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# A Study of the Effect of Substrate Composition on the Microbial Ecology of Activated Sludge

by

# **Raymond A. Noble**

A Doctoral Thesis

Submitted in partial fulfilment of the requirements for the award of the Degree of Doctor of Philosophy of Loughborough University

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#### <u>ABSTRACT</u>

Eighty percent of all biologically treated waste waters in Europe are oxidised by the activated sludge process. Bulking sludge caused by the proliferation of filamentous organisms is the primary cause of failure of this system. The effect of various substrates in both laboratory scale, fully mixed and sequencing batch (SBR) reactor configurations were used to assess their combined effect on activated sludge microbial ecology and hence sludge settlement. Five different substrate types were used ; synthetic sewage, a basic monosaccharide, disaccharides, polysaccharides and amino acids. In all cases using the fully mixed reactor, bulking occurred while, good settling sludge was produced in the sequencing batch reactor. The cause of this bulking was deemed to be due to the lack of so called "selector effect" within the fully mixed reactor characterised by :-

- i) high rates of substrate consumption
- ii) high oxygen (or generally : electron acceptor) up take rate
- iii) enhanced growth of zoogleal bacteria
- iv) increased metabolic diversity

This laboratory work was compared and contrasted with a pure oxygen activated sludge (VITOX) system treating a high strength pea processing waste water. This fully mixed system had proved difficult to operate since its installation and in the first two years of this study suffered bulking caused by low dissolved oxygen levels. In the third year a combination of a hydraulic problem and subsequent lack of control led to filamentous bulking. This particular bulking incident was controlled by the addition of chlorine to the aeration tank which was selectively toxic to the filamentous organisms present. Due to the studies carried out at both laboratory and full scale an initial contact zone was installed within the main aeration tank prior to the 4th year of this study so as to create an area of high floc loading and high substrate uptake. This initial anoxic contact zone proved successful in preventing the development of a poorly settling sludge and is in line with common practice for the elimination of filamentous bulking reported in the literature.

Oxidation Reduction Potential (ORP) proved a reliable and appropriate monitor of conditions of low to zero D.O. experienced in the laboratory scale reactors and at full scale in the anoxic contact zone. It was also found that ORP could be used to detect when D.O. levels became completely depleted and monitor reductions in nitrate levels.

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#### **CHAPTER 1 INTRODUCTION**

Since the development of urbanisation, the treatment and disposal of sewage has required the oxidation of carbonaceous and nitrogenous waste products to be carried out both efficiently and cost effectively. A major breakthrough came about with the introduction of the activated sludge process. The activated sludge process was developed early this century at the Davyhulme sewage treatment works in Manchester. The originators of this process being Dr E. Arden, Mr W.T. Lockett and Dr G.S. Fowler. Their crucial discovery was that by returning settled solids from the treated water back to the aeration vessel, resulted in a substantial improvement in the rate of purification. Since the introduction of Arden and Lockett's batch reactor and the first full-scale activated sludge plant in the U.K. installed at Worcester in 1916 the process has been refined and developed into various forms but still central to the process is aeration, settlement and sludge recycle. A comprehensive review of the history of the process to the mid-1970's has been produced by Stanbridge (1977) and the proceedings of a fortieth anniversary symposium in 1954 summarised the state of development at that time.

The activated sludge process is now extensively used for the treatment of domestic sewage and industrial waste water, with 80% of all the treated waste water in Europe being oxidised by the activated sludge process. The process has been successfully applied to a wide range of industrial waste waters including, paper making, vegetable processing, brewing, tanning and the petrochemical industry. Activated sludge is now the method used to treat 50% of the biologically treated waste waters in the U.K. In addition it has also been developed not only to remove carbonaceous pollutants but also to oxidise ammonia, reduce total nitrogen, remove phosphate and concentrate heavy metals in sludge.

The mechanism of the process is that the waste water containing biodegradable pollutants is mixed with activated sludge and aerated by diffusion or air/gas injection to support aerobic growth and respiration. After the required period of treatment (typically 8-24h) the mixed liquor is allowed to stand unaerated in a separate vessel

1

(clarifier) where the biomass flocculates and settles. The resultant supernatant is discharged either to a water course or to tertiary treatment while the settled solids are returned to the aeration vessel. A certain portion of the settled solids is wasted from the system as this is the sludge growth on each cycle and is usually 5-20% of the total recycled.

In spite of the wide use of the activated sludge process throughout the world and many of the problems encountered being of a microbiological nature, the microbiological aspect of the system are still not fully understood. By far the most serious and widespread problem of the activated sludge process is "bulking sludge". This term "bulking sludge" is used to describe an instability which can occur in the form of poor sludge settlement. This results in settled sludge occupying an increasingly large volume leading to difficulties in the separation of biomass from the treated effluent. In some severe cases of sludge bulking this can lead to the carry over of biomass into the final effluent discharged from the plant increasing both its turbidity and oxygen demand.

Workers originally hoped that a better understanding of activated sludge settlement would lead to process design modifications giving optimal and reliable solids separation. This has however, not happened, but laboratory work and observations made on full-scale plants has led to the realisation that certain reactor configurations are associated with good settlement. The degree of mixing can be crucial in determining the quality of sludge settlement and there is also evidence that anoxic and anaerobic zones prior to aeration in some cases can lead to improvements in settlement. Despite these improvements, activated sludge bulking, particularly that caused by filamentous organisms, remains a severe problem and a topic worthy of further research.

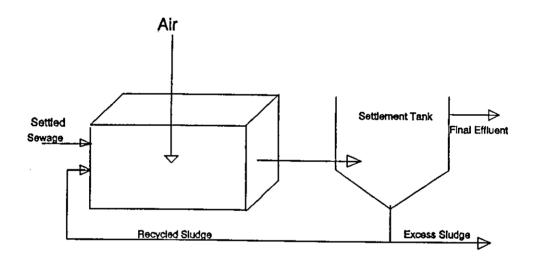
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#### CHAPTER 2 LITERATURE REVIEW

#### 2.1 Activated Sludge

2.1.1 Since the introduction of Arden and Lockett's batch reactor in 1914 (Arden and Locket, 1914) the activated sludge process has been refined and developed into various forms, but its basic principles remain unchanged and are shown in Figure 2.1.

Figure 2.1 Schematic Diagram of the Activated Sludge Process



The activated sludge process consists of two phases, aeration and sludge settlement. The first phase of this process relies on a dense microbial population being mixed in suspension with the wastewater under aerobic conditions. Wastewater is introduced at a rate which is designed to allow for effective treatment of the waste by the complete replacement of the tanks contents within the period of a few hours (normally 8 to 24 hr). During this aeration period, with unlimited food and oxygen, high rates of microbial growth and respiration can be achieved, resulting in the complete utilisation of organic matter present in the wastewater with the end products being carbon dioxide, water, simple inorganic compounds and a considerable quantity of newly synthesised cells and absorbed substrate. As the settled wastewater enters the aeration tank it displaces the mixed liquor (the mixture

of wastewater and microbial biomass) into the sedimentation tank. This is the second stage of the activated sludge process, where the flocculated biomass is settled from the suspension to form a sludge blanket and a clarified effluent. The final or clarified effluent is required to be virtually free from solids and normally of a quality to discharge directly into a receiving water course i.e. 95% removal of organic carbon. An essential feature of the activated sludge process is that the majority of the settled organic solids are returned to the main aeration tank (Return Activated Sludge).

The reactor is typically loaded with substrate at a rate of 1 kg/m<sup>3</sup>.d measured as biochemical oxygen demand (B.O.D.). Oxidation of carbonaceous and nitrogenous waste products are typically achieved with a biomass concentration of between 2.0 and 5.0 g/l mixed liquor suspended solids (MLSS), a biological loading rate or food to micro-organism ratio (F.M.) of between 0.02 and 1.8 kg B.O.D./kg MLSS.d and an oxygen supply of up to 2 kg O2/m3.d dependant on the degree of treatment required. Dissolved oxygen concentrations range from 0.5-2.5 mg/l. Returned activated sludge (RAS) is recycled at a rate of 60-100 % of the influent. Biomass yield (Y), is a function of feed rate, specific growth rate of the micro-organisms present in the reactor and the cell "maintenance energy" requirements. The average time that a cell remains in the system is measured in days either as a mean cell residence time or sludge age (SA). Excess biomass or surplus activated sludge (SAS) is generally wasted from the system and disposed of to land, after typically anaerobic digestion. Other alternative treatments are aerobic digestion, disposal to landfill sites or incineration. High quality effluents, with discharge consents of 5 mg/l ammonia-N (NH<sub>a</sub>), 15 mg/l BOD and 25 mg/l suspended solids can consistently be achieved from domestic sewage using the activated sludge process. The process requires that the complex biological medium is capable of performing consistently, whilst at the same time being subjected to changing conditions such as flow, substrate concentration and temperature. Some of the most recent general reviews of the process are shown in Table 2.1.

Title	Authors
The Activated Sludge Process:	Jenkins, R., Deeny, K. and Eckbot, F.T.
Fundamentals of Operation	(1983)
Biological Wastewater Treatment Systems:	Horan N.J. (1990)
Theory and Operation	
Biology of Wastewater Treatment	Gray N.F.(1989)
Activated Sludge: Theory and Practice	Gray N.F.(1990)

Table 2.1 Recent reviews of The Activated Sludge Process.

## 2.2 <u>Reactor Types</u>

Activated sludge reactors are designed to provide facilities for mixing the waste with the micro-organisms, aeration and an even flow rate through the plant. The exact configuration of the tank selected will have a profound affect on many aspects of plant performance and economy. Activated sludge systems are divided into three well recognised bands of loading rates :-

a) Conventional aeration

b) Extended aeration

c) High-rate aeration

The mode depends on the loading these plants operate at (see Table 2.2).

Table 2.2 Operating Parameters in Activated Sludge Plants. Horan (1990)

Treatment Mode	Sludge Loading Rate	MLSS
	(kg BOD/kg MLSS.d)	(mg/l)
Conventional Aeration	0.15-0.50	2000-3000
Extended Aeration	0.05-015	2000-5000
High Rate (or partial treatment)	>1	<2000
Conventional with Nitrification	0.15	3000-4000

These various modes of operation are well reviewed in the recent publications listed in Table 2.1

Further categorisation of activated sludge processes is based on mixing pattern in the aeration tank, feeding pattern and the method of aeration.

#### 2.3 Conventional Activated Sludge Reactors

Within this category is a wide variety of systems both in structure and operation. The most important difference is the mixing regime used with conventional activated sludge being nominally plug-flow or nominally completely mixed. The majority of sewage plants are of a plug-flow configuration because this has been shown to produce better sludge settling properties (section 2.3.2). Plug-flow plants are twice as expensive to construct than completely mixed reactors and it is for this reason that completely mixed configurations are more popular in the treatment of industrial waste waters.

#### 2.3.1 Batch Reactors

The original activated sludge process was operated as a batch reactor and was known as a fill and draw process. This involved filling a reactor with settled sewage and aerating it for a sufficient period of time to oxidise the majority of the BOD present (generally 8 hr). Following this the aeration is switched off and there was a period where the reactor solids were allowed to settle and the treated supernatant discharged to the receiving water course. A portion of the settled solids were wasted and the whole process repeated. Fill and Draw reactors as they became known require a large amount of operator control and hence lost favour. More recently modifications of the original Fill and Draw process, known as a Sequencing Batch Reactor (SBR) is once again gaining popularity (Irvine and Davis 1971, Irvine and Bush 1979). An SBR allows several processes, such as carbonaceous oxidation, nitrification, denitrification and phosphorus removal to be carried out in the same reactor at different times (Alleman and Irvine 1980, Chin and Wun-Jern 1985, Goronszy and Rigel 1991). The SBR can cope with highly variable hydraulic

and organic loads and only limited operating skills are now required because of the availability of automatic control. This system is also more resistant to the effects of any toxic substances present compared to conventional activated sludge, due to the large dilution of the influent sewage afforded by the aeration tank acting as a buffer.

## 2.3.2 Plug Flow Reactors

In conventional plug flow reactors both the influent wastewater and the return activated sludge (RAS) are introduced at the same point into a tank of high length to breadth ratio (>5). The tank is usually equipped either with diffusers or less commonly surface mechanical aerators to provide oxygen to the mixed liquor along the length of the tank. As the mixed liquor proceeds along the tank the organic matter is utilised with the desired level of treatment being achieved by the time the mixed liquor reaches the outlet at the far end of the tank. A gradient of decreasing BOD and oxygen demand is achieved longitudinally across the tank. These reactors in practice consist of a number of tanks or pockets in series, each equipped with its own aerator and hence each behaves as a separate complete mix reactor. Due to there being an area of relatively high BOD concentration at the inlet to the reactor, this represents an area of high organic loading. Problems of sludge settlement are much reduced with plug flow reactors, however, toxic or shock loads are not diluted or buffered as in completely mixed systems, but pass through the system as a discrete plug which could result in a serious deterioration in effluent quality. As the load and oxygen demand are not evenly distributed along the reactor, oxygen supply may be deficient at the inlet where demand is greatest and be in excess at the outlet where demand is lowest. Lister and Boon (1973) calculated that the oxygen requirement in a plug flow tank was twice as high in the first half of the tank compared to the second half.

Two similar variations of the conventional plug flow reactor are now in use:-

a) Step or Tapered Aeration - where constant aeration is provided along the length of a plug flow system, problems may arise with under aeration at the inlet and over aeration at the outlet. It is now usual to match aeration to demand by tapering the aeration along the length of the tank such that it matches the demand and hence the respiratory requirements of the mixed liquor. As the cost of aeration can account for up to  $\frac{1}{2}$  the total plant running costs, then the potential for savings in this area are large.

b) Step or Incremental Feeding - is another possibility for providing the same "evening out" of oxygen demand along the aeration tank by introducing the waste water incrementally at several points along the length of the tank. However, if too many increments of feed are employed, then the reactor will resemble a complete mix system rather than plug flow as the gradient of substrate has been reduced. Consequently it is unwise to provide more than two increments or the disadvantages associated with a complete mix system may be encountered. This modification of the conventional activated sludge system has not proved to be as popular or successful as tapered aeration.

#### 2.3.3 Biosorption or Contact Stabilisation

Contact stabilisation was developed independently by Ulrich and Smith and also Eckenfelder and Grich in the 1950's (Ulrich and Smith 1951, Eckenfelder and Grich 1955). Contact stabilisation provides a separate zone with very high floc loading by the provision of a small tank in which return activated sludge and settled sewage are mixed. The residence time in the contact zone is 0.5-1 hr. During this short contact period the organic material present is absorbed into and adsorbed onto the activated sludge flocs, a process known as biosorption (discussed later in section 2.10.6). The mixed liquor is then settled, with the supernatant being discharged into the receiving water course and the settled sludge pumped into the sludge aeration tank where it is aerated for 5-6 hours, so that the adsorbed organic material can be fully oxidised. By having a short HRT in the contact tank, it is not necessary to provide aeration capacity for the entire waste flow. In fact approximately 50% less aeration capacity is required compared with other conventional activated sludge processes (Eikelboom 1982, Rensink and Donker 1991). In some designs the aeration tank is as large as that for conventional plants. This leads to long SRT and results in the micro-organisms being in endogenous respiration leading to lower sludge yields (Moore and Todd 1968).

#### 2.3.4 Completely Mixed Systems

The theoretical basis of a completely mixed system is that the influent wastewater and returned activated sludge (RAS) are rapidly distributed throughout the tank such that a sample, taken from any point in the reactor should yield identical values for MLSS, BOD and oxygen concentration. The immediate dilution effect afforded by the aeration tank, buffers the system against shock loadings of strong organic or toxic wastes. Another advantage of this system is the uniform distribution of load throughout the tank, which should ensure an efficient use of aerators. However, while short circuiting may be a problem in badly designed fully mixed reactors, the main problem encountered with completely mixed systems is their tendency to produce low density sludge which is difficult to settle.

### 2.4. Extended Aeration

The extended aeration process is similar to the conventional plug-flow process. The difference being the low sludge loading level, between 0.03-0.15 kgBOD /kgMLSS.d, which results in the process being food limited, forcing the microorganisms making up the activated sludge into the endogenous respiration phase of growth. The sludge age as expected is quite long, greater than 5-6 days and sludge yield low.

#### 2.4.1 Oxidation Ditches

The first extended aeration system was developed in the Netherlands by Pasveer in 1953. Known as the Pasveer or oxidation ditch, (Pasveer 1959) the system originally had no provision for screening, grit removal or primary sedimentation and comprised a continuous unlined channel (1-3.5m in depth) in which mixed liquor was circulated and aerated by means of a horizontally mounted rotor which maintained a flow velocity sufficient to prevent the settlement of sludge. Oxidation ditches may be operated either as intermittent flow (fill and draw) or continuous

flow units. The former usually serving populations of up to 500.

Oxidation ditches are inexpensive to build and require little maintenance compared to conventional or high rate activated sludge systems. They are reported to suffer less from sludge bulking or odour problems. It is for this reason that they are particularly suited to small or rural regions. The lower loading levels give a far greater reserve of dissolved oxygen to cope with surges in organic load. While the settleability of activated sludge tends to improve with increased SRT, after a critical period so much of the microbial mass has been broken down during endogenous respiration that the floc particles become too small to settle efficiently and thus cause problems. This basic system represents approximately 95% of the oxidation ditches operating in the UK (Gray 1990) and they are now utilised in most parts of the world.

#### 2.5 Advanced Activated Sludge Systems

Certain recent developments of the activated sludge process have been introduced as a result of advances in technology and increased understanding of process fundamentals. Some modifications are concerned with what could be described as process intensification, and others provide a much greater degree of process control and flexibility. Process intensification has been achieved by increasing the rate of oxygen transfer from the gas phase to the liquid phase and so increasing the saturation concentration of dissolved oxygen. Two advanced activated sludge systems have received considerable commercial attention, the pure oxygen activated sludge system and the deep shaft process.

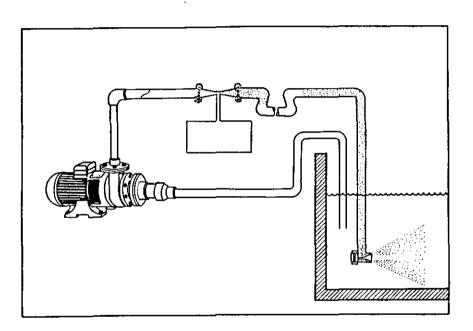
#### 2.5.1 Pure Oxygen Activated Sludge Systems

Aeration with pure oxygen is a possible method of increasing oxygen utilisation efficiency. Atmospheric air contains only 21% oxygen, so by using pure oxygen instead of air, a five fold increase in the oxygen content available for transfer, thereby significantly increasing the oxygen transfer rate.

Pure oxygen activated sludge systems have been developed by various companies

and marketed under various names. The most common of these being the Wimpey Unox, BOC Vitox and the Megox systems. Oxygen can be used in two ways: one is in conjunction with conventional aeration or two as the sole method of supplying the necessary oxygen. The former case is an excellent method of coping with seasonal overloads. For example pure oxygen systems have been adopted at a number of holiday resorts (Rees 1978) and is widely used to treat seasonal fluctuations in the food processing industry (Gostick 1990).

Figure 2.2 Side Stream Injection (Vitox Process)



The Vitox (side stream injection) aeration system, developed by the British Oxygen Company (BOC), was originally developed for re-aerating lakes and rivers that had become oxygen depleted. The potential of the system to uprate overloaded activated sludge plants was also realised with new specifically designed pure oxygen systems being designed. Since its development in 1972, the Vitox process has become the most widely used pure oxygen treatment system.

The process requirements other than a supply of oxygen, are relatively simple (Figure 2.2); consisting of a pump, a venturi and dispersal jets. The pump draws mixed liquor directly from the aeration tank, this sidestream is injected with pure

oxygen as it passes through the venturi and is then returned to the aeration tank via an expansion nozzle. The pressure regime and turbulence is such that 25% of the gas dissolves in the venturi. Further dissolution takes place in the pipework so 50% of the gas is dissolved before the mixture is discharged through the jets. The high velocity of the discharge causes considerable turbulence ensuring the oxygen is rapidly mixed with the contents of the aeration tank (Boon 1978). The experimentally measured oxygen transfer capacity of the Vitox system, has been demonstrated to be as high as 3kg.kWh<sup>-1</sup> (Kite and Garrett 1983). A further development of the original side steam injection technique, has been the BOC Vitox 2 system which employs a low pressure recycle flow passing through a bell-diffuser contact chamber.

#### 2.5.2 The Deep Shaft Process

The deep shaft process is also a form of high intensity activated sludge treatment. The process was developed by ICI as a part of research on gas lift in tube fermenters which also led to single cell protein production. The deep shaft process consists of a "U" tube divided by a single central division or central core. Thus the shaft is split into a downflow and an upflow zone. The circulation within the shaft is brought about by differential densities of mixed liquor in the riser and downcomer columns. Initially, air is injected into the riser at a relatively shallow depth, with this air initiating the circulation. The liquid velocity is of the order 1-2ms<sup>-1</sup> and at this velocity the downward flow of liquid has a greater speed than the rise rate of the air bubbles. Air is thus carried down the 20-50m deep shaft.

The oxygen transfer is a key feature in the advantages claimed for this process. The high liquid velocities within the shaft also give rise to high Reynolds numbers. Bubble coalescence is therefore minimised and there is a high rate of surface renewal, both these are factors which give promote high levels of oxygen transfer. In addition, the driving force for the transfer of oxygen is significantly increased since the pressure at the bottom of the shaft can result in the solubility of oxygen in water being increased 5-10 fold. The overall result is that transfer efficiencies of 3-

4.5 kgO<sub>2</sub>kWh<sup>-1</sup> have been reported together with oxygen utilisations of greater than 85% (Hemming et al 1977). While there is a high degree of mixing in the process there is little dispersion or back mixing, making the process essentially plug flow in nature (Dunlop 1976).

One of the other benefits for this process because of its tube shape is that it occupies a fraction of the land area that a conventional activated sludge plant would. The power requirement is low, approximately 0.85kWh/kg.BOD which includes both aeration and sludge recirculation. The considerable saving in land is particularly important in densely populated regions such as Japan where most Deep Shaft (approximately 40) are located. There are two Deep Shaft units in the U.K. at Tilbury which is the largest of its kind in the world and Southport . The plant at Tilbury is operated by Anglian Water who have found the deep shaft process particularly useful in treating variable high strength wastewaters (Annon 1987b, Irvine et al. 1989). However, few new Deep Shaft systems will be built until some of its associated problems are overcome. For example high capital costs are incurred in sinking the deep shaft. Operational problems include foam production (Wheatland and Boon 1979), degassing, the possibility of a reversal of flow in the shaft and the accumulation of dense solids at the bottom of the shaft.

### 2.6 Microbial Ecology

In spite of its wide use and many of the problems encountered being of a microbiological nature, the microbiological aspects of activated sludge are still not fully understood. Like all biological treatment processes, the activated sludge system relies on a mixed culture of bacteria to carry out the basic oxidation of the substrate present, with higher grazing micro-organisms present removing colloidal substrate and unflocculated bacteria thus forming a complete ecosystem with various trophic levels

#### 2.6.1 Species Profile

Biological sewage treatment plants can be regarded as an artificial complex ecosystem (Hawkes 1978). As in many other biological systems, a vast number of individual species are represented in any activated sludge system and most aspects of microbial ecology of the process are pertinent to the problem of sludge bulking. It has been suggested that the primary factors selecting a species present in any activated sludge is the composition of the waste water being treated (Banks et al 1976). However this is an oversimplification and other classically recognised ecological determinants such as pH, substrate concentration, temperature and redox potential will also play an augmenting or modifying role (Hawkes 1968).

For the establishment of any species within an activated sludge system there is an essential requirement that their growth rate is equal to or greater than the hydraulic retention time of that system or that they settle within the clarifier from which they can be recycled to the aeration tank. If either of these two criteria are not met the species will be washed out of the system. As a consequence of the activated sludge process operating at high biomass concentrations, substrate concentrations are low within the reactor. In this growth limiting environment the growth rate of a species is unlikely to match the hydraulic retention time (usually 8-24 hours) but can become established if its growth rate is greater than or equal to the solids retention time (sludge age) of the system (5-10 days).

Within the reactor, flocculated unicellular bacteria are held together in an extracellular, bio-polymeric glycoprotienaceous, felt like envelope or glycocalyx (Costerton and Irvin 1981). These flocculating bacteria competitively co-exist with filamentous organisms such as bacteria, actinomycetes and fungi. Also present are grazing protozoa, metazoa and nematodes which live and feed on the floc forming and free bacteria. The general microbial population, which consists of mainly Gram negative rod bacteria, is highly diverse but can be divided into two process groups. The removal of BOD, by heterotrophic bacteria and the reduction of ammonia by autotrophic nitrifiers. Other genera, which perform specific functions such as

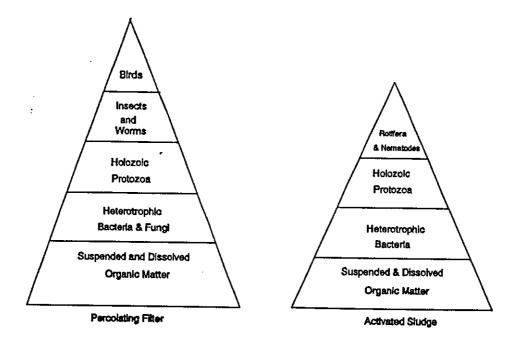
denitrification (Achromobacter) are listed by Horan 1990 and reproduced in Table 2.3.

Table 2.3 Reactions Carried Out By Principle Genera Of Bacteria Found In Activated Sludge (Horan 1990)

Genus	Function
Zooglea	Removal of carbohydrate, slime production, denitrification
Bacillus	Protein degradation
Athrobacter	Carbohydrate degradation
Microthrix	Lipid degradation, filamentous growth
Nocardia	Filamentous growth, Foam formation
Acinetobacter	Phosphorus removal
Nitrosomonas	Nitrification
Achromobacter	De-nitrification

Activated sludge is a dispersed growth system with the microbial biomass present as discrete flocs and individual bacteria, which are suspended in the waste water and mixed together by the aeration system. The activated sludge system is totally aquatic and fewer trophic levels are present within the process than percolating filters (Figure 2.3). The larger macro-invertebrate grazers associated with percolating filters are largely absent from the activated sludge process as they cannot be supported within the floc.

Figure 2.3 Comparison Of The Food Pyramid For A Percolating Filters And Activated Sludge Treatment Systems (Wheatley 1985)



#### 2.3.2 Protozoa Within Activated Sludge

Protozoa demonstrate a wide range of feeding modes and are capable of feeding on soluble and particulate organic matter, as well as bacteria and other protozoa. They form an integral part of the food chain of waste water treatment plants. Extensive work carried out by Curds and Cockburn (Curds and Cockburn 1970 I and II) in the seventies, showed that over 100 species of protozoa can be found in activated sludge.

The ciliated protozoans are a microscopically obvious component of the biomass and these can be split into three distinct groups; crawling ciliates (Spirotrichs), stalked ciliates (Peritrichs) and free swimming ciliated protozoa (Holotrichs). The ciliated protozoa play little or no part in the oxidative processes, but they have a major role in the final clarification of the effluent by preying on the free swimming bacteria. This has been proved by Curds et al. (1968) who showed that in the absence of ciliated protozoa a turbid, poor quality effluent was produced, but within a short period of time after the addition of ciliates there was a rapid improvement in the effluent quality. This is in full agreement with the findings of Curds (1975), Gerardi (1986) and Lana -Pabello et al. (1990). With the main reason for this improvement in effluent quality being a reduction in the number of free swimming bacteria present in the effluent. In addition to this role, it has been shown (Curds 1963) that ciliated protozoa are able to cause the fixation (flocculation) of small particle in suspension.

It has also been shown that protozoa can also be used as biotic indicators (Curds 1982a, Sartory 1976, Madoni 1981). This is due to their sensitivity to many bulk solution properties such as the presence of toxic compounds within an effluent, quality of sewage, temperature, D.O., pH and other operating parameters, which makes it possible for them to be used as indicators of plant performance (Al-shahwani and Horan 1991, Esteban et al. 1991).

#### 2.7 Flocculation and Settlement

The bioflocculated microbial aggregates, known as flocs, are the essential component of the activated sludge process. The primary objectives of the process i.e. the transformation of organic matter into carbon dioxide and cell material, as well as the incorporation of colloidal matter into settleable solids, are ultimately carried out by the process of flocculation. However, most of the major operating problems of the process, such as those which occur in solid-liquid separation, can be attributed to the properties of the flocs. Indeed almost all the basic principles of the activated sludge process are related to or dependant on the physical characteristics of the flocs.

#### 2.7.1 Measurement of Sludge Settlement

During the settlement of activated sludge in a cylindrical glass or clear plastic vessel, a distinct supernatant interface can be seen. A plot of the position of the interface with time gives a settlement curve from which settlement rates can be determined. The initial part of the curve may show a short lag periods during which there is an aggregation of adjacent flocs. This is followed by a linear phase of unhindered settlement where the solids concentration remains almost constant. The next distinct part of the curve is the transition zone where the rate of settlement progressively decreases due to the influence on settlement of increasing sludge concentration and the increasing interaction between flocs. The final part of the curve is the compression zone where the weight of the sludge forces water out of the sludge matrix.

Determination of settlement behaviour of activated sludge at a predicted solids and hydraulic loading aids the sizing and design of final clarifiers. For this purpose a sludge volume index (SVI) is determined by a settlement test. The SVI is defined as the volume in millimetres occupied by one gram of activated sludge solids following thirty minutes of settlement, usually in a one litre measuring cylinder. A refinement of this test was introduced by White (1975) whereby the sludge is stirred at a low speed in a specialised apparatus to give the stirred sludge volume index (SSVI). The SSVI is considered to be a better measure of the behaviour of activated sludge within the clarifier as it overcomes wall effects in the test cylinder such as bridging and streaming (White 1975, Rachwal et al. 1982). In practice both tests are still used and both give a reasonable indication of the sludge settlement properties (Daigger and Roper 1985). Both indexes can be dependent in an unpredictable way on the solids concentration in the sample. Ideally the settlement index should be determined at two different concentrations and the index at 3.5 gdm<sup>-3</sup> calculated by interpolation.

Other settlement tests have been introduced to improve the predictability of activated sludge settlement within clarifiers. The Dilute Sludge Volume Index (DSVI) is similar to the SVI test but is determined at a much lower solids concentration (Koopman and Cadee 1983, Ekama and Marais 1984) with the DSVI test now being more commonly used as a measure of settlement than the SSVI test within Germany and South Africa. Ekama and Marais (1986) measured DSVI and SSVI values on sludges from thirteen plants in the Western Cape of South Africa but could not establish a relationship between these measurements of sludge settlement. However, they recommended that both the tests were reliable and could

be used for estimating flux constants. Wherever possible one or both these measurements of sludge settlement should be made. Due to the influence of solids concentration on SVI measurement, SVI is considered to be to unreliable for the purpose of designing final settlement tanks (Ekama and Marais 1986). The Zone Settling Velocity (ZSV) is another similar test (Catunda and Van Haandel 1987, Ekama and Marais 1986). All these tests have been incorporated into design procedures for activated sludge clarifiers (Anderson 1981, Telcippe and Bender 1987, Pitman 1984).

#### 2.7.2 Floc Structure

Under the guiescent conditions of the secondary sedimentation tank (clarifier) a well flocculated activated sludge can settle out and thicken to give a four fold increase in solids concentration. This settling ability is a direct result of the capacity of the micro-organisms and colloidal material which make up the mixed liquor suspended solids, to agglomerate into flocs with a high zone-settling velocity (White 1975). Activated sludge flocs are semi permanent structures of bacteria immobilised in a matrix of bacterially derived extracellular polymeric material, which is predominantly carbohydrate. Bacteria initially aggregate and the floc becomes a discrete structure which may be stabilised by filamentous organisms growing within the floc to form a rigid backbone or skeleton (Sezgin 1980, Urbain et al 1993) which gives the floc its strength and structure. The filaments have the ability to extend out into the bulk medium and form a site for subsequent attached growth. Young flocs are comprised of actively growing heterotrophic bacteria with a high rate of metabolism. In contrast, older flocs contain a lower proportion of viable cells, being made up of mainly non viable cells surrounded by a viable bacterial layer. Older flocs therefore have a lower rate of metabolism when compared to young flocs. In addition, due to their large size they settle more rapidly than younger flocs which are often associated with poor settleability. Viability of the micro-organisms making up the floc has been estimated to be as low as 5-20% (Weddle and Jenkins 1971).

The surface area of the flocs has been found to range between 40-140 m<sup>2</sup>/g dry solids, which indicates the porous nature of the floc. This porous nature explains why flocs are so good at absorbing particulate matter and also why diffusion rates of nutrient and oxygen into the centre of the floc is greater than if the flocs were homogeneous masses of bacteria (Smith and Coackley 1983, Coackley 1985).

Of the organisms isolated from activated sludge by Shimuz and Odorawa (1986) 80% were found not to be floc formers and Ueda and Earle (1972) found only four floc formers among fifty four isolated strains. However, in both of the above cases the ability to flocculate was tested in batch culture or by classical microbiological isolation so possibly under different conditions the isolates may have expressed the ability to flocculate. Furthermore isolation and culture of organisms from environmental samples does not reflect their active growth or viability in the original environment. Shimuz and Odorawa (1986) did find that the addition of a floc forming bacteria to activated sludge improved settlement which would support their theory that many organisms in activated sludge do not flocculate in pure culture. Observations on industrial effluents suggest that the presence of certain groups of bacteria were necessary for good floc formation. These species being Klebsiela sp., Escherichia coli and Streptococcus sp. (Al-Shahwani 1986). However, just the simple addition of these species to a mixed culture is probably not effective at improving flocculation and to simple control method (Cook 1990). As these selected bacteria were found to be specialised and their capabilities changed quickly when added to mixed microbial cultures or different environments. Ultimately DNA and RNA analysis suggested that there may be significant polymorphation according to conditions of growth (Cook 1990).

### 2.7.3 Reasons For Flocculation

Floc formation is not a general property of micro-organisms, but has been found to be a response of some species to low substrate levels and energy levels within the cell (Kiff 1978). Miskell (1984) found that flocculated cells have lower maintenance energy requirements than non-flocculated cells, so flocculation could be an adaptation for survival in environments of low substrate concentration. Logan and Hunt (1988) concluded there was a common condition inducing flocculation, that being starvation. They also hypothesised that when subjected to conditions of low substrate availability, substrate uptake is limited by flow-through kinetics. The rate and pattern of the flow through the permeable flocs as they undergo gravitational movement could result in increased substrate uptake. Therefore, this could lead to a selective advantage for flocculant growth. This theory for the advantages of flocculant growth are backed up by the experimental evidence of flow through flocs (Li and Ganczarczyk 1987, Li and Ganczarczyk 1988, Li and Ganczarczyk 1990).

#### 2.7.4 The Mechanism Of Floc Formation

The exact mechanism of floc formation is far from being understood. Originally it was thought due to the slime-forming bacterium Zooglea ramigera, however, many other bacteria and protozoa are now known to be associated with floc-formation. Bacteria, protozoa and detritus are either attached to the surface of the floc or embedded in some form of material forming a matrix. This material can readily be extracted from activated sludge (Brown and Lester 1979) and constitutes up to 10% of the dry weight of the sludge. This material, extracellular polymer (ECP) has two different origins: (i) From metabolism or lysis of micro-organisms (protein, DNA, polysaccharides and lipids) and (ii) from the waste water itself (e.g. cellulose, humic acids etc.). The influence of ECP's on sludge settlement has been widely studied, but since there is no standard method for their extraction, it is sometimes difficult to compare results from different studies. Thus relationships between sludge settlement and ECP (Magara et al. 1976, Choa and Kienath 1979) and data about the ECP composition are sometimes contradictory (Sato and Ose 1980, Horan and Eccles 1986). Each polymer will have varying surface properties and charges which will influence not only the settling characteristics but also the water binding properties. The polymer is not only giving the floc components cohesion, it is also allowing suspended particles in the waste to bind to the floc by adsorption.

Since bacterial surfaces, ECP and inorganic particles provide negative adsorption sites, the role of divalent cations in floc stability must be emphasised. Divalent cations such as  $Ca^{2+}$  and  $Mg^{2+}$  are known to be involved in the structure of chemical aggregates (Belcourt et al 1974, Eriksson and Alm 1991), because of their ability to bind negatively charged chemical groups. Steiner et al. (1976) showed that different types of adsorption isotherms can be obtained with cations such as cobalt, calcium, or copper and with a higher affinity for ECP alone than for the whole sludge. In spite of the importance of divalent cations in the flocculation process, there is a lack of information about either their accumulation in extra cellular structures of sludge flocs or their affinity with specific constituent of ECP.

Activated sludge is a highly hydrated structure. However, many studies on sedimentation properties have been based on the colloid chemistry of the flocs and this has subsequently been related to surface charge, extracellular polymers and hydrophobicity all of which may play a part in the process of flocculation (Magara 1976, Steiner 1976). Erikssen and Axberg (1981) showed the hydrophobic-hydrophilic balance should be considered an important factor in the process of bioflocculation. Cell surfaces exhibit hydrophobic areas (Magnusson 1980) and hydrophobic molecules such as lipids and proteins from cells are trapped in the flocs. The contact angle technique has been used in an attempt to relate the flocculation performance of activated sludge to its hydrophobic character (Valin and Sutherland 1982). However, due to the need to study hydrophobicity on diaggregated samples, this area remains difficult to investigate.

#### 2.8 Activated Sludge Bulking

Sludge bulking is perhaps the most common cause of waste water treatment plant failure (i.e. excedence of discharge permit limitations by excess solids). Bulking sludge is characterised by the loss of settleability, leading to much thinner sludge being returned to the aeration tank. This in turn will lead to a dilution of the mixed liquor suspended solids (MLSS) and result in overloading of the solids handling processes and produce effluents with a high solids and BOD concentration. In

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severe cases this may lead to solids washout from the system. A number of surveys have been carried out to establish the extent of the problem of activated sludge bulking. Tomlinson (1976) reported a survey of 65 activated sludge plants in Britain, of which 63% experienced problems with sludge settlement and 52% had experienced bulking to such an extent that serious loss of solids in the final water had occurred. By 1982 the situation had not changed with an estimated 50% of treatment plants in the U.K. experiencing bulking problems regularly (Tomlinson 1982). There has been an improvement in this situation over the past few years, but Pujol and Canler (1989) still reported 25% of activated sludge plants in France experiencing bulking problems.

#### 2.8.1 Types Of Sludge Settlement Problems

Activated sludge solids separation problems can be summarised into several types (Jenkins 1992). These are dispersed growth of micro-organisms, non-filamentous bulking, filamentous bulking, fungal bulking, pin or pin-point floc, denitrification, foam and scum formation. The occurrence of any of the above phenomena can severely effect the functioning of the secondary settling tank and hence the final discharge. A brief summary of each type is given below.

i) Denitrification:- Denitrification in the secondary clarifiers is widely encountered. The sludge initially settles, but due to continued microbial respiration dissolved oxygen becomes depleted. This can become a problem when the sludge residence time in the secondary clarifier is too long and the effluent has been fully nitrified. Under these conditions the majority of facultative micro-organisms have to rely on fermentation to regenerate NAD<sup>+</sup>. However, certain chemorganotrophs are capable of utilising NO<sub>3</sub> as a terminal electron acceptor and respiration can proceed with the reduction of nitrate through the following pathway.

