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The Effects of Shear and Mixing on a Continuously Fed Stirred Tank Reactor for Aerobic, Biological Wastewater Treatment

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PHD THESIS

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The Effects of Shear and Mixing on a Continuously Fed Stirred Tank Reactor for Aerobic, Biological Wastewater Treatment

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This thesis is submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy.

ABSTRACT

Treatment of domestic wastewater in a 9 L well defined conventional biotechnology type reactor was investigated over a range of stirrer speeds (8.3 to 16.7 s^{-1}) and retention times (8 to 12 h). Parameters of reactor oxygen transfer coefficient and shear were found to be close to conditions used for pure cell culture in industrial applications rather than typical wastewater treatment conditions.

The major treatment effects measured were carbonaceous load removal and nitrification. Carbonaceous load removal was found to be highest at low stirrer speeds with short retention times. Ammonia removal was greatest at stirrer speeds of 15 s^{-1} with 12 h retention time. Most of the ammonia was converted to nitrite, this agreed with reports in the literature of temperature; retention time and free ammonia inhibition promoting nitrite build up. Specific nitrification rates of up to 35 $mg(N)g^{-1}h^{-1}$ (at 15 s^{-1} 10 h retention time) were achieved in the reactor, found to be close to those observed in pure culture experiments. An inverse correlation was observed between ammonia and $CBOD_5$ removal.

The temperature increased with stirrer speed and also had a strong effect in the nitrification rate. The interaction between temperature and stirrer speed was investigated using a control unstirred reactor and multiple linear regression technique. It was found that while the temperature and stirring were correlated, separate effects could be discerned. The stirrer effects were further investigated by varying the impeller type. Tip speeds were matched to the disk turbine for a low and a high shear impeller. The lower shear LE20 impeller gave promising results that required a much lower power input to achieve the treatment.

Finally an anoxic reactor was added to denitrify the stirred tank effluent. It was found to successfully denitrify when sufficient nitrite and nitrate were supplied by the stirred tank. The combination of a stirred nitrifying tank followed by a denitrifying stage could make be an attractive alternative wastewater treatment method providing the stirred tank power requirements can be reduced.

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This Thesis is dedicated to

Joyce Berry

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1 INTRODUCTION

In the UK over 11 billion litres of wastewater is collected in sewers each day (D.E.F.R.A. 2002). Wastewater exerts a large biological oxygen demand (BOD) on the receiving watercourse as it is degraded by bacteria. Eutrophication is caused by the addition of large quantities of nitrogen and phosphorus also present in wastewater. Further damage is caused by toxic compounds and pathogens entering the watercourse. These factors decrease the viability of receiving waters as a potable water source, recreational facility, fishery or ecological resource.

In Europe the problems associated with untreated wastewater became notoriously acute in 19th century. The growth of industrial cities urban populations overloaded the rivers abilities to cleanse themselves leaving dirty, species poor, rivers carrying pathogens and toxic compounds (Mason 1996). This drove the first wastewater treatment facilities to be built along with sewer systems piping the wastewater away from urban areas. Since wastewater treatment was first introduced river quality has improved. This has been driven by legislation since the first parliamentary act to control water pollution was passed in 1873; the Rivers Pollution Prevention Act (Wolf and White 1997). Current legislation in the UK is dominated by the results of the 1991 Water Acts and by European Community environmental directives particularly the Urban Wastewater Directive, Bathing Water directive and Shellfish Waters directive (D.E.F.R.A. 2002). The requirements for treatment are set for each wastewater treatment works according to the watercourse sensitivity and the size of both the receiving water and the population discharging to a treatment plant.

Biological wastewater treatment is used to oxidise the pollutants in the wastewater building biomass and producing CO_2. It is usually based on biological processes that would naturally occur in a stream being intensified and controlled within the treatment works (Moss 1988). Organisms at a number of trophic levels form a complex food web, each with a function in the treatment works. Biological systems are controlled by providing the best physical environment for the bacteria and higher organisms involved in treatment.

Biological trickling filters use an attached growth system with a biofilm of bacteria growing on media. The wastewater is sprayed over the surface and percolates through

the gaps in the media, air enters passively through the gaps. Bacteria use the wastewater trickling over the support material as a food source, removing the carbonaceous load to grow and respire producing biomass and CO_2. Nitrifying bacteria autotrophs that use ammonia as an energy source, oxidizing it to nitrate and nitrite may also develop. A microscopic grazing fauna (ciliates, rotifers etc.) develops that crawls over the surface of the biofilm and feeds on the bacteria and particles from the wastewater. A further trophic level of insect larvae and worms graze on the microscopic biofilm, maintaining the gaps between the media and allowing air to circulate and the wastewater to flow through (Mason 1996). Some of the biofilm and grazers are washed off, these are settled out of the water as humus sludge and the water is released into the receiving environment.

Activated sludge is also based on a bacterial community fed on by a grazing fauna. In activated sludge the bacteria are suspended in the wastewater and the mixed liquor is aerated either mechanically or by diffusion. Activated sludge processes require the separating of biomass from the effluent this occurs in a number of ways, through settling in a separate basin (conventional activated sludge), settling in the same tank (sequencing batch reactor, SBR) or retention of solids and removal of liquid by a physical barrier (membrane bioreactor, MBR) (Metcalf and Eddy Inc. 2003). In all cases the biomass is reused and retained for longer than the liquid. In conventional activated sludge a proportion of the settled solids are returned to the start of the aeration tank. This maintains a bacterial community suited to the wastewater source and the physical reactor conditions. In these processes the bacteria tend to form aggregates in response to the grazing pressure from the higher organisms (Güde 1979 and Jurgens et al. 1997 found that ciliates and rotifers fed on free swimming bacteria and those on the outside of the flocs, promoting denser floc formation). In SBR and conventional activated sludge processes aggregate formation is further promoted by the settling and reuse of the biomass.

Process such as these have been widely used, as well as a number of biological processes that cross over the main types of reactor. Examples include packed bed reactors, media submerged in wastewater and aerated, supporting biofilm and rotating biological contactor (RBC), a series of biofilm covered turning disks that are partially submerged in the wastewater (Metcalf and Eddy Inc. 2003).

Factors of importance with biological wastewater treatment are the ability of the system and organisms to deal with fluctuations in the influent quality and quantity, the nature of the influent, the site, the cost of treatment and the disposal of waste products (sludge).

A number of researchers have attempted to reduce the sludge production or speed up the reaction process by using dispersed bacteria rather than film or floc based treatment. In such a reactor the bacteria are under a selection pressure for rapid growth but not to form flocs. Flocs do not form because the grazing fauna have been removed from the reactor and there is no recycle of settled biomass. A number different methods of further treatment were used to remove the remaining solids from the influent and bacteria washing out of the first reactor. Lee and Welander (1996) used a predatory reactor as a second stage in which they encouraged the growth of the grazing fauna. Other research into disersed bacterial wastewater treatment has used activated sludge and MBRs for further treatment (Table 1.1)

Table 1.1 Suspended growth reactors and possible further treatment stages.

Reference	Wastewater	Retention time	Further treatment
Ghyoot and Wouter (2000)	Synthetic wastewater	34 – 8 h	MBR and conventional activated sludge
Chang and Alvarez-Cohen (1997)	Chlorinated organic solvent waste	5 d	Plug flow
Lee and Welander (1996)	Papermill waste	3 – 10 h	Grazing fauna
Ratsak *et al.* (1994)	Mineral synthetic	16.7 – 5.56 h	Grazing fauna

In this study treatment of a typical domestic wastewater was investigated with the aim of increasing the process rate by preventing floc formation and achieving a rapidly growing, dispersed bacterial culture. This was to be achieved by increasing the power input, giving an increase in mass transfer so that the dispersed culture would have availability of all possibly limiting substrates.

2 LITERATURE SURVEY

2.1 SHEAR EFFECTS IN AEROBIC WASTEWATER TREATMENT

Cell culture is widespread and has many applications from the production of food and drugs (e.g. penicillin and Quorn) to wastewater treatment. In submerged culture the cells' primary needs are provided by the medium. Mixing promotes homogeneity, transferring nutrients and oxygen from the bulk medium to the cell and removing waste products (Joshi *et al.* 1996) (step 5, Figure 2.1). In well mixed media concentration gradients are minimized and resistance to mass transfer in the bulk liquid is reduced (Doran 1995). The relatively thicker films occurring around larger cells or aggregates cause major resistance to mass transfer (step 6, Figure 2.1). Cell aggregates require diffusion of a substance through the solid mass of cells before the substance reaches a specific cell, e.g. activated sludge flocs, extra-cellular polymer matrix (Doran 1995). Steps 8, 7, 5 and 4 in Figure 2.1 have been found to be the most significant resistances to mass transfer (Doran 1995). Diffusion through the floc matrix was found to be the rate-controlling process by Benefield and Molz (1983) limiting cell metabolism.

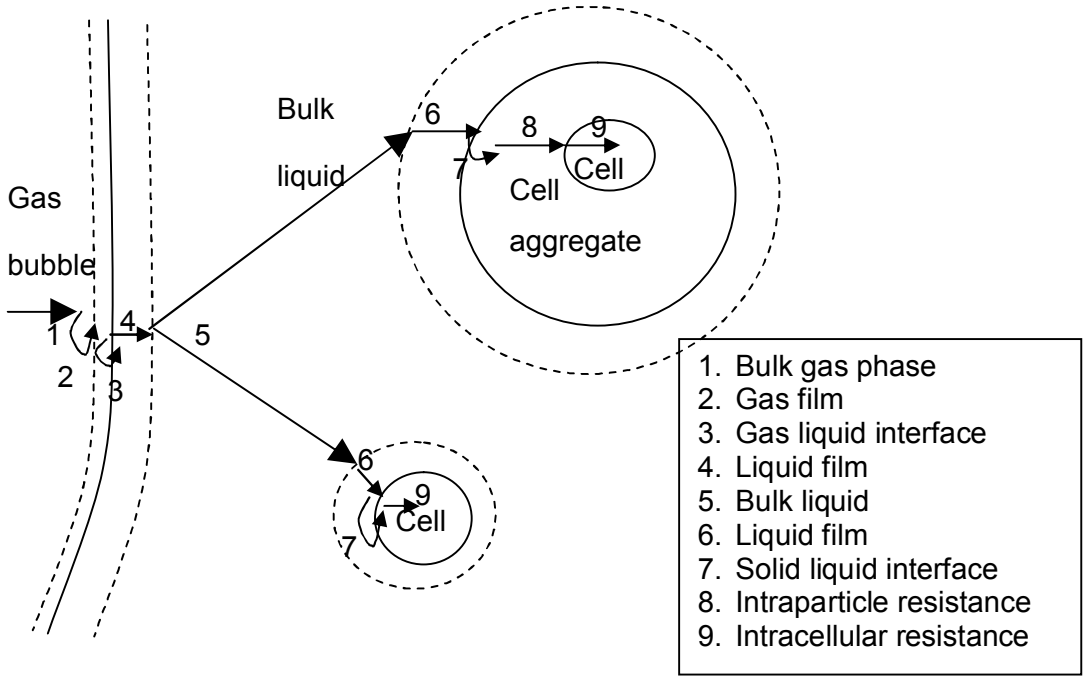

Figure 2.1 Oxygen transport mechanisms from a gas bubble to the cell (Doran, 1995)

Mass transfer can be improved by maintaining a homogenous culture medium, mixing is generally achieved through mechanical agitation (stirred vessel), gas bubbling

(bubble column), flow induced mixing (packed bed) or a combination of the above (air lift reactor) (Mann 1983; Mersmann *et al.* 1990). Agitation exerts a shear stress on cultured cells; this can damage cell structure and function. Shear stress can also have positive effects on cell culture, promoting mass transfer. Afschar *et al.* (1986) found that passing *Clostridium acetobutylicium* through a capillary reactivated the cells by removing a slime layer and increasing degassing ability.

A cell's ability to withstand a shearing force is dependent on its mechanical strength, morphology and the shearing forces applied to the cell (Thomas 1990). Mammalian cell cultures are particularly susceptible to shear as they are large and lack cell walls (Thomas 1990). Microbial cells are less susceptible to shear due to their small size relative to the turbulent micro-scale and protection offered by the cell wall.

Joshi *et al.* (1996) reviewed the effects of hydrodynamic shear on a range of organisms, covering single cells in suspension and filamentous fungi, pellet and hyphal forms, but neglected other cell aggregates. Hua *et al.* (1993) reviewed shear effects including bubble cell interactions, focussing on animal and plant cells, investigating shear effects on microcarrier and suspended culture. These topics are not covered here in detail as this research does not deal with pure culture of fungi, animal or plant cells.

Shear has been described by a number of methods for the wide variety of applications it affects. The most common parameters and the context in which they have been used is discussed below.

2.2 SHEAR PARAMETERS AND MEASUREMENT

The strict definition of shear stress is the force per unit area acting parallel to the surface of a body (Thomas 1990). The term shear has been used to represent the combined mechanical forces in bioreactors including surface tension, collisions and normal stresses (Hua *et al.* 1993); this broad definition has been adopted as used by Thomas (1990). The methods of describing shear and mechanical forces used in the literature are discussed below.

The simplest parameters used to describe mixing in stirred tank reactors are the impeller rotational speed (N_i, s^{-1}) and impeller tip speed (T_i, ms^{-1}):

Equation 2.1 $$T_i = N_i \pi D_i$$

Where D_i is the impeller diameter (m).

These simple representations of mixing describe the speed of mixing well but cover no other aspects; they only allow comparison between one particular impeller and tank geometry. Thomas (1990) and Joshi *et al.* (1996) described the integrated shear factor (ISF, s^{-1}), the impeller tip speed corrected for impeller: tank diameter (D_t, m):

Equation 2.2 $$ISF = \frac{2\pi N_i D_i}{\left(D_t - D_i\right)}$$

Power per unit volume (Wm^{-3}) and average shear rate (G) (Equation 2.3) have been used to describe the energy dissipation. Most commonly the shearing or mixing intensity has been described in terms of shear rate (G, s^{-1}), the root-mean-square velocity gradient (Equation 2.3) was developed in by Camp and Stein (1943) and is commonly used to describe water and wastewater treatment flocculation processes.

Equation 2.3 $$G = \sqrt{\frac{P}{\mu V}}$$

Where V is volume (m^3), μ is viscosity (Nms^{-1}) and P is power (W)

Cleasby (1984) and others have criticised the use of G for flocculation, as viscosity is temperature dependent and orthokinetic flocculation is not. Velocity gradient can only reasonably be used for the flocculation of particles smaller than the Kolmogorov micro-scale of turbulence, when Brownian motion is the main mechanism for agglomeration. Cleasby (1984) suggested the use of the energy dissipation per unit mass (ε,Wkg^{-1}) Equation 2.4 to the two-thirds power ($\varepsilon^{2/3}$) as a valid replacement for G:

Equation 2.4 $$\bar{\varepsilon} = \frac{P}{\rho V}$$

where ρ is density (kgm^{-3}).

The Kolmogorov micro-scale of turbulence has been widely linked to shear effects (Joshi *et al.* 1996 and Hua *et al.* 1993). The micro-scale (η, m) is defined as:

Equation 2.5 $$\eta = \sqrt[4]{\frac{\nu^3}{\varepsilon}}$$

where ν is kinematic viscosity (m^2s^{-1})

Cell damage and aggregate breakage have been found to be relative to the micro-scale of turbulence. Biggs and Lant (2000) used the Kolmogorov micro-scale of turbulence to describe activated sludge floc breakage. Flocs larger than the turbulence micro-scale undergo fracture, while primary particle erosion is the predominant breakage mechanism for particles smaller than the micro-scale (Thomas *et al.* 1999).

Shear stress (τ, Nm^{-3}) is used where shear is well defined. Equations for shear stress in turbulent flow regime used by Mersmann *et al.* (1990) are described below:

Equation 2.6 $$\tau = \frac{\rho}{2}(u')^2$$

Equation 2.7 $$\tau \approx \frac{\rho}{2}\left(\frac{\bar{\varepsilon} D_i}{6}\right)^{\frac{2}{3}}\left(\frac{\varepsilon}{\bar{\varepsilon}}\right)^{\frac{2}{3}}$$

Where u′ is dimensionless velocity.

The shear stress associated with dissipative eddies was estimated by Oh *et al*. (1989):

Equation 2.8 $$\tau = \rho(\varepsilon\nu)^{0.5}$$

Equation 2.8 was altered by Cherry and Kwon (1990) in Joshi *et al*. (1996) to describe the maximum shear stress as 5.33 times the calculated dissipative eddy shear stress.

Shear parameters describe the mean or maximum conditions to which cells are subject. Stirred tank reactors have a region of high-energy dissipation around the impeller; in this area good mixing occurs. Cutter (1966) found that only 30% of the energy supplied to a tank reached outside the impeller zone. Tomi and Bagster (1978) observed that a zone of extreme turbulence occupied just 5% of the impeller zone. The remainder of the tank may suffer poor mass transfer and low oxygen levels, which has been found to cause problems in large fermenters (Makagiansar *et al.* 1993). There is still scope to increase understanding of shear and mixing in the wide range of bioreactors used for a variety of applications.

2.3 BIOREACTORS

Stirred tank reactors have been used for culturing bacteria in suspension. The Rushton turbine has generally been fitted as standard due to the efficient gas liquid contacting achieved in the standard reactor configuration (Lawford and Rousseau 1991). The Rushton turbine is an example of a radial flow device, which in the turbulent range it has an equal power consumption to an equivalent diameter fan turbine with blades of the same height and a paddle of 3 times the height (Nagata 1975). Other radial flow devices include curved blade turbines and anchor and gate type impellers. Axial flow type impellers give flow parallel to the shaft and include propellers, pitched blade turbines, hydrofoil impellers, helical ribbons and screw impellers (Figure 2.2). Axial impellers give good pumping and have a lower power draw than the radial flow impellers (Lawford and Rousseau 1991, Doran 1999). Axial flow impellers give uniform circulation when used within a draft tube (Nagata 1975). A combination of axial and radial flow can be achieved with impellers such as the lightnin' A310 and Ekato Intermig (Mann 1983).

The impeller shear is related to the impeller diameter, power input and impeller geometry. Sweep volume and trailing vortex structure are important for shear conditions in the micro-mixing zone around the impeller where high energy dissipation and most damage occurs (Doran 1999 and Lawford and Rousseau 1991). Macro-mixing and efficiency circulation define how frequently the cells are brought to the micro-mixing high shear zone (Doran 1999). Rushton turbine impellers have generally been termed as high shear mixing devices, but Justen *et al*. (1996) observed greater damage to *Penicillium chrysogenum* with pitched blade turbines, hydrofoils and propellers; generally termed low shear devices. Doran (1999) explained this phenomenon as being due to more better power dissipation; more trailing vortices and wider impellers can spread a given power input more evenly through the reactor.

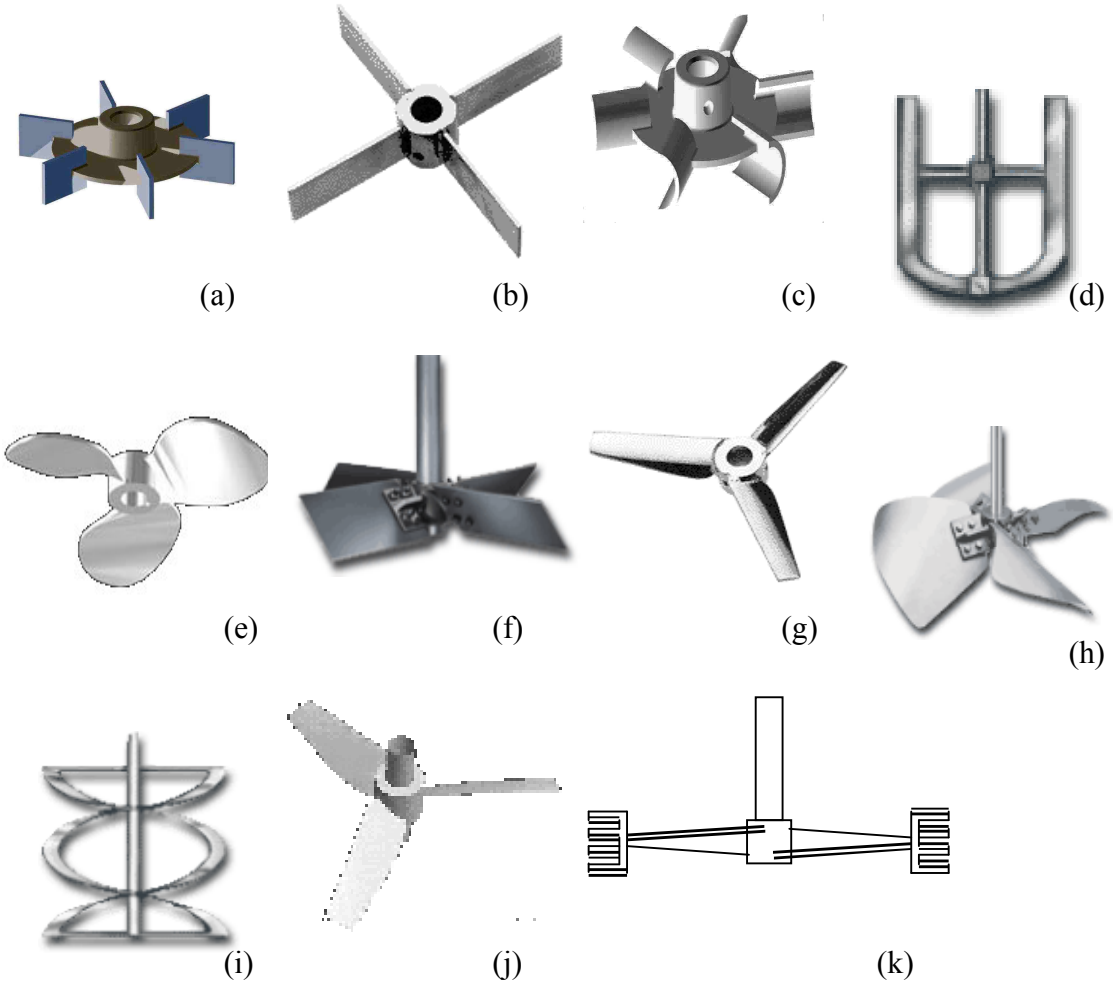

Figure 2.2 Impeller types, (a) disk turbine, (b) flat bladed turbine, (c) concave turbine, (d) anchor type impeller, (e) marine propeller, (f) pitched blade turbine, (g) hydrofoil impeller, (h) hydrofoil impeller, (i) helical ribbon, (j) A310 and (k) Intermig.

A number of researchers have investigated the use of different impeller configurations for a variety of operations, often to reduce the damage to cells. Justen *et al*. (1998) found that higher agitation rates caused increased specific growth rates in *Penicillium chrysogenum* thought to be through increased fragmentation. However, the Penicillin production rate decreased with increasing stirrer speed for all impellers. The impellers tested at the same specific power imput were ranked; the least shear damage was sustained by the culture with the paddle (D_i/D_t = 0.65), next the Rushton Turbine impeller (D_i/D_t = 0.33) and the Pitched Blade (D_i/D_t = 0.4) impeller caused the most damage. Lawford and Rousseau (1991) replaced a Rushton turbine impeller with different configurations of impeller and aeration device to try and recreate the hydrophobic exo-polymer product quality produced at small scale in shake flasks. They found that the most effective oxygen transfer was achieved using the Rushton

turbine impeller, but that the reactor set up damaged cells and reduced the product quality. Axial flow impellers yielded higher product quality despite the lower oxygen mass transfer coefficient. A wide "Dumbo ear" impeller was compared to a high efficiency A310 of equal diameter by Menisher *et al*. (2000). They found that the Dumbo ear while consuming more power for a specific stirrer speed, gave a shorter blend time at the same power consumption. The results of Menisher *et al*. (2000) also suggested that higher stirrer speeds would be required for the "Dumbo ear" to create eddies of a turbulent microscale (likely to damage cells). Unfortunately the study did not report performance within a bioreactor.

Gibbs *et al*. (2000) reviewed problems of growing filamentous fungi in submerged culture. They found that more effective impeller configurations than the widely used Rushton turbine had been reported for bulk mixing in viscous filamentous fermentations, particularly the Prochem hydrofoil. Gibbs *et al*. (2000) also cited an account of a Helical Ribbon run at the same oxygen mass transfer coefficient (K_La) as a Rushton turbine that did not give sufficient shear for the exo-polymer product to be removed from the cell. Huang *et al*. (2002) cultured *Stizolobium hassjoo* using a number of impellers, a flat blade turbine, a pitched blade turbine, a 2 impeller system of a flat blade and pitched blade turbine and a maxblend (gate type) impeller. It was found that mixing time, low shear stress, and high oxygen transfer coefficient could not be achieved using one impeller, and that compromises had to be made to meet the requirements of culture systems.

High shear sensitivity cells such as mammalian and plant cells have been cultured successfully using stirred tanks by protecting cells in a number of ways. The addition of an anionic surfactant was found to be commonly used to protect mammalian cells by Milward *et al*. (1994) although they did not know the mechanism of protection. Bovine serum has been used to protect mammalian cells, and was found by Butler *et al*. (1999) to effectively protect hybridoma cells along with a number of additives tested. Gum encapsulation has been used to protect cells. Huang *et al*. (1990) immobilized *Escherichia coli* cells in carrageen gel and found with high dissolved oxygen conditions the immobilized cells product (plasmid pTG201) remained more stable than in free cells. However, cell entrapment had disadvantages as it lowers mass transfer to the cell and increased stirring requirement and so shear rate, encouraging cell release from the immobilizing complex (Arnaud *et al*. 1992).

Alternatives to stirred tanks are often useful for suspended cell culture (Figure 2.3). Commonly cells are cultured in unstirred tanks such as bubble columns and gaslift reactors where the aeration provides the mixing. These systems have lower shear than stirred tanks and do not have any moving parts making them less susceptible to contamination or breakdown. Doran (1999) found a number of accounts of airlift reactors not providing sufficient mixing to allow cultivation of high density cultures. Better production of neomycin (Ohta *et al*. 1995) and growth of *Tetrahymena thermophila* (Hellenbroich *et al*. 1999) were found in stirred tank reactors than in airlift reactors due to the superior mixing performance. Wang and Zhong (1996) added an impeller to pump upwards through a draft tube. The aeration took place in the down-comer around the draft tube preventing the impeller from cavitating. This was found to increase mixing but could be used to culture shear sensitive cells at low impeller speeds.

A further alternative for suspended cell culture is the membrane bioreactor (MBR) they are made up of a bubble column reactor, with either a submerged membrane or a side-stream membrane unit. Membranes always require some level of shear across the surface to reduce membrane fouling, which can be supplied through bubble or liquid flow. Kim *et al*. (2001) found that the pump type used to supply mixed liquor to a side-stream membrane module had an effect on the biology and effluent quality in a wastewater treatment MBR. The centrifugal pump was found to give a lower shear, cause less damage and produce a higher quality effluent than the rotary pump tested.

Cells have also been cultured with a solid immobilising matrix. Fluidized bed reactors rely on liquid or gas phase being pushed through a bed of cells immobilised within or growing as biofilm on a solid medium. Cells have also achieved self immobilization growing as granules held in suspension (Shin *et al*. 1992, Tay *et al*. 2001), the flow rate of the passing gas and fluid phases determines the growth and texture of such granules with high flow and turbulence required to produce small, dense regularly shaped granules (Tay *et al*. 2001). Similar findings were made for biofilms in fluidized bed reactors. Wagner and Hemple (1988) added additional sand particles to increase the shear sufficiently to prevent high biomass accumulation. Guo and Rathor (1997) found that gas lift reactors could damage mammalian and insect cells around the sparger, they combined a gas lift reactor with a fluidized bed to lower the liquid shear rate and damage to the cells.

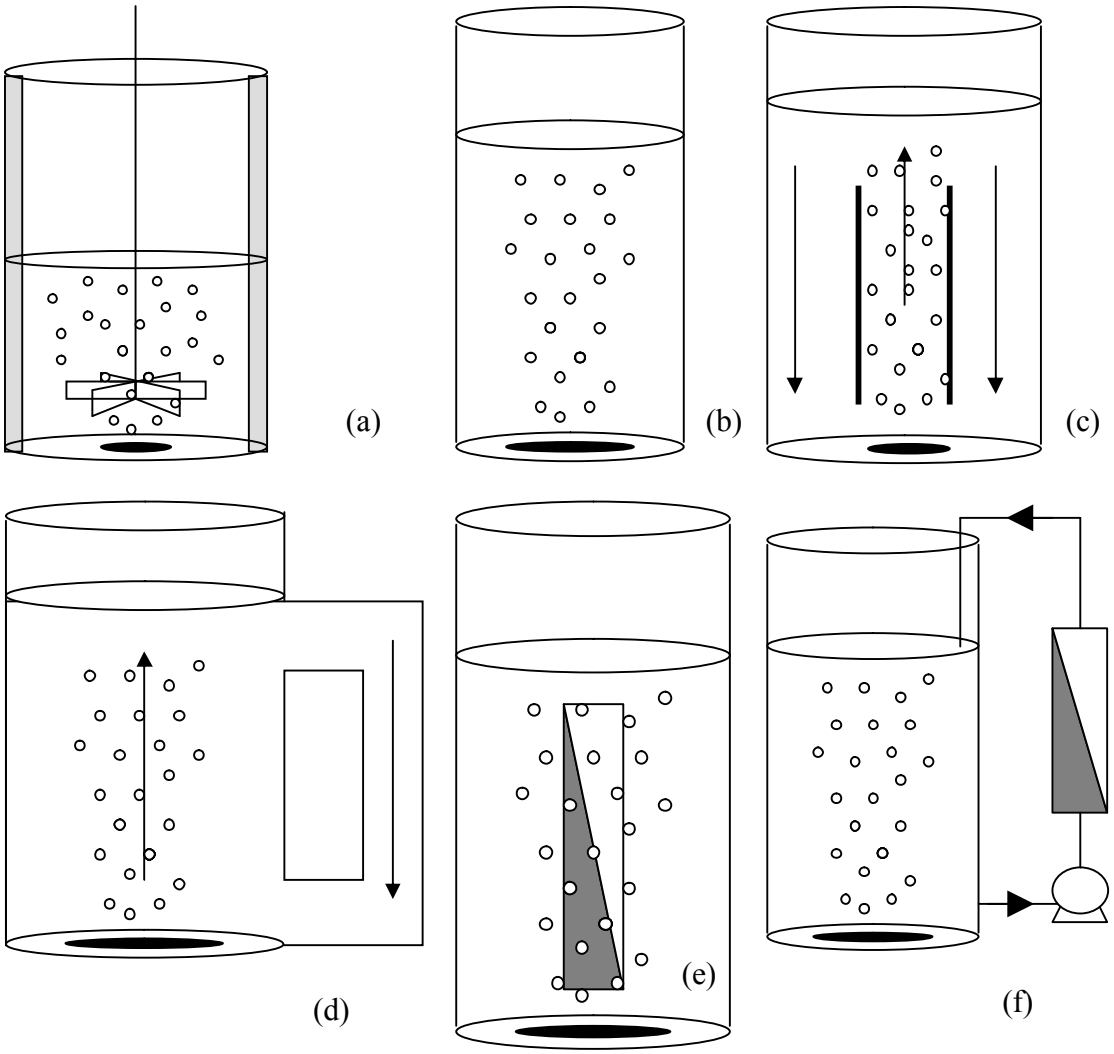

Figure 2.3 Various bioreactors for suspended culture growth (a) stirred tank impeller with flat bladed impeller or paddle, (b) bubble column, (c) internal loop gas lift reactor, (d) external loop gas lift reactor, (e) submerged MBR and (f) sidestream MBR.

