ACTIVATED SLUDGE TREATMENT
OF DAIRY PROCESSING WASTEWATERS:

THE ROLE OF SELECTORS FOR THE
CONTROL OF SLUDGE BULKING.

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ABSTRACT

The typical wastewater from a milk processing facility producing butter and milk powder was treated in a modified activated sludge system in order to establish process characteristics and investigate operational problems.

A synthetic wastewater was developed with similar average physical and chemical characteristics to that from a full scale facility. The relative biodegradability of the wastewater fractions was assessed and basic microbial growth parameters also determined. A laboratory scale activated sludge reactor configuration was then established and its performance monitored. Although effective treatment was achieved in terms of suspended and soluble organic matter removal, the use of a completely mixed reactor resulted in the system becoming inoperable due to the excessive growth of filamentous microorganisms, with Type 0411 being the dominant filament.

In order to inhibit filamentous bulking, various selector reactor configurations were trialed. As nitrification of feed stream proteins had been indicated, unaerated selectors were used with the intention of effecting anoxic substrate removal in the initial selector zone; but due to the limited supply of oxidised nitrogen, insufficient substrate removal occurred in the selectors to prevent filamentous bulking, with Type 021N becoming dominant.

The next series of trials used aerated selectors, with some configurations demonstrating the ability to both prevent and cure filamentous bulking. The unsuccessful trials resulted in the proliferation of *Haliscomenobacter hydrossis*. From selector trials conducted it was established that the requirements for successful suppression of filamentous growth were the incorporation of an initial selector zone in which greater than 95% of removable soluble substrate was removed and the bulk solution was maintained in a fully aerobic state. Serial selector configurations demonstrated improved performance over a single selector.

From observations of the physical conditions and substrate concentrations in the reactor configurations employed, a correlation of filament type to environmental condition can be tentatively made: Types 0411 and 021N were indicated to be low organic loading type filaments, whereas *H. hydrossis* was indicated to be a low dissolved oxygen filament.

Rapid substrate removal rates were attributed to biosorption, accumulation and storage mechanisms, increasing as the selector configuration trials progressed. In general floc formers possessed a higher specific growth rate and substrate affinity than the filamentous microorganisms. Filament Type 021N was indicated to lack biosorptive capacity, however *H. hydrossis* was indicated as having a greater biosorptive capacity than the floc formers present.

The highly degradable nature of the substrate and high substrate concentration gradients imposed by the selector configuration caused rapid oxygen uptake rates; resulting in aerobic, anoxic and anaerobic substrate removal mechanisms all occurring in the initial selector zone. The occurrence of simultaneous nitrification, denitrification and phosphorus accumulation resulted in significant nutrient removals from the aerated selector reactor system, with influent nitrogen and phosphorus levels each reduced by up to 96% in the effluent stream.

This study found that an activated sludge process was an appropriate method for the effective treatment of milk processing wastewaters, as effluent suspended solids of less than 10 $g.m^{-3}$ and soluble COD of less than 30 $g.m^{-3}$ were consistently obtained, however a modified configuration would be required to prevent the growth of filamentous microorganisms and attendant operability problems. Due to the nature of dairy processing wastewaters, a selector reactor configuration could be employed not only to overcome potential filamentous bulking problems, but also to provide an opportunity for biological nutrient removal without the inclusion of dedicated anoxic / anaerobic reaction steps or the complex flow regimes conventionally employed for nutrient removal activated sludge systems.

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ABBREVIATIONS AND NOMENCLATURE

AS	Activated Sludge
ATU	Allylthiourea
BOD	Biochemical Oxygen Demand $(g.m^{-3})$
COD	Chemical Oxygen Demand $(g.m^{-3})$
DO	Dissolved Oxygen $(g.m^{-3})$
DRI	NZ Dairy Research Institute, Palmerston North, NZ.
DRP	Dissolved Reactive Phosphorus $(g.m^{-3})$
EBPR	Enhanced Biological Phosphorus Removal
HRT	Hydraulic Retention Time (d)
K_s	Substrate half saturation co-efficient $(g\ COD\ .m^{-3})$
μ_{max}	Maximum specific growth rate (d^{-1})
N	Nitrogen
NTU	Nephelometric Turbidity Units
OUR	Oxygen Uptake Rate $(g\ O_2\ .m^{-3}\ .\ min^{-1})$
O_x	COD content of biomass $(g\ COD.g\ TSS^{-1})$
PAO	Phosphorus Accumulating Organism
P	Phosphorus
RAS	Return Activated Sludge
RBCOD	Readily Biodegradable Chemical Oxygen Demand
sCOD	Soluble COD $(g\ COD\ .m^{-3})$. Defined as GFC filterable COD for this study.
S	Substrate concentration $(g\ COD\ .m^{-3})$
S_I	Soluble Inert substrate concentration $(g\ COD\ .m^{-3})$
S_H	Rapidly hydrolyseable substrate $(g\ COD\ .m^{-3})$
S_O	Initial substrate concentration $(g\ COD\ .m^{-3})$
S_S	Readily biodegradable substrate $(g\ COD\ .m^{-3})$
SMP	Soluble Microbial Products
SpOUR	Specific Oxygen Uptake Rate $(g\ O_2\ .\ g\ VSS^{-1}\ .\ d^{-1})$
SRT	Solids Retention Time (d)
SVI	Sludge Volume Index $(ml\ .\ g^{-1})$
TKN	Total Kjehldahl Nitrogen $(g\ .m^{-3})$
TN	Total Nitrogen $(g.m^{-3})$
TP	Total Phosphorus $(g.m^{-3})$
TS	Total Solids $(g.m^{-3})$

TSS   Total Suspended Solids $(g.m^{-3})$

VSS   Volatile Suspended Solids $(g.m^{-3})$

X   Biomass concentration $(g.m^{-3})$

X_I Particulate inert substrate concentration $(g\ COD\ .m^{-3})$

X_0 Initial biomass concentration $(g.m^{-3})$

X_S Slowly biodegradable substrate $(g\ COD\ .m^{-3})$

Y   Cell growth yield $(g\ cell\ mass\ .\ g\ substrate\ COD^{-1})$

Y_H Heterotrophic growth yield $(g\ cell\ COD\ .\ g\ substrate\ COD^{-1})$

CHAPTER 1

INTRODUCTION

1.1   Background

The dairy industry is one of New Zealand's major industries, contributing significantly to many local economies as well as being one of the country's largest export earners. The extent of the milk processing capability is still increasing with over 8 million m^3 of milk being handled each year, predominantly into products such as whole milk or milk component powders, cheese and butter.

The manufacture of dairy products results in an effluent stream comprised mainly of diluted milk or milk products, which can possess a considerable oxygen depleting effect if discharged into natural waters and therefore require treatment before disposal. As the continuing trend is toward fewer but larger milk processing installations, an efficient treatment system will be required for future facilities, which needs to be capable of handling large volumes of a wastewater that may be highly variable in both flowrate and composition.

Most New Zealand milk processing facilities are positioned at a location central to the milk supply and due to the requirement for a significant supply of fresh water, are usually positioned near a river. Discharge of wastewaters has traditionally been to natural waterway, or by irrigation on to land. The most important parameters for disposal into a natural waterway are removal of organic matter, suspended solids and nutrients, in particular nitrogen and phosphorus.

As dairy processing wastewaters are essentially readily biodegradable and non-toxic, they are amenable to biological treatment methods, however due to the move towards facilities with larger processing capacities, higher rate treatment systems are being sought due to the concurrently larger land area requirements if spray irrigation or aerated ponds are used.

One of the options for higher rate biological treatment is an activated sludge system, as these have been successfully used for many years to produce a high quality effluent from a wide range of wastewaters. Activated sludge systems are more compact than the

aerobic treatment systems already in use by the NZ dairy industry, and have the opportunity to be modified for a variety of treatment objectives including nutrient removal, which is of importance as the treatment requirements for dairy effluents are likely to include nitrogen and phosphorus removal in the future.

This study was undertaken to establish the treatability of milk processing wastewaters, such as that produced from a butter and milk powder production facility, in an activated sludge system, to assist in the evaluation of the appropriateness of such systems for future processing installations.

1.2   Approach taken in this study

In order to establish the important requirements for an effective activated sludge treatment system, a laboratory scale reactor was established for this study utilising a typical dairy processing wastewater.

The study was undertaken in stages, with results from each stage being used to determine the direction and focus of subsequent study. Chapter 2 reviews literature that has been published relevant to the milk processing industry on methods utilised for the treatment of its wastewaters. A review is also made of activated sludge treatment systems, including modifications used to address specific operability problems or treatment objectives, in particular the removal of nutrients and the prevention of filamentous bulking.

The methodology utilised for the various experiments conducted and parameters determined in this study is outlined in Chapter 3. The first series of experiments as detailed in Chapter 4, was conducted to devise and characterise a synthetic wastewater representative of the 'typical' effluent produced by a butter and milk powder production facility. Substrate characterisation was performed in a manner consistent with current research directions for activated sludge systems, to assist with future design or modelling information requirements.

Treatability of the wastewater was initially evaluated in a conventional activated sludge configuration as described in Chapter 5. Although a high quality wastewater was obtained, filamentous bulking problems were encountered which rendered the system inoperable. This has been found in practice to be a common operational problem encountered in the aerobic treatment of diary processing wastewaters using activated

sludge type systems. The next stage of the study therefore concentrated on the establishment of a reactor configuration which could prevent the growth of problematic microorganisms, and the determination of critical parameters for reactor design. The use of unaerated selector reactors in an attempt to prevent bulking is detailed in Chapter 6 and subsequent aerated selector trials are described in Chapter 7.

During the evaluation of various selector reactor configurations, it became evident that significant biological nutrient removal was also occurring. Although the removal of nitrogen was expected during the unaerated selector trials, the removal of phosphorus was not anticipated and the continuing improvement in nutrient removal under the fully aerated reactor configurations was also not expected. As a result, a subsequent set of trials at an increased level of substrate nitrogen content was conducted, as detailed in Chapter 8, to further investigate nutrient removal relationships and refine reactor requirements for the prevention of filamentous growth.

A comparison between results obtained for the different sets of trials in the study and an evaluation of wastewater treatability, substrate removal mechanisms and the extent of nutrient removal observed is given in Chapter 9; together with a discussion of implications for full scale systems and recommendations for areas of further research.

CHAPTER 2

DAIRY PROCESSING WASTEWATERS

AND THEIR TREATMENT

2.1   Introduction

The dairy industry is important to the economy of New Zealand and its continued success in the future will see new processing facilities being built or processing capacities of existing plants being expanded to handle the increased milk volumes becoming available. This will increase the requirements for an effective wastewater treatment method capable of efficiently treating the variable effluents typically produced by such facilities. The initial aim of the project was to investigate the feasability of using an activated sludge system for the treatment of dairy processing wastewaters.

Activated sludge treatment is a well proven technology, however operability problems are commonly reported. Many variations on the conventional activated sludge configuration have also been developed to enhance the removal of specific wastewater constituents, in particular nitrogen and phosphorus which can otherwise cause nutrient enrichment problems in receiving waters. However, the treatment conditions reported as being required for overcoming operability problems or enhancing nutrient removal are widely varied and in some cases, conflicting.

2.2   The Dairy Processing Industry

The processing of milk into a variety of dairy products is a significant activity in New Zealand, with a total of 8633 million m^3 of milk processed in 1994/95 (NZ Dairy Board, 1995). Milk production and hence milk processing is seasonal in nature, with products manufactured being destined predominantly for overseas markets. The main products include cheese, butter, milk powders and milk component powders.

Individual processing facilities tend to be very large, in 1995 there were a total of 32 manufacturing units, with the largest being a multi-product plant capable of processing 7000 $m^3.d^{-1}$ of milk (Russell, 1996). The trend over the past few decades has been towards fewer plants with a larger individual processing capacity (Barnett *et al.*, 1982; Marshall and Harper, 1984; NZ Dairy Board, 1995).

2.3   Dairy Processing Wastewaters

Dairy processing plants are large consumers of fresh water and producers of wastewater. Dairy processing effluents are comprised primarily of diluted milk, milk components or milk products (Carawan *et al.*, 1979; Barnett *et al.*, 1982; Marshall and Harper, 1984; Strydom *et al.*, 1993) and require some form of treatment before disposal.

2.3.1   Characteristics of Dairy Processing Wastewaters

As wastewaters contain predominantly water and diluted milk or milk products, the main components of milk are also the main components of the wastewater, however the composition may be highly variable, depending on the products manufactured at the given processing facility.

The main components of milk are water (85-87%), fat (3-7%), protein (3-5%), lactose (4-5%), minerals (0.7-0.8%), and vitamins. The milkfat exists as globules, forming an emulsion in the milk serum. Milk proteins are either caseins (80%) or whey proteins (20%) the former being present as micelles while the latter are soluble and therefore dissolved in the milk serum along with the lactose and minerals (Marshall and Harper, 1984; Walstra and Jenness, 1984).

Water is removed from milk during concentration or evaporation stages in the production of various milk products such as butter, cheese and milk powder, so the effluent contains not only process water but also milk derived water. The dairy processing industry is a large user of water, predominantly for washing and cooling purposes, with specific water consumption ratios (water consumed: raw milk processed) of 1.0 to 4.0 reported in NZ (Russell, 1996) and 1.4 to 9.6 in South Africa (Strydom *et al.*, 1993)

Main sources of wastewater constituents include: rinsings and washings from tanks, pipelines, processing equipment, vats and delivery tankers; carryover in separation processes; startup or shutdown residues, spills and leaks from the process and unusable by-products such as the condensate from evaporators and permeates from membrane plants. Other significant components of the wastewater may include detergents, sanitisers, lubricants and boiler or process water treatment chemicals (Carawan *et al.*, 1979; De Haast, 1984; Marshall and Harper, 1984).

The general characteristics of dairy processing wastewaters are: high organic content, as measured by wastewater biochemical oxygen demand (BOD) or chemical oxygen demand (COD); rapid biodegradability; low to negligible toxicity; potential for significant nitrogen contents due to milk proteins; a significant fat content; and high turbidity (Barnett *et* al., 1982; Marshall and Harper, 1984; Fang, 1990; Strydom *et al.*, 1993).

Whole milk has a BOD of approximately 100,000 $g.m^{-3}$ and COD of around 200,000 $g.m^{-3}$ (De Haast *et al.*, 1984; Marshall and Harper, 1984). Average COD values of the wastewaters from milk powder type processing plants range between 3500 $g.m^{-3}$ to 4000 $g.m^{-3}$ (Marshall and Harper, 1984; Strydom *et al.*, 1993); although one of the salient features of these wastewaters has been a highly variable composition and concentration (Jones, 1974; Barnett *et al.*, 1982; Marshall and Harper, 1984; Fang, 1990).

Due to the highly degradable nature of the wastewater, initial substrate removal is very rapid, with deoxygenation rates reported as being twice that observed for domestic wastewaters (Marshall and Harper, 1984), and in some instances in excess of the possible oxygenation rate (Adamse 1968c). Of the soluble components of the substrate, milk proteins are more slowly removed (Adamse 1968c; Jones, 1974); however of the total milk derived constituents, the insoluble milk fats are the most slowly degraded due to their size, but are still highly biodegradable (Marshall and Harper, 1984). The presence of fats have been associated with other problems in activated sludge systems such as foaming and the proliferation of filamentous microorganisms such as *Nocardia sp.* and *Microthrix parvicella* (Forster, 1992). Foaming has also been associated with the whey protein fraction of milk (Jones, 1974; Marshall and Harper, 1984).

As well as significant N contents of up to 190 $g.m^{-3}$ due to milk proteins, and in some cases due to nitric acid cleaners (IDF, 1985); dairy processing wastewaters have also been reported to have significant phosphorus contents, with values averaging 10 $g.m^{-3}$

being commonly reported (Marshall and Harper, 1984), ranging from 2 to 120 $g.m^{-3}$, and due mainly to the use of P containing detergents or boiler water treatment chemicals (Jones, 1974; IDF, 1985). The pH of the wastewater can vary widely and is mainly dependent on whether the cleaning compounds used are acid or alkali (Marshall and Harper, 1984).

2.3.2   Methods of Treatment for Dairy Processing Wastewaters

As wastewaters are derived primarily from milk components, the greatest gains in reducing the impacts of processing effluents have been through in-plant waste minimisation. However, due to technological, financial and product quality constraints, a waste stream is still currently produced; which must be treated prior to disposal into the receiving environment to remove organic, turbidity causing or nutrient containing constituents.

The reported methods commonly used for the treatment of dairy processing wastewaters include: discharge into municipal sewers (Carawan et al., 1979; Barnett et al., 1982; Elkin, 1984; Marshall and Harper, 1984; IDF, 1985; Fang, 1990; Strydom et al., 1993); irrigation onto land and pasture (Carawan et al., 1979; Barnett et al., 1982; Marshall and Harper, 1984; Strydom et al., 1993); chemical precipitation (Marshall and Harper, 1984; IDF 1985; Honer 1992); anaerobic biological treatment (Jones, 1974; Middlebrooks, 1979; Elkin, 1984; Marshall and Harper, 1984; Honer, 1992; Kilani, 1993); and aerobic biological treatment (IDF, 1985; Honer, 1992).

The most often reported means of aerobic treatment include: activated sludge (Jones, 1974; Middlebrooks, 1979; Elkin, 1984; Hung, 1984; Marshall and Harper, 1984; Fang, 1990; Fang 1991); trickling filters (Jones, 1974; Middlebrooks, 1979; Barnett et al., 1982; Elkin, 1984; Marshall and Harper, 1984); oxidation ditches (Marshall and Harper, 1984); aerated lagoons (Jones, 1974; Carawan et al., 1979; Middlebrooks, 1979; Barnett et al., 1982; Marshall and Harper, 1984) and rotating biological discs (Jones, 1974).

In New Zealand the treatment methods used generally include either chemical or biological treatment followed by discharge to land or to natural waterways. The various methods of treatment currently employed in NZ are listed in Table 2.1 (Russell, 1996) with some processing sites utilising more than one of the methods outlined.

For many years land based systems such as spray irrigation have been the favoured means of treatment in New Zealand, however this method is dependent on the availability of suitable land and favourable weather conditions to be effective. As processing sites become larger, irrigation schemes need to become correspondingly larger and hence more expensive to install and operate. Therefore treatment processes with a smaller land requirement may need to be considered.

Table 2.1 Dairy processing wastewater treatment methods employed in New Zealand.

Wastewater Treatment Method	No. of sites
Land disposal	4
Dissolved air flotation followed by land disposal	11
Dissolved air flotation followed by aerobic treatment	1
Aerobic biological treatment only	1
Anaerobic / aerobic biological treatment	1
Discharge to river or sea	11
Discharge to municipal treatment system	6
Application of high strength waste to land	14

The readily biodegradable nature of dairy processing wastewaters means that they are amenable to biological treatment and due to the high initial oxygen demand, high rate / short hydraulic residence time systems can be used, as long as a longer sludge age is employed to degrade the non - soluble milk derived material such as milk fat. Of the commonly employed aerobic biological treatment options, activated sludge requires the shortest hydraulic residence time and therefore a smaller space requirement than the currently used aerated lagoon configuration.

2.4   Activated Sludge Treatment

The activated sludge process has been used for many years, being first developed by Ardem and Lockett in England in 1914 (Metcalf & Eddy, 1991). Activated sludge (AS) treatment systems utilise a high concentration of suspended biomass in the form of flocculated microorganisms or 'flocs'. The conventional system configuration is comprised of an aeration tank followed by a settler for the gravity separation of biomass

flocs from the liquid to produce a clarified effluent. The flocs are recycled to the aeration tank to maintain elevated concentrations of biomass, generally resulting in a mixed liquor suspended solids level of between 1500 and 8000 $g.m^{-3}$ (Marshall and Harper, 1984; Metcalf and Eddy, 1991), which allows for more rapid biodegradation and a smaller sized treatment plant. This type of treatment process also allows for variation between the time spent by solid and liquid phases in the system.
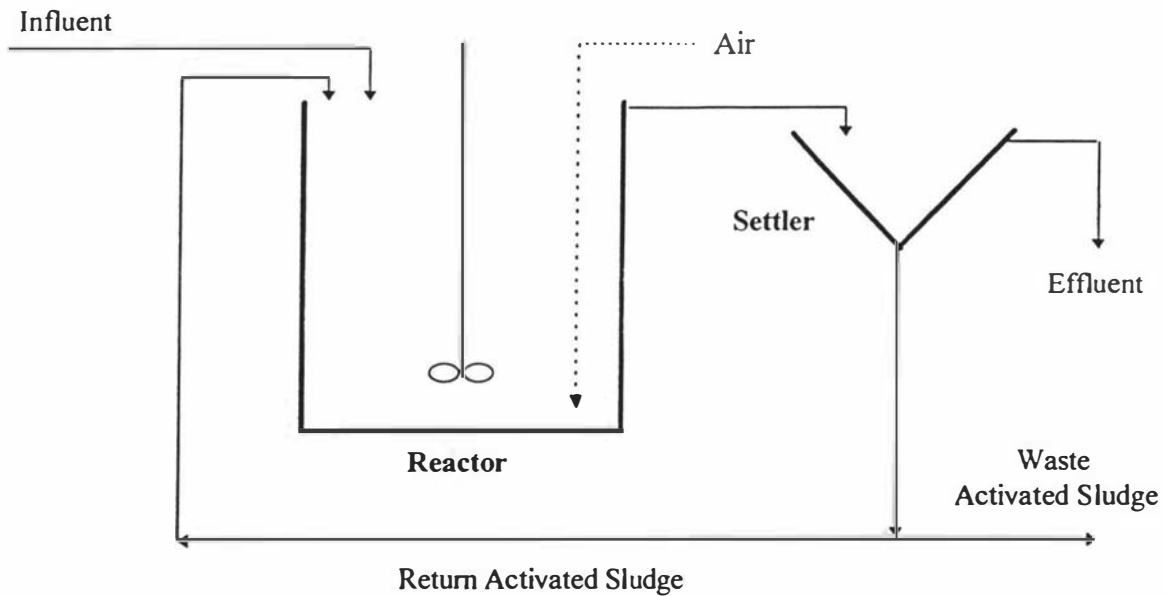
Figure 2.1:   Activated Sludge Process Schematic

The conventional AS system configuration has been modified in a variety of ways to either overcome problems such as poor solids settling due to sludge bulking, or to achieve additional or enhanced treatment performance, such as biological nutrient removal (Metcalf and Eddy, 1991). Types of modified AS configurations that have been utilised for the treatment of dairy processing wastewaters include: fill and draw, high rate, extended aeration, contact stabilisation and deep shaft (Marshall and Harper, 1984).

The use of activated sludge to treat dairy processing wastewaters has resulted in efficient removal of organic matter, with removals of up to 99% of the influent BOD being reported (Marshall and Harper, 1984; Fang, 1990); although Jones (1974) and Hung (1984) reported that effluent BOD concentrations of below 30 $g.m^{-3}$ were difficult to consistently achieve. Orhon et al. (1993) achieved similar results but found that the

effluent sCOD was due to the formation of soluble microbial products rather than residual undegraded substrate components.

2.4.1   Biomass Characteristics in Activated Sludge Systems

The operability of an activated sludge system is most often limited by the physical characteristics of the biomass, in particular sludge settleability. The biomass exists in a flocculated form in activated sludge systems, as cell aggregates connected by an exopolymer gel consisting of generally proteins, polysaccharides and DNA (Urbain *et al.*, 1993; Jorand *et al.*, 1995). The sludge settleability is related to the size and density of the flocs, which can be affected by biomass composition, filament abundance or the mass and type of polymeric substances present; and is generally measured as initial settling velocity (ISV); zone settling velocity (ZSV); or as sludge volume index (SVI) which is usually determined as diluted (DSVI) or stirred (SSVI) sludge volume indices (Hultman *et al.*, 1991; Catunda and van Haandel, 1992; Jenkins *et al.*, 1993).

Adamse (1968a) identified the main bacterial flora of an dairy processing wastewater activated sludge biomass to be coryneform bacteria, Psuedomonadaceae and Achromobacteraceae. The biomass had a brown / orange colour which was attributed mainly to the presence of *Flavobacterium sp.*

2.4.2   Substrate Characterisation for Activated Sludge Treatment Systems

Wastewaters to be treated by activated sludge type treatment systems are currently commonly characterised with respect to biodegradablity and microbial response rather than the chemical composition of its constituents. This form of characterisation was first proposed by Dold *et al.* (1980); and was further developed in computer modelling developments such as the IAWPRC Activated Sludge Model No.1 (Henze *et al.*, 1987) and IAWQ Activated Sludge Model No.2 (Henze *et al.*, 1995).

Initially four categories were proposed: soluble readily biodegradable (S_S); soluble inert (S_I); particulate slowly biodegradable (X_S); and particulate inert (X_I) material. However, this has since been further refined by dividing the degradable material into three fractions: S_S as before; rapidly hydrolysable (S_H) and slowly hydrolysable (X_S) material (Sollfrank and Gujer, 1991). The S_H fraction was previously included in the initial definition of X_S due to its degradability rather than its physical form. The S_S and

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S_H fractions are physically 'soluble' and the X_S 'insoluble' as defined by molecular size or filtration tests (Henze, 1992; Henze et al., 1994). The nitrogenous fractions of a wastewater have also been characterised due to biodegradability, using similar classifications as those adopted for carbonaceous substrate.

The types of compounds that constitute the various degradable fractions of a substrate have been outlined predominantly for municipal wastewaters (Henze, 1992; Henze et al., 1994), from a knowledge of the classes of compounds involved and their molecular weight. The S_S fraction is comprised predominantly of volatile fatty acids (VFAs); the S_H fraction of higher VFAs, simple carbohydrates, lower alcohols and amino acids; and the X_S fraction of higher carbohydrates, proteins and fats.

Some of the earlier methods proposed for the complete characterisation of a wastewater, as outlined in Ekama et al. (1986) and Henze et al. (1987) have since been considerably developed and refined. Many methods for determining the various biodegradability fractions of a wastewater have been proposed, as summarised by Henze (1992), consisting of either batch or continuous tests with the wastewater and acclimated biomass. The parameters that have been utilised for the determination of wastewater characteristics include: specific substrate components; soluble and total chemical oxygen demand (COD); oxygen uptake rate (OUR), specific oxygen uptake rate (SpOUR) and nitrate uptake rate (NUR).

2.5   Filamentous Bulking in Activated Sludge Systems

Although activated sludge treatment can result in a high quality effluent in terms of low levels of organic matter and suspended solids, there have been a very large number of reports of operational difficulties due to the proliferation of filamentous microorganisms and the subsequent interference with biomass settling and recycling in the system. Though a common problem, specific management strategies which prevent the growth of filaments have been difficult to define, due to the wide range of causative conditions proposed to result in their proliferation.

2.5.1   Causes of Filamentous Bulking

From the very large number of reports of filamentous bulking problems published, the most commonly proposed factors influencing the growth of microorganisms include:

- reactor configuration
- substrate type
- substrate concentration
- dissolved oxygen concentration
- availability of nutrients
- biomass age
- biomass concentration
- pH
- temperature

The extent of filamentous growth and effect on sludge settleability is measured as the Sludge Volume Index (SVI) of the mixed liquor, which is the volume that a unit mass of mixed liquor solids occupies after a defined settling period. A bulking sludge is characterised by a high SVI value, with SVIs of greater than 150 ml.g^{-1} being indicative of filamentous bulking, and increases in SVI having been found to reflect increases in filament abundance (Palm *et al.*, 1980; Strom and Jenkins, 1984; Surucu and Cetin, 1990; Jenkins *et al.*, 1993).

2.5.1.1   Influence of substrate on filamentous bulking

Both the substrate type and concentration have been proposed to influence the ability of filamentous microorganisms to proliferate in an activated sludge system. The influent carbonaceous substrate is generally a combination of soluble and particulate substrates, with the extent of each form present considered to have an influence on the tendency for a system to bulk (Wanner, 1994).

Low molecular weight substrates, such as simple sugars are readily biodegradable as they can be directly utilised by microorganisms. Wastewaters with a high carbohydrate substrate fraction are commonly reported to have resulted in bulking (Rensink, 1974; Chudoba, 1985a; Strom and Jenkins, 1984; Jenkins *et al.* 1993; Wanner, 1994); and dairy processing wastewaters or lactose containing substrates have often been specifically cited as being problematic (Adamse, 1968a; Adamse, 1968b; Jones, 1974; Rensink, 1974; Van den Eynde *et al.* 1982; Marshall and Harper, 1984; Strom and

Jenkins, 1984; Chudoba, 1985a; Goronszy *et al.* 1985; Hoffman, 1987; Rensink and Donker, 1990).

Higher molecular weight substrates are more slowly degraded and often particulate in nature, but have also been implicated in the growth of filamentous microorganisms. The modelling of filamentous bulking on particulate substrates usually assumes that the hydrolysis products of particulate substrates are released back into solution where they are available for consumption by filamentous organisms (Henze *et al.*, 1987). This implies that particulate substrate can be a supply of soluble substrate for the filaments via hydrolysis products (Wanner and Novak, 1990; Albertson, 1991; Wanner, 1994), which may result in bulking. As bulking is not always observed when the substrate has a significant fraction of particulate matter, a second theory proposes that the readily biodegradable hydrolysis products remain on the floc surface and are therefore not made available to filaments (Wentzel *et al.*, 1992); or that the availability of such products in bulk solution to filaments is limited (Gujer and Kappeler, 1992).

The origin of the wastewater will also influence availability of inorganic nutrients, particularly N and P, which have been known to affect bulking. It is not only the concentration, but also the form of nutrients present that is of importance (Jenkins *et al.*, 1993) as it is the fraction of bioavailable N and P that is critical. Adamse (1968a,c) proposed that the combination of a readily biodegradable C source in the form of lactose and less degradable N source in the form of milk proteins can cause problems.

The substrate concentration has also been indicated as affecting filament growth (Chudoba *et al.*, 1973b; Hoffman 1987; Albertson, 1991; Jenkins *et al.*, 1993). Filamentous bulking is commonly reported in systems with low F/M ratios, or in completely mixed AS systems which maintain a low substrate concentration in bulk solution at all times; although some CSTR systems have been reported that did not bulk below a critical substrate loading (Rensink, 1974; Lee *et al.*, 1982). It is the concentration of soluble degradable substrate in particular that has been found to be important, with a relationship commonly reported between SVI and the maximum concentration of sCOD that the biomass is exposed to (Chudoba *et al.*, 1973b; Chudoba, 1985a).

2.5.1.2   Influence of reactor conditions on filamentous bulking

The dissolved oxygen (DO) concentration in the various zones of a reactor system has been found to affect filamentous growth, with low DO often reported as promoting some filament types. Chudoba (1985a) proposed that the aeration tank DO must be kept above 2mg/l to keep the flocs aerobic, otherwise only the protruding filaments would see aerobic conditions. Palm *et al.* (1980) deduced that the required DO concentration depends on the substrate loading and listed various DO limits for a range of Food : Microorganism ratio (F/M) conditions. However, the presence of aerobic reactor conditions has not been found to be essential, as Wanner *et al.* (1987) ran an anoxic CSTR without an aerated stage which still maintained control over filament growth, although it did result in dispersed flocs. Higher organisms such as rotifers and cilitated protozoa also disappeared in the totally anoxic system.

The use of plug flow reactors or reactor configurations which result in a substrate gradient have been shown to result in a more well settling biomass than that from a completely mixed reactor receiving the same influent (Chudoba *et al.*, 1973a; Chudoba *et al.*, 1973b; Rensink, 1974; Azimi and Horan, 1991). Some authors have illustrated a relationship between the Dispersion Number through the system and the resultant biomass SVI (Chudoba *et al.*, 1973b; Salmeh and Malina, 1989). The use of a batch feeding regime can also be used to introduce a substrate gradient in a CSTR system and prevent filament growth (Rensink, 1974; Houtmeyers *et al.*, 1980; Van den Eynde *et al.*, 1983; Chiesa *et al.*, 1985; Goronszy *et al.*, 1985).

The use of a substrate gradient in the system exposes the floc to an initially higher substrate concentration, with lower SVIs resulting from higher substrate floc loadings in the first selector compartment (Chudoba *et al.*, 1973b). However, high floc loadings have also been found to result in the promotion of filament growth, with Chudoba *et al.* (1974) determining a loading limit of 0.5 g BOD .g $MLVSS^{-1}.d^{-1}$ above which bulking occurred.

2.5.1.3   Identification of Causative Filamentous Microorganisms

Due to difficulties in isolating and identifying individual bacterial species from the activated sludge biomass, filamentous microorganisms have been classified due to morphological characteristics and staining reactions in the mixed culture. This classification system was first proposed by Eikelboom (1977) and has been further

developed into comprehensive keys such as that stated in Jenkins *et al.* (1993) and WPCF (1990). Many of the filaments identified are classified by 'type number' rather than genus and species, and it has been found that the occurrence of a specific type can often be related to various operating conditions. Therefore identification of the causative organism may be of use in identifying a successful bulking control strategy.

Strom and Jenkins (1984) grouped the filaments according to growth environment as either low organic loading, low DO, or low inorganic nutrient; as well as summarizing those commonly due to various specified substrate types such as food processing, pulp/paper or chemical wastewaters. Further examination of filament growth requirements led Wanner and Grau (1989) to propose three classes of bulking microorganisms, which have been further refined to four classes by Jenkins *et al.* (1993) as listed in Table 2.2. Jenkins *et al.* (1993) also devised control strategies after categorising the main causes of bulking as either: nutrient deficiency; low DO; aeration basin configuration, or feeding strategy.

Filaments that have been specifically associated with dairy processing wastewaters include: Nocardia (Strom and Jenkins, 1984); Type 0092 (Eikelboom, 1977; Strom and Jenkins, 1984;); *Haliscomenobacter hydrossis* (Eikelboom, 1977); Type 021N (Eikelboom, 1977); Type 0041 (Eikelboom, 1977); *Sphaerotilius natans* (Adamse, 1968b; Van den Eynde *et al.*, 1982; Rensink and Donker, 1990); *Leucothrix sp.* (Van den Eynde *et al.*, 1984a); and *Nostocoida limicola* (Van den Eynde *et al.*, 1984a).

2.5.2   Control of Filamentous Bulking

The two main approaches to control filamentous bulking are to either inhibit all biomass growth in a non-specific manner, or to specifically suppress the growth of filamentous microorganisms. Non-specific control measures usually involve dosing the mixed liquor with a biocidal chemical such as chlorine or hydrogen peroxide (Jenkins *et al.*, 1993). By carefully controlling the dose rate, protruding filaments will be affected to a greater extent than the floc formers.

Specific control of filamentous bacteria involves altering the growth conditions in the reactor system so as to confer a selective advantage on the floc forming fraction of the population. This results in the bulking microorganisms being unable to compete for substrate, therefore filament abundance in the mixed liquor will decline and their future

proliferation will be prevented. Specific control is generally implemented by imposing carefully controlled environmental conditions for substrate removal.

Table 2.2: Categorisation of filaments as proposed by Jenkins *et al.* (1993):

	Requisite conditions	Control strategy	Major types
Group I: Low DO oxic zone growers - range of SRT - low DO - readily degradable substrate		- aerobic / anoxic or anaerobic selectors - increased DO - increased SRT	*S.natans,* Type 1701, *H.hydrossis*
Group II: Mixotrophic oxic zone growers - mid to high SRT - readily degradable substrate - nutrient deficient		- aerobic / anoxic or anaerobic selectors - increased nutrients	Type 021N, *Thiothrix* sp.
Group III: Other oxic zone growers - mid to high SRT - readily degradable substrate		- aerobic / anoxic or anaerobic selectors - reduced SRT	Type 1851, *N. limicola*
Group IV: Aerobic, Anoxic, Anaerobic zone growers - nutrient removal systems - high SRT		- stage the aerobic zone - maintain uniform DO in aerobic zone	Types 0041, 0675 and 0092, *M.parvicella*

2.5.2.1   Use of a Selector Reactor to Suppress Filamentous Organism Growth

From observations of bulking and non-bulking activated sludge systems, it has been proposed that in order to provide conditions that favour the growth of floc formers, a concentration gradient must be imposed. This may be accomplished by using an

intermittent feed regime, or in continuously fed systems by using a plug flow configuration or a selector reactor (Wanner, 1994). A 'selector' can be defined as the inlet part of the reactor system in which higher concentrations of substrate are maintained in order to support the growth of faster growing floc formers and to suppress the growth of slow growing filaments (Chudoba, 1985a).

The success of the concentration gradient in the prevention of filamentous growth has been attributed to filaments generally having a lower maximum specific growth rate (μ_{MAX}) and substrate half saturation co-efficient (K_S) than floc formers. This 'kinetic selection theory' was first presented by Chudoba *et al.* (1973b) and has been experimentally verified by a number of researchers (Van den Eynde *et al.*, 1983; Chudoba *et al.*, 1985a; Chiesa *et al.*, 1985; Daigger *et al.*, 1985; van Niekerk *et al.*, 1987b; Chudoba *et al.*, 1991). Selectors are therefore used to alter the F/M ratio, or substrate concentration experienced by the mixed liquor when it first comes into contact with the wastewater, providing an environment which can select for the growth of organisms which grow fastest at increased substrate concentrations.

The kinetic selection theory would explain the ability of filaments to dominate under conditions of low organic loading, however to explain the occurrence of bulking also at high organic loadings, Chiesa and Irvine (1985) proposed that μ_{MAX} and K_S of floc formers was DO dependent. They proposed that there was a third type of micro-organism - a fast growing, starvation susceptible filament which had a μ_{MAX} and K_S that was higher than that of the floc formers under low DO conditions. The kinetic selection theory is therefore useful in the inhibition of low F/M type filaments, although other types of filaments may require different strategies (Chudoba and Pujol, 1994).

Generally the propagation of filaments proceeds faster than their suppression (Chudoba *et al.*, 1973b; van Niekerk *et al.*, 1988) with selection of floc forming microorganisms and a return to non-bulking conditions commonly taking three SRTs or longer (Linne and Chiesa, 1987; van Niekerk *et al.*, 1988; Salameh and Malina, 1989; Foot, 1992; Pujol and Canler, 1994). The use of shorter SRTs also results in lower MLSS concentrations, and therefore generally higher substrate loading rates and bulk DO levels which favour floc formers. It was observed by van Niekerk *et al.* (1988) that the SVI continued to increase for up to 1 SRT after the installation of an appropriately sized selector, before the desired reduction was observed.

2.5.2.2   Requirements for selector type and configuration

Bulk conditions in the selector zone can be maintained in either an aerobic, anoxic or anaerobic state. A commonly reported selector type is an aerated selector (Chudoba *et al.*, 1973b; Daigger *et al.*, 1985; Daigger and Nicholson, 1990) and is termed a 'kinetic selector' due to the relative ability of different microorganisms present to rapidly accumulate and store substrate being the sole selection criteria.

Anoxic selectors have been used successfully to suppress bulking (Hoffman, 1987; Wanner *et al.*, 1987 Shao and Jenkins, 1989; Brenner and Argaman, 1990b; Foot, 1992); particularly that due to Type 021N and *S. natans*. Wanner *et al.* (1987) found that filamentous cultures had rates of nitrate utilisation that were an order of magnitude lower than non-filamentous mixed cultures and concluded along with others (Shao and Jenkins, 1989; Brenner and Argaman, 1990b) that some filamentous microorganisms did not have the ability to denitrify and were therefore unable to use substrate under anoxic conditions, or could only partially denitrify nitrate to nitrite (Shao and Jenkins, 1989; Casey *et al.*, 1994).

Casey *et al.* (1994) found that bulking in anoxic selector systems was related to nitrate and more particularly, nitrite concentrations leaving the anoxic selector zone. It was hypothesized that floc formers would be inhibited in subsequent aerobic stages by the presence of intracellular nitrification intermediates, while the filaments which could only denitrify to nitrite were not affected.

The use of anaerobic selector in a full scale application was reported by Daigger and Nicholson (1990) and on a laboratory scale by Wanner and Novak (1990). Daigger and Nicholson (1990) compared the performance of several full scale plants with differing selector types and concluded that rather than selector type, the success of a particular configuration was dependent on aeration in the main reactor and the extent of substrate storage as compared to oxidation in the selector zone. Both anoxic and anaerobic selectors are termed 'metabolic selectors' as they select for the ability of the microorganisms to denitrify or accumulate phosphorus respectively, in addition to the substrate affinity or 'kinetic' selection imposed due to the 'feast / famine' conditions (Jenkins *et al.*, 1993). The added advantage of these type of selectors is therefore the incorporation of a biological N or P removal step in the activated sludge process.

Even though the selector may be aerated and have appreciable DO in bulk solution, Albertson (1991) suggested that at high selector F/M ratios (>3kg/kg.d) floc centres are

likely to be devoid of either O_2 or NO_3 and therefore experience anoxic or anaerobic conditions respectively, a situation that was also proposed by Hoffman (1987). This could result in aerobic, anoxic and anaerobic metabolisms active simultaneously in various regions of the same reactor zone (Jenkins *et al.*, 1993).

The reactor configurations employed have been as varied as the selector types. The use of a single selector reactor has been commonly reported (Daigger and Nicholson, 1990; Foot, 1992; Pujol and Canler, 1994); however serial selector configurations have also been frequently utilised as this configuration would have a greater capacity to handle variations in the influent flowrate and substrate concentration (Van den Eynde *et al.*, 1984a; Chudoba *et al.*, 1973b; Daigger *et al.*, 1985; Hoffman, 1987; van Niekerk *et al.*, 1988; Linne *et al.*, 1989; Salmeh and Malina, 1989). The use of serial selectors is also more desirable for aerobic selectors, where kinetic mechanisms provide the sole basis for microbial selection (Jenkins *et al.*, 1993; Wanner, 1994), due to the ability of such a configuration to maintain a concentration gradient even under varying substrate conditions.

The residence time in the selector zone is relatively short, with residence times for aerobic selectors of 11 to 15 minutes commonly used (Daigger and Nicholson, 1990; Linne *et al.*, 1989; Daigger *et al.*, 1985), but of up to 25 minutes also reported (van Niekerk *et al.*, 1988). Longer residence times of between 25 and 40 minutes are generally employed for anoxic selectors (Shao and Jenkins, 1989; Foot, 1992), and durations of up to 99 minutes reported for anaerobic selectors (Daigger and Nicholson, 1990). Lee *et al.* (1982) proposed that the fraction of system volume in the selector zone should form the dominant design parameter, with V_{total} / $V_{selector}$ values of greater than 40 necessary for successful operation, however Wanner (1994) suggests that values of 10 should be sufficient.

2.5.2.3   Requirements for selector substrate concentration

To be effective the selectors must maintain a sharp concentration gradient in the system, imposing higher F/M ratios in the selector zone than in the main aerated reaction zone (Linne and Chiesa, 1987; Albertson, 1991); usually with a greater gradient being reported as providing better suppression of filamentous growth (Chudoba *et al.*, 1973b; Hoffman 1987). The substrate gradient also results in high oxygen uptake rates (OURs) in the initial selector zone (Chiesa *et al.*, 1985; Linne and Chiesa, 1987; Linne *et al.*,

1989); with values as high as 60 $mgO_2.gMLVSS^{-1}.h^{-1}$ being reported (Daigger $et\ al.$, 1985).

The substrate removal requirement for successful selector operation depends on the substrate type, with the most important fraction being the soluble readily biodegradable substrate. A considerable level of removal is required to prevent significant quantities of substrate entering the reactor zone, to prevent the growth of microorganisms with low μ_{MAX} and K_S values.

Some researchers have defined successful selector performance in terms of degradable COD concentration exiting the selector zone, with Chudoba $et\ al.$ (1985a) stating that this must be less than 30 $g.m^{-3}$ to give biomass SVI values of less than 100 $ml.g^{-1}$. Shao and Jenkins (1989) proposed a limit of less than 100 $g.m^{-3}$ sCOD, with an additional requirement of less than 1 $g.m^{-3}$ of readily biodegradable sCOD (RBCOD) leaving the selector zone.

Other researchers have stated limits in terms of fraction of substrate removal in the selector zone, with recommendations of the extent required for the successful suppression of bulking varying widely. Suggestions have ranged from at least 60% (Linne $et\ al.$, 1989; Daigger and Nicholson, 1990); to at least 80% (Foot, 1992); to greater than 90% of soluble organics removed in the selector zone (Linne and Chiesa, 1987).

The contact time in the selector must be sufficient to allow the required substrate removal to be achieved, with recommended contact times varying between 10 and 18 minutes in aerobic selectors; 10 to 30 minutes in anoxic selectors; and 30 to 60 minutes or longer in anaerobic selectors as summarised by Wanner (1994). Residence times in anoxic and anaerobic selectors may be greater than that necessary for substrate removal alone due to nutrient removal performance requirements. Due to the added metabolic selection pressures in these types of selectors, the contact time is less critical than for aerobic selectors which rely solely on kinetic selection (Jenkins $et\ al.$, 1993; Wanner, 1994).

Substrate loadings in the first selector are usually reported as a F/M ratio, with values of up to 7.2 $gBOD.gMLSS^{-1}.d^{-1}$ used (Pujol and Canler, 1994) although 3 to 5 $gBOD.gMLSS^{-1}.d^{-1}$ are generally recommended for design (Wanner, 1994). Lower selector F/M ratios, from 3 $gBOD.gMLSS^{-1}.d^{-1}$ (Foot, 1992) down to 0.7 $gBOD.gMLSS^{-1}.d^{-1}$ (Daigger and Nicholson, 1990) were possible when anoxic or

anaerobic selectors were used due to the additional selective pressures afforded by these systems.

The requirements for both high substrate concentration gradients and high substrate removal efficiencies in the same selector zone represent conflicting conditions, particularly when a single selector configuration is employed. Patoczka and Eckenfelder (1990 and 1991), developed a model for optimising selector design and suggested that the best recycle rate is that which results in a substrate concentration in the selector equal to 50% of the influent concentration. Wanner (1994) recommends a recycle rate of less than unity in order to maintain the necessary selective pressures.

2.5.2.4   Substrate removal mechanisms in Selector Reactor Configurations

Selector reactor configurations provide a zone of high floc loading followed by a longer period of aeration with little or no residual substrate in solution, resulting in different substrate removal mechanisms to those exhibited in CSTR configurations. Substrate removal by biosorption has been commonly documented (Marshall and Harper, 1985; Chiesa *et al.*, 1985; Goronszy and Eckenfelder, 1986; Bunch and Griffin, 1987; Hoffman, 1987; Kohno *et al.*, 1991; Rensink and Donker, 1991; Eckenfelder and Grau, 1992; Pujol and Canler, 1992) especially for modified activated sludge systems that incorporate selector reactors or intermittent feeding patterns. Substrate removal is proposed to involve adsorption onto the cell surface, transport across the cell wall, accumulation and conversion into storage compounds.

Evidence for substrate accumulation and storage has been obtained from monitoring the changes in substrate concentration and respiration rates in the selector zone. It has been found that substrate was removed from bulk solution sooner than the OUR had declined to a stable level (Chudoba *et al.*, 1973; Chudoba *et al.*, 1982; Chiesa *et al.*, 1985; Goronszy *et al.*, 1985; Shao and Jenkins, 1989). Linne *et al.* (1989) found that less than 20% of the system O_2 requirement was used in the selector zone indicating that substrate uptake and oxidation processes were not coupled, an effect also observed by Chudoba *et al.* (1991). Further evidence was reported by Van den Eynde *et al.* (1984b) who measured instantaneous substrate absorption amounting to 7 mg of glucose per g MLVSS.

The rate of substrate removal from bulk solution via biosorption has been observed as rapid, usually being completed within 5 to 15 minutes of contact with the biomass

(Chiesa *et al.*, 1985; Eckenfelder, 1987; Eckenfelder and Grau, 1992). The biosorption ability of biomass has been positively correlated to the substrate loading experienced (Rensink and Donker, 1991; Novak *et al.*, 1995) up to a maximum of 0.5 gCOD.gVSS^{-1} (Pujol and Canler, 1992).

The period of initial biosorption is followed by a period of first order substrate removal, although Grau *et al.* (1975) and van Niekerk *et al.* (1987a) propose that this is actually a 'pseudo' first order effect resulting from the summation of different zero order removal rates for each of the individual substrate components. Low SVI sludges have been shown to exhibit higher specific OURs and first order substrate removal rates (Houtmeyers *et al.*, 1980; Chiesa *et al.*, 1985; van Niekerk *et al.*, 1987b; Chudoba *et al.*, 1991). Chiesa *et al.* (1985) found that bulking sludges did not exhibit an initial period of biosorption, however Pujol and Canler (1992) detected filamentous populations exhibiting higher biosorptions than non-filamentous ones. Andreadakis and Chatjikonstantinou (1994) demonstrated similar substrate removal curves under anoxic conditions and concluded that similar substrate accumulation and storage effects occurred with nitrate as with oxygen as the electron acceptor.

Chudoba *et al.* (1982) proposed a model for substrate accumulation and storage, defining the accumulation capacity (AC) as the quantity of substrate which can be accumulated per unit weight of cells, and found that with glucose as a substrate, AC's 0.3 to 0.4 g/g could be measured. It was also proposed that the initial F/M ratio would indicate whether cell replication could be expected to occur. With systems having an initial F/M ratio below 2, the main synthesis products were accumulation and storage compounds, but if the F/M was greater than 2, then cell replication occurred before the removal of exogenous substrate was completed.

Requirements determined for substrate accumulation included the presence of a suitable concentration gradient and of a regeneration period for accumulation capacity (AC) restoration, with at least 50 % of the substrate removed needing to be oxidised if a low SVI was to result (Chudoba *et al.*, 1982). During the regeneration period accumulated substrate was oxidised and storage compounds produced, resulting in the regeneration of accumulation capacity, the extent of which could be tracked using respiration rate measurements. Drtil *et al.* (1993) determined that the regeneration phase may be equally accomplished under either aerobic or anoxic conditions. If the regeneration period was long enough to restore the ACs of all the species in the mixed culture, then the predominant species would be that with the highest capacity for and rate of substrate accumulation, and generally floc formers have been found to possess a higher AC than

filaments (Chudoba *et al.*, 1982; Van den Eynde *et al.*, 1983 and 1984b; Chudoba 1985a).

The period of aeration without exogenous substrate also aids in the selection against microorganisms without biosorption ability, as these species will starve in this 'famine' phase of feast / famine environments (Verachtert *et al.*, 1980). In general, filaments have been found to more susceptible to starvation than floc formers (Cheisa and Irvine, 1985; Chiesa *et al.*, 1985).

2.6   Measurement of Biokinetic Parameters in Activated Sludge

In order to characterise an activated sludge biomass and to design a full scale treatment system, estimated values for the kinetic parameters of biomass growth and decay need to be obtained.

Growth rates of microorganisms, including mixed cultures used in wastewater treatment are commonly described by Monod type kinetic expressions where the characteristic parameters are a maximum specific growth rate (μ_{MAX}) and substrate affinity in terms of a half saturation co-efficient (K_S). Methods for the measurement of parameters μ_{MAX} and K_S vary from direct measurement of microbial growth (Peil and Gaudy, 1971) to the use of surrogate growth measures such as oxygen consumption or substrate removal (Williamson, 1975; Cech *et al*, 1985; Gaudy *et al.*, 1987; Grady *et al.*, 1989; Kappeler and Gujer, 1992). The various means of determination have included both batch and continuous tests; monitoring changes in characteristics such as suspended solids, turbidity, dissolved oxygen and substrate components. Decay rates are generally determined using a batch test conducted over a period of several days (Marais and Ekama, 1976; Henze *et al.*, 1987).

As well as defining system design aspects such as solids residence time and sludge production rates, kinetic parameters are also of interest due to the variations that have been attributed to the dominant microorganism type present and reactor configuration imposed. The differences in μ_{MAX} and K_S between floc forming and filamentous bacteria has already been mentioned in Section 2.5.2.1. Differences in biomass decay rates have been observed for differing AS configurations, with Shao and Jenkins (1989) finding decay rates for selector systems lower than for comparable CSTR systems. Rates of $0.11d^{-1}$ were measured in conventional AS systems, with a lower rate of 0.063

d^{-1} recorded for biomass from biological nutrient removal (BNR) systems (McClintock *et al.*, 1993).

2.7 Nutrient Removal in Activated Sludge Systems

In conventional activated sludge reactor systems, nitrogen and phosphorus removal from the influent stream occurs only to the extent necessary to satisfy biomass growth requirements. Biomass N contents that have been measured in activated sludge systems include: an average of 9.8% of VSS during both conventional and BNR systems (McClintock *et al.*, 1993); and ranging between 8.7 and 10.2%, with an average of 9.7% in long SRT conventional AS experiments (Suwa *et al.*, 1992). These measured values are lower than the empirical value of 12.4% proposed in Metcalf and Eddy (1991). The normal P content of biomass is considered to range from 2 to 3% (Tetreault *et al.*, 1986; Yoeman *et al.*, 1988b).

2.7.1 Nitrogen removal in Activated Sludge Systems

The removal of substrate organic nitrogen in activated sludge is a staged process requiring a variety of microorganisms and environmental conditions. The steps involved include: ammonification, the conversion of organic nitrogen compounds such as proteins to ammonia; nitrification, the conversion of ammonia to nitrite and then nitrate; and denitrification, the conversion of nitrate to nitrogen gas. Activated sludge systems are able to be modified so that the entire sequence of reactions occurs within a single sludge system (Henze, 1991; Metalf and Eddy, 1991).

2.7.1.1 Ammonification

The first stage of nitrification, the conversion of organic nitrogenous materials to ammonia, is considered to be the rate limiting step in the reaction pathway and has been determined to be a first order reaction (Wong-Chong and Loehr, 1975) with an average rate constant at 20°C and pH 6.5 to 9.0 of 0.111 h^{-1}, reaching a maximum of 0.240 h^{-1} at pH 8.0. The rate of ammonification measured was dependent on both the concentration and form of organic nitrogen supplied.

2.7.1.2   Nitrification

Nitrification, the conversion of ammonia to nitrate, involves oxidation: firstly to nitrite by *Nitrosomonas sp.*; then to nitrate by *Nitrobacter sp.*, with the former reaction generally considered to be the rate limiting step (Wong-Chong and Loehr, 1975; Antoniou *et al.*, 1990; Stenstrom and Song, 1991) although some researchers have suggested otherwise (Munch *et al.*, 1996).

Oxygen is required for the conversion of ammonia to nitrate and nitrite, with the minimum DO levels recommended ranging between 0.5 and 2.5 $g.m^{-3}$ under steady state conditions, depending on mass transfer considerations in the flocs (Metcalf and Eddy, 1991; Stenstrom and Song, 1991). Munch *et al.* (1996) determined a considerably higher half saturation coefficient for nitrification of 4.5 g $DO.m^{-3}$; stating that this value would be a system constant and dependent on the floc size, mixing intensity and rate of oxygen diffusion into the flocs. Nowak *et al.* (1995) found a decrease in the nitrite oxidation capacity of activated sludge subjected to anaerobic conditions, whereas the ammonia oxidation capacity remained constant.

Autotrophic nitrifiers have been found to require more favourable conditions than heterotrophs, being less competitive under adverse conditions such as low DO (Stenstrom and Song, 1991). Temperatures of between 15 and 25 °C were found to best support the growth and activity of nitrifiers (Antoniou *et al.*, 1990). A sludge age of greater than 2.5 days also needed to be maintained to sustain a population of nitrifying bacteria at 20°C (Argaman and Brenner, 1986; Azimi and Horan, 1991), with the SRT requirement increasing at lower mixed liquor DO concentrations (Stenstrom and Song, 1991). Nitrification efficiencies have been found to be greater in plug flow or selector reactor configurations than in CSTRs (Chudoba *et al.*, 1985; Linne *et al.* 1989; Azimi and Horan, 1991) and for longer SRTs when DO is limited (Stenstrom and Song, 1991).

Both the ammonia and nitrite oxidation reactions have been determined to be zero order when the ammonia concentration is above 2 $g.m^{-3}$ (Wong-Chong and Loehr, 1975; Argaman and Brenner, 1986; Harremoes and Sinkjaer, 1995); the ammonia oxidation rate constant being a maximum at pH 7.5 to 7.8 (Wong-Chong and Loehr, 1975; Antoniou *et al.*, 1990), while the nitrite oxidation rate constant increased up to a pH of 9. The optimum pH for nitrification has been variously reported to be between 7.5 and 8.6 (Metcalf and Eddy, 1991); and between 7.0 and 8.2, with a maximum at 7.9 (Antoniou *et al.*, 1991). Alkalinity is required during nitrification, with values of 7.14

to 8.64 g alkalinity. g NH_3^{-1} reported (Argaman and Brenner, 1986; Metcalf and Eddy, 1991).

As the ammonium oxidation reaction is generally considered the rate limiting step, significant nitrite concentrations are not normally observed; however the combination of high pH and ammonium concentrations (Wong-Chong and Loehr, 1975; Azimi and Horan, 1991, Munch et al, 1996) or transient increases in the ammonia oxidation rate (Stenstrom and Song, 1991) have been suggested as being responsible for nitrite accumulation in solution. The inhibition effect of ammonia was observed to be less in reactors with a plug flow rather than a completely mixed reactor configuration (Chudoba et al., 1985) suggested as being due to differences in the distribution of nitrifiers and mass transfer resistances through the floc structure.

A range of nitrification rates have been measured in practise with Argaman and Brenner (1986) having recorded a specific nitrification rate of 2.3 $gN.gMLVSS^{-1}d^{-1}$; and Metcalf and Eddy (1991) of between 0.05 and 0.6 $gN.gMLVSS^{-1}d^{-1}$. Specific nitrification rates of between 1.3 and 7.8 $mgN.gMLVSS^{-1}h^{-1}$ were measured by McClintock et al. (1993), with the rate generally found to decrease as the SRT was increased.

2.7.1.3   Denitrification

The removal of N from activated sludge systems occurs via the conversion of oxidised N compounds to nitrogen gas. Denitrification proceeds in anoxic zones when a readily assimilable carbonaceous substrate is present, with specific bacteria being able to utilise nitrate as an electron acceptor during substrate removal, thereby reducing nitrate or nitrite present in solution. Henze (1991) reported typical effluent N concentrations of between 6 and 10 $gN.m^{-3}$ from biological N removal plants.

Denitrification occurs in activated sludge systems which incorporate anoxic zones. These conditions can be specifically provided for in modified activated sludge system configurations, or can arise in inadequately aerated reactors (Suwa et al., 1992), or non aerated zones such as the secondary clarifier (Cizinska et al., 1992; Henze et al., 1993; Siegrist and Gujer, 1994; Siegrist et al., 1995). Denitrification in the secondary clarifier can interfere with system operation by causing rising sludge, unless the effluent nitrate concentration is kept below 6 to 8 $gNO_3-N.m^{-3}$ (Henze et al., 1993; Siegrist and Gujer, 1994).

Oxygen has been found to exert a negative influence on denitrification, (Henze *et al.*, 1993); even at very low levels (Lie and Welander, 1994). However, denitrification in aerobic reactors has been recorded by Suwa *et al.* (1992) when the bulk liquid DO was maintained at above 3 $gO_2.m^{-3}$, with the extent of N removal increasing as the COD:N ratio was increased, due to the provision of anoxic regions within the flocs at higher readily biodegradable COD (RBCOD) concentrations. Munch *et al.* (1996) described the effect of oxygen on denitrification using a Monod type expression and determined half saturation co-efficients of between 0.4 and 0.67 g $DO.m^{-3}$. Denitrification also results in the release of alkalinity, with measurements of 3.57 g alkalinity. g NO_3 removed^{-1} being reported (Argaman and Brenner, 1986).

Denitrification requires the presence of a carbonaceous substrate and the main factors affecting N removal have been summarised as being the COD:N ratio, recycle ratio and aerobic volume fraction of the system (Brenner and Argaman, 1990a). The presence or addition of RBCOD has been found to result an immediate increase in the rate of denitrification, with the type of substrate also affecting the resultant rate (Clayton *et al.*, 1991; Carucci *et al.*, 1996; Henze *et al.*, 1994; Siegrist and Gujer, 1994; Isaacs and Henze, 1995). The mass of substrate required for denitrification has been measured as 6.0 to 6.7 g $sCOD.gNO_3-N^{-1}$ (Shao and Jenkins, 1989); and 7 to 8 $gCOD.gN^{-1}$ (Argaman and Brenner, 1986; Henze, 1991; Siegrist and Gujer, 1994; Wanner, 1994; Isaacs and Henze, 1995).

The denitrification reaction has been reported as zero order with respect to both carbon and nitrogen substrates (Suwa *et al.*, 1992; Carucci *et al.*, 1996); although Argaman and Brenner (1986) found that this was the case only when COD concentration was high, otherwise denitrification was first order and governed by the COD removal reaction. Clayton *et al.* (1991) found the denitrification rate to be proportional to the active fraction of the biomass, the rate remaining constant until RBCOD in bulk solution was consumed. Carucci *et al.* (1996) reported the use of endogenous or stored substrate for denitrification, though at considerably lower rates than when a readily degradable C source was supplied.

Denitrification rates reported include: up to 0.11 $gNO_3.gVSS^{-1}.d^{-1}$ (Argaman and Brenner, 1986); between 0.024 and 0.12 $gNO_3.gVSS^{-1}.d^{-1}$ on whey (Henze, 1991); 0.096 $gNO_3-N.gVSS^{-1}d^{-1}$ (Wanner, 1994); 0.075 to 0.115 $gNO_3-N.gVSS^{-1}d^{-1}$ at 20°C, with the denitrification rate decreasing as the sludge age increases (Metcalf and Eddy, 1991); and from 0.25 $gNO_3-N.gVSS^{-1}d^{-1}$ (Carucci *et al.*, 1996) up to 0.48 $gNO_3-N.gVSS^{-1}d^{-1}$ when readily biodegradable substrate was used (Henze *et al.*, 1994). Suwa

et al. (1992) recorded an endogenous denitrification rate of 0.0122 g oxidised $N.gVSS^{-1}d^{-1}$, while Carucci *et al.* (1996) measured a higher rate 0.07 $gNO_3-N.gVSS^{-1}d^{-1}$. Clayton *et al.* (1991) found increased rates of denitrification in combined N and P removal systems as compared to systems removing N only.

Although denitrification is said to require the absence of oxygen, while nitrification requires the presence of oxygen, simultaneous nitrification and denitrification in the same reactor zone has been suggested by Suwa *et al.* (1992), and by Szpyrkowicz and Zilio-Grandi (1995a) and Munch *et al.* (1996) during periods of low reactor DO. The main explanations given were that low DO conditions would allow the interior of the flocs to be anoxic while the exterior remains aerobic; or that dentrification enzymes were only gradually repressed under continued aeration rather than immediately inhibited by DO. Munch *et al.* (1996) recorded a maximum specific denitrification rate of 0.06 g $N.gVSS^{-1}d^{-1}$ under aerated conditions in a sequencing batch reactor, not dissimilar to the rates recorded by others under anoxic conditions.

2.7.2   Phosphorus Removal in Activated Sludge Systems.

Conventional activated sludge systems remove in the order of 10% to 40% of influent P for normal biomass metabolic requirements (Yeoman *et al.*, 1988a; Metcalf and Eddy, 1991), however additional phosphorus removal can be incorporated into activated sludge systems by using either chemical or biological removal processes.

Methods for chemical removal of P include precipitation, either with: calcium salts, usually lime, at a pH of between 8 and 11; iron (III) ions around pH 5.3; or aluminium ions, usually added as aluminium sulphate at around pH 6.3 (Yeoman *et al.*, 1988b; Metcalf and Eddy, 1991; Eckenfelder, 1989). Precipitation within the activated sludge system can also occur due to the presence of calcium and magnesium ions in solution at a pH of between 7.5 and 8.5. This P precipitation effect can be increased under favourable conditions including increased P concentration provided by anaerobic P release, or increased pH which can result from the occurrence of denitrification. (Tetreault *et al.*, 1986; Yeoman *et al.*, 1988b; Szpyrkowicz and Zilio-Grandi, 1995b)

The P content of activated sludge effluents can also be reduced by enhanced biological phosphorus removal (EBPR), due to the activity of phosphorus accumulating organisms (PAOs). It is generally considered that for biological P removal, the system must have alternating anaerobic and aerobic zones with a readily degradable substrate present in

the anaerobic zone. The PAOs can store low molecular weight fatty acids in the substrate as cellular polyhyroxybutyrate (PHB), causing a release of phosphorus into bulk solution, due to the degradation of stored polyphosphate (polyP) to provide energy for PHB storage (Comeau *et al.*, 1987). Under aerobic conditions the PAOs utilise the stored PHB and remove phosphorus from bulk solution as polyP is resynthesised. However recent studies (Knight *et al.*, 1995) indicate that synthesis of PHB and polyP can occur under a range of environmental conditions and that the synthesis of both may occur under the same conditions.

The bacteria responsible for such a metabolism are usually considered to be *Acinetobacter* species, and in plants with EBPR, it has been claimed that up to 50% of the heterotrophic biomass may be *Acinetobacter* species (Yoeman *et al.*, 1988b), generally found clumped together due to the presence of extracellular material. The ability of various *Acinetobacter* strains to accumulate polyP has been demonstrated to be highly variable (Knight *et al.*, 1995). Other researchers have found P accumulating bacteria other than *Acinetobacter* to be present (Yoeman *et al.*, 1988a,b), or dominant (Kavaugh and Randall, 1994; Szpyrkowicz and Zilio-Grandi, 1995b) in EBPR systems, including *Aeromonas*, *Pseudomonas* and *Klebsiella* species.

Conditions generally stated as being required for biological P removal include: an anaerobic zone followed by an aerobic zone; the initial anaerobic zone to be plug flow; anaerobic detention times of up to 12 hours or longer, although 1 to 2 hours is more commonly used; COD: P ratios of greater than 100:3; a pH of less than 6 and the absence of nitrates in the anaerobic zone; (Yoeman *et al.*, 1988b). Conditions required in the aerobic zone for optimum P uptake include: a pH of between 6 to 8 (Deinema *et al.*, 1985); a DO of between 2 to 6 $g.m^{-3}$; and a temperature of between 20 and 30 degrees C. (Tetreault *et al.*, 1986; Yoeman *et al.*, 1988a,b; McClintock *et al.*, 1993). System SRTs of greater than 5 days with an aerobic SRT greater than 2.7 days have been found to be successful, with a further decrease in SRT reducing P removal (McClintock *et al.*, 1993). Chudoba *et al.* (1974) found that plug flow configurations had a greater P demand than CSTR systems.

Several modified 'single sludge' activated sludge configurations have been developed to enhance the maintenance of conditions required for EBPR. The most common of these include the 'Bardenpho', 'UCT' and 'A/O' processes although numerous variants of these systems also exist (Tetreault *et al.*, 1986; Yoeman *et al.*, 1988b; Eckenfelder, 1989; Metcalf and Eddy, 1991; Wentzel *et al.*, 1992). All these systems feature alternating

anaerobic and aerobic zones, often with internal recycle to minimize the concentration of nitrates in the anaerobic zone.

Biological P removal occurs due to the luxury uptake of P by cells, transferring the P from influent substrate to mixed liquor biomass. Therefore P is removed from the system in the wasted biomass which has an elevated P content. An activated sludge biomass with more than 2% to 3% is generally considered to be due to luxury P uptake (Tetreault *et al.*, 1986; Yoeman *et al.*, 1988b) with biomass P contents as high as 11% having been reported (Appeldoorn *et al.*, 1992). The highest possible P content is dependent on the ratio of carbonaceous substrate to phosphorus in the influent, as shown by Tetreault *et al.* (1986) who illustrated a decrease in sludge P content as the influent sBOD:sTP ratio increased.

For efficient biological P removal and to obtain a final effluent with a low final P concentration, a relatively high COD:P ratio is required, reported as ranging from above 64:1 to above 100:1 (Tetreault *et al.*, 1986; Yoeman *et al*, 1988b; Szpyrkowicz and Zilio-Grandi, 1995a). The overall substrate requirement for P removal has been reported as 0.1 gP per g influent RBCOD (Clayton *et al.*, 1991) and P release in response to substrate uptake has been reported as 0.5 $gP.gCOD^{-1}$ (Mamais *et al.*, 1993; Isaacs and Henze, 1995) and 0.78 $gP.g Acetate^{-1}$ (Comeau *et al.*, 1987).

The preferred substrates for PAOs have been determined to be short-chain fatty acids, alcohols and lactic acids (Deinema *et al.*, 1985; Mino *et al.*, 1994; Knight *et al.*, 1995) and a high acetate:glucose ratio (Appeldoorn *et al.*, 1992). When comparing dissolved and particulate carbon sources, Jing *et al.* (1992) found more consistent and greater P removal when using glucose rather than starch, and that the type and cellular location of P storage compounds also differed with substrate type. Wanner and Novak (1990) also found improved EBPR with soluble rather than particulate substrates. Heymann and Potgieter (1989) found that sewage substrates that were supplemented with milk protein demonstrated improved P removals, although lactose was also present in that substrate. The anaerobic uptake of substrate in EBPR systems can also occur due to the presence of competing non poly-P accumulating bacteria, termed 'G bacteria' (Cech and Hartman, 1993), which predominated when glucose was present in addition to acetate. P release in the absence of readily degradable material has also been observed (Carlsson *et al.*, 1996)

There is conflicting evidence on the effect of the presence of oxidised N compounds on biological P removal. Previously it has been generally accepted that the presence of

oxidised N inhibits P release due to denitrifiers competing successfully with PAOs for substrate (Tetreault *et al.*,1986); or PAOs being unable to store substrate under anoxic conditions (Comeau *et al.*, 1987). Hoffman (1987) and Appeldoorn *et al.* (1992) also demonstrated a negative effect, recording a reduction in biomass P content when reactor nitrate and nitrite concentrations increased. However Isaacs and Henze (1995) and Chuang *et al.* (1996) reported P release under anoxic conditions, and Gerber *et al.* (1987) proposed that nitrate in the 'anaerobic' zone did not inhibit P release if the concentration of short-chain fatty acids was high enough.

Tetreault *et al.* (1986) found that for one particular operationally modified plant, the initial stage was possibly anoxic rather than anaerobic, but that P removal still occurred and suggested that if sufficient substrate was available (influent BOD:TP > 25) then simultaneous denitrification and P release could occur, with denitrification occurring outside the flocs and anaerobic conditions occurring inside the floc. However the effluent nitrate concentration was still positively correlated with effluent soluble P concentration.

Szpyrkowicz and Zilio-Grandi (1995a,b) found no influence of nitrate concentrations up to $8gN.m^{-3}$ on P removal and further concluded that as the bacterial culture had not changed during process start-up, that denitrifiers could also carry out biological P removal. P release was also observed in anoxic zones when substrate was still available in solution after a previous anaerobic stage (Isaacs *et al.*, 1995), with anoxic P release showing a greater dependence on the availability of substrate than denitrification rate.

Kerrn-Jespersen and Henze (1993), Barker and Dold (1996), and Sorm *et al.* (1996) reported that some P accumulating bacteria were able to utilise nitrate as well as oxygen as an oxidant, therefore indicating that the occurrence of P accumulation could be accompanied by denitrification; although Knight *et al.* (1995) found that *Acinetobacter* strains could only reduce nitrate to nitrite, not to nitrogen gas. The effect of denitrifying PAOs has also been included as a modification to the IAWQ Activated Sludge Model No.2 (Henze *et al.*, 1995) by Mino *et al.* (1995) and Isaacs *et al.* (1995), to account for observations of decreasing phosphate concentrations under anoxic conditions.

The majority of the research into nutrient removal in activated sludge systems has been progressed using domestic wastewater as a substrate, however the principles and meachanisms discussed would also have relevance to dairy processing wastewaters due to the biodegradability of carbonaceous substrates and similar origins of N and P compounds in the wastewater as compared to domestic sewage substrates.

2.8   Proposed project.

From the previous studies of both laboratory and full scale activated sludge systems, it can be concluded that the removal of substrate components including nutrients is highly dependent on the microbial species present in the activated sludge biomass, which in turn is dependent on the environmental conditions prevailing in the system. The effectiveness of a treatment system, particularly with respect to operability and nutrient removal was not easily defined and many of the reported findings conflict in their recommendations.

The objectives of the project were to define the critical operating parameters that would result in the effective treatment of dairy processing wastewaters, where 'effectiveness' of the system was assessed in terms of:

* removal of undesirable wastewater components: primarily carbonaceous substrate fractions (cabohydrates, proteins and fats); but preferably also removal of nutrients (nitrogen and phosphorus).
* production of an effluent low in turbidity and suspended solids.
* biomass stability and robustness, development of a population with desirable floc and hence sludge settleability characteristics.
* process operability, having a system configuration and operating strategy suitable for large scale use.

The definition of a successful treatment strategy for dairy processing wastewater therefore was to be established through a series of laboratory scale experiments with a typical effluent. The means and extent of removal of substrate components was to be monitored as well as the physical characteristics of the biomass, in order to define the requisite environmental conditions for an effective and operable treatment system.

CHAPTER 3

METHODOLOGY FOR THE ANALYSIS OF
TREATMENT SYSTEM PERFORMANCE

3.1   Introduction

This chapter describes the methods and materials that were used for the determination of parameters identified or measured in this study.   These methods were used to characterise the reactor system performance and biomass present under varying feed and reactor conditions.

3.2   Determination of Biomass Concentration.

The biomass concentration was measured as being equivalent to the volatile suspended solids (VSS) concentration of the mixed liquor suspended solids. There was some error associated with this assumption as not all the VSS present would have been due to active biomass. As well as inactive or dead cells, the VSS measurement could also include substrate derived material.   The substrate to be used contained suspended organic matter in the form of milkfat and milk proteins, which could be present in the mixed liquor either in bulk solution, adsorbed onto cell surfaces, or enmeshed in biomass flocs.

As there was no rapid method that could be routinely used to distinguish between cell derived VSS and substrate derived VSS, it was assumed for this study that the substrate contribution to mixed liquor VSS was small and that all the VSS could be attributed to biomass. This may have lead to an overestimation in the value used for biomass concentration, affecting both the performance data and biokinetic constants calculated, however results obtained during this study indicate that the above assumption did not introduce significant errors.

The types of solids determinations that were carried out during this study included total solids and suspended solids, both of the substrate and various reactor streams.

3.2.1 Determination of Total and Volatile Suspended Solids

The total suspended solids (TSS) and volatile suspended solids (VSS) were determined according to the procedure in '*Standard Methods*' (APHA 1992) Sections 2540D and 2540E. All analyses were performed in duplicate, with the average of the two values taken as the result.

Specific materials and equipment used were:

1. Analyses were carried out using glass fibre filters (Whatman, GFC, 4.7cm diameter filter circles).

2. Filters were prepared by placing in a NEY M525 Series II muffle furnace at 525 (+/- 25) $^\circ$C, for 20 minutes then cooled and stored in a desiccator containing silica gel (BDH, Poole, England) until needed.

3. Filters and dried residue were weighed on a Mettler AE200 balance to four decimal places.

4. Filters were dried at 104 (+/- 1) $^\circ$C in a Contherm Series Five oven, for between one and 24 hours.

5. Dried filters were cooled in a desiccator containing silica gel before being weighed.

6. For VSS analysis, filters were ignited in the muffle furnace at 525 (+/- 25) $^\circ$C, for one hour, or until a stable weight was obtained.

7. TSS and VSS were calculated as follows:

$$\text{TSS (g.m}^{-3}) = \frac{(B - A) \times 1000}{V} \qquad (3.1)$$

$$\text{VSS (g.m}^{-3}) = \frac{(B - C) \times 1000}{V} \qquad (3.2)$$

where:
A = Weight of filter (**mg**)
B = Weight of filter plus residue dried at 104°C (**mg**)

C = Weight of filter plus residue after ignition (mg)

V = volume of sample (ml)

3.2.2   Determination of Total Solids

The total solids (TS) of the substrate was determined according to the procedure in Section 2540B of '*Standard Methods*' (APHA 1992) using the same apparatus as outlined for TSS analysis.

3.3   Determination of Chemical Oxygen Demand

Chemical Oxygen Demand (COD) was determined using the closed reflux, colorimetric method as given in Section 5220D of '*Standard Methods*' (APHA 1992).

Both total (tCOD) and soluble (sCOD) Chemical Oxygen Demand determinations were carried out. Soluble COD was defined as the COD of a filtrate that had passed through a GFC grade filter. All analyses were performed in duplicate, with the average of the two values taken for the result.

COD analyses were conducted as follows:

1. A standard curve was prepared using potassium hydrogen phthalate standards in the range from 0 to 500 mg/l COD. Samples were diluted before analysis as necessary to give a final COD in the required range. Reactor and selector mixed liquor samples were usually analysed using a 1:20 dilution whereas effluent and filtered reactor samples were usually undiluted.

2. 2.5 ml of sample, 1.5 ml of digestion solution and 3.5 ml of catalyst solution were added to 16 x 100 mm Kimax culture tubes with teflon lined caps.

3. Samples were digested for 2 hours at 150 °C in a HACH COD Reactor of 25 tube capacity.

4. Absorbances of the cooled samples were read at 600nm on a Philips PU 8625 UV/VIS Spectrophotometer , using a glass cuvette with a 1cm path length. A reagent blank was used to zero the spectrophotometer.

5. COD was calculated as follows:

$$COD \ (g.m^{-3}) = A \ x \ F \ x \ D \qquad (3.3)$$

where:

A = absorbance at 600nm

F = conversion factor calculated from the calibration curve

D = dilution factor of the sample

3.4   Determination of Sludge Volume Index

The method used determining sludge volume was the Diluted Sludge Volume Index (DSVI) as described in Jenkins *et al.* 1993. This is essentially the same as the method outlined in '*Standard Methods*' (APHA 1992) Section 2710D, except that the mixed liquor was diluted so that the final settled sludge volume did not exceed 250 ml per litre of sample volume.

The method used was as follows:

1. Mixed liquor, or mixed liquor diluted to a 1/2, 1/4 or 1/8 dilution with tap water, was poured into a one litre measuring cylinder.

2. The measuring cylinder was inverted several times to ensure that the biomass was evenly distributed throughout the liquid volume, then allowed to stand undisturbed for a period of 30 minutes.

3. The volume of the cylinder occupied by sludge at the end of the settling period was recorded. If the settled sludge volume was more than 250 ml the test was repeated using greater dilution of the mixed liquor.

4. The SVI was then calculated as in Equation (3.4):

$$SVI \ (ml.g^{-1}) = \frac{S \ x \ 1000}{TSS \ x \ V} \qquad (3.4)$$

where:

S = volume occupied by sludge after settling $(ml.l^{-1})$

TSS = TSS of the mixed liquor $(g.l^{-1})$

V = volume of mixed liquor used in 1 litre total of sample settled (ml)

3.5   Determination of Dissolved Oxygen and Oxygen Uptake Rate

Dissolved Oxygen (DO) was measured using a Model 57 YSI Dissolved Oxygen Meter connected to a YSI Model 5750 BOD bottle probe that was fitted with a YSI standard membrane. The meter was air calibrated according to the manufacturers instructions.

The Oxygen Uptake Rate (OUR) was measured on the mixed liquor in the reactor and selectors, and also measured during various batch respirometric tests to determine kinetic parameters.

Measurements were made either in stirred 10 l bioreactor vessels, or in a 1 litre working volume respirometer placed on a Heidolph MR3001 magnetic stirrer. The reaction vessels are described in more detail in Section 3.13 of this chapter.

The DO meter was connected to a Sekonic SS250F Chart Recorder. The recorder range was set at 0.1mV and chart speed at 600 mm/hr. The DO meter was set at the 0-10 mg/l range scale, which corresponded on the chart to a width of 30.5mm per mg DO.

The OUR was calculated from the slope of the line recorded on the chart:

$$\text{OUR } (gO_2.m^{-3}.s^{-1}) = \frac{\Delta \text{ DO } (gO_2.m^{-3})}{\text{time elapsed (s)}} \qquad (3.5)$$

Both total OUR and carbonaceous OUR measurements were made. In the heterotrophic bacteria OUR determinations, nitrifying bacteria were inhibited with allylthiourea (BDH, Poole, England) at a concentration of approximately 1 $g.m^{-3}$ as recommended by Cech *et al.* (1984).

3.6   Determination of pH

pH measurements on samples from the reactors were made using a Radiometer PHM61 Laboratory pH meter fitted with a Philips CE 50 electrode.

The pH meter was calibrated before use at pH 7 and pH 4 or pH 10 as appropriate, with colour key buffer solutions (BDH, Poole, England).

3.7   Determination of Nitrogen Concentrations

The nitrogen compounds of interest were ammonia, nitrate, nitrite and total Kjeldahl nitrogen (TKN). NH_3, NO_3 and NO_2 were measured on GFC filtered samples, while TKN was measured on the whole sample. The methods used for the determination of each nitrogenous compound follow.

3.7.1   Ammonia

Ammonia was analysed using a Technicon Autoanalyser (Technicon (Ireland) Ltd, Dublin) continuous flow analytical instrument using a modification of the automated phenate method as outlined in Section 4500-NH_3-H of '*Standard Methods*' (APHA, 1992). The absorbance of the solutions was detected in the Technicon colorimeter fitted with a 660 nm filter and recorded as a peak on a Technicon chart recorder.

Standards of 0, 0.25, 0.5, 1.0, 2.0, 2,5 and 3.0 $g.m^{-3}$ ammonia were analysed to construct a calibration curve of peak height versus ammonia concentration.

Samples were diluted with deionised water as necessary to give peak heights in the required range.

3.7.2   Nitrate

Nitrate was analysed at the same time as ammonia on the Technicon Autoanalyser. An automated hydrazine reduction method as outlined in Section 4500-NO_3- H of '*Standard Methods*' (APHA 1992) was used, the absorbance of the resultant solution being measured at 520 nm and recorded on the chart recorder at the same time as the ammonia results.

A range of nitrate standards from 0 to 3.0 $g.m^{-3}$ was used to calculate nitrate concentrations in the same manner as for the ammonia determination. Samples were diluted as necessary prior to analysis with deionised water to give peaks in the required range.

3.7.3   Nitrite

Nitrite concentration was determined using a HACH DR/2000 Direct Reading Spectrophotometer. The nitrite determination performed was Method No. 371 in the DR/2000 Handbook, which is a diazotisation method.

One HACH Nitriver 3 reagent powder pillow was added to 25 ml of filtered sample. The sample was mixed to dissolve the reagents then allowed to stand for 15 minutes before reading the absorbance at 507 nm.

The spectrophotometer uses a preprogrammed calibration curve and reports the result as $g.m^{-3}$ NO_2^--N. The instrument calibration was checked using a standard nitrite solution as outlined in the DR/2000 Handbook.

3.7.4   Total Kjeldahl Nitrogen

TKN was determined using a micro Kjehldahl method as follows.

1. A 5 ml sample of mixed liquor or 50 ml sample of whole effluent measured into a 100ml digestion tube, acidified with 1 drop of concentrated H_2SO_4 then dried overnight in a Contherm Series Five oven at 105 °C.

2. 4 ml of digest acid containing concentrated H_2SO_4, K_2SO_4 and selenium powder, was added to the dried sample in the digestion tube, then the tube heated in a digestion block at 350 °C for four hours or until the solution had become colourless.

3. The digested sample was cooled to room temperature, then made up to 50 ml with deionised water. A Labline Instruments Inc. (Illinois, USA) Supermixer No.1291 vortex mixer was used to ensure that the resultant solution was well mixed.

The TKN content of the sample was then analysed using a Technicon Autoanalyser continuous flow analytical instrument. The method used was a modified phenate method with colorimetric determination at 630 nm by a Technicon Autoanalyser II colorimeter. The resultant absorbance was measured as a peak on a Technicon Chart Recorder. Standard solutions in the range from 5 to 150 $g.m^{-3}$ N were analysed so that a calibration curve relating peak height to TKN could be constructed.

3.8   Determination of Phosphorus Concentrations

Two different phosphorus analyses were performed: Total Phosphorus and Dissolved Reactive Phosphorus. Both sets of results are reported as $g.m^{-3}$ P.

3.8.1   Total Phosphorus (TP)

The TP was analysed on digested samples at the same time as the TKN. P concentration was measured by the Technicon Autoanalyser using a modified vanadomolybdophosphoric acid method with colorimeters at 400nm. Standards in the range of 0.5 to 15 $g.m^{-3}$ P were analysed to provide a linear calibration curve, from which measured sample peak height could be converted to P concentration.

3.8.2   Dissolved Reactive Phosphorus (DRP)

DRP was determined primarily using the Vanadomolybdophosphoric Acid Colorimetric method as outlined in Section 4500-P C. of *Standard Methods* (APHA 1992). The HACH DR/2000 Direct Reading Spectrophotometer was used for a small number of analyses. Similar results to the standard method were obtained using the DR/2000 spectrophotometer.

3.8.2.1   DRP determination by the Vanadomolybdophosphoric Acid Method.

1. The sample was filtered using Whatman GFC filter paper prior to analysis.

2. 18 ml of undiluted sample was pipetted into a 25 ml volumetric flask, 5ml of vanadate reagent was added, then the flask made up to the mark with deionised water. The sample was inverted several times to mix then allowed to stand for at least 10 minutes.

3. The absorbance of the resultant solution at 400nm was measured on a Shimadzu UV-1201, UV-Vis Spectrophotometer. A reagent blank was used to zero the instrument.

4. A calibration curve was constructed in the range of 0 to 18 $g.m^{-3}$ P using an anhydrous KH_2PO_4 standard solution.

3.8.2.2   DRP determination using the HACH DR/2000 Spectrophotometer

1. The amino acid method, No. 485 in the DR/2000 Manual was used. The sample was filtered using Whatman GFC filter paper prior to analysis.

2. Then 1ml of HACH amino acid reagent and 1 ml of HACH molybdate reagent was added to 25 ml of sample. The sample was inverted several times to mix then allowed to stand for at least 10 minutes.

3. The absorbance of the resultant solution was measured on the DR/2000 Spectrophotometer at 530nm. A reagent blank was used to zero the instrument.

4. The result was reported in $g.m^{-3}$ PO_4 which was converted to $g.m^{-3}$ P by dividing the PO_4 value by 3.07.

3.9   Determination of Biomass Floc Morphology

The physical appearance of the biomass in the mixed liquor was examined microscopically as outlined in Jenkins *et al.*, (1993). Observations included: floc size and morphology; abundance of filaments; presence of other significant microorganisms and reaction to various staining procedures.

Most observations were made at 100x magnification on a Olympus BH2 light microscope with DPlan objective lenses. Photographs were made using a Leitz Ortholux II microscope with an Leitz manual camera and Kodak 35mm Technical pan film. Stained preparations were viewed at 1000x on one of the above microscopes. Phase contrast observations were made on a Nikon Optiphot microscope.

Filamentous microorganism identification was carried out using the morphology and staining techniques and identification keys outlined in Jenkins *et al.*, (1993) and LaTrobe University (1993). The microbial stain solutions required were prepared as described in Jenkins *et al.*, (1993) or in Harrigan and McCance (1966).

3.10   Determination of Lactose Concentration

Lactose concentration was measured using a Waters HPLC consisting of: a Model 590

pump and controller; a Model R401 differential refractometer and a Model 740 data module. The HPLC was fitted with a Waters Sugar-Pak column and used an aqueous mobile phase containing 50 g.m^{-3} CaEDTA . A set of standard solutions containing from 100 to 1000 g.m^{-3} lactose was used to construct a standard curve relating peak area to lactose concentration.

3.11   Determination of Turbidity

Turbidity of the reactor feed solution was measured using a HACH 2100P Turbidimeter, calibrated with manufacturer supplied standards.

3.12   Determination of DNA Content

Total DNA content of the activated sludge mixed liquor was measured using the method outlined in Marshall *et al.*, (1981).

3.13   Reactor Vessels

Three main types of reaction vessel were used in this study: a 10 litre working volume completely mixed reactor with external settler, a 6.25 l working volume completely mixed reactor with an internal settler and a 1 litre working volume respirometer.

3.13.1   Respirometer Design

The 'respirometer' used for many batch tests and kinetic parameter estimations consisted of a cylindrical 0.1m internal diameter perspex vessel with a working volume of 1 litre as detailed in Figure 3.1 and based on the design described by Cech *et al.* (1984). These respirometers were constructed specifically for this use in the Department of Process and Environmental Technology (PET) workshop, Massey University.

Constant temperature conditions were maintained by placing the respirometer in a waterbath at the desired temperature, or placing the stirred respirometer on a Heidolph MR 3001 heated plate magnetic stirrer.

Top view

Port for DO probe

ort for thermometer

Port for substrate addition
and for air supply line

Side view

O-ring seal
between lid and
body of
'respirometer'

Internal   wall
baffles
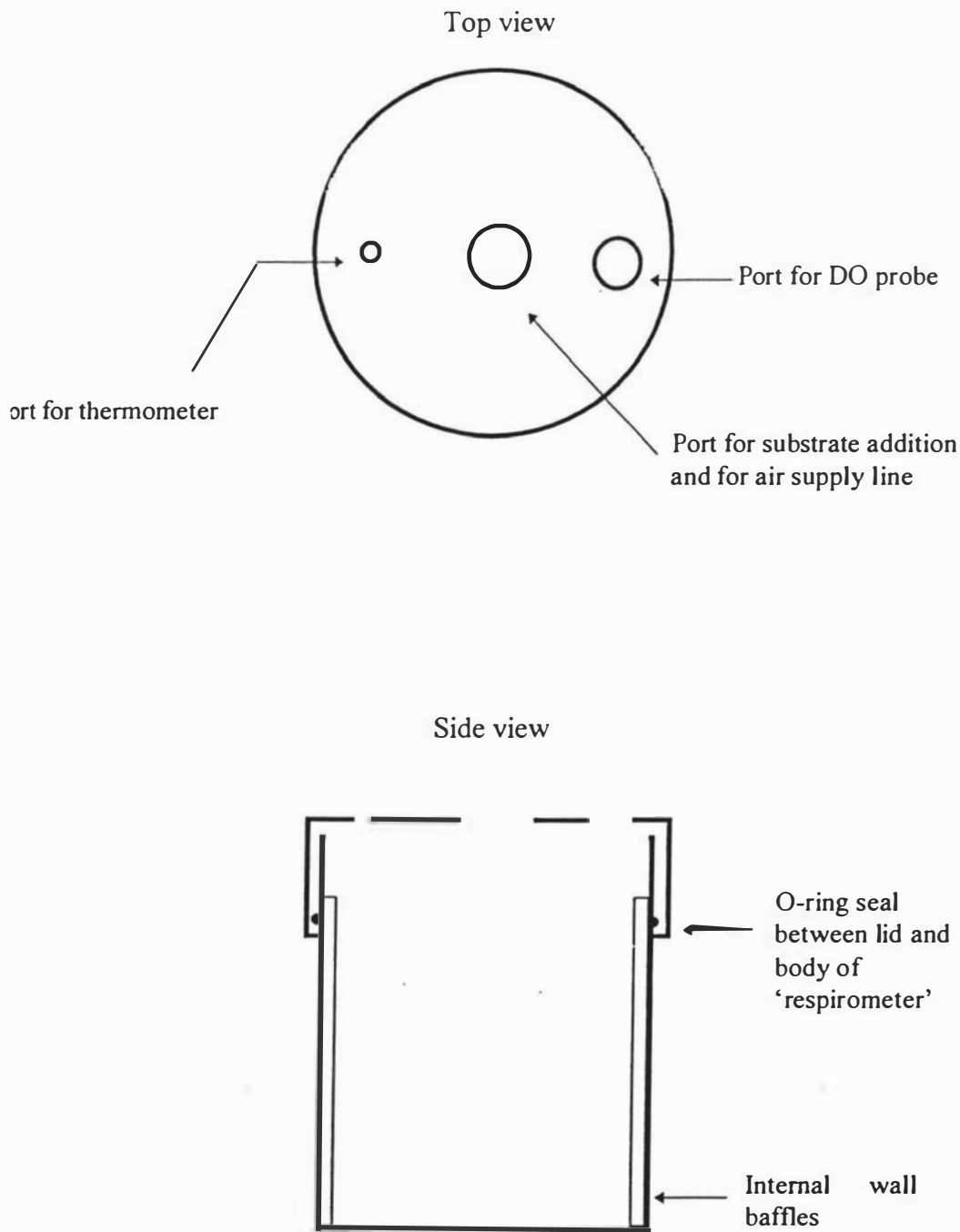
igure 3.1:   'Respirometer' vessel schematic.

Aeration was supplied via a length of flexible tubing connected to a filtered, pressurised air supply. The tubing was immersed in the respirometer contents through the air port in the respirometer lid and air flowrate was adjusted as needed using a screw clamp on the tubing.

A DO probe could be immersed directly into the respirometer contents via a specifically sized port, and a third sealable port was available for the addition of chemicals and for exit airflow during aeration.

3.13.2   6.25 litre Completely Mixed Reactor with Internal Settler

This reactor was used for initial biodegradability studies and to supply acclimated biomass for batch tests, as described in Chapter 4. The rectangular reactor was constructed of perspex by the PET Department, having a total volume of 10 litres and liquid level control via a stainless steel overflow pipe through the reactor base.

A porous stone diffuser located centrally at the base of the reactor provided both aeration and mixing in the vessel. The overflow pipe was positioned to maintain a working volume in the reactor of 6.25 litres. Feed to the reactor was delivered via a Cole Parmer Model 7554-30 Masterflex pump and controller, fitted with a 7013-20 pump head and Norprene Masterflex tubing.

3.13.3   10 litre Completely Mixed Reactor with External Settler

The continuous activated sludge trials were conducted using a New Brunswick Scientific Co. Model MF-14 bioreactor with a 10 l working volume glass vessel. The bioreactor had adjustable stirrer, air flowrate and temperature control. The stirring rate in the reactors was maintained at 100 rpm, air flowrate at 7 l/min and temperature at 25 °C for all activated sludge trials.

The reactor used an external settler, which was an 0.11 m internal diameter cylindrical perspex container with a sloping base, notched overflow weir and 1.75 litre working volume. A wire stirrer rotated at approximately 4 rpm at the base of the settler to prevent floc bridging at the settler outlet.

Flowrates in and out of all the activated sludge system vessels were controlled using Cole Parmer Model 7554-60 or Model 7554-30 Masterflex pumps fitted with 7014-20

or 7016-20 pump heads and Norprene Masterflex tubing.

3.14   Chemicals

All chemicals used were analytical reagent grade, unless specified.

3.14.1   Reactor Feed

Whole Milk Powder	'Anchor' brand, NewZealand Dairy Foods Ltd, Auckland, NZ.
Skim Milk Powder	'Anchor' brand, NewZealand Dairy Foods Ltd, Auckland, NZ.
Butter	'Anchor' brand, NewZealand Dairy Foods Ltd, Auckland, NZ.
Lactose	BDH, Poole, England.
NaOH	'Prolabo' brand, Rhone Poulenc ltd, Paris, France.

3.14.2   Chemical Oxygen Demand:

Potassium hydrogen thalate	BDH, Poole, England.
$K_2Cr_2O_7$	'Univar' brand, Ajax Chemicals, Auburn, Australia.
$HgSO_4$	BDH, Poole, England.
H_2SO_4	'Univar' brand, Ajax Chemicals, Auburn, Australia.
Ag_2SO_4	BDH, Poole, England

3.14.3   Phosphorus Determination

$(NH_4)_6Mo_7O_{24}.7H_2O$	'Univar' brand, Ajax Chemicals, Auburn, Australia.
NH_4VO_3	'Univar' brand, Ajax Chemicals, Auburn, Australia.
HCl	'Univar' brand, Ajax Chemicals, Auburn, Australia.
KH_2PO_4	'Univar' brand, Ajax Chemicals, Auburn, Australia.
HACH Amino Acid Reagent	HACH Cat. No. 1943-32
HACH Molybdate Reagent	HACH Cat.No. 2236-32

3.14.4   Ammonia Determination

$NaKC_4H_4O_6.4H_2O$	'Univar' brand, Ajax Chemicals, Auburn, Australia.

NaOH	'Prolabo' brand, Rhone Poulenc ltd, Paris, France.
Disodium hydrogen orthophosphate. $12H_2O$	'Univar' brand, Ajax Chemicals, Auburn, Australia.
Sodium salicyiate	'Univar' brand, Ajax Chemicals, Auburn, Australia.
$Na_2(NO)Fe(CN)_5.2H_2O$	BDH, Poole, England
NaOCl(4% solution)	'Janola' brand, Reckitt and Coleman, Auckland, New Zealand
NaCl	'Prolabo' brand, Rhone Poulenc ltd, Paris, France.

3.14.5   Nitrate Determination

$CuSO_4.5H_2O$	'Univar' brand, Ajax Chemicals, Auburn, Australia.
$ZnSO_4.7H_2O$	'Univar' brand, Ajax Chemicals, Auburn, Australia.
NaOH	'Prolabo' brand, Rhone Poulenc ltd, Paris, France
Hydrazine sulphate	BDH, Poole, England
Sulphalinimide	BDH, Poole, England
N-(1-naphthyl) ethylene diamine dihydrochloride	BDH, Poole, England
Merckoquant 10 020 Nitrate test strips	MERCK

3.14.6   Nitrite Determination

HACH Nitriver 3 Reagent Powder Pillows.   HACH Catalogue No. 14065 - 66.

3.14.7   Total Kjeldahl Nitrogen Determination

NaOH	'Prolabo' brand, Rhone Poulenc ltd, Paris, France.
Phenol	BDH, Poole, England
$NaKC_4H_4O_6.4H_2O$	BDH, Poole, England

3.14.8   Digestion Acid for TKN and TP Determination

H_2SO_4	'Univar' brand, Ajax Chemicals, Auburn, Australia.
K_2SO_4	'Univar' brand, Ajax Chemicals, Auburn, Australia.
Selenium powder	BDH, Poole, England

3.15   Determination of System Steady State

All trials were carried out for a period of at least three solids retention times (SRTs) where possible as it is widely reported (Linne and Chiesa, 1987; van Niekerk *et al.*, 1988; Salameh and Malina, 1989; Foot, 1992; Pujol and Canler, 1994) that changes in the biomass mixed culture population require that order of time to reach a new 'pseudo steady state' after a change in system parameters.

Some of the trials failed within the 3 SRT interval and others demonstrated a continuing change in measureable parameters after the 3 SRT period had passed. Results for each trial are therefore reported as trends during the period of the trial, and averages at the end of the trial where measured system parameters appear to have stabilised. In general it could not be concluded that systems were operating at 'steady state' during the trials, but system status after at least 3 SRTs was taken as an indication of the system response to a change in operating conditions.

3.16   Determination of Measurement Variability

Where possible and appropriate, 95% confidence intervals on average measured values are provided. However, in most cases estimates of measurement variability are not stated as it was deemed inappropriate to do so.

To be a true replicate, the individual values needed to be determined using the same biomass population reacting under the same conditions. Many of the results of repeated tests for which an average value is given were performed using biomass with a differing population composition and therefore could not be considered true replicates. Likewise it was inappropriate to provide confidence intervals on most average system parameters measured during a trial, as the system was not at steady state over that period, with changing trends readily observable. For such average values, a range is provided rather than a confidence interval.

All analytical tests were performed in duplicate, with the average value being recorded.

CHAPTER 4

SUBSTRATE BIODEGRADATION STUDIES

4.1 Introduction

The first section of experimental work was carried out to define then characterise the substrate to be used in this study. The substrate to be used was to represent the typical wastewater from a milk processing facility producing butter and milk powder, so a synthetic substrate was designed using average effluent characteristics measured on effluent from a full scale operation. The important physical and chemical characteristics of the real wastewater were defined, then reproduced in the synthetic substrate using a combination of milk derived products.

To assess the relative impact of the various milk constituents on the overall wastewater biodegradability, the synthetic substrate was further characterised using biodegradability definitions as developed for the modelling of activated sludge systems. This involved a series of specifically designed batch tests with acclimated biomass; monitoring changes in substrate, oxygen and biomass concentrations with time. Biodegradability assessment of the substrate also included obtaining estimates for the important biokinetic parameters such as biomass growth and yield.

To provide acclimated biomass for the batch studies, a small continuous reactor was operated at a 2.5 day HRT and SRT using the synthetic substrate under study. The treatment performance of this bioreactor was also monitored periodically to give an initial assessment of effluent treatability.

Results from the biodegradability studies could also be used subsequently to provide information required for the next phase of the study - a laboratory scale activated sludge system - as suitable values for operating parameters such as hydraulic residence time and organic loading rate could be determined from the data obtained as well as an estimate of the degree of treatment achievable with the defined substrate.

4.2   Substrate Characterisation

The substrate used as the reactor feed stream was to be representative of the typical effluent from a milk processing facility producing butter and milk powder. As these effluents consist primarily of diluted milk and milk products, the wastewater to be used in further studies was decided to be a 'synthetic' one, with similar physical and chemical characteristics as the 'typical' wastewater. The use of a synthetic effluent would ensure that a consistent substrate composition and quality would be available for all tests, and would also allow the impact of variations in reactor operating conditions to be distinguished without interference from variations in substrate.

4.2.1   Substrate Definition

The typical effluent characteristics of a butter and milk powder production facility were supplied by the New Zealand Dairy Research Institute (NZDRI) as listed in Table 4.1.

Table 4.1   Typical Effluent Characteristics of Butter and Milk Powder Production Facility

Characteristic	Average Value	Typical Range
COD $(g.m^{-3})$	2000	1000 - 4000
BOD$_5$ $(g.m^{-3})$	1150	NS
TSS $(g.m^{-3})$	3000	NS
pH	11.2	8.3 - 12.6
Temperature (^{o}C)	35	20 - 50
Total N $(g.m^{-3})$	29	4 - 80
Total P $(g.m^{-3})$	18	9 - 31
Lactose $(g.m^{-3})$	600	NS
Fat $(g.m^{-3})$	300	100 - 700

NS = not supplied

As dairy processing wastewaters are noted for their variability in both concentration and composition with time, the 'synthetic' wastewater was to comprise of a mixture of milk

products that would give the same COD, lactose, N and fat content as the average value determined from analyses of a large number of actual wastewater samples.

The milk products used as ingredients of the synthetic effluent were: whole powder (WMP); skim milk powder (SMP) and full cream salted butter (B). These ingredients had compositions determined by the NZDRI as given in Table 4.2.

Table 4.2   Compositions of Butter and Milk Powder Products

Constituent	g /g WMP	g / g SMP	g / g Butter
COD	1.5	1.1	2.1
Fat	0.257	0.008	0.82
Lactose	0.37	0.523	0.0064
Total N	0.045	0.0445	0.0008
Total P	0.058	0.058	0

The combination of milk products that resulted in the desired level of wastewater constituents is given in Table 4.3. The resultant COD, lactose and fat concentrations matched the average value for typical dairy effluent constituents very closely.