NO<sub>3</sub> ----> NO<sub>2</sub> ----> NO ----> N<sub>2</sub>O ----> N<sub>2</sub> NITRATE NITRITE NITRIC NITROUS GAS OXIDE OXIDE

This process is known as anoxic or nitrate respiration and is carried out by a variety of bacteria such as Spirillum sp., Xanthomonas sp., Achromobacter sp., Micrococcus sp. and Pseudomonas sp. (Painter 1970). Barnes and Bliss (1983) and Payne (1981) both review the general process of de-nitrification and the associated problems this can cause in secondary clarifiers. Not all these genera are capable of complete oxidation to nitrogen and a variety of gaseous products can be produced (Wagner et al. 1987). Of these gaseous products, oxygen is utilised but the nitrogen forms small bubbles that attach to the sludge flocs and buoy them to the surface. Rising sludge can be overcome by ensuring that the settled sludge is not retained too long in the secondary clarifier before being recirculated or wasted. Flatbottomed tanks with a central sludge take-off are particularly susceptible to denitrification problems as the sludge at the periphery of such tanks may remain there for long periods of time. Crabtree (1984) had some success in reducing the problems of denitrification by lowering the concentration of oxidised nitrogen (TON) in the mixed liquor. While Chambers and Tomlinson (1981) found denitrification could also be controlled by increasing the sludge recycle rate or ensuring the mixed liquor is well aerated before it enters the secondary clarifier.

ii) Foaming or Scum Formation: Since the ban on the use of non-degradable detergents, white frothy foam often covering the entire aeration basin is now rarely encountered at activated sludge plants. However, a denser foam or mouse of a light brown colour, occasionally occurs on the aeration tank and it is this phenomena which is referred to as activated sludge foaming. From one of the earliest reports of foam formation in the so called "Milwaukee Mystery" (Anon. 1969) over two decades ago numerous surveys and observations into activated sludge foaming have been carried out through out the world. In North America by Al-Diwany and Cross (1978), Pipes 1978b, Dhaliwhal (1979) and more recently by Pitt and Jenkins (1990) where 66% of the sites surveyed experienced some type of foaming. Also in Europe (Segerer 1984, Lemmer 1986, Tandoi 1991, Goddard and Forster 1987) in Japan (Hiraoka and Tsumura 1984 and Mori et al. 1988), in Australia (Geenfield et al. 1985 and Seviour 1990). These stable foam are another microbially induced

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problem associated with excessive growth of Nocardia, Rhodococcus and Microthrix sp. (Dhaliwhal 1979, Lemmer and Popp 1982, Blackbeard et al. 1986). The amount of foam present on an aeration tank can vary from 2-3cm to 50-60cm and this foam is comprised of an almost pure culture of the organism involved at a concentration of 5-6g (dry solids) per 100g of foam. The foam appears to have little effect on the performance of the aeration tank but does severely effect the operation of the final settlement tanks, as the foam causes solids flotation which over flows the clarifier weirs leading to solids loss in the final effluent. The conditions leading to excessive growth of foam forming species and hence stable foam formation are not fully understood, with contradictory evidence relating to such factors as temperature (> 18°C) (Pitt and Jenkins 1990), high loading levels and long sludge ages (>9 days) (Pipes 1978b). Experience in Europe and Australia suggests that the presence of emulsifiable fatty material may be significant (Eikelboom 1975, Pipes 1978b, Greenfield et al. 1985, Slijkhuis 1988). The actual formation of a foam depends on the mixed liquor containing adequate suspended solids (MLSS > 5000mg/l) (Wheeler and Rue 1980, Wheatley et al. 1988). The amount of foam produced has been found to be directly proportional to the concentration of the causative organism present based on a total filament length basis (Rodriguez 1983). The most widely used method of control to cope with foam formation in activated sludge is to reduce the sludge age. By increasing the sludge wastage rate it is possible to washout the causative organism from the system. Other control method used are high pressure sprays, which can be an effective method of preventing the stabilisation of foams, the addition of hypochlorites and physical removal which are limited in their effectiveness, costly and time consuming.

iii) Deflocculation or Dispersed Growth: This is sometimes described as pin-point floc, the final effluent is turbid and contains suspended solids. This is caused by dispersed growth or poorly flocculating growth in the aeration tank (Bisogni and Lawrence 1971). There are two documented causes, one is the absence of filamentous micro-organisms, creating the so called backbone of the flocs (Jenkins et al. 1986) leading to the formation of small weak flocs. And two, the sludge is subjected to high shear forces caused by ill suited (high speed) pumping regimes may suffer floc destruction, which inturn may lead to dispersed growth (Parker 1983; Tuntoolavest et al. 1983; Konicek and Burdych 1988)

iv) Non-Filamentous Bulking Sludge: Non-filamentous bulking; in the past termed viscous bulking (Hale and Garver 1983, Jenkins et al. 1986) or zoogleal bulking (Eikelboom and van Buijsen 1981) has rarely been reported and only confirmed by microscopic examination on a few occasions. The cause of non-filamentous bulking is predominantly an excessive production of extracellular polymer by Zooglea type micro-organisms, apparently due to severe nitrogen depletion (Lau et al. 1984), deficiency phosphorus or trace nutrients such as iron (Wood and Tchobanolgous 1974) a high content of fatty and oleic compounds (van Leeuwen 1988; Novak et al. 1993), in some cases selector systems stimulating growth of zoogleal colonies (Gabb et al. 1991). The very poor compaction of Zooglea-like flocs is caused by the large amount of water held in the extracellular polymer, which prevents rapid settlement. This leads to the settled sludge occupying a large volume which may fill the clarifier resulting in solids loss in the final effluent.

v) Filamentous Bulking: Filamentous growth causing bulking was first described by Donaldson in 1932 as "the weeds of activated sludge". The evidence to support the role of filamentous organisms in bulking is compelling, with a number of surveys carried out to establish the extent of the problem of sludge bulking. Tomlinson (1976) reported a survey of 65 activated sludge plants in Britain, of which 63% experienced problems with sludge settlement and 52% had experienced bulking to such an extent that serious loss of solids in the final effluent had occurred. While Pipes (1979) studied 94 sets of samples from 32 different plants and found a strong correlation between the SVI and the number of filaments present. These studies confirmed earlier work by Finstein and Heukelekian 1965, Chudoba et al. 1973a, Sezgin et al 1978 who found similar correlations. By 1982 this situation had not changed with an estimated 50% of treatment plants in the U.K. experiencing bulking conditions regularly (Tomlinson and Chambers 1982). This situation has improved over the past two decades, however, Pujol and Canler (1989) still reported 25% of activated sludge plants in France experiencing bulking problems. This dominance within activated sludge by filamentous species hinders settlement and holds the flocs apart. The main causes of this type of bulking are thought to be low dissolved oxygen concentration, low carbon substrate concentration or inorganic nutrient deficiency (Eikelboom 1975). In addition to the conditions mentioned above further work by Strom and Jenkins (1984) have associated certain filamentous organisms with various operating parameters shown here in Table 2.4.

Table 2.4 Filamentous Organisms Associated With Certain Operating Parameters. (Strom and Jenkins 1984)

Operating Parameter	Filamentous Organism	
Low Dissolve Oxygen Concentration	Type 0710, S. natans, H. hydrosis	
Low F:M Ratio	M. parvicella, H. hydrosis, Nocardia sp. Types 021N, 0041, 0675, 0092, 0581, 0961, 0803	
Septic Wastewater	Thiothrix sp., Beggiatoa, Type 021N	
Nutrient Deficiency	Thiothrix sp., S. ntaans, Type 021N and possibly H. hydrosis, Types 0041 and 0675	
Low pH	Fungi	

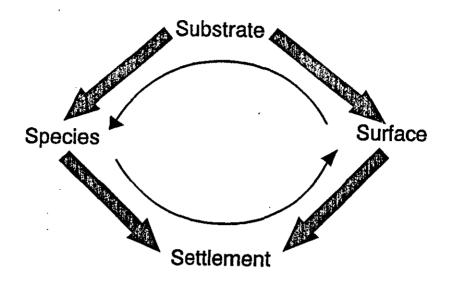
# 2.8.2 Mechanisms Of Sludge Bulking

Two mechanisms have been proposed to explain sludge bulking; one suggests that bulking results from an increased floc charge brought about by changes in floc surface chemistry, and the other suggests that bulking is caused by a dominance of activated sludge flora by filamentous bacteria. The sludge surface model, was based on the observation that the magnitude of the floc surface charge affects sludge settlement behaviour, both thickening and filtration properties. The magnitude of surface charge is governed by the exact chemical composition and the extra cellular polymer produced by micro-organisms in activated sludge (Forster 1971b, Steiner et al.1976, Goodwin and Forster 1985).

The second model is based on the observation that the onset of bulking is almost always accompanied by an increase in the number of filamentous bacteria in the sludge floc. The model suggests that there are two groups of bacteria in activated sludge which are responsible for flocculation: Filamentous bacteria provide a rigid backbone which gives the floc its structure and strength, allowing colonisation by a second group of organisms, the floc formers. These are typical Zooglea-type organisms which attach to the filaments to form a gelatinous matrix. The outgrowth of excessive quantities of filaments from the floc can be correlated with sludge bulking, quantitative measures have been suggested by Baker and Veenstra (1986), Palm et al. (1980), Lee et al.(1982). Thus the settling, compaction and separation properties of an activated sludge are related to the relative numbers of filamentous and floc forming organisms. The absence of filamentous organisms leads to the production of small, weak flocs which settle poorly (pin-point floc). Therefore an ideal sludge is produced when there is a balance between filamentous and floc forming organisms.

However, although these two theories have been maintained as separate concepts, it is most likely that the real cause of bulking is an interaction of both models in some way (Forster 1985a). This is best summarised in what is now known as the S-Hypothesis which shows the interaction between the floc surface, substrate, species profile and settlement.

Figure 2.4 S-Hypothesis as an explanation of bulking.(Forster 1985)



A positive correlation between total filament length and settlement was found by Eriksen and Hardin (1984). However when they compared stirred and non-stirred settlement tests they considered that bulking was not caused by filaments extending from the floc but instead claimed that filaments outside the floc were proportional to those inside the floc and it was these filaments that affected settlement by altering the size, shape and strength of the flocs.

# 2.8.3 Filametous Identification

After the discovery that filamentous growth caused bulking, early researchers initially attributed the poor settling properties of the sludge to the sheathed bacterium *Sphaerotilus natans* (Eikelboom 1974) which led to an over simplification of both the identification of filaments and their requirements for growth and competition. Later a more detailed investigation of filamentous organisms by Eikelboom (1975) led to the publication of an identification key. Since this publication more attention has been paid to the numerous other genera involved in bulking and now approximately twenty five different filamentous bacteria are known to be able to cause activated sludge bulking.

A further development in filament identification was made by Eikelboom and Buijsen (1981). This they did by adding metabolic abilities reflected in staining properties, to the morphological features and dispensed with traditional species classification by typing the filament accordingly, using numbers. These individual types were then correlated with operational conditions and sewage characteristics. Jenkins et al. (1987) later grouped these into five causative groups. Wanner and Grau (1989) were able to use four groups according to morphological, physiological and metabolic similarity but more importantly, similar operational conditions and problems were reflected in their groupings.

Table 2.5 Classification of filamentous organisms from operational conditions(Wanner and Grau 1989)

S	Sphaerotilus-like oxic zone growers
<u> </u>	Cyanophyte-like oxic zone growers
<u> </u>	Microthrix parvicella type all zone growers
F	Foam forming filaments

#### 2.8.4 The Causative Organisms

Since the publication of the survey results and the identification key of filamentous organisms in activated sludge by Eikelboom (1975), more attention has been paid to identification. Eikelboom studied 1900 samples collected from two hundred different activated sludge plants in the Netherlands. Twenty six different types of filamentous organism were identified which he assigned to groups in an identification key. Subsequent work by Eikelboom (1975) established that *Microthrix parvicella* and Type 021N were the most frequently observed filamentous organisms. Strom and jenkins (1984) were also able to identify filamentous organisms associated with particular operating conditions (shown in Table 2.4)

The waste composition was also found to exert an influence on the filamentous

organisms present. Filamentous bulking occurred more frequently in plants receiving industrial effluents and the species *H. hydrosis*, Type 021N, Type 0041 and Type 0092 were more likely to be the cause.

The same bacterial species have been observed causing bulking throughout the world with approximately ten bacterial species accounting for at least 90% of the bulking incidents. The frequency of occurrence and frequency of dominance of filamentous micro-organisms has been measured in the United States (Richard et al. 1981; Strom and Jenkins 1984) in South Africa (Blackbeard and Ekama 1984; Blackbeard et al. 1986; Ekama et al. 1985) and in Europe (Eikelboom 1977; Wagner 1982,; Byron 1987; Pujol and Canler 1989). All the above studies found comparable results. However, differences were found in the relative ranking of the dominant filament type. The most frequently encountered species in different countries are summarised in Table 2.6 The results from the United States stand out as being notably different, particularly the relative ranking of *Nocardia* sp. and *M. parvicella*. These differences were suggested by Strom and Jenkins to be due to the way in which activated sludge plants are operated in each country and in particular the use of lower loading rates in the Netherlands.

Table 2.6 Filamentous organisms in bulking activated sludge from five separate surveys of activated sludge plants. Hierarchal classification was used with each type ranked according to the frequency of its occurrence as the dominant filamentous organism in bulking sludge (Pujol and Canler 1989).

Type of Filament	South	U.S.A.	Netherlands	Germany	France
	Africa				
Type 0092	1	11	4	-	2
M.parvicella	2	. 7	1	2	3
Type 1851	3	9	12	· -	10
Type 0675	4	6	-	-	8
Type 0914	5	-	-	-	-
Type 0041	6	5	6	3	1
Nocardia sp.	6	1	14	5	-
Туре 0805	7	7	9	10	-
Type 1701	8	2	5	8	7
Nostciodas Limicola	9	8	11	7	9
Type 021N	-	3	2	1	5
Haliscomenobacter Hydris	9	9	3	6	-
S.natans	-	6	7	4	6
Thiothrix sp.	9	4	17	-	-
Type 0581	9	12	8	-	-
Туре 0961	-	10	10	9	-
Beggiatoa sp.	-	12	-	-	-
Number of Plants	56	190	200	315	50
Number of Observations	56	300	1900	3500	50

# 2.9 Factors Affecting Sludge Settlement

A number of theories explaining the predominance of filaments in the aeration tank have been proposed. These can be loosely categorised as either being related to process operation or wastewater characteristics. Either one of the above factors or combination of factors may have a detrimental affect on the settling characteristics of the sludge. The major causes of bulking are thought to include low dissolved oxygen concentration, sludge loading, pH, high temperature and nutrient deficiency.

2.9.1 The Nature Of Wastewater

The strength of a wastewater is expressed in terms of the biochemical oxygen demand (BOD), but this single parameter neither takes account of the different sources nor the changes which may occur within a wastewater. This is especially true for industrial wastes, where products change and factory output fluctuates dramatically.

If effluents are to support the growth of micro-organisms then they must contain a wide range of certain nutrients in a form available for assimilation by these organisms. The ratio of the major macronutrients also need to be within a certain range to provide for both the growth and the right environmental conditions e.g. alkalinity. Domestic sewage is generally carbon limited for bacterial growth, which is an ideal situation for water treatment bioreactor design as it means that the major polluting component is the first to be exhausted. However, many industrial wastes, for example, brewery wastes, paper mill waste and fruit and vegetable processing waste, are high in carbon and when they form a large component of the effluent, then nutrients such as nitrogen or phosphorus may become limiting for growth.

Apart from nutrient levels and ratios other contents of the wastewater may have a detrimental effect on the process :-

a) Synthetic Detergents - Synthetic detergents along with other surface active substances can affect oxygen transfer by aeration equipment.

b) The presence of heavy metals or their compounds :- Heavy metals such as chromium, nickel, mercury or copper which may be present in sewage in industrial area, may exert a toxic effect on the biomass if concentrations are high enough. Along with heavy metals a toxic effect may also be induced if sulphide

concentrations are high.

c) Free oil or fat :- Abnormal quantities of fat and free oil can interfere with oxygen transfer and lead to dispersion of activated sludge flocs.

Stale or septic sewage :- Sewage which has become anaerobic during its passage through the sewage system or during storage in balancing tanks contains significant concentrations of sulphides and a range of volatile organic acids (i.e. acetic acid, proprionic acid) which are intermediate breakdown products of anaerobic metabolism. Septicity may also occur due to oversized primary tanks. Abattoir, tannery waste and leachate from waste tips are all associated with septicity, which is a known cause of activated sludge bulking (White et al. 1980).

# 2.9.2 Macronutrients

The macronutrients are the major biochemical elements, carbon, hydrogen, nitrogen, phosphorus and sulphur. Deficiencies of carbon (BOD) and oxygen will be dealt with in more detail in sections 2.10 and 2.9.7 respectively, while sulphur is rarely limiting. In order for a wastewater to support microbial growth it must contain sufficient quantities of macronutrients in an available form. The optimum C:N:P weight ratio for biological treatment is 100:5:1 (Pipes 1975, IWEM Manual 1987). The C:N:P ratio for raw domestic sewage is approximately 100:17:5 and 100:19:6 for settled sewage, both containing abundant nutrients for growth. Certain trade wastes however may well be nutrient deficient, as are wastes containing a high proportion of fats, oils and hydrocarbons.

The effect of deviation from the appropriate nutrient ratio (100:5:1) is not a well recorded area. Sludge settleability has been related to various ratios of these nutrients (Forster and Dallas-Newton 1980, Wagner 1982a). Jones (1965) found filamentous bulking associated with high carbohydrate, nutrient deficient wastewater. Hattingh (1963) also showed that when activated sludge was fed with synthetic feed with a BOD:N:>22:1 and BOD:P>168:1 the sludge showed a tendency to bulk due to the proliferation of filamentous organisms. The filamentous

organism *S. natuns* was noted to favour low nitrogen conditions by Dias et al.(1968). Other workers who have noted an deterioration in settlement due to low nitrogen conditions are:- Thompson and Forster (1983), Wu et al. (1982), Strom and Jenkins (1984), Wu et al (1984), Mavinic (1986), Tien Huang (1987), Novak (1988) and Richards (1985).

Fewer workers have carried out research into the affect of phosphorus deficiency. Wu and Okrutny (1982) showed that biomass growth and soluble BOD uptake was severely restricted by phosphorus deficiency. This restriction in growth may also promote filamentous growth by restricting the otherwise more rapid growth rate of the floc forming bacteria (Stoveland et al. 1979). A severe bulking problem caused by filamentous bacteria was reported by Ericsson and Eriksson (1988) in an activated sludge plant preceded by ferric chloride precipitation to remove phosphate.

Nutrient deficiency can be detected by analysing the final effluent. If no excess N or P is present then addition is suggested. Additions of solid urea and Ammonium phosphate are frequently used to correct deficiencies in nutrient levels as are a range of agricultural fertilisers (Kiuri 1991). Due to the handling difficulties involved in the use of these products along with inaccuracies in dosing, specifically formulated products are gaining favour in the United Kingdom (Gostick 1991). There is concern that nutrient emissions into the environment should be kept to a minimum. For this reason nutrient additions should be carefully controlled by monitoring nutrient concentrations in the final water (Mobius 1991, Grau 1991).

# 2.9.3 Micronutrients

In addition to the macronutrients bacteria also have a requirement for a range of micronutrients (Pirt 1975). These are listed in Table 2.7. The role of micronutrients within the activated sludge system has rarely been investigated and is frequently ignored entirely. A deficiency of micronutrients will have a detrimental effect on the performance of an activated sludge plant and may also lead to bulking (Gostick 1990). It has been claimed that due to filamentous organisms having a higher

surface area to volume ratio than floc forming bacteria, they are better able to take up the micronutrients. Therefore the filamentous organisms may have a growth advantage when concentrations of micronutrients are growth limiting (Wood and Tchobanoglous 1975). The transition metals are of particular interest, which although only required in trace quantities have vital functions as catalytic centres of metaloenzymes which participate in many of the cells redox reactions (Wackett et al. 1989). The transition metals are very susceptible to being precipitated from wastewater as sulphides, phosphates and hydroxides and therefore become unavailable for utilisation by the biomass. Simpson et al. (1990) reported a number of cases where filamentous bulking has been controlled by the addition of a mixture of essential trace metals. The accessibility of any added metals to the biomass is also of importance (Gostick 1991). The transition metal iron has been found to be inhibitory to the filamentous organism S. natans both in pure culture (Chang et al. 1980, Kato and Kazama 1991) and in bulking laboratory scale activated sludge systems (Al-shahwani et al. 1987). The effect of calcium, copper, nickel and zinc on Thiothrix, 021N and Type 1701 was studied by Shuttleworth and Unz (1991), while the effect of chromium on activated sludge was investigated by Cokcay and Yetis (1990).

Macronutrients	Micronutrients	
Carbon	Calcium	
Hydrogen	Magnesium	
Oxygen	Sodium	
Nitrogen	Potassium	
Phosphorus	Iron	
Sulphur	Copper	
	Cobalt	
	Nickel	
	Manganese	
	Molybdenum	
	Vanadium	
	Boron	
	Chlorine	
	Iodine	
	Zinc	

## 2.9.4 pH

For most bacteria and thus for most wastewater processes, the pH range for growth is given as between 4 and 9 (Benefield and Randall 1980). Besides the growth rate, pH also effects enzyme activity. Studies by Suruco and Cetin (1989) investigated the effect of pH (range 5.7-9.0) on activated sludge. From this work it was concluded that as the operating pH was increased so did the zone settling velocities. At pH values above 7.2 a sharp decrease in SVI was observed which was due to the proliferation of undesired micro-organisms below this pH. The optimum range for operating an activated sludge plant is between 6.5 and 8.0, pH values lower than 5.0 may lead to excessive growth of fungi (Strom and Jenkins 1984). Periodic fluctuations of high and low pH value can upset the balance of micro-organisms in the plant and lead to a deterioration in effluent quality.

### 2.9.5 The Effect Of Temperature

The majority of biological treatment systems operate in the mesophilic temperature range, growing best between 20-40°C. However, aeration tanks normally operate within the range 12-20°C. Higher temperatures result in increased biological activity which in turn increases the rate of substrate removal. This increased metabolism at higher temperatures can lead to problems with oxygen limitation. During periods of warmer weather there is a significant increase in the incidence of bulking. Lin and Heinke (1977a,b) considered temperature to be of major importance in explaining the variation in performance of activated sludge systems. Pipes (1987) found that temperature in excess of 30°C in aeration tanks increased the tendency for the formation of a bulking sludge. This work was further backed up by the findings of Suruco and Cetin (1990) who found sludge grown at temperatures of 35°C and above led to the proliferation of filamentous organisms.

# 2.9.6 The Importance Of Dissolved Oxygen Concentration

In aerobic metabolism oxygen is required as terminal electron acceptor. Therefore a certain dissolved oxygen concentration is required in the aeration tank if efficient

treatment is to be achieved. This level is generally accepted as being > 1mg/l for a non nitrifying plant and 2mg/l for a nitrifying plant (Horan 1990) but can also be dependent on the solids retention time and temperature.

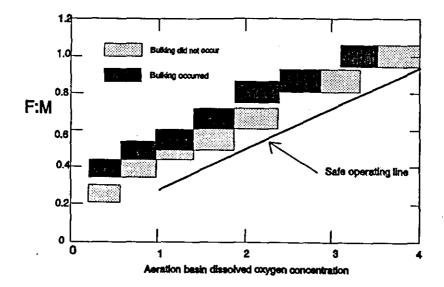
Benjes (1980) gives a typical value of 1.1kg of oxygen per kgBOD at solids retention of 7 days at 10-20°C. If the solids retention time is long enough for nitrification a further 4.3kg of oxygen will be required to oxidise each kg of ammoniacal nitrogen (Lister and Boon 1973).

The observation that under-aeration and low dissolved oxygen levels favour the growth of filamentous bacteria (Adamse 1968) brought about the proposition of a unified theory of sludge bulking (Sezgin et al. 1978). This unified theory suggests that filamentous organisms are an inherent component of activated sludge but that in a "healthy" good settling sludge they are confined to the central regions of the floc where due to diffusion gradients dissolved oxygen is limiting and filaments will have a growth advantage over flocculant organisms. The floc surface in contrast to this is not oxygen limited, so flocculant organisms have a growth advantage and hence out compete and prevent filaments from extending out of the floc. However, if for any reason the dissolved oxygen concentration falls within the reactor, then oxygen levels at the floc surface will be reduced giving filamentous organisms a growth advantage and hence they are able to grow out from the floc and hinder sludge settlement.

There has been some debate as to whether high dissolved oxygen levels or the use of pure oxygen can improve settlement. Evidence has been produced both to support (Andreakis 1987, Chapman et al. 1976) and refute (Houtmeyers 1980) the argument. Cycling between high and low levels of dissolved oxygen has also been suggested to have an effect on settlement (Ip et al. 1987). This has been put forward as an explanation to the claimed improvement in sludge settlement associated with the use of anoxic zones which were initially included in reactor design to promote denitrification. White (1975) did however, point out that in achieving an anoxic zone a substrate gradient is inevitable and it is this that promotes good settlement.

From the work of Palm et al. (1980) it is clear that the minimum dissolved oxygen concentration required to prevent bulking is a function of sludge loading, increasing as the sludge loading increases (see figure 2.5)

Figure 2.5 The minimum dissolved oxygen concentration in the aeration tank required to prevent bulking as a function of sludge loading (Palm et al. 1980)



Houtmeyers et al. (1980) while working on the effect of mixing patterns using intermittently fed reactors, found that low dissolved oxygen concentration could lead to bulking in intermittently fed reactors which previously had been producing good settling sludge, (when adequately aerated). However, high dissolved oxygen concentrations did not prevent bulking in continuously fed reactors which were producing bulking sludge at normal dissolved oxygen concentrations.

A study undertaken by Strom and Jenkins (1984) attempted to find a correlation between the excessive growth of various types of filamentous organisms and the plant operating conditions associated with sludge bulking. From this work filamentous types were identified into groups based on the conditions with which they were associated. The organisms Type 1701, 021N, *S. natans* and Type 1863 were indicative of low dissolved oxygen concentrations. Low dissolved oxygen bulking can therefore be identified by the identification of the above filamentous types. For example Type 1701 was the most commonly observed filamentous organism in bulking sludges in the United States (Jenkins et al. 1984) indicating low dissolved oxygen to be a major cause of bulking in the United States.

More evidence to support this work on the effect of dissolved oxygen levels came from studies of the growth kinetics of the filamentous organisms that had been found to be associated with low dissolved oxygen concentrations. Type 1701 has a lower half saturation coefficient for dissolved oxygen ( $K_{DO} = 0.014$  mg/l) than a representative floc-former ( $K_{DO} = 0.073$  mg/l). This accounts for its competitive advantage at low dissolved oxygen concentrations(Hao et al. 1982, Richard et al. 1982). S. natans ( $K_{DO} = 0.033$  mg/l) successfully competed with a floc-former at low dissolved oxygen concentrations (Lau et al. 1980, Lau et al. 1984). The relative magnitude of the K<sub>DO</sub> values suggests that Type 1701 may occur at severe dissolved oxygen limitation while S. natans may occur at modest dissolved oxygen limitation (Richard et al. 1985). The mixing conditions in the aeration basin are also of importance in low dissolved oxygen bulking since large flocs exacerbate low dissolved oxygen problems due to reduced oxygen diffusion to the floc interior (Richard et al. 1982). The above work shows that filamentous species may also show cross-over Monod kinetics in relation to dissolved oxygen as well as the carbon/energy source.

## 2.9.7 Dissolved Oxygen Control

Due to the importance of dissolved oxygen concentrations and the critical levels required to prevent bulking, control of the aeration system is essential. Oxygen consumption is independent of dissolved oxygen concentration until shortly before its complete disappearance and the oxygen transfer rate is proportional to the oxygen deficit in the mixed liquor. This makes it logical to operate the aeration tank as close to the critical minimum dissolved oxygen concentration as possible (Gray 1990). To maintain this critical oxygen concentration in aeration tanks the dissolved oxygen concentration should be automatically maintained by oxygen electrodes, which control the aeration rate to match the rate of oxygen utilisation by the

biomass. The positioning of the oxygen probes is of great importance if the critical dissolved oxygen is to be maintained throughout the tank. The oxygen electrodes are normally positioned where the lowest dissolved oxygen concentrations can be expected e.g. in the corners of square tanks or near the inlet where oxygen demand is greatest, to ensure the rest of the tank is maintained at dissolved oxygen levels above the level achieved in the low spot (Lewin and Henley 1972). The dissolved oxygen concentration tends to increase towards the saturation concentration as the tank outlet is neared. Therefore, if energy is to be saved and efficiency improved it is vital to match oxygen supply with demand by treating aeration tanks as a series of independently controllable zones, each with its own dissolved oxygen probe with appropriate control settings (Barnard and Meiring 1988).

## 2.9.8 Redox Potential

The oxidation-reduction potential (ORP), or redox potential, is a measure of the activity of electrons involved in oxidation-reduction reactions within an aqueous environment. In most complex biological systems, such as encountered in waste water treatment applications, the many chemical and biological oxidation-reduction reactions taking place are not in equilibrium and the observed ORP cannot be interpreted thermodynamically (Kjaergaard 1977, Koch and Oldman 1985).

The earliest reference to oxidation-reduction potential (ORP) measurement relating to waste water treatment dates back to 1906 (Dirasin et al. 1963). However, it was not until the 1940's that interest in ORP became more widespread and it was thought by many as showing great promise as an operational tool (Dickinson 1940, Rohlich 1944, Hood 1948). Hewitt (1950) provides an excellent summary of this early work along with a review of the many aspects and intricacies of ORP measurement and its interpretation. This much maligned and often controversial parameter has rarely been the subject of extensive or continuous research. In practise development work was concentrated on the D.O. probe and ORP has rarely extensively or continuously recorded. In fact, the use of ORP as a monitoring or control parameter has almost completely disappeared. The general reluctance to fully explore the suitability of ORP in design, monitoring and control of waste water treatment facilities may stem from the widely held opinion that ORP measurements are difficult to obtain and unambiguous interpretations are not possible. Some papers cite the seemingly impossible task of being able to fully rationalise and understand the theoretical aspects of ORP in complex biological systems as a reason to suggest that ORP as an environmental factor cannot be successfully applied and its use should not be considered (Stumm 1966, Morris and Stumm 1967).

Extensive work on ORP was carried out between the 1940's and 1960. Dickinson (1940), Moore, Ruchoft and Wattie (1942), Moore and Ruchoft (1943), Rohlich (1944), Eliasen (1945), Hood (1948), Rohlich (1948), Hood and Rohlich (1949), Eckenfelder and Hood (1951), Nussberger (1955) independently and jointly examined ORP in numerous sewage treatment works at different stages of purification. The many observations made during these studies both on domestic sewage and industrial waste water treatment plants provided extensive documentation on ORP. However, widely differing values were obtained from highly variable conditions with nearly as many anomalies revealed as similarities between measurements taken from different treatment works. All the above studies did indicate that there was a recognisable pattern of ORP change over the course of aerobic treatment. Although not definitive with respect to actual value, measurable increases in ORP provided a general indication of carbon oxidation and the degree of waste stabilisation that had been achieved. Claims made as a result of these early investigations into ORP were: That it could be used to detect if a plant was overloaded, underloaded, underaerated or overaerated; detect the presence of industrial wastes and toxic conditions; to examine influent and effluent quality and to provide a method for odour control. Without exception, all researchers advocated optimistically that ORP be hailed as the "new" process control parameter. Some researchers, see (Nussberger 1953) for example, proposed bar charts and rational diagnosis tree methods so that ORP monitoring could be used for interpreting operating problems, presenting guidelines for controlling plant

operation, specifically aeration control.

Even though all the above workers reported that ORP monitoring showed great promise as an operational tool, little further work into ORP was ever conducted beyond 1960. This may have been due to the development and widespread use of D.O. as a successful and convenient process control parameter in the activated sludge process. A few isolated instances of the practice of ORP monitoring as a process control parameter has continued with some references in obscure journals (Rudd et al. 1961, Roberts and Rudd 1963, O'Rourke et al. 1963, Burbank 1981) with its use being mainly practical and diagnostic and seldom any truly scientific data collected. However, there has been a resurgence in interest linked to a more extensive use of different redox potentials for waste water treatment. In Japan, in the early eighties there have been a number of applications for several patents (Miendensha Electric 1980, Kankyo Engineering 1983) which involve the use of ORP as a more sensitive method of controlling D.O. and preventing sludge bulking in activated sludge.

ORP has been the subject of more recent research on anaerobic biological waste treatment which produced definitive and unambiguous results. Pioneering work in this area was carried out at the university of Michigan by Grune and Chuch (1958) with their results being subsequently confirmed by Hartz and Kountz (1966). Further investigations at New York University (Blanc 1969, Blanc and Molof 1968,1973,1974) demonstrated that anaerobic sludge digestion is particularly amenable to ORP monitoring and control. From these studies, optimum conditions for methane production was observed to occur in a narrow range of electrode potentials ( $E_c$  values between -520 and -530mV). However, gas production was observed to occur over a wider range of electrode potentials ( $E_c$  values between - 495 and -555mV). Outside this range there was a marked reduction in gas production and process failure occurred. Further work by Borchardt (1970) at the University of Michigan found that fatty acid production also occurred at a defined optimum ORP regime ( $E_c$  values of -500 to -520mV). The general conclusions reached in these investigations was that ORP was an invaluable process monitoring

and control parameter in anaerobic digestion.

Due to the above work carried out on anaerobic treatment, there has been recent suggestions that ORP may be a possible control method for biological nutrient removal plants. ORP was potentially of use in determining the degree of anaerobiosis in the so called "anaerobic" or "anoxic" zone located at the head of most biological phosphorus removal plants (Barnard 1976). At the process level, ORP was found to be correlated with non zero nitrate concentrations in the anoxic zone. However, Randall et al. (1970) had earlier concluded that phosphate release was not a function, nor was it dependant on observed ORP levels. Further studies into the effect of ORP were conducted by Koch and Oldman (1985) who conducted their research at laboratory, pilot and full scale nutrient removal plants. They concluded ORP to be very useful as a monitor of biological systems and ORP could be correlated with phosphorus release in the "anaerobic" zone. ORP should be successful as a process control parameter for nitrate removal in anoxic zones. Sekine et al. (1985) for example proposed a control system based on the measurement of ORP for controlling nitrification, suggesting that nitrification reactions occurring in the aeration tank are some of the most sensitive of all the biological oxidation reactions. Therefore, the rate of nitrification indicating the state of reaction could be detected in terms of ORP. Similarly for denitrification ORP could also be used to detect the state of reaction. This work however does not seem to have been followed up with reports on more extensive full scale applications as yet.

### 2.10 Mixing Patterns and Filamentous Bacteria

Mixing patterns and oxidation gradient have also been linked to with sludge settlement for a long time. Donaldson (1932) was the earliest worker to suggest reactor compartmentalisation as a method of controlling filamentous bulking. Completely mixed activated sludge systems began to be studied in more detail at the end of the fifties and early sixties (Stack and Conway 1959, Kalinske 1960, McKinney 1960,1962). Initially good mixing was thought to confer advantages to the activated sludge system. These were listed as follows:-

a) The concentration of substrate is uniform throughout the aeration tank and consequently the oxygen demand is also uniform throughout the tank.

b) The biomass is under favourable conditions, not being exposed to periodic changes in organic substrate concentrations.

c) The entire contents of the aeration tank are utilised for the dilution of the raw waste water. This buffers the biomass against the effects of toxic substances which may be present in the incoming wastewater.

d) The cost of construction of circular fully mixed aeration tanks without complex baffles is approximately half that of a comparable size plug- flow tank.

However, it became apparent particularly when treating industrial waste waters that completely mixed systems promoted the growth and proliferation of non-desirable filamentous micro-organisms leading to sludge bulking (Chudoba et al. 1973a). This would far outweigh most of the advantages of well mixed systems.

The British Water Pollution Laboratory Annual Report (1969) demonstrated that staging the aeration zone improved settlement. A survey of 65 activated sludge plants in England and Wales (Tomlinson 1976) found not only that 63% of the plants surveyed had experienced serious bulking problems, but also that plants employing plug-flow configuration were less prone to bulking than those with complete mixing. The findings of this study were confirmed by Tomlinson and Chambers (1979) who characterised the mixing of more than 20 full scale activated sludge plants in the United Kingdom and correlated the mixing characteristics with sludge settleability. Results again confirmed that completely mixed aeration tank configurations are more prone to producing sludges with poor settling characteristics. In addition to the information referred to above, there is also extensive evidence from laboratory scale experiments to support this argument(Chudoba et al. 1973; Humphries 1982) and further examples will be described in sections 2.10.3 and 2.10.4.

#### 2.10.1 Completely Mixed Systems

The most commonly used model, relating microbial growth to substrate utilisation, is that of Monod (1949). He observed that the growth rate dx/dt was not only a function of micro-organism concentration but also of some limiting substrate or nutrient concentration. He described the relationship between the residual concentration of the growth limiting substrate or nutrient and moreover the form of the relationship was similar to the effect of substrate concentration on the rate of an enzyme catalysed reaction (i.e. first order). The growth of a micro-organism can be described by an equation analogous to the Michaelis-Menten equation:-

 $\mu = \underline{\mu}_{\underline{m}} \underline{S} \\ K_{\underline{s}} + S$ 

where

 $\mu$  = Specific Growth Rate

 $\mu_m$  = Maximum Specific Growth Rate

S = Concentration Of Growth Limiting Substrate

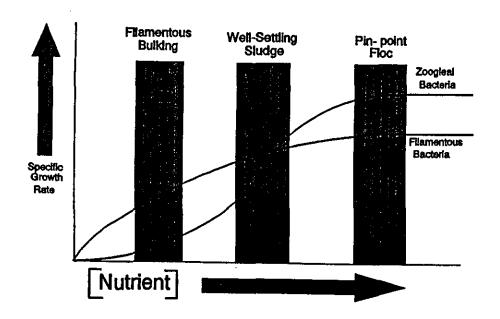
 $K_s$  = Saturation Constant (growth limiting substrate concentration that allows the organism to grow at half the maximum specific growth rate)

The saturation constant is the most important parameter in determining the out come of competition between different micro-organisms for a limited supply of food. It is a measure of the affinity an organism has for the growth limiting substrate, hence the lower the  $K_s$  value then the greater the organisms affinity for that substrate. In an open environment with low substrate concentrations (such as a waste water treatment process) organisms exhibiting the lowest saturation coefficients will poses the greatest capacity for growth.

In "completely-mixed" aeration tanks running at low substrate concentrations filamentous organisms have a high affinity for the available substrate and hence have

a higher growth rate than the floc forming organisms with their lower affinity for the available substrate. This leads to a proliferation of filamentous organisms within the sludge and hence poor sludge settlement. Conversely, at higher substrate concentrations, floc forming organisms will have a faster growth rate resulting in better settlement. This phenomenon has been described as cross-over Monod Kinetics, and an example was reported by Van Nierkirk et al.(1987). They determined Zooglea ramigera had a maximum specific growth rate of  $5.5d^{-1}$  and a half saturation coefficient for acetate of 0.3mg dm<sup>-3</sup>, and Type 021N had a maximum specific growth rate of  $3.75d^{-1}$  and a half saturation coefficient for acetate of 0.07mg d<sup>-3</sup>, showing cross-over Monod kinetics with respect to substrate concentration. Endogenous decay rates of both these organisms was also considered by these workers. Type 021N was shown to be able to economise on maintenance energy requirements at the low growth rates associated with extreme substrate depletion. These observations are shown in Figure 2.6 showing the predicted out come of competition between floc-formers and filamentous organisms at various substrate concentrations.

Figure 2.6 Plot of growth rate against substrate concentration for two hypothetical organisms showing Cross-over Monod kinetics.



#### 2.10.2 Completely-Mixed or Plug-Flow

Although the terms "Plug-flow" and "Completely-mixed" are used to describe flow patterns, the terms actually describe ideal situations which are rarely experienced in practice. In all real situations flow characteristics or mixing patterns are more correctly described by reference to the degree back mixing, longitudinal mixing or dispersion.