Membrane Aerated Biofilm Reactors (MABRs) have been used to prevent shear and bubble damage to shear sensitive biofilms. Cells colonise one side of the membrane while air or oxygen remains on the other side. The membrane allows gaseous exchange, so that one limiting substrate is fed through the membrane and any soluble nutrients are obtained from the other side, allowing a thin biofilm to develop. Casey *et al*. (2000) found that in a MABR supporting *Vibrio natriegens* the flow velocity had an effect on the mass transfer, detachment rate and maximum biofilm thickness.

Other reactor types have been used to achieve extreme shear or defined shear for analysis of shear effects. Generally laminar shear has been achieved using a

viscometer or rheometer run at a variety of well defined shear forces to compare effects. These experiments have been carried out for a range of organisms e.g. Midler and Finn (1966) and Arnaud *et al*. (1993) used constant shearing devices based on Couette viscometers to investigate shear effects on *Tetrahymena pyriformis* and *Lactobaccillus delbrueckii* respectively. Chamsart *et al*. (2001) used laminar shear results from a concentric cylinder rheometer to aid selection of operating conditions within a stirred tank. Oh *et al*. (1997) produced a multi-disk impeller with a large well defined region of high shear; this device was used to test shear effects on high viscosity polysaccharide production. From the results a novel bioreactor with a spiral impeller reaching almost to the vessel wall and 2 turbine impellers within the spiral was designed to effectively mix the high viscosity polysaccharide.

Other short term methods of testing well defined shear include capillary tubes (Augenstine *et al*. 1971) and free jet experiments (Bronnenmeier and Markl 1982). Hua *et al*. (1993) also listed a number of other methods of shear stress investigation less commonly employed. Sowana *et al*. (2001) tested a device aimed to improve upon these methods. They made a thin ring for the culture through which a rod was rotated. The torque was measured and accurate modelling of the reactor hydrodynamics was carried out.

Very high shear levels are normally avoided for bioreactors. However some enzyme reactions take place under very strong mechanical forces, examples of such reactors include batch stirred tank reactor, semicontinuous ultrafiltration, column plug flow attrition or ball mill reactors. Gusakov *et al*. (1996) proposed the use of an intensive mass transfer reactor stirred with ferromagnetic particles for cellulose hydrolysis. They found that with the intensive shear fields in this reactor deactivation of enzyme was significant, and it would be unlikely to be suitable for cell culture.

2.4 SHEAR EFFECTS ON MICRO-ORGANISMS

Microbial cultures often have an optimum mixing level: where micro-organisms are supplied with nutrients and have waste products removed, but are not adversely affected by the shearing forces.

Bronnenmeier and Markl (1982) found that in a 2 L stirred tank reactor unrestricted growth continued in *Chlorella vulgaris* and *Anacystis nidulans* up to the maximum stirrer speed of 50 s^{-1}. In the same study it was found that wild type *Chlamydomas reinhardii* was unaffected by shear produced by stirrer up to 40 s^{-1}, while growth was severely restricted by 16.7 s^{-1} in a wall defective mutant. This illustrated the importance of the cell wall as structural protection against shear stress.

2.4.1 Protozoa

Protozoa are similar to mammalian cells as they are large and have no cell walls, they are however free living, and must have developed some mechanisms to limit mechanical damage. Mammalian cells have been studied more frequently than protozoa because they are more widely cultured. Midler and Finn (1966) carried out work looking at shear sensitivity of protozoa. They used a 2.3 L stirred tank reactor with a 4-bladed turbine impeller at stirrer speeds of 1.67 to 20 s^{-1}. At the low stirrer speed slight damage was sustained, while the higher stirrer speed reduced the population of *Tetrahymena pyriformis* to one-fifth of its original number. Hellenbroich *et al.* (1999) found that at stirrer speeds between 0.833 to 2.3 s^{-1}, in a 13 L stirred tank reactor with a 6 bladed paddle impeller, the highest concentration of *Tetrahymena thermophila* was achieved at the higher stirrer speed. Broudiscou *et al.* (1997) found that in a 1.1 L stirred tank reactor decreasing the speed of a marine impeller from 4.33 to 3.83 s^{-1} improved numbers of a cultured rumen protozoan. These results indicate that there is an optimum shear level associated with protozoan culture.

2.4.2 Bacteria

Death due to shear has rarely been observed with bacterial cultures. However, changes in cell productivity and structure have been reported (Joshi *et al.*, 1990). Fowler and Robinson (1991) forced growth medium past *Escherichia coli* immobilized on a membrane. Mechanical stress of 300 000 Nm^{-2} measured with a pressure transducer was reported to be orders of magnitude lower than was required to inhibit growth. Wase and Ratwatte (1985) and Fowler and Robinson (1991) both discovered an osmo-regulatory response to high shear stress. The former noticed that in a 1 L fermentor, *Escherichia coli* increased in volume but not dry weight when the stirrer speed was increased from 10 to 25 s^{-1}. The size increase was an osmotic

response to increased intracellular salt concentrations caused by a change in cell membrane transport due to shear. Further literature examples of shear effects on bacteria are summarised in Table 2.1.

Table 2.1 Effects of shear on Bacteria grown in submerged cultures.

Reference	System	Organism / culture	Tip Speed (ms^{-1})	τ Nm^{-2}	Effects
Joshi *et al.* 1996	(REVIEW PAPER)	*Lactobaccillus debruchii*		36 – 54	Improved metabolism, short lag.
				72	Damage, long lag time
Arnauld *et al.* 1992	Entrapped cells in gel beads, four bladed stirrer	*Lactobaccillus casei* Anaerobic	0.173		Increasing agitation increased mass transfer coefficient, growth rate, metabolism and cell release rate from immobilising medium, (disrupts downstream processing)
			0.346		
			0.518		
Toma *et al.* 1991	5L STR 2 turbines & sparger	*Brevibacterium flavum*	3.11		Optimum O_2 uptake and growth rate
			6.22		Turbulence induced shear stress
Fowler & Robertson 1991	Cells on membrane	*Escherichia coli*		300000	Maximum force exerted orders of magnitude lower than required to inhibit growth. Increase mechanical stress increase catabolic activity caused by osmo-regulatory response
Paul *et al.* 1990	Batch fermentation Rushton turbine, aerated	*Azospirrillum lipoferum*		Change in shear	Changes extra-cellular polymer production
					Changes rheology
Edwards *et al.* 1989	Novel device for assessment of shear effects.	*Escherichia coli*		10.8	Optimum growth
				10.9 –14.5	Increase in cell length, asymmetrical cell division.
Yerushalmi & Volesky 1985	14L Microferm 2 6-bladed impellers	*Clostridium acetobutylicium* Anaerobic	0.419		Gas production peak
			0.506		Reduced
			0.691		Ceased

2.5 MICROBIAL AGGREGATES

Microbial aggregates form in single species and heterogeneous cell cultures. Aggregation may be stimulated by a number of factors including grazing pressure (Güde, 1982; Macek *et al.* 1993) and the nature of the cultured cells. Aggregation occurs by a number of mechanisms, more complex than those of simple chemical flocculation. Fungal hyphae and pellets have been cultivated widely and are not of particular relevance to this work. Shear effects on fungal fermentations have been extensively reviewed by Gibbs *et al*. (2000).

2.5.1 Flocculation

Bratby (1980) described flocculation as the process whereby destabilised particles come together, make contact and form larger agglomerates. Simons (1996) further claimed that a balance between binding and destructive forces governs flocculation, and floc characteristics. Li and Ganczarczyk (1986) found floc formation reached a dynamic equilibrium floc size, termed the maximum stable floc diameter, calculated as an exponential function of G. As discussed previously, Cleasby (1984) disputed the relevance of G for flocculation. Despite the work of Cleasby (1984) G has continued to be used for flocculation due to "… its ease of determination, simplicity and widespread acceptance" (Wahlberg *et al,* 1994). Kawamura (1996) and McConnachie and Lui (2000) found Gt to be the controlling factor for flocculation.

Mechanisms and kinetics of flocculation have been covered thoroughly by Gregory (1989). Optimum conditions for floc formation rely on G, t and a number of other factors relating to the specific system and the primary particle type.

Spicer and Pratsinis (1996) and Matsuo and Unno (1981) found increasing shear decreased floc sizes for polystyrene particles between 63 and 540 s^{-1} and fine clay from 90 to 220 s^{-1} respectively. Gmachowski (2002) found that increasing shear rate led to a narrower aggregate size distribution; high shear rates resulted in breakage of weak aggregates and increased the number of strong, high fractal dimension aggregates. This was in agreement with an earlier study that demonstrated smaller, more dense flocs forming after shearing (Spicer *et al*. 1998)

2.5.2 Floc Measurements

Floc size has been measured in a number of ways which has made comparisons more difficult. Maximum floc diameter is a common descriptor of floc size as it is easy to

measure (Li and Ganczarczyk 1986). Floc breadth or width, measured as the shortest dimension (Li and Ganczarczyk 1986), and the volume diameter and cross section diameter are also used to measure floc size (Kavanaugh *et al.* 1980).

Particle description requires a measure of shape and texture (Kaye 1992). Mandelbrot (1982) determined a fractional number to describe the space filling ability of a shape, the fractal dimension. Simons (1996) described fractal dimension as an attempt to quantify how densely a fractal occupies the space in which it lies. The coalesced fractal sphere (Lee *et al.* 2000) and average local volume fraction (Sonntag and Russel 1987) are measures of particle size relating to fractal dimensions. Attempts have been made in water and wastewater treatment to correlate fractal dimensions to floc settling ability. Snidaro *et al.* (1997) characterised the 3 dimensional fractal numbers of sonicated activated sludge flocs, relating 3 dimensional fractal dimensions larger than 2 to increased settling velocity and decreased activity of bacteria inside the floc due to impeded mass transfer.

2.5.3 Micro-organisms

Breakage of aggregates can occur in high shear environments; the maximum aggregate size is a balance between breakage and growth. The dynamic equilibrium point is dictated by a number of variables, including shear, and the biological and physical characteristics of the primary particles and bonding material. Mikkelsen and Nielsen (2001) used the adhesion erosion model of floc formation, describing it as allowing estimation of a floc "enthalpy" of cell adhesion. Mikklesen and Keiding (2002) found that G was very clearly linked to primary particle erosion in activated sludge, finding that they were able to successfully extrapolate data about shear effects from laboratory tests to full-scale treatment plants.

Bubbles can strongly affect viability of cultures; the proposed mechanisms were comprehensively reviewed by Hua *et al.* (1993). Illing and Harrison (1999) observed that for C*orynebacterium glutaminicium* more severe aggregate breakage occurred when sparged than in bubble free culture. Factors such as physical and chemical conditions of the culture medium can also affect the shear sensitivity. Wilen *et al.* (2000) found that cycled anaerobic conditions decreased the ability of sheared activated sludge to reflocculate compared to aerobic conditions.

2.5.4 Extra-Cellular Polymer

Extra-cellular polymer (ECP) is required by many micro-organisms to form aggregates. Microbial bonding differs from many flocculation mechanisms as once broken the ECP molecules will not reform (Spicer *et al.* 1998). The production of ECP has been related to shear intensity (Joshi *et al*. 1996). Wang and Wang (1990) found that increasing shear stress acting on immobilised *Acinetobacter calcoaceticus* from 0 to 0.5 Nm^{-2} decreased cell bound polymer to dry weight ratio from 1.6 to 0.2. Funahashi *et al*. (1987) also found that ECP removal occurred when the cells were subject to shear stress, and speculated that ECP layer removal stimulated an increase in polymer production. Conversely Gibbs and Seviour (1996) found reduced ECP production in *Aureobasidium pullans* with increasing shear. They went on to link this to an increase in oxygen concentration and not shear damage as earlier studies had assumed. The study highlighted the complexity of interactions in such biological systems.

2.6 ACTIVATED SLUDGE FLOCS

Activated sludge treatment of wastewater is reliant on flocculation for biomass separation through settling. Floc formation is favoured biologically through recycle of settled sludge and pressure from the protozoan grazing community (Güde 1979; Macek *et al.* 1993; Jurgens *et al.* 1997). Activated sludge flocs have been described in a variety of ways (Table 2.2).

Activated sludge requires a certain level of shear to reflocculate; brownian aggregation is only important for primary particles. The predominant mechanism of flocculation, cluster – cluster aggregation (Chaignon *et al.* 2002) is facilitated through the mechanisms of differential settling and fluid shear (Oles 1992; Gmachowski 2002).

For activated sludge typical flocculation process ranges are G 20 -150 s^{-1} (Metcalf and Eddy Inc. 2003). A number of investigations have been carried out into activated sludge flocculation, looking at optimum settling characteristics and improvement of plants (Table 2.3). Li and Ganczarkyk (1993) found that increasing shear rate from 23 to 102 s^{-1} decreased floc size, Mikkelsen and Keiding (1999) found that an increase

from 500 to 1000 s^{-1} caused total deflocccculation. The main sources of disruptive energy in activated sludge plants are jet aerator pumps, bubble motion (entrainment and bubble bursting), liquid transfer to the clarifier and induced velocity gradients (fluid motion and mechanical rakes) (Glasgow *et al.* 1983).

Table 2.2 Morphological models describing activated sludge flocs.

Reference	Floc type and system
Biggs and Lant 2002	Filamentous backbone a framework onto which primary flocs attach Floc formed of 3 major components, Bacteria surrounded by ECP matrix and water
Abbassi *et al*. 2000	3 zone floc, outer region oxygen and substrate saturated inner region oxygen saturated, substrate limited. Core oxygen limited, substrate saturated
Snidaro *et al*. 1997 and Jorand *et al*. 1995	Sonicated sludge revealed 3 size classes and levels of organisation: 2.5 μm bacteria, 13 μm micro-colonies of bacteria embedded in ECP and 125 μm tertiary structures - ECP linked micro-colonies.
Sanin and Vesilind 1996	Synthetic sludge required both polymer and calcium ions to form flocs, suggest calcium ions used as bridging ions between particles or particles and polymer.
Eriksson *et al*. 1992	Filamentous micro-organisms aid floc formation. Old flocs are smooth and slow growing embedded in an ECP matrix, young flocs are fluffy and grow rapidly using polymer bridging. Change in conditions stimulates rapid growth on floc exterior – fluffy "young" floc growth.
Li and Ganczarczyk 1990	Flocs cut into 3-6μm slices, viewed under microscope. Non-uniform distribution of cells, water gaps and extra-cellular polymer. Characterisation by fractal dimension. Water channels and reservoirs allow flow through flocs.
Parker *et al*. 1972	Filamentous backbone and primary particles.

Often investigations into shear in activated sludge have looked only at batch reactors and not the effects of shear rate over a number of retention times on population pressure. For example, Biggs and Lant (2000) measured floc agglomeration during reflocculation at various shear rates and Mikkelsen and Keiding (1999) measured turbidity compared to shearing.

Table 2.3 Effects of shear on activated sludge flocs.

Reference	System	τ Nm^{-2}	G s^{-1}	Effects
Biggs and Lant 2000	Standard fermentor vessel re-flocculation after destruction to primary particles by sonication		19.4 37 113 346	Correspond to floc sizes: 120 - 130μm (Primary particle size 100 - 110μm 14μm) 60 - 70μm 20 – 30 μm
Mikkelsen and Keiding 1999	1 L baffled tank single bladed paddle, in ice bath to minimise bacterial activity		500 800 1700	Increasing turbidity with increasing shear rate, levels off beyond 1000 s^{-1} i.e. total deflocculation
Clauss *et al.* 1998	Passing culture through pump	>3X10^6		Total deflocculation
Mikkelsen *et al*. 1996	Activated sludge from treatment plant to reaction chamber		530	Shear sensitivity and G give quantity of fine particles present in effluent at this velocity gradient due to floc damage.
Li and Ganczarkyk 1993	Activated sludge plants		102 23	Decrease in floc size from 102 to 23 s^{-1} due to shear.
Tuntoolavest *et al.* 1983	Activated sludge with aeration providing shear.		140	Average turbulence used in practice.
			162	Minimum in this experiment as below this dissolved oxygen is limiting.
Parker 1983	Survey of 19 full scale activated sludge plants		88 – 220	Favours floc breakup rather than flocculation
	Achieved though experiment		20 - 70	Values resulting in lowest values of dispersed primary particles

2.6.1 Flocculation and Breakage

Glasgow *et al*. (1983) found that flocs in a jet broke by a combination of deformation and rotation, causing erosion of subunits from the floc surface. Parker *et al*. (1972) stated that there were two possible mechanisms of floc breakage, surface erosion of primary particles and bulgy deformation-floc splitting with filament breakage in activated sludge flocs, these main mechanisms continue to be used to model floc breakup.

Shear damage to activated sludge has been found to be generally reversible. Certain conditions have appeared to limit the ability of activated sludge to re-flocculate. Wilen *et al*. (2000) found that anaerobic shearing induced deflocculation was less reversible than aerobic shearing, in the absence of nitrate, and thought this was due to an absence of bacterial activity, including facultative anaerobic activity.

2.6.2 Flocculation Summary

From Table 2.3 and the preceding discussion we can determine that the there is a lack of consensus regarding shear effects and limitations are added by the varied models and methods of quantification used to describe flocculation. All biological wastewater treatment requires a balance between cell growth and removal, shear forces have a role in moist systems.

2.7 Shear and Other Biological Wastewater Treatment Methods

Bacterial granules are formed in upflow anaerobic sludge blanket reactors (UASB). Attempts have been made to stimulate aerobic granule formation, Beun *et al*. (1999) cited a number of examples where aerobic granulation had been achieved. The paper hypothesised that in an aerobic sequencing batch reactor (SBR) reduced settling times would favour granule rather then floc formation, as flocs would be washed out. This hypothesis was proved, but elevated shear levels were required to prevent large substrate limited granules forming. Tay *et al.* (2001) attempted to quantify the factors required for granule formation. They used a sequential aerobic sludge blanket reactor fed with synthetic media using various air flow rates to give different shearing

properties. At low air flow velocities typical activated sludge flocs remained, while at higher air flow velocities granules appeared, becoming more compact and dense with increasing shear.

Biofilm reactors are used widely in waste water treatment. Liu and Tay (2001) used an annular biofilm reactor to correlate metabolic response to shear rate (tip velocity ranging between 0.48 and 1.45 ms^{-1}). Biofilm thickness was monitored; increased shear stress resulted in a thinner and denser biofilm. Kwok *et al*. (1998) presented a change in biofilm morphology as the result of changed shear, with smooth dense biofilm being formed under high shear conditions. These results can be related to the findings of Eriksson *et al.* (1992) who propose that "young floc" protrusions are removed with elevated shear, reinforcing the "old floc" state by encouraging more compact growth (Table 2.2). Peyton and Characklis (1993) found that high shear rate did not increase detachment in a RotoTorque reactor when constant or gradually changing. They proposed that the shear decreased the resistance to mass transfer, increasing substrate utilization rate; any rise in detachment was in due to the elevated growth rate and not a direct effect of shear.

The breakage behaviour of biofilm was investigated by Stoodley *et al*. (2001); they measured biofilm deformation in a glass flow cell, finding that biofilm colonies behaved elastically until they finally broke off at the critical yield fluid shear stress.

2.8 CONCLUSION

The heterogeneity of the activated sludge system has made it more difficult to understand the complex shear effects. Parker *et al*. (1992) found that the water industry had a limited ability to predict and model bioflocculation.

While bacteria can survive high shear, cell aggregates are affected at much lower levels. It is clear from the literature cited above that bio-flocculation is a sensitive process, that maintains biological treatment but reduces mass transfer and with it the treatment rate. It would appear sensible to investigate the possibility of increasing treatment rate through using a dispersed bacterial culture rather than aggregates. The shear rates reported in Table 2.3 can be used as a guide to the levels of shear required to break flocs, information about protozoan and bacterial sensitivity will also serve to direct limits the shear rate employed and aid analysis of results.

3 TYPICAL ACTIVATED SLUDGE PLANT POWER REQUIREMENT

Information kindly provided by Yanmin Zhang and Elizabeth Wood, Yorkshire Water Plc.

Information from a nitrifying activated sludge plant run by Yorkshire Water treating 200 000 m^3 of wastewater per day. The current power consumptions were given for the plant as 30369 $kWhday^{-1}$ for aeration and 6705 $kWhday^{-1}$ for pumping (Table 3.1). The pumping was broken down as flows of 3600 m^3day^{-1} settled activated sludge (RAS) and 5164 m^3day^{-1} scrubber and sludge liquors

Table 3.1 Power requirement to treat 200 000 m^3day^{-1} wastewater

	Power ($kWhday^{-1}$)	Per unit volume ($kWhm^{-3}$)
Aeration	30369	0.152
Pumping	6705	0.034
	(For the actual 8764 m^3 pumped	0.765)
Total for influent		0.189

The activated sludge process nitrified but did not denitrify. There was no internal recycle and less than 2 % of the flow to the aeration basin made up of returned settled activated sludge.

This information is used to compare power results of the stirred tank reactor with a typical activated sludge plant in the Section 7, Page 108.

4 AIMS AND OBJECTIVES

The aim of the project was to develop a single pass continuous fed stirred tank reactor for wastewater treatment. The underlying concept was that it may be possible to apply principles used in biotechnology to achieve an elevated growth rate with bacteria feeding on the wastewater as dispersed single cells rather than flocs, granules or biofilm, thereby maintaining a more active biomass and allowing free mass transfer.

4.1 OBJECTIVES

- To design and build a single pass continuously fed stirred tank reactor suitable for real domestic wastewater treatment.
- Characterise the reactor properties of oxygen mass transfer, shear and mixing.
- Test the effects of increasing the mixing and shear rates beyond those typically used in wastewater applications with relation to the effects on reactor community and effluent quality.
- Separate the effects of mixing and shear types to better understand and optimise the system.
- Investigate possible applications, further treatment requirements and feasibility of the reactor system.

5 MATERIALS AND METHODS

5.1 EXPERIMENTAL RIG

5.1.1 Stirred Rig

The primary experimental rig used was a 9 L continuous stirred tank reactor (CSTR) built as a standard disk turbine, baffled reactor (Figure 5.1, Figure 5.2 and Figure 5.3). The reactor was made up of a glass pipe section (229 by 500 mm internal diameter and height) (P59/500, QVF, Stone, UK) connected to a purpose built stainless steel base plate and lid using a flange with inserts (CT9 kit, QVF,) and sealed using gaskets (TMP9, QVF).

The reactor was built with a standard aspect ratio following Wu (1995) and Gibilaro *et al*. (1985) (Table 5.1). A peristaltic pump (603s, Watson Marlow, Falmouth, UK) was used to control the feed rate, according to the gas hold up and required retention time, Neoprene and Marprene pump tubing was used to deliver feed (903.0032.016, Watson Marlow).

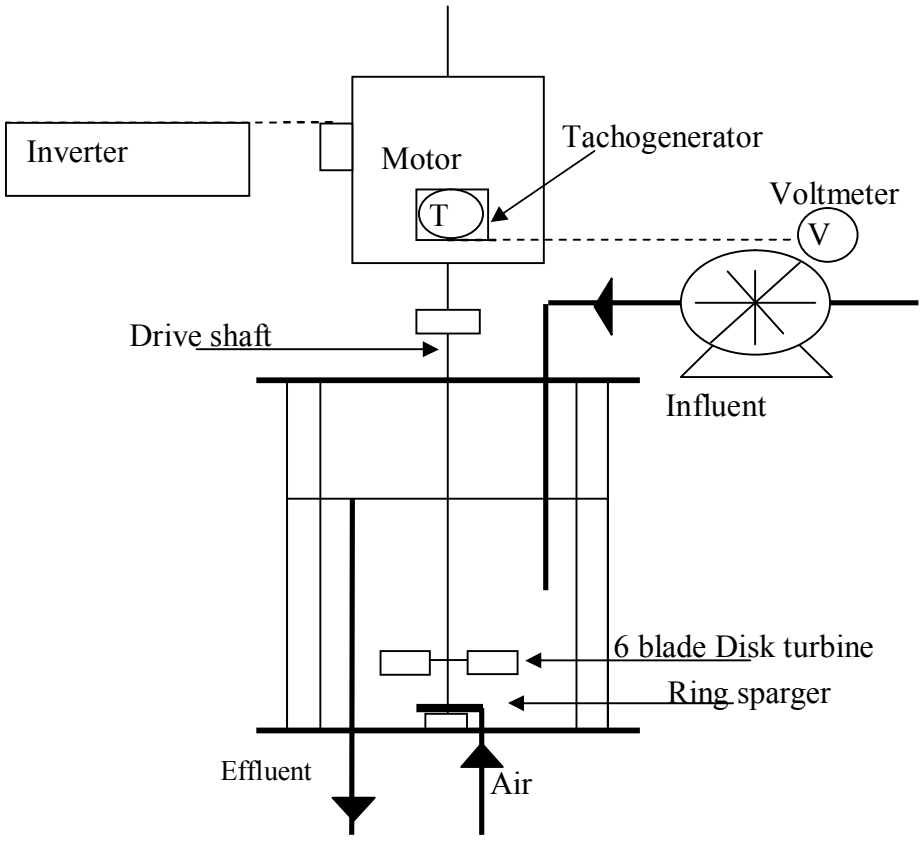

Figure 5.1 Schematic diagram of reactor set up (not to scale)

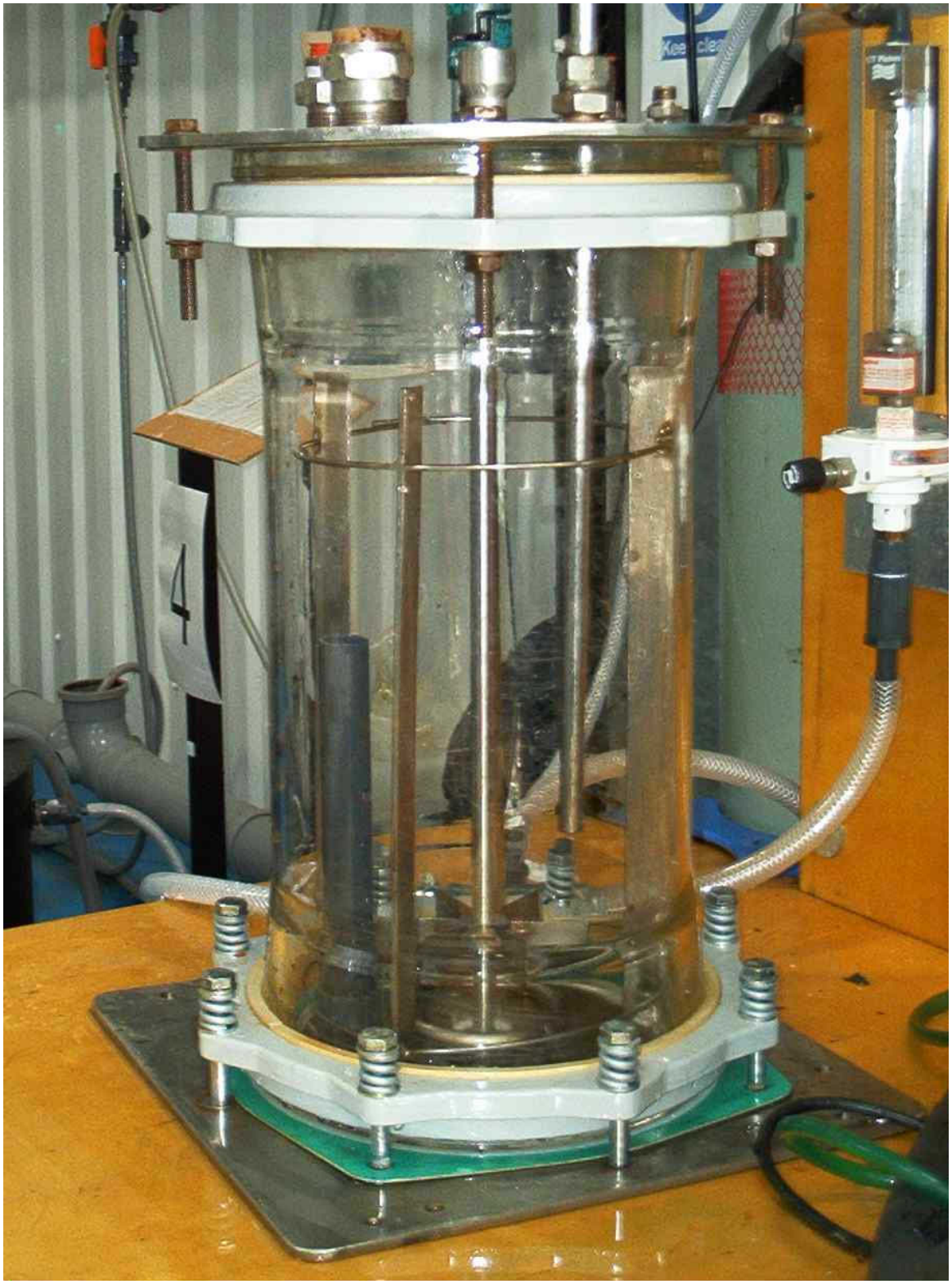

Figure 5.2 Stirred rig with Disk turbine installed

A six bladed Disk turbine impeller was used; this was made for the reactor from stainless steel, and built in house. The aspect ratio of the impeller is given in Table 5.1.

The inverter and program control module (605, Eurotherm Drives Ltd. Littlehampton, UK) were used to control the motor (FFIAC 2K2, Brook Hansen, Huddersfield, UK) power input. Rotational speed was measured using a tachogenerator (DC GT7.08, Brook Hanson) which gave a DC output of 3.6 V for each revolution per second (s^{-1}) (3.6 VHz^{-1}). The output voltage of the tachogenerator was monitored using a digital multi-meter (NLDMLC, Newlec, Edgebaston, UK).

Aeration was provided by a ring sparger made from 12.7 mm stainless steel tube with a 40 mm outside diameter with 5 6.35 mm holes cut into the underside, made in house. The coarse bubbles were broken up by the stirring. The airflow rate was controlled using an automatic airflow controller (MNBB21 l, Platon, Basingstoke, UK) and a float type flow meter (1 – 10 L GTV, Platon) measured the airflow rate.

