addition to any S_{bs} required for the removal of extraneous nitrate and dissolved oxygen.

If it is assumed that there is 3 mg N/ℓ as nitrate and 3 mg/ℓ of dissolved oxygen in the return sludge, the additional S_{bs} required is 3(8,6+1) = 28,80 say 30 mg/ℓ, giving a total requirement for S_{bs} of 130 mg/ℓ while only 100 mg/ℓ is available for P removal.

Assume a domestic sewage of the following composition:

$$S_{ti} = S_{bpi} + S_{bsi} + S_{upi} + S_{usi}$$

where S_{ti}	= total influent COD	= 500 mg/ℓ.
S_{bpi}	= particulate biodegradable COD = 0,6 S_{ti}	= 300 mg/ℓ.
S_{bsi}	= readily biodegradable COD 0,2 S_{sti}	= 100 mg/ℓ.
S_{upi}	= particulate unbiodegradable COD = 0,13 S_{ti}	= 65 mg/ℓ.
S_{usi}	= soluble unbiodegradable COD =0,07 S_{ti}	= 35 mg/ℓ.

By sludge recirculation to the head of the primary sedimentation tanks and maintaining a sludge retention time of 4 days, approximately 50% of the particulate biodegradable COD can be converted to S_{bs}, producing about 150 mg/ℓ S_{bs} in the above case and an excess of 120 mg/ℓ S_{bs} over and above that required for P removal.

The additional S_{bs} added to the process will increase operating costs as a result of :-

- additional aeration requirements and
- greater sludge masses resulting in larger clarifiers and greater costs in the treatment and disposal of the sludge.

Thus any system intended to supplement S_{bs} must be designed to permit the minimum required mass of S_{bs} to be added to avoid additional operating costs, and possibly capital costs. The same principles apply in respect of each method proposed above.

Additional Aeration Equipment

Additional air is required only for the metabolism of excess S_{bs} added to the process.

$M(O_c) = (1-f_{cv}\ Y_h)\ M(S_{bi}) + f_{cv}\ (1-f)\ b_h\ M\ (X_a)$.

where :

f_{cv} = COD to VSS ratio of volatile sludge mass (1,48 mg COD/mg VSS)

f = unbiodegradable fraction of active mass (0 in this case))

Y_h = heterotrophic organism yield coefficient (0,45 mg VSS/mg COD)

S_{bi} = influent biodegradable COD concentration.

S_{ti} = influent total COD concentration.

b_h = endogenous mass loss rate for heterotrophic organisms at T°C

$= b_{h20}\ (1,029)^{T-20} = 0,24$ at 20 °C.

For all soluble biodegradable substrate; f = 0.

$$M(O_c) = M(S_{bi})\ (1-f_{cv}\ Y_h) + f_{cv}\ (1-f)\ b_h \frac{Y_h\ R_s}{(1 + b_h\ R_s)}$$

$$= M(S_{bi})\ (1 - 1,48 \times 0,45) + 1,48\ (1,0) \frac{0,24\ .0,45\ .15}{1 + 0,24\ .15}$$

$$= M(S_{bi})\ (0,334 + 0,521) = 0,855$$

$M(O_c) = 0,855\ M\ (S_{bi})$.

$M(S_{bi}) = 0,12 \times 50\ 000 = 6\ 000$ kg O_2/day.

Based on an oxygen transfer efficiency of 1 kg O_2/kWh (typical for mechanical surface aerators on the Witwatersrand), additional energy requirements are 6 000 kWh/day or 2 190 000 kWh/annum.

Additional Cost of Electricity

Demand charge :	278 x 365 x 0,4428	R 44 931
Energy charge :	2 190 000 x 0,0 271	R 59 349
		R104 288 say 105 000.
ADDITIONAL UNIT COST OF ELECTRICITY		0,575 c/kℓ

Sludge Disposal

Additional waste activated sludge (WAS) resulting from supplementary COD (at 0,28 g/g COD) = 0,28 x 0,120 x 50 000 = 1 680 kg/d.