Table 4.3   Synthetic Wastewater Composition Based on Ingredient Composition

	WMP	SMP	Butter	Synthetic Effluent Total $(g.m^{-3})$	Desired Value $(g.m^{-3})$
Mass of ingredient $(g.m^{-3})$	650	700	150	1500	
COD $(g.m^{-3})$	975	770	315	2060	2000
Lactose $(g.m^{-3})$	241	366	1	608	600
Fat $(g.m^{-3})$	167	6	123	296	300
N $(g.m^{-3})$	29	21	0	60	29
P $(g.m^{-3})$	38	40	0	78	18

The value for total N was above the average value, but well within the typical range specified and so considered acceptable. Although the expected P value was apparently higher than desired, it could not be reduced using the ingredients available and subsequent analysis of the synthetic wastewater gave total P values very close the typical effluent value, as shown in Table 4.4. This indicated that the supplied P value for the milk powders shown in Table 4.2 may have been overestimated.

All ingredients were dissolved in a small volume of warm tap water then made up to the required volume with tap water. Sufficient NaOH was then added to the wastewater to give a solution of pH 11.

4.2.2 Physical and Chemical Characteristics of the Substrate

The physical and chemical characteristics measured on the wastewater as defined in Table 4.3 are listed in Table 4.4.

Table 4.4   Characteristics Measured on the Synthetic Wastewater

Characteristic	Whole Wastewater	GFC filtered (< approx. 1μm)	Membrane filtered (< 0.45 μm)
TS $(g.m^{-3})$	1500	-	-
TSS $(g.m^{-3})$	150	-	-
VSS $(g.m^{-3})$	150	-	-
COD $(g.m^{-3})$	2200	1650	1250
Turbidity (NTU)	442	218	77
Total P $(g.m^{-3})$	14	13	-
Total N $(g.m^{-3})$	89	72	-

(- : not measured)

It can be seen that the largest proportion of the solids and organic matter (90% of TS and 75% of COD) is less than 1μm in size, with 57% of COD attributed to solids less than 0.45 μm, indicating that the substrate is predominantly in a colloidal or dissolved form. The turbidity values also indicate that there is significant colloidal matter in the wastewater. In all further experimental work the term 'soluble' refers to that which

passes through a GFC filter, which includes both colloidal and truly soluble material.

4.2.3   Readily Biodegradable Fraction of Substrate

The wastewater characterisation protocol described by Henze *et al.* (1987) divides the total COD into four fractions: inert and biodegradable fractions of both particulate and soluble COD. The terms 'soluble' and 'particulate' were defined so that they represented biodegradability to microorganisms rather than actual physical state of the organic matter.

The soluble inert (S_I) fraction of milk processing wastewaters has been reported previously (Germirli *et al.*, 1991; Orhon *et al.*, 1993) to be negligible and so was not measured, as the composition of the synthetic wastewater was not expected to differ in this regard. As the VSS/TSS ratio measured was equal to 1, the inert content due to ash components of the substrate was also considered to be negligible.

The methodology given for estimating particulate COD fractions, both inert (X_I) and slowly biodegradable (X_S) is by curve fitting and difference techniques once the soluble proportion is known (Henze *et al.*, 1987).

The remaining COD fraction, readily biodegradable (S_S), was expected to be the most significant due to the origin of the organic matter in the wastewater and the high proportion of soluble or colloidal COD. Methods described for estimating S_S are summarised by Henze (1992), and most commonly involve measuring the change in OUR in response to either feed cessation in continuous reactors (Ekama *et al.*, 1986; Sollfrank and Gujer, 1991) or feed addition in batch reactors (Ekama *et al.*, 1986; Kappeler and Gujer, 1992). The batch method outlined in Ekama *et al.* (1986) was initially chosen for estimating S_S due to the suitability of the method and biomass availability at this stage of the project.

A respirometer containing mixed liquor from the 2.5 day SRT/HRT reactor was injected with varying volumes of concentrated feed so that the feed volume added was less than 1% of the volume of mixed liquor in the respirometer. The OUR was measured during the interval required for it to return to a constant rate or plateau, which Ekama *et al.* (1986) attributes to the hydrolysis rate of X_S once all S_S has been removed. The experiment was repeated using different added volumes of substrate to give a range of initial substrate concentrations in the respirometer.
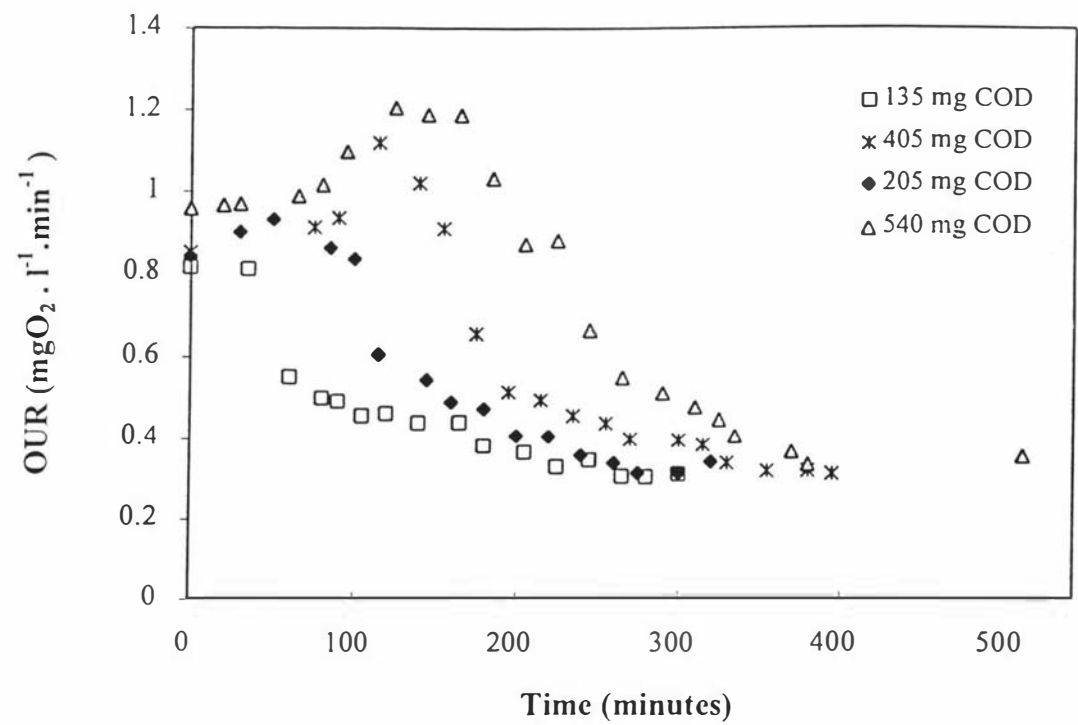
Figure 4.1: Estimation of S_S - measurement of the OUR after substrate addition.
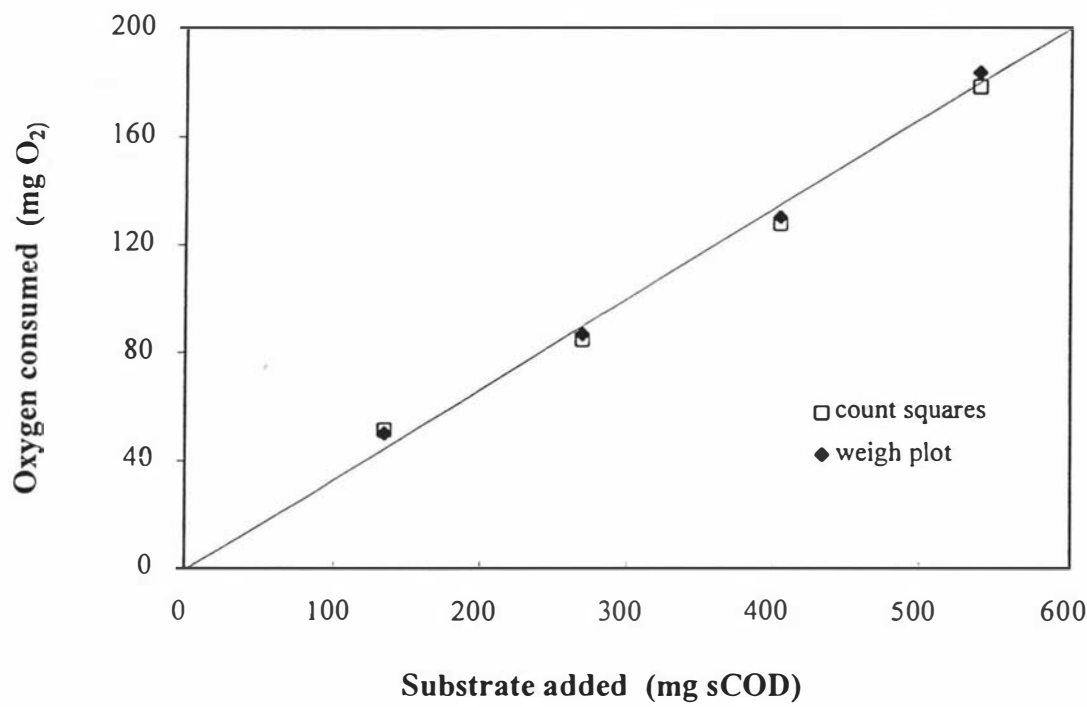
Figure 4.2: Estimation of S_S - determination of the ratio of oxygen consumed to substrate added.

Typical OUR profiles recorded during the experiment are shown in Figure 4.1. The area under the curve, above the final stable OUR baseline, represents the O_2 consumed in response to the substrate COD added. From the areas under the curves for a set of experiments, a mass ratio of O_2 consumed to COD added was calculated as illustrated in Figure 4.2. Ekama *et al.* (1986) suggest that this is related to the readily biodegradable fraction (f S_S) of the substrate as follows:

$$f S_S = \frac{1}{1 - Y_H} \cdot \frac{\text{g } O_2 \text{ consumed per g COD added}}{\text{g COD added}} \tag{4.1}$$

where:

$fS_S = S_S/S_O$ = COD fraction of readily biodegradable substrate.

S_S = concentration of readily biodegradable COD $(g.m^{-3})$

S_O = initial substrate COD concentration in the respirometer $(g.m^{-3})$

Y_H = yield coefficient (g.cell COD per g.substrate COD)

Using this relationship, the results obtained from different replicates of the trial are summarised in Table 4.5. The substrate used in these experiments was the GFC filtered portion of the feed, therefore the readily biodegradable fraction of the wastewater was estimated to be 75% of the experimental estimate, given the soluble and total COD results reported earlier in Table 4.4. A value of $Y_H = 0.68$ g cell COD produced per g substrate COD consumed was used in calculating the values of S_S, the choice of Y_H value used being discussed later in this chapter.

More recently (Sollfrank and Gujer, 1991) the biodegradable fraction of the wastewater has been divided into three, rather than two fractions, based on observed OUR responses with activated sludge biomass. The fractionation, based on rate of biodegradation of the substrate components is now thought to be more accurately represented by:

S_S: readily biodegradable material

S_H: rapidly hydrolysable material

X_S: slowly hydrolysable material

The new fraction S_H is mostly the physically soluble, more degradable fraction of the previously defined X_S, but may also contain some of the more rapidly degraded particulate material (Henze, 1992; Henze *et al.*, 1994).

The area under the OUR curve obtained by Sollfrank and Gujer (1991) from similar batch tests is considered to contain both S_S and S_H, with the degradation of S_H being indicated by a linear decline in OUR at the end of the test. A similar shaped curve that includes a high OUR plateau, followed by a rapid decrease and then a linear decrease in OUR, is indicated in this study as can be seen in Figure 4.1. Using this analysis, the fraction of readily biodegradable substrate calculated in Table 4.5 would then be the sum of both S_S and S_H fractions.

Table 4.5   Estimation of Biodegradable Fractions S_S and S_H of the Soluble Wastewater.

Date	No. of tests	$\dfrac{g\ O_2\ consumed}{g\ COD\ added}$	$f\,S_S + f\,S_H$ of Soluble Wastewater	$f\,S_S + f\,S_H$ of Whole Wastewater
17/3/93	4	0.33	1.03	0.77
18/3/93	4	0.31	0.97	0.73
25/3/93	4	0.26	0.81	0.61
31/3/93	4	0.31	0.97	0.73
Average		0.30	0.94	0.71

From the results in Table 4.5, it appears that almost the entire soluble substrate COD was either readily biodegradable or rapidly hydrolysable. Using batch test OUR data as shown in Figure 4.1, Kappeler and Gujer (1992) estimate S_S as being the area under the curve with a baseline equal to the final linear OUR decline rather than a final stable OUR baseline as described in Ekama *et al.* (1986). This distinction as shown in Figure 4.3 allows a separation of the oxygen consumed during the batch test into that due to the presence of S_S and that due to S_H. A similar analysis of the OUR graphs from the tests listed in Table 4.5 resulted in the fractionation given in Table 4.6.
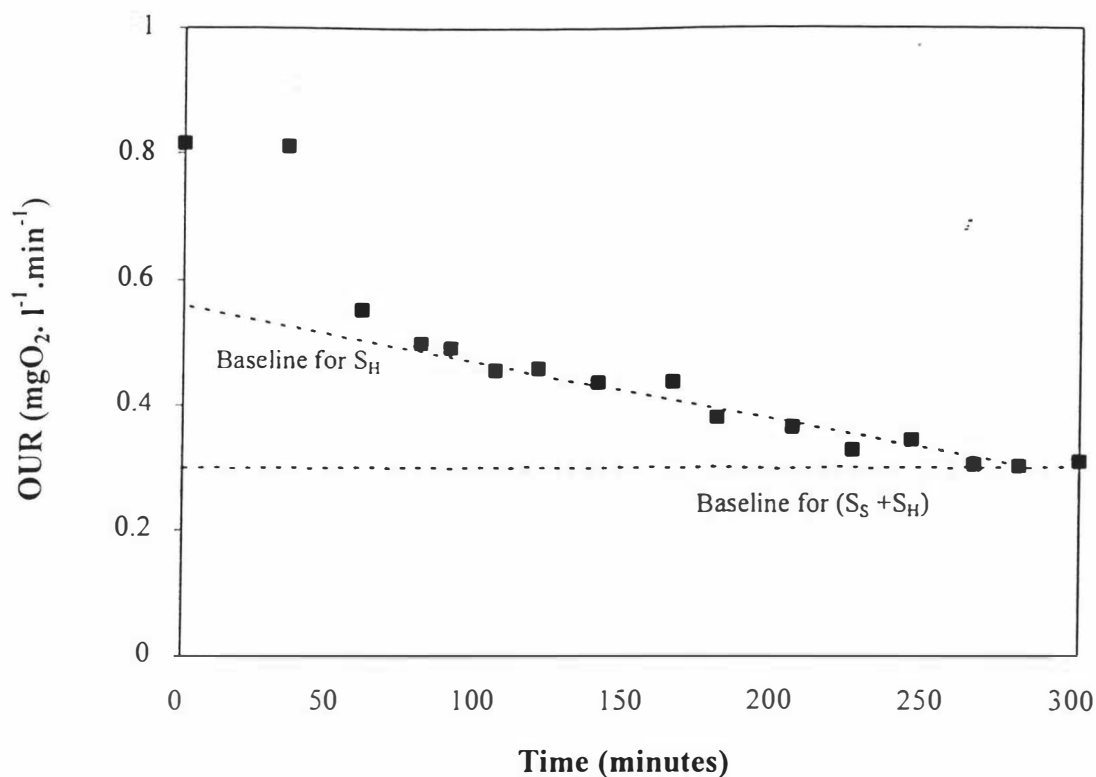
Figure 4.3:   Determination of S_S and S_H fractions from OUR data.
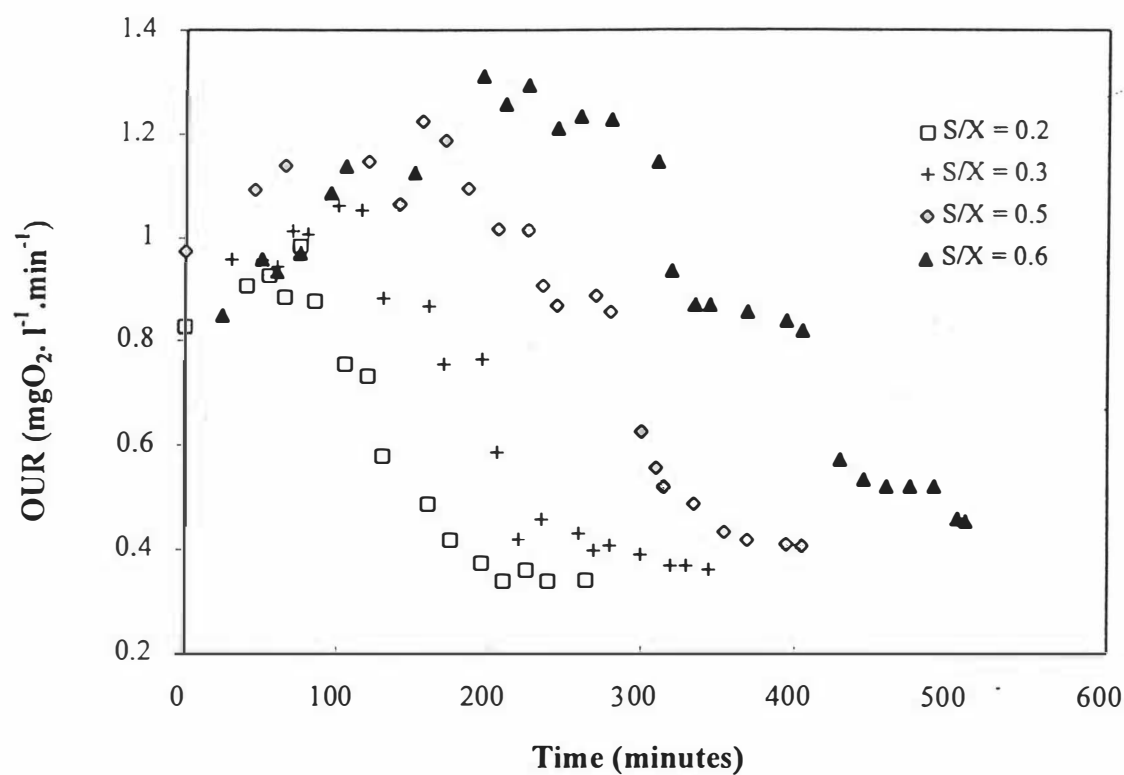
Figure 4.4:   Effect of variation in S/X ratio on the interval of elevated OUR.

Table 4.6 Division of Readily Biodegradable Fraction of the Wastewater into S_S and S_H

Date	S_S Fraction	S_H Fraction	f S_S of Whole Wastewater	f S_H of Whole Wastewater
17/3/93	0.57	0.43	0.44	0.33
18/3/93	0.61	0.39	0.45	0.28
25/3/93	0.60	0.40	0.37	0.24
31/3/93	0.60	0.40	0.44	0.29
Average	0.60	0.40	0.425	0.285

From these results the size distribution of the colloidal material in the membrane filtered fraction of the substrate can also be roughly estimated. The respirometric tests indicated that of the GFC filterable material, 60% was soluble and 40% colloidal. As 24% of the COD passing through a GFC filter did not pass through the smaller 0.45 μm pore size, it is estimated that 60% of the colloidal materials in the S_H fraction were between 0.45 μm and approximately 1 μm in size.

Both Ekama *et al.* (1986) and Kappeler and Gujer (1992) specify fairly closely the interval of elevated OUR in the batch test for the determination of S_S, the former suggesting a period of approximately 1 to 2 hours and the latter of 30 minutes, with a total measurement time of 4 to 5 hours. This was recommended to ensure that heterotrophic growth did not occur and so that the amount of hydrolysed substrate would not interfere with the interpretation.

In the batch tests performed, a wide range of initial substrate to biomass (S_0/X_0) ratios were used so that the influence of a varying elevated OUR interval could be assessed. The average TSS concentration in the respirometers was 1700 g.m^{-3}, resulting in S_0/X_0 ratios in the order of 0.04 to 0.65 g.COD per g.TSS. The curves as shown in Figure 4.1 and Figure 4.4 illustrate that the period of elevated OUR lasted between one and eight hours. As each set of results gave a constant ratio of O_2 consumed to COD added over the entire F/M range, there did not seem to be any effect of prolonged OUR elevation on the ratio obtained. No significant increase in TSS concentration was measured over the duration of the tests, indicating negligible cell growth, as would be expected since the S_0/X_0 ratio was less than 2 (Chudoba *et al.*, 1992).

The biodegradable fractions S_S and S_H have been related to the kinds of organic molecules present in wastewaters. Henze (1992) describes the readily biodegradable fraction (S_S) as being limited to small molecules of volatile fatty acids, carbohydrates, alcohols and amino acids, with a molecular weight below about 1000. The rapidly hydrolysable matter S_H, is described as that which would be hydrolysed under aerobic conditions within a few hours, and a general approximation for its estimation is given as:

$$S_H = \text{Total soluble COD} - S_I - S_S \qquad (4.2)$$

It can be seen from the results calculated in Table 4.5 that the above approximation held for the substrate used in this study, given that it was assumed that S_I was negligible. As the determination method used for estimating S_S involved time periods from one to nine hours, and considering the results gained in Table 4.5 and Figures 4.1 and 4.4, it seems most likely that the value initially obtained for the biodegradable fraction did in fact include both readily biodegradable and rapidly hydrolysable material.

Using the descriptions of Henze (1992) for S_S and S_H, the COD fractionation can be related to the substrate components. All the following milk component data was taken from Walstra and Jenness (1984), unless otherwise stated. Whole milk comprises of approximately 87% water and 13% solids, as a non-homogenous solution, with almost all of the solids being lactose, milk proteins or milk fat. Using the expected composition data for the synthetic wastewater given in Table 4.3, an estimate of the COD contribution from each of the lactose, protein and fat fractions can be made.

For the synthetic substrate, the readily biodegradable fraction would be expected to comprise of lactose, which represents 36% of the dry matter in milk. Lactose has a molecular weight of 342, so would be expected to be readily biodegradable given the definition of a molecular weight lower than 1000 (Henze 1992; Henze *et al.*, 1994). The chemical composition of lactose is $C_{12}H_{22}O_{11}$, therefore a theoretical COD value of 1.12 gCOD.g lactose^{-1} can be calculated (Metcalf & Eddy, 1991). The synthetic substrate lactose concentration of 608 g.m^{-3} would then be expected to provide 681 gCOD.m^{-3}, which represents 34% of the total substrate COD and 80% of the readily biodegradable COD fraction.

Fat molecules make up 31% of the dry matter in milk, being found as globules of varying sizes between 0.1μm and 10 μm. Approximately 97% of the fat globules are greater than 1μm in size, so most of the COD associated with fat would be insoluble

and would form the X_S fraction of wastewater organic matter. From the data given in Marshall and Harper (1984), milkfat has an average BOD_5 of 0.89 kg/kg and an average BOD_5/COD ratio of 0.79. The expected 296 $g.m^{-3}$ of fat would then represent 333 $g.m^{-3}$ of COD, or 16.7% of the total COD.

The remaining major class of component, protein, comprises a further 26% of the dry matter in milk, 80% of which is casein proteins with molecular weights between 19,000 and 25,300 and the remainder mostly whey proteins with the greatest proportion having a molecular weight up to 18,500 (Walstra and Jenness, 1984). Due to their size, proteins are expected to form the rapidly hydrolysable fraction of the wastewater. An approximation of the proteinaceous COD in the synthetic substrate can be made from the following data: proteins contribute to 95% of the N content (Walstra and Jenness, 1984); there is approximately 6.38 g protein per g N ('*NZ Dairy Industry Standard Methods..*', 1993); the average BOD_5 of milk proteins is 1.03 kg/kg and the BOD_5/COD ratio is 0.46 for casein and 0.23 for whey protein (Marshall and Harper, 1984). Therefore the wastewater would be expected to contain 364 $g.m^{-3}$ of protein with a COD of 977 $g.m^{-3}$, representing 48.9% of the total COD.

Casein exists predominantly as a fine dispersion of micelles having a diameter of between 10nm and 300nm (Walstra and Jenness, 1984), whereas whey proteins exist as a colloidal solution with particle diameters of 3-6nm. Therefore the whey proteins are expected to be more rapidly degraded than the caseins. Using the protein data already stated, of the estimated 977 $g.m^{-3}$ of proteinaceous COD, 326 $g.m^{-3}$ would be expected to be associated with the soluble whey proteins and 651 $g.m^{-3}$ associated with casein micelles, representing 16.3% and 32.6% respectively of the total substrate COD.

The above calculations suggest that the COD contributions due to lactose, fat and protein are 681, 333 and 977 $g.m^{-3}$ respectively, giving a total component COD of 1991 $g.m^{-3}$ compared to the total expected COD of 2000 $g.m^{-3}$ calculated from ingredient data. This supported the assumption made that the amount of 'inert' non-biodegradable COD was not expected to be significant, as the difference between total component and ingredient COD values was less than 0.5%.

A comparison of substrate COD fractionation and component COD data is given in Figure 4.5, where fractionation is given by:

$$\text{Total substrate COD} = X_S + X_I + S_I + S_S + S_H \qquad (4.3)$$

It has already been stated that S_I was assumed to be negligible, and due to the total COD contribution calculated for lactose, protein and fat; X_I can also be assumed to be small. Figure 4.5 suggests that most of the S_S fraction is due to the lactose content in the wastewater, just over half of X_S is due to fat, and that the milk proteins contribute to all three fractions: approximately 17% of S_S, possibly almost all of S_H, and just under half of X_S.
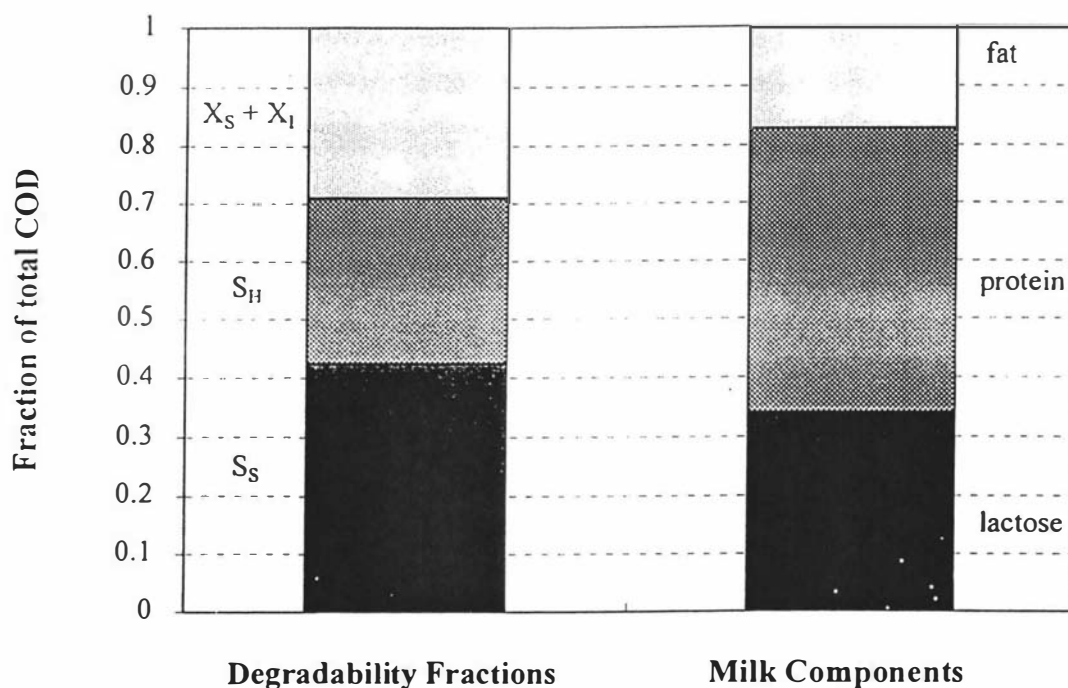
Figure 4.5: Fractionation of substrate COD by degradability and milk components.

4.3   Estimation of Kinetic constants

In order to characterise the response of the biomass to the substrate, several kinetic parameters were measured. Monod type kinetics have been used previously for the biodegradation of dairy wastewaters (Orhon *et al.* 1993), so were assumed to adequately describe the relationship between growth rate and substrate concentration in this case. Therefore the parameters of interest were: the maximum specific growth rate of biomass, μ_{max} (d^{-1}); the substrate half saturation co-efficient, K_S ($gCOD.m^{-3}$) and the yield co-efficient, Y_H (g cell COD. g substrate COD^{-1}). These parameters were subsequently used to design continuous reactor studies.

4.3.1   Selection of Methodology

All the methods selected for the determination of biokinetic constants used COD as a measure of substrate concentration, as this is now the common basis for activated sludge models (Henze *et al.*, 1987) and allows an oxygen balance to be performed over the system. The biomass concentrations were measured gravimetrically and also as COD, after the oxygen equivalence of cells, O_X (g COD. g biomass^{-1}) had been established from COD and TSS/VSS data.

As the substrate in this case was turbid, conventional methods for measuring biokinetic constants involving relating cell concentration to optical density at a specified wavelength could not be used. Other methods which measure concentrations of substances specific to biological growth such as DNA or ATP are not suited to routine application. Therefore respirometric and gravimetric methods were employed in this study.

4.3.2   Measurement of μ_{max} and K_S.

The estimation of μ_{max} and K_S requires determination of the biomass growth response to various initial substrate concentrations. This can be done either: directly by following biomass growth as an increase in cell number, optical density or cell mass; or indirectly by measuring substrate removal as a change in component or COD concentration, or from oxygen consumption data. The indirect methods relate substrate or oxygen removal to an increase in biomass through a yield factor, Y (mass of cells produced per unit of substrate removed), assuming that all substrate is either used for the production of new cells or oxidised to provide energy. In this study respirometric methods were used, which relate oxygen uptake rates to cell growth.

There are two main types of experiments used for the respirometric determination of μ_{max} and K_S : those that employ a high S/X ratio (Gaudy *et al.*, 1987; Kappeler and Gujer, 1992) and those using a low S/X ratio (Cech *et al.* 1984). The high S/X ratio tests measure 'growth' as an increase in cell numbers, while the low S/X methods measure 'growth' as a storage or accumulation response to substrate addition.

Both high and low S/X methods were assessed for further use in this study. The substrate used in both methods was the GFC filtered fraction of the feed so that cell concentration could be estimated by TSS analysis.

4.3.2.1   Measurement of μ_{max} and K_S Using a High S/X Ratio.

The methodology outlined by Gaudy *et al.* (1987) was used to determine μ_{max} and K_S under cell replication conditions. Biomass from the chemostat was used to seed a series of respirometers with initial soluble substrate concentrations of between 255 and 2900 $gCOD.m^{-3}$. The initial S/X ratio was greater than the 2 - 4 range needed to ensure that cell replication would take place (Chudoba *et al.* 1992). The respirometers were aerated, stirred and maintained at 20 °C for the duration of the test. OURs in each respirometer were measured every 10 to 20 minutes for a period of approximately 8 to 12 hours until a decrease in OUR was observed. TSS and COD concentrations were measured at the beginning and end of the test so that Y_H and O_X could be estimated.

The change in biomass concentration (X) is related to the change in oxygen concentration (O) via Y, the cell yield ($g.m^{-3}$ cells formed per $g.m^{-3}$ COD removed) as follows:

$$\frac{dX}{dt} = \frac{1}{\frac{1}{Y} - O_X} \cdot \frac{d\,O_2}{dt} \qquad (4.4)$$

The ($1/Y - O_X$) expression represents the mass of O_2 consumed per mass of biomass produced and is known as the respirometric ratio (R), that is the ratio of respiration to synthesis (Gaudy *et al.*, 1987) and is a constant for any particular system. Using this substitution, Equation (4.4) can be integrated to provide the following equation for biomass:

$$X(t) = X(0) + \frac{\Delta O_2(t)}{R} \qquad (4.5)$$

where: $X(t)$ = biomass concentration at time, t

               $\Delta O_2(t)$ = cumulative oxygen uptake at time, t

The OUR versus time data measured during a test was used to calculate the accumulated oxygen uptake. Using the Y and O_X values calculated from test TSS and COD data, the increase in cell concentration was calculated using Equation 4.5. The logarithm of the resultant cell concentration in each respirometer was plotted versus time as shown in Figure 4.6 and the specific growth rate (μ) estimated from the slope of the straight line portion. This gave an estimate of μ for each respirometer at a different
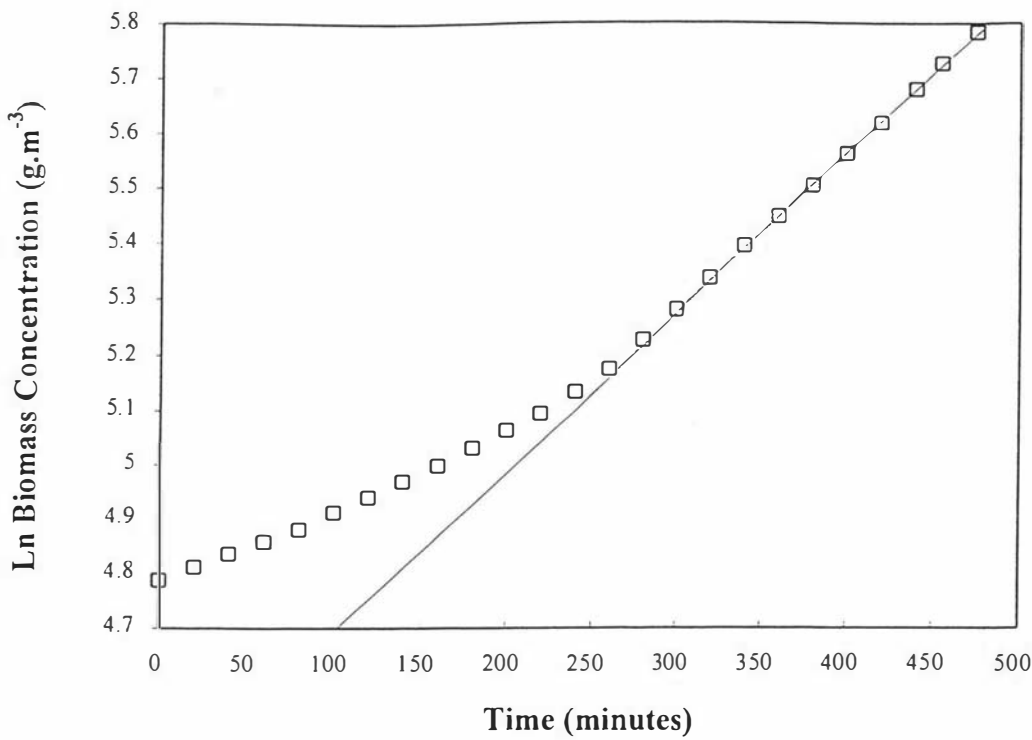
Figure 4.6:   Calculated biomass concentration versus time for the estimation of μ_{max} using the high S/X method.

S_O value in the series.

The values of S_O and μ were then plotted on a modified linear plot as shown in Figure 4.7 to estimate μ_{max} and K_S. A measure of the accuracy of the result can be determined from the size of the triangle defined at the intersection point by the series of lines. The values obtained from various repetitions of the procedure are listed in Table 4.7.

Table 4.7   Estimation of μ_{max} and K_S Using High S/X Ratios at 20 °C.

Date	S_O range (g.m^{-3} COD)	S_O/X_O range (gCOD .gTSS^{-1})	R	μ_{max} (d^{-1})	K_S (g.m^{-3} COD)
2/12/92	180 - 365	5.9	0.99	2.7	60
8/12/92	255 - 390	2.6 - 3.8	0.67	2.4	68
30/12/92	455 - 2600	15	0.71	3.6	90
8/1/93	680 - 2900	5.7 - 24.1	0.71	5.0	380
Average:				3.4	150
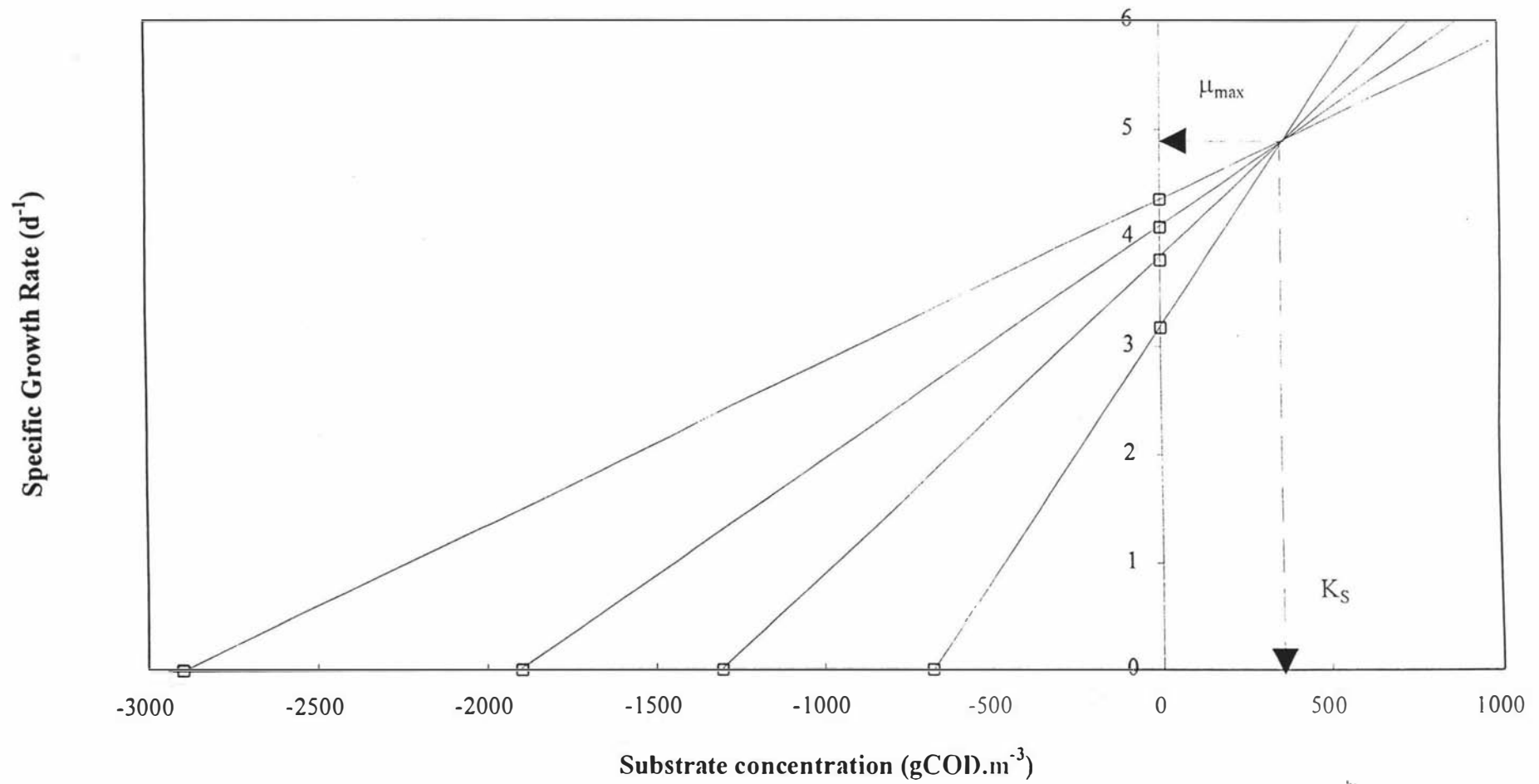
Figure 4.7   Modified linear plot for the estimation of μ_{max} and K_S using the High S/X ratio method.

The value of Y used was taken as the average Y calculated from COD and TSS data for the series of respirometers in each test, where $Y = Y_H / O_X$. The values for Y_H are summarised in Table 4.11. The value used for O_X was 1.42 g ceil COD / g TSS, also as measured during the test from final TSS and COD data. The series of respirometers employed for each determination used either a constant X_O value or a constant S_O/X_O ratio, both experimental approaches gave good results.

4.3.2.2 Measurement of μ_{max} and K_S Using a Low S/X Ratio.

The low S/X ratio method used to determine μ_{max} and K_S was that outlined in Cech *et al.* (1984). This is a respirometric method in which the biomass growth rate is related to the immediate change in OUR measured in response to the addition of a known amount of substrate.

One litre of mixed liquor from the 2.5 day HRT/SRT reactor was aerated in a respirometer for approximately 30 to 60 minutes or until a stable endogenous OUR was recorded. The respirometer was reaerated until a dissolved oxygen concentration of between 6 and 8 $g.m^{-3}$ was reached, then the aeration was stopped and the OUR recorded on a chart recorder. Once a measurable straight line was obtained, a known amount of concentrated substrate was added, and the new OUR recorded. The change in OUR was determined from the change in slope of the lines recorded before and after substrate addition. At the end of the test, the respirometer was reaerated and after the OUR had returned to a stable endogenous rate, the process was repeated for other initial substrate concentrations. At least three runs with different substrate concentrations were performed so that at least three estimates of μ were obtained.

Temperature of the mixed liquor was controlled at a constant value ($\pm$ 0.5 $^\circ$C) throughout the experiment. As the biomass used had an SRT of 2.5 days, inhibition of nitrification during the tests was not considered necessary, as nitrifier growth was not expected at this short sludge age.

The OUR is related to μ through an oxygen balance as follows:

$$\mu = \frac{Y_H}{1 - Y_H} \cdot \frac{\Delta \, OUR}{VSS \cdot O_X} \qquad (4.6)$$

where:

VSS = volatile suspended solids concentration in the respirometer ($g.m^{-3}$)

ΔOUR = change in OUR in response to the addition of substrate ($g\ O_2.m^{-3}.d^{-1}$)

A specific ΔOUR ($g\ O_2.g$ cell $COD^{-1}.d^{-1}$) can be calculated from the following group of variables in Equation 4.6:

$$Sp\Delta OUR = \frac{\Delta OUR}{VSS.O_x} \tag{4.7}$$

The value of μ_{max} was determined from a modified linear plot as in the high S/X method. An example of the results gained from this method is shown in Figure 4.8. One advantage of this method is that since ΔOUR is proportional to μ for a given respirometer, a modified linear plot of ΔOUR versus volume of substrate injected could be constructed as the set of runs progressed and the experiment could be stopped as soon as the required number of intersecting lines was obtained.

Values estimated for μ_{max} and K_S over a period of several months are summarised in Table 4.8. A value of $Y_H = 0.68$ g cell COD per g of substrate COD was used in all calculations, as determined by respirometric evaluation and discussed in Section 4.3.3.

Either a set of replicate respirometers or a single respirometer reinjected several times was used, both techniques gave conclusive results. In any given respirometer, the OUR response to additions of a defined amount of substrate was found to be stable over a period of at least six hours and with as many as ten reinjections of substrate. In this study the culture response did not seem to be particularly sensitive to aeration or feed history over a period of several hours, a possible concern that has been raised with this method (Grady and Philbrook, 1984).

It can be seen that the results gained seemed to be variable over the period of study, indicating that the microbial composition of the mixed culture was changing with time. As the SRT of the biomass used was relatively short at 2.5 days, and due to the multicomponent nature of the substrate, significant population shifts could be possible over a period of several months. It can be seen from results gained on consecutive days however, as on 16, 17 and 18/12/92 at 20 °C, that relatively consistent results could be gained from daily measurements, as results for μ_{max} ranged from 0.97 to 1.05 d^{-1} and K_S from 10.1 to 17.3 $g.m^{-3}$ COD over the three days.
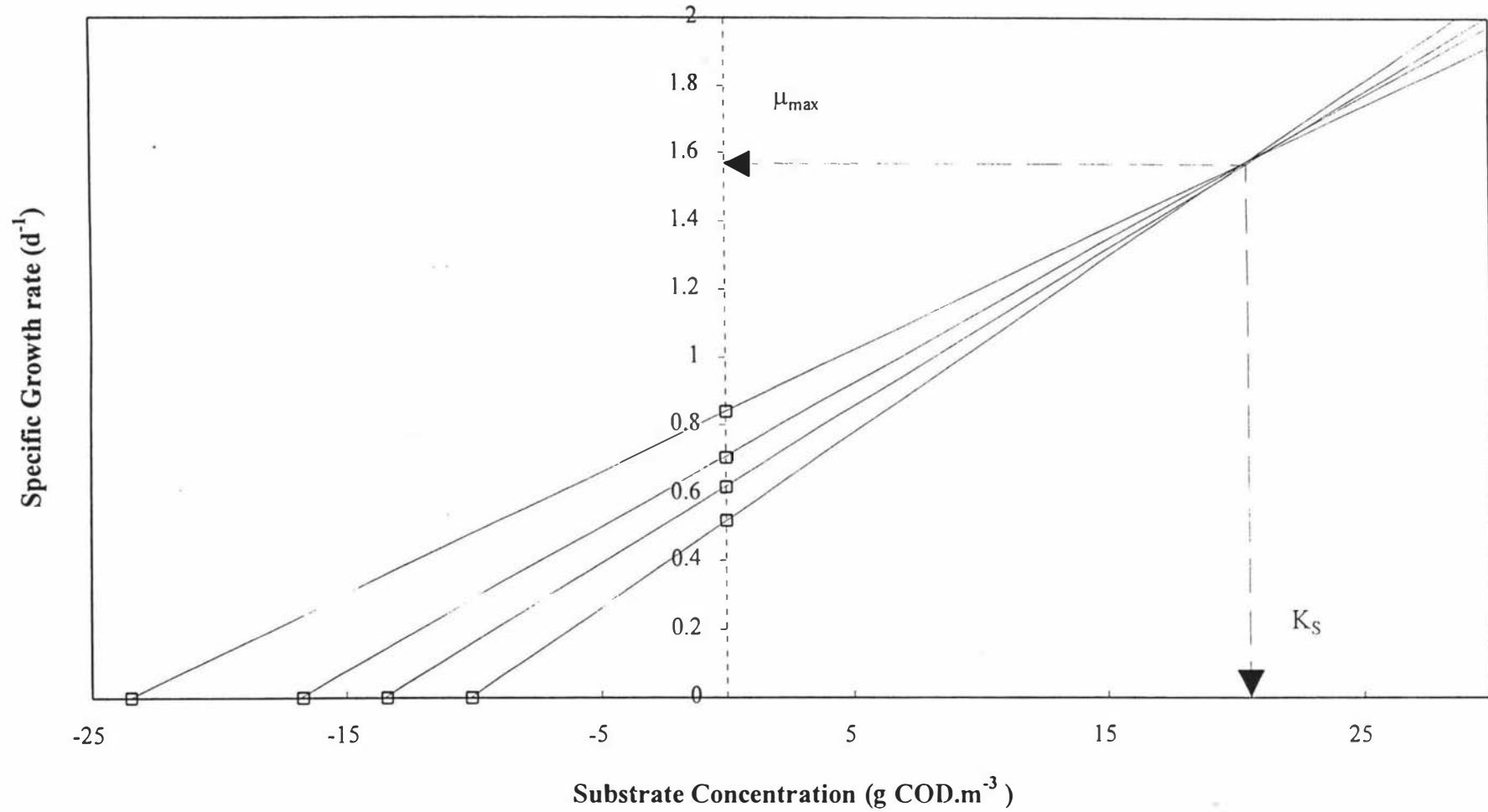
Figure 4.8   Modified linear plot for the estimation of μ_{max} and K_S using the Low S/X ratio method.

Table 4.8   Estimation of μ_{max} and K_S Using Low S/X Ratios.

Date	Temp °C	S_O Range (g.m^{-3} COD)	$\Delta SpOUR_{max}$ (gO$_2$.gCOD^{-1}.d^{-1})	μ_{max} (d^{-1})	K_S (g.m^{-3} COD)
10/12/92	24	5.3 - 63.6	0.51 - 0.55	1.09 - 1.16	14.2 - 17.3
16/12/92	19	4.55 - 54.6	0.47 - 0.50	1.00 - 1.05	15.5 - 17.3
17/12/92	20	8.4 - 33.6	0.47	1.00	10.1
	24.5	8.4 - 33.6	0.60 - 0.63	1.28 - 1.33	8.4 - 9.9
18/12/92	20	9 - 36	0.46 - 0.47	0.97 - 1.00	8.1 - 9.9
	25	9 - 36	0.75 - 0.80	1.60 - 1.70	11.7 - 15.3
7/1/93	20	6.6 - 46.2	1.02 - 1.06	2.17 - 2.26	33 - 35.6
27/1/93	20	13.8 - 26.8	0.44	0.94	5.5
	25	10.4 - 26.8	0.91	1.93	17.9
29/1/93	18	10 - 23.5	0.57	1.20	19.1
	20	10 - 23.5	0.72	1.53	19.4
	23	10 - 23.5	0.83	1.75	21.1
	25	10 - 23.5	0.83	1.76	15.4
	27	10 - 26.8	0.98 - 1.01	2.09 - 2.14	19.4 - 20.8
16/3/93	20	33.8 - 270	0.235 - 0.245	0.50 - 0.52	35.4 - 43.9
18/3/93	25	70 - 280	0.31 - 0.32	0.65 - 0.68	10.7 - 21.3
11/6/93	20	12.6 - 75.6	0.43 - 0.44	0.91 - 0.94	15.6 - 17.5
	21	50.4 - 63.0	0.38	0.81	3.4
Average	20 °C		0.54	1.15	16.9
Average	25 °C		0.69	1.47	14.4

The temperature dependence of μ_{max} and K_S was assessed using a series of respirometers maintained at different temperatures. At temperatures lower than 17 °C and higher than 27 °C, conclusive results were difficult to obtain.   The continuous reactor that was used as a biomass source was operated at ambient temperatures in the laboratory, usually between 20 and 25 °C, so extremes in batch test temperature may

have affected the ability of the culture to utilise the substrate. From the set of data collected on 29/12/92 at temperatures between 18 and 27 °C, it can be seen that μ_{max} did increase with increasing temperature. Using the following relationship commonly applied for the dependence of biological processes on temperature (Metcalf and Eddy, 1991):

$$\mu_{max}(T) = \mu_{max}(20) \; \Theta^{(T-20)} \tag{4.8}$$

where:

       T   = temperature (°C), with 20°C as the reference point temperature.

       Θ = temperature activity co-efficient.

An average value of $\Theta = 1.063$ was obtained, which compares well to the typical value of 1.04 ± 0.04 given in Metcalf and Eddy (1991) for activated sludge biomass. No obvious temperature relationship was observed for K_S.

4.3.2.3 Effect of S/X Ratio on the Measurement of μ_{max} and K_S.

The averages of estimates obtained for μ_{max} and K_S at 20 °C by the high S/X and low S/X methods are summarised in Table 4.9. It can be seen that the magnitude of the result appears to be dependent on the estimation method used.

The major differences between the two approaches were the S/X ratio and the initial substrate concentration range used. The S/X ratio affected whether cell replication would occur or not and the initial substrate concentration would be important if high substrate concentrations inhibited cell growth. The ranges of these parameters used in the various methods are also listed in Table 4.9.

Each method had an inherent limit in the S_O range that could be used, due to the accuracy of respirometric measurements. For the high S/X method, using low S_O concentrations such as those below 200 gCOD.m^{-3}, required that very low biomass concentrations be used to maintain the required initial S/X ratio. These very low biomass concentrations resulted in a very low OUR, which was difficult to determine accurately. For the low S/X method, the largest value feasible for S_O was determined by the ability of the measuring apparatus to accurately detect the large resultant ΔOUR.

Table 4.9   Effect of S/X Ratio on the Estimation of μ_{max} and K_S at 20 °C.

	High S/X Method	Low S/X Method
μ_{max}: average (d^{-1})	3.4	1.2
μ_{max}: range (d^{-1})	2.4 - 5.0	0.5 - 2.3
K_S : average (gCOD.m^{-3})	150	17
K_S : range (gCOD.m^{-3})	60 - 380	8.1 - 44
Number of tests performed	4	7
Initial Substrate COD, S_O (g.m^{-3})	180 - 2900	4.5 - 270
S_O/X_O Ratio (g COD / g VSS)	2.6 - 24	0.003 - 0.15

To discount the effect of initial substrate concentration, the low S/X method was repeated using a larger range of S_O so that highest concentration used was in the same order as the concentrations used in the high S/X method, and so that the range spanned a tenfold increase in S_O concentration. The results of this test using S_O values from 33.8 to 270 g.m^{-3} soluble COD are shown in Figure 4.9 and it can be seen that all the concentrations used gave a common intersection point. Therefore the discrepancy in μ_{max} and K_S results given by the two different methods is unlikely to be caused by the choice of S_O.

It is then indicated that the values obtained for μ_{max} and K_S are affected by whether cell replication occurs or not. This is in agreement with the results obtained by Chudoba *et al.* (1992), who reported higher values for both μ_{max} and K_S when a high S/X ratio method was used. The reported magnitude of difference between values estimated by the two methods using various pure substrates, is also similar to the results found in this study.

The reason proposed for the higher values of μ_{max} and K_S obtained from a high S/X method, is that only faster growing species in the mixed culture will replicate within the time frame of the test (Chudoba *et al.*, 1992; Grady *et al.*, 1996). The estimates of kinetic parameters gained will therefore pertain to a culture with a higher proportion of faster growing species than the original mixed culture. It is therefore recommended that low S/X methods be used for obtaining representative data on mixed cultures.
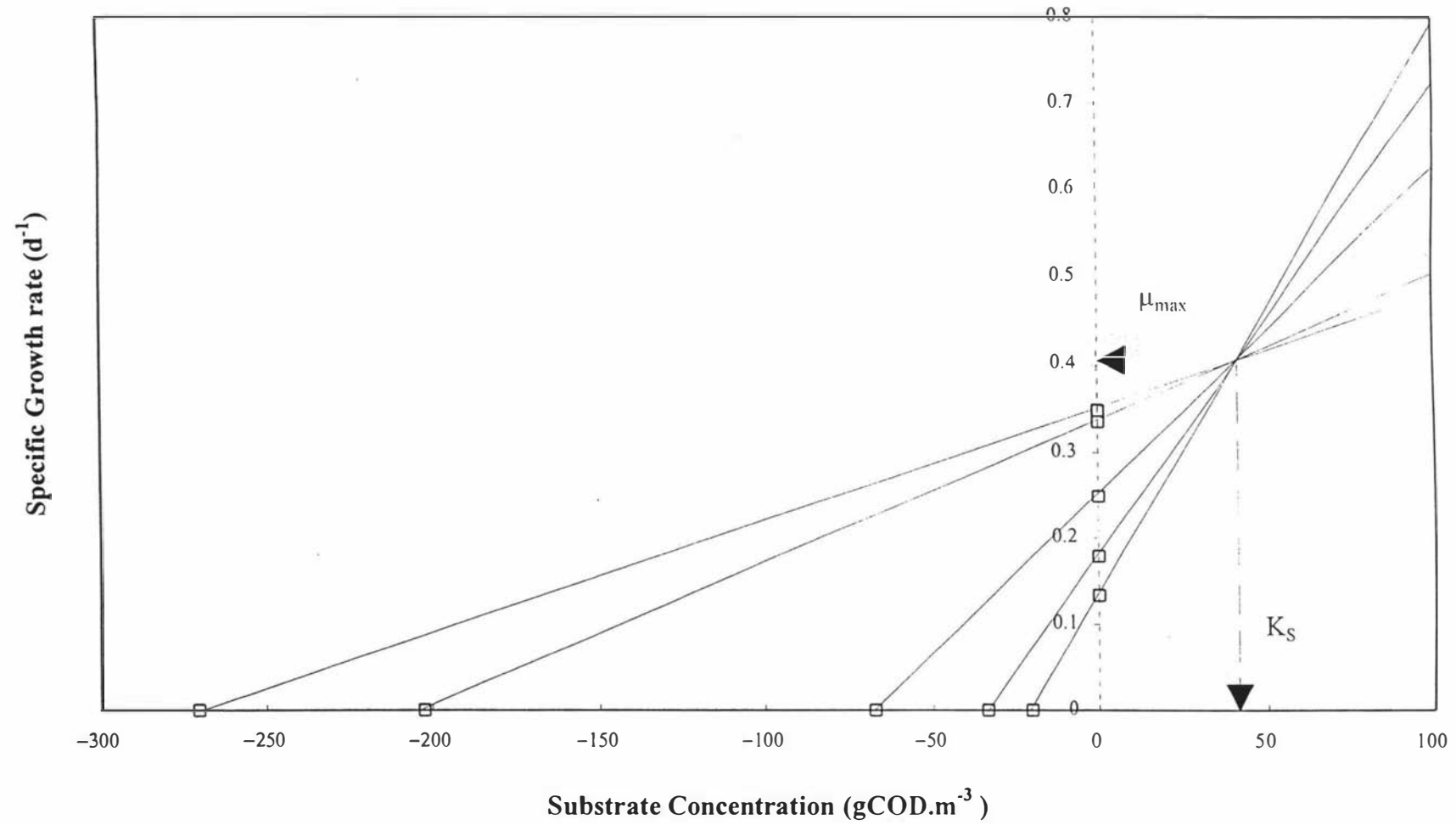
Figure 4.9   Use of a wide range of initial substrate concentrations to determine U_{max} and K_S using the Low S/X ratio method.

4.3.3 Measurement of Growth Yield, Y_H

Several methods are reported for the estimation of heterotrophic biomass growth yield, Y. All the estimated yield values made in this study were calculated on a COD basis (Y_H), where Y_H is equal to g.cell COD produced per g.substrate COD consumed.

4.3.3.1 Y_H from Batch Growth Tests

The traditional growth test involved measuring the increase in cell mass and corresponding decrease in substrate concentration. This is the basis of the method outlined in Henze *et al.* (1987). In this method a small amount of settled biomass is used to seed a known volume of filtered wastewater. The total and soluble COD are measured periodically and the cell yield calculated from the following two equations:

$$\text{Cell COD (g.m}^{-3}) = \text{Total COD (g.m}^{-3}) - \text{Soluble COD (g.m}^{-3}) \qquad (4.9)$$

$$Y_H = \frac{\Delta \text{ cell COD}}{\Delta \text{ soluble COD}} \qquad (4.10)$$

Batch tests were carried out in a series of respirometers at either 20°C or 25°C with varying initial COD concentrations. The initial substrate concentration in the respirometers varied between 225 and 1500 g.m^{-3} soluble COD and the initial S/X ratio ranged from 2.6 to 25.6, to ensure that cell replication would occur. There was not expected to be any effect of batch test temperature on the value obtained for Y_H, as Y_H has not been found to be significantly dependent on temperature or sludge age (Henze *et al.* 1987, Sollfrank and Gujer, 1991). The values that were obtained for Y_H using batch growth tests are listed in Table 4.10.

The results ranged between 0.48 and 0.71 with an average calculated to be 0.62 ± 0.11 g cell COD/g substrate COD. These results were comparable to those reported elsewhere using different substrates and biomass compositions: Orhon *et al.* (1993) reported values of Y from 0.41 to 0.538 g VSS/g COD using a high S/X method and dairy processing wastewaters, which is equivalent to Y_H = 0.58 to 0.76 g cell COD/g substrate COD consumed, if a value of 1.42 g COD per g VSS is assumed. Sollfrank and Gujer (1991) obtained an average value of Y_H = 0.64 ± 0.4 (low S/X method) and Henze *et al.* (1987) suggest a typical value of Y_H = 0.67 (no method specified), both for domestic wastewater substrates.

Table 4.10   Estimation of Y_H from batch growth tests.

Time (hours)			4	5	6	7	8	24	Average
Date	S_O	S_O/X_O							Y_H
30/11/92	550	6.0	0.67	0.59	0.57	0.64	0.62		0.62
2/12/92	350	3.9	0.58	0.65	0.57		0.57		0.59
8/12/92	400	2.6		0.65	0.68	0.70	0.65		0.67
8/12/92	320	2.7		0.68	0.70	0.71	0.68		0.69
30/12/92	1350	11.6			0.58	0.66	0.68		0.64
8/1/93	990	8.6				0.66	0.68		0.67
1/4/93	1300	10				0.48	0.66	0.48	0.54
1/4/93	900	10				0.60	0.48	0.50	0.53
1/4/93	620	10				0.63	0.57	0.54	0.58
25/5/93	1500	25					0.67	0.68	0.68
25/5/93	1500	25					0.57	0.59	0.58
25/5/93	740	13				0.68	0.63	0.54	0.62
25/5/93	740	13				0.62	0.58	0.59	0.60
Average:									0.62 ±0.11

Some uncertainty may have been introduced in this method of estimation for Y_H due to the nature of the substrate. From Equation 4.9 it is assumed that all the insoluble COD is due to biomass, but from batch COD removal test data reported later in this chapter, it is apparent that some of the insoluble COD may be due to substrate adsorbed onto the cells. This would result in an overestimation of the value of Y_H, however as no clear increasing trend is observed in the value obtained for Y_H at increasing S_O concentrations, the effect of adsorbed substrate was assumed to be not significant.

4.3.3.2 Y_H Estimated From Batch Respirometric Tests

Both Cech *et al.* (1984) and Sollfrank and Gujer (1991) recommend measuring Y_H from an indirect method relating oxygen consumption to COD degraded in a batch test as follows:

$$Y_H = \frac{\text{biomass produced}}{\text{substrate consumed}} = 1 - \frac{O_2 \text{ consumed}}{\text{substrate COD consumed}} \qquad (4.11)$$

Conditions in the batch tests are such that cell replication does not occur. The above methods outline how Y_H can be determined at the same time as μ_{max} and K_S or S_S are determined.

Generally the amount of substrate added in the low S/X determination of μ_{max} and K_S was such that the culture did not return to an endogenous OUR before the DO in the respirometer had dropped to 1 $g.m^{-3}$. Therefore the method illustrated in Cech *et al.* (1984) was not used at this stage.

If the data in Table 4.5 from the estimation of S_S are used and COD degraded is assumed to be equivalent to COD added, then the method outlined by Sollfank and Gujer (1991) can be used to obtain the values for Y_H listed in Table 4.11. This method gave a value for Y_H of 0.70 ± 0.05 (g cell COD produced per g substrate COD consumed). The average value of the three closest replicates, $Y_H = 0.68$ was used in other respirometric test calculations.

Table 4.11   Estimation of Y_H from Oxygen Consumption During S_S Determination.

Date	$\dfrac{\text{g } O_2 \text{ consumed}}{\text{g COD added}}$	Y_H (g cell COD.g substrate COD^{-1})
17/3/93	0.33	0.67
18/3/93	0.32	0.68
25/3/93	0.25	0.75
31/3/93	0.30	0.70

The assumption that COD degraded is equivalent to COD added seems reasonable, as only soluble COD was added in the S_S test, and if the COD degraded was less, then the

resultant value for Y_H would be even higher, which seems unlikely considering other published literature values already stated. Also the wastewater characterisation studies implied that virtually all the soluble wastewater was degraded during the batch test.

4.3.3.3   Comparison of Y_H Estimates From Different Methods

The method for estimation of Y_H seemed to affect the value obtained. As for the μ_{max} and K_S results, batch tests in which cell replication occur seemed to give different results to those in which replication did not occur. In this case the average Y_H value of 0.61 from batch growth tests was lower than the value of $Y_H = 0.68$ obtained from oxygen consumption data. Chudoba *et al.* (1992) also found that Y_H depended on S_O/X_O ratio, with lower Y_H values as S_O/X_O increased. This was explained as being due to more substrate being oxidised for energy and therefore less substrate being channelled into cell mass during cell replication 'growth' than during substrate accumulation and storage 'growth'.

The Y_H value that was used in the calculations for S_S, μ_{max} and K_S depended on the type of experiment used. For the respirometric estimation of S_S, μ_{max} and K_S at low S/X ratios, the respirometrically determined value of $Y_H = 0.68$ was used. For the determination of μ_{max} and K_S at high S/X ratios, the average value of Y_H measured on the set of respirometers from COD and TSS data was used, as listed in Table 4.10. In this manner the 'growth' yield value that was used in the calculation corresponded to the 'growth' response measured in the test.

4.4   Lactose Removal Rate

As the readily biodegradable fraction of the wastewater was estimated to be due to the presence of lactose, the ability of the mixed culture to remove lactose was assessed.

The rate of removal of lactose by acclimated biomass was measured by adding known amounts of lactose to a series of aerated respirometers, each containing one litre of continuous reactor mixed liquor and maintained at 20 °C. Initial target concentrations of between 200 and 800 g lactose.m^{-3} and 1530 to 1620 g VSS.m^{-3} were used. Samples were withdrawn every 60 minutes and immediately filtered through a 0.45 μm membrane filter, the lactose content being determined using HPLC.
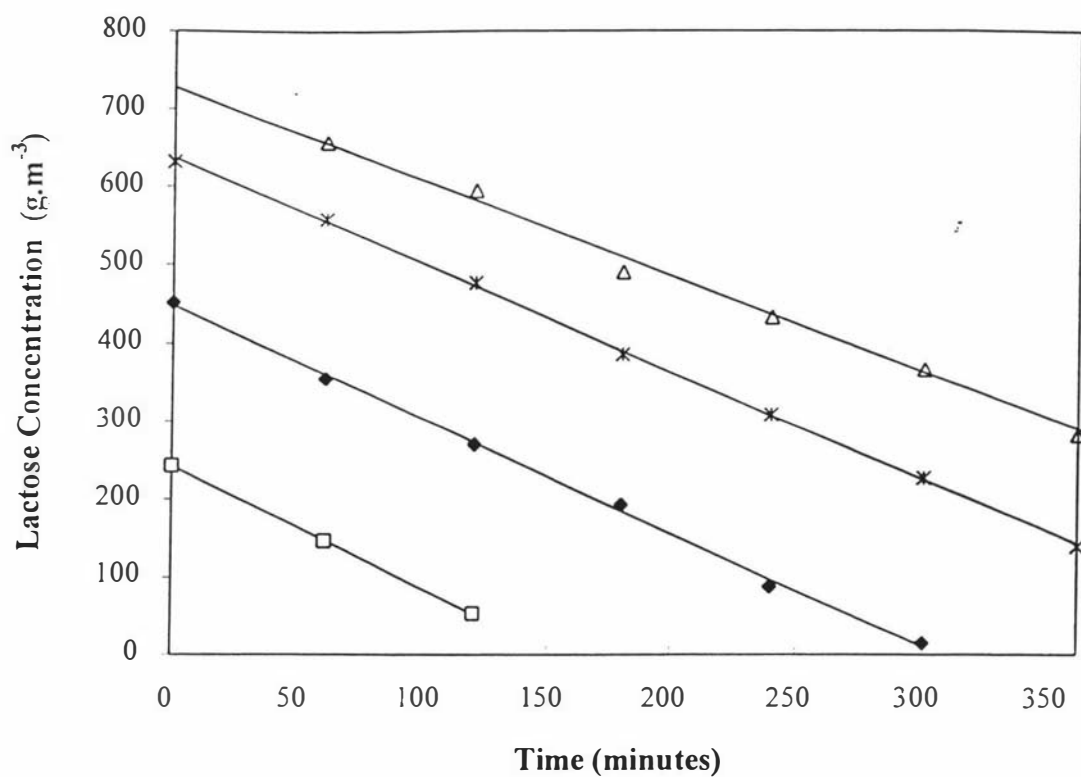
Figure 4.10: Lactose removal rates during batch tests with varying initial lactose concentrations.
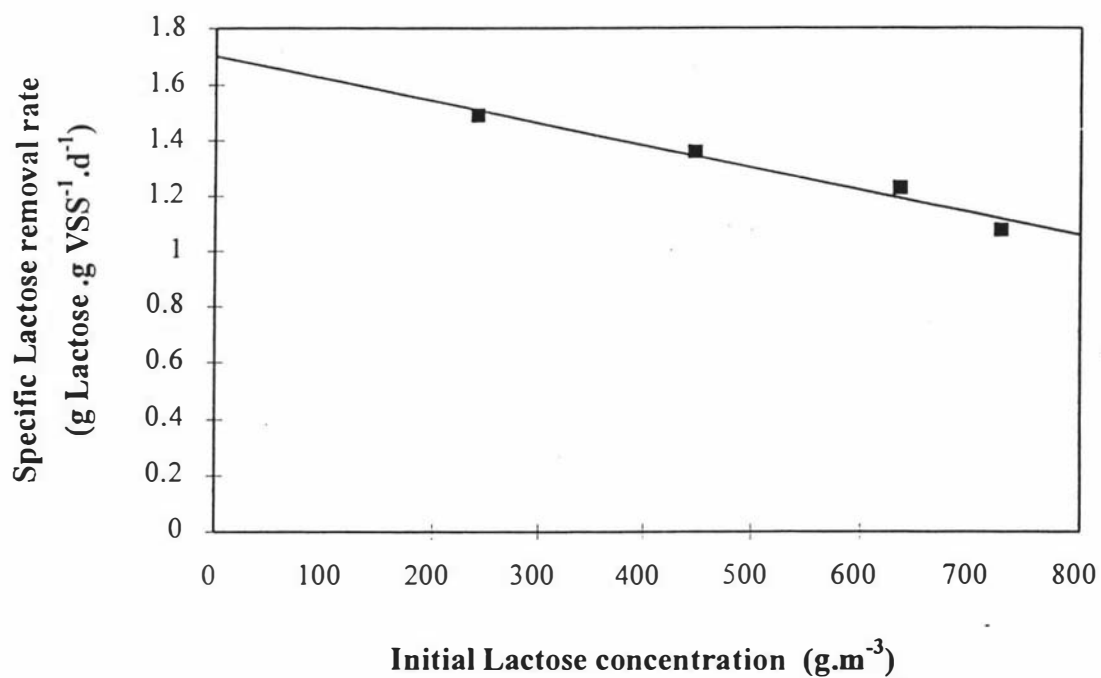
Figure 4.11: Effect of initial lactose concentration on removal rate in batch tests.

A plot of lactose concentration versus time for each batch test is shown in Figure 4.10. From a regression analysis of the data, it was found that the data were best described using a zero order equation, hence linear lactose removal rate constants were calculated as listed in Table 4.12.

Table 4.12   Lactose Removal Rates at Varying Initial Concentrations.

Target Initial Lactose Concentration ($g.m^{-3}$)	200	400	600	800
Initial VSS in Respirometer ($g.m^{-3}$)	1530	1550	1610	1620
Removal Rate ($g.lactose.m^{-3}.min^{-1}$)	1.58	1.46	1.37	1.21
Specific Removal Rate ($g\ lactose.\ g\ VSS^{-1}.d^{-1}$)	1.49	1.36	1.23	1.08
R^2 for linear regression	0.99	0.99	0.99	0.99
R^2 for log regression	0.96	0.83	0.95	0.94

It is indicated that removal rate was dependent on initial lactose concentration with faster removals being observed at lower initial substrate levels. The reduction in removal rate appeared to be a linear function of initial lactose concentration as shown in Figure 4.11. Using a lactose COD content of 1.12 g COD/g lactose, the above data corresponds to COD removal rates of between 1.2 and 1.7 g COD.g $VSS^{-1}.d^{-1}$.

4.5   COD Removal Rate

The rate of soluble COD removal by the acclimated microorganisms was also determined by batch tests. A known amount of concentrated, GFC filtered substrate was added to a series of respirometers to give initial COD concentrations of between 400 and 1600 $g.m^{-3}$. Samples were withdrawn approximately half hourly and immediately filtered using GFC filters, the COD was then determined on the filtrate. The resultant COD profiles are shown in Figure 4.12, with the values at time t=0 minutes representing the theoretical initial soluble COD concentration in solution due to the volume of concentrated substrate injected.

It can be seen that a greater proportion of the COD appeared to be removed in the first 30 minute time interval, followed by a linear removal rate until a level of approximately 100 g.m^{-3} soluble COD was reached. A regression analysis was performed using the data during the linear phase only, resulting in the removal rates listed in Table 4.13. A comparison of R^2 values indicated that COD removal was best described as a zero order process, although the data could also have been adequately described by a first order relationship. From the results obtained, there was no definite effect seen of initial substrate concentration on removal rate. When the COD remaining in solution had decreased to about 100 g.m^{-3}, the removal rate declined significantly, indicating that different removal mechanisms were operating at low COD concentrations.
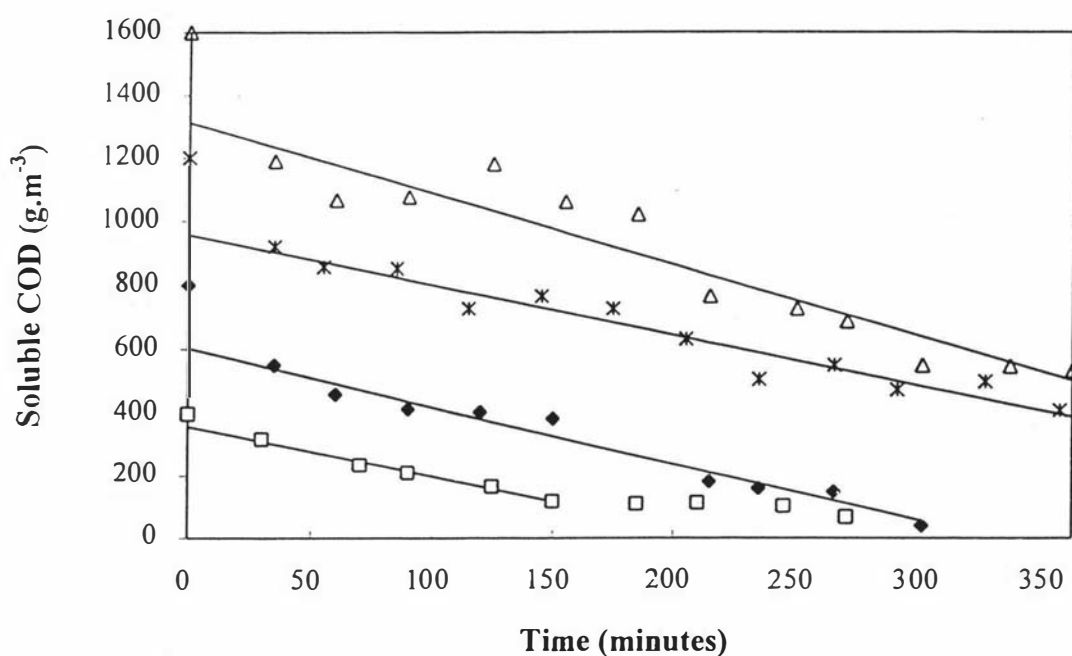
Figure 4.12: Soluble COD removal rates during batch tests at varying initial soluble COD concentrations.

The greater mass of COD appearing to be removed from solution in the first 30 minute time interval was thought to be due to the adsorption of substrate onto the biomass when initially mixed in the respirometer. This mechanism of COD removal has been observed to be significant by other researchers (Chiesa *et al.*, 1985; Bunch and Griffin, 1987; Goronszy and Eckenfelder, 1986; Rensink and Donker, 1991; Pujol and Canler, 1992). If the regression line is used to predict a soluble COD concentration at t=0 minutes, then the amount of COD initially removed by adsorption can be calculated in

each test. It can be seen from Table 4.13 that the mass of COD adsorbed per mass of suspended solids in the respirometer increased as the initial COD concentration increased, which would be expected as generally the amount adsorbed per unit weight of adsorbant is a function of the concentration of adsorbate (Metcalf & Eddy, 1991).

The substrate COD removal rate was lower than the lactose removal rate, as would be expected due to the presence of other more slowly degraded compounds present in the substrate. The specific removal rates determined above indicate that the substrate would be amenable to activated sludge treatment given the usual design loadings of 0.2 to 0.6 g BOD. g $TSS^{-1}.d^{-1}$ suggested in Metcalf and Eddy (1991), assuming an average BOD:COD ratio of around 0.5 (Marshall and Harper, 1984).

Table 4.13 Soluble COD Removal Rates at Varying Initial Concentrations.

Soluble COD added (g sCOD.m^{-3})	400	800	1200	1600
VSS in Respirometer (g.m^{-3})	2490	2630	2930	2850
Initial Biosorption (gCOD.gVSS^{-1})	0.016	0.075	0.083	0.101
Removal Rate (g.sCOD.m^{-3}.min^{-1})	1.62	1.82	1.56	2.24
Specific Removal Rate (g sCOD. g VSS^{-1}.d^{-1})	0.94	1.00	0.77	1.13
R^2 for linear regression	0.99	0.97	0.95	0.89
R^2 for log regression	0.98	0.84	0.94	0.89

4.6 Continuous Reactor.

To provide acclimated biomass for the batch tests described in this chapter, a small scale continuous reactor without cell recycle was operated for the duration of the biokinetic investigations. The reactor was originally seeded with a mixed culture obtained from a full scale dairy wastewater treatment facility and its performance in terms of biomass concentration and COD removal was monitored during two intervals of its operation.

4.6.1   Continuous Reactor Operation.

The reactor comprised of a 6.25 litre working volume, mixed and aerated vessel with a SRT and HRT of 2.5 days. It was continuously fed with the synthetic substrate as defined in Table 4.3, except that the feed stream was more concentrated, having a total COD of 3750 g.m^{-3} to provide a loading rate of 1.5g COD per litre of reactor volume per day. At lower feed concentrations the resultant biomass concentration in the reactor was not sufficient to provide the required mass of cells for batch tests.

The continuous reactor was operated at a short SRT so that the growth of nitrifiers was likely to be negligible at the initial level of organic carbon used. Therefore inhibition of nitrification was not considered necessary in the batch tests described in this chapter and all of the OUR in the batch tests was assumed to be due to the activity if heterotrophic microorganisms.

4.6.2   Continuous Reactor Treatment Performance.

The performance of the reactor in terms of COD removal and mixed liquor concentration was monitored during two intervals of its operation. The results obtained are listed in Table 4.14.

Similar results were seen for the two periods monitored, the TSS levels being slightly higher in the second period due to a small increase measured in influent COD concentration. The overall soluble COD removals were very high, up to 93%. This was to be expected considering the highly degradable nature of the wastewater. The effluent produced was turbid due to the presence of dispersed suspended biomass, which did not readily settle out of the liquid. The mixed liquor was a predominantly a yellow colour, but varied occasionally through to a more orange hue, indicating that the composition of the culture was probably changing.

Table 4.14   2.5 day HRT/SRT Continuous Reactor Performance

	November 1992	January 1993
Reactor feed characteristics		
pH	11.0	11.0
Flowrate $(m^3.d^{-1})$	2.5×10^{-3}	2.5×10^{-3}
Total COD $(g.m^{-3})$	3700	3900
Soluble COD $(g.m^{-3})$	2750	2900
COD Loading Rate $(g\ COD.\ l^{-1}.d^{-1})$	1.48	1.56
Reactor mixed liquor / effluent characteristics		
pH	8.3	7.4
TS $(g.m^{-3})$	1640	1820
TSS $(g.m^{-3})$	1220	1470
VSS $(g.m^{-3})$	1100	1440
Total COD $(g.m^{-3})$	2100	2450
Soluble COD $(g.m^{-3})$	270	210
Overall performance		
F/M $(gCOD.gVSS^{-1}.d^{-1})$	1.35	1.08
Soluble COD removal (%)	90	93

4.7   Discussion

This chapter investigated the biodegradability of a wastewater designed to be representative of that from a typical milk processing facility involved in the manufacture of butter and milk powder. A substrate with a COD, lactose, fat and N content very similar to the typical 'real' wastewater could be achieved using a mixture of milk powders and butter, as the actual wastewaters comprise predominantly of diluted milk and milk products.

The wastewater had an total COD of 2200 $g.m^{-3}$, of which 75% was attributable to GFC filterable organics. The high turbidity and significant soluble COD fraction between

0.45 and 1μm (400 $gCOD.m^{-3}$) indicated the presence of dispersed and colloidal material as well as dissolved organic matter.

The wastewater degradability was characterised according to the oxygen uptake rate response of an acclimated mixed culture with almost all the GFC filterable organics being degraded within a matter of hours. The wastewater characterisation results could be provisionally related to substrate components on the basis of molecular weight.

The organic matter in dairy processing wastewaters consists almost entirely of either lactose, milk proteins or milk fats (Walstra and Jenness, 1984). In the synthetic wastewater the lowest molecular weight fraction, lactose, comprised about 80% of the readily biodegradable COD fraction (S_S) which is able to be directly metabolised by cells (Henze, 1992). The fat content of milk exists as micelles, with particle sizes covering a wide range from 0.1 μm to 10μm. Due to their size distribution, approximately 97% of the fat content of milk would be 'insoluble' when GFC filtered, and would be expected to form part of the 'particulate' slowly hydrolysable fraction, X_S. Over half of the COD of the X_S fraction (57%) could be attributed to milk fats.

The remaining major milk component, protein, consists of casein micelles in dispersion (80%) and soluble whey proteins (19%) with molecular weights ranging from 18,500 to 23,500. The proteins were expected to contribute to both the S_S, X_S and rapidly hydrolysable (S_H) fractions. The whey proteins probably contributed to the more biodegradable fractions due to their smaller size and soluble nature. The inert fractions, both soluble and particulate were assumed to be not significant due to the nature of the waste and a COD balance of milk components and negligible concentration of non-volatiles concurred with this assumption.

Typical values of fS_S and fS_H for raw domestic wastewater COD have been reported by Henze (1992) to be 10-15% readily biodegradable and 15-25% rapidly hydrolysable. The substrate used in this study was found to have an average fS_S of 42.5% and f S_H of 28.5%, therefore this dairy wastewater has a significantly higher readily biodegradable fraction than typical domestic wastewaters.

The performance of both batch and continuous tests in terms of soluble substrate removal was measured. Up to 93% of influent soluble COD was removed in a 2.5 day HRT/SRT continuous reactor. The total influent COD removal could not be assessed due to the high level of suspended solids in the effluent. Batch lactose and soluble substrate removal rate tests resulted in specific COD removal rates of up to 1.69 and

0.89 gCOD.gTSS^{-1}.d^{-1} respectively. The lactose removal rates were higher than for the substrate as expected, because lactose forms the most readily biodegradable fraction of the substrate, as already discussed. These removal rates indicate that the wastewater is amenable to activated sludge treatment and other high organically loaded/ high rate methods of aerobic treatment.

Estimates were gained for the biokinetic parameters Y_H, μ_{max} and K_S of the acclimated biomass. The values gained depended on the initial S/X ratio in the batch test used for the determination.

Average values of μ_{max} and K_S ranged from 1.2 d^{-1} and 17 g.m^{-3} COD at low S/X ratio, to 3.4 d^{-1} and 150 g.m^{-3} COD at high S/X ratio. In a summary paper, Henze *et al.* (1987) gave typical values for μ_{max} and K_S of 6 d^{-1} and 20 g.m^{-3} COD respectively for activated sludge treatment of domestic wastewater (no method specified). Sollfrank and Gujer (1991) gave values of 1.5 d^{-1} and 5 g COD.m^{-3} using a low S/X method on domestic wastewater. Dairy wastewater values (Orhon *et al.*, 1993) have been reported as 3.3 d^{-1} and 74 g.m^{-3} COD using a high S/X method. The average results from this study, depending on determination method used, are therefore not substantially different to other published values and the magnitudes of μ_{max} and K_S for dairy processing wastewaters seem to be similar to those obtained for domestic wastewaters.

The average yield value in COD terms, Y_H, was calculated to be 0.61 ($\pm$ 0.08) g cell COD per g substrate COD when high S/X methods were used and 0.68 when low S/X methods were used. These results are similar to other published values for Y_H (Henze *et al.*, 1987; Sollfrank and Gujer, 1991).

As the values obtained for kinetic parameters were dependent upon the estimation method used, there must be some question about the appropriateness of the methods used. Several authors (Chudoba, 1990; Peil and Gaudy, 1971) have questioned the validity of using Monod kinetics at all for mixed cultures growing on multicomponent substrates. Also the concept of measuring 'growth' rate as a response which does not involve cell replication has been a matter of much debate, as summarised in Chudoba *et al.* (1992) and Grady *et al.* (1996).

Although 'Monod' kinetics may not be strictly applicable to mixed cultures and multicomponent substrates, the fact that conclusive results could be obtained for μ_{max} and K_S from the methodology employed, indicate that a Monod type hyperbolic expression does indeed describe the relationship between biomass 'growth' response and

substrate concentration in this case.

The argument of whether the methods used are valid or not, should really depend on what the end user wishes to utilise the kinetic data for. In activated sludge modelling it is important to be able to measure the response of an acclimated mixed culture to various substrate concentrations under typical activated sludge operating conditions. The kinetic values are of use only in being able to accurately predict the most important plant performance characteristics: particularly the oxygen requirement, and to a lesser extent, the mixed liquor suspended solids concentration.

The actual value obtained for μ_{max} is not critical for the operation of the activated sludge plant as it is general practice to operate at SRTs much greater than the minimum value indicated by μ_{max}. Being able to detect significant culture shifts or changes in affinity of the biomass for substrate is more important than the actual numerical value gained. The actual S/X ratio experienced by the biomass in a conventional activated sludge system is also very low. So it would seem reasonable that a low S/X method would be more appropriate for determining the response of the culture in a state more closely matched to actual treatment plant conditions.

At S/X ratios below 2, the 'growth' response measured is due to energy utilised for the accumulation and storage of substrate by the biomass (Chudoba *et al.*, 1992). As the initial S/X ratio rises above the 2-4 range, substrate is utilised for cell replication, process which requires more energy to be expended than for substrate storage. Therefore the cell yield value obtained will depend on initial S/X ratio in the test. In the short period of time employed in a batch growth test, only the faster growing microorganisms will multiply, so that at the end of the test the mixed culture will have a different composition from the original culture, with a higher proportion of faster growing species. Therefore the value of μ_{max} and K_S obtained will also depend on the initial S/X ratio. As it is the response of the original mixed culture to applied substrate that is of interest, a low S/X method is the only manner in which the biomass can be studied without population shifts occurring.

This study used respirometric methods for the estimation of kinetic constants, that is, the parameters of interest were determined from the oxygen uptake rate of the biomass in response to different substrate concentrations. Other commonly used methods either measure the increase in cell mass, or the decrease in substrate concentration. Respirometry may be an indirect method of measuring the cell 'growth' response, however it was deemed to be appropriate in this case as the oxygen consumption rate is

more critical in the operation of real activated sludge systems than substrate removal rates (Henze *et al.*, 1987), and more representative of the mixed culture under study.

Therefore, it is believed that the methods utilised for the estimation of biokinetic parameters were appropriate and valid for the proposed end use of the data in this study.

The values obtained for the kinetic constants can only really be considered as a range of values, rather than an absolute value due to the multicomponent nature of the substrate and mixed microbial composition of the biomass. The results gained for μ_{max} and K_S by the low S/X method varied over a period of 6 months from 0.5 to 2.3 d^{-1} and 8.1 to 44 $g.m^{-3}$ COD respectively. The colour of the mixed liquor in the continuous reactor also varied over the period of time that the experiments were carried out, indicating that a stable 'steady' state had not been reached even after 70 SRTs. The occurrence of population shifts with time was probably contributed to by the lack of temperature control in the reactor which was operated at ambient temperatures in the laboratory, varying between 20 and 25 $^{\circ}C$.

The consistently high substrate removal performance in the batch reactor supported the assessment of a high degree and rate of biodegradability made in the substrate characterisation and removal tests. The dairy processing wastewater resulted in biomass with similar biokinetic parameters as found for domestic wastewaters, but improved biodegradability indicated that treatment systems with shorter residence times and producing a higher quality effluent in terms of organics removal may be possible with dairy processing effluents than those of domestic origin.

4.8   Conclusions

A synthetic substrate was designed for use in this study, which had a similar COD, lactose, fat and N content as a typical wastewater from a butter and milk powder production facility.

Initial biodegradability studies demonstrated that the substrate to be used was easily and rapidly biodegraded. The organic matter in the substrate was determined to be predominantly soluble: with 42.5% of the total COD being readily biodegradable, mostly due to lactose; and a further 28.5% of the COD being rapidly hydrolysable, due to the presence of the milk proteins. The remaining 29% of COD was more slowly biodegradable, due to milk fat and larger milk proteins in the substrate.

The biokinetic parameters measured for microbial growth on the substrate also indicated a highly degradable wastewater. Respirometric methods were used to determine average values of $\mu_{max} = 1.15$ d^{-1}, $K_S = 17$ g.m^{-3} COD and $Y_H = 0.68$ g cell COD per g substrate COD. Both low S/X and high S/X methods were used to determine biokinetic parameters, the low S/X method being preferred as it would not involve population shifts in the mixed culture.

Substrate removal rates in both batch and continuous tests indicated that a good quality effluent in terms of COD removal was achievable in a relatively short time period, suggesting that relatively highly loaded systems were an option with this wastewater. Specific substrate removal rates of up to 1.4 g lactose.gTSS^{-1}.d^{-1} and 0.9 g soluble COD.gTSS^{-1}.d^{-1} were measured with a total of 93% soluble COD removal being achieved in a 2.5d HRT/SRT continuous reactor.

Substrate characterisation tests using acclimated biomass suggested that dairy processing wastewater was highly and rapidly biodegradable, with negligible inert or recalcitrant organic matter. It is indicated that the wastewater would be amenable to activated sludge treatment and that a high quality effluent in terms of organic matter removal should be readily achievable.

CHAPTER 5

MODEL ACTIVATED SLUDGE PERFORMANCE

5.1   Introduction

Having defined and characterised the substrate to be used, the next stage of the study was to develop a laboratory scale activated sludge reactor for the treatment of the synthetic wastewater. As the wastewater had a high proportion of readily biodegradable substrate, a relatively short hydraulic residence time would be possible. Biokinetic data had established a maximum specific growth rate of 1.15 d^{-1}, which would also have allowed a very short solids residence time, however more conventional SRTs were considered to be more appropriate, especially as this would permit nitrification to also take place.

The reactor configuration to be used was a conventional completely mixed reactor with external settler, and although some other researchers have reported filamentous bulking problems with dairy processing wastewaters, the suspended growth treatment plants currently operating in NZ also employ this configuration.

The treatment performance and reactor operation at different reactor solids retention times (SRT) was investigated, as well as the effect of variations in organic loading. The main features to be studied for the various operating regimes were: treatment efficiency in terms of COD and TSS removal; substrate removal rates; and biokinetic parameters of the mixed culture.

5.2   Reactor Configuration and Operating Characteristics.

A conventional activated sludge reactor configuration was used, as shown in Figure 5.1, consisting of a completely mixed aerated reactor with a working volume of 10 litres and a 1.75 l settler. The wastewater flowrate to the system was maintained at 10 $l.d^{-1}$,
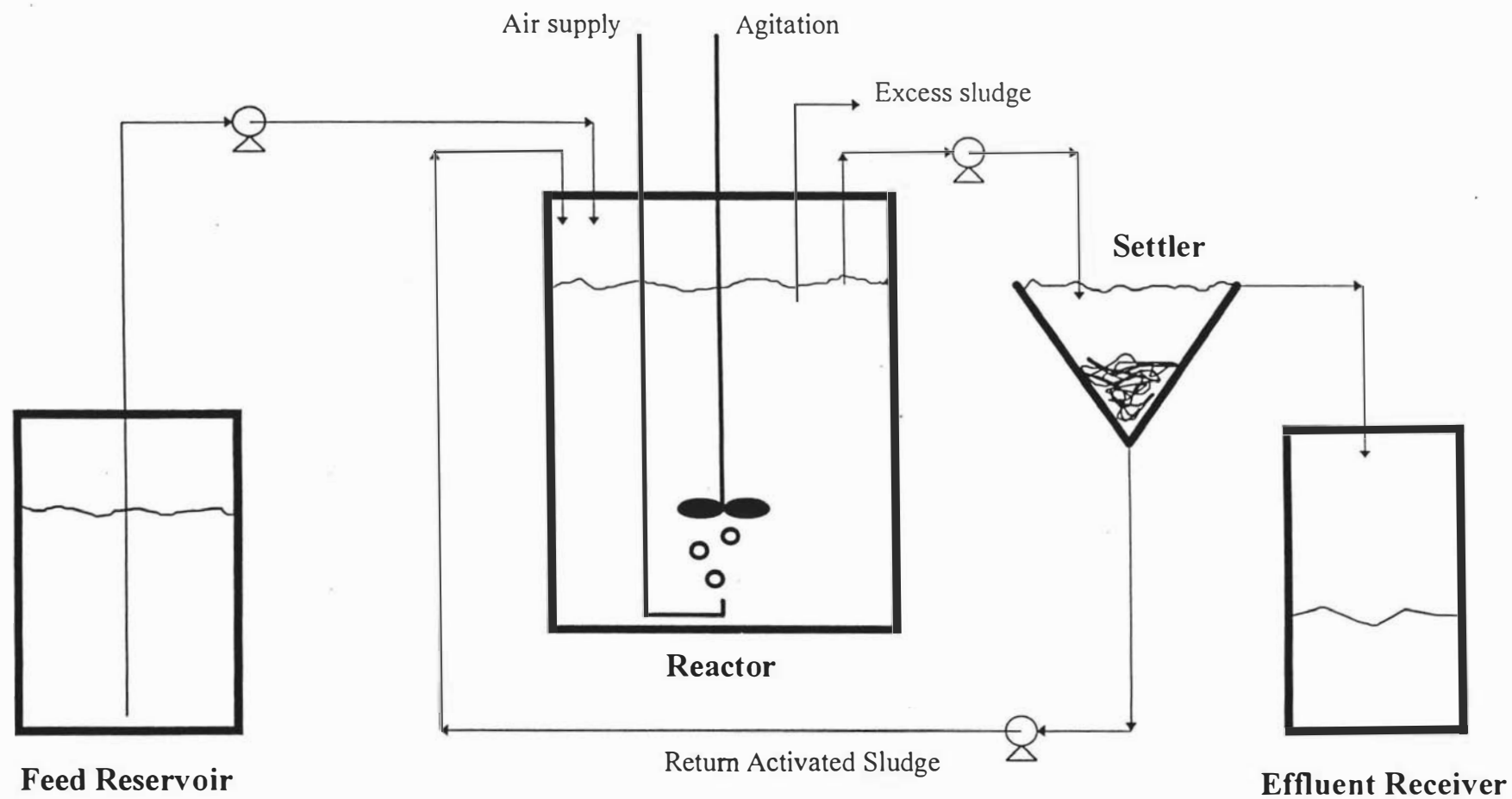
Figure 5.1   Reactor configuration during the conventional CSTR Trials.

giving a HRT of 1 day and hydraulic loading of 2.2 $kgCOD.m^{-3}d^{-1}$ at the average measured feed concentration of 2200 $g.m^{-3}$ COD. Temperature in the aerated reactor was controlled to 25 ($\pm$ 0.1) $^{\circ}C$ and an air flowrate of 7 $l.min^{-1}$ was maintained throughout all subsequent trials.

A range of SRTs from 5 to 30 days were used, with the sludge age being based on the reactor contents only, as generally the mass of microorganisms in the clarifier was negligible compared to that in the reactor. The average value obtained for μ_{max} of 1.2 d^{-1} in initial batch studies indicated that quite short SRTs were possible with this wastewater, however more conventional sludge ages were chosen that would support a nitrifying biomass. The sludge wastage regime used to maintain the desired SRT was dependant on the length of the sludge age. For the 5 day and 10 day SRT trials, sludge wastage was achieved by continuously withdrawing mixed liquor from the reactor vessel. For the 20 and 30 day SRT trials, the required biomass was removed once daily from reactor. The wastage regimes were in accordance with the recommendations made by Marais and Ekama (1976) and were calculated taking the suspended solids in the effluent stream into account:

$$\text{SRT (days)} = \frac{V_R X_R}{V_E X_F + V_W X_R} \tag{5.1}$$

where:

V_R = Volume of reactor (m^3)

X_R = TSS in reactor ($g.m^{-3}$)

V_E = Volume of effluent produces per day (m^3)

X_E = TSS of effluent ($g.m^{-3}$)

V_W = Volume of reactor mixed liquor wasted per day (m^3)

The reactor feed was made up daily, consisting of 1.5g of butter, 6.5g of whole milk powder and 7.0g of skim milk powder in 10 litres of tap water. The wastewater was adjusted to a final pH of 11.0 using 0.5N NaOH solution. Refrigeration of the feed was not deemed necessary as measurement of the pH and COD over a 24 hour period indicated that the wastewater was stable at room temperature for that length of time. The feed containers were washed thoroughly each day to prevent microbial contamination and all pump tubing and reactor walls were regularly cleaned to remove wall growth.

The return activated sludge (RAS) flowrate was varied as necessary to maintain a minimum volume of settled sludge in the clarifier.

5.3   Activated Sludge Reactor Start-up Procedure.

The activated sludge reactor used in each SRT trial was seeded with biomass from the 2.5 day HRT/SRT reactor. The reactor was usually batch fed for up to five days to increase the mass of cells in the reactor and to encourage flocculant growth, then continuously fed at the desired flowrate of 10 l/d. Sludge wastage began after the suspended solids in the reactor reached about 3000 g. m^{-3} and effluent TSS had dropped below approximately 100 $g.m^{-3}$ TSS, indicating that a well flocculating biomass had been formed.

5.4   Reactor Performance at Varying SRT

A total of four trials were conducted with SRTs at 5, 10, 20 and 30 days. Reactor contents, feed and effluent streams were monitored daily. Each trial was continued for as long as possible until system failure occurred, which in each case was due to filamentous bacterial growth causing excessive washout of biomass in the effluent stream. It was intended to operate each reactor for approximately 3 SRTs until a 'psuedo steady state' was reached prior to assessing performance, however most trials failed before this length of time, so the data is presented as the number of days of operation at the target SRT.

5.4.1   Reactor Conditions.

The average reactor TSS concentration was dependent on system SRT, with an increase in TSS accompanying an increase in SRT. The average mixed liquor concentrations that were achieved after TSS had stabilised and before system failure occurred can be seen in Figure 5.2 and are summarised in Table 5.1. The average VSS/TSS ratio measured during each trial appeared to have a decreasing trend with increasing SRT, however the results were not significantly different when 95% confidence intervals were compared. The resultant substrate loading, or food:microorganism (F/M) ratio in each trial was calculated from the following equation as given in Metcalf & Eddy (1991):
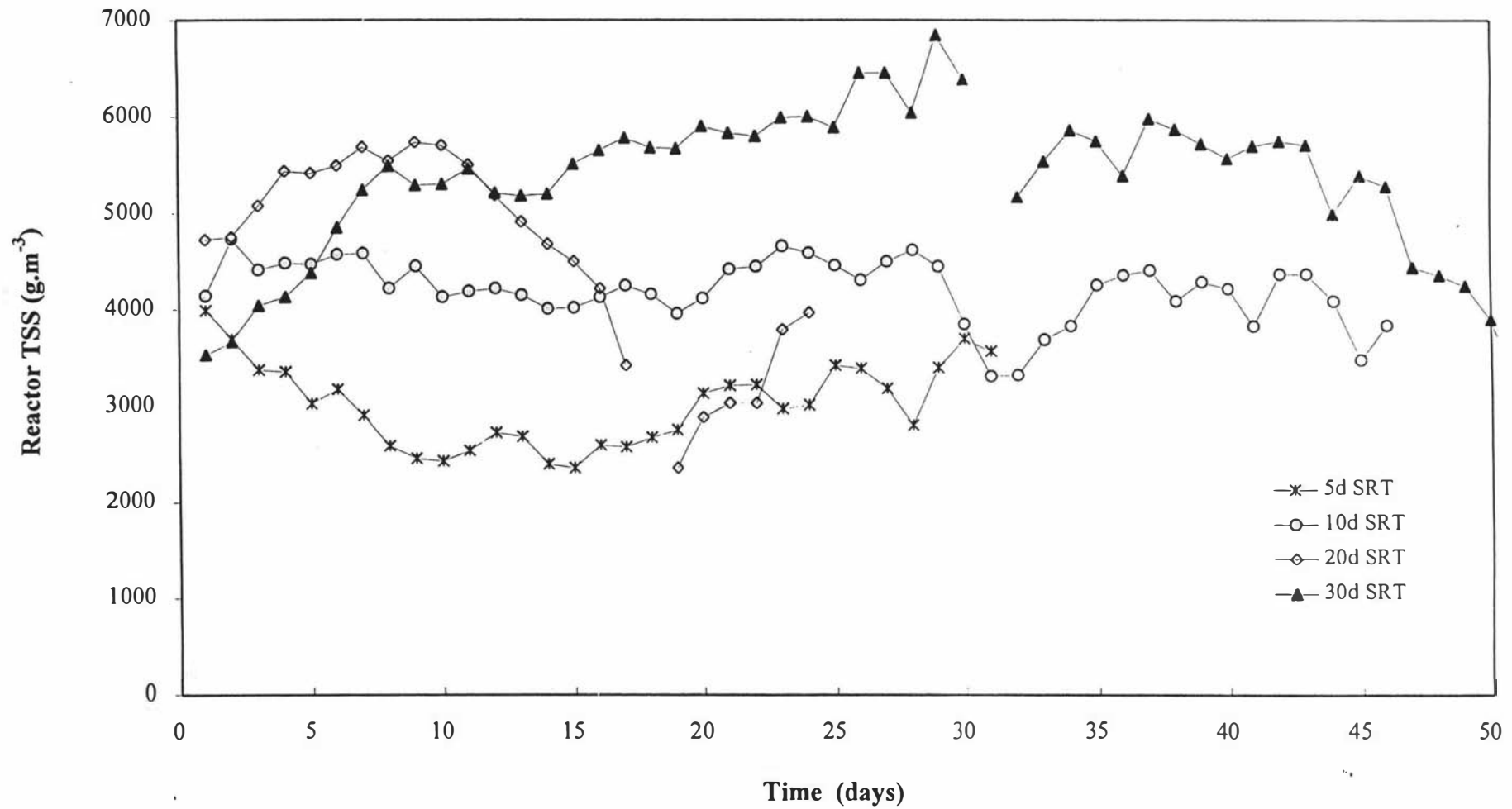
Figure 5.2:   Reactor mixed liquor suspended solids concentration during the CSTR trials at various SRT.

$$F/M \; = \; \frac{S_O \, Q}{X_R \, V}$$

(5.2)

where:

> S_O = influent COD concentration $(g.m^{-3})$
>
> Q = influent flowrate $(m^{-3}.\,d^{-1})$
>
> X_R = VSS concentration in the aeration tank $(g.m^{-3})$
>
> V = volume of the aeration tank (m^3)

At an HRT of 1 day and influent COD concentration of 2200 $g.m^{-3}$, average F/M ratios of between 0.46 and 0.82 $gCOD.gVSS^{-1}.\,d^{-1}$ were calculated, with the F/M ratio increasing as SRT and reactor VSS decreased. These F/M ratios represent typical design values for completely mixed activated sludge systems as given in Stevens and Elkin (1984) and Metcalf and Eddy (1991).

The average pH in the reactors ranged from 7.60 to 7.75, similar to that found for dairy wastewater treatment by Adamse (1968c), indicating that there was sufficient buffering capacity in the system. Biomass concentrations and average substrate loadings during each trial are listed in Table 5.1.

Table 5.1   Reactor Mixed Liquor Conditions at Different SRTs.