Table 5.1 Dimensions of the stirred reactor vessel components (Disk turbine).

Reactor Component	Relation to vessel diameter (Dt)	Size in reactor (mm)
Maximum liquid depth	= Dt	229
Impeller diameter	= Dt/2	115
Impeller to tank bottom	= Dt/3.5	65
Baffle width	= Dt/10	23
Impeller blade height	= Dt/10	23
Impeller blade length	= Dt/8	29
Impeller disk width	= Dt/3	76

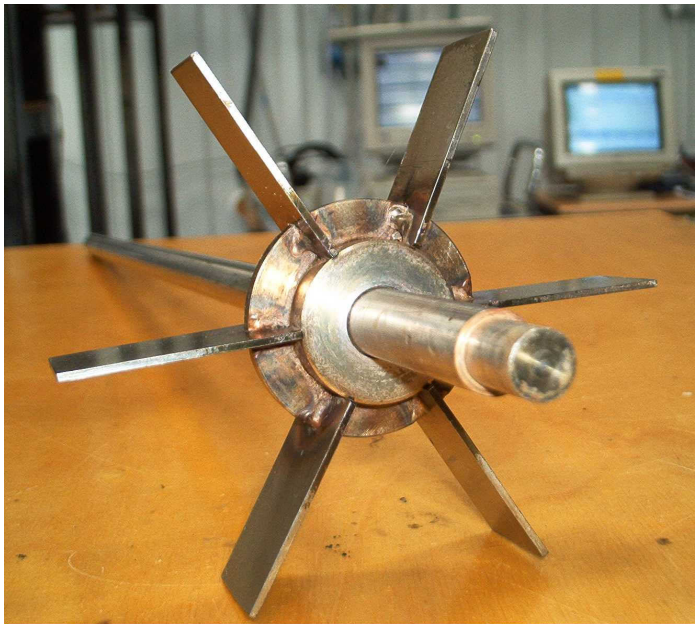

Figure 5.3 Disk turbine Impeller

For Run 8 2 different impellers (Figure 5.4) were used to try to separate the effects of mixing and shear. The impellers were a high shear disk (HSD), power number 0.1 – 0.15 (Brown D. 2002) and an LE20, power number 0.35 for size in reactor (FMP, 2001) (Figure 5.4).

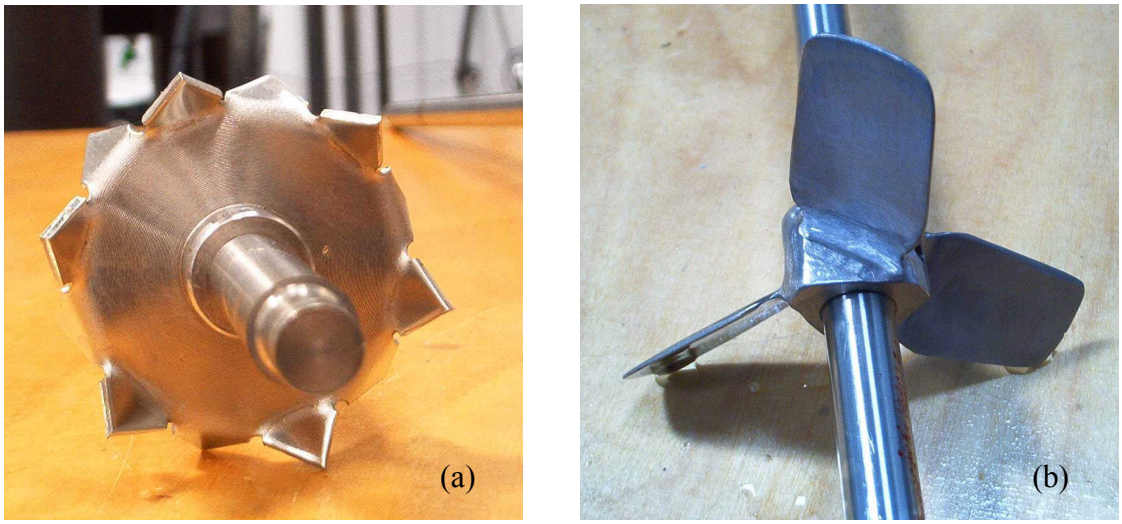

Figure 5.4 Alternative impellers used (a) High Shear Disc and (b) LE20.

Table 5.2 Impeller properties

Parameter	Disk turbine	High Shear Disk	LE20
Di (mm)	115	62	104
Np	4.22	0.1 – 0.15	0.35

A simple cooling coil and insulating jacket provided temperature control for the stirred reactor. The equilibrium temperature was elevated by the insulation; a capillary thermostat (250-6106, RS Components Ltd., Corby, UK) turned the centrifugal coolant pump (LMR 71/4, Lafert, Venice, Italy) on and off. Coolant water was circulated through the cooling coil to a heat exchanger (APV, Derby, UK) connected to a chiller (IPE81, Coldbox Ltd., Poole, UK) (Figure 5.5).

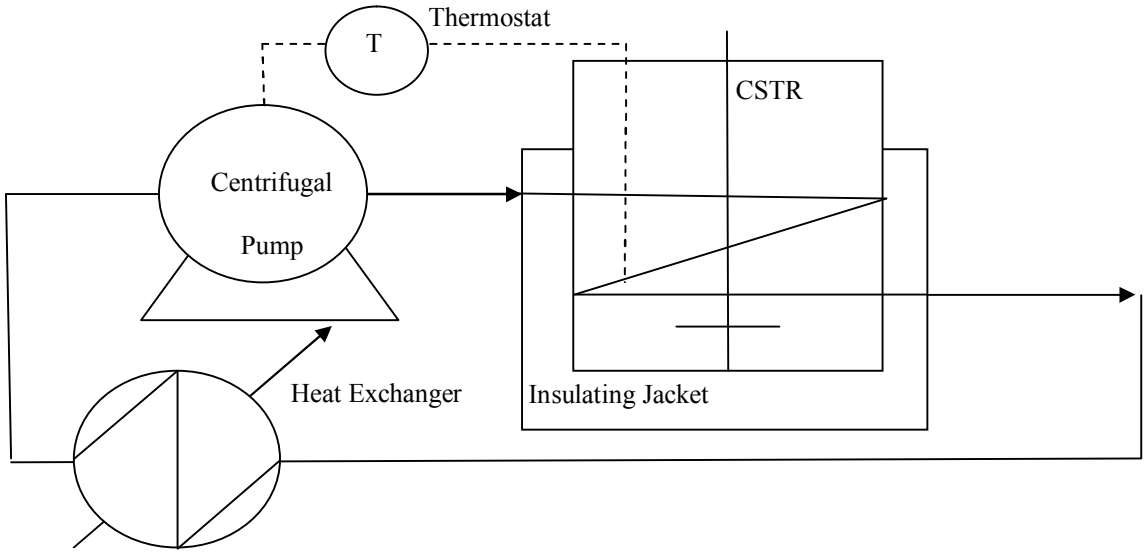

Figure 5.5 Stirred reactor temperature control system schematic (not to scale)

5.1.2 Unstirred Rig

An unstirred reactor was set up to test if the nitrification in the stirred tank was entirely due to temperature or if mechanical agitation had an important effect. Temperature control was provided by a heated water bath (Immersion circulator, C1, Haake, Karlsruhe, Germany)) (Figure 5.6). The influent and effluent were pumped using peristaltic pumps (603s, Watson Marlow). The reactor was a white 8 L polypropylene bucket (Lucy housewares, Portsmouth, UK) aerated using fish tank aerator stones (Aqua Air Jazz cylinder stone 14 X 25 mm, Interpet Ltd., Dorking, UK) at 2 $Lmin^{-1}$, measured using a float type flow meter (1 – 10 L GTV, Platon).

5.1.3 Anoxic Rig

The anoxic reactor was a fixed film submerged reactor, run as a secondary reactor to the stirred tank. The reactor was used to test if the effluent from the stirred tank could be denitrified. The effluent from the stirred reactor flowed down the overflow pipe, and into the top of the secondary reactor. The Perspex reactor had internal dimensions of 100 mm by 100 mm by 360 mm, (Model Products, Bedford, UK). PVC structural fill media (BIOdek FB10.12, Munters Euroform, Aachen, Germany, Figure 5.7) was cut to fit the vessel to a height of 300 mm, the liquor flowed down through this to the effluent tube at the bottom. The reactor volume without biomass was 2.5 L (Figure 5.8).

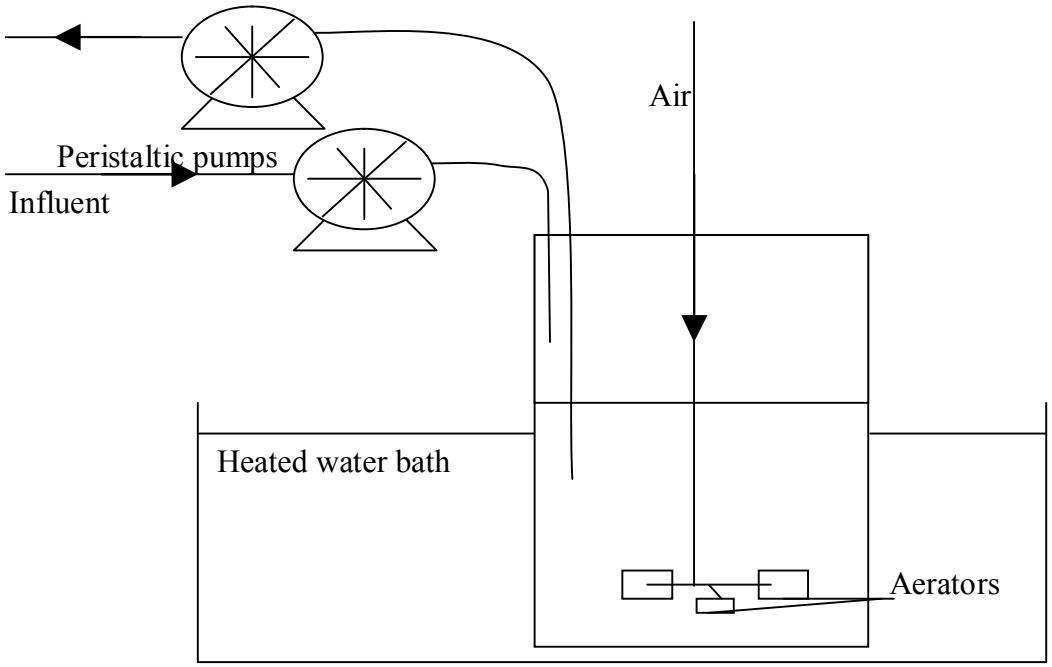

Figure 5.6 Unstirred reactor (not to scale)

Figure 5.7 BIOdek media showing two sides and the structure with diagonal corrugation in opposite directions.

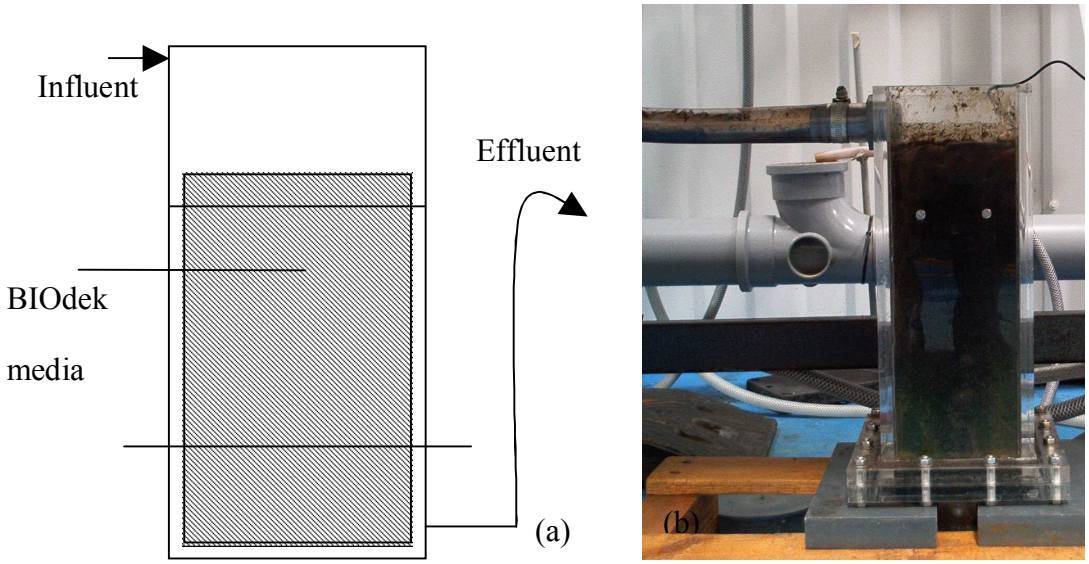

Figure 5.8 Anoxic reactor: (a) schematic (not to scale) and (b) photograph.

5.2 REACTOR OPERATION

5.2.1 Feed

The reactor was fed using settled sewage from the Cranfield University Wastewater Treatment Works. The sewage was taken from the pyramidal primary sedimentation tank to a holding tank (1 m^3). From the holding tank sewage was pumped around a ring main from which the feed was taken for the experimental rig. The influent characteristics were measured for every sample.

5.2.2 Reactor Maintenance

The stirred reactor was emptied daily; the reactor walls, baffles, overflow tube, temperature probes, air sparger and impeller were scrubbed using a burette brush (BUR-440-070M B68-145, Fisher Scientific, Loughborough, UK) and rinsed with tap water to prevent an accumulation of wall growth distorting the results. During cleaning the reactor liquor was aerated in a 10 L polyethylene narrow neck jerrican (BTK-525-070A, Fisher Scientific) reserved for this purpose, it was returned to the reactor once the cleaning was complete.

The same process was used to keep the unstirred reactor relatively biofilm free. The reactor was emptied daily into an 8 L polypropylene bucket (Lucy housewares) and

scrubbed clean. This reactor contained much greater quantities of solids, the wall growth was greater and cleaning could be considered a form of biomass wastage.

The influent flow rate was checked daily. The influent pipe was transferred to a 250 mL measuring cylinder. The time taken for a set volume to flow into the cylinder was measured; any adjustments to the flow were made by changing the pump tubing or pump rate.

The influent pipe was scoured daily to remove excess build up of biofilm by transferring the influent tube to a bucket opening the pump head, and allowing the pressure from the ring main to force any solids through the pipe. The influent was run into the bucket until it flowed free from gross solids.

5.2.3 Sampling

Samples for analysis were taken a minimum of one retention time apart, at least 6 retention times were allowed for acclimatisation to steady state after each change of conditions before a sample was taken. Samples were collected in acid washed, 250 mL high density polyethylene, wide mouth square bottles (2114 – 0008, Nalgene, Rochester NY, USA).

Influent samples were taken by removing the feed pipe from the reactor and continuing to pump into a sample bottle at the same rate, this was necessary to prevent a change in the influent composition. Samples of 200 ml – 250 ml were taken to minimise the time the feed was disconnected from the reactor. One influent sample was taken for the stirred and unstirred reactor as the feed was from the same source and pumped through the same tubing to the reactor (Marprene, Watson Marlow)

Stirred tank samples were taken directly from the stirred reactor so that there was no interference from any wall growth in the effluent pipe. Unstirred reactor samples were taken in the same way, after mixing the reactor contents.

Samples of effluent from the anoxic rig were, collected from the effluent pipe at the natural rate of emergence. Movement of the pipe was avoided as it caused solid to be drawn through the tubing.

at 4 °C. Turbidity, suspended solids and pH were tested in the laboratory within 30 min of collection to limit the effects of flocculation on the results.

General glass and plastic ware used for analysis and sample collection was cleaned by soaking in 5 % acid (Nitric acid, Certified AR, Fisher Scientific, Loughborough, UK) overnight and rinsing 3 times in deionised water, except BOD incubation bottles where the acid solution was substituted for an acidified iodine solution in accordance with the standard methods (APHA 1998). TOC glassware was washed using hydrochloric acid solution according to manufacturers instructions.

5.2.4.1 Quality Control

During runs 1 and 2 all analysis was carried out in triplicate for every measurement. This gave a background level of error for each method. During all other runs one sample was analysed in triplicate for each measurement. The repeated measurements confirmed the level of error was still comparable to the standard error measured during Runs 1 and 2.

5.3 EXPERIMENTAL PROTOCOL

The main conditions altered in the stirred reactor were stirrer speed (power, mixing and shear) and hydraulic retention time. The measured temperature in the reactor varied with stirrer speed, this was controlled for certain stirrer speeds. The main running conditions are given in Table 5.3 below.

Table 5.3 Reactor run conditions

Run (part)	Samples	Stirrer speed (RPM)	(s^{-1})	Tip speed (ms^{-1})	Retention time (h)	Additional equipment	Impeller
1	Varied	500	8.3	3	12		DT
2 (1)	10	500	8.3	3	12		DT
2 (2)	10	500	8.3	3	10		DT
2 (3)	10	500	8.3	3	08		DT
2 (4)	10	600	10	3.61	08		DT
2 (5)	10	500	8.3	3	08		DT
3 (1)	10	600	10	3.61	08		DT
3 (2)	10	700	11.7	4.23	08		DT
3 (3)	10	900	15	5.42	08		DT
3 (4)	10	900	15	5.42	10		DT
3 (5)	10	900	15	5.42	08		DT
4	10	900	15	5.42	10		DT
5 (1)	20	900	15	5.42	10		DT
5 (2)	20	1000	16.7	6.03	10		DT
5 (3)	20	1000	16.7	6.03	12		DT
5 (4)	18	900	15	5.42	12		DT
6	14	900	15	5.42	10	C 34 ºC, U	DT
7 (1)	10	900	15	5.42	10	C 34 ºC, A	DT
7 (2)	10	900	15	5.42	10	C 31 ºC, A	DT
7 (3)	10	700	11.7	4.23	10	C 27 ºC, A	DT
7 (4)	10	700	11.7	4.23	10	C 31 ºC, A	DT
8 (1)	10	1302	21.7	4.23	10	A	HSD
8 (2)	10	924	15.4	3	10	A	HSD
8 (3)	10	551	9.18	3	10	A	LE20
8 (4)	10	776	12.9	4.23	10	A	LE20

C = Cooling, U = Unstirred Rig, A = Anoxic Rig, DT = Disk turbine. HSD = High Shear Disk

5.4 REACTOR MEASUREMENTS

5.4.1 Power Input

The power input of the Disk turbine reactor was calculated at various stirrer speeds (N_i) with a constant gas flow rate (Q_G) of 2 $Lmin^{-1}$. The impeller power number (N_p) was calculated as 4.218 for the reactor set up using Equation 5.1. The Reynolds number (N_{Re}) and ungassed power input (P_o) and G were calculated using standard equations (Equation 5.2, Equation 5.3 and Equation 2.3 respectively). The gassed power input (P_a) was calculated with an equation derived using data from aerated Disk turbine reactors (Equation 5.4).

Equation 5.1

$$N_P = 5.17\left(\frac{C}{D_i}\right)^{0.29}$$

System: Flat bottomed vessel. 4 baffles, 1 impeller, 6 flat bladed turbine. Water and air 0.232 m^3 (Gray *et al*. 1982)

When C is height off tank bottom (m)

Equation 5.2

$$N_{\mathrm{Re}} = \frac{D_i^2 N_i \rho}{\mu}$$

(Metcalf and Eddy Inc. 2003)

Equation 5.3

$$P_o = N_P \rho N_i^3 D_i^5$$

(Metcalf and Eddy Inc. 1991)

Equation 5.4

$$P_a = 0.83\left(\frac{P_o N_i D_i^3}{Q_G^{0.56}}\right)^{0.45}$$

System: flat bottomed, 5.5L, 4 baffled, Impeller 1 Rushton turbine. Diameter T/3 C = T/3 (Loiseau *et al*. 1977)

5.4.2 Mixing Measurements

Tracer studies were used to describe mixing and flow characteristics of the stirred reactor. The tests were used to check that dead zones and short circuiting were not occurring within the reactor. The test was carried out as a clean water test in the stirred tank. Sodium chloride was used as the tracer, dissolved in water (Levenspiel 1999). The tracer emergence was measured with a conductivity meter (4071, Jenway Ltd., Dunmow, UK). This method was only carried out for clean water tests as the conductivity of sewage varied. The tests were carried out at stirrer speeds intended to be used for later experiments. The retention time was decreased to allow samples to be taken more rapidly (it would take a number of minutes for each measurement if 8 h retention time was used).

The influent rate, stirrer speed and aeration rate were set and the vessel was run for 10 min under these conditions to normalise the flow. 75 ml (0.8% reactor volume) of 1 molar NaCl (4.38 g Analar grade NaCl, BDH, Poole, UK) solution in tap water was added to the vessel over 15 s (1.7% retention time) while temporarily stopping the influent following the method of Mendoza Espinoza *et al*. (1997). The effluent was then collected in 100 mL sample bottles at 30 s intervals, and a conductivity meter was used to test the conductivity of each sample. The conductivity recorded represented the effluent cumulative sample collected over 30 s leading to the recorded time. The conditions tested are given in Table 5.4 below.

Table 5.4 Conditions tested using salt tracer studies

Impeller	Stirrer speed (s^{-1})	Aeration rate ($Lmin^{-1}$)	Retention time (min)
RT	0	0	15
RT	0	2.5	15
RT	0	10	15
RT	4.17	0	15
RT	4.17	2.5	15
RT	4.17	10	15
RT	8.33	0	15
RT	8.33	2.5	15
RT	8.33	10	15
HSD	15.4	2	15
HSD	21.7	2	15
LE20	9.18	2	15
LE20	12.9	2	15

The theoretical residence time was calculated, taking into account the gas hold up at different stirrer speeds and aeration rates. The reactor was filled with water and run under the appropriate conditions for a 20 min or until the liquid level appeared stable; the liquid level was then measured. The results of the tracer study were analysed using the methods of Levenspiel (1999) (Table 5.5).

Table 5.5 Calculations used in determining mixing regime.

Mean Residence time (t)			Flow Modelling		
Calculated (t_c)	$t_c = \frac{V}{v}$	Equation 5.5	Variance (σ^2)	$\sigma^2 = \frac{\sum t_i^2 C_i}{\sum C_i} - \bar{t}^2$	Equation 5.6
Observed (t_o)	$t_o = \frac{\sum t_i C_i}{\sum C_i}$	Equation 5.7	Tanks in series (N)	$N = \frac{\left(\bar{t}_{out} - \bar{t}_{in}\right)^2}{\sigma^2_{out} - \sigma^2_{in}}$	Equation 5.8
			Dispersion model	$\sigma_\theta^2 = \frac{\sigma^2}{\bar{t}^2} = 2\frac{D}{uL} - 2\left(\frac{D}{uL}\right)^2\left(1 - e^{-ul/D}\right)$	Equation 5.9

C_i = concentration (gL-1)
v = flow rate (L/min)
D/uL = Vessel dispersion number (-)
V = reactor liquid volume (L)
t_i = time (s)

5.4.3 Oxygen Transfer Coefficient (K_La)

The KLa was measured for clean water using the dynamic gassing out method (Dursan *et al.* 1999). The oxygen concentration in the reactor liquor was reduced to zero by purging with nitrogen (Low Oxygen Nitrogen, BOC, Guildford, UK) and then the

aeration was resumed and the dissolved oxygen concentration increase was measured using a data logging dissolved oxygen probe (Oxyguard Oxylog, Partech Electronics Ltd., St. Austell, UK). The conditions under which the Experiment was carried out are in Table 5.6. The K_La was calculated using the method in Doran (1995) using Equation 5.10 below.

Table 5.6 Experimental conditions used to test K_La

Impeller	Stirrer Speed (s-1)	Aeration rates (Lmin-1)
RT	0.83	2.5, 5, 7.5 and 10
RT	1.67	2.5, 5, 7.5 and 10
RT	4.17	2.5, 5, 7.5 and 10
RT	8.33	2.5, 5, 7.5 and 10
RT	12.5	2.5, 5, 7.5 and 10
HSD	15.4	2
HSD	21.7	2
LE20	9.18	2
LE20	12.9	2

$$K_L a = \frac{\ln\left(\frac{\overline{C}_{AL} - C_{AL1}}{\overline{C}_{AL} - C_{AL2}}\right)}{t_2 - t_1}$$

Equation 5.10 (Doran 1995)

C_{AL} = dissolved oxygen concentration t = time

5.5 ANALYTICAL METHODS

5.5.1 Biochemical Oxygen Demand (C and N)

The quantity of oxygen required to biologically stabilise organic matter present in a sample is measured using BOD_5 (Metcalf and Eddy Inc. 2003). To achieve biological stabilisation bacteria need to be present in the sample. If they are not present, the solution can be seeded with bacteria from a known source.

The BOD_5 test was carried out by incubating a solution in the dark for 5 d at 20°C. A sample was diluted by a known ratio, with standard, aerated, nutrient rich, dilution water to prepare the solution. The diluted sample was transferred to a bottle and the dissolved oxygen (DO) was measured, the bottle was refilled and sealed to prevent gaseous oxygen dissolving into the solution. After 5 days the DO was measured again.

The BOD_5 was calculated from the oxygen used and the sample dilution ratio (APHA 1998).

The BOD_5 test is subject to interference from nitrifying bacteria. Nitrifying bacteria utilise ammonia present in both the sample and included in the dilution water, exerting a nitrogenous BOD_5 ($NBOD_5$). This would not necessarily be exerted outside the wastewater treatment works (Carter 1984). The $NBOD_5$ was only observed in samples containing numerous active nitrifying bacteria; $NBOD_5$ was usually only measurable after more than 5 days incubation (Figure 5.9).

Carbonaceous BOD_5 ($CBOD_5$) has been quoted for water quality rather than total BOD_5 as it measured only heterotrophic activity and did not include $NBOD_5$. $CBOD_5$ was measured by adding a nitrification inhibitor to the prepared solution. Allylthiourea (ATU) has been found to be 96% effective at preventing ammonia oxidation (Sharma and Ahlert 1977), and was used to inhibit nitrification in this study. Boyd and Gross (1999) separated the various constituents of BOD_5 using Equation 5.11.

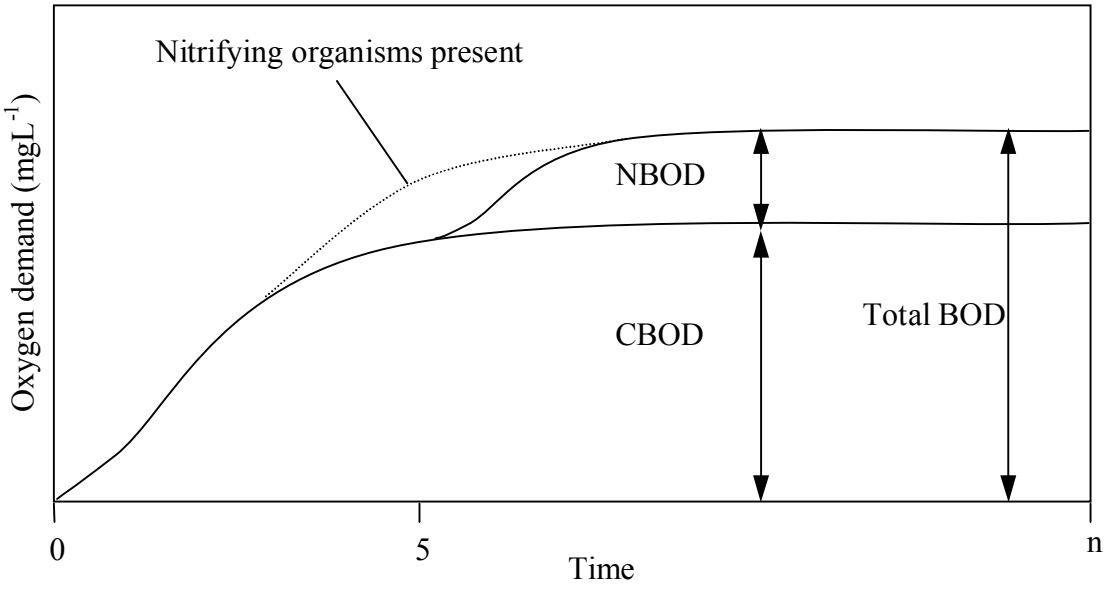

Figure 5.9 Sketch defining the exertion of the carbonaceous and nitrogenous BOD in a wastewater sample (Metcalf and Eddy Inc. 2003)

Equation 5.11 $BOD_5 - CBOD_5 = NBOD_5$

Strotmann and Windecker (1997) used a similar equation for respirometry measuring a carbonaceous oxygen uptake rate (OUR) for an ATU inhibited sample (Equation 5.12)

Equation 5.12 $OUR - C.OUR = N.OUR$

Both BOD_5 and $CBOD_5$ were measured in this study so that $NBOD_5$ could also be evaluated to show the levels of active nitrifying bacteria present in the reactor liquor. Nitrification in the reactor was measured by analysing ammonia, nitrate and nitrate; such measurements could not indicate where in the reactor the nitrification was occurring. Wall growth has been believed to be the source of nitrification in some suspended growth reactors (Lee and Welander 1996). Measuring the $NBOD_5$ of a sample illustrates the presence of nitrifying bacteria in the sample, in this case the bulk reactor liquor.

5.5.2 Routine Analysis

The routine laboratory analyses carried out on the samples were 5 day Biochemical Oxygen Demand (BOD_5) (carbonaceous and nitrogenous), suspended solids (SS) volatile suspended solids (VSS) and nitrite using standard methods (APHA 1998). Chemical Oxygen Demand (COD), ammonia and nitrate were determined using photometric test kits (Hach, Loveland, USA), measured using a spectrophotometer (DR/2010, HACH, USA) using the methods in the handbook supplied with the equipment. Turbidity was measured using a turbidimeter (2100N, Hach). Total Organic Carbon (TOC) was determined using a standard catalyst TOC analyser (5000a, Shimadzu Europa, Milton Keynes, UK), by the total carbon minus inorganic carbon method. Total phosphorus was analysed using an inductively coupled plasma (ICP) with atomic absorption spectroscopy (AAS) detector (Atomscan 16, Scitech instruments, Olney, UK). Raw samples were used for turbidity, pH, total COD, ammonia and BOD_5 (C and N). Samples for TOC, soluble COD, nitrate and nitrite were prepared by filtering through glass fibre filter papers (GF52 Schleicher and Schuell, Dassel, Germany). Samples for ICP analysis were filtered as above and acidified to 0.5% (Nitric acid, Certified AR, Fisher Scientific, Loughborough, UK).

The reactors were monitored for pH, dissolved oxygen concentration and temperature. These parameters were tested each time the reactor was cleaned and when samples were taken using an electronic pH meter (Hanna HI8424 Patterson Scientific, Luton) with a flat surface probe (DirectION epoxy bodied electrode, Patterson Scientific), a dissolved

oxygen probe (Oxyguard Handy Beta, Partceh Electronics Ltd.) and a max-min electronic thermometer (427-461, RS components Ltd.).