The degree of longitudinal mixing in a flow system can be determined by tracer studies (Levenspiel 1962). The results from these studies can then be utilised as a comparison with theoretical models of non-ideal flow. Many mathematical models of non-ideal flow are complex but two are relatively simple and have been used previously to describe the behaviour of full-scale activated sludge aeration tanks (Tomlinson and Chambers 1979). These are the so called "dispersion" and "tanks in series" models.

The "dispersion" model assumes longitudinal mixing occurs in a manner analogous to the diffusion process. It is possible to quantify non-ideal flow using this model in terms of the dimensionless Dispersion Number which varies from nought for plugflow to infinity for complete mixing.

The "tanks in series" model assumes that non-ideal flow may be represented by a system consisting of an appropriate number of equal sized "completely mixed" tanks. Here the degree of longitudinal mixing is dependent on the theoretical number of tanks in series. The number of tanks can vary between one for "complete mixing" and infinity for "plug-flow".

# 2.10.3 Mixing Characteristics

Chudoba and his co-workers (1973a) were the first to attempt to quantify the mixing characteristics of activated sludge aeration tanks and to relate this to the settling characteristics of the sludge produced. Chudoba et al. (1973a) conducted laboratory experiments on four activated sludge systems with various flow patterns. The four systems were operated under similar conditions, a hydraulic retention time

of 8 hours and approximately the same sludge loading were applied. Dispersion numbers for the four systems were infinity, 1.06, 0.17 and 0.033. This was produced by each system being formed of an increasing number of fully mixed tanks in series (1 x 4 litre tank, 4 x 1 litre tanks in series, 8 x 0.5 litre tanks in series, 16 x 0.25 litre tanks in series). From this work Chudoba et.al. showed that the degree of dispersion affected the settling characteristics of the sludge produced. With the systems with the highest degree of dispersion (fully mixed) producing poorly settling sludge and systems with a low degree of dispersion (plug-flow) gave zoogleal forms a selective advantage over filamentous types. These results were later confirmed by Chambers (1982) at pilot plant scale consisting of 24 compartments in series produced a good settling sludge under all operating conditions investigated. It is felt that the primary cause of the selection of flocculating organisms in mixed cultures is the actual concentration of substrate at the inlet of a given system and the systems with a low degree of dispersion and hence high concentration gradients of substrate along the first parts of the system produce growth of zoogleal forms and suppress the growth of filamentous organisms (Humphries 1982, Tomlinson and Chambers 1979b).

# 2.10.4 Feed Patterns

A new hypothesis about the cause of activated sludge bulking was developed by Rensink (1966+1968) and Chudoba (1973a, 1973b,1974). Rensink et.al found that bulking sludge from oxidation ditches could be cured in the laboratory when the sludge was fed in a batch system. Kiff (1978) found settlement improved when the feeding pattern was changed from continuous to an intermittent feed pattern. Houtmeyers (1980) also obtained better sludge settlement using intermittent feeding and discovered that intermittently fed reactors produced sludges with higher glucose uptake rates than sludges from continuously fed systems. The first activated sludge plants were intermittently fed and these sequencing batch reactors are again gaining in popularity due to the stability of the sludge settlement properties produced by such systems (Ainsworth and Gill 1987).

#### 2.10.5 The Use Of Selectors

Chudoba et al. (1973b) followed their earlier work on mixing patterns by recommending that a selector (pre-mixing tank) or area of high substrate concentration be incorporated into plant design to encourage the growth of zoogleal types and hence promote good settlement. Other workers followed and built on this basic theory of plug-flow and selectors to control bulking and made recommendations to their size (Jones and Franklin 1985, Van Nierkirk et al. 1988, Shao and Jenkins 1989, Pujol and Boutin 1989, Rensink and Donker 1991).

The IAWPRC hydrolysis model (Ekama and Marais 1986) delimits the advantage of longitudinal mixing by suggesting that hydrolysis of the biomass towards the end of extended aeration plants can also promote the growth of filamentous organisms. Parallel to this work, there were other studies on fed-batch systems by Gabb et al. (1988) which showed that filamentous growth could be controlled by using a two stage anaerobic or anoxic initial contact zone. Initial contact zones or selectors are small chambers preceding the aeration tank in which thickened RAS from the final settlement tanks is mixed with the incoming wastewater before it enters the main aeration tank. These form areas of high substrate concentration (3-12 kgBOD/kgMLSS.d), which permits soluble substrate (>80%), to be adsorbed onto the biomass prior to oxidation. It is this adsorption of the bulk of the substrate under high substrate concentrations which favours the floc forming bacteria. The size of the contact chamber has been estimated at between 0.01 and 0.06 the size of the aeration tank although the precise design criteria had not been developed by the late 1980's (Lee et al. 1982, Ekama and Marais 1986). More recently, Pujol and Canler (1994) recommended sizing of initial contact zones at a hydraulic retention period of ten minutes at peak flow rate plus sludge return flow with an average retention time within the contact zone of about 1/2 hour. These figures are also now considered good design practice in the United states (Hsu and Wilson 1992). Wakefield and Slim (1988) used a selector to successfully restore a badly bulking sludge at a municipal sewage treatment works, while Ruider et al. (1988) successfully used a selector at a plant treating sugar beet wastewater. In a recent

study Rissler (1991) considered that a horizontal flow velocity of 0.25m/s was sufficient to maintain good mixing and prevent settlement in anoxic zones. Hydraulic retention times (HRT's) are generally greater than thirty minutes. The aerobic/redox status of initial contact zones were well defined by Albertson (1991) (see Table 2.8). Albertson (1991) reviewed the evolution of bulking control concepts from when batch and semi-continuous selectors were employed to recent developments in anaerobic, anoxic, oxic and high F:M biological selectors. In addition to this the paper also summarises recent results obtained from 12 United States treatment facilities employing various selectors. From this study Albertson concludes that high to low F:M gradients are the dominant factor in the effectiveness of selectors. Along with this it is recommended that the available oxygen in the initial contact zone is substantially lower than the oxygen demand in the aeration zone to ensure that anaerobic functions occur within the cell mass.

 Table 2.8 Quantification criteria for initial contact zone (ICZ) modified by Albertson

 (1991)

Environment	Criteria
Anaerobic (An)	No added air or oxygen,
	$D.O = 0$ , total influent $NO_X N < 0.5 mg/l$
	Oxidation/reduction potential <-150mv,
Anoxic (Ax)	No added air or oxygen,
	D.O = 0,
	total influent NO <sub>X</sub> N ≥0.5mg/l,
	Oxidation/reduction
	potential > -150 to <+50
Oxic (Ox)	Addition of air or oxygen, D.O.≥1.0mg/l,
	oxidation/reduction potential > +50mv
High F:M Ratio	≥3kgBOD/kgMLSS.d in ICZ
Low D.O. (Al)	≤0.3mg/l D.O. in ICZ
High D.O (AH)	≥2.0mg/l D.O. in ICZ

Originally, initial contact zones or "selectors" as they are more commonly known in Europe, were effectively used in the control of filamentous bulking (Tomlinson and Chambers 1979, Chambers 1981, Hoffman 1987). These were used mainly against oxic zone growers such as *S. natans*, Types 1701 and 021N, but some microorganisms (e.g. *M.parvicella* and *N. limicola*) grow at all redox potentials but have still been controlled effectively by this means. Suppression of filamentous growth is achieved by placing them at a competitive disadvantage to floc-forming organisms, in terms of either oxygen (metabolic) or growth (kinetic). Metabolic de-selection of oxic growers is achieved because they are unable to utilise nitrate bound oxygen (Wanner et.al 1987). Shao and Jenkins (1989) found that two types of filamentous organism, *Thiothrix* sp. and Type 021N exhibited much lower rates of denitrification than *Z. ramigera* a flocculating organism and could only denitrify to nitrite. *Z. ramigera* therefore had a competitive advantage in the anoxic selector.

The difficulty lies in controlling filaments which can obtain oxygen from this source (nitrate), such as *M. parvicella*, which has very similar physiological requirements to that of floc-forming bacteria (Wanner 1991). Restricting the growth of these types of filaments will rely on more subtle principles of kinetic and metabolic microbial selection. These are currently the subject of investigation in design and operating strategies.

## 2.10.6 Biosorption and the prevention of bulking

The excessive growth of filamentous bacteria in a completely mixed system may be prevented by conversion to a process with a high loading during the mixing of wastewater and sludge. To achieve this two options are available :-

a) a "plug-flow" configuration.

b) a separate mixing tank for waste water and returned sludge.

There are no significant loading differences between these two options as the

loading in both is high during the initial mixing of wastewater and recycled sludge. Different theoretical explanations for the prevention of excessive growth of filamentous organisms are postulated:

1) At low substrate concentrations the maximum growth rate of filamentous bacteria would exceed that of floc-forming organisms. At high substrate levels the reverse would be the case (Chudoba et al. 1973a).

2) A separation of exogenous and endogenous phase would favour the growth of floc-forming micro-organisms (Rensink et al. 1977, Houtmeyers 1978)

3) Floc-forming bacteria have a higher substrate removal capacity than filamentous organisms. However, they can only fully utilise this feature at high loading rates (Houtmeyers 1978).

Prevention and control of filamentous bulking by means of a selector can only be effective if a large part of the available substrate is actually taken up by the floc during its residence within the initial mixing zone. Only in this way can the exogenous and endogenous phases be clearly separated. This rapid removal of organic compounds can only take place if the sludge possesses an adequate biosorption capacity.

The notion of biosorption was introduced by Eikelboom in 1982: Immediately after contact between sludge and wastewater there is a direct substrate uptake by the bacterial cells in which these compounds may be stored as reserve material. Biosorption includes the adsorption and absorption phenomena occurring at the interface of the two phases. It is defined by the amount of assimlable organic matter retained by micro-organisms during the short time period following sludge and effluent contact.

Biosorption reflects bacterial behaviour in relation to a given substrate ; it integrates the notions of uptake rate and accumulation capacity which play a major role in biological competition (Chiesa and Irvine 1985). Biosorption is linked with bacterial metabolism, substrate bio-availability, floc loading and contact time (Eikelboom

#### 1982, Pujol 1987, Rensink and Donker 1991).

Parallel studies into micro-organism selection using pre-mix selectors were carried out by Grau et al. (1982) and Tomlinson and Chambers (1984). This work raised another factor that may have an influence on the selection of floc-forming organisms, this factor being the regeneration time of activated sludge. The term regeneration time has been used to designate the period of sludge aeration occurring after the biosorption of exogenous removable substrate. Regeneration is usually carried out in separate tanks known as stabilisation tanks. Many investigators (Rideau et al. 1975, Jenkins and Orhon 1973, Morfax et al. 1975) observed that separate sludge regeneration has improved sludge settlement. This theory of accumulation and regeneration is based on the accumulation, storage and metabolism concept rather than the growth-death concept. Grau et al.(1982) found cell replication took place in the stabilisation tank with only substrate storage (biosorption) taking place in the contact tank. These findings are in full agreement with those of Gujer and Jenkins (1975) who stated "COD removed in the contact basin is not entirely associated with cell growth. The COD removed is associated with the activated sludge in a partially unassimilated form that has a low COD equivalent and only becomes fully assimilated in the stabilisation basin." Grau et al. (1982) concluded that a regeneration period long enough to result in at least 65%oxidation of the total substrate removed in the contact basin result in lower SVI values being obtained. In addition they found that selectors with a "plug-flow" pattern and low dispersion number are more efficient in the suppression of filamentous bulking.

#### 2.10.7 Influence of the Organic Fraction of the Feed

Variations in the relative frequency of occurrence of filamentous types in plants treating domestic waste and those treating industrial effluent were identified by Eikelboom (1977) and Strom and Jenkins (1984). In addition these authors were able to identify filament types that were associated with the treatment of particular industrial effluents. These differences were concluded to be due to variation in the

composition of the organic fraction present in the waste water being treated. The organic fraction of a waste water is often only regarded in terms of oxygen demand even though it may be composed of a variety of different organic compounds. Protein, fat, carbohydrate and other hydrocarbons may be present and serve as substrates for the biomass. Bacteria do not have universal abilities to degrade organic compounds and different species can have a marked differences in their metabolic profiles. The filamentous organism *Microthrix parvicella* for example has a high specific growth requirement for fatty acids and has been shown to grow well in activated sludge systems containing fat (Slijkhuis and Deinema 1983). However the growth of *M. parvicella* on fatty acids is also dependant on dissolved oxygen concentration and has been shown to become the dominant organism in activated sludge when oxygen supply is limited (Slijkhuis and Deinema 1988). These workers suggested that the organism required reduced forms of nitrogen and sulphide which would be oxidised when dissolved oxygen was at high concentrations.

In spite of the above evidence that particular types of substrate can influence the population dynamics of activated sludge systems, few studies have concentrated on the composition of substrate rather than simple strength and loading rate. In studying the influence of substrate feeding pattern on activated sludge systems Verachtert et al. (1980) and Van den Eynde et al. (1982) compared the effects of different substrates. These included, glucose, nutrient broth, acetate, starch and casein. When compared all produced a sludge with better sludge settling characteristics when fed intermittently than when fed continuously. In the continuously starch fed reactor settlement rates deteriorated rapidly, in addition when fed intermittently the initial rate of settlement was good but eventually deteriorated. It was shown that the intermittently fed system showed a higher rate of substrate uptake, but both systems showed similar rates of oxygen uptake. This led Verachtert et.al. (1980) to propose that starch was adsorbed onto the floc surface and was slowly hydrolysed resulting in a slow release of substrate at low concentration and therefore led to conditions favouring the growth of filamentous organisms. Buali and Horan (1989) grew a pure culture of Type 0092 on various

monosaccharide and disaccharide carbon sources and observed changes in morphology depending on the carbon source.

Progress in activated sludge modelling is carried out by introducing new concepts into the existing sludge biokinetic models. Early activated sludge mathematical models based on the single-component substrate approach attempted to incorporate some kinetics of adsorption and storage mechanisms (Marais and Ekama 1976, Ekama and Marais 1977,1979). This theory was developed when the Bi-substrate hypothesis was introduced (Dold 1980). The IAWPRC Task Group on modelling proposed that the organic fraction of a waste water applied to a treatment system can be split into the readily biodegradable (soluble) fraction and the particulate biodegradable fraction. This model was advanced by Dold et al. (1980) who proposed that particulate biodegradable COD is adsorbed onto the heterotrophic biomass. Enzymes, attached to the organisms then hydrolyse this adsorbed COD to a readily biodegradable (soluble) form which can then be directly absorbed by the organisms and then used to synthesise new cell mass. In 1985 this model was further modified by Dold and Marais. This new model described the fate of the particulate biodegradable fraction in the following series of steps:-

a) particulate biodegradable COD is enmeshed in the sludge mass.

b) heterotrophic organisms release hydrolytic enzymes into the bulk liquid that hydrolyse the particulate biodegradable COD to a readily biodegradable soluble form.

c) this adds to the pool of readily biodegradable COD in the bulk liquid.

d) heterotrophic organisms absorb the readily biodegradable COD from the liquid and utilise this to synthesise new cell mass.

The bi-substrate principle divides substrate into two groups, easily metabolisable soluble materials and particulates. The high easily assimlable substrate concentrations following feeding stimulates a high specific growth rate in the floc formers which gives them a competitive advantage. The floc formers also generate sufficient adsorption surface to remove other particulate substrate. Then even when the readily metabolisable substrate has been used there is sufficient flocculated biomass to absorb the substrate released by the hydrolysis of particulate matter. Bulking sludge might still occur however, in a selector system which is fed on wholly particulate substrate. This would result in slow substrate release and no stimulation of high specific growth rates in floc-formers. The overall reaction rate for particulate substrate breakdown is limited by extracellular enzymatic hydrolysis in the multi-step path "adsorption-breakdown-storage-synthesis" which has been modelled as the slowest step. There has been significant further research in this area due to interest in biological nutrient removal systems (Marais et al. 1983, Siebritz et al. 1983, Wentzel et al. 1984). Mechanisms for substrate enmeshment, adsorption and storage have been incorporated into nitrification-denitrification systems (Dold et al. 1991) and into the model for biological phosphorus removal (Wentzel et al. 1992). Wanner and Novak (1990) demonstrated that biological phosphorus removal systems would produce a filamentous bulking sludge when fed on soluble or particulate (egg based) substrate. This bulking was a result of the proliferation of oxic zone growing filamentous organisms (e.g. Sphaerotilus sp. and Type 021N) when the oxic zone was supplied with either soluble substrate generated by fermentation in the proceeding anaerobic zone or with the products of the hydrolysis of enmeshed particulate substrate. Their findings were, however, questioned by Chudoba (1991) who stated that a selector should have at least eight compartments (Wanner and Novak's had three) to prevent bulking, therefore filamentous bulking was due to an insufficient substrate concentration gradient.

Both of the above models when applied to activated sludge give excellent agreement with simulated and observed responses (Dold and Marais 1985). Ekama and Marais (1986) used the Dold and Marais (1985) classification of COD load to develop an alternative selection criteria for low F:M bulking to that of Chudoba et al. (1973a and b). They reported that theoretical simulations using the IAWPRC model and kinetic constants for filaments and floc-formers consistent with Chudoba's selection criteria predicted the eventual dominance of filamentous forms in selector and intermittently fed systems. This prediction was not observed in practice. They therefore proposed that an intermittent (selector or plug-flow) feeding pattern resulted in a sludge that contained floc-formers with higher specific growth rates than filamentous organisms at all concentrations of readily biodegradable substrate.

The role of soluble biodegradable substrate, its importance and appropriate prediction by means of mathematical modelling have further been developed since the first theories on activated sludge bulking (Ekama and Marais 1986). For the purpose of a better estimation of the activated sludge biocenosis composition even two fractions of biodegradable organic substrate were proposed; a readily biodegradable and a rapidly hydrolysable fraction (Sollfrank and Gujer 1991, Henze 1992). Subsequently, the first population dynamics models including floc formers and filamentous organisms have been constructed (Gujer and Kappler 1992).

## 2.11 Controlling Bulking Sludge

There is now a better understanding of the detailed microbiology of the activated sludge process, particularly of the competition between filamentous and flocforming organisms and this has yielded some improvements in design and operation of treatment systems (Albertson 1987). But as discussed in section 2.7, filamentous bulking is still a frequent and serious problem of the activated sludge process. For the operator of an activated sludge system with a bulking problem the immediate problem is how to maintain the operation of the plant and avoid the loss of suspended solids in the final water from the plant. Even if the filamentous organism responsible is known, there is no specific control method for bulking sludges. Therefore, operators must try a series of corrective options, starting by correcting the possible cause as indicated by the filamentous organism present until the problem disappears (Waller and Hurley 1982).

The most cited causes of filamentous bulking are low dissolved oxygen concentration, low organic loading (F:M), septic influent, nutrient deficiency and low pH and these are therefore usually the first parameters which are investigated.

Tomlinson and Chambers (1984) proposed the use of an "action flow sheet" to not only help diagnose the cause of bulking but to remedy the problem. However, this approach in practice is extremely time consuming, as various options are investigated while all the time solids are lost from the system and are impractical in an emergency. A number of quick remedial actions are also available to the plant operator to provide a rapid improvement in settlement and avoid solids contamination of the final effluent. These methods, detailed below, involve the use of chemicals, either biocides such as chlorine or hydrogen peroxide or coagulants (or flocculants). This approach is usually expensive but often unavoidable. This may also require capital expenditure for the installation of storage, mixing and dosing equipment. They are therefore not appropriate solutions to long term persistent filamentous bulking problems. In these cases the actual design of the plant must be carefully be re-evaluated by something like the Tomlinson and Chambers method.

### 2.11.1 Treatment With chemicals

Chemicals used in the control of activated sludge bulking can be categorised by their mode of action within the process. The modes of action being toxic (biocides) or coagulants (flocculants).

a) Biocides - Surprisingly this approach does have a record of success particularly in the United States. The assumption is that chemical agents are selectively toxic to the filamentous organisms. This selectivity comes from the fact that filaments associated with bulking, grow out from the floc into the surrounding liquid. The filaments are therefore more exposed than the floc-forming organisms to any biocides added to the mixed liquor. The objective is to selectively kill the filaments present while having a minimum toxic effect on the floc-forming organisms.

An alternative explanation not previously discussed in the literature is that the metabolically active population in most activated sludge is so small (< 10%) that the biocides can only have a marginal effect in the short term.

Two biocides are in general use. Chlorine and chlorine based compounds such as

sodium hypochlorite which are very toxic to micro-organisms at low concentrations. Hydrogen peroxide and ozone are also very reactive and biocidic and also add oxygen to the mixed liquor as they decompose. Much work has been done using chlorine (Tapelshay 1945, Pipes 1974, Jenkins et al. 1982, 1983, 1984, Lackay 1988, Wakefield and Slim 1988). Jenkins et al. (1984) sited a case where RAS chlorination had been effective as a method of bulking control in a nitrifying activated sludge system. Dose rates of 2-4g Cl<sub>2</sub> per kg VSS.d<sup>-1</sup> were successful in combating Type 0041 and Type 1701. In addition Jenkins et al. also claimed that nitrification efficiency remained unaffected during the period of chlorine dosing. A number of studies have also been published on the use of hydrogen peroxide (Cole et al. 1973, Keller and Cole 1973, Strunk and Shapiro 1976), although the use of chlorine appears to be more widespread, Leeuwen and Pretorius (1988) have successfully used ozone to control bulking at a continuous dose rate of 4g per kg sludge in the aerobic zone of a three stage activated sludge process. Such control measures, however, can only be temporary, since continuous dosing is reported to eventually lead to acclimatisation. The cost of such chemical treatment is reviewed by Chambers and Tomlinson (1982) who provide nomographs for estimating probable costs of dosing various chemicals at a range of dosing rates at plants of different sizes.

### **B)** Flocculation

An alternative chemical approach is to dose an agent that will promote flocculation, increase floc strength and improve settleability. Inorganic coagulants, such as lime, aluminium sulphate as well as polyelectrolytes have often been used to improve settlement (Carter and McKinney 1973, Rensink et.al. 1979, Thomanetz and Bardtke 1977, Demel and Mobius 1988). Metal salts, which can be dosed directly into the aeration basin exert their metabolic effect on micro-organisms in the sludge at low concentrations. A major problem with the use of metal salts is the significant increase in sludge production and possible sludge de-watering problems.

Polyelectrolytes are flocculating agents which have be dosed into the overflow from

the aeration tank as it passes to the final settling tanks. As with metal salts, dosing level should commence at a low concentration and if no improvement in sludge settleability is observed then the dosage can be increased. If there is still no improvement then an alternative polyelectrolyte should be used.

## 2.11.2 Biological Methods

Over the past ten years several companies have commercialised special innoculums or supplementation products that are designed to improve the performance of biological waste water treatment processes. Sometimes called "bioaugmentation" or "bacterial augmentation", these products are essentially a lyophilised active culture of bacteria, possibly with some supporting nutrients. They can also contain some quantities of extracellular enzymes. Bacterial innoculum products can be used in a number of situations apart from bulking (Stephenson and Gerrard 1990). These include :-

- 1) aiding rapid start up of a treatment plant.
- 2) improving COD and BOD removal.
- 3) removal of odours.
- 4) eliminating foaming and scum formation.
- 5) achieving efficient nitrification.
- 6) improving xenobiotic removal.

All of the above are applicable to activated sludge. Articles written by employees of the manufacturers and distributors have appeared in trade journals describing the effectiveness of commercially available bacterial innocula (Saunders 1985, Jones 1988). Independent reports from researchers investigating such products have been rare. Qasim and Stinehelfer (1982) studied the effect of a commercial bacterial innoculum product on the COD removal of laboratory scale continuous flow activated sludge units. It was concluded that the product would have little or no effect on the performance of a well designed and operated activated sludge plant but may be of use in an overloaded plant. An innoculum designed to correct bulking had no effect on the main cause of poorly settling activated sludge due to the growth of filamentous bacteria (Hirt et al. 1980). However, not all studies have indicated that innoculum products have no effect. Some products have been found to be successful in biodegrading chlorinated hydrocarbons (Ying et al. 1986). Successful applications of bioaugmentation in the control of filamentous bulking have also been reported (McDowell and Zitrides 1979, Chambers 1981). However, the reproducible effectiveness of bioaugmentation has still to be proved.

Reports on microbially derived substances that cause filament cells to lyse, have also become more prevalent recently. These substances can be added directly to the activated sludge system or the micro-organisms which produce them added or incorporated into the biomass (Yaguchi et al. 1991). The most effective organism the Yaguchi et.al. were able to isolate was a soil isolate identified as *Xanthamonas maltophilia* which produced lysis activity against Type 021N.

Protozoa form an integral part of the biomass in activated sludge systems. In addition they are often used as indicators of the operational performance of activated sludge and there have been many attempts to relate the physio-chemical parameters of effluent or activated sludge biomass with species of ciliated protozoans present (Curds 1973, Al-Shahwani and Horan 1991, Esteban 1991). It has been observed that two species of ciliated protozoa, *Trithigmostoma cucullus* and *Trochilioides recta* predate then filamentous bacterium Type 021N (Inamori et al. 1991). These workers suggested that it might be possible to cultivate these organisms and the add them to activated sludge systems to control bulking. However, it does not appear that this work has ever been followed up.

#### 2.12 Industrial Waste Water Treatment

The wide range of industrial manufacturing processes generate an equally diverse range of waste waters. Industrial waste waters tend to be characterised by great variability in flow and composition. Thus the industrial waste water treatment plant must be able to accommodate a range of flow and load variations far greater than those encountered at a domestic treatment. Equalisation is therefore required to dampen these fluctuations and maintain a stable operation of the treatment process and hence as a general rule, the equalisation basin (balance tank) should be designed to provide an effluent load peaking factor of 1.2 (Eckenfelder and Musterman 1995).

The capital costs of water treatment plant does not improve manufacturing efficiency and the plant incurs additional operating, maintenance and running costs. Therefore, while it is easy to advocate stringent water pollution control measures, these represent an economic disadvantage in many cases. Until recently, industrial waste water treatment plants were installed on the basis of lowest capital cost and were then expected to operated with the minimum attention from unskilled or untrained operators. Such an approach, when combined with the erratic and difficult nature of many industrial waste waters, has led to poor performance from many plants.

Another influence upon industry's attitude to pollution control is the potential volatility of any manufacturing activity. While municipal engineering tends to anticipate many decades as the potential life of a given facility, manufacturing can change very rapidly as new products are developed or science and technology offer a novel approach to a product. Pollution control equipment must either be sufficiently flexible to accommodate these changes or must be expandable. Thus while the majority of municipal waste water treatment plants are built as massive structures of in-ground reinforced concrete, industrial plants tend to be of a less permanent nature and constructed, often above ground, of steel or glass fibre.

It is only by adopting an integrated approach to combine the manufacturing and pollution control operations that the most cost effective form of effluent treatment can be selected. In this way the requirements for effluent treatment can be constructive, in that they encourage good house keeping within the production area, provide an impetus to recycle and recover materials and minimise the environmental impact of the whole operation.

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#### 2.12.1 The Treatment of Waste Waters from the Vegetable Processing Industry

Pollution control is becoming an increasing problem for both fruit and vegetable processing plants. The reasons for this growing problem vary, depending on the circumstances of the individual processor. Most often however these industries are rural and suitably sized municipal waste water treatment works are not available. Recently the effluent quality requirements from industrial treatment plants have changed increasing the need for better waste water treatment and led to many vegetable processors re-evaluating their existing treatment systems. For processors constructing new plant, there is an increased awareness of the impact of waste water on the overall cost of processing food. Although modifications to reduce product loss, such as changes in peeling systems, new processing methods, water conservation and reuse systems have all combined to change waste characteristics. These changes in waste water characteristics have sometimes reduced the efficiency of previously effective waste treatment hardware. In view of these technological changes and the implications of the increased cost and environmental requirements, there is ample reason to examine the technologies currently in use in the industry.

#### 2.11.2 Waste Characteristics

The composition and source of waste water from the vegetable processing industry is largely determined by the industrial end product and by the manufacturing process used. Major waste loads are usually generated by washing, peeling, blanching, cooling and general plant hygiene. Although manufacturing and product differences can cause considerable variation in waste characteristics, all food processing plants share common characteristics and associated waste water treatment problems.

In general, waste waters from the processing of vegetables contain compounds that are mainly organic, primarily soluble and highly reactive when contacted with bacteria. Waste from vegetable processing are amenable to biological treatment by conventional methods used for treating domestic waste water. However, a combination of factors unique to vegetable wastes have resulted in major problems in their treatment. Such factors include the rapid demand for oxygen necessary for aerobic treatment, seasonal load variations, high variations in waste strength and volume, low pH, lack of nutrients and changes in waste characteristics associated with product changes. Major pollutants of concern include biochemical oxygen demand (BOD), total suspended solids, settleable solids, waste water pH and nitrogen.

Many fruits and vegetables have a significant proportion of fermentable sugars in their make up. This may not only cause problems with rapid oxygen demand in secondary biological treatment but also may cause pH problems due to anaerobic fermentation of these sugars in primary settlement tanks and the formation of organic acids which may greatly lower the pH of the waste water. A large percentage of the dry solids within the vegetables are made up of starch and dextrin's. This may produce settlement problems within an activated sludge plant due to the starch being a slowly biodegradable substrate resulting in a slow release of readily assimilated substrate giving filamentous organisms a selective advantage (Dold and Marais 1986).

Only generalisations can be made concerning food processing wastes, due to the following factors causing variation in waste strength:-1)the commodity being produced, 2) the product mix, 3) storage requirements for materials, 4) the field conditions at time of harvest, 5) the method of harvest and transport, 6) the fruit and vegetable maturity. However, the EPA (1977) lists pollutant loads commonly found, on the basis of quantity of raw product used by the typical fruit and vegetable processor. These are listed in Table 2.9

	Flow (litres/tonne	BOD	TSS
Crop	Raw Product)	(kg/Tonne Raw	(kg/Tonne Raw
		Product)	Product)
Brocoli	38	10	8.5
Brussel Sprouts	34	3.8	7.5
Carrots	14	15	8.5
Cauliflower	71	8	3
Peas	23	19	5.5
Potatoes	1.5	42	64

 Table 2.9 Average Waste Characteristics of Vegetables (EPA 1977)

It can be seen from Table 2.9 that most waste waters from the processing of vegetables have a considerably higher BOD per unit volume than domestic sewage and other waste waters. Underestimation of the high rate of BOD oxidation has resulted in problems because aeration systems were undersized for peak oxygen demands, with conditions of low dissolved oxygen concentration leading to the growth and proliferation of filamentous organisms. Due to the high loads produced during the processing of fruit and vegetables pure oxygen plants which can support higher biomass concentrations per unit volume of aeration tank, may be beneficial in reducing the size of aeration basin needed to treat these highly concentrated wastes. It has also been reported that a pure Vitox activated sludge plant proved very versatile in the treatment of a highly variable vegetable waste water (Gostick et al. 1990).

The nitrogen content of fruit and vegetables occurs in various forms; however the majority is usually present as complex organic nitrogen. Unless these wastes undergo anaerobic digestion to break or hydrolyse this complex organic nitrogen, most such wastes are deficient in the ammonia nitrogen necessary for biological treatment. This lack of available nutrient and especially nitrogen has historically been a problem in the treatment of fruit and vegetable processing wastewaters.

## 2.13 Summary

The activated sludge process is a suspended growth system comprising of a mixed culture of micro-organisms constantly supplied with organic matter and oxygen. There is a continuous interaction between the different species present and between the biomass and the engineered conditions created by the plant. These interactions are critical to the operation of the system and many of the problems associated with the activated sludge process are due to these interactions and their effect on the microbiology of the system. The major source of variability in effluent quality produced by an activated sludge plant is due to the loss of settleability of the biological solids leading to loss of these solids from the system. The settling ability is a direct result of the ability of the micro-organisms and colloidal matter which comprise the reactor mixed liquor to agglomerate into large flocs with high zone settling velocity. This agglomeration process known as flocculation is essential if efficient waste water treatment is to be achieved. The loss of sludge settleability and associated problems (e.g. solids loss, dilute returns and hydraulic overloading of solids handling processes) is commonly termed bulking.

There is no indication in the literature that suggests problems with sludge settleability were experienced when the first experiments using activated sludge were performed at the start of this century. However, by the early 1920's a number of problems had been reported and from that time onwards it has become apparent that these problems can be serious and are widespread. Since then our understanding of the process through operational experience and experimentation has improved. However, the problem still exists and bulking is reported in 25% of activated sludge plants in France.

Two mechanisms have been proposed to explain sludge bulking; one suggests that bulking results from increased floc surface charge brought about by changes in floc surface chemistry. The other proposes that bulking is caused by the dominance of the activated sludge microbial flora by filamentous species of bacteria. The relationship between substrate, species, floc surface and settlement is described by

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the S-hypothesis which attempts to simplify the complex interactions occurring in activated sludge.

The observation that the onset of bulking is almost inevitably accompanied by an increase in the number of filamentous organisms within the sludge has led to more recent work concentrating on the specific problem of filamentous bulking. The outgrowth of excessive quantities of filaments from the floc is correlated with bulking, whereas the absence of filaments from the floc leads to the production of small, weak flocs which also settle poorly. An ideal sludge is found when there is a balance between filamentous and floc forming organisms.

Traditionally filamentous bulking has been attributed to an over growth of the organism *Sphaerotilus natans*, however since the publication by Eikelboom in the mid 1970's of a key for the identification of filamentous organisms within activated sludge more attention has been paid to other genera involved. Many studies have now been carried out to find the most commonly occurring filamentous organisms in activated sludge and associated them with various operating conditions. From this work a number of conditions that promote the growth of filamentous organism have been identified:

- 1. Low bulk dissolved oxygen concentration in the aeration basin.
- 2. Low substrate concentration (F:M) resulting in low growth rates and intense competition for available substrate.
- 3. Deficiency of nutrients essential for bacterial growth.
- 4. Septic incoming waste water containing reduced sulphur and nitrogen compounds.
- 5. Low pH promoting the growth of filamentous fungal species. Of the conditions above low F:M and low dissolved oxygen are the major causes of bulking.

As previously mentioned low dissolved oxygen concentration has been shown to lead to the proliferation of filamentous organisms. This is because some filamentous organisms show a great affinity to dissolved oxygen at low concentrations because of low values of half saturation constant  $K_{DO}$ . The boundary between "bulking" and "non bulking" D.O. concentrations is not fixed because this value depends on the actual value of activated sludge loading. Due to this a bulk dissolved oxygen of above 2.0mg/l is generally recommended. The relationship between dissolved oxygen concentration and bulking is further complicated by the use of anoxic and anaerobic selectors for control of settlement and in biological nutrient removal plants. There has also been some debate as to whether high dissolved oxygen concentrations or the use of pure oxygen can improve settlement with there being evidence to both support and repute the argument.

It is largely recognised now that mixing patterns and oxidation gradients can be linked to sludge settlement, with bulking being less prevalent in systems employing a concentration by intermittent gradient either feeding, plug-flow or compartmentalisation. Such aeration systems characterised by a low degree of axial mixing result in higher substrate concentration gradient promoting the growth of flocculant species. This is explained by supposing a different kinetic behaviour of filamentous and floc forming bacteria. It is proposed that the two organisms exhibit cross over Monod kinetics with respect to a mutually limiting substrate. At low substrate concentrations (e.g. fully mixed systems) filamentous organisms have a higher specific growth rate than floc forming organisms. While at high substrate concentrations floc forming organisms have a higher specific growth rate so dominate the resultant sludge population. Due to this, introducing an area of high substrate concentration at the front end of an aeration lane and hence a substrate gradient over the length of the aeration tank has been advanced as a general remedy for avoiding the proliferation of filamentous organisms.

This intermittent exposure to high substrate concentrations stimulates higher rates of substrate uptake in the biomass. Floc formers have been identified as having a greater capacity to accumulate food reserve in the form of internal storage products while filamentous organisms accumulate substrate at a much slower rate. By presuming that different organisms posses different growth constants and,

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consequently different specific growth rates in relation to substrate concentration it may be possible to control filamentous bulking by means of a selector. A selector was originally defined as the initial part of a biological reactor, characterised by a low value of dispersion number and by an adequate substrate concentration gradient. This term selector is now sometimes also used to describe "initial contact zones" and "premixing zones". A substrate gradient whether produced by plug-flow design or by the installation of a selector creates conditions for so called unbalanced growth when the phases of substrate (both soluble and particulate) and nutrient uptake and the phase of cell growth are partially or fully separated. Again, the conditions of unbalanced growth are suitable more for floc formers than for filamentous organisms. The concentration gradient therefore leads to a combined effect of kinetic and metabolic selection.

Waste water is the source of substrates, nutrients and micronutrients for the microbial biomass of activated sludge. Because of this the waste water make up must influence the microbial selection processes in activated sludge systems. The distinction between readily biodegradable soluble substrate and the more slowly biodegradable particulate substrate was introduced in the IAWPRC model of activated sludge. This distinction is important in understanding how substrates react with the flocs and has implications on the use of concentration gradients in the control of bulking.

Readily biodegradable substrates can be directly used by the bacterial cells and are normally low molecular weight compounds such as monosaccharides, alcohols, volatile fatty acids and amino acids. The molecules represent approximately 10-20% of COD in common municipal waste waters but can be significantly higher in industrial wastes from the food industry. While particulate slowly biodegradable substrate forms the majority of substrate in domestic waste waters. This substrate whether in colloidal form or as true suspended solids require enzymatic hydrolysis and breakdown prior to becoming available for assimilation by the biomass. Hydrolysis is however, a surface phenomenon and is connected with the flocs. Therefore, if the main aeration tank is plug flow these substrates released by hydrolysis are available more to the floc formers than filamentous organisms.

The above distinction between readily available substrate and slowly degradable particulate substrate is important in the understanding of how substrate reacts with the biomass and this may have major implications on the use of concentration gradients in the control of bulking. In addition this categorisation may also lead to the explanation of frequent cases of bulking in biological phosphorus removal plants. The control basis for bulking sludge has always been based around the concept of competition between filamentous and floc forming organisms. However, it is now being questioned by some workers whether cross over Monod kinetics adequately explains the predominance of one or other group with doubts being raised as to whether competition can be considered to be between only two uniform groups of filamentous and non filamentous organisms.

Over the past twenty years numerous studies have concluded that filamentous bulking is a serious and widespread problem of the activated sludge process. Despite considerable advances in the understanding of the process and the phenomenon of bulking, recent surveys have still shown little improvement with the situation in France being reported by Pujol and Canler (1989) that 25% of plants surveyed reported settlement problems. This led Albertson (1991) to conclude "In spite of all we know and understand some sludges will still bulk" and this is why filamentous bulking remains a topic worthy of further research.

#### **CHAPTER THREE : OBJECTIVES OF THIS STUDY**

3.1 To investigate the effect of substrate composition on the settlement of activated sludge at both laboratory and full scale.

3.1.1 Different substrates vary with respect to molecular weight and hence solubility. As complexity of the substrate molecule increases so do the number of steps required to breakdown the molecule to a form available for assimilation by the biomass. It is only recently that further categorisation of substrate has been introduced to the activated sludge process through the IAWPRC Model. This model categorises substrate as either easily metabolisable (soluble) and slowly biodegradable (particulate). The range of organic substrates present in waste waters is enormous and the balance between readily metabolisable and particulate substrate may therefore influence microbial selection and hence affect sludge settleability

3.1.2 The creation of substrate gradients within or outside the main aeration vessel has become common practise in the control of filamentous bulking. This however, may not be a universal solution to all bulking problems and the effect of substrate availability may play a major role in either the success or failure of such systems.

3.1.3 With legislation becoming ever tighter activated sludge plants can now be required to perform biological nutrient removal. The removal of nitrogen or phosphorus requires the biomass to experience alternating anoxic, aerobic and or anaerobic conditions. Under conditions of low or zero dissolved oxygen concentration (D.O.) the measurement of D.O. becomes unreliable. For this reason an alternative monitoring or control method is required. Oxidation Reduction Potential (ORP) is just such a tool and should be assessed as a possible parameter for process control.