Target SRT (days)	5	10	20	30
Reactor pH	7.60	7.60	7.75	7.60
Average TSS $(g.m^{-3})$	3020	4220	4570	5470
TSS Range $(g.m^{-3})$	2360 - 4000	3320 - 4740	2360 - 5740	3540 - 6850
VSS/TSS Ratio	0.92 ± 0.06	0.92 ± 0.05	0.90 ± 0.05	0.89 ± 0.05
Average F/M $(gCOD.gVSS^{-1}.\,d^{-1})$	0.82	0.57	0.57	0.46

5.4.2   Reactor Effluent Quality.

A high quality effluent in terms of COD and TSS concentrations was achieved during all four SRTs trialed, as shown in Figures 5.3 to 5.6. Total COD removed by the
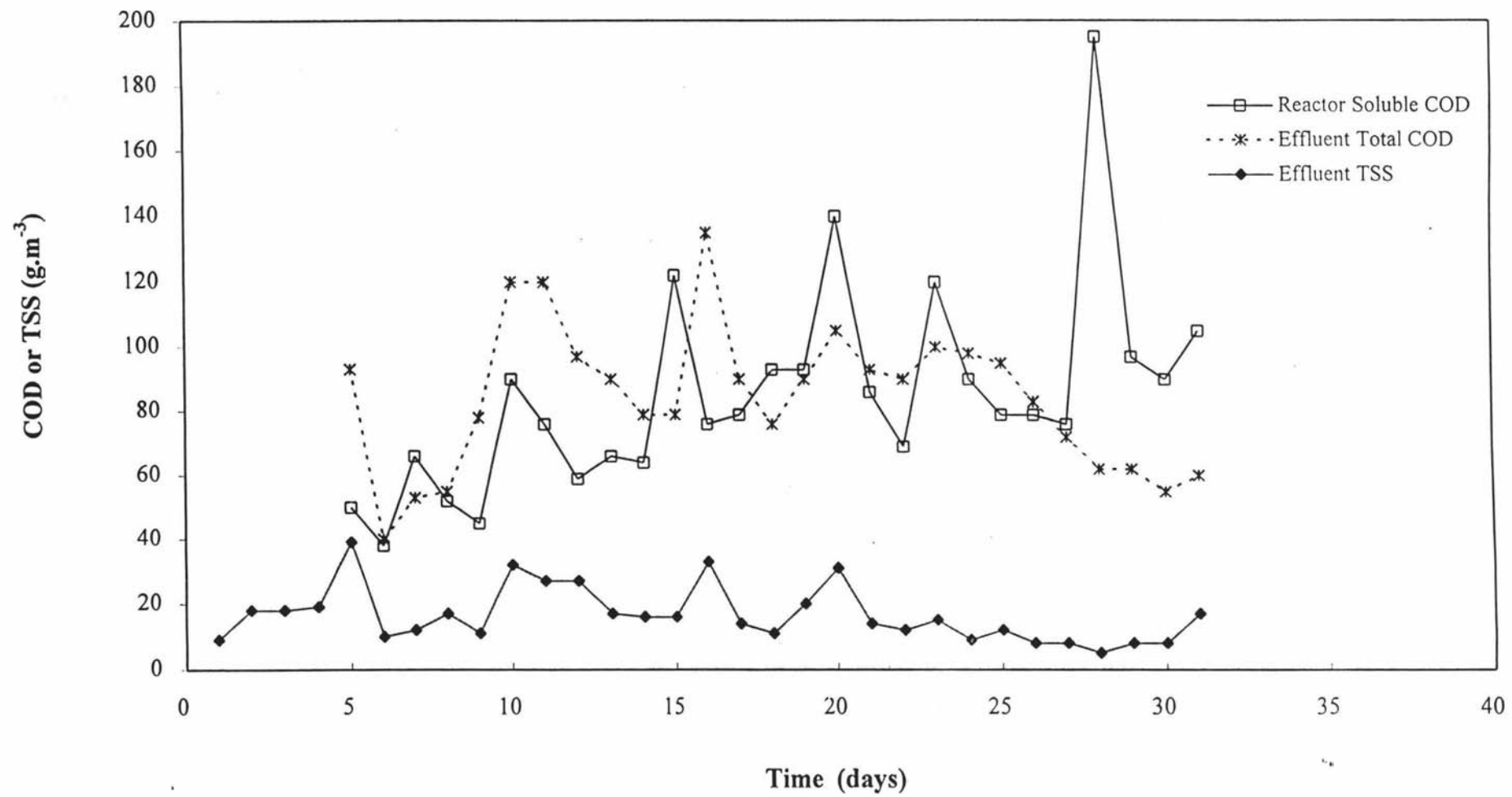
Figure 5.3: Reactor effluent quality during the 5 day SRT trial.
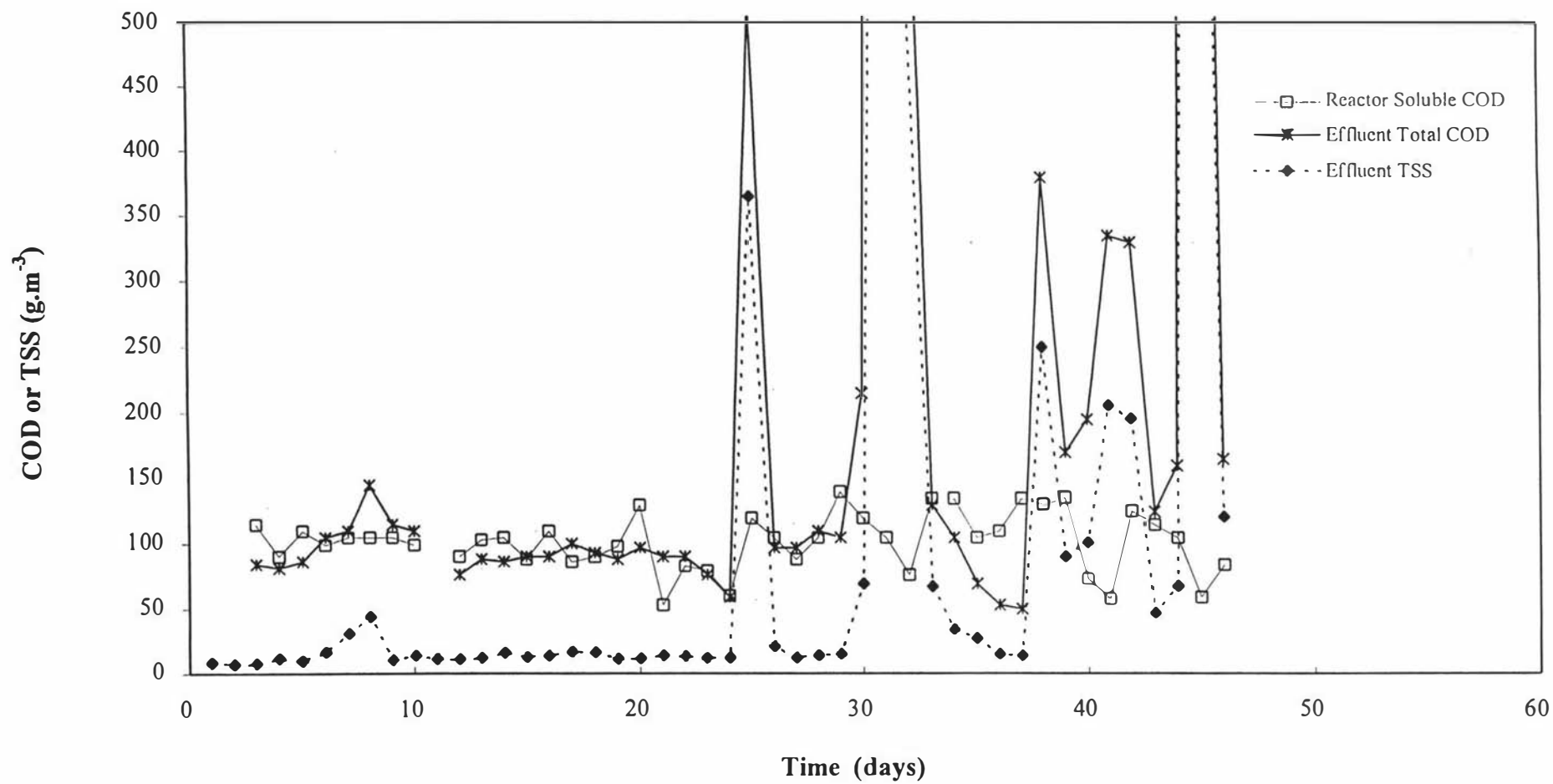
Figure 5.4:   Reactor effluent quality during the 10 day SRT trial.
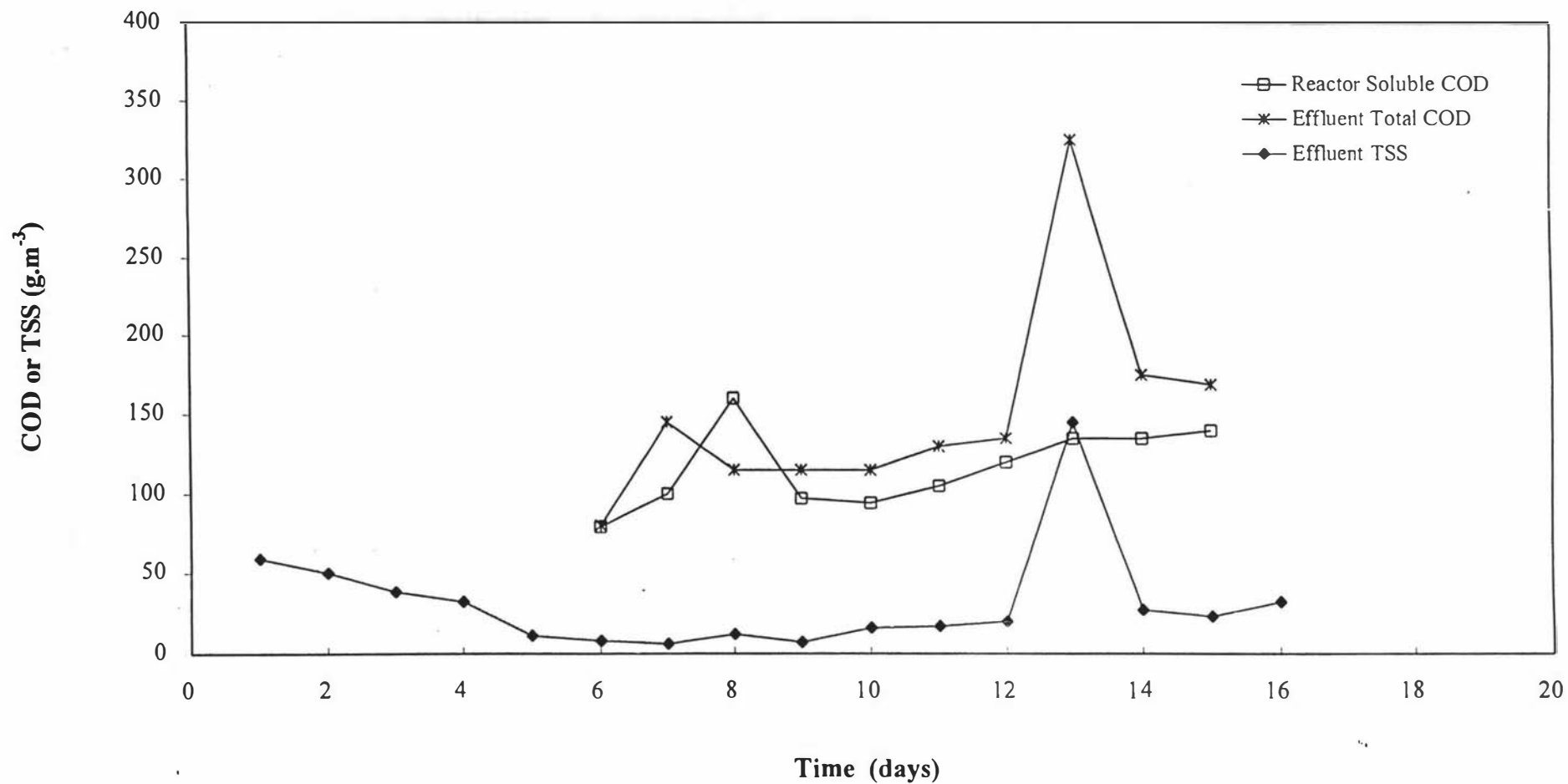
Figure 5.5: Reactor effluent quality during the 20d SRT trial.
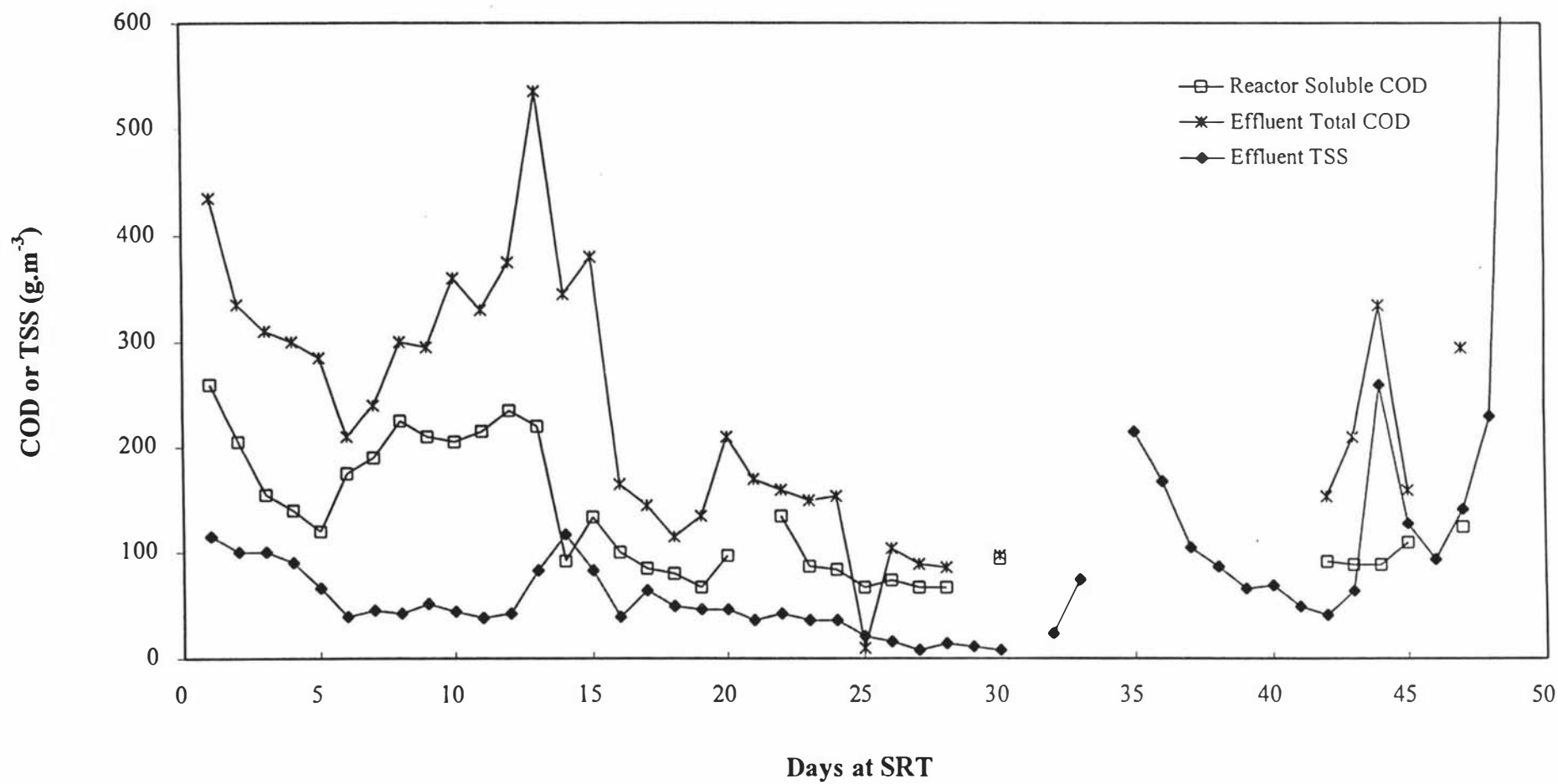
Figure 5.6: Reactor effluent quality during the 30d SRT trial

activated sludge systems ranged from 93% to 96%, with an average of up to 34% of the effluent COD due to the presence suspended solids. The reactor soluble CODs were decreased from a feed strength of 1650 g.m^{-3} to averages ranging from 79 g.m^{-3} to 117 g.m^{-3} as listed in Table 5.2. This represented average sCOD removals of 93% to 95% through the systems.

Low effluent TSS levels were achieved, averaging between 15 and 44 g.m^{-3} and the filtered effluent showed no residual turbidity or colour. There was no apparent effect of reactor SRT on effluent quality, although the 20d and 30d SRT reactors failed before a minimum of two solids retention times had passed, so it was difficult to predict whether an improved effluent quality would have been achieved had the system been able to operate for a longer period.

A summary of the effluent quality and reactor performance for each trial is listed in Table 5.2, the data used being taken from the period of stable reactor operation before filamentous bulking started to interfere with effluent quality. The results indicate that activated sludge systems could provide an acceptable treatment method for the dairy processing wastewater, assuming that operability problems in terms of filamentous bulking could be overcome.

Table 5.2   Reactor Effluent Quality at Different SRTs.

Target SRT (days)	5	10	20	30
Reactor Soluble COD (g.m^{-3})	80	95	115	85
Soluble COD Removal Efficiency (%)	95	94	93	95
Effluent Total COD (g.m^{-3})	90	95	150	130
Total COD Removal Efficiency (%)	96	96	93	94
Effluent TSS (g.m^{-3})	18	15	28	44
Effluent COD due to TSS (%)	10	-	10	34
Days at SRT used in data analysis	5 - 27	5 - 24	6 - 16	16 - 35
Days at SRT to Failure	31	45	24	48

5.4.3   Kinetic Parameters

The low S/X method for the estimation of μ_{max} and K_S as outlined in Chapter 4 was repeated with reactor mixed liquor from each SRT trial. The respirometers were maintained at 25°C during the test and nitrification was inhibited using allylthiourea, so that the OUR response measured would be due to heterotrophic biomass only. The results obtained for μ_{max} and K_S using a value of $Y_H = 0.68$ are listed in Table 5.3, and are at the low end of the 0.65 to 1.93 d^{-1} range measured at 25°C during biodegradability studies. There was no obvious effect of SRT on the values of μ_{max} and K_S apparent from the limited data obtained.

Table 5.3   Estimation of μ_{max} and K_S for mixed liquors of various SRT.

Reactor SRT (days)	5	10	30
Days at SRT when tested	16	30	22
ΔSpOUR $_{max}$ (gO_2.g cell COD^{-1}. d^{-1})	0.32	0.28	0.41
μ_{max} (d^{-1})	0.67	0.60	0.87
K_S (g.m^{-3} COD)	2.2	4.0	3.5

5.4.4   Decay Rate

The heterotrophic biomass endogenous decay rate was estimated using a respirometric batch test method as outlined in Marais and Ekama (1976) and Henze *et al.* (1987). Nitrification was inhibited by the addition of 20 g.m^{-3} of allylthiourea to the mixed liquor in each respirometer. The respirometer was aerated and maintained at 25°C for the duration of the test. Any evaporative losses were replaced with distilled water daily, before OUR measurements were taken.

The decay rate was calculated from a plot of OUR against time as shown in Figure 5.7 and represents the traditional decay rate coefficient, b. The modelling decay rate co-efficient used by Henze *et al.* (1987), b_H, is determined from b in the following manner: -

$$b_H = \frac{b}{1 - Y_H (1 - f_P)} \tag{5.3}$$

where:

f_P = the fraction of biomass that ends up as inert particulate products after decay

= 0.08 using the decay process specified by the IAWPRC model.

A summary of all the results obtained at various SRT is given in Table 5.4.

Table 5.4   Decay Rate Co-efficients Estimated at Various SRT.

SRT of biomass	Days at SRT at start of decay test	Traditional Decay Co-efficient, b (d^{-1})	IAWPRC Model Decay Co-efficient b_H (d^{-1})
5 days	10	0.27	0.91
	12	0.26	0.87
	24	0.23	0.78
10 days	13	0.24	0.82
	16	0.22	0.75
	18	0.19	0.66
	27	0.22	0.75
	41	0.22	0.76
5d SRT biomass average:		0.25	0.85
10d SRT biomass average:		0.22	0.75
Overall average:		0.23 +/- 0.05	0.79 +/- 0.19

The decay rate is not expected to vary with SRT (Ekama and Marais, 1976) and apart from the initial value obtained from the 5d SRT mixed liquor, the results remained relatively constant over the duration of the both trials. The average values obtained were 0.23 +/-0.05 d^{-1} for the traditional decay co-efficient and 0.79 +/-0.19 d^{-1} for the IAWPRC Model decay co-efficient, using a value of Y_H = 0.68 g cell COD.g substrate COD^{-1}.
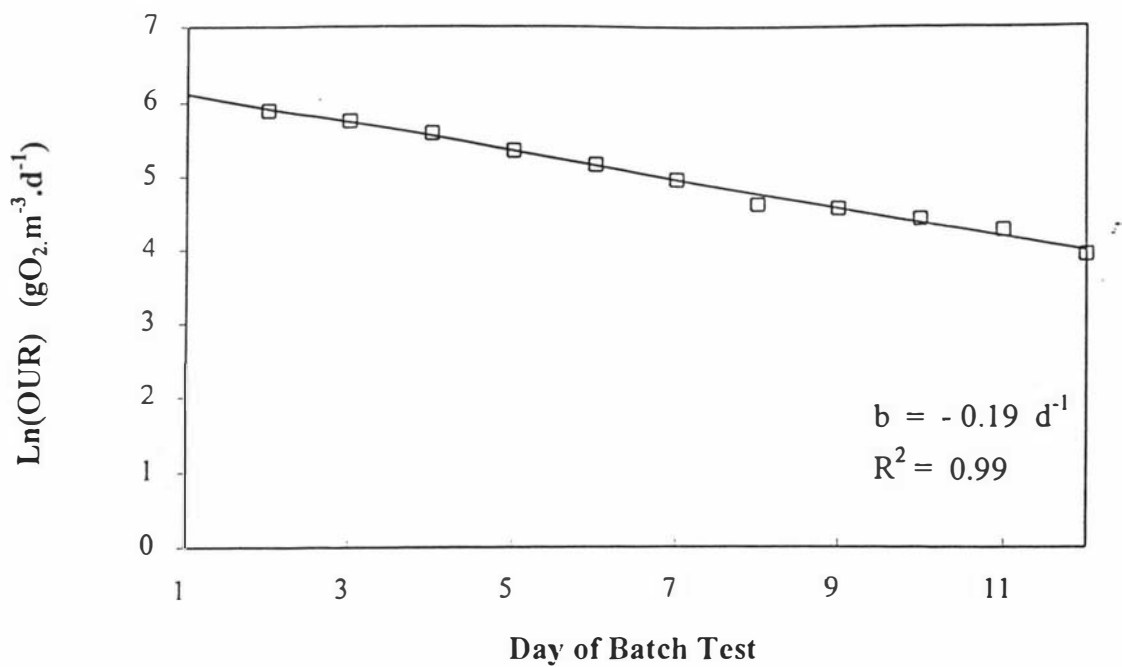
Figure 5.7: Determination of decay rate using biomass from the 10 day SRT trial, starting after 18 days at target SRT.

5.4.5 Batch COD Removal Rate Tests.

A batch test to measure specific COD removal rates was performed using mixed liquor from both the 5d SRT and 10d SRT reactors. Two tests were performed at different times during the reactor trial. A portion of the soluble COD added appeared to be immediately removed on contact with the biomass, then as can be seen from Figure 5.8 and Table 5.5, the remaining sCOD was removed at a linear rate until a sCOD concentration of approximately 100 $g.m^{-3}$ was reached.

The proportion of sCOD added that was removed 'immediately' from solution by the biomass, that is within 20 seconds of substrate addition, ranged from 20% to 54% in the batch tests performed. It was assumed that this removal was due to substrate adsorption onto the biomass, although it was not possible to directly measure such adsorption from the tests performed, and therefore not possible to verify this assumption.
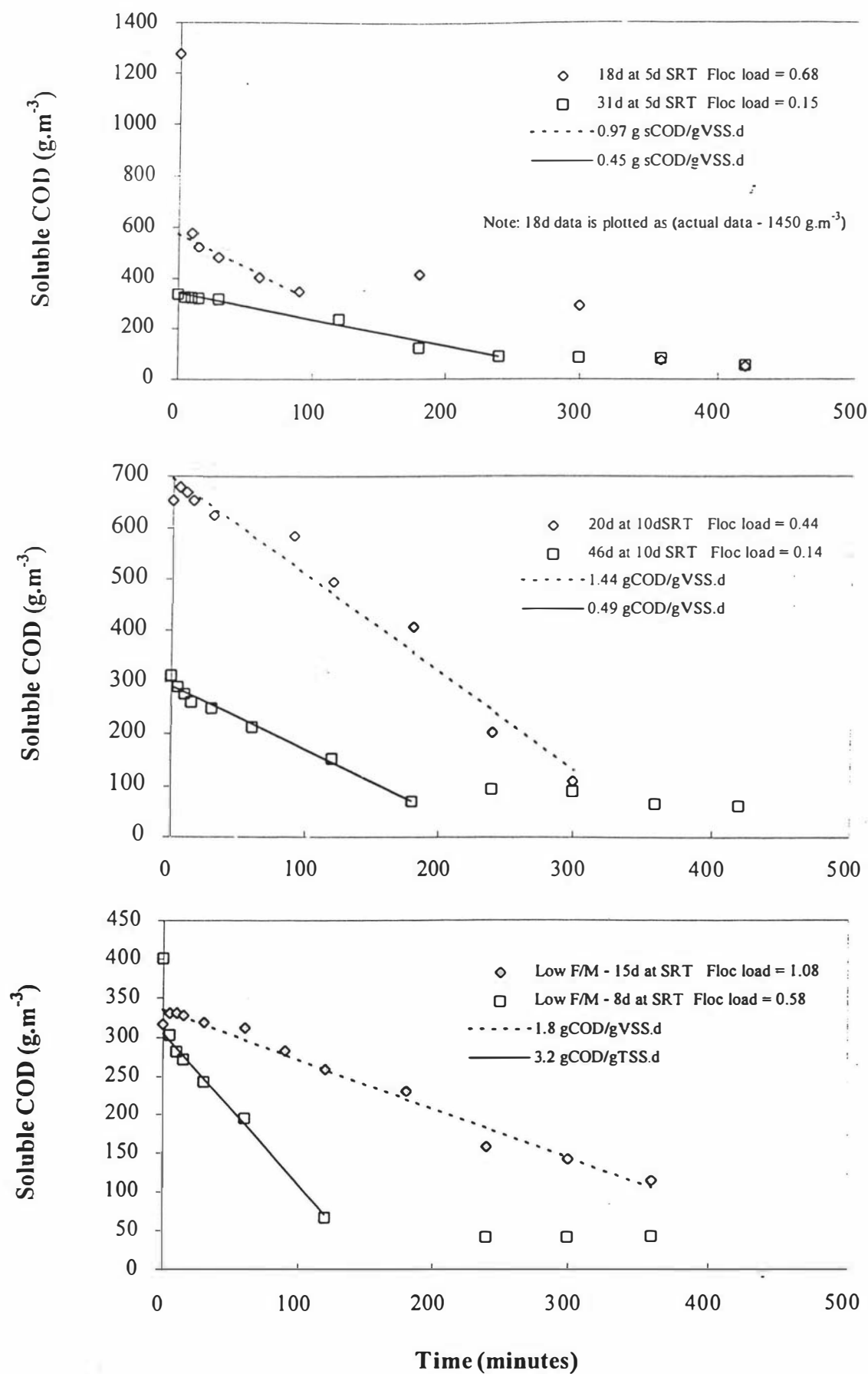
Figure 5.8:   Batch soluble COD removal rates during the various CSTR trials

Table 5.5 Soluble COD removal rates in batch tests using biomass at various SRT.

Substrate concentration in the CSTR trial	Reactor Feed $So = 2200$ gCOD.m^{-3}				Reactor Feed $So = 440$ gCOD.m^{-3}	
Biomass SRT	5	5	10	10	20	20
Days at SRT when tested	18	31	20	46	8	15
Floc loading during test (g sCOD.g VSS^{-1})	0.68	0.15	0.44	0.14	0.58	1.08
COD adsorption onto biomass (g sCOD. gVSS^{-1})	0.138	0.081	0.129	0.076	0.151	0.546
COD immediately adsorbed (% of added)	20%	54%	29%	26%	26%	51%
R^2 (linear fit)	0.96	0.97	0.97	0.98	0.996	0.98
Linear COD removal rate (gCOD.g VSS^{-1}. d^{-1})	0.97	0.45	1.44	0.49	3.19	1.82

The apparent biosorptive capacities measured during the tests ranged from 0.076 to 0.138 gCOD.gVSS^{-1}, with the amount of COD adsorbed onto the biomass increasing as the floc loading used in the test increased. Further substrate removal could be modelled by either a zero or first order equation. however, the data points were more closely fit by a linear removal rate, indicating that substrate removal rate was independent of substrate concentration. The COD removal rates observed after initial biosorption decreased as the trial progressed, suggesting that the mixed liquor population was changing to a higher proportion of species with a lower substrate removal rate capability.

5.4.6   Extent of Nitrification at Various SRT.

Effluent samples were taken during some of the trials to evaluate the extent of nitrification occurring. As can be seen from the ammonia and nitrate concentrations measured as listed in Table 5.6, nitrification was occurring, with the effluent being almost completely nitrified after the trial had operated for more than one SRT.

Table 5.6   Effluent Ammonia and Nitrate Concentrations at Various SRT.

Reactor SRT (days)	Number of Days at SRT	Ammonia (g.m^{-3})	Nitrate (g.m^{-3})
5	8	12.1	9.3
5	16	0.7	10
10	16	0.6	24
10	24	0.4	16
10	31	0.2	13.4
30	30	23.5	2.8
30	42	0.6	16
30	43	0.6	13.5

This effect would have been due to the time required for a significant proportion of nitrifiers to develop in the biomass, as the CSTRs were seeded with biomass from the 2.5d SRT reactor, which would have had a negligible nitrifier content due to the short mean cell residence time.

5.5 Reactor Performance at Varying F/M ratio

A trial was also performed at a low F/M ratio to investigate whether a lower F/M ratio and lower biomass concentration would affect treatment performance or filamentous bulking tendency. The same substrate was used but at a 1:5 dilution, so that the influent stream COD was now 440 $g.m^{-3}$. The reactor was started up in the same manner as for the other reactors, and after the mixed liquor had reached a level of approximately 3000 $g.m^{-3}$ TSS the feed concentration was reduced and SRT set at 20 days. The reactor performance at 20d SRT and low F/M conditions is given in Table 5.7.

Table 5.7   Reactor Performance at Low F/M and 20d SRT.

	Reactor	Effluent
Average TSS $(g.m^{-3})$	1460	51
TSS Range $(g.m^{-3})$	1250 - 1780	10 - 288
Average VSS $(g.m^{-3})$	1330	48
VSS/TSS Ratio	0.91	0.94
Total COD $(g.m^{-3})$	1880	92
Soluble COD $(g.m^{-3})$	44	-
pH	8.05	8.10
Total COD Removal (%)	-	82
Soluble COD Removal (%)	89	-
F/M Ratio	0.33	-
Days at SRT to failure	31	-

The system failed after 31 days at SRT due to filamentous bulking as for the trials with higher influent COD. As both total and soluble COD removal efficiencies of 82% and 89% respectively were lower than for the trials with a more concentrated influent of 93-96% and 93-95% respectively, and as the system still failed due to bulking, further trials with the diluted substrate were abandoned.

Batch soluble COD removal rate tests were performed on the mixed liquor earlier in the trial while the reactor was operating at 10d SRT. The same pattern of initial biosorption

followed by a linear COD removal rate was observed, as shown in Figure 5.8. The two tests after 8 and 15 days at 10d SRT gave specific COD removal rates of 3.19 and 1.82 $gCOD.gVSS^{-1}.d^{-1}$ respectively, again showing a decrease in specific removal rate as the culture spent longer at a given SRT.

5.6   Filamentous Bulking

All of the conventional completely mixed activated sludge reactor trials conducted failed due to filamentous bulking. As the sludge volume index increased, the return activated sludge recycle rate needed to be increased to retain the biomass in the reactor system. Eventually system failure occurred when the SVI increased, as shown in Figure 5.9, to such an extent that the biomass overflowed from the settler into the effluent.

5.6.1   Microscopic observations.

Microscopic observations were made regularly on the activated sludge as outlined in Jenkins *et al.* (1993) and WPCF (1990). General observations of floc structure and filament abundance were made on wet mounts at 100x magnification.

In all trials the mixed liquor started off as a well settling flocculant biomass, with few visible protruding filaments as seen in Figure 5.10. As each trial progressed, filaments started to protrude from the flocs and increase in abundance until they dominated the biomass and caused system failure. The progression from well settling to bulking mixed liquor suspended solids is outlined for the 10d SRT trial in Table 5.8 and illustrated in Figures 5.10 to 5.13.

The increase in biomass SVI and filament abundance was accompanied by a colour change in the mixed liquor: from a yellow/tan colour at low initial SVI through to a red /orange colour which intensified as bulking progressed. This same colour change was observed in all conventional activated sludge trials performed, suggesting that the same microorganism was becoming dominant in all cases. The red/orange colour was associated with the biomass only, as the filtered effluent remained uncoloured at all times.
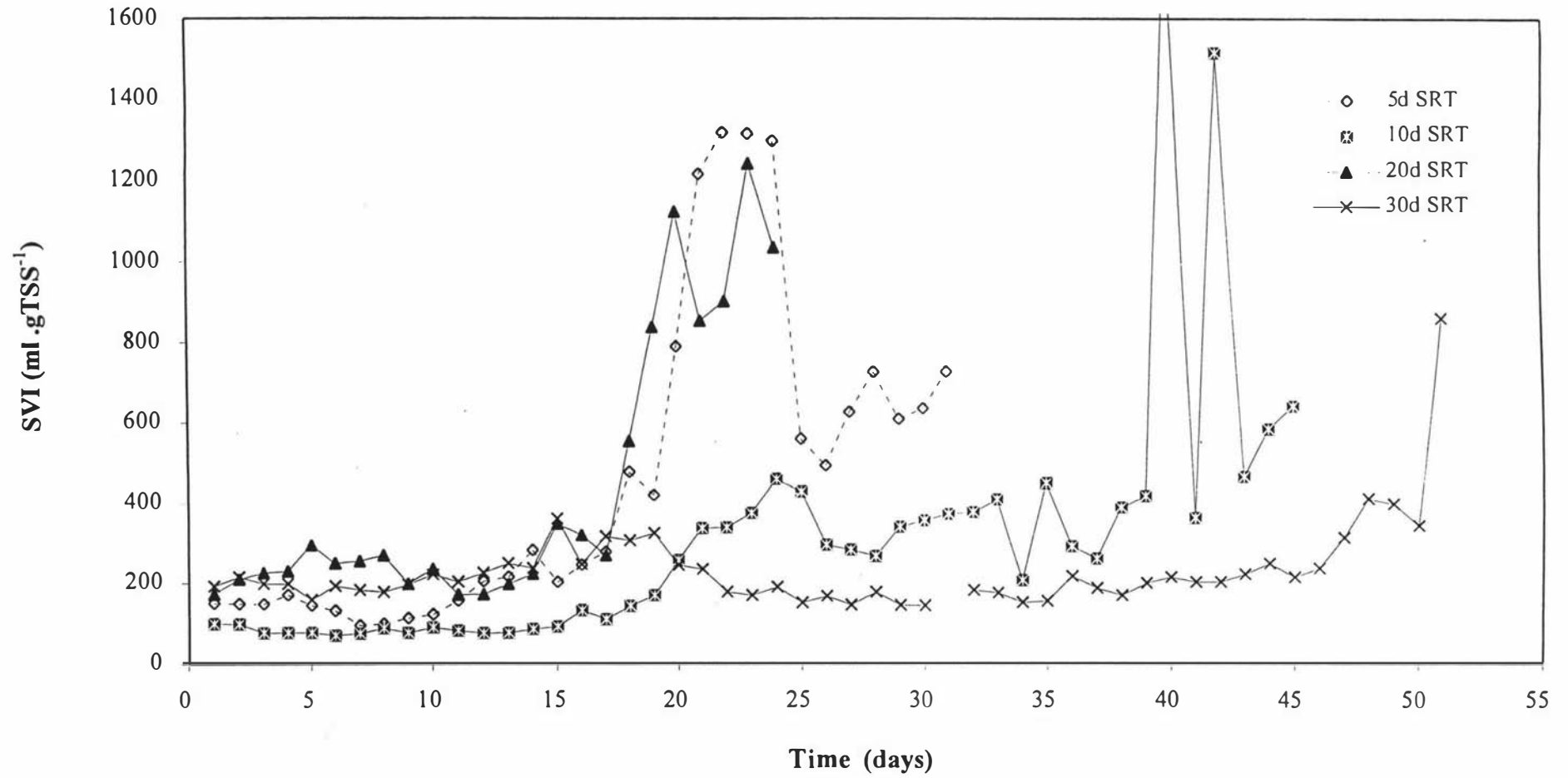
Figure 5.9   Change in Sludge Volume Index during the conventional CSTR trials at various SRT.

Other activated sludge microorganisms that were observed included rotifers, stalked ciliated protozoa and nematodes. These groups of organisms are predominant in low F/M and higher SRT systems (Jenkins *et al.*, 1993).

Table 5.8   Microscopic Observations of Filament Abundance During 10d SRT Trial.

Days at SRT	SVI $(ml.g^{-1})$	Observations
2	83	Flocs large and dense, no filaments visible. (Fig. 5.10)
16	133	Few long filaments protruding out from the flocs in long 'ropes' or 'bundles'.
19	170	Filament bundles becoming longer and more extensive, protruding from small irregular, dense flocs. (Fig. 5.11)
23	376	Filament bundles still increasing, and wrapping around flocculant biomass, increasing 'floc' size' but giving a less dense structure. (Fig. 5.12)
35	450	Excessive filamentous growth dominates the biomass as bundles around and between flocs. (Fig. 5.13)
45	640	Filamentous growth still excessive, loss of biomass in the effluent stream.

Rotifers were frequently observed in the mixed liquor of all the trials. The dominant type seen were the *Philodina sp.* (Figure 5.14), although a few *Lecaninae* type rotifers (WPCF, 1990) were occasionally seen in the 10d SRT biomass. Population densities for rotifers usually ranged from 6000 to 30 000 per ml of mixed liquor, although they were present at up to approximately 120 000 rotifers per ml in the early stages of the 10d SRT trial, reducing to the normal range after 2 SRT. Rotifers in activated sludge systems consume both bacteria growing at the floc surface and free floating bacteria, reducing the effluent suspended solids. During the period of high rotifer populations in the 10d SRT trial, the effluent TSS was very low, between 10 and 16 $g.m^{-3}$.

Stalked ciliated protozoa were also commonly seen, becoming more abundant and with increasing colony size as the trial progressed. The colonial protozoa observed were tentatively identified as *Opercularia sp.* (Figure 5.15). As for rotifers, the presence of protozoan species enhances effluent quality by removing suspended bacteria and stimulating active floc bacteria. Madoni *et al.* (1993) found that *Opercularia sp.* were associated with high organic loadings, low DO, high effluent BOD and non-nitrifying conditions. This does not seem to be true in this case however, as ammonia and nitrate

analyses indicated that nitrification was occurring, DO concentrations were maintained above 4.5 $g.m^{-3}$ in the reactor and F/M ratios were not above average for activated sludge systems.

5.6.2   Identification of filamentous bacteria

Using the morphology and staining techniques as outlined by Eikelboom (1981), and Jenkins *et al.* (1993), identification of the dominant filament was tentatively made. The same filament was dominant in all the various SRT trials, although secondary filaments were also observed and identified where possible.

The dominant filament protruded initially from the flocs as 'ropes' or 'bundles' of a few filaments. As filament abundance increased the bundles became longer and thicker, extending further out into solution until they surrounded the flocs entirely. Visual observations of the filaments at 400x and 1000x under phase contrast highlighted the morphology and staining reactions listed in Table 5.9.

Table 5.9:   Microscopic observations of the dominant filamentous microorganism

Filament appearance:
- irregularly bent bundles of filaments both protruding from flocs and free floating
- no attached growth or branching
- long, thin filament; approximately $0.5\mu m$ x $150\mu m$
- indents at septa seen at 1000x, but not at 400x

Staining reactions and observations on stained samples:
- Gram negative, long chain of cells
- cells approximately 2-5 μm long
- Sulphur test negative / inconclusive
- Crystal violet sheath stain inconclusive
- India ink stain for exocellular polymers negative
- Neisser stain negative
- PHB stain negative
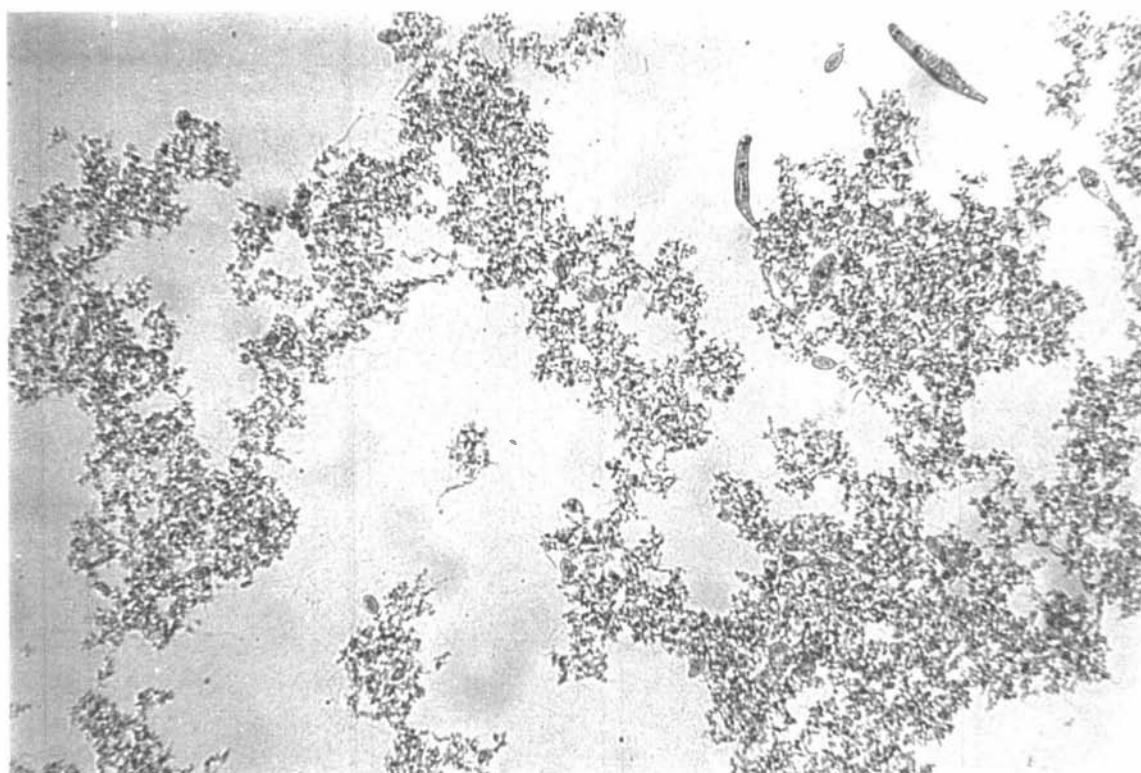
Figure 5.10:   Well settling biomass. 10d SRT trial after 2 days at SRT, non-bulking SVI of 83 ml.g^{-1}. (100x magnification)
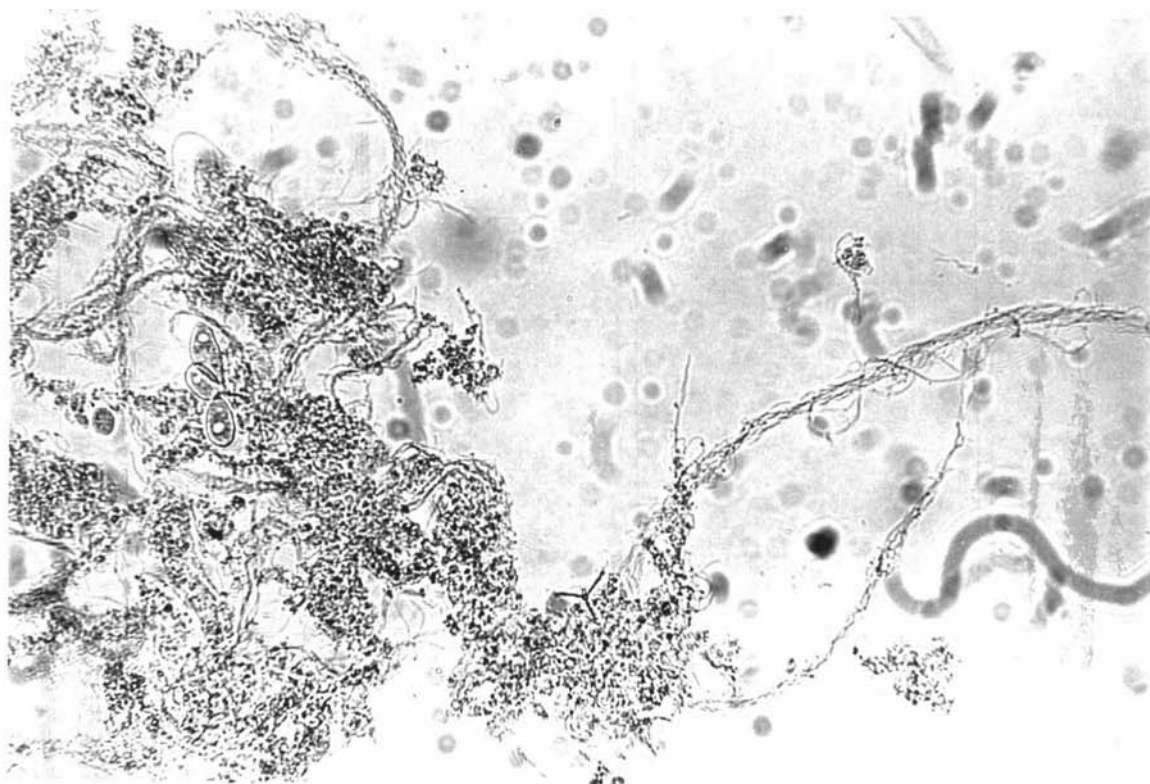
Figure 5.11:   Filaments protruding from the flocs. 10d SRT trial after 19 days at SRT, SVI had increased to 170 ml.g^{-1}. (100x magnification)
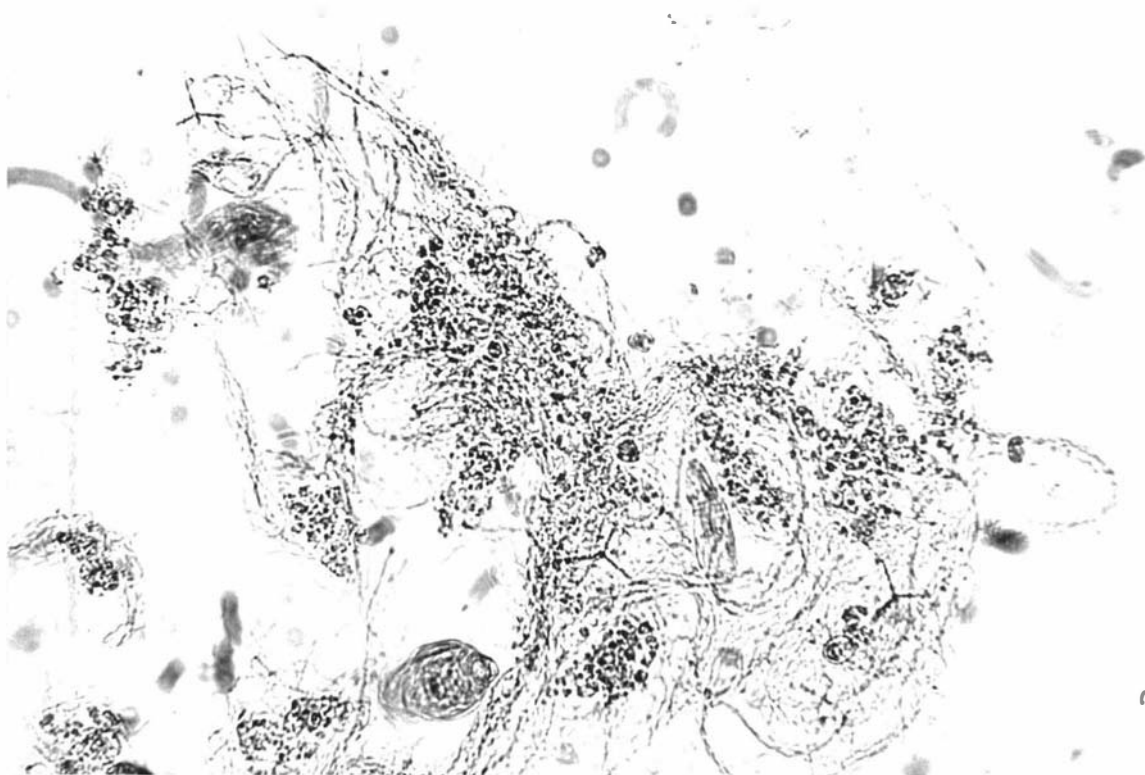
Figure 5.12: Abundant filament growth. 10d SRT trial after 23 days at SRT, SVI had continued to increase to 376 ml.g^{-1}. (100x magnification)
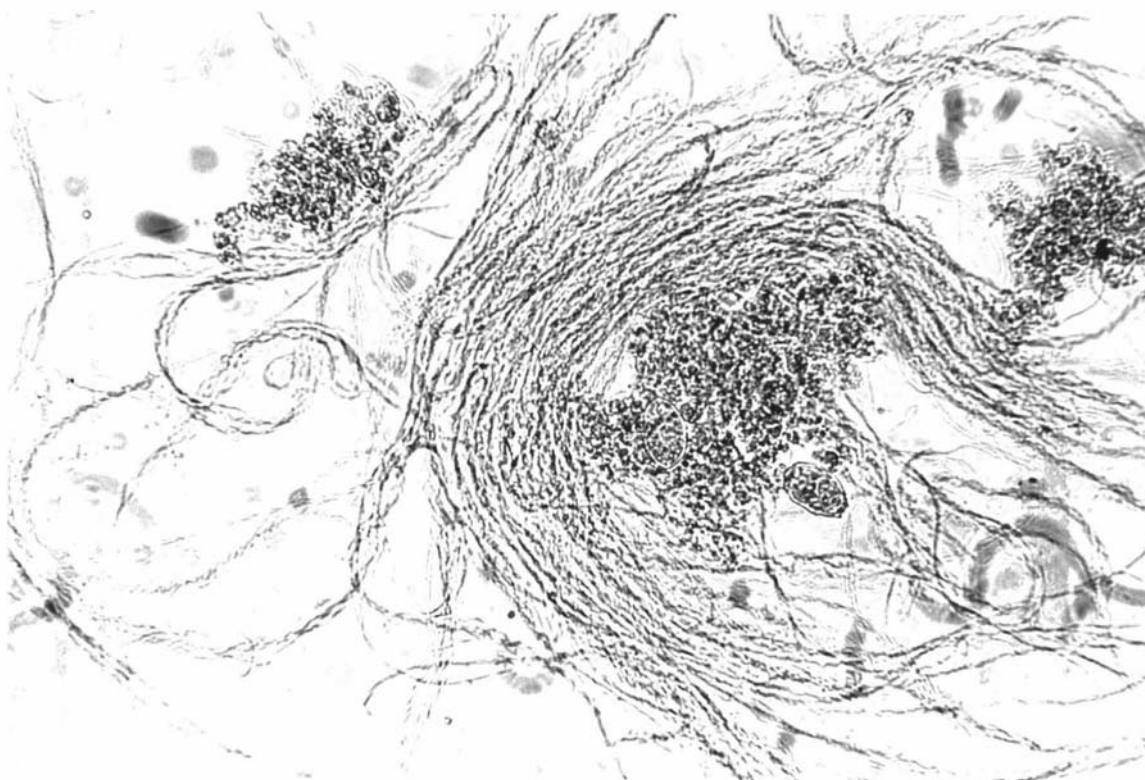
Figure 5.13: Excessive filament growth. 10d SRT trial after 35 days at SRT, SVI had increased still further to 450 ml.g^{-1}. (100x magnification)
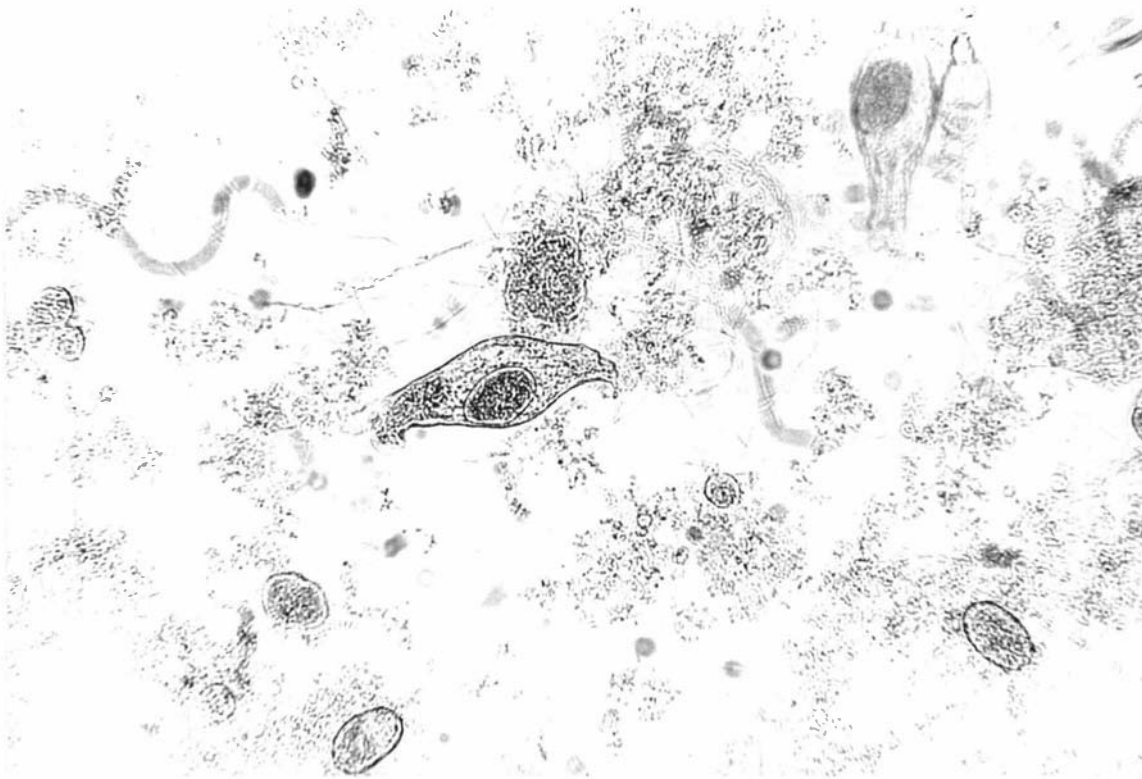
Figure 5.14:   Dominant rotifer type observed throughout all trials.
(100x magnification)
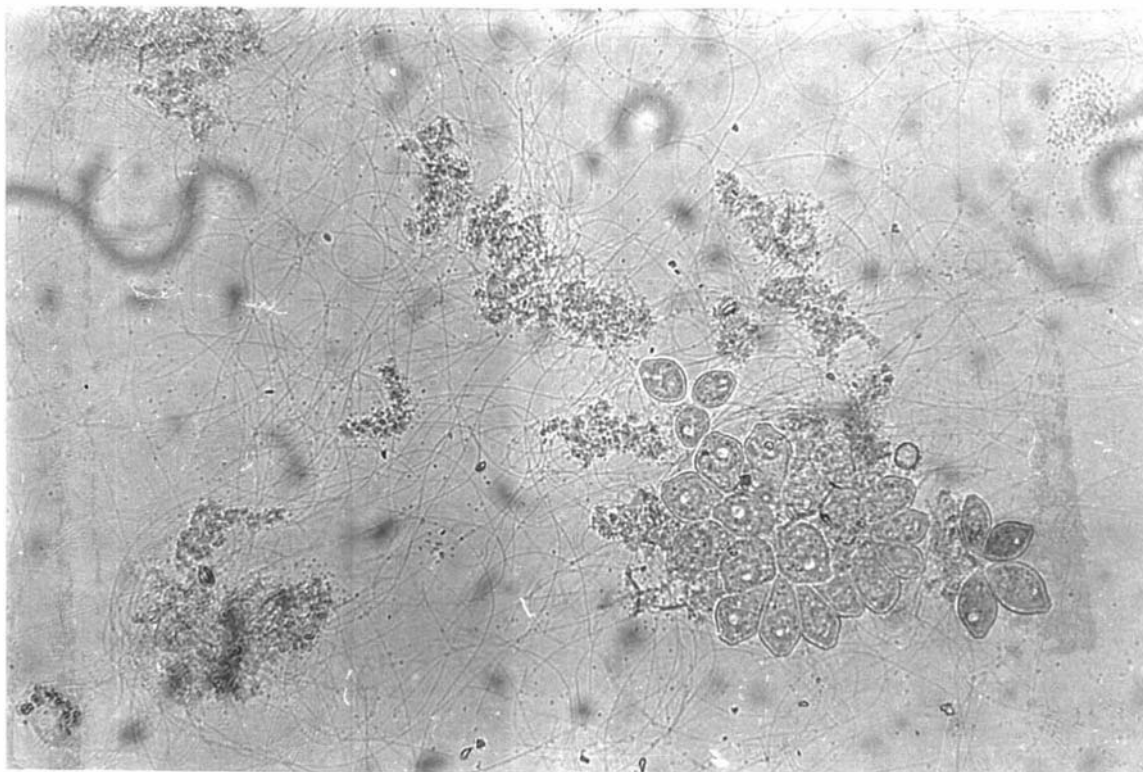
Figure 5.15:   Stalked ciliated protozoa commonly observed in the CSTR trials.
(100x magnification)

Using the identification key in Jenkins *et al.* (1993), the above results lead to the identification of the dominant filament as Type 0411. Eikelboom (1980) found that the Type 0411 microorganism produced yellow, orange or red coloured colonies when isolated as plate cultures and proposed that this type may belong to the genus *Flavobacterium.* It is therefore possible that the colour change observed in the mixed liquor may be due to this organism.

Secondary filaments were observed for the 5d SRT and 10d SRT trials, in each case at a greatly reduced frequency than the dominant filament. In the 5d SRT reactor after 2 SRTs had passed, a filament composed of a short gram negative rod, PHB positive, with much attached growth was observed, identified as probably Type 1701. A 1µm diameter, long, gram negative, PHB positive filament was observed in both cultures near the end of the trials. This filament was present both inside and protruding from the flocs, being more curled inside the floc, and was identified as being either Type 0914 or Type 021N. *Nocardia sp.* was also observed in stained preparations from the 10d SRT reactor mixed liquor near the end of the trial, which was not unexpected as foaming at the inlet to the settler had been observed for the 6 days prior to microscopic examination.

5.6.3 Possible Filamentous Bulking Control Strategies

Much work has been done in relating the dominant filament in a bulking sludge with reactor operating conditions in order to enable implementation of the appropriate remedial action, as summarised in Jenkins *et al.*, (1993). However, the dominant filament in this case, Type 0411, is not a commonly reported filament, and has not been related to a particular system operating condition. Therefore no indication can be made of the most likely course of action to be taken for the prevention of filamentous bulking in this case.

The secondary filaments identified have been associated with a wide range of conditions: Type 1701 - low DO at low to moderate SRT; Type 0914 - high SRT nutrient removal systems; Type 021N - readily metabolisable substrates, low F/M, nutrient deficiency; *Nocardia sp.* - slowly hydrolysed substrates, low F/M, low DO. (Richard *et al.*, 1985; Jenkins *et al.* 1993; Strom and Jenkins, 1984). Therefore the secondary filaments did not point to a single cause for the filamentous bulking either.

5.7 Discussion

A range of SRTs were chosen for initial activated sludge studies so that the effect of SRT on treatment efficiency could be evaluated. The SRTs used were those commonly found in full scale nitrifying activated sludge plants: between 5 and 30 days.

As the SRT was increased, an increase in the average TSS and apparent decrease in VSS/TSS was observed, with TSS ranging from an average of 3020 $g.m^{-3}$ at 5d SRT to 5530 $g.m^{-3}$ at 30d SRT. This was as expected due to the lower biomass wastage rate at higher SRT. The inert fraction of the mixed liquor suspended solids will increase with increased SRT due to the accumulation of any inert solids in the influent stream (X_I) and of inert particulate products arising from microbial decay. This was indicated by a decrease in the reactor VSS/TSS ratio with increasing SRT, but the change seen was not deemed significant, probably due to the low value of X_I (assumed negligible from results in the previous chapter) and the relatively short time of system operation before failure.

A high quality effluent in terms of COD and TSS concentrations was achievable using the activated sludge systems. Average soluble COD removals of between 92 and 95% were measured, with the 5, 10 and 30d SRT trials all giving efficiencies of about 95%. The 20d SRT reactor showed a lower COD removal efficiency, but this was most probably due to the fact that it failed after only one SRT, so did not reach optimum operating conditions. The proportion of effluent total COD that was attributable to suspended solids was low, generally less than 10%. The final effluent soluble COD of approximately 100 $g.m^{-3}$ would have been predominantly due to the presence soluble microbial products rather than substrate components, as virtually all the soluble substrate had been estimated to be readily biodegradable or rapidly hydrolysable. Again, no conclusive effect of SRT on effluent COD could be discerned due to the relatively short period of system operation in the trials.

From effluent ammonia and nitrate concentrations it was determined that the effluent was fully nitrified after the system had operated for at least one SRT indicating that a significant nitrifying biomass had developed within this timeframe.

A trial conducted at a lower F/M ratio by using a 1:5 diluted feed showed significantly lower COD removal efficiencies, with 87% of the soluble COD and 79% of the total COD removed at 20d SRT. The F/M ratio in this trial was 0.33 $gCOD.gVSS^{-1}. d^{-1}$, compared to between 0.45 and 0.80 for the full strength effluent trials, therefore

operating at very low F/M ratios did not seem to be effective for the treatment of this wastewater. This trial also failed due to filamentous bulking of the biomass.

The decay rate co-efficient was also measured on the reactor mixed liquor. The value measured seemed to decrease as the trial progressed for the 5d SRT mixed liquor, this could be due to a higher proportion of undegraded adsorbed organic matter such as fats on the biomass at the beginning of the trial. The remaining results were relatively constant and the average value measured for b of 0.23 +/- 0.05 d^{-1} compared favourably to other activated sludge system decay rate co-efficients reported. Using the temperature relationship reported in Ekama and Marais (1974) other values for b have been reported at 25°C as: 0.28 d^{-1} (Ekama and Marais, 1976; municipal wastewater), 0.22 to 0.32 d^{-1} (Orhon et al., 1993; dairy processing wastewaters) and 0.22 d^{-1} (Henze et al., 1987; typical domestic wastewater value).

All trials failed in less than 50 days, or between 1 and 6 SRTs due to the sludge bulking to such an extent that biomass was lost in the settler overflow. Microscopic observations confirmed that the increase in SVI was due to the increase in filamentous microorganism abundance. At the end of each trial the excessive filamentous growth dominated the activated sludge biomass.

Chudoba et al. (1973a) found that activated sludge system configuration and more importantly, the concentration of substrate first in contact with the biomass affected filamentous bulking. These observations were used to propose a theory for filamentous bacterial growth. The kinetic selection theory first presented by Chudoba et al. (1973b) was based on the Monod equation and proposed that activated sludge flocs were comprised of two types of microorganisms, filaments with a low μ_{max} and K_S and nonfilamentous floc formers with a higher μ_{max} and K_S.

This theory was experimentally verified by Chudoba et al. (1985) using the same low S/X method for the determination of μ_{max} and K_S as used in this study. A decrease in the values obtained for μ_{max} and K_S would then be expected as the culture became more filamentous. Only one test was performed during each SRT trial, but in each case the mixed liquor had become filamentous when the test was done. The filamentous cultures gave μ_{max} values of 0.67 to 0.87 d^{-1} compared to the average value of 1.2 d^{-1} measured from studies on the non-filamentous 2.5d HRT/SRT reactor, therefore the results obtained are in line with the kinetic selection theory.

Chudoba (1982) also proposed that floc formers had a higher accumulation capacity for

substrate than filaments, allowing them to store substrate when the biomass was at higher substrate concentrations and oxidise the substrate later when the exogenous substrate had been removed from solution. This was also reviewed by Goronszy and Eckenfelder (1986), who discussed the relatively slower substrate removal abilities of filamentous microorganisms compared to floc forming microorganisms.

In batch tests performed with the mixed liquor biomass, between 20% and 54% of the soluble substrate added was immediately adsorbed on mixing with the biomass, indicating that this was a significant mechanism for substrate removal, but also that a large fraction of the substrate was remaining in solution for the growth of other microorganisms. As the batch tests were not conducted at a constant floc loading, the effect of SVI on sorptive capacity of the biomass was not able to be assessed. However, as the batch tests indicated that substrate removal rates were zero order, and that they decreased as the trial progressed and biomass SVI increased, it is indicated that the filaments had a decreased substrate removal capacity as compared to the floc formers.

The change in kinetic constants and substrate removal ability with SVI are summarised in Table 5.10, with the μ_{max} and COD removal rate values plotted against SVI in Figure 5.16. These results support the theory that filamentous microorganisms have lower growth and substrate removal rates than floc formers.

Table 5.10 Summary of Biomass Characteristics at Various SRT and SVI.

SRT during trial	5	5	5	10	10	10	30
Days at SRT when tested	16	18	31	20	30	46	22
SVI (ml.g^{-1})	246	478	726	258	357	550	179
μ_{max} (d^{-1})	0.67				0.60		0.87
K_S (g.m^{-3} COD)	2.2				4.0		3.5
Substrate removal rate (gCOD.g VSS^{-1}. d^{-1})		0.86	0.43	1.35		0.46	

The most commonly proposed reactor conditions that affect filamentous bacterial growth are: substrate type, reactor configuration, DO concentration, substrate concentration and nutrient deficiency (Chudoba, 1985; Jenkins et al., 1993).
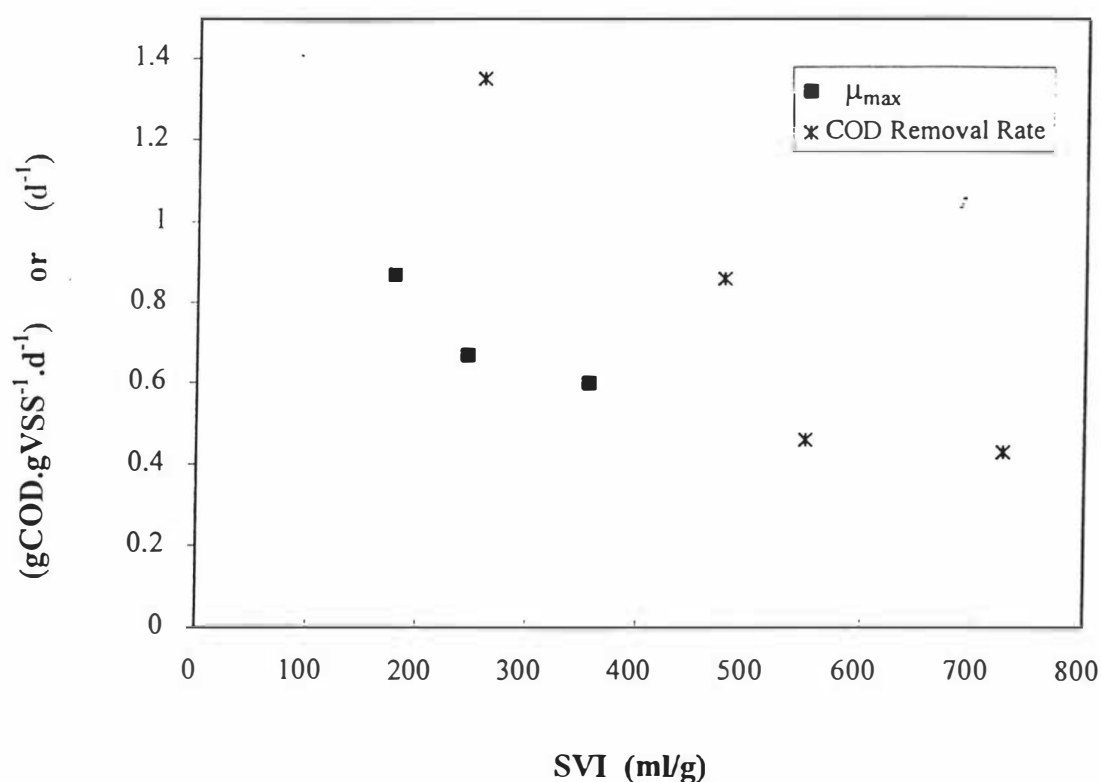
Figure 5.16: Change in substrate removal rate and maximum specific growth rate with SVI.

Substrate type is known to influence the tendency of an activated sludge system to bulk, especially the presence of readily metabolisable substrates. A high proportion (36%) of the substrate COD was due to the presence of lactose, a readily biodegradable sugar and substrate known to promote filamentous bulking (Chudoba, 1985). Dairy processing wastewaters in general have been widely reported as supporting filamentous growth (Adamse, 1968a and b; Rensink, 1974; Van den Eynde et al. 1982; Goronszy et al., 1985; Rensink and Donker, 1990; Mino, 1993).

All the trials described in this chapter used a conventional completely mixed activated sludge reactor configuration. This configuration results in a low substrate concentration at all times in the reactor, a factor known to support filamentous growth (Chudoba, 1973a; Palm et al., 1980; Chudoba 1985; and many others). This is commonly referred to as low F/M bulking, but it is the lack of a substrate concentration gradient that is more important than the actual organic loading rate, or F/M ratio.

The 'ideal' nutrient ratio for organic carbon removal in terms of BOD:N:P for activated sludge, is usually quoted as being 100:5:1 (Eckenfelder and Grau, 1992; Jenkins et al.,

1993). The COD:N:P ratio measured for this substrate is 100:4:0.6, and as the COD:BOD ratio is approximately 2, this indicates that there should be sufficient N and P available for preventing nutrient deficient bulking. However as Jenkins *et al.* (1993) states, there may still be a nutrient deficiency if the C source is readily metabolizable but the N source is organically bound, as it was in this case where a third of the COD was present as lactose but all of the nitrogen was in the form of milk proteins (Adamše, 1968a).

Low dissolved oxygen in the aeration basin is also known to be associated with filamentous growth, as the filaments protruding from the flocs would have a competitive advantage for the oxygen present in solution over the floc formers inside the flocs. Chudoba (1985) suggests that the aeration basin DO be kept above 2 g.m^{-3} to prevent this, however Jenkins *et al.* (1993) suggest that the reactor DO level required to keep the floc biomass aerobic is dependent the applied F/M ratio.

At the F/M ratios used in these trials of between 0.45 and 0.80 gCOD.g VSS^{-1}. d^{-1}, the DO concentrations given in Jenkins *et al.* (1993) required to prevent bulking ranged from 1.9 to 3.7 g.m^{-3} respectively. Actual reactor DO levels were maintained above 4.5 g.m^{-3} for all trials, well above the theoretical 'safe operating line' for the F/M ratios used (Palm *et al.*, 1980). The high DO levels are also evidenced by the abundant growth of rotifers, which are strict aerobes and require environments of at least 3 g.m^{-3} of oxygen (WPCF, 1990) to survive. Therefore it was unlikely that the filamentous bulking could be attributed to low DO conditions.

It was therefore surmised that the growth of filamentous microorganisms was most likely due to the readily biodegradable nature of the substrate, the low substrate concentrations in the reactor due to its completely mixed nature, or nutrient limitation effects.

As activated sludge bulking has many causes, identification of the dominant filament can be of use in distinguishing the cause of bulking (Eikelboom 1977, Jenkins *et al.*, 1993). In this case, the dominant filament in all trials was Type 0411, which is not a commonly reported filament and has not been definitively associated with a particular reactor or substrate condition. The secondary filaments identified did not point to a particular cause for bulking either.

Cases of filamentous bulking due to readily biodegradable substrates and low substrate concentrations can be overcome by the use of a selector reactor - a small reactor

upstream of the aeration tank in which the return sludge and incoming wastewater are mixed, providing a substrate concentration gradient through the activated sludge system. Therefore, if the growth of filamentous microorganisms is caused by either of these two factors, then the use of a selector should be able to overcome the activated sludge system operability problems experienced.

5.8   Conclusions

A conventional completely mixed activated sludge reactor configuration could produce a high quality effluent but the system failed due to filamentous bulking of the biomass. Total COD removals of 93% to 96% and soluble COD removals of 92% to 95% were seen at SRTs ranging from 5 to 30 days. There was no obvious effect of SRT on effluent quality, but the longer SRT trials failed too soon to make conclusive findings.

The kinetic parameters measured as the trials at different SRT progressed indicated that both the specific growth rate and substrate removal rate capacities decreased as the trials progressed and the SVI increased. This supports the theory that filamentous organisms have slower growth and substrate removal rates than floc forming bacteria.

The dominant filamentous microorganism was the same in all trials, tentatively identified at Type 0411. Although this filament can not be positively associated with any particular condition causing bulking, the trials conducted did use a highly degradable carbonaceous substrate and a low reactor substrate concentration, two commonly quoted factors associated with filamentous bulking that can be remedied by the use of a selector reactor configuration.

CHAPTER 6

UNAERATED SELECTOR REACTORS

6.1   Introduction

The work presented in the previous Chapter demonstrated that a conventional completely mixed activated sludge reactor configuration could not be used for the treatment of the defined dairy processing wastewater, due to operability problems caused by the proliferation of filamentous bacteria.

The use of a selector reactor in which the return activated sludge (RAS) and influent are mixed prior to the main aeration basin is widely documented as being able to prevent the growth of many types of filamentous organisms (Chudoba, 1985; Jenkins *et al.*, 1993; Chudoba and Pujol, 1994). Selector types utilised include those with aerobic, anoxic or anaerobic conditions; which may be configured either singly or in series. The selector type and configuration that would be most effective for the prevention of bulking is dependent on the causative filament.

The dominant filament associated with the CSTR studies was identified as Type 0411, a filament type not commonly reported the literature, or positively associated with any particular substrate or operating condition. Therefore the appropriateness of various selector types could not be assessed from identification of the causative organism.

The remaining work in this study involved trials conducted with both unaerated and aerated selectors of various configurations in an effort to produce a modified activated sludge system without filamentous bulking problems. The important factors affecting the ability of a selector system to prevent bulking and possible implications for selector design criteria were also assessed.

This chapter discusses the trials that were performed with unaerated selectors.

6.2   Selector Design

There has been a great volume of literature published on the use of selector reactors for the prevention of filamentous bulking on a wide variety of wastewaters (Albertson, 1991; Jenkins et al., 1993; Chudoba and Pujol, 1994; Wanner, 1994). As the successful operation of a selector is very dependant on substrate composition and system operating variables, selector design is not straightforward.

The critical factors in selector design include:
- selector type: aerobic, anoxic, anaerobic.
- selector configuration: a single selector, a series of one type of selector, or a series of a combination of selector types.
- selector size and loading rate.

6.2.1   Choice of Selector Type

The use of anoxic or anaerobic selectors is generally favoured over aerobic selectors (Hoffman, 1987; Shao and Jenkins, 1989; Daigger and Nicholson, 1990; Wanner et al., 1987) due to the additional selective pressures imposed in the former types. Aerobic selectors are known as 'kinetic' selectors as they confer an advantage to microorganisms with a high substrate uptake rate and storage capacity due to the alternating 'feed - starve' conditions imposed. In either anoxic or anaerobic selector types, there is an additional metabolic selection occurring because only microorganisms which can either denitrify or utilise intracellular polyphosphate can remove substrate from solution in the respective selector types (Jenkins et al., 1993).

In addition, there are practical advantages of anoxic selectors over aerobic selectors for implementation in full scale systems, including the opportunity for biological N removal due to denitrification and the absence of selector aeration costs. Therefore it was decided to trial anoxic selectors first.

Anoxic selectors require the absence of DO and presence of nitrate, as substrate removal occurs via denitrification and storage (Jenkins et al., 1993). The CSTR trials gave a fully nitrified effluent, so the return activated sludge would provide the source of nitrate and the absence of aeration in the selectors would ensure the absence of DO.

6.2.2 Selector Configuration

Jenkins *et al.* (1993) states that usually a single anoxic selector is sufficient for the prevention of filamentous bulking, so the first two trials used single selectors of different sizes. The third trial used three equally sized selectors in series when it became obvious that additional selection pressure was required.

6.2.3 Selector Size and Organic Loading Rate.

There are two conflicting principles when designing selectors, as discussed in Patoczka and Eckenfelder (1990). The first is that the higher the substrate gradient in the system, that is, the higher the substrate concentration (S) in the selector, the greater the advantage conferred on floc formers that have a faster growth rate at higher S. The second principle is that high substrate removal efficiencies are required in the selector to ensure that the amount of available substrate passing into the aeration tank is low. High selector efficiencies result in low selector substrate concentrations, which reduces the substrate gradient in the system.

Two of the most commonly used operating parameters used in selector design are floc loading and F/M ratio, defined as follows:

$$\text{Floc Loading (gCOD.gVSS}^{-1}) \; = \; \frac{Q_F \, S_O}{Q_R \, X_R} \tag{6.1}$$

$$\text{F/M (gCOD.gVSS}^{-1}.\text{d}^{-1}) \; = \; \frac{\text{substrate mass utilised (d}^{-1})}{\text{reactor cell mass}} \; = \; \frac{S_O \, Q_F}{X \, V} \tag{6.2}$$

where:

Q_F = feed flowrate ($m^3.d^{-1}$)
S_O = feed concentration ($gCOD.m^{-3}$)
Q_R = RAS flowrate ($m^3.d^{-1}$)
X_R = RAS solids concentration ($g.m^{-3}$ VSS)
θ = Hydraulic residence time (d^{-1}) = V/Q
X = Biomass concentration ($gVSS.m^{-3}$)

Values for all of the above parameters can be obtained either from the system definition or from component balances around the settler. The feed flowrate was 10^{-2} $m^3.d^{-1}$ and

all remaining trials were operated at a 10 day SRT. As it is the removal of the soluble fraction of substrate COD (sCOD) that is of interest in selectors, 'S_0' in equations 6.1 and 6.2 was equivalent to 1650 $gCOD.m^{-3}$. From the data obtained during the CSTR trial at 10d SRT, the MLVSS could be expected to be about 3900 $g.m^{-3}$ and Q_R would be approximately 15×10^{-3} $m^3.d^{-1}$ for a well settling biomass. Therefore X_R would have a value of about 6500 $gVSS.m^{-3}$ calculated from a solids balance around the settler assuming negligible solids in the effluent. The total flowrate through the selector would be about 25×10^{-3} $m^3.d^{-1}$.

The first approach was to use a desired selector F/M to calculate the required selector size. Different authors have specified various selector substrate removal efficiencies that would be required to prevent bulking, ranging from 60% of influent sCOD (Daigger and Nicholson, 1990) to a level that would give a selector effluent of 60 $g.m^{-3}$ sCOD (Shao and Jenkins, 1989). Using the above parameter values and a recommended F/M of 2 (Jenkins et al., 1993), the resultant selector volume could be calculated using Equation 6.2, for 60% sCOD removal:

$$V = \frac{S_0 Q_F}{(F/M) . X} = \frac{(1650 \times 0.6) . 10^{-2}}{2 . 3900} = 1.27 \times 10^{-3} \ m^3$$

For substrate removal resulting in a sCOD concentration of 60 $g.m^{-3}$:

$$V = \frac{S_0 Q_F}{(F/M) . X} = \frac{(1650 - 60) . 10^{-2}}{2 . 3900} = 2.04 \times 10^{-3} \ m^3$$

Therefore a selector size of between 1.3×10^{-3} m^3 and 2×10^{-3} m^3 was indicated. The selector size could also be estimated from batch sCOD removal test data obtained at a similar floc loading to that expected in the selector, as calculated from Equation 6.1:

$$\text{Floc Loading} = \frac{Q_F S_0}{Q_R X_R} = \frac{10 . 1650}{15 . 6500} = 0.17 \ (gCOD.gVSS^{-1})$$

Data from batch soluble COD removal rate tests performed at a similar floc loading of 0.14 $gCOD.gVSS^{-1}$ on the 10d SRT mixed liquor, as described in Section 5.4.5, was therefore used to estimate an appropriate selector size. Removable sCOD was depleted after a period of 200 minutes, but this was using a filamentous biomass which had a specific substrate removal rate approximately one third of that of the well settling biomass. Therefore a nonfilamentous biomass at the same floc loading should be able to remove the sCOD in approximately 67 minutes. At the estimated flowrate through the
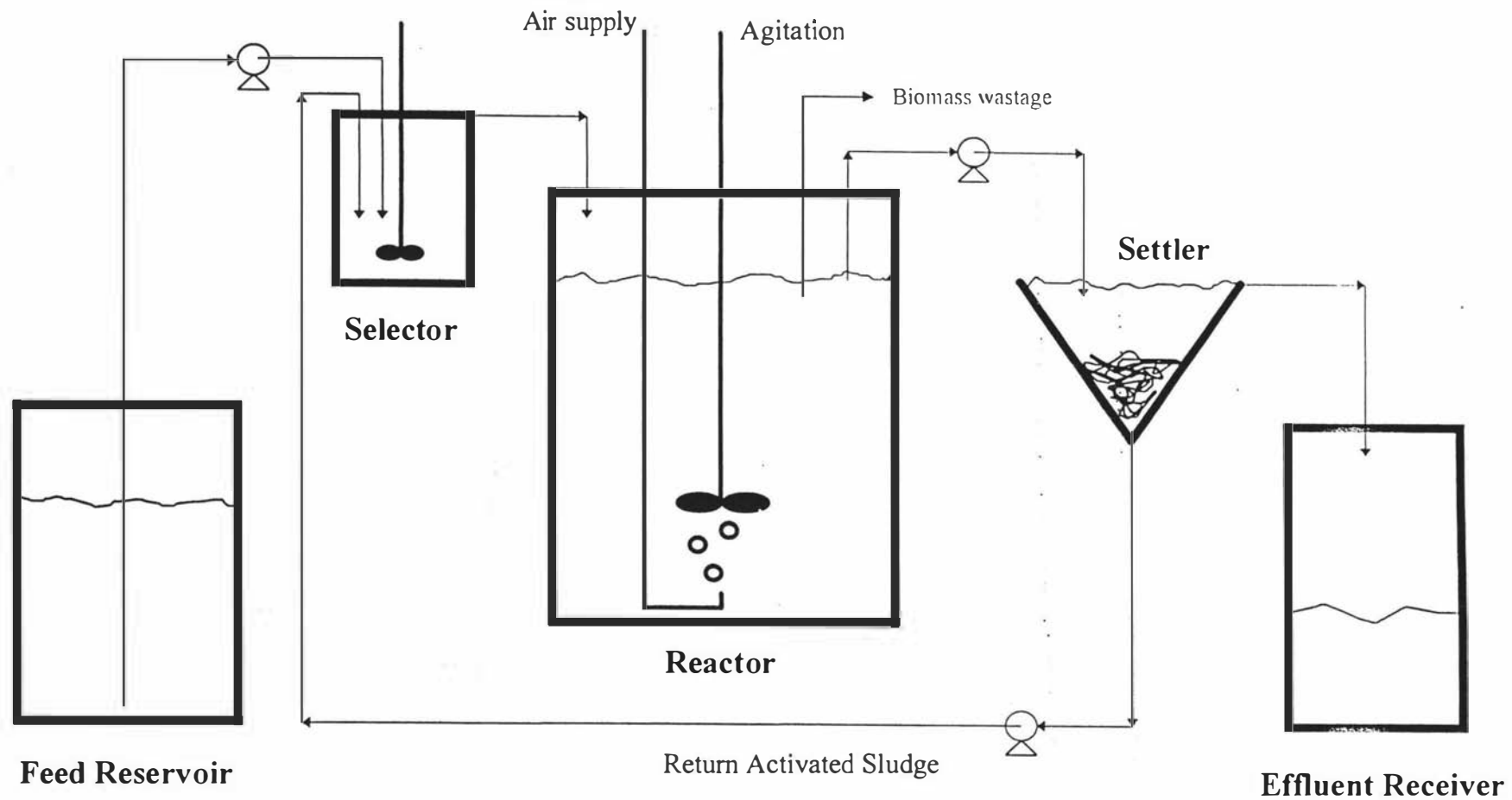
Figure 6.1   Reactor system configuration during the selector reactor trials.

selectors of 25 $\times 10^{-3}$ m³.d⁻¹, to give a residence time of 67 minutes, the selector volume would need to be 1.16 $\times 10^{-3}$ m³.

From the above calculations, a selector volume of 1.2 litres was chosen for use in the first trial, in the configuration outlined in Figure 6.1. After the results of the first trial became apparent it was decided firstly to increase the selector size and secondly alter the selector reactor configuration. It was arbitrarily decided to double the initial selector volume in the first instance and use three selector in series with a total volume of 1.5 times the initial selector volume in the second instance.

The unaerated selector trials that were conducted are as follows:

Trial AN1: 1 x 1.2 l unaerated selector
Trial AN2: 1 x 2.4 l unaerated selector
Trial AN3: 3 x 0.6 l unaerated selectors in series

6.3   Unaerated Selector Reactor Treatment Performance

Each unaerated selector trial used the same substrate, aeration tank and settler as the conventional activated sludge trials, the SRT being maintained at 10 days and HRT at 1 day, both parameters being calculated on the 10 litre aeration tank volume only. The return activated sludge (RAS) flowrate was adjusted as necessary to maintain a low solids inventory in the settler, and therefore was increased if the SVI increased. Data was recorded with Day 1 of the trial being the first day of reactor operation with the specified selector configuration and target SRT.

The reactor treatment performance in terms of COD removal, and effluent quality in terms of TSS and COD remained high, the average results obtained during each trial being listed in Table 6.1. Total COD removals were very similar in all three trials, varying between 96.5% and 96.7%, and similar to the 96% removal observed for the CSTR reactor trial operating at 10d SRT.

Reactor sCOD concentrations averaged between 46-60 g.m⁻³ compared to 91 g.m⁻³ in the 10d SRT CSTR trial, resulting in system removals ranging between 96.4% and 97.2% sCOD. This increased removal efficiency is suggested to be due to the production of a lower concentration of soluble microbial products (SMP) in the selector

Table 6.1   Unaerated Selector Reactor Treatment Performance

Trial	AN1	AN2	AN3
Selector configuration (l)	1 x 1.2	1 x 2.4	3 x 0.6
Selector outlet conditions:			
pH	6.95	6.90	6.90
TSS $(g.m^{-3})$	3360	3250	3590
VSS $(g.m^{-3})$	2970	2960	3280
Total COD $(g.m^{-3})$	5300	5100	5100
Soluble COD $(g.m^{-3})$	270	305	135
Reactor outlet conditions:			
pH	7.65	7.65	7.70
TSS $(g.m^{-3})$	3770	3460	3700
VSS $(g.m^{-3})$	3440	3130	3400
Total COD $(g.m^{-3})$	5300	4900	5100
Soluble COD $(g.m^{-3})$	57	60	46
Soluble COD Removal (%)	96.5	96.4	97.2
SVI Range	109 - 333	92 - 244	267 - 357
SVI Trend	increasing	increasing	stable
Effluent conditions:			
pH	7.80	7.85	7.70
TSS $(g.m^{-3})$	25	17	36
VSS $(g.m^{-3})$	23	16	32
Total COD $(g.m^{-3})$	77	75	73
Total COD Removal (%)	96.5	96.6	96.7
Proportion of effluent COD due to TSS (%)	25	20	37

trials. Orhon *et al.* (1993) found that the effluent sCOD from activated sludge treatment of similar wastewaters at SRTs longer than 2 days was due almost entirely to SMP and therefore that the overall system sCOD removal was determined by SMP production. The SMP production levels reported by Orhon *et al.* (1993) ranged from 3.1% to 6.8% of influent sCOD, which would have corresponded to effluent sCOD concentrations of 51 to 112 $g.m^{-3}$.

Effluent TSS levels were similar for all the 10d SRT reactors, averaging 12 $g.m^{-3}$ for the CSTR trial and between 17 and 36 $g.m^{-3}$ when unaerated selectors were used. This resulted in a higher proportion of the effluent COD being attributable to suspended solids in the unaerated selector reactor trials, due to the lower sCOD and the higher TSS concentrations obtained.

Although an acceptable effluent quality was obtained, filamentous bacterial growth still occurred with the unaerated selector configurations trialed, as evidenced by the increase in SVI with time shown in Figure 6.2.

The extent of sCOD removal occurring in the selector differed for each trial. In selector systems it is the 'removable' soluble COD that is of importance, defined as the difference between the substrate influent and reactor effluent sCOD concentrations. The theoretical sCOD of the selector influent (S_O) due to the combined RAS and feed streams could be calculated, assuming that the RAS sCOD concentration was equivalent to the reactor sCOD concentration. Average selector substrate removal efficiencies, measured as the proportion of 'removable' sCOD consumed in the selectors, varied from 47% in Trial AN1 to 70% in Trial AN3, as listed in Table 6.2.

The floc loading in the selector varied both during and between the different trials as the RAS flowrate was altered in response to changing sludge settleability. Using equation 6.1, the floc loading in the selector during each trial was calculated, as listed in Table 6.2. The RAS VSS concentration at a given RAS flowrate was calculated from a solids balance around the settler using the average mixed liquor and effluent VSS values over the relevant period.

As the mixed liquor SVI increased, the RAS flowrate increased and the RAS VSS concentration decreased, resulting in a decrease in selector floc loading which according to Patoczka and Eckenfelder (1990), should have improved the substrate removal efficiency in the selector.
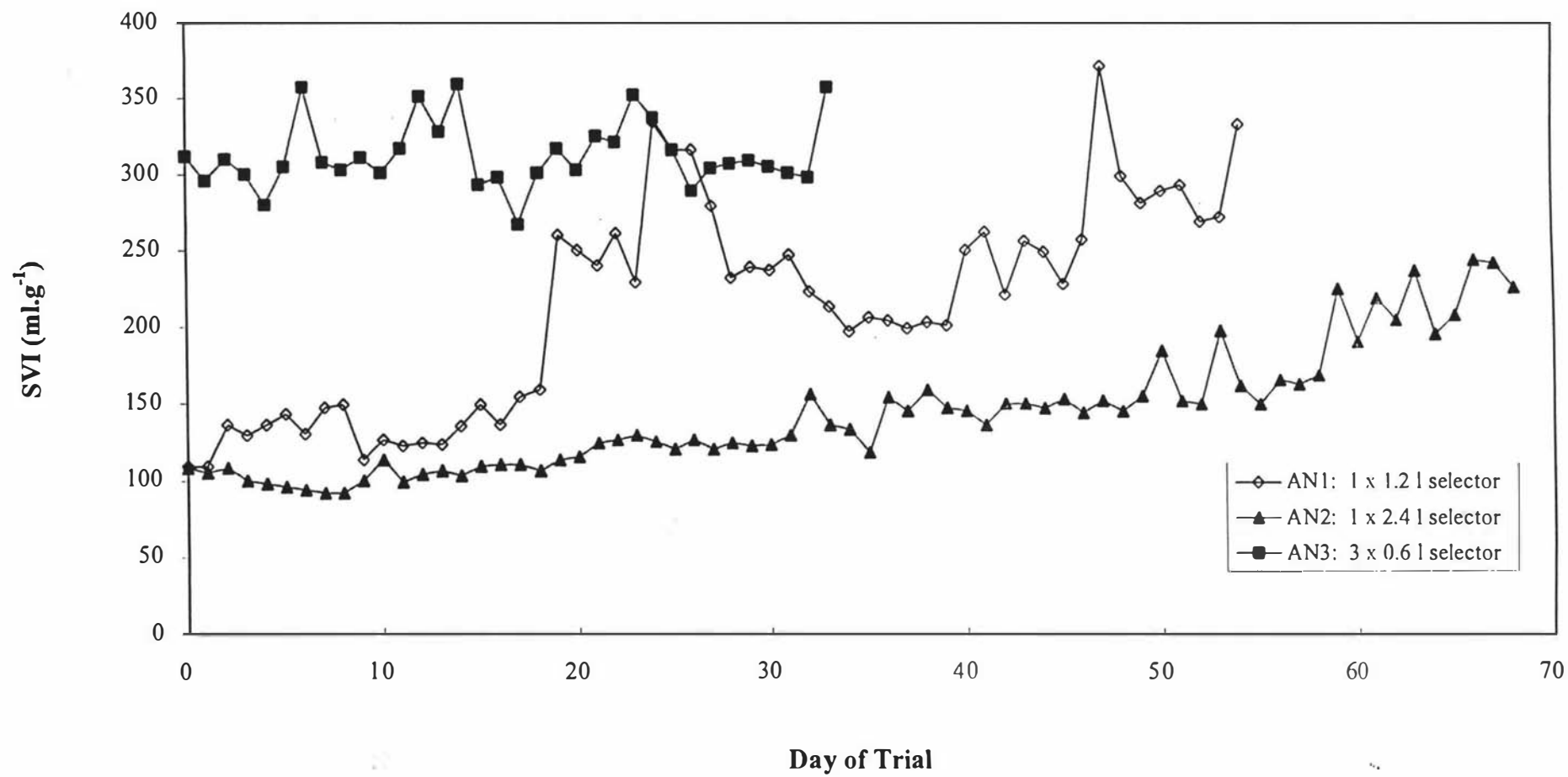
Figure 6.2: Change in Sludge Volume Index during Trials AN1, AN2 and AN3.

Table 6.2   Selector performance and operation during the unaerated selector trials

Trial	Days at SRT	RAS Flowrate $10^{-3}m^3d^{-1}$	HRT min^{-1}	Floc Load $\frac{g\ sCOD}{g\ VSS}$	S_O $g\ sCOD$ m^{-3}	Selector sCOD $g\ sCOD.m^{-3}$	removable sCOD, % consumed
AN1	1 - 23	11.5	80	0.24	800	390	55.3
	24 -31	30.3	43	0.11	450	250	50.3
	32 -38	34.6	39	0.10	405	235	47.4
	39 -50	37.5	36	0.10	395	205	57.3
	51 -56	42.8	33	0.09	360	205	51.3
AN2	1 - 62	16.4	131	0.20	665	310	58.5
	63 -70	21.0	111	0.15	565	265	57.5
AN3	1 - 12	46.2	46	0.09	335	210,200,175	56.1
	13 -24	53.4	41	0.07	300	140,135,110	74.7
	25-31	44.5	48	0.09	345	160,155,140	69.4
	32-34	56.2	39	0.07	285	125,120,80	83.9

A decrease in selector effluent COD was observed with increasing RAS flowrate, as illustrated in Figure 6.3 for Trial AN1, however due to the decreased theoretical initial substrate concentration in the selector at higher RAS flowrates, the overall sCOD removal efficiencies in the selector did not increase, varying between 47% and 57% over the trial. The SVI of the mixed liquor continued to increase even as selector effluent COD decreased, indicating that sufficient substrate was still flowing into the aeration tank to support filamentous growth.

The rate and extent of filamentous bacterial growth during Trial AN1 was much lower than that observed in the conventional activated sludge trials, evidence that the use of some type of selector may be appropriate for solving the sludge bulking problem. The next two unaerated selector trials used slightly different approaches, Trial AN2 used a larger selector, and Trial AN3 used three smaller selectors in series.
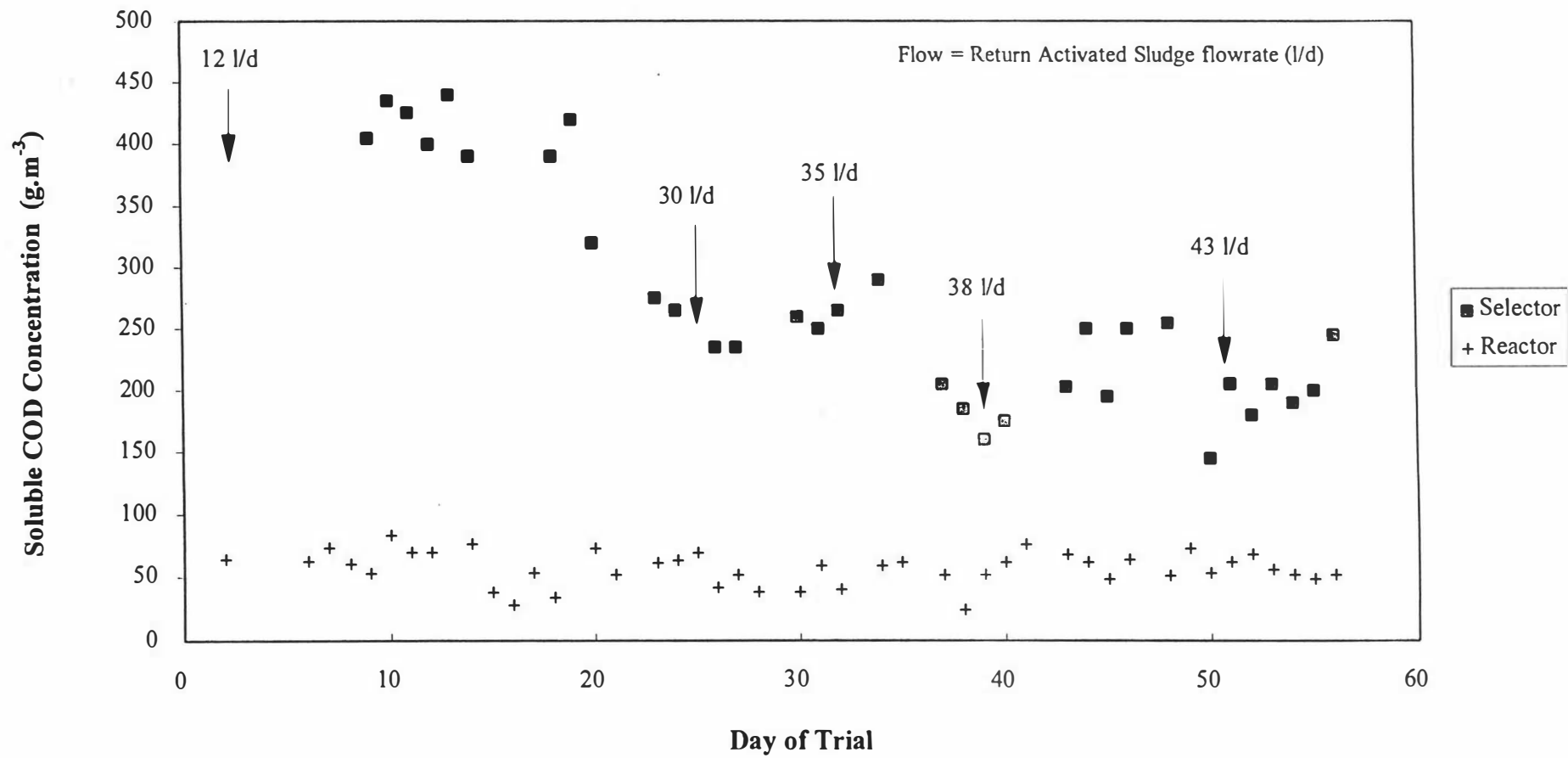
Figure 6.3:   Change in selector soluble COD concentration with increasing recycle rate during Trial AN1.

Trial AN2 was conducted with a 2.4 l selector in the expectation that increased residence time in the selector would increase selector sCOD removal efficiency. As can be seen from the data in Table 6.1, the average 'removable' COD consumed in the selector did not change significantly with the doubling of selector size. The actual selector effluent COD concentration was higher in Trial AN2 than in Trial AN1, but RAS flowrates were lower in Trial AN2, resulting in a higher theoretical S_O in the larger selector.

Figure 6.2 indicates that the rate of proliferation of filamentous microorganisms was much slower when a 2.4 l selector was used rather than a 1.2 l selector, even though the concentration of selector effluent COD was higher. This indicated that the success of the selector system was predicted more accurately by soluble substrate removal efficiency than by sCOD concentration entering the reactor.

Trial AN3 was conducted using three selectors in series to increase the substrate gradient through the system. The sCOD removal efficiency in the selector was improved using this selector configuration, increasing the average removable COD consumed to 69.7%. Trial AN3 was conducted on the filamentous biomass resulting from Trial AN1 and seemed to prevent any further increase in SVI.

The RAS flowrate was constant throughout Trial AN3 and Figure 6.4 shows that there was an initial improvement in selector performance after 10 days, then average sCOD concentrations remained constant in the selector system at 148, 144 and 122 $gCOD.m^{-3}$ for selectors A, B and C respectively during that period. Most of the sCOD removal occurred in the first selector, with 88% of the sCOD removed in the selector system being consumed in the first selector, a further 2% in the second selector and 10% in the last selector. This represents specific sCOD removal rates of 5.6 g $sCOD.g\ VSS^{-1}.d^{-1}$ in the first selector.
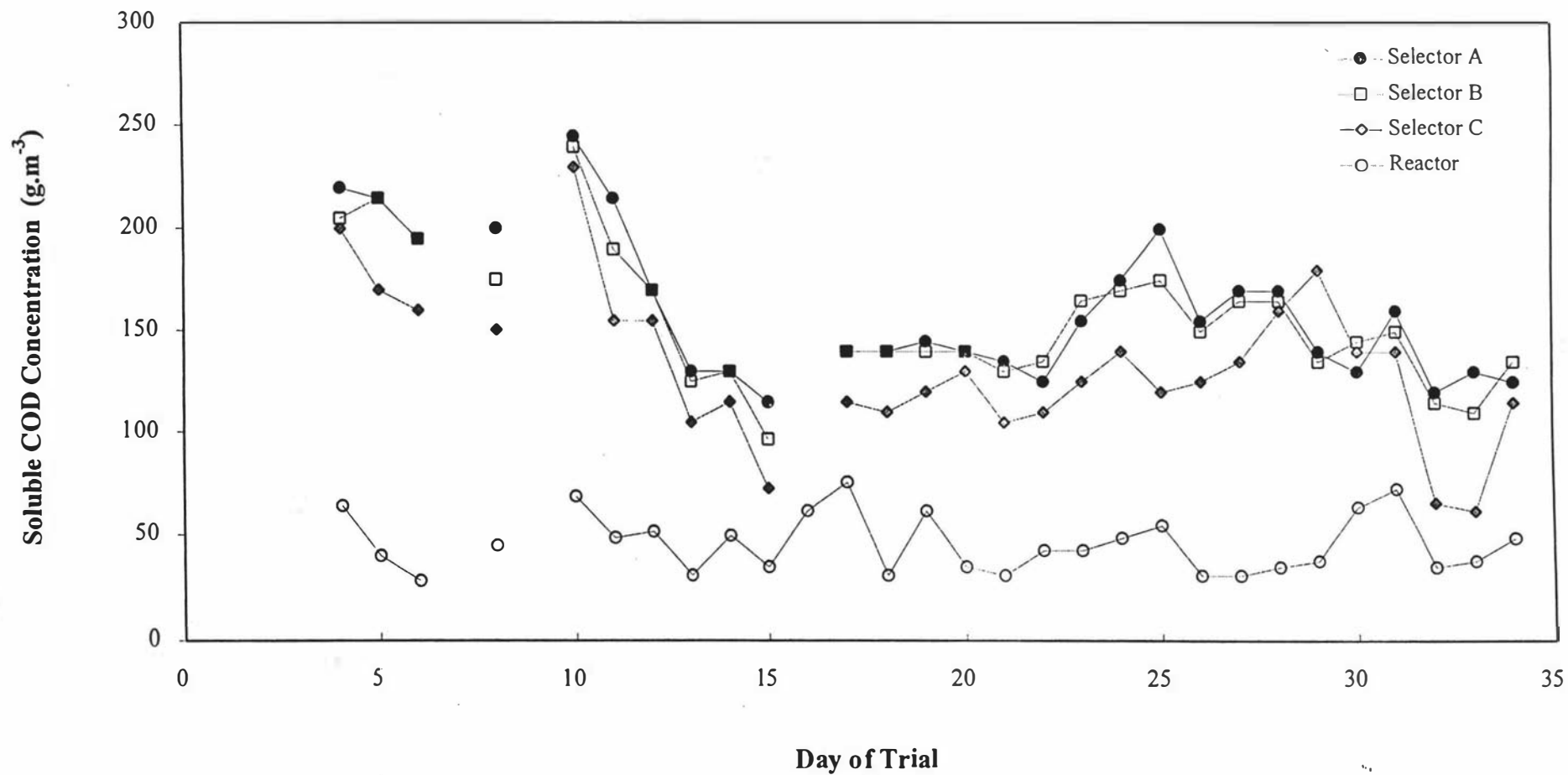
Figure 6.4   Selector soluble COD concentrations during Trial AN3.