5.5.3 Statistical analysis of results

The Student t test and Wilcoxon Signed Rank Test for paired samples were used to compare paired data. The test for skewness, Shapiro-Wilk test for normality and F-test to compare variances were used to fulfil the requirements of the student t test; reasonable normality of distribution and equal variances. When the requirements for the Student t test were not fulfilled the Wilcoxon Signed Rank Test was used - a non parametric hypothesis test which compares the medians rather than the means and variance. Non parametric hypothesis tests do not assume a known data distribution, giving a less powerful test that is more applicable to the small data sets with non-normal distribution obtained. The Mann Whitney U test was used to compare independent groups (analysis ideally carried out using an independent t test). The alternative nonparametric methods for hypothesis testing have the same structure as a t test as they use a null hypothesis that the samples are the same and compare a test statistic to a proven value.

The Wilcoxon test compares the data sets by ranking the differences and disregarding the sign and then comparing the positive and negatively signed results. The Mann Whitney U test ranks the samples as one group, the ranks of the two data sets are then compared. The smaller result of the calculated U is then compared to the standard U value of the appropriate significance level (Rees 1995). The analysis was carried out using Statsoft Statistica.

Linear regression, multiple regression and curve fitting were used to analyse relationships between variables and to test their statistical significance. These techniques rely on the least squares method to derive linear relationships of the form y=a+bx+error. The ordinary least squares seeks to find the values (a and b) in this formula that minimises the deviation of observations from the model. This is calculated by measuring the sum of the squared deviations in the sample (Fleming 2000). Analysis was carried out using the data analysis add-in for Microsoft Excel. Multiple linear regression carries out this analysis by changing one variable at a time and fitting a line to the data while maintaining the other variables at the same level.

The coefficient of determination (R^2) is used to describe the extent to which the model explains variation from the sample mean. It is based on the fact that deviation from the mean is explained in part by the model and in part by random error. The ratio of variance described by the model to the sample variance gives the R^2. As R^2 tends to 1 it implies the model more accurately fits the data and less variation is explained by error (Fleming 2000).

The t tests used in linear regression test the significance of the coefficients by comparing them to 0 (the null hypothesis). This gives the significance of relationships found within the sample (to the required significance level) using the t distribution (Fleming 2000).

6 RESULTS

6.1 REACTOR SET UP

6.1.1 Power input

The reactor liquid volume was found to vary due to gas entrainment in the reactor. The liquid volume (V m^3) was observed for a number of stirrer speeds using settled sewage for each of the impellers. The results were used to calculate the average power input per unit volume ($\overline{P}$) for the stirred reactor under all operating conditions. The stirrer speeds used for the LE20 and HSD were matched tip speeds to the Disk turbine at 8.3 and 11.7 s^{-1} (Table 6.1). Ideally the power input would have been matched, this was not possible as the motor used was not able run at high enough speeds to produce the necessary power for the LE20 and High Shear Disk impellers. The tip speed was matched because this parameter has been widely used to compare shear. The power information was calculated (using Equations 5.1 to 5.4 and Equations 2.1 to 2.5 and 2.8) for liquid properties equal to those of water at 20 (Dynamic viscosity (μ) = 1.002 x 10^{-3} Nsm^{-2}, Kinemaic viscosity (ν) = 1.003 x 10^{-6} m^2s^{-1}, Density (ρ) = 998.2 kgm^{-3}) and 30 ºC. (μ = 7.98 x 10^{-2} Nsm^{-2}, ν = 8 x 10^{-5} m^2s^{-1}, ρ = 995.7 kgm^{-3})

The Reynolds number was greater than 10 000 for all of the experimental conditions, meaning that the reactor was always turbulent. As no data was available for the LE20 and HSD impellers under gassed conditions the ungassed power was used for further power and shear calculations. Gas entrainment significantly decreased the Disk turbine power input, and the influence of gassing increased with stirrer speed (Table 6.1); this was also observed during the clean-water experiments (Appendix B CD-ROM). The highest power inputs were achieved with the Disk turbine, as up to 9142 Wm^{-3} was achieved at a stirrer speed of 16.7 s^{-1} under gassed conditions (2 $Lmin^{-1}$ air). The Disk turbine under gassed conditions had a theoretical power input 4 times that of the ungassed LE20 and 30 times that of the HSD at tip speed 3 ms^{-1}.

Temperature was found to have little effect on power input or energy dissipation per unit mass. However, the Reynolds number and shear rate increased with temperature and the eddy dissipative length and associated shear stress decreased with the increase in temperature (Table 6.1).

Table 6.1 Calculated power and shear for the stirred reactor under all operating conditions, calculated for water at 20 and 30 ˚C.

Impeller	Tip speed (ms^{-1})	Ni (s^{-1})	Volume (m^3)	N_{Re} (-)	P_o (W)	P_a (W)	P (Wm^{-3})	ISF (s^{-1})	G (s^{-1})	ε (Wkg^{-1})	η (μm)	τ (Nm^{-3})
20 ˚C	Eqn 2.1			5.2	5.3	5.4		2.2	2.3	2.4	2.5	2.8
Disk turbine	3.0	8.3	0.0075	109400	50.3	9.1	1212	52.6	1099	1.21	30.2	1.10
	3.6	10.0	0.007	131700	88.0	12.7	1816	63.4	1346	1.82	27.3	1.35
	4.2	11.7	0.006	154100	140.9	16.9	2811	74.2	1674	2.82	24.5	1.68
	5.4	15.0	0.004	197600	296.9	26.4	6593	95.1	2565	6.61	19.8	2.57
	6.0	16.7	0.0035	220000	409.7	32.0	9142	105.8	3020	9.16	18.2	3.03
LE20	3.0	9.2	0.0085	91400	2.7		318	44.7	563	0.32	42.2	0.56
	4.2	12.9	0.0085	128900	7.6		891	63.0	943	0.89	33.6	0.94
HSD	3.0	15.4	0.0085	59000	0.3		39	35.9	198	0.04	71.2	0.20
	4.2	21.7	0.0085	83100	0.9		110	50.6	331	0.11	55.0	0.94
30 ˚C												
Disk turbine	3.0	8.3	0.0075	137000	50.2	9.1	1211	52.6	1232	1.22	25.5	0.98
	3.6	10.0	0.007	165000	87.8	12.7	1814	63.4	1508	1.82	23.0	1.20
	4.2	11.7	0.006	193100	140.5	16.8	2807	74.2	1876	2.80	20.6	1.50
	5.4	15.0	0.004	247500	296.2	26.3	6586	95.1	2873	6.61	16.7	2.29
	6.0	16.7	0.0035	275600	408.7	32.0	9131	105.8	3383	9.17	15.4	2.70
LE20	3.0	9.2	0.0085	114500	2.7		317	44.7	630	0.32	35.6	0.50
	4.2	12.9	0.0085	161500	7.6		888	63.0	1055	0.89	27.5	0.84
HSD	3.0	15.4	0.0085	73900	0.3		39	35.9	222	0.04	60.1	0.18
	4.2	21.7	0.0085	104100	0.9		110	50.6	371	0.11	46.4	0.30

6.1.2 Mixing measurement

Salt tracer studies were carried out for all of the impeller configurations at a number of stirrer speeds and aeration rates. The retention time was reduced to around 15 minutes to shorten the test length. A wide variety of stirrer speeds and aeration rates were tested for the disk turbine, as the tests were carried out before the stirrer speed and retention time experiments, including a number of test with lower stirrer speeds and higher aeration rates than were used in the later experiments. The LE20 and high shear disk tracer studies were carried out under the conditions used for the stirrer speed and retention time tests. The reactor showed a large deviation from plug flow in all cases (D/uL was > 0.01, Table 6.2 and Table 6.3). The tanks in series model confirmed that the reactor was well mixed and was represented by approximately one tank in series except when the reactor was unstirred and unaerated. For all of the impeller configurations there appeared to be some level of short circuiting, as the observed retention time dropped below the theoretical retention time.

Table 6.2 Summary of salt tracer studies results for Disk turbine CSTR at a variety of stirrer speeds and aeration rates.

Stirrer speed (s^{-1}), aeration rate ($Lmin^{-1}$)	0, 0	4.2, 2.5	8.3, 10
Theoretical RT (t_t) (min)	14.58	13.56	12.07
Observed RT (t_o) (min)	23.88	14.5	10.94
Variance (σ^2)	252	170	115
Dispersion model (D/uL)	0.32	1.43	8.84
Tanks in series model (N)	2.2	1.2	1.0

Table 6.3 Summary of salt tracer studies results for HSD and LE20 at 2 $Lmin^{-1}$ aeration.

Stirrer speed (s^{-1})	HSD		LE20	
	15.4	21.7	9.178	12.94
Theoretical RT (t_t) (min)	17	17	14.5	14.5
Observed RT (t_o) (min)	12.2	11.2	12.4	19
Variance (σ^2)	136	106	78.8	351
Dispersion model (D/uL)	3.80	1.89	0.412	15.0
Tanks in series model (N)	1.08	1.17	1.93	1.02

6.1.3 Oxygen Transfer Coefficient

The Oxygen transfer coefficient was calculated for all of the impeller conditions and at various stirrer speeds and gas flow rates (Table 6.4 and Table 6.5). The Disk turbine impeller appeared to have an optimum oxygen transfer at a stirrer speed of 4.1 s^{-1} and

10 $Lmin^{-1}$ air. A decrease in K_La was observed at high stirrer speed and aeration rate (Figure 6.1), which may be explained by the frequency of bubbles flooding the dissolved oxygen probe with air and apparently slowing the return to oxygen saturation. The reading from the meter did not reach 100 % smoothly, but fluctuated at the end point (Figure 6.2). The K_La calculated was lower for all of the air flow rates tested at 12.5 s^{-1} than at 8.33 s^{-1}. The K_La was determined for the HSD and LE20 impellers at the stirrer speeds and aeration rates used in experimental run 8 (Table 6.5). The HSD and LE20 produced smaller bubbles, decreasing the flooding of the probe. The HSD produced some small bubbles, although the ring sparger was a little too large for the impeller and it did not manage to consistently break up all of the bubble streams (Appendix B CD ROM).

The choice of method was made for the ease of measurement rather than accuracy. Linek et al. 1992 reported discrepancies of up to 94 % between various methods of K_La determination; the K_La results are useful for comparison.

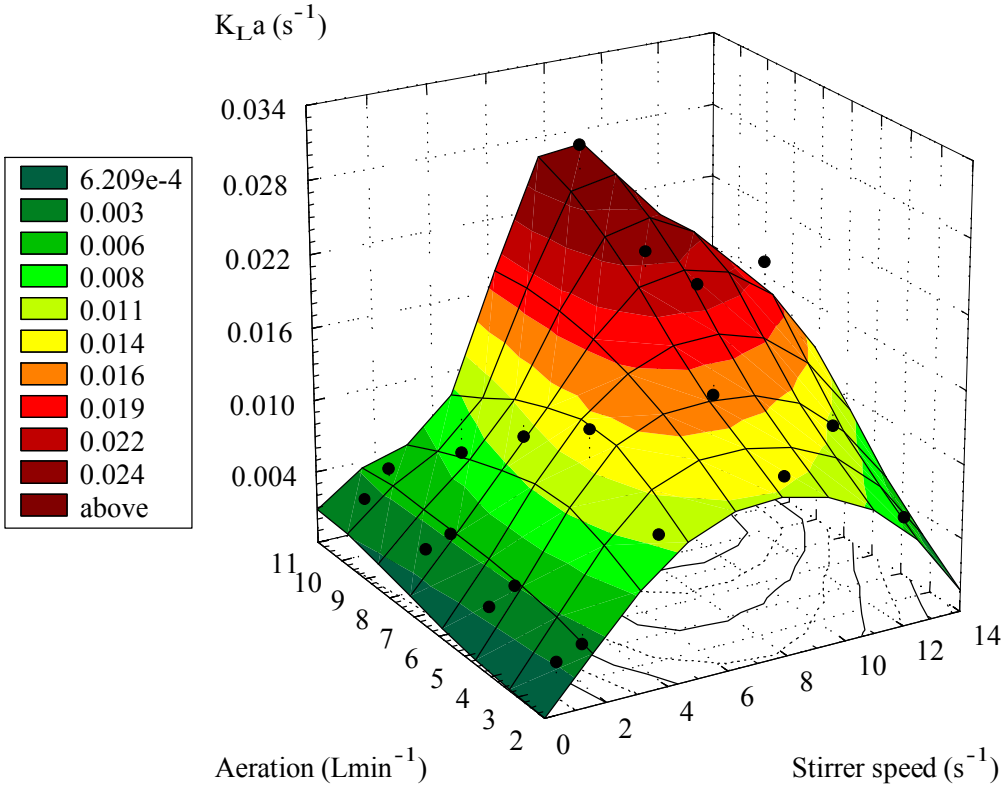

Figure 6.1 K_La values plotted against stirrer speed and aeration rate for disk turbine impeller. Surface plotted with Statistica using least squares, two cuts per data point smoothing.

Table 6.4 Oxygen transfer results (K_La) for each stirrer speed and aeration rate tested

Aeration Rate ($Lmin^{-1}$)	N_i (s^{-1}) 0.83	1.67	4.17	8.33	12.5
2.5	0.0011 s^{-1}	0.002 s^{-1}	0.0092 s^{-1}	0.0115 s^{-1}	0.0058 s^{-1}
5	0.0015 s^{-1}	0.0027 s^{-1}	0.0139 s^{-1}	0.0144 s^{-1}	0.0097 s^{-1}
7.5	0.0023 s^{-1}	0.0031 s^{-1}	0.0097 s^{-1}	0.0227 s^{-1}	0.0198 s^{-1}
10	0.0027 s^{-1}	0.0048 s^{-1}	0.0479 s^{-1}	0.0283 s^{-1}	0.0147 s^{-1}

Table 6.5 K_La for all impellers at matched tip speed to the disk turbine (aeration 2 $Lmin^{-1}$, 3 ms^{-1} = disk turbine 8.3 s^{-1} and 4.23 ms^{-1} = 11.7 s^{-1}).

Impeller	Tip speed (ms^{-1}) 3	4.23
HSD	0.0075 s^{-1}	0.0114 s^{-1}
LE20	0.0113 s^{-1}	0.0237 s^{-1}
Disk turbine	0.0131 s^{-1}	0.0112 s^{-1}

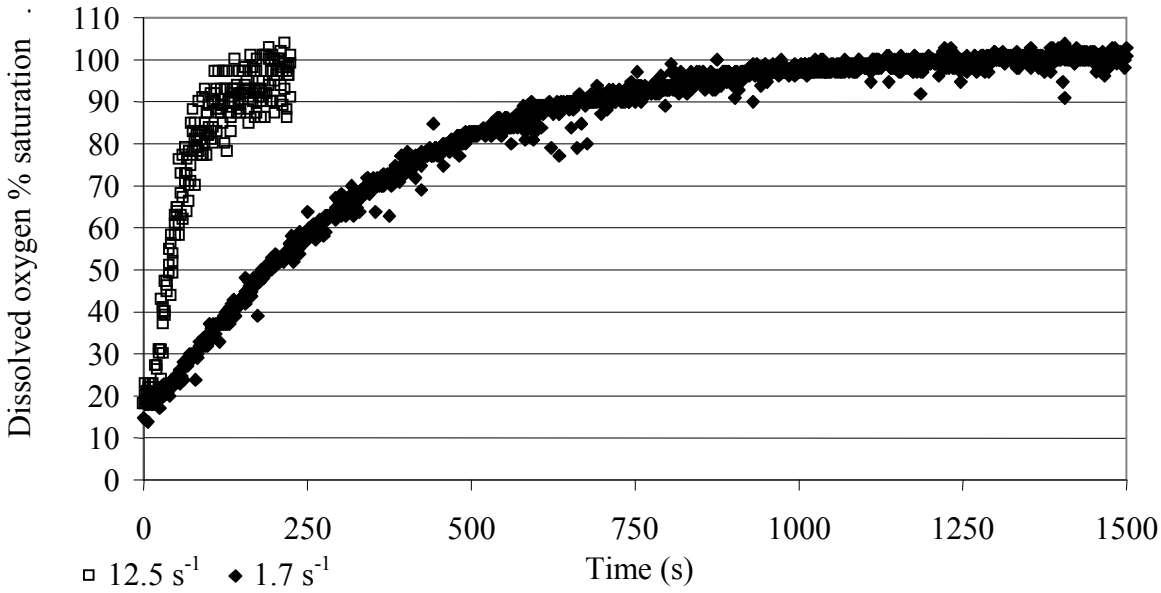

Figure 6.2 Oxygen concentration recovery at 2 stirrer speeds with the Disk turbine impeller, aerated at 7.5 $Lmin^{-1}$.

6.2 INFLUENT QUALITY

The influent was settled sewage from a real domestic wastewater. The average influent total COD loading was 221 mgL^{-1}, soluble COD 82 mgL^{-1}, soluble TOC 20.9 mgL^{-1} and the influent total $CBOD_5$ 78.9 mgL^{-1}. The average ammonia loading was 27.9 mgL^{-1}.

The average results represent a domestic wastewater well; there was however a large amount of variation as can be seen from the data from runs 1 to 7 summarised in Table 6.6. The table shows the variation in terms of standard deviation of the influent over all data points. The average COD:$CBOD_5$ ratio was 3.07 with a standard deviation of 1.45.

Table 6.6 Typical feed characteristics, runs 1 – 7 (number of samples (n) ≈ 280).

Parameter	Average value	Standard deviation	Minimum	Maximum
COD total (mgL^{-1})	221	118	40.0	1080
COD soluble (mgL^{-1})	82.3	40.6	13.0	(OR >150)
$CBOD_5$ (mgL^{-1})	78.9	39.3	3.6	178
$NBOD_5$ (mgL^{-1})	3.19	11.3	-82.5	55.9
TOC (mgL^{-1})	20.9	9.20	2.83	65.8
Ammonia (mgL^{-1})	27.9	12.1	0	(OR > 50)
Nitrate (mgL^{-1})	2.29	3.09	0	(OR > 30)
Nitrite (mgL^{-1})	0.07	0.27	0	2.64
SS (mgL^{-1})	91.5	60.1	10	620
VSS (mgL^{-1})	86.0	60.1	20	580
Phosphorus (mgL^{-1})	4.81	1.28	1.16	10
Turbidity (NTU)	167	78.8	6.77	398
pH	7.66	0.14	7.18	8.05

OR= over range, the maximum value within the range is given.

6.3 STIRRER SPEED AND RETENTION TIME EFFECTS ON TREATMENT

6.3.1 Carbonaceous Load Removal

6.3.1.1 Run 1: scoping run, carbonaceous load.

Run 1 was conducted with the Disk turbine impeller at a stirrer speed of 8.3 s^{-1} and 12 h retention time. The reactor was run for 171 retention times between September and December 2000 with samples taken for analysis 3 times per week. During this run the dissolved oxygen concentration fell below 80 % on only one occasion. Reactor pH was between 8 and 9.8 and temperature between 7.9 and 30 ˚C (Figure 6.3).

Over this run a small decrease in stirred tank concentration of both total and soluble COD was observed compared to the influent (Figure 6.4 and Figure 6.5). The mean percentage COD reduction over the period was 24 % for total and 22 % for soluble COD. Samples were all analysed in triplicate, with an average sample standard

deviation of 4.19 and 5.09 mgL^{-1} for influent and stirred tank soluble COD respectively and 13.08 and 9.11 mgL^{-1} for total COD (Table 6.7).

Total and soluble $CBOD_5$ were analysed initially during run 1. The Total $CBOD_5$ displayed a similar trend to the total COD with an average of 38 % removal occurring (Figure 6.6). The peak influent $CBOD_5$ concentrations occurred at the same time as the total COD peaks (Figure 6.4). The total $CBOD_5$ analysis average sample standard deviation was of 5.9 and 7.6 % of the influent and effluent sample concentration. A difference of over 95 % significance was found between the $CBOD_5$ influent and effluent, using the Wilcoxon matched pair test due to the non normal distribution of the data.

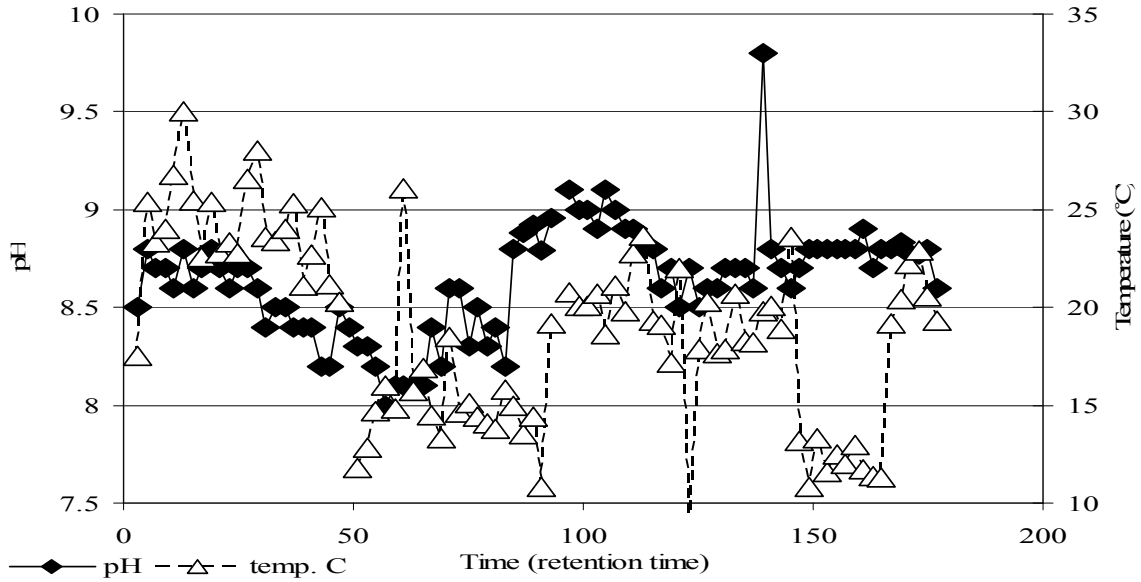

Figure 6.3 Run 1 reactor temperature and pH plotted against time.

The average removal of soluble $CBOD_5$ was found to be much higher at 78 %. The standard deviation of this analysis was found to be unacceptably high (16 % and 49 % of the average influent and effluent concentrations, plotted as error bars on Figure 6.7). This analysis was discontinued after Run 1, retention time 109.

The total COD:CBOD5 ratio was found to be 3.2 (standard deviation 0.7) for influent and 4.1 (standard deviation 2.3) for the effluent, with effluent standard deviation over 50 % of the result this ratio was not clear enough to discontinue either set of analysis and base results on the assumption that the ratio would remain the same.

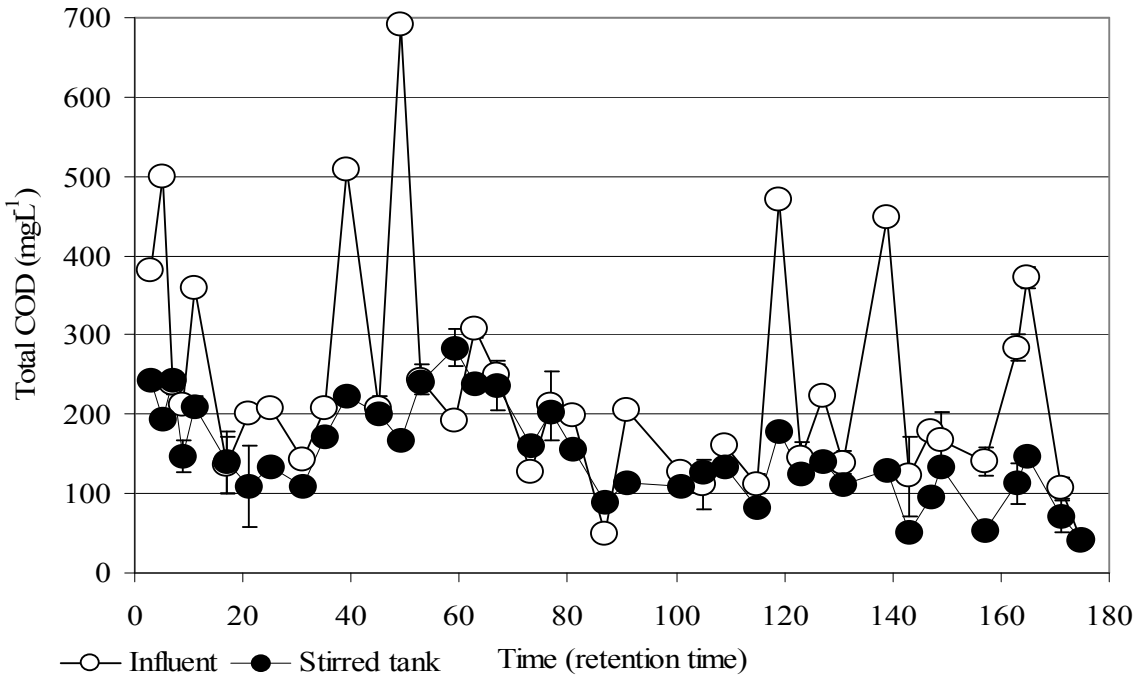

Figure 6.4 Run 1, total COD plotted against time for influent and stirred tank samples. Error bars show standard deviation for sample.

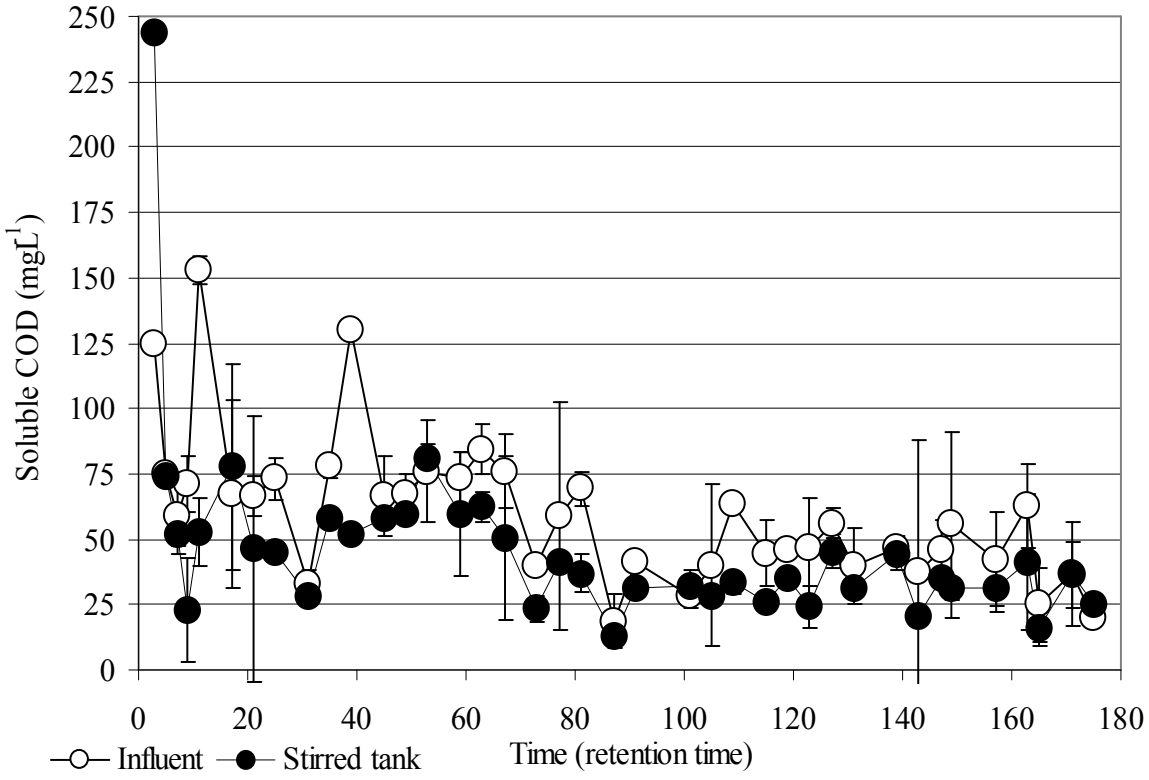

Figure 6.5 Run 1, soluble COD plotted against time for influent and stirred tank samples. Error bars show standard deviation for sample.

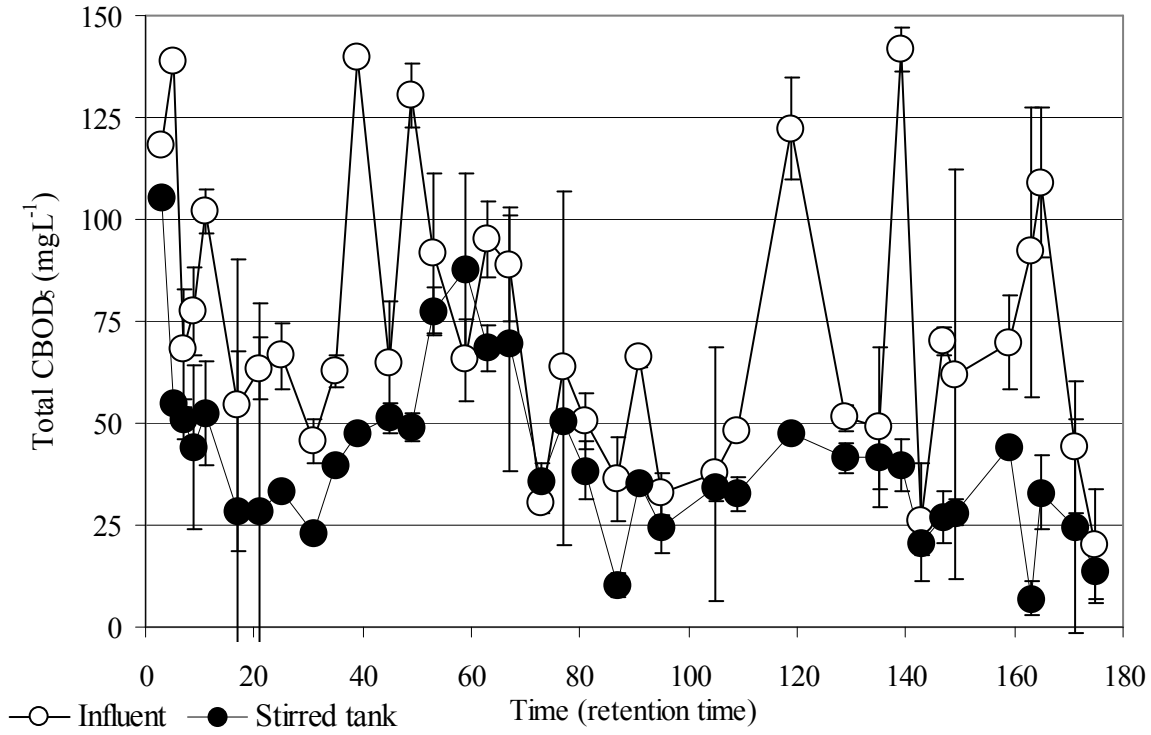

Figure 6.6 Run 1, total $CBOD_5$ plotted against time for influent and stirred tank samples. Error bars show standard deviation for sample.