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3.1.4 Control of filamentous bulking is not only associated with kinetic selection (substrate uptake) of floc forming organisms. Metabolic selection must also have an influence on the biomass present as it passes through various different environmental conditions during the course of the treatment process.

3.2 To investigate the performance of a pure oxygen (VITOX) plant used for the treatment of waste water arising from vegetable processing and relate any laboratory scale findings to the full scale plant which may lead to improved efficiency and performance.

3.2.1 It has previously been claimed that one of the benefits of the use of pure oxygen as a method of aerating activated sludge is improved sludge settlement. The relationship between dissolved oxygen concentration and filamentous bulking is complex and has been related to such parameters as organic loading and substrate availability. Due to the higher rates of oxygen transfer made possible by the use of pure oxygen this system may behave differently.

3.2.2 Mixing patterns and oxygen transfer efficiencies have been associated with filamentous bulking. Both these parameters can be assessed through the study of the full scale plant and related to the findings of the laboratory scale experimentation.

3.2.3 Oxygen uptake rates can be used as an indicator of substrate removal with increased substrate removal rates under conditions of high substrate concentration favouring the selection of floc forming organisms. This parameter can be measured in situ in the Vitox plant and can be related to similar measurements made on the laboratory scale apparatus.

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#### CHAPTER 4 : EQUIPMENT, MATERIALS AND METHODS

The laboratory scale experimentation reported here was conducted within the Department of Civil Engineering, Loughborough University of Technology, with full scale investigations being carried out on a pure oxygen activated sludge plant (VITOX) at Salvesen Food Services Ltd., Bourne, Lincolnshire. This plant was extensively studied over four pea processing seasons.

### 4.1 Full Scale Pure Oxygen Activated Sludge Reactor.

The use of pure oxygen is a radical alternative to conventional methods of aeration in the activated sludge process. Advantages claimed for pure oxygen systems are: that higher biomass concentrations can be supported and hence a greater organic load (BOD) per unit volume can be treated. This is due to better oxygen transfer efficiency which may also prevent low dissolved oxygen conditions and hence afford protection against bulking. However, pure oxygen cannot be used within a conventional aeration system as much of the oxygen will be lost from the mixed liquor without dissolving, in addition the volume of gas is inadequate to mix the reactor and keep the biomass in suspension. The British Oxygen Company Vitox System was developed to solve both these problems but also with an aim of intensifying the activated sludge process so that space and capital expenditure requirements are reduced without significantly raising costs of operation (Gould and Stringer 1986). The system also has advantages for research purposes as it allows a precise measurement of oxygen supplied at a given organic loading. In addition it allows the respiration rate of the biomass to be determined *in situ*.

The pure oxygen (Vitox) activated sludge plant at Salvesen Food Services Ltd, Bourne was constructed in 1986 to cope with the increasing loads produced at the factory. The plant receives effluent over most of the year (10 months) but the main organic load to the plant is produced over the six to eight week pea harvesting season. The effluent arises from the washing and blanching of vegetables for freezing. In addition water from defrosting freezers and general hygiene wash waters around the factory are included in the effluent to be treated.

The effluents from various parts of the factory drains to a single sump from which it is pumped to the treatment plant. Initially the effluent is screened through 0.25 mm parabolic screens before the flow is split between two parallel balancing tanks and primary settlement tanks (see Figure 4.1). One stream is then passed through a high rate biotower system (this can be by-passed) and onto the activated sludge system, while the other stream by-passes the biotower and is pumped directly from the primary settlement tank to the activated sludge reactor.

The activated sludge tank is an enamelled pre-fabricated steel tank with a depth of 5m and a capacity of 3136 m<sup>3</sup> (the capacity was reduced to 2863 m<sup>3</sup> prior to the 1994 season). Activated sludge is separated from the final water in two parallel flat bottomed clarifiers each 16m in diameter and 2.5m deep, with a single parabolic scraper rotating at two revolutions per hour (Inka Systems, Glasgow). Oxygen is supplied to the aeration vessel by three British Oxygen Company (BOC) Vitox Units (2 x 75 kW, plus 1 x 55 kW originally designed as duty/duty and assist). These work by drawing mixed liquor from the aeration tank and forcing it through a venturi. Pure oxygen is introduced into the throat of the venturi and the aerated mixed liquor returned to the aeration vessel by means of a sparge nozzle system which serves to mix the contents of the tank. The dissolved oxygen concentration was maintained between set points by a programmable logic controller which operates solenoid valves altering the rate of oxygen injection. The dissolved oxygen concentration is measured by three D.O. probes (pHOX Instruments) positioned around the perimeter of the aeration tank. A continuous chart record of dissolved oxygen concentration was also produced. Measurement of pH is carried out by a single pH probe (pHOX Instruments). Previous works (Gostick 1991) have shown that carbon dioxide stripping is necessary to control pH, this is carried out by a submerged drilled-frame. This operates by pumping air through the mixed liquor

and hence stripping out any dissolved carbon dioxide present. The liquid flow is metered at three points through the plant (ABB Kent Taylor):

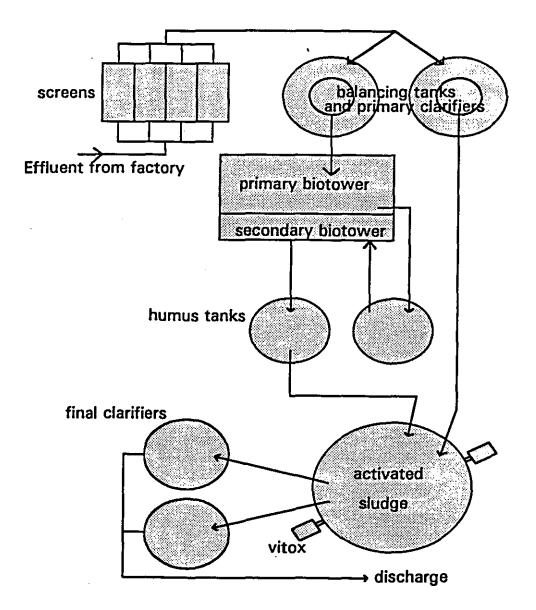
- 1. Inlet flow (Flow from factory to the screens)
- 2. No. 1 Feed (Flow to activated sludge exiting biotower)
- 3. No. 2 Feed (Flow to activated sludge exiting primary settlement tank two)

Daily flows were recorded and together with analytical results used to calculate loading rates. Flow metering on the return activated sludge became available prior to the 1994 pea season.

The oxygen activated sludge plant was monitored over the 1991, 1992; 1993 and 1994 pea harvesting seasons. Prior to the beginning of the 1991 season, the performance of the effluent plant over the two previous seasons was analysed. From this data it was decided that due to the excellent performance of the plant in 1990, to run the plant without the biotower and use the activated sludge alone to cope with the hydraulic and organic loads produced at the factory.

Running the plant by-passing the biotower in 1990 proved very successful in terms of sludge settlement. This was mainly due to the resultant increase in F:M from 0.2 - 0.47 kgCOD/kg MLSS.d. This increase in F:M ratio was thought to have prevented the formation of a poorly settling bulking sludge arising from excessive growth of filamentous bacteria (Strom and Jenkins 1984) by increasing the growth rate of the flocculant bacteria.

Figure 4.1 Schematic Diagram of the Full scale Plant at Salvesen Food Services Bourne, Lincs.



## 4.1.1 Tracer Study

Attempts were made to classify the mixing characteristics of the activated sludge plant using an inert tracer technique. Although reactors are often designated "plug flow" or "completely mixed", such standard flow characteristics are rarely experienced in practice. In reality flow patterns fall somewhere between the two ideals. Analysis of the flow or mixing characteristics of the activated sludge plant can be useful in terms of economy and process efficiency.

The hydraulic profile of the tank was established using Manganese sulphate (Mn  $SO_4.H_2O$ ) as an inert tracer material. Manganese was chosen as a tracer on the following criteria :-

- 1) not normally found in activated sludge.
- 2) not detrimental to the environment.
- 3) easy to analyse.
- 4) not too expensive.

The more traditional Lithium, Rhodamine or Flourescene tracers would have been more costly particularly for regular assessment of mixing characteristics. In addition, with respect to the two coloured tracers mentioned above the fact that the treatment plant makes a major contribution to the flows within the receiving water course (especially during summer months) it was decided that any addition of coloured species would have an undesirable effect on the water course. Preliminary tests were made within the laboratory too characterise the behaviour of manganese as a tracer in activeated sludge. Lumley and Horkeby (1989) also report on the use of Manganese as a tracer to measure hydraulic retention time of the liquid phase in settlement tanks. These workers measured retention time using both Lithium and Manganese in parallel which both gave identical effluent distributions.

The knowledge of the real flow pattern and the active volume of reactors in biological wastewater treatment processes, can be of significant importance. Both the flow pattern and the active volume can be determined by using stimulus - response techniques for the analysis of Residence Time Distribution curves (RTD). Interpretation of RTD curves is performed by fitting them to some theoretical models. The complexity and number of variables for each model is related to the level of accuracy to which the engineer wants to reproduce the process. The use of models with many parameters to design chemical and biological reactors is risky

because of the lack of means with which to evaluate them accurately. Therefore the use of ideal flow patterns has become common practice.

Models of varying complexity have been used to assess mixing coefficients within waste water treatment systems. However, three commonly used models which predict arbitrary flow performance are :-

- 1) the axial dispersion model
- 2) the N tanks in series model
- 3) the Cholette Cloutier model

For the axial dispersion model, a degree of backmixing is superimposed on the plugflow regime. The N-tanks in series model predicts the concentration of tracer for a series of equally sized and completely mixed reactors. The model developed by Cholette and Cloutier (1959) assumes a portion of the total flow into the reactor short circuits to the outlet. Each of the above models since its introduction have under gone various developments by various authors in attempts to gain a better prediction of flow characteristics.

The dispersion model has been used by Murphy and Boyko (1970) to investigate mixing in spiral flow aeration tanks, by Thirumurthi (1969) to establish the effects of geometric design on hydraulic efficiency of sedimentation tanks, and Tomlinson and Chambers (1979) to relate the settling properties of mixed liquor to the degree of mixing within the aeration vessel. Murphy and Wilson (1974) studied mixing in aerated lagoons by applying both the dipersion and N-tanks in series models. While the Colette - Cloutier model was used by Monteith and Stephenson (1981) to estimate mixing and short circuiting in full scale anaerobic digester and by Hall (1982) to estimate biomass accumulation in pilot scale anaerobic filters. Due to it common use for the analysis of flow petterns within wastewater treatment plants and the availability of a computer progreamme for analysing results of exit curve tracer distributions, it was decided to use the axial dispersion model in this study.

A pulse dose of the tracer was dissolved up in the tank liquor and rapidly pumped into the aeration tank. The weight of Manganese sulphate added was enough to give a concentration of 5 mg/l of manganese for the whole working volume. Samples were collected at the aeration tank outlet every 30 minutes for four hours and every hour for the next thirty hours, with a similar sampling regime being employed on the RAS and influent flow mixed stream over the same 30 hour period. Manganese concentrations were measured on an atomic absorption spectrometer after filtration and the resultant data analysed by the residence time distribution (RTD) technique.

The concept of residence time distribution, which is a statistical approach to fluid flow, was used as a simple dispersion model to analyse the tracer results (Levenspiel 1972). Using this model, for a closed vessel, the relationship between the dispersion number (D/µl) and variance ( $\delta^2$ ) of a theoretical "C" curve is given as-:

$$\delta^2_{\varrho} = 2 (D/\mu l) - 2(D/\mu l)^2 [1 - e^{-\mu l/D}]$$

In practice the distribution curve is recorded as a series of discrete values of concentration and time, a good approximation is given by the expression:

$$\delta^2 = (\sum ti^2 Ci / \sum Ci) - t^2$$

and converting to dimensionless values

$$\delta^2 = \delta^2 / t^2$$

where

$$\mathbf{t}^2 = [\sum \mathbf{ti} \mathbf{Ci} / \sum \mathbf{Ci}]^2$$

Here t is the mean residence time (h): ti is the time (h) and Ci is the tracer concentration in the effluent at time i (mg/l).

The dimensionless group  $(D/\mu I)$ , is called the vessel dispersion number and is the parameter which gives a direct estimate of the axial dispersion. Thus:

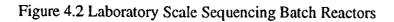
when  $D/\mu l \rightarrow 0$  negligible dispersion (plug-flow) is indicated.

when  $D/\mu l \rightarrow \infty$  large dispersion (complete mixing) is indicated.

## 4.2 Laboratory Scale Reactors

### 4.2.1 Sequencing Batch Reactors

The sequencing batch reactors (SBR's) were manufactured to a laboratory design by Island Engineering Ltd. Hitchin (Figure 4.2 and Plate 4.1). The vessels were made from perspex tubing with a capacity of thirty litres. The reactor operates on a fill and draw principle controlled by timers and solenoid valves. It simulates a plugflow reactor by creating a temporal rather than a spatial substrate concentration gradient. The reactor was fed by a peristaltic pump (Watson Marlow) and to maintain a good substrate concentration gradient, the fill period was rapid (5 minutes). This fill period was followed by a period of aeration and mixing after which the aeration and mixing were stopped and the sludge solids allowed to settle in the main aeration vessel. Following this period a solenoid valve opens allowing the supernatant (final effluent) to flow to a collection vessel. Activated sludge was wasted on a daily basis via a second solenoid valve during the aeration cycle which drains a portion of the mixed liquor to another collection vessel. The amount of sludge wasted was controlled by altering the volume of feed added to the reactor during each feed cycle. Air flow to the reactor was controlled via a needle valve in the air supply line, with air flow to each reactor being monitored via a rotamator in the supply to each vessel. Aeration was monitored regularly during all experimental periods and dissolved oxygen concentrations maintained at or slightly above the minimum level required for nitrification (2.0mg/l) within the mixed liquor. Settlement in the aeration vessel eliminates the need for a separate settlement vessel and sludge return system which are difficult to operate at laboratory scale and in addition allows a more accurate determination of the total amount of solids in the system. The SVI was measured in situ and samples of mixed liquor could be removed from the reactor during the aeration period without effecting the process operation or settlement. The pH of the mixed liquor was measured daily and adjusted to pH 7.0 if necessary by the addition of small amount of Sodium bicarbonate.



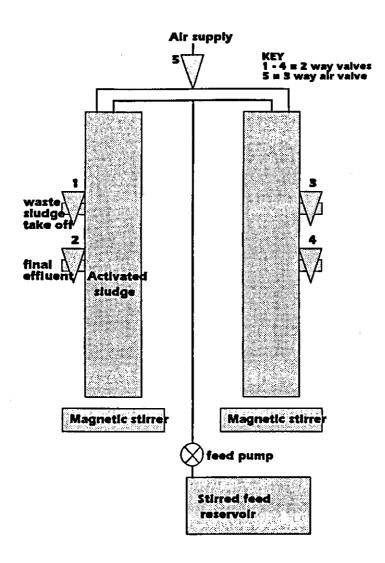
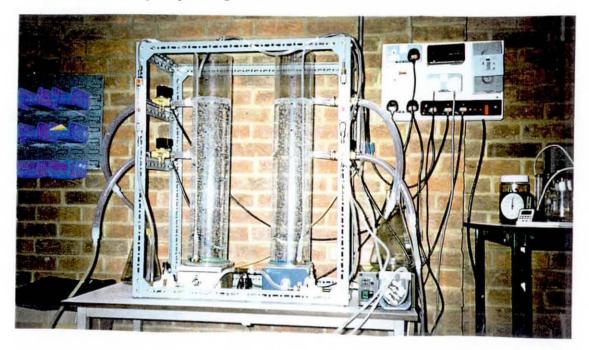


Plate 4.1 Laboratory Sequencing Batch Reactors



4.2.2 Fully Mixed Reactor

The fully mixed reactor was constructed by technical staff within the department of Civil Engineering. The aeration tank of this reactor has a working volume of 3.56 litre (diameter 8.5 cm, height 12.5 cm). The reactor was aerated by a surface aerator (diameter 1.5 cm) powered by a variable speed electric stirrer motor (Citenco) and was fed continuously by a peristaltic pump (Watson Marlow). Dissolved oxygen concentrations within the mixed liquor were controlled by altering the level of imersion of the surface aerator and altering the speed of rotation via the motor. As with the sequancing batch reactor dissolved oxygen concnetrations were monitored regularly and D.O. levels maintained at or above the minimum level required for nitrification (2.0 mg/l). The mixed liquor flows into a 2 litre settlement vessel from which activated sludge is returned to the aeration vessel via a paddle linking the settlement tank and the aeration vessel (see Figure 4.3). The return paddle (diameter 15cm) was powered by a separate variable speed electric stirrer (Citenco) allowing an appropriate return rate of activated sludge to the aeration vessel to be set. Final effluent overflows the settlement tank to a collection vessel. Activated sludge was wasted manually on a daily basis to ensure steady operating conditions.

Figure 4.3 Laboratory Scale Fully Mixed Reactor (Sectional View)

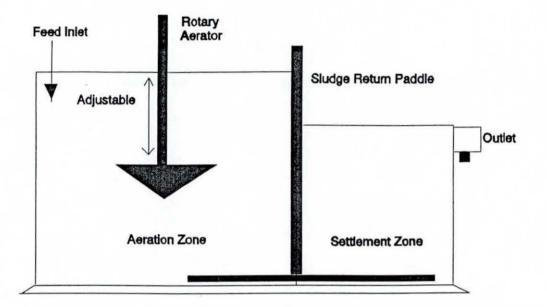
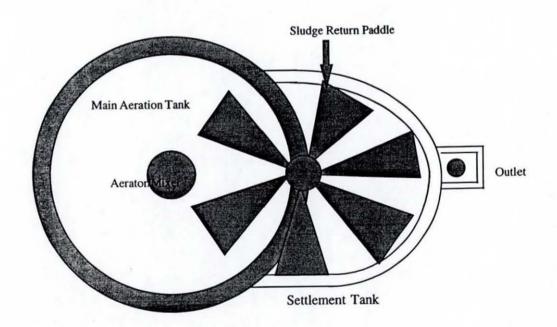


Figure 4.3 B Laboratory Scale Fully mixed Reactor (Plan View)



#### 4.3 Measurement of Settlement

Two measures of sludge settleability were used in this study, the Sludge Volume Index and the Stirred Sludge Volume Index (SSVI) (White 1975). The SSVI is reported to give a better correlation with sludge settlement characteristics at full scale as the SVI test is prone to interference from wall effects in the test vessel (White 1975, Ekama and Marais 1986). In the unstirred test, water may stream up the sides of the vessel or sludge may bridge across the vessel causing the SVI to vary in an inconsistent way. Despite these disadvantages of the SVI test, it was used as a measure of settleability in the laboratory SBR work due to the considerable advantages of being able to measure settlement without removing the sample from the reactor. The wall effects which occur in the SVI test were kept to a minimum by the large diameter of the SBR reactor. At full scale and fully mixed laboratory work, sludge settlement was measured in the WRC Stirred Jar Apparatus (Triton). Both the SVI and SSVI give a reliable indication of settlement trends when measured on a regular basis and both tests gave an adequate measure of settlement for this study.

#### 4.4 Microscopic Investigation

Samples of activated sludge were collected for microscopic analysis to coincide with routine chemical analysis and specific tests such as respirometry. The samples were then viewed under a binocular light microscope (Olympus) with 100 x magnification, 400 x magnification and 1000 x magnification with oil immersion. Observations were made mainly according to the "Sludge Investigation Manual" by Eikelboom and Van Buijsen (1981) but also as modified by Strom and Jenkins (1984). Filamentous micro-organisms were tentatively identified from wet mounts using the x 400 objective. Rapid routine assessment of filament abundance was performed using a subjective scoring system (e.g. absent, rare, common, frequent, abundant) where abundance of filaments can be compared with a set of photographs at x100 magnification (Forster and Dallas-Newton 1980, Jenkins et al. 1984, Eikelboom 1982). Slides were also prepared as thin smears, air dried and stained

stained with methylene blue, Neisser or Gram stains. Further identification of filamentous micro-oganisms were made under oil immersion (x1000) according to morphological and staining characteristics described by Eibelboom and Van Buijsen (1981).

In addition to the microscopic investigations performed with regard to filamentous organisms. Protozoan numbers were also checked at both laboratory and full-scale plants. As recommended by Al-Shahwani and Horan (1990) any alteration in diversity and numbers were noted and if possible, related to plant operational conditions and performance.

4.4.1 Gram Stain Technique

A drop of sludge was are placed on a slide and heat fixed by allowing water to evaporate from the sample by gentle heating. The heat fixed smears are then treated as follows:

1. Stained with crystal violet for 30 seconds and then rinsed with water.

2. Sample then covered with dilute iodine solution, allowed to stand for 30 seconds and then rinsed with water.

3. Decolorised drop by drop with ethanol (95% w/v) for 25 seconds and then rinsed with water.

4. Counter stain with safranin for 30 seconds, rinsed with water, blot dried and examined under 1000X magnification under oil immersion.

5. Gram +ve cells are stained blue, Gram -ve red.

4.4.2 Neisser Stain Technique

Sludge samples were collected and heat fixed onto a slide as described for the Gram stain technique. The heat fixed samples are then treated as follows :

1. Sample stained with acid Methylene blue and then rinsed with water.

2. Sample counter stained with aqueous Bismark brown for 30 seconds and rinsed with water, blot dried and then observed under 1000X magnification under oil immersion.

3. Neisser +ve are stained greyish blue.

4.4.3 Methylene Blue Stain

Sludge samples were collected and heat fixed onto a slide as described for the Gram stain technique. The heat fixed smears are then treated as follows :

1. Stain with 0.1% Methylene blue (BDH) solution for ten minutes then rinse with distilled water.

2. Blot dry then observe under 1000X magnification under oil immersion.

## 4.5 Respirometry

By measuring the respiration of the activated sludge, and reactions caused by adding waste water to activated sludge, some parameters can be determined, such as: reaction time, biodegradation curve, maximum substrate respiration rate, toxicity of waste water, adaptation of activated sludge to new waste water and oxygen consumption for the treatment plant.

#### 4.5.1 Rank Oxygen Electrode

The specific oxygen uptake rate was measured using the Rank oxygen electrode (Rank, Cambridge). Setting up instructions recommended by the manufacturer were followed. A saturated solution of Potassium chloride was added to the base of the respirometer to wet the silver and platinum electrodes. A 1cm<sup>2</sup> piece of tissue was cut and at its centre a 1mm diameter hole was made. The tissue was placed over the platinum electrode, care being taken to ensure that the hole was at the centre of the electrode. A 1cm<sup>2</sup> piece of Teflon membrane was cut and placed over the tissue (care being taken to keep the membrane straight to avoid trapping air bubbles), and then secured in place putting back the base and screwing down the locking nut.

The Rank cell was zero calibrated by filling the cell with 0.6% Sodium sulphite solution along with a magnetic stirrer. The cell was stoppered, the polarising voltage set to 0.6V and the stirrer motor switched on. The recorder was operated at a speed of 5mm/min and its zero offset was adjusted so that the chart recorder pen was set at a suitable zero position. A constant temperature of 15<sup>o</sup>C was maintained in the cell by circulating temperature controlled water

The cell was air saturated water calibrated using tap water that had been aerated for one hour at a constant temperature of  $20^{\circ}$ C. The dissolved oxygen concentration was determined by means of a dissolved oxygen meter (Yellow Springs Instruments). After thoroughly cleaning the cell with distilled water, air saturated water was added and the same procedure for the zero calibration was repeated. An optimum stirring speed was necessary to avoid fluctuations on the recorder. This was achieved by gradually increasing the speed of the stirrer from zero until there was a uniform trace on the chart recorder.

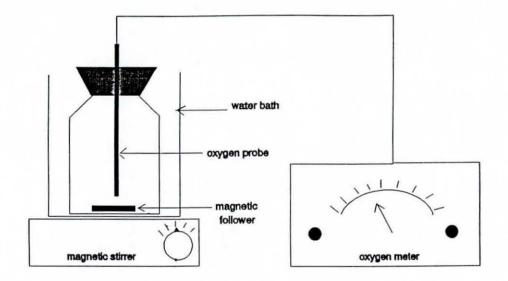
Oxygen Uptake Rates were determined by placing an acclimatised  $(15^{\circ}C)$  sample of air saturated activated sludge  $3 \text{cm}^3$  in the reaction cell. The cell was then stoppered with care being taken not to trap any air bubbles. The stirrer and chart recorder were switched on simultaneously, their operational speeds being held the same as during the calibration. A chart recorder displaying the linear trace of declining oxygen concentration within the sample were obtained and used to calculate the oxygen uptake rate.

## 4.5.2 Winkler's Bottle Respirometer

Due to the limited sample volume size studied using the above apparatus, later respiration rates were carried out using an alternative apparatus to determine oxygen uptake rate which afforded a larger sample volume size. The rate of oxygen consumption was determined in a Winkler's bottle. Dissolved oxygen concentration in a known volume was measured with an oxygen selective electrode. Activated sludge was transferred into the bottle and continuously stirred by a magnetic stirrer (Figure 4.4). The temperature of the sample was held at 15<sup>o</sup>C by placing the

apparatus in a temperature controlled bath and the oxygen concentration versus time was measured (Apha 1989)

Figure 4.4 Winkler Bottle Respirometer



#### 4.5.3 Oxygen Uptake Rate At Full Scale

In the full scale oxygen activated sludge system it was possible to determine the oxygen uptake rate in situ. This was done by allowing the dissolved oxygen concentration to rise to above 5.0mg/l at which point all oxygenation was ceased and the reduction in dissolved oxygen concentration with time was measured. A sample of mixed liquor taken at the same time was analysed for suspended solids so that the OUR could be calculated.

4.6 Chemical Analysis of Feeds and Effluents

Table 4.1 Chemical parameters measured

Parameter	Summary of Method	<b>Reference</b>
BOD <sub>5</sub> (ATU)	Oxygen required to satisfy heterotrophic respiration of pollutants during five days of incubation at 20°C Dissolved oxygen determination by Winkler titration.	Standard Methods (1985)
COD	Oxygen required to oxidise organic pollutants during boiling under reflux in acidified dichromate in the presence of catalysts (COD reagent BDH). Residual dichromate determined by titration with ferrous ammonium sulphate.	Standard Methods (1985)
Total Suspended Solids	Dry weight at 103 - 105 °C of solids retained by a glass fibre filter paper (Whatman GF/C).	Standard Methods (1985)
рН	Determined by pH probes of various manufacture.	
Dissolved Oxygen Concentration	Determined with portable dissolved oxygen probes (pHox), (Yellow Springs Instruments).	
Oxidation Reduction Potential(ORP)	Determined with combination oxidation - reduction potential probes (Orion) reference electrode Ag/AgCl.	

## 4.7 Media

## 4.7.1 BOD Nutrients

Table 4.2 composition of BOD Nutrients.

COMPONENT	SUPPLIER	gdm <sup>3</sup>
Fe Cl <sub>3</sub> . 6H <sub>2</sub> 0	(BDH)	0.125
CaCl <sub>2</sub>	(BDH)	27.5
Mg $SO_4$ . $7H_20$	(BDH)	25.0
Phosphate Buffer		
- KH <sub>2</sub> PO <sub>4</sub>	(BDH)	42.5
- Na OH	(BDH)	8.8
- (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	(BDH)	2.0

Table 4.3 Artificial Feed No. 1

COMPONENT*	SUPPLIER	QUANTITY
Ammonium chloride	(BDH)	100 mg
D - Glucose	(BDH)	400 mg

\*+ 1.0 ml of each BOD nutrient made up to 1 litre with tap water.

Table 4.4 Artificial Feed No. 2

\*+ 1.0 ml of each BOD nutrient made up to one litre with tap water.

COMPONENT*	SUPPLIER	QUANTITY
Yeast Extract		110 mg
Bacteriological	oxoid	160 mg
Peptone	(BDH)	30 mg
Urea	(BDH)	7.0 mg
Na Cl	(BDH)	4.0 mg
Ca Cl <sub>2</sub> . 2 H <sub>2</sub> 0	(BDH)	2.0 mg
Mg SO <sub>4</sub> . 7 H <sub>2</sub> O	(BDH)	28 mg

Table 4.5 Artificial Feed No. 3 Artificial Sewage

\*Made up to one litre with tap water.

Table 4.6 Artificial Feed No. 4

COMPONENT*	SUPPLIER	QUANTITY
L - Lysine	(BDH)	200 mg

\*+ 1.0 ml of each BOD nutrient made up to one litre with tap water.

Table 4.7 Artificial Feed No. 5

COMPONENT*	SUPPLIER	QUANTITY
L - Alanine	(BDH)	200 mg

\*+ 1.0 ml of each BOD nutrient made up to one litre with tap water.

Table 4.8 Artificial Feed No. 6

COMPONENT*	SUPPLIER	QUANTITY
Amylopectin	(BDH)	300 mg

\*+ 0.1 ml of each BOD nutrient made up to one litre with tap water.

## Table 4.9 Artificial Feed No. 7

COMPONENT*	SUPPLIER	QUANTITY
Amylose	(BDH)	300 mg

\*+ 1.0 ml of each BOD nutrient made up to 1 litre with tap water.

Table 4.10 Artificial Feed No. 8

COMPONENT*	SUPPLIER	QUANTITY
Sucrose	(BDH)	300 mg
Ammonium chloride	(BDH)	100 mg

\*+ 1.0 ml of each BOD nutrient made up to 1 litre with tap water.

Table 4.11 Artificial Feed No. 9

COMPONENT*	SUPPLIER	QUANTITY
Lactose	(BDH)	300 mg
Ammonium chloride	(BDH)	100 mg

\*+ 1.0 ml of each BOD nutrient made up to 1 litre with tap water.

#### 4.7.2 Artificial Feed No. 10 (Pea Starch)

An attempt was made to formulate a feed to mimic that of the waste stream arising from the production of frozen peas. This was done by boiling 1kg of frozen unprocessed peas in 2.5 litres of water for 10 minutes. The resulting liquid was then sieved (1.18 mm) and the mix made up to 20 litres with tap water. Three different strength of pea starch feed were used in this study as shown in Table 4.12.

# Table 4.12 Artificial Feed No. 10

Strength	Feed Make Up
x 3	1 kg boiled in 2.5 I made up to 20 I
x 2	1 kg boiled in 5.01 made up to 201
x 1	1 kg boiled in 7.5 I made up to 20 I

#### CHAPTER 5 : RESULTS AND DISCUSSION

## 5.0 Introduction

This thesis examines the ecology of activated sludge populations and the mechanisms which increase the potential for filamentous bulking, with the objective of providing component data upon which settlement control strategies can be based. Normally activated sludge cultures are not grown under strict control conditions (steady state) as laboratory cultures in this study have. It is for this reason that data from these studies should be regarded as indicative of microbial behaviour rather than absolute. What this data does provide are physiological and metabolic pointers, suggesting how filamentous organisms can be better underdstood and ultimately be controlled. To overcome the differences between laboratory and full scale, data has also been obtained from practical observations on full scale plant and related laboratory studies.

#### 5.1 Laboratory Scale Reactor 1 (Fully Mixed Reactor)

The major problems associated with laboratory scale reactors are that of clarification and sludge return. The problems of clarification are associated with the small scale of the reactor making it difficult to minimise wall effects in any settlement chamber. In addition, sludge bridging across the whole of the settlement chamber can occur. This leads to a dilute return or any pumped return drawing only clear liquor and not sludge.

The early stages of this research were spent designing and testing reactors that minimised these problems. The final fully mixed reactor design was described in Chapter 4. The main problems with this reactor were still associated with sludge return and clarification. Sludge return volumes could only be estimated from the RPM of the sludge return paddle and an estimate of the returned sludge concentration. In addition to this some back mixing was evident between the aeration chamber and the settlement zone. This back mixing was reduced by altering the return paddle but however was never completely eliminated. The problem of back mixing and the small size of the settlement chamber reduced the rate of settlement and hence the concentration of return activated sludge. There were other problems that were never solved with this reactor these included the build up of sludge solids above the water level on the reactor walls and periodic blocking of the final effluent overflow.

In spite of these problems associated with this reactor, these were improvements compared with previous designs tried. For this reason it was this reactor design that was selected to carry out all the fully mixed reactor experimentation reported in this study.

### 5.1.1 Tracer Study

The first experiments were made to classify the mixing characteristics of the laboratory scale fully mixed reactor using an inert tracer technique (Chambers 1982, Levenspiel 1962). This was carried out to establish if the desired completely mixed flow pattern had been achieved within the laboratory designed and built reactor.

In order to express the degree of longitudinal mixing exhibited by the laboratory scale reactor a Dispersion Number Technique (see Chapter 3) as described by Levenspiel (1962) was used. For ideal cases of "plug-flow" and "complete mixing" the dimensionless Dispersion Number has values of 0 and  $\infty$  respectively. All real systems have values between these two extremes.

Exit manganese concentration (mg/l) was measured with respect to time. This data was normalised and replotted to give the curve shown in Figure 5.1. From this it was possible to calculate the Dispersion Number  $(D/\mu l) = 0.36$ . This relatively large Dispersion Number indicates a substantial degree of mixing within the single aeration vessel and hence the desired flow characteristics within the reactor.

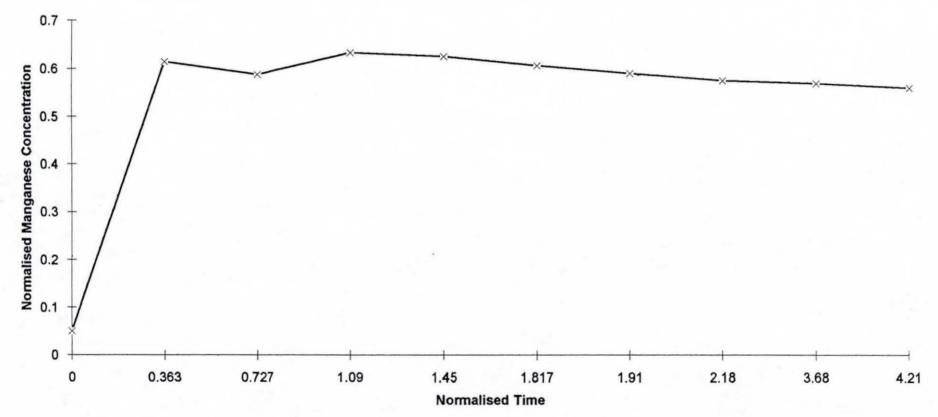


Figure 5.1 Normalised Exit Manganese Concentration Vs. Normalised Time

#### 5.2 Laboratory Reactor 2 (Sequencing Batch)

The Sequencing Batch Reactor (SBR) was considered the best solution when attempting to mimic the conditions of conventional plug flow reactor. The SBR is again described in detail in Chapter 4. The SBR produces a temporal rather than spacial concentration gradient within the reactor which resembles the conditions encountered by an individual floc in an ideal Plug-Flow system, but eliminates the need for compartmentalisation. The problem of back mixing is also eliminated as the reactor is completely mixed during the aerobic phase of the process and due to the lack of baffles the surface area in contact with the mixed liquor is kept to a minimum. This minimised the build up of attached growth on the submerged surfaces and above the liquid level which could lead to interference with experimental results. Settlement was carried out in the aeration vessel which alleviated the need for any sludge return and the SVI could be measured in situ avoiding the disturbance of removing a large sample for settlement tests.

Some problems with this reactor were still encountered. Sludge wasting was to be achieved on an automatic basis, by filling the reactor above the upper discharge point then opening this discharge prior to the settlement phase. In practise the lack of any significant head pressure meant that this discharge point was unreliable and was prone to air-locks and blocking. Sludge wastage was therefore carried out by manually removing a portion of the mixed liquor.

## 5.3 The Effect Of Various Carbon Sources On Sludge Settlement

In an attempt to gain a better understanding of the effect of various substrates on sludge settleability, a series of experiments utilising different carbon sources ranging from directly absorbable (monosaccharides and disaccharides) to polymeric substrates requiring enzymatic hydrolysis prior to absorbtion were carried out using both reactor configurations. Artificial feeds containing these different carbon sources were fed to each reactor at three different loading rates, this was achieved by altering the concentration of the feed. The loading rates for Reactor 1 (Fully Mixed Reactor) and Reactor 2 (SBR) are shown in Tables 5.1 and 5.2 respectively.

## 5.3.1 Monosaccharide Feed

The composition of a glucose feed is detailed in Chapter Four. The reactor loading rates are reported in Tables 5.1 (Fully Mixed) and 5.2 (SBR). Three different loading rates were employed, this was achieved by altering the concentration of the feed. Volumetric loading and sludge age were kept constant in both reactor configurations and the MLSS concentration allowed to fluctuate. This feeding pattern was adopted for all the artificial feeds used throughout the laboratory scale work.

Substrate	Volume of	Load	BOD	MLSS	F:M Ratio	Yield
	Feed (l/d)	(gBOD/d)	(mg/l)	(g/l)	(gBOD/gMLSSd)	(gMLSS/gBOD)
Monosaccharide Feed			1			State Anderson
Glucose (x1)	5.5	1.45	263	1.8	0.23	0.35
Glucose (x1.5)	5.5	2.08	379	2	0.30	0.31
Glucose (x2.0)	5.5	2.87	522	2.3	0.36	0.29
Disaccharide Feed		12523434	10.000	1.1.1.1.1.1		
Sucrose (x1)	5.5	1.45	263	2.85	0.15	0.38
Sucrose (x1.5)	5.5	2.82	512	3	0.27	0.34
Sucrose (x2)	5.5	4.12	749	3.2	0.37	0.3
Lactose (x1)	5.5	1.41	256	2.5	0.16	0.41
Lactose (x1.5)	5.5	2.61	475	2.7	0.28	0.35
Lactose (x2.0)	5.5	3.23	587	3	0.31	0.34
Polysaccharide Feeds		The second second			hand bandstalling	North States
Amylose (x1.0)	5.5	1.58	287	1.8	0.25	0.35
Amylose (x1.5)	5.5	2.00	364	2.1	0.27	0.34
Amylose (x2.0)	5.5	2.54	462	2.2	0.33	0.31
Amylopectin (x1.0)	5.5	1.65	300	2	0.24	0.32
Amylopectin (x1.5)	5.5	2.43	442	2.2	0.32	0.3
Amylopectin (x2.0)	5.5	3.14	570	2.3	0.39	0.27
Pea Effluent (x1.0)	5.5	11.55	2100	3.8	0.87	0.14
Pea Effluent (x1.5)	5.5	15.19	2760	4.1	1.06	0.12
Pea effluent (x2.0)	5.5	15.95	2900	4.5	1.01	0.11
Amino Acid Feeds	Contract Star Big	WHEN THE				
Glutamic acid (x1.0)	5.5	1.51	274	1.3	0.33	0.29
Glutamic Acid (x1.5)	5.5	1.97	359	1.5	0.38	0.27
Glutamic Acid (x2.0)	5.5	2.18	396	1.6	0.39	0.26
L-Lysine (x1.0)	5.5	1.42	259	1.7	0.24	0.31
L-Lysine (x1.5)	5.5	2.00	364	2.31	0.25	0.27
L-Lysine (x2.0)	5.5	2.63	479	2.3	0.33	0.24
L-Alanine (x1.0)	5.5	1.16	210	1.5	0.22	0.31
L-Alanine(x1.5)	5.5	1.48	269	1.7	0.25	0.3
L-Alanine (x2.0)	5.5	1.92	349	1.7	0.32	0.27
Complete Feeds	1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1	- Allent	New!	1.		
Artificial sewage	5.5	1.73	314	1.5	0.33	0.26
Artificial sewage	5.5	2.61	475	2.0	0.37	0.23
Artificial sewage	5.5	3.99	726	2.4	0.48	0.18

Table 5.1 Loading data For Reactor 1 (Fully Mixed)

Note the intended loading rate increases contained in Table 5.1 do not always match the actual increase in concentration (e.g. Pea Effluent x 2.0 =only 1.4 x pea effluent x 1.0 in concentration).