6.4   Identification of Activated Sludge Microorganisms.

The use of an unaerated selector did not prevent filamentous growth as evidenced by microscopic observations and the SVI increase shown in Figure 6.2. The single selector trials both commenced with a well settling mixed liquor having an SVI of approximately 100 ml/g. Within 3 SRTs the SVI had increased to around 250 ml/g in Trial AN1, but only to 125 ml/g in Trial AN2. After a further 3 SRTs, the SVI had increased slowly to 300 ml/g in Trial AN1 and to about 200 ml/g in Trial AN2. Both trials were concluded at this stage as the selectors were obviously not able to prevent filamentous growth.

The increase in filamentous bacterial abundance during Trial AN1 is illustrated in Table 6.3 and Figures 6.5 to 6.7. All photographs were of wet mounted mixed liquor samples at 100x magnification.

Table 6.3   Microscopic Observations of Filament Abundance During Trial AN1.

Days at SRT	SVI $(ml.g^{-1})$	Microscopic Observations
3	110	No filaments observed protruding from flocs.
16	135	Few filaments observed protruding from floc surface.
23	240	Filaments common, protruding a short distance out from the flocs. (Figure 6.5)
49	280	Filaments very common, both protruding and free floating, predominantly in bundles extending from the flocs. (Figure 6.6)
56	300	Abundant filamentous growth, both protruding and free floating. Filaments exist both singly and in bundles. (Figure 6.7)
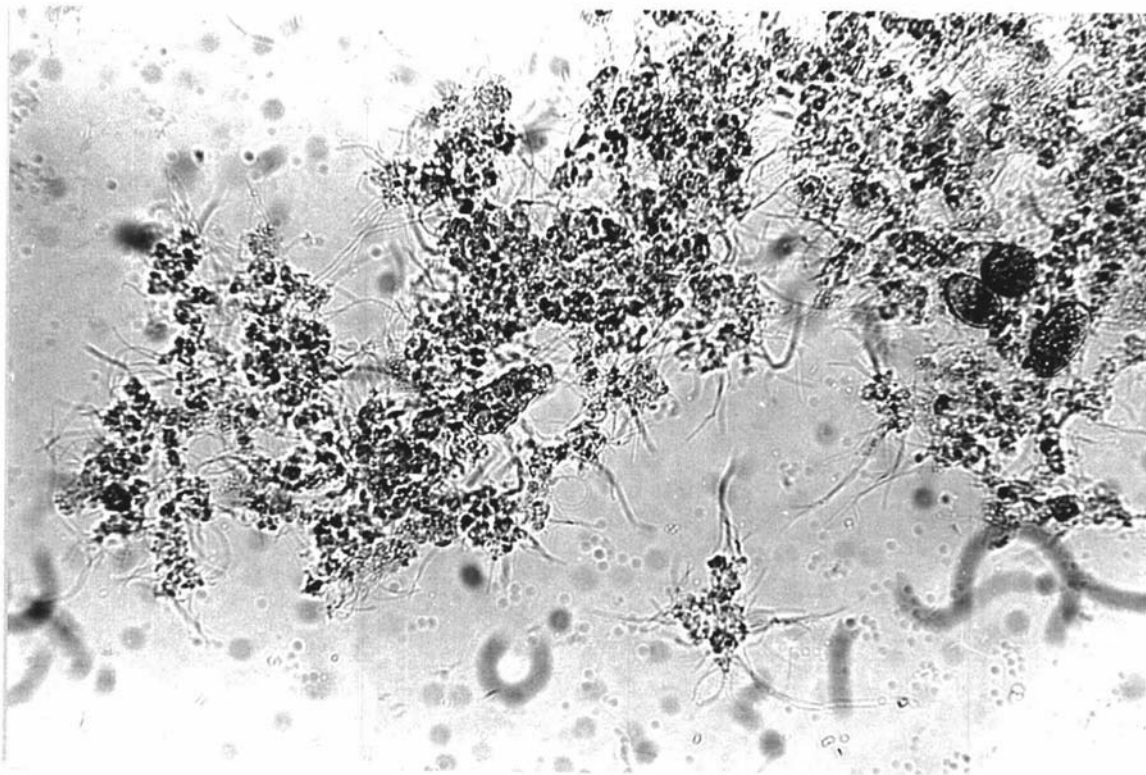
Figure 6.5: Trial AN1 after 23 days at SRT. SVI = 240 ml.g^{-1}. (100x magnification)

Figure 6.6: Trial AN1 after 49 days at SRT. SVI = 280 ml.g^{-1}. (100x magnification)
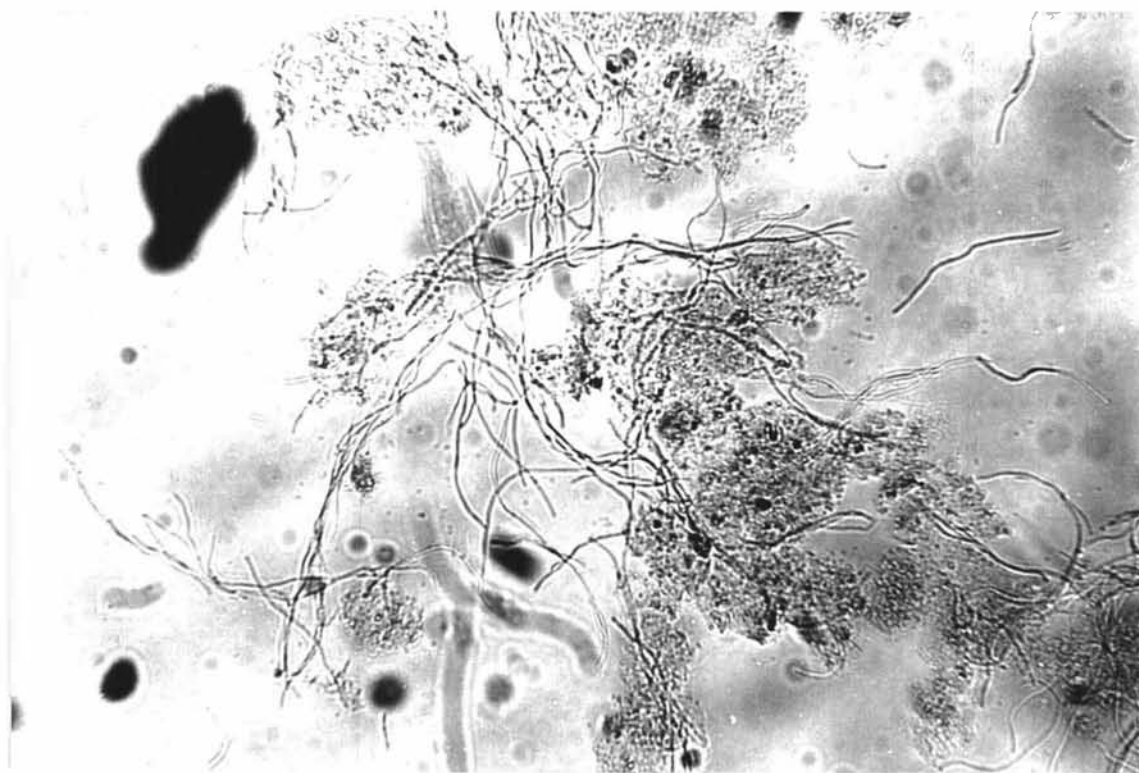
Figure 6.7: Trial AN1 after 56 days at SRT. SVI = 300 ml.g^{-1}. (100x magnification)
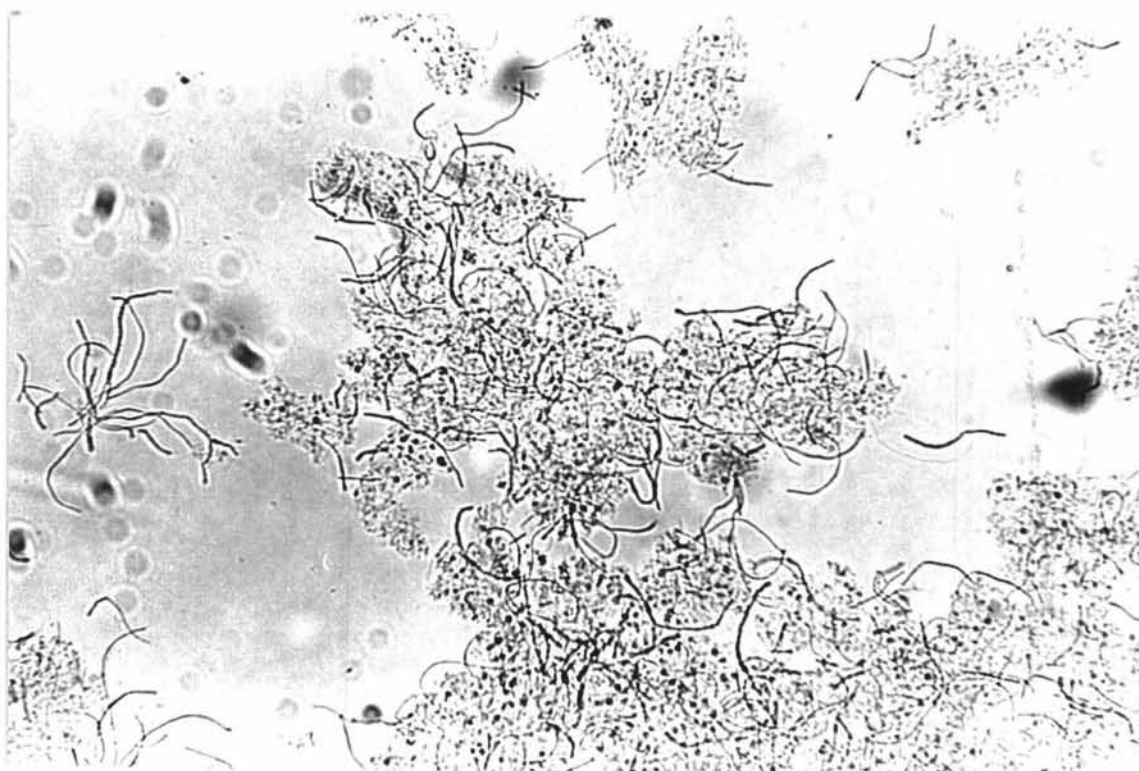
Figure 6.8: Trial AN3 after 18 days at SRT. SVI = 300 ml.g^{-1}. (100x magnficiation)

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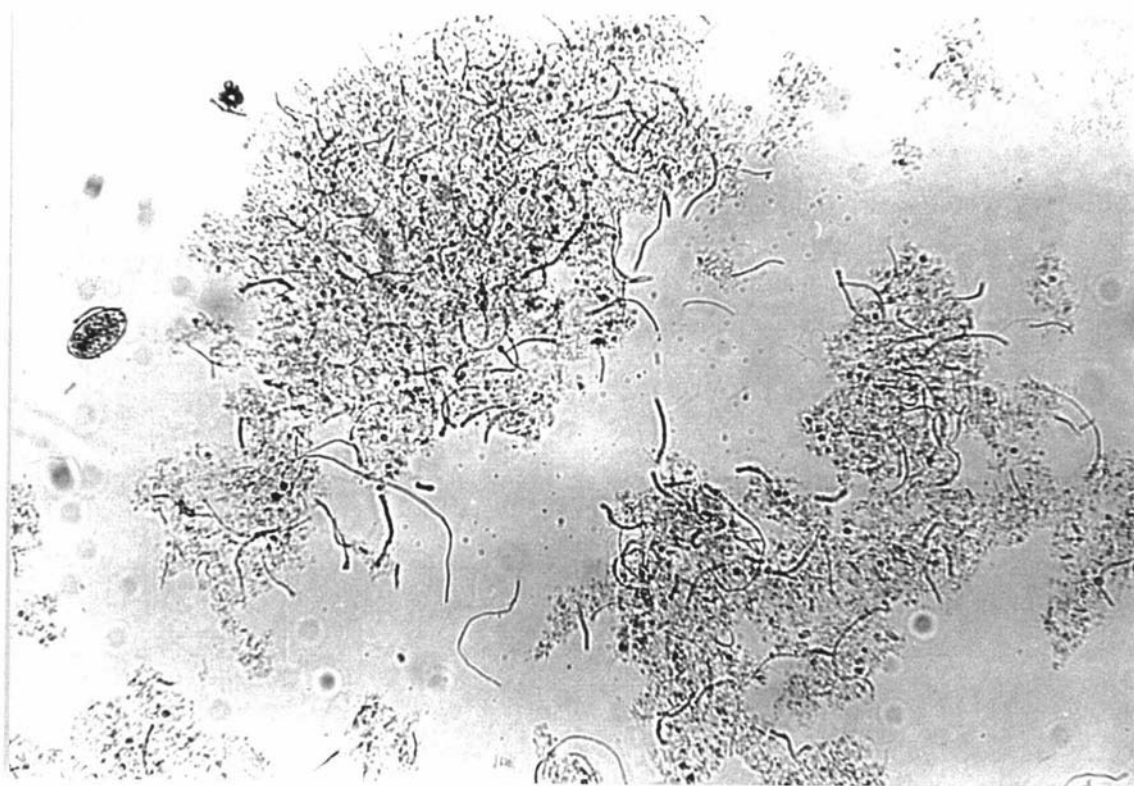
Figure 6.9:   Trial AN3 after 25 days at SRT. SVI unchanged from Figure 6.8.
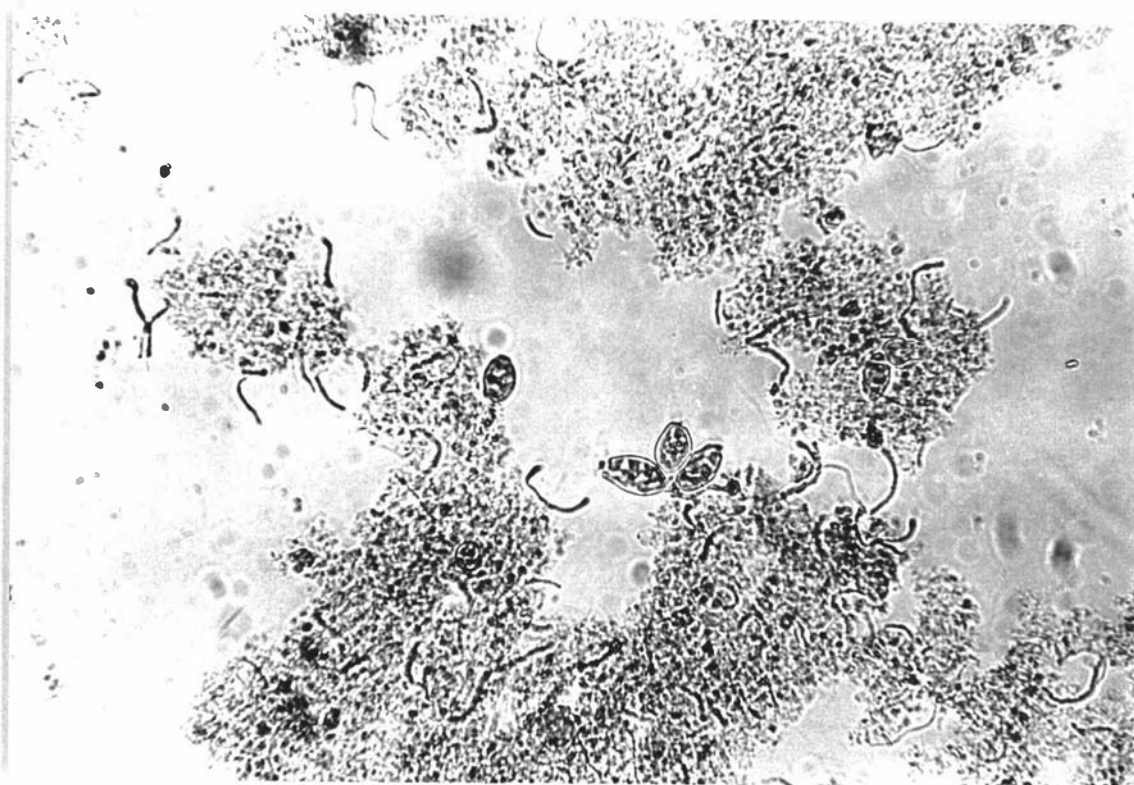
Figure 6.10:   Trial AN3 after 34 days at SRT. SVI unchanged from Figure 6.8.

The dominant filament observed in both trials was observed as being:

- both protruding and free floating; singly and in bundles.
- filament diameter about 1 μm, length about 40 - 400 μm.
- individuals cells square to disc shaped, indentations at cell septa.
- no attached growth.
- non branching, non motile.
- no sulphur granules.

Using the keys and descriptions in Jenkins *et al.* (1993) and LaTrobe University (1993), the filament was identified as being Type 021N. This type is commonly associated with readily metabolizable substrates at moderate to high SRT and with nutrient deficiency (Jenkins *et al.*, 1993).

A secondary filament present was observed as being:

- filament present mostly within the floc
- trichome coiled,
- cells rectangular, constrictions at septa, about 0.8 μm diameter.
- non branching, non motile.
- Gram stain negative.
- Neisser stain negative.
- no PHB granules observed.

This filament was identified as either Type 1701 or Type 0803 using the key in Jenkins *et al.* (1993), however from a comparison with other published photographic descriptions (LaTrobe University, 1993) it was considered to be most probably the former. Type 1701 is characterised by Jenkins *et al.* (1993) as low DO aerobic zone grower, present at conditions of low DO and a readily metabolizable substrate.

Unaerated selectors were therefore successful in preventing the proliferation of Type 0411, but provided an environment in which other undesirable microorganisms could flourish. As the single selectors obviously gave an improved performance over the CSTR trials, Trial AN3 was conducted with a series of selectors, using the bulking mixed liquor from Trial AN1.

The average SVI remained at 300 ml/g for a period of over 3 SRTs during Trial AN3, indicating that this selector configuration could prevent further proliferation of Type 021N. Microscopic observations indicated that the filamentous microorganisms became less extensive and bundles of filaments were no longer observed. Figure 6.8

was taken on Day 18 after the serial selector system was put in place and although the SVI remained at 300 ml/g, a decrease in filament abundance from Figure 6.7 can be observed. Figure 6.9 was taken on Day 25 and Figure 6.10 on Day 34, illustrating a continuing decrease in filament abundance, however the SVI still had not changed significantly. The dominant filament was still Type 021N. As the SVI had still not decreased after 3.5 SRTs, Trial AN3 was discontinued.

6.5 Measurement of Kinetic Constants

Tests were conducted during each trial to assess microbial growth and decay kinetic constants as well as substrate removal rates.

6.5.1 Soluble COD Removal Rates

Batch tests were performed to measure the biosorption capacity and substrate removal rates of the mixed liquor during the selector reactor trials. Each batch test was conducted by adding 15 ml of concentrated soluble feed with a COD of approximately 0.5 g, to one litre of reactor mixed liquor at $25^{\circ}C$. This resulted in a floc loading of approximately 0.15 g sCOD per g VSS, similar to that applied in the selectors.

6.5.1.1 Aerobic Batch Tests

A portion of the COD, between 38% and 61% of that added, was again removed immediately upon contact with the biomass, with the remaining sCOD being removed within 2 hours as shown in Figure 6.11. The period of sCOD removal was best fitted by a first order type relationship, and Figure 6.12 illustrates that a plot of the logarithm of soluble COD concentration against time indicated a linear relationship until the COD concentration reached approximately 100 $g.m^{-3}$.

It is thought that this apparent 'first order' removal rate was in fact a 'pseudo first order' effect resulting from a series of concurrent zero-order removal rates for the various substrate components (Grau *et al.*, 1975; van Niekerk *et al.*, 1987a). The resulting apparent first order removal rate constant however would still be useful as a means of comparing substrate removal between different mixed cultures.
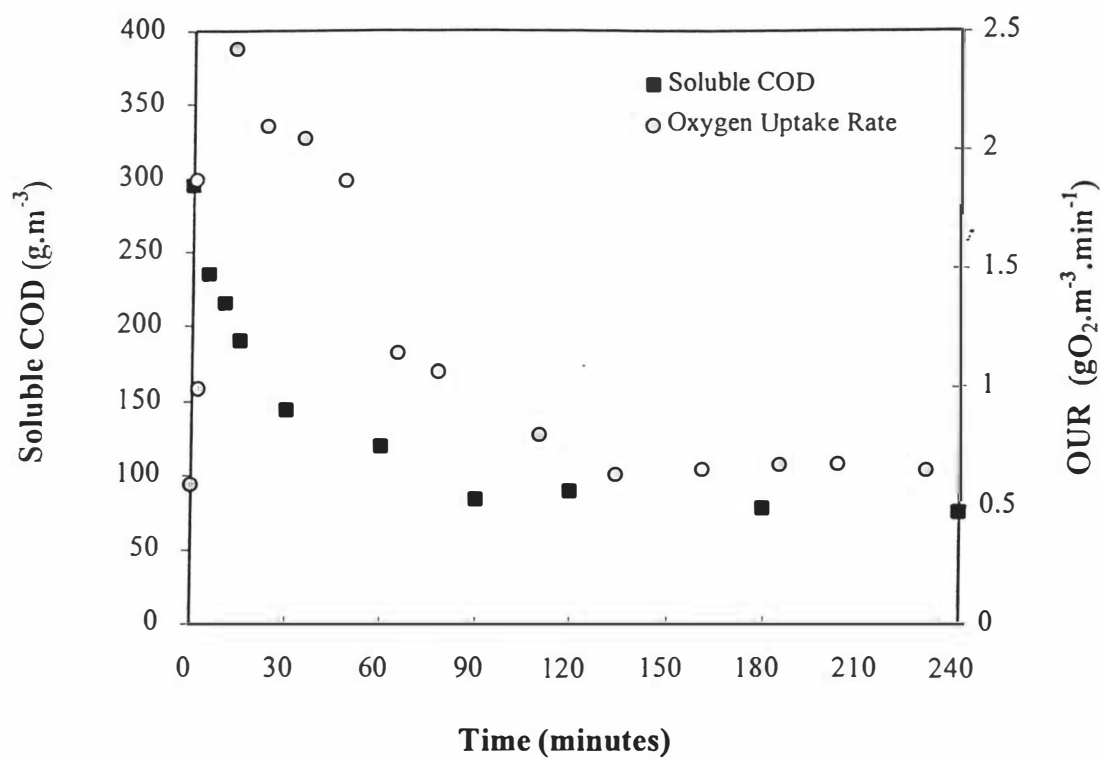
Figure 6.11: Soluble COD removal and OUR during batch tests with AN2 biomass.
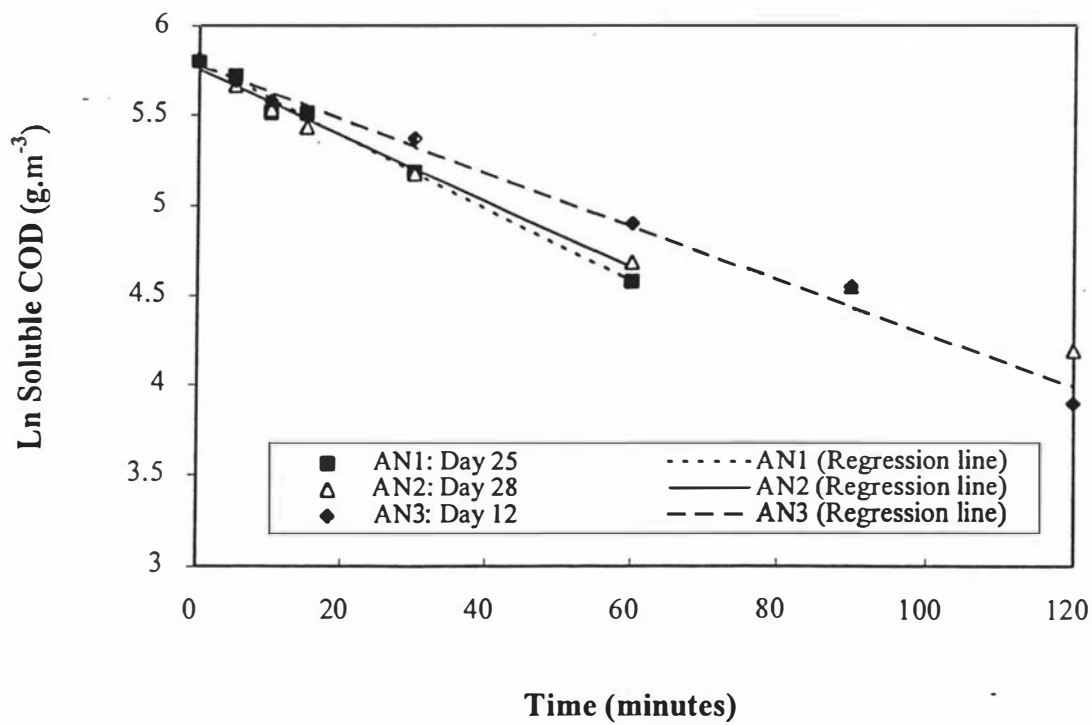
Figure 6.12: Initial soluble COD removal rates during Trials AN1, AN2 and AN3.

Table 6.4   Soluble COD Removal During Aerobic Batch Tests.

Trial	Days at SRT	Floc loading (gCOD.gVSS^{-1})	Biosorption (gCOD.gVSS^{-1})	k (d^{-1})	gO$_2$ consumed / g sCOD added	%O$_2$ consumed when COD consumed
AN1	5	0.19	0.09	23	-	-
	12	0.18	0.09	12	-	-
	19	0.18	0.07	21	-	-
	25	0.13	0.06	29	-	-
	32	0.17	0.07	36	-	-
	54	0.16	0.07	32	-	-
AN2	10	0.16	0.09	32	0.20	96
	28	0.18	0.09	26	0.18	87
	58	0.14	0.07	19	0.19	89
AN3	12	0.25	0.06	21	0.21	83
	13	0.15	0.06	38	0.17	81
	31	0.16	0.10	-	0.20	72

The values obtained for the apparent first order removal rate constant, k, from a regression analysis of the straight line portion of the Ln COD concentration versus time plot for each batch test are given in Table 6.4. Regression R^2 values of 0.954 to 0.998 were obtained in each case, except for the final test on the 3 x 0.6 l selector configuration, where the plot of LnS versus time still resulted in a curve.

As the floc loadings at the beginning of the batch tests were fairly constant, changes in the apparent biosorption capacity of the biomass could be related to the abundance of filamentous microorganisms. In trials AN1 and AN2 the biosorption capacity of the biomass decreased as the trial progressed and the SVI increased. The decrease in filament abundance observed in trial AN3 was accompanied by an increase in the measured biosorptive capacity. Biosorptive capacities measured during selector reactor trials were lower than for the CSTR trials at comparable floc loading and SVI,

indicating that a different mixed culture composition was present in the unaerated selector trials.

The apparent removal rate constant (k), tended to increase during the AN1 trial but decrease during the AN2 trial. As the dominant filament was the same in both trials, a similar trend would have been expected. The trend from the AN3 trial was inconclusive as a value could not be obtained for k in the third batch test. The removal rates also did not appear to be related to the initial floc loading in the batch test.

A comparison of the batch sCOD removal rates from unaerated selector reactor biomass and CSTR biomass is illustrated in Figure 6.13. The increased rate of sCOD removal by biomass which experiences feast / famine conditions or intermittent feeding, such as that imposed by the selector reactor, as compared to a CSTR biomass, has been reported by others and has been dubbed the 'selector effect' (Houtmeyers *et al.*, 1980; van den Eynde *et al.*, 1984; van Niekerk *et al.*, 1987; Chudoba *et al.*, 1991; Jenkins *et al.*, 1993).
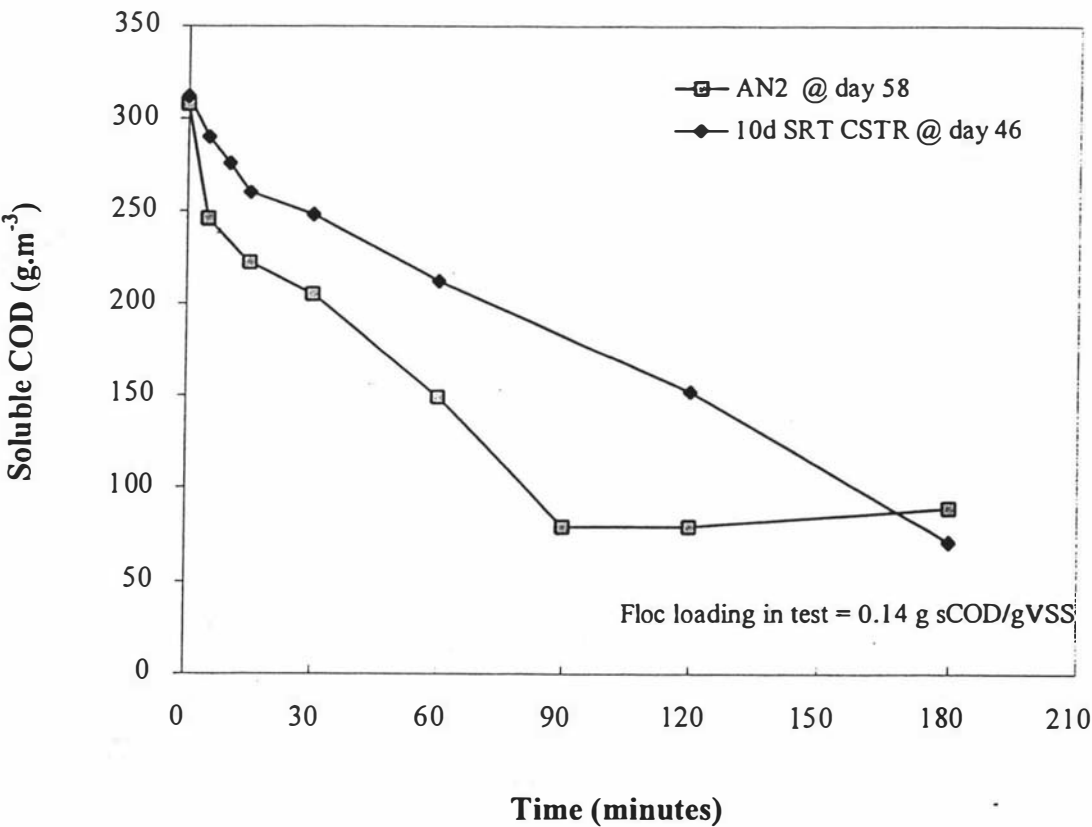
Figure 6.13: Comparison of batch soluble COD removal rates between biomass from CSTR and unaerated selector trials.

The oxygen uptake rate was also measured during batch sCOD removal tests with AN2 and AN3 mixed liquor, an example of which is shown in Figure 6.11. The OUR initially increased for the first 10 to 20 minutes and then decreased slowly over a period of approximately two to four hours until a stable OUR was again recorded. The concurrent COD removal from solution was accomplished in a much shorter time, usually in less than two hours, an effect also reported by Chudoba *et al.* (1973); Chudoba *et al.* (1982); Chiesa *et al.* (1985); Goronszy *et al.* (1985) and Shao and Jenkins (1989).

The ratio of mass of oxygen consumed to mass of COD added was 0.19 ± 0.02 for both trials, much lower than the 0.32 value measured during initial biodegradability studies for the estimation of S_S and Y_H. Jenkins *et al.* (1993) also reported that the oxygen consumed during COD removal is less in a selector system than a completely mixed system, indicating that the substrate is being stored by the microorganisms rather than being oxidised and used for cell growth.

The elevated metabolic rate as evidenced by the measured OUR persisted for a longer period than it took to remove substrate from bulk solution. The proportion of the oxygen consumed in response to COD added that coincided with the period of COD removal was higher in Trial AN2 (86.5% to 96.4%) than in Trial AN3 (71.5% to 83.2%). This indicated that different oxygen consuming processes such as substrate accumulation, production of storage compounds and possibly cell replication, must have been occurring at the same time as sCOD removal from bulk solution during the aerated batch tests. It was indicated that substrate removal from solution occurred at a greater rate compared to the production of storage compounds during trial AN3 than during Trial AN2.

6.5.1.2 Anoxic Batch Tests

Several sCOD removal batch tests were performed in which the respirometer was not aerated, simulating selector conditions. It can be seen from Figure 6.14 that COD removal mechanisms were similar: biosorption followed by a period of first order substrate removal. Andreadakis and Chatjikonstantinou (1994) also found similar removal curves under both aerobic and anoxic conditions.

The sCOD removal rates were initially similar, but after a period of about 15 minutes, the sCOD in the unaerated respirometer began to increase rather than decrease. Tests

for the presence of nitrate and nitrite indicated that there was no nitrate or nitrite remaining in solution after 15 minutes. The increase in sCOD concentration indicated that substrate that had been accumulated by the biomass under anoxic conditions was now being released back into solution, an effect that had also been observed by Shao and Jenkins (1989) and Foot (1992).

The residence time in the selector system varied between 34 and 140 minutes depending on the return activated sludge (RAS) flowrate and the selector configuration in use, as listed in Table 6.2. The actual selector residence time at the same floc loadings of 0.13 to 0.16 g sCOD.gVSS^{-1} as used in the batch test was at least 55 minutes, indicating that all nitrate and nitrite would have been removed well within the residence time of the selector. Conditions in the selector would then have been anaerobic rather than anoxic and any further COD removal would have had to occur via anaerobic mechanisms.
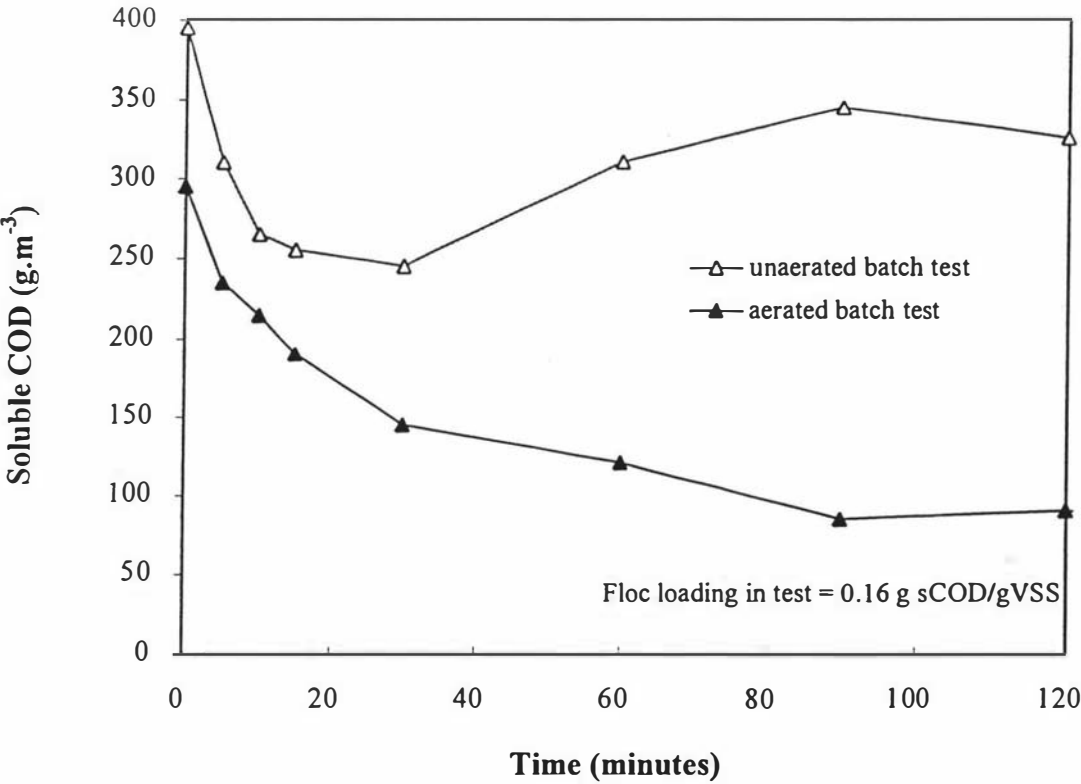
Figure 6.14: Soluble COD removal in aerated and unaerated batch tests during Trial AN2.

6.5.2 Biomass Decay Rates

The traditional decay rate co-efficient, b was estimated periodically from aerated batch tests using the reactor biomass as previously described in Section 5.4.4. The results as listed in Table 6.5 were obtained using biomass with added ATU, so the decay rate measured was due to the activity of heterotrophic organisms only. The values obtained for b are similar to those obtained during CSTR trials ranging 0.19 to 0.27 with an average of 0.23.

Shao and Jenkins (1989) reported that the first order decay rates from prolonged aeration in batch tests were lower for anoxic selector system biomass (0.22 d^{-1}) than for CSTR biomass (0.38 d^{-1}) and Chiesa et al. (1985) reported higher decay rate constants for bulking sludges. Neither of these trends were obvious in the above results.

Table 6.5   Estimation of Decay Rate Co-efficient during unaerated selector trials.

Trial	Days at SRT	SVI (ml.g^{-1})	Decay Rate, b (d^{-1})
AN1	26	334	0.21
AN2	29	124	0.23
AN3	32	301	0.26

Shao and Jenkins, (1989) suggest that the decay rate of biomass taken from an anoxic selector system was lower than for a completely mixed system due to storage compounds present in the anoxic selector system biomass. The duration of the batch decay tests was not specified. Chiesa et al. (1985) found that filamentous mixed cultures exhibited a diphasic decay response composed of a much faster decay rate for the first 24 hours, followed by a decay rate only slightly faster than that of non-filamentous biomass for the next 2 days. This indicated that any significant differences in b may be due to the period of measurement being relatively short, ie. over only one day. Any effect due to stored substrate would also be expected to be exhibited over a period of hours rather than days. The decay rates in this study were estimated over a period of approximately 10 days as outlined in Henze et al. (1987), so as implicated from the results of Chiesa et al. (1985), significant variations in the decay rate may not be observed.

The use of ATU may also affect the results obtained as only the respiration rate of heterotrophic organisms is monitored, whereas nitrifiers would have been expected to contribute to a significant proportion of anoxic selector reactor biomass.

6.5.3   Estimation of Biokinetic Parameters μ_{max} and K_S.

A respirometric estimation of μ_{max} and K_S was made several times during the single selector trials and at the conclusion of the serial selector trial. The results from these tests is given in Table 6.6. The value used for Y_H was that obtained from a measurement of the oxygen consumed in response to the addition of small amounts of substrate, as outlined by Cech *et al.* (1984). Values of between 0.62 and 0.68 g cell COD per g substrate COD were measured, being similar to the $Y_H = 0.68$ value obtained from initial biodegradability studies.

Nitrification was inhibited with ATU so that only the heterotrophic biomass 'growth' response was measured. Again, the general trend was for a decrease in $\Delta SpOUR_{max}$ and hence the calculated value for μ_{max} , with increasing SVI in trials AN1 and AN2. The value measured for $\Delta SpOUR_{max}$ at the conclusion of the serial selector trial (AN3) had not changed significantly from that at the end of the AN1 trial which proceeded it. The SVI of the biomass was also unchanged for the duration of the AN3 trial.

Table 6.6   Values Calculated for μ_{max} and K_S During Unaerated Selector Trials

Trial	Days at SRT	SVI $(ml.g^{-1})$	$\Delta SpOUR_{max}$ $\left(\dfrac{gO_2}{g\ cell\ COD.d}\right)$	Y_H	μ_{max} $(d-1)$	K_S $(gCODm^{-3})$
AN1	11	113	0.35	0.63	0.60	15.5
	24	261	0.31	0.62	0.50	3.1
	31	239	0.34 - 0.35	0.63	0.58 - 0.61	4.3 - 5.0
	55	272	0.32	0.63	0.54	5.5
AN2	30	124	0.30	0.68	0.65	7.7
	59	163	0.23	0.63	0.40	2.8
AN3	33	298	0.26	0.68	0.56	8.6
Ave:					0.52	6.5

The range of values obtained for μ_{max} during the unaerated selector trials of 0.40 to 0.65 d^{-1} were lower than the 0.60 to 0.87 d^{-1} range measured in the conventional activated sludge trials, although the SVI ranges of 298 - 113 ml/g and 357 - 179 ml/g respectively were similar. The dominant filament in the selector trials was Type 021N compared to Type 0411 for completely mixed systems, indicating that Type 021N may have a lower maximum specific growth rate than Type 0411.

6.6   Nutrient Removal

The concentrations of various nitrogen and phosphorus species were measured on the selector mixed liquor, aeration basin mixed liquor and settler effluent. Nitrogen concentrations that were measured included Total Kjeldahl Nitrogen (TKN), ammonia (NH_3), nitrate (NO_3) and nitrite (NO_2). Phosphorus was measured as Total Phosphorus (TP) and Dissolved Reactive Phosphorus (DRP). The N and P concentrations were measured over a period of at least 10 days, in the later stage of each trial when the reactor configuration had been in operation for at least two SRTs. The average concentration of the various species measured during each trial is given in Tables 6.7 to 6.9.

Table 6.7   Average N and P concentrations during Trial AN1: 1 x 1.2 l selector.

(Days 46 to 56)	Selector	Reactor	Effluent
TKN ($gN.m^{-3}$)	430	481	5.46
NH_3 ($gN.m^{-3}$)	1.92	0.49	0.43
NO_3 ($gN.m^{-3}$)	< 0.1	4.83	3.88
NO_2 ($gN.m^{-3}$)	0.04	3.91	3.03
TP ($gP.m^{-3}$)	51.3	58.9	7.64
DRP ($gP.m^{-3}$)	8.5	6.8	6.9
Organic N /VSS ($g.g^{-1}$)	0.136 ± 0.018	0.137 ± 0.026	-
Organic P /VSS ($g.g^{-1}$)	0.0138 ± 0.0021	0.0149 ± 0.0029	-

Table 6.8   Average N and P concentrations during Trial AN2: 1 x 2.4 l selector.

(Days 17 to 26)	Selector	Reactor	Effluent
TKN (gN.m^{-3})	420	420	-
NH$_3$ (gN.m^{-3})	6.09	<0.1	0.10
NO$_3$ (gN.m^{-3})	<0.1	6.36	5.76
NO$_2$ (gN.m^{-3})	0.04	4.80	3.87
TP (gP.m^{-3})	55	58	-
DRP (gP.m^{-3})	6.7	4.7	4.8
Organic N /VSS (g.g^{-1})	0.139 ± 0.023	0.136 ± 0.017	-
Organic P /VSS (g.g^{-1})	0.0161 ± 0.0024	0.0172 ± 0.0022	-

Table 6.9   Average N and P concentrations during Trial AN3: 3 x 0.6 l selectors.

(Day 20 to 34)	Selector A	Selector B	Selector C	Reactor	Effluent
TKN (gN.m^{-3})	382	409	443	416	-
NH$_3$ (gN.m^{-3})	0.39	0.83	1.30	<0.1	<0.1
NO$_3$ (gN.m^{-3})	<0.1	<0.1	<0.1	2.92	2.79
NO$_2$ (gN.m^{-3})	0.008	0.009	0.015	0.78	0.60
TP (gP.m^{-3})	51	56	59	59	-
DRP (gP.m^{-3})	6.2	6.8	6.6	4.8	5.2
Organic N/VSS (g.g^{-1})	0.129 ± 0.016	0.128 ± 0.033	0.131 ± 0.018	0.127 ± 0.028	-
Organic P/VSS (g.g^{-1})	0.0152 ± 0.0018	0.0154 ± 0.0028	0.0157 ± 0.0029	0.0164 ± 0.0024	-

During Trial AN1 the nitrate and nitrate concentrations in the selector zone were negligible, indicating that all the available oxidised N in the RAS had been utilised for substrate removal. Effluent ammonia concentrations were also low, but not negligible, suggesting that nitrification was not quite complete in the reactor zone. The average DRP concentration in the selector zone was almost 2 $gP.m^{-3}$ higher than that in the reactor and the biomass P content was indicated as possibly being higher in the reactor.

The reactor and effluent ammonia concentrations were negligible during Trial AN2, indicating that the effluent was now fully nitrified. The selector oxidised N compound concentrations were again negligible in the selector and the same differences in DRP and biomass P contents in the various zones were observed as in the previous trial.

In Trial AN3 the nitrate and nitrite concentrations were negligible in all three selector zones, but an increase in ammonia concentration was observed as the mixed liquor progressed through the selectors. The average DRP concentration was increased in the second selector, however differences in DRP and biomass P contents between the selectors and reactor were similar to those noted previously in AN1 and AN2.

The nutrient content of the biomass in each trial was similar to that quoted elsewhere for conventional activated sludge. Biomass N contents averaged between 12.7% and 13.9%, close to the value of 12.4% based on an analysis of cellular components given in Metcalf & Eddy (1991). P contents of biomass have been reported to be 1.5% to 2% (Tetreault *et al.*, 1986; Yeoman, 1988); therefore the averages of 1.4 to 1.7% measured during trials AN1 to AN3 were similar to those being quoted for conventional activated sludge.

6.6.1 Ammonification.

For the selector trials conducted, average ammonia concentrations of between 1.9 and 6.1 $gN.m^{-3}$ were measured in the mixed liquor flowing out of the selector zones. The ammonia concentrations in the effluent and hence the RAS stream were negligible and there was negligible ammonia in the feed stream, indicating that the presence of significant ammonia concentrations in the selectors was due to ammonification of the feed stream proteins.

As can be seen in Figure 6.15, the selector ammonia concentrations were highest during Trial AN2 which had the highest selector substrate concentration, and hence organic N
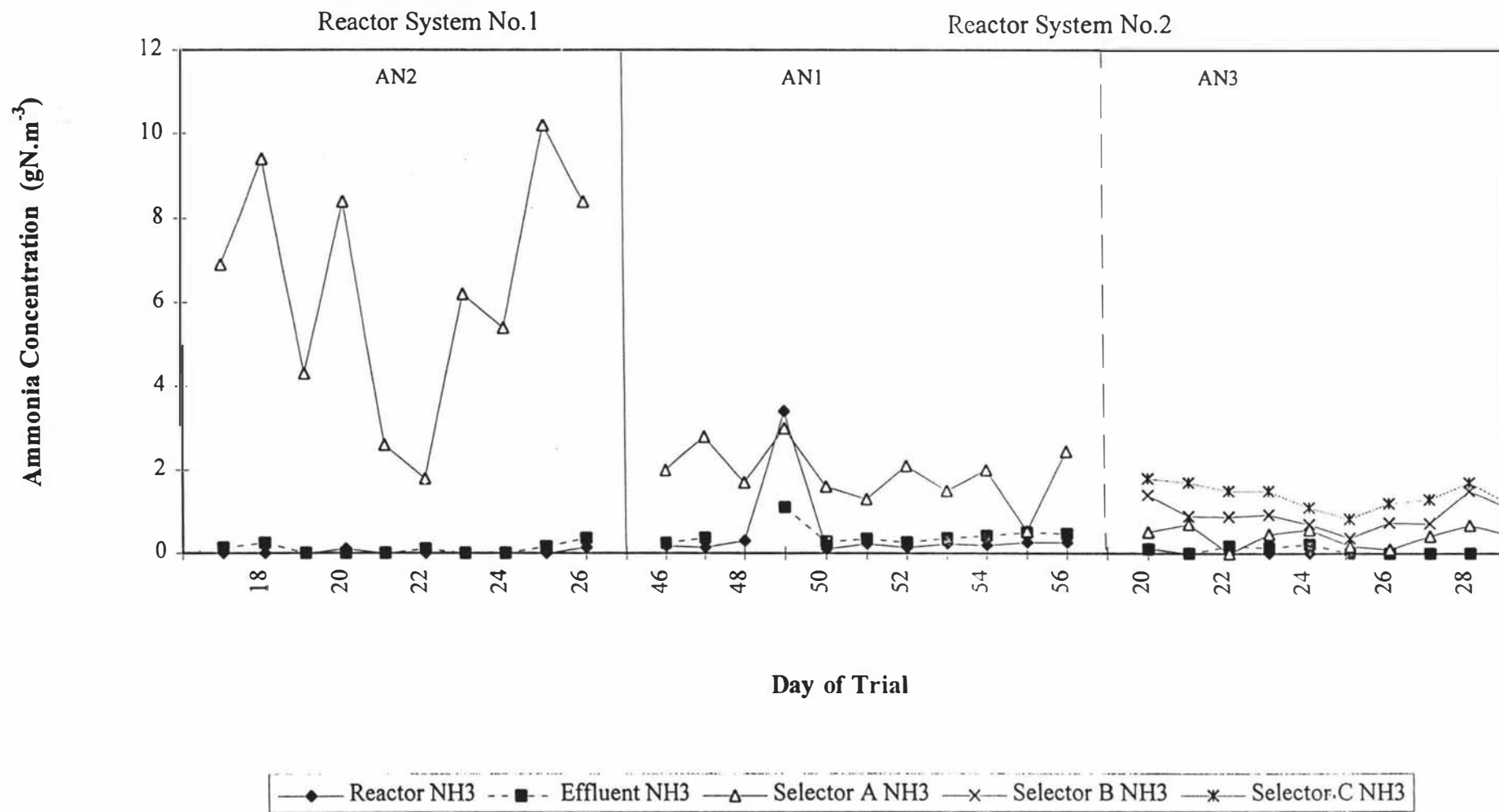
Figure 6.15: Ammonia concentration in the reactor zones during Trials AN1, AN2 and AN3.

concentration, as well as the longest selector residence time. In all three trials the ammonia concentration reached in the first selector was found to be proportional to the COD concentration in that zone, as would be expected since ammonification is reported to be a first order reaction with respect to organic N (Wong-Chong and Loehr, 1975).

The ammonia profiles through the serial selectors in Trial AN3, as illustrated in Figure 6.15, resulted in average concentrations of 0.39, 0.83, 1.30 gNH_3-$N.m^{-3}$ as the mixed liquor passed through the selector sequence. The stepwise increases of 0.39, 0.44 and 0.47 gNH_3-$N.m^{-3}$, indicated an increasing ammonification rate through the selectors suggesting that only a small portion of the feed proteins had been degraded in the selector zone of the system.

The ammonification rate constant could not be assessed as it was not possible to distinguish between the organic N of mixed liquor cells and the organic N due to proteins in the feed stream from TKN analysis data. An estimate of the ammonification rate through the selector system in trial AN3 can be gained from a plot of ammonia production (Ln NH_3-N) against time as shown in Figure 6.16. Values of the rate constant for ammonia production, K_{NH3} averaged 0.029 min^{-1} with a maximum of 0.047 min^{-1}, and were considerably higher than that reported for organic N removal of up to 0.004 min^{-1} by Wong-Chong and Loehr (1975).

The reactor ammonia concentration was less than 0.1 $gN.m^{-3}$ in Trials AN2 and AN3, and usually below 0.3 $gN.m^{-3}$ during AN1. As ammonium oxidation is considered to be the rate limiting step in the conversion of organic nitrogen to nitrate (Wong-Chong and Loehr, 1975), one would expect the lack of NH_3-N in the reactor to be a sign of complete ammonification. A comparison of the concentration of NH_3-N at the selector outlet to that of oxidised N (NO_3 + NO_2) at the aerated reactor outlet, indicated that a minimum of 21%, 55% and 35% of ammonification occurred in the selectors for trials AN1, AN2 and AN3 respectively.

6.6.2   Nitrification.

As the selectors were unaerated with an absence of both oxygen and nitrate in bulk solution, it can be assumed that no nitrification occurred in the selector part of the system. This would have resulted in all of the nitrification reactions proceeding in the aerobic reactor, to produce the nitrate concentrations illustrated in Figure 6.17.
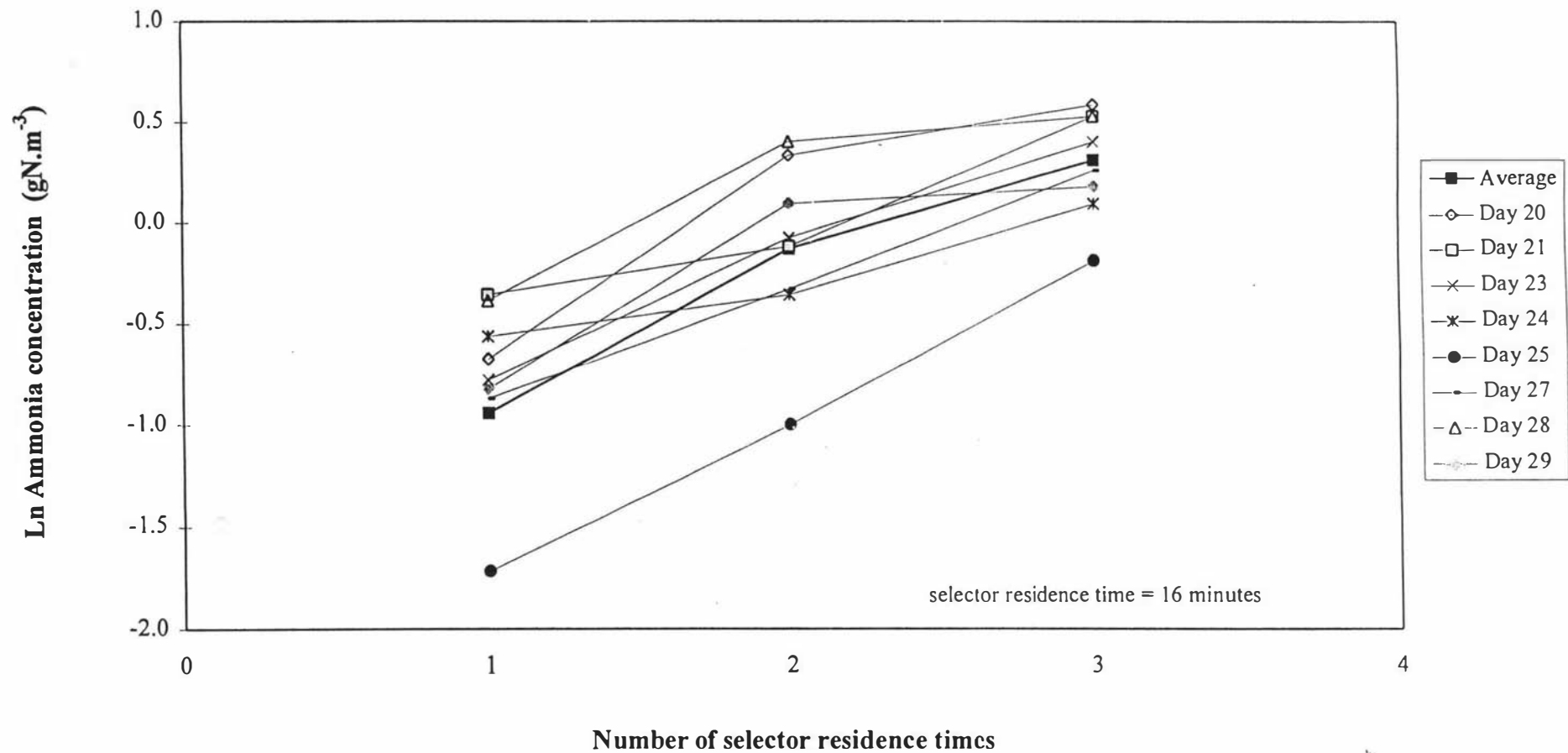
Figure 6.16: Plot of ammonia concentration against selector residence time during Trial AN3.
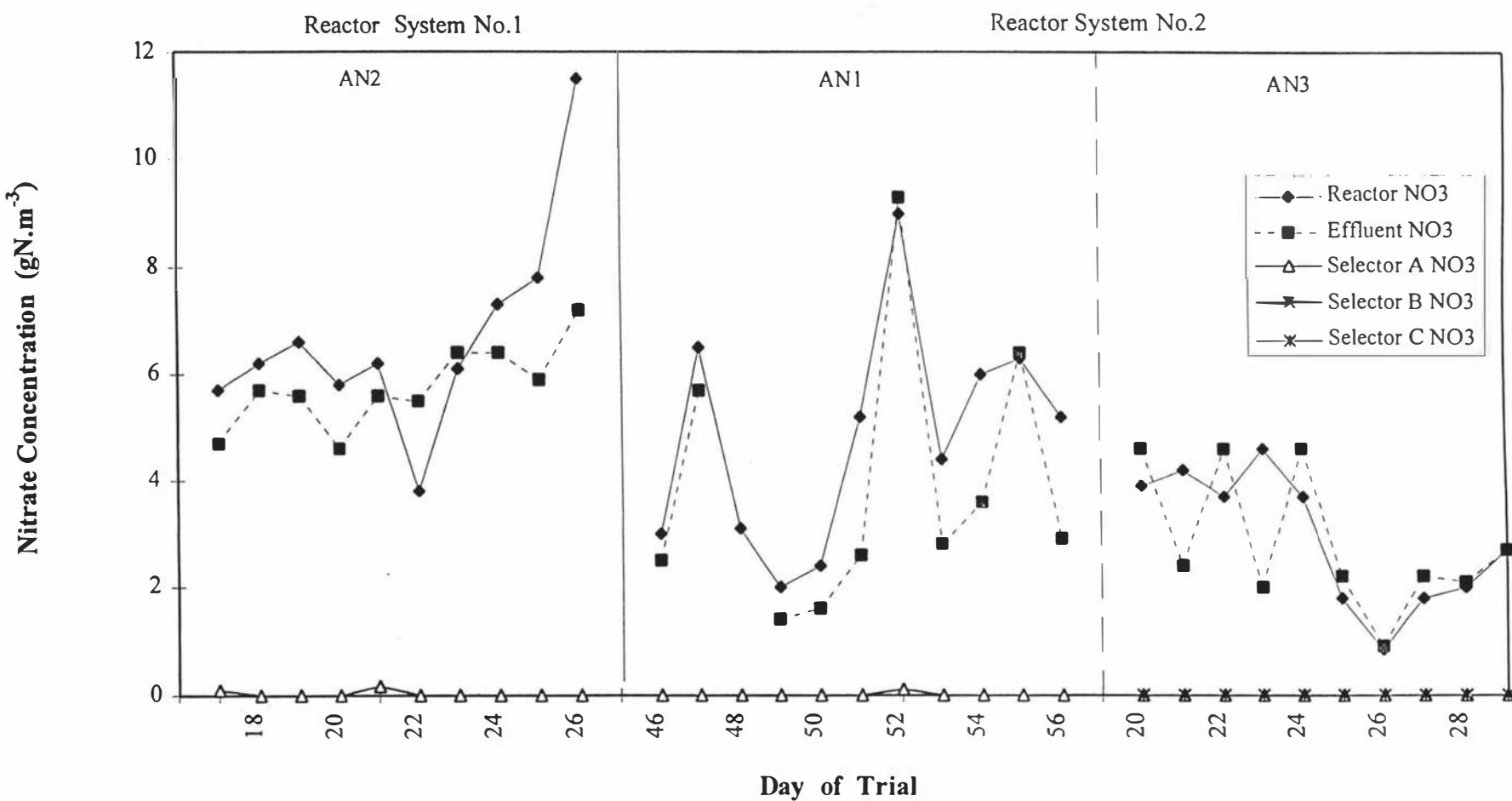
Figure 6.17: Nitrate concentration in the reactor zones during Trials AN1, AN2 and AN3.
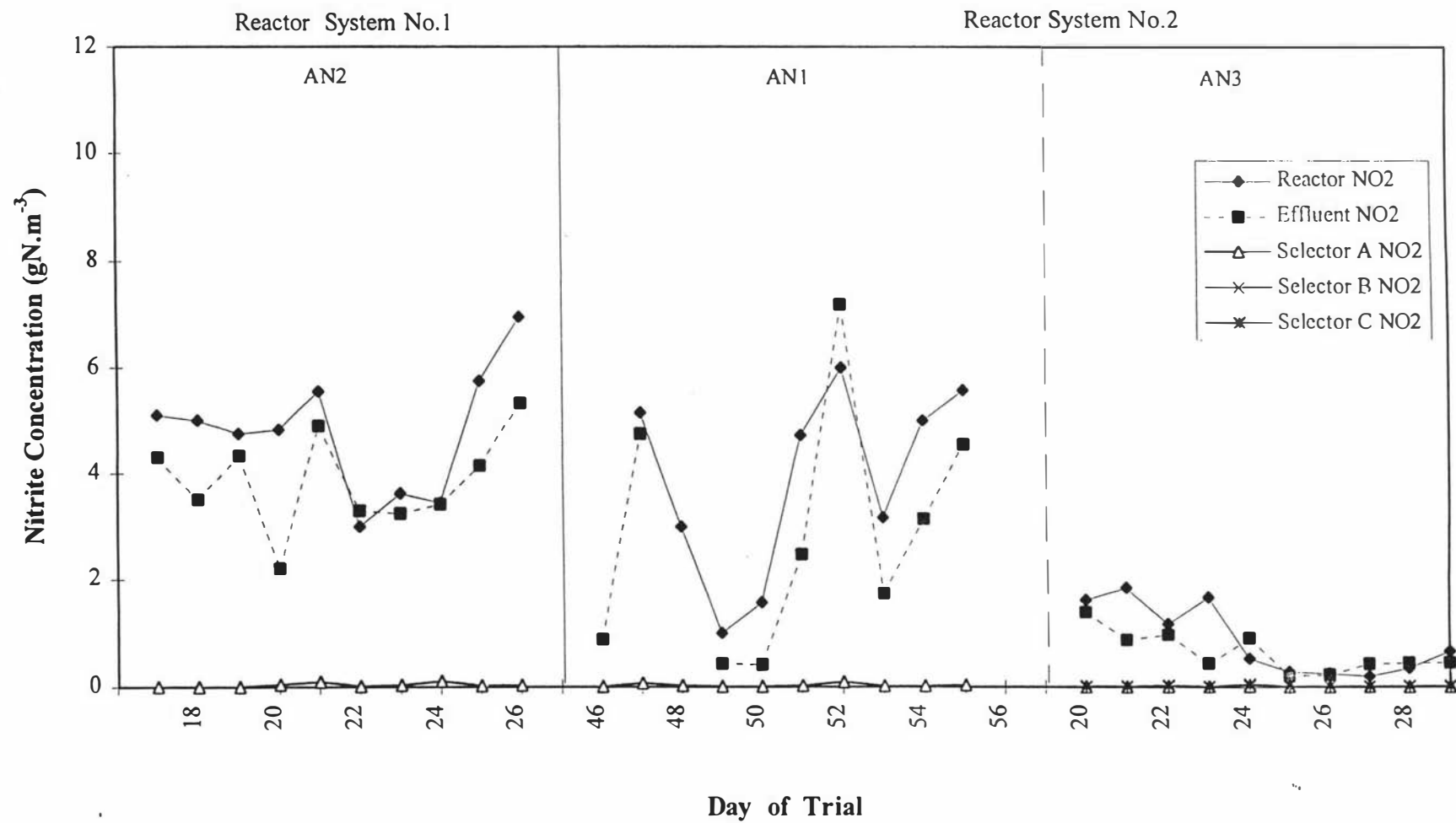
Figure 6.18: Nitrite concentration in the reactor zones during Trials AN1, AN2 and AN3.

The ammonia concentrations in the reactor were below the detection limit (<0.1 g.m^{-3} NH$_3$-N) during the AN2 and AN3 trials, indicating that the wastewater was fully nitrified. During trial AN1 the reactor ammonia concentration was below 0.3 g.m^{-3} NH$_3$-N for all but one sample, and considerably lower than the selector ammonia concentration, indicating that nitrification was also almost complete in this trial.

Both ammonium and nitrite oxidation are reported by Wong-Chong and Loehr (1975) to be zero order reactions, with the formation of nitrate being essentially equal to the rate of ammonium oxidation, however significant nitrite concentrations were also measured in the reactor during all three trials. The actual nitrification rate could not be calculated as it was indicated that both ammonification and nitrification were occurring simultaneously in the aeration tank, and the ammonification rate could not be estimated due to the reasons already given in Section 6.6.3. However, to produce the oxidised N concentrations measured, specific nitrification rates of at least 0.021, 0.016 and 0.011 gN oxidised.gVSS^{-1}.d^{-1} must have occurred in Trials AN1, AN2 and AN3 respectively. As these 'worst case' values were considerably lower than those rates determined by other researchers of 0.03 to 0.19 gN.gVSS^{-1}.d^{-1} (McClintock *et al.*, 1993) and 2.3 gN.gVSS^{-1}.d^{-1} (Argaman and Brenner, 1986), it can be concluded that nitrification was not limited by the residence times provided in the reactor zones.

Significant nitrite concentrations were observed in the reactor zone, as shown in Figure 6.18, particularly in Trials AN1 and AN2 where the average nitrite concentrations were 80% and 75% of the nitrate concentration respectively. Trends in the reactor nitrite concentration were matched by variations in reactor nitrate concentrations during Trials AN1 and AN3, suggesting that nitrite was not accumulating due to inhibition of *Nitrobacter sp.* activity. Also the ammonia concentrations were negligible and pH levels not increased, ruling out two factors commonly reported to result in nitrite accumulation (Wong-Chong and Loehr 1975; Azimi and Horan, 1991). Hoffman (1987) also reported the appearance of nitrite in selectors, which disappeared in the reactor zone, and Nowak et al (1995) found a decrease in nitrite oxidation capacity for activated sludge subjected to anaerobic conditions, such as would have been the case in these trials.

6.6.3   Denitrification.

One of the contributing factors in the decision to trial 'anoxic' selectors was the potential for N removal, due to denitrification when nitrate and nitrite are used as electron

acceptors for exogenous substrate removal and storage. An overall mass balance for total nitrogen in the system, assuming 'steady state' had been reached at the time of N measurements, and therefore assuming no accumulation of N in the system or loss of N by volatilization, is as follows :

N_{FEED} = N in the effluent + N in the wasted mixed liquor + N lost by denitrification.

Total N in each stream can be measured as:

Total N = organic N + ammonia + nitrate + nitrite

where organic N can be calculated from:

organic N = TKN - ammonia.

The total nitrogen content of the feed stream was measured as 89 $gN.m^{-3}$, therefore the mass of N supplied to the reactor system each day, N_{FEED}, was:

89 $gN.m^{-3}$ x 10^{-2} m^3d^{-1} = 0.89 g $N.d^{-1}$.

The SRT of each system was based on aeration tank volume only, so the N content of cells wasted per day was equal to the organic N content of one litre of mixed liquor. Using the data stated in Tables 6.7 to 6.9, the nitrogen content of the wasted mixed liquor (WML) and effluent cells were calculated, as listed in Table 6.10.

The results from the nitrogen balance in Table 6.10 indicate that significant N removals were occurring from the reactor system. A comparison of the nitrate and nitrite concentrations in the reactor and in the effluent stream as listed in Tables 6.7 to 6.9 indicates that denitrification was occurring in the settler as well as the selector zones. This was also indicated by occasional rising sludge in the settler after sludge accumulation during periods of high SVI. The concentration of oxidised N in the effluent was near or above the critical limit of 6 to 8 $gN.m^{-3}$ suggested by Henze *et al.* (1993) to prevent the interference of settling by denitrification, particularly in Trials AN1 and AN2.

An estimate of the N removed by denitrification in the settler and selector parts of the system can be made, assuming that the fate of the nitrate and nitrite flowing out of the reactor is either:

- to flow out in effluent stream
- to be removed by denitrification in the settler

- to flow out in the RAS and be removed by denitrification in the selectors.

The total oxidised N compounds removed by denitrification in the settler and selector is therefore the difference between the mass of (nitrate + nitrite) flowing out of the reactor and the mass of (nitrate + nitrite) in the effluent stream.

Table 6.10   Estimation of N Removal during the Unaerated Selector Reactor Trials

Trial	AN1	AN2	AN3
WML volume (10^{-3} $m^3.d^{-1}$)	0.93	0.95	0.90
N content of cells in WML and effluent ($gN.d^{-1}$)	0.48	0.42	0.42
WML: $NH_3 + NO_3 + NO_2$ ($gN.d^{-1}$)	0.009	0.011	0.003
Effluent: $NH_3 + NO_3 + NO_2$ ($gN.d^{-1}$)	0.07	0.09	0.03
N_{OUT} : WML + Effluent ($gN.d^{-1}$)	0.55	0.52	0.46
N_{FEED} - N_{OUT} : N removed by denitrification ($gN.d^{-1}$)	0.34	0.38	0.44
N removed by denitrification (%)	38	42	49
System N removal (%) (N_{FEED} - N_{EFF}) / N_{FEED}	91	88	92

Table 6.10 indicated an increasing extent of denitrification with increasing ability to suppress filamentous growth, however the data in Table 6.11 indicates that less of the N assumed to be removed by denitrification could be attributed to the removal of oxidised N flowing out of reactor as the extent of denitrification increased.

The amount of N estimated to be removed by denitrification in the settler and selectors was 115% of the estimate of system N removal for trial AN1. Barker and Dold (1995) found that N balances of between 95 to 108% could be obtained for anoxic-aerobic AS systems and 92 to 110% for AS systems with anaerobic zones. As the mass balances used in Tables 6.10 and 6.11 were calculated using average N values over 10 days of operation, the agreement between the two values was not considered to be inconsistent with other reported findings.

Table 6.11   Estimation of N removed in the settler and selector zones.

Trial	AN1	AN2	AN3
Oxidised N out of reactor zone (gN.d^{-1})	0.46	0.30	0.20
Oxidised N in effluent (gN.d^{-1})	0.07	0.10	0.03
Oxidised N out of reactor removed by denitrification in selector and settler zones (gN.d^{-1})	0.39	0.20	0.17
Estimated total gN.d^{-1} removed by denitrification (from Table 6.10)	0.34	0.38	0.44
% of denitrification due to removal of oxidised N flowing out of reactor zone.	115	53	39

However, the difference between the two N removal values obtained for Trials AN2 and AN3 was outside that expected to be explained by measurement variation. It is proposed that significant N removal was occurring by either denitrification occurring simultaneously with nitrification in the reactor zone, or by nitrification occurring simultaneously with denitrification in the selector zones; but as the selectors were unaerated the latter scenario was unlikely.

Denitrification rates in the reactor systems could not be accurately determined as the nitrate concentration in the selectors was generally below the detection limit and any denitrification in the reactor zone would have been occurring simultaneously with nitrification reactions. An estimate of the minimum denitrification rate can be obtained during Trial AN3, where the maximum concentration of 4.6 g.m^{-3} NO$_3$-N measured in the RAS stream at a flowrate of 44.5 x10^{-3}m^{3}d^{-1} must have been removed at a rate in excess of 0.15 gNO$_3$-N.gVSS^{-1}.d^{-1} to maintain a negligible nitrate concentration in the selector. This value was slightly above the figures quoted by other researchers of up to 0.11gNO$_3$-N.gVSS^{-1}.d^{-1} (Argaman and Brenner, 1986; Metcalf and Eddy, 1991); however high rates would have been expected in this first selector zone as the denitrification rate has been found to be proportional to readily biodegradable COD concentrations (Argaman and Brenner 1986, Clayton *et al.* , 1991; Isaacs and Henze, 1995).
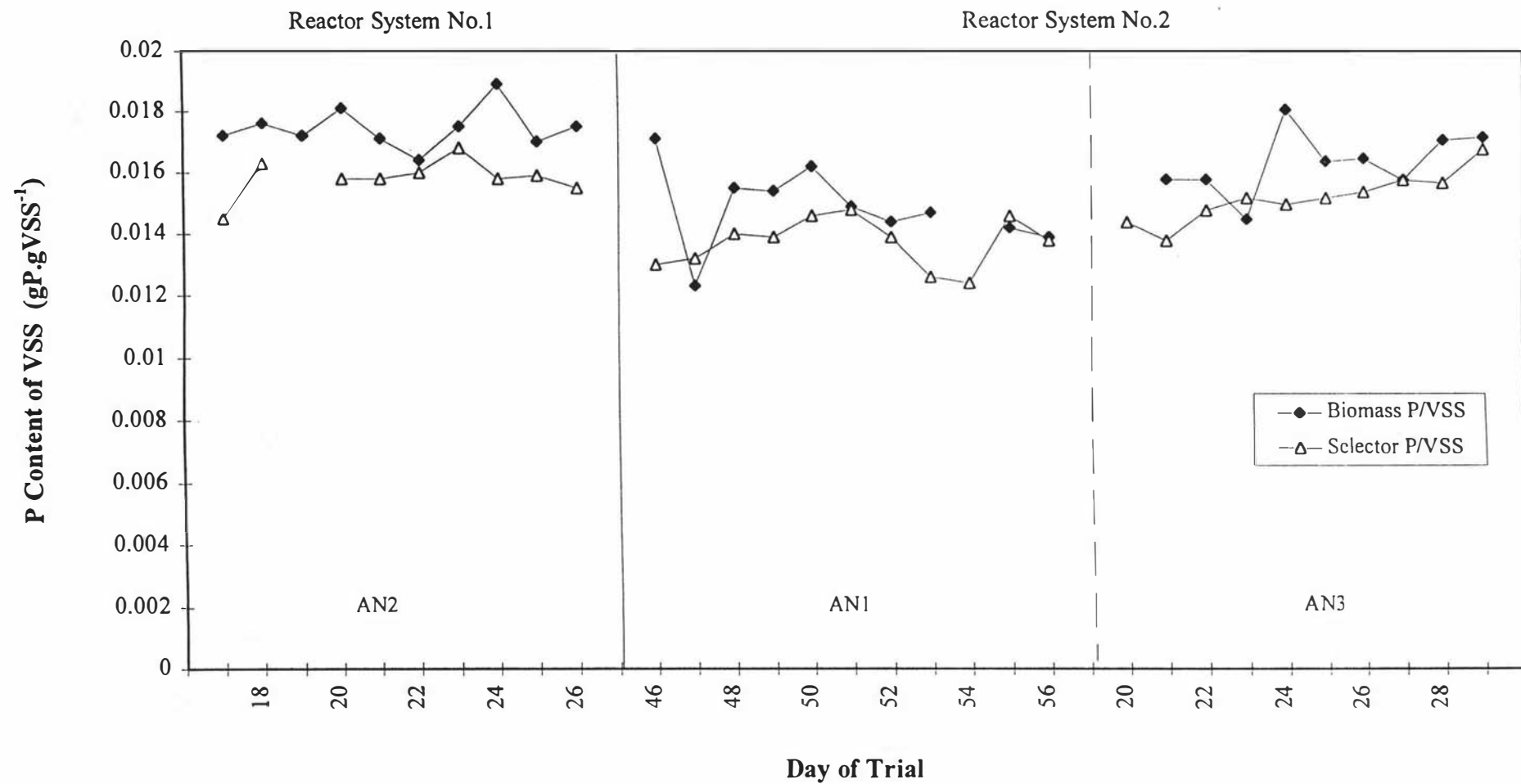
Figure 6.19: Phosphorus content of biomass in the reactor and selector zones during Trials AN1, AN2 and AN3.
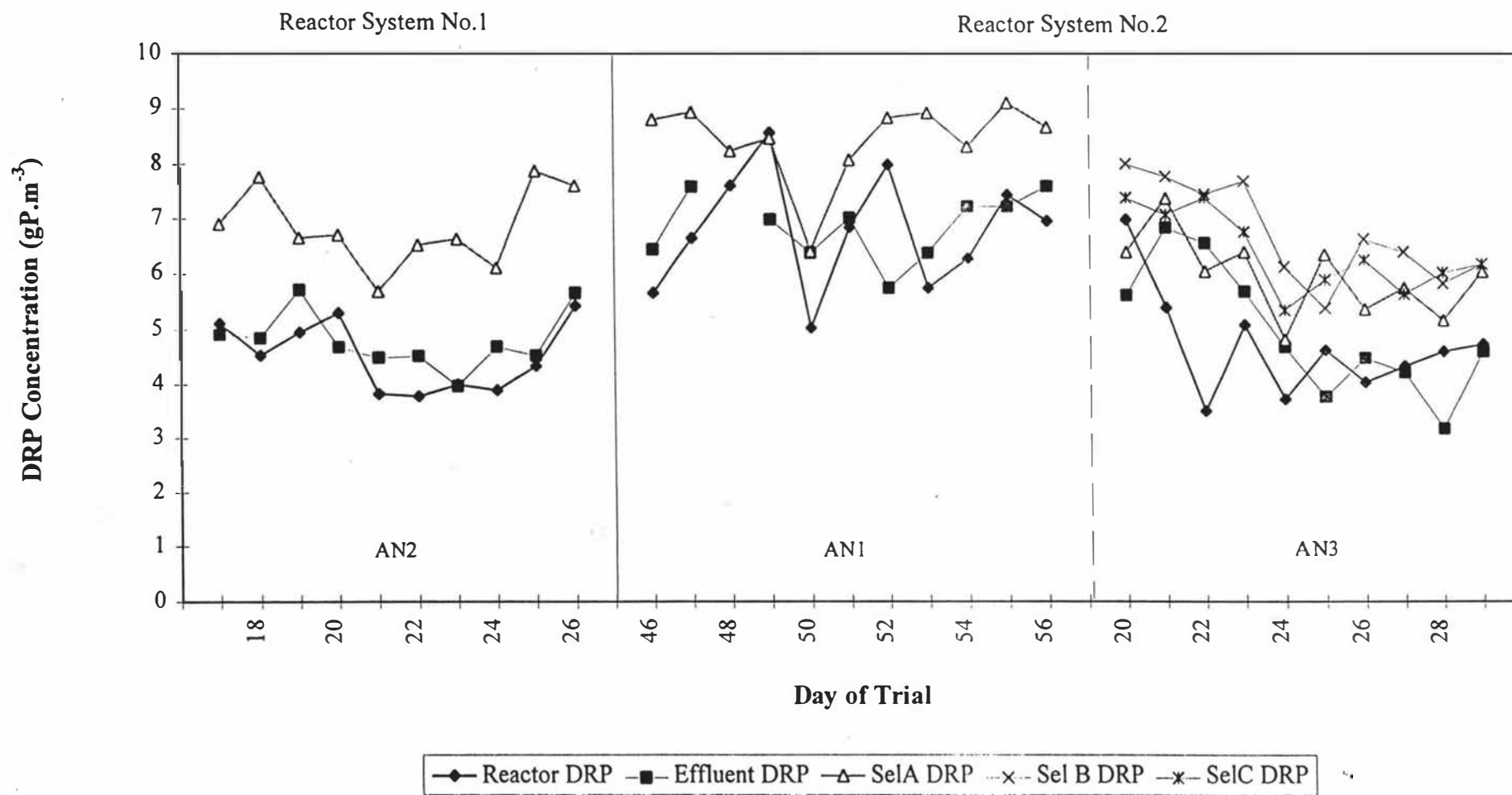
Figure 6.20: DRP concentration in the reactor zones during Trials AN1, AN2 and AN3.

6.6.4 Phosphorus Removal

The effluent DRP concentrations ranged from 3.2 to 7.6 $gP.m^{-3}$ with averages of 6.9, 4.8 and 5.2 in Trials AN1, AN2 and AN3 respectively. Average DRP concentrations measured in the reactor zones were slightly lower at 6.8, 4.7 and 4.8 $gP.m^{-3}$ respectively. As these values were lower than the 7.5 $gP.m^{-3}$ measured during the 10d SRT CSTR trial, it was indicated that P removal mechanisms may have been active under the unaerated selector conditions. Due to the negligible nitrate concentrations indicated, conditions in the selectors could have supported anaerobic activities such as biological P removal via phosphorus accumulating organisms (PAOs).

The variation in total P and DRP levels in the reactor zones as listed in Tables 6.7 to 6.9 also indicated that anaerobic substrate removal may be occurring in the selectors via the activity of PAOs. Although not significantly different at a 95% confidence level, the organic P/VSS ratios measured indicate a possible trend. As seen in Figure 6.19, the average ratio of organic P/ VSS for the mixed liquor was higher in the reactor than it was in the selectors, indicating the possibility of P accumulation by the biomass under aerobic conditions.

The DRP concentrations in the selector zones were higher than that in the reactor, as illustrated in Figure 6.20; which would be consistent with P release during substrate uptake by PAOs under anaerobic conditions. A DRP balance around the first selector could not be performed as substrate DRP was not able to be assessed due to the turbid nature of the feed stream. All reactor system filtrates were free of such turbidity. In most cases, the DRP increased slightly in the settler, which may have been due to release of P in the sludge layer which could be expected to have been anaerobic.

As any phosphorus removed from solution is incorporated into biomass, a P balance should be able to be performed, where:

P_{FEED} = P in the effluent + P in the waste mixed liquor (WML).

The total phosphorus in the substrate was measured as 14 $gP.m^{-3}$. At an influent flowrate of 10 $l.d^{-1}$, the total mass of P entering the system was therefore 0.14 $gP.d^{-1}$. The total mass of P leaving the system in the effluent and in the wasted mixed liquor was calculated for the periods represented in Tables 6.7 to 6.9, the results of which are listed in Table 6.12. A consistent shortfall was observed in the P balance for all trials, ranging between 14% and 28% .

Table 6.12   Phosphorus Removals in the Unaerated Selector Systems

Trial	AN1	AN2	AN3
TP in the influent: P_{FEED} (gP.d^{-1})	0.14	0.14	0.14
TP in WML + effluent biomass (gP.d^{-1})	0.052	0.053	0.054
DRP in WML (gP.d^{-1})	0.006	0.005	0.005
DRP in effluent (gP.d^{-1})	0.063	0.043	0.043
TP in effluent + WML: P_{OUT} (gP.d^{-1})	0.121	0.101	0.102
P_{OUT} / P_{FEED}	0.86	0.72	0.73

6.7   Discussion

The use of various unaerated selector configurations gave slightly increased soluble COD removals than the CSTR trials. The average reactor sCOD concentrations of 57, 60 and 46 g.m^{-3} for trials AN1, AN2 and AN3 respectively were considerably lower than the average 10d SRT CSTR value of 91 g.m^{-3}, increasing the average sCOD removal efficiency to 96.5% - 97.2% from 94.5%. Although the HRT was increased by 6, 12 and 18% respectively in the three selector trials, the batch sCOD removal tests reported in Chapter 4 indicate that all the sCOD would have been easily biodegradable within the CSTR HRT of 24 hours, so the increase in residence time would not have been expected to affect sCOD removal.

The lower effluent sCOD is more likely to have been attributable to a decreased concentration of soluble microbial product derived sCOD. Orhon *et al.* (1993) reported that the sCOD of AS treated dairy wastewaters was due to SMP generated in the reactor, at a level of 3 to 7% of the influent sCOD. Assuming all effluent sCOD was due to SMP, the production levels in the unaerated selector trials would have represented 2.8% to 3.6% of the influent sCOD, in agreement with the lower level of that found by Orhon *et al.* (1993).

Substrate removal rates for the selector reactor biomass differed to that observed for CSTR biomass, with a 'pseudo first order' substrate removal rate observed. The rates

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of COD removal were greater and evidence of substrate accumulation and storage was observed. These effects are commonly reported for sludges exposed to feed / starve situations (Houtmeyers *et al.*, 1980; Verachtert *et al.*, 1980; Chiesa *et al.*, 1985; Chudoba *et al.*, 1991), as would be effected by the use of a selector reactor. A decrease in substrate removal rates and μ_{max} was again generally seen accompanying increase in biomass SVI, in agreement with other reported results (Chudoba *et al.*, 1973b; Van den Eynde *et al.*, 1983; Chudoba *et al.*, 1985; Chiesa *et al.*, 1985).

The rate and extent of proliferation of filamentous microorganisms was lower for the various selector reactor configurations than for the conventional activated sludge configurations reported in Chapter 5. The 10d SRT CSTR trial failed after 45 days at an SVI of 640 ml.g^{-1}, whereas trials AN1 and AN2 only reached SVIs of approximately 250 ml.g^{-1} and 150 ml.g^{-1} respectively for the same operating period.

The SVI remained stable at about 300 ml/g during trial AN3, although a visible decrease in filament abundance was observed during the 34 days of reactor operation and it was expected that if this trial had been continued for a longer period, a decline in the SVI would have been eventually observed. Trial AN3 was abandoned after 3 SRTs as filament abundance was still significant and it was considered that any system that took longer than this time period to demonstrate a decrease in SVI would be of limited use in a full scale system.

The dominant filament observed in the unaerated selector trials differed to that in the CSTR trials. Type 021N was determined to be the primary organism responsible for the high biomass SVI and a secondary filament present was identified as being Type 1701. Therefore the incorporation of a selector zone seemed to prevent the proliferation of Type 0411, which had been the dominant filament in the CSTR trials. There was also no red/orange colour change of the biomass as the SVI increased as was observed in the CSTR trials, indicating that a different filament was responsible for bulking in the unaerated selector trials.

Both filament types have been associated with readily metabolisable substrates (Jenkins *et al.*, 1993), so their dominance in this series of trials was not surprising as the results detailed in Chapter 4 indicate that this substrate could be characterised as such. Type 021N has also been commonly reported in nutrient deficient systems (Richard *et al.*, 1985; Jenkins *et al.*, 1993) and as was proposed in Section 5.6., a N deficiency effect may be induced due to the proteinaceous form of substrate nitrogen in combination with the readily biodegradable carbon source.

Richard *et al.*, (1985) conducted a survey of almost 400 Type 021N bulking incidents and concluded that this organism was associated with a variety of conditions. Type 021N has been associated with low F/M bulking (Richard *et al.*, 1985; Shao and Jenkins, 1989), but also termed a high organic loading organism by others (Chiesa and Irvine, 1985). Its growth is reported to be inhibited by anoxic or anaerobic conditions (Wanner and Grau, 1989), however the use of increased aeration to remove septicity has also been used as a control strategy for this filament (Richard *et al.*, 1985; Jenkins *et al.*, 1993).

Growth of the secondary filament, Type 1701, has been attributed to low DO conditions (Jenkins *et al.*, 1993), which would have been provided in these trials by the unaerated selector zones. The dissolved oxygen in the aerated reactor was maintained in the 3.5 to 5.0 $g.m^{-3}$ DO range, so 'low DO' bulking would not be expected due to conditions in the reactor (Palm *et al.*, 1980).

Both Type 021N (Wanner *et al.*, 1987; Shao and Jenkins, 1989; Jenkins *et al.*, 1993) and Type 1701 (Goronszy *et al.*, 1985; Jenkins *et al.*, 1993) have been successfully controlled by selectors, therefore the unaerated selector configurations trialed in this study must not have been able to provide the required selective pressure. The ability of the various selector configurations to suppress the growth of filamentous bacteria was found to be related to the soluble COD removal efficiency in the selector zone rather than the concentration of sCOD leaving the selector zone or selector floc loading.

The selector sCOD removal efficiency can be measured as fraction of 'removable' sCOD consumed in the selector portion of the system, and it can be seen that the greater the proportion removed in this zone, the slower the increase in SVI. The fraction of removable sCOD consumed in the selector zone varied between 47% to 57% in AN1 and 58% to 59% in AN2. Even though the increased removal in AN2 was only slight, there was a marked improvement in the suppression of filamentous growth as seen in Figure 6.2. The increase in removable sCOD fraction consumed in the selectors to 70% as achieved in Trial AN3, was able to reduce the visible abundance of filaments, but not decrease SVI in a practical time frame. This agrees with the findings of Linne and Chiesa (1987) who reported than selectors needed to remove more than 80% of soluble organic material to be effective in preventing filamentous growth. Richard *et al.* (1985) reported that Type 021N isolates had a high affinity for glucose and lactate, as indicated by K_S values of less than 1 $g.m^{-3}$; suggesting that removals in the selector zone would have needed to be very high.

Significant increases in selector volume did not result in the expected increase in sCOD removal, indicating that selector substrate removal was not limited by reaction time. The batch sCOD removal tests exhibited similar initial substrate removal rates for both aerated and unaerated reactors, as long as oxidised N compounds were available in solution in the unaerated test. Once all the nitrate and nitrite had been consumed in the unaerated reactor, soluble substrate removal from bulk solution ceased, in fact COD measurements indicated that substrate was being released back into the bulk solution when all oxidised N compounds had been removed.

Substrate removals in the unaerated selectors was therefore limited by the amount of nitrate and nitrite present. Conditions in the selectors were originally intended to be anoxic, but due to the rapid removal of available oxidised N compounds, predominant selector conditions would have been anaerobic, if the operationally defined criteria of both DO and oxidised N compound concentrations being less than 0.2 g.m^{-3} (Tetreault *et al.* 1986) is used.

The COD removed from solution via denitrification can be calculated knowing the oxygen reduction equivalent (R_{EQ}) of the oxidised N species to be reduced and the yield co-efficient Y_H (Siegrist and Gujer, 1994; Barker and Dold, 1995):

$$\text{g COD required per g N oxidised} = \frac{R_{EQ}}{1 - Y_H} \qquad (6.4)$$

The reduction equivalents of nitrite and nitrate are 1.71 and 2.86 respectively and the average value of Y_H during the unaerated selector trials was 0.63. Denitrification therefore requires 7.7 g COD per g NO_3-N and 4.6 g COD per g NO_2-N. These values are consistent with the substrate requirement values of 7 to 8 g COD.g NO_3-N^{-1} reported by Argaman and Brenner (1986); Siegrist and Gujer (1994); and Isaacs and Henze (1995).

The value of ($1 - Y_H$) represents the mass of O_2 consumed per mass of substrate removed and using a value of $Y_H = 0.63$ g cell COD. g substrate COD^{-1}, is equal to 0.37. However the batch sCOD removal tests indicated that this value decreased for the selector reactor biomass, as the substrate removed from solution was being stored rather than oxidised for biomass growth. The average value obtained during batch tests was 0.19 g O_2.g sCOD removed^{-1}, resulting in increased values of 15.1 g COD per g NO_3-N and 9.0 g COD per g NO_2-N consumed.

The amount of sCOD expected to be removed in the selectors due to denitrification using both values for $(1-Y_H)$ was calculated and compared to the total sCOD removal, as listed in Table 6.13. The results indicated that the majority of substrate removed in the selectors was by mechanisms other than denitrification of RAS nitrate and nitrite.

The mass of sCOD removed due to the reduction of RAS nitrate and nitrite during trial AN1 was approximately twice that for trials AN2 and AN3, with the proportion of sCOD removal attributable to denitrification of RAS compounds decreasing as the total mass of sCOD removed increased. This was due to the decrease in reactor nitrate and nitrite concentrations through the series of trials conducted.

Table 6.13   Selector soluble COD removal attributable to denitrification.

Trial:	AN1	AN2	AN3
Mass sCOD removed in selectors $(g.d^{-1})$	8.2	9.3	11.2
Mass RAS nitrate removed $(gN.d^{-1})$	0.17	0.09	0.12
Mass RAS nitrite removed $(gN.d^{-1})$	0.13	0.06	0.03
Total mass of sCOD removed due to denitrification $(g.d^{-1})$ $(1-Y_H = 0.37)$	1.9	1.0	1.1
% COD removal in selectors attributable to denitrification $(1-Y_H = 0.37)$	23	11	10
Total mass of sCOD removed due to denitrification $(g.d^{-1})$ $(1-Y_H = 0.19)$	3.7	2.0	2.1
% COD removal in selectors attributable to denitrification $(1-Y_H = 0.19)$	45	21	19

The mass of oxidised N species required per mass of substrate removed under anoxic conditions may have been even less than that estimated from aerobic batch sCOD removal data, as the results in Table 6.4 indicated that an average of only 90.5% and 78.5% of the O_2 consumed in response to sCOD addition was utilised during the period of sCOD removal, in trials AN2 and AN3 respectively. This indicated that not all the O_2 consumed in response to sCOD addition was utilised for substrate removal from bulk solution, O_2 was also being utilised for other reactions, probably the production of storage compounds and other increased metabolic activities occurring in response to the

sorption of available substrate. Assuming that the biomass was able to utilise oxidised N species in the same manner, a greater proportion of the sCOD removed in the selectors may have been removed due to denitrification than estimated in Table 6.13, however the existence of other significant sCOD removal mechanisms would still have been required.

Due to the rapid removal of oxidised N compounds, anaerobic conditions prevailed in the selector zones during all three trials, providing conditions conducive to anaerobic substrate removal mechanisms, such as that exhibited by PAOs. Average pH values recorded in the selectors were 0.7 to 0.8 units lower than in the reactor, and as the incoming feed had a pH of 11, the pH drop in the selector zone indicated the production of acidic compounds as a result of anaerobic activity. The suggestion of PAO activity was supported by P measurements at various points on the reactor system: the possible trend in organic P:VSS ratios and the change in DRP through the selector reactor system are both indicators that P release in the selectors and uptake in the reactor may have been occurring.

The nutrient content of the biomass in each trial was similar to that quoted elsewhere for conventional activated sludge. Biomass N contents averaged between 12.7% and 13.9%, slightly above the value of 12.4% given in Metcalf & Eddy (1991). Normal P contents of biomass have been reported to be 1.5% to 2% (Tetreault et al., 1986; Yeoman, 1988); therefore the averages of 1.4% to 1.7% measured during trials AN1 to AN3 were similar to those being quoted for conventional activated sludge. However, although the P content of the VSS appeared to be still in conventional AS range; it has been reported (Tetreault et al., 1986) that as the influent sBOD:P ratio increases, the P content of sludge decreases due to the limited amount of P available per unit of biomass. As the soluble COD:P ratio was 1650:13 and assuming an average BOD:COD ratio of around 0.5 (Marshall and Harper, 1984), the resultant sBOD:P ratio of 63 would have resulted in a TSS with a P content of less than 2% (Tetreault et al., 1986), no higher than that commonly reported for conventional AS levels.

Induction of PAO activity has been stated by Ubukata and Takii (1994) to occur after at least two anaerobic/aerobic cycles of the biomass, however as the biomass used in the reactors had not been subjected to anaerobic conditions until the commencement of Trials AN1 and AN2, several SRTs would have been required to increase the fraction of PAOs in the mixed culture. The development of an increasing proportion of PAOs can be seen in Figures 6.19 and 6.20, where the reactor DRP concentrations decline from Trial AN1 levels throughout subsequent trial AN3, accompanied by an increasing

P/VSS ratio. The data in Table 6.13 also suggests an increase in the extent of substrate removal by anaerobic means from Trial AN1 through to AN3, as a smaller proportion of sCOD removal could be attributed to anoxic activity as the series of trials progressed.

Total N removals from the unaerated selector reactor systems ranged from 38% in AN1 to 49% in AN3, indicating that significant denitrification was occurring. However, a comparison of the oxidised N species removed in the settler and selector zones to the total N removed from a mass balance indicated that simultaneous nitrification and denitrification must have been occurring in one or more of the reactor system zones during Trials AN2 and AN3. As there was no oxygen supply to the selectors, nitrification in these zones can be assumed to be non-existent, so it is suggested that denitrification must have been occurring in the reactor zone, simultaneously with ammonification and nitrification reactions.

It is generally accepted that denitrification does not occur if dissolved oxygen is present, however Jenkins *et al.* (1993) suggest that the activated sludge flocs may provide an environment in which different types of metabolism can occur concurrently. In this case, even though the bulk liquid DO concentration was maintained between 3.5 - 5 $g.m^{-3}$ and therefore aerobic, conditions inside the floc may have been devoid of DO and therefore anoxic or even anaerobic. Simultaneous nitrification and denitrification has also been reported by other researchers: Suwa *et al.* (1992) reported denitrification in aerated reactors, especially for substrates with a BOD:TKN ratio of higher than 12:1 - a value similar to that provided by the substrate used in these trials. Szpyrkowicz and Zilio Grandi (1995a) also reported simultaneous nitrification and denitrification during periods of low reactor DO which allowed the interior of the flocs to become anoxic.

The suggestion of denitrification in the reactor is also supported by the decreasing mass of nitrate and nitrite flowing out of the reactor in Trials AN2 and AN3. As it was indicated that both ammonification and nitrification were complete in the reactor zone, a similar mass of oxidised N species would have been expected to produced in the reactor during all three trials. The data in Table 6.11 indicates that the extent of denitrification in the reactor zone increased from below 50% in Trial AN2, up to a level of 60% of total denitrification in Trial AN3.

No conclusions can be made on the nutrient removal ability of the filamentous microorganisms that dominated during this set of trials. During AN1 and AN2 both the population of filamentous organisms and of organisms with denitrification and P accumulation abilities increased. However as Trial AN3 progressed, N and P removal

increased whereas the visible abundance of Type 021N declined. This suggests that Type 021N was not the prime microorganism in denitrification and P accumulation activities, however the ability of either filament type to utilise such metabolic pathways can not be inferred from the results obtained.

The unaerated selector set of trials demonstrate that the rate and extent of proliferation of filamentous microorganisms observed in a CSTR configuration could be reduced by the incorporation of a selector zone. However as results indicated that insufficient substrate removal may have been occurring in the selectors under unaerated conditions to completely inhibit the undesirable filamentous growth, it was considered that aerated selectors may provide a solution to the bulking problem.

6.8   Conclusions

The use of unaerated selectors decreased both the rate and extent of proliferation of filamentous microorganisms, however high biomass SVIs still occurred within the timeframe of the trials, with the predominant filament being identified as Type 021N. The ability of a selector configuration to control filamentous bulking was related to the proportion of removable sCOD that was consumed in the selector zone of the reactor system. At least 70% of the sCOD removed in the system needs to be consumed in the unaerated selector zone to suppress filamentous growth.

The selector reactor biomass exhibited higher substrate removal rates than biomass from CSTR studies and OUR data indicated that significant substrate storage was occurring with biomass that was exposed to an intermittent feed regime. Decreasing substrate removal rates and μ_{max} values were again observed with increasing biomass SVI.

Significant overall system N removals of up to 92% of influent values were observed, with between 38% and 49% of system N indicated as being removed via denitrification which was occurring in all zones of the reactor system. However, the mass of nitrate and nitrite present in the selector zone was insufficient to provide the required level of soluble substrate removal via anoxic mechanisms. Denitrification was estimated to only account for approximately 20 to 50% of the sCOD removal occurring in the selectors during the three trials.

Due to negligible nitrate and nitrite concentrations in the unaerated selectors, anaerobic conditions resulted in these zones. Substrate removal by anaerobic mechanisms via phosphate accumulating microorganisms was indicated, with the extent of phosphorus removal from solution increasing as the trials progressed. However, the extent of anaerobic substrate removal was not sufficient to prevent soluble substrate carryover into the reactor zone and subsequent filamentous bacterial growth.

CHAPTER 7

AERATED SELECTOR REACTORS

7.1   Introduction

Activated sludge systems treating dairy processing effluents have been reported to result in proliferation of filamentous bacteria (Eikelboom, 1977; Van den Eynde *et al.*, 1982; Rensink and Donker, 1990), and the results presented in the two previous Chapters illustrated that both conventional and unaerated selector reactor configurations could result in bulking problems. The use of a selector reduced the rate and extent of proliferation of filamentous microorganisms, but could not prevent or cure bulking under the conditions provided.

The conditions reported as resulting in the suppression of filamentous growth are diverse (Albertson, 1991; Jenkins *et al.*, 1993; Chudoba and Pujol, 1994) and at times conflicting, however the provision of a substrate gradient which allows the generally faster growing floc formers to predominate is a commonly proposed strategy, which in a continuous system may be provided by the use of a selector system. The extent of substrate removal in the selector zone is also proposed to be an important factor (Chudoba *et al.*, 1973b; Chudoba *et al.*, 1985a; Linne and Chiesa, 1987; Shao and Jenkins, 1989).

The first series of selector configurations trialed were unaerated in an attempt to impose a defined period of anoxic conditions, however experimental results indicated that the mass of oxidised nitrogen compounds in the return activated sludge was insufficient to remove the required amount of influent substrate, or to maintain anoxic conditions in the selector zone. The next step was therefore to try aerated selector configurations so that selector zone soluble substrate removal would not be limited by the amount of electron acceptor present. As the filament type that had dominated during CSTR trials was inhibited by unaerated selector reactor conditions, it seemed likely that those filaments that proliferated under the selector trial conditions could also be inhibited when exposed to the appropriate selective pressures.

The use of unaerated selectors in the previous Chapter resulted in significant nitrogen (N) and phosphorus (P) removals from the wastewater. The use of a totally aerated selector system was therefore not expected to result in noticeable nutrient removal due to the reliance of N and P removal on the existence of defined anoxic and anaerobic zones respectively.

7.2   Selector Design

The same experimental equipment and operating conditions were used as for the unaerated selector trials. The first aerated selector trial was initiated by introducing aeration to the selectors at the conclusion of Trial AN3. Aeration rate was not measured, but was supplied at a rate that was sufficient to provide vigorous agitation and intended to provide aerobic conditions in bulk solution.

After it became apparent that the serial aerated selector configuration was able to remove nearly all of the 'removable' sCOD(rsCOD) in the selector zone, various combinations of selector sizes and configurations were trialed in an attempt to establish the critical parameters for the prevention of filamentous bulking.

The configurations that were used are:

Trial AE1: 3 x 0.6 l aerated selectors
Trial AE2: 1 x 1.2 l aerated selectors
Trial AE3: 2 x 0.6 l aerated selectors
Trial AE4: 1 x 0.6 l aerated selectors
Trial AE5: 3 x 0.6 l aerated selectors

Two identical reactor vessels were used as shown in Figure 7.1, with the trials being conducted sequentially as follows: Trials AE1, AE3 and AE5 in one reactor and Trial AE2 and AE4 in the other reactor. Changing from one trial to the next simply involved changing the selector configuration, with the biomass used being that which resulted from the previous trial in that reactor. The aeration rate to the selectors was not measured or controlled, except to ensure that vigorous bubbling and complete mixing in the selector vessel was maintained.