This is assumed to be a high SVI sludge (150 mℓ/g) with the appropriate cost of dewatering and disposal given in Table 9.2.

Cost of sludge disposal = 1,68 x 87,30	R146,66 /day.
ADDITIONAL UNIT COST RESULTING FROM THIS SLUDGE	0,293 c/kℓ.

N.B. No account is taken of the cost savings resulting from the reduced mass of sludge that must be anaerobically digested.

TOTAL ADDITIONAL UNIT COST	0,868 c/kℓ.

9.3.5 Case 5:
50 000 m³/d 5 stage Bardenpho process without primary sedimentation with facilities to modify sewage characteristics.

In this case it is assumed that the original process had no primary sedimentation with the total sewage load being treated in the reactor. A primary sedimentation tank with sludge recirculation is added to the configuration to generate readily biodegradable COD, with both the sedimentation tank overflow and underflow being treated in the reactor.

The costs of chemical addition would be similar to those given for Case 2.

If the total COD load removed in primary sedimentation is not all returned to the reactor, it may be possible to increase the volumetric throughput of the plant at the additional cost of providing primary sludge treatment facilities. This alternative has not been analysed.

Capital Costs

Primary sedimentation tank and associated pipework and pump station	R485 000
Mechanical and electrical equipment	R110 000
Total capital costs	R595 000
	SAY R600 000

Operating Costs

Interest and redemption on capital (6% p.a.)	R 52 320

Electricity	R 2 290
Maintenance : 1% of R600 000	R 6 000
Total Operating Costs	R 60 610
TOTAL ADDITIONAL UNIT COST	0,332 c/kℓ.

9.3.6 Case 6:
50 000 m^3/d Two stage biological filtration plant with chemical addition.

The costs estimated in this section are for the retro fitting of chemical dosing equipment to a 2 stage biological filter plant. The following assumptions are made in this analysis:-

- the loading rate on the secondary humus tanks is acceptable as a result of polyelectrolyte addition prior to the tanks, and no additional secondary clarifiers are required;
- the chemical rich humus sludge is returned to the primary sedimentation tanks where it is thickened with the raw sludge prior to anaerobic digestion;
- no additional digestion capacity is required, the digesters being operated at a reduced solids retention time consistent with the greater mass and volume of sludge resulting from chemical addition;
- there is no recirculation;
- provision is made for both primary and secondary addition of chemicals for P removal; and
- lime addition is necessary to restore the alkalinity lost as a result of nitrification and chemical addition and to maintain a neutral pH.

Capital Costs

Chemical dosing equipment	R270 000
Lime dosing equipment	R184 000
TOTAL CAPITAL COSTS	R454 000

Operating Costs

Chemical addition		
Interest and redemption on capital costs (6% p.a.)		R 39 600
Chemicals :	Fe^{3+}	R1 017 400
(See Appendix 9.3)	Lime	R260 000
	Polyelectrolyte	R 45 600
Electricity		R 2 030
Maintenance : 3% of R454 000		R 13 620
TOTAL OPERATING COSTS		R1 378 250

ADDITIONAL UNIT COST 7,552 c/kℓ.

Sludge Disposal

Typically the addition of ferric ions for PO_4 precipitation in this type of plant results in an increase of approximately 30% in the sludge to be disposed of. The additional sludge is estimated at 3 600 kg/d.

Additional cost of sludge disposal based on irrigation to land at R10,00/t DS is :-

3 600 x 10 x 365	R 13 140/annum
	0,072 c/kℓ
TOTAL ADDITIONAL UNIT COST	7,624 c/kℓ.

9.4 SUMMARY OF COSTS FOR VARIOUS P REMOVAL OPTIONS

The estimated additional costs over and above the base case (Case 1) associated with the various options for removing phosphate are given in Table 9.1.