Substrate	Volume	Load	BOD	MLSS	F:M Ratio	Yield
	of Feed (l/d)	(gBOD/d)	(mg/l)	(g/l)	(gBOD/gMLSSd)	(gMLSS/gBOD)
Monosaccharide Feed			BRE	NG AND		
Glucose (x1)	10.5	2.76	263	1.2	0.24	0.37
Glucose (x1.5)	10.5	3.98	379	1.5	0.28	0.31
Glucose (x2.0)	10.5	5.48	522	2.4	0.24	0.36
Disaccharide Feed			A. Car			Constant and the
Sucrose (x1)	10.5	2.76	263	2.70	0.11	0.61
Sucrose (x1.5)	10.5	5.38	512	3	0.19	0.52
Sucrose (x2)	10.5	7.86	749	3.4	0.24	0.39
Lactose (x1)	10.5	2.69	256	2.5	0.11	0.61
Lactose (x1.5)	10.5	4.99	475	2.7	0.19	0.52
Lactose (x2.0)	10.5	6.16	587	3	0.22	0.39
Polysaccharide Feeds		1.000	1.50	1-10.50		
Amylose (x1.0)	10.5	3.01	287	1.8	0.18	0.59
Amylose (x1.5)	10.5	3.82	364	2.1	0.19	0.57
Amylose (x2.0)	10.5	4.85	462	2.2	0.23	0.46
Amylopectin (x1.0)	10.5	3.15	300	2	0.17	0.56
Amylopectin (x1.5)	10.5	4.64	442	2.2	0.22	0.49
Amylopectin (x2.0)	10.5	5.99	570	2.3	0.27	0.45
Pea Effluent (x1.0)	10.5	16.49	2100	4.0	0.43	0.29
Pea Effluent (x1.5)	10.5	21.67	2760	4.3	0.53	0.24
Pea effluent (x2.0)	10.5	22.77	2900	4.5	0.53	0.19
Amino Acid Feeds	11.1.22.70		1. 1. Start			A CARLES AND A CARLES
Glutamic acid (x1.0)	10.5	2.15	274	1.3	0.17	0.48
Glutamic Acid (x1.5)	10.5	2.82	359	1.5	0.20	0.42
Glutamic Acid (x2.0)	10.5	3.11	396	1.7	0.19	0.4
L-Lysine (x1.0)	10.5	2.72	259	1.7	0.17	0.57
L-Lysine (x1.5)	10.5	3.82	364	2.1	0.19	0.54
L-Lysine (x2.0)	10.5	5.03	479	2.3	0.23	0.46
L-Alanine (x1.0)	10.5	2.21	210	1.5	0.15	0.51
L-Alanine(x1.5)	10.5	2.82	269	1.7	0.17	0.5
L-Alanine (x2.0)	10.5	3.66	349	1.7	0.23	0.42
Complete Feeds	6120 Jack 840					
Artificial sewage	10.5	2.86	314	1.5	0.20	0.46
Artificial sewage	10.5	4.0	475	2.0	0.21	0.45
Artificial sewage	10.5	5.98	726	2.4	0.26	0.42

Table 5.2 Loading data from Reactor 2 (SBR)

Note the intended loading rate increases contained in Table 5.1 do not always match the actual increase in concentration (e.g. Pea Effluent x 2.0 =only  $1.4 \times$ pea effluent x  $1.0 \times 1.0 \times$ 

## 5.3.2 Reactor 1 (Fully Mixed)

A series of experiments to investigate the effect of using glucose as the sole carbon source were conducted in Reactor 1 (Fully Mixed). Data for the glucose feed within Reactor 1 are reported in Table 5.1. At the lowest feed strength employed (BOD = 263mg/l) the MLSS concentration stabilised at 1.8g/l resulting in an F:M Ratio of 0.23gBOD/gMLSSd<sup>-1</sup> and a yield of 0.35mgMLSS/mgBOD. SSVI values from daily sludge settlement tests are shown in Figure 5.2 with an initial value of 50ml/g remaining relatively constant until day 14 of the study. From this point SSVI values rose steadily until day 25 when SSVI values were in excess of 250ml/g and the experiment was terminated due to solids loss from the system. (An example graph showing the spread of SSVI values gained during this experiment are shown in Appendix 1.0.)

The intermediate and high strength feeeds with BOD's of 379mg/l and 522mg/l respectively were capable of supporting 2.0g/l biomass resulting in an F:M Ratio of 0.30 gBOD/gMLSSd<sup>-1</sup> and a yield of 0.31 mgMLSS/mgBOD for the intermediate strength feed and 2.3g/l biomass resulting in an F:M Ratio of 0.36 gBOD/gMLSSd<sup>-1</sup> and a yield of 0.29mgMLSS/mgBOD for the highest strength feed. Both these higher feed concetrations employed resulted in a steady increase in SSVI values from their initial starting values below 60ml/g and rising to 260ml/g for the intermediate strength feed and 280ml/g for the highest strength feed on day 25 of the study.

Microscopic examination over the 25 day period of all the above studies revealed a gradual increase in filamentous organism numbers within the biomass which corresponded to the deterioration in sludge settlement. In the case of the lower strength feed the generation of a bulking population of filamentous organisms took rather longer. The causative organism appeared to be identical in all three cases but was not identified.

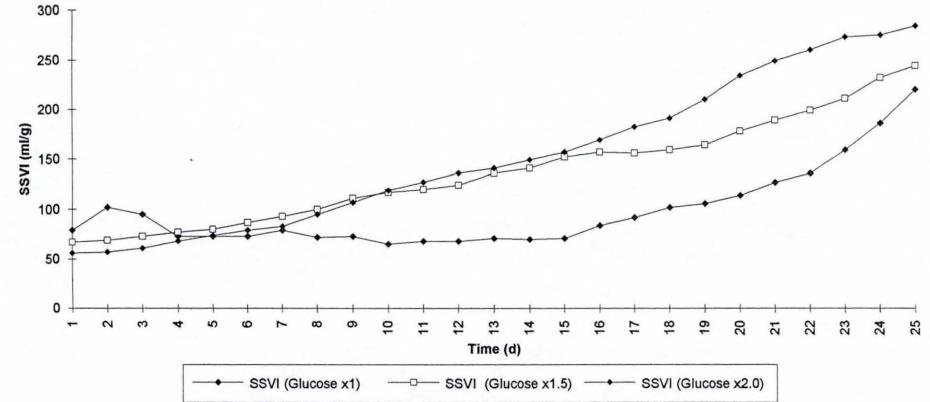


Figure 5.2 Glucose Feed In Reactor 1 (Fully Mixed)

## 5.3.3 Reactor 2 (SBR)

A similar series of experiments using the glucose feed were also carried out in Reactor 2 (SBR). A feed pattern similar to that used in Reactor 1 was adopted i.e. a constant volumetric loading rate and sludge age (10 days) using the same three different concentrations of glucose and mineral base. The loading rates employed are shown in Table 5.2. At the lower feed concentration (BOD = 263mg/l) the biomass concetration stabalised at 1.2 g/l leading to an F:M Ratio of 0.24 gBOD/gMLSSd<sup>-1</sup> and a yield of 0.37mgMLSS/mgBOD. SVI values recorded throughout the studies using glucose within the laboratory scale SBR are shown in Figure 5.3. When using the lowest feed concetration SVI values remained constant until day 14 of the study when, due to problems with air supply SVI values rose to 200ml/g when using the lower strength feed. Following the restoration of the correct operation of the system SVI values decreased at a constant rate for the rest of the experimentation period. The intermediate feed concentration (BOD = 379mg/l) supported a biomass concentration of 1.5g/l resulting in an F:M Ratio of 0.28gBOD/gMLSSd<sup>-1</sup> and a yield of 0.31mgMLSS/mgBOD while the highest strength feed (BOD = 522mg/l) supported 2.4g/l of biomass at an F:M of 0.24gBOD/gMLSSd<sup>-1</sup> with a yield of 0.36mgMLSS/mgBOD. Both these higher strength feeds showed a similar pattern of decreasing SVI values from initially high values of above 150ml/g over the 25 day period of the studies. These initially high sludge settlement values were a result of problems being experienced at the local sewage works, the source of all seed sludge for the laboratory scale work reported here. (An example graph showing the spread of SVI values gained during this experiment are shown in Appendix 1.0)

Microscopic examination revealed a substantial decrease in the filamentous population within the sludge over the 25 day period of the studies employing the two higher concentration feeds. This decrease in the filamentous population within the biomass corresponded with the decrease in SVI values measured throughout the experimentation. Aeration problems experienced in the study using the lowest feed strength caused filamentous numbers within the biomass to increase and hence led to a corresponding increase in SVI values measured. Microscopic examination and

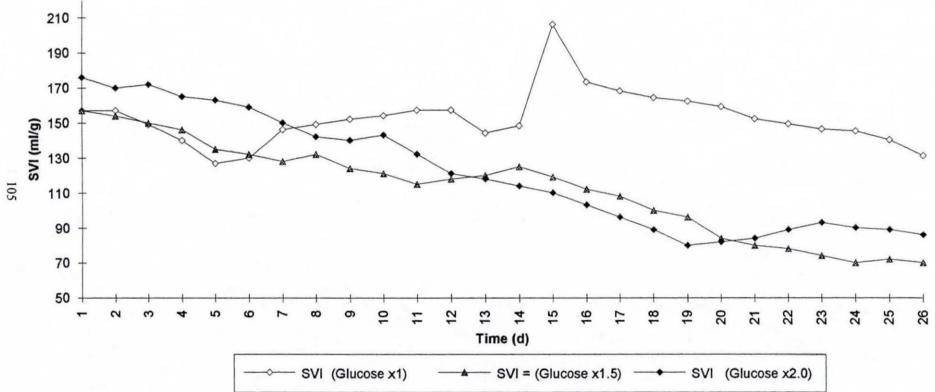


Figure 5.3 Glucose Feed In Reactor 2 (SBR)

morphological characteristics revealed the causative organism to be unbranched, non-motile with cross walls Neisser negative and Gram negative leading to this organism being identified as Type 021N whose numbers decreased once correct operation and aeration had been restored.

Associated along with the reduction in SVI values during this study however, was an increase in the turbidity of the supernatant (final effluent). Microscopic examination of the supernatant revealed the high turbidity to be due to nonflocculated bacteria. These results are similar to those reported in a study by Kiff in 1978, who found that when activated sludge was batch fed with a 50% dissaccharide carbohydrate/settled sewage feed instead of settled sewage, lead to an increase in turbidity of the supernatant along with increased SVI values. However, when the same apparatus was fed with a 75% carbohydrate feed, resulting in a higher loading rate, there was a decrease in SVI values with a considerable increase in supernatant turbidity. This increase in turbidity was reversed by the addition of urea to rebalance the C:N ratio leading to a reduction in the production of extractable extracellular polymer. Further studies during the 1980's by various workers produced relationships which have related some ratios of the main nutrients (carbon, nitrogen and phosphorus) to settlement (Forster and Dallas-Newton (1980); Wagner (1982a and 1982b); Clark and Forster (1983); Wu et al. (1982)). Of these the most significant results were those reported by Wu et al. (1982). These workers revealed that settlement, surface charge, floc ecology and the amounts of biopolymer in the sludge (both protien and carbohydrate) all of which are dependant on the balance of nutrients in the substrate and the rate at which the substrate was fed to the reactor. Hence the nutritional balance can affect the two parameters which are thought of as influencing the settlement characteristics; the species and the surfaces. The present results indicate that the presence of readily biodegradable substrate may effect a change in the nature of the extracelluluar polymeric material produced by the bacterial biomass. The above results were concluded to be due to enduced changes in extracellular polymer production in response to nutrient levels in the floc environment and energy levels

within the cells. Increases in loading rate and readilly available substrate could result in a tendancy to produce extra capsular slime as a storage product. It is now evident from this that up to a certain increase in the production of extracellular polymer will favour settlement but however, above this level may result in the reverse i.e. a dispersing effect especially if the polymer produced are hydrophilic or highly charged in nature (Becarri 1980; Jenkins et al. 1986).

5.4 Disaccharide Feeds (sucrose and lactose)

## 5.4.1 Sucrose

Sucrose, an important disaccharide containing glucose and fructose was also investigated. About ninety million tons of sucrose are produced annually in the world and forms a common component of wastewaters arising from the soft drinks, confectionery and baking industries. Due to the nature of sucrose and its more complex structure when compared to glucose, an additional step is required in its breakdown before any products can be assimilated by the biomass. This step involves the cleaving of the glycosidic linkage by invertase enzymes to liberate an equimolar mixture of D-glucose and D-fructose. A similar feeding pattern to that adopted with the glucose feed was used for both reactor configurations (Fully Mixed and SBR).

## 5.4.2 Reactor 1 (Fully Mixed)

The effect of sucrose as the sole organic substrate on activated sludge settlement was studied through a series of experiments using Reactor 1 (Fully Mixed). As with the studies utilising glucose feed the volumetric loading and sludge age were kept constant and the MLSS concentration allowed to fluctuate. The loading rates employed with the sucrose feed in Reactor 1 are shown in Table 5.1 and the effect of the three feed strengths on sludge settlement in Figure 5.4. At the lowest loading rate (BOD = 263mg/l) the biomass concentration stabalised at 2.7g/l which resulted in an F:M Ratio of 0.11gBOD/gMLSSd<sup>-1</sup> and a yield of 0.61mgMLSS/mgBOD. As with the glucose feed SSVI values steadily increased over the duration of the

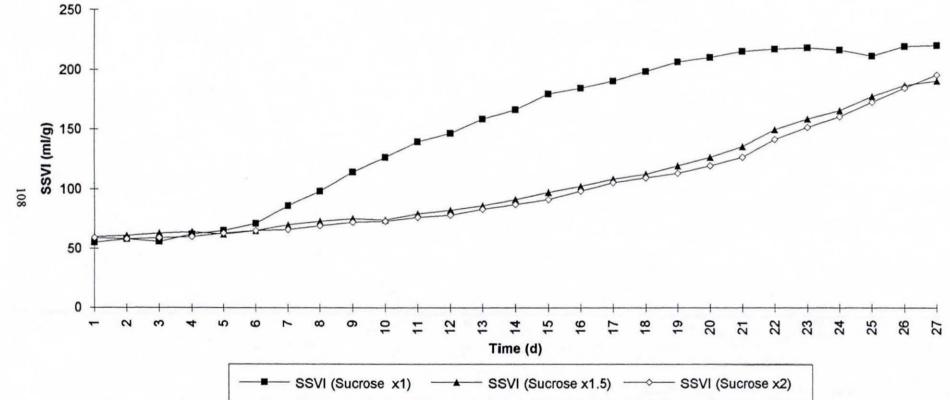


Figure 5.4 Sucrose Feed In Reactor 1 (Fully Mixed)

experiment with initial values rising from 55ml/g on day one too 220ml/g on day 27 of the study. The intermediate and highest strength feed (BOD = 512mg/l and 749mg/l) were able to support biomass concentrations of 3.0g/l and 3.4g/l and resulted in F:M Ratios of 0.19 and 0.24 mgBOD/mgMLSSd<sup>-1</sup> respectively. These two higher strength feeds showed a similar pattern of increasing SSVI values over the 27 day period of the experiment with initial values rising from 60ml/g to approximately 190ml/g for both feed strengths when the experimentation was terminated.

Daily microscopic examination revealed a substantial increase in filament numbers present within the the biomass at all feed strengths used in the fully mixed reactor. The causative organism appeared to be identical in all three cases exhibiting false branching. This led to this organism being identified as as a *Sphaerotilus Type*.

## 5.4.3 Reactor 2 (SBR)

The above experimentation using sucrose as the sole organic substrate were repeated in Reactor 2 (SBR). The loading data for the three concentrations of sucrose feed within the sequencing batch reactor are shown in Table 5.2 with sucrose x1.0, x1.5 and x2.0 being able to support biomass concentrations of 2.7, 3.0 and 3.4g/l leading to F:M Ratios of 0.11, 0.19 and 0.24g BOD/gMLSSd<sup>-1</sup> respectively. Similar patterns of sludge settlement were produced with all three sucrose feed concentrations (Figure 5.5) with SVI values remaining relatively constant throughout the period of experimentation. Microscopic examination of the biomass throughout the study period revealed no discernable increase in the number or length of any filamentous organisms present.

A problem of pH control was exerienced when using sucrose as the sole organic substrate. This had been anticipated by the findings of Gostick (1991) who when using a sucrose based feed had experienced sludge settlement problems caused by filamentous fungi when no pH correction had been carried out. As with the work carried out by Gostick (1991) pH levels were maintained above 6 by the addition of Sodium hydroxide.

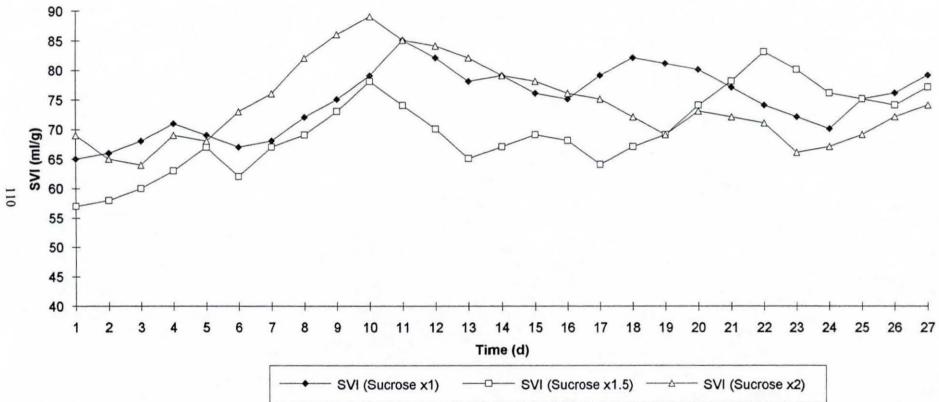


Figure 5.5 Sucrose Feed In Reactor 2 (SBR)

## 5.4.4 Lactose

The effect of lactose on sludge settlement was also investigated in both reactor configurations. Lactose is a disaccharide consisting of a molecule of glucose and a molecule of galactose joined by a glycosidic linkage. The steriochemistry of this glycosidic bond is ß-linkage . This steriochemistry is of importance due to the breakdown of lactose being carried out by the enzyme ß-galactosidase, which catalyzes the hydrolysis of this ß-glycosidic linkage; this hydrolysis allows lactose to act as a source of glucose. Lactose is principally found in milk and is present to the extent of about 4.5% in cows milk. Due to this, waste waters arising from milk processing and dairies contain lactose and have been recognised as generally producing poorly settling sludges (Marshall 1992, Beetschen and Eichelberger 1992).

## 5.4.5 Reactor 1 (Fully Mixed)

The next set of experiments were conducted using lactose as the sole organic substrate at three different concentrations within Reactor 1 (SBR). The three strengths (x1.0, x1.5 and 2.0) of lactose were able to support biomass concentrations of 2.5, 2.7 and 3.0g/l respectively resulting in F:M Ratios of 0.16, 0.28 and 0.31g BOD/gMLSSd<sup>-1</sup> (see Table 5.1). A similar pattern of deteriorating sludge settlement as that found when using sucrose feed was also found when using lactose. SSVI values were initially low (below 40ml/g) and rose steadily to values in excess of 210ml/g by the end of the experimentation period for all three feed concentrations used (Figure 5.6).

Again the lowest strength feed showed the most rapid deterioration in sludge settlement when compared with the other feed concentrations used. As with sucrose microscopic exmination revealed the deterioration in sludge settlement in all three cases to be due to the proliferation of a filamentous organism exhibiting false branching leading it to be identified as a *Sphaerotilus Type*.

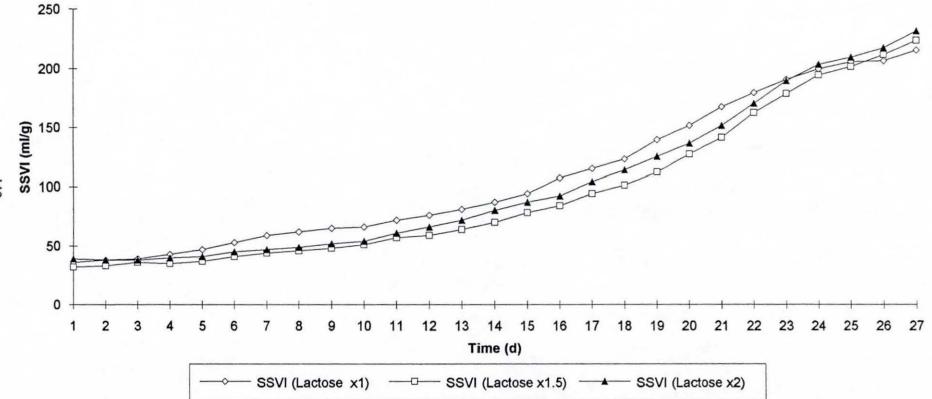
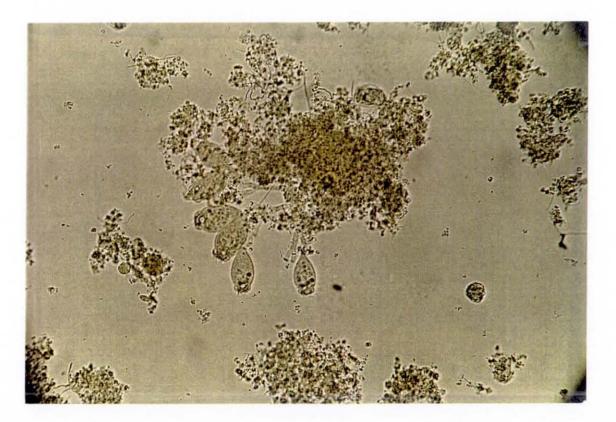


Figure 5.6 Lactose Feed in Reactor 1 (Fully Mixed Reactor)

## 5.4.6 Reactor 2 (SBR)

The three feed strengths (x1, x1.5 and x2) of lactose were able to support biomass concentrations of 2.5, 2.7, and 3.0 g/l respectively at F:M Ratios of 0.11, 0.19 and 0.22 in the laboratory scale SBR reactor (full loading data is shown in Table 5.2). pH was (as in all the experimentation reported) measured on regular basis during this study and pH correction with sodium hydroxide was necessary to prevent any settlement problems associated with low pH values. The SVI of the seed activated sludge was initially between 70 and 85ml/g in all three studies involving lactose (Figure 5.7). At all three loading rates employed this figure showed a slight improvement with SVI values decreasing by approximately 25% over the period of the experiment. In all three cases, microscopic examination revealed a healthy sludge containing a diverse population of both crawling and stalked ciliates and only a few filaments protruding outside the flocs (see Plate 5.1).

Plate 5.1 Biomass Produced in Reactor 2 (Lactose Feed x1.5)



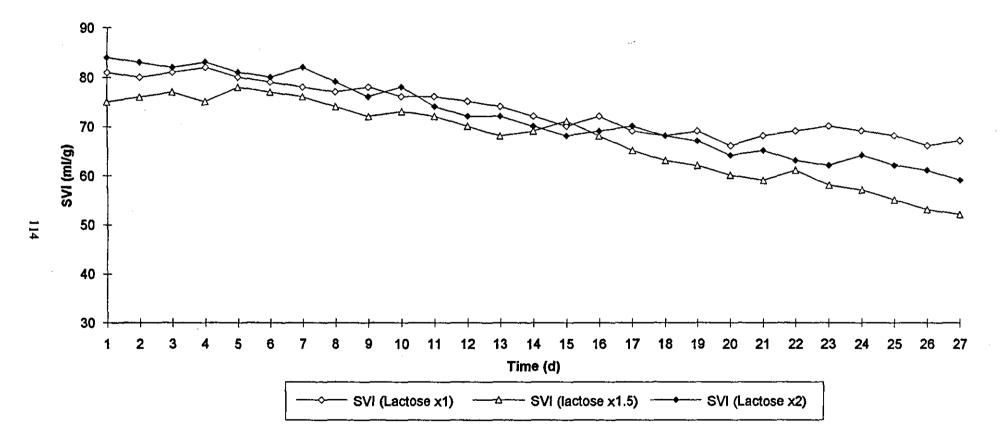


Figure 5.7 Lactose Feed In reactor 2 (SBR)

The experimentation carried out using simple soluble carbohydrate substrates demonstrated two contrasting results in the two different reactor configurations used in this study. In all the above cases using Reactor 1 (Fully Mixed) sludge settlement rates deteriorated due to the proliferation of filamentous organisms within the biomass. While Reactor 2 (SBR) produced a good settling sludge with few filaments when fed with simple carbohydrates.

The above results demonstrate that reactor configuration can have a significant effect on sludge settrlement when fed with substrates of similar solubility and biodegrability. These results are similar to those found by Verachtert et al. (1980) and Van den Eynde et al. (1982) who while studying the effect of various feed compositions demonstrated sludge settlement rates improved regardless of substrate used when fed intermittently rather than continuously. Both these workers attributed these findings to the different growth rates of floc forming and filamentous organisms at different substrate concentrations. Under regimes with a period of high substrate concentration, floc forming organisms have a greater growth rate than filamentous organisms. While at lower substrate concentrations (as found in fully mixed, continuously fed systems) filamentous organism with a higher substrate uptake rate will out compete floc formers. These results utilising simple soluble carbohydrate substrates demonstrate the phenomenon of cross over Monod kinetics (Section 2.10.2) as reported by Van Nierkirk (1987). This follows the standard pattern of sludge settlement noted with the other substrates used in the laboratory scale experimentation and is discussed in more detail in section 5.7.3.

## 5.5 Polysaccharide Feeds (Starch, Amylose and Amylopectin)

Starch is an important storage polysaccharide found in all plants and forms a major constituent of corn, potatoes and other vegetables. Because of the size and complexity of the starch molecule it cannot be absorbed directly into the bacterial cell so it must first be hydrolysed by extracellular enzymes. Starch is a polymer of glucose. Infact, starch is a mixture of two different types of glucose polymers. In one, amylose, the glucose residues are connected by  $\alpha$ -1,4-glycosidic linkages. The

other constituent of starch is amylopectin, a branched polysaccharide. Amylopectin contains relatively short chains of glucose residues in  $\alpha$ -1,4-linkages and in addition it contains branches that involve  $\alpha$ -1,6-glycosidic linkages.Verachtert et al. (1980) reported that an intermittently fed activated sludge system fed with soluble starch had produced a poorly settling sludge when the same system fed with glucose had produced a good settling sludge with few filaments. The next group of experiments were carried out using polysaccharides as the sole carbon source. These included the constituents of starch amylose and amylopectin along with pea effluent whose major constituent is starch.

#### 5.5.1 Amylose

As previously mentioned amylose a constituent of starch is made up of glucose residues connected by  $\alpha$ -1,4-glycosidic linkages. Before absorption the glycosidic links have to be broken by the secretion of amylase resulting in the breakdown of amylose to glucose. As before this substrate was used at three different concentrations to form an artificial feed used in both reactor types (Fully Mixed, SBR).

#### 5.5.2 Reactor 1 (Fully Mixed)

The three different strengths of amylose feed (x1, X1.5 and X2) were able to support biomass concentrations of 1.8, 2.1 and 2.2g/l (similar to the simple sugars) respectively leading to F:M Ratios of 0.25, 0.27 and 0.33 in the Fully Mixed laboratory scale reactor (full loading data is shown in Table 5.1). The SSVI values during all three runs using amylose rapidly increased from the initial low starting values of approximately 60ml/g (Figure 5.8). This initial value rose to above 150ml/g in all three cases within the first seven days of the experiment and with the highest strength feed had reached values above 276ml/g by day twenty of the experiment. Solids losses from the reactor the at this point meant that the experiment was terminated. At the other two remaining feed strengths (BOD = 287mg/l and 364mg/l) SSVI values reached 230ml/g and 240ml/g respectively by

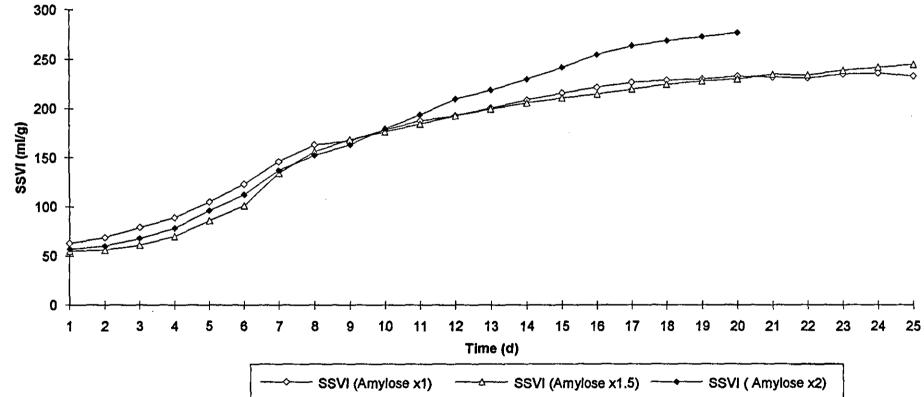
day 25 when the experiment was terminated (Figure 5.8) Microscopically the sludge from the amylose fed fully mixed reactor showed extensive filamentous growth. An explanation of the observation of a rapid deterioration in the settlement characteristic of the sludge is that the continued slow hydrolysis of amylose resulted in the slow release of easilly assimilatable BOD creating a low uniform concentration of available substrate throughout the reactor. This low uniform concentration of substrate gave the filamentous organisms a selective advantage and allowed them to flourish (Thomlinson 1982, Eikelboom 1982).

#### 5.5.3 Reactor 2 (SBR)

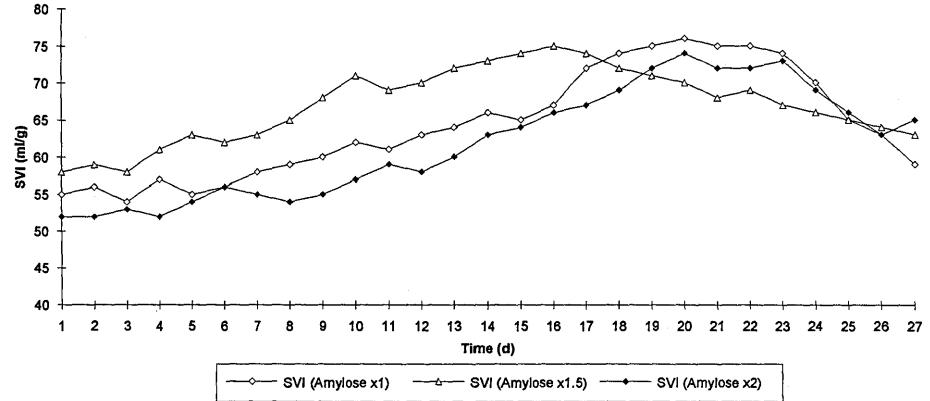
When Reactor 2 (SBR) was fed with the three different strengths of amylose feed, biomass concentrations of 1.8, 2.1 and 2.2g/l were supported resulting in F:M Ratios of 0.18, 0.19and 0.24 respectively (full loading data is shown in Table 5.2). SVI values at all three feed concentrations remained relatively constant throughout the 27 day experiment (Figure 5.9). At the lowest feed concentration (BOD = 287mg/l) settlement, from the initial SVI value of 55ml/g on day one of the experiment was terminated on day 27. A very similar pattern of sludge settlement was repeated at the two higher feed strengths (BOD = 364mg/l and 462mg/l) with only minor fluctuations occuring during the experimental period. Again there was no observable alterations in the number of filamentous organisms within the sludge make up at any of the three feed concentrations used. This result again shows that changes in sludge settlement may be attributal to both a combination of reactor configuration and the type of substrate.

## 5.5.4 Amylopectin

As previously mentioned amylopectin is a constituent of starch containing relatively short chains of glucose residues in  $\alpha$ -1-4-linkages. In addition to this, it contains branches that involve  $\alpha$ -1-6-glycosidic linkages. It is these glycosidic likages that have to be hydrolysed before amylopectin can be utilised as a source of glucose. Experiments using amylopectin as the sole carbon source were carried out again at three different feed concentrations in both reactors 1 + 2.



## Figure 5.8 Amylose Feed In Reactor 1(Fully Mixed)



# Figure 5.9 Amylose Feed In Reactor 2 (SBR)

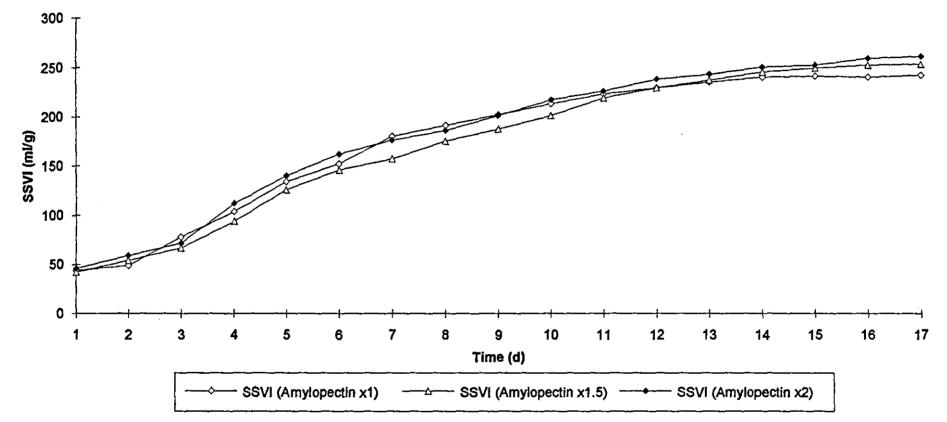


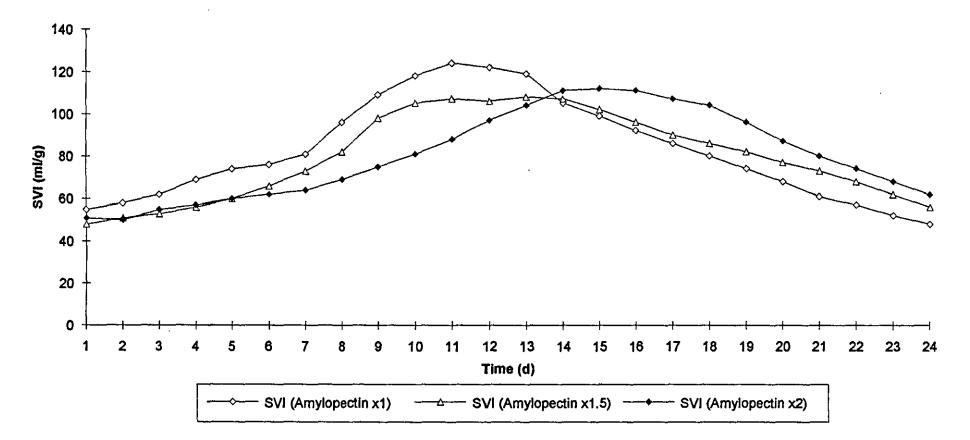
Figure 5.10 Amylopectin Feed In Reactor 1 (Fully Mixed)

#### 5.5.5 Reactor 1 (Fully Mixed)

The three different strengths of amylopectin feed (X1, X1.5 and X2) were able to support biomass concentrations of 2, 2.2 and 2.3 g/l MLSS respectively leading to F:M ratios of 0.24, 0.32 and 0.39 within the fully mixed reactor. The effect of the amylopectin feed at all three strengths on settlement is shown in Figure 5.10. At all three feed concentrations SSVI values rose rapidly from their initial low starting values of approximately 45ml/g. to above 150ml/g within the first five days of the experiment with both the highest and intermediate strength feeds producing SSVI values in excess of 250ml/g by day seventeen of the experiment when the tests were terminated due to excessive solids loss from the reactor.

5.5.6 Reactor 2 (SBR)

These experiments using amylopectin were then repeated in the Sequencing Batch Reactor and resulted in biomass concentrations of 2, 2.2 and 2.3 at the three increasing feed strengths respectively (full loading data is shown in Table 5.2). In contrast to the effect of this feed in the Fully Mixed Reactor sludge settlement rates did not deteriorate and no observable increase in the numbers of filamentous organisms present in the sludge occured. Sludge settlement rates deteriorated initially and at the lowest strength feed reached 124ml/g before gradually decreasing until day 24 when the SVI value had reduced to 48ml/g when the experiment was terminated (see Figure 5.11). This pattern of deteriorating sludge settlement rates in the early stages of the experiment followed by a gradual improvement in sludge settlement until the end of the experimental period was repeated at the two higher feed strengths. These may be linked to the induction of enzyme production before rapid adsorption can take place.



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Figure 5.11 Amylopectin Feed In Reactor 2 (SBR)

#### 5.5.7 Pea Effluent (starch)

The next experiments were carried out using with pea effluent ( a description of how this was made up is described in Chapter 4). This feed was used to mimic the effluent being produced at the full scale plant reported in this study. In addition to this Verachtert et al.(1980) reported that an intermittently fed activated sludge system when fed with soluble starch, sludge settlement rates deteriorated while when the same system was fed with glucose a good settling sludge was produced. The main constituent of this feed was starch which is released from the peas while being boiled. As previously mentioned, because of the size of the starch molecule it cannot be absorbed directly into the bacterial cell so it first must be hydrolysed by extracellular enzymes.

## 5.5.8 Reactor 1 (Fully Mixed)

The next series of experiments were performed to study the effect of pea effluent (starch) on sludge settlement within Reactor 1(Fully Mixed reactor). Loading data for pea effluent at the three different strengths used are shown in Table 5.1, with pea effluent x1.0, x1.5 and x2.0 being able to support biomass concentrations of 3.8, 4.1 and 4.5 g/l respectively leading to F:M ratios within the reactor of 0.87, 1.06 and 1.01. In all the experimentation using pea effluent in the continuously fed fully mixed reactor a rapid deterioration in sludge settlement occcured. SSVI values from daily sludge settlement tests are shown in Figure 5.12 with initial values of approximately 55ml/g for each of the three fed strengths used, rapidly increasing to above 200ml/g by day eight of the studies. These values were allowed to continue to increase until day 14 when solids started to be lost from the system and the experiments were terminated. Microscopic examination of the biomass from all the studies using pea effluent showed a corresponding increase in filamentous bacterial numbers within the biomass as sludge settlement deteriorated. Morphological characteristics along with staining techniques were used to show the causative organism showed no-branching, was coiled, non-motile, Gram positive and Neisser positive leading this organism to be identified as *M. parvicella*. The

results found when using pea effluent as a feed source are similar to those found by Verachtert et al. (1980) who found sludge settlement deteriorated rapidly when a starch feed was used in a continuously fed system. Verachtert and his co-workers attributed this to the slow hydrolysis of starch and hence the absence of any concentration gradient of substrate that could be absorbed by the biomass. Unfortunately the causative organism in the study carried out by Verachtert et.al. was not identified as *M. parvicella* has been identified to be associated with low F:M bulking (i.e. plants lacking any substrate concentration gradient).

#### 5.5.9 Reactor 2 (SBR)

The above experiments using pea starch feed were then repeated in the sequencing batch reactor. Loading data for the pea effluent at the three different strengths used are shown in Table 5.2. with pea effluent x1.0, x1.5, and x2.0 being able to support biomass concentrations of 4.0, 4.3 and 4.5g/l leading to F:M ratios of 0.43, 0.53 and 0.53. As in the experimentation using amylose and amylopectin in the SBR, sludge settlement values remained low except for a brief increase in sludge settlement values over the first nine days of the study (Figure 5.13). Following this sludge settlement remained low with SVI with values by day 22 reaching approximately 70ml/g some ten units below the initial settlement levels at the start of the experiment. Daily microscopic observations were made throughout the above studies and no disernable increase in the number of filamentous organisms within the biomass were observed. These results appear to contradict those reported by Verachtert et al. (1980) who found when fed with starch intermittently fed systems produced a sludge dominated by filamentous organisms well within the duration of the experiments above. However, as Gostick (1991) who studied both the effects of boiled starch and raw starch on sludge settlement points out even the same starch can have a varying biodegrability depending on its physical state. Similar results were achieved with pea effluent (starch) which was of course extracted by boiling as Gostick (1991) reported when using boiled maize starch.