Trial AE5 employed the same selector configuration as AE1, and was used as a check on the ability of that configuration to consistently cure bulking after Trial AE3 had resulted in filamentous growth.
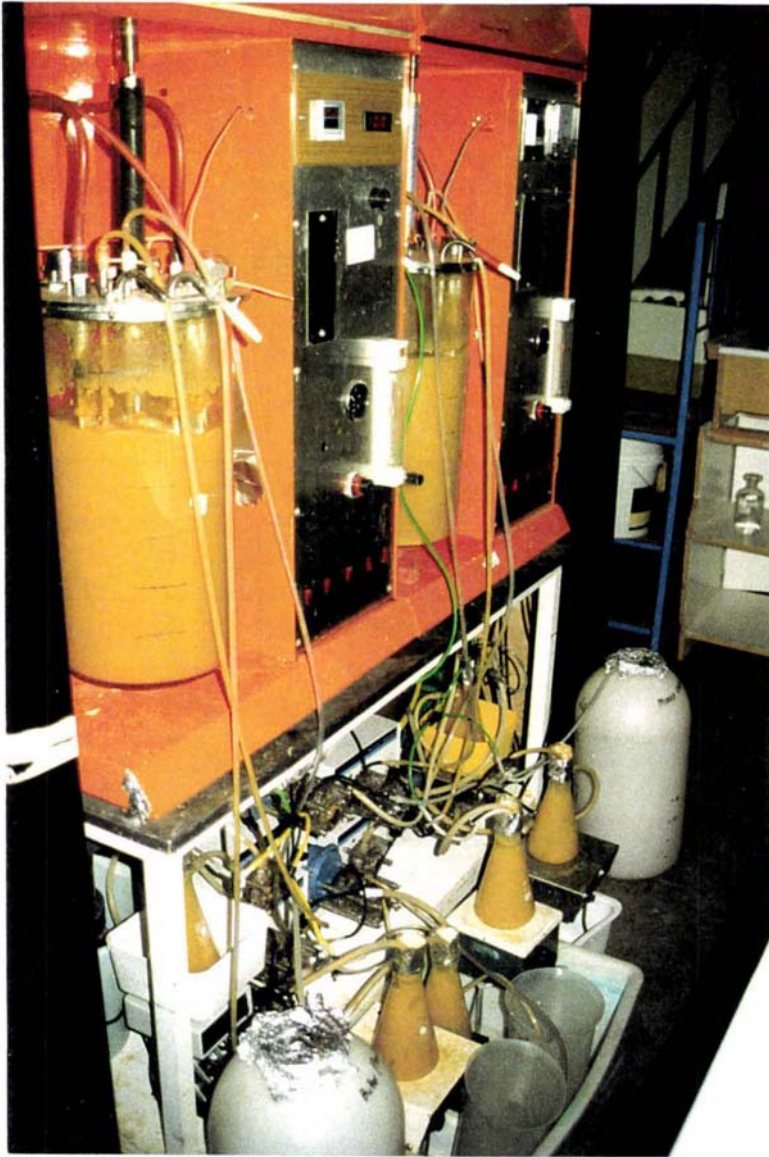
Figure 7.1:   Aerated selector reactor equipment configuration.

7.3   Reactor Treatment Performance

The average reactor performance measured during the five aerated selector trials is listed in Table 7.1. Overall system soluble COD removal efficiencies ranged from 97 to 98%, with average reactor sCOD concentrations ranging between 29 and 46 $g.m^{-3}$.

Table 7.1   Aerobic Selector Reactor Treatment Performance

Trial	AE1	AE2	AE3	AE4	AE5
Selector configuration (l)	3 x 0.6	1 x 1.2	2 x 0.6	1 x 0.6	3 x 0.6
Days at SRT	65	70	46	22	30
Selector outlet conditions:					
pH	7.50	7.50	7.55	7.80	7.65
TSS $(g.m^{-3})$	4290	4020	4830	4870	4980
VSS $(g.m^{-3})$	3860	3670	4340	4420	4450
Total COD $(g.m^{-3})$	5800	5870	6550	6450	5500
Soluble COD $(g.m^{-3})$	47	62	53	57	34
Reactor outlet conditions:					
pH	7.60	7.75	7.75	7.90	7.80
TSS $(g.m^{-3})$	3940	3890	4590	4840	4410
VSS $(g.m^{-3})$	3530	3560	4130	4400	3940
Total COD $(g.m^{-3})$	5450	5270	6050	6700	5850
Soluble COD $(g.m^{-3})$	46	46	37	29	33
sCOD Removal (%)	97.2	97.2	97.8	98.2	98.0
SVI Range	113 -351	136 -441	100 -175	136 -344	200-123
SVI Trend during Trial	decrease	decrease	increase	increase	decrease
Effluent conditions:					
pH	7.90	7.85	7.90	7.80	7.95
TSS $(g.m^{-3})$	27	43	16	17	19
VSS $(g.m^{-3})$	24	41	14	16	18
Total COD $(g.m^{-3})$	86	94	62	51	73
Total COD Removal (%)	96.1	95.7	97.2	97.7	96.7
% effluent COD due to TSS	46.5	51.1	40.3	43.1	54.8

Table 7.2   Selector performance and operation during the aerobic selector trials

Trial	Days at SRT	RAS Flowrate $(10^{-3}m^3d^{-1})$	Total Selector HRT (min)	Floc Load $(\frac{g sCOD}{g VSS})$	S_O into Selector $(gsCODm^{-3})$	Selector sCOD $(g sCOD.m^{-3})$	% rsCOD consumed in selector
AE1	1 - 20	56.2	39	0.071	296	92, 58, 53	100
	21 -31	54.8	40	0.076	297	92, 63, 46	100
	32 -34	32.2	61	0.109	422	84, 51, 39	100
	35 -39	37	55	0.095	380	85, 41, 45	98.3
	40 -42	28.2	68	0.114	457	47, 42, 40	98.6
	43 -44	25.0	74	0.131	499	76, 54, 50	97.4
	45	18.1	92	0.150	613	135, 58, 58	96.9
	46 -65	13.0	113	0.195	749	185, 73, 44	100
AE2	1 - 7	23.8	51	0.154	528	105	90.0
	8 - 14	28.2	45	0.134	472	92	90.9
	15 -18	23.8	51	0.142	532	73	97.7
	19 -35	38.2	45	0.100	385	68	95.8
	36 -58	10.2	86	0.217	835	44	99
	59 -70	14.3	71	0.167	691	37	98.9
AE3	1 - 9	13.0	75	0.178	737	110, 55	97.2
	10 -29	10.2	86	0.197	837	120, 59	97.4
	30 -46	12.6	76	0.176	750	120, 43	99
AE4	1 - 18	14.3	36	0.153	691	59	95.6
	19 -22	44.7	16	0.069	320	41	93.6
AE5	1 - 30	12.6	115	0.186	750	69, 42, 34	99.9

The average sCOD concentration in Trial AE1 appears higher than that in AE3, however as can be seen in Figure 7.2, the sCOD declined during AE1, from an initial
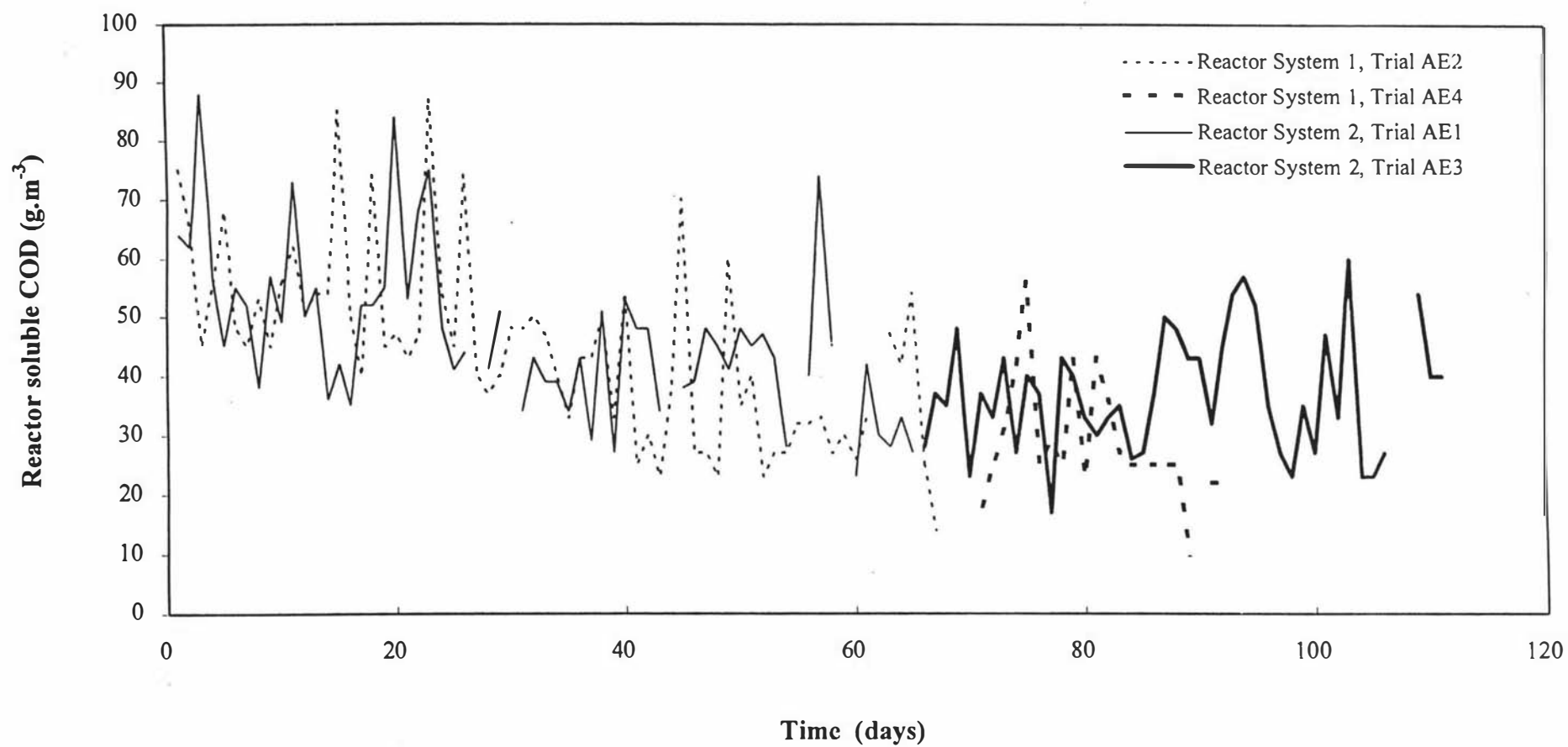
Figure 7.2 Reactor soluble COD concentrations during aerated selector trials AE1 to AE4.

average of 63 g.m^{-3} (days 1-5) to an average of 30 g.m^{-3} at the end of the Trial (days 60 - 65). The average sCOD also declined during Trial AE2 - the first aerated selector trial in the second reactor, from an initial average sCOD of 57 g.m^{-3} to a final average sCOD (days 59 - 70) of 33 g.m^{-3}. The average reactor sCOD values in Trials AE3, AE4 and AE5 remained constant for the duration of the trial. 'Steady state' reactor sCOD values during all five trials ranged from 29 g.m^{-3} to 37 g.m^{-3}, indicating little effect of selector configuration on final sCOD removal efficiency or SMP formation.

The average TSS concentrations in the effluent ranged from 16 to 43 g.m^{-3}, but as for reactor sCOD, the higher averages were associated with the first aerobic selector trial in each reactor. The average effluent TSS concentrations at the end of the trial were 12 and 18 g.m^{-3} for AE1 and AE2 respectively. The effluent total COD values for Trials AE1 and AE2 also appear higher for the same reasons.

As in the previous trials, RAS flowrates were adjusted in response to SVI so as to maintain a minimum level of biomass in the settler. The performance of the selector zone in each trial during varying flow conditions in summarised in Table 7.2. The increase in RAS flowrate is accompanied by a decrease in floc loading and theoretical initial sCOD (S_0) in the selectors. The sCOD concentrations observed during Trial AE5 represent an average value of 6.5 g sCOD. gVSS^{-1}.d^{-1} for substrate removal in the initial selector zone.

The proportion of removable sCOD (rsCOD) consumed is calculated using the average reactor sCOD concentration during the same period. The percentage of removable sCOD consumed in the selector zone was above 90% in all the trials, considerably higher than the 47% to 70% values measured in the unaerated selector trials at similar selector HRTs. The addition of oxygen therefore enabled the removal of almost all rsCOD from solution in the selector zone.

7.4   Filamentous Microorganism Growth

Two of the aerobic selector reactor configurations trialed were able to cure filamentous bulking as evidenced in the decrease in SVI shown in Figures 7.3 and 7.4.

Trial AE1 directly followed Trial AN3 and began to demonstrate a consistent decline in SVI after almost 3 SRTs at the new selector configuration. The SVI took 20 days to decline from an average of 310 ml.g^{-1} to a stable level of below 130 ml.g^{-1}. The trial was continued for a further 2 SRTs and remained constant in the 113 to 136 ml.g^{-1} range.
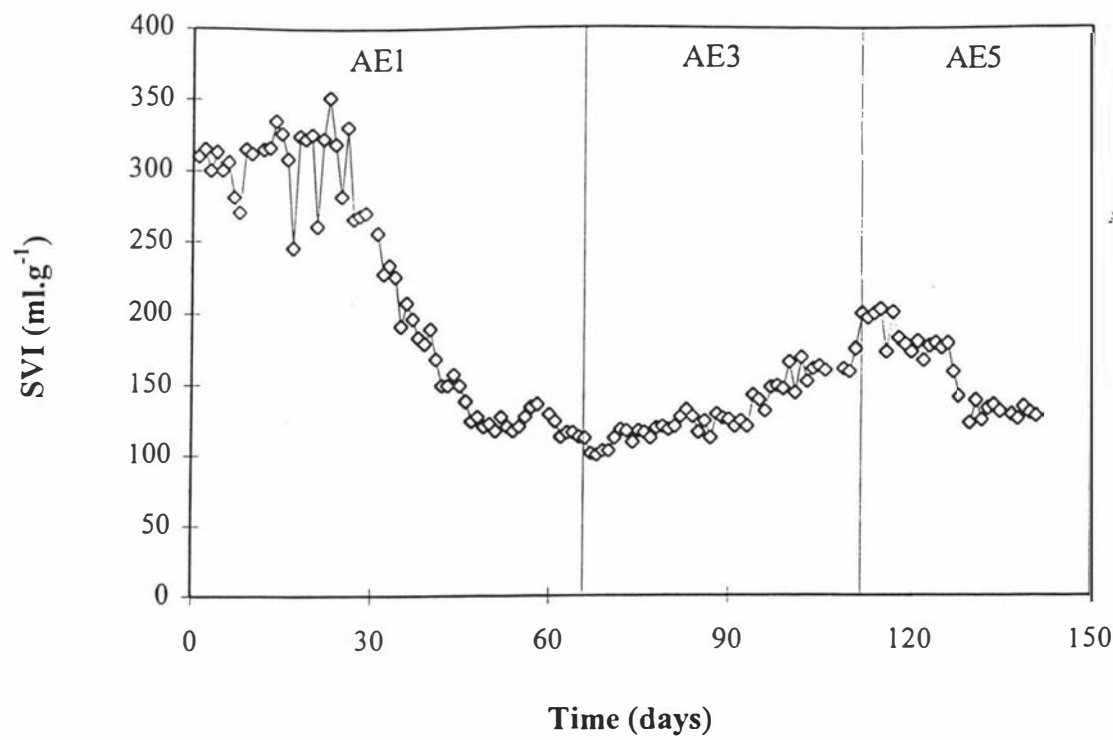
Figure 7.3: Change in Sludge Volume Index during trials in Reactor System 2.
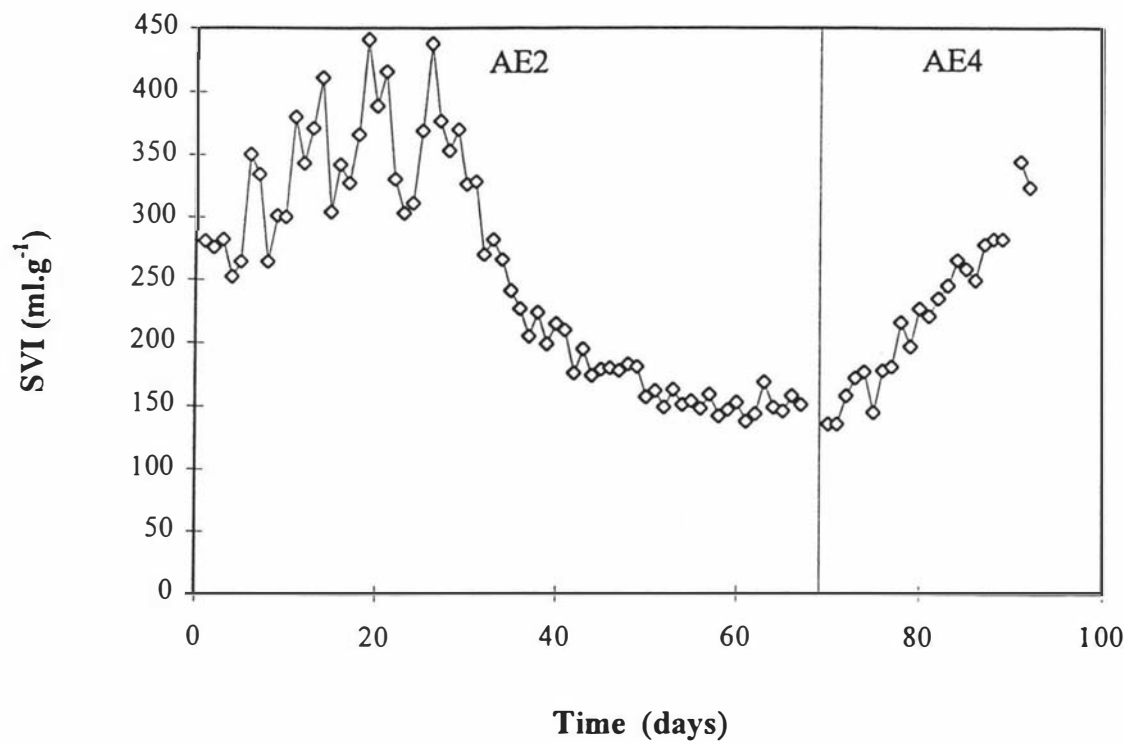
Figure 7.4: Change in Sludge Volume Index during trials in Reactor System 1.

Trial AE2 directly followed Trial AN2 and the initial SVI of around 280 ml.g^{-1} increased to 440 ml.g^{-1} by day 26 of the trial. The SVI then began to decrease rapidly and took 26 days to drop to below 150 ml.g^{-1}, remaining in the 140 - 160 ml.g^{-1} range for a further 2 SRTs.

Although both AE1 and AE2 resulted in a decrease in selector SVI, the serial selector configuration of AE1 resulted in a lower final stable SVI. In both cases the decline in SVI began after nearly 3 SRTs at the new reactor conditions, a time frame commonly considered as that needed to reach a new 'steady state' with activated sludge systems.

The next pair of selector configurations trialed: 2x 0.6l and 1x 0.6l, resulted in the proliferation of filamentous microorganisms and increases in SVI. It was expected that if the 1x 1.2l selector (AE2) could prevent bulking then so would the 2x 0.6l configuration (AE3) as the volume of the selector zone was the same in both cases, however the SVI during Trial AE3 steadily rose from 100 to 175 ml.g^{-1} over a 46 day period. The use of a single 0.6l selector in AE4 resulted in a rapid increase in SVI and the trial was abandoned after 22 days by which time the SVI had increased from 136 to 323 ml.g^{-1}. Figure 7.5 was taken at the end of the trial and clearly demonstrates the open floc structure resulting from filamentous growth.

Trial AE5 used the same selector configuration as AE1 and was implemented to confirm the ability of this configuration to cure bulking. As can be seen from Figure 7.3, the SVI decreased slowly for the first 15 days, then more rapidly to stabilise between 120 and 140 ml/g after less than 2 SRTs from the beginning of the trial. The accompanying reduction in the abundance of filamentous bacteria can be seen from the condition of biomass in Figure 7.6, taken on Day 22 when the SVI had declined to 133 ml/g.

Morphology and staining observations on the biomass indicated that the dominant filament causing bulking in trials AE3 and AE4 differed to those previously observed in either the conventional CSTR or anoxic selector trials.

The dominant filament was observed as being:
- short, fine (0.5 μm diameter), straight filament protruding from floc surface
- cell septa not discernible
- Gram stain -ve
- Neisser stain -ve
- no granules

Using the keys in Jenkins *et al.* (1993) and La Trobe University (1993) this filament was identified as *Haliscomenobacter hydrossis*.

Type 021N was also present, but as a minor secondary filament, both protruding from the flocs and free floating. A few filaments of *Nocardia sp.* were also seen in some trials, during periods when intermittent foaming was observed at the inlet to the settler. Two other very minor filaments were also present, tentatively identified as Types 0041 and 0092.

The activated sludge mixed liquor changed in hue to a pale yellow when an abundance of *H. hydrossis* was present. Well settling sludges with a low SVI were still characterised by a deep gold-yellow colour.

The predominance of *H. hydrossis* has been associated with low DO at low to moderate SRT (Jenkins *et al.*, 1993; La Trobe University, 1993); low F/M (La Trobe University, 1993; Chiesa and Irvine, 1985); soluble, readily metabolizable substrates (Jenkins *et al.*, 1993) and has been proposed to belong to the 'Low DO Aerobic Zone' group of filamentous organisms (Jenkins *et al.*, 1993).

Observations made during morphology and staining procedures also suggested the presence of biological phosphorus removal processes, as a pink colour was observed in the flocs when stained with methylene blue, which indicates the occurrence of polyphosphate accumulation (La Trobe University, 1993).

The photograph in Figure 7.5 illustrates the very fine nature of the *H. hydrossis* filament and the resultant open floc structure. The ability of the serial selector configuration to cure bulking is shown in Figure 7.6 where the disappearance of filaments and restoration of denser floc units is observed. Both photographs were of wet mounts at 100x magnification.
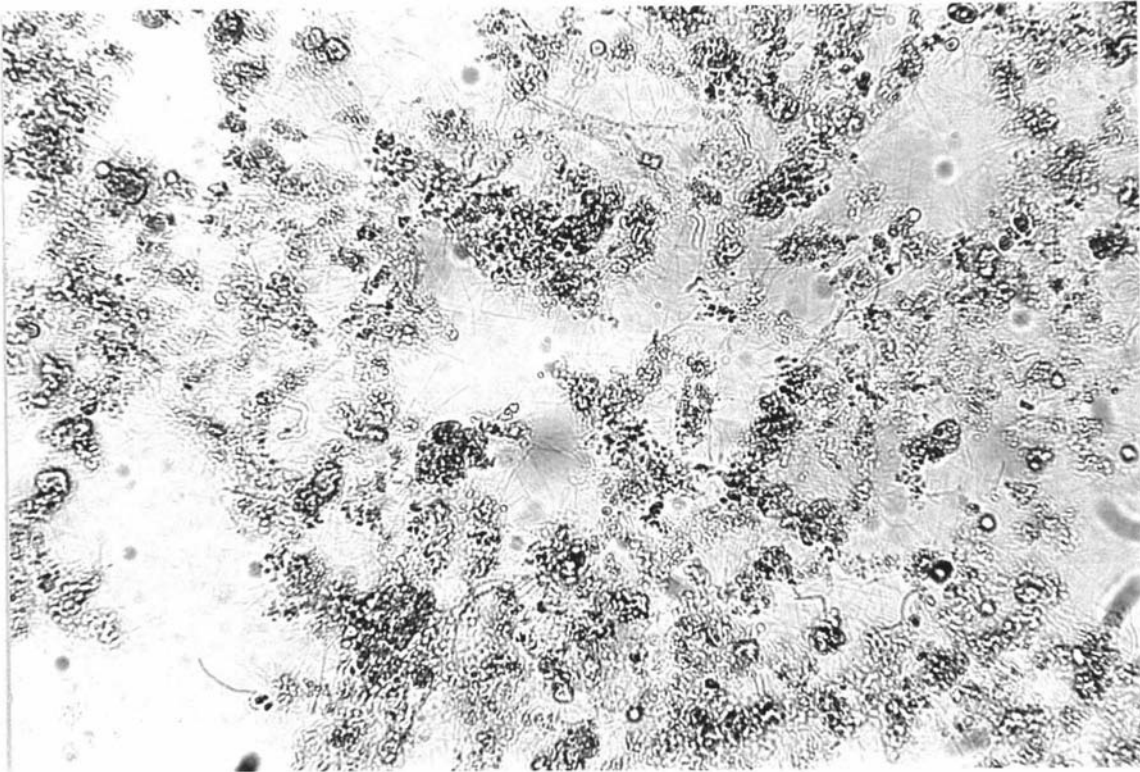
Figure 7.5:   Filamentous bulking at the end of Trial AE4. SVI = 323 ml.g^{-1}.
(100x magnification)
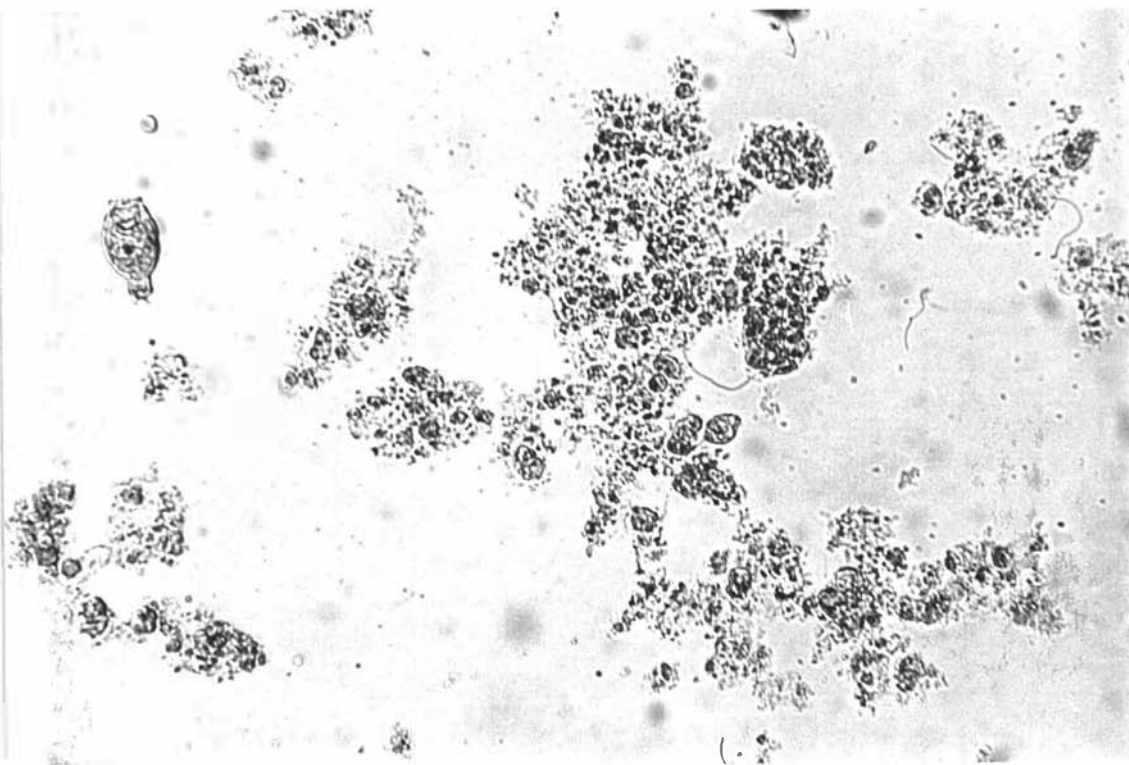
Figure 7.6:   Reduction in filament abundance and SVI during Trial AE5; by Day 22 of
the trial the SVI had decreased to 133 ml.g^{-1}. (100x magnification)

7.5   Measurement of kinetic constants

Substrate removal rates and biomass oxygen uptake rates in response to soluble substrate addition was again measured during the trials using batch tests.

7.5.1   Soluble COD Removal Rates

An increased rate of substrate removal was expected to be obtained in batch tests on biomass from the aerated selector configurations due to the high rates indicated from reactor performance data. The very rapid removal of sCOD can be observed in Figure 7.7 which shows the sCOD profile through the reactor system in Trial AE5. It can be seen that 95% the rsCOD removal from bulk solution occurs within the 38 minute residence time in the first selector.
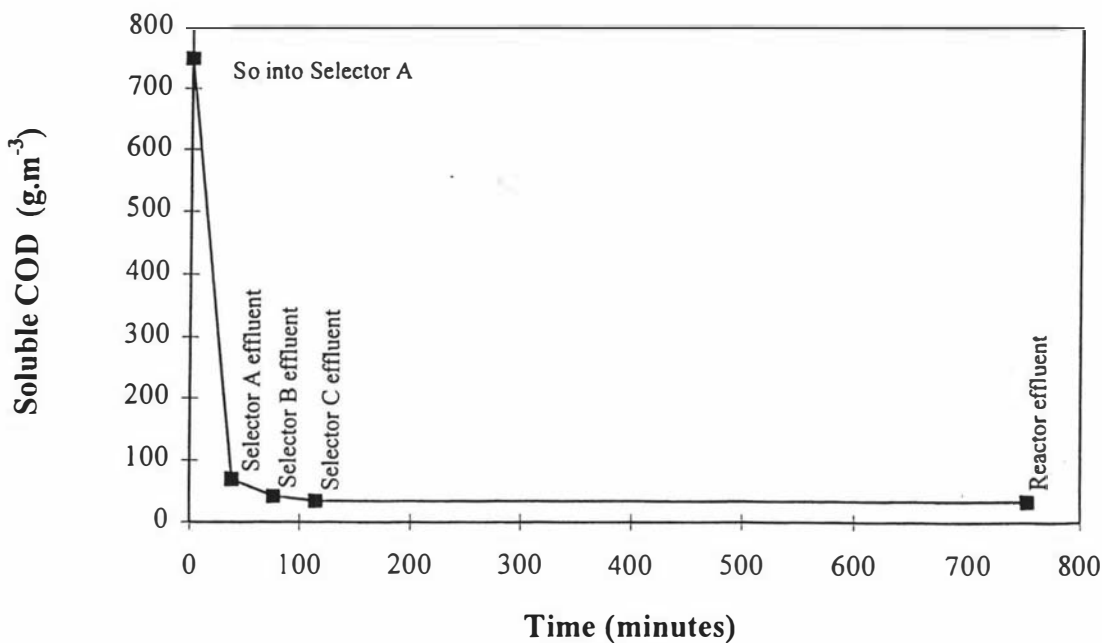
Figure 7.7:   Soluble COD concentration in the reactor system zones during Trial AE5.

Batch sCOD removal rate tests were conducted in the same manner as for the unaerated selector reactor trials. A volume of concentrated GFC filtered substrate containing approximately 500 mg sCOD was added to one litre of biomass that had been aerated

until a constant endogenous respiration rate had been recorded. Initial substrate concentrations ranged from 435 to 650 g $sCOD.m^{-3}$ resulting in floc loadings from 0.10 to 0.17 g $sCOD.gVSS^{-1}$, similar to values obtained in the first selector zone of the reactors.

The same trend of initial biosorption followed by a log decay in sCOD concentration, as seen in tests on biomass from unaerated selector reactor configurations, was observed as shown in Figure 7.8. A summary of the results obtained from batch tests performed, including the apparent first order removal rate constant (k), are listed in Table 7.3.

Table 7.3   Batch Soluble COD Removal Rate Tests

Trial	Days at SRT	SVI $(ml.g^{-1})$	Floc loading ($\frac{g\ sCOD}{gVSS}$)	Biosorption ($\frac{g\ sCOD}{gVSS}$)	k (d^{-1})	$\frac{g\ O_2\ consumed}{g\ sCOD\ added}$	%O_2 consumed when sCOD removed
AE1	28	267	0.13	0.08	41	0.16	70
	48	127	0.13	0.05	68	0.12	44
	61	124	0.17	0.09	92	0.13	70
AE3	17	127	0.13	0.07	105	0.22	62
	45	159	0.12	0.08	130	-	-
AE5	29	130	0.15	0.07	100	0.15	69
AE2	43	195	0.16	0.08	145	0.15	34
	67	151	0.10	0.04	180	0.18	54
AE4	22	323	0.12	0.08	97	0.31	64

Comparing tests performed during the same trial and considering the floc loading in each test, it appeared that there was an increase in the apparent substrate removal rate constant as the trials progressed, indicating that the culture in each case was developing into one with a greater ability for rapid substrate removal. The biosorption capacity of biomass subjected to similar floc loadings seemed to now increase as the SVI and filament abundance increased, indicating that *H. hydrossis* may also have biosorptive capacity.

The mass of oxygen consumed in response to mass of sCOD added ranged from 0.12 to 0.22 $g.g^{-1}$, with the exception of one higher value being recorded during the bulking

conditions. The values were generally lower than those measured during the unaerated selector trials, reflecting more efficient substrate removal and storage mechanisms.
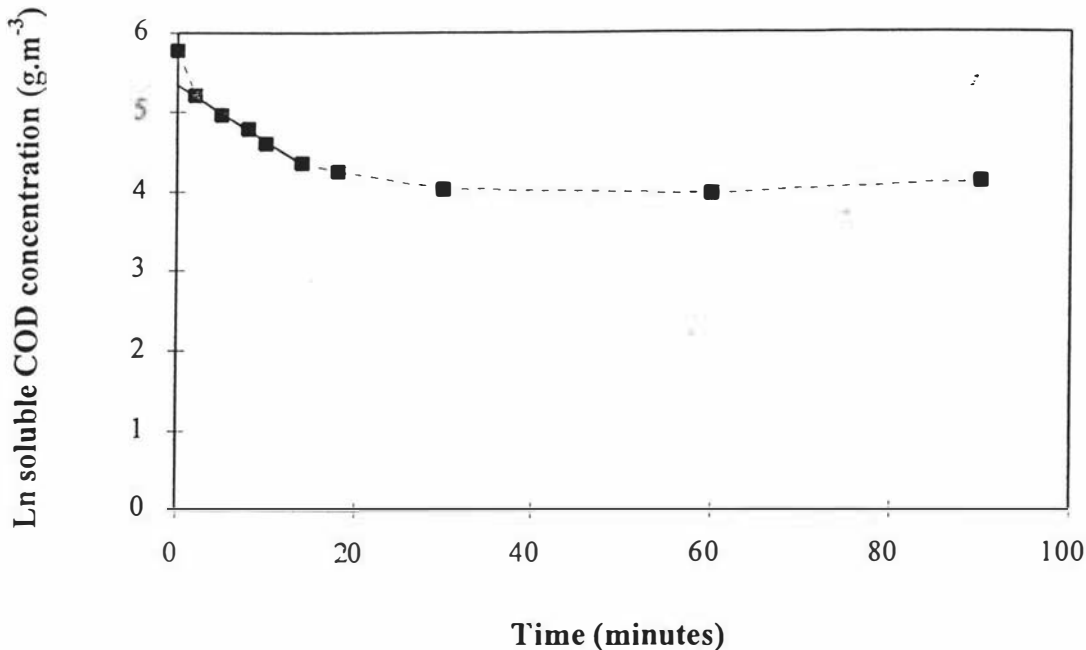
Figure 7.8:   Soluble COD concentration during batch tests using biomass from Day 29 of Trial AE5.

The fraction of the total oxygen consumption that occurred during the period of sCOD removal from bulk solution was also lower in this set of trials, ranging from 0.34 to 0.70 compared to 0.72 to 0.96 in the anoxic selector trials. This indicated that processes involved in substrate removal from bulk solution had increased more rapidly than the rate of substrate storage processes. The increased rate of removal from bulk solution would then have been largely responsible for the dramatic increase in apparent k values observed when changing from unaerated to aerated selectors.

Similar values for oxygen consumption in response to sCOD added can be calculated from actual reactor performance data measured during trial AE5. The selector DO and OUR values were used to calculate the results listed in Table 7.4.

As virtually all the rsCOD is removed from bulk solution in the selector zone, the specific OUR (SpOUR) in the reactor can be taken as approximating the endogenous·OUR. The difference between the selector OUR and reactor OUR therefore approximates the increased oxygen consumption in response to exogenous substrate removal as measured in the batch tests. The average mass of oxygen consumed per mass of sCOD removed

from bulk solution in the selector zone was 0.10 $gO_2.g$ $sCOD^{-1}$, the same value as that obtained in Table 7.3 for O_2 consumed in response to sCOD added, during the period of exogenous substrate removal:

$$0.15 \text{ g } O_2.g \text{ } sCOD^{-1} \times 0.69 = 0.10 \text{ g } O_2 \text{ consumed.g } sCOD \text{ removed}^{-1} \qquad (7.1)$$

Table 7.4   Oxygen consumption during substrate removal in the selector zone during Trial AE5.

Trial AE5:	Reactor	Sel. A	Sel. B	Sel. C	Total Selector
DO $(gO_2.m^{-3})$	6.50	1.35	5.4	4.68	
OUR $(gO_2.m^{-3}min^{-1})$	0.47	1.54	1.17	0.95	
SpOUR $(10^{-3} gO_2.gVSS.min^{-1})$	0.12	0.40	0.25	0.20	
Elevated SpOUR due to sCOD addition $(10^{-3} gO_2.gVSS.min^{-1})$	-	0.28	0.13	0.08	
sCOD removed $(g.d^{-1})$	0.07	14.97	0.71	0.17	15.92
Total O_2 consumed $(g.d^{-1})$	6.49	1.33	0.82	0.75	2.90
Increased g O_2 consumed in response to g.sCOD removed		0.061	0.65	1.19	0.10

7.5.2   Estimation of Biokinetic Parameters

Decay rates were not measured during the aerobic selector trials, as no significant change in biomass decay rate co-efficients were seen between the CSTR and anoxic selector trials.

The biokinetic parameters μ_{max} and K_S were estimated using the low S/X respirometric method as previously defined with ATU added to inhibit nitrification reactions. The values calculated for μ_{max} and K_S using $Y_H = 0.68$ g cell COD. g substrate COD^{-1} are listed in Table 7.5 below. Although both Trials AE1 and AE5 utilised the same reactor configuration, the values measured for both $\Delta SpOUR_{max}$ and K_S were considerably higher during the later trial.

It can be seen from the results in Table 7.4 that the mass of oxygen consumed per mass of sCOD removed increased through the selector system, indicating 'unbalanced growth' and that substrate was being removed via accumulation and storage rather than mineralisation. As the ratio of increased mass of O_2 consumed in response to mass of sCOD removed was so low in the first selector, the presence of substrate removal mechanisms other than purely aerobic mechanisms is demonstrated.

Table 7.5   Kinetic constants determined during aerated selector trials

Trial	Days at SRT	SVI (ml.g^{-1})	ΔSpOUR$_{max}$ (gO$_2$.gcell COD^{-1}.d^{-1})	μ_{max} (d^{-1})	K_S (gCOD.m^{-3})
AE1	28	267	0.22	0.47	11.8
	49	120	0.25 - 0.28	0.53 - 0.58	9.5 - 12.9
	62	113	0.28	0.60	28.4
AE3	45	159	0.78	1.66	26.6
AE5	30	128	0.88	1.87	40.9
AE2	67	151	0.86	1.83	23.2

The values determined for ΔSpOUR$_{max}$ increased as the trials in Reactor 2 progressed, from 0.22 to 0.88 gO$_2$.g cell COD^{-1}.d^{-1}, the final values being considerably higher than the 0.23 to 0.35 gO$_2$.g cell COD^{-1}.d^{-1} values measured during the unaerated selector trials. The values obtained for K_S also increased as the trials progressed. The results during Trial AE1 again indicated that the value of μ_{max} and K_S increased as the SVI decreased, however only single results were obtained during subsequent trials, so this effect could not be investigated further for the aerated selector configurations.

The very rapid OUR observed in response to substrate addition during these trials was obviously a result of substrate accumulation and storage processes rather than assimilation and replication. The increased values of ΔSpOUR$_{max}$ as the trials progressed was then to be expected as the substrate removal rates increased significantly in the same manner. The use of the low S/X respirometric method for estimating traditional 'growth' parameters μ_{max} and K_S is therefore limited, being only a reflection of traditional growth parameters when substrate removal is for balanced growth. However the principle of comparing ΔSpOUR$_{max}$ as a means of determining the response of biomass to substrate addition and comparing mixed cultures was still considered to be useful and valid.

7.5.3   Measurement of Biosorbed Substrate

As the batch sCOD removal rate tests and $\Delta SpOUR_{max}$ determinations indicated that significant substrate biosorption and accumulation was occurring, an attempt was made to assess whether the cellular and substrate components of the flocs could be easily distinguished.

The two indicators of floc biomass content to be investigated were variations in the COD/VSS ratio and in the DNA/TSS ratio.

It can be assumed that the VSS fraction of floc solids would be comprised of microbial cells and substrate components. The COD content of cells, (O_X) has been measured at around 1.42 gCOD.g cells^{-1}. Batch tests outlined in this section were performed using soluble substrate, therefore the COD fraction that would be adsorbed would be the colloidal components (S_H), represented mainly by the protein fraction. Using the data stated in Section 4.1.3, the COD of milk proteins is in the order of 2.5 gCOD.g protein^{-1}, considerably higher than the COD content of cell mass. Therefore it could be expected that if a significant quantity of floc VSS was derived from biosorption of substrate, the COD/VSS ratio would be higher than 1.42; and higher in the selectors than in the reactor. The data calculated from the various zones in the aerated selector trials and the value measured during the conventional 10 day SRT CSTR trial are listed in Table 7.6.

Table 7.6   Mixed liquor suspended solids COD / VSS Ratios

	Reactor	Selector A	Selector B	Selector C
10d SRT	1.48 ± 0.24	-	-	-
AE1	1.51 ± 0.24	1.55 ± 0.20	1.54 ± 0.16	1.53 ± 0.21
AE2	1.48 ± 0.22	1.53 ± 0.29	-	-
AE3	1.46 ± 0.24	1.55 ± 0.25	1.51 ± 0.23	-
AE4	1.52 ± 0.08	1.60 ± 0.19	-	-
AE5	1.58 ± 0.22	1.59 ± 0.23	1.57 ± 0.24	1.56 ± 0.24

Although there are trends in average values calculated, due to the variability in the data values were not significantly different at a 95% CI, so no conclusion about the possibility of the presence of adsorbed substrate onto the floc biomass can be made. The COD/VSS

ratios in the reactor zone during the selector trials were similar to that measured in the conventional CSTR trial at the same SRT.

The second method attempted was to measure the DNA content of mixed liquor solids from various zones in the reactor system. Samples were taken from each selector and the reactor on day 27 of Trial AE5, with a duplicate analysis being carried out on the sample from Selector C. The results obtained are listed in Table 7.7 and implied that the DNA/VSS ratio was higher in the selectors than in the reactor, the opposite of what would have been expected. However, as the two values obtained for Selector C duplicate samples indicated, the analysis was not accurate enough to enable the DNA/VSS ratio to be detected at the level of precision that would be required.

Table 7.7   DNA content of biomass at different locations in the reactor system.

	Reactor	Sel. A	Sel. B	Sel. C (1)	Sel. C (2)
DNA/VSS (g/g)	0.046	0.082	0.077	0.075	0.084

The reactor value of 4.6% compared favourably to the results reported by Speece et al. (1973) of MLSS DNA contents ranging from 1.3 to 5.6%, with increasing DNA content as the F/M ratio increased.

7.6   Nitrogen and Phosphorus Removal

The concentrations of various nitrogen and phosphorus compounds were measured for a period of approximately one SRT towards the end of each trial, except during Trial AE4 where only limited measurements could be obtained before the trial was abandoned due to filamentous growth. The average concentrations measured during each trial are listed in Tables 7.8 to 7.12, indicating that both nitrogen and phosphorus removal was occurring in the aerated selector reactor systems. The TKN value reported is for the unfiltered mixed liquor sample and it can be seen that the biomass N content dominated the result, with variations in TKN reflecting variations in zone MLSS concentration. Ammonia, nitrate and nitrite concentrations in the various reactor zones during the trials are illustrated in Figures 7.9 to 7.14.

During Trial AE1 the levels of nitrate and nitrite were negligible in the first selector, even though significant quantities of nitrate were supplied via the return activated sludge. This

indicated that denitrification was occurring in the first selector even though aeration was being supplied to that zone. The nitrate concentration remained negligible in the second selector, but a measurable nitrate concentration was detected in the third selector, evidence that nitrification was also occurring in the final selector zone.

Table 7.8   N and P concentrations during Trial AE1: 3x0.6 l selector configuration

(Days 49 - 64)	Selector A	Selector B	Selector C	Reactor	Effluent
TKN ($gN.m^{-3}$)	454	485	568	470	3.72
NH_3 ($gN.m^{-3}$)	1.91	1.93	0.74	<0.1	0.17
NO_3 ($gN.m^{-3}$)	< 0.1	< 0.1	0.63	6.91	5.76
NO_2 ($gN.m^{-3}$)	0.02	0.05	0.35	0.17	0.17
TP ($g.m^{-3}$)	95	100	126	86	2.46
DRP ($g.m^{-3}$)	3.43	2.00	1.35	1.76	1.68
Organic N / VSS	0.122 ± 0.016	0.121 ± 0.028	0.121 ± 0.007	0.128 ± 0.026	-
Organic P / VSS	0.025 ± 0.006	0.026 ± 0.007	0.026 ± 0.007	0.023 ± 0.002	-

Nitrite concentrations remained very low throughout all reactor zones. The negligible reactor ammonia concentrations indicated that nitrification was again completed within the reactor residence time. DRP concentrations showed a marked decrease through the selector zones, with the value in the third selector being lower than that in the reactor for some periods in the trial. Low effluent TP values reflected the low TSS concentrations in the settler overflow.

The data from Trial AE2 also indicated that denitrification was occurring in the selector zone, although both the nitrate and nitrite concentrations coming out of the selector zone were higher in Trial AE2 than AE1. Reactor and effluent DRP concentrations were slightly higher than for AE1, indicating a lower P removal efficiency.

As trials AE2 and AE3 both used a total selector volume of 1.2 l, similar results were expected, however filamentous bacterial growth occurred during Trial AE3 whereas Trial AE2 was able to cure filamentous bulking.

Table 7.9   N and P concentrations during Trial AE2: 1x 1.2 l selector configuration.

(Day 48 -59)	Selector	Reactor	Effluent
TKN $(gN.m^{-3})$	457	482	3.5
NH$_3$ $(gN.m^{-3})$	2.86	0.12	0.22
NO$_3$ $(gN.m^{-3})$	2.36	6.80	5.36
NO$_2$ $(gN.m^{-3})$	0.34	0.04	0.11
TP $(g.m^{-3})$	79	81	2.7
DRP $(g.m^{-3})$	2.3	1.9	2.0
Organic N / VSS	0.116 ± 0.029	0.120 ± 0.013	-
Organic P / VSS	0.020 ± 0.004	0.020 ± 0.002	-

Table 7.10   N and P concentrations during trial AE3: 2x 0.6 l selector configuration.

(Days 32 - 41)	Selector A	Selector B	Reactor	Effluent
TKN $(gN.m^{-3})$	492	520	504	6
NH$_3$ $(gN.m^{-3})$	1.36	1.61	< 0.1	< 0.1
NO$_3$ $(gN.m^{-3})$	< 0.1	< 0.1	1.93	1.89
NO$_2$ $(gN.m^{-3})$	0.01	0.06	0.16	0.16
TP $(g.m^{-3})$	93	99	98	1.57
DRP $(g.m^{-3})$	2.8	1.5	1.1	0.7
Organic N / VSS	0.120 ± 0.031	0.117 ± 0.012	0.120 ± 0.020	-
Organic P / VSS	0.022 ± 0.004	0.023 ± 0.002	0.023 ± 0.010	-

The significant differences observed between AE2 and AE3 included:

- a higher selector ammonia concentration in Trial AE2 which suggested a greater extent of ammonification than in AE3.

- negligible concentrations of oxidised N compounds in the selectors during Trial AE3, which maintained anaerobic bulk liquid conditions in the entire selector zone.

- a peak in reactor system nitrite concentrations in the selector zone during Trial AE2, but in the reactor zone during Trial AE3.

- lower DRP concentrations and indications of a higher biomass P content during Trial AE3, suggesting that more substrate was being removed via anaerobic mechanisms than in AE2.

The single 0.6 l selector used in Trial AE4 also failed to prevent bulking, the rate of filament proliferation being so rapid that only six sets of N and P analyses were performed before the Trial was abandoned. Low concentrations of oxidised N compounds were measured in the mixed liquor leaving the selector zone, similar to that recorded in AE1, however the reactor nitrate and nitrite concentrations were also lower than in other trials except for AE3. The low effluent DRP values and low nitrate out of the selector again indicated that substrate was being removed by both anaerobic and anoxic mechanisms as well as aerobically.

Table 7.11   N and P concentrations during Trial AE4: 1x 0.6 l selector configuration.

(Days 16 - 22)	Selector	Reactor	Effluent
TKN $(gN.m^{-3})$	528	515	1.8
NH_3 $(gN.m^{-3})$	1.48	< 0.1	< 0.1
NO_3 $(gN.m^{-3})$	0.64	2.65	2.32
NO_2 $(gN.m^{-3})$	0.50	0.03	0.12
TP $(g.m^{-3})$	98	95	1.24
DRP $(g.m^{-3})$	1.43	0.74	0.73
Organic N / VSS	0.12 ± 0.03	0.12 ± 0.05	-
Organic P / VSS	0.021 ± 0.007	0.021 ± 0.007	-

Trials AE1 and AE5 both used the same selector configuration, so were expected to exhibit the same trends in nutrient reactions and concentrations throughout the reaction zones. Both trials resulted in a fully nitrified effluent and a peak ammonia concentration in Selector B. System nitrate concentrations were highest in the reactor during both AE1 and AE5, with denitrification obviously occurring in the first selector and to a lesser extent, in the settler. During Trial AE1 the nitrate concentration was negligible in Selectors A and B, then increased to 0.6 $g.m^{-3}$ in Selector C, and although the nitrate concentrations also increased throughout the selector system in Trial AE5, they were

maintained at a significantly higher level. Nitrite concentrations were very low but increasing sequentially through the selector system in Trial AE1, slightly higher and decreasing through the selectors in Trial AE5.

Table 7.12   N and P concentrations during Trial AE5: 3x 0.6 l selector configuration.

(Days 22 - 30)	Selector A	Selector B	Selector C	Reactor	Effluent
TKN (gN.m^{-3})	473	449	509	460	4.53
NH$_3$ (gN.m^{-3})	1.07	1.73	1.59	< 0.1	< 0.1
NO$_3$ (gN.m^{-3})	2.80	3.35	3.8	7.85	7.03
NO$_2$ (gN.m^{-3})	0.70	0.54	0.53	0.04	0.09
TP (g.m^{-3})	97	93	105	93	0.6
DRP (g.m^{-3})	1.27	0.59	0.52	0.28	0.38
Organic N / VSS	0.118 ± 0.034	0.113 ± 0.011	0.116 ± 0.019	0.115 ± 0.014	-
Organic P / VSS	0.024 ± 0.002	0.024 ± 0.003	0.024 ± 0.003	0.024 ± 0.004	-

Even though the flowrate through the system remained constant during the period of N species determinations for both trials, different trends were observed in both cases. During Trial AE1 ammonia concentrations decreased in the selectors, while in AE5 they increased, reflecting changes in selector sCOD concentrations during the same period. Both nitrate and nitrite concentrations decreased in the reactor during Trial AE1 and in all zones during AE5, possibly reflecting decreasing nitrification and increasing denitrification respectively. During the period of increased denitrification in Trial AE5, selector pH also decreased, which would be consistent with increased anaerobic activity as well. Any effects due to variations in the selector DO levels could not be checked as these values were not regularly monitored until Trial AE5, where the Selector A DO concentrations were found to be highly variable, ranging from 0.2 to 3.0 g.m^{-3}.
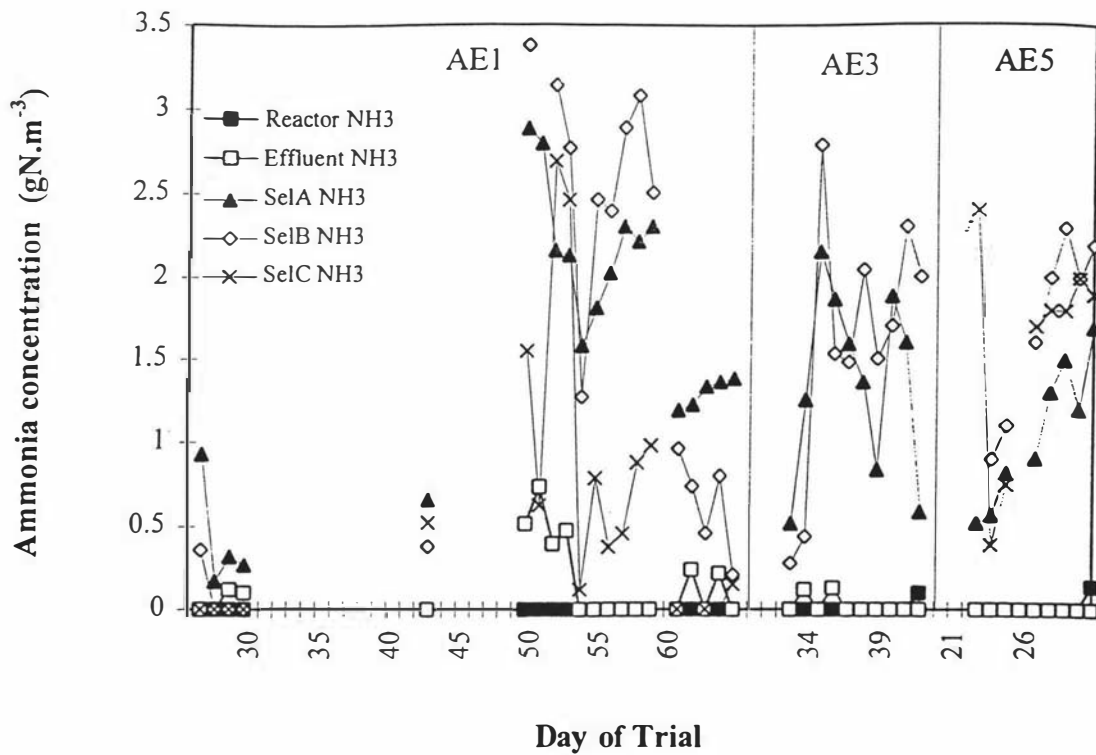
Figure 7.9: Ammonia concentration trends during periods of Trials AE1, AE3 and AE5.
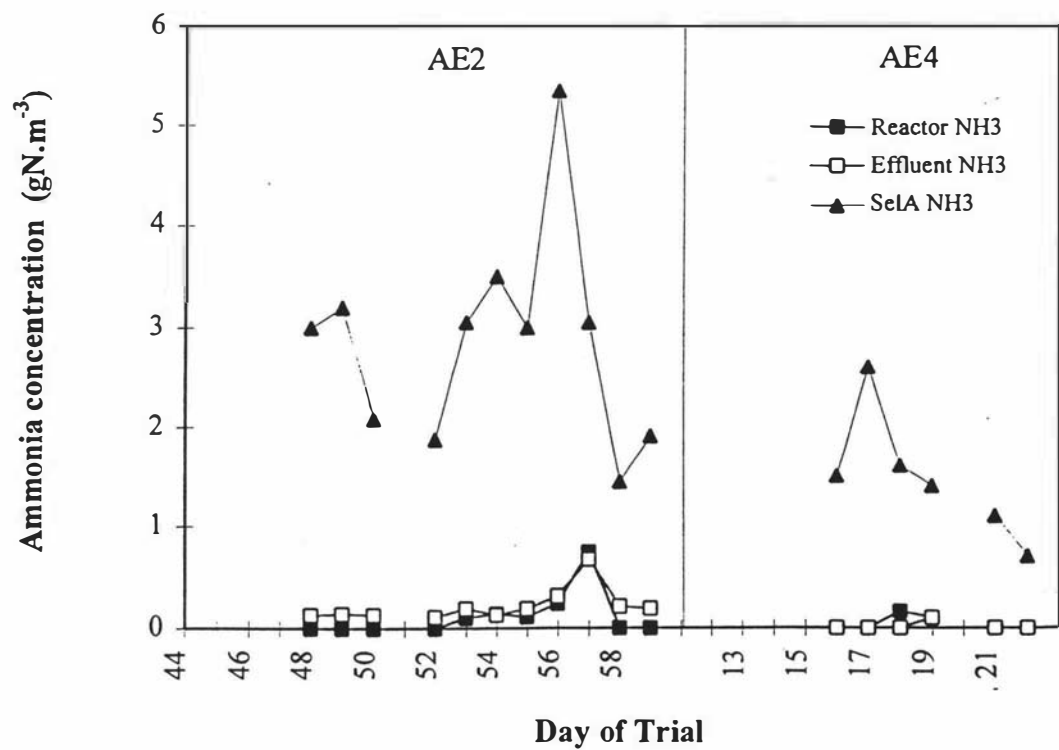
Figure 7.10: Ammonia concentration trends during periods of Trials AE2 and AE4.
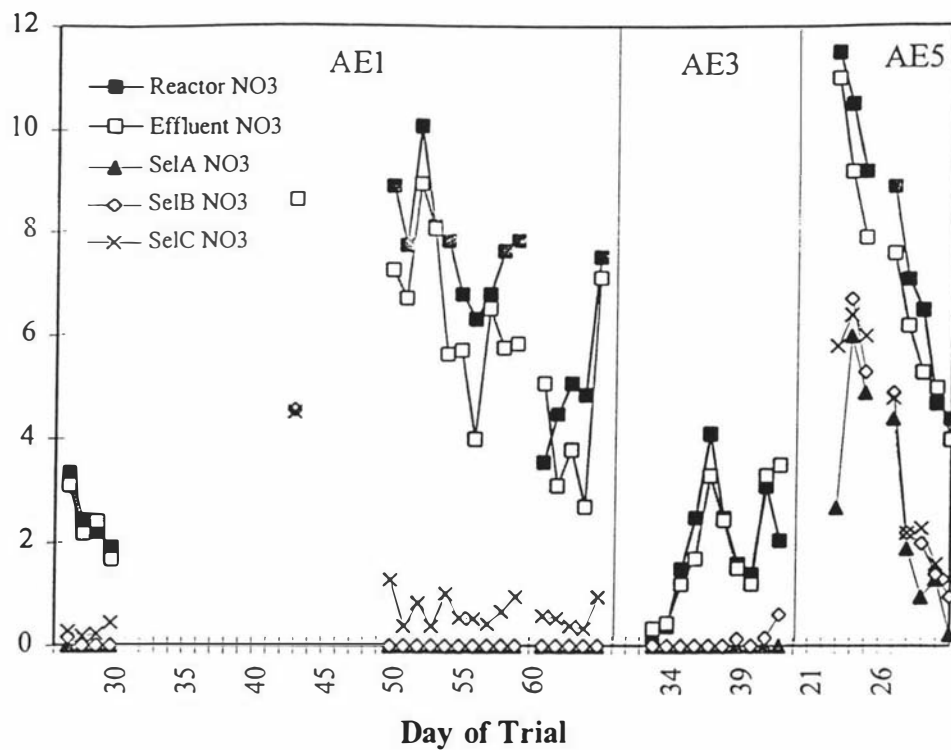
Figure 7.11:   Nitrate concentration trends during periods of Trials AE1, AE3 and AE5.
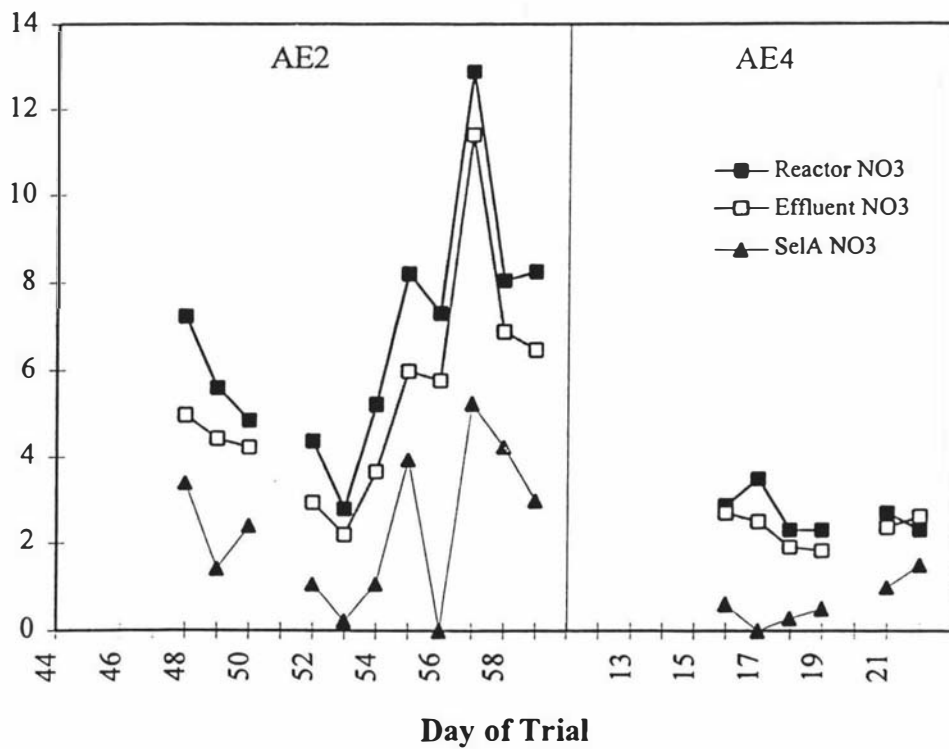
Figure 7.12:   Nitrate concentration trends during periods of Trials AE2 and AE4.
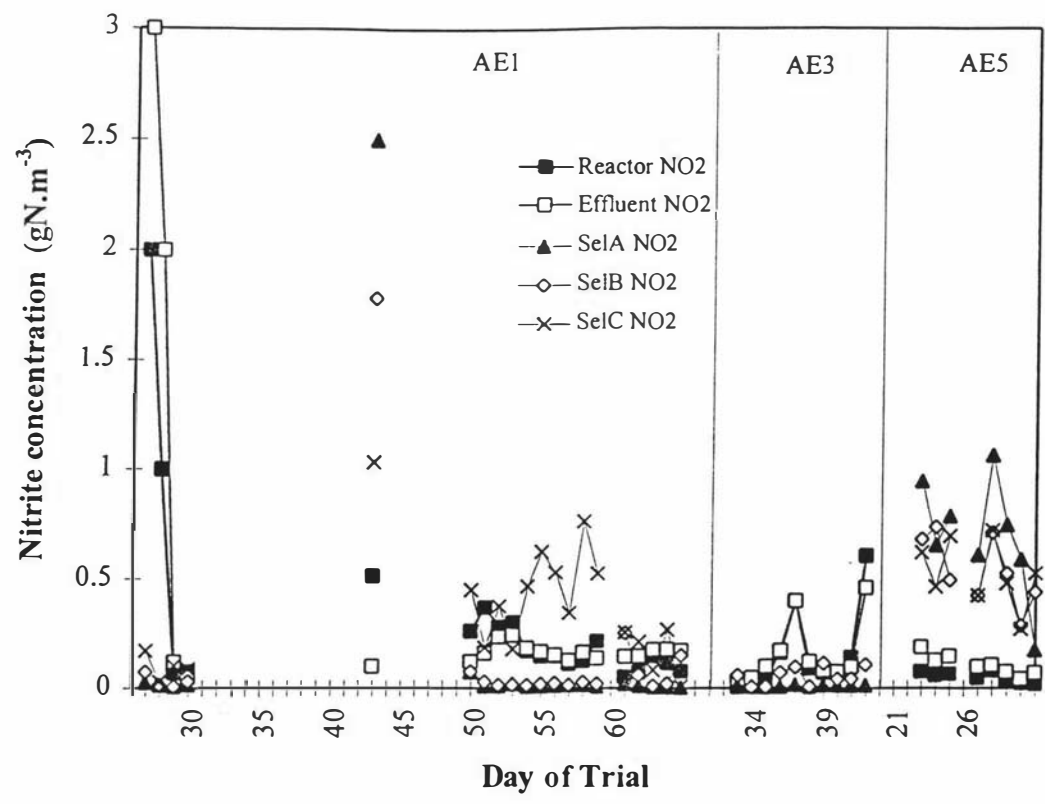
Figure 7.13: Nitrite concentration trends during periods of Trials AE1, AE3 and AE5.
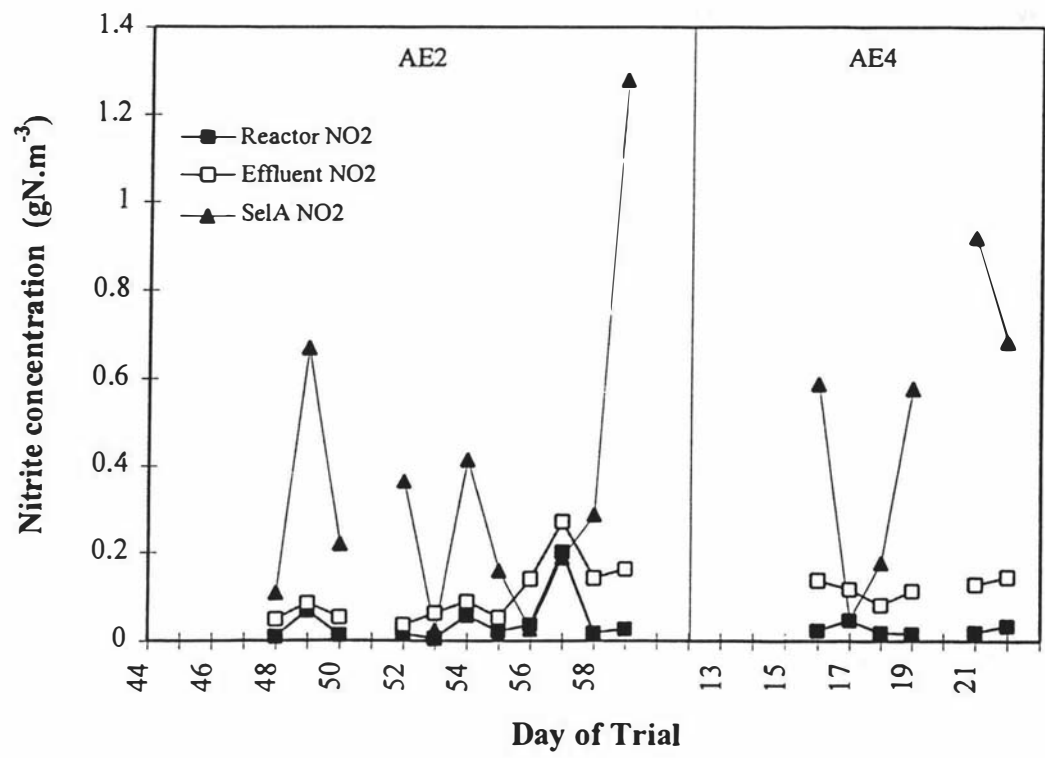
Figure 7.14: Nitrite concentration trends during periods of Trials AE2 and AE4.
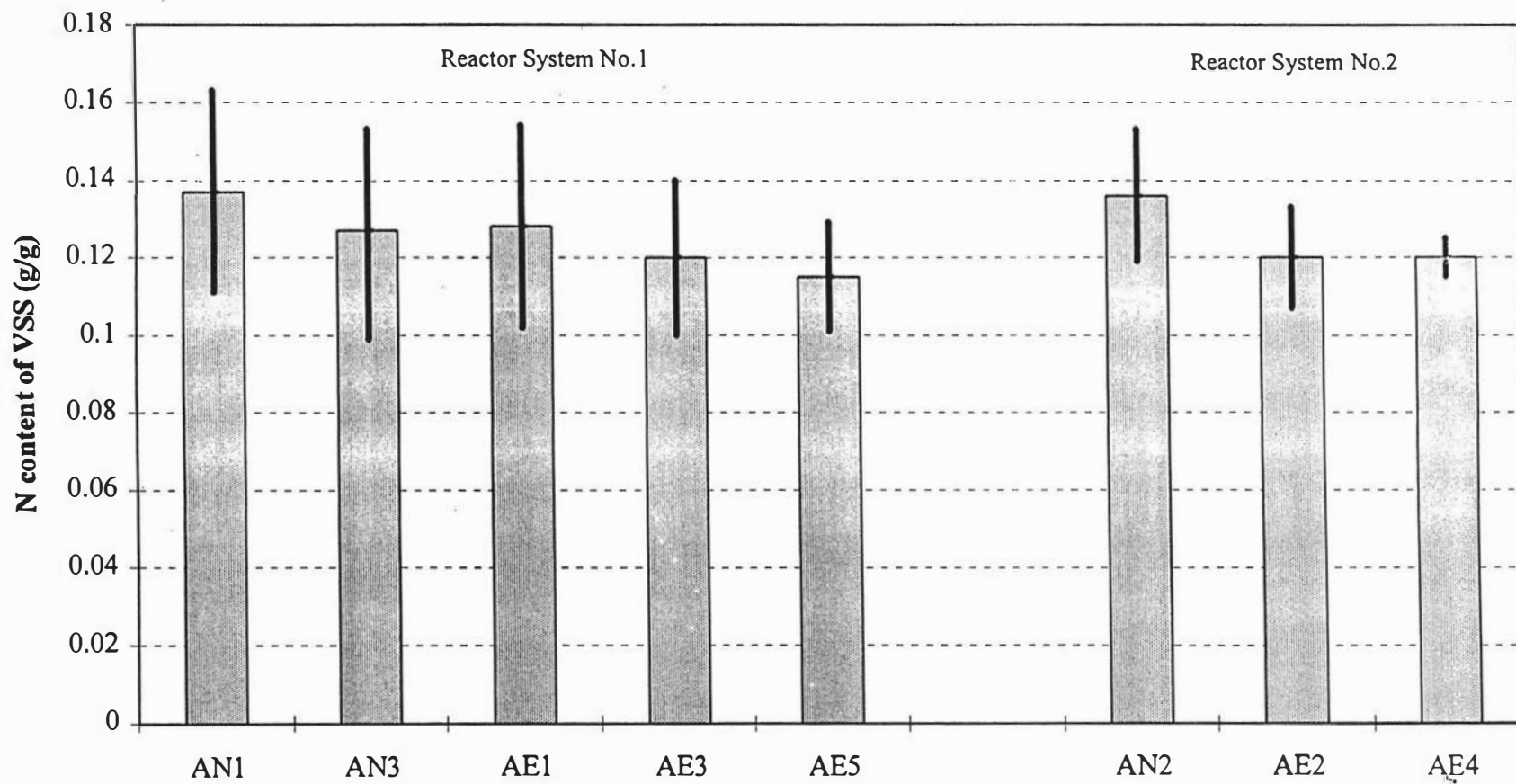
Figure 7.15   Nitrogen content of VSS during selector trials

The average N content of biomass declined as the selector trials progressed, as shown in Figure 7.15, to a consistent average value of 12.0 to 12.2 % which is slightly below the empirical value for the N content of biomass at 12.4% (Metcalf & Eddy, 1991) but still higher than average values reported for AS biomass of 9.7% (Suwa *et al.*, 1992) to 9.8% (McClintock *et al.*, 1993).

7.6.1   Ammonification

As the rate of ammonification has been found to be faster than the rate of ammonia oxidation (Wong-Chong and Loehr, 1975), ammonification can be considered to be completed in the reactor as no ammonia was detectable in that zone.   Ammonia concentrations in the RAS and feed were negligible, but as seen in Figures 7.9, 7.10 and 7.16, were detectable in the selectors, indicating that ammonification was occurring. As in the unaerated selector trials, the ammonia concentration in the first selector was proportional to the sCOD concentration and hence the substrate organic N concentration, as well as the selector residence time.   In the triple serial selector trials, the ammonia concentration was highest in the second selector, the decreasing ammonia concentration being accompanied by an increasing concentration of oxidised N compounds, which indicated that nitrification was also occurring in this third selector zone.

As discussed in the previous chapter, it was not possible to measure the ammonification rate directly and in this set of trials it was indicated that both ammonification and nitrification were occurring in the same zones. However an estimate can be obtained using the data from trials AE1 and AE3 which had negligible oxidised N levels in the first selector, if it assumed that nitrification was also negligible in this zone. By a similar analysis to that used in Section 6.6.1, the ammonification rate constant ranged from 0.005 to 0.028 min^{-1} during Trial AE1 and 0.005 to 0.020 min^{-1} during Trial AE3. These estimated values are lower than the 0.024 to 0.048 min^{-1} range estimates obtained during Trial AN1, which may be due to a lower actual ammonification rate or to the assumption of negligible nitrification not being valid.
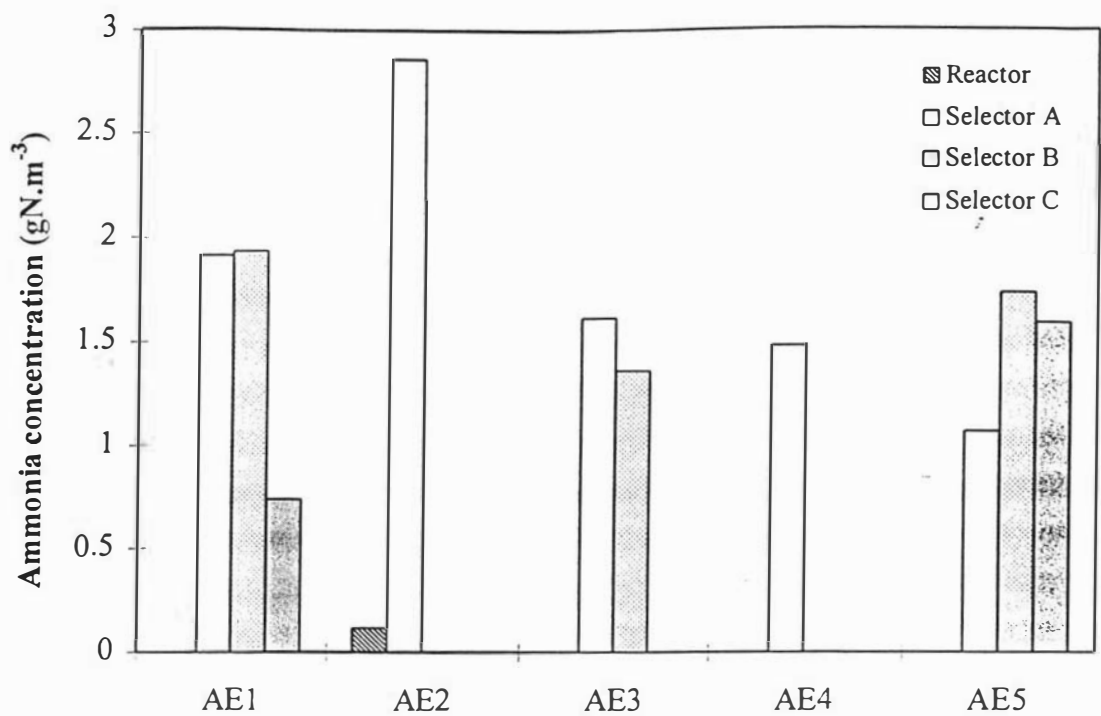
Figure 7.16:   Average ammonia concentrations in the reactor zones during the aerated selector trials.
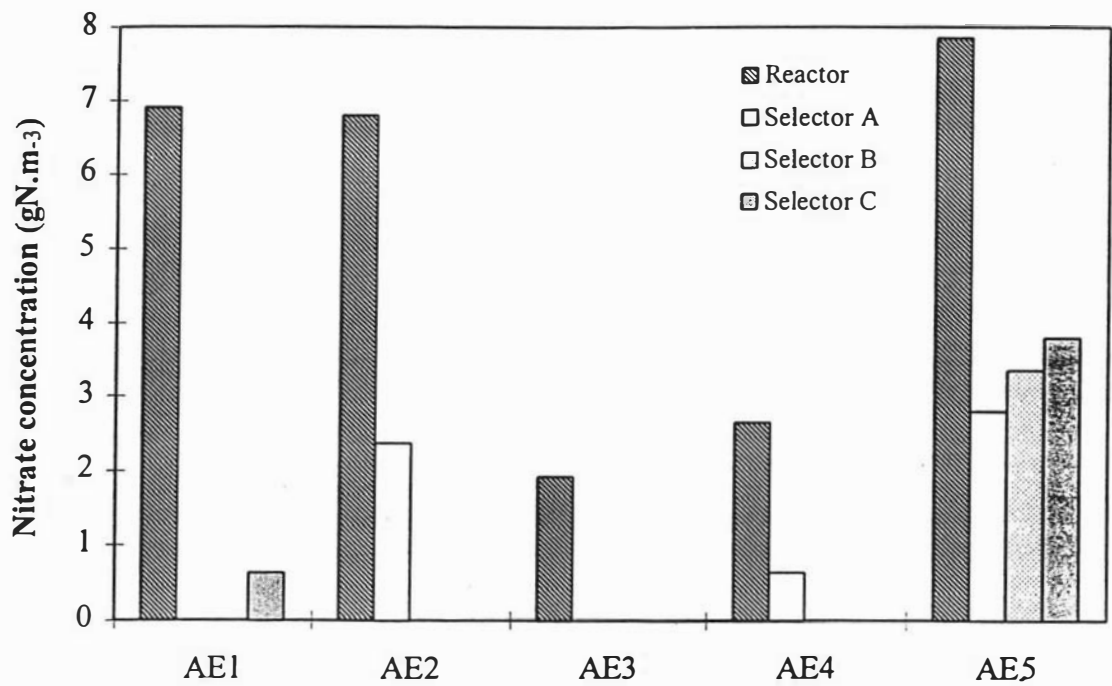
Figure 7.17:   Average nitrate concentrations in the reactor zones during the aerated selector trials.

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7.6.2   Nitrification

Nitrification was occurring in the reactor zone during all trials and in the selector zone in some trials as evidenced by an increasing concentration of nitrate or nitrite after the first selector zone. As illustrated in Figures 7.11, 7.12 and 7.17, the concentration of oxidised N compounds remained undetectable in the first two selector zones in Trials AE1 and AE3, but both nitrate and nitrite were present in the selector during trials AE2, AE4 and AE5. It is generally accepted that the DO must be at a level of greater than 1 $g.m^{-3}$ for nitrification to proceed (Metcalf & Eddy, 1991), therefore nitrification would not be expected to have occurred in the first two zones of the selector system as the rapid substrate removal processes resulted in a lack of oxygen in bulk solution. However the reduction in estimated ammonification rates determined in the preceding section indicate that some nitrification may have been occurring. Significant nitrite concentrations were measured in the reactor during the unaerated selector trials but were negligible during this set of trials, as seen in Figures 7.13 and 7.14.

Table 7.13   Nitrification rates estimated from reactor NO_2 and NO_3 concentrations

Trial:	AE1	AE2	AE3	AE4	AE5
Average ($gN.gVSS^{-1}.d^{-1}$)	0.004	0.002	0.001	0.002	0.002
Minimum ($gN.gVSS^{-1}.d^{-1}$)	0.002	0.001	0.001	0.0002	0.002
Maximum ($gN.gVSS^{-1}.d^{-1}$)	0.006	0.004	0.002	0.004	0.003

An estimate of the minimum specific nitrification rate can be made by calculating the increase in oxidised N compound concentrations across the reactor, the values determined during each trial being listed in Table 7.13. These values are approximately one tenth of those estimated in the unaerated selector trials, suggesting that all available ammonia is easily oxidised within the reactor residence time available, although it is suspected that these values are artificially low due to denitrification also taking place in the reactor zone.

7.6.3   Denitrification

The concentrations of nitrate and nitrate were either low or negligible in the first selector zone during each trial, indicating that denitrification was occurring in this zone. As effluent nitrate concentrations were lower than reactor nitrate concentrations, it was indicated that denitrification was also occurring in the settler. The average mass of N
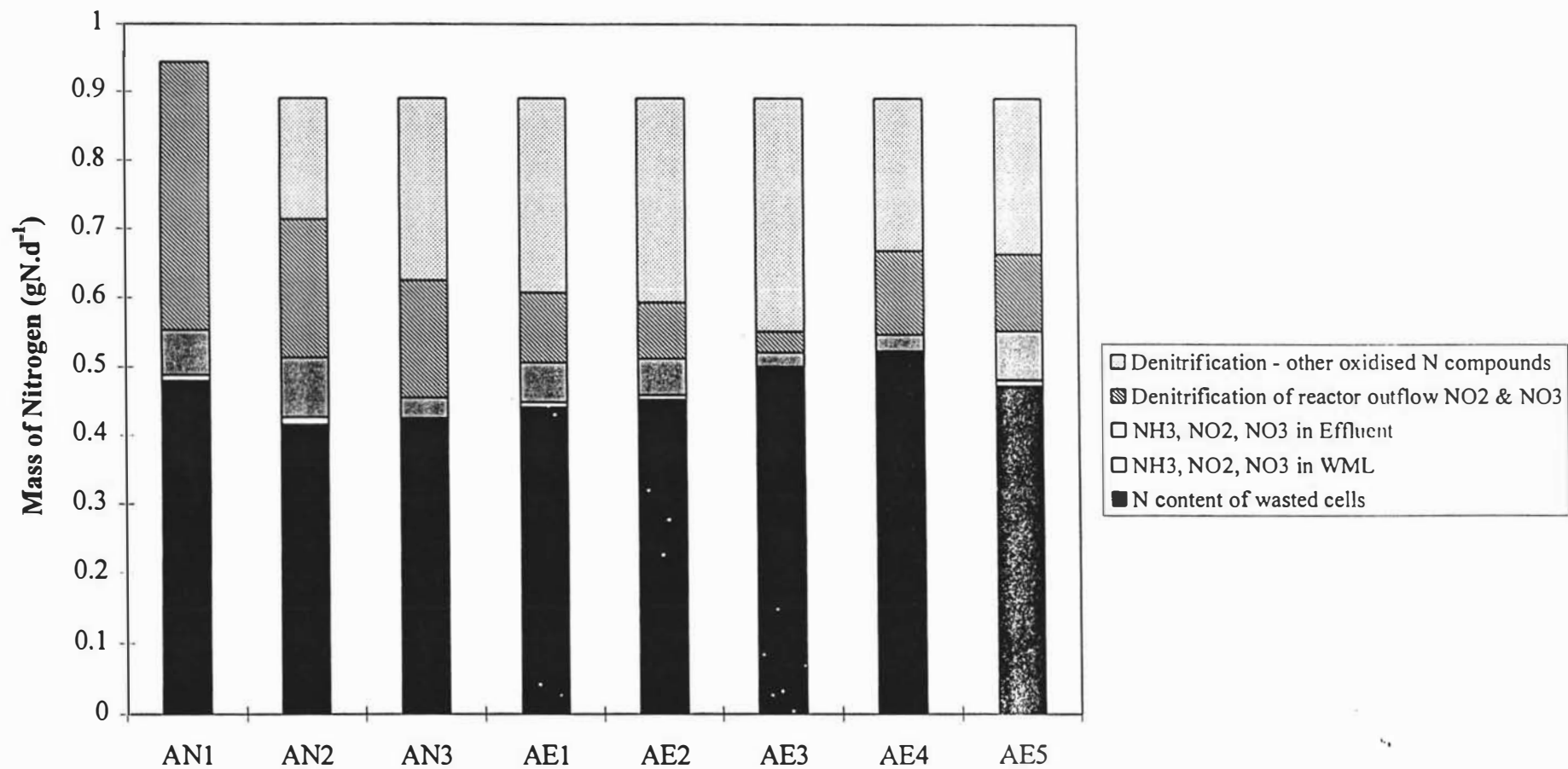
Figure 7.18: Nitrogen balance during the various unaerated and aerated selector reactor trials

removed by denitrification during each trial was calculated from a mass balance as in Section 6.6.3, the results being listed in Table 7.14 and illustrated in Figure 7.18. The mass of nitrate and nitrate flowing out of the reactor that is removed by denitrification in the settler and selector zones is also calculated and compared to the estimated N removal by denitrification calculated from the mass balance.

The amount of N removed by denitrification in the aerobic selector system was constant at 37% to 43%. This was similar to the 37% to 49% N removals observed for the anoxic selector trials, indicating that although the entire reaction system was aerated, a similar mass of substrate was still being removed by anoxic activity.

Table 7.14   Estimation of N removal during aerobic selector trials.

Trial:	AE1	AE2	AE3	AE4	AE5
WML volume ($10^{-3}m^3d^{-1}$)	0.97	0.98	0.93	0.98	0.98
N content of cells in WML and effluent ($gN.d^{-1}$)	0.441	0.452	0.499	0.530	0.473
WML: $NH_3 + NO_3 + NO_2$ ($gN.d^{-1}$)	0.007	0.007	0.002	0.003	0.008
Effluent: $NH_3 + NO_3 + NO_2$ ($gN.d^{-1}$)	0.059	0.054	0.021	0.025	0.071
N_{OUT}: WML + Effluent ($gN.d^{-1}$)	0.508	0.512	0.521	0.558	0.552
N_{FEED} - N_{OUT} : N removed by denitrification ($gN.d^{-1}$)	0.382	0.378	0.369	0.332	0.338
% N removed by denitrification	43	42	41	37	38
NO_X in reactor outflow denitrified in Sel.A and settler zones ($gN.d^{-1}$)	0.103	0.082	0.027	0.047	0.107
% of denitrification due to removal of NO_3 and NO_2 in reactor outflow	27	22	7	14	32
System N removal (%) (N_{FEED} - N_{EFF}) / N_{FEED}	92	88	96	95	90

The mass of N removed by denitrification in the settler and first selector of oxidised N compounds flowing out of the reactor ranged from 0.03 to 0.12 $gN.d^{-1}$, considerably lower than the 0.17 to 0.39 $gN.d^{-1}$ values obtained in the unaerated selector trials. This indicated that simultaneous nitrification and denitrification in the selector and reactor zones occurred to a greater extent in the aerated selector trials. The nitrification rates estimated

in Table 7.13 were also very low to those estimated in the previous set of trials, suggesting that the necessary assumption of negligible denitrification in the reactor was not valid.

Indication of simultaneous nitrification and denitrification in the selector zones was also supported by occasional DO measurements in the selectors during AE3, where DO concentrations of 1-2 $g.m^{-3}$ were recorded, but the nitrate concentration at that time was negligible. The mass of oxidised N compounds flowing out of the reactor was considerably lower in the two trials resulting in the growth of filamentous microorganisms, but as the overall level of N removal was similar, it was indicated that the extent of simultaneous nitrification and denitrification was much greater during these two trials.

7.6.4 Phosphorus removal

The average DRP concentration in the effluent decreased as the aerobic selector trials progressed, as shown in Figures 7.19 and 7.20, indicating an increase in the activity of phosphate accumulating microorganisms in the biomass with continuing operation. As can be seen from the data in Tables 7.8 to 7.12 and Figure 7.21, the P content of the VSS had also increased, up to 2.0 - 2.4% compared with 1.4 - 1.7% in the unaerated selector trials. This confirmed the occurrence of luxury P uptake as the biomass P content was now significantly above the 1.5 - 2% level reported for conventional AS biomass, and was at the maximum that would be expected considering the high substrate COD:P ratio (Tetreault et al., 1986). Microscopic observations of the stained biomass also indicated the presence of polyphosphate accumulation. No difference could be discerned between P contents for biomass in different zones of the reactor system.

It can be seen from Figure 7.19 that the DRP concentrations were fairly constant throughout the reactor zones after 25 days from the beginning of Trial AE1, but after another two SRTs there was a distinct DRP profile through the system. In all trials, the DRP was highest in the first selector then generally decreased through the subsequent selectors and reactor, consistent with anaerobic substrate uptake and concurrent P release in the first selector, and aerobic P uptake in the subsequent selectors and reactor. It can be seen in Figures 7.19 and 7.20 that for a short period during trials AE1, AE2 and AE5 the final selector DRP concentration was in fact lower than that in the reactor, suggesting that P release may have also been occurring in the reactor zone under some circumstances. Reactor and effluent DRP concentrations generally declined as the trials progressed,
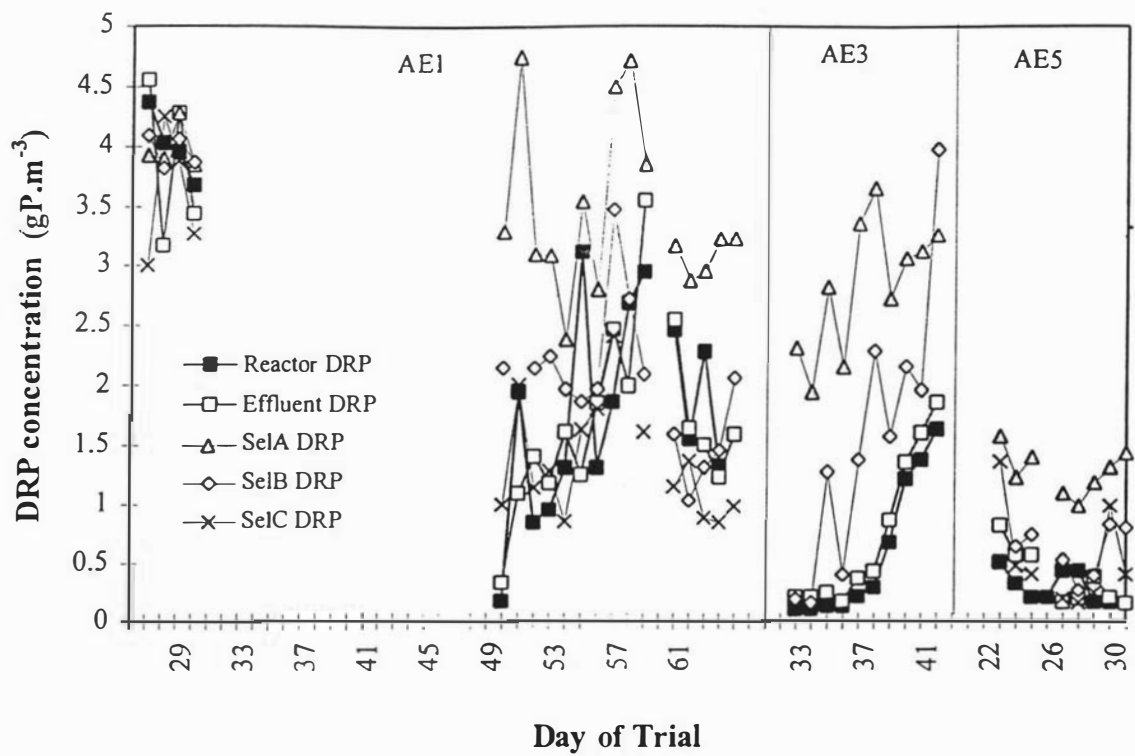
Figure 7.19: DRP concentration trends during periods of the trials conducted in Reactor System 2.
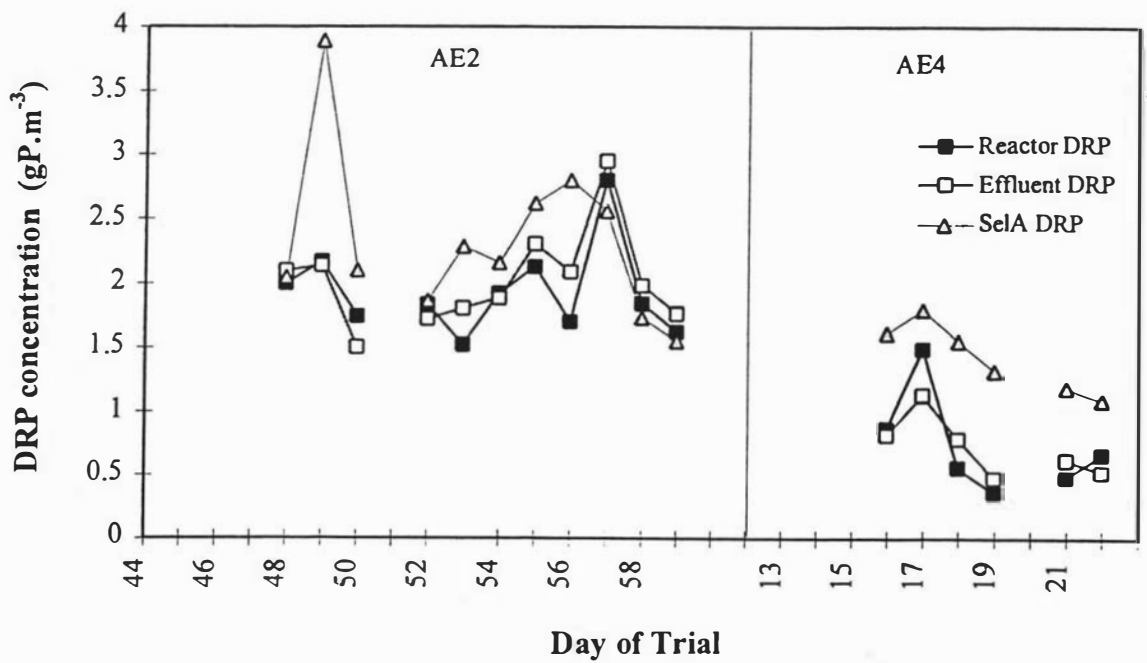
Figure 7.20: DRP concentration trends during periods of the trials conducted in Reactor System 1.
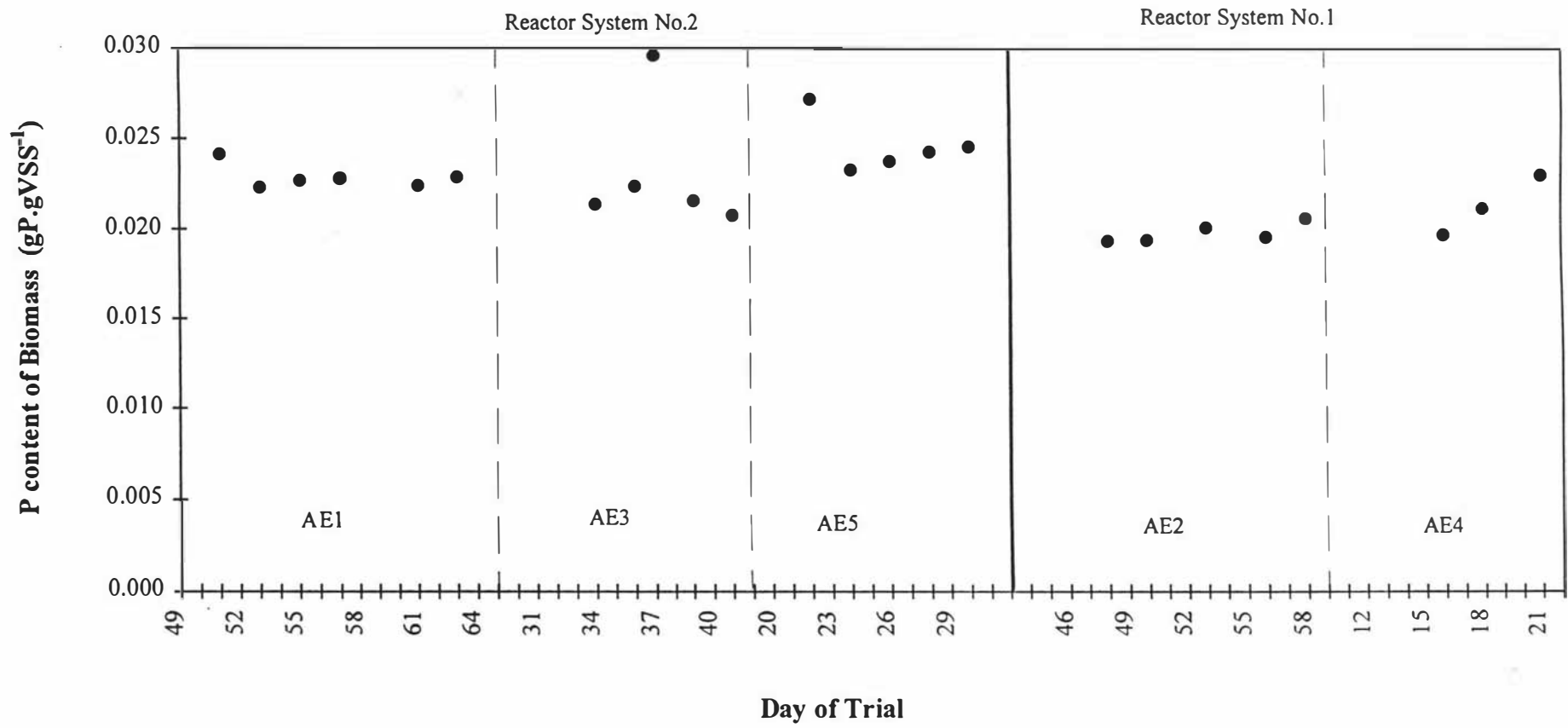
Figure 7.21 P content of the biomass in the aerated selector trials

except for Trial AE3 where the DRP showed an increasing trend. There was no obvious trend in any of the other parameters with fluctuations in reactor DRP.

The nitrate concentration was negligible in the first selector during Trials AE1 and AE3, so anaerobic conditions would have existed in bulk solution, providing the required anaerobic conditions for PAO activity. However during Trials AE2 and AE5, significant nitrate concentrations were maintained in the first selector, so anaerobic conditions were no longer sustained in the bulk liquid. As the sCOD concentration was highest at this point in the system, conditions existing in the floc could be expected to have been anaerobic, so would have continued to provide the appropriate conditions for the growth of PAOs.

A mass balance was calculated for total P across the reactor system, as summarised in Table 7.15. A consistent shortfall of 26 to 31% was obtained in the P balance, similar to that determined from Trial AN2 and AN3 data. The total P removal for the system was estimated from the difference between the influent and effluent stream TP contents.

Table 7.15   Phosphorus Mass Balance for the Aerated Selector System Trials

Trial:	AE1	AE2	AE3	AE4	AE5
TP in influent, P_{in} (gP.d^{-1})	0.140	0.140	0.140	0.140	0.140
TP in WML and effluent biomass (gP.d^{-1})	0.084	0.075	0.096	0.092	0.095
DRP in WML (gP.d^{-1})	0.002	0.002	0.001	0.001	0
DRP in effluent (gP.d^{-1})	0.017	0.020	0.007	0.007	0.004
TP_{out} (gP.d^{-1})	0.103	0.097	0.104	0.100	0.099
TP_{out} / TP_{in}	0.74	0.69	0.74	0.71	0.71
% P removal (effluent only)	82	81	89	91	96

7.7   Discussion

The use of aerated selectors provided a reactor configuration that could both prevent and cure filamentous bulking. Two of the reactor configurations trialed: 3 x 0.6l and 1 x 1.2l could cure bulking due to Type 021N and prevent the growth of other filamentous bacteria, whereas two other configurations trialed: 2 x 0.6l and 1 x 0.6l, allowed the proliferation of *H. hydrossis* filaments.

Soluble COD concentrations in the mixed liquor flowing out of the reactor were consistently lower in the aerated selector trials. The highest reactor sCOD concentrations were observed at the beginning of the first aerated selector trial in each reactor vessel apparatus, but steadily declined and remained consistently lower in subsequent trials. The change in microbial population resulting from imposing anoxic or aerobic rather than anaerobic conditions in selector zone, must have altered the SMP formed to that having a lower overall sCOD than when unaerated selectors were used.

The ability of the reactor configuration to prevent the growth of filamentous bacteria did not seem to relate definitively to either substrate loading or substrate removal criteria as most often proposed. The anoxic selector trials had indicated that ability to suppress filamentous growth could be correlated with the fraction of rsCOD consumed in the selector zone, however this was not observed in the aerobic selector trials. In fact the results presented in Table 7.2 indicated that the rsCOD removal efficiency occurring in the selectors during bulking trial AE3 was greater than during non-bulking trial AE2.

As listed in Table 7.2, the proportion of removal soluble COD consumed in the selector zone varied between 90% and 100%, being above 94% in all trials, except during the first 14 days of Trial AE2, when removals averaged 90- 91%. This was considerably higher than the 50% to 70% removals observed in the unaerated selector trials, indicating that the addition of aerobic substrate removal mechanisms allowed almost all exogenous substrate to be removed in the selector zone.

The trials indicated that for successful selector operation virtually all rsCOD must be removed in that zone. The SVI in Trial AE2 continued to increase at the beginning of the trial when rsCOD removals only averaged 90-91% but started to steadily decline after 26 days at SRT, when the removal efficiencies had increased to 96%. However the failure of Trial AE4 at consistently high rsCOD removal efficiencies demonstrated that substrate removal in the selector zone was not the sole criteria for success.

Trials AE2 and AE3 both had a selector zone with a total volume of 1.2l and were expected to demonstrate a similar performance, with AE3 possibly exhibiting better performance due to an increased substrate gradient through the system. Both resulted in sCOD removal efficiencies of greater than 96%, but only AE2 could prevent filamentous growth. The rate of SVI increase was reasonably slow, only increasing from 100 to 175 ml.g^{-1} over a period of 46 days, however the trial was abandoned at that stage as the conditions imposed during this trial were obviously not able to prevent the growth of filaments.

Floc loadings in Trials AE2 and AE3 were not significantly different either, indicating that substrate concentration effects were not responsible for causing bulking in AE3 in this instance. The major difference between the two trials was that the aeration rate to the selectors may have been lower during Trial AE3, as the absence of nitrate or nitrate in both selectors and pH drop through the selector zones in Trial AE3 indicated that anaerobic conditions prevailed in bulk solution. Aeration rates in the selectors were not automatically controlled and selector DO was not routinely monitored until Trial AE5. During Trial AE2 both nitrate and a very low level of nitrite were measurable, indicating that anoxic or aerobic conditions prevailed in the selector. This suggested *H. hydrossis*, the dominant filament in Trial AE3, may have been a 'low DO' type filament.

Trial AE4 also resulted in bulking due to *H. hydrossis*, with the rate of proliferation being much more rapid than in AE3. The HRT in the selector zone was shortest in this trial, but the rsCOD removal efficiency at 94% to 96%, was only very slightly lower than other trials where removal efficiencies of between 96% and 100% were maintained. The selector HRT was therefore possibly near the minimum required for the removal of all rsCOD under the conditions imposed. The presence of measurable nitrate and nitrite concentrations in the selector indicated that the conditions in bulk solution were either anoxic or oxic, however it can be seen in Figure 7.18 that both bulking trials had a lower concentration of oxidised nitrate compounds entering the selector zone in the RAS than non-bulking trials.

The use of aeration in the selector zones altered the dominant filament observed. It is suggested that Type 021N could no longer proliferate under aerated selector conditions either because there were no longer significant quantities of substrate getting through to the reactor zone, or because bulk conditions in the selectors were no longer anaerobic, indicating that for this substrate, Type 021N was either a low F/M filament or utilised low molecular weight acids. *H. hydrossis* only proliferated in what was indicated to be low

DO conditions or when there were low nitrate concentrations flowing into the selector zone, suggesting that *H. hydrossis* was a low DO filament.