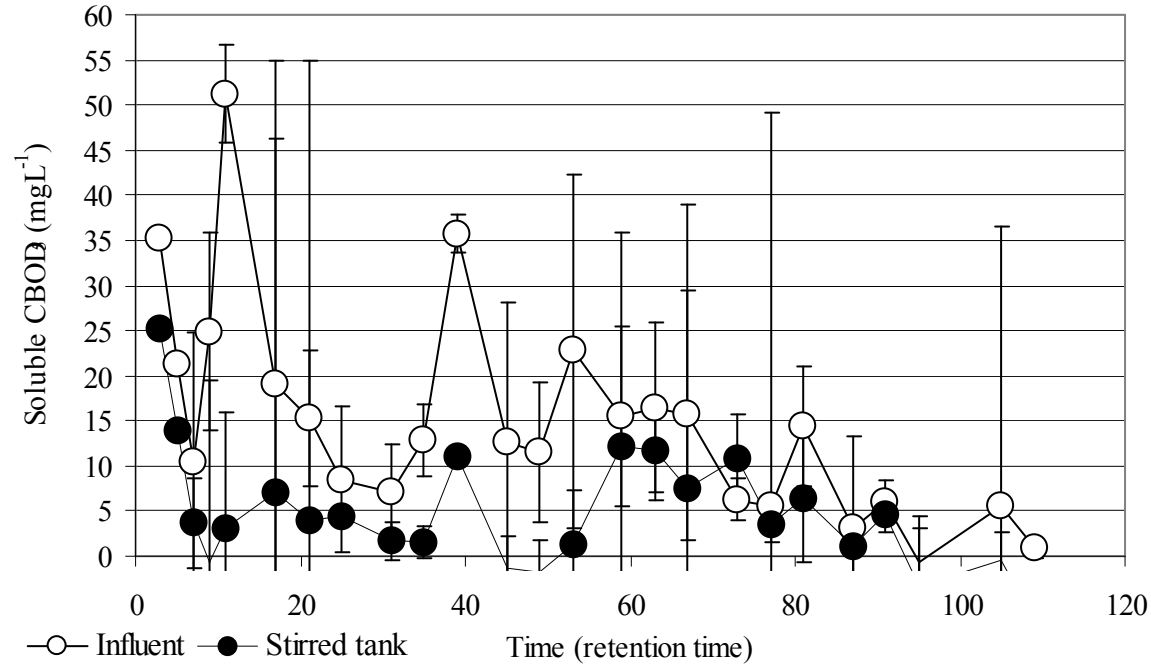

Figure 6.7 Run 1, soluble $CBOD_5$ plotted against time for influent and stirred tank samples. Error bars show standard deviation for sample.

The soluble TOC was measured for the influent and stirred tank sample, and appeared to have less variation associated with the measurement and less variation over time compared to the $CBOD_5$ (Figure 6.8). The average soluble TOC percentage removal during this run was 31 %. The Wilcoxon matched pair test showed a significant difference to 95 % confidence between the influent and stirred tank sample.

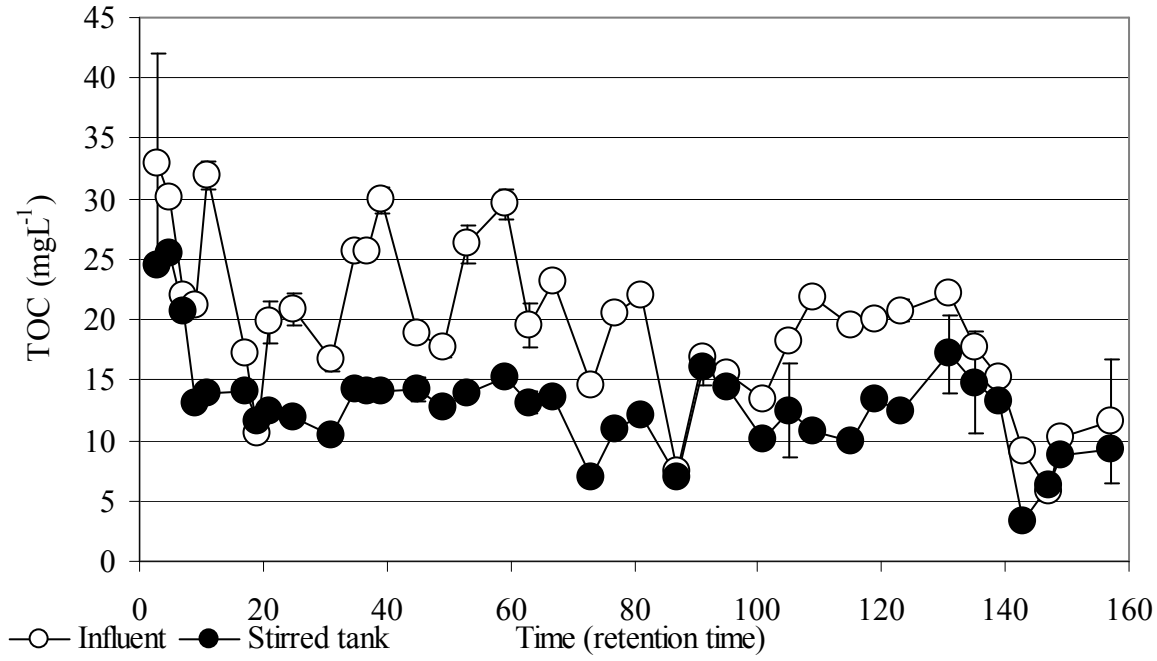

Figure 6.8 Run 1, soluble TOC plotted against time for influent and stirred tank samples. Error bars show standard deviation for sample

Suspended solids and volatile suspended solids were also analysed for the influent and stirred tank (Figure 6.9 and Figure 6.10). A small increase in reactor suspended solids was noticed compared to the influent (significant to 95 % using Wilcoxon matched pair). The suspended solids contained on average 82 % volatile suspended solids (standard deviation of 11%) for the influent and 83 % (standard deviation of 12 %) for the effluent. This demonstrated that there was little biomass build up in the reactor. The relative error observed was less for the solids measurements than for total $CBOD_5$ and soluble COD allowing reasonable assurance that a result from a single measurement was reliable.

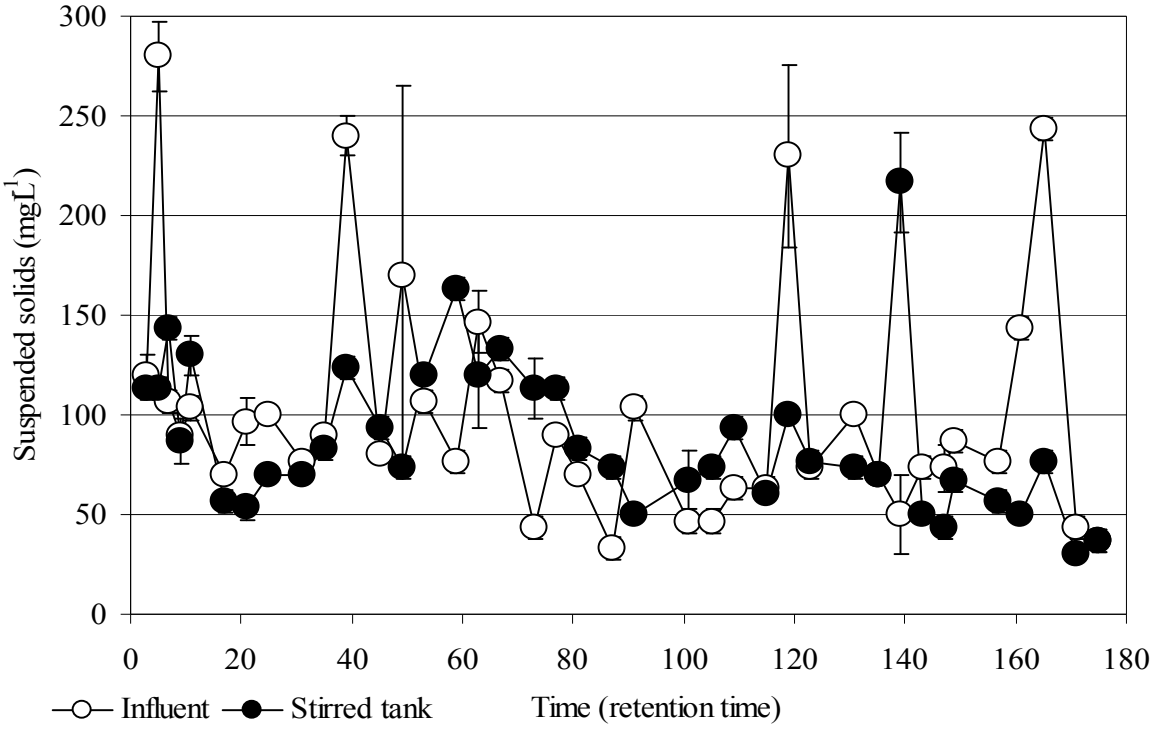

Figure 6.9 Run 1, suspended solids plotted against time for influent and stirred tank samples. Error bars show standard deviation for sample.

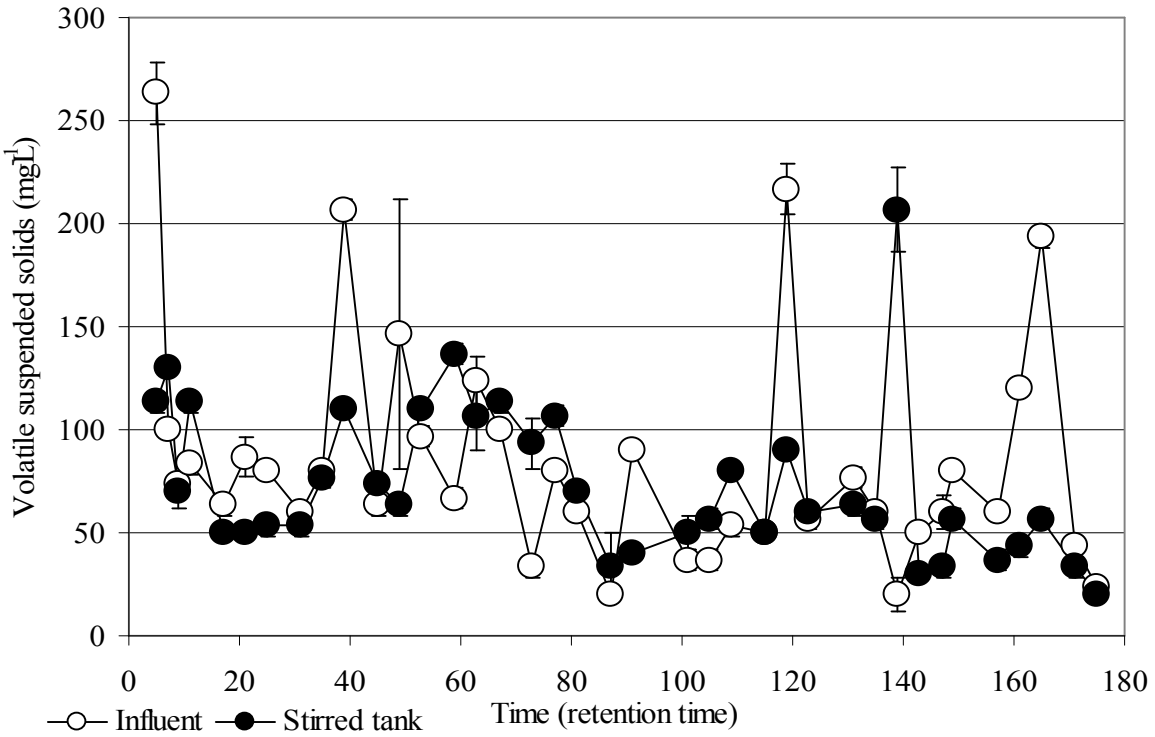

Figure 6.10 Run 1, volatile suspended solids plotted against time for influent and stirred tank samples. Error bars show standard deviation for sample.

Suspended solids and volatile suspended solids were not repeated after run 1, with a 100 mL filtered sample the results were found to be quite repeatable (average sample standard deviation: influent 8.74 mgL^{-1} and stirred tank 6.32 mgL^{-1} with an average result of 92.2 mgL^{-1} and 84.4 mgL^{-1} respectively). To carry out three repeats (300 mL) would have required a total sample collection of 500 mL for analysis, 10 % of reactor liquid volume when the gas hold up was large (e.g. stirrer speed 16.7 s^{-1}). Without repeats the analysis could be carried out with a 250 mL sample. Decreasing the filtered volume would have provided more data on reproducibility, while decreasing the quality of each measurement; therefore a single 100 mL measurement was used.

Table 6.7 Sample standard deviation and percentage standard deviation, run 1.

Parameter	n	Influent st. dev. (mgL^{-1})	Influent % st. dev.	Stirred tank st. dev. (mgL^{-1})	Stirred tank % st. dev.
S COD	38	4.19	7.39	5.09	11.25
T COD	38	13.08	6.61	9.11	6.62
T CBOD	35	4.15	6.23	3.09	8.457
S CBOD	20	2.45	22.50	2.80	72.45
TOC	34	1.08	5.86	0.78	6.30
SS	39	9.18	9.07	6.06	6.79
VSS	38	5.44	7.09	4.55	7.08

6.3.1.2 Runs 1 to 7: stirrer speed and retention time experiments, carbonaceous load.

The initial trial run allowed plans to be made for further analysis. A number of retention times and stirrer speeds were tested for their effectiveness. The scoping run had indicated that it would take a long time to collect sufficient results to compare different conditions, therefore the sampling frequency was increased to every retention time where practical, with 10 samples being taken for each set of conditions. After runs 2, 3 and 4 it was noticed that the results were not generally normally distributed making statistical analysis more difficult. During run 5 the number of samples taken was increased to 20 for each set of conditions. These results were no closer to normality and the sample number was returned to 10 for runs 6, 7 and 8.

Percentage removal of $CBOD_5$ varied little (Table 6.8, Figure 6.11). A decrease in performance was seen on the surface at 8.3 s^{-1}, 8 h and 15 s^{-1}, 10 h retention time. Stirrer speed 15 s^{-1}, 10 h retention time represented the poorest $CBOD_5$ performance during the experiments, with only 7 % removal achieved.

Comparing the results for the same run conditions, a positive correlation between influent $CBOD_5$ and percentage removal was seen (Figure 6.12). All of the data for 15 s^{-1} and 10 h retention time was plotted in Figure 6.12, omitting 3 data points for influent concentrations lower than 10 mgL^{-1} and removals of less than -70 %. The data deviated significantly from a horizontal line (99 % using t test) confirming the trend towards greater removal with higher influent concentration. When the 6 average data points for each run were plotted an R^2 value of 0.97 is achieved improving the fit by normalising the data. This indicated that the removal pattern observed for $CBOD_5$ related to the influent concentration and the run conditions used (Figure 6.11).

Table 6.8 Summary of total $CBOD_5$ results for all run conditions used.

Stirrer speed (s^{-1})	Retention time (h)	n	Average influent (mgL^{-1})	Average stirred tank (mgL^{-1})	St. dev. Influent (mgL^{-1})	St. dev. stirred tank (mgL^{-1})	% removal
8.3	8	10	66.9	37.9	39.6	14.0	33.0
8.3	8	10	99.2	70.7	31.2	19.2	25.9
8.3	10	10	83.6	38.1	43.3	19.7	49.4
8.3	12	35	72.8	41.7	33.2	20.2	37.7
8.3	12	10	65.9	25.2	28.4	6.98	54.2
10	8	10	74.6	46.8	32.5	9.58	29.1
10	8	10	101	62.7	19.4	12.0	38.3
11.7	8	10	105	64.7	35.0	14.6	31.9
11.7	10	10	106	56.7	15.4	6.84	46.1
11.7	10	10	126	58.6	19.1	29.9	52.8
15	8	10	75.9	40.1	25.8	7.74	44.0
15	8	10	96.0	48.5	31.2	8.10	46.1
15	10	10	55.4	33.9	20.5	9.58	34.4
15	10	10	82.5	46.7	25.1	10.4	41.2
15	10	20	41.9	28.1	15.4	10.5	32.0
15	10	14	103	50.1	45.2	32.8	42.9
15	10	10	119	58.3	19.4	10.6	51.0
15	10	10	146	63.9	18.6	10.5	55.1
15	12	18	49.8	30.3	42.6	17.6	7.39
16.7	10	20	55.9	30.6	22.7	12.2	37.1
16.7	12	20	44.0	25.2	19.7	11.0	38.2

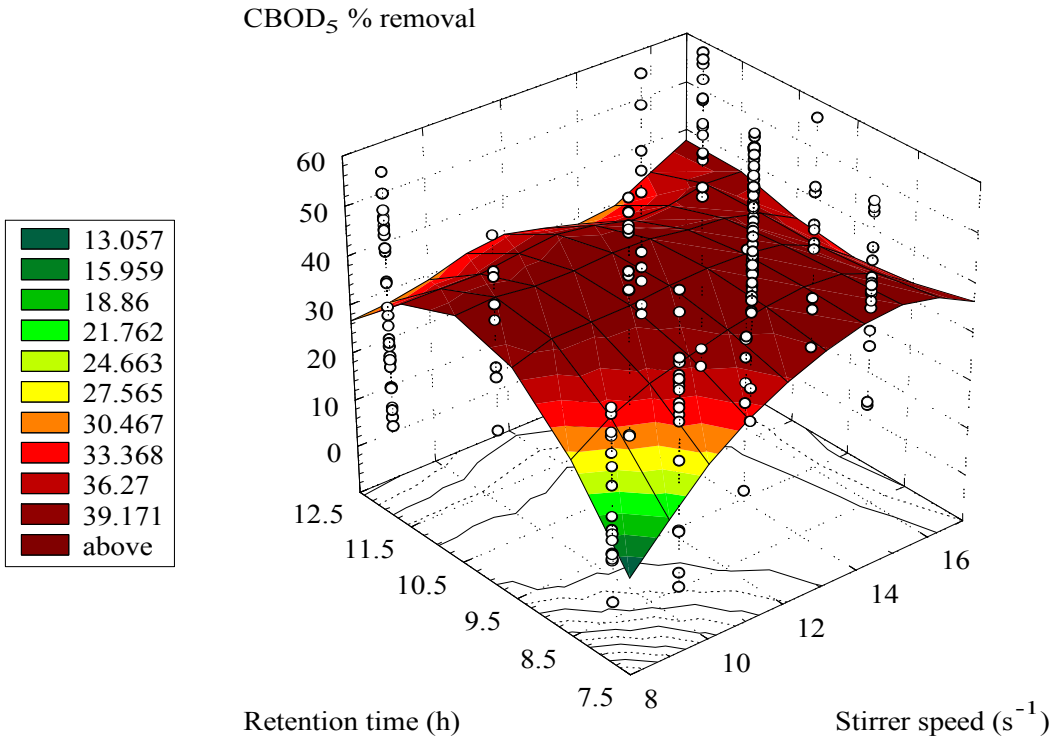

Figure 6.11 $CBOD_5$ percentage removal plotted against stirrer speed and retention time. Surface smoothed with Statistica using least squares, 2 cuts per data point smoothing.

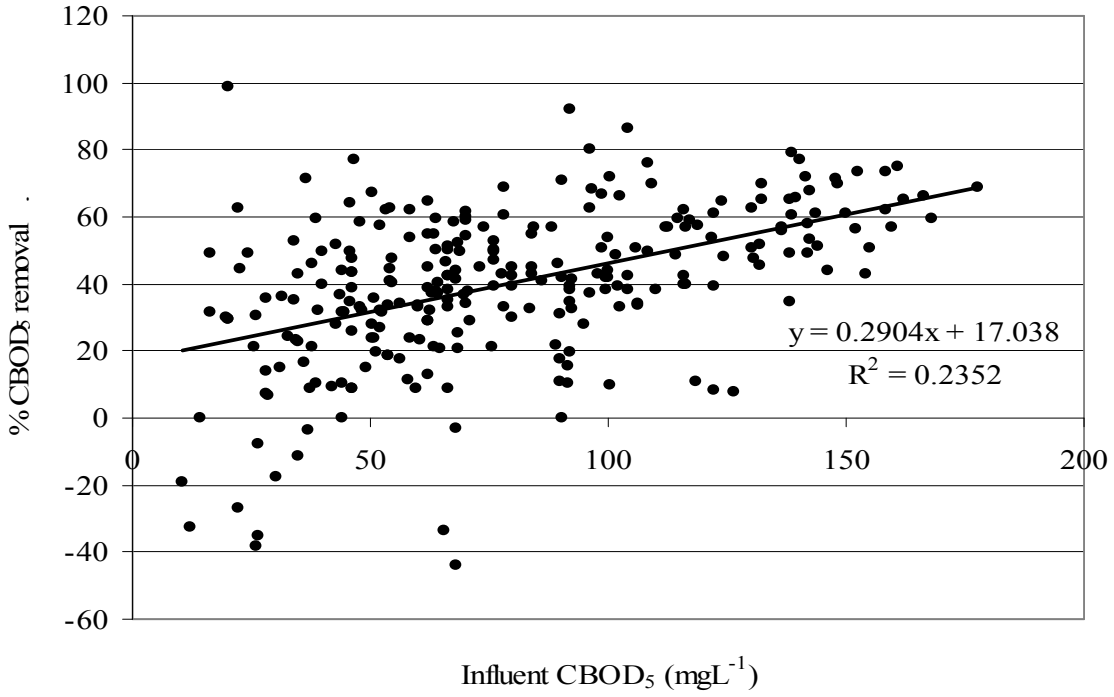

Figure 6.12 $CBOD_5$ percentage removal plotted against influent concentration for a stirrer speed of 15 s^{-1} and 10 h retention time.

The trend in total COD removal compared to retention time and stirrer speed showed greatest removal at low stirrer speed and long retention time (Figure 6.13). The trend appeared more pronounced than for $CBOD_5$ (Figure 6.11). There was a trough and a small peak at the rear corner, denoting high stirrer speed and long retention time, due to the poor removal (-6%) at 15 s^{-1} 12 h retention time. The trend of poor performance under these conditions was also observed for soluble COD and TOC removal (Figure 6.15 and Figure 6.16 respectively). Carrying out regression analysis for total COD gave a significant correlation between influent concentration and percentage removal.

Table 6.9 Total COD results for all stirrer speeds and retention times tested

Stirrer speed (s^{-1})	Retention time (h)	n	Average influent (mgL^{-1})	Average stirred tank (mgL^{-1})	St dev influent (mgL^{-1})	St dev stirred tank (mgL^{-1})	% removal
8.3	8	10	209	121	132	40.2	30.7
8.3	8	10	243	190	67.8	61.9	20.8
8.3	10	10	222	121	140	49.4	33.3
8.3	12	38	234	150	13.1	9.11	24.0
8.3	12	10	176	102	60.1	40.7	37.6
10	8	10	241	143	152	24.1	28.3
10	8	10	297	198	62.4	25.3	31.6
11.7	8	10	253	192	78.8	27.3	0.115
11.7	10	10	269	177	26.4	25.9	34.1
11.7	10	10	331	249	137	158	15.4
15	8	10	211	152	46.6	15.4	26.2
15	8	10	277	208	78.0	16.2	20.9
15	10	10	163	143	52.7	27.8	4.56
15	10	10	213	162	45.6	32.0	22.7
15	10	20	140	137	54.7	53.2	1.09
15	10	14	296	233	62.1	36.3	17.9
15	10	10	336	264	86.8	48.7	18.7
15	10	10	279	179	52.6	36.6	35.3
15	12	18	172	120	233	43.5	-6.18
16.7	10	20	147	124	35.7	85.8	10.2
16.7	12	20	136	108	44.5	24.1	14.3

The total COD: $CBOD_5$ ratio was quite variable during the runs and the data was skewed (Figure 6.14) which meant that it was necessary to continue measuring the influent and effluent COD and $CBOD_5$ throughout the experiments. The percentage removal of $CBOD_5$ was higher than removal of total COD (Table 6.8, Table 6.9). COD removal had a greater range and was more skewed than $CBOD_5$ removal. Soluble COD

had a narrower range than total COD or $CBOD_5$ and with a slightly lower percentage removal than total COD (Table 6.10).

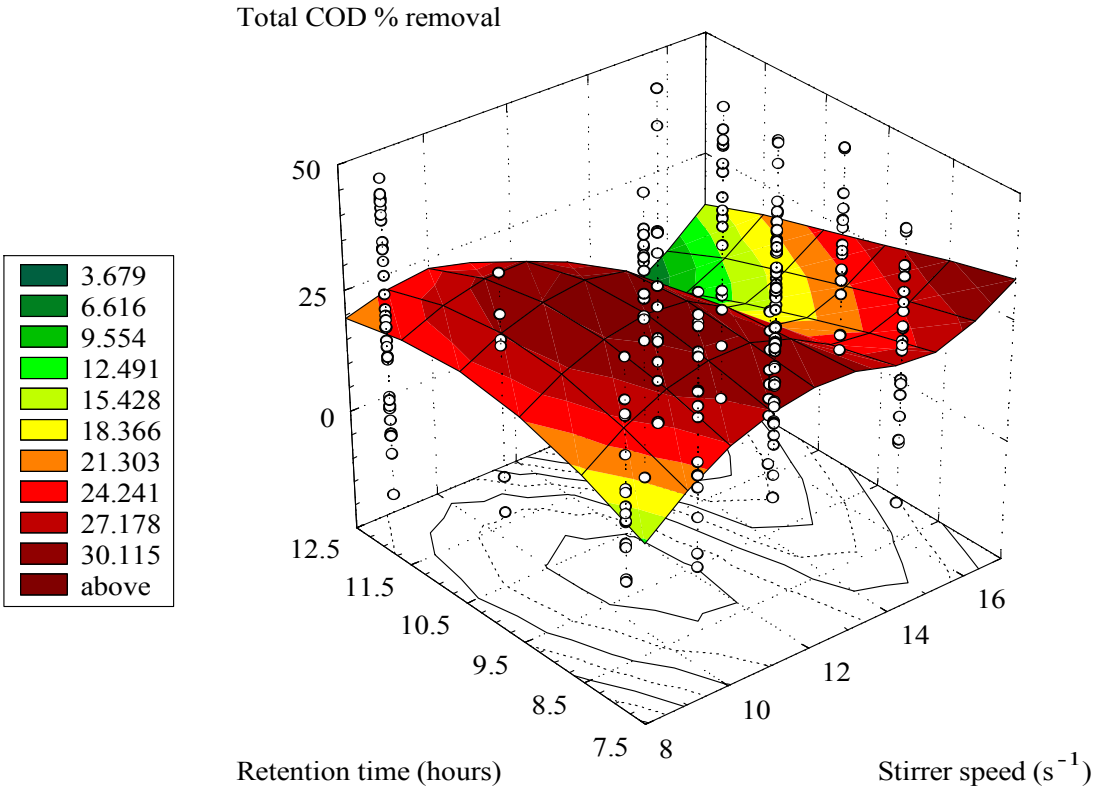

Figure 6.13 Total COD percentage removal plotted against retention time and stirrer speed. The surface represents the trend using the least squares method with 2 cuts per data point smoothing.

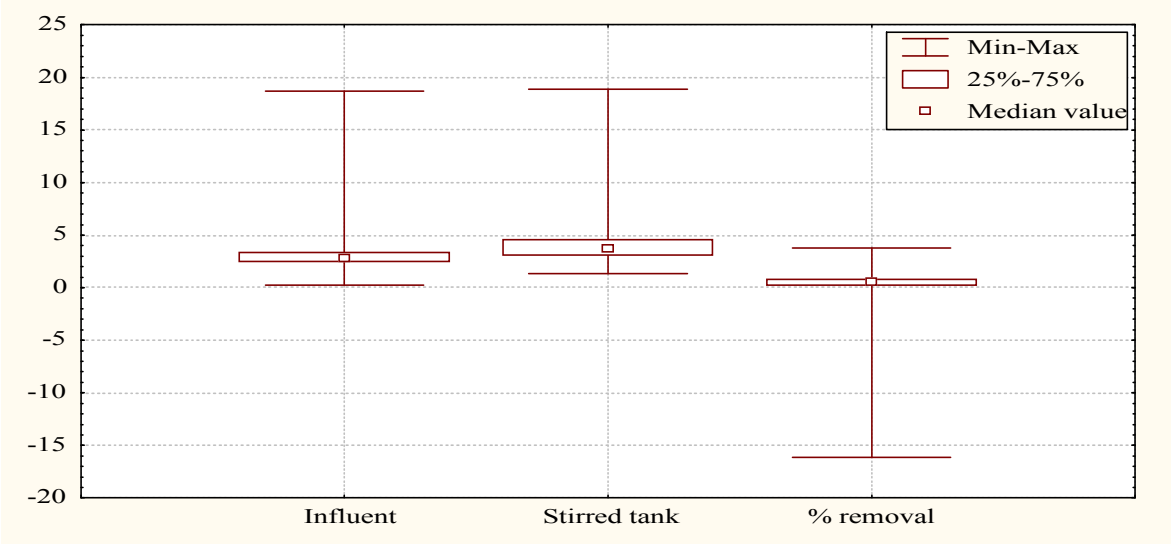

Figure 6.14 Total COD: $CBOD_5$ ratio for the influent, stirred tank and percentage removal as box and whisker plots showing minimum, maximum and 25 and 75 percentile ranges.

Table 6.10 Soluble COD results for all stirrer speeds and retention times tested

Stirrer speed (s^{-1})	Retention time (h)	n	Average influent (mgL^{-1})	Average stirred tank (mgL^{-1})	St dev Influent (mgL^{-1})	St dev stirred tank (mgL^{-1})	% removal
8.3	8	10	61.5	41.2	26.2	16.3	31.8
8.3	8	10	94.3	48.6	31.6	8.52	44.7
8.3	10	10	53.1	36.3	11.1	6.47	30.2
8.3	12	38	61.5	36.8	20.6	9.59	34.8
8.3	12	10	59.9	46.4	4.19	5.09	22.3
10	8	10	58.2	40.3	17.8	3.99	23.4
10	8	10	109	60.2	20.0	9.95	44.0
11.7	8	10	121	77.3	22.0	19.9	35.7
11.7	10	10	131	72.8	15.6	10.5	44.2
11.7	10	10	149	84.0	19.2	23.1	43.0
15	8	10	94.6	54.7	27.4	12.4	38.9
15	8	10	114	83.6	34.5	8.68	20.1
15	10	10	62.6	56.1	28.3	9.13	-10.7
15	10	10	85.5	73.1	26.9	14.8	10.6
15	10	20	56.2	66.6	28.6	25.9	-26.8
15	10	14	116	69.3	29.6	45.6	36.9
15	10	10	155	90.1	8.43	9.64	41.8
15	10	10	135	81.9	30.5	18.0	38.4
15	12	18	52.0	59.2	25.9	17.4	-31.0
16.7	10	20	53.7	52.9	16.7	25.8	5.01
16.7	12	20	58.6	53.7	21.1	12.7	2.13

All of the measures of carbonaceous load removal showed a peak performance at high stirrer speed, long retention time. Good soluble COD removal was also achieved at low stirrer speed, low retention time (Figure 6.15), the other measures show decreased performance at 8 h retention time 8.3 s^{-1} stirrer speed. Least variation was noticed in $CBOD_5$.