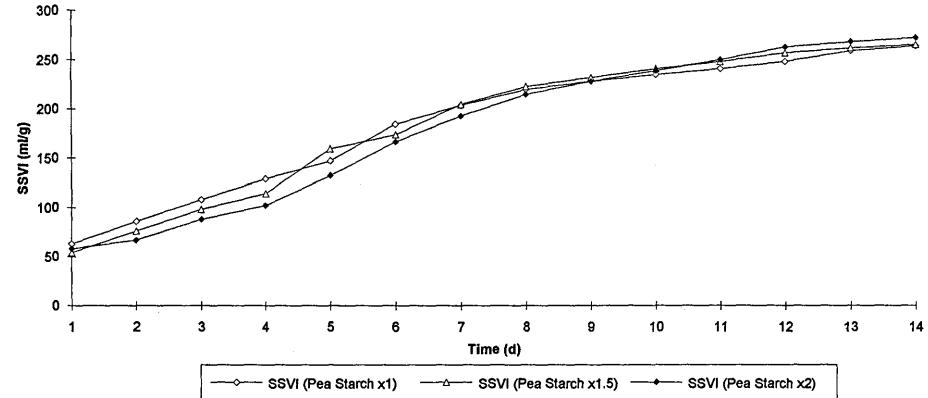
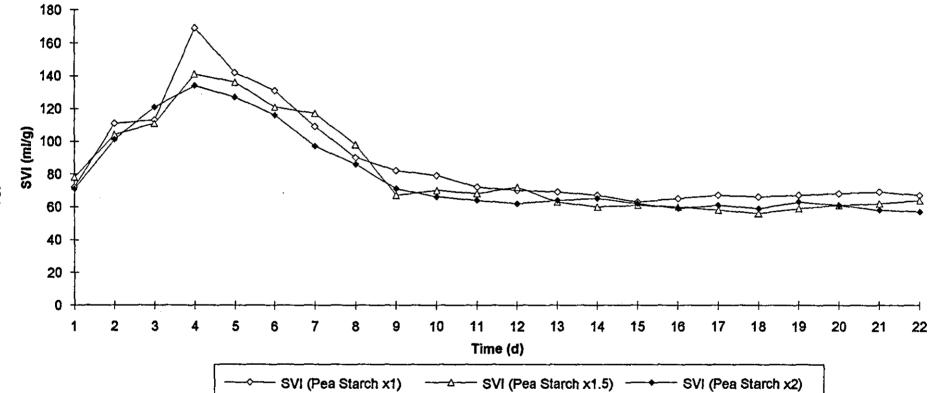


Figure 5.12 Pea Starch in Reactor 1 (Fully Mixed)



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Figure 5.13 Pea Starch In Reactor 2 (SBR)

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#### 5.6 Amino Acid Feeds

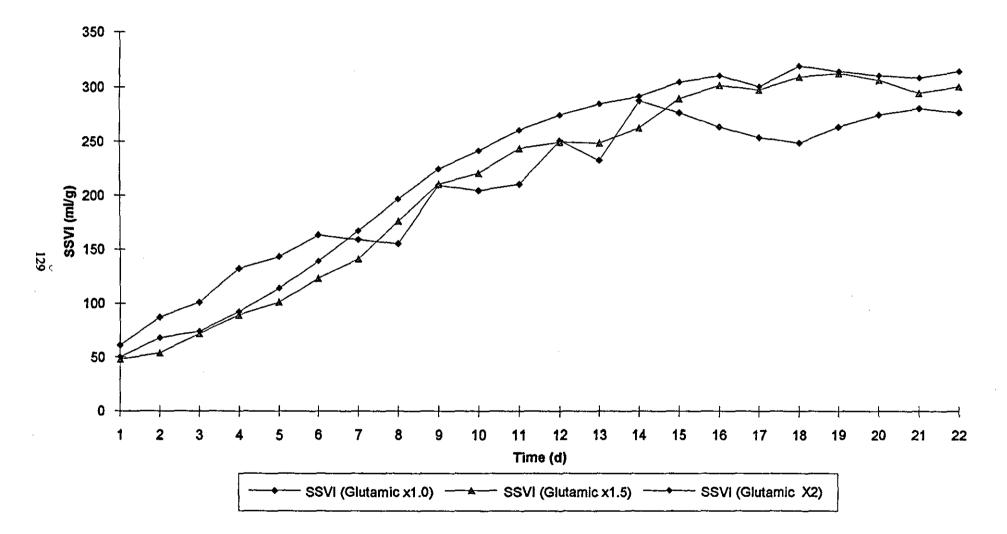
5.6.1The effect of amino acids on sludge settlement was studied in a series of experiments utilising Alanine, Glutamic Acid and Lysine, three of the twenty common naturally occuring amino acids which hence form building blocks in proteins present in dairy produce, blood products and agricultural crops (cereals and vegetables). Due to this these amino acids form part of the protein component of waste waters from the dairy, abattoir and vegetable processing industries. The three amino acids chosen in this study are  $\alpha$  amino acids which all have the same general structure, differing only in the identity and nature of the side chain. Alanine being the simplest with a simple aliphatic side chain (-CH<sub>3</sub>), Glutamic acid containing a carboxylic acid side chain (-CH<sub>2</sub>CH<sub>2</sub>-CO<sub>2</sub>H) and Lysine containing a strongly basic side chain (-(CH<sub>2</sub>)<sub>4</sub>-NH<sub>2</sub>).

#### 5.6.2 Reactor 1 (Fully Mixed Reactor)

A series of experiments were carried out to investigate the effect of amino acids as the sole organic substrate within Reactor 1 (Fully Mixed Reactor). Loading data for each amino acid at the three different strengths used are show in Table 5.1 along with the results of daily sludge settlement tests in Figure 5.14, 5.15 and 5.16 for Glutamic acid, Lysine and Alanine respectively. In all the experimentation using the three amino acids within the continuously fed fully mixed reactor a rapid deterioration in sludge settlement occured. This result was repeated at all three feed strengths for each amino acids (Alanine, Glutamic acid and Lysine ) with SSVI values reaching above 150ml/g within the first eight days of the experiment. These values continued to increase until day 22 of the experiment when SSVI values under all conditions tested reached above 250ml/g when the studies were terminated. Daily microscopic examination of the biomass from all the runs using amino acid feeds revealed a corresponding increase in filamentous bacteria number within the sludge as sludge settlement deteriorated. These observations in conjunction with staining techniques revealed the causative filamentous organism in all the above cases was unbranched, non-motile, with cross walls clearly visible,

Neisser negative and Gram negative leading this organism to be identified as *M. parvicella*. In addition to the problem of filamentous bulking, higher living organisms (Ciliates, Rotifera etc.) disappeared completely and this coincided with a cloudiness in the supernatant following settlement. This appeared to be due to deflocculated bacteria in suspension in the supernatant. This result is in disagreement with the findings of Verachtert et al.(1980) who when using casien as the sole organic substrate within a continuously fed system reported no increase in filament numbers within the biomass over the 17 day duration of their study. However, during this study these workers did report the development of a cloudiness in the effluent from the reactor. This was reported to be as the result of deflocculation which they attributed to be due to high protease activity of the sludge, induced by the casein feed.

Figure 5.14 Glutamic Acid In Reactor 1 (Fully Mixed)



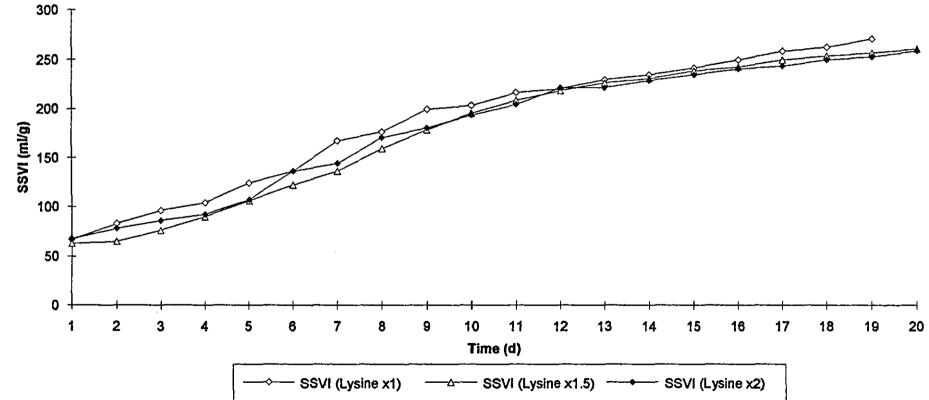
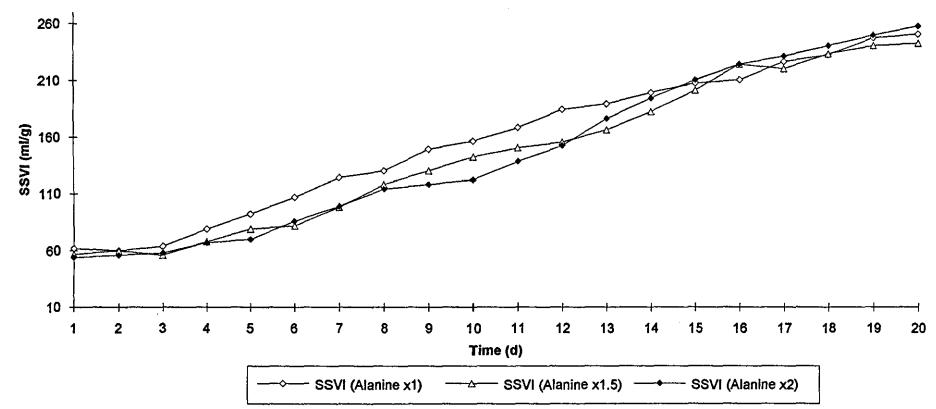


Figure 5.15 L-Lysine In Reactor 1 (Fully Mixed)





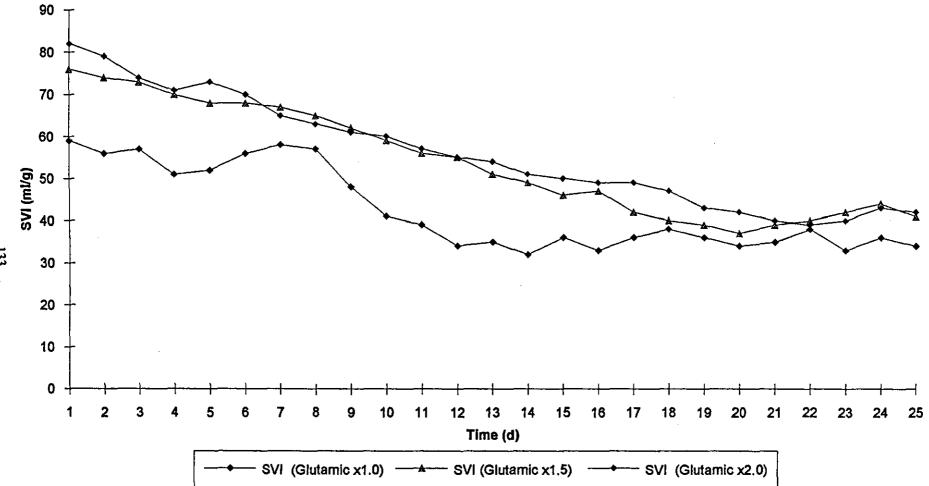
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#### 5.6.3 Reactor 2 (SBR)

The effect of amino acids as the sole organic substrate on sludge settlement was also studied in reactor 2 (SBR). Loading data for each amino acid studied at three different strengths are shown in Table 5.2 along with the results of daily sludge settlement tests in Figures 5.17, 5.18 and 5.19 for Glutamic acid, Lysine and Alanine respectively. As reported for Reactor 1 (fully mixed reactor) similar results were gained throughout all the experimentation using amino acids in the sequencing batch reactor. For both Glutamic acid (Figure 5.17) and Lysine (Figure 5.18) a slight improvement in sludge settlement was seen throughout the 22 days of the experiments, with SVI values reducing from 75-85 ml/g to approximately 40ml/g for Glutamic acid and approximately 55ml/g for Lysine. SVI values remained constant (Figure 5.19) for the entire study using Alanine as the sole organic substrate. Daily microscopic investigation of the biomass showed no increase in filament numbers from their initial low numbers at the the start of all the experimentation utilising amino acids in Reactor 2. However, as in the studies carried out in the continuously fed fully mixed system using amino acids higher living organisms dissappeared from the biomass and effluent became cloudy due to dispersed bacteria being washed from the system. These results are similar to those reported by Verachtert et al. (1980) who when studying the effect of casien as the sole organic substrate within an intermittently fed system found no discernable increase in filament numbers throughout the 17 day duration of their study. Defloculation was also reported by these workers which resulted in an opaque effluent from the reactor. However, no mention is made of higher living organism numbers within the biomass and deflocculation was attributed to high protease activity.

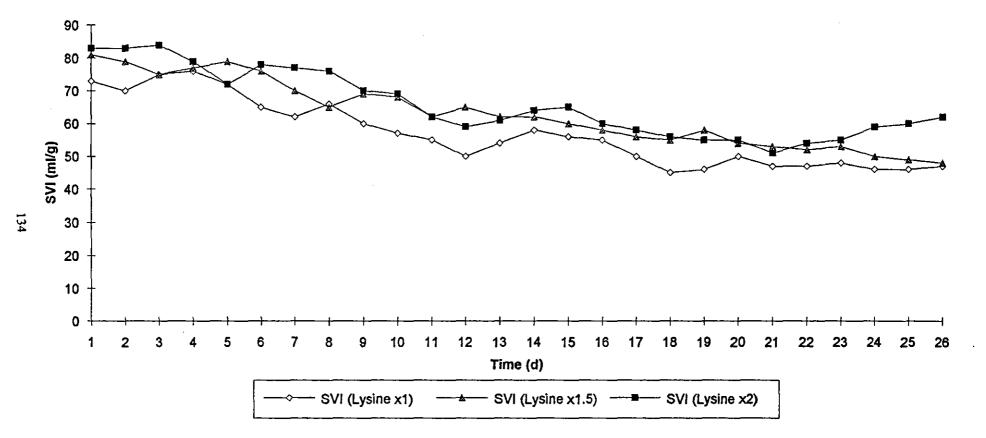
Sludge settlement from experiments using Amino acids as the sole organic substrate follow the standard pattern noted with other substrates used in this study and will be discussed in further detail in Section 5.7.3.

Figure 5.17 Glutamic Acid In Reactor 2 (SBR)



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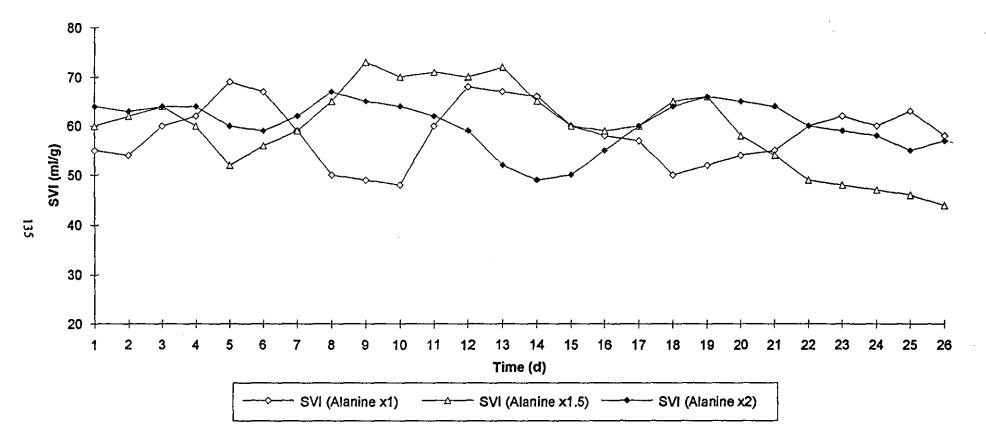


Figure 5.19 L - Alanine In Reactor 2 (SBR)

#### 5.7 Complete Feed

The composition of an artificial feed is detailed in Chapter Three. It's composition is based on OECD Standards Methods composition for an artificial sewage. This feed was used in an attempt to study the effect of a complete feed in the two reactor configurations used in this study. The reactor loading rates are shown in Tables 5.1(Fully Mixed) and 5.2 (SBR). As with all the previous feeds used, three different loading rates were employed by altering the the concentration of the feed, maintaining a constant volumetric loading rate and sludge age.

# 5.7.1 Reactor 1 (Fully Mixed Reactor)

The effect of this feed on sludge settlement is shown in Figure 5.20. At the lowest loading rate (BOD = 364mg/l) the biomass concentration stabalised at a concentration of 1.5g/l which resulted in an F:M ratio of 0.33gBOD/gMLSSday<sup>-1</sup> and a yield of 0.26 mgMLSS/mg BOD. As with all the experiments using the fully mixed reactor SSVI values steadily increased over the duration of the study with initial SSVI values rising from 56ml/g to 150 ml/g on day 25 of the experiment.

The intermediate and high strenghth feeds with BOD concentrations of 475 and 726 mg/l respectively were able to support biomass concentrations of 2 and 2.4 g/l and gave rise to the reactor operating at F:M ratios of 0.37 and 0.48mgBOD/mgMLSSday<sup>-1</sup>. These two higher feed concentrations produced similar results with sludge settlement deteriorating slightly more rapidly than with the lower strength feed. Using the intermediate strength feed SSVI values rose from 61ml/g on day one to 175ml/g on day 25 of the experiment while the high strength feed produced a slightly worse settling sludge (SSVI 190ml/g) by the end of the experiment. Microscopic examination of the biomass from the fully mixed reactor at all three feed strengths used in the study above revealed a gradual increase in the numbers of filamentous organisms within the sludge over the 25 day period of the experiment. the organism associated with the increase in sludge settlement values in this case was unbranched, non-motile with cross walls Neisser negative and

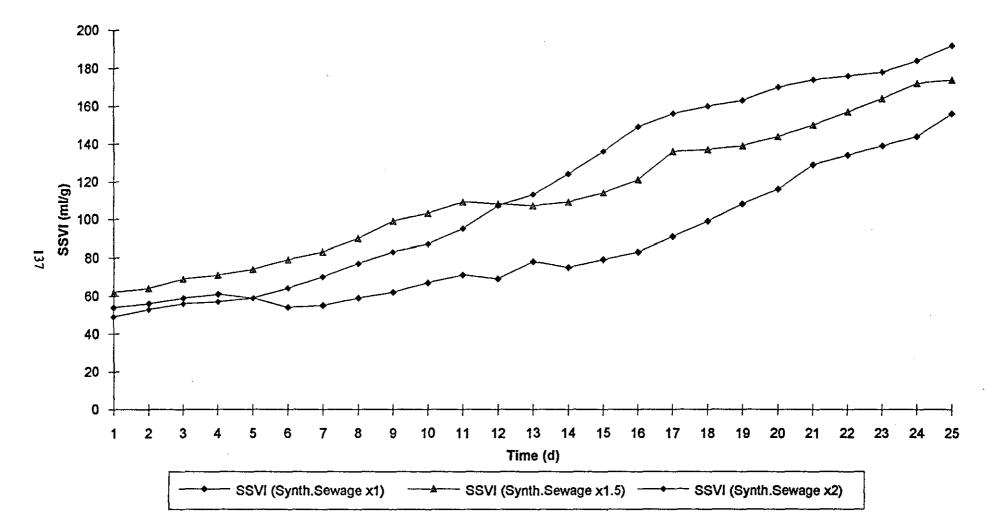


Figure 5.20 Synthetic Sewage In Reactor 1 (Fully Mixed)

Gram Negative leading this organism to be identified as Type 021N. The artificial sewage produced similar result to that of glucose in the fully mixed reactor which may not be unexpected as the main constituents in the artificial sewage are glucose and peptone. Although the causative organism was not identified with the glucose feed Type 021N did produce a deterioration in sludge settlement in the glucose fed SBR when aeration problems did ocurr.

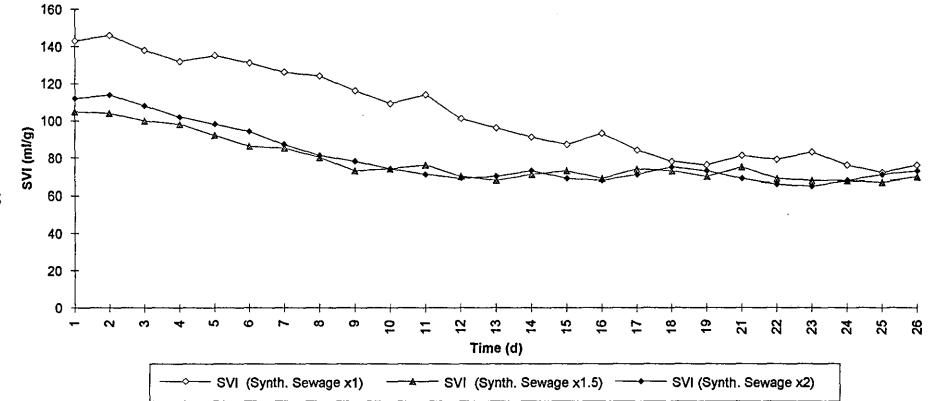
## 5.7.2 Reactor 2 SBR

The effect of the complete feed (synthetic sewage) on sludge settlement was also studied in Reactor 2 (SBR). Loading data, MLSS concentrations and yields for this experiment is shown in Table 5.2 along with the results of daily sludge settlement tests in Figure 5.21. Initial SVI values were high especially at the start of the run using the single strength synthetic sewage (SVI = 142ml/g) As in all the experiments in the sequencing batch reactor these initially high SVI values gradually fell throughout the length of the experiment with SVI values at all three strengths of feed being approximately 80ml/g by day 26 of the experiments.

Microscopic examination of the biomass was caried out on a daily basis. This again revealed a similar pattern at all three feed strengths with filament numbers reducing throughout the period of the experiment, which correlates with the improved rates of sludge settlement. At the end of the experiment little difference could be distinguished between the sludges produced at all three feed strengths, with all having good flocculent growth few filaments and abundant stalked and crawling cilliated protozans.

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Figure 5.21 Synthetic Sewage In Reactor 2 (SBR)



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#### 5.7.3 Comparison Between Reactor 1 (Fully Mixed) and Reactor 2 (SBR)

The parallel experiments carried out in the sequencing batch reactor and the fully mixed reactor reported above reveal a single clear result. That reactor configuration can have a significant effect on sludge settlement regardless of the substrate used as feed. As long ago as 1932 Donaldson noted that the degree of longitudinal mixing had an effect on sludge settlement. Since that time, many studies have further established the link between sludge settleability and reractor configuration (Chambers 1982, Chodoba et al. 1973a, Chiesa et al. 1985). As previously mentioned Verachtert et al. (1980) and Van den Eynde et al. (1982) observed sludge settlement rates improved regardless of substrate when fed intermittently rather than continuously. Both these workers attributed these findings to the varying growth rates of floc forming and filamentous organisms at different substrate concentrations. The present study has involved the use of both a continuously fed fully mixed system and a Sequencing batch reactor. As reported in section 5.1.1 the first experiments were carried out to confirm the degree of mixing within the laboratory scale fully mixed reactor. As a result of this it was possible to calculate a dispersion number for this reactor  $(D/\mu l = 0.36)$ . This relatively large dispersion number indicates a substantial degree of mixing and hence little chance of the formation of a substrate concentration gradient in the aeration zone. Section 5.2.1 describes how the sequencing batch reactor mimics the conditions of a plug flow reactor by creating a temporal rather than spacial substrate concentration gradient within the reactor. These two mixing extremes therefore create uniform or continually decreasing conditions with regard to substrate which the floc experiences during its passage through the aeration zone. The results gained using the fully mixed reactor and the SBR therefore confirm substrate concentration in the aeration zone may govern the composition of activated sludge on the basis of kinetic selection. This kinetic selection is based around the phenomenon of cross over Monod Kinetics as reported by Van Nierkirk (1987a) with this being further developed into a kinetic selection theory by Chudoba et al. (1973b). This kinetic selection theory would predict that if the return activated sludge is mixed in such a

way that a substrate concentration gradient is formed between the inlet and outlet of the reactor, then floc formers can gain an advantage over filamentous organisms. It would appear from the results gained that the two reactor configurations used in this study confirm part of the basis of this kinetic selection theory, that reactor configuration creating a substrate gradient rather than a uniform substarte concentration within the aeration zone enhances the growth of zoogleal bacteria.

The activated sludge culture in reactors with substrate concentration gradients gain certain features which have been described as the "selector" effect by Gabb et al. (1988). These include:-

1. High rates of substrate consumption.

- 2. High oxygen (or generally:electron acceptor) uptake rate.
- 3. Enhanced growth of zoogleal bacteria.

Because of floc stratification a fourth feature can be added.

4. Increased metabolic diversity.

The next set of experiments reported were carried out in an attempt to establish if any of the above phenomenon were taking place and that by the use of the sequencing batch reactor the so called "Selector" effect had been created.

### 5.8 Oxygen Utilisation

As discussed in Section 2.10.5 selector theory revolves around the removal of substrate under conditions of high substrate concentration. The examination of nutritional relations in activated sludge cannot be dissociated from the wastewater/microrganism pair. In the food uptake process by bacteria, two phases can be distinguished :

• firstly the exogenous phase : contact between sludge and wastewater takes place, at this time, the substrate is outside the bacterial cell wall.

• secondly the endogenous phase : The substrate undergoes several metabolic alterations.

Pujol and Canler (1991) used biosorption as a guide to the relevance of installing a contact zone. Biosorption as previously mentioned in section 2.10.5 includes both adsorption and absorption phenomena occuring at the interface between floc and wastewater. This integrates the notion of uptake rate and accumulation capacity which play a major role in biological competition (Chiesa and Irvine 1985) and is linked to bacterial metabolism, substrate availability, floc loading and contact time. This measure of biosorption is a further development of substrate removal rate (SRR) which Van Nierkirk et al. (1987) related to sludge settleability. With readily biodegradable soluble substrates such as those used in this study the rapid uptake induces an increase in metabolic activity and increased oxygen or electron acceptor uptake rates. Measures of biosortion, substrate uptake rate and oxygen utilisation rate are all significant as measures of selector effect. However, it was decided to limit measurements to oxygen utilisation as a measure of substrate removal. This was due to OUR being a more sensitive measure of substrate uptake as substrate that has merely been adsorbed onto the floc rather than taken up by the biomass can be released back into solution. It is likely that this substrate would only be released into the microenvironment of the floc before absorption and would therefore not be detectable in the bulk liquor. It is also possible to compare OUR rates measured both in situ and in the laboratory from full scale experimentation carried out in this study which further supports the use of OUR.

Oxygen utilisation rates recorded in both the fully mixed and sequencing batch reactor are shown in Table 5.3. and Table 5.4 Table 5.3 shows a comparison between the peak OUR induced following feed cycle and the OUR measured at the end of the eight hour aeartion period (endogenous).

Substrate	Peak OUR (mgO <sub>2</sub> /gMLSS.h)	EndogenousOUR (mgO <sub>2</sub> g/MLSS.h)		
Glocose Feed (x1)	39.6	7.2		
Glucose Feed (x1.5)	36.4	6.4		
Glucose Feed (x2.0)	35.2	6.0		
Sucrose (x1.0)	38.4	5.2		
Sucrose (x2.0)	36.1	5.1		
Lactose (x1.0)	38.1	4.9		
Amylose (x1.5)	27.9	5.8		
Amylose (x2.0)	26.7	5.1		
Amylopectin (x1.0)	23.4	4.9		
Pea Effluent (1.0)	19.6	6.2		
Pea Effluent (x2.0)	21.2	5.9		

Table 5.3 Peak and Endogenous Oxygen Utilisation Rates of Sludges From The Sequencing Batch Reactor.

For comparison Table 5.4 shows oxygen utilisation rates of sludges from the fully mixed reactor using various feeds.

Substrate	Peak OUR (mgO <sub>2</sub> /gMLSS.h)				
Glocose Feed (x1)	10.4				
Glucose Feed (x1.5)	11.2				
Glucose Feed (x2.0)	9.9				
Sucrose (x1.0)	8.9				
Sucrose (x2.0)	9.2				
Lactose (x1.0)	10.5				
Amylose (x1.5)	8.2				
Amylose (x2.0)	8.4				
Amylopectin (x1.0)	7.6				
Pea Effluent (1.0)	6.6				
Pea Effluent (x2.0)	7.1				

Table 5.4 OURs Of Sludges from Reactor 1 (Fully Mixed Reactor)

All the above results for OURs gained with sludges from the fully mixed reactor are significantly lower than the peak OUR measured in the SBR for the same substrates. A common method of calculating the daily oxygen requirement for carbonaceous

removal is an equation in the form :

$$R = a B + b MV$$

where R is the daily oxygen requirement (kg/d), B is the substrate removed (kg BOD/day), M is the mixed liquor concentration and V is the aeration tank volume. The coefficient "a" is a measure of the mass of substrate oxidised and "b" is a measure of sludge respiration (Boon 1983). A similar method for estimating oxygen required in an oxidation ditch, which incorporated nitrification and denitrification, was developed by Johnstone and Carmichael(1982). The problem of selecting values for "a" and "b" have been reviewed by Vosloo (1973), who argued that both coefficients can vary between wide limits which depend on the sludge loading rate employed. Johnstone and Carmichael (1982) estimated the measure of sludge respiration to be equal to:

# 0.024rVM

Where r is equal to the endogenous respiration rate. This respiration rate they measured during the early hours of the morning on an oxidation ditch when incomming load fell to negligable quantities, was calculated to be 2.0 mgO<sub>2</sub>/gMLSS.hour at 10°C. The respiration rates measured at the end of the aeration period in the present study, although not as low as Johnstone and Carmichael's calculated endogenous respiration rate are within one or two orders of it. Randall et al. (1991) investigated OUR in a batch test in which a slug of raw sewage was added to activated sludge. Following the OUR over 24 hours with a respirometer of their own devising they reported that a slug of sewage enduced a peak OUR of  $15mgO_2/gMLLSS$ .hour which had dropped by approximately 50% after 6 hours and declined to  $3mgO_2/gMLSS$ .hour after 24 hours aeration. These observations are in close agreement with the endogenous respiration rates measured during this study at the end of the 8 hour aeration period within the sequencing batch reactor.

The above results are also in good agreement those reported by Van Nierkirk et al. (1987a) who also found differences in OUR between fully mixed systems with a substrate concentration gradient (SBR and Selector) systems. Biomass from SBR and selector systems showed peak oxygen utilisation rates (OUR) in excess of  $60 \text{mgO}_2/\text{gMLSS.h}$ , while biomass from their fully mixed system showed no such induced peak in oxygen utilisation.

Verachtert et al. (1980) reported similar findings when studying substrate removal rate and the oxygen utilisation rate in intermittently fed and completely mixed activated sludge systems when fed easily metabolisable substrates such as glucose and nutrient broth. However, with a starch substrate that promoted bulking even in an intermittenetly fed system they found a high SRR but the OUR only increased to 11.4 mgO<sub>2</sub>/gMLSS.hour from an endogenous respiration rate of 6.0 mg O<sub>2</sub>/gMLSS.hour. They considered this to be further evidence of the slow release of soluble BOD from adsorbed substrate. These observations are similar to the results gained during this study using pea effluent where peak OUR to endogenous OUR ratio was approximately 3:1.

From these results it is concluded that the low uniform subsrate concentration in the fully mixed system is insufficient to promote the growth of floc-forming organisms and hence they are out competed for this available substrate leading to the proliferation of filamentous organisms seen in all cases using the fully mixed apparatus. It can be concluded therefore that one of the criteria specified by Gabb et al. (1988) as an indicator of selector effect (that of high oxygen uptake rate) has been induced by the use of intermittent feeding using various feed substrates.

# 5.9 Boisorption and Accumulation Capacity

In an attempt to confirm a second feature of selector effect soluble COD was measured in both feed solutions and an filtered liquor samples following the short five minute feed cycle in the SBR. The results of this are shown in Table 5.5.

Substrate	Soluble COD in	Soluble COD	%age Soluble		
	Feed (mg/l)	Following Feed Cycle	COD Removal		
		(mg/l)			
Glucose(x1.5)	510	81	84.1		
Sucrose (x1.5)	684	114	83.3		
Lactose (x1.5)	596	123	80.4		
Amylose (x1.5)	542	159	71.7		
Amylopectin (x1.5)	491	167	66.0		
Pea Effluent (x1.5)	3248	1209	63.8		
Glutamic Acid (x1,5)	428	98	77.2		
Artificial Sewage (x1.5)	603	124	79.4		

 Table 5.5 Soluble COD Concentrations in Feeds and Following Five Minute Feed

 Cycle

It can be seen from the results above that a substantial amount (63-84%) of soluble substrate is removed from the bulk liquid even in the short time period of the feed cycle for all substrates used. It is also evident that the simple biodegradable substrates produce the highest soluble COD removal rates during the feed cycle. The monosaccharide feeds along with the disaccharides are absorbed by the biomass most rapidly as is the synthetic sewage which has a similar soluble substrate make up (glucose and peptone) to the monosaccharides. As molecular complexity increases the amount of soluble substrate removed decreases and hence the passage of soluble substrate onto the aeration phase is also decreased. The removal rates of soluble substrate for the simple degradable substrates are similar to those recommended by Wanner (1992a) who stated that 80-90% of soluble COD should be removed within a selector zone. The figures gained in the present study may also be distorted by the short time span of the feed period and given a longer period may lead to a higher percentage removal of the soluble substrate. It is evident from this study that even the less degradable substrates used are able to produce high substrate uptake levels in the sludge and may remove sufficient soluble substrate to indicate selector use as an appropriate control for filamentous bulking in plants treating such substrates.

Also of importance in the selection of floc forming organisms is the restoration of accumulation/storage capacity (Chudoba 1973a + b). To restore this accumulation/storage capacity there has to be sufficient retention time within the aeration zone for all adsorbed and absorbed material to be hydrolysed and assimilated by the biomass. It is for this reason that it is recommended that loading across the whole system does not exceed 0.3-0.5 kgBOD/kg MLSS.d (Chudoba and Wanner 1989, Eikelboom 1991). All laboratory experiments were carried out at loading rates in this range and therefore complete restoration of the accumulation capacity should have ocurred. Wanner (1994) further states that for aeration zones with a high dispersion number that soluble COD exiting the selector zone should not exceed the soluble COD concentration in the final effluent by more than 20-30mg/l. Soluble COD levels were never measured on final effluent in this study but total COD's of the final effluent were low with total COD removal in the systems being approximately 80-85%.

The above results all demonstarte the main principles of kinetic selection theory first proposed by Chudoba et al. in the 1970's, who states that the factors affecting the domination of a system by filamentous organisms are:

- composition of wastewater.
- actual concentration of dissolved oxygen in the aeration tank
- actual concentration of soluble substrate under which microorganisms grow
- hydraulic regime of the aeration tank (degree of axial mixing)
- degree of accumulation capacity regeneration
- control parameters of the system (sludge loading and age)

As reported in the literature review some studies have confirmed this kinetic selection theory in practice at full scale (Wheeler et al. 1984, Matsche 1982 Albertson 1991), while other workers have disputed it. For example Gabb et al. (1991) concluded that a selector had no effect on the supression of filamentous organisms on an intermittently fed system fed on a synthetic subtstrate with a high

proportion of glucose. This would also appear to contradict the findings of the current study as glucose when used in the intermittently fed system produced a good settling sludge with few filaments. The system used by Gabb et al. was however, grown on a feed composed of 75% glucose. Dohanyos et al. (1970) has previously shown that glucose removal capacity above 600mg/l (present study 400mg/l) stays constant and this may have affected the results reported by Gabb et al.(1991).

5.10 Metabolic Selection.

There are three main phases during the eight hour cycle of a sequencing batch reactor. These being the fill phase, the aeration phase and the settlement phase. During each of these separate phases the biomass present in the system experiences different conditions which may have an influence on the floc make up and hence sludge settlement. It was decided to monitor these conditions during each phase of sequencing batch reactor cycle and the parameter measured was oxidation reduction potential (ORP). The decision to use ORP was made due to the various conditions in each cycle. Two of the phases involve periods of low dissolved oxygen concentration. During these phases D.O. levels fall below 0.5 mg/l under which D.O. measurement becomes unreliable. Koch and Oldman (1985) noted ORP determinations to be easy to make, reliable and can be used to distinguish between different levels of repiratory acivity and also to identify the onset of nonrespiratory activity. ORP was monitored on a continuous basis within the reactors using a Ag/AgCl Redox electrode, calibrated according to the manufacturer's instructions and standard solutions with redox levels noted at various stages of the sequencing batch cycle. Table 5.6 shows ORP levels measured during the aeration phase, at the end of the settlement phase and the average reading at the end of the fill phase.

Substrate	ORP During	ORP at End Of	ORP at the End of the		
	Aeration Phase	Settlement (MeV)	Fill Phase (MeV)		
Glucose(x1.5)	177	166	98		
Sucrose (x1.5)	134	128	76		
Lactose (x1.5)	191	174	86		
Amylose (x1.5)	186	170	92		
Amylopectin (x1.5)	144	153	84		
Pea Effluent (x1.5)	186	159	73		
Glutamic Acid (x1,5)	182	175	96		
Artificial Sewage (x1.5)	148	146	72		

Table 5.6 ORP Levels During Various Phases in the SBR Cycle

It is obvious from the results shown in Table 5.6 that ORP levels remain relatively constant during both the aeration and the settlement phases of the SBR cycle. However, following the start of the fill cycle there is a sudden drop in ORP levels recorded. This is shown in Figure 5.22 which shows ORP measured against dissolved oxygen levels measured in the reactor during the three phases of the SBR.

Figure 5.22 and Table 5.6 show that ORP seems to be relatively independant of D.O. until D.O. concentration is completely depleted. This phenomenon was repeated with all substrates used in the present study. This suggests that ORP may be an appropriate measure to detect when respiration changes from aerobic to anaerobic. The results gained using ORP produced similar results to those of Koch and Oldman (1985) who during the pre-conditioning of sludge from the aerobic zone of a nitrifying plant noted a similar breakpoint when D.O. levels become completely depleted. It has been noted in sewage treatment plants with nitrification, denitrification and enhanced biological phosphorus removal, ORP can be used to assay the various states of the sludge (aerobic, anoxic and anaerobic) and for optimising aeration rates (Koch and Oldman 1985, Charpentier et al. 1989, de la

Menardiere et al. 1991). In the present study it was concluded that the increase in available substrate during the fill period produces an increase in aerobic respiration which led to the depletion of D.O. levels which was detected within the mixed liquor by a marked drop in ORP.

From the studies carried out and reported above it is evident that the biomass during the individual phases of the SBR is exposed to high and low substrate concentrations and to conditions of varying D.O. which has been indicated to produce an increase in the diversity of metabolic processes ocurring in the floc and hence unbalanced growth (Wanner 1994). This high substrate gradient induces high oxygen demand during the the fill period which lead to anoxic conditions in the reactor. Albertson (1987) concluded that a significant fraction of this available substrate can be utilised under anaerobic or anoxic conditions within the internal structure of the floc. And that these anaerobic and anoxic conditions within the internal structure of the floc will prevail even in aerated systems due to the high oxygen demand lowering the bulk D.O. outside the floc. It is this substrate gradient and hence high oxygen uptake and the creation of anoxic or anaerobic conditions that consequently lead to a combined effect of kinetic and metabolic selection.