TABLE 9.1
COMPARISON OF COSTS FOR VARIOUS P REMOVAL OPTIONS

Case	Description	Total Additional Cost (c/kℓ)
1	Base Case	0
2	Case 1 + chemical addition	2,47
3	Case 1 + anaerobic zone	0,33
4	Case 3 + sewage character modification	1,60
5	Additional cost of primary sedimentation	0,33
6	Two stage biofiltration plus chemicals	7,62

9.5 REFERENCES

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APPENDIX 9.1

COST OF GRAVITY THICKENING

Flow = 50 000 m^3/d

Mass of sludge	: without chemicals	6 440 kg/d
	: with chemicals	7 440 kg/d

Capital Costs

Mechanicals	R130 000
Civils	R130 000
	R260 000

Operating Costs

Interest and redemption on capital (6% p.a.)	R 22 672
Electricity	R 234
Maintenance 1% of R260 000	R 2 600
Labour	R 6 000
	R 31 506

SAY R31 500 PER ANNUM

TOTAL UNIT COST : 0,173 c/kℓ

APPENDIX 9.2

COST OF ACTIVATED SLUDGE DEWATERING AND DISPOSAL

Operating experience of sludge thickening and dewatering on Johannesburg's works using DAF units and belt presses suggests that:-

- the float concentrations from a dissolved air flotation unit at equivalent loading rates depends on the sludge volume index (SVI) of the sludge, the approximate relationship being shown in Figure 9.2:-

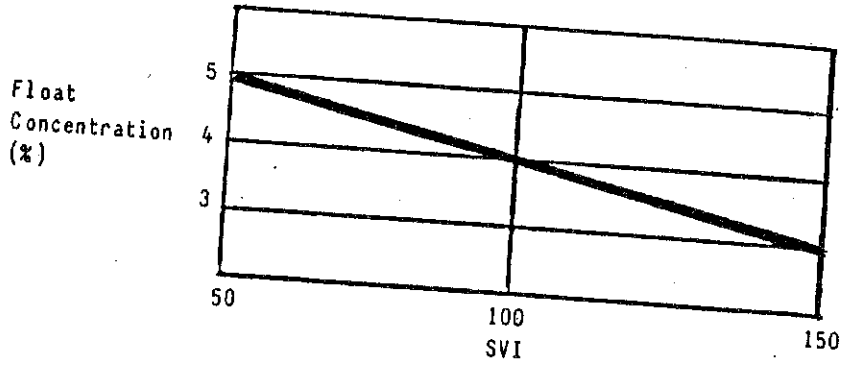

Figure 9.2: Effect of SVI on float concentration.

- the loading rate on belt presses is also dependent on the sludge SVI. For sludges with a high SVI the practical loading rate for maximum cake solids concentration is substantially lower than for low SVI sludges.

Experience suggests that for low SVI sludges (SVI ≈ 70-80) loading rates of up to 7 m^3/m width of belt can be handled on a belt press, whereas for high SVI sludges (SVI ≈ 150-200), loading rates must be limited to about 3,5 m^3/m belt width/h.

Waste sludge from an activated sludge plant with chemical addition to the reactor for P removal would be classed as a low SVI sludge.

The costs of sludge dewatering and disposal are estimated in Table 9.2.

TABLE 9.2
BELT PRESSING OF THICKENED WASTE ACTIVATED SLUDGE (WAS)

	COST FACTOR	COSTS	COST PER DAY	
		Independent of SVI	3,5 m³/m/h High SVI sludge	7 m³/m/h Low SVI Sludge
	SLUDGE DEWATERING			
Variable Costs	Chemicals (Poly: 2,4 kg/t DS (c/kg))	1,68		
	Make up water (0,2% solution) (c/kg)	0,09		
Fixed Costs	Electricity (1 300 kWh/d @ 0,047)		61,10	30,60
	Maintenance (3% x R1 054 000)		86,63	86,63
	Labour (75% of R205,20 per day)		153,90	153,90
	Interest & redemption on capital (R105 718 per annum)		289,64	289,64
	Belts (R10 300 p.a. per machine)		84,66	42,33
	SLUDGE DISPOSAL			
Variable Costs	Landfilling (c/kg)	0,10	-	-
Fixed Costs	Front-end loader (R193,60 per day)		193,60	193,60
	Truck (1x10t/day = R271,60 per day)		271,60	271,60
	TOTALS	1,87 c/kg	1 141,13	1 068,30
	Practical Loading Rate		3,5 m³/m/h	7,0 m³/m/h
	Concentration of thickened WAS		3%	4%
	Mass of sludge per day		16 632 kg	44 352 kg
	Unit Cost	1,87 c/kg	6,86 c/kg	2,41 c/kg
	TOTAL COST		1,87 + 6,86 = 8,73 c/kg or R87,30/t	1,87 + 2,41 = 4,28 c/kg or R42,80/t