Soluble COD and TOC were both measured using the filtered sample, and had a smaller range than the total COD. The percentage removal of carbonaceous load was largest when measured using $CBOD_5$; this was intuitively the case as the BOD_5 test relies on biological sample degradation. Soluble COD and TOC had smaller ranges than total COD and $CBOD_5$, this also followed due to the samples being normalised by removing inhomogeneous solids. The results were skewed for percentage removal, limiting the statistical hypothesis tests available to use with this data (Figure 6.17).

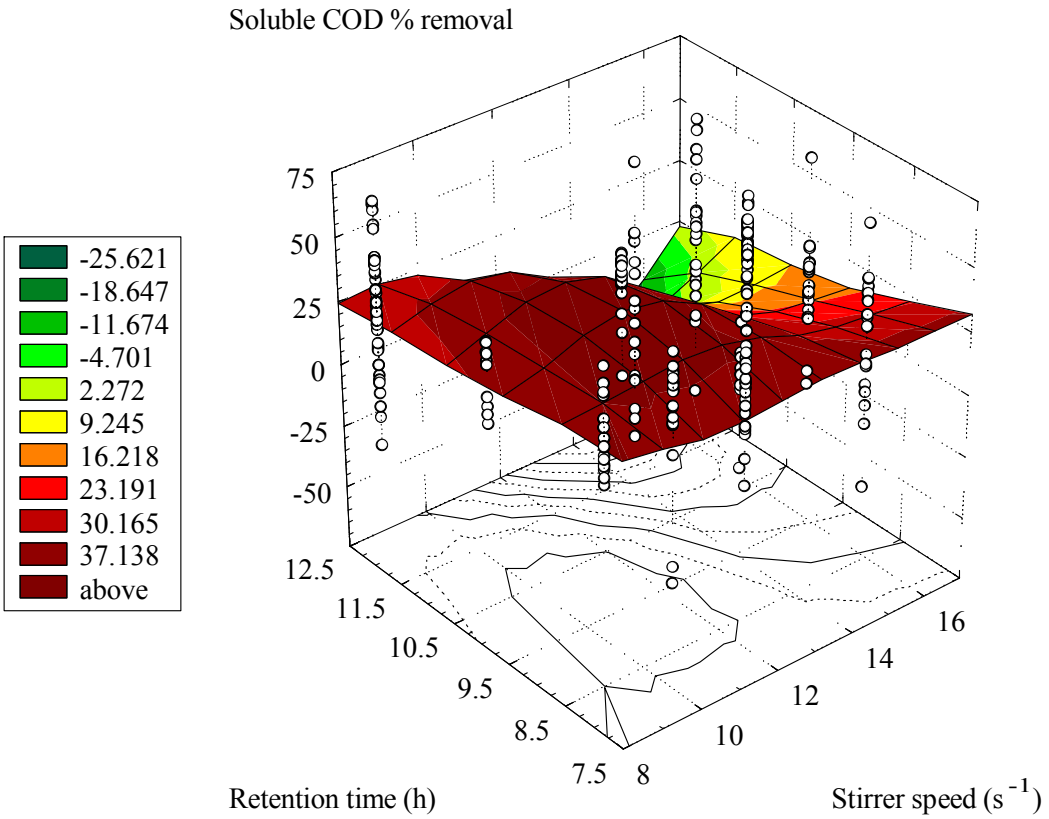

Figure 6.15 Soluble COD percentage removal plotted against retention time and stirrer speed. The surface represents the trend using the least squares method, 2 cuts per data point smoothing

Table 6.11 Soluble TOC results for all stirrer speeds and retention times tested

Stirrer speed (s^{-1})	Retention time (h)	n	Average influent (mgL^{-1})	Average stirred tank (mgL^{-1})	St dev influent (mgL^{-1})	St dev stirred tank (mgL^{-1})	% removal
8.3	8	10	7.6	7.0	1.2	0.2	5.7
8.3	8	10	5.2	5.0	1.3	0.5	-2.9
8.3	12	34	19.5	13.0	6.6	4.3	30.8
8.3	12	10	10.5	10.4	2.1	1.2	-4.2
10	8	10	19.5	15.7	3.5	3.7	16.8
10	8	10	14.4	9.6	6.5	2.0	25.5
11.7	8	10	25.2	21.2	4.0	3.4	14.8
11.7	10	10	25.3	19.3	11.3	1.1	15.6
11.7	10	10	25.5	18.4	3.4	2.5	26.0
15	8	10	31.9	22.3	12.4	5.1	24.4
15	8	10	33.6	28.5	9.0	3.5	9.0
15	10	10	28.0	22.4	8.6	4.7	13.5
15	10	10	24.5	21.1	10.4	8.0	12.2
15	10	20	22.2	15.1	7.1	2.3	25.5
15	10	14	18.7	17.0	4.2	4.6	8.9
15	10	10	26.6	19.0	12.2	4.5	21.2
15	10	10	21.1	20.3	3.8	8.2	4.4
15	12	18	18.4	16.0	6.3	5.9	16.0
16.7	10	20	21.1	16.3	2.7	3.3	22.1
16.7	12	20	22.0	16.4	6.5	5.8	16.4

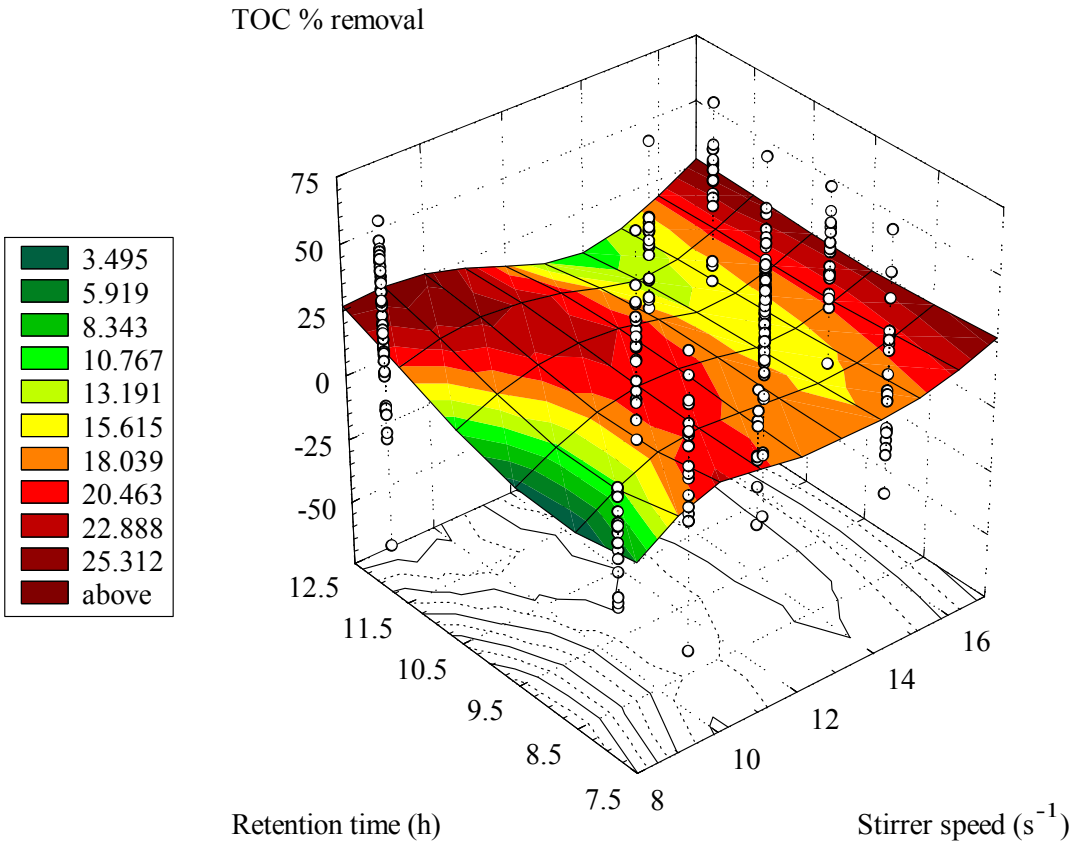

Figure 6.16 Soluble TOC percentage removal plotted against retention time and stirrer speed. The surface represents the trend using the least squares method. 2 cuts per data point smoothing.

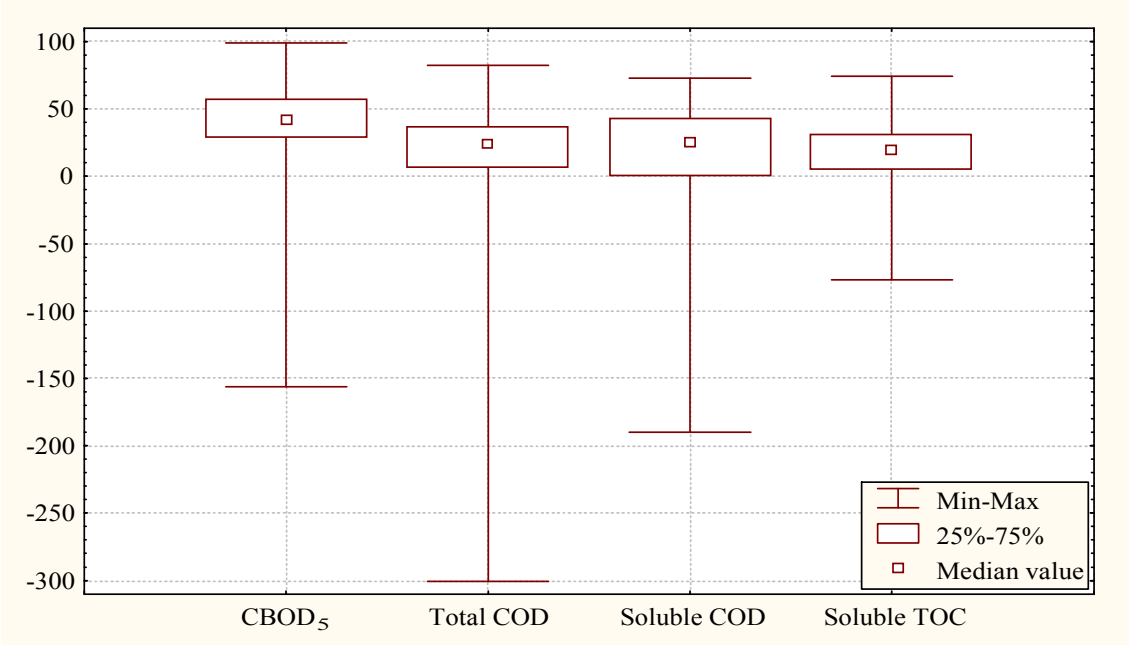

Figure 6.17 Box and whisker plot showing median and 25 and 75 percentile regions for percentage removal of the analysed components of carbonaceous load removal used.

The suspended solids and volatile suspended solids concentration remained low throughout the experimental runs, with values close to those of the influent found in the stirred tank (Table 6.12). Some solids removal occurred with stirrer speed 8.3 s^{-1} and 10 h retention time (Figure 6.18 and Figure 6.19).

The stirred tank was capable of some carbonaceous load removal, with low biomass accumulation; an increase in volatile suspended solids compared to influent was generally measured (Figure 6.19). The stirred tank also nitrified; the ammonia levels and nitrification products were monitored throughout the study and this aspect is presented in the next section.

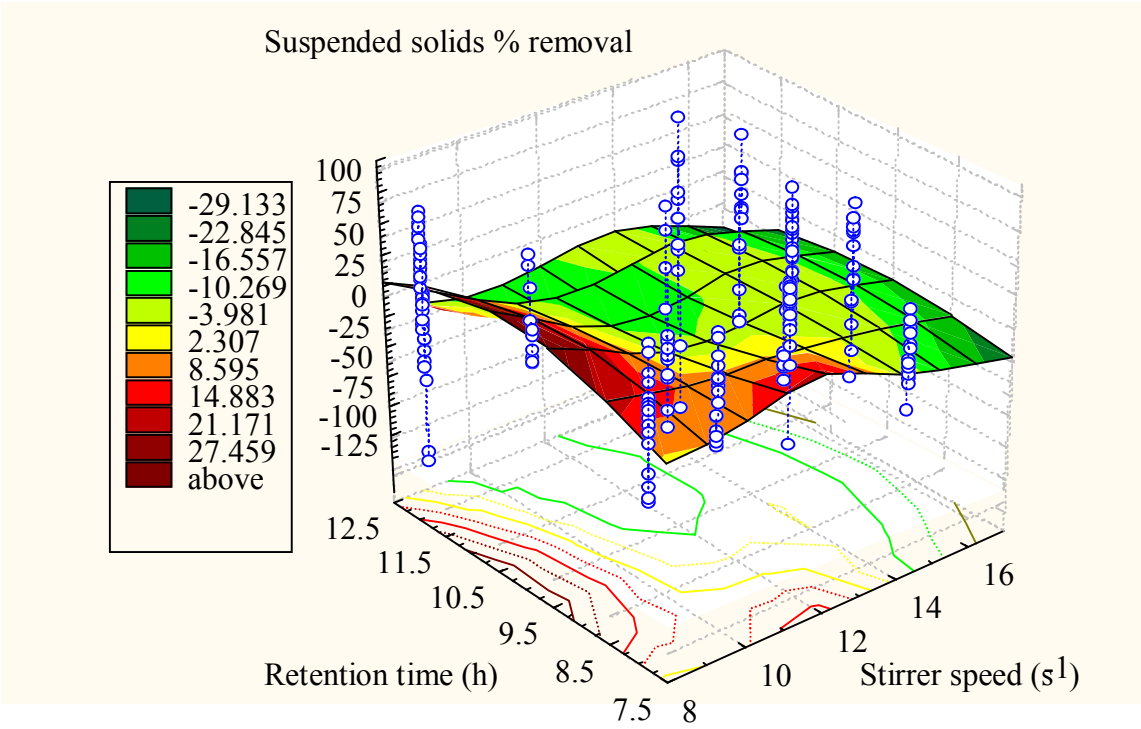

Figure 6.18 Suspended solids percentage removal plotted against retention time and stirrer speed. The surface represents the trend using the least squares method. 2 cuts per data point smoothing.

Table 6.12 Summary of solids results for all stirrer speeds and retention times tested

Stirrer speed (s^{-1})	Retention time (h)	n	Influent	Stirred tank	St dev influent	St dev stirred tank	% removal
Suspended solids			(mgL^{-1})				
8.3	12	39	101	87.7	59.0	37.9	-7.68
8.3	12	10	81.7	53.0	26.6	11.5	30.1
8.3	10	10	100	54.0	69.2	16.8	32.8
8.3	8	10	116	87.3	95.5	80.0	24.2
10	8	10	90.7	76.3	32.4	12.9	7.19
8.3	8	10	100	102	29.5	26.1	-7.73
10	8	10	110	101	21.9	13.6	5.76
11.7	8	10	127	92.0	60.6	12.3	18.2
15	8	10	80.0	93.0	13.3	18.3	-17.7
15	10	10	85.6	82.0	28.3	19.3	-0.08
15	8	10	101	106	15.2	15.1	-5.94
15	10	10	91.0	78.0	19.7	6.32	11.1
15	10	20	56.0	63.0	29.3	22.0	9.17
16.7	10	20	68.0	75.5	23.3	27.8	-16.9
16.7	12	20	61.0	72.0	27.1	18.2	-37.2
15	12	18	81.7	68.9	101	26.1	-45.8
15	10	14	111	122	22.8	21.2	-12.9
15	10	10	95.0	105	37.8	19.0	-26.6
15	10	10	89.0	79.0	16.6	24.2	11.1
11.7	10	10	79.0	102	57.4	22.0	5.55
11.7	10	10	161	104	176	16.5	-5.86
Volatile suspended solids			(mgL^{-1})				
8.3	12	38	84.6	73.4	55.3	37.8	-22.1
8.3	12	10	67.0	43.7	25.0	8.1	26.8
8.3	10	10	88.3	45.3	58.7	16.0	35.6
8.3	8	10	90.7	68.0	95.6	78.2	24.8
10	8	10	76.3	63.0	24.1	8.4	9.8
8.3	8	10	86.3	86.3	29.9	23.1	-6.3
10	8	10	105.3	94.7	17.3	12.0	6.3
11.7	8	10	113.0	83.0	52.3	8.2	17.3
15	8	10	75.0	77.0	12.7	13.4	-5.9
15	10	10	72.0	71.0	39.1	15.2	75.9
15	8	10	94.0	91.0	11.7	11.0	2.6
15	10	10	73.0	86.0	31.3	20.7	139.2
15	10	20	63.0	64.0	30.8	19.6	23.0
16.7	10	20	71.5	67.0	24.3	15.6	-0.2
16.7	12	20	61.0	66.0	21.3	12.3	-20.3
15	12	18	87.1	68.2	81.2	28.6	2.7
12	10	14	92.1	98.6	19.7	14.6	-15.9
15	10	10	107.0	126.0	22.1	43.5	-29.9
15	10	10	98.9	85.6	18.3	26.5	14.6
11.7	10	10	108.0	109.0	51.2	19.7	-11.0
11.7	10	10	162.0	94.0	157.0	79.9	24.8

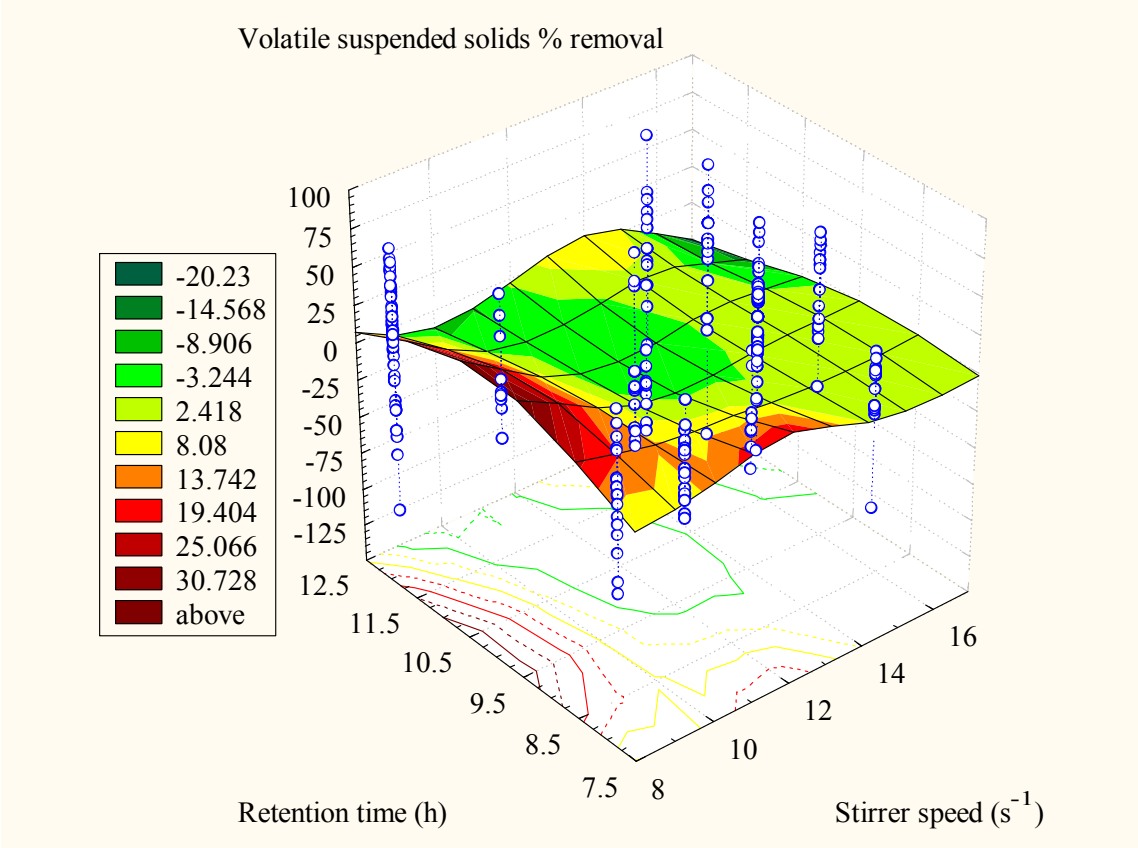

Figure 6.19 Volatile suspended solids percentage removal plotted against retention time and stirrer speed. The surface represents the trend using the least squares method.

6.3.2 Nutrient removal

6.3.2.1 Run1: scoping run, nutrient removal

Initially the ammonia and phosphorus concentrations were monitored to see if any nutrient removal occurred. The pattern of ammonia removal was quite striking (Figure 6.20). Too much ammonia appeared to be removed to be simple incorporation into the biomass, and therefore additional nitrate and nitrite measurements were made to allow a nitrogen balance to be carried out. The results of nitrate and nitrite measurements also confirmed that nitrification and not ammonia stripping was taking place (Figure 6.21 and Figure 6.22 respectively). It was seen from the shape of the graphs that nitrification was occurring. At retention time 120 there was marked ammonia removal (Figure 6.20) the removal decreased by retention time 140. The shape of the ammonia graph between retention times 120 and 140 was the opposite of the nitrate and nitrite graphs (Figure 6.21 and Figure 6.22 respectively) as less ammonia was converted to nitrite and nitrate.

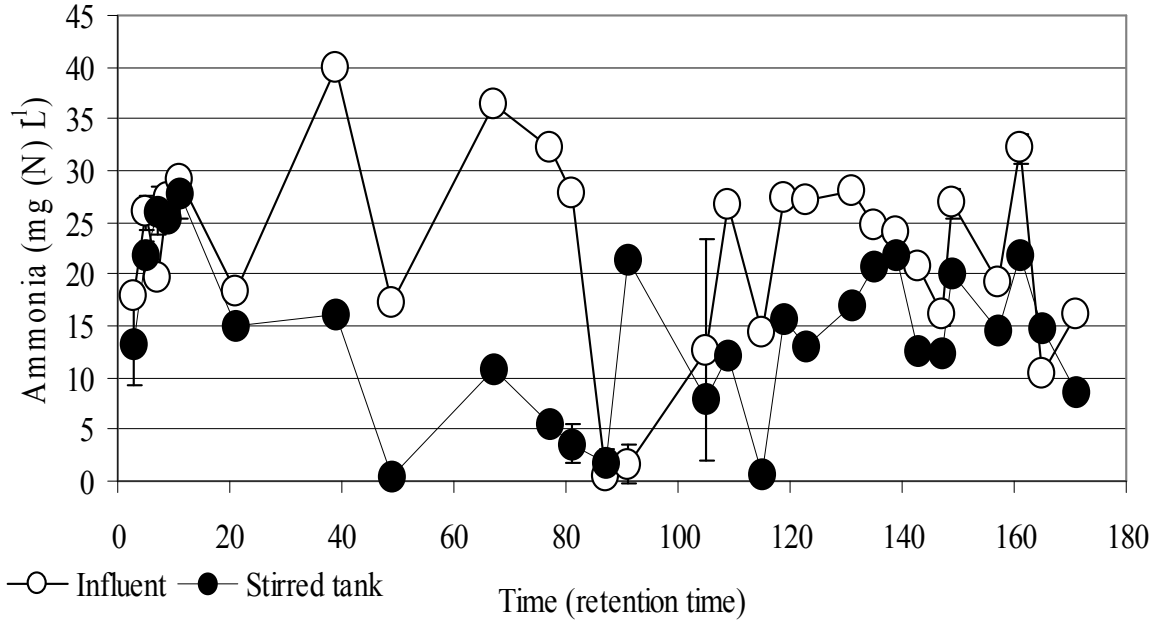

Figure 6.20 Run 1, influent and stirred tank ammonia (nitrogen) concentration plotted against time.

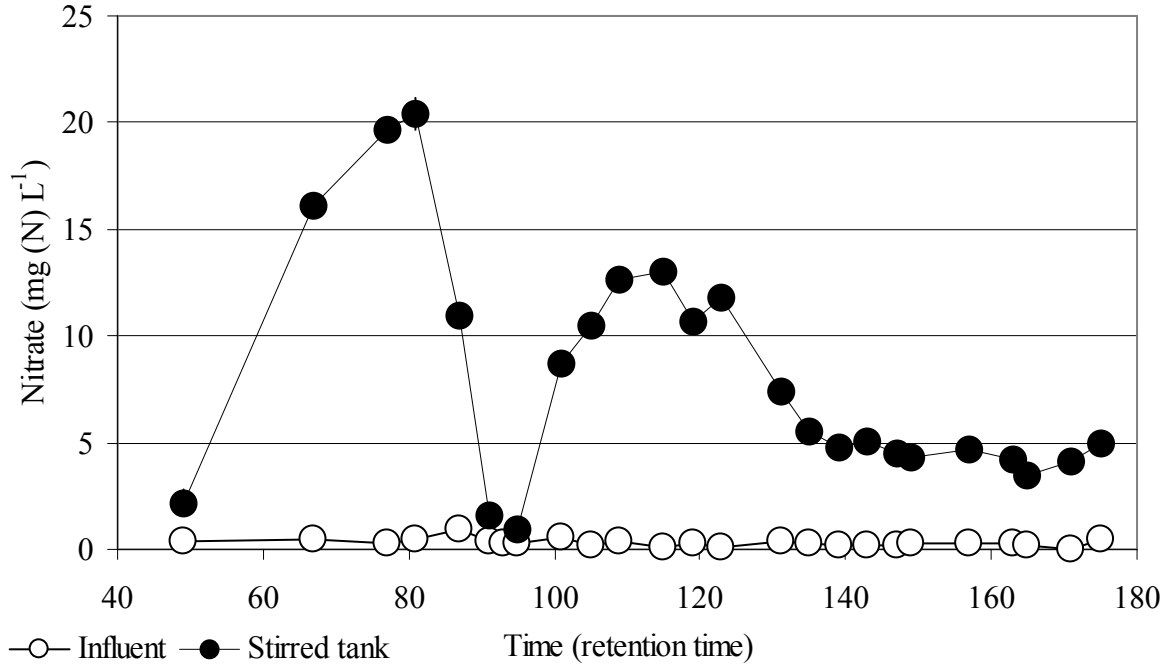

Figure 6.21 Run 1, influent and stirred tank nitrate (nitrogen) concentration plotted against time.

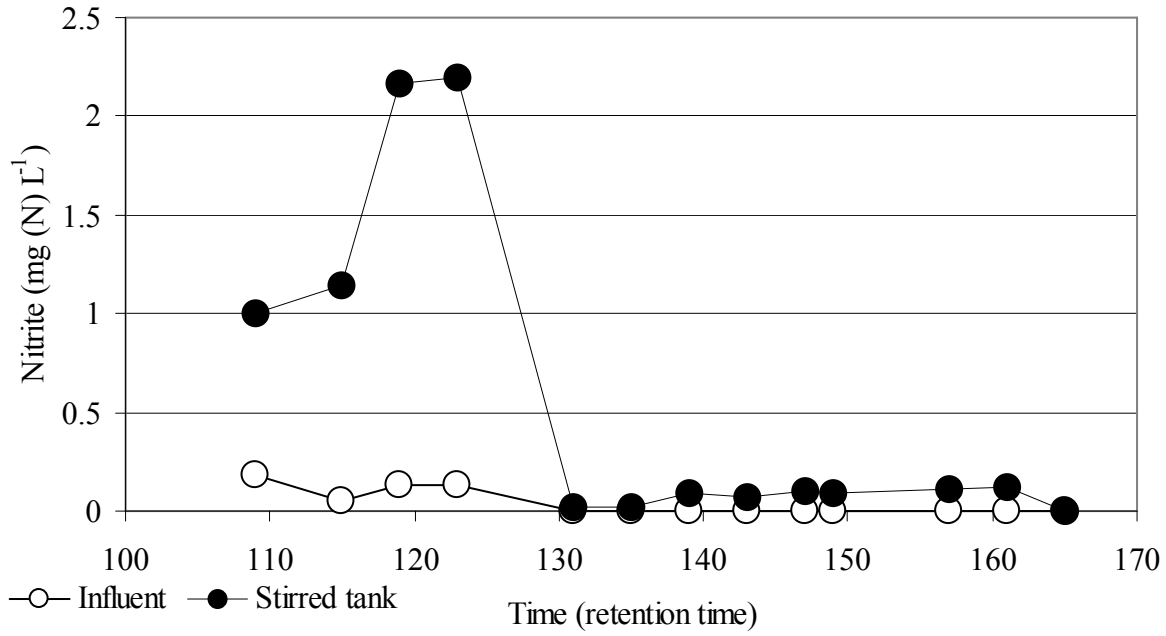

Figure 6.22 Run 1, influent and stirred tank nitrite (nitrogen) concentration plotted against time.

An additional test was added to determine where the nitrification was occurring. If a high concentration of nitrifying bacteria were present in the reactor a $NBOD_5$ would be exerted additional to the $CBOD_5$. If the bacteria were being fed into the reactor at high concentrations the influent would also exert a $NBOD_5$. Similarly, if the bacteria were all growing as biofilm the $NBOD_5$ should not be exerted unless there was a constant washing off of bacteria. The results showed little $NBOD_5$ in the influent, with more $NBOD_5$ than $CBOD_5$ measured in the stirred tank sample (Figure 6.23).

A nitrogen balance was carried out comparing the influent and effluent concentrations of the inorganic nitrogen species and organic nitrogen incorporated in the volatile suspended solids (assuming the empirical formula for biomass $C_5H_7NO_2$ given in Metcalf and Eddy, Inc. (2003)). The balance is shown below in Table 6.13. Using this balance 105 % of the nitrogen in the influent was recovered in the effluent. Using the average of the sample balances 98 % of the influent nitrogen was recovered in the effluent.