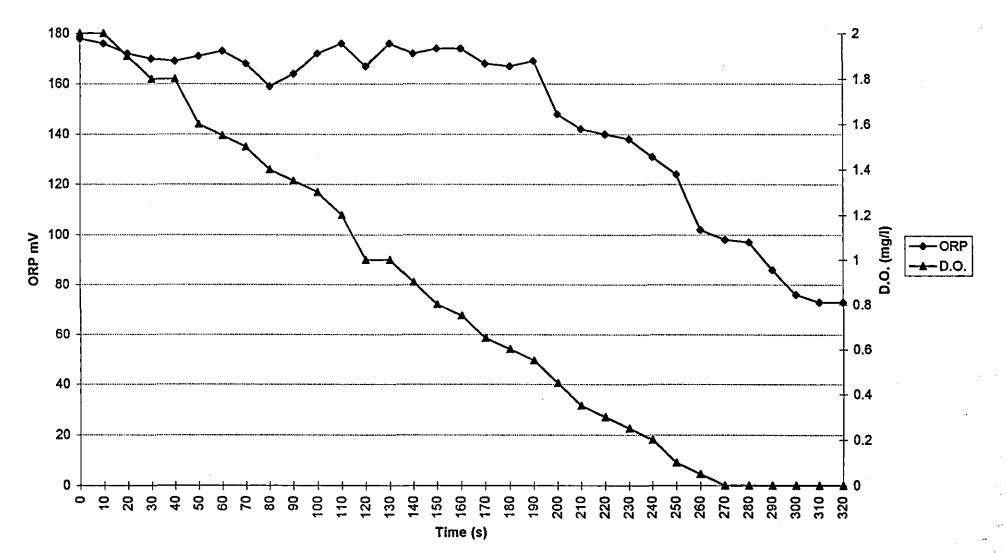


Figure 5.22 ORP Vs. D.O. During Feed Cycle of SBR

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### 5.11 Summary of Laboratory Results

At all feed concentrations and all different substrates used during this study the fully mixed reactor produced a poorly settling sludge due to the proliferation of filamentous organisms while the sequencing batch reactor produced a sludge that settled well with few filaments. This has been attributed to the different conditions that the biomass experiences in both these reactor systems. Substrate concentration is uniform within the fully mixed reactor, known to promote filamentous growth, while the SBR gave rise to a substrate gradient and hence altering conditions with respect to D.O.and substrate concentration during the three different phases of the SBR cycle. It can be concluded from the work above that all the substrates above were capable of producing the features known as the "selector" effect such as high substrate consumption, high oxygen uptake, enhanced growth of zoogleal bacteria and increased metabolic diversity. Due to these findings it can be concluded that the installation of an initial contact zone was the correct method for curing the bulking problems experienced anually at the full scale plant monitored as part of this study.

# 5.12 Full Scale Oxygen (VITOX) Activated Sludge Plant

Pea processing effluents present a particular problem as the onset of the short, ten to twelve week season is rapid and the effluent produced particularly strong (3-10gCOD/l). Start up of the plant and operation through the season requires constant monitoring of process performance parameters. This monitoring data from 1991, 1992, 1993 and 1994 pea processing seasons form part of this study.

5.12.1 Prior to the 1990 season

Prior to the beginning of the 1991 season, the performance of the plant over the previous two seasons were analysed. From this data it was decided that due to the excellent performance of the plant in 1990 (when no sludge settlement problems were experienced) to run the plant in a similar manner i.e. without the Biotower and use the pure oxygen activated sludge plant alone to cope with the hydraulic and organic loads produced by the factory.

Running the plant in 1990, by-passing the Biotower proved very successful with no filamentous bulking being observed throughout the season. This was thought to be mainly due to the resultant increase in F:M Ratio (0.22-0.47 kgCOD/kgMLSS.d<sup>-1</sup>) within the activated sludge plant. This increase in F:M Ratio was thought to have lead to increased growth rates of flocculant bacteria and the de-selection of filamentous organisms (Forster 1985).

There were additional factors considered when selecting this mode of operation these were as follows:

- The VITOX plant can degrade up to 1kgCOD/kgMLSS.d<sup>-1</sup> and consumes approximately 1-1.6 kgO<sub>2</sub>/kgCOD (0.62-1.0 kgCOD/kgO<sub>2</sub>).
- The pure oxygen (VITOX) activated sludge plant can effectively inject 5.9kgO2/m<sup>3</sup>.d<sup>-1</sup>.
- Peak production of 400 Tonnes of product per day and loads of 35kgCOD/Tonne result in a total waste load to plant of 14 TonneCOD.d<sup>-1</sup>.

- Primary treatment could reduce the organic load by 22% (average organics removal in primary settlement over past two seasons).
- Therefore, the expected loading rates to the VITOX plant would be 3.64kgCOD/m<sup>3</sup>.d-1 which could effectively be degraded by the available capacity of the activated sludge tank alone.
- 5.13. Seasons 1991-1994
- 5.13.1 The 1991 Season

In 1991 the activated sludge plant was operated in the same manner as the successful 1990 season. This ensured conditions that maintained an adequate dissolved oxygen concentration (1-3mg/l) a high F:M Ratio (average 0.79 kgCOD/kgMLSS.d-1) good mixing characteristics (All three Vitox pumps on) and a sludge age of 7 days. The plant was seeded prior to the start of the season with twenty loads  $(27m^3/load)$  of surplused activated sludge from the Potato and Allied Services plant Easton, Lincs. giving rise to a biomass concentration of 1.5mg/l.

Settlement indices (SSVI) for the 1991 season are shown in Figure 5.23. Although the SSVI values of the imported seed sludge were initially high (in excess of 100ml/g) these fell to approximately 60ml/g for the first 10 days of the season. From day 12 of the season sludge settlement indices start to rise rapidly with SSVI values being in excess of 150ml/g from approximately day 17 of the season. This rise in SSVI values coincided with a problem with dissolved oxygen control. This first occasion occurred on day 12 of the season when the biomass was starved of oxygen for about 7 hours (see chart recordings Figure 5.24). The effect on settlement was progressive and settlement indices continued to rise even after satisfactory concentrations of D.O >2mg/l had been restored. Previous work by Strom and Jenkins (1984); Wagner (1982); Pujol and Canler (1989) has already shown that dissolved oxygen concentration is one of the most important factors causing filamentous bulking. Oxygen levels within the activated sludge tank also became depleted for about five hours on day 18 and 7 hours on day 23 of the season.

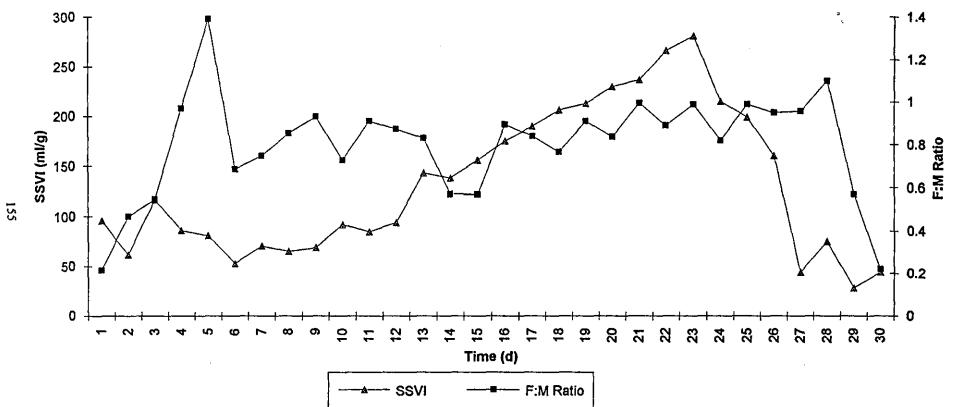


Figure 5. 23 SSVI and F:M Ratio Full Scale Plant (1991)

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Figure 5.24 Dissolved Oxygen Trace From D.O. Probe Showing Periods Of Oxygen Limitation.

Chart Speed=1cm per30min.

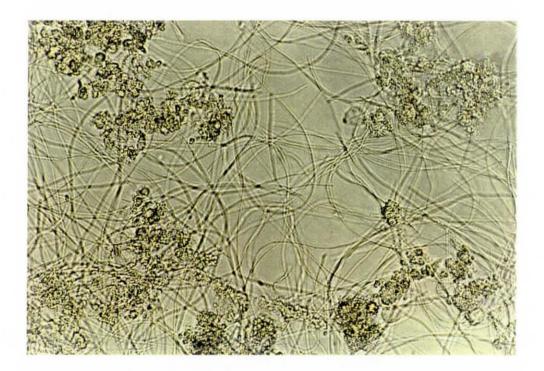
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However, on both of these latter two occasions oxygen deficit did not appear to have such a marked affect on sludge settlement. This was probably due to the already poor settlement characteristics of the sludge when these particular periods of oxygen starvation occurred.

Microscopic examination of the sludge was carried out on a daily basis throughout the season which confirmed the problem of poor sludge settlement to be due to the proliferation of a filamentous organism within the biomass. The organism was identified as Type 021N using the Eikelboom key (Eikelboom 1977). The bacteria was non-motile, sheathless slightly curled multi cellular bacteria. Plate 5.2 is a photo micrograph and shows the worst extent of the bulking during the 1991 season when SSVI values were in excess of 200ml/g with a large proportion of the biomass being composed almost solely of filamentous growth. Eikelboom (1977) reported in his study of some 200 activated sludge plants in the Netherlands that 021N was one of the most commonly observed filamentous organisms found in these plants. Eikelboom (1977) also associated Type 021N growth with industrial waste waters from the potato processing industry which produces a similar waste water to pea processing effluent with a high particulate starch content. This is further enforced by the work of Strom and Jenkins (1984) who studied 78 activated sludge plants in the United States. They found 021N to be one of the indicative filamentous organisms of low dissolved oxygen concentration and again one of the most common filamentous organism in activated sludge plants treating food processing waste waters. Gostick (1991) during studies also carried out on this plant found the causative organism in a bulking incident during 1988 to be Type 0092. However, this organism formed rope like structures when loading to the plant was reduced. These rope like structures have only previously been reported by Ziegler et al. (1990) in pure cultures of Type 021N. This indicates the difficulties in identifying filamentous organisms which is often a time consuming process. The proliferation of Type 021N in the current study was attributed to mixing and retention times within the system which gave rise to low instantaneous floc loading and a low general F:M ratio. This was confirmed by the low respiration rates recorded (Table 5.8). Following the 1991 season the following recommendations were made to improve process control of the pure oxygen activated sludge plant:

- Dissolved Oxygen concentrations within the activated sludge tank during the 1990 season were exercised through a single D.O. probe (No. 1 nearest the tank exit). D.O. control should be controlled through the average reading of the three D.O. probes situated within the tank. This would lead to a more representative measurement and better control of D.O. level within the activated sludge tank.
- Further mixing studies should be carried out on the activated sludge tank to assess the extent of mixing and pursue a better design and possible relocation of the H-Frame CO<sub>2</sub> stripper.
- Rates of sludge return and sludge wastage (RAS and SAS) should be accurately measured. This would lead to a more accurate control of sludge wastage and measurement of sludge age.

Plate 5.2 A Photomicrograph Showing the Extent of Filamentous Growth During 1992



# 5.13.2 The 1992 Season

Only one of these recommendations had been implemented by the start of the 1992 season. That being the alteration in D.O. control to run from an average of the three readings from the three D.O. probes within the activated sludge tank. As in the 1991 season it was intended to operate the plant under conditions that maintained an adequate dissolved oxygen level, a high F:M Ratio, good mixing and low sludge age. Figure 5.25 shows the SSVI values recorded throughout the 1992 season. It can be seen from this that sludge bulking occurred again during 1992 with SSVI values exceeding 150-ml/g by day 8 of the season. This again coincided with a period of oxygen limitation (about two hours due to operator error). From this point onwards SSVI values rarely fell below 200ml/g. These poor levels of sludge settlement caused many problems in operation and led to the final clarifiers being operated on a batch system to try to prevent solids loss from the system.

Due to poor sludge settlement there was a risk of river pollution due to a loss of solids from the system and a number of actions were consequently taken in an attempt to control filamentous growth within the biomass and restore normal operating procedures.

# 5.13.3 Reduced Sludge Age

The first of these was to increase sludge wastage from the system to reduce sludge age and increase the loading onto the plant in an attempt to give the flocculating organisms within the biomass a selective advantage over the filamentous organisms. This had no detectable effect on sludge settlement but was not aided by a reduced levels of production at the factory. In addition to the volumes of sludge wasted were restricted due to the low solids content of returned activated sludge and hence surplused activated sludge leading to the overloading of sludge handling facilities at the plant.

### 5.13.4 Addition of Trace Elements

Due to the successful use of D-Bulk (E and A West) during the 1988 season reported by Gostick (1991) and Simpson et al. (1990) it was decided to dose this

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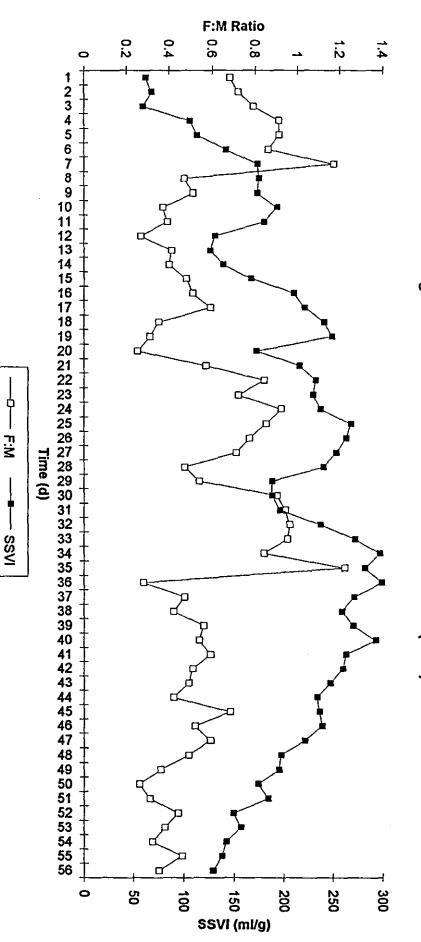


Figure 5.25 SSVI and F:M Ratio Full Scale Plant (1992)

. 091 product in an attempt to control filamentous growth. D-Bulks mode of action as suggested by its manufacturers is that it is correcting a trace element deficiency in the activated sludge. This is further elaborated by Simpson et al. (1990) that this trace nutrient deficiency gives filamentous bacteria a selective advantage as they are better able to absorb and assimilate these scarce nutrients due to their greater surface area. Hence when these trace elements are supplied this selective advantage is removed. Pujol and Canler (1989) also report on ten other occasions when Iron salts have been used to control filamentous bulking along with Al-Shawanni et al. (1987) and Chang et al. (1985) who have reported that Iron is inhibitory to the filamentous organism *Sphaerotilus natans*.

D-Bulk is mostly composed of Ferrous sulphate  $[Fe_2(SO_4)_3]$  which is likely to act as a coagulant with trace elements either added or present as contaminants from ferrous sulphate. D-Bulk was supplied in liquid form and dosed at a rate of 8mg Fe per litre activated sludge. Dosing with D-Bulk commenced on day 20 of the season and had little effect on sludge settlement or the number of filamentous organisms within the biomass. This is in total contrast with the results of both Simpson et al. (1990) and Gostick (1991) who both reported the successful use of D-Bulk in controlling filamentous bulking caused by Type 0092. In the study reported by both Simpson et al.(1990) and Gostick (1991) reporting on data collected in 1988, settlement indices were already reducing before D-Bulk was dosed and the further improvement in sludge settlement was attributed to D-Bulk. The data shown here as part of this research (Figure 5.25) from the 1992 season recorded on the same plant as Gostick reveal a similar rapid reduction in settlement indices at the end of the season. This rapid improvement in sludge settlement remains unexplained as condition were not altered prior to this reduction. However, organic loading rates on to the plant did reduce along with there being a more even pattern of loading as production at the factory dropped coinciding with this sudden reduction in SSVI values. No D-Bulk was dosed during this period of the 1992 season but reveals a similar rapid improvement in sludge settlement that was attributed to the dosing of D-Bulk by Simpson et al. (1990) and Gostick(1991).

# 5.13.5 Addition of Flocculants

The third action taken, was to dose flocculents. Polyelectrolyte was dosed into the overflow chamber from the Vitox plant prior to entry into the final clarifiers. The first polyelectrolyte to be used was Zetag 78 which was dosed to give an active concentration of 3ppm. This appeared to have little or no effect on sludge settlement within the plant although it improved sludge settlement dramatically in the laboratory. This polyelectrolyte was the changed to Magnafloc 1690 a cationic polyelectrolyte which again proved successful in improving sludge settlement within the laboratory, but again had little or no effect on the full scale works.

5.13.6 Recommendations Following the 1992 Season

Due to the poor performance of the activated sludge plant over these two seasons (1991 and 1992) caused by bulking sludge and the extra costs incurred due to the problematic operation of the works, further recommendations were made to improve the performance of the plant. These included :-

- 1. That a separate anoxic mixed selector zone be put in place prior to the completely mixed aeration tank. The retention time in this zone, based on waste water flow should be 30 minutes (60m<sup>3</sup>). This separate mixing zone will create an area of high floc loading and hence give floc forming organisms a selective advantage over filamentous organisms within the system. This has now become common practice within the waste water industry for the prevention of filamentous bulking within fully mixed activated sludge systems (Chambers 1982; Pujol and Canler 1992, Chudoba 1985)
- 2. No accurate measurement of sludge return or sludge wastage could be made on the works. This led to inaccurate measurement of sludge age an important control parameter within activated sludge systems. It was therefore recommended that flow measuring devices be installed to measure surplused and returned activated sludge.

The decision to implement the recommendations above came too late for them to be implemented for the start of the 1993 season.

### 5.13.7 The 1993 Season

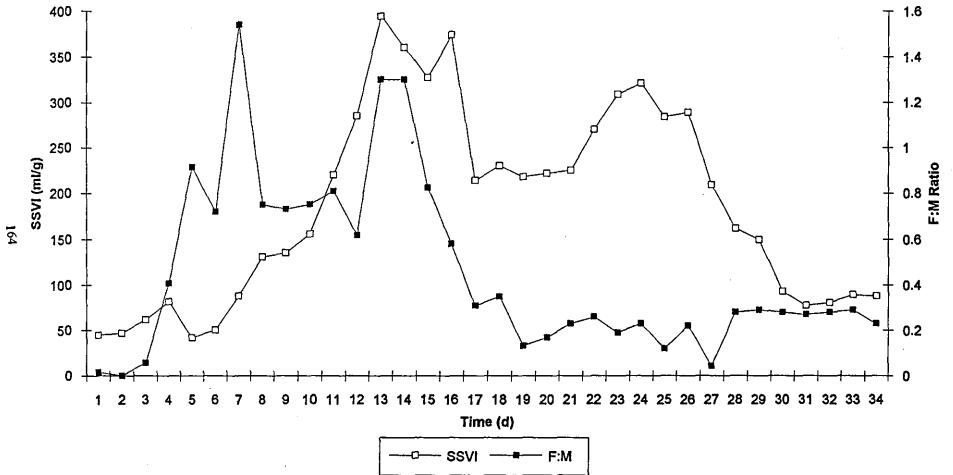
The performance of the activated sludge plant during the 1993 season was worse than in the previous two seasons. The season began with a problem of floating sludge in the final clarifiers, a problem not experienced before at the plant. Difficulties appeared to be caused by a hydraulic restriction in the pipes delivering activated sludge from the Vitox unit to the final clarifiers. This hydraulic problem caused the flow into the clarifiers to occur in surges rather than even flow. These surges were powerful enough to cause entrapment of air in the sludge entering the clarifiers. D.O. measurements taken within the clarifier revealed high dissolved oxygen levels (saturation in the stilling ring and 1.5-2.0 mg/l at the weir side). Attempts to improve this situation were made with air release pipes being fitted to the exits from the Vitox unit, polymers of various types being dosed. These measures reduced the problem with floating sludge but never eliminated it completely especially in periods of high flow.

Trying to cope with the hydraulic problems meant that less operator time was given to operating the Vitox unit and SSVI levels soon reached unprecedented levels. SSVI values recorded over the 1993 season along with the daily F:M Ratios are shown in Figure 5.26. From approximately day six of the season sludge settlement indices started to rise with SSVI values peaking at just below 400ml/g on day 13 of the season. No one factor could be attributed as a cause of this bulking incident but the combination of a bulking sludge and the hydraulic problem which was still being experienced made operation of the plant near impossible. Because of this it was decided to tanker waste water off site and treat it at other works in the area. This operation started on day 13 of the season and dramatically reduced the organic and hydraulic loading onto the plant. This can be seen in Figure 5.26 by the sudden drop in F:M ratios measured which however did not produce any significant alteration in sludge settlement.

## 5.13.8 Chlorine Dosing

As in the previous two seasons when filamentous bulking had become a problem various measures were taken in an attempt to de select the filamentous organisms

Figure 5.26 SSVI and F:M Ratio Full Scale Plant (1993)



causing the problem. In 1993 when SSVI value had reached an unprecedented level a decision was made to dose the activated sludge system with chlorine to selectively kill off the filamentous organisms present in the sludge (Jenkins et al. 1982). The use of chlorine has a record of success combating filamentous bulking particularly in the United States where most larger plants include the capability to chlorinate return sludge. Chlorination has been used in Europe (Bode 1983, Anon:Thames Water 1983) although only on a retrofit basis. The assumed mode of action is that chlorine is selectively toxic to filamentous organisms. This selectivity comes from the fact that filaments associated with bulking, grow out from the floc and into the surrounding liquid and due to their greater surface area are therefore more exposed and hence more rapidly impaired than the desirable floc formers (Chambers and Tomlinson 1981 ; Jenkins et al. 1982,1983 ; Wakefield and Slim 1988).

Chlorine, in the form of hypochlorite was dosed into the return activated sludge pumping station, one of the four recommended dosing points suggested by Jenkins et al. (1982). Excellent initial mixing of hypochlorite solution and RAS is vitally important. Reactions of chlorine with RAS can be rapid so without good mixing some parts of the RAS may not come in contact with any chlorine whilst a small part may become over dosed. Prolonged dosing at a point of poor mixing may result in the production of a turbid effluent and a reduction in treatment efficiency by killing off protozoa and other desirable organisms. The dosing commenced on the day 12 of the season. The initial dose was at a low concentration as recommended by Jenkins et al (1982, 1984) and Gray (1990). This was to prevent over dosing and the impairment of the metabolically active biomass within the sludge. The initial dose was set at 4mg/l active chlorine within the vitox plant and final clarifiers (the whole treatment plant volume) (2.67kgCl/tonne MLSSd<sup>-1</sup>). This appeared to have little effect on sludge settlement and the dose rate was increased to 6mg/l.(4kgCl/Tonne MLSSd<sup>-1</sup>). This was still a low dose when compared to the maximum recommended dose advised by Jenkins et al.(1982) of 15kgCl/Tonne d<sup>-1</sup>. The problem with dosing such a high concentration was that by dosing into a pump

sump, local chlorine concentrations could become too high and the selectivity of the dose could be lost. Nitrification rates and protozoan numbers were monitored during the dosing of chlorine as a reduction in these along with the production of a turbid effluent is indicative of overdosing and inhibition. The dose was increased on day 15 of the season and SSVI values rapidly dropped from in excess of 350 to 220ml/g. This dose was subsequently reduced because of fears that permanent damage could be caused to the biomass, but sludge settlement again began to rise and the dosing rate of 6mg/l was restored. By day 30 of the season SSVI values fallen below 100ml/g and the dosing of chlorine was terminated.

The effect of the chlorine on the extended filaments could be assessed microscopically. Cells could be seen to become detached from the cell walls and lysis of the cells occurred. Jenkins et al.(1982) report many occasions where chlorination has successfully controlled bulking at plants treating both domestic and industrial waste waters and due to a variety of filamentous organisms. The organisms combated in this way include Type 0041,Type 0092,Type 01701, Type 021N, Type 0581, *Spaerotilus natans, Microthrix parvicella* and *Thiothrix* sp. This list includes some of the causative organisms from previous years at this plant. Chlorine has been reported to be a universal biocide and can be used to combat most filamentous bulking incidents and proved extremely successful in rectifying the poor sludge settlement values experienced during the 1993 season.

5.13.9 Recommendations Following the 1993 Season

Following the 1993 season recommendations similar to those made after the 1992 season were made. These included:-

- 1. A separate anoxic mixed selector zone be installed to create an area of high floc loading prior to the completely mixed Vitox tank.
- 2. To reduce the surging problems experienced in the final clarifiers the head in the Vitox tank should be reduced.
- 3. Flow meters should be fitted so as to make it possible to measure return and surplused activated sludge rates.

### 5.13.11 The 1994 Season

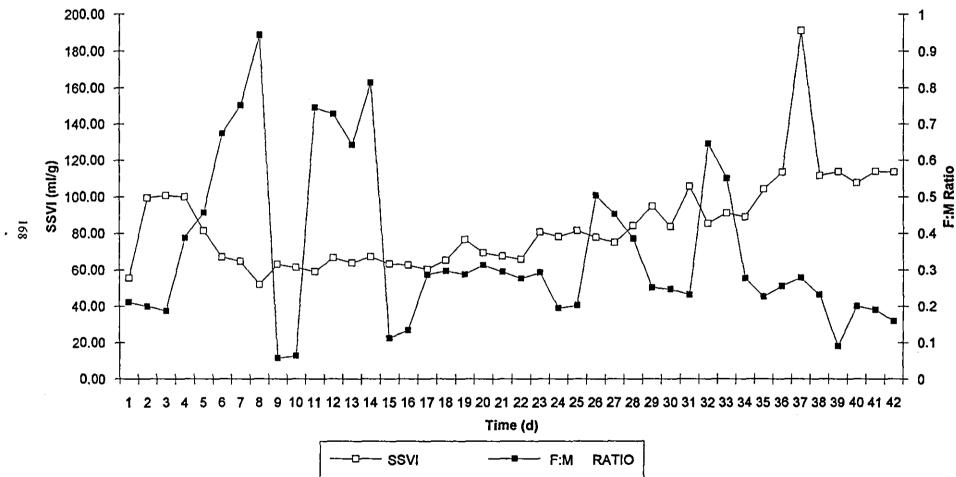
Due to the poor performance of the plant during the 1993 season and the recommendations made over the previous two years some major work was carried out prior to the 1994 season. This work included :-

- A separate anoxic mixed selector zone (60m<sup>3</sup>) was installed within the main aeration tank. All flow (forward feed and RAS) to the aeration tank passes through this selector
- Metal work within the primary settlement tanks had become severely corroded and needed replacing. New metal work was installed in an attempt to maximise the treatment capacity of the primary tanks.
- A magnetic flow meter was installed on the sludge return pipework.
- The head in the aeration tank was reduced by approximately 1 metre reducing the volume of the tank from 3136m3 to 2865m3.

Figure 5.27 shows sludge settlement in the form SSVI (ml/g) and F:M Ratios measured over the 1994 season. SSVI values rose from day 1 to peak at 120ml/g on day 12 of the season after which sludge settlement improved to between 70 and 80ml/g for the remainder of the season. Daily microscopic examination of the sludge revealed a health biomass with few filaments with a diverse population of higher life forms being present. This good settling sludge was maintained throughout the season and gave rise to good settling sludge. This was the first season since 1990 that bulking sludge had not been experienced at the Bourne plant.The successful operation of the plant during the 1994 season could be attributed to:-

- 1. The installation of the anoxic mixed selector.
- 2. Better plant monitoring ; monitoring was carried out on a four hourly basis during the 1994 season aiding in better control of operational parameters.
- 3. The installation of a flow measuring device on the sludge return pipework meant that sludge return rates could be monitored and controlled. This has never before been possible on this plant.

Figure 5.27 SSVI and F:M Ratio Full Scale Plant (1994)



4. No periods of oxygen limitation were experienced throughout the season. Oxygen limitation has been highlighted as the trigger of bulking in two of the previous three seasons.

# 5.14 Investigations Carried Out At Full Scale

Over the four seasons monitored for this thesis various additional controlled experiments and investigations were carried out into different aspects of the activated sludge process. These included mixing studies, dissolved oxygen surveys, oxygen transfer and utilisation studies. These were carried out in an attempt to gain a better understanding of the fundamental processes within the Pure oxygen (Vitox) activated sludge plant.

# 5.14.1 Dissolved Oxygen Survey

Numerous authors have reported that filamentous organisms have a growth advantage over floc forming organisms at low dissolved oxygen concentrations (Strom and Jenkins 1984, Hao et al. 1983,Lau et al. 1984, Richard et al. 1985, Palm et al. 1980). While Gabb et al. (1988 and 1991) demonstrated at laboratory scale that alternating anaerobic and aerobic conditions over short time scales consistently caused bulking. It was for this reason and that dissolved oxygen limitation had been indicated as the cause of bulking in two of the previous three seasons prior to the commencement of this study that a dissolved oxygen survey of the activated sludge tank was carried out. The aim of this study was to assess the dissolved oxygen concentration within the activated sludge tank and establish if any areas of low dissolved oxygen concentration exist within the tank and their extent.

The survey was conducted when the plant was operating at approximately 75% of its peak hydraulic and organic load. All available Vitox pumps were running continuously (total power input 205kW,  $55W/m^3$ ). Oxygen input from Vitox pumps 1 and 2 was D.O. controlled between 2-3 mg/l with an injection rate of 350kg O<sub>2</sub>/hour. Vitox pump No. 3 can not be D.O. controlled and was running continuously with an oxygen input of 80kg O<sub>2</sub>/hour.

Dissolved oxygen measurements were taken on a transect across the tank and then at a distance of approximately 1m from the tank wall. Measurements were taken at 1m intervals down to a maximum depth of 5m except in the conventionally aerated zone where the maximum depth sampled was limited to 3m.

Figure 5.28 shows the conditions found to prevail in the tank, with no indication of any low spots present with all measurements taken being above or within the range set on the control system of 2-3mg/l. This suggests an adequate level of both aeration and mixing throughout the tank. However, most sampling points had a lower dissolved oxygen concentration at increasing depth. This depth gradient implies that a significant amount of oxygen dissolution was occurring due to bubbles rising through the mixed liquor.

# 5.14.2 Oxygen Transfer Efficiency

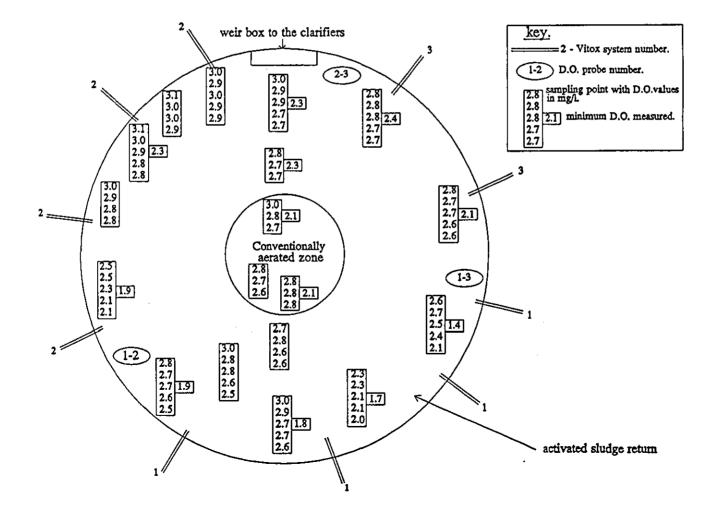
Kite and Garrett (1983) discussed the advantages of an oxygen venturi system. They describe a number of methods for the determination of oxygen transfer efficiency. Of these the most practical measure of efficiency of oxygen usage (o/c ratio) is a comparison of oxygen used and the biological treatment performance of the plant (Johnstone 1984; Kite and Garrett 1983). Oxygen usage and organic load removed within the Vitox system for 1990-1994 are presented in Table 5.7

Year	Average Daily Oxygen Usage (kg)	Average Daily COD Load Removed (kg)	% Efficiency (Use Of Oxygen) kgCOD/kgO <sub>2</sub>
1991	13.71	10.87	83
1992	10.28	6.71	70
1993	4.68	3.43	79
1994	4.01	3.72	93

Table 5.7	Average	Efficiency	of Oxygen	Usage
				- 0.

The results reveal some interesting trends with the second highest oxygen usage efficiency being measured during the1991 season.BOC design figures for the Vitox plant are 1kgO<sub>2</sub>/kgCOD removed (Kite and Garett 1983).The figure recorded in

# Figure 5.28 : Survey Of Dissolved Oxygen Conditions In The Activated Sludge Tank August 91



1991 represents 79% with respect to BOC design figures. The explanation for this high efficiency of oxygen usage is the high load and oxygen demand leading to increased efficiency of the equipment. The 1994 season reveals an even higher efficiency with 0.93 kgCOD removed/kgO<sub>2</sub> consumed. Most filamentous microorganisms are not able to use nitrate nitrogen as a terminal electron acceptor, at least with a rate enabling them to compete with floc-formers for substrate under anoxic conditions. It is evident from the higher efficiency recorded during the 1994 season that the use of nitrate, present in the return activated sludge, as a terminal electron acceptor within the anoxic contact zone did occur. The reduction of nitrate under anoxic conditions leading to a selective advantage of floc-formers was verified experimentally for the so called Oxic Zone Growers by Wanner and Grau (1989) and may be one of the factors preventing filamentous growth at this plant following the installation of the selector zone in 1994.

The problem with this method of assessing oxygen usage efficiency is that it takes no account of any oxygen given off from the top of the tank or the amount of oxygen used to oxidise ammonia to nitrate. A gas phase material balance procedure for oxygen transfer measurement was introduced by Redmon et al. (1983) and this method was used on the Vitox plant at Bourne in 1988 (Gostick et al. 1991). This indicated an oxygen transfer efficiency of 69% which is more comparable with observed nitrification rates measured during this study, when endogenous respiration and COD load treated by the plant are taken into account.

# 5.14.3 Respiration Rates

Respiration rate or oxygen utilisation rate (OUR) is a common method of measuring microbial activity at laboratory scale but may also give a guide to oxygen demand at full scale and has recently been recommended as a suitable parameter for process control (Shamas and Englande 1992). Two methods of measuring respiratation rates were used. These being an in situ measurement determined in the main oxygen activated sludge tank by raising the dissolved oxygen concentration to above 5.0mg/l then with no further oxygenation, measuring the reduction in dissolved oxygen with time and the other method using

a laboratory type respirometer previously described in Chapter 4. The results obtained from these studies are shown in Table 5.8

YEAR	Oxygen Uptake Rate (mgO <sub>2</sub> /gMLSS/h)
1991 Week 1,2,3,4,5,6,7, and 8	10.8, 21.5, 32.4, 27.9, 31.7, 24.6, 25.1, 17.8
1991 Average of 8 Readings	23.98
1992 Week 1,2,3,4,5,6,7, and 8	36.2, 32.8,42.2, 36.4, 31.0, 29.5, 28.4, 34.7, 39.8
1992 Average of 8 Readings	27.5
1993 Week 1,2,3,4,5,6,7 and 8	26.3, 28.7, 32.2, 28.9, 30.0, 24.4, 29.7, 20.
1993 Average of 8 Readings	38.8
1994 Week 1,2,3,4,5,6,7 and 8	27.5, 22.4, 28.7, 24.3, 21.1, 19.7, 27.5, 22.3
1994 Average of 8 Readings	24.2

 Table 5.8 In Situ Oxygen Usage Rates

Table 5.9 Oxygen Uptake Rate Measured in Laboratory Respirometer (1990-1994)	Table 5.9 Oxygen	I Uptake Rate Measured in	n Laboratory Respiromet	er (1990-1994)
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Sample Point	1991 (mgO <sub>2</sub> /gMLSS/h)	1992 (mgO <sub>2</sub> /gMLSS/h)	1993 (mgO <sub>2</sub> /gMLSS/h)	1994 (mgO <sub>2</sub> /gMLSS/h)
Adjacent to Ras	267.8	332.1	264.3	NA
and Feed Inlet	301.4	285.9	287.5	
2m From RAS and	36.4	20.6	32.1	NA
Feed Inlet	28.9	18.6	35.7	
4m From RAS and Feed Inlet	9.6	7.6	8.9	12.8
	7.8	6.9	7.2	14.7
6m From RAS and Feed Inlet	7.4	6.8	6.8	11.7
	6.9	7.9	5.1	10.7
8m From RAS and Feed Inlet	5.6	5.7	6.3	9.7
	5.4	6.1	7.2	8.9
10m From RAS and Feed Inlet	5.7	6.4	5.9	9.5
	6.3	5.8	4.8	10.7

The first point to be made from the results in Tables 5.8 and 5.9 is that the oxygen uptake rate determined in situ are on average 4-6 times greater than those determined by the conventional laboratory respirometer. This finding is similar to those presented by Erasin et al. (1992) who also found differences in results measured in situ and results measured in the laboratory. There are three principle reasons for this, firstly losses of oxygen from the system when measuring respiration in situ are not taken into consideration and of course do not occur in the laboratory respirometer, secondly the in situ measurements are averages of a large range of respiration rates taking place throughout the tank volume and thirdly removing biomass from its normal environment will reduce its activity.

The large range of respiration rates from various sites around the perimeter of the tank (Table 5.9) may suggest that the mixing within the tank is not perfect. However, exit curve distribution studies (Levenspiel 1962 ; Chambers and Thomlinson 1982) were carried out in both 1992 and 1994 and revealed the tank to be well mixed (see section 5.15.4). Many authors have identified filamentous types that are associated with the treatment of particular industrial effluents (Eikelboom 1977; Strom and Jenkins 1984) who concluded these differences to be due to variation in the organic fraction of the waste water being treated. It has also been proved that some kinds of particulate substrates can support the growth of filamentous organisms. For example fats and greases support the growth of nocardioform actinomycetes and long chain fatty acids have been reported to support Spaerotilus natans (Slijkhuis and Deinema 1982). This theory was further developed by Dold (1980) who introduced the Bi-substrate hypothesis which divided the organic substrate into two groups. These being the easily metabolisable soluble materials and the particulate materials which comprise of large-molecule organic compounds which are present in waste waters as colloids or suspended solids.

The results gained during this study may have been distorted by the ratio of easily metabolised substrate exerting an instantaneous oxygen demand compared to adsorbed particulate substrate exerting a lower oxygen demand due to its slow

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break down and assimilation. The notion of biosorption was introduced by Eikelboom in 1982, who suggested that immediately after contact between sludge and waste water there is a direct substrate uptake (easily metabolised substrate) which may exert a large oxygen demand and in addition to this other physical processes such as adsorption by the floc or entrapment of larger particles in the open structure of the floc. These physical processes are the first stage in the slow breakdown and assimilation of this particulate material and exert a reduced but sustained oxygen demand throughout the sludge's time within the aeration tank.

The governing characteristic of particulate substrates in terms of the IAWPRC Task Group Model (Dold and Marais 1986) is that they need to be hydrolysed prior to being utilised by the cell. Starch is just such an organic substrate. It can be seen that there is a substantial oxygen demand created in the area adjacent to the RAS and feed inlet to the aeration tank. This would suggest that the effluent does contain a considerable amount of easily assimilated material. However, due to the complete mixing characteristics of the plant this easily assimilated material is rapidly dispersed throughout the tank volume and high biosorption rates are not sustained to give floc forming organisms a growth advantage. It has been suggested on work carried out on contact zones (Pujol and Boutin 1989) that biosorption rates in excess of 100mgCOD/gSS are required to suppress filamentous growth and biosorption measurements can serve as an indicator to the relevance of installing a contact zone.

It is evident from the respiration rates recorded that the aeration tank prior to the 1994 season was as designed, a fully mixed tank and as such provided an almost uniform concentration of substrate throughout the whole tank volume. In addition, due to the nature of the effluent being treated there was a slow breakdown of the particulate substrate in the aeration tank and hence maintaining a low concentration of substrate within the floc environment throughout the aeration tank leading to filamentous organisms having a growth advantage.