APPENDIX 9.3

COST OF BIOLOGICAL FILTRATION WITH CHEMICAL ADDITION

PO_4 Precipitation

Assume: (a) 10 mg P/ℓ to be removed from the effluent.
(b) Molar ratio Fe:P = 2,5:1.
Mass ratio Fe:P = 4,5:1.

Mass of P = 50 000 x 0,01 = 500 kg/d.
Mass of Fe = 500 x 4,5 = 2 250 kg/d.
Annual cost of chemicals = 2 250 x 365 x R1,267 = R1 017 400

Polyelectrolyte

Assumed dosage rate of polyelectrolyte = 0,5 mg/ℓ.
Mass of polyelectrolyte = 50 000 x 0,0005 = 25 kg/d.
Annual cost = 25 x 365 x 5,00 = R45 600

Lime

By titration of a lime solution with ferric sulphate solution to pH = 7, 1t of lime is required to neutralize 4t of ferric sulphate.

Mass of ferric sulphate to precipitate PO_4 = 22,5 t/day.
Mass of lime required at 1t/4t $Fe_2(SO_4)_3$ = 5,63 t/day.
Cost of lime = R125,66.
Cost of lime per annum = 5,63 x 125,66 x 365 = R258 225
R260 000

CHAPTER TEN

Conclusions and Recommendations

10.1 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 >70 mg/ℓ as acetic acid and an S_{bs} >120 mg/ℓ, has been successfully treated in the Johannesburg five stage Bardenpho plants, to give an effluent having very low concentrations of both orthophosphate (<1,0 mg o-P/ℓ) and total nitrogen (<5,0 mg N/ℓ).

Precise quantification of the amount of S_{bs} required in relation to other pertinent parameters has not been attempted. It is believed that the University of Cape Town (UCT) mathematical model will ultimately provide the required information.

10.2 MODIFICATION OF INFLUENT SEWAGE CHARACTERISTICS

The production of volatile fatty acids either by fermentation in the primary sedimentation tanks, or by the fermentation of primary sludge in high rate anaerobic digesters operated in a batch mode, was examined. In both cases it was necessary to maintain an SRT of 3 to 4 days and to ensure that 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.

Where it is necessary to produce additional quantities of VFA's to improve biological phosphorus removal, this should be done by fermentation of sludge in the PST's. Optimal retention time of sludge is in the region of 3 to 4 days, but this may also be dependent on tank design, sewage sludge characteristics and temperature. Individual tanks should be **completely drained of sludge** at fixed intervals based on experience, in order to prevent methane producing bacteria gaining a foothold. Tank contents should not be allowed to turn black.

10.3 TRANSFER OF FERMENTATION PRODUCTS TO BARDENPHO PLANT

VFA's produced by the fermentation of sludge under hydraulically static conditions tend to remain adsorbed to the sludge and must be "washed" off or elutriated into the liquid phase, if they are to serve any useful purpose in the biological reactor. It is therefore recommended that designers take cognisance of the following elutriation techniques :-

10.3.1 Primary Sludge Accumulation and Recycle

The simplest form of elutriation is to recycle a portion of the accumulated sludge to the incoming sewage, as depicted in Figure 10.1. Under these conditions the volume of sludge collected is lower, but a lot more dense. Smaller digester capacity will be required and less sludge gas will be produced, when compared to non-recycle conditions.