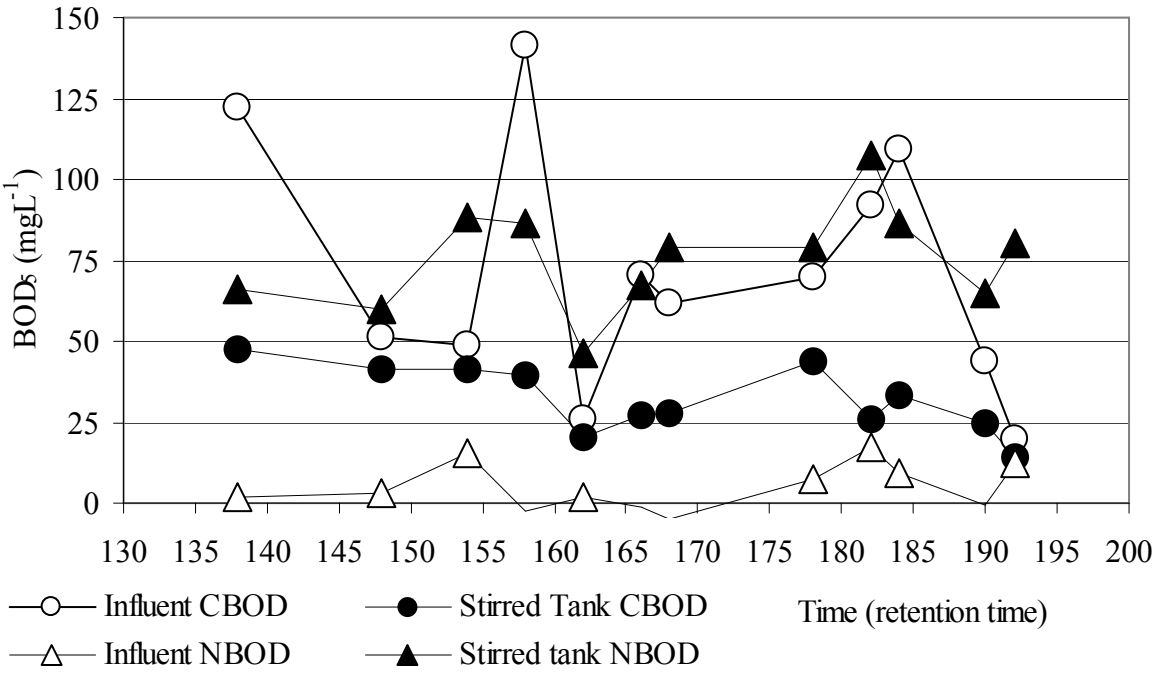

Figure 6.23 Run 1, influent and stirred tank carbonaceous and nitrifying BOD_5 plotted against time.

Table 6.13 Nitrogen balance of mean results.

	Influent	Stirred tank
Ammonia (mg (N) L^{-1})	22.15	14.36
Nitrate (mg (N) L^{-1})	0.41	10.35
Nitrite (mg (N) L^{-1})	0.04	0.55
VSS (mg (N) L^{-1})	10.47	9.48
Total nitrogen (mg (N) L^{-1})	33.07	34.74

The phosphorus results did not show a positive removal or change (Figure 6.24). The changes in phosphorus concentration were not easily linked to the volatile suspended solids concentration in the reactor, assuming 2 % volatile solids phosphorus composition quoted in Metcalf and Eddy (2003)(Figure 6.25)

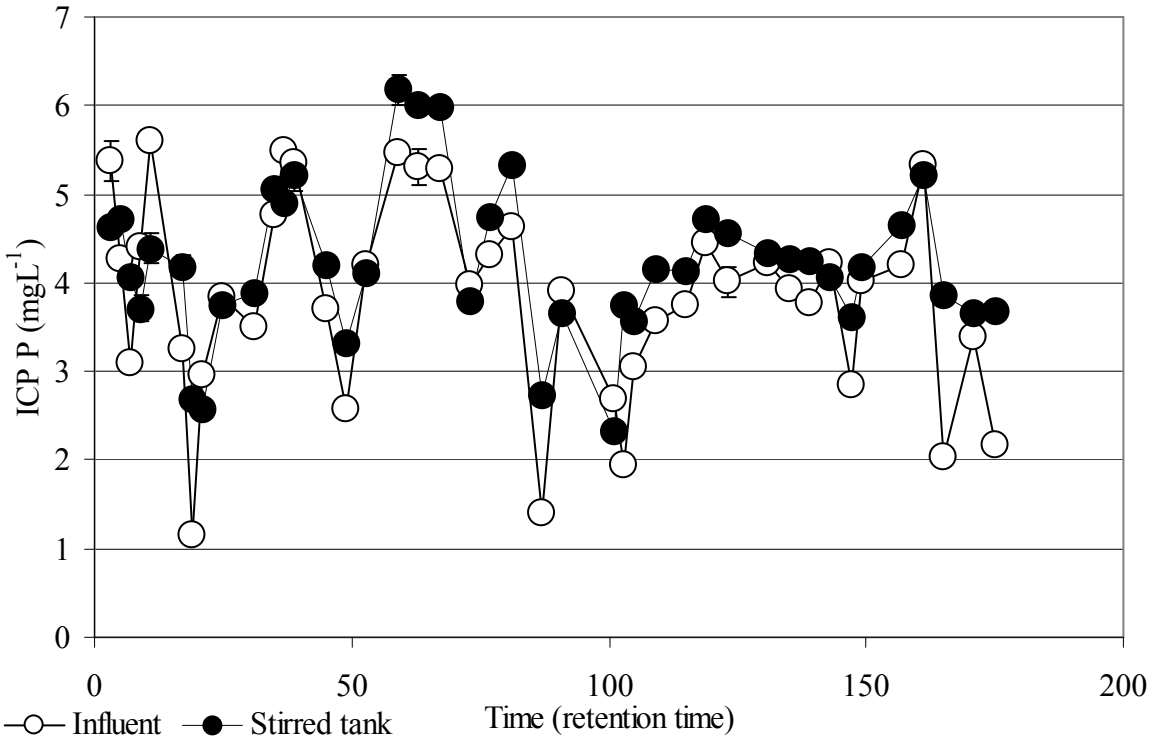

Figure 6.24 Run 1, influent and stirred tank phosphorus concentration plotted against time

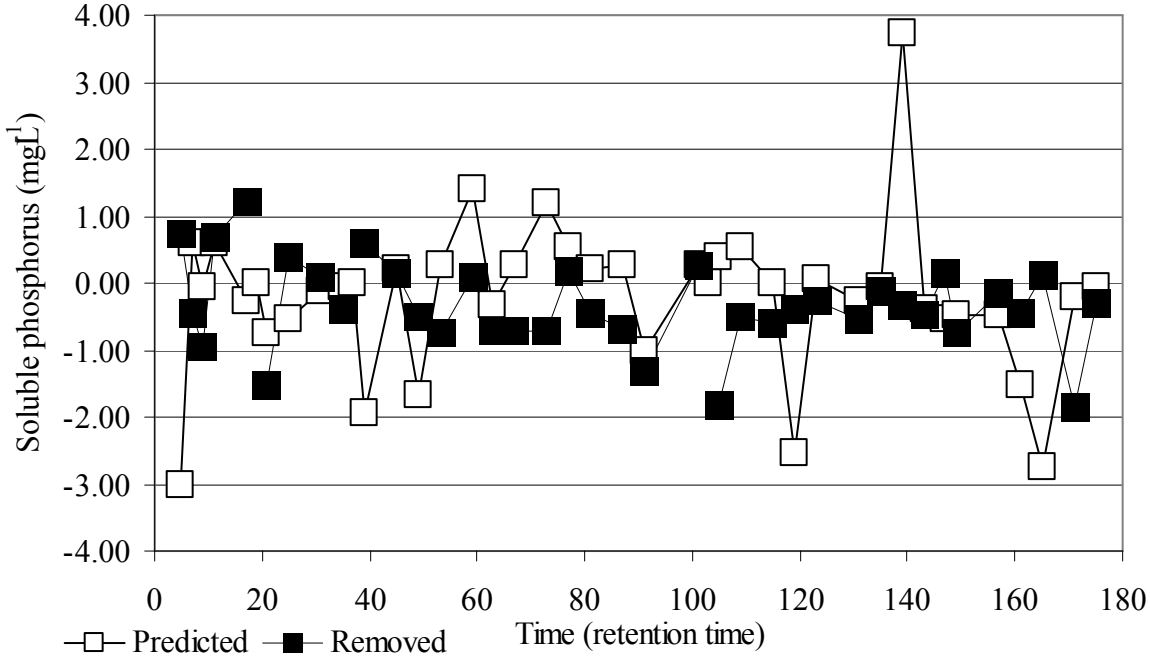

Figure 6.25 Run 1, phosphorus removal from liquor predicted values (calculated from volatile suspended solids) compared to actual quantity removed.

6.3.2.2 Runs 1 to 7: stirrer speed and retention time experiments, nutrient removal

The ammonia removal was subsequently monitored for all run conditions and there appeared to be peak ammonia removal at a stirrer speed of 15 s^{-1} and 10 h retention time, with a decrease of performance when the stirrer speed was increased to 16.7 s^{-1} (Table 6.14 and Figure 6.26). Removal of ammonia was accompanied by an increase in nitrate and nitrite in the stirred tank indicating nitrification was occurring (Figure 6.27). Summarised results of nitrite and nitrate for each individual run are given in Table 6.15.

Table 6.14 Summary of ammonia results for all stirrer speeds and retention times tested. (Data points showing < -100 % ammonia removal have been ignored.)

Stirrer speed (s^{-1})	Retention time (h)	n	Average influent ($mg(N)L^{-1}$)	Average stirred tank ($mg(N)L^{-1}$)	St dev influent (mgL^{-1})	St dev stirred tank (mgL^{-1})	% removal
8.3	12	26	22.2	14.4	9.25	7.64	35.9
8.3	12	10	20.8	15.5	5.25	2.10	22.0
8.3	10	10	22.1	15.7	6.06	4.05	25.8
8.3	8	10	23.0	19.2	6.78	6.24	15.9
10	8	10	23.6	18.1	4.27	2.59	21.0
8.3	8	10	30.2	23.9	7.13	5.98	19.9
10	8	10	34.6	18.6	4.76	3.12	46.5
11.7	8	10	37.0	21.5	6.76	4.58	40.4
15	8	10	30.0	20.5	3.19	2.15	30.9
15	10	10	23.8	3.08	6.64	2.55	88.2
15	8	10	33.7	12.7	4.83	3.58	62.7
15	10	10	35.3	15.1	6.93	5.14	58.0
15	10	20	27.4	6.29	10.3	4.93	79.8
16.7	10	20	29.3	12.3	6.46	7.55	61.2
16.7	12	20	31.9	11.4	5.28	5.89	69.6
15	12	18	26.4	4.88	4.98	2.68	82.2
15	10	9	36.8	7.12	6.09	11.5	83.1
15	10	10	41.0	7.82	6.93	4.57	81.7
15	10	10	37.4	11.6	10.2	6.24	71.4
11.7	10	10	33.4	14.7	3.84	1.43	55.7
11.7	10	10	43.2	9.20	8.11	3.02	78.4

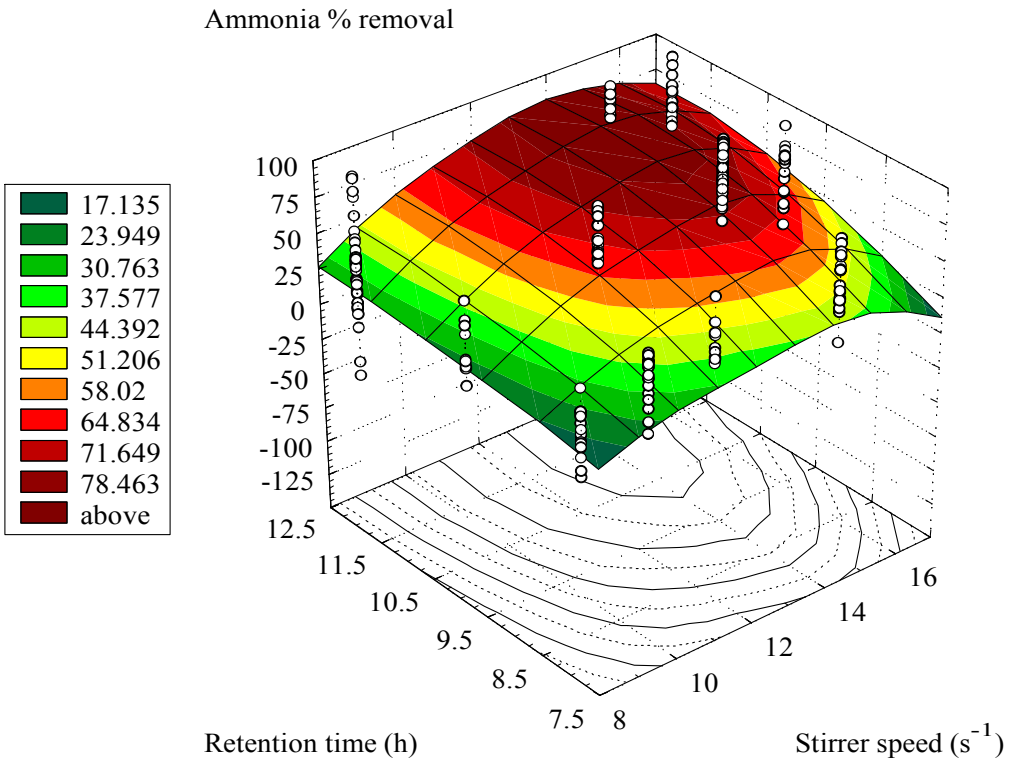

Figure 6.26 Ammonia percentage removal plotted against retention time and stirrer speed. The surface represents the trend using the least squares method. 2 cuts per data point smoothing.

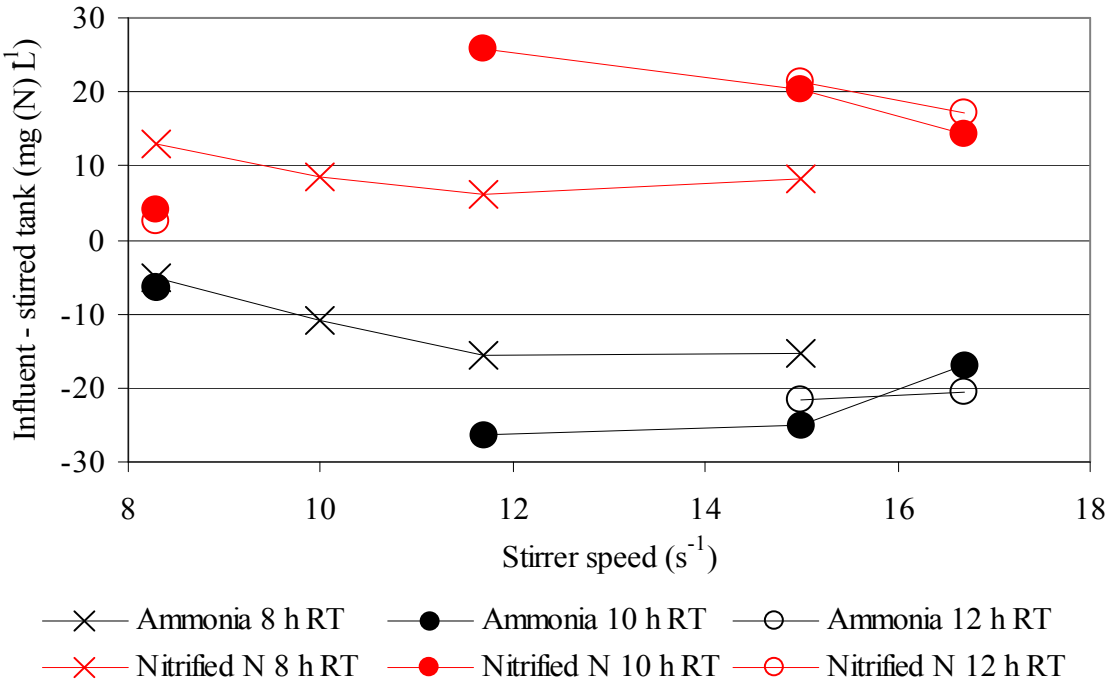

Figure 6.27 Mean change (influent – stirred tank)in ammonia and nitrified nitrogen (nitrate and nitrite) plotted for each retention time and stirrer speed tested.

Table 6.15 Summary of nitrite and nitrate results for all conditions tested.

Stirrer speed (s^{-1})	Retention time (h)	n	Average influent	Average stirred tank	St dev influent	St dev stirred tank	% increase
Nitrite Results			($mg(N)L^{-1}$)				
8.3	12	13	0.04	0.55	0.06	0.82	92.16
8.3	12	10	0.04	0.95	0.08	0.59	89.84
8.3	10	10	0.03	1.29	0.05	0.79	96.60
8.3	8	10	0.14	0.88	0.17	0.35	86.32
10	8	10	0.05	1.90	0.08	0.35	97.16
8.3	8	10	0.01	1.38	0.01	0.10	99.16
10	8	10	0.01	10.98	0.01	10.98	99.93
11.7	8	10	0.01	5.54	0.01	1.58	99.82
15	8	10	0.08	0.72	0.17	0.79	16.53
15	10	10	0.27	16.74	0.68	2.71	98.60
15	8	10	0.01	11.90	0.00	1.98	99.93
15	10	10	0.07	12.58	0.17	1.33	99.44
15	10	20	0.01	18.05	0.01	5.25	99.92
16.7	10	20	0.00	11.69	0.01	3.66	99.96
16.7	12	20	0.01	14.82	0.01	6.14	99.94
15	12	18	0.04	18.01	0.12	3.48	99.78
15.0	10	14	0.26	12.01	0.50	7.19	87.70
15.0	10	10	0.01	30.44	0.01	3.62	99.96
15.0	10	10	0.02	19.45	0.04	1.33	99.89
11.7	10	10	0.13	14.34	0.37	1.73	98.99
11.7	10	10	0.40	26.18	0.84	4.90	98.35
Nitrate Results							
8.3	12	13	1.28	24.03	1.09	21.37	89.51
8.3	12	10	0.50	2.53	0.34	0.68	79.59
8.3	10	10	0.51	3.35	0.52	0.61	85.39
8.3	8	10	0.40	2.23	0.36	0.82	83.08
10	8	10	0.29	2.65	0.23	0.30	89.54
8.3	8	10	0.34	1.56	0.19	0.37	76.08
10	8	10	0.23	1.92	0.23	1.92	88.55
11.7	8	10	0.94	1.65	0.95	0.58	47.59
15	8	10	2.44	3.17	1.43	3.05	-19.73
15	10	10	2.55	5.33	1.33	1.73	49.71
15	8	10	2.11	5.18	1.58	2.67	48.85
15	10	10	3.03	4.82	1.41	2.40	34.60
15	10	20	2.54	5.73	2.35	2.11	51.42
16.7	10	20	3.83	6.52	4.10	3.13	45.77
16.7	12	20	2.42	6.28	1.27	1.90	58.25
15	12	18	4.74	8.09	7.09	3.57	42.03
15	10	14	2.98	6.16	1.96	4.31	34.39
15	10	10	4.56	23.92	3.40	8.73	78.29
15	10	10	2.07	7.26	1.59	4.25	65.41
11.7	10	10	2.14	15.81	1.16	9.22	80.67
11.7	10	10	17.89	15.60	43.31	7.18	-3.41

The rate of nitrification related to biomass was calculated to allow comparison to wastewater treatment and pure culture nitrification. The total volatile suspended solids content was used to calculate the mg ammonia (N) removal per g volatile suspended solids per hour. The results are presented for all stirrer speeds and retention times tested in Table 6.16 below. The optimum rate of nitrification was achieved at a 12 h retention time and stirrer speed of 15 s^{-1}. The shape of the surface plot (Figure 6.28) corresponded well to the ammonia percentage removal surface plot (Figure 6.26).

Table 6.16 Ammonia removal rate calculated from influent and effluent ammonia concentration and reactor volatile suspended solids.

Run	Stirrer speed (s^{-1})	Retention time (h)	n	Ammonia removal rate (mg (N) g^{-1} h^{-1})		(mg (N) g^{-1} h^{-1})	
1	8.3	12	28	9.39	13.8 st. dev	0.016	0.021 st. dev.
2(1)	8.3	12	10	10.29	9.49 st. dev	0.011	0.009 st. dev.
2(2)	8.3	10	10	17.51	18.1 st. dev	0.015	0.012 st. dev.
2(3)	8.3	8	10	16.81	25.3 st. dev	0.011	0.012 st. dev.
2(4)	10	8	10	11.24	10.5 st. dev	0.016	0.014 st. dev.
2(5)	8.3	8	10	9.58	8.14 st. dev	0.019	0.015 st. dev.
3(1)	10	8	10	21.29	2.66 st. dev	0.048	0.008 st. dev.
3(2)	11.7	8	10	23.32	13.3 st. dev	0.047	0.026 st. dev.
3(3)	15	8	10	15.62	6.44 st. dev	0.028	0.011 st. dev.
3(4)	15	10	10	30.51	9.93 st. dev	0.050	0.013 st. dev.
3(5)	15	8	10	28.92	3.73 st. dev	0.063	0.010 st. dev.
4	15	10	10	24.85	6.95 st. dev	0.049	0.010 st. dev.
5(1)	15	10	20	33.25	6.51 st. dev	0.051	0.017 st. dev.
5(2)	16.7	10	20	25.97	7.30 st. dev	0.041	0.011 st. dev.
5(3)	16.7	12	20	27.54	7.70 st. dev	0.042	0.007 st. dev.
5(4)	15	12	18	30.77	14.6 st. dev	0.043	0.007 st. dev.
6	15	10	14	34.39	11.1 st. dev	0.073	0.016 st. dev.
7(1)	15	10	10	28.50	8.68 st. dev	0.080	0.012 st. dev.
7(2)	15	10	10	34.56	20.0 st. dev	0.062	0.013 st. dev.
7(3)	11.7	10	10	17.30	2.73 st. dev	0.045	0.009 st. dev.
7(4)	11.7	10	10	24.84	16.8 st. dev	0.082	0.018 st. dev.

Nitrifying bacteria can suffer from substrate and product inhibition, the quantities of free ammonia and free nitrous oxide were calculated (related to ammonia concentration, temperature and pH). It was found that the free ammonia levels reached 15.8 mgL^{-1}, 16 % of samples were found to be above 5 mgL^{-1} free ammonia. Free nitrous acid concentrations remained low throughout the experiments (Table 6.17).

Table 6.17 Free ammonia and free nitrous acid concentrations for each set of operating conditions.

Stirrer speed (s-1)	Retention time (h)t	n	Free ammonia (mgL^{-1})	Free nitrous acid (mgL^{-1})
8.3	12	10	1.7	1.13×10^{-13}
8.3	10	10	1.7	1.64×10^{-13}
8.3	8	10	1.4	4.17×10^{-12}
10	8	10	3.2	2.15×10^{-14}
8.3	8	10	2.8	1.28×10^{-13}
10	8	10	2.7	4.91×10^{-16}
11.7	8	10	8.7	1.83×10^{-19}
15	8	10	12.6	1.76×10^{-21}
15	10	10	0.5	2.35×10^{-18}
15	8	10	6.1	4.82×10^{-21}
15	10	10	6.2	2.64×10^{-19}
15	10	20	1.9	3.62×10^{-19}
16.7	10	20	3.9	1.13×10^{-12}
16.7	12	20	2.2	4.19×10^{-15}
15	12	18	0.8	8.41×10^{-16}
15	10	14	0.7	1.19×10^{-17}
15	10	10	0.4	1×10^{-16}
15	10	10	1.4	3.76×10^{-16}
11.7	10	10	1.1	3.05×10^{-08}
11.7	10	10	0.4	8.34×10^{-16}

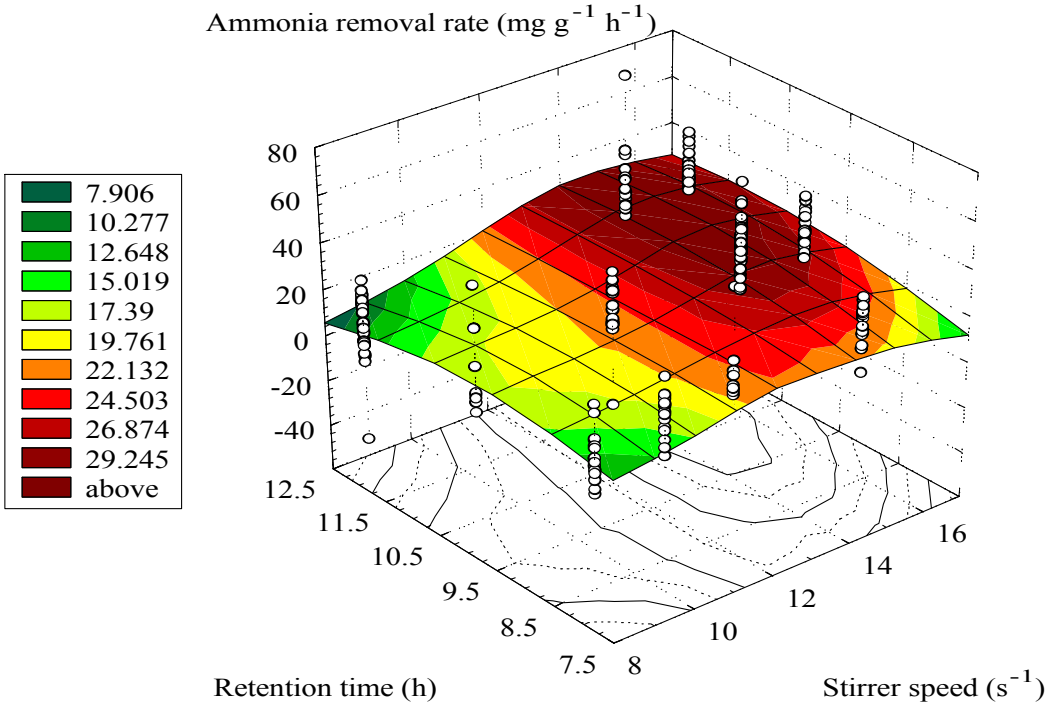

Figure 6.28 Ammonia removal rate plotted against stirrer speed and retention time. The surface represents the trend using the least squares method. 2 cuts per data point smoothing.

During this investigation most of the ammonia was nitrified to nitrite rather than nitrate. With 8 hours retention time nitrification was less effective at all stirrer speeds. Little nitrite or nitrate was present in the stirred tank, presumably because the retention time was too short for nitrifying bacteria to grow(Figure 6.29).

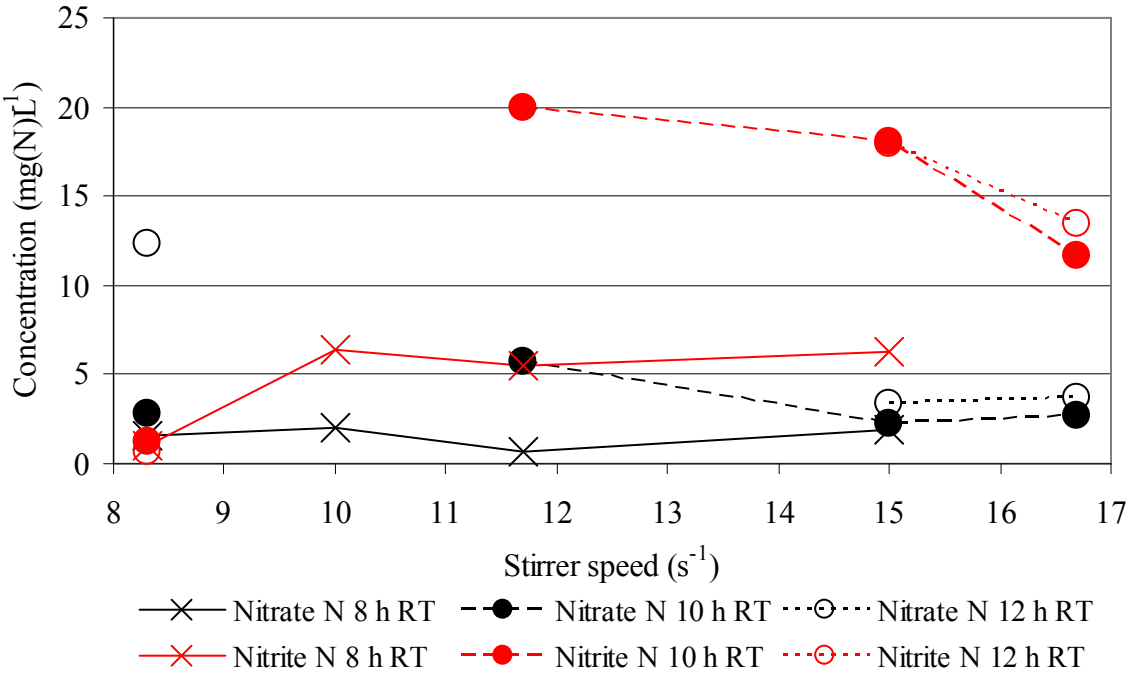

Figure 6.29 Nitrified nitrogen species plotted against stirrer speed for each retention time tested.

Phosphorus removal showed a trend towards greater removal (from the filtrate) at higher stirrer speed, low retention time (Figure 6.30). The graph would be expected to link to the volatile suspended solids (VSS), as phosphorus was incorporated into biomass. There was some correlation as a negative percentage VSS removal can be seen on the surface plot at high stirrer speed short retention time (Figure 6.19)where the peak phosphorus removal was seen (Figure 6.30). This was by no means conclusive.

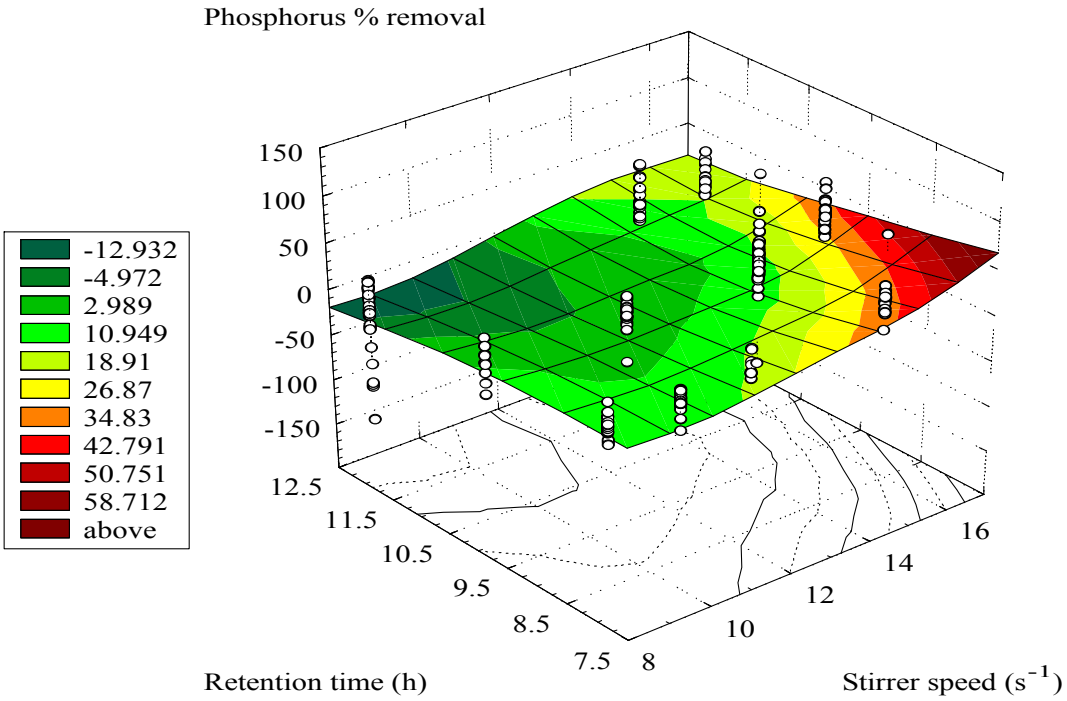

Figure 6.30 Phosphorus removal plotted against stirrer speed and retention time for all data points tested. Surface plotted using least squares method, smoothed to 2 cuts per data point.