Batch substrate removal rate tests indicated considerably higher 'psuedo first order' substrate removal rates for the biomass growing in the aerated selector trials than during unaerated selector trials, with values for the apparent removal rate constant increasing from 10 - 40 d^{-1} to 100 - 180 d^{-1}. The aerated selectors therefore promoted the growth of bacteria with increased substrate removal rate capabilities. The biosorption capacity of the cultures did not change significantly between the two types of trials, remaining in the 0.05 - 0.1g sCOD.g VSS^{-1} range, but biosorption capacity had become positively correlated with SVI, indicating that *H. hydrossis* may also possess biosorptive capacity.

A comparison of oxygen uptake data with substrate removal data suggested that the rate of substrate accumulation as opposed to substrate storage occurred more rapidly in the aerated selector trials, indicating that the increased substrate removal rate was due to more rapid substrate accumulation, but that the utilisation of accumulated substrate for storage compounds or metabolic processes had not been increased to the same extent. A lower oxygen requirement in response to substrate addition indicated that the substrate accumulation and storage processes were more efficient in terms of electron acceptor requirement for the culture grown under aerated selector conditions. It can be seen from Tables 7.3 and 7.16 that during the two trials when filamentous bulking occurred, the biomass had a lower substrate removal efficiency with respect to oxygen utilised, suggesting that *H. hydrossis* had a reduced substrate accumulation and storage ability compared to non-filamentous organisms under aerobic conditions.

Although the selectors were aerated, there was evidence that a combination of aerobic, anoxic and anaerobic substrate removal mechanisms were present. The DO levels and oxygen uptake rates in the selectors were not routinely monitored except during Trial AE5, where the results demonstrated that the first selector zone possessed the lowest DO concentration and highest OUR while removing the greatest mass of substrate. From calculations of mass of oxygen consumed in the selectors per mass of substrate removed, it was indicated that not all the substrate removal observed could have been due to aerobic processes.

An estimation of the substrate removed via each mechanism can be made assuming the substrate removed aerobically is represented by the data in Table 7.3; the anoxic substrate removal requirement is 7 gCOD.gN^{-1} (Henze, 1991; Siegrist and Gujer, 1994; Isaacs and Henze, 1995); and the anaerobic substrate removal requirement is 2 gCOD.gP^{-1} (Isaacs

and Henze, 1995). The OUR data in the selector was not regularly measured until Trial AE5, so the oxygen consumption value used for AE4 is an average of limited data points obtained. The value for DRP released is made assuming that the DRP in the substrate was negligible.

Table 7.16   Estimation of substrate removal processes in the first selector zone.

Trial	AE1	AE2	AE3	AE4	AE5
sCOD removed $(g.d^{-1})$	13.0	16.0	14.2	15.3	15.0
O_2 consumed $(g.d^{-1})$	ND	ND	ND	1.8	1.33
RAS $NO_3 + NO_2$ consumed $(g.d^{-1})$	0.077	0.054	0.026	0.035	0.089
DRP released $(g.d^{-1})$	0.058	0.026	0.054	0.024	0.025
$gCOD.gO_2^{-1}$ from Table 7.3	11.0	10.3	7.3	5.0	9.7
sCOD removed aerobically @ rate given above $(g\ sCOD.d^{-1})$	ND	ND	ND	9.0	12.85
sCOD removed by denitrification @ 7 $gCOD.gN^{-1}$ $(g\ sCOD.d^{-1})$	0.54	0.38	0.18	0.25	0.62
sCOD removed via P release @ 2 $gCOD.gP^{-1}$ $(g\ sCOD.d^{-1})$	0.12	0.05	0.11	0.05	0.05
Total sCOD removed: aerobic + anoxic + anaerobic $(g\ sCOD.d^{-1})$				9.3	13.52
% of sCOD removal accounted for				61	90

ND = could not be determined from the data collected.

The results in Table 7.16 for Trial AE5 support the proposition that the dominant substrate removal mechanism was aerobic substrate accumulation. The removal of RAS oxidised N compounds in the first selector zone indicated that anoxic substrate removal was occurring, however the mass of oxidised N compounds flowing out of the reactor per day was lower than that in the unaerated selector trials, resulting in a lower mass of oxidised N compounds removed by denitrification in the settler and selector zones. However as the total mass of N removed from the reactor system by denitrification was not different from the previous series of trials, and the N mass balance implied that only between 7% and 36% of the denitrification could be accounted for by the removal of oxidised N compounds from the RAS, it is indicated that both nitrification and denitrification would have been occurring simultaneously in the selector and reactor zones.
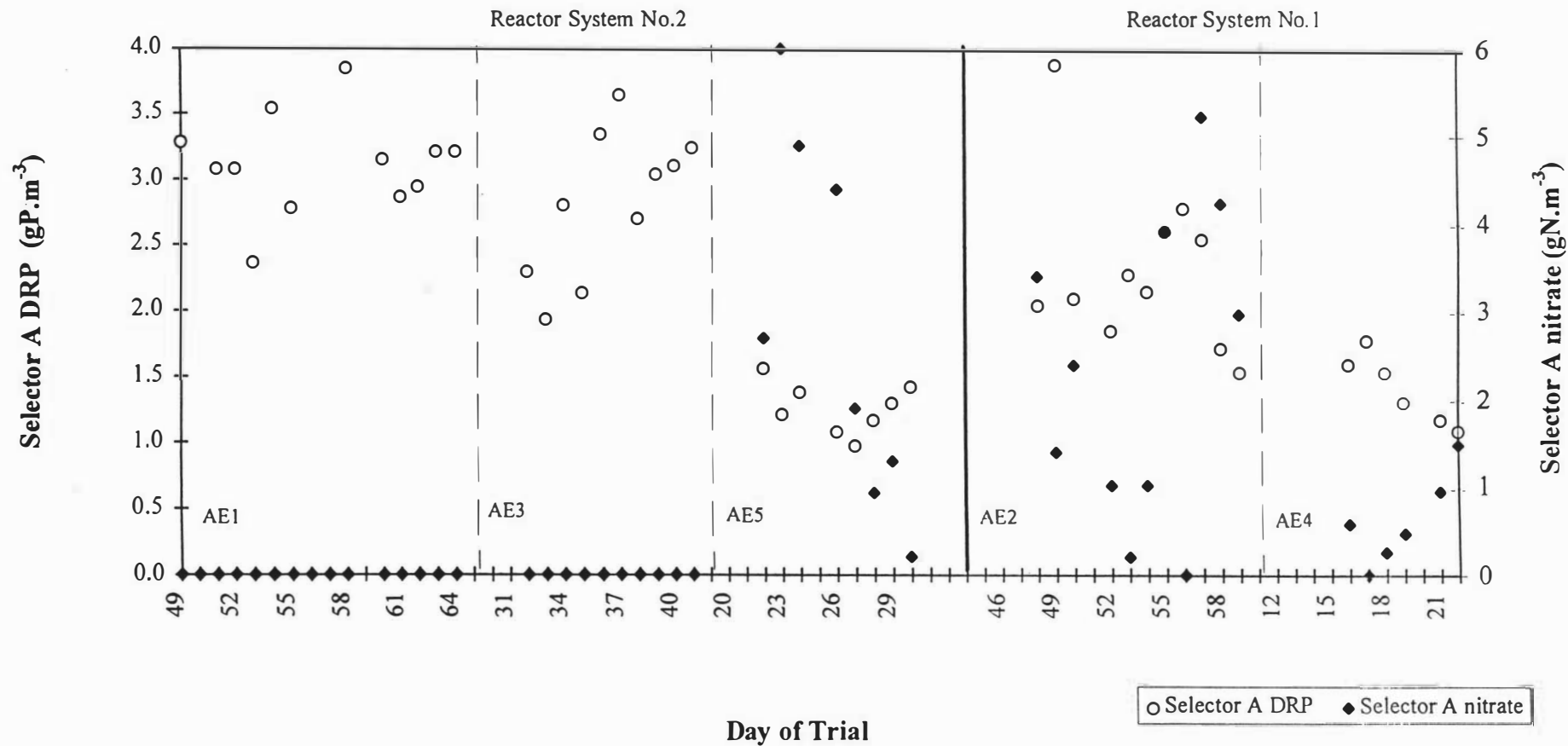
Figure 7.22   Reactor DRP and nitrate concentrations during the aerated selector trials
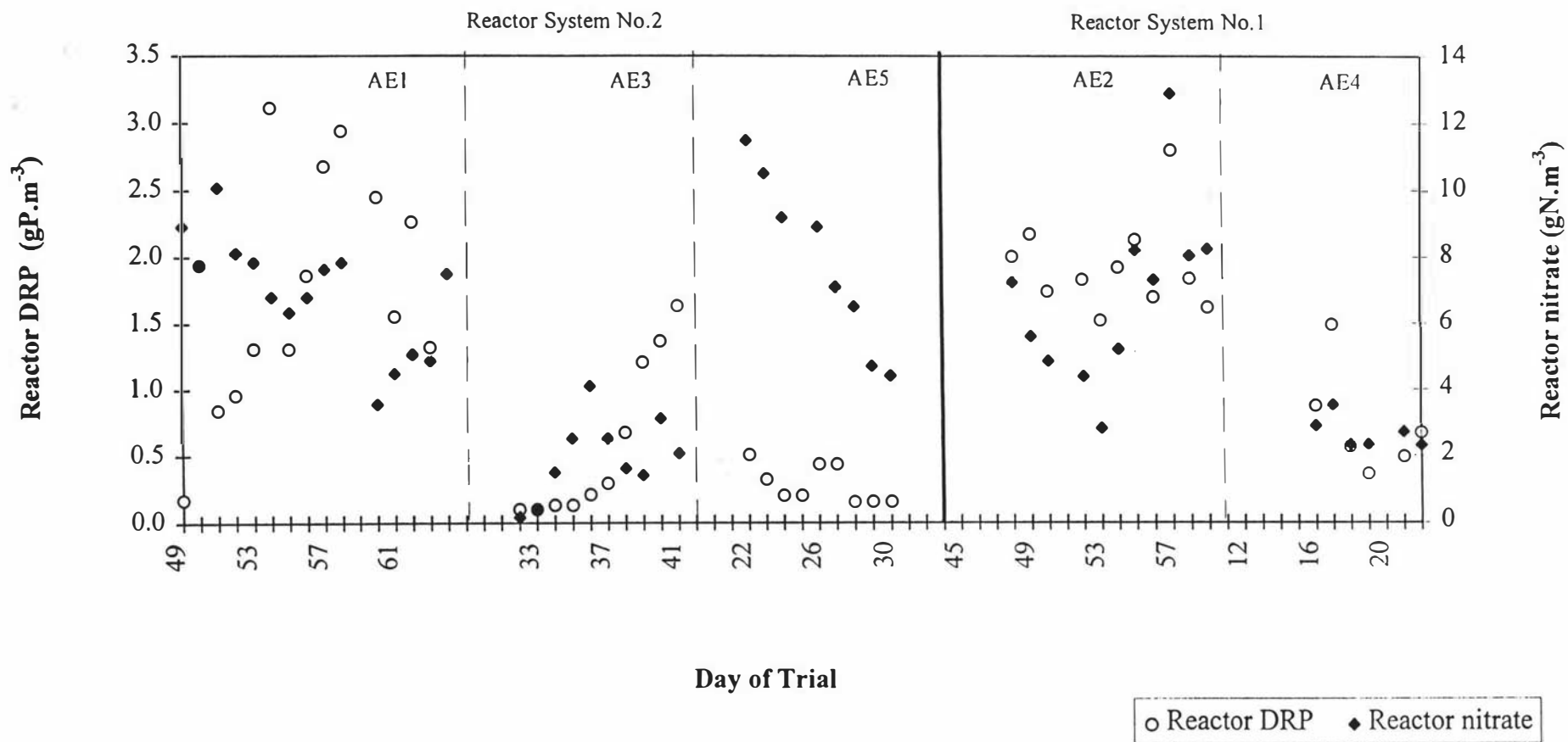
Figure 7.23   Reactor DRP and nitrate concentrations during the aerated selector trials

The bulk liquid DO in the Selector A ranged between 0.2 and 3.0 $gO_2.m^{-3}$ and the nitrate between 0.2 and 6.0 $gN.m^{-3}$; but due to the very rapid substrate removal rates observed, even though the bulk liquid conditions may have been aerobic, conditions inside the floc could have been expected to be oxygen deficient (Jenkins *et al.*, 1993), providing adequate conditions for anaerobic activity.

Anaerobic activity seems to have been confined to the initial selector zone as DRP concentrations declined through the subsequent selector and reactor zones, consistent with P uptake under aerobic conditions. The extent of biological P removal increased as the trials progressed: evidenced by a continued decrease in effluent DRP concentration, a concurrent increase in biomass P content and the microscopic observation of staining reactions which indicate accumulated polyphosphate in the mixed liquor.

During the unaerated selector trials the pH of bulk solution dropped in the selector zones, which may have been due to the production of acidic compounds as a result of anaerobic activity. However, during all five aerated selector trials, the average pH in the selectors was only 0.1 to 0.2 pH units lower than in the reactor, indicating that the bulk solution in these zones did not contain significant quantities of fermentation products and therefore were not predominantly anaerobic even though nitrate and oxygen deficient conditions were maintained in the first selector during Trials AE1 and AE3.

It is generally accepted that the presence of oxidised N compounds inhibits P release (Tetreault *et al.* 1986; Comeau *et al.*, 1987), and although the selector data shown in Figure 7.22 may seem to support this, the presence of nitrate in the initial selector zone did not seem to affect the overall extent of EBPR. Trials AE3, AE4 and AE5 all had significant concentrations of oxidised N compounds in the first selector, however the reactor DRP still declined from that seen in AE1 and AE2. Figure 7.23 demonstrates that no positive correlation could be seen between reactor zone nitrate and DRP as was suggested by Tetreault *et al.* (1986); nor did the increase in selector nitrate concentration adversely affect biomass P content as has been previously reported (Appeldoorn *et al.*, 1992).

The occurrence of both anoxic and anaerobic substrate removal processes in the same reactor have been proposed by other researchers. Tetreault *et al.* (1986) found that P removal still occurred in the initial 'anaerobic' stage of a full scale system which contained nitrate, and suggested that if sufficient substrate was available (influent BOD:TP > 25) then simultaneous denitrification and P release could occur, with denitrification occurring outside the flocs and anaerobic conditions occurring inside the floc. Other researchers

(Szpyrkowicz and Zilio Grandi, 1995a,b; Kerm-Jespersen and Henze, 1993) have also suggested that some PAOs have the ability to denitrify, so the presence of nitrate would not have been inhibitory.

7.8   Conclusions

The use of aerated selectors could both prevent and cure bulking due to Type 021N filamentous bacteria, however if the bulk solution in the selector stage of the reaction system was not aerobic, the growth of *H. hydrossis* was promoted. Therefore for successful filamentous bulking control two conditions needed to be fulfilled: virtually all removable soluble COD must be consumed in the selector zone and bulk liquid conditions for substrate removal must be aerobic.

Substrate removal rates increased significantly over that observed with biomass from the unaerated selector trials, with indications that the substrate accumulation rates had increased to a greater extent than substrate storage reaction rates. Even though the selectors were aerated, the extremely rapid rate of exogenous substrate removal often resulted in predominantly anoxic or anaerobic conditions in bulk solution in the initial selector zone.

A combination of substrate removal mechanisms were operating simultaneously in the selectors. Although the substrate was predominantly removed via aerobic mechanisms, removal via anoxic and anaerobic mechanisms was also observed. The extent of denitrification in the reactor systems was similar for the unaerated and aerated selector trials, but the extent of biological phosphorus removal continued to increase as the aerated selector trials progressed. The occurrence of simultaneous nitrification and denitrification was indicated in most, if not all the reactor zones, whereas anaerobic activity seemed to be confined to the initial selector zone.

CHAPTER 8

EFFECT OF INFLUENT NITROGEN CONTENT

8.1   Introduction

The studies presented in the previous chapter demonstrated that aerated selectors were able to prevent filamentous bulking provided that all of the removable substrate was consumed in the selector zone under aerobic bulk solution conditions. Even though the entire reactor system was aerated, significant anoxic and anaerobic activity was indicated, with simultaneous nitrification and denitrification being indicated in all reactor system zones.

The role of substrate nitrogen in the performance of the activated sludge system utilising dairy processing wastewater was seen as important for a variety of reasons including:

• the possible advantage conferred to filamentous microorganisms due to N limited conditions. The nitrogen in the original substrate was in the form of milk proteins, which only became readily available to the biomass for growth after ammonification had occurred (Jenkins et al., 1993). This may result in N deficient conditions if the carbonaceous fraction of the substrate is readily metabolizable, and can result in the proliferation of Type 021N (Richard et al., 1985; Jenkins et al., 1993), which was observed in both the anoxic and aerobic selector trials.

• the provision of oxidised N in selectors for anoxic substrate removal. Due to the very rapid rates of substrate removal observed, an adequate supply of oxygen and oxidised N compounds would have been required to prevent anaerobic conditions in bulk solution.

• the ability of various types of microorganisms to use nitrate as an electron acceptor during substrate removal, including filaments (Wanner et al., 1987; Shao and Jenkins, 1989; Casey et al., 1994); and P accumulating bacteria (Szpyrkowicz and Zilio-Grandi, 1995a,b; Kerrn-Jespersen and Henze, 1993; Knight et al. (1995).

In order to more clearly define the role of N in the activated sludge treatment of the defined dairy processing wastewater, it was decided to increase the N content of the

feed and observe the resultant change in N species through the system. The supplemented N was in the form of ammonia, which could be directly utilised by the biomass for growth and nitrification reactions.

8.2 Trials at Increased Substrate Nitrogen Content

All the trials used the 3 x 0.6l aerated selector configuration as this was the most successful reactor configuration from the previous set of trials for the prevention of filamentous bulking and allowed the progression of various reactions through the selector zone to be more easily defined. The additional N was added in the form of ammonium sulphate, providing a readily available form of N for cell growth or nitrification processes. The levels of increased substrate nitrogen were calculated to provide an increase of 50% and 100% in total N content from that initially estimated in Table 4.3 based on ingredient composition. That initial value was estimated to be 60 $gN.m^{-3}$, so 143 and 286 $g(NH_4)_2SO_4.m^{-3}$ were added to the substrate in each case to provide an extra 30 and 60 $gN.m^{-3}$ respectively. As the actual N content of the substrate was later measured to be 89 $gN.m^{-3}$, the increased N contents represented increases of 34% and 67% in the nitrogen content respectively.

One trial was conducted with the 34% increase in substrate N, while two trials were conducted at the highest level of feed N content: one with a well settling biomass, and the other with a biomass having a 'very common' (Jenkins *et al.*, 1993) abundance of filamentous microorganisms, as indicated by initial SVIs of 110 and 180 $ml.g^{-1}$ respectively. The trials conducted were:

Reactor System 2:	Trial AE6, feed N increased by 30 $gN.m^{-3}$, starting with the well settling biomass remaining from Trial AE5.
Reactor System 2:	Trial AE7, feed N increased by 60 $gN.m^{-3}$, starting with the biomass remaining from Trial AE6.
Reactor System 1:	Trial AE8, feed N increased by 60 $gN.m^{-3}$, started with biomass in a bulking condition with *H. hydrossis* as the dominant filament.

The SRTs were maintained at 10 days, based on reactor volume only, as in previous trials, with all trials being conducted for a period of 3 SRTs as this is commonly cited as

the length of time required for mixed liquor biomass to reach a new 'psuedo steady state'. Concentrations of the various N species and DRP were measured every second day over the entire period of the trial.

8.3   Reactor Treatment Performance

The average reactor sCOD concentration during Trial AE6 remained unchanged from the level achieved during the preceding trial with normal substrate N content (AE5); however as can be seen in Table 8.1 and Figure 8.1, the reactor sCOD declined from an average of 35 g.m^{-3} during Trial AE6 to an average of 28 g.m^{-3} during Trial AE7.
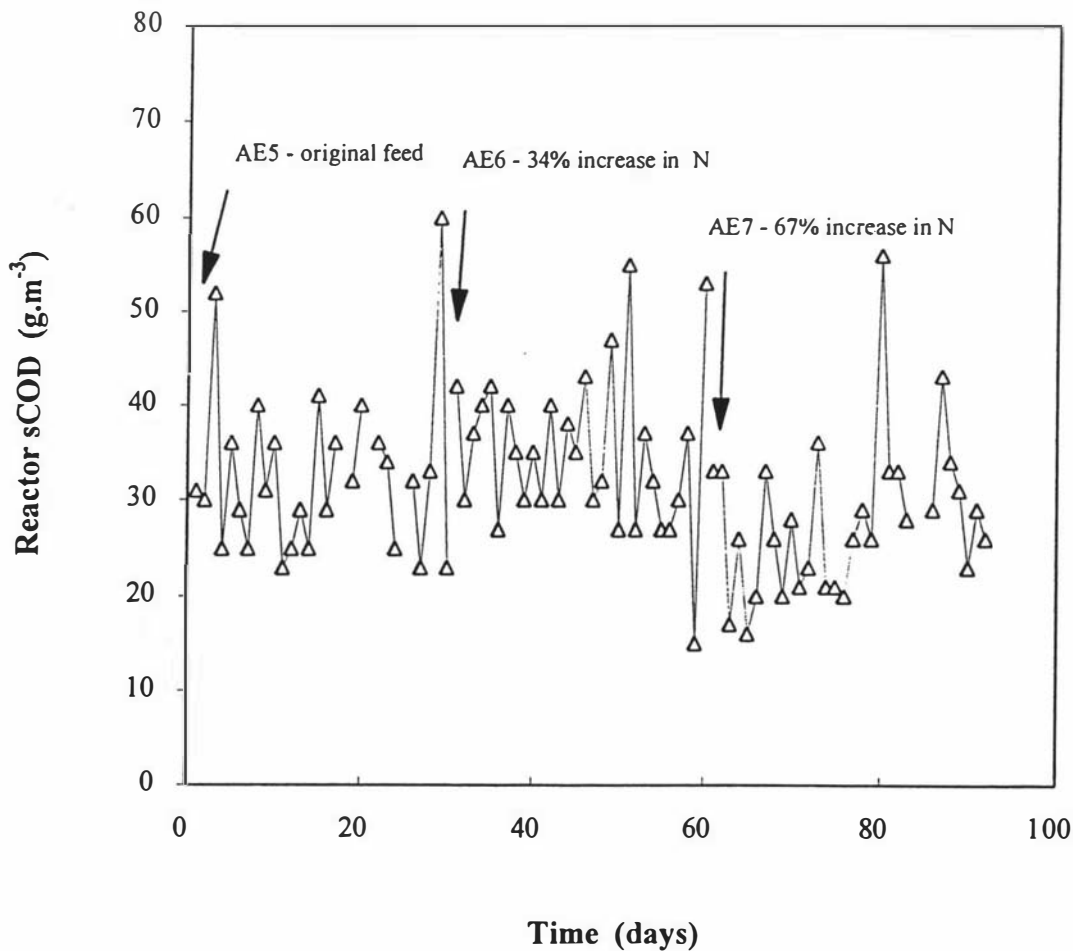
Figure 8.1:   Reactor soluble COD concentrations during Trials AE5, AE6 and AE7.

Table 8.1   Treatment performance at increased substrate N content.

Trial	AE6	AE7	AE8
N level in feed (gN.m^{-3})	119	149	149
Selector outlet conditions:			
pH	7.60	7.25	6.95
TSS (g.m^{-3})	5750	5450	4500
VSS (g.m^{-3})	5070	4810	3940
Total COD (g.m^{-3})	7400	7260	6050
Soluble COD (g.m^{-3})	38	35	31
Reactor outlet conditions:			
pH	7.60	6.10	6.25
TSS (g.m^{-3})	4600	4820	4280
VSS (g.m^{-3})	4060	4260	3740
Total COD (g.m^{-3})	5650	5950	5750
Soluble COD (g.m^{-3})	35	28	28
Soluble COD Removal (%)	97.9	98.3	98.3
SVI Range	110 - 163	54 - 281	94 - 656
SVI Trend	increasing	decreasing	decreasing
Effluent conditions:			
pH	7.70	6.65	6.65
TSS (g.m^{-3})	17	16	32
VSS (g.m^{-3})	15	15	29
Total COD (g.m^{-3})	63	49	53
Total COD Removal (%)	97.1	97.8	97.5
Proportion of effluent COD due to TSS (%)	44.4	42.8	47.2

The same lower reactor sCOD concentration was obtained during AE8, the other high substrate N level trial, as shown in Figure 8.2. Effluent TSS concentrations were variable, between 5 and 90 $g.m^{-3}$, but on average were unchanged from the previous set of trials.

A second difference observed at the 150 $gN.m^{-3}$ feed level but not at the 120 $gN.m^{-3}$ feed level was a change in pH periodically through the reactor system as seen in Figure 8.3. The average final selector pH values were 0.10 to 0.25 units below the average reactor pH value during Trials AE1 to AE5, and equal during trial AE6, but during periods of Trial AE7 and AE8 the reactor pH was as much as 2 pH units lower than the selector value. The pH drop in the reactor during Trial AE7 was observed as soon as the N content was increased, dropping to pH 5.50 within two days of the beginning of the trial. A similar effect was observed during Trial AE8, although it took 12 days for the pH to drop below a value of pH 6.0.
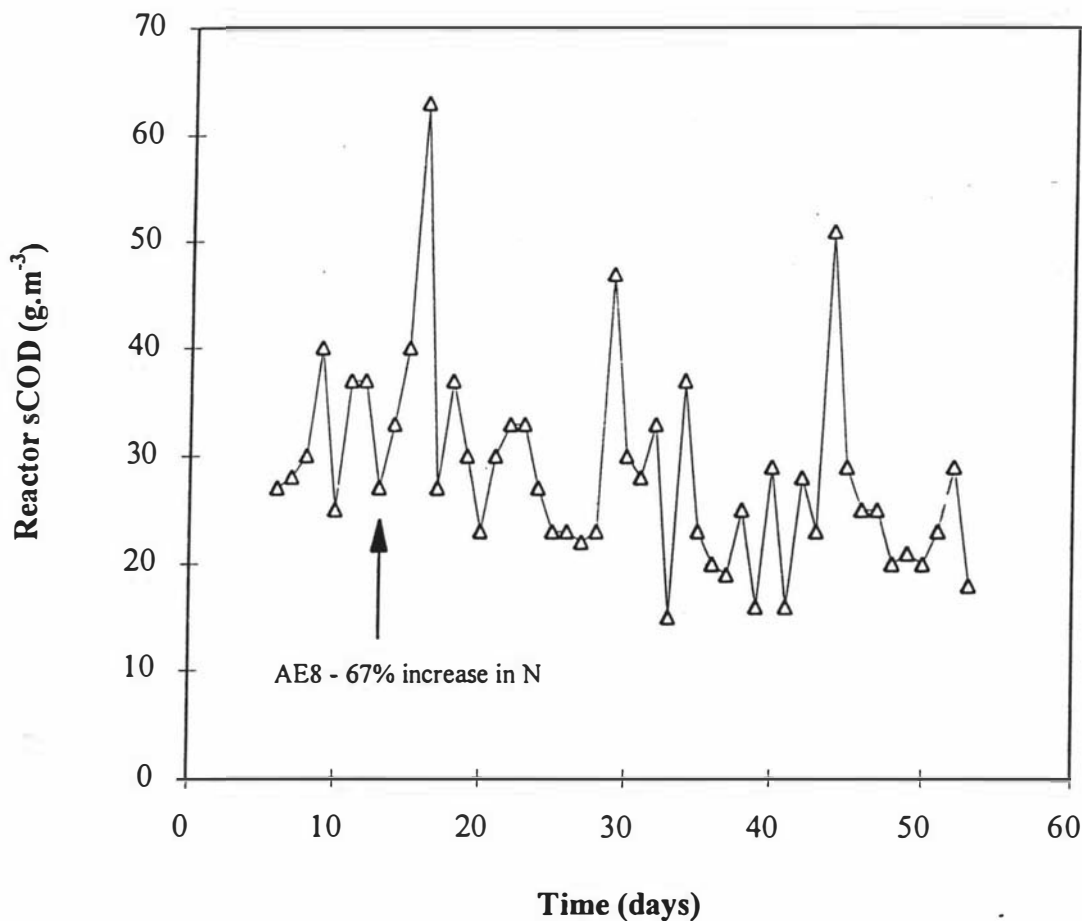
Figure 8.2:   Reactor soluble COD concentrations during Trial AE8.

Table 8.2 outlines the selector performance during Trials AE6 to AE8. The low SVI allowed the recycle rate to remain low during trials AE6 and AE7, but it was much higher during Trial AE8 as this trial began with a filamentous biomass. The decreased SVI and increased solids settling rate obtained at the end of Trial AE7 allowed the RAS flowrate to be reduced to a value lower than the feed flowrate for the first time in this study. The low recycle rate resulted in floc loading rates of over 0.2 g sCOD.g VSS^{-1} and theoretical initial substrate concentrations of over 900 g $sCOD.m^{-3}$ in the first selector. At higher floc loading rates a higher substrate gradient was observed through the selector system, but overall selector zone removable sCOD removals were maintained at above 98.8%. The change in sCOD concentrations through the initial selector zone represented average removals of 6.0, 5.1 and 6.1 g $sCOD.g$ $VSS^{-1}.d^{-1}$ in Trials AE6, AE7 and AE8 respectively.

Table 8.2   Selector performance and operation at increased substrate N content.

Trial	Days at SRT	RAS Flowrate ($10^{-3}m^3d^{-1}$)	Total Selector HRT (min)	Floc load (g sCOD) g VSS	S_0 into selector (g $sCODm^{-3}$)	Selector sCOD (g $sCODm^{-3}$)	% rsCOD consumed in selectors
AE6	1 -10	12.3	116	0.195	760	90,55,40	99.4
	11 -30	15.9	100	0.152	660	71,43,37	99.7
AE7	1 - 16	15.9	100	0.147	650	91,40,32	98.8
	17 -29	9.6	132	0.199	860	142,68,40	99.2
	30 -32	8.2	142	0.222	915	175,67,31	99.4
AE8	1 - 3	32.5	61	0.093	400	50,37,36	99.2
	4 - 50	47.6	45	0.077	310	45,32,31	98.9
	51-53	54.8	40	0.064	275	56,42,32	96.4
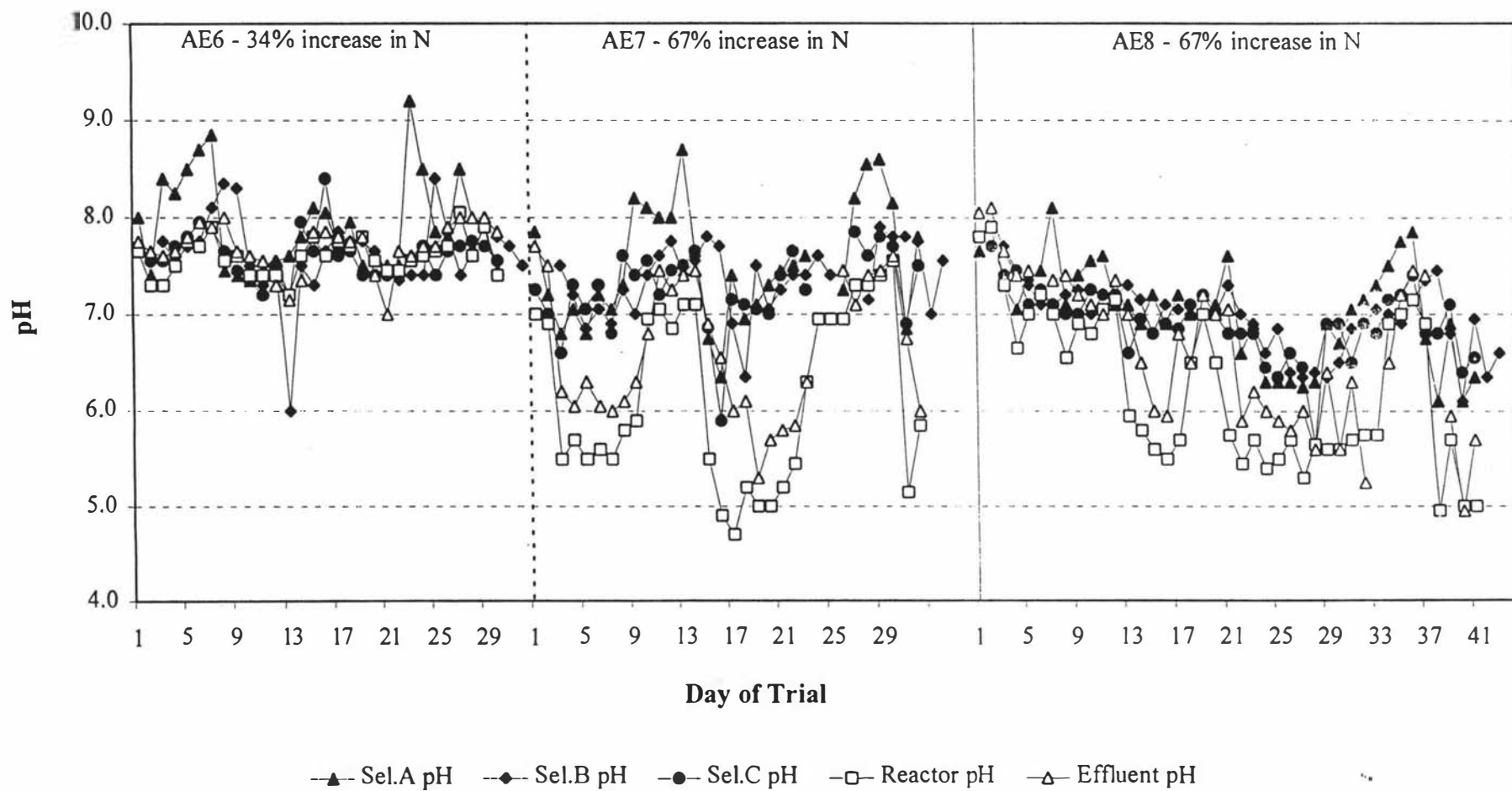
Figure 8.3   pH in the reactor zones during trials AE6, AE7 and AE8.

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8.4   Filamentous microorganism growth

Although the reactor configuration remained unchanged from the preceding successful trial, altered feed conditions resulted in changes in the biomass SVI. The changes during Trials AE5, AE6 and AE7 are shown in Figure 8.4. Trial AE5 had demonstrated the ability of the 3x 0.6l serial selector configuration to cure bulking due to *H. hydrossis* and to prevent the growth of other filamentous microorganisms. After the change to a substrate with a 33% higher N content, the SVI continued to decline slightly from the initial value of 130 ml.g^{-1} to an average of 115 ml.g^{-1} over a period of two SRTs, but the SVI then steadily increased to 163 ml.g^{-1} by the end of the third SRT of Trial AE6.

Trial AE7 was begun after 3 SRTs had elapsed for AE6, by further increasing the substrate N content to 67% above the original level. This resulted in a continued increase in SVI which varied between 174 and 209 ml.g^{-1} for the next SRT, before declining once more to a stable level around 60 ml.g^{-1}. Figure 8.5 shows the floc structure on Day 2 of Trial AE7 when the SVI was 215 ml.g^{-1} and although the flocs were quite dense, the occurrence of very fine protruding filaments was very common. The rapid disappearance of filaments can be observed in Figures 8.6 and 8.7 taken on Days 8 and 18 of Trial AE7 respectively.

The biomass as shown in Figure 8.7 was observed when the SVI had declined to 93 ml.g^{-1}, but four types of filaments could still be observed in the mixed liquor as follows:
- The dominant filament was identified as *H. hydrossis* from the following characteristics:
    - protruding from flocs
    - very thin, approximately 0.5 μm in diameter, no indentations at cell septa
    - stained as: gram -ve, Neisser -ve, PHB -ve
- The secondary filament was identified as Type 021N from the following:
    - protruding from flocs and free floating
    - knots and loops in trichome
    - discoid shaped cells
    - stained as: gram -ve; Neisser -ve; PHB +ve granules
- A minor filament comprised of short rectangular gram -ve cells was observed and tentatively identified as Type 0803:
- A further minor filament with attached growth was observed in a wet mount preparation at 400x , but could not be identified as it was not observed in any of the stained preparations.
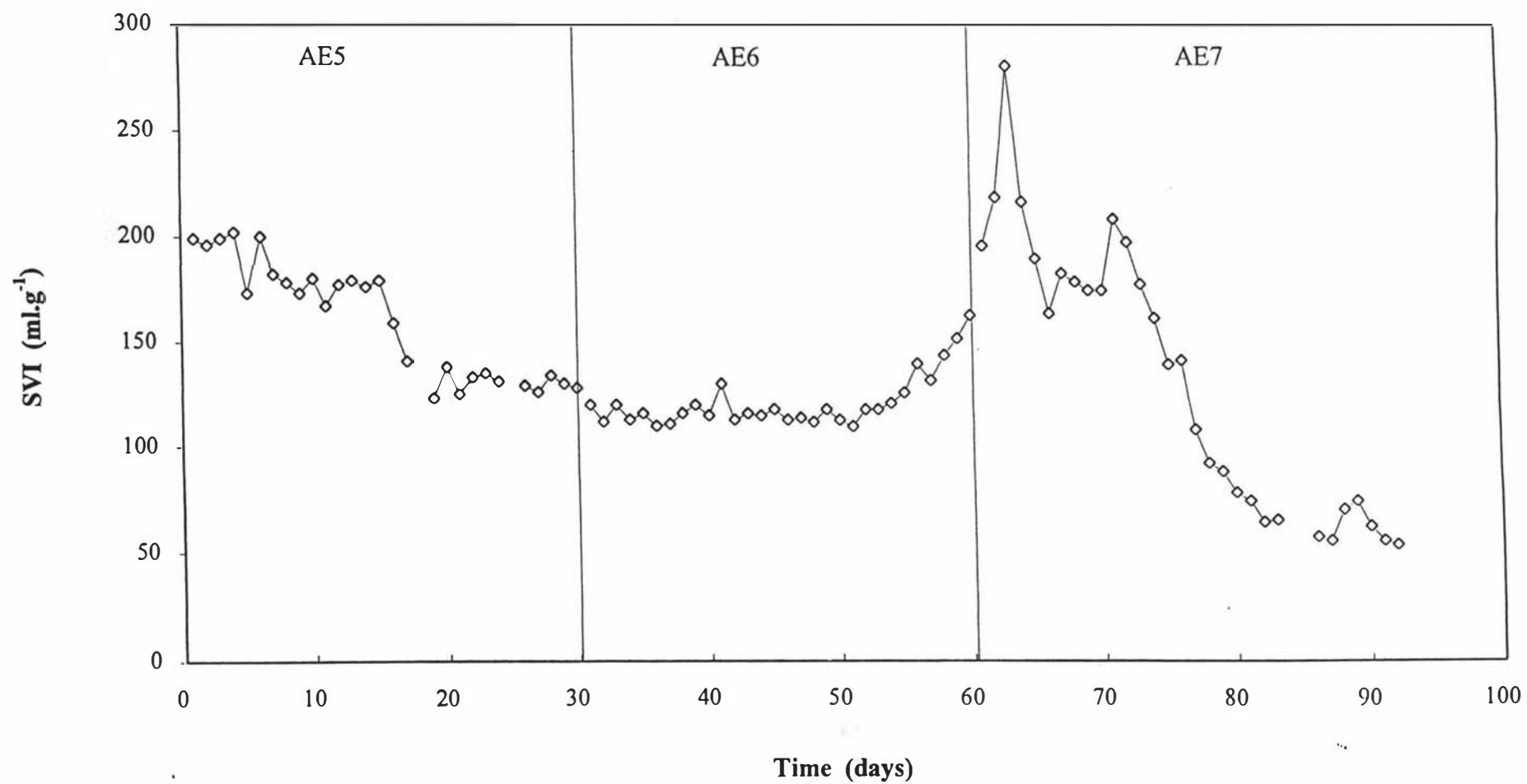
Figure 8.4   Change in Sludge Volume Index during Trials AE5, AE6 and AE7.

The final average SVI in Trial AE7 declined to around 60 ml.g^{-1}, considerably lower than the levels reached at the end of Trials AE1 and AE5 of 120 and 130 ml.g^{-1} respectively. In addition to the lower SVI, the biomass at the end of Trial AE7 settled at a considerably faster rate, with the final sludge volume being reached in the SVI test well before the 30 minute settling period had concluded. The floc size had decreased and small but very dense units were now observable as shown in Figure 8.8. The filterability of the mixed liquor had also increased markedly and the dried residue from the TSS determinations formed a crumbly mass rather than the coherent disc that had been observed on the filter during all previous trials. This seemed to indicate that the extent of extracellular polymeric substances in the floc had decreased.

The reactor used for Trial AE8 was seeded with biomass containing some filamentous bacteria, as evidenced by the SVI of about 180 ml/g when the trial began. The reactor was operated for a total of 12 days at SRT with the originally defined substrate before the N content of the feed was increased, by which time the SVI of the mixed liquor had increased to 340 ml.g^{-1} as shown in Figure 8.9. The SVI continued to increase, peaking at 656 ml.g^{-1} after 10 days of operation at increased substrate N concentration, then declined to 94 ml.g^{-1} after a further 31 days. The dominant filament at high SVI during Trial AE8 was again observed to be *H. hydrossis*, the same as that occurring during the higher SVI period in Trial AE7.

Figure 8.10 was taken on Day 24 when the SVI was 301 ml.g^{-1}, illustrating the very fine protruding filaments which resulted in an open and disperse floc structure. Figures 8.11 and 8.12 show that as the trial progressed the abundance of filaments and SVI decreased, and the floc structure became more dense. Figure 8.12 was taken on Day 40 of Trial AE8 when the SVI had declined to 116 ml.g^{-1}, but microscopic observations still identified the dominant filament as *H. hydrossis*. Type 021N was also present, but as a minor secondary filament. A very minor gram -ve filament with long rectangular cells of approximately 0.8 μm diameter was also observed, but could not be identified further as it was not seen in all the stained preparations. Microscopic observations of methylene blue stained preparations from both AE7 and AE8 mixed liquor highlighted the presence of pink coloured cell clusters, indicating the occurrence of accumulated polyphosphate.

All photographs were taken of wet mounted mixed liquor samples at 100x magnification.
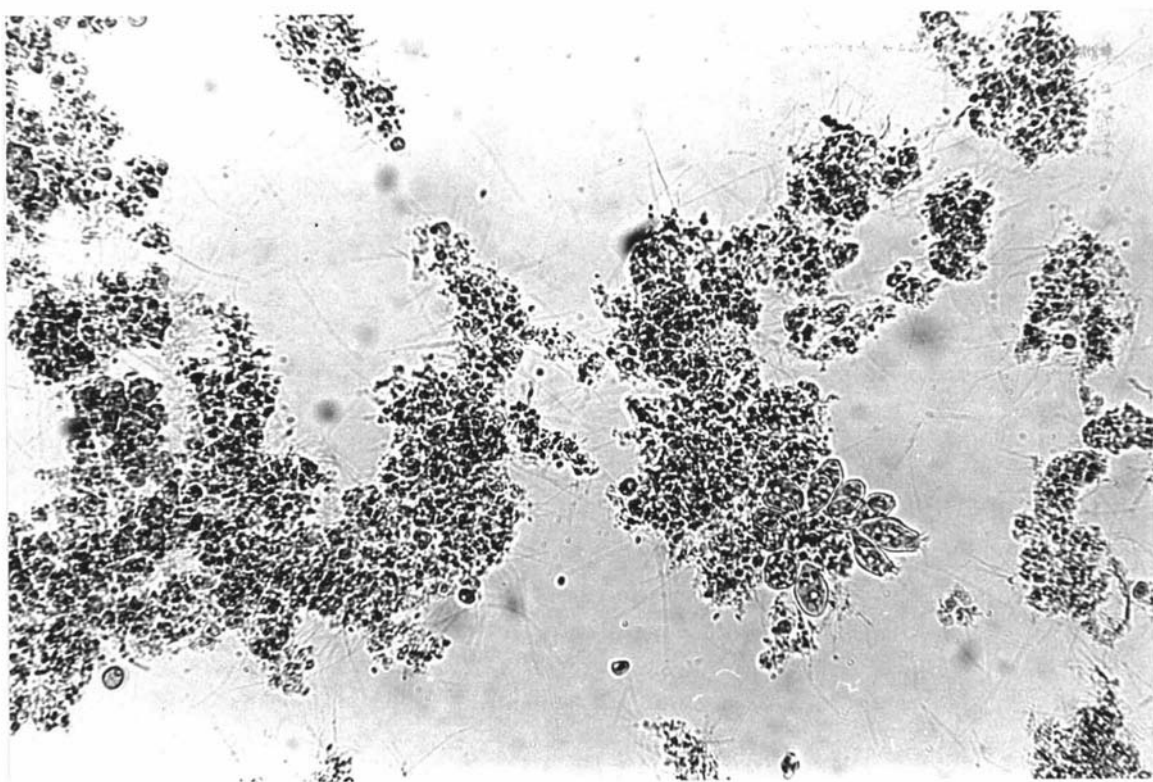
Figure 8.5:   Filament abundance on Day 2 of Trial AE7, SVI = 219 ml.g^{-1}.
(100x magnification)
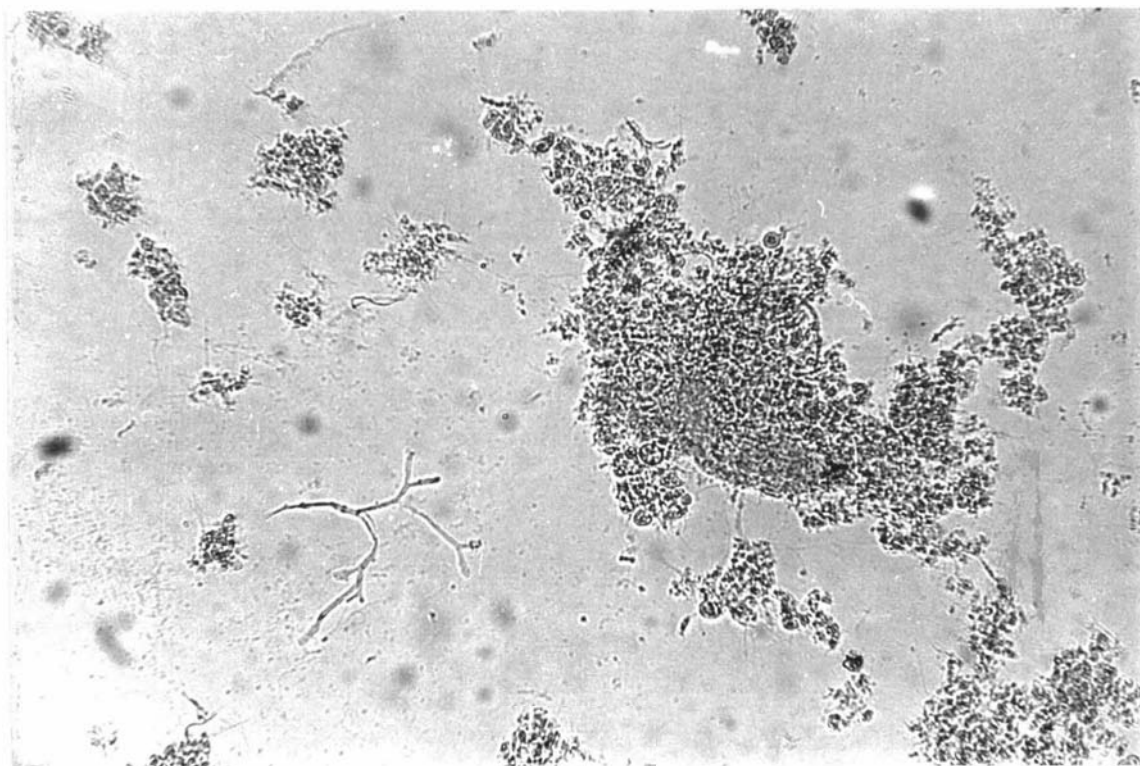
Figure 8.6:   Filament abundance on Day 8 of Trial AE7, SVI = 179 ml.g^{-1}.
(100x magnification)
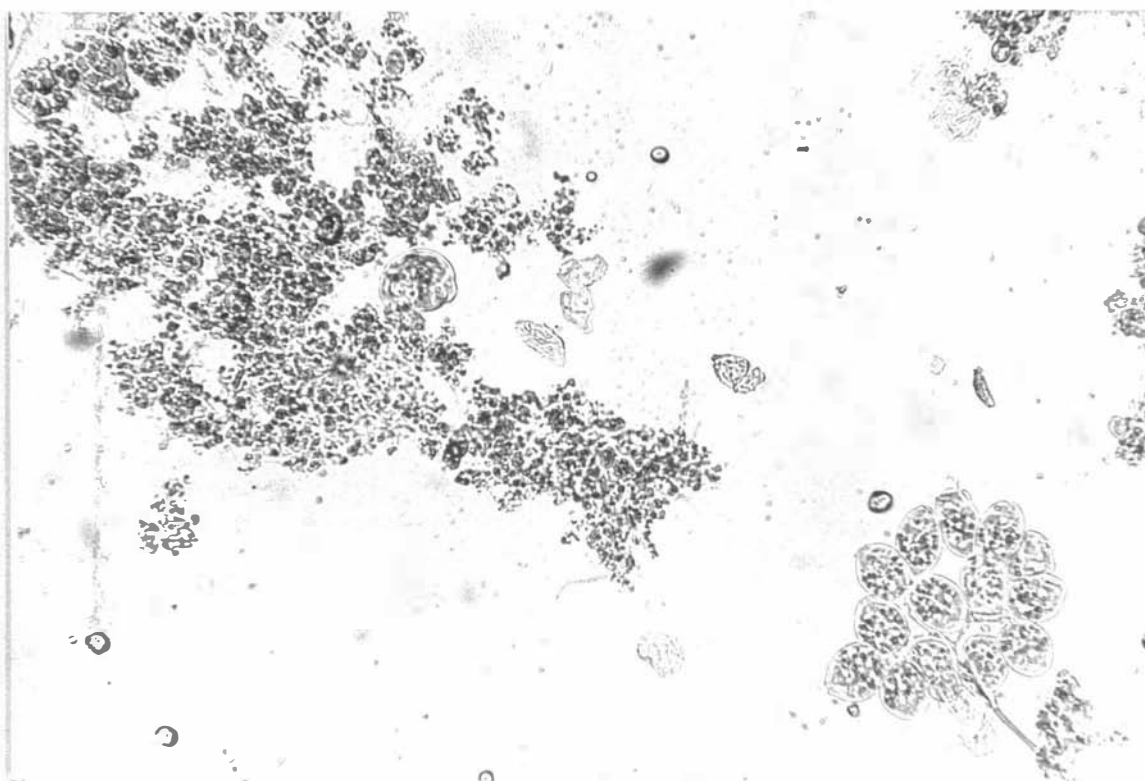
Figure 8.7:   Filament abundance on Day 18 of Trial AE7, SVI = 93 ml.g^{-1}.
(100x magnification)
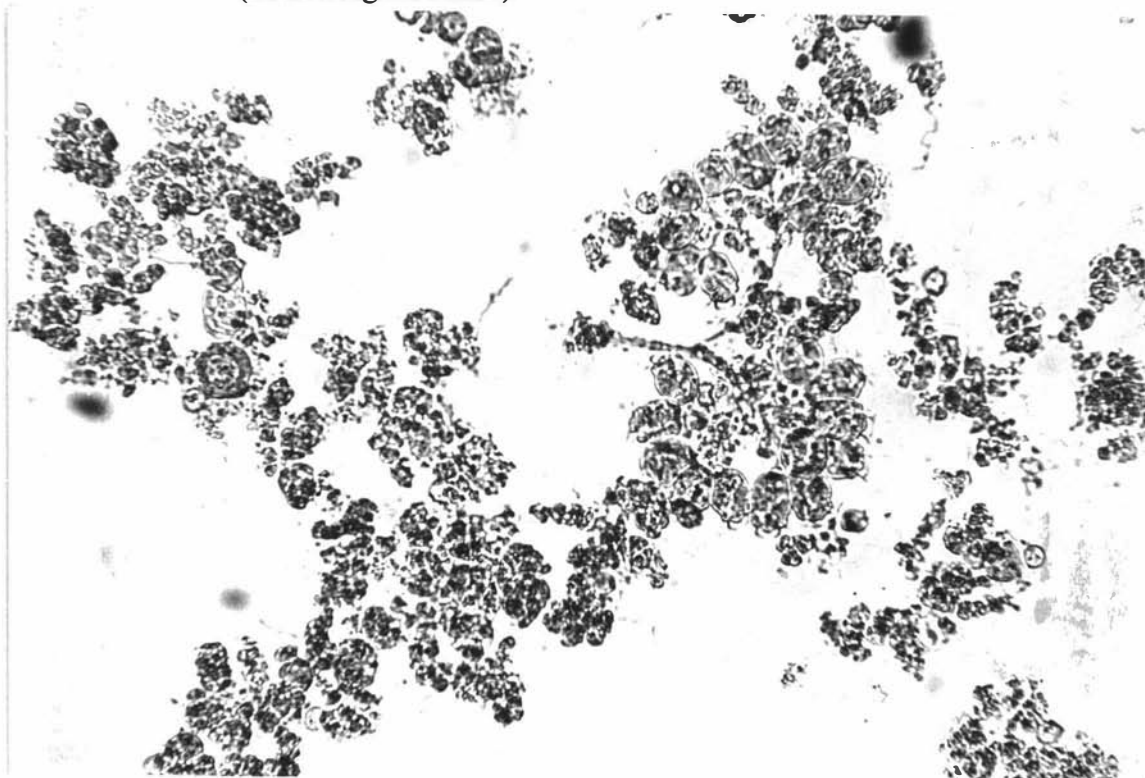
Figure 8.8:   Filament abundance on Day 30 of Trial AE7, SVI = 63 ml.g^{-1}.
(100x magnification)
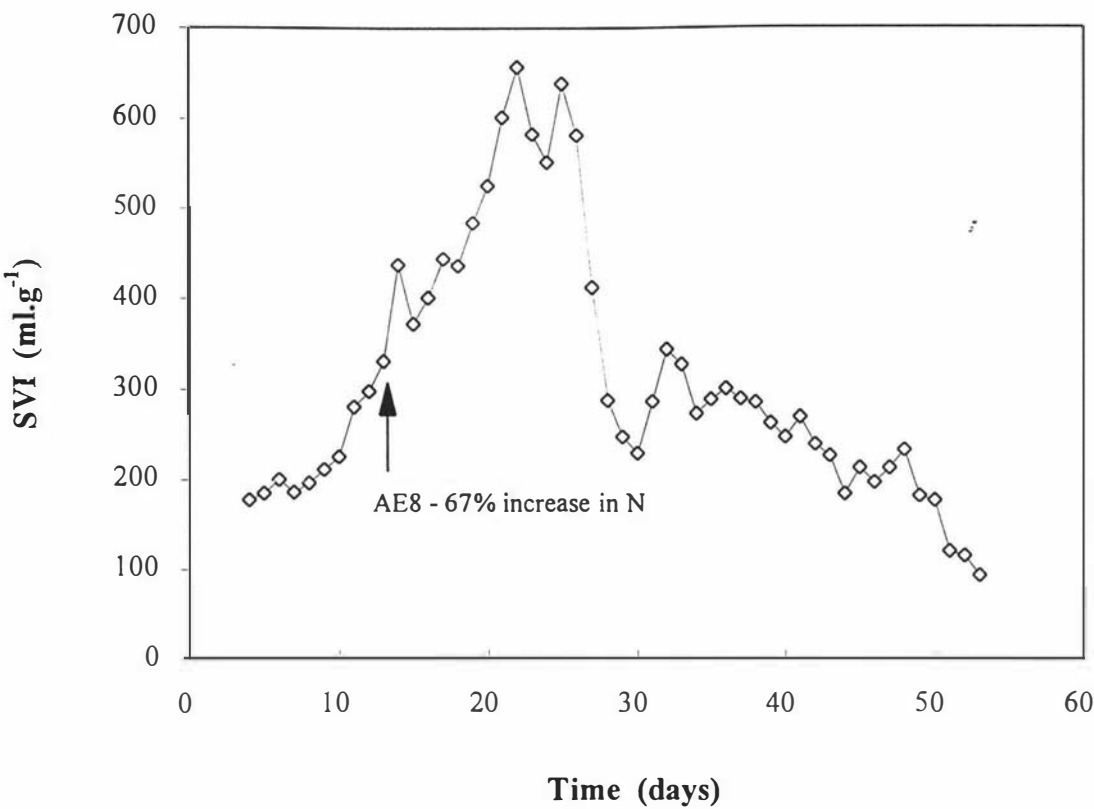
Figure 8.9:   Change in SVI during Trial AE8.
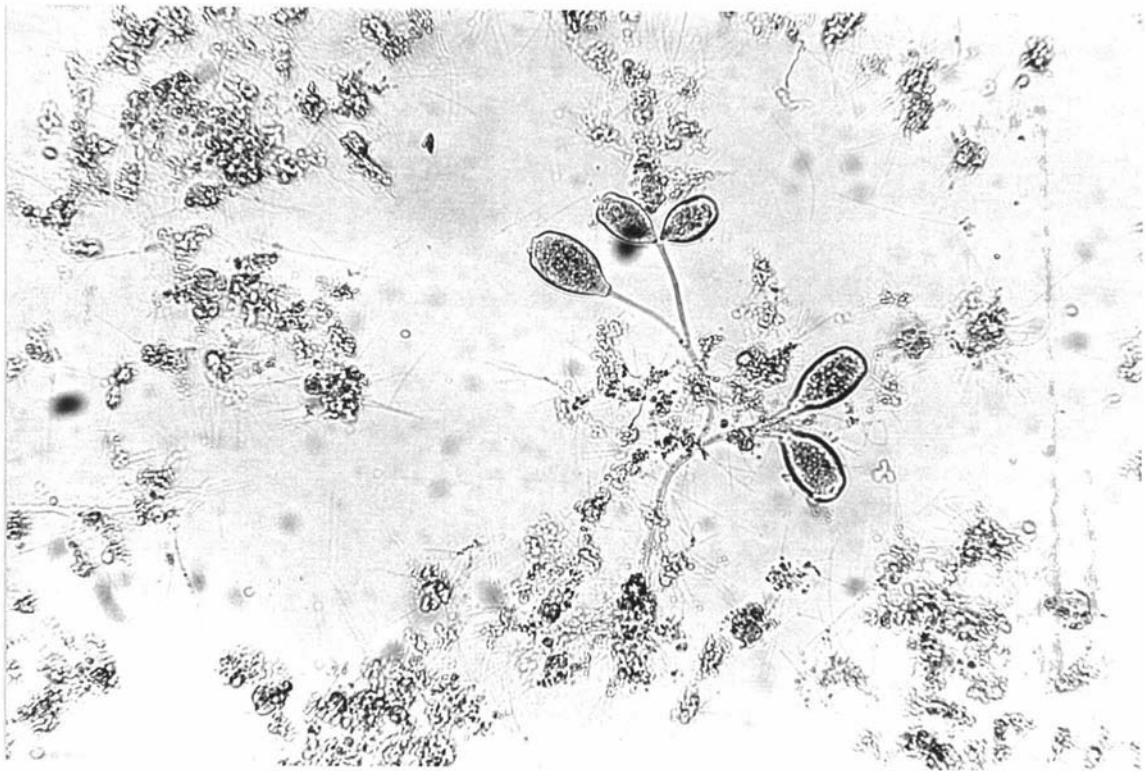
Figure 8.10:   Filament abundance on Day 24 of Trial AE8, SVI = 301 ml.g^{-1}.
(100x magnification)
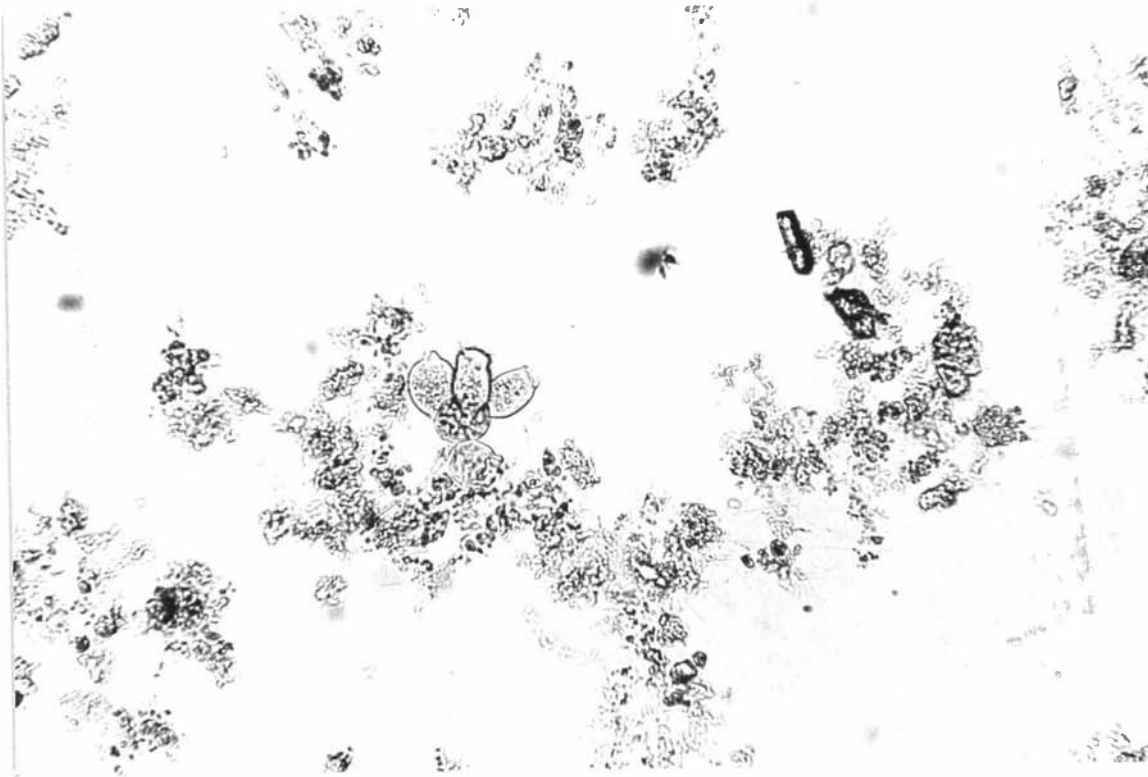
Figure 8.11:   Filament abundance on Day 37 of Trial AE8, SVI = 183 ml.g^{-1}.
(100x magnification)
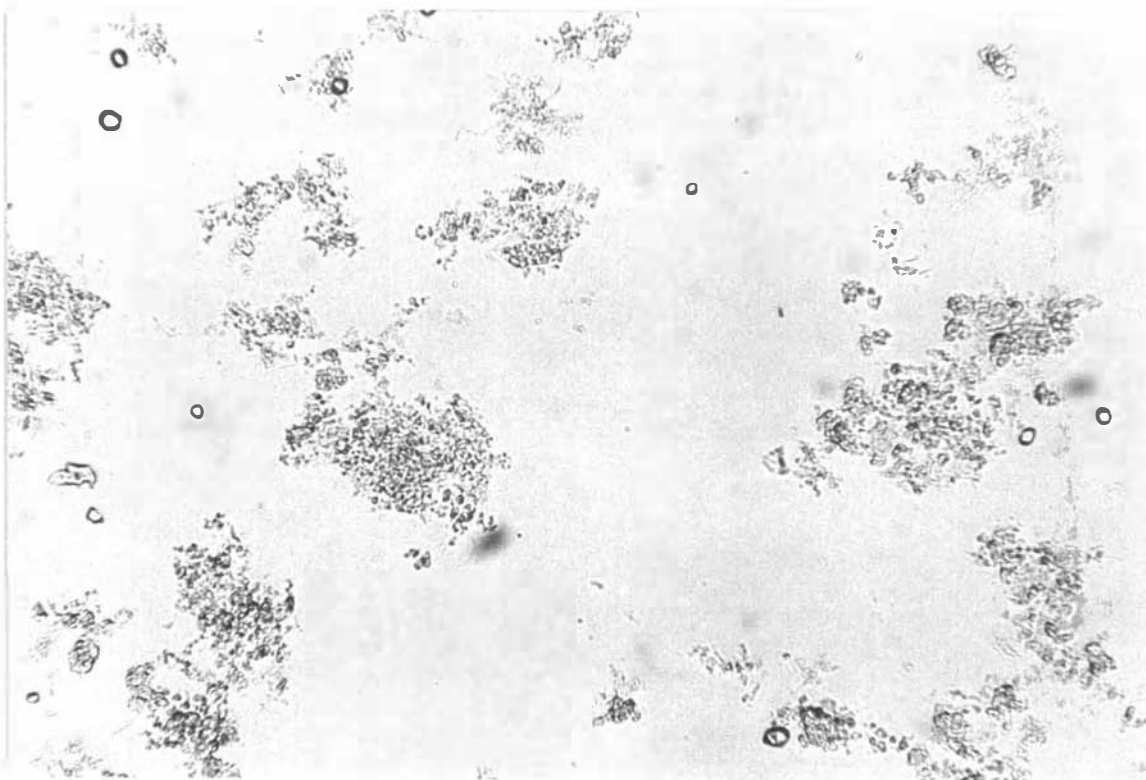
Figure 8.12:   Filament abundance on Day 40 of Trial AE8, SVI = 116 ml.g^{-1}.
(100x magnification)

8.5   Measurement of Kinetic Constants

8.5.1   Soluble COD Removal Rates

Batch soluble COD removal tests were performed as for the preceding trials, but a change in the substrate removal mechanisms operating was indicated as the shape of substrate removal rate curves differed from that previously observed. As shown in Figure 8.13, the initial biosorption phase was followed by a substrate removal curve that was now best fit as a series of two phases: a log removal phase followed by a linear removal phase; rather than a single log removal phase as observed in Trials AE1 to AE5. The removal rates calculated from the batch tests conducted are summarised in Table 8.3.

Table 8.3   Results from batch soluble COD removal rate tests.

Trial	Days at N level	Floc load (gCOD.gVSS^{-1})	Biosorption (gCOD.gVSS^{-1})	log removal rate, k (d^{-1})	Time at rate change (min)	linear removal rate (gCOD.gVSS^{-1}d^{-1})
AE6	10	0.11	0.07	75	12	0.85
	20	0.14	0.10	89	18	0.12
	28	0.11	0.06	86	15	0.76
AE7	4	0.12	0.06	193	6	0.74
	12	0.13	0.10	107	14	0.56
	18	0.11	0.06	223	7	0.38
	30	0.13	0.09	83	21	0.06
AE8	2	0.11	0.08	110	10	0.04
	12	0.16	0.10	189	11	0.44
	26	0.10	0.05	225	5	0.52
	30	0.12	0.07	-	10	0.50
	34	0.14	0.10	107	14	0.59
	40	0.12	0.07	136	7	0.29
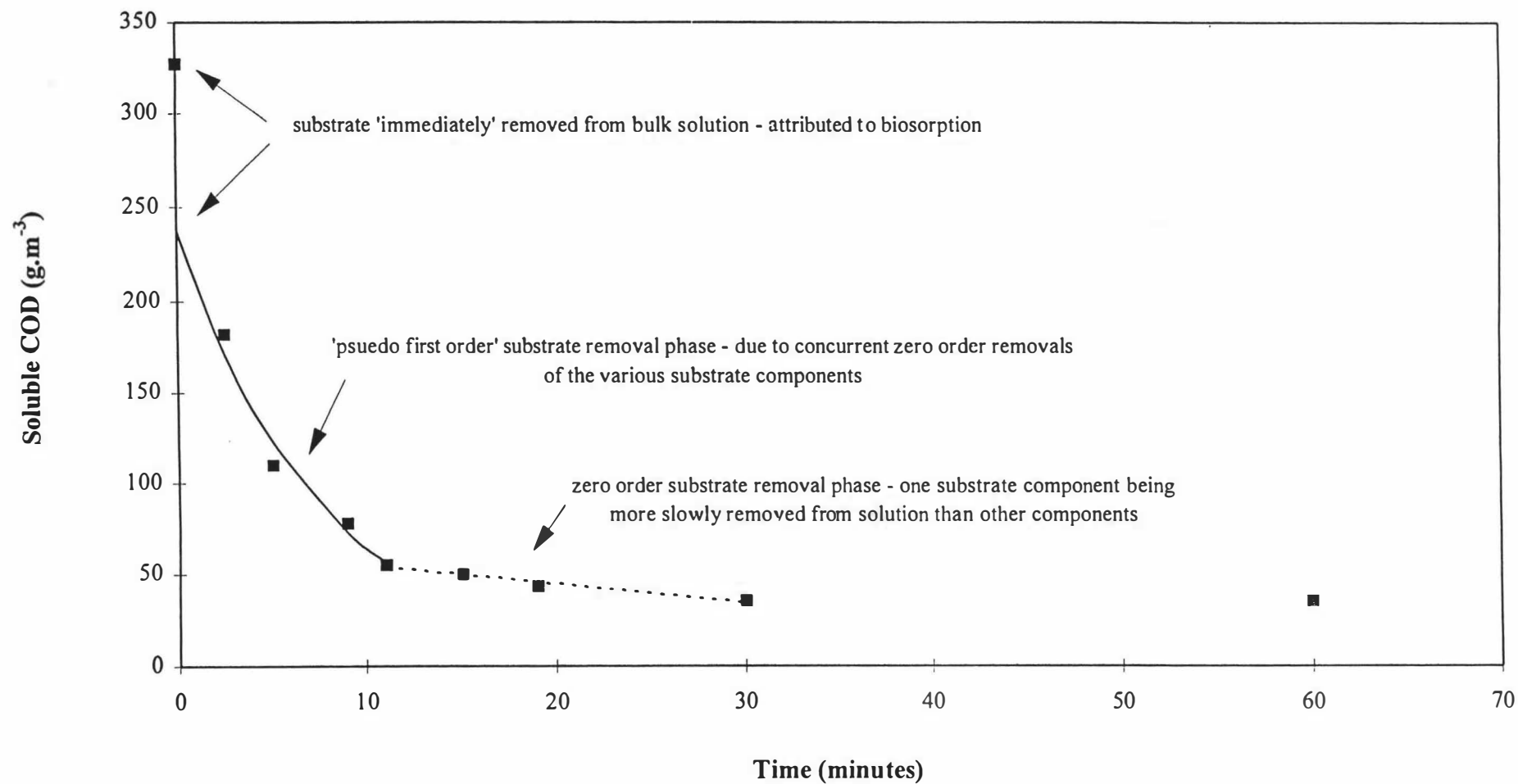
Figure 8.13   Batch soluble COD removal, Day 12 of Trial AE8.

As the 'pseudo first order' removal apparent in previous trials was thought to be due to the concurrent zero order removals of the various substrate components.   The appearance of a distinct zero order removal period at the end of the test indicated that removal of one component had either been inhibited or not increased in the same manner as other substrate components and was now removed after, rather than at the same time as the other components.

Soluble substrate being removed immediately from solution and attributed to biosorption ranged between 0.05 and 0.11g sCOD.gVSS^{-1}, not significantly different from the 0.05 to 0.09 range measured with the originally defined feed. The apparent log removal rates were also in similar ranges, but again with some higher values being measured during the increased feed N content trials. The major difference observed with the use of the modified substrate was therefore the loss of relative removal ability from bulk solution for one of the substrate components.

The oxygen consumed in response to substrate added could not be accurately determined from the above batch tests due to the very high initial OURs resulting in low bulk liquid DO concentrations. This relationship was determined by a second set of batch tests conducted under similar conditions, but with a considerably lower floc loading rate so that initial OURs could still be obtained. The results are listed in Table 8.4 and are slightly lower than the 0.15 values recorded during AE5.

Table 8.4   Oxygen consumption in response to soluble substrate addition.

Trial	Days at SRT	g O_2 consumed per g COD added	
		Average	Range
AE7	4	0.13	0.122 - 0.141
AE7	31	0.14	0.129 - 0.144
AE8	33	0.11	0.114

These figures agree closely with the calculated mass of additional oxygen consumed in the selector system listed in Table 8.5 as determined from OURs that were measured daily in the selector and reactor zones during Trials AE6 to AE8.

Table 8.5   Dissolved oxygen concentrations and uptake rates in the reactor system.

	Trial AE6					Trial AE7					Trial AE8				
	Reactor	Sel A	Sel B	Sel C	Total Selector	Reactor	Sel A	Sel B	Sel C	Total Selector	Reactor	Sel A	Sel B	Sel C	Total Selector
DO ($gO_2.m^{-3}$)	6.6	2.3	3.7	4.3	-	6.3	3.3	3.9	5.2	-	6.3	1.7	3.7	4.3	-
sCOD removed ($g.d^{-1}$)	0.1	15.1	0.7	0.2	16.0	0.2	14.1	1.5	0.4	16.0	0.1	15.2	0.7	0.3	16.2
Based on Total Oxygen Consumption:															
SpOUR (gO2.gVSS.d-1)	0.17	0.56	0.35	0.29		0.16	0.58	0.37	0.34		0.17	0.61	0.38	0.31	
Total O2 consumed (g.d-1)	6.96	1.41	0.92	0.86	3.19	6.69	1.63	1.06	0.98	3.67	6.41	1.49	0.92	0.75	3.15
gO2 used/ g sCOD removed		0.09	1.23	3.92	0.19		0.11	0.73	2.05	0.23		0.09	1.4	2.44	0.18
Based on Oxygen Consumption above endogenous rates: *															
Elevated SpOUR	-	0.38	0.18	0.12		-	0.42	0.21	0.18		-	0.44	0.21	0.14	
gO2 used/ g sCOD removed	-	0.06	0.95	2.68	0.11	-	0.08	0.54	1.76	0.14	-	0.07	1.18	1.16	0.12

* Assumes that the OUR in the reactor is close to endogenous rates due to negligible substrate removal from solution in this zone.

8.5.2   Measurement of Biokinetic Constants

Batch tests to determine K_S and the maximum ΔSpOUR were conducted several times during the two trials at the highest substrate N level, the results obtained are listed in Table 8.6. ATU was added to inhibit nitrification and ensure that OUR was due to the activity of heterotrophic biomass. It can be seen that both ΔSpOUR$_{max}$ and K_S could again be related to SVI with the values of both parameters increasing as the SVI decreased. A value was calculated for μ_{max} using $Y_H = 0.68$ g cell COD. g substrate COD^{-1}, although again the ability of this μ_{max} value to reflect actual microbial growth rates is questionable. However the values obtained were of use for comparing mixed cultures exposed to differing substrate conditions, with the ΔSpOUR$_{max}$ response values being considerably higher than those obtained at a similar SVI with the originally defined substrate.

Table 8.6   Kinetic constants determined during increased feed N content trials.

Trial	Days at SRT	SVI ml.g^{-1}	ΔSpOUR$_{max}$ (gO$_2$.gcell COD.d^{-1})	μ_{max} (d^{-1})	K_S (gCOD.m^{-3})
AE7	4	217	1.49 - 1.54	3.2 - 3.3	87-96
	10	175	1.79	3.8	60
	16	142	1.83	3.9	120
	31	56	3.59	7.6	270
AE8	2	435	0.67	1.4	27
	33	214	1.81	3.8	51
	38	178	1.71	3.6	91

8.5.3   Decay Rate

The biomass decay rate was measured from the decrease in OUR over a 10 day period as in Sections 5.4.4 and 6.3.4. The results listed in Table 8.7 show a possible trend of increasing decay rate with decreasing SVI, which would represent the opposite effect to that reported by Shao and Jenkins (1989) and Chiesa et al. (1985). The actual values obtained were slightly higher than those measured in previous trials.

Table 8.7   Biomass decay rates measured during increased substrate N content trials.

Trial	Days at Feed N Level	SVI (ml.g^{-1})	Decay rate Co-efficient, b (d^{-1})
AE6	21	110	0.26
AE7	20	71	0.30
AE8	13	638	0.24
AE8	41	94	0.33

8.6   Nitrogen and Phosphorus removal.

Concentrations of various N and P species in the biomass and liquid phases were measured every second day for the duration of the higher substrate N trials.   The average concentrations observed during each trial are shown in Tables 8.8 to 8.10.

Ammonia concentrations in the selector zones were higher than those measured during Trial AE5 with the originally defined substrate, as was expected due to the increased ammonia loading in the substrate.   A low but detectable ammonia concentration of between 0.1 and 0.2 gN.m^{-3} was measured in the reactor zone in just under half of the data collected, suggesting that reactor residence time was close to the minimum required for complete nitrification in this trial, assuming that ammonification of feed stream proteins was complete.

The lower concentration of nitrate in the selectors and effluent compared to that in the reactor indicated that denitrification was still occurring in the former zones, however a nitrate concentration of above 6 gN.m^{-3} was consistently measured in the first selector, considerably higher than the 2.8 gN.m^{-3} level recorded for Trial AE5.   Nitrite concentrations showed an immediate increase in all reactor zones, declining within 5 days to low levels in the reactor, but remaining higher in all three selector zones.
Variations in the mixed liquor TKN values again reflected variations in the MLSS concentrations, with the N content of the biomass remaining constant in all reactor zones.

DRP concentrations decreased through the selector and reactor zones, and a decline in effluent DRP was observed as the trial progressed indicating a continued increase in the extent of biological P removal. During Trial AE6 the SVI remained at low levels until Day 25, when an increase in SVI from around 120 to 160 ml.g^{-1} was observed. No obvious trends in the concentration of any N or P species was observed at this time.

Table 8.8   N and P concentrations during Trial AE6 at 33% higher substrate N.

(Days 1 - 30)	Selector A	Selector B	Selector C	Reactor	Effluent
TKN (gN.m^{-3})	505	546	566	478	2.7
NH$_3$ (gN.m^{-3})	9.8	11.4	10.7	0.07	0.04
NO$_3$ (gN.m^{-3})	12.4	11.3	13.5	27.6	22.6
NO$_2$ (gN.m^{-3})	2.7	2.0	1.9	0.98	0.85
TP (gP.m^{-3})	97	105	111	94	0.70
DRP (gP.m^{-3})	0.94	0.50	0.34	0.36	0.37
Organic N / VSS	0.114 ± 0.027	0.116 ± 0.017	0.113 ± 0.013	0.118 ± 0.014	-
Organic P / VSS	0.022 ± 0.004	0.023 ± 0.003	0.022 ± 0.002	0.023 ± 0.003	-

Trial AE7 introduced a further increase in substrate ammonia concentration, which resulted in an almost immediate shift to higher ammonia and nitrate concentrations throughout the whole reactor system, as shown in Figures 8.14 and 8.15. The nitrate concentrations were similar in the three selector zones and significantly greater in the reactor, with variations in the selector nitrate levels during the trial being mirrored in the reactor nitrate variations. Figure 8.16 shows that nitrite concentrations remained unchanged on average from that measured in the previous trial, although nitrite levels in all zones demonstrated an increasing trend as the trial progressed.

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Table 8.9   N and P concentrations during Trial AE7: at 67% higher substrate N.

(Days 1 to 32)	Selector A	Selector B	Selector C	Reactor	Effluent
TKN $(gN.m^{-3})$	618	618	604	506	13
NH$_3$ $(gN.m^{-3})$	27.6	29.6	27.2	9.5	12.8
NO$_3$ $(gN.m^{-3})$	23.7	25.5	27.6	47.7	47.5
NO$_2$ $(gN.m^{-3})$	2.7	1.8	1.9	1.1	1.2
TP $(gP.m^{-3})$	114	113	111	102	0.85
DRP $(gP.m^{-3})$	0.75	0.53	0.26	0.12	0.24
Organic N / VSS	0.118 ± 0.012	0.118 ± 0.013	0.118 ± 0.017	0.116 ± 0.020	-
Organic P / VSS	0.023 ± 0.002	0.023 ± 0.003	0.023 ± 0.003	0.024 ± 0.007	-
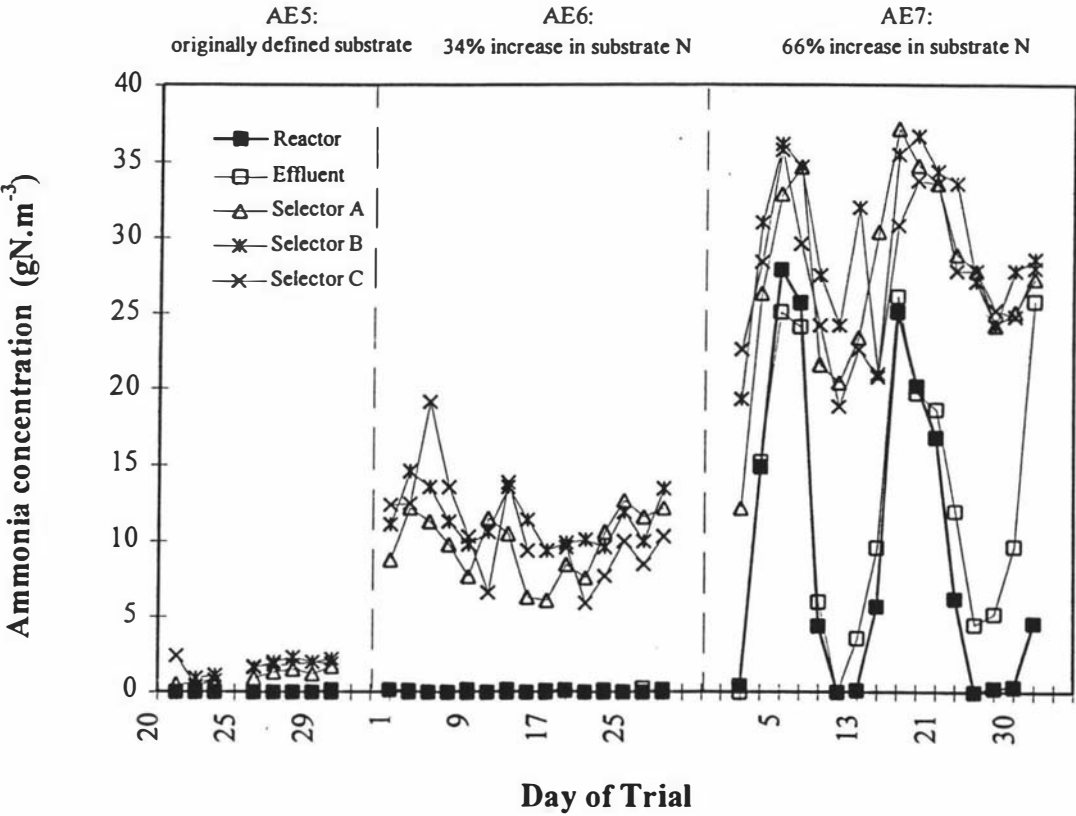
Figure 8.14:   Ammonia concentration trends during the trials in Reactor System 2.
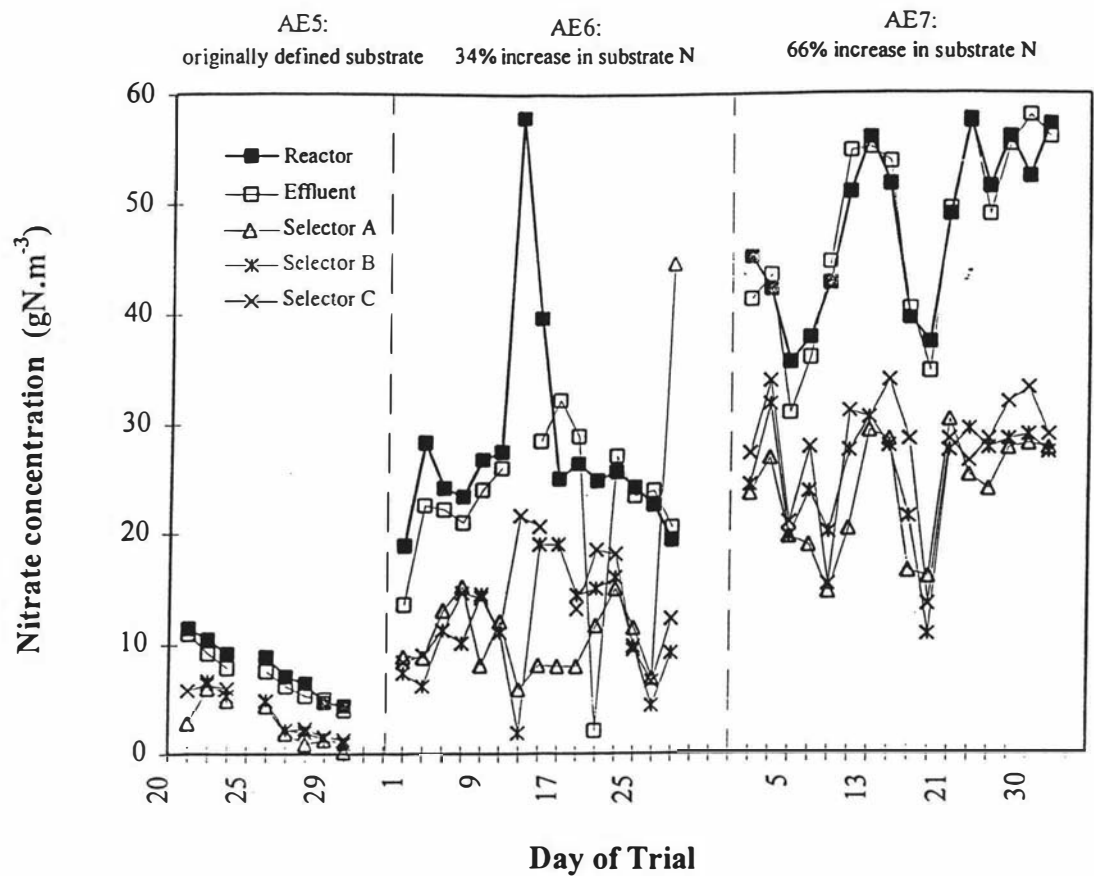
Figure 8.15: Nitrate concentration trends during the trials in Reactor System 2.
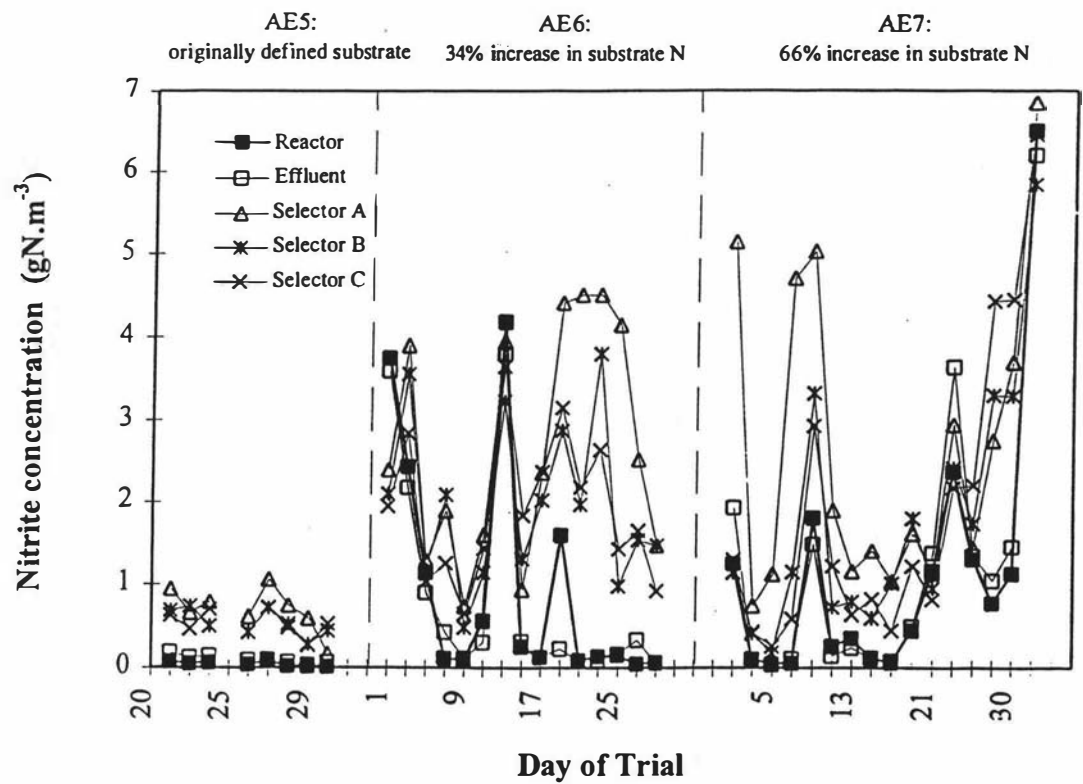
Figure 8.16: Nitrite concentration trends during the trials in Reactor System 2.

The reactor ammonia concentration increased to significant levels during two periods of AE7, reaching a maximum of 28 $gN.m^{-3}$ on Day 5 and of 25 $gN.m^{-3}$ on Day 17. Ammonia concentrations in the selectors also increased during the same periods, but not to the same extent as in the reactor. Nitrate concentrations in the reactor decreased by a similar magnitude when the ammonia levels increased, suggesting that nitrification was being suppressed during those periods.

Table 8.10   N and P concentrations during Trial AE8: 67% higher substrate N.

(Days 11 - 41)	Selector A	Selector B	Selector C	Reactor	Effluent
TKN $(gN.m^{-3})$	560	516	490	459	14.7
NH_3 $(gN.m^{-3})$	18.06	17.49	18.4	10.78	10.7
NO_3 $(gN.m^{-3})$	34.63	41.64	42.7	50.55	47.9
NO_2 $(gN.m^{-3})$	5.43	3.67	3.86	2.66	2.0
TP $(gP.m^{-3})$	113	101	101	99	2.6
DRP $(gP.m^{-3})$	2.27	2.06	1.96	1.36	1.7
Organic N / VSS	0.126 ± 0.027	0.121 ± 0.020	0.118 ± 0.020	0.121 ± 0.014	-
Organic P / VSS	0.026 ± 0.005	0.026 ± 0.005	0.026 ± 0.005	0.026 ± 0.005	0.026 ± 0.005

Trial AE8 used the same substrate as AE7, but both the biomass SVI and recycle rate in AE8 were higher. As can be seen in Figure 8.17, two periods of high reactor ammonia were also seen during this trial, however the difference between final selector and reactor ammonia concentration remained fairly constant during Trial AE8 unlike in AE7. The reactor nitrate concentration again showed a decrease during the high reactor ammonia events, suggesting inhibition of nitrification.   Although average nitrate concentrations in the reactor were similar, concentrations in the selector zones were significantly higher during Trial AE8 than AE7, reflecting the higher recycle rate and indicating that the degree of denitrification in the selector zones may have decreased. The concentrations of nitrite were also considerably higher throughout the reactor system in AE8 than in AE6 or AE7, again showing a very high peak in the selectors after the change in substrate, but settling to lower levels within one SRT.
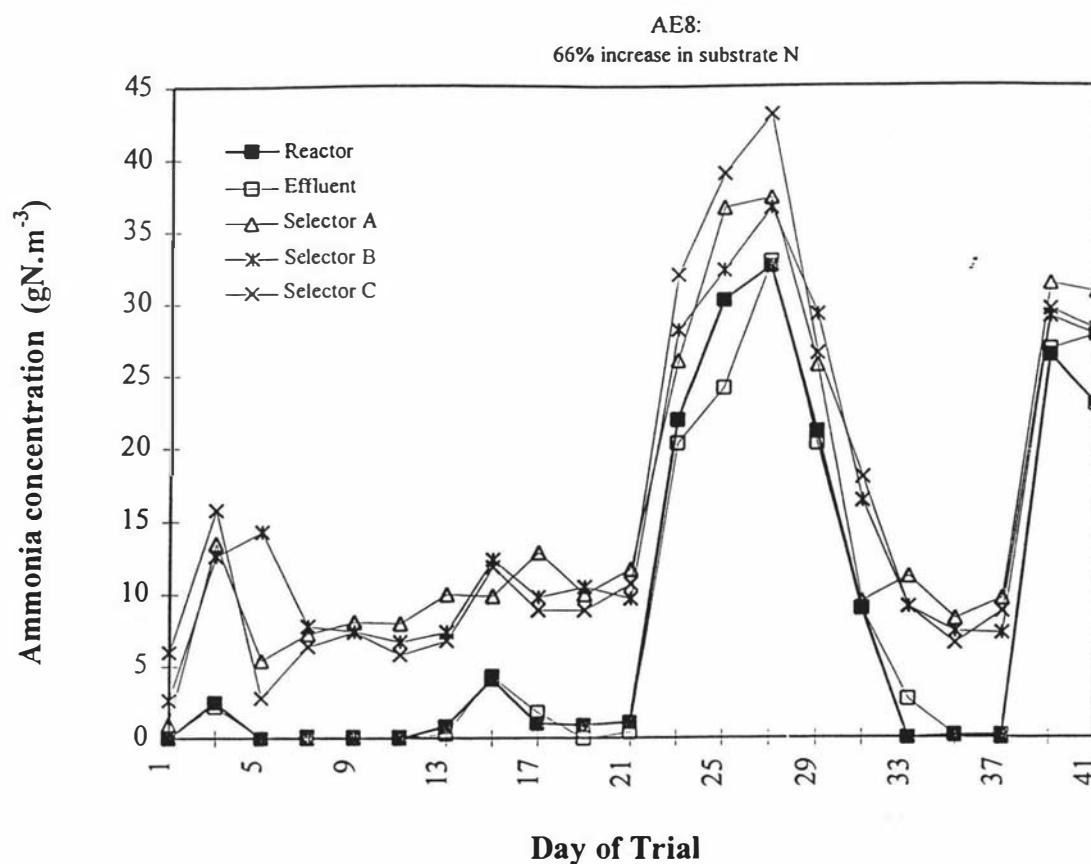
Figure 8.17:   Ammonia concentration trends during the trials in Reactor System 1.
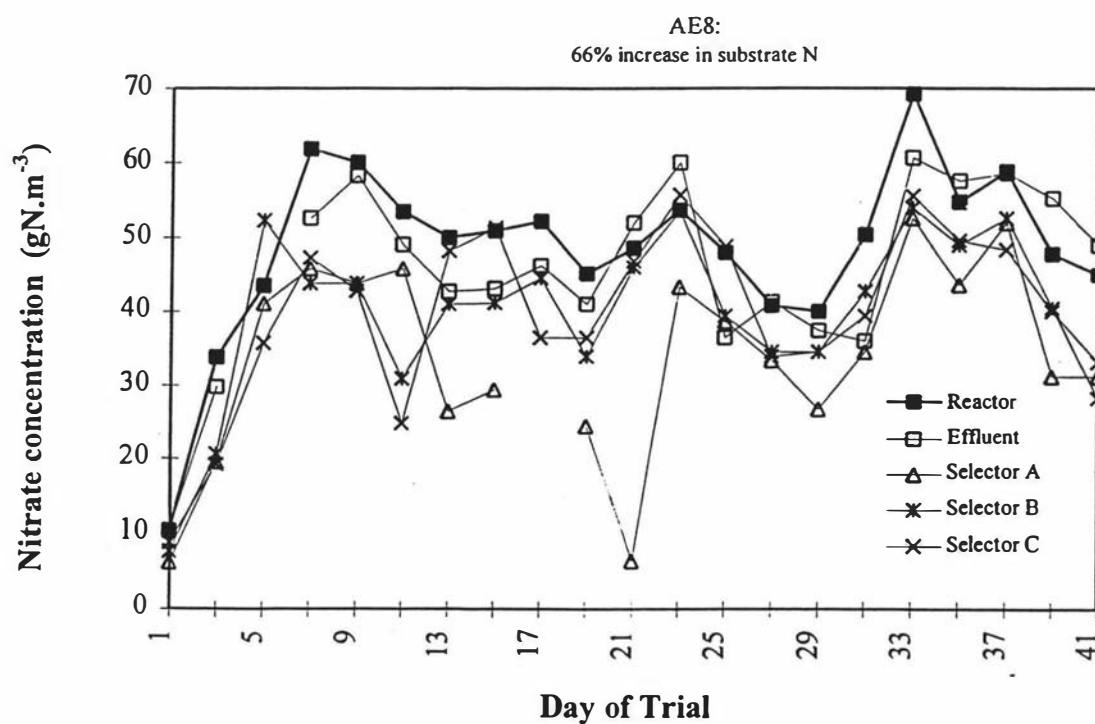

Figure 8.18:   Nitrate concentration trends during the trials in Reactor System 1.
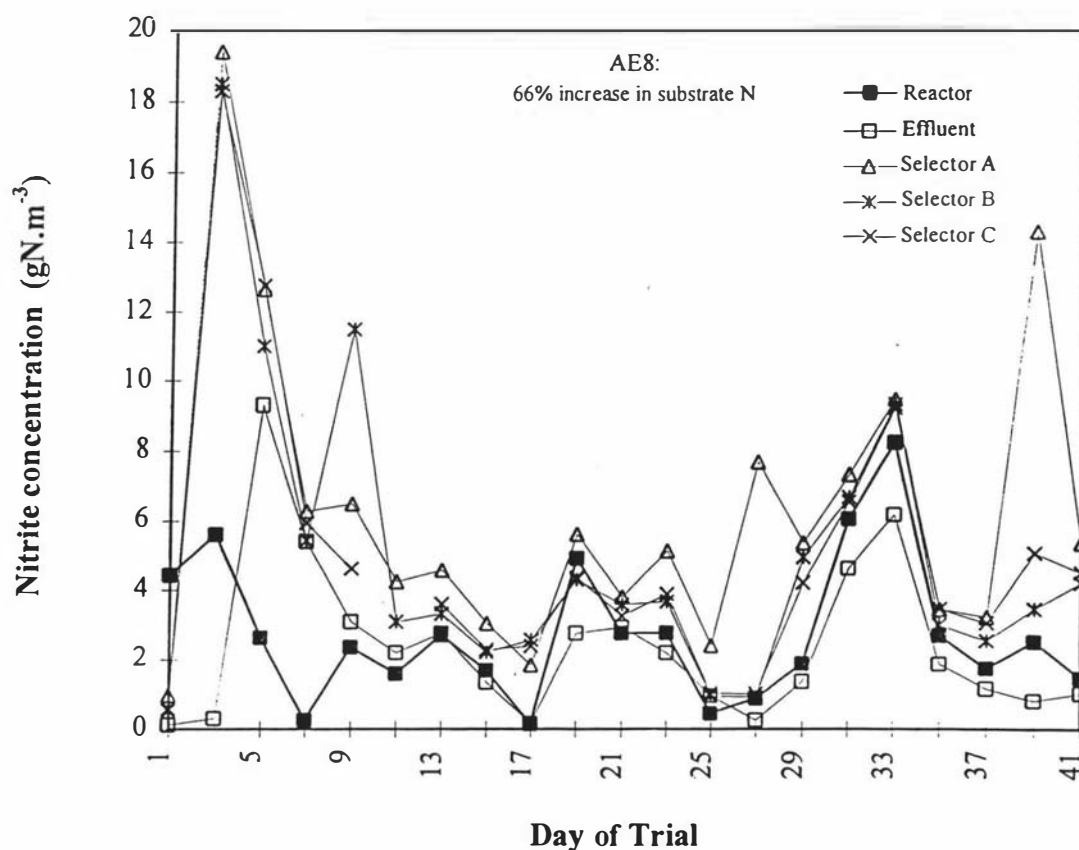
Figure 8.19:   Nitrite concentration trends during the trials in Reactor System 1.

8.6.1   Ammonification

The ammonia concentrations in the various reactor system zones are shown in Figures 8.14, 8.17 and 8.21. The concentrations measured were similar in all the three selector zones, generally peaking in Selector B during Trials AE6 and AE7, and in Selector C during AE8, indicating that ammonification was occurring in the selectors. Figure 8.22 illustrates that the ammonia concentration leaving Selector A was lower than that calculated to be entering that zone via the substrate and RAS streams, indicating that nitrification was also occurring.