If an additional boost in the mass of VFA's transferred to the bio-reactor is required during certain defined but limited periods, the PST's may be overloaded to transfer some particulate sludge to the settled sewage line, or as a last resort, the actual underflow sludge may be added to the bio-reactor, as indicated by the dotted line in Figure 10.1.

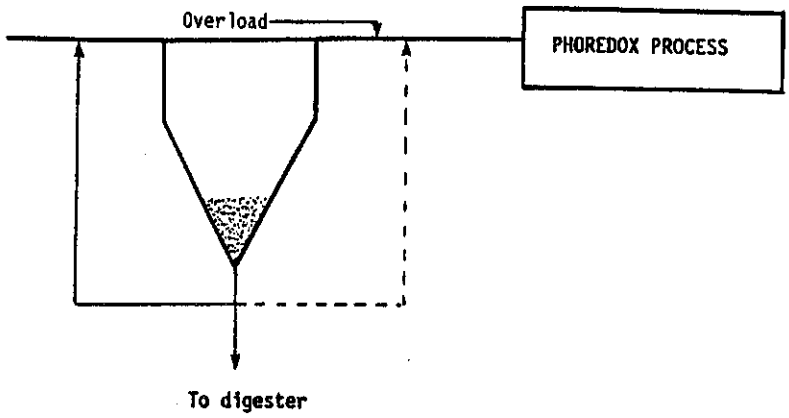

Figure 10.1 : Primary sludge accumulation and recycle

Such additions should desirably be made at times of low load as they result in an increase in oxygen demand and will ultimately build up increased levels of MLSS.

10.3.2 Primary Sludge Elutriation Incorporating a Thickener

In this option PST sludge with an SRT of 3 to 4 days is combined with a volume of elutriation liquid (settled sewage) and passed to a thickener (see Figure 10.2). The thickener overflow is rich in VFA's and is bled into the influent to the bio-reactor, and the underflow is a thickened sludge suitable for anaerobic stabilisation. Provision as under 10.3.1 can be made to return the thickened sludge to either the influent or effluent of the PST's, to cater for specific localised high demand periods for VFA's. As in 10.3.1 above, whole acid sludge would only be added to the process during periods of low incoming load.

It is further recommended that designers eliminate or minimise where possible, opportunities for oxygen to gain entry to the influent to the bio-reactor, thus preventing facultative anaerobes from utilising some of the S_{bs} prior to its entry to the anaerobic zone where it is required as a substrate for phosphorus accumulating bacteria.

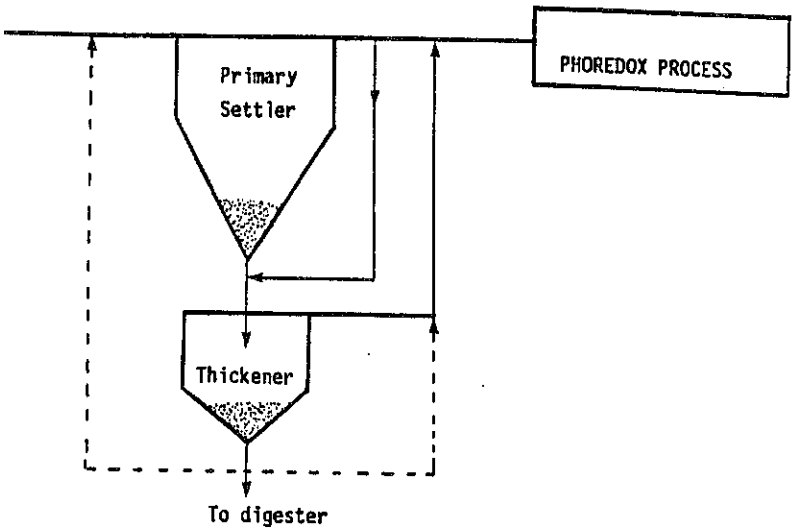

Figure 10.2 : Primary sludge elutriation incorporating a thickener.