It can be concluded from the results above that :-

- 1. The tank side respiration rates measured conventionally did not compare well with the in situ measurements made.
- 2. The low tank side respiration rates measured indicate a low concentration of available substrate. While the high activity region around the feed and sludge inlet gave insufficient protection against bulking.
- 3. Due to the large size of the Vitox plant and large variations in flow being treated, hydraulic retention times within the system are high (> 24 hours), which subsequently produces a low instantaneous floc loading.
- 5.14.4 The Initial Contact Zone (ICZ)

As previously stated, due to the poor performance of the activated sludge plant over the first two seasons reported in this study, it was recommended that a Initial Contact Zone (ICZ) be installed prior to the main activated sludge aeration tank. The installation of initial contact zones has now become common practice in the control of filamentous organisms with Pujol and Canler (1994) reporting a 91% success rate following the installation of contact zones for the control of filamentous bulking in 12 extended aeration plants treating population equivalents ranging from 600 - 30,000 in France.

The initial contact zone was installed prior to the 1994 season. It was decided that the contact zone would be installed within the main aeration tank and all the flow entering the Vitox tank (RAS and waste flow) would pass through this zone. Following the recommendations of Albertson (1991) it was decided that this initial contact zone should be operated at substantially lower available oxygen levels than the oxygen demand within the aeration zone. Hence, no aeration was supplied to this tank and according to design criteria the selector zone installed would fall into the anoxic category as stipulated by Albertson (1991). The initial contact zone was sized to afford a hydraulic retention time within the zone of 10 minutes (volume 60m3) at peak flow rate plus sludge return flow with the retention time in this initial contact zone being about 1/2 hour on average. These figures correspond well to those recommended by Pujol and Canler (1994) and are now considered to be good design practice in the United Sates (Hsu and Wilson 1992). However, the main design criteria remains that the contact time within the ICZ should be sufficient to allow the absorption of the majority of soluble substrate and prevent its subsequent breakthrough to the main aeration vessel where it may support the growth of filamentous organisms. While if the contact time is too long floc-forming and filamentous organisms have equal opportunity to compete for this readily available substrate in the contact zone.

# 5.15 Studies on the Initial Contact Zone (ICZ)

Following the installation of an initial contact zone within the main aeration tank prior to the 1994 season it was decided to carry out various studies on various parameters within the ICZ to gain a better understanding on the processes occurring.

# 5.15.1 Redox Potential

The initial contact zone installed prior to the 1994 season was designed to have no added air and operate under anoxic conditions. "Anaerobic", "anoxic" and "aerobic" are only quantitative descriptors and it is clear that the ORP of the system is a more precise measure of "anaerobicity" or "aerobicity". At low dissolved oxygen concentrations D.O. measurements become unreliable and at these levels a clear advantage in the use of ORP as a monitor may be gained.

During the 1994 season the oxidation reduction potential within the ICZ was monitored. The Redox potential was measured using a Phox instruments combination probe(Ag/AgCl-Redox electrode) suspended to a depth of 2 metres 3m from the inlet within the ICZ. Results from these measurements are shown in Figure 5.29. This figure shows redox potentials measured between 1st and 3rd of August with recording being taken every hour. The oxidation reduction potentials vary between -236 and -68 mV These are mainly within the specified range for an anoxic selector zone (-150 to 50mV). However, problems did occur with the probe due to it requiring frequent cleaning due to sludge becoming attached and blinding the probe. This attached growth may have lead to recorded readings being lower than the true readings as it was evident that the recorded redox potentials were

always higher following cleaning of the probe. This finding is similar to those of Koch and Oldman (1985) who recommended that cleaning of the probe should take place at least twice weekly and more frequently if fouling was suspected. This may

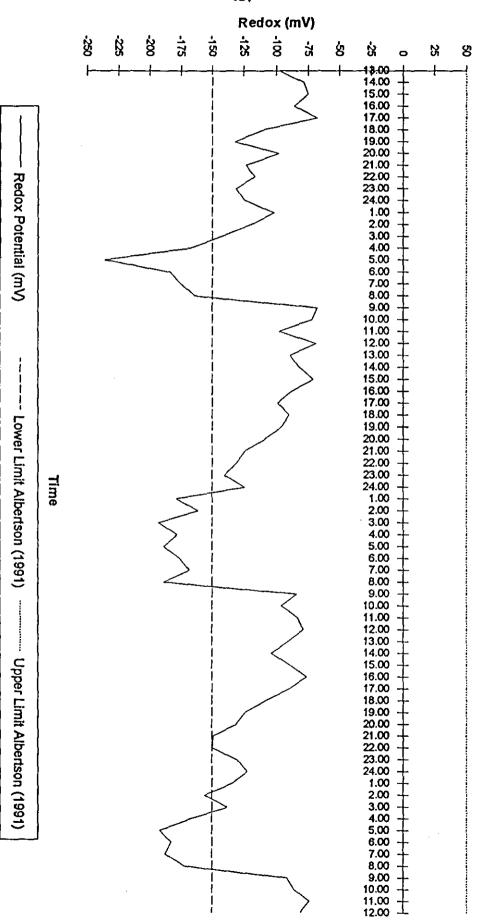


Figure 5.29 Redox Potential Within ICZ (01/08/94-03/08/94)

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go some way to explaining the low readings recorded which were below that of a normal anoxic contact zone. The majority of the results recorded confirm that the ICZ as designed meets the specification with regard to redox potential to be classed as anoxic (Albertson 1991). Wanner and Grau (1989) grouped filamentous organisms into assembliges according to their morphilogical, physiological and metabolic similarity, and describe their preferred envionment, the results gained by Wanner and Grau (1989) are summarised in Table 5.10

Table 5.10 Response of filamentous microorganisms to various kinetic and metabolic conditions (Wanner and Grau 1989).

Group	Organism Type	Oxic	Anoxic	Anaerobic
Oxic Zone Growers (S)	S. natans and Types 1701, 0041, 0675	~	~	×
Oxic Zone Grower (C)	Type 021N, Leucothrix, Thiothrix,	~	~	<b>√</b>
All Zone Growers (A)	Microthrix parvicella, Type 0092, Nostocodia limicola	0	0	0/+
Foam Forming Organisms	Nocardia	?	?/ ✓	√/?

 $\checkmark$  = Suppression 0 = No Effect ? = Effect Uncertain + = Stimulation

As previously stated the ICZ installed during this study meets the specification with regard to redox potential to be classed as anoxic (Albertson 1991). This categorization would according to Wanner and Grau (1989) suppress the both oxic zone growers (Sphaerotilus - like organisms) and oxic zone growers (Cyanophyte like organisms). The filamentous organism identified as the causative organisms during bulking periods in the years prior to the installation of the ICZ (Type 021N and a Sphaerotilus type organism) fall into both these categories. Since the installation of the ICZ within the aeration unit at Bourne there have been no

reported incidences of bulking, therefore supporting the findings of Wanner and Grau (1989) that anoxic selectors are capable of suppressing both these two types of organism.

The Oxidation Reduction Potential measurements recorded during the 1994 season are similar to those recorded by Schon et al. (1994) who studied the effect of ORP and D.O. on phosphate release and concluded that D.O. was the determining factor for phosphate release in the anaerobic stage of their laboratory scale reactor. Koch and Oldman (1985) have suggested that ORP can be used to detect the point that nitrate conditions become zero revealed by a sudden drop in ORP levels from approximately -100 to -300 mV. In addition ORP levels were shown to gradually decrease with decreasing nitrate concentrations. Experiments were carried out in the initial contact zone to see if any detectable change could be detected in ORP from the inlet to the outlet of the contact zone with the average results from five studies results being shown in Table 5.11.

Sample Point	ORP (mV)
At inlet to Contact Zone	-81
1 Metre From Inlet	-87
2 Metres From Inlet	-94
3 Metres From Inlet	-101
4 Metres From Inlet	-109
5 Metres From Inlet	-121

Table 5.11 ORP Measurements Across the Initial Contact Zone

It is evident that there is a drop in redox recordings across the tank which may correlate to an increase in anaerobic conditions brought about by a reduction in nitrate concentration across the contact tank. If this is a correct assumption then it may be possible to use ORP as a control for the optimisation of nitrate removal within anoxic contact zones and may be an area worthy of further research.

# 5.15.2 Substrate Take Up

As previously stated the main design criteria with regard to contact time within an initial contact zone is to ensure sufficient soluble substrate is absorbed in the contact zone and soluble substrate does not penetrate the main aeration tank and support filamentous growth. Because of this it was decided to monitor substrate levels before and after the initial contact zone. This was done by measuring filtered COD levels prior to the ICZ and in samples exiting the ICZ. Table 5.12 shows the filtered COD levels in both the influent to the ICZ and the filtered COD of the discharge from this tank. Filtered COD's entering the Initial contact zone ranged between 2364 and 826mg/l and averaged 1852mg/l revealing that approximately 65% of the substrate entering the contact zone was in a soluble form. Filtered COD's measured on flow exiting the ICZ ranged from 445 and 97mg/l with an average of 193mg/l. This corresponds to approximately 89.5 % removal of soluble COD within the initial contact zone. This equates to approximately 98mg/l of readily degradable COD within the contact zone. Chudoba et al. (1973b) recommended that to produce mixed cultures with SVI values below 100ml/g that the minimum values of removable COD within a selector was 25mg/l while Patoczka and Eckenfelder (1990) and Wanner (1992a) recommended that at least 80-90% of the applied soluble substrate be removed in the contact zone. The figures measured on the studies carried out on the initial contact zone are in a similar range to these.

Soluble COD In (mg/l)	Soluble COD Out (mg/l)	% age Removal Within ICZ
2364	445	81.2
826	97	89.3
1597	109	93.2
2289	143	93.6
2184	171	92.2

 Table 5.12 Soluble COD Concentrations In and Out of the Initial Contact Zone

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Biosorption is defined as the mg BOD or COD removed/g VSS within the initial contact zone and the figures above give rise to a biosorption rate of 65mg soluble COD/g VSS within the initial contact zone and are similar to those quoted by Eckenfelder and Grau (1992) who found COD removal within a contact zone affording 15 minutes contact time to be 80% when treating a food processing waste water at similar floc loading levels. Pujol and Canler found biosorption levels to be in a similar range 51mg COD/g MLSS.h with a contact time of approximately 30 minutes in their 1987 study.

Wanner (1994) selected activated sludge loading (kgBOD/kgMLSS.d) as a design criteria as an alternative to floc loading. Table 5.13 shows the activated sludge loadings quoted by Wanner (1994) along with the sludge loadings measured in this study for comparison. The Bx values quoted are related to the whole volume of the contact zone, so that for compartmentalised contact zones the actual loading in the first compartment is correspondingly higher.

Source	Bx kg/kg.d <sup>-1</sup> based on	
	BOD	COD
Albertson (1991)	3.0	
Chudoba and Wanner (1989)	>3.0	
Daigger and Nicholson (1990)	3.2-4.9	
Eikelboom	2.0-5.0	
Linne et al. (1989)	5.0-6.0	
van Nierkirk et al. (1987)		20-30
This Study		20-26

Table 5.13 Activated Sludge Loading in Contact Zones

It is evident from Table 5.3 (sample points 4m and above in 1994 season) that following exit from the contact zone into the aeration zone there is little or no sudden increase in oxygen demand. This would appear to confirm the above result that the majority of soluble readily degradable substrate has been absorbed and stored by the biomass within the contact zone and any particulate matter present has been entrapped within the floc structure prior to hydrolysis. This stored substrate is utilised during the sludge particles passage through the aeration basin. It has been claimed that this use of accumulated and stored substrate within the aeration zone is essential if the installation of an initial contact zone is to be successful in combating bulking (Chudoba 1973b). In the present study hydraulic residence times within the aeration tank were in the range 24-30 hours (extended aeration). This is an ample time period for the complete restoration of the accumulation/storage capacity when the sludge loading of the whole system did not exceed 0.3-0.5kgCOD/kgMLSS.d-1. These figures again are similar to those reported by Chudoba and Wanner (1989) and Eikelboom (1991). Wanner (1994) recommended that for aeration zones with a high dispersion number (complete mixing) that soluble COD levels exiting the contact zone should not exceed the final effluent soluble COD by more than 20-30mg/l. Filtered COD's were not measured on samples of final effluent. However, total COD's were. These averaged 72mg/l and ranged between 39 and 218mg/l. The figures for soluble COD exiting the contact zone are within 20-30 mg/l of the total COD figures for final effluent exiting the plant. It is difficult to estimate the concentration of soluble COD within the final effluent although measurements made over this four year study suggest a COD:BOD ratio of approximately 6:1. However this ratio may lead to an under estimate of the soluble fraction of the COD present as much of this may be in a recalcigent form and not susceptible to biological break down in the five day BOD test but may oxidise under the strong oxidising conditions present in the COD test. This would lead to false high measurements of COD and low measurements of BOD levels.

The presence of a substrate concentration gradient in each environment within an activated sludge system has been recommended (Albertson 1991) as to provide the optimal opportunity for the selection of non filamentous organisms and control bulking. It can be seen from the respiration rates measured in the aeration tank in 1994 that the oxygen uptake rates measured at various distances from the ICZ's exit are almost uniform across the tank. This confirms that the aeration tank mixing characteristics remain unaltered and hence there is little substrate concentration gradient created as would normally be desired. This however, may not be as important as Albertson makes out in systems with long retention times and high dispersion. Chudoba et al. (1973a+b) developed the kinetic selection theory (KST) from which they concluded that the selection of non filamentous organisms does not only occur in the contact zone but that the conditions within the aeration zone form an integral part of the selection process. Therefore, the term selector could be used to describe the whole configuration and not only the contact zone. It has been found however, that when contact zones are operated at high sludge loadings, a good selection of non filamentous micro-organisms cannot be ensured (Rensink 1979, Gabb et.al. 1991, Wanner 1992a, b). This was explained by Chudoba (1985)and Chudoba and Wanner (1989) who concluded that substrate concentration alone was not sufficient to eliminate filamentous organisms but that a starvation (endogenous) period was also necessary to successfully control bulking by the restoration of accumulation capacity. The existence of accumulation capacity (AC) was demonstrated for a pure culture by Rickenberg et al. (1956) and Kepes and Cohen (1962), and for mixed culture by Dohanyos et al. (1970) and Cech and Chudoba (1983). This assumed that filamentous organisms cannot accumulate a considerable amount of substrate whereas non-filamentous organisms can. This accumulation does not mean synthesis of reserve polymers but represents the capability of micro-organisms to consume some amount of substrate prior to its use for oxidative and synthetic purposes. Chudoba (1985) concluded that the primary factors influencing bulking are an actual concentration of substrate and the degree of AC exhaustion prior to the solids being returned to conditions of high substrate concentration. Numerous laboratory studies (van den Eynde et al. 1982, Chudoba 1985, 1991) pilot plant experiments (Chudoba 1991) and large scale treatment plant achievements (Matsche 1982, Wheeler et al. 1984, Albertson 1991) confirmed the kinetic selection theory in practise. While Pujol and Boutin (1989) developed a French modification- a contact zone which also demonstrated the positive effect of this technique on suppression of low F:M filaments such as *Microthrix parvicella*, Type 0092, and Type 0041. Under these conditions which are similar to those of this study and with an adequate retention time in the aeration zone complete restoration of the accumulation/storage capacity should take place.

### 5.15.3 Mixing and Aeration Characteristics

The term selector is now sometimes used instead of terms such as "pre-mixing zone" (Chambers 1982) or "initial contact zone" (Pujol and Boutin 1989). However, it is important to stress that the main difference between these systems cited in the literature is related to substrate gradient and the degree of axial or longitudinal mixing. Chudoba et al. (1973b) defined a selector as the initial part of a biological reactor, characterised by a low value of dispersion number, desirably below 0.2 and an adequate substrate concentration gradient. It has been further recommended by Chudoba and Wanner (1989), Rensink (1974) and Wanner (1994) that to provide the recommended degree of dispersion and hence the substrate gradient that the selector should be divided into at least 2-4 separate compartments. It was however difficult if not impossible to fabricate a baffled tank within the main aeration tank at Bourne due to the small size and surface area of the contact zone installed due to the requirement of a residence time of 10 minutes at peak flows. This case may also be true for many full scale plants since this leads to a recommended volume ratio (Vcz/Vtotal) of about 1/10 (Wanner 1994). It is due to this lack of compartmentalisation that the contact zone installed does not fall into the original description (Chudoba 1973a) of a selector but should be described as an in initial contact zone a modification of the selector developed by the French authors Poujol and Boutin (1989).

Attempts were made however, when installing the contact zone to avoid short circuiting and attempt to create some extent of plug-flow characteristics. This was done by extending the inlet pipework (both RAS and waste water flow) to approximately 1/2 the depth of the contact zone and directing these flows vertically down wards away from the exit of the ICZ. Although this was only a crude attempt to avoid short circuiting within the contact zone an exit curve distribution study carried out on the contact zone during 1994 revealed a dispersion number of 0.19 (Section 5.15.4) which falls into the desirable range below 0.2 as recommended by Chudoba (1973b) and indicates that some extent of plug flow was created within the contact zone. This flow characteristic is likely to create the high to low F:M gradient across initial contact zone recommended by Albertson (1989), Chudoba (1989) and Patoczka and Eckenfelder (1990) and hence provided the optimal conditions for non filamentous organisms to flourish.

During the discussions regarding the design of the contact zone the hardest decision was over whether the contact zone should be aerated or not. Due to difficulties of control and expense and the evidence in relevant literature that both low and high D.O. contact zones have been successful in combating bulking (Pujol and Boutin 1989), Albertson 1991, Pujol and Canler 1994) it was decided that the contact zone would have no added air but would rely on mixing from the pump flows entering it and nitrate in the return activated sludge to provide sufficient molecular oxygen in the contact zone. Albertson (1991) concluded the use of high F:M contact zones possibly negates the effects of aeration and the presence of nitrate nitrogen and also stated that dissolved oxygen and NOxN concentrations were not a good measure of the conditions within the floc itself. This employment of high F:M in the initial contact zone probably assured that the cells pass through an anaerobic stage which Albertson (1991) saw as a conditioning stage possibly necessary in controlling filamentous bulking. From the results recorded in this study it is evident that the contact zone fits nicely into Albertson's definition of an anoxic zone and as such conditions within the floc during its passage through the contact zone will be anaerobic.

Another physiological characteristic which may affect population dynamics in activated sludge processes is the denitrifying ability, i.e. the ability to oxidise organic carbon utilising nitrate and or nitrite as an electron acceptor. Wanner (1987) found severely bulking sludge had maximum rates of nitrate respiration one order of magnitude lower than non filamentous mixed cultures cultivated. While further studies by Shao and Jenkins found that nitrate removal by Type 021N was some five times less than that of *Thiothrix* sp. while *Zooglea ramigera* had a nitrate removal rate of almost two orders greater than that of *Thiothrix* sp. Further selection may come from the fact that *Zooglea ramigera* has the ability to convert nitrate into nitrite and then onto atmospheric nitrogen, while *Thiothrix* sp. and Type 021N can only reduce nitrate to nitrite.

Peas being legumes naturally have a high nitrogen content in their processing waste water. During passage through the treatment process this ammonia is oxidised to form nitrate some of which is returned to the contact zone in the returned activated sludge. Nitrate levels in the final effluent over the 1994 season ranged between 4.3mg/l and 47.4mg/l and averaged 24.1mg/l. While nitrate in the flow exiting the initial contact zone were some six times less than those entering ranging between 0.8 and 9 mg/l and averaging 3.1mg/l. It is evident from these that denitrification did occur within the contact zone a fact that is backed up by the improvement in oxygen usage efficiency also observed in 1994. This improvement in efficiency may have been due to this utilisation of nitrate as a terminal electron acceptor during the oxidation of organic material in the initial contact zone and may also have been indicated by the reduction in ORP levels recorded across the contact zone. As prior to this season the only denitrification taking place was under anoxic conditions in the final clarifiers and hence most of the nitrate formed was lost out of the plant in the final effluent and the oxygen used to form this accounted for in the removal of organics rather than oxidation of ammonia to nitrate. This along with substrate gradient and complete exhaustion of accumulation capacity may singularly or in combination be one of the determining factor in the selection of floc-forming organisms and the successful operation of the pure oxygen Vitox plant in 1994.

# 5.15.4 Tracer Studies

The degree of longitudinal mixing in a flow system can (as previously mentioned in Chapter 4) be determined using tracer tests (Levenspiel 1962). Such tracer tests were carried out on the pure oxygen activated sludge plant in the 1992 season and in the 1994 season. Due to the long retention time within the aeration tank and hence the effect of tracer returns wihin the RAS system manganese concentrations were also measured within the mixture of incomming RAS and settled effluent. This manganese concentration against time was graphed and the area under the graph subtracted from the area under the graph for Manganese concentrations exiting the aeration tank hence eliminated any effect the tracer (manganese) substance may have had within the return system. This also made it possible to calculate the percentage recovery of the tracer and hence validate the tests.

Figure 5.30 shows the variation of normalised exit manganese concentration against normalised time of the first tracer study performed in 1992. From this it was then possible to calculate the dispersion number ( $D/\mu/I = 0.27$ ) and a theoretical residence time of (t = 13.5 hours). The relatively high value for dispersion indicates a substantial degree of mixing. However, the difference between the calculated residence time (13.5 hours) and the actual residence time (23 hours) suggests the presence of possible dead zones or pockets where mixing is poor or non existent.

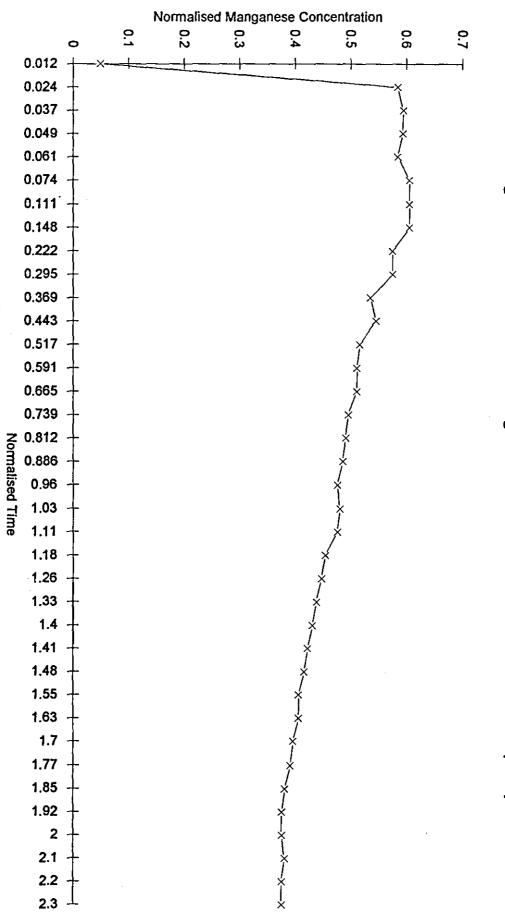


Figure 5.30 Normalised Exit Manganese Concentration Vs. Time Vitox Plant (1992)

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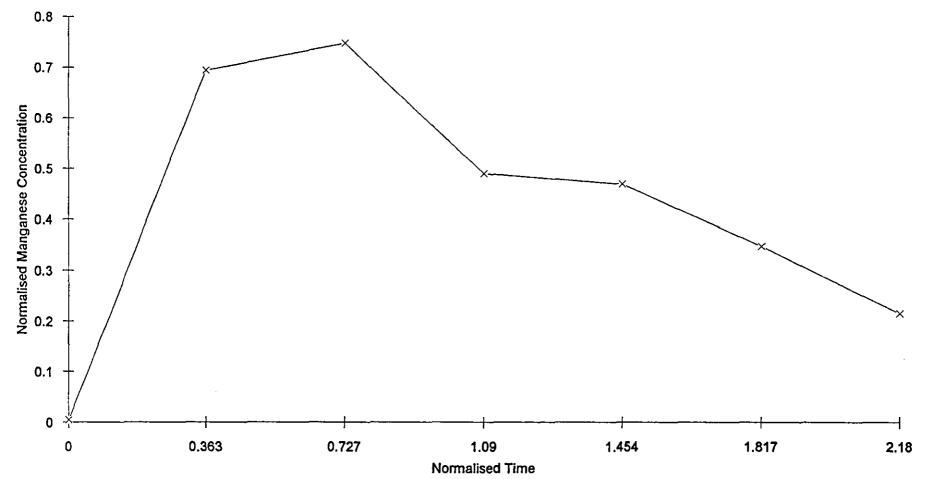
A second tracer study was carried out on the activated sludge plant during the 1994 season following the installation of the contact zone within the aeration tank. This was carried out to establish if the installation of this tank had created any change in the mixing characteristics within the main tank and confirm if the desired mixing characteristics had been created within the initial contact zone.

Figure 5.31 shows the normalised data from exit concentrations of manganese from the initial contact zone. From this it was possible to calculate the dispersion number  $(D/\mu l = 0.1870$  this indicates a degree of longitudinal mixing within the contact zone and is below 0.2 the level for dispersion recommended by Chudoba (1973b) to create a good substrate gradient across the contact zone. This degree of dispersion achieved in the contact zone may go some way in explaining its success in combating the growth and proliferation of filamentous organisms during the 1994 season.

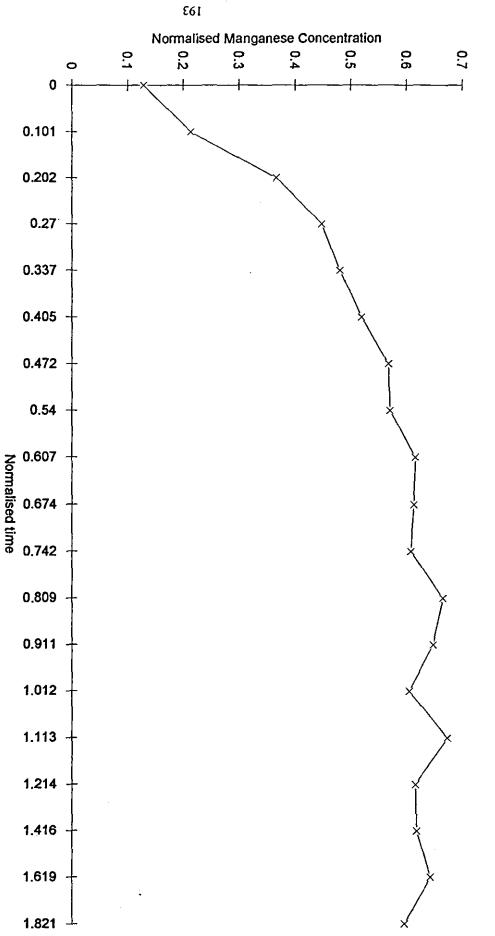
A tracer study was also carried out over the whole system (contact zone and aeration tank) in 1994. This was carried out to establish what changes the installation of the contact zone had created on the dispersion within the whole system. Figure 5.32 shows the normalised data gained from the exit manganese concentration from the aeration tank. This was then used to calculate the dispersion number (D/ul = 0.134). From this it can be seen that due to the installation of the contact zone the overall characteristic of the system has been altered from that of a fully mixed tank to a system exhibiting some degree of plug flow. This change corresponds to the creation of an area of high substrate loading (ICZ) and hence the selection of floc forming organisms rather than filamentous organism.

As previously mentioned in Chapter 4, this is only one of a number of possible models used to describe mixing within biological reactors and it may be possible to look towards these alternative models to confirm the findings of the present study.

Figure 5.31 Normalised Exit Manganese Concentration Vs. Time ICZ (1994)



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# **CHAPTER 6 : CONCLUSIONS**

6.1 The control of filamentous activated sludge bulking is based on substrate gradient and the so called "selector effect" (Gabb et al. 1988). This "selector effect" has been shown to be produced using substrates of various levels of solubility and rates of biodegradation.

6.1.1 When parallel experiments were carried out in both a laboratory scale sequencing batch reactor (SBR) and a continuously fed fully mixed reactor sludge with, good settling characteristics were produced with all substrates used within the SBR. While the fully mixed reactor produced poorly settling sludge due to the proliferation of filamentous organisms. These results are in good agreement with much of the published work in this area and the general finding that plug flow and intermittently fed or selector system produce sludges with good settling characteristics.

6.1.2 Oxygen utilisation following the addition of substrate and through the aeration phase of the SBR was monitored. The addition of substrate in all cases was able to induce high peak oxygen utilisation rates within the sludge. With peak uptake rates being 3-8 times greater than endogenous uptake rates measured at the end of the aeration phase.

6.1.3 Peak oxygen utilisation rates varied for each substrate used with the simple readily degradable substrates inducing the highest oxygen uptake rate. However, the higher molecular weight substrates (amylopectin, amylose and starch) still induced a peak oxygen uptake rate approximately four times greater than endogenous rates measured during this study. This indicates that even the less readily degradable substrates used may be able to produce the selector effect in systems employing a substrate gradient.

6.1.4 No such induction of high peak oxygen utilisation rates occurred in the fully mixed reactor were oxygen utilisation rates measured were approximately 1.5 - 2 times that of the endogenous oxygen utilisation rates measured in the SBR. This finding reveals that due to the reactor configuration being fully mixed the biomass experiences a uniform substrate gradient as it passes through the fully mixed reactor. It is this lack of substrate gradient that has been linked to the poor performance of fully mixed systems with respect to filamentous bulking.

6.1.5 Accumulation of substrate under conditions of high loading may have contributed to the suppression of filamentous growth in the sequencing batch reactor. Filamentous bacteria have been frequently reported as having a growth advantage at low substrate concentrations. In the sequencing batch reactor the process of biosorption removes substrate during the initial rapid feed period and prevents the passage of substrate onto the subsequent aeration phase. In the present study 63-84% of the soluble substrate applied to the biomass was removed during the rapid 5 minute feed period. This finding further supports results gained using oxygen uptake rate as an indicator of substrate utilisation.

6.1.6 A further selective pressure which may promote the growth of zoogleal bacteria is varying conditions of D.O. leading to an increase in metabolic processes and hence unbalanced growth (Wanner 1994). Oxidation reduction potential measurements were monitored during the three phases of the SBR and from these it is evident that during the feed period the biomass experiences changes with regard to D.O. levels. From this it was concluded that a proportion of the available substrate could be used under anaerobic conditions within the floc.

6.2 The pure oxygen (Vitox) activated sludge plant prior to the installation of an initial contact zone performed well with respect to organics removal but regularly suffered from filamentous bulking. 6.2.1 The pure oxygen, Vitox activated sludge system at Salvesen Food Services Ltd. Bourne Lincs. was capable of treating a high/variable strength effluent arising from the processing of peas. The use of pure oxygen has been claimed to produce better sludge settlement. However, filamentous bulking occurred in three out of the four years reported in this study. Several factors may make this plant susceptible to filamentous bulking. These are the plant is fully mixed, treats a strong carbohydrate based effluent, the plant operates at a high hydraulic retention time (>24hrs) and has a long sludge age.

#### 6.3 Dissolved oxygen limitation contributed to filamentous bulking.

Problems with dissolved oxygen control were experienced during both the 1991 and 1992 season. Following these periods of D.O. limitation sludge settlement rapidly deteriorated and in 1992 the causative organism was identified as Type 021N a filamentous organism commonly associated with low dissolved oxygen concentrations (Gabb et al. 1988). Low dissolved oxygen concentration is one of the known causes of filamentous bulking (Palm et al. 1980).

#### 6.4 The dosing of trace element solutions had no effect on sludge settlement

6.4.1 A number of control methods were tried to control the filamentous bulking at the full scale plant. The addition of D-Bulk (E&A West) a proprietary trace element had previously been successfully applied at this plant to control filamentous bulking (Gostick 1991) . This however, proved unsuccessful in controlling the bulking incident during the 1992 season.

# 6.5 The dosing of chlorine caused a rapid improvement in sludge settlement.

6.5.1 Chlorine has a record of success in combating filamentous bulking (Jenkins 1982, Annon. Tames Water 1983). Chlorine in the form of hypochlorite was dosed into the return activated sludge initially at a concentration of 4mg/l active chlorine

(for the whole plant volume). This initial dosing rate had little or no effect and the rate was subsequently increased to 6mg/l. This concentration of chlorine produced a rapid improvement in sludge settlement (SSVI's reduced from 350ml/g to 100ml/g in 14 days). During this period of dosing microscopic examination revealed lysis of filamentous organisms occurring which supports the theory that chlorine can be used at low doses to selectively kill filamentous organisms.

# 6.6 The installation of an initial contact zone proved successful in preventing the formation of a bulking sludge during the 1994 season.

6.6.1 The installation of an initial contact zone altered the reactor configuration from fully mixed with an almost uniform substrate concentration to a selector system with an area of high floc loading. Experiments performed on the contact zone revealed high levels of substrate removal taking place in the contact zone, preventing the subsequent passage of low levels of available substrate on to the aeration tank where this could lead to low substrate concentrations giving filamentous organisms a growth advantage. This contact zone also operated under anoxic conditions which may have given rise to metabolic selection in conjunction with a kinetic selection of floc forming organisms.

# 6.7 Oxidation reduction potential proved a good monitor of conditions of low or zero dissolved oxygen concentration.

6.7.1 At low dissolved oxygen concentrations D.O. measurements become unreliable and at these levels a clear advantage is gained. From the studies carried out at both laboratory and full scale ORP can be used to detect the point when D.O. levels become completely depleted and biomass respiration alters from aerobic to anaerobic. ORP levels also fall in relation to nitrate levels and show a similar second rapid reduction when nitrate levels become completely depleted. These conclusions indicate that ORP may be a suitable control parameter in biological nutrient removal nutrient removal plants where anoxic and anaerobic conditions form sections of the treatment process.

# 6.8 Respiration rates measure in situ and measured in the laboratory did not compare well.

6.8.1 Oxygen uptake rates determined in situ were on average 4-6 times those determined by conventional laboratory respirometer. This finding is similar to that of Erasin et al. (1992) who also found differences between results obtained in situ and laboratory. there are three possible reasons for this, firstly losses of oxygen from the system when measuring in situ (e.g. gas escaping from the top of tank), secondly in situ measurements are an average of a large range of respiration rates occurring throughout the tank and thirdly removing biomass from its normal environment may reduce its activity.

6.9 On the basis of the data presented and the success of the initial contact zone, the following design criteria are concluded to be appropriate for selector design.

	Retention Time (sewage flow + RAS)	Aeration	Environment	Redox Potential	Floc Loading mgCOD/g MLSS
Minimum	10 minutes	no	Anoxic	- 150 to < +50 mv	50
Average	20 minutes	no	Anoxic	- 150 to < +50 mv	75
Maximum	40minutes	no	Anoxic	- 150 to < +50 mv	150

In addition to the above criteria the selector zone should be either compartmentalised (2-4 compartments) or serpentined to create a concentration gradient across the selector zone. However, it must be emphasised that this design is only based on the results gained during this study and there is no universal method of control, applicable to all filamentous species.

#### **CHAPTER 7: RECOMMENDATIONS FOR FURTHER WORK**

7.1 Little information is available on the conditions within the internal structure of the floc. It has been indicated that due to diffusion rates and mass transfer limitations that the internal structure of the flocs experience are markedly different from those conditions that prevail in the bulk media. This field of study is difficult complex and due to probes of a suitable size or reliability not being available to monitor these microenvironments. ORP may however, form an appropriate tool for measuring conditions in these micro environments.

7.2 ORP has been shown to be a suitable monitoring parameter for environments where D.O. measurements become unreliable and can be used to indicate when D.O. or Nitrate become completely depleted. The challenge is now to establish if ORP can be used as a suitable control parameter for the optimisation of nitrate removal within anoxic contact zones or if it may have further applications within biological nutrient removal plants.

7.3 The installation of selectors/contact prior to the main aeration tank is now becoming common practice in the design of activated sludge plants as a measure to prevent bulking. The wide variety of substrates in industry may not lend themselves to this possible form of preventing bulking. It is known that particulate high molecular weight slowly degradable substrates are associated with filamentous bulking. Substrates of higher molecular weight and complexity need to be examined in more detail to establish if these are suitable for treatment in selector type treatment plants.

7.4 The competition between floc-forming and filamentous organisms is explained by crossover monod kinetics and explains the competition between these two types of organism for a growth limiting substrate. However, competition between only two uniform groups of filamentous and non filamentous organisms is unrealistic and may only go some way to solving low F:M bulking. Competition for macronutrients and micronutrients must also take place and be subject of competition between all organisms present. More information is required with regard to macro and micronutrient requirements especially in the field of industrial waste water treatment where it is more likely that these are limited.

7.5 The fate of adsorbed particulate material is ill defined. With increasing complexity the time to hydrolyse this substrate and subsequently absorb it must increase. Therefore, the required time for restoration of these adsorption sites must increase and hence long retention times in the system will be required. Estimation of the time period required to completely restore these adsorption sites with various substrates could lead to a further optimisation of the process.

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## Appendix 1.0

During single substrate experimentation using both Reactor 1 (fully mixed) and Reactor 2 (sequencing batch reactor, SBR) sludge settlement was measured using two different indices.

a) The Stirred Sludge Volume Index was used in association with Reactor 1

and

b) The Sludge Volume Index was used in association with Reactor 2.

The reasons for using both these indices has been reported in Chapter 4 and both give a reliable indication of settlement trends when measured on a regular basis.

Due to the cyclic nature of the operation of Reactor 2 (SBR) it was possible to gain up to three measurements of SVI per day which were averaged to form the graphs shown in Chapter 5. While SSVI within Reactor 1 (fully mixed) was measured twice daily with the results again being averaged to form the graphs shown in Chapter 5. Due to the graphs in Chapter 5 containing the settlement indices gained at three different strengths of substrate it was difficult to indicate any error onto them without making them unintelligable. For this reason two example graphs showing sludge settlement within Reactor 1 (fully mixed) and Reactor 2 (SBR) when fed with Glucose X 1.0 feed are shown here. The limited number of results gained per day (2 or a maximum of 3) does not lend it self to calculating experimental errors. Therefore it has been decided to show the spread of results via bars showing the maximum and minimum values recorded each day.



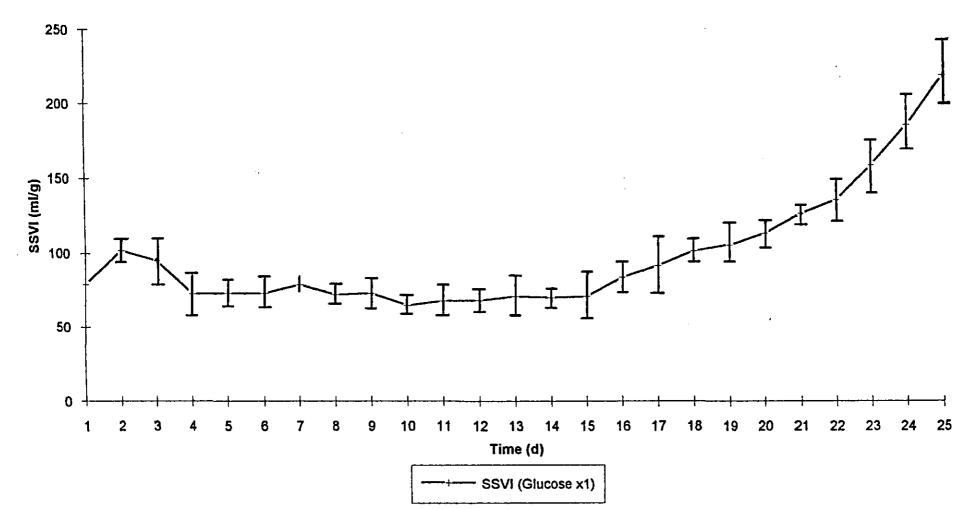


Figure 1b Glucose x 1.0 Feed In Reactor 2 (SBR)