As the level of organic N in the substrate was unchanged, the ammonification rate would have been expected to depend on the substrate concentration in the selectors, as determined by the recycle rate. From the data given in Table 8.2, the highest rate of ammonification would have been expected in AE7 and the lowest in AE8, however due to the presence of ammonia in the modified substrate and the occurrence of
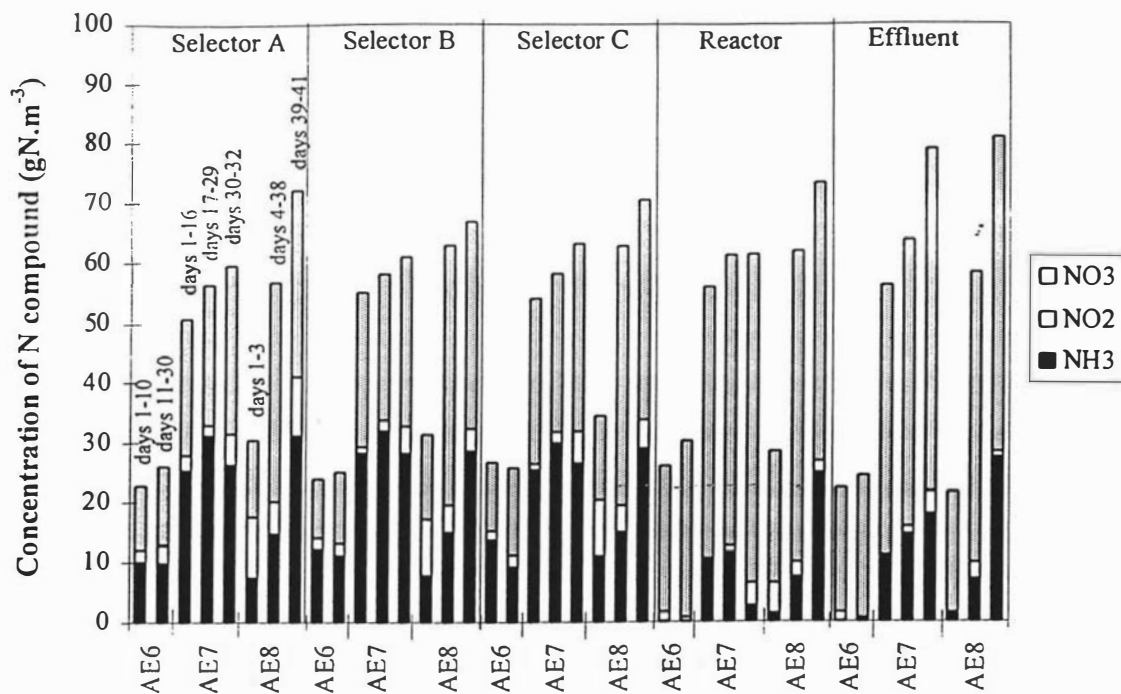
Figure 8.20: Average concentrations of ammonia, nitrate and nitrite during Trials AE6, AE7 and AE8, by reactor zones.
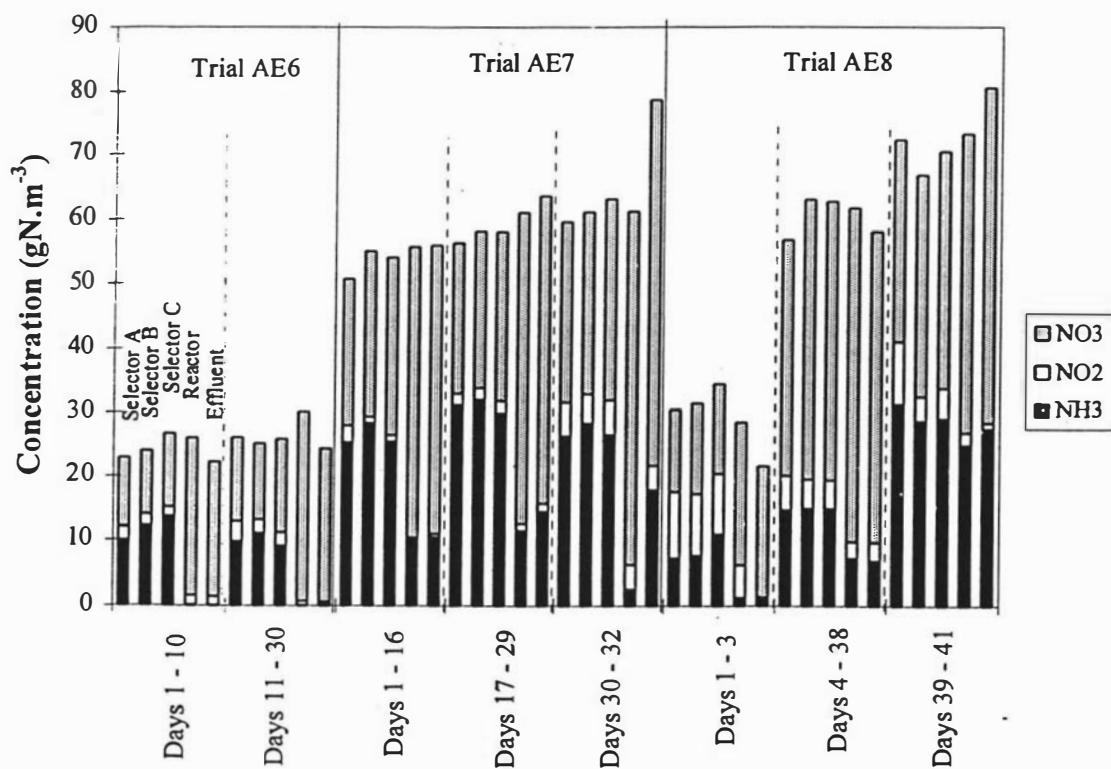
Figure 8.21: Average concentrations of ammonia, nitrate and nitrite in reactor zones during Trials AE6, AE7 and AE8.
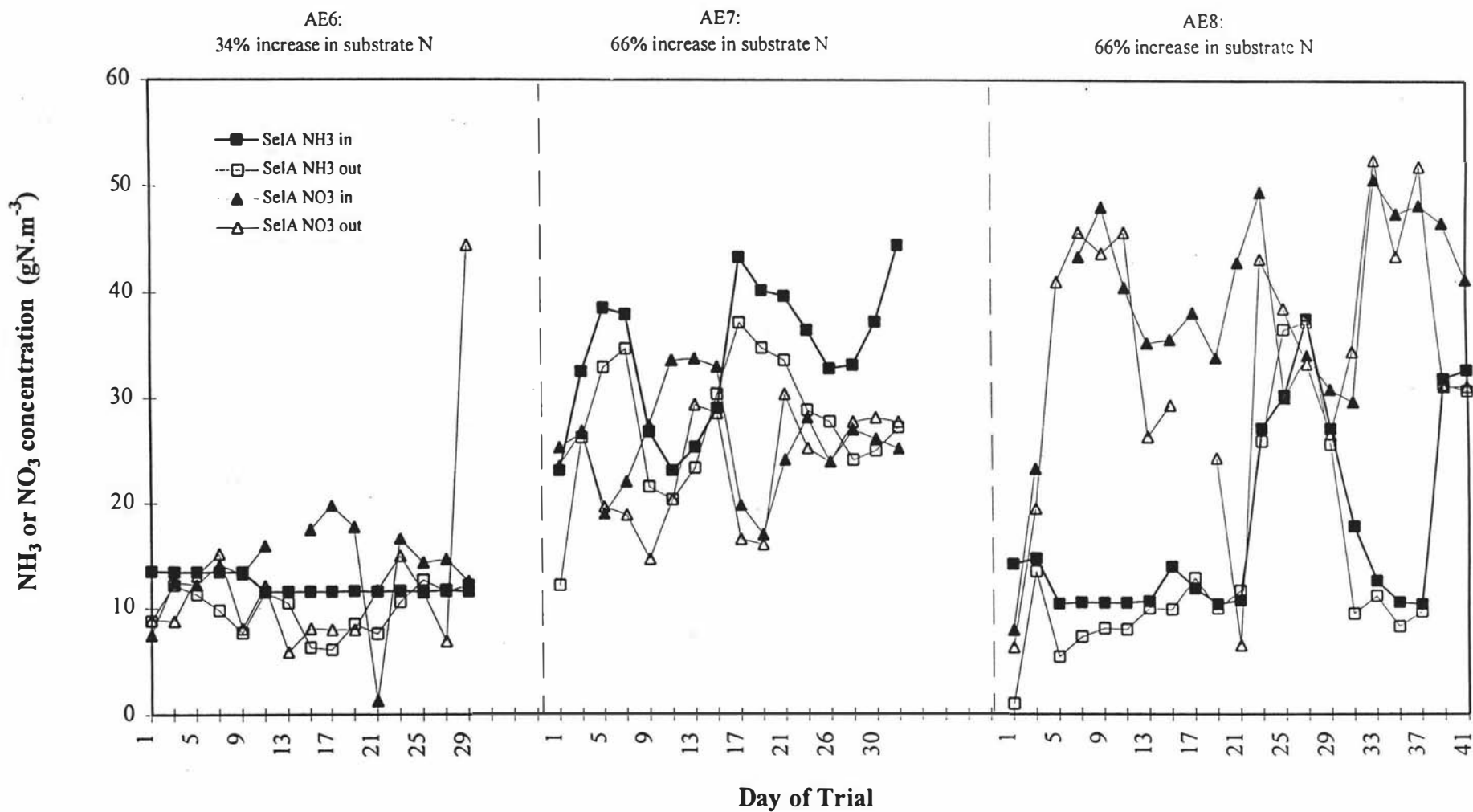
Figure 8.22   Concentration of ammonia and nitrate flowing into and out of the first selector zone during Trials AE6, AE7 and AE8.

simultaneous nitrification in the selectors, estimates of the ammonification rate and any evidence of variations in that rate would have been difficult to determine.

The data in Table 8.11 lists the change in ammonia concentration through each reactor zone during the various recycle rates in each trial, where the change in ammonia concentration represents the ammonia generated via ammonification minus the ammonia utilised by nitrification. The consistent negative values for Selector A and the reactor indicate that nitrification exceeded ammonification in these zones during all trials. The value became less negative as each trial progressed, indicating that either the ammonification rate increased or nitrification rate decreased in each case. As the change in Selector A sCOD and reactor residence time did not consistently increase, increases in the ammonification rate or extent of ammonification could not be assumed.

8.6.2   Nitrification

In each case, an increase in substrate ammonia concentration resulted in an increased N compound concentration for all reactor zones. It can be seen from Figures 8.16 and 8.19 that nitrite concentrations, particularly in the selector zones, increased immediately in response to an increase in substrate N content, then declined from the initial peak values to a more stable level within 5 to 10 days. This indicated that the increase in substrate ammonia content may have inhibited *Nitrobacter* activity for a short period, causing an accumulation of nitrite.

Increasing the concentration of ammonia in the substrate also resulted in an immediate increasing trend in effluent nitrate concentration, which reached a stable new level within the same 5 to 10 day period. This gradual increase to a higher effluent nitrate level indicated there must have been an increase in the number of nitrifying bacteria in the reactor biomass, otherwise an instantaneous increase to a new higher effluent nitrate level would have been observed. Azimi and Horan (1991) suggested that the maximum specific growth rate of nitrifiers could be estimated from a plot of the logarithm of effluent oxidised N species against time. As shown in Figure 8.23, such an analysis resulted in an estimate of 0.6 to 1.2 d^{-1} for the nitrifier maximum specific growth rate, which compared well to the values of 0.89 to 1.17 d^{-1} reported by Azimi and Horan (1991) and 0.3 to 3.0 stated in Metcalf and Eddy (1991), considering the limited data available. The value at 'Day 0' was derived as the average value of the last five daily measurements taken before the substrate was changed.

Table 8.11: Change in mass of oxidsed N and ammonia through the various reactor zones during Trials AE6, AE7 and AE8.

Trial	AE6	AE6	AE7	AE7	AE7	AE8	AE8	AE8
Days at SRT	1 - 10	11 - 30	1 - 16	17 - 29	30 - 32	1 - 4	4 - 38	39 - 41
Oxidised N in effluent $(gN.d^{-1})$	0.225	0.248	0.455	0.492	0.606	0.210	0.519	0.527
Change in oxidised N in Selector A $(gN.d^{-1})$	0.015	0.036	-0.065	0.023	0.106	0.327	-0.007	-0.257
Change in oxidised N in Selector B $(gN.d^{-1})$	-0.024	-0.057	0.035	0.019	-0.009	0.026	0.340	-0.182
Change in oxidised N in Selector C $(gN.d^{-1})$	0.028	0.068	0.049	0.040	0.070	-0.009	-0.017	0.201
Change in oxidised N in Reactor $(gN.d^{-1})$	0.286	0.347	0.444	0.418	0.401	0.161	0.382	0.457
Change in oxidised N in settler $(gN.d^{-1})$	-0.083	-0.152	-0.008	-0.008	0.04	-0.302	-0.185	0.313
Ammonia in effluent $(gN.d^{-1})$	0.0004	0.0005	0.104	0.141	0.162	0.012	0.069	0.270
Change in ammonia in Selector A $(gN.d^{-1})$	-0.078	-0.048	-0.113	-0.128	-0.268	-0.318	-0.086	-0.081
Change in ammonia in Selector B $(gN.d^{-1})$	0.048	0.032	0.079	0.016	0.036	0.017	0.014	-0.017
Change in ammonia in Selector C $(gN.d^{-1})$	0.033	-0.049	-0.075	-0.041	-0.033	0.144	0.001	0.026
Change in ammonia in Reactor $(gN.d^{-1})$	-0.301	-0.234	-0.400	-0.360	-0.436	-0.427	-0.439	-0.272
Change in ammonia in settler $(gN.d^{-1})$	-0.001	-0.0003	0.015	0.057	0.279	-0.004	-0.021	0.168

Note: 1. negative values indicate removal and positive values indicate accumulation.
2. change in oxidised N = (nitrification - denitrification)
3. change in ammonia = (ammonification - nitrification)
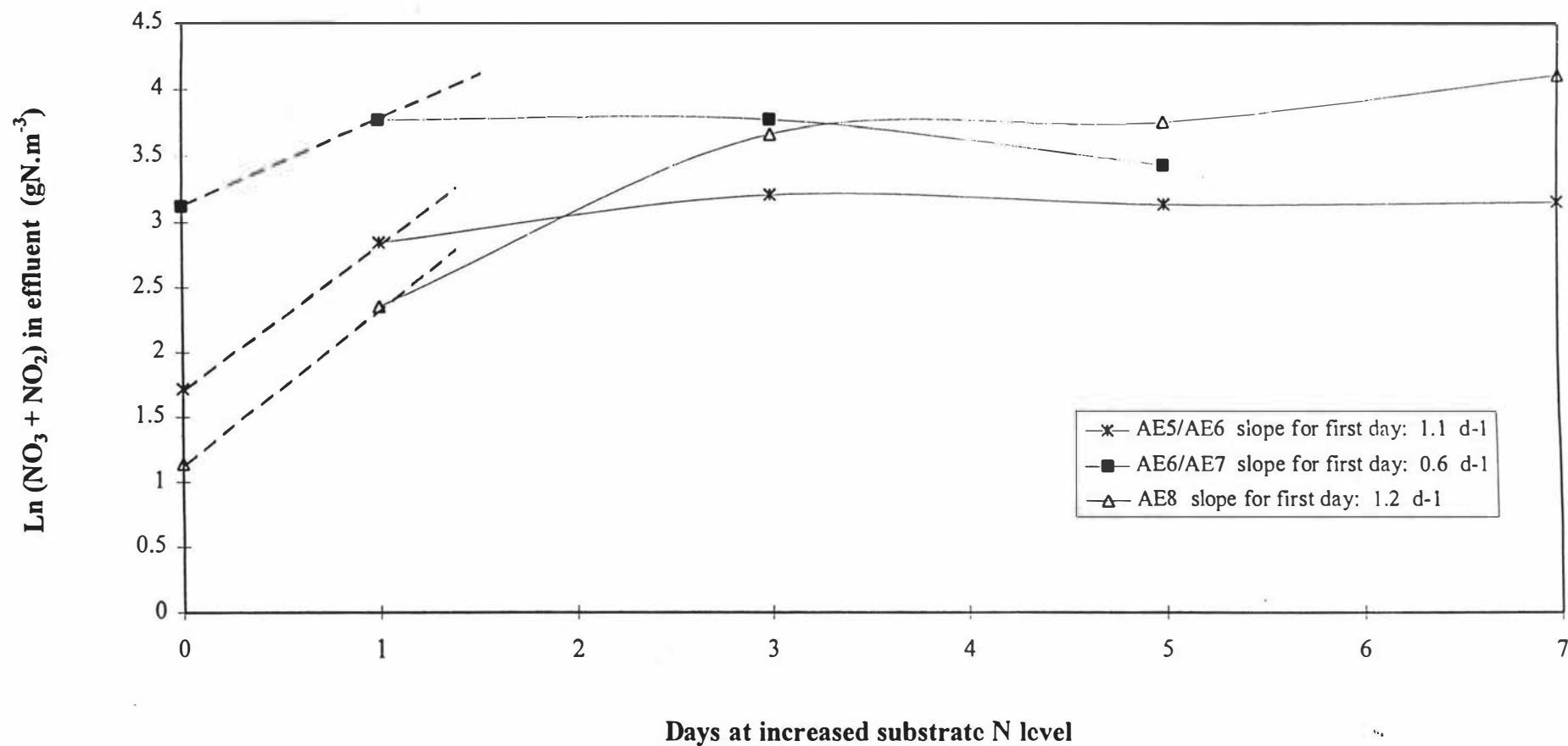
Figure 8.23   Estimation of maximum growth rate of nitrifiers from effluent nitrate and nitrite concentrations after a change in substrate N level.

The ammonia concentrations into and out of Selector A as depicted in Figure 8.22 show a decrease through this zone which indicated that nitrification was occurring, with the overall mass of ammonia removed being listed in Table 8.11. The concentrations of ammonia flowing into Selector A increased during Trials AE7 and AE8 due to increasing ammonia in the RAS, and although selector nitrate levels also increased, they did so to a lesser extent, indicating that the high ammonia levels may have had an inhibitory effect on nitrification rates.

Nitrification in the initial selector zone was expected due to the high ammonia concentrations, with the bulk liquid DO concentration averaging 2.2, 3.3 and 1.7 in the first selector during Trials AE6, AE7 and AE8 respectively. These values were above the minimum DO concentration generally reported as necessary for nitrification to occur (Stenstrom and Song, 1991; Metcalf and Eddy, 1991). As nitrification was occurring in the first selector zone, it would have also been occurring in Selectors B and C where conditions were more fully aerobic, although the slight increase in ammonia concentrations through these zones indicated that the rate of nitrate accumulation was less than the rate of ammonia accumulation in Selector B for all trials and in Selector C for some trials.

The data in Table 8.11 indicate that the greatest mass of ammonia was consumed and oxidised N compounds accumulated in the reactor zone, therefore nitrification was the predominant reaction in this zone. The reactor ammonia concentration was less than $<0.1 \text{ gNH}_3\text{-N.m}^3$ consistently during Trial AE6, periodically during Trial AE7, and very occasionally during Trial AE8; indicating complete nitrification during the trial at the first level of increased substrate N, but intervals of incomplete nitrification when the substrate ammonia loading was increased further.

As the ammonia concentration in the reactor was negligible in just over half of the results obtained, it was possible that the reactor residence time was now close to the minimum required for complete nitrification of incoming ammonia and substrate proteins undergoing ammonification in the reactor zone. As for previous sets of trials, an estimate of the minimum nitrification rates can be calculated from the increase in concentration of oxidised N species across the reactor if it is assumed that denitrification in the reactor zone is negligible. The results obtained from such an analysis are given in Table 8.12.
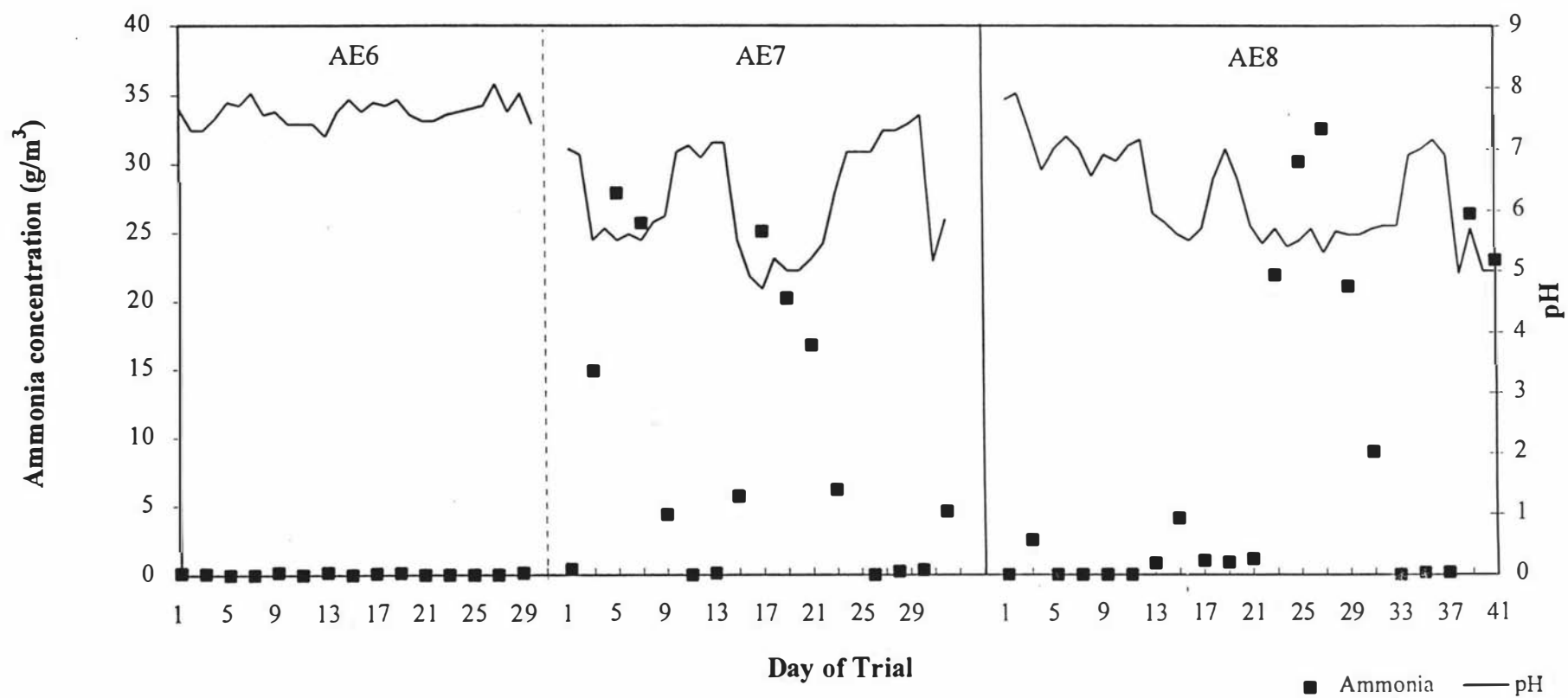
Figure 8.24 Ammonia concentration and pH in the reactor zone during Trials AE6, AE7 and AE8.

Table 8.12   Estimation of nitrification rates from reactor NO_3 and NO_2 concentrations.

Trial	AE6	AE7	AE8
Average estimated rate ($gN.g\ VSS^{-1}.d^{-1}$)	0.005	0.01	0.01
Highest estimated rate ($gN.g\ VSS^{-1}.d^{-1}$)	0.02	0.02	0.04

The specific nitrification rates estimated from reactor nitrate and nitrite levels were higher than those in Trials AE1 to AE5 due to the increased ammonia loading, but again considerably below those reported in the literature (Argaman and Brenner, 1986; Metcalf and Eddy, 1991; McClintock et al., 1993), of between 0.03 and 2.3 $gN.gMLVSS^{-1}d^{-1}$ suggesting that either nitrification was being inhibited or that denitrification was still occurring in the reactor.

The periods of high ammonia concentration in the reactor zone during Trials AE7 and AE8 were accompanied by a lower mixed liquor pH as shown in Figure 8.24. As nitrification reactions consume alkalinity, a low bulk solution pH would have hindered further nitrification. The optimal pH for nitrification has been found to be around 7.9 (Wong-Chong and Loehr, 1975; Antoniou et al., 1990) with a decrease in nitrification resulting when the pH drops below 7.2 (Metcalf and Eddy, 1991); therefore the reactor pH values recorded of below pH 6 would have had a severe negative effect. The nitrification process involves the activity of both *Nitrosomonas sp.* and *Nitrobacter sp.*, and in this case the activity of both microorganisms must have been inhibited as no nitrite accumulation occurred, as can be expected during elevated ammonia concentrations (Wong-Chong and Loehr, 1975; Azimi and Horan, 1991). The high ammonia concentrations seen to therefore have been a result of, rather than the cause of the decrease in nitrification efficiency.

8.6.3   Denitrification

The mass of nitrogen removed from the system per day by denitrification was calculated from a mass balance across the entire system as previously, with the results obtained being shown in Table 8.13 and Figures 8.25 and 8.26. It can be seen that as the mass of N removed by denitrification remained relatively constant during Trial
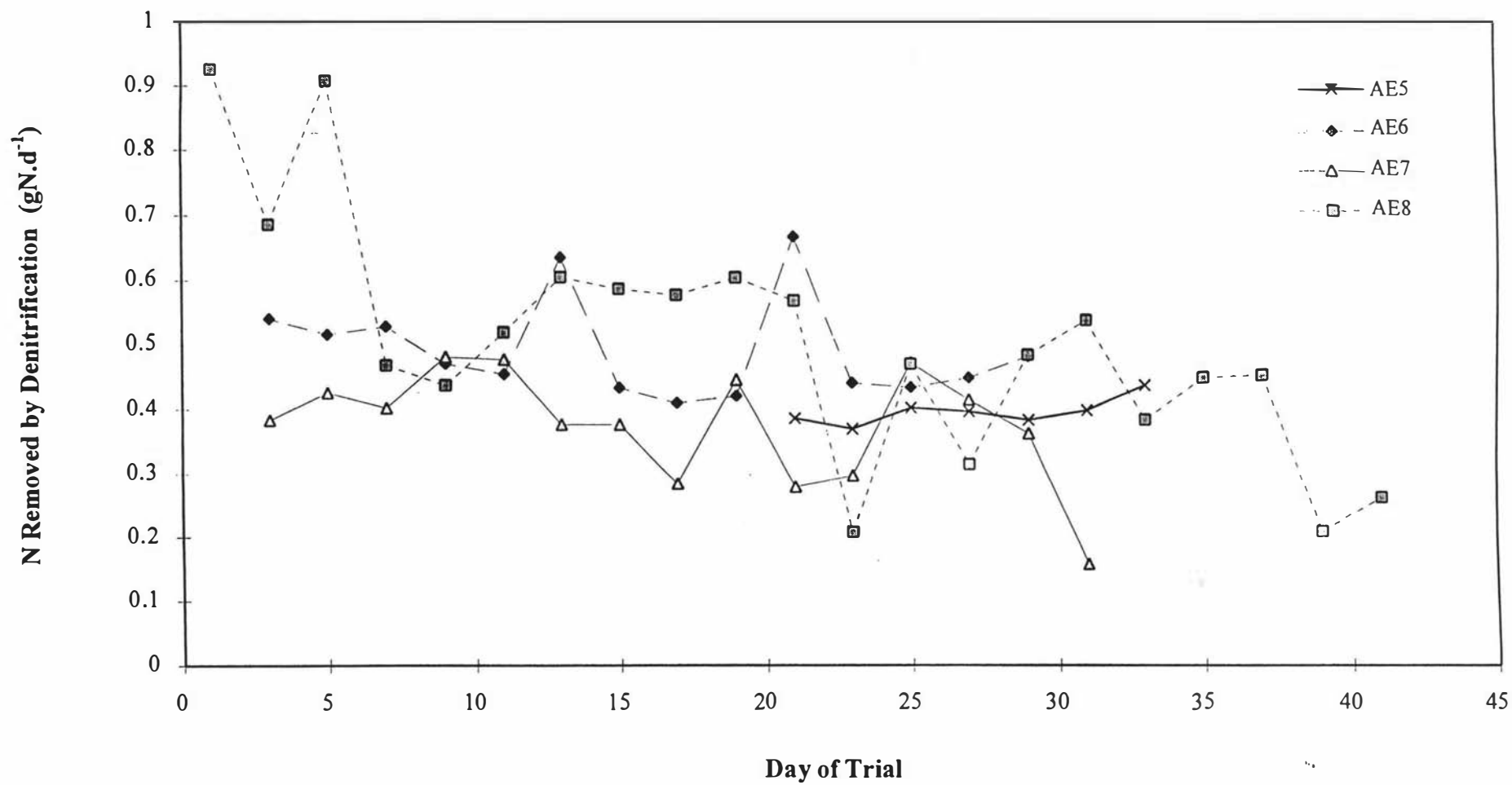
Figure 8.25 Comparison of nitrogen removals via denitrification during Trials AE5 to AE8.

Table 8.13　　　　Reactor system nitrogen balance during Trials AE6, AE7 and AE8.

Trial	AE6	AE6	AE7	AE7	AE7	AE8	AE8	AE8
Days at SRT	1 - 10	11 - 30	1 - 16	17 - 29	30 - 32	1 - 3	4 - 38	39 - 41
N_{FEED} (gN.d^{-1})	1.2	1.2	1.5	1.5	1.5	1.5	1.5	1.5
N content of cells in WML and effluent (gN.d^{-1})	0.432	0.478	0.493	0.481	0.463	0.456	0.422	0.453
WML: $NH_3 + NO_3 + NO_2$ (gN.d^{-1})	0.026	0.030	0.055	0.061	0.061	0.028	0.061	0.073
Effluent: $NH_3 + NO_3 + NO_2$ (gN.d^{-1})	0.199	0.218	0.503	0.571	0.707	0.193	0.522	0.725
Total N_{OUT}: WML + Effluent (gN.d^{-1})	0.658	0.726	1.052	1.113	1.232	0.677	1.005	1.250
$N_{FEED} - N_{OUT}$: N removed by denitrification (gN.d^{-1})	0.532	0.464	0.438	0.377	0.258	0.813	0.484	0.240
% influent N removed by denitrification	45	39	29	25	17	55	33	16
% System N removal: $(N_{FEED} - N_{EFF})/N_{FEED}$	82	80	65	61	52	87	62	50
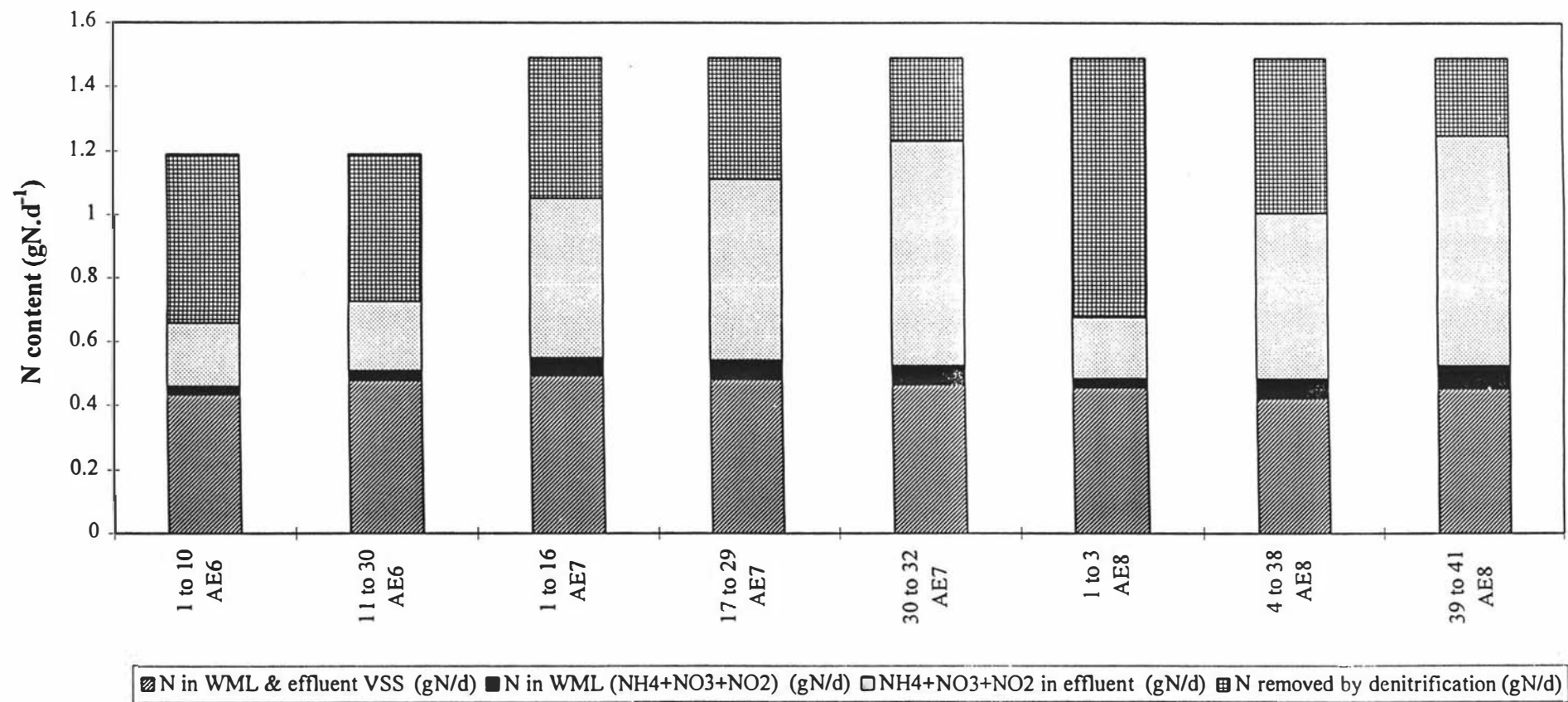
Figure 8.26   Nitrogen removal balance during the increased feed N content trials.

AE6, but decreased as Trials AE7 and AE8 progressed, the decrease in N removal producing an increase in the soluble N species in the effluent stream. The increased concentrations of nitrate and nitrite in the RAS were sufficient to enable anoxic or aerobic conditions to be maintained in bulk solution in Selector A at all times.

The change in the mass of oxidised N compounds across Selector A per day, as listed in Table 8.11, shows decrease during some periods, indicating that denitrification was occurring in this zone. The ammonia balance over the same zone demonstrated that nitrification had been occurring, however as the decrease in ammonia was greater than the increase oxidised N compounds, it is concluded that both nitrification and denitrification were occurring concurrently in the first selector. Figure 8.3 indicates a significantly higher pH in Selector A than in other zones during some periods of the trials and as denitrification releases alkalinity, it is suggested that these periods reflected intervals when the denitrification rate was in excess of the nitrification rate in the first selector zone.

As can be seen from Figure 8.21, the concentrations of nitrate and nitrite remained relatively constant through the subsequent selectors, and as nitrification was occurring, it was again indicated that both nitrification and denitrification must have been proceeding simultaneously in these zones as well, although Table 8.11 demonstrates that the dominant reaction varied throughout the trials.

The extent of denitrification, if any, in the reactor zone was difficult to assess due to the inhibition of nitrification at low reactor pH, although the concentration of nitrate and nitrate dropped across the reactor during some periods of Trial AE8 when reactor pH was low; suggesting that denitrification may have been occurring. In the settler zone, the average concentrations of both nitrate and nitrite decreased, indicating that denitrification was still occurring in this zone. The results in Tables 8.11 and 8.13 indicated that the decrease in oxidised N compound concentrations in the settler zone contributed to a varying degree to the total system denitrification, up to a maximum of 38% in Trial AE8. Results during the final period of both AE7 and AE8 indicated that either no denitrification was occurring in the settler at that point in the trial, or that the extent of denitrification was less than the extent of nitrification. As the settler pH was greater than that in the reactor during the low pH periods, nitrification would have been able to resume once the mixed liquor flowed into the settler zone.

8.6.4   Phosphorus removal

The effluent DRP decreased during Trial AE6 and stayed below 1 gP.m^{-3} during AE7, but increased during AE8 after staying at or below 1 gP.m^{-3} for the first 20 days of the trial. DRP concentrations in Trial AE6 were unchanged from the preceding trial with originally defined feed, but declined during Trial AE7, from an average of 0.37 gP.m^{-3} to 0.24 gP.m^{-3}, as shown in Figure 8.27. During Trial AE8, the effluent DRP declined for the first 13 days to a minimum of 0.18 gP.m^{-3}, then increased to above 4 gP.m^{-3} by the end of the trial 20 days later.
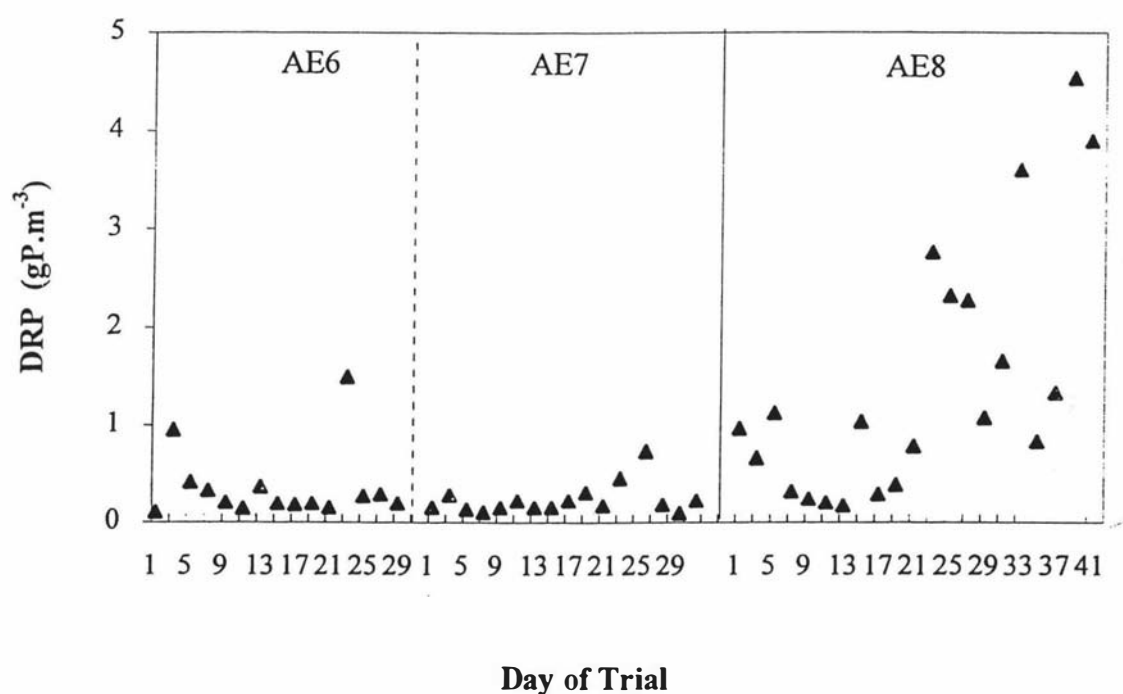
Figure 8.27:   Effluent DRP concentrations during Trials AE6, AE7 and AE8.

Within the reactor system, the DRP concentration was generally highest in the first selector then declined to a minimum in the reactor, as shown in Figure 8.28, indicating P release in Selector A and P uptake in the subsequent selector zones and reactor zone. The DRP concentration in the effluent was increased from that measured in the reactor, suggesting release of P from biomass in the settler. It was therefore indicated that anaerobic zones were maintained in the flocs, both in the initial selector zone and in the settler sludge layer.
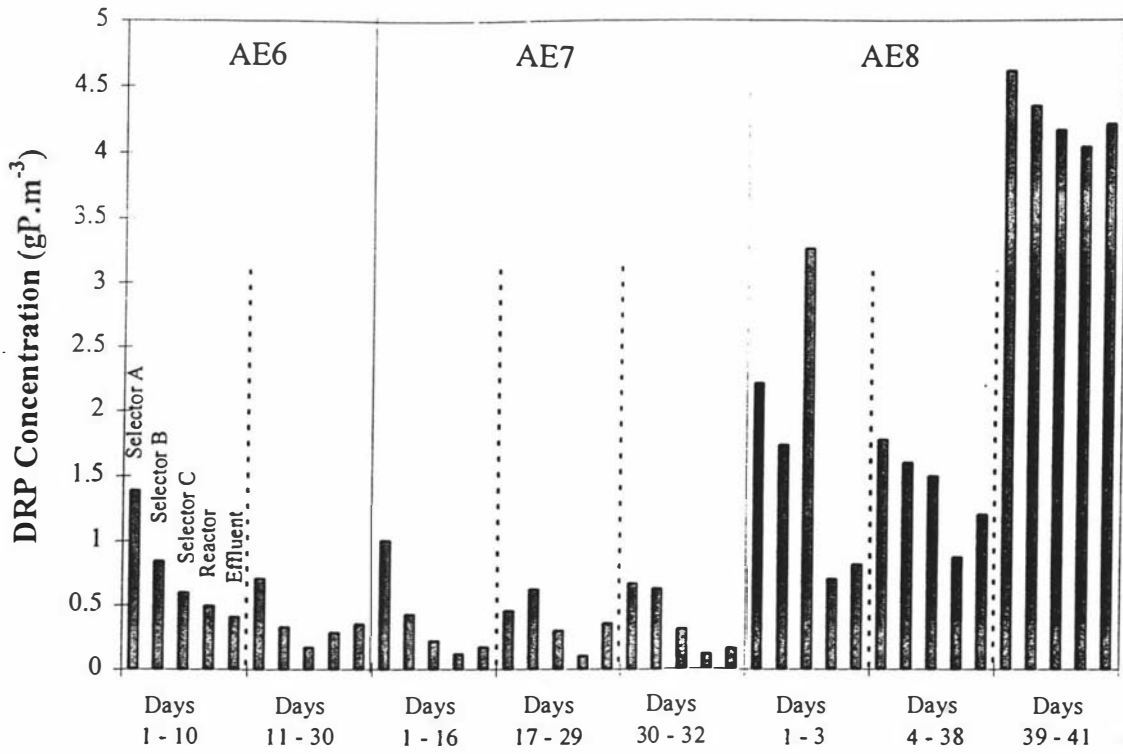
Figure 8.28: DRP concentrations in the various reactor zones during Trials AE6, AE7 and AE8.

Although utilising the same substrate and reactor system configuration, the extent of P removal was quite different between Trials AE7 and AE8, and the reasons for changes in effluent DRP levels were investigated. Tetreault *et al.* (1986) and Appeldoorn *et al.* (1992) reported a positive correlation between effluent soluble P and effluent oxidised N concentrations, due to a reported inhibition of PAO activity with nitrate, however as can be seen from Figure 8.30, this effect was not observed during these trials. The fluctuation in effluent DRP levels was not mirrored by any similar fluctuation in reactor nitrate or ammonia concentrations.

The main differences between the two trials were: that the biomass in AE7 had been acclimated for a longer period in a series of prior experiments, and had began with a higher proportion of floc forming microorganisms than that in AE8; and that the recycle ratio was lower in AE7, resulting in a longer residence time in each reactor zone. By the end of Trial AE8, the SVI was similar to that in AE7, but the DRP concentrations in all reactor zones had continued to increase to a level above that observed in any of the other aerated selector reactor trials. As can be seen from Figure 8.29, the nitrate concentrations in the first selector zone were considerably higher in Trial AE8 than
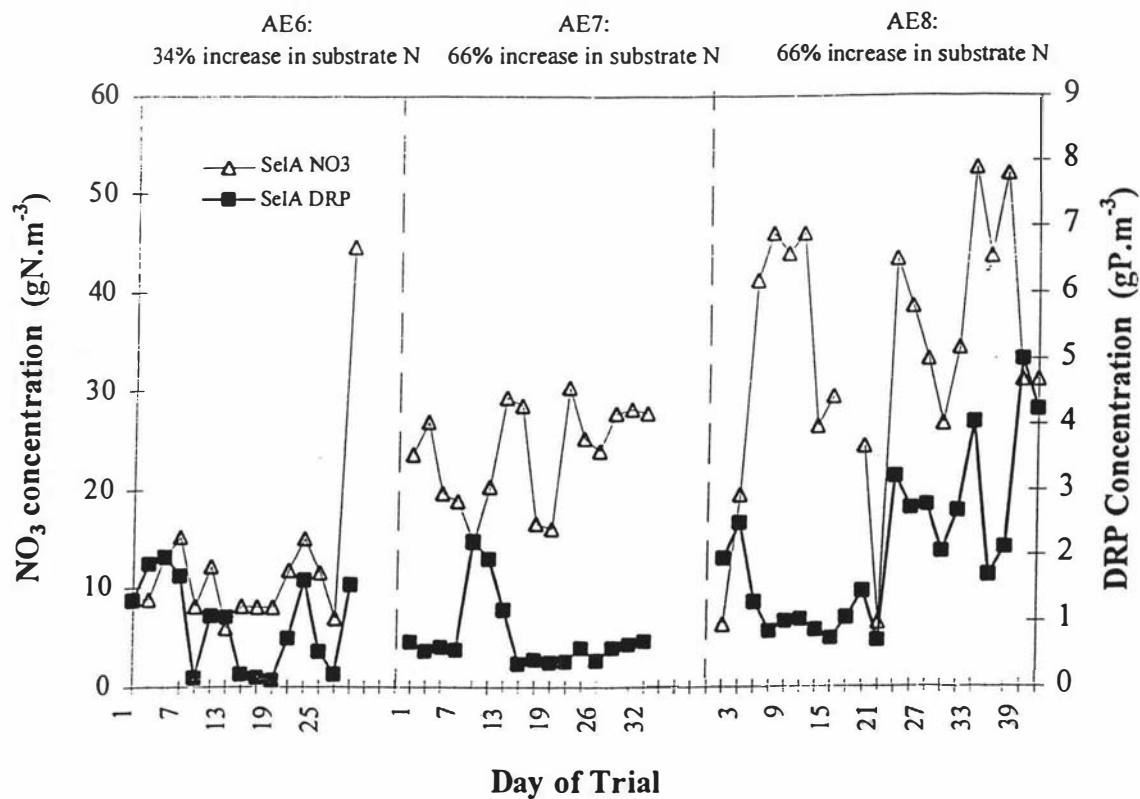
Figure 8.29:   Selector A nitrate and DRP concentrations during Trials AE6, AE7 and AE8.
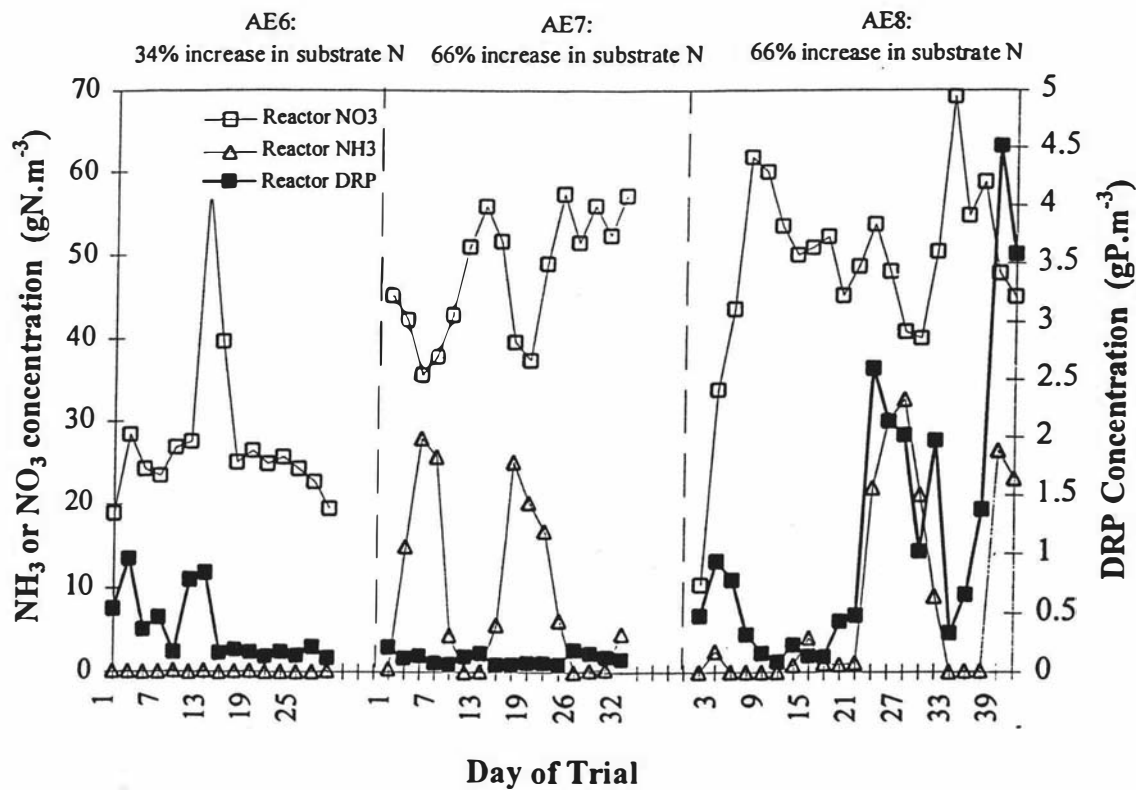
Figure 8.30:   Reactor ammonia, nitrate and DRP concentrations during Trials AE6, AE7 and AE8.

AE7, which would have decreased the extent of any anaerobic regions in the flocs during AE8. The higher recycle rate also resulted in a lower floc loading in the initial selector zone in AE8 which would have resulted in less substrate being available to micro-organisms in the centre of the floc. Therefore diminishing PAO activity was thought to have been attributable to a decrease in the necessary environmental requirements of a readily metabolizable substrate being available under anaerobic conditions.

Biomass P content remained constant at 2.2% to 2.4% indicating that luxury P uptake was continuing, even though bulk conditions in the first selector zone were no longer anaerobic. Figure 8.31 demonstrates that the P content of the biomass remained stable during Trials AE6 and AE7 but increased up to a maximum of 3% on Day 19 of Trial AE8. The biomass P content in AE8 began to decline after Day 21 when the effluent DRP concentration increased, further evidence of a decline in PAO activity.

The review paper by Yeoman *et al.* (1988) summarised the aerated zone requirements for P uptake to include a pH of between 6 and 8 and a DO of between 2 and 6 $g.m^{-3}$, however the periods of decreased reactor pH as shown in Figure 8.18 did not appear to interfere with system P removal. As the DRP concentration in the final selector zone was usually only slightly above that in the reactor, it was indicated that the majority of P uptake had occurred prior to the reactor zone, so fluctuations in pH would not have been expected to have had an effect on P uptake as long as they were confined to the reactor zone.

A phosphorus balance over the reactor system was calculated as for previous trials, by summing the P content of wasted cells and the DRP in the effluent and WML volumes, the results being listed in Table 8.14. The shortfall in the P balance during trials AE6 and AE7 was similar to that reported in Sections 6.6.4 and 7.6.4, but was considerably lower in Trial AE8 which had a higher effluent DRP concentration, resulting in a P balance of 95% during the final stage of Trial AE8. Measurements of the total P in the mixed liquor and effluent were also made throughout Trials AE6 to AE8, therefore a P balance could also be calculated from the total P data rather than from summing P in the solid and liquid phases as previously. The results agreed to within 4% for all trial periods, except for the last period in AE7 in which there were only two data points; indicating that the method of calculation used in Chapters 6 and 7 was valid.

Table 8.14   Reactor system phosphorus mass balance during Trials AE6 to AE8.

Trial	AE6	AE6	AE7	AE7	AE7	AE8	AE8	AE8
Days at SRT	1 - 10	11 - 30	1 - 16	17 - 29	30 - 32	1 - 4	4 - 38	39 - 41
P in effluent + WML biomass (gP.d^{-1})	0.091	0.096	0.100	0.106	0.090	0.091	0.098	0.092
DRP in effluent and WML (gP.d^{-1})	0.004	0.003	0.002	0.003	0.002	0.008	0.012	0.042
TP$_{out}$ from Biomass P + DRP (gP.d^{-1})	0.095	0.100	0.102	0.109	0.092	0.099	0.110	0.134
Ratio TP$_{out}$ / TP$_{in}$	0.68	0.71	0.73	0.78	0.66	0.71	0.79	0.95
% P removed (TP$_{in}$ - Effluent TP)	93	96	96	93	90	91	86	66
TP$_{out}$ from WML TP + Effluent TP (gP.d^{-1})	0.098	0.097	0.101	0.114	0.102	0.100	0.108	0.136
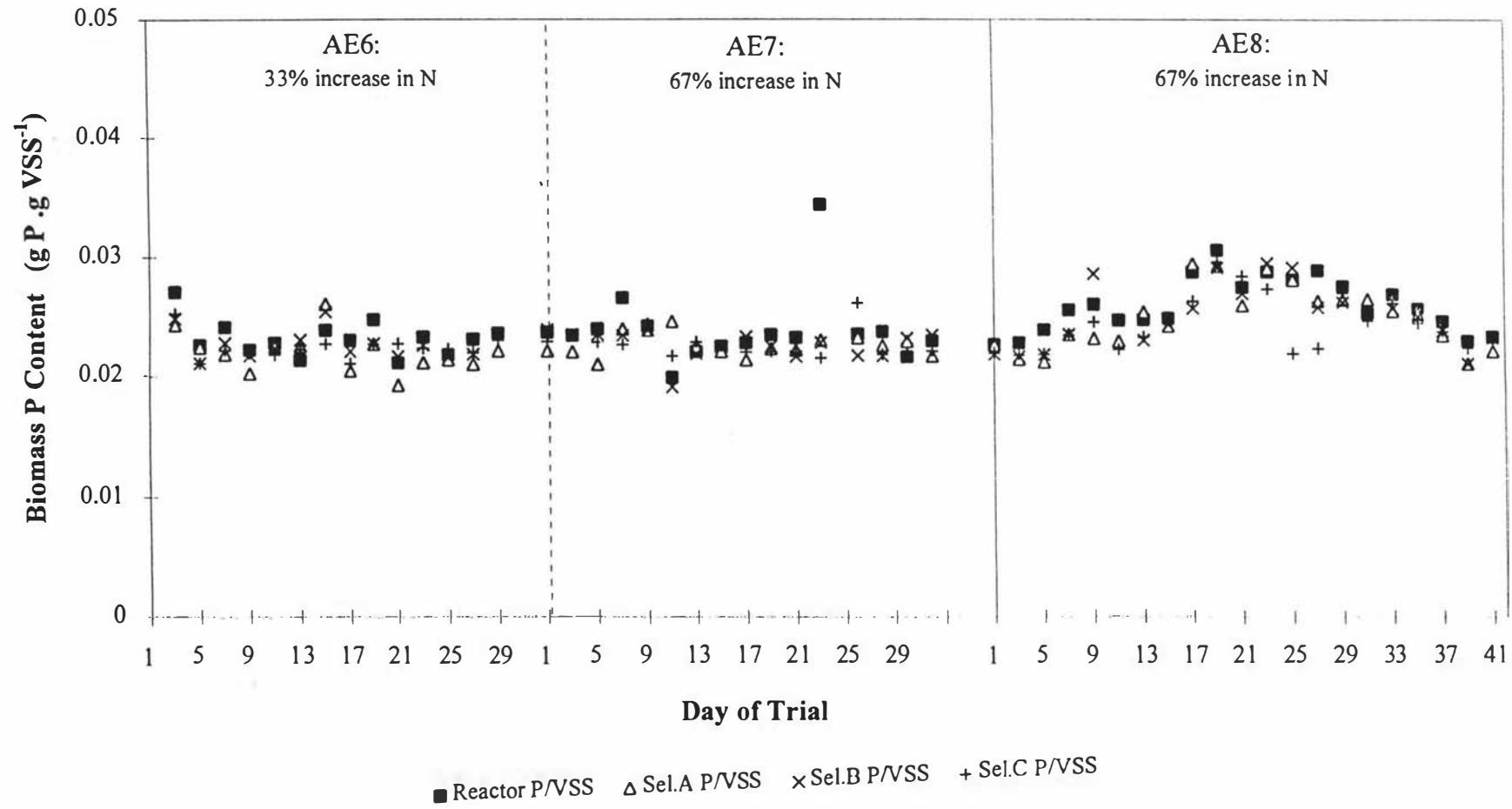
Figure 8.31   Phosphorus content of VSS during Trials AE6, AE7 and AE8.

8.7   Discussion

The reactor performance in terms of effluent quality was unchanged with respect to TSS concentration but improved in terms of COD removal at the highest substrate N concentrations. The 34% increase in feed N did not result in any improvement in COD removal performance, but a decrease in effluent sCOD was seen during both trials at the 120 $gN.m^{-3}$ feed level, falling to 80% of previous levels. This suggested that the microbial growth processes resulting from biomass grown at the higher substrate N contents resulted in a lower level of persistent SMP generation.

The ammonia loading on the system was increased by 33% in Trial AE6 and 67% in Trials AE7 and AE8. All of the additional substrate ammonia, as well as ammonified substrate proteins were completely removed by nitrification during Trial AE6, but ammonia was detectable in the effluent during periods in both AE7 and AE8. Calculated nitrification rates in the reactor were considerably lower than other published values, so the reactor residence time was considered sufficient, even at the higher substrate ammonia loadings, for complete nitrification to occur.

During periods of increased reactor ammonia concentration in Trials AE7 and AE8, the reactor nitrate concentration decreased, indicating that nitrification had been inhibited. The ammonia concentrations in the selectors reflected the variations in the reactor zone, as would be expected due to the influence of ammonia being recycled back to the selectors in the RAS. The difference between selector and reactor zone ammonia concentrations remained relatively constant during AE8, but varied during AE7, with the difference being greater during periods of negligible reactor ammonia concentrations.

When the concentration of ammonia leaving the selector zone increased to above approximately 10 $g.m^{-3}$ there was an observed decrease in reactor pH, to a level which would have been inhibitory to further nitrification. It is suggested that the lower initial selector DO levels which occurred periodically through the trials, limited the extent to which nitrification could occur in the selector zone, thereby increasing the mass of ammonia to be nitrified in the subsequent reactor zone. It is generally accepted that the DO must be greater than 1 $g.m^{-3}$ for nitrification to occur (Metcalf and Eddy, 1991) and although the average DO in the first selector zone was well above this level, Figure 8.32 illustrates that this minimum was not maintained during some periods during each trial.

As nitrification consumes alkalinity, it is suggested that the drop in reactor pH was due to limitations in the available alkalinity, especially when the extent of alkalinity-releasing denitrification reactions in the system deceased through Trials AE7 and AE8. This limitation, resulting in a decrease in reactor pH to well below the optimum of 7.5 to 8.6 for nitrification (Metcalf and Eddy, 1991), would have been the main inhibitory factor to further nitrification, rather than the high accumulated ammonia concentrations. Zhang and Bishop (1996) have also reported inhibition of nitrification in biofilms when pH decreased due to low alkalinity to oxygen supply ratios.

The predominant substrate removal mechanisms were again biosorption followed by rapid accumulation and storage. The additional mass of oxygen consumed in response to substrate added was similar to the lowest values obtained during the aerobic selector trials with the originally defined substrate, indicating that substrate removal efficiency in terms of oxygen consumption had not declined. However the substrate removal rates and $SpOUR_{max}$ values observed in the batch tests indicated that both had increased slightly from previous trials. Batch substrate removal curves also indicated that removal could now be characterised by a 'psuedo first order' phase followed by a zero order phase, rather than a single log phase.

Grau *et al.* (1975) and Van Niekerk *et al.* (1987a) proposed that the apparently first order substrate removal for multicomponent substrates was due to the summation of zero order removals for the individual components, therefore the appearance of a diphasic response in this set of batch tests could have been due to one component of the substrate being now removed at a slower rate relative to the remaining components. It is indicated then that the biomass population had adapted under the conditions of higher N concentrations to enable more rapid substrate accumulation of selected components. The fastest removed fraction is assumed to have been lactose, followed by the soluble whey proteins, with the colloidal casein micelles expected to constitute the more slowly removed components.

The same 3x 0.6l selector reactor configuration was used in all three trials due to the previously demonstrated ability of this arrangement to prevent the growth of filamentous bacteria. However, a period of increased biomass SVI was observed during all trials in this series: towards the end of AE6 / beginning of AE7, and at the beginning of AE8. In both cases the SVI declined again, stabilising at a new lower level of 60 ml/g in Trial AE7 and 90 ml/g in Trial AE8. Increases in SVI were again caused by *H. hydrossis*, the same filament that had been dominant in some of the aerated selector trials with originally defined feed.
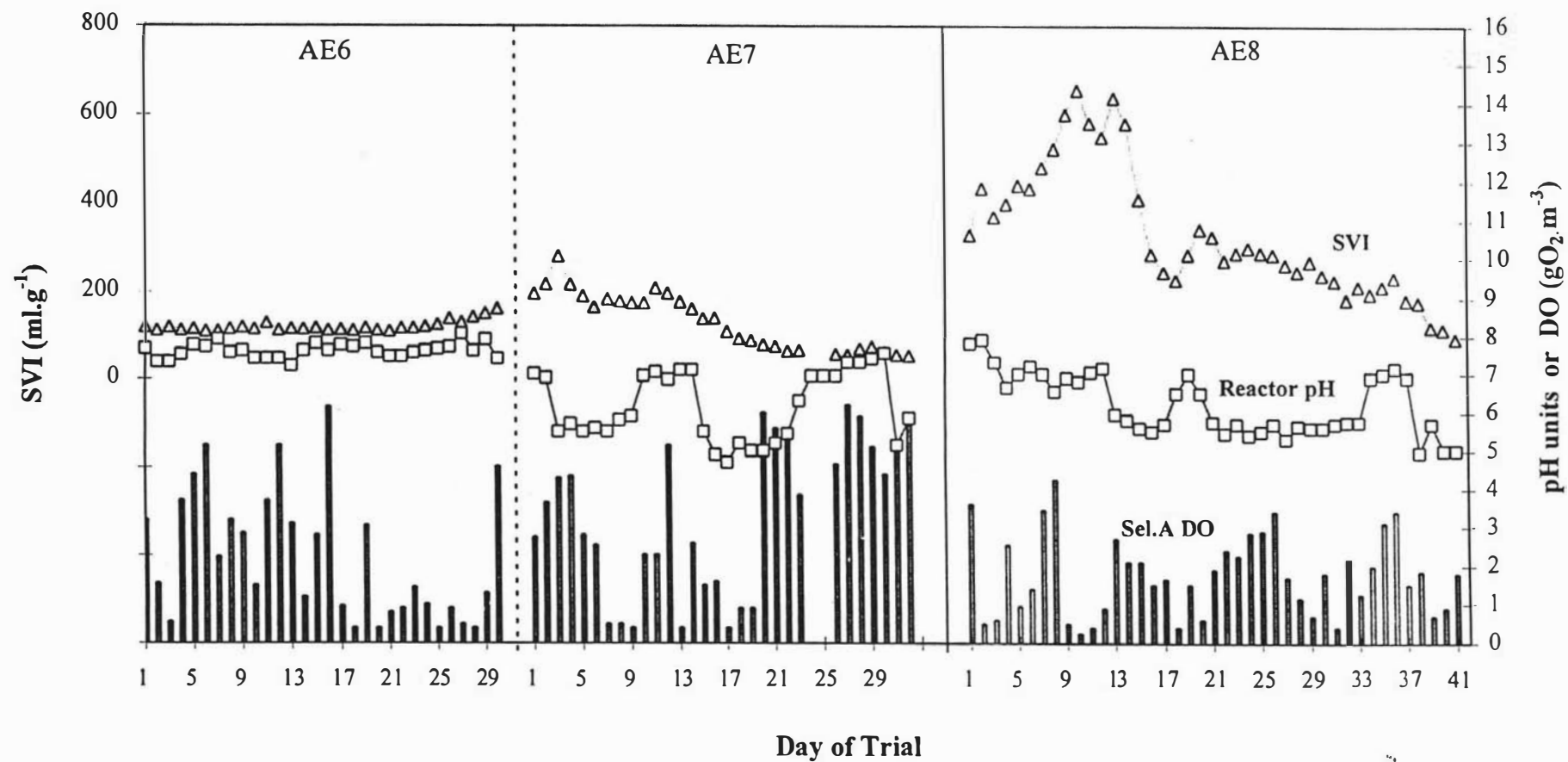
Figure 8.32   Biomass SVI, reactor pH and Selector A DO during Trials AE6, AE7 and AE8.

The increases in SVI could not be closely correlated to changes nitrate or nitrite concentrations in any particular reactor zone. Although a decrease in nitrate concentrations was observed when the reactor pH dropped and ammonia levels increased, Figures 8.15 and 8.18 show that significant nitrate concentrations were maintained in all selector zones due to high nitrate concentrations in the RAS and concurrent nitrification of substrate ammonia. This resulted in conditions in bulk solution being maintained in at least an anoxic, and usually an aerobic state. The nitrate concentrations were also significantly above the levels produced in Trial AE5 which had no periods of increasing SVI. Figure 8.31 indicates that the increases in SVI occurred after periods of low DO in the initial selector zone, and decreases in SVI coincided with periods of lower pH in the reactor zone.

It was therefore again indicated that a *H. hydrossis* could be classified as a 'low DO' filament for growth on this substrate. It has been demonstrated by researchers as early as Adamse (1968c), that the degradability of dairy processing wastewaters is very rapid, resulting in very high initial oxygen consumption rates. Along with the oxygen demand for the nitrification of additional substrate ammonia and in some periods, significant RAS ammonia loadings, the overall oxygen requirement in the initial selector zone was considerable, and aerobic conditions would have probably been restricted to bulk solution and the outer surfaces of the flocs.

The drop in SVI and concurrent reactor pH decrease were accompanied by an increase in ammonia and decrease in nitrate in all reactor zones, due to inhibition of nitrification at low pH. The pH did not drop to the same extent in Selector A, however ammonia and nitrite concentration effects were similar to the remainder of the reactor zones. It is then indicated that *H. hydrossis* may have been either inhibited by high ammonia concentrations, lower nitrate concentrations, or by decreases in reactor pH. In the previous set of trials, *H. hydrossis* proliferated at low nitrate concentrations in the initial selector zone where the majority of substrate was being removed, so this did not seem to be the likely cause.

Batch substrate removal tests were performed over the same period and the data as summarised in Table 8.3 illustrates that the higher first order removal rates were accompanied by the earliest appearance of a change to a linear removal rate in the batch test. The biomass used in these tests was taken directly from the reactor zone, aerated, then injected with a concentrated aliquot of the originally defined substrate. The fastest k values and earliest change to a linear rate occurred when the test was performed during periods of the trial when the reactor zone had a low pH.

This indicated that for biomass regenerating at the lower pH values of approximately pH<6, substrate accumulation of the most readily removed substrate components was able to occur at a faster rate, while the rate of removal of the more slowly accumulated substrate fraction did not increase to the same extent. Adamse (1968c) also indicated the more efficient substrate removal in terms of oxygen consumption for dairy wastewaters under conditions of lower pH. The floc formers may have therefore been able to compete more favourably with *H. hydrossis* by more rapid substrate accumulation after regeneration at a reduced pH.

It was indicated that *H. hydrossis* had substrate biosorption and accumulation ability and was also able to utilise nitrate as an electron acceptor; attested by its proliferation under conditions of limited DO. However the floc forming bacteria were able to compete more successfully for substrate when bulk solution conditions in the initial selector zone were fully aerobic and were able to accumulate lactose and soluble proteins more efficiently than the filamentous bacteria after regeneration at decreased pH. The apparent selective inhibition of filaments may have been due to the distribution of filaments in the mixed liquor, differences in enzymatic activity when subjected to low pH or variations in the type of preferred substrate for each microorganism.

Filamentous bacteria were observed protruding from the flocs, therefore the proximity of the most filaments to the lower pH bulk solution may have been closer than for the majority of the floc formers. The inner regions of the floc may have maintained a higher pH environment when the bulk liquid pH dropped, due to diffusional resistance in the floc or any localised release of alkalinity due to denitrification as it was also indicated that denitrification was occurring simultaneously with nitrification in the reactor zone.

Adamse (1968c) reported that for dairy substrates the activity of many proteolytic enzymes is depressed at low pH. The activity of proteolytic enzymes utilised by *H. hydrossis* may be more susceptible to changes in pH than those of the floc formers, or the filament may have had a higher accumulation capacity for specific proteinaceous components of the substrate than the floc formers. Either situation would have resulted in the observed selective inhibition of the filamentous microorganisms at the lower pH conditions in the reactor zone, by having restricted the extent of substrate hydrolysis and regeneration of accumulation capacity possible.

Towards the end of both trials at the highest substrate N level the SVI declined to very low levels. This was due to changes in both the biomass composition and the floc structure. The abundance of the filamentous microorganism *H. hydrossis* decreased and the average size of the floc units also decreased. It was indicated that the composition of the floc exopolymers had also changed as the mixed liquor filterability increased markedly. Analysis of floc biopolymers (Urbain *et al.*, 1993; Jorand *et al.*, 1995) have found that the major components were proteins, polysaccharides and DNA; and as exopolymer production occurs under endogenous respiration (Surucu and Cetin, 1990), which would correspond to the reactor zone in these trials, it is indicated that the high ammonia concentrations and / or lower pH affected the type of exopolymers produced.

Surucu and Cetin (1989 and 1990) reported reduced settling and a higher turbidity, but a decreased resistance to filtration when the pH was reduced to 5.7, which was explained as being due to the reduction of exopolymer charge and size. Jorand *et al.* (1995) indicated that the flocs were assemblages of smaller subunits held together by polymeric substances, so a reduction in exopolymer production or activity would reduce the average floc size. The conditions in these trials were obviously severe enough to reduce exopolymer abundance or bridging ability, but were not severe enough to disrupt flocculation to the extent observed by Surucu and Cetin (1990) at similar pH values.

Even though the selectors were aerated, substrate removal via anoxic and anaerobic means were again observed. The oxygen supplied was consumed in three main processes: substrate removal and oxidation; biomass growth and maintenance; and nitrification. Measurements of oxygen and substrate consumption in each of the selector zones were made as listed in Table 8.5 and the oxygen uptake rates in each zone are also shown in Figure 8.33, indicating a similar declining trend through the selector zones to the reactor, with higher rates observed in the increased substrate N trials. This corresponded to a total oxygen consumption in each zone as given in Figure 8.34.

As virtually all of the exogenous substrate was removed in the selector zones, it was previously assumed that the specific OUR in the reactor was approximately equal to that due to endogenous respiration, however due to the increased ammonia in the substrate in trials AE6 to AE8, a significant quantity of oxygen would have also been consumed for nitrification processes. A summary of the oxygen requirement for nitrification and the total mass of oxygen consumed in each Trial is listed in Table 8.15, but as O_2 consumption did not increase to the same extent as that required to nitrify the increased ammonia loading, it is suggested that a greater proportion of the substrate was
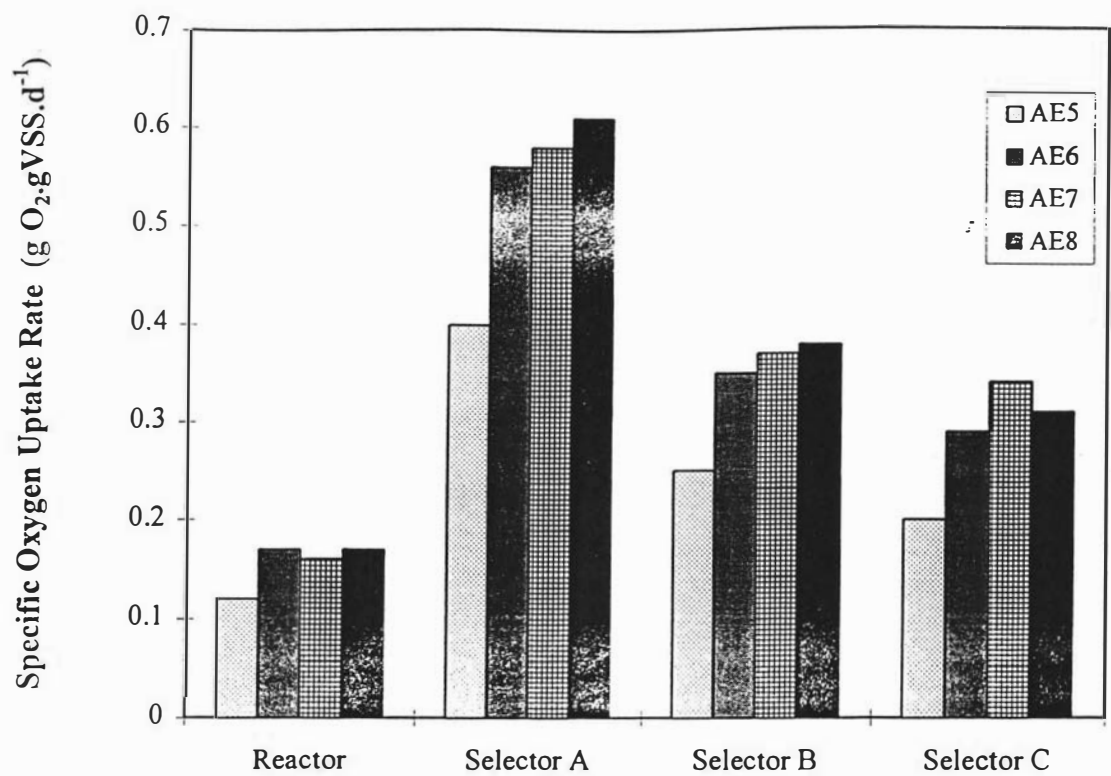
Figure 8.33:   Specific oxygen uptake rate in the various reactor zones.
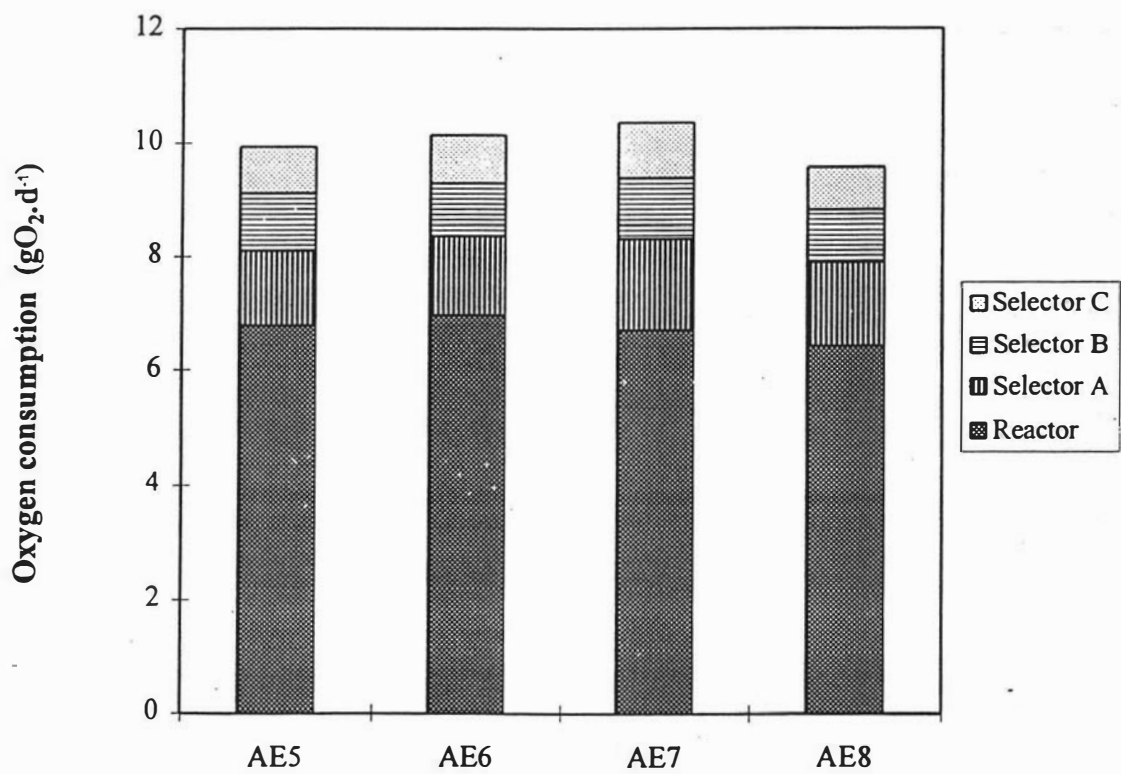
Figure 8.34:   Oxygen consumption in the various reactor zones.

removed by means other than aerobic processes, as evidenced by the continued decrease in effluent DRP from Trial AE5 through to AE7.

The actual substrate removal due to each type of process in the selector zones could not be estimated due to the extent of simultaneous ammonification, nitrification and denitrification, and P release and uptake, indicated as occurring in each zone.

Table 8.15   Estimation of oxygen consumption for nitrification and substrate removal.

	AE5	AE6	AE7	AE8
N in feed due to milk proteins (gN.m^{-3})	90	90	90	90
N in feed due to NH$_3$ added (gN.m^{-3})	0	30	60	60
Total N in substrate (gN.d^{-1})	0.9	1.2	1.5	1.5
N in wasted cells (gN.d^{-1})	0.47	0.48	0.50	0.44
NH3 in effluent	0	0	0.14	0.08
Total N nitrified (N supplied - N in wasted cells - NH3 in effluent) (gN.d^{-1})	0.43	0.72	0.70	0.76
Total O$_2$ consumed (gO$_2$.d^{-1})	9.34	9.79	10.27	9.82
Total O$_2$ required for nitrification @ 3.43 gO$_2$.gN^{-1} (gO$_2$.d^{-1})	1.49	2.47	2.40	2.60
O$_2$ consumed for processes other than nitrification	7.85	7.32	7.87	7.22
N removed by denitrification (gN.d^{-1})	0.40	0.49	0.37	0.51
DRP in effluent (gP.d^{-1})	0.004	0.003	0.002	0.014
Average total MLVSS in reactor and selector zones (g.VSS)	47.4 ± 2.5	49.1 ± 3.4	51.2 ± 2.5	44.6 ± 2.5

The mixed liquor suspended solids levels as indicated in Figures 8.35 and 8.36 can be also used as an indication of predominant substrate removal mechanisms. Towards the end of AE5 the MLVSS declined which would have been consistent with a greater proportion of substrate removal being due to anaerobic activity which has a lower biomass yield than aerobic means. During Trial AE6 the total MLVSS demonstrated a continual increase from 43 to 53 g VSS in the reactor system, and although the effluent
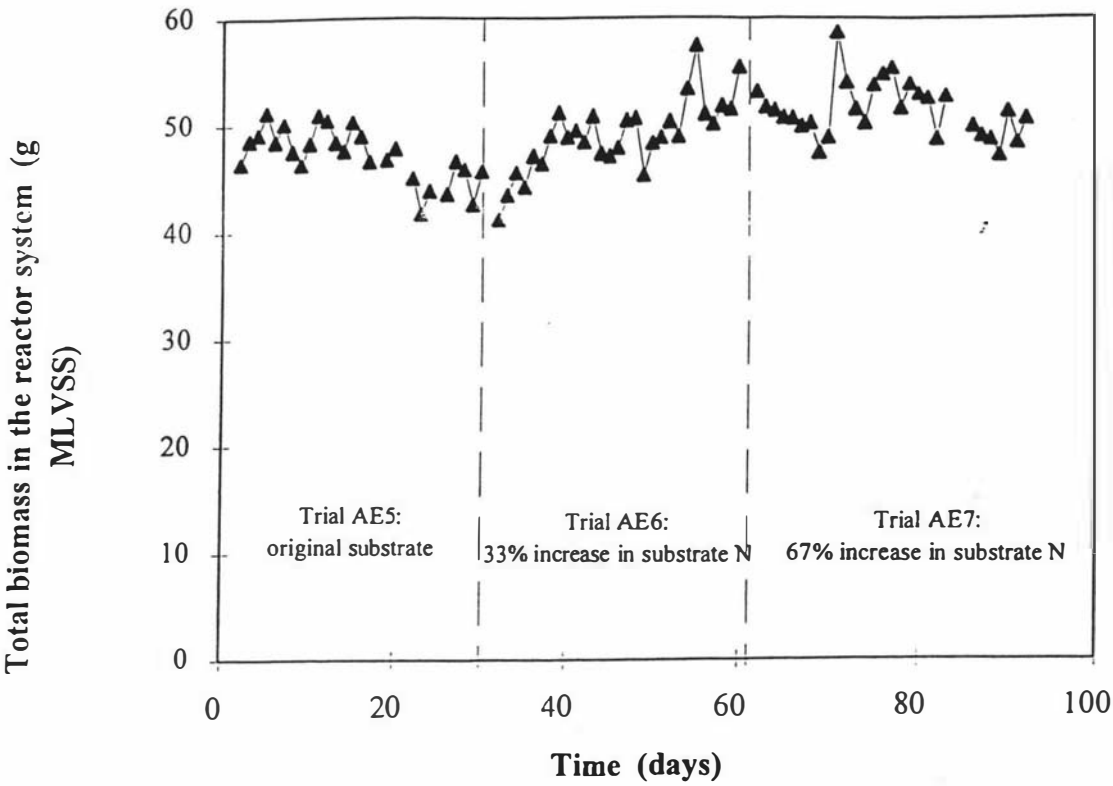
Figure 8.35: Total mass of mixed liquor suspended solids in the reactor system during the trials in Reactor System 2.
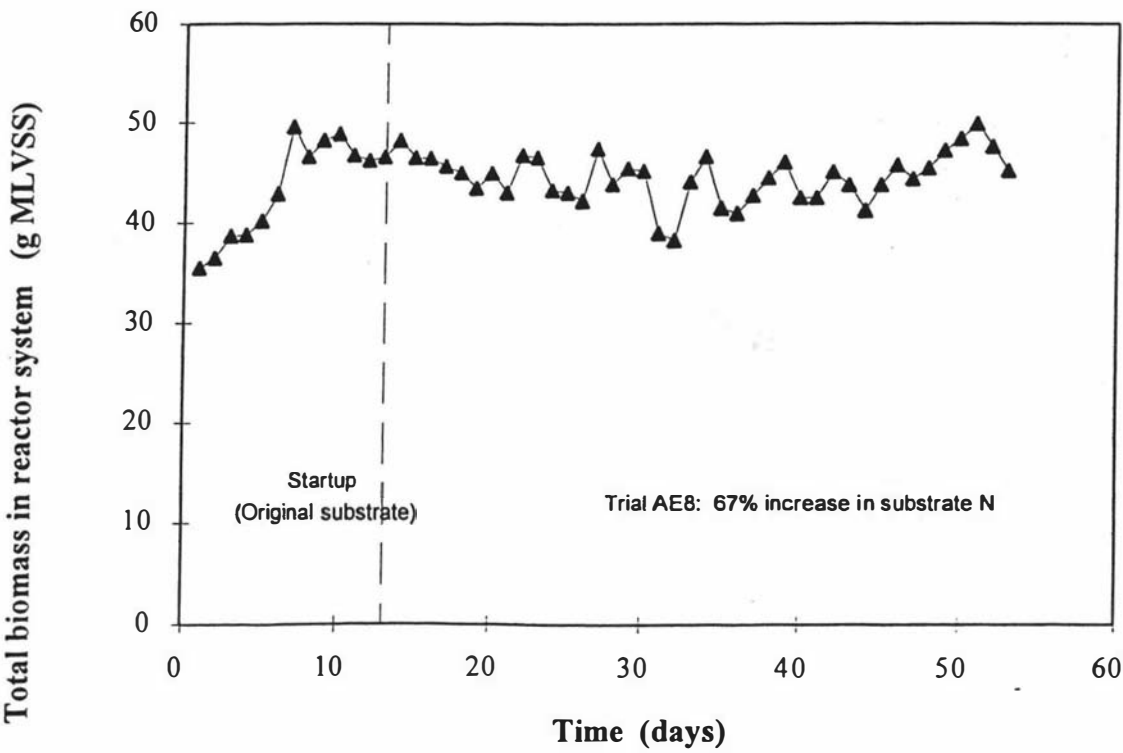
Figure 8.36: Total mass of mixed liquor suspended solids in the reactor system during the trials in Reactor System 1.

DRP remained low a decrease was observed in the extent of denitrification, as was illustrated in Figure 8.25. It was then indicated that the extent of anaerobic substrate activity has remained unchanged, but that there was a slight decline in anoxic removal and concurrent increase in aerobic removal. The increase in MLVSS may have also been partly due to improved cell yield owing to the availability of substrate ammonia in the initial selector zones. In all previous selector trials, substrate proteins required ammonification before a readily metabolizable form of N was available for biomass growth.

Total MLVSS levels remained stable during AE7 and unchanged from that recorded at the end of AE6 although denitrification showed a continued declining trend from that initially observed in AE6. The effluent DRP showed a slight decrease, indicating that substrate removal via anaerobic means had not declined. The mass of oxygen consumed in the selectors as illustrated in Figure 8.34 had increased from Trial AE6, however it seems that this was not used to nitrify any of the additional ammonia as Table 8.15 lists a decreased oxygen requirement for nitrification; therefore it is suggested that more substrate was removed via aerobic mechanisms in AE7 than in AE6.

The mass of MLVSS in the reactor system during Trial AE8 was stable as shown in Figure 8.36, but at a significantly lower level than for Trial AE7 which utilised the same substrate. Figure 8.25 indicated a considerably higher mass of N removed via denitrification, so the lower MLVSS level would probably have been due to a greater extent of anoxic substrate removal in AE8. The effluent DRP concentration increased markedly during the second half of the trial indicating loss of PAO activity and anaerobic substrate removal, and this was accompanied by a decline in denitrification, however no significant MLVSS was observed as would have been expected due to the considerable increase in substrate removal via aerobic means.

8.8   Conclusions

An increase in the substrate nitrogen concentration from 90 to 150 $g.m^{-3}$ by the addition of ammonia resulted in a fully nitrified effluent with a decreased effluent soluble COD concentration and a well settling biomass, as long as conditions in bulk solution were maintained in a fully aerobic state for the first selector zone.

When the dissolved oxygen concentration was not sufficient to maintain adequate nitrification in the selector zones, the increased nitrification load in the reactor zone was such that alkalinity was limited and a decrease in pH was observed in that zone. This acted to inhibit further nitrification and resulted in significant ammonia concentrations in the effluent.

Filamentous bulking due to the proliferation of *H. hydrossis* was observed after periods of low dissolved oxygen in the initial selector zone, reinforcing the suggestion that this filament be classified as a 'Low DO' type filament and that fully aerobic conditions need to be maintained in bulk solution to prevent filamentous growth with dairy processing wastewaters. *H. hydrossis* was inhibited by low reactor zone pH and this was considered to be due to the diminished ability of this filament to regenerate substrate accumulation capacity under such conditions, compared to the floc formers present in the mixed culture.

Although aerobic conditions were maintained in bulk solution during most of the trials, significant anoxic and anaerobic substrate removals were again observed, resulting in considerable nutrient removals. Decreases of up to 87% and 96% from influent levels were observed in the effluent stream for nitrogen and phosphorus respectively.

Simultaneous nitrification and denitrification was observed in all of the selector zones and indicated during in the reactor and settler zones during some periods; however the extent of denitrification decreased as the highest level substrate N content trials progressed. Effluent DRP concentrations continued to decline, with concentrations of well below 1 $g.m^{-3}$ consistently obtained for two of the three trials. Biological P removal decreased during one of the trials at the highest substrate N level, when higher recycle rates provided increased oxidised N compound concentrations and lower floc loadings in the initial reactor zone, restricting the opportunity for PAO activity.

CHAPTER 9

SUMMARY OF RESULTS AND DISCUSSION

9.1   Introduction

The project was undertaken to study the treatability of dairy processing wastewaters in an activated sludge system. With the trend in the NZ dairy industry being towards fewer, but larger processing facilities, the use of reliable and operable higher rate and more compact treatment facilities will be required for future developments.

Dairy processing effluents are readily biodegradable and have been successfully treated in activated sludge systems (Jones, 1974; Middlebrooks, 1979; Hung, 1984; Marshall and Harper, 1984; Fang, 1990; Fang 1991). However there have been numerous reports of operability problems due to filamentous bulking when using such substrates (Adamse, 1968a; Adamse, 1968b; Rensink, 1974; Van den Eynde et al., 1982; Strom and Jenkins, 1984; Chudoba, 1985; Goronszy et al., 1985; Hoffman, 1987; Rensink and Donker, 1990) which has tended to deter some processors from using activated sludge systems. The growth of filamentous bacteria can be discouraged by either using non-specific methods, or more desirably by selectively inhibiting the growth of the problematic species.

The trials conducted during this study concentrated on developing a reactor configuration that would prevent the proliferation of filaments, and on defining the operating parameters necessary to prevent bulking from occurring. Many conflicting theories have been proposed as to what the cultural requirements are to ensure non-bulking conditions, but as there are many different types of filamentous bacteria with different growth requirements, no single theory can be used for all substrate types.

A selector reactor configuration was employed to overcome bulking problems, but in addition to certain configurations preventing filamentous growth, significant nutrient removal was observed. As dairy processing wastewaters can contain significant quantities of N and P, and processing facilities in NZ commonly discharge into a river environment, the biological removal of nutrients would be a desirable feature of any new treatment system contemplated. As this additional feature was found in the

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successful reactor configurations, the conditions under which N and P removal occurred were also studied.

9.2   Overview of trials conducted.

A summary of the trials conducted in this study are listed in Table 9.1. The 1 day HRT and 10 day SRT values in the 'AN' and 'AE' series trials were based on the main 10 l reactor volume only. The CSTR trials at varying SRT and initial 'AN' trials were all seeded using biomass from the 2.5d SRT reactor, and the biomass was wasted at the end of the trial. The remaining trials were conducted in one of two identical reactor systems utilising the biomass in the reactor remaining from the previous trial as follows:

Reactor 1: AN2, AE2, AE4, AE8 (AE8 was seeded from AE5 biomass)

Reactor 2: AN1, AN3, AE1, AE3, AE5, AE6, AE7

Table 9.1   Summary of reactor configurations and conditions for the trials conducted.

Trial	HRT (d)	SRT (d)	Substrate	Reactor conditions and configuration
2.5d SRT	2.5	2.5	original[*1]	Aerated 6.25 l reactor
5d SRT	1	5	original	Aerated 10 l reactor
10d SRT	1	10	original	Aerated 10 l reactor
20d SRT	1	20	original	Aerated 10 l reactor
30d SRT	1	30	original	Aerated 10 l reactor
20d SRT	1	20	1:5 dilution of original	Aerated 10 l reactor
AN1	1	10	original	Unaerated 1.2 l selector followed by aerated 10 l reactor.
AN2	1	10	original	Unaerated 2.4 l selector followed by aerated 10 l reactor.
AN3	1	10	original	Unaerated 3 x 0.6 l selectors in series followed by aerated 10 l reactor.
AE1	1	10	original	Aerated 3 x 0.6 l selectors in series followed by aerated 10 l reactor.
AE2	1	10	original	Aerated 1.2 l selector followed by aerated 10 l reactor.
AE3	1	10	original	Aerated 2 x 0.6 l selectors in series followed by aerated 10 l reactor.
AE4	1	10	original	Aerated 0.6 l selector followed by aerated 10 l reactor.
AE5	1	10	original	Same as for AE1.
AE6	1	10	Increase N content in original by 34% as NH_3	Same as for AE1 and AE5.
AE7	1	10	Increase N content in original by 66% as NH_3	Same as for AE1 and AE5.
AE8	1	10	Increase N content in original by 66% as NH_3	Same as for AE1 and AE5.

Note: [*1]   original substrate as defined in Table 4.3

9.3   Effect of reactor configuration on effluent quality

The filterable or 'soluble' COD concentration in the stream flowing out of the reactor zone declined as the trials progressed. The levels measured in each reactor system during the subsequent trials are shown in Figure 9.1. As initial substrate biodegradability studies indicated that the soluble substrate would have been easily degraded in the 1 day HRT maintained all trials, the difference in effluent sCOD are suggested to have been due to a decrease in the production of soluble microbial products. Orhon *et al.* (1993) determined that for configurations with an SRT greater than 2 days operating on similar dairy processing substrates, the effluent sCOD was due almost solely to SMP generation. The consistent decrease in sCOD indicated that the reduction may not have been due to reactor configuration effects, but rather to the continuing selection of microorganisms which were able to more efficiently use the substrate available.

Average effluent sCOD concentrations of less than 30 $g.m^{-3}$ were obtained in the final trials, representing sCOD removals of greater than 98%. This is similar to other minimum effluent COD levels observed when treating dairy processing wastewaters of 30 $g.m^{-3}$ (Hung 1984) to 39 $g.m^{-3}$ (Orhon *et al.*, 1993); and consistent with a report made by Jones (1974) that effluent BOD concentrations of consistently below 30 $g.m^{-3}$ were difficult to obtain with this type of wastewater. The effluent sCOD achieved represents a lower level of SMP generation than that observed by Orhon *et al.* (1993); who determined a SMP concentration co-efficient (Y_P) of 0.031 for whey-washwater and 0.068 for general dairy effluent, as compared to the Y_P values of as low as 0.018 resulting from these trials.

The effluent TSS values during the various trials are shown in Figure 9.2, with higher values resulting from solids carryover from the settler zone, predominantly during periods of filamentous bulking, or of rising sludge due to denitrification in the settler. Effluent TSS levels of less than 10 $g.m^{-3}$ were consistently obtained when non-bulking conditions were experienced, confirming that the appropriate activated sludge configurations could result in a high quality effluent in terms of both suspended and dissolved organic matter.

Soluble COD concentration out of the reactor zone (g.m⁻³)
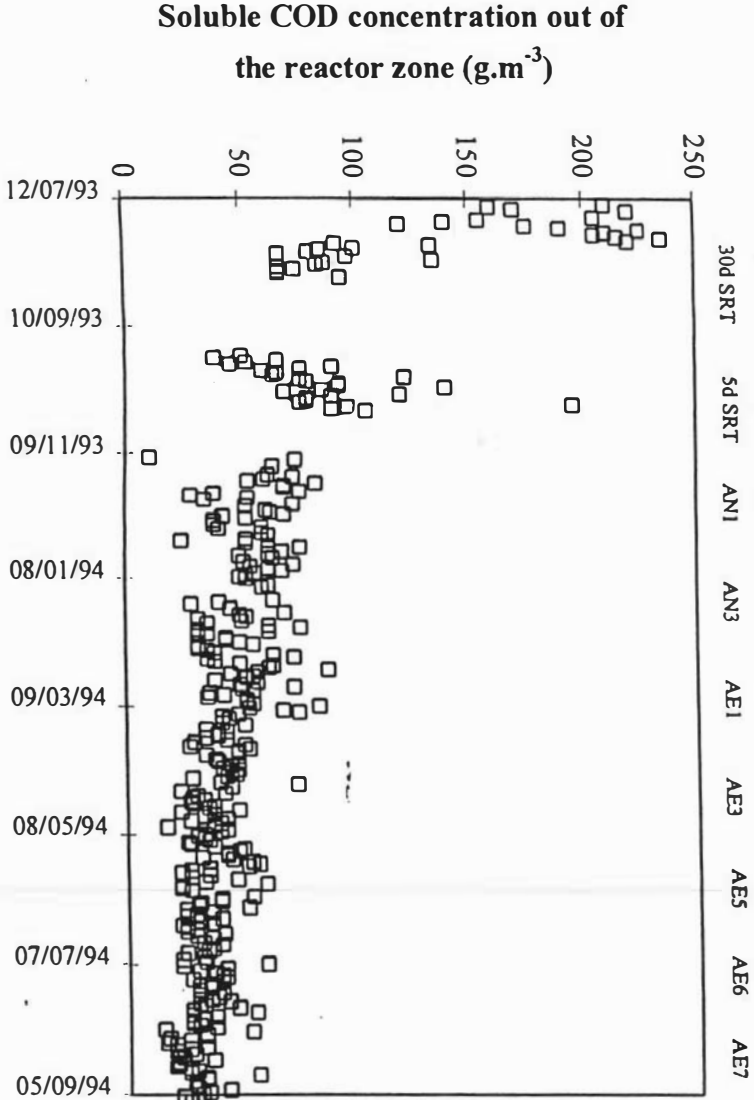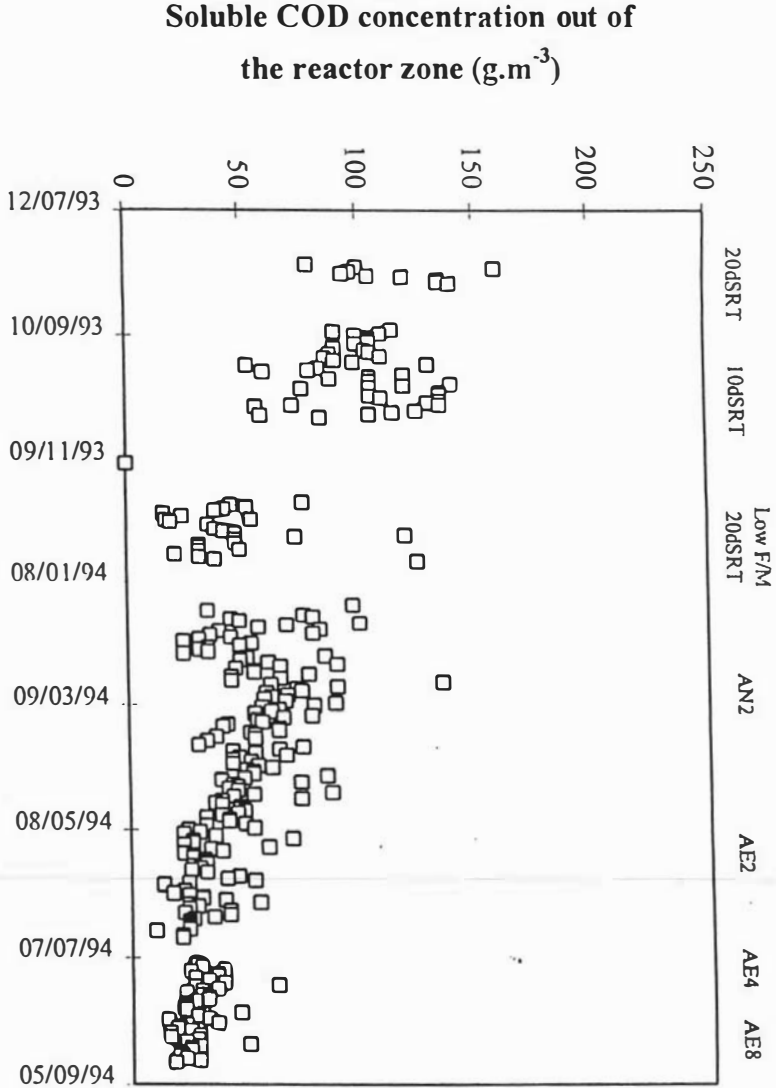
Figure 9.1: Effluent soluble COD concentrations obtained during the various trials in each reactor system.
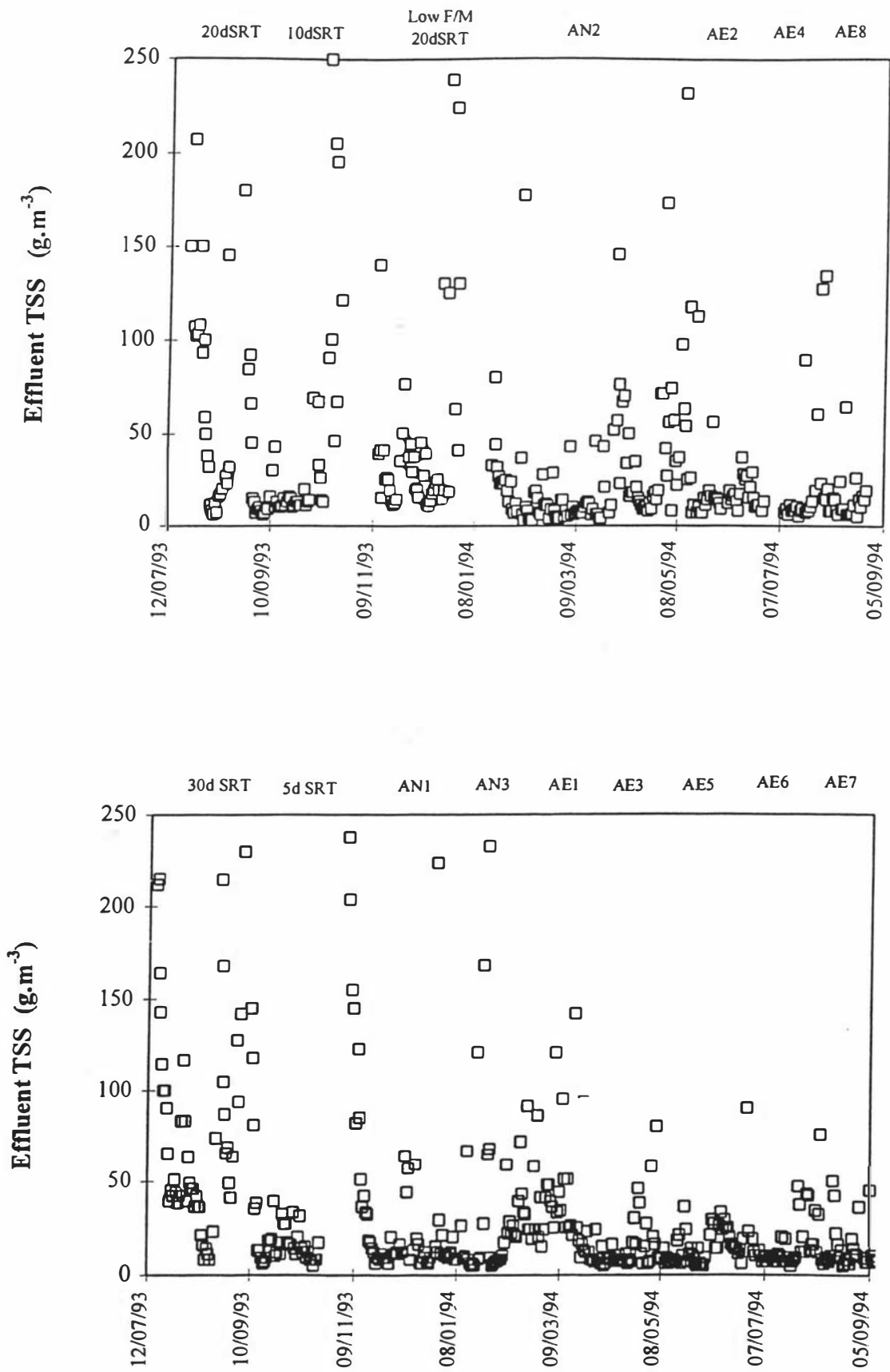
Figure 9.2: Effluent suspended solids concentrations obtained during the various trials in each reactor system.

9.4   Substrate removal mechanisms

A variety of substrate removal mechanisms were indicated in the various reactor configurations trialed, with the use of selector configurations increasing the rate at which the biomass removed substrate from bulk solution. The rapid removal rates occurring in the zones with high floc loadings resulted in a range of environmental conditions for substrate removal with aerobic, anoxic and anaerobic removals all being observed, even when the entire reactor configuration was aerated.

9.4.1   Comparison of Substrate Removal Mechanisms.

The GFC filterable or 'soluble' fraction of the substrate consisted of lactose and milk proteins, and batch sCOD removal tests were employed throughout the study to determine the means and rates of soluble substrate removal. A portion of the substrate was always removed immediately on contact with the biomass, regardless of the reactor configuration from which the cells were taken. This initial period of biosorption during the first few minutes of contact with substrate, as recorded by other researchers (Chiesa et al., 1985; Eckenfelder, 1987; Hoffman, 1987; Eckenfelder and Grau, 1992; Pujol and Canler, 1992), was followed by a longer period of removal in a zero or first order manner until a stable lower sCOD was reached.

Similar substrate removal processes of biosorption followed by first order removal were observed under both aerobic and anoxic conditions, as evidenced during unaerated batch tests conducted with biomass from the unaerated selector trials. This supports the findings of Andreadakis and Chatjikonstantinou (1994), who concluded that substrate removal via accumulation and storage mechanisms occurred with nitrate as the electron acceptor, as it did with oxygen. Soluble substrate removal continued until the oxidised N in bulk solution was exhausted, then a slight increase was observed, which seemed to indicate a release of stored substrate. There was a lack of continued substrate removal by anaerobic means during these tests, most probably as a significant population of anaerobic microorganisms had not yet developed in the earlier stages of the 'AN' series trials.