It is recommended that the system detailed in 10.3.1 continue to be practised at the Northern Works and be extended to the Bushkoppie Works to gain further experience with this system under different climatic conditions, in particular, wet weather conditions with associated lower strength sewages. Proposals under 10.3.2 will be partially implemented in the near future when it becomes possible to commission sludge thickeners combined with newly constructed digesters. Limitation in Northern Works pump capacity prevents any elutriation of PST underflow with settled sewage prior to delivery to the thickeners.

10.4 DENITRIFICATION

Experience in Johannesburg (and elsewhere) has shown that when dealing with predominantly domestic sewages 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. However, the original anaerobic zone was enlarged 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. The successful operation of the plant under these coditions 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 bio-reactor. Under such circumstances, the concentration of denitrifiers is likely to be about double that present in conventional anoxic zones.

This modification appeared to confer additional stability to the biological removal of phosphorus and it is recommended that this process configuration be monitored for a further extensive period. It is proposed to make a similar modification to the three stage Bushkoppie Plant, which is based on diffused air aeration and a comparison of the performance of this works with the mechanically aerated three stage module of the Northern Works, should provide an insight into the effects of these different modes of aeration.

10.5 COMPARTMENTALISATION

This study indicates that compartmentalisation of the various zones, particularly at the head of the biological reactor, can benefit plant performance. Experience at the Northern (and Bushkoppie) Works has shown that partitioning of the anaerobic zone to encourage plug flow can increase the potential to release phosphorus and hence, to take up phosphorus again under aerobic conditions.

Recent work at UCT has shown that semi-plug flow conditions in the aerobic zones, improves phosphorus uptake. This has been tentatively corroborated at the Johannesburg plants, where there are indications that compartmentalisation of the aerobic zone improves both phosphate removal and nitrification, and also helps to suppress the growth of filamentous organisms.

Designers should take note of the apparent advantages to be gained by the introduction of plug flow conditions and incorporate this feature in future designs.

10.6 AERATION

It has been demonstrated that aeration has a marked effect on nutrient removal processes. Aeration levels should be such as 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 excess nitrification will occur and increase the denitrification requirements of the process.

Operators should refrain from the practice of switching off surface aerators to improve denitrification, as this is likely to impair phosphorus uptake and encourage the growth of filamentous organisms. Furthermore, plant designers should ensure that it is not possible for surface aerators to force highly aerated liquor into adjacent non-aerated zones.

Particular attention should be given to providing an unrestricted surface pathway across the entire biological reactor, including the anaerobic zone, to permit the free flow of scum to the final clarifiers for ultimate collection and removal.

If sludge age control is to be achieved by wasting sludge from the aerobic zones, designers must ensure that the withdrawal system can cope hydraulically with the increased volumes of liquor that are required to be discharged when a bulking sludge situation prevails.

10.7 COMPARISON OF THREE AND FIVE STAGE BARDENPHO PROCESSES

A reliable comparison of the three and five stage modules at the Northern Works was not possible at this stage, as modifications were being made to the final clarifier inlet systems of both units and operation at full load was not feasible. The presence of filamentous growths also increased operational problems. Moreover, with decreased load on the three zone Module 2, additional aerators were switched off to conserve power, thereby creating anoxic conditions in what should have been a totally aerobic zone. The presence of enhanced and compartmentalised anaerobic facilities in

Module 2 also invalidated direct comparison.

Nevertheless, it was very evident that provided the nitrates in the recycle stream to the anaerobic reactor could be controlled, the feed to both the processes had an overriding influence on biological phosphorus removal.

In future experiments, Module 2 will be operated with all aerators operational and the performance will be monitored with particular reference to the growth of filamentous organisms. Comparison with a diffused air oxygenated module at the Bushkoppie Works, where problems with filamentous growths are minimal, should also be enlightening.

10.8 ON-LINE MONITORING

Considerable delays have been experienced in the commissioning of this equipment, but initial results have provided some useful information, particularly with regard to oxygen utilisation rates.

It is recommended that further experience be gained with the existing equipment and that OUR measurements be extended to monitor the situation in the three stage Module 2 at the Northern Works. Interim indications are that this may be a better technique for plant control than those based on DO measurement. Designers should make provision for differential oxygen demand between the inlet and outlet of the aerobic zone, which occurs even in the presence of high MLSS recycle ratios.