9.4.1.1   Substrate Biosorption

The mass of substrate removed via biosorption per unit mass of MLVSS generally increased with increased floc loading in the batch test, as shown in Figure 9.3. This is in agreement with the results reported by other researchers (Pujol and Canler, 1992; Novak, 1995) and the principles of adsorption (Metcalf and Eddy, 1991). From the sets of data represented it is indicated that the substrate adsorbed at a given floc load was lower in the 'AN' series trials than in the 'AE' trials, suggesting that the latter trials selected for microorganisms with a higher biosorption capacity.
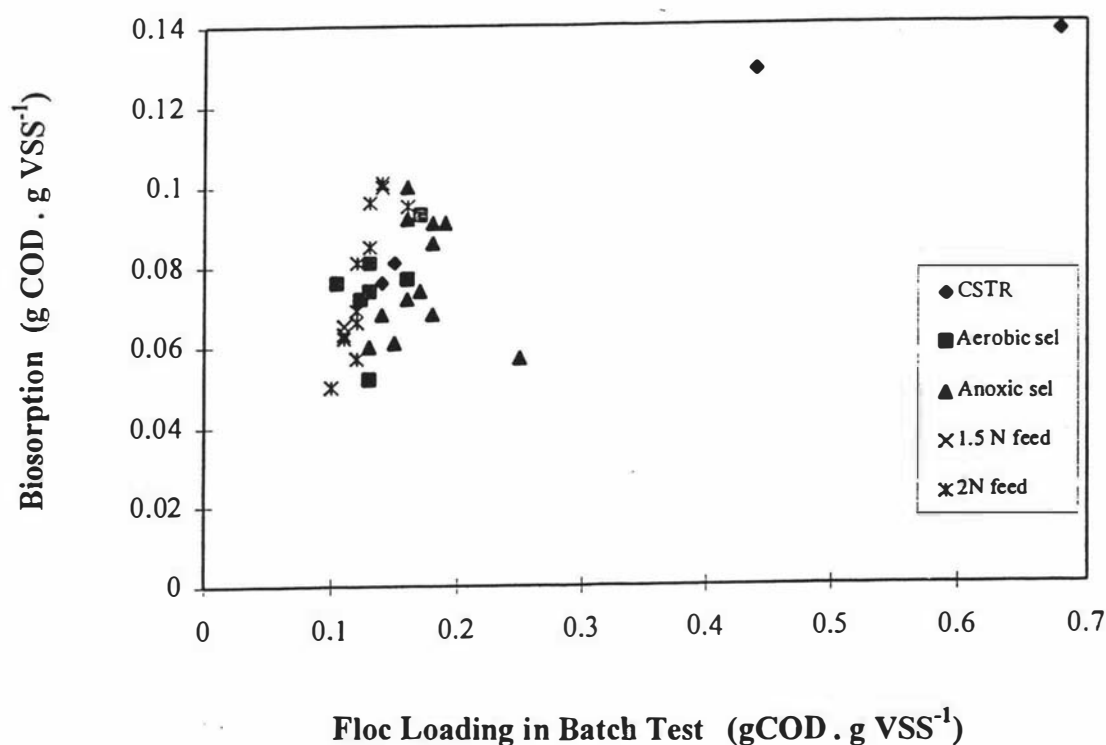
Figure 9.3:   Effect of floc loading in the batch test on substrate biosorption

The effect of SVI on biosorption can be made by comparing batch tests conducted at the same initial floc loading, during the same trial. The results for the unaerated selector trials are presented in Figure 9.4 and indicate that biosorption was negatively related to SVI. It is therefore suggested that the dominant filament during that set of trials, Type 021N, had a lower biosorption capacity than the floc formers, an effect similar to that observed by Chiesa *et al.* (1985). However as can be seen from Figure 9.5, the opposite effect was observed during the aerobic selector trials. This indicated that *H. hydrossis* possessed significant biosorption ability, as the filamentous populations exhibited a
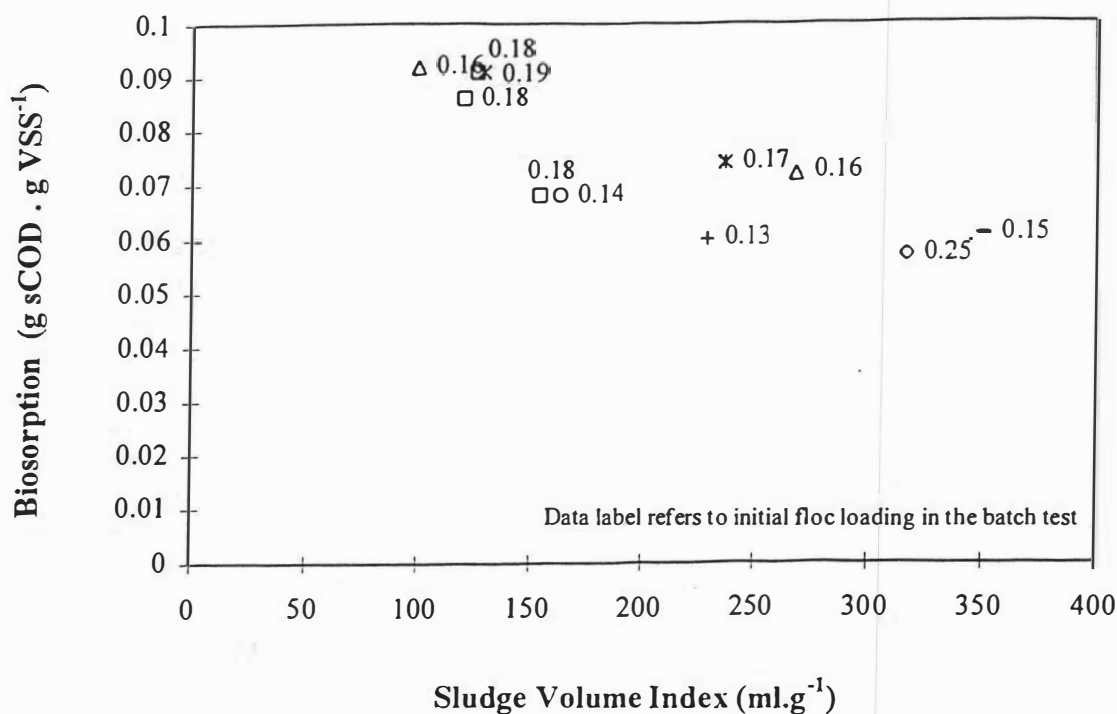
Figure 9.4: Effect of SVI on biosorption during batch substrate removal tests utilising biomass from unaerated selector trials. Dominant filament Type 021N.
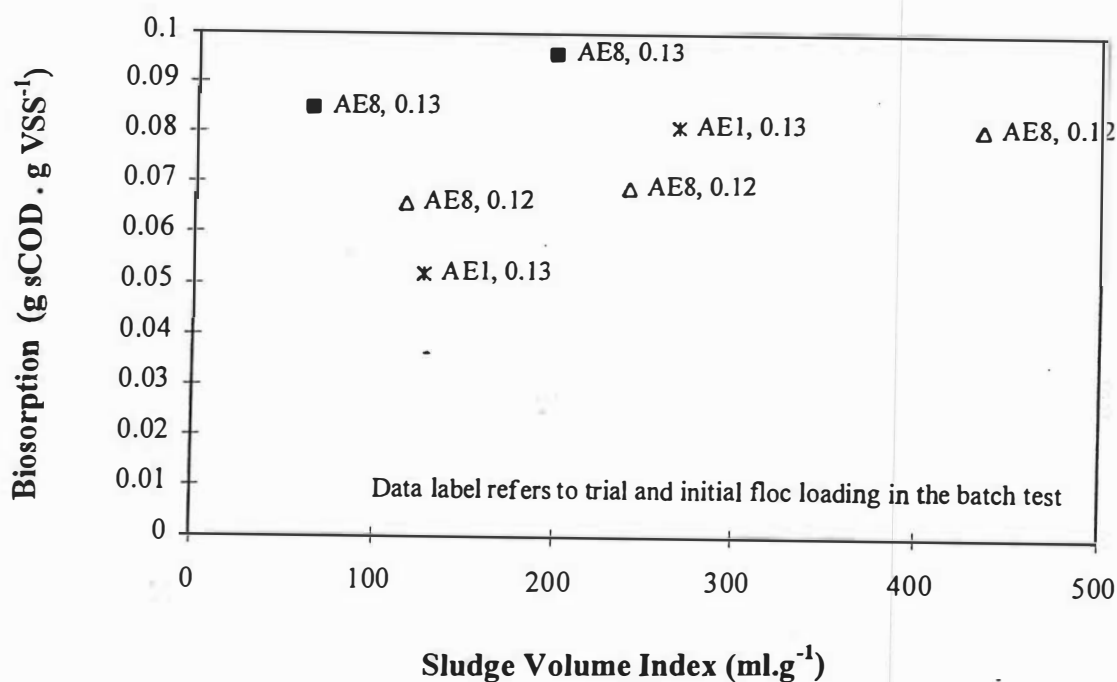
Figure 9.5: Effect of SVI on biosorption during batch substrate removal tests utilising biomass from aerated selector trials. Dominant filament *H. hydrossis*.

higher biosorption at a constant floc load than non-filamentous ones, a trend that was in agreement with observations made by Pujol and Canler (1992). Therefore there was no singular effect of SVI on biosorption, as it was dependent on the type of filament present.

9.4.1.2 Substrate Removal Rates

Batch soluble COD removal tests demonstrated that after a portion of the substrate was immediately removed on contact with the biomass, remaining substrate was removed gradually over a 30 to 90 minute period. In the initial biodegradation studies and in trials using a conventional CSTR configuration, the sCOD removal observations were best fit by a linear, or zero order relationship. While most of the data could have also been adequately described by a first order relationship, regression analyses indicated that a linear relationship better described the results obtained. After selector reactor configurations were introduced, the data were clearly best fit as a log removal relationship.

It has been proposed (Grau *et al.*, 1975; van Niekerk *et al.*, 1987) that first order substrate removal rates observed when using multicomponent substrates were due to the cumulative effect of zero order removals for each of the individual components. This concept was supported by the observations that during a set of batch removal tests initially performed using lactose alone, substrate removal was best fit as zero order. The changes observed in substrate removals during batch tests for all subsequent trials, as indicated in Table 9.2, would therefore have been due to changes in the relative removals of the different substrate components. All batch tests, including those using biomass from the later trials with modified substrate, were performed using the substrate as originally defined in Table 4.3.

The 'psuedo first order' removal rates increased as the trials progressed, indicating that the use of a selector configuration favoured the growth of microbial species with a faster substrate accumulation capacity. This agreed with a previous report by Van den Eynde *et al.* (1982) who compared dairy wastewater COD uptake rates for continuously and intermittently fed systems.

Table 9.2   Substrate removal observed in batch soluble COD removal tests.

Series of Trials:	Removal Rate best fit as:	Biosorption (g sCOD. g VSS^{-1})	Psuedo First order Removal Rate constant, k (d^{-1})	Specific Zero order Removal Rate (g sCOD. g VSS^{-1}.d^{-1})
2.5 d SRT	linear	0 *lactose	-	1.08 - 1.49 *lactose
2.5 d SRT	linear	0.016	-	0.77 - 1.13
5 - 30 d SRT	linear	0.076 - 0.081	-	0.43 - 0.46
AN1 - AN3	log	0.060 - 0.100	12 - 38	-
AE1 - AE5	log	0.052 - 0.093	40 - 180	-
AE6 - AE8	log, then linear	0.057 - 0.101	75 - 225	0.04 - 0.84

*lactose = tests performed using lactose only as the substrate.

The individual substrate components were removed at a greater rate during the aerated selector trials than during the previous unaerated selector trials with the same substrate, but were removed in a similar manner, with a smooth logarithmic decay continuing to be observed until a stable lower plateau was reached. This indicated that the individual substrate components were all removed at either a faster or slower rate to a similar extent, so as to maintain the same first order sCOD removal curve.

In the final set of trials with biomass exposed to an elevated substrate N content, a similar or slightly faster logarithmic removal was initially observed, but this was then followed by a distinct period of linear substrate removal. This suggested that under the conditions imposed by increasing the feed N level, most of the components were removed in the same manner but at an increased rate, except for one major component. The majority of this component was now taken up after the other components had been removed from bulk solution. As the sCOD components were known to be lactose and milk proteins, it is indicated that the lactose and soluble whey proteins were able to be more rapidly removed, but the more slowly removed component, suggested to be the casein micelles, were not removed at a similar increased rate.

This diphasic substrate removal response was more pronounced when the reactor zone experienced lower pH (<6) conditions and it is proposed that some proteolytic enzymes could have been inhibited by the decreased pH, preventing regeneration of the

organisms accumulation capacity for casein, and therefore resulting in lower removal of that protein fraction when the biomass was next exposed to exogenous substrate.

Batch removal tests with soluble substrate were also conducted to determine average biomass kinetic parameters for the mixed culture. An estimate of the maximum specific growth rate was obtained from tests conducted at low initial S_O/X_O ratios (Cech *et al*, 1984). A comparison was made between low and high S_O/X_O ratio methods for the estimation of biokinetic constants during the initial biodegradation testing phase of the study, and it was decided that even though low S_O/X_O tests did not actually measure 'growth' as cell replication (Chudoba *et al*. 1992; Grady *et al*., 1996), it was the preferred method as it measured the response of the mixed culture as it existed at the time of the test.

Table 9.3   Respirometric estimation of biomass kinetic parameters.

Trial	S_O/X_O (g sCOD.gVSS^{-1})	SVI (ml.g^{-1})	$\Delta SpOUR_{MAX}$ (gO$_2$.gVSS.min^{-1})	K_S (g sCOD.m^{-3})
2.5d SRT	0.003-0.15	-	0.2 - 1.1	3.4 - 44
5 d SRT (16)	0.007 - 0.012	246	0.32	2.2
10 d SRT (30)	0.005 - 0.012	357	0.28	4
30 d SRT (22)	0.0003 - 0.0007	179	0.41	3.5
AN1	0.003 - 0.016	113 - 272	0.31 - 0.35	4.3 - 16
AN2	0.004 - 0.007	124 - 163	0.23 - 0.30	2.8 - 7.7
AN3	0.003 - 0.007	298	0.26	8.6
AE1	0.001 - 0.014	113 - 267	0.22 - 0.28	2.6 - 28
AE2	0.01 - 0.02	151	0.86	23
AE3	0.02	159	0.78	27
AE5	0.01 - 0.02	128	0.88	41
AE7	0.03 - 0.06	56 - 217	1.5 - 3.6	87 - 272
AE8	0.006 - 0.06	178 - 435	0.67 - 1.8	27 - 91
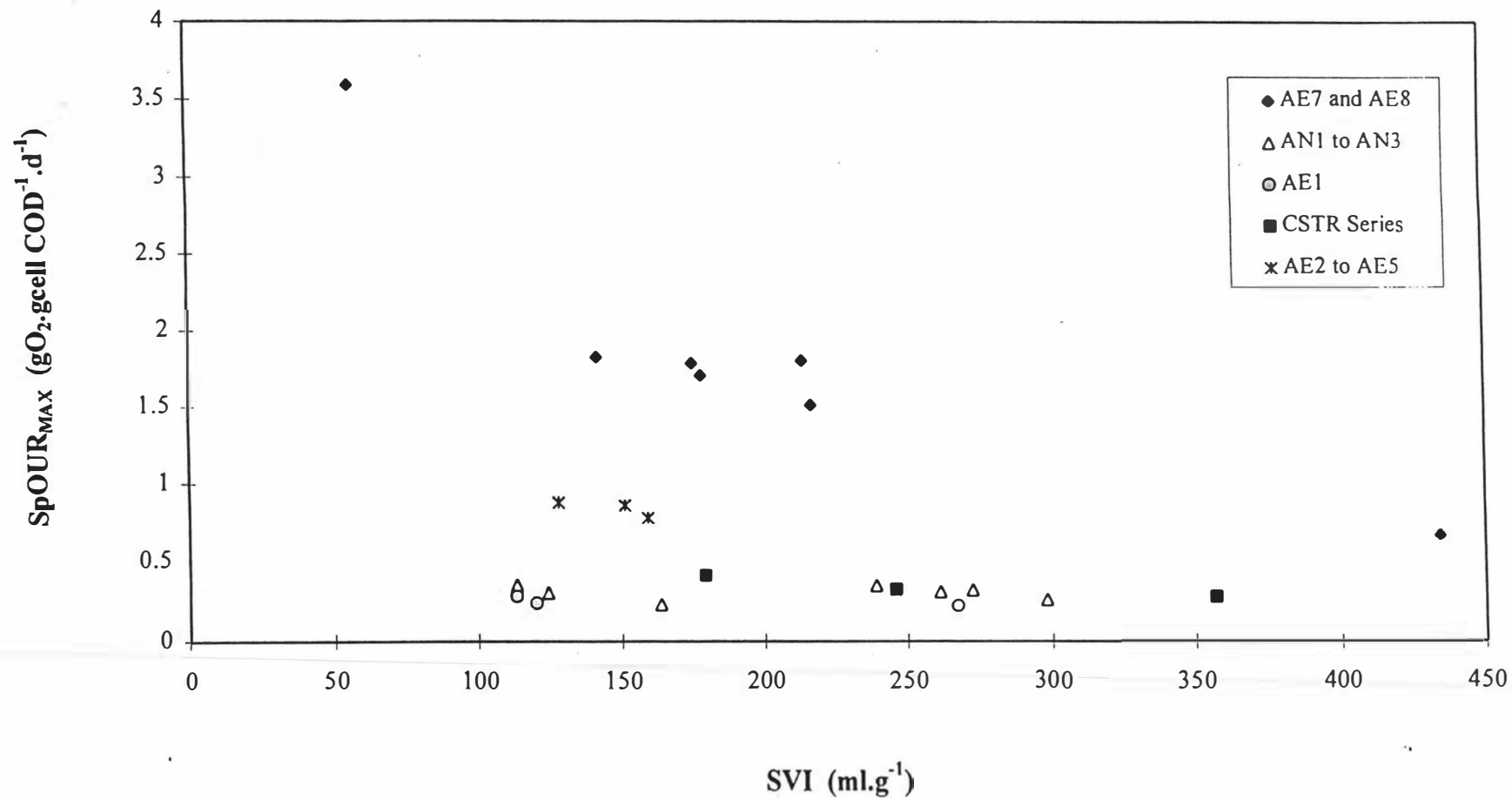
Figure 9.6   Change in maximum SpOUR with SVI during the various series of trials.
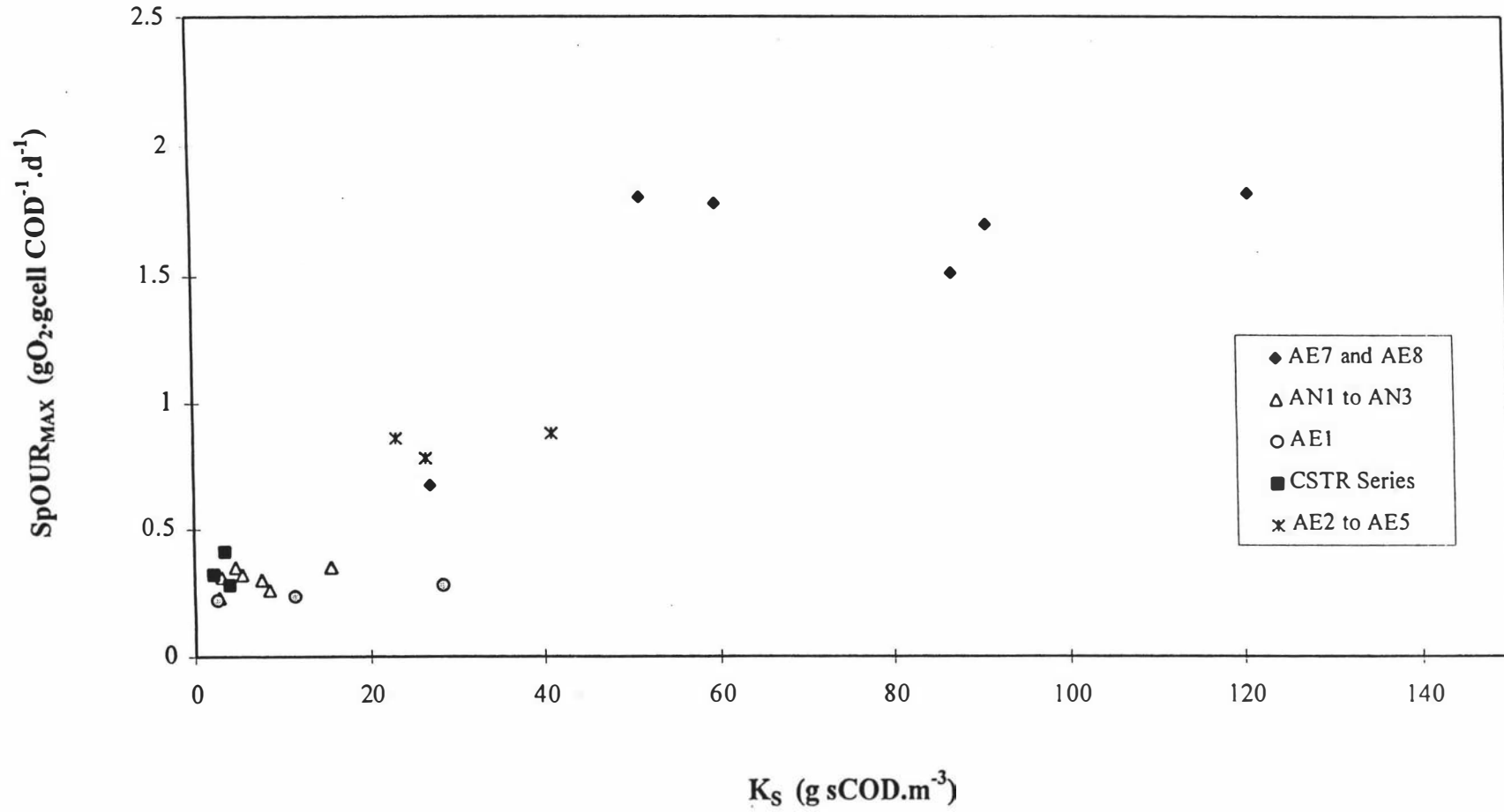
Figure 9.7   Relationship between maximum SpOUR and Ks during the various series of trials.

The maximum value of specific OUR was used to determine μ_{max} which relates to the specific growth rate during periods of 'balanced growth'. However as the selector trials progressed it became evident that the immediate maximum oxygen uptake response of the biomass ($\Delta SpOUR_{max}$) to added substrate was more a measure of a substrate storage response rather than a 'growth' response (μ_{max}). The maximum rate, calculated as either $\Delta SpOUR_{max}$ or μ_{max} was still measured in subsequent tests, but was used more as a comparison of the biomass response to substrate addition than a measure of cell growth or replication. The values obtained for $\Delta SpOUR_{max}$ and K_S during the various reactor configurations trialed are summarised in Table 9.3.

Figures 9.6 and 9.7 illustrate that in general $\Delta SpOUR_{max}$, which is directly proportional to μ_{max}, and K_S were positively correlated and that the maximum $\Delta SpOUR$ decreased as the SVI increased, in agreement the 'kinetic selection theory' as first proposed by Chudoba et al., (1973b); and verified by a number of other researchers since that time (Van den Eynde et al., 1983; Chudoba et al., 1985; Chiesa et al., 1985; van Niekerk et al., 1987b; Chudoba et al., 1991). It can also be seen that as the reactor configuration was changed to impose a greater concentration gradient on the system, selection for micro-organisms with a more rapid substrate accumulation capacity occurred, with a marked increase in the values obtained for $\Delta SpOUR_{max}$ and K_S.

Biomass decay rates were also determined from batch tests in which the $\Delta SpOUR$ was followed, but over a longer period. There was a significant increase in the decay rate when selector reactor configurations were employed and nutrient removal was observed; opposite to the results reported by Shao and Jenkins (1989) and McClintock et al. (1993) when comparing biomass from CSTRs to that from selector systems and Biological Nutrient Removal (BNR) systems respectively.

Table 9.4   Trend in biomass decay rates during the study.

Trial	Average Decay co-efficient, b (d^{-1})
10d SRT CSTR	0.22
AN1	0.21
AN2	0.23
AN3	0.26
AE6	0.26
AE7	0.30
AE8	0.29

9.4.1.3   Substrate presence in the biomass flocs

Batch tests only determined the removal of filterable substrate components. The removal of the insoluble components, namely the larger milk proteins and milk fat, was difficult to assess as these fractions could not be easily differentiated from the biomass in the analyses conducted. As the liquid phase of the mixed liquor was not turbid in any of the reactor zones, it was indicated that the insoluble substrate components were rapidly adsorbed onto or enmeshed in the floc structure.

Evidence of substrate presence in the floc mass, either as adsorbed filterable components or enmeshed insoluble components, was suggested from biomass N content data and the COD content of the MLVSS. Substrate N was in the form of milk proteins, existing as either colloidal material in the filterable fraction of the substrate or as larger insoluble material. The average N content of activated sludge biomass has been reported as ranging from 8.7% to 10.2%, averaging 9.7% to 9.8% (Suwa *et al.*, 1992; McClintock *et al.*, 1993) as compared to an empirical value of 12.4% (Metcalf and Eddy, 1991). The average N:VSS ratio measured during the various trials ranged from 11.3% to 13.9%, as shown in Figure 9.8, considerably above those levels reported by other researchers, and measuring above the theoretical limit in the earlier AN series trials.

The non-filterable TKN fraction of substrate VSS was determined to be 11.3%, higher than typical biomass N contents; therefore the higher biomass N contents measured in this study may have been due to substrate proteins being adsorbed onto or enmeshed in the flocs. The differences in average N contents measured between the various reactor zones were not significant at a 95% confidence level, however there was a significant decline in biomass N contents as the series of trials progressed, as shown in Figure 9.8.

The COD content of the MLVSS also suggested that substrate was being adsorbed as the COD content of the substrate VSS was significantly higher at 3.67 than the levels of around 1.42 measured on MLVSS. Figure 9.9 illustrates that the average COD:MLVSS ratios were higher in the selector zones than the reactor zone for all trials, which would be consistent with higher levels of substrate adsorption or enmeshment in the selector zones; although again the data were not significantly different at the 95% confidence level of ± 0.2 gCOD.gVSS^{-1}. McClintock *et al.* (1993) observed higher biomass COD contents during nutrient removal trials than CSTR trials, but no such difference could be detected in these trials.
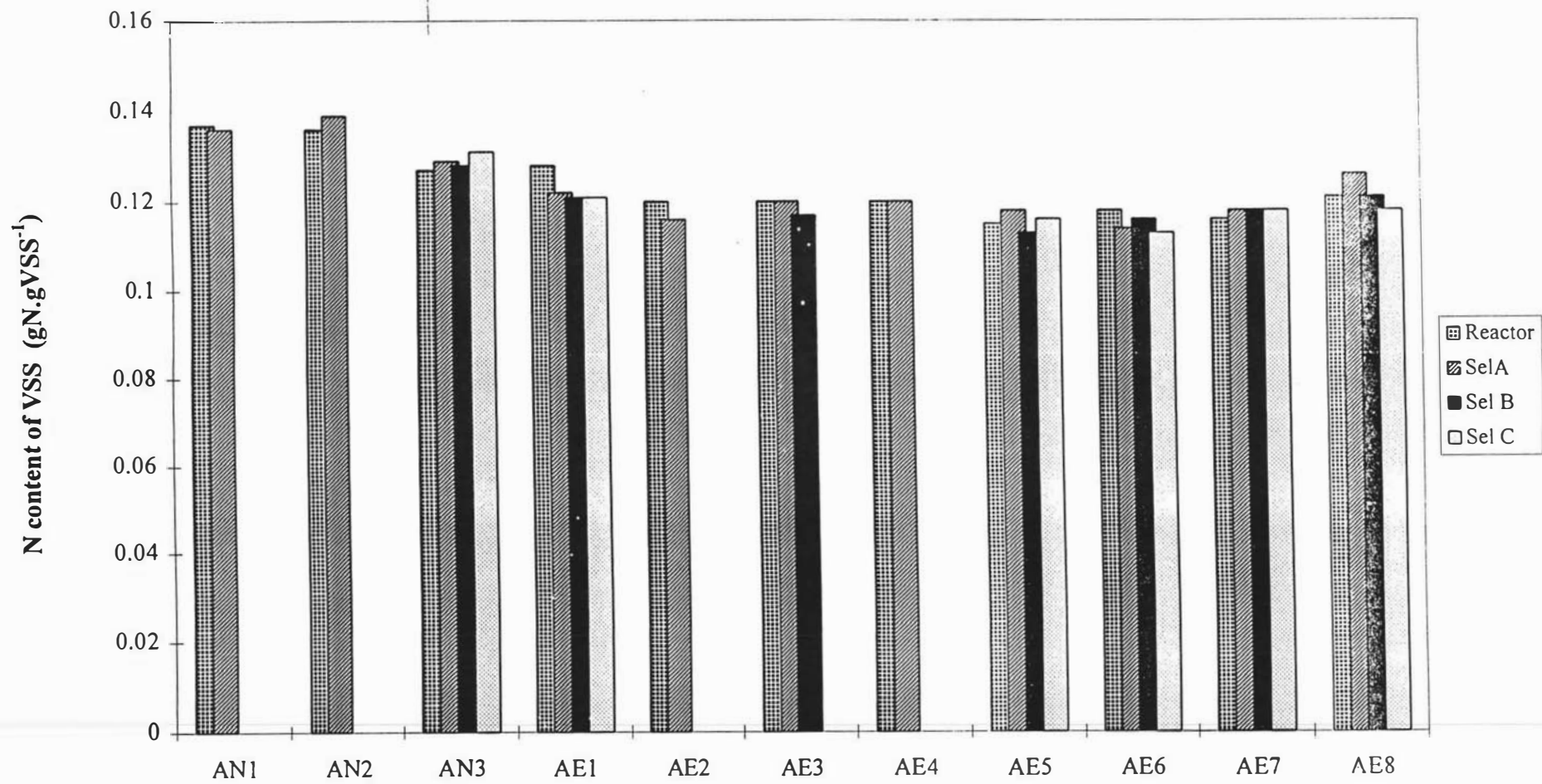
Figure 9.8   N content of VSS in the various reactor zones during the selector configuration trials
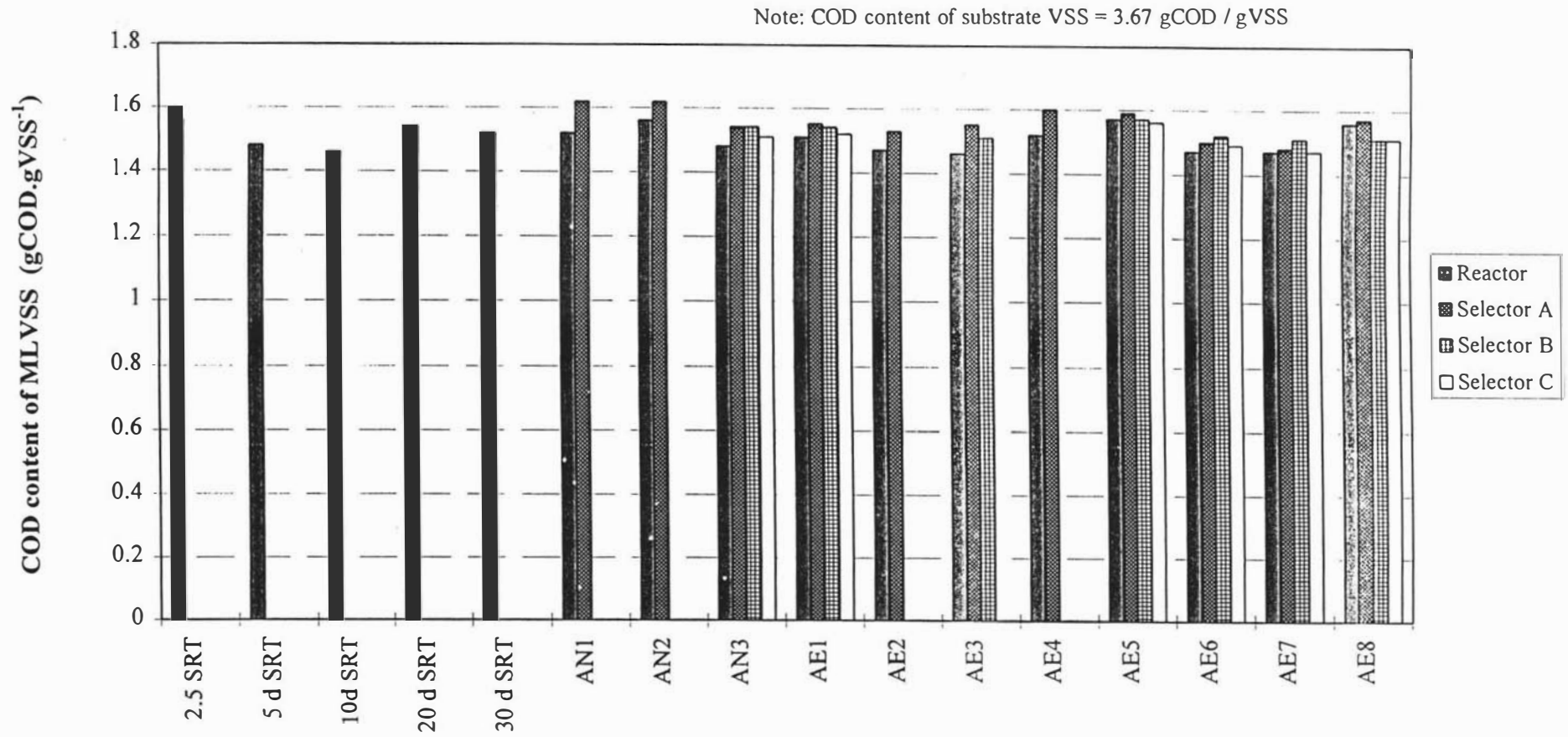
Figure 9.9   COD content of Mixed Liquor Volatile Suspended Solids in the various reactor zones during each trial.

9.4.2 Comparison of substrate removal under differing environmental conditions

A range of microbial metabolism's was observed in the trials, with anaerobic, anoxic and anaerobic substrate removals all indicated; sometimes as occurring simultaneously in a single aerated reactor zone. The initial CSTR trials were fully aerated and only aerobic substrate removal mechanisms were detected. In the first series of selector trials the selectors were not aerated as anoxic substrate removals were anticipated due to the presence of significant quantities of nitrate in the return activated sludge. However by the end of the trial some anaerobic activity was also indicated. The next two sets of trials utilised aerated selectors, but the level of anoxic and more particularly anaerobic activity increased substantially.

The occurrence of anoxic and anaerobic activity in an aerated system was due to the degradable nature of the substrate: with just over 40% being determined to be readily biodegradable and almost another 30% rapidly hydrolysable; the rate of initial immediate oxygen demand was very high, and for some microorganisms in the system, higher than the rate of supply. Under conditions imposed in this study, it is possible that the availability of substrate in floc inner regions was greater than that of either oxygen or nitrate, however the extent of this effect would have been dependant on the level of substrate loading in the initial selector zone and floc characteristics such as floc size and density. This would have resulted in inner regions of the floc being progressively anoxic and then anaerobic, supporting the growth of denitrifiers and P accumulating organisms under aerated reactor conditions.

The evidence for substrate removal under the various environmental conditions was obtained from measurements of substrate removal, oxygen consumption and biological nutrient removal. The oxygen and substrate consumption in the selector zone, during which almost all exogenous substrate was removed from solution, was monitored during the aerated selector trials. The mass of oxygen consumed above endogenous rates per mass of substrate removed, was determined from batch tests in trials AE1 to AE5 and from daily OUR and sCOD data in trials AE5 to AE8. From the low ratios obtained as listed in Table 9.5, it was concluded that the oxygen was used for substrate accumulation and storage rather than for substrate oxidation. The very low ratio in Selector A indicated that substrate removal by means other than aerobic mechanisms was also taking place.

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Table 9.5   Oxygen and substrate consumption in the aerated selector zones.

Trial	Elevated $gO_2.gsCOD^{-1}$ in Selector A	Elevated $gO_2.gsCOD^{-1}$ in Selector B	Elevated $gO_2.gsCOD^{-1}$ in Selector C	Elevated Ave. $gO_2.gsCOD^{-1}$ in Selector Zones	Total $gO_2.gsCOD^{-1}$ in Selector Zones
AE1	ND	ND	ND	0.05 - 0.11	ND
AE2	ND	-	-	0.05 - 0.10	ND
AE3	ND	ND	-	0.13	ND
AE4	ND	-	-	0.20	ND
AE5	0.06	0.65	1.2	0.10	0.20
AE6	0.06	0.95	2.7	0.11	0.19
AE7	0.08	0.54	1.8	0.14	0.23
AE8	0.07	1.2	1.2	0.12	0.18

ND   = not determined

-    = not applicable in this trial

Although the selector zone OURs indicated no significant difference in overall oxygen consumption per mass of substrate removed, both the batch tests to determine sCOD removal rates (Table 9.2) and $\Delta SpOUR_{max}$ (Table 9.3), indicated significant increases in substrate removal ability as the trials progressed.   Of the two types of batch tests, the latter measured oxygen consumption of heterotrophs alone, while the former did not differentiate between heterotrophic and nitrifier activity. As the proportion of nitrifiers would have been expected to increase as substrate N concentration increased, it is indicated that the activity of both types of organisms were similarly affected.

The actual mass of substrate removed under each type of environmental condition was difficult to assess due to the occurrence of simultaneous nitrification and denitrification in the initial selector zone and also the inability to distinguish influent DRP from released P in that zone. In the aerated selector trials utilising the original substrate it was possible to more closely define the zones in which exogenous substrate removals via denitrification were occurring, however in the higher substrate N content trials, this was made difficult by the presence of ammonia in the RAS and simultaneous ammonification, nitrification and denitrification.

As the various electron acceptors were being consumed during utilisation of both exogenous and stored or endogenous substrate, prediction of substrate removal via the different metabolic pathways was impossible to determine from the parameters measured during the tests conducted. Some indication of the relative importance of aerobic, anoxic and anaerobic removals can be gained from mass balances and the changes in the total reactor system biomass as discussed in the previous chapter. however further more detailed experimentation would be required to define the extent of and critical parameters for the various removal mechanisms more clearly.

9.5 Causes, cures and prevention of filamentous bulking

The operability of activated sludge systems can be severely affected by high SVIs, caused by the proliferation of filamentous microorganisms. Bulking sludge was observed in all three major types of reactor configuration trialed, with a different microorganism being dominant in each case.

During the first set of trials with a completely mixed reactor configuration, Type 0411 was identified as being the primary filament, increasing in abundance until the trial failed due to loss of biomass from solids carryover in the effluent. Very little is recorded in the literature about this filament, as it obviously not commonly encountered as the dominant filament in full scale systems. Eikelboom (1975) makes reference to this filament possibly belonging to the genus Flavobacterium, and Adamse (1968) also refers to *Flavobacteria* in activated sludge utilising dairy wastewater. Both authors refer to red / orange / brown colouration associated with *Flavobacteria*, which fits well with the change in colour of the mixed liquor to a red / orange colour once this offending filament had become abundant in the trial. As the DO concentration was maintained above 4.5 $gDO.m^{-3}$ in the reactor throughout the trials, DO deficiency is unlikely to be the cause and it is suggested that Type would be classified as a low F/M, or Group III (Jenkins *et al.*, 1993) filament in this situation.

A different filamentous microorganism was responsible for increased SVIs in the unaerated selector trials. This was evidenced by both microscopic examinations and the observation that bulking events were no longer accompanied by a change in mixed liquor colour. Although the use of an unaerated selector inhibited the proliferation of Type 0411, Type 021N was now able to dominate the mixed liquor under the prevailing

conditions. A secondary filament was also commonly observed, but to a far lesser extent than Type 021N and was identified as Type 1701.

Wanner and Novak (1990) found that filamentous bulking due to oxic zone growers such as Type 021N resulted when substrate was still available after the selector zone. This could be due to either incomplete soluble substrate removal in the selectors, or the hydrolysis of particulate and colloidal material in the main reactor zone, especially if the selectors were anaerobic. During the unaerated selector trials the conditions in bulk solution were anaerobic, therefore the opportunity for hydrolysis of the colloidal and particulate substrate, which constituted 59% of influent COD, may have been limited. Type 021N proliferated in the unaerated selector trials, however the rate and extent of filamentous growth could be correlated to the extent of removable sCOD consumption in the selector zone; so it appears that the filament utilised soluble substrate being carried over to the reactor, rather than that from hydrolysis of particulate substrate.

Type 021N is also reported to proliferate under conditions of N deficiency (Jenkins *et al*., 1993), which may have been a factor in the trials with originally defined feed, due to the N source being milk proteins which required ammonification before a directly utilisable N source was available to the biomass. A significant proportion of the C source was readily degradable and the RAS ammonia concentrations were negligible, so this may have led to N deficiency problems in the initial selector zones. However Type 021N, although still present, was not dominant in the aerated selector trials which experienced the same conditions of bioavailability of C and N sources. Therefore although possibly a contributing factor, the proliferation of Type 021N seems to be due mainly to the maintenance of low F/M conditions in the reactor rather than a possible N limitation effect.

The unaerated selectors were intended to be anoxic selectors as it was determined that there was a significant concentration of nitrate in the effluent during the previous CSTR trials. However, substrate removal in the unaerated selector zone was limited by the mass of oxidised N compounds available, and batch tests indicated that sCOD removal from bulk solution ceased once oxidised N compounds were exhausted. Although the conditions in bulk solution were anaerobic, it is suggested that at this stage in the trials a significant population of anaerobic microorganisms had not yet developed and therefore substrate removal was halted until the mixed liquor passed into the aerated reactor zone. To improve substrate removal in the selectors, these zones of the reactor configuration were also aerated in the remaining trials, with average removals obtained being illustrated in Figure 9.10.
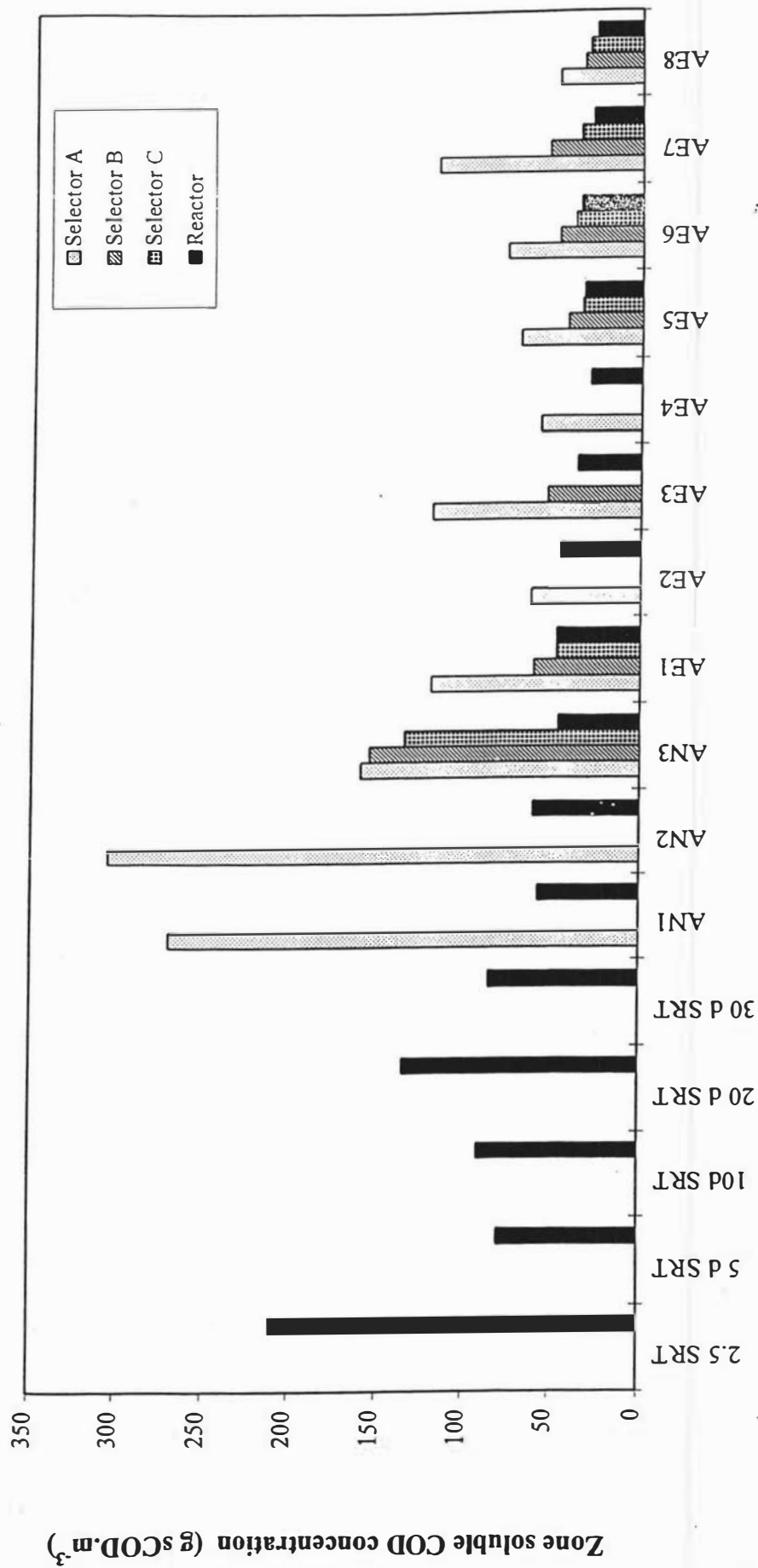
Figure 9.10 Soluble COD concentration in the various zones of the reactor system.

The use of aerated selectors also resulted in filament growth in some cases, with the dominant organism this time being identified as *Haliscomenobacter hydrossis*. The colour of the mixed liquor could again be associated with changes in SVI, as bulking incidents attributable to *H. hydrossis* resulting in the mixed liquor changing from a deep yellow colour to a paler hue. Substrate removal efficiencies in the selector zone were very high, at above 95%, and could not be directly related to bulking incidents; however significantly lower concentrations of nitrate and nitrite were recorded in the selector zones during the two unsuccessful trials with original substrate, indicating that *H. hydrossis* must have been able to compete in low DO conditions. DO had not been regularly recorded in earlier 'AE' series trials, but would have been negligible in zones where the nitrate concentration was also low.

Trials conducted at elevated substrate N levels also demonstrated periodic increases in SVI due to the proliferation of *H. hydrossis*. The increases in filament abundance occurred after periods of low DO in the initial selector zone, even though significant nitrate concentrations were observed at all times, reinforcing the 'low DO' or Group I (Jenkins *et* al., 1993) classification of this filament.

A decline in the abundance of *H. hydrossis* was observed when the ammonia concentrations in the reactor rose above around 10 $g.m^{-3}$, and pH in the reactor declined below pH 6. It is proposed that *H. hydrossis* was inhibited by either high ammonia concentrations or its ability to restore accumulation capacity under lowered pH conditions, and although Kampfer *et al*. (1995) found inhibition of *H. hydrossis* under high ammonia concentrations, it was at significantly higher concentrations than those recorded in these trials. Batch sCOD removal tests also indicated a change in substrate removal ability for biomass regenerated under lowered pH, indicating that it was the pH effect that probably had the greater influence.

It is suggested that the low reactor zone pH interfered with the regeneration of substrate accumulation capacity for *H. hydrossis* to a greater degree than for the floc formers present. This may have been due to the closer proximity of the filaments to the lower pH bulk solution than for the floc formers, as the latter could then have been protected by diffusional resistance or greater buffering within the floc structure; or it may have been due to differing susceptibility of the various microbial enzymes to lower pH conditions.

The substrate removal rate curves indicated that elimination of the more slowly removed filterable substrate components were affected to a greater degree under conditions of

biomass regeneration at lower pH, and it is proposed that this fraction would be represented by the casein micelles. The lower pH may have interfered with filament enzyme activity, in particular proteolytic enzymes responsible for substrate hydrolysis, which would have rendered the filament unable to restore accumulation capacity in the reactor zone.

The substrate removal conditions provided that prevented proliferation of the filaments types observed is therefore indicated as being: Type 0411 was inhibited by maintenance of a substrate gradient; Type 021N by efficient substrate removal in the selector zone; and *H. hydrossis* by well aerated selectors and low pH. It is generally stated that the use of selectors is effective against both 021N and *H. hydrossis*, particularly if anoxic or anaerobic selectors are used (Jenkins *et al.*, 1993), however it was found in this study that the selector performance requirements for the successful prevention of filamentous growth were more specific and restricted to:

- greater than 95% removable sCOD consumption, and
- fully aerobic conditions in bulk solution.

Due to the limited supply of oxidised N compounds, unaerated selectors were unable to remove sufficient substrate to prevent bulking. However this type of selector may have proved successful if the trial had been continued until a significant proportion of PAO's had developed to consume the remaining removable sCOD in the selectors.

Both single and serial selector configurations were successful, however the serial selectors were more so, resulting in a more rapid reduction of SVI to a lower level. The use of serial selectors would also allow greater flexibility and assurance of the required selector performance under the fluctuating substrate conditions that would be encountered in full scale systems.

The other commonly reported operability problem due to biomass composition is foaming. This phenomena was only observed occasionally during the study; resulting in scum accumulation and biomass carryover in the settler zone. During these periods some *Nocardia* was observed to be present in the mixed liquor, but removal of the accumulated scum on the settler tended to overcome foaming incidents.

Other activated sludge microorganisms commonly seen under the 100x and 400x magnification predominantly used for biomass observations included: rotifers; protozoa, particularly attached ciliates; and more infrequently, nematodes. The rotifers present were primarily *Philodina sp.*, although a few *Lecaninae sp.* were occasionally observed;

the colonial stalked ciliated protozoa were tentatively identified as *Opercularia sp.*, *Epistylis sp.*, and *Vorticella sp.*. It has been recently proposed by a number of researchers (Madoni *et al.*, 1993; Salvado and Gracia, 1993; Salvado *et al.*, 1995) that the protozoan species present can be used as an indicator of activated sludge operational conditions, and those identified in this study are commonly associated with high organic loadings, as were provided in this study by the use of a selector configuration.

Small free swimming ciliates were also often observed, but further identification of these organisms was not attempted. Rotifers and nematodes were indicated by Salvado *et al.* (1995) as being associated with AS plants with a good effluent quality in terms of BOD, in agreement with the results in this study. The diversity of higher organisms including free swimming and stalked ciliated protozoa, rotifers and nematodes were as expected for a SRT of 10 days (Jenkins *et al.*, 1993); and would have contributed to the low effluent TSS levels regularly obtained, by consuming unflocculated or dispersed bacteria.

9.6   Nutrient removal

Due to the rapid rate of substrate removal in the initial selector zone, anoxic and anaerobic zones were maintained in the floc, even under aeration in that zone. This resulted in the growth of a significant population of microorganisms that were able to utilise substrate under anoxic and anaerobic conditions, resulting in denitrification and P accumulation in the biomass respectively. The ability to achieve significant removal of nitrogen and phosphorus from the liquid effluent stream was considered an additional benefit of the selector reactor configuration. Average system nutrient removal of up to 96% N and P were measured, due to low soluble N and P concentrations combined with low effluent TSS levels, with the wasted biomass becoming a significant mechanism for both N and P removal from the reactor system. Denitrification was estimated to account for up to 49% of system N removals.

9.6.1   Nitrogen Removal

Substrate nitrogen was supplied in the form of milk proteins, which required ammonification to take place before a readily utilisable form of N was available to the biomass for growth or nitrification. The total BOD:N ratio of the substrate was around
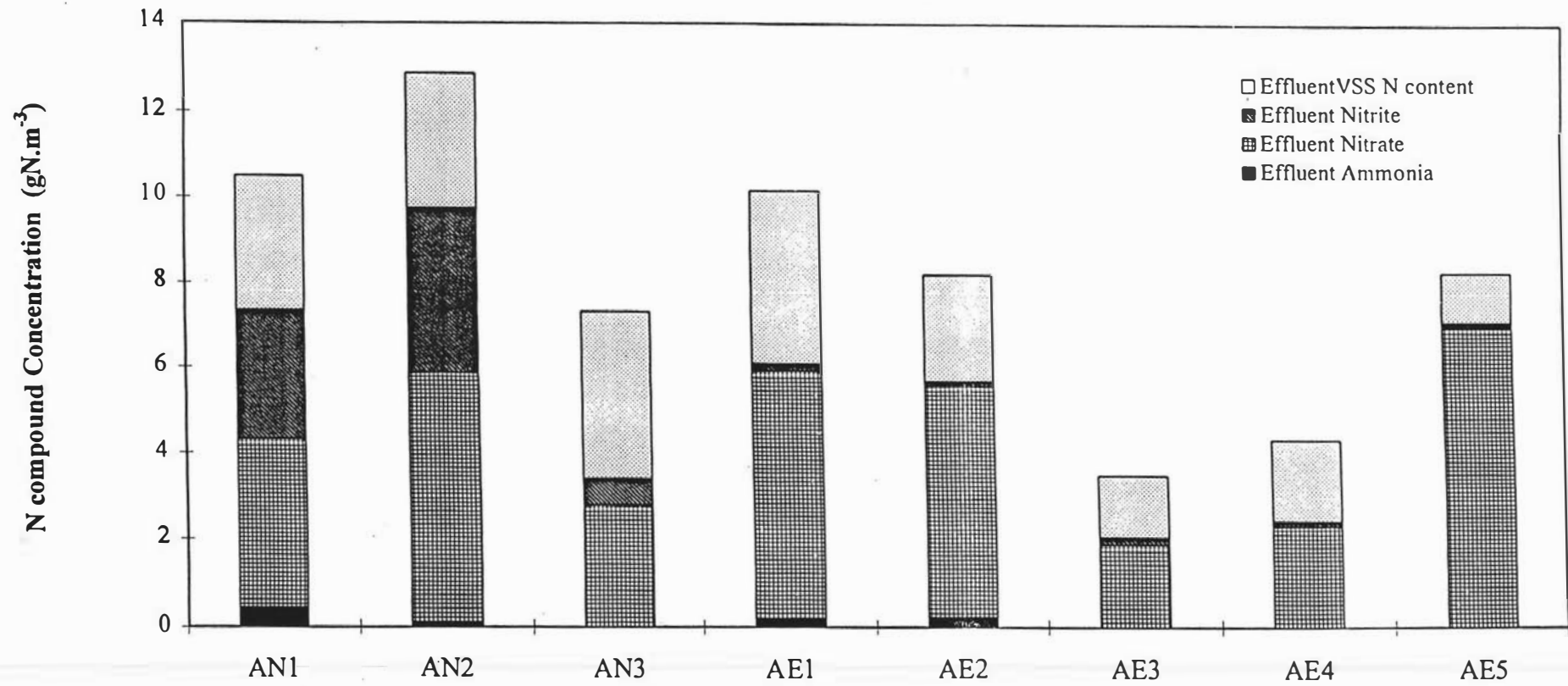
Figure 9.11   Nitrogen compounds in the effluent stream during Trials AN1 to AE5.

12, a value below the ideal ratio of 20 commonly proposed by a BOD:N:P ratio of 100:5:1 for biomass growth (Metcalf and Eddy, 1991; Jenkins *et al.*, 1993); however nitrogen removal was observed during all of the selector reactor configuration trials, with average N removals via denitrification estimated to range between 16% and 49%.

Nitrogen in the influent stream was either removed via denitrification in the reactor system, flowed out in the waste activated sludge stream, or flowed out in the effluent stream. The N content of the liquid effluent stream was due to the presence of ammonia, nitrate, nitrite and biomass cellular nitrogen; and the contribution of each is shown for the trials with original substrate in Figure 9.11. It can be seen that the effluent VSS content can have a significant effect on the total effluent N, emphasising the importance of good floc structure and settleability on achieving a high quality effluent.

Henze (1991) reported typical effluent N concentrations of between 6 and 10 $gN.m^{-3}$ for biological N removal plants, therefore the results in Figure 9.11 indicate that the aerated selector trials performed as well as could be expected from a specialised BNR configuration in terms of effluent quality. The best N removal performances were recorded during Trials AE3 and AE4, which were indicated as having low selector DO and therefore more substrate removal via anoxic and/or anaerobic mechanisms; however both these configurations permitted the proliferation of *H. hydrossis* and consequently would have resulted in operability problems.

9.6.1.1   Ammonification

In the originally defined substrate, N was supplied at a rate of 0.89 $gN.d^{-1}$ in the form of milk proteins. Therefore ammonification needed to occur before the N was in a readily available form to the activated sludge microorganisms, either for cell growth or for nitrification.

Ammonification of feed stream proteins occurred in the selector under all environmental conditions, as evidenced by the appearance of detectable concentrations of ammonia in the selector zones during both the aerated and unaerated selector trials. The RAS ammonia concentration was negligible but ammonia concentrations were observed to increase through subsequent selector zones. It was indicated that up to 55% of ammonification occurred in the selector zones, although this would have been

dependant on the RAS recycle rate which determined both floc loading and residence time in the selector zones.

Ammonia concentrations in the initial selector correlated well to the substrate loading to that zone, supporting the theory that ammonification is a first order reaction with the rate being dependant on organic N concentration (Wong-Chong and Loehr, 1975). During the unaerated selector trials, no nitrification was possible in the selector zones, so an estimate of the ammonification rate constant was possible from ammonia production data and ranged between 0.029 to 0.047 min^{-1}, about ten times greater than values reported by Wong-Chong and Loehr (1975) on a variety of substrates.

In the aerated selector trials utilising serial selectors the ammonia concentration reached a peak in the first or second selector, indicating that in the final selector zone the nitrification rate exceeded the ammonification rate. Exogenous substrate removal was almost complete in this zone, so more oxygen would have been available for nitrification reactions. During the final series of aerated selector trials simultaneous ammonification and nitrification were observed in all selector zones, which made further estimates of the ammonification rate difficult to determine.

9.6.1.2   Nitrification

During the trials with the originally defined substrate, complete nitrification occurred consistently in the reactor zone resulting in negligible effluent ammonia concentrations as illustrated in Figure 9.11. Although nitrification was determined to be occurring in all aerated zones during the trials performed, the greatest mass of ammonia was oxidised in the reactor zone. An estimation of the minimum nitrification rates required to produce the oxidised N compound concentrations observed in the reactor zone indicated that nitrification was easily completed within the reactor residence times provided.

Significant nitrite concentrations were recorded during some of the trials. The unaerated selector trials produced significant nitrite concentrations in the reactor zone, which could not be linked to commonly reported causative conditions such as high ammonia and low pH. Trends in the concentrations of nitrite mirrored those for nitrate which also seemed to indicate that nitrite accumulation was not caused by the inhibition of *Nitrobacter sp.* However it has been reported by Nowak *et al.* (1995) that exposure of activated sludge microorganisms to anaerobic conditions did not affect ammonia oxidation capacity but resulted in a decreased nitrite oxidation capacity.

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As the unaerated selector trials progressed the concentration of nitrite in the reactor declined, which would have been expected as the biomass became acclimated by repeated exposure to the anaerobic selector conditions. During the aerated selector trials the presence of measurable nitrite concentrations was confined to the selectors, and to a much lower level than that observed in the series of unaerated selector trials, which also seemed to indicate that the effect was due to the extent of exposure of the flocs to anaerobic conditions.

Immediately after the substrate N concentration was increased, a period of nitrite accumulation was observed which may have been due to the inhibition by the increased ammonia concentrations provided via the substrate. An estimate of the maximum specific growth rate of nitrifiers was obtained from the reactor nitrification response when the substrate N concentration was increased, and calculated to vary between 0.6 to 1.2 d^{-1}, within the range of values reported by other researchers (Azimi and Horan, 1991; Metcalf and Eddy, 1991).

Although complete nitrification was observed with the originally defined substrate, when substrate N levels were increased, periods of inhibited nitrification were recorded which resulted in significant effluent ammonia concentrations. Increasing the substrate ammonia concentration required an increase in aeration to respond to the increased nitrification load. When the aeration was at lower levels in the first selector, sCOD removals did not seem to be adversely affected, suggesting that the availability of oxygen for nitrification would have been reduced. This increased the nitrification load in the subsequent reactor zone, to the extent that all available alkalinity was consumed, thereby reducing the reactor pH and causing an accumulation of ammonia. Therefore care must be taken to not exceed the system alkalinity if periods of high N loadings in the influent are possible.

9.6.1.3 Denitrification

The extent of denitrification during each trial was estimated from a N balance as outlined in Barker and Dold (1995), however the mass of N removed via denitrification was calculated over the entire reactor system and not just unaerated zones, as denitrification was indicated as occurring in all reactor zones, whether unaerated or aerated. Bulk solution conditions in the initial selector zones during the 'AN' trials were anaerobic rather than anoxic due to the supply of oxidised N compounds being insufficient for complete substrate removal. The aerated selector trials had selector
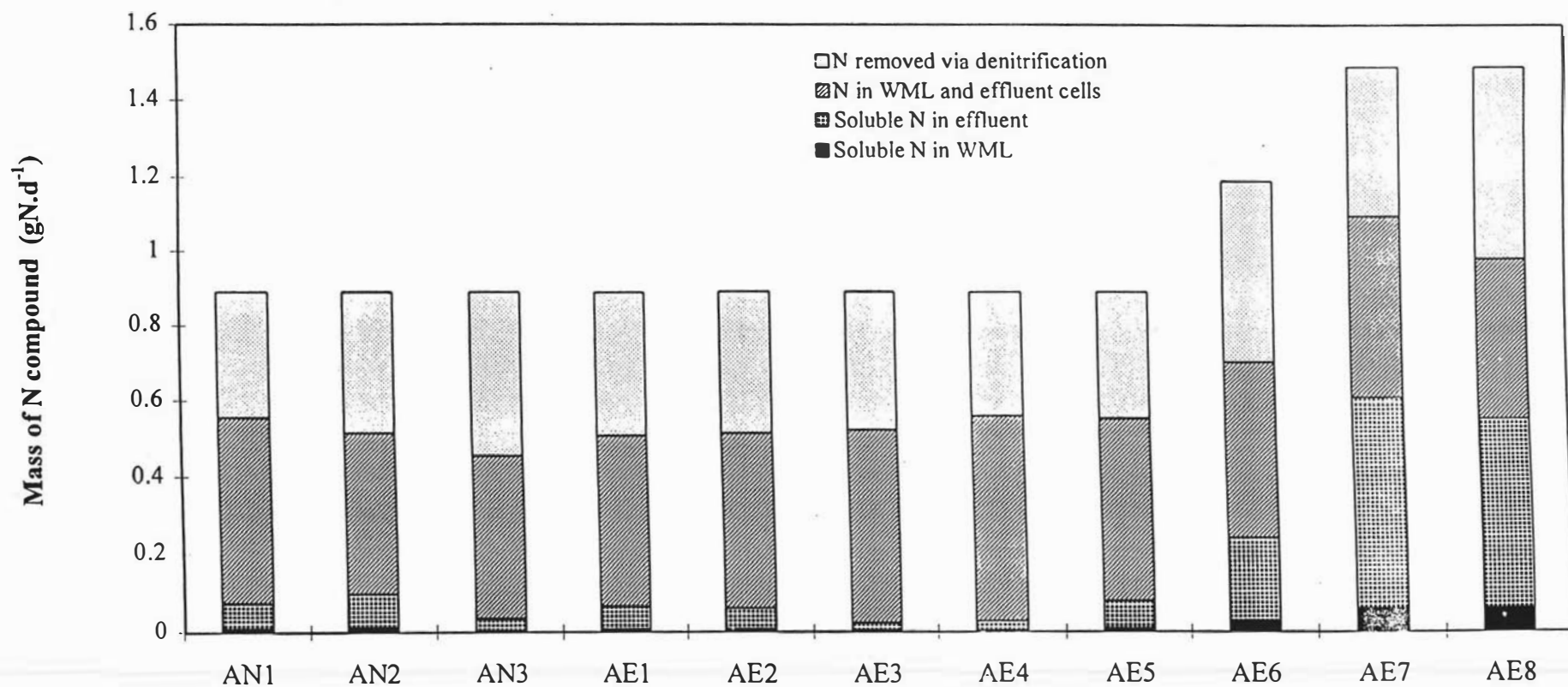
Figure 9.12   Reactor system nitrogen balance and trend in extent of denitrification during Trials AN1 to AE8.

zones with bulk solutions that were variously anaerobic, anoxic and aerobic, with evidence of denitrification processes occurring in each instance.

The average extent of denitrification in each trial, including those at increased substrate N content is illustrated in Figure 9.12. Of the N removed as biomass, only approximately 2% of this value was associated with the effluent stream and the remainder with the WAS stream. It can be seen that denitrification was a significant means of N removal, both in the unaerated and aerated selector trials. The estimated N removals when using the originally defined substrate averaged from 37% in Trial AE4 to 49% in Trial AN3; and while at increased substrate N levels ranged from 16% to 55% during Trial AE8.

Denitrification rates could only be estimated during the unaerated selector trials. Rates of at least 0.15 gNO_3-$N.gVSS^{-1}.d^{-1}$ must have been achieved to remove all the nitrate and nitrite in the RAS, which is slightly above values commonly reported (Argaman and Brenner, 1986; Henze, 1991; Metcalf and Eddy, 1991; Wanner, 1994) but below maximum levels more recently reported for experiments with readily biodegradable substrate (Henze *et al.*, 1994; Carucci *et al.*, 1996).

Denitrification was first observed during the unaerated selector trials, but a N balance performed around the reactor system indicated that as the trials progressed the amount of N removed by denitrification became greater than that accounted for by the removal of oxidised N compounds in the RAS alone. This indicated that denitrification must have been occurring together with nitrification in some reactor zones, a situation that has also been more recently reported by other researchers (Suwa *et al.*, 1992; Szpyrkowicz and Zilio-Grandi, 1995a; Munch *et al.*, 1996).

In the unaerated selector trials nitrification was unable to proceed in the selector zones, which indicated that to account for the N removals observed, denitrification must have been taking place in the reactor zone. During the aerated selector trials the mass of influent N removed remained similar at between 37 to 49%, however the proportion of denitrification that could be attributed to removal of RAS compounds further decreased, averaging between 7% and 32%. Therefore it was indicated that the extent of simultaneous nitrification and denitrification had continued to increase, with its occurrence suggested in all reactor system zones. Although there was no exogenous substrate in solution in the reactor zone, Carucci *et al.* (1996) reported that endogenous or stored substrate was also able to be utilised for denitrification. Other researchers (Isaacs and Henze, 1995; Barker and Dold, 1996; Sorm *et al*, 1996) have concluded that

PAO's can utilise nitrate to restore poly-P reserves. Therefore denitrification may have been occurring both a means of substrate removal and also as a result of PAO activity.

The DO concentration in the reactor zone was maintained at above 4.5 $g.m^{-3}$, however the internal regions of the floc must still have been anoxic for denitrification to occur as oxygen is generally believed to be inhibitory; although Munch *et al.* (1996) indicated that denitrification may still proceed at appreciable rates under low, but measurable, DO concentrations. Due to the appreciable levels of stored and enmeshed substrate to be utilised in the reactor zone, and the significant nitrate concentrations, especially during trials at increased substrate N levels; conditions of limited oxygen but consequential nitrate in the inner regions of the floc could have been easily achieved.

Denitrification was also a significant reaction in the settler zone, as has been reported by other researchers (Cizinska *et al.*, 1992; Henze *et al.*, 1993; Siegrist and Gujer, 1994; Siegrist *et al.*, 1995), and has been reported to cause problems with rising sludge when the nitrate concentration was above 6 to 8 gNO_3-$N.m^{-3}$. A decrease in the concentration of oxidised N compounds through the settler zones was observed in most trials and although the nitrate concentration was as high as 60 gNO_3-$N.m^{-3}$ in Trial AE8, rising sludge was not observed in the later trials. Rising sludge had however been a problem towards the end of earlier CSTR trials when bulking biomass resulted in long residence times in the settler zone.

The extent of denitrification decreased in the final aerated selector trials with the increased substrate N content. It is proposed that this was due to changes in the diffusional resistances in the flocs. Microscopic observations of floc size and visual observations of mixed liquor filterability indicated that the flocs became smaller and the extent of extracellular polymeric substances declined as both Trials AE7 and AE8 progressed; which would have improved the diffusion of oxygen, nitrate and substrate into the centre of the floc.

It is indicated that the loss of denitrification occurred primarily in the reactor zone as the extent of denitrification decreased before loss of biological P removal was observed. Increased oxygen penetration into flocs would have meant that PAOs were then able to utilise oxygen rather than nitrate for P uptake in the reactor zone. As Trial AE8 progressed, an increase in effluent DRP concentration was observed which indicated that the extent of anaerobic floc regions in the initial selector had declined, and a parallel reduction in the reactor zone anoxic regions would also have been expected.

It is therefore suggested that a careful balance between floc characteristics, substrate loading and environmental conditions needs to be maintained to ensure the occurrence of simultaneous nitrification and denitrification in a fully aerated system.

9.6.2   Phosphorus Removal

It became evident that phosphorus removal, in excess of that expected for normal growth requirements, was occurring in the system to an increasing extent during the selector reactor trials. The trend of declining DRP concentration in the effluent was accompanied by an increase in biomass P as shown in Figures 9.13 and 9.14. The P content of the biomass was not measured in the conventional CSTR configurations, but averaged 0.015 $gP.gVSS^{-1}$ in the first unaerated selector trials and increased to a maximum of 0.026 $gP.gVSS^{-1}$ which was observed during Trial AE8.

The time to initiate biological P removal has been observed to be as short as two anaerobic / aerobic cycles (Ubukata and Takii, 1994), however the gradual decline in effluent DRP and increase in biomass P content observed in Figures 9.13 and 9.14 indicated that selection for PAOs, and their increase to a significant proportion of the biomass took place over a number of SRTs.

It has been found that the ratio of BOD to P is important in determining the extent of P removal achievable. A comparison of the substrate requirements detailed by published reviews of biological P removal (Tetreault *et al.*, 1986; Yeoman *et al.*, 1988; Metcalf and Eddy, 1991) to that supplied in this study, reveals that the ratio of carbonaceous substrate to phosphorus in the wastewater was higher than recommended, to the point of being possibly P deficient. This implies that the P content of the biomass and extent of P removal achievable would have been limited, in fact the ratio of P removed to substrate removed was 0.006 $gP.gCOD^{-1}$ or approximately 0.012 $gP.gBOD^{-1}$; considerably lower than published values of 0.04 to 0.08 $gP.gBOD^{-1}$ (Tetreault *et al.*, 1986;) due to the high initial COD:P ratio of the substrate.

The P content of enhanced biological phosphorus removal (EBPR) biomass has been reported to be as high as 11% (Appeldoorn *et al.*, 1992), however using the relationship between substrate COD:P ratio and biomass P content reported in Tetreault *et al.* (1986), a maximum biomass P content of about 2% would be expected for this substrate. Therefore P contents observed in the later aerated selector trials did represent
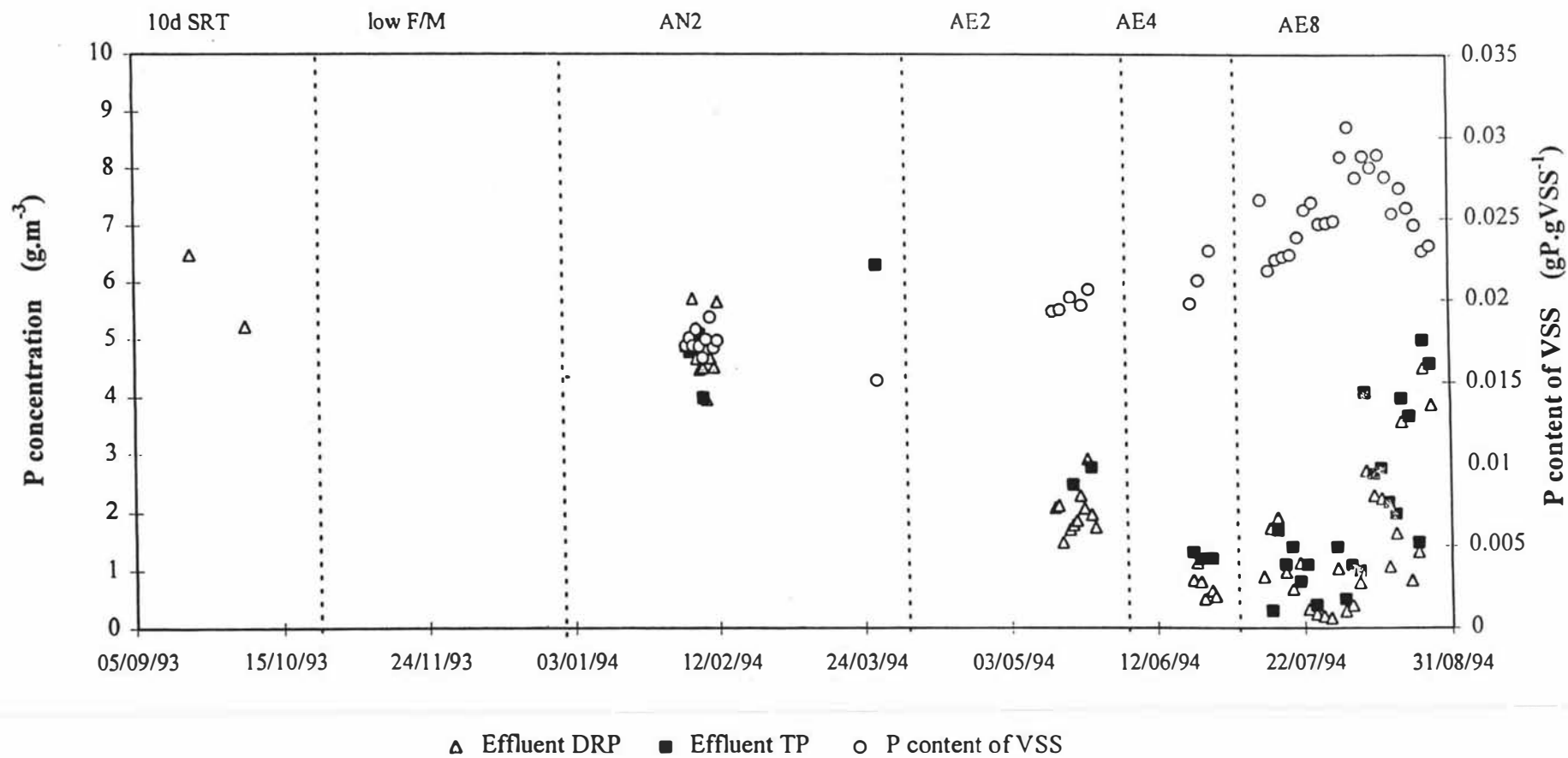
Figure 9.13   P content of biomass and effluent during trials in Reactor System 1.
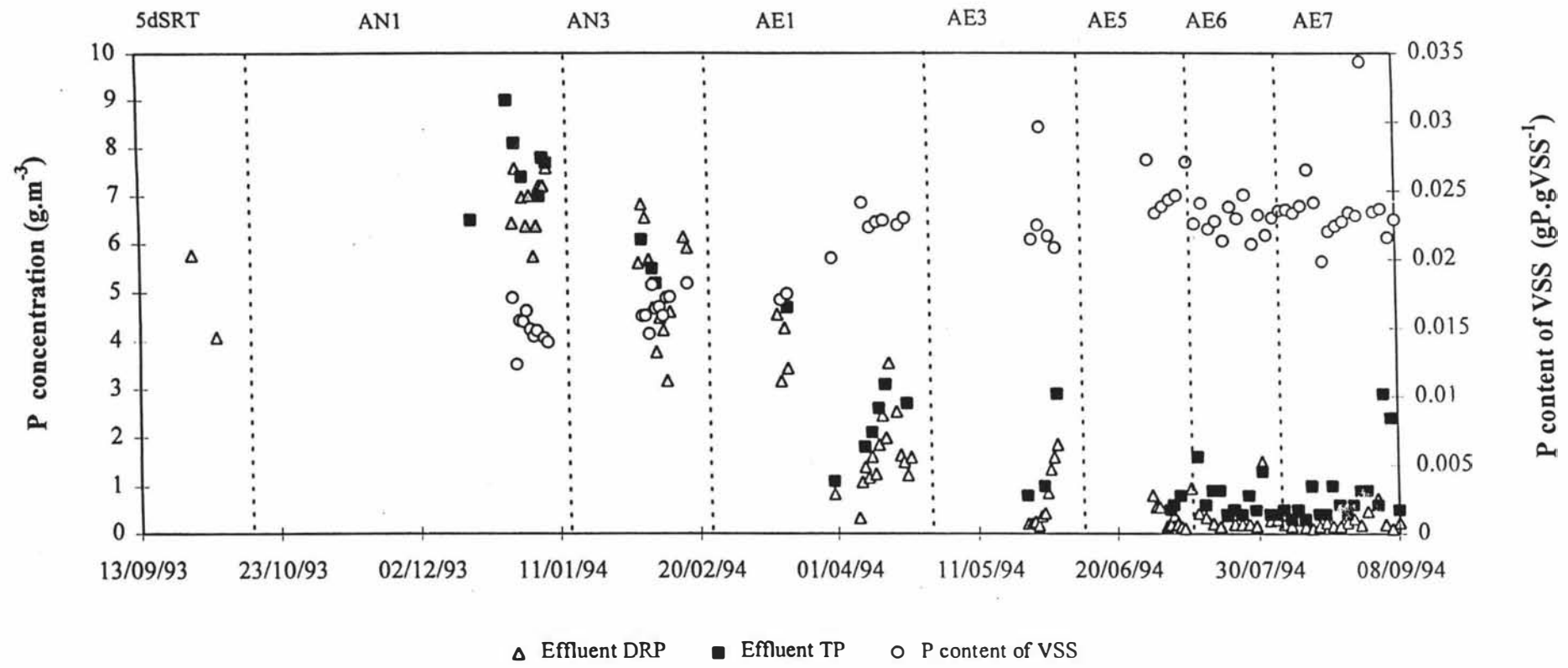
Figure 9.14   Trend in effluent P concentrations and the P content of biomass during trials in Reactor System 2.

luxury P uptake even though they were considerably lower than that observed in conventional EBPR systems.

Due to the accumulation of P in the biomass, it has also been observed that as the extent of P removal increased, the VSS/TSS ratio of the MLSS decreased (Heymann and Potgieter, 1989). As can be seen in Table 9.6 the VSS/TSS ratio is indicated to have possibly decreased slightly as the trials progressed, which may have been due to the increasing fraction of inorganic P in the biomass, from 1.8% to 2.6% in Reactor 1 trials, and 1.5% to 2.5% in Reactor 2.

As well as P removal due to the activity of poly-P accumulating microorganisms, additional removal may occur via precipitation of P due to calcium ions in the wastewater. Heymann and Potgieter (1989) indicate that this effect may be significant at elevated pH when the calcium concentration is above 50 $g.m^{-3}$; but as the Ca concentration in the substrate was estimated to be 17 $g.m^{-3}$, and the mixed liquor pH remained less than 9, the opportunity for chemical precipitation of P onto flocs would have been minimal, as supported by the limited extent of decrease in the VSS/TSS ratio.

Table 9.6   VSS/TSS ratio of the reactor suspended solids.

R1: Trial	VSS/TSS	R2: Trial	VSS/TSS
CSTR trials:			
20d SRT	0.91 ± 0.06	30d SRT	0.88 ± 0.06
10d SRT	0.92 ± 0.05	5d SRT	0.92 ± 0.06
Selector trials:	(10d SRT)		
		AN1	0.91 ± 0.04
AN2	0.90 ± 0.04	AN3	0.92 ± 0.05
		AE1	0.90 ± 0.04
AE2	0.91 ± 0.03	AE3	0.90 ± 0.06
AE4	0.91 ± 0.03	AE5	0.89 ± 0.03
		AE6	0.88 ± 0.03
AE8	0.87 ± 0.04	AE7	0.88 ± 0.05

Figure 9.14 illustrates that the average total P concentration in the effluent stream was 0.6 $gP.m^{-3}$ in Trials AE5 and AE6, which represented a maximum average P removal of 96 % for the trials conducted. The aerated serial reactor configuration was therefore efficient in supporting biological P removal even though anaerobic conditions were not maintained in bulk solution at any point in the system. By the end of trial AE5 the soluble P in the effluent was consistently below 1 $g.m^{-3}$ and due to the low level of suspended solids in the effluent, the total P concentration was also generally below 1 $g.m^{-3}$. Due to the relatively low phosphorus content of the biomass, effluent TSS levels had a lower effect on total effluent P than commonly reported for EBPR systems.

An increase in the average P content of biomass as it passed from selector to reactor zones was indicated during most of the selector reactor trials as shown in Figure 9.15, however the difference was not significant at the 95% level of confidence. This would have been consistent with P release for substrate accumulation in the first selector zone and subsequent P uptake in downstream zones.

The increase in average DRP concentrations between the reactor and effluent zones would be consistent with P release into solution in the settler, which would have been possible as anaerobic conditions would have existed in the settled sludge layer, the necessary substrate may have been provided by the hydrolysis of enmeshed particulate substrate. Carlsson *et al.* (1996) has also reported P release in zones without RBCOD.

The accepted mechanisms for biological P removal require that readily biodegradable substrate be available to the bacteria in an anaerobic environment: a condition that was provided in all of the trials possessing a selector configuration. During trials AN1 to AN3 anaerobic conditions prevailed in the selector zones, however during Trials AE1 to AE8, bulk conditions in the selector zone were either anoxic or aerobic and the anaerobic conditions are indicated as having existed within the flocs in the first selector zone only.

Generally aerobic conditions are required to follow the anaerobic zone, to allow the PAO's to regenerate substrate accumulation capacity by restoring P reserves. The indication of simultaneous nitrification and denitrification in all reactor zones suggests that floc interiors may have been oxygen deficient even in the reactor zone, however Comeau *et al.* (1987) and Kerrn-Jespersen and Henze (1993) demonstrated that some P accumulating bacteria were able to utilise nitrate to oxidise stored substrate and regenerate poly-P reserves. This would have allowed P uptake to occur along with denitrification in the reactor zone.
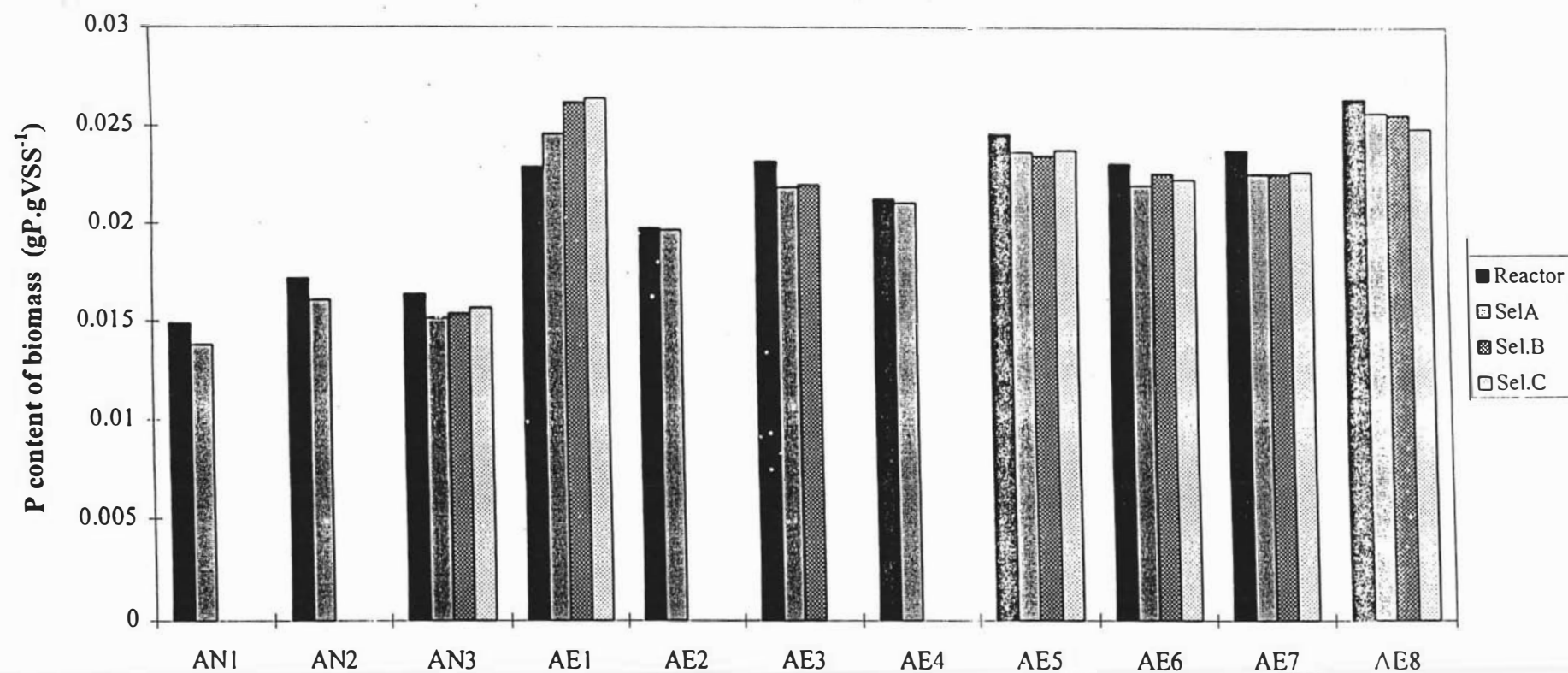
Figure 9.15   Average phosphorus content of biomass in the various reactor zones during Trials AN1 to AE8.
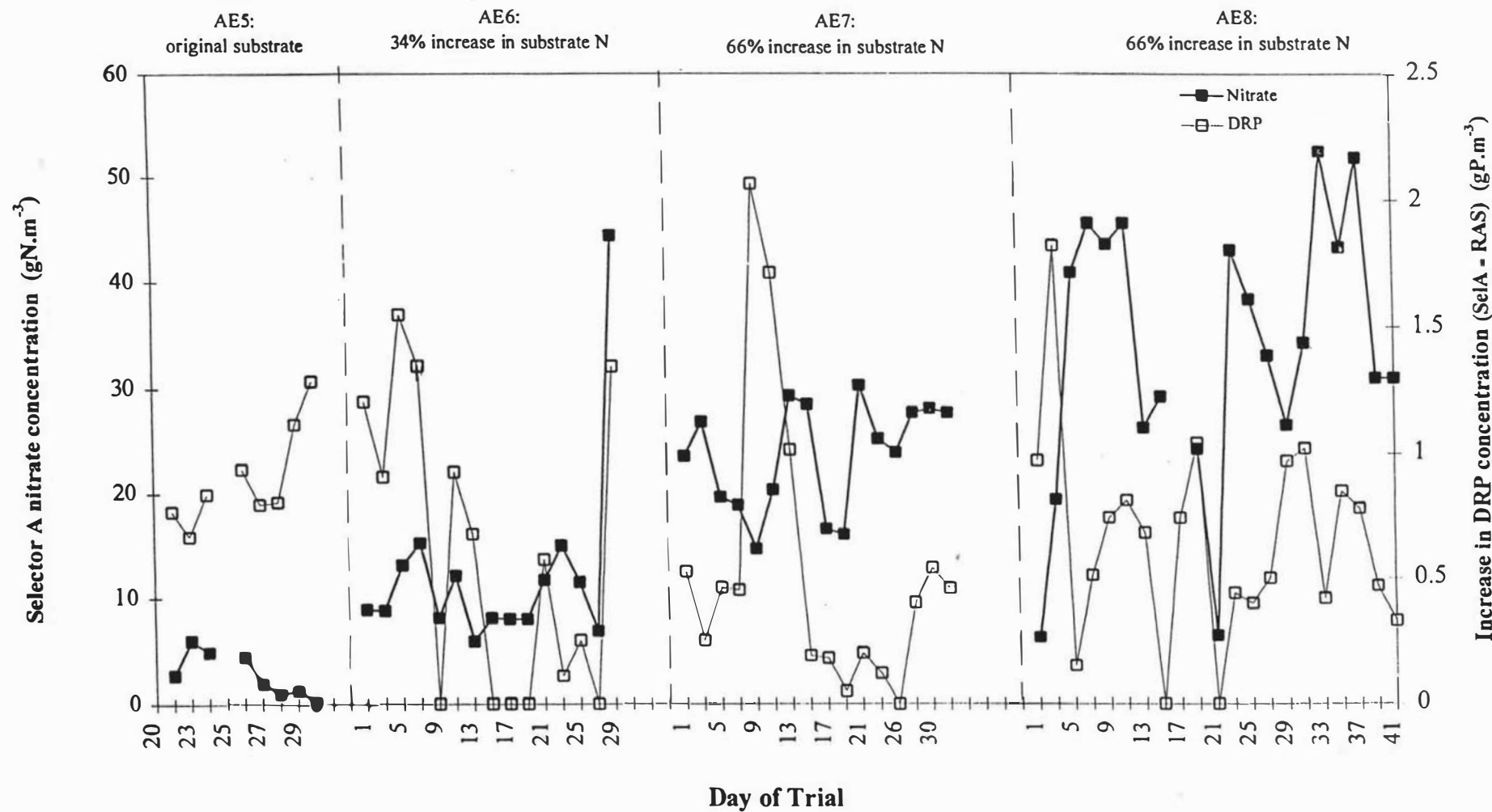
Figure 9.16   Change in initial selector zone DRP with increasing nitrate concentration during Trials AE5 to AE8.

An increase in initial selector zone nitrate concentrations as the higher substrate N trials progressed did not seem to adversely affect overall system P removal as has been proposed (Tetreault *et al.*, 1986; Appeldoorn *et al.*, 1992). The nitrate concentrations increased substantially during Trials AE6 to AE8, but as can be seen from Figure 9.16, there was no concurrent decrease in biomass P content, although a declining trend in first selector zone DRP concentration may have been indicated. However, as the overall extent of EBPR increased during Trials AE6 and AE7 even though the nitrate levels in all zones increased, it is indicated that the presence of nitrate alone does not inhibit PAO activity. This seems to support the suggestion that the presence of nitrate allows microorganisms to utilise oxidative pathways in preference to fermentative pathways (Lotter, 1985), rather than acting as an inhibitor P release.

An increasing extent of EBPR was observed as the trials progressed, except for during AE8, the second trial with a high level of substrate N. The flocs were smaller and contained less viscous polymeric material at the end of Trials AE7 and AE8, so penetration of oxygen and oxidised nitrogen compounds to the interior of the flocs would have been facilitated and the extent of any anaerobic regions in the floc decreased. Trials AE7 and AE8 were operated under similar conditions except that the recycle rate was higher in AE8. It is proposed that the lower resultant floc loading rate in AE8, coupled with a higher bulk nitrate concentration in the first selector zone and lower floc resistance to mass transfer, eliminated the required combination of adequate substrate supply and anaerobic conditions in the centre of the flocs, and as a result the EBPR declined.

9.7   Implications for Design of Full Scale Systems

This study has demonstrated that an activated sludge system can be used to effectively treat the wastewater from a dairy processing facility, however careful design is required to ensure that operational problems due to filamentous bulking do not result. It was demonstrated that the use of a selector configuration to impose a concentration gradient in the system could have a number of benefits: more efficient substrate removal; the prevention of filamentous microorganism growth; and the opportunity to incorporate biological N and P removal.

Due to the readily biodegradable nature of the substrate, under high floc loading conditions of around 0.1 to 0.2 g sCOD. gVSS^{-1} rapid rates of substrate accumulation

result in anoxic and anaerobic zones being maintained in the biomass flocs, even though the bulk solution is aerated and may be maintained in an aerobic condition. This effect can possibly be used to advantage to accomplish biological N and P removal from the wastewater, without the need to incorporate defined anoxic and anaerobic reactor system zones or utilise complicated internal recirculating flow regimes.

Although the use of unaerated selectors was not successful within the timeframe of this study, it is believed that this mode of selector operation could still provide successful bulking control for dairy processing wastewaters. The 'AN' series of trials still resulted in bulking as a population of PAOs sufficient to remove the substrate remaining after the consumption of oxidised N compounds had not yet developed by the end of the trial. Further study with combinations of anoxic / aerobic selectors, or the strategy of starting up the process in this configuration, then converting to completely unaerated selectors once the slower developing PAO's and denitrifiers were sufficiently developed, should be investigated

Serial selector configurations were found to be more successful than single selectors and would provide better assurance of required substrate removal efficiencies under variable substrate conditions. The use of serial selectors would also allow greater flexibility in maintenance of anoxic and anaerobic zones within the floc to maximise the opportunity for providing N and P removal in the selector zone.

The extent of denitrification observed was due not only to anoxic substrate removal mechanisms in the initial selector zone, but also denitrification in all the other reactor zones. Simultaneous nitrification and denitrification in zones with appreciable dissolved oxygen contents suggest that anoxic zones are maintained in the inner regions of the flocs. Denitrification in the reactor zone may have also been due to PAO's utilising nitrate to restore poly-P reserves, therefore the maintenance of these anoxic zones was important for both N and P removal performance. The ability to maintain anoxic regions in the floc would have been dependant upon controllable reactor characteristics such as DO concentration and floc loading, but also in the less easily controlled parameters such as floc size, floc density and floc polymer composition.

Conditions for maximum N and P removal required that high substrate loadings be maintained in the initial selector zone; however filamentous bulking occurred when conditions of bulk solution in the initial selector were not maintained in an aerobic state. Therefore a balance between sufficient DO supply to prevent bulking and maintain low

RAS recycle rates; but restricted electron acceptor supply to maintain anoxic and anaerobic regions in the floc must be struck.

Very high N and P removals from the liquid effluent stream can be achieved, however in addition to maintaining the requisite floc conditions for biological nutrient removal, some other contributing factors were highlighted. If the influent is highly variable with regard to N content and full nitrification is essential, the system alkalinity may need to be monitored. Trials at the highest substrate N content were affected by alkalinity limitations which resulted in inhibition of nitrification and accumulation of significant effluent ammonia concentrations. The wasted mixed liquor removal from the system contributes significantly to the system N removal and is the sole point of P removal, therefore the conditions under which the biomass is removed and point of withdrawal from the system is important. Settler conditions also affect overall P removal and in order to maintain low effluent soluble P levels, anaerobic conditions in the settler should be minimised.

The effluent total N and P levels obtained for the most successful configuration in this study of less than 10 $g.m^{-3}$ N and less than 1 $g.m^{-3}$ P, are close to those that would generally be required for effluents discharging to freshwater receiving waters. The NZ Ministry for the Environment (1992) specifies N and P concentrations in freshwater systems to prevent undesirable biological growth, in particular benthic algae; and the effluent N and P levels achieved in this study would require receiving water dilution of approximately 100 times and 30 times to achieve the desired N and P levels respectively. As the N level achieved is likely to be closer to any nutrient limits imposed than the P level, further optimisation of the system to reduce effluent nitrate concentrations may be of benefit. Effluent ammonia concentrations of <0.1 $g.m^{-3}$ were consistently obtained and therefore did not contribute to the effluent nitrogen load.

As the trials in this study were conducted with the prevention of filamentous growth being the primary concern, and the subsequent high degree of N and P removal occurring as an unexpected bonus, some of the information required to more adequately detail the relationships between N, P and substrate compounds was not foreseen. The critical parameters for maintaining a balance between the needs for a non bulking biomass and reliable nutrient removal need closer definition before a full scale system with a variable influent can be considered. In general, a system such as a sequencing batch reactor (SBR) could provide the flexibility required to maintain appropriate conditions for achieving both biomass settleability and nutrient removal objectives, with the extent of substrate variability for which dairy processing wastewaters are notorious.

CHAPTER 10

SUMMARY AND CONCLUSIONS

1. A modified activated sludge treatment system was successfully used to treat a synthetic wastewater simulating that arising from a milk processing facility producing butter and milk powder. A high quality effluent with a TSS of less than 10 $g.m^{-3}$ and soluble COD of less than 30 $g.m^{-3}$ was achieved, representing both soluble and total COD removals of greater than 98%.

2. Biodegradability of the defined substrate was investigated, and determined to be 42.5% readily biodegradable and 28.5% rapidly hydrolysable. Of the remaining 29%, the majority was concluded to be slowly biodegradable, with an almost negligible non-biodegradable or inert fraction. Approximately 80% of the readily biodegradable fraction was determined to be due to lactose, and just over half of the slowly degradable fraction due to milk fat. Milk proteins were present in all three fractions: comprising virtually all of the rapidly hydrolysable fraction, 20% of the readily biodegradable fraction, and just under half of the slowly degradable fraction.

3. Conventional completely mixed reactor configurations could not be successfully used due to operational problems resulting from the excessive growth of filamentous microorganisms. The most successful reactor configuration trialed included a selector zone comprising three aerated compartments in series, with greater than 95% of removable COD being consumed in the selector zone. As well as substrate removal efficiency considerations, conditions in the selector were also important for the suppression of filamentous growth, with fully aerobic conditions in bulk solution required in the initial selector zone.

4. It was considered that other serial selector configurations could also be viable, such as completely unaerated zones, or initial unaerated zones followed by aerated zones. However the success of these configurations would be dependent on the rapid development of a slower growing population of anaerobic microorganisms, due to the restricted opportunity for adequate anoxic substrate

removal possible with the carbonaceous substrate to nitrogen ratio typically provided by the wastewater.

5. Different types of filamentous bacteria proliferated under different substrate removal conditions. In a completely mixed reactor configuration, Type 0411 dominated the biomass, which was classified as being a low F/M filament. When unaerated selectors were used, Type 021N was the dominant filament. Proliferation under these conditions was considered to be due to readily biodegradable substrate entering the reactor zone rather than anaerobic conditions in the selector zone, and hence Type 021N was also indicated as being a low F/M filament. Increases in SVI during trials with aerated selectors were due primarily to *Haliscomenobacter hydrossis*; with the proliferation of this filament being related to periods of low dissolved oxygen in the initial selector zone, therefore indicating that this microorganism could be classified as a low DO filament.

6. Substrate removal from bulk solution occurred by biosorption, followed by a period of 'psuedo first order' substrate removal. Biosorption capacity of the biomass increased as the floc loading in the initial contact zone was increased, but was also dependant on the mixed culture composition. Biomass resulting from the aerated selector trials had a higher biosorption capacity than that from the unaerated selector trials. The filamentous bacteria demonstrated variable biosorption abilities; with Type 021N indicated as having a lower biosorption capacity than non-filamentous bacteria, however *H. hydrossis* appeared to have a higher biosorption capacity than the floc formers present in the mixed culture.

7. Very high substrate removal rates were observed with apparent first order substrate removal rates of up to 180 d^{-1} being measured in batch tests using biomass from the successful aerated selector reactor trials. During reactor operation specific removals averaging 6.5 g $sCOD. gVSS^{-1}.d^{-1}$ were recorded in the initial selector zone, at floc loadings of 0.19 g $sCOD. gVSS^{-1}$.

8. Due to the readily biodegradable nature of the substrate and the very high substrate removal rates achieved by the biomass that were able to proliferate in the selector system; aerobic, anoxic and anaerobic substrate removal mechanisms were observed simultaneously in the initial selector zone. Conditions in bulk solution were maintained in aerobic state, but significant anoxic and anaerobic activities were also indicated, therefore conditions in the internal regions of the floc must have been progressively less oxic, being firstly anoxic and then

anaerobic. The high substrate loadings in the initial selector zone resulted in sufficient substrate diffusing into the internal regions of the floc, whereas both oxygen and nitrate availability became increasingly limited.

9. Considerable biological nitrogen and phosphorus removals were observed due to anoxic and anaerobic substrate removal mechanisms respectively. Removal of up to 49% of influent N via denitrification was indicated. Effluent stream nutrient concentrations represented decreases as high as 96% of both influent nitrogen and phosphorus levels.

10. Phosphorus removals occurred via phosphorus accumulating microorganisms which developed in the mixed culture during the unaerated selector trials and whose activity continued even when aerated conditions were imposed in the selector zones. Due to the low nutrient : organic matter ratio in the substrate, biomass phosphorus contents remained below 3% and effluent total phosphorus concentrations of less than 1 $g.m^{-3}$ were consistently achieved.

11. Nitrogen removal via denitrification was indicated as occurring simultaneously with nitrification in all of the aerated reactor system zones. Nitrate was utilised for exogenous substrate removal in the initial selector zone, but was also consumed in the reactor zone where no exogenous substrate was present. Reactor zone consumption may have been due to phosphorus accumulating organisms utilising nitrate to restore polyphosphate reserves.

12. Wastewater nitrogen and phosphorus removal efficiencies declined at high substrate nitrogen levels when a combination of reduced floc diffusional resistance and size, combined with high nitrate concentrations and decreased floc loadings, resulted in the loss of anaerobic floc zones in the initial selectors and anoxic floc zones in the reactor. Denitrification declined before phosphorus removal, indicating that the extent of reactor zone denitrification declined before selector zone denitrification.

13. The use of a selector reactor configuration in an activated sludge system was therefore beneficial for a number of reasons as it resulted in the control of filamentous bulking, improved overall substrate removal efficiency and effective biological nutrient removal. The latter effect was particularly desirable as high levels of both nitrogen and phosphorus removals were achieved without defined

anaerobic or anoxic reactor zones, or complicated internal recirculation strategies both of which are commonly employed for biological nutrient removal systems.

14. The results of this study have several implications for the design of future full scale treatment systems for dairy processing wastewaters. Conditions for the control of filamentous growth were defined, however those for efficient nitrogen and phosphorus removal were found to be a more complex interaction of substrate, environmental and floc physical conditions. This interaction requires closer definition so that the nutrient removal processes within an aerated activated sludge system can be controlled and optimised, especially under the conditions of variable substrate composition for which dairy processing wastewaters are well known.

APPENDIX

All the reactor operation data, data analysis methodology and calculated results are included as spreadsheet files in MICROSOFT EXCEL for WINDOWS (Version 5.0) format. A directory of the file names and file contents on the two data disks is given in Table A1 and Table A2.

TABLE A1:   Directory of Appendix Files, Data Disk No.1

Directory	Filename	Contents
Chapter 4	CODrem4.xls	Estimation of lactose and substrate removal rates using biomass from the 2.5d HRT / SRT reactor
	Kinest4.xls	Estimation of biokinetic parameters using both High S/X and Low S/X methods.
	Substrat.xls	Estimation of biodegradable fractions S_S and S_H for the defined substrate.
	Trials4.xls	Reactor performance parameters measured during reactor operation at 2.5d HRT / SRT.
Chapter 5	CODrem5.xls	Estimation of substrate removal rates during CSTR trials at varying SRT
	Decay5.xls	Estimation of biomass decay rates during CSTR trials at varying SRT
	Kinest5.xls	Respirometric estimation of biokinetic parameters during CSTR trials at varying SRT
	Trials5.xls	Reactor performance parameters measured during CSTR trials at varying SRT: 5d, 10d, 20d, and 30d.

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Directory	Filename	Contents
Chapter 6	CODrem6.xls	Estimation of substrate removal rates during unaerated selector trials: AN1, AN2 and AN3.
	Decay6.xls	Estimation of biomass decay rates during unaerated selector trials: AN1, AN2 and AN3.
	Kinest6.xls	Respirometric estimation of biokinetic parameters during unaerated selector trials: AN1, AN2 and AN3.
	Trials6.xls	Reactor performance parameters measured during unaerated selector trials: AN1, AN2 and AN3.
Chapter 7	CODrem7.xls	Estimation of substrate removal rates during aerated selector trials: AE1, AE2, AE3, AE4 and AE5.
	Kinest7.xls	Respirometric estimation of biokinetic parameters during aerated selector trials: AE1, AE2, AE3, AE4 and AE5.
	MASSbal7.xls	Calculation of mass removals and mass balance for substrate parameters and oxygen during aerated selector trials: AE1, AE2, AE3, AE4 and AE5.
	Trials7.xls	Reactor performance parameters measured during aerated selector trials: AE1, AE2, AE3, AE4 and AE5.

TABLE A2:   Directory of Appendix Files, Data Disk No.2

Directory	Filename	Contents
Chapter 8	CODrem8.xls	Estimation of substrate removal rates during aerated selector trials at high substrate N content: AE6, AE7 and AE8.
	Decay8.xls	Estimation of biomass decay rates during aerated selector trials at high substrate N content: AE6, AE7 and AE8.
	Kinest8.xls	Respirometric estimation of biokinetic parameters during aerated selector trials at high substrate N content: AE6, AE7 and AE8.
	MASSbal8.xls	Calculation of mass removals and mass balance for substrate parameters and oxygen during aerated selector trials: AE1, AE2, AE3, AE4 and AE5.
	Trials8.xls	Reactor performance parameters measured during trials at increased substrate N content: AE6, AE7 and AE8.
Chapter 9	Summary.xls	reactor diary; summary of biosorption results; summary of biokinetic parameter estimation.

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