10.9 FINAL CLARIFIERS

The success of Bardenpho type nutrient removing plants is critically dependent on the correct functioning of the final clarifiers.

The sludge settling characteristics are optimal when SVI values are low, i.e. <150 mg/ℓ. Experience has shown that high SVI's require high recycle ratios of return sludge, which adversely affects the operation of the anaerobic zone due to lowering of the anaerobic mass fraction, and the introduction of nitrate and dissolved oxygen into this zone. High SVI's have also been shown to adversely affect the downstream operation of sludge

thickening by flotation and belt pressing.

The various process configurations, for example, anaerobic selectors, experimented with at the Northern Works, have been shown to have very little effect on the sludge settling characteristics, suggesting that some external factors which may include temperature and oxygenation, appear to play a controlling role.

A better understanding of the factors controlling the growth of filamentous organisms, particularly Microthrix parvicella, is urgently required and it is recommended that the current research programme being carried out by UCT be accelerated.

It is urged that designers give increased attention to the method of collecting and removing settled activated sludge from secondary clarifiers, while bearing in mind the difficulties which have been experienced at the Northern Works. Provision should also be made to withdraw additional waste activated sludge from the clarifier underflow when necessary. Air entrapment in the return sludge en route to the anaerobic zone should be minimised.

10.10 FUNDAMENTAL STUDIES

10.10.1 Analytical Procedures

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 paramaters were successfully determined during the Contract, 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, polyhydroxybutyrate was successfully determined in mixed liquor.

Attempts to determine activated sludge energy levels by analysis for ATP were unsuccessful. Further work is required in this regard.

A satisfactory method for fractionation of intracellular phosphorus compounds has not been developed. Future fundamental studies will have to address this aspect.

10.10.2 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. Acinetobacter spp repeatedly dominated the phosphorus removing organism population in the aerobic zone under the test conditions applied.

The predominant acid producing organisms are Aeromonas punctata in primary sedimentation tanks and Klebsiella spp in acid digesters. The more efficient acid production observed in primary sedimentation tanks could well be linked to the type of organisms present. Further investigations into this aspect are required for optimum acid production.

The accumulation of phosphate in the form of polyphosphate is an integral part of successful biological phosphate removal. The determination of polyphosphate is a time-consuming task and has to date, not been undertaken successfully on activated sludge samples. Intracellular polyphosphate can however, be readily estimated by microscopic observation, after specific staining. This microscopic technique allows routine assessment of polyphosphate accumulation in activated sludge to be undertaken.

10.10.3 Bacterial Metabolism

A number of investigations into the cellular metabolism of Acinetobacter spp have allowed researchers at UCT, in collaboration with Johannesburg, to propose a biochemical model that explains the behaviour of Acinetobacter spp in enhanced biological phosphate removal activated sludge systems. In this model, two key parameters are identified in controlling polyphosphate and polyhydroxybutyrate synthesis and degradation, namely, the ATP/ADP and NADH/NAD ratios.

A number of hypotheses in the model require further experimental investigation. It is recommended that future research concentrates on elucidating enzymatic control systems which exist in Acinetobacter spp as this is crucial to unravelling the mechanism of biological phosphate removal.

While many of the fundamental studies described here are only exploratory in nature, it is clear that the study of enzymatic control mechanisms holds considerable promise for future research.

10.11 FINANCIAL ASPECTS

A 50 000 m^3/d four-stage Bardenpho process with primary sedimentation and flow balancing, was used as a base 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 phosphate removal by chemical addition would incur an additional cost of 2,47 c/kℓ.

In contrast to the above, the retro-fitting 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 amortised, might not be an economical proposition.

10.12 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 main-frame computer. During this study, the model equations have been successfully applied to a spread sheet, used in conjunction with a microcomputer.

The use of this technique has permitted the sophisticated UCT model to become available to wastewater plant management staff, allowing 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.

CHAPTER ELEVEN

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 of these appears below for easy reference.

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