6.4 REACTOR ENVIRONMENTAL CONDITIONS

6.4.1 Temperature Control

Temperature effects are reported here because increasing stirrer speed (power input) was found to cause an increase in temperature (Figure 6.31 energy balance Appendix C). Bacterial growth rates and reactions are more rapid at higher temperatures so temperature control was used during runs 5 to 8. The effects on carbonaceous load removal are described below.

Little correlation was found between temperature and percentage $CBOD_5$ removal for the overall (Figure 6.32) or for individual conditions (Table 6.18). The data indicated a poor fit to the regression line (R^2 = 0.01) and no significant evidence that $CBOD_5$ removal was linked to temperature (using t test, comparing data to a straight horizontal line). The results suggested that factors other than temperature had a greater influence on the effluent quality (e.g. influent quality, see Figure 6.12). For this analysis 3 data points that were less than -75 % removal were taken out of the data set as outliers, these occurred when the influent concentration was less than 10 mgL^{-1} $CBOD_5$.

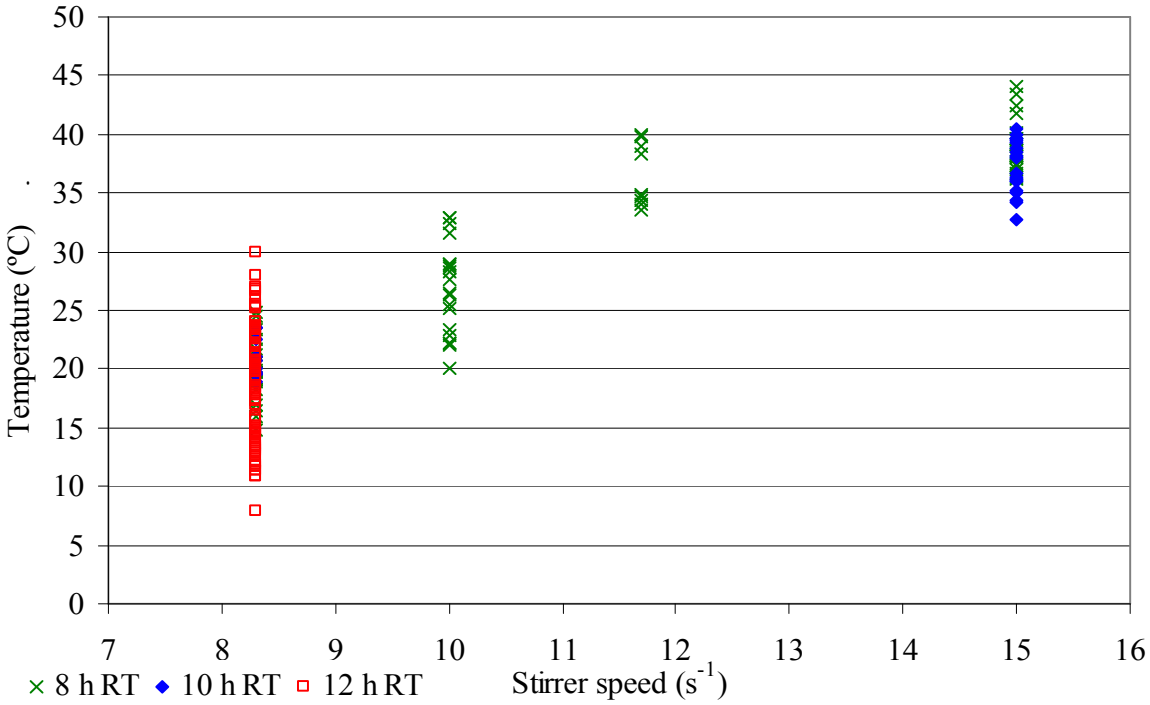

Figure 6.31 Temperature plotted against stirrer speed, for each set of conditions without temperature control (Runs 1 – 5).

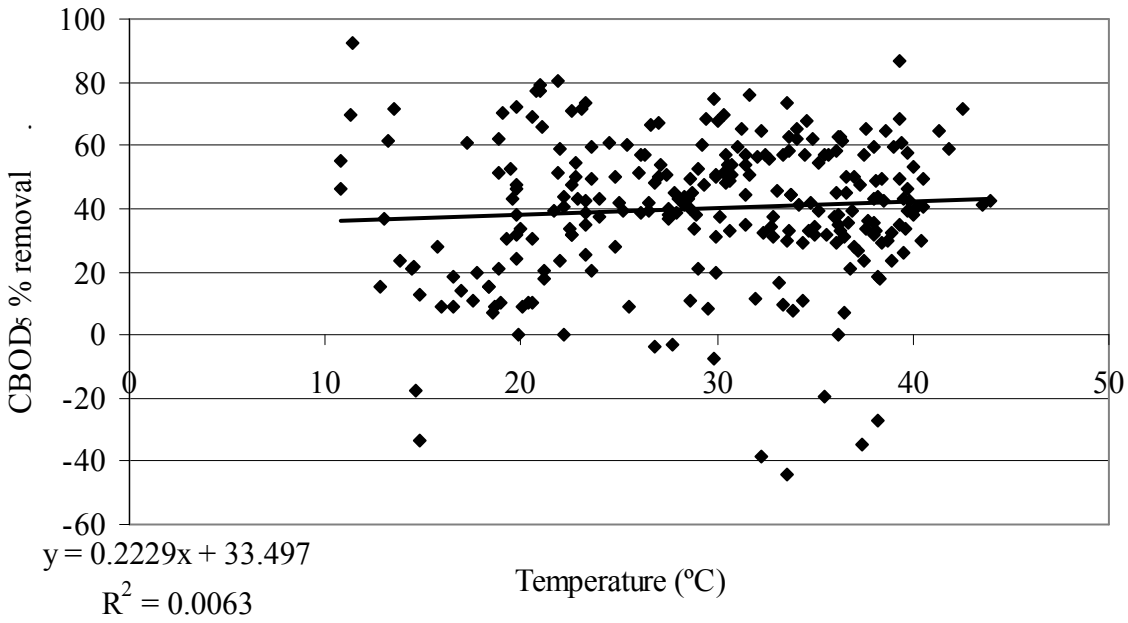

Figure 6.32 Percentage $CBOD_5$ removal plotted against temperature, all data.

Table 6.18 Summary data for linear regression analysis comparing temperature and percentage $CBOD_5$ removal for all conditions without temperature control (runs 1 – 5). Significance level for correlation was calculated using a t test.

Stirrer speed (s^{-1})	Retention time (h)	n	Linear regression	R^2 value	Significance level
8.3	12	44	$y = 1.16x + 19.12$	0.04	< 90 %
8.3	10	9	$y = -2.30x + 97.58$	0.04	< 90 %
8.3	8	19	$y = 3.21.x - 34.27$	0.16	92 %
10	8	19	$y = 0.78x + 12.75$	0.03	< 90 %
11.7	10	19	$y = 0.59x + 32.32$	0.01	< 90 %
11.7	8	8	$y = 4.94x - 149.6$	0.17	< 90 %
15	12	16	$y = 4.48x + 147.4$	0.04	< 90 %
15	10	63	$y = -1.21x + 85.30$	0.06	95 %
15	8	20	$y = 1.00x + 6.17$	0.027	< 90 %
16.7	12	18	$y = 0.25x + 44.28$	0.00	< 90 %
16.7	10	19	$y = 1.48x - 10.68$	0.62	< 90 %
All data		257	$y = 0.205x + 33.49$	0.01	< 90 %

Nitrification was found to be more sensitive to temperature change than carbonaceous activity (compare Figure 6.32 and Figure 6.33). To separate the effects of the different stirrer speeds and retention times from the temperature effects these were again analysed separately (Figure 6.34); a summary of the linear regression analysis carried out for each set of conditions is given in Table 6.19. The results for each set of conditions confirmed there was a correlation between increasing temperature and treatment rate. The results gave an overall trend with a poor fit (R^2 0.32), but good correlation tested by using a t test with the null hypothesis that the slope of the data is zero. The results show almost all of the correlations being significant, to the 95 % level (Table 6.19). For 12 h retention time, 8.3 s^{-1} stirrer speed the correlation appeared to be in the opposite direction to all of the other conditions, with a negative correlation between temperature and ammonia removal. The correlation was quite significant, however, the data fitted poorly to the linear regression with an R^2 value of just 0.11 and it comprises of data taken during the 1st run when the reactor community was developing (Table 6.19, Figure 6.34 data represented by unfilled blue squares on this plot).

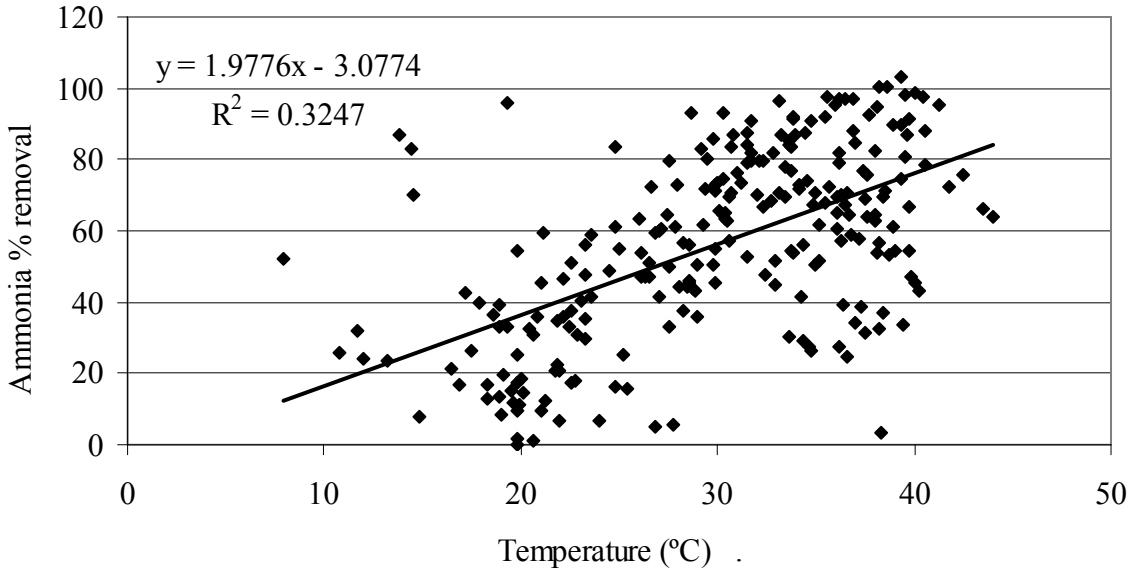

Figure 6.33 Ammonia removal plotted against temperature. The linear regression was carried out using Excel linear regression, the correlation and the coefficient of determination were automatically calculated using Excel.

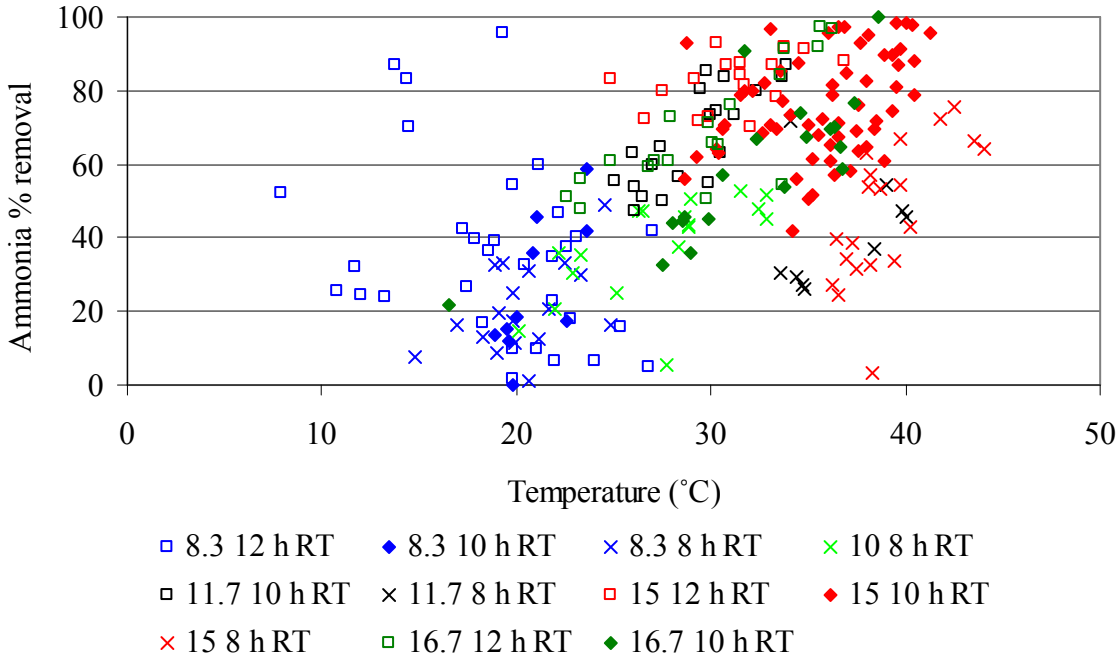

Figure 6.34 Ammonia percentage removal plotted against temperature for each of the run conditions tested. The first number in the legend for each data set is the stirrer speed (s^{-1}).

Table 6.19 Summary data for linear regression analysis comparing temperature and percentage ammonia removal for all conditions. Significance level that the data is related to the line calculate using t test.

Stirrer speed	Retention time	n	Linear regression	R^2 value	Significance level
8.3	12	32	$y = -1.17x + 68.12$	0.11	94 %
8.3	10	10	$y = 7.77x - 136.96$	0.53	98 %
8.3	8	18	$y = 2.18.x - 23.12$	0.51	95 %
10	8	19	$y = 2.43x - 30.31$	0.34	99 %
11.7	10	20	$y = 3.00x - 19.65$	0.65	>99 %
11.7	8	9	$y = 1.63x - 18.41$	0.08	<90 %
15	12	17	$y = 1.01x + 51.11$	0.18	90 %
15	10	62	$y = 1.61x + 18.71$	0.13	99 %
15	8	20	$y = 5.50x - 167.89$	0.45	99 %
16.7	12	19	$y = 3.00x - 19.64$	0.65	99 %
16.7	10	20	$y = 3.33x - 46.69$	0.67	99 %
All data		245	$y = 1.98x - 3.08$	0.32	99 %

Despite the temperature control runs there were no runs of the same average temperature, using different stirrer speed that allowed conclusions to be drawn about the temperature and stirrer speed effects by a straight forward hypothesis test. The results of the entire investigation were compared using a multiple linear regression with Microsoft Excel Data Analysis add-in. The results of the multiple linear regression showed that all of the parameters tested as relating to ammonia removal (stirrer speed, retention time and temperature) had a separate significant effect on ammonia removal. The linearity of the temperature and stirrer speed (Figure 6.31) was found not to prevent the regression analysis separating these effects, presumably due to the large sample (n = 252) and the temperature control results providing data points of the same temperature for various stirrer speeds.

The multiple linear regression gave positive correlation for temperature, stirrer speed and retention time all giving 99 % confidence that there was a relationship between the variable and the ammonia removal (Table 6.20). The R^2 value for the overall model was 0.55 showing that the model accounted for 55 % of the variance.

Table 6.20 Multiple linear regression results for stirrer speed, temperature and retention time effects on the % ammonia removal, with significance to the 99 % level.

Variable	Coefficient	Standard error	Significance	R^2
Stirrer speed (s^{-1})	1.9	0.62	99 %	
Retention time (h)	6.7	0.93	99 %	
Temperature (˚C)	1.9	0.27	99 %	
Whole regression			99 %	0.55

6.4.2 pH

pH appeared to have less of an influence on ammonia removal than temperature. The data was scattered (overall R^2 0.23) but did vary significantly from a horizontal line on the graph (99 % significance using student t test) when all of the data was plotted, with a tendency towards greater ammonia removal at lower reactor pH (Figure 6.35, Table 6.21). The $CBOD_5$ removal was not significantly related to pH. This showed that other reactor factors were more important in determining levels of $CBOD_5$ removal (overall R^2 0.0039). The regression results for all $CBOD_5$ removal under all conditions are presented in (Table 6.22). Stirrer speed 15 s^{-1} with 10 h retention time shows an increase in $CBOD_5$ removal with increasing pH, to 90% confidence level (Figure 6.36).

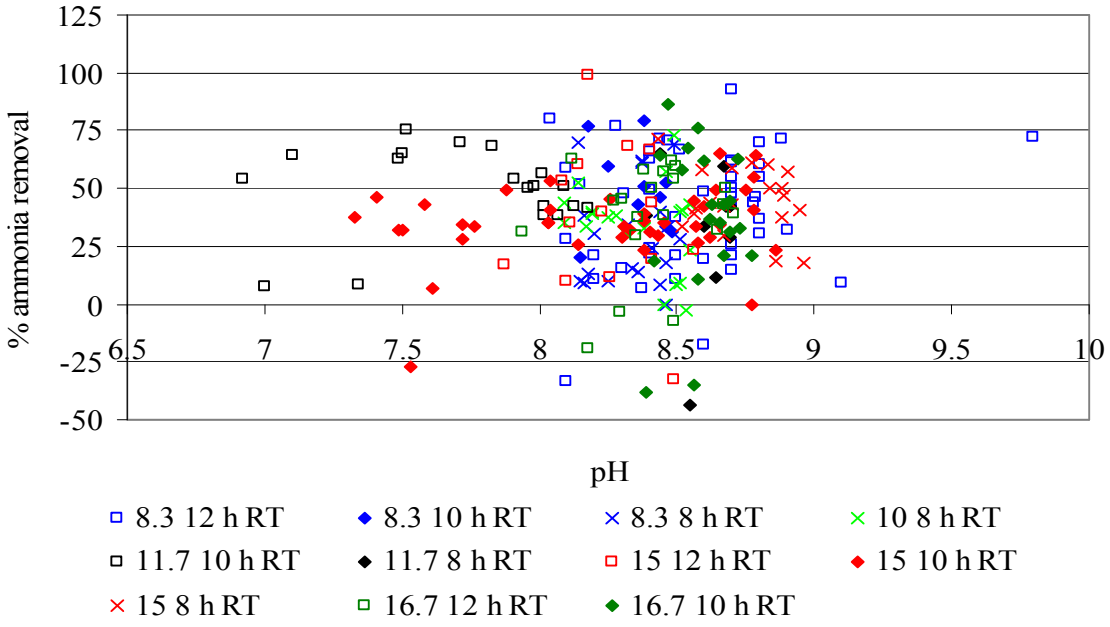

Figure 6.35 Percentage ammonia removal plotted against pH for all run conditions, with the first number in the legend as the stirrer speed (s^{-1}).

Table 6.21 Summary data for linear regression analysis comparing pH and percentage ammonia removal for all conditions. Significance level that the data is related to the line calculate using t test.

Stirrer speed	Retention time	n	Linear regression	R^2 value	Significance level
8.3	12	36	$y = -13.77x + 150.4$	0.02	<90 %
8.3	10	10	$y = -104.1x + 895.8$	0.45	94 %
8.3	8	20	$y = 22.04.x - 165.8$	0.04	<90 %
10	8	20	$y = -69.27x + 612.7$	0.43	99 %
11.7	10	18	$y = -29.77x + 297.1$	0.77	99 %
11.7	8	10	$y = -59.62x + 553.4$	0.20	<90 %
15	12	17	$y = -5.756x + 130.19$	0.02	<90 %
15	10	69	$y = -16.71x + 210.8$	0.37	99 %
15	8	20	$y = -100.1x + 921.7$	0.69	99 %
16.7	12	20	$y = -61.47x + 586.4$	0.58	99 %
16.7	10	20	$y = -72.37x + 683.4$	0.13	<90 %
All data		261	$y = -30.98x + 312.4$	0.23	99 %

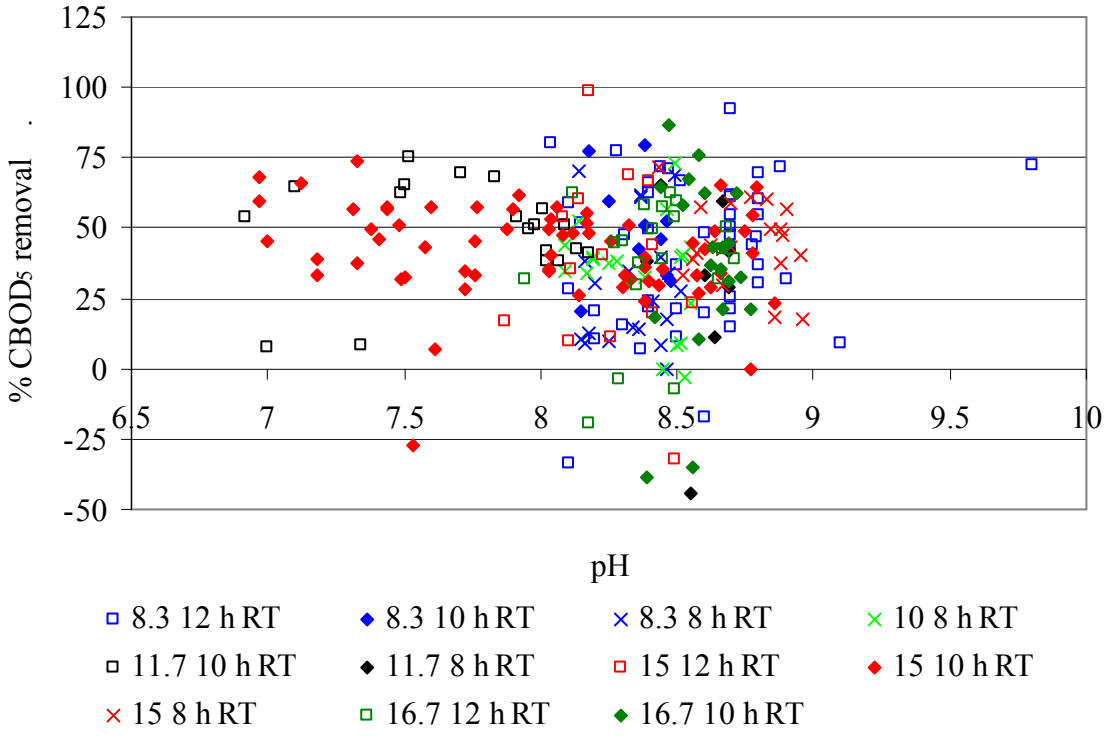

Figure 6.36 Percentage $CBOD_5$ removal plotted against pH for all run conditions with the first number in the legend as the stirrer speed (s^{-1}).

Table 6.22 Summary data for linear regression analysis comparing pH and percentage $CBOD_5$ removal for all conditions. Significance level that the data is related to the line calculate using t test.

Stirrer speed	Retention time	n	Linear regression	R^2 value	Significance level
8.3	12	46	$y = 15.20x - 87.94$	0.03	<90 %
8.3	10	10	$y = -23.94x + 249.3$	0.02	<90 %
8.3	8	20	$y = 10.14.x - 55.06$	0.00	<90 %
10	8	20	$y = -32.01x + 301.3$	0.08	<90 %
11.7	10	19	$y = 3.687x + 20.91$	0.01	<90 %
11.7	8	10	$y = 5.77x - 17.68$	0.00	<90 %
15	12	17	$y = -88.32x + 739.5$	0.05	<90 %
15	10	57	$y = 8.733x - 36.92$	0.06	91 %
15	8	20	$y = -22.93x + 245.5$	0.07	<90 %
16.7	12	20	$y = 24.32x - 166.3$	0.04	<90 %
16.7	10	20	$y = 30.57x - 225.7$	0.01	<90 %
All data		242	$y = -3.170x + 66.78$	0.00	<90 %

6.5 TEMPERATURE AND STIRRING

Run 6 used an unstirred rig with the same run parameters as the stirred rig (temperature (34 ˚C), retention time (10 h) and air flow rate (2 $Lmin^{-1}$ dissolved oxygen mean concentration of 87.3 % compared to 90 % in the stirred reactor, student t test did not find a significant difference)). The unstirred reactor was seeded with the effluent from the stirred tank. Samples were taken less frequently during this run to observe the changes in treatment from the stirred tank. The influent to the rigs was treated in the same way (pumped the same distance, at the same rate using the same tubing) therefore only 1 influent sample was analysed.

The stirred reactor had a problem during retention time 15/16 as the peristaltic pump tubing had moved, this increased the feed rate, significantly reducing the retention time. The community appeared to have been washed out as nitrification ceased, recovery took approximately 7 retention times (retention time 16 – 23 Figure 6.37).

The $CBOD_5$ removal did not change noticeably in the unstirred tank over more than 30 retention times (Figure 6.38). A paired student t test was conducted; a difference of less than 80 % significance between the stirred and unstirred tank results was found.

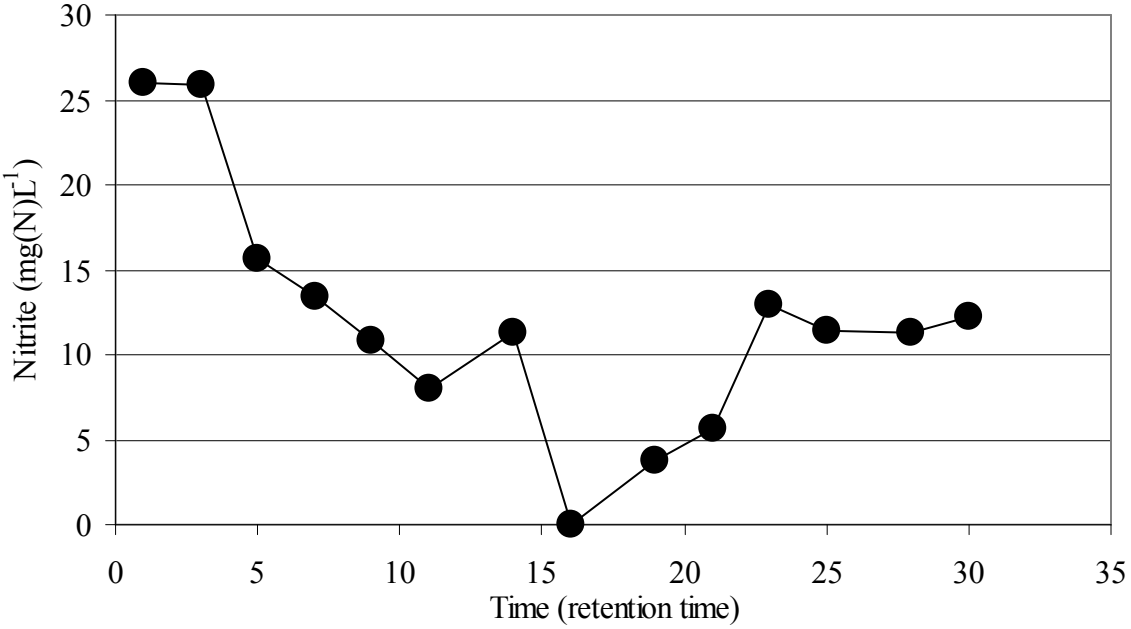

Figure 6.37 Run 6, stirred tank nitrite concentration plotted against time. During retention time 15/16 the feed rate had been elevated due to a problem with the pump.

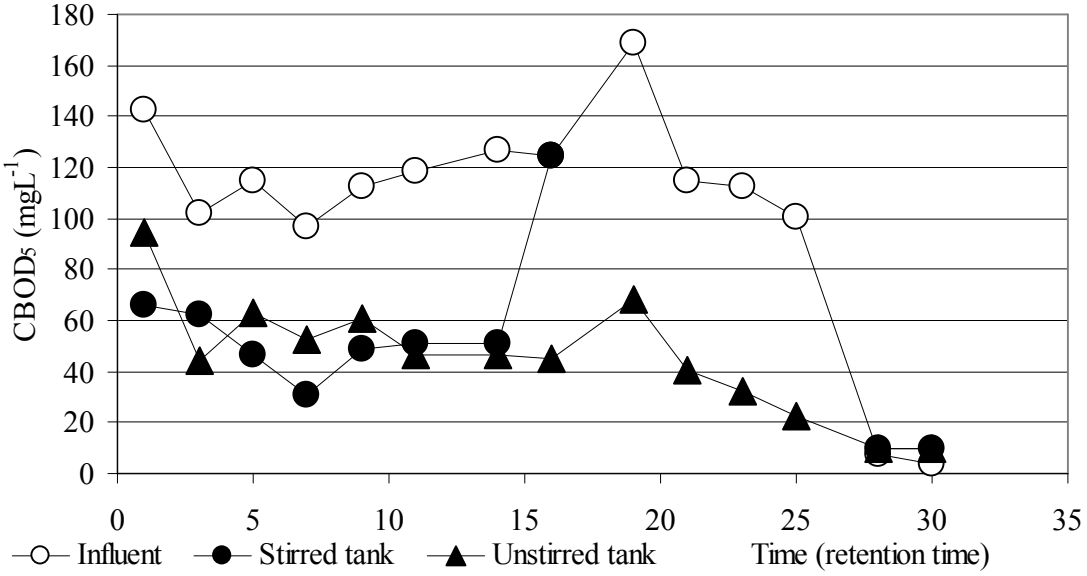

Figure 6.38 Run 6, stirred tank and unstirred tank $CBOD_5$ plotted against time.

In contrast the nitrification decreased from the first retention time in the unstirred rig (Figure 6.39). There was a significant difference (>95 %) between the stirred and unstirred tank samples using a paired t test. Again the effect of the pump malfunction

can be seen on the stirred tank plot at 16 retention times (Figure 6.39) as the ammonia level increased to meet the influent and unstirred tank ammonia concentrations

The solids retention time in the unstirred tank may have been raised, as a build up of biomass was observed. The mixing due to aeration may not have been sufficient to maintain homogeneity as these conditions allowed bacteria to attach to the wall between cleanings. However, the wall growth was removed daily as with the stirred tank. The solids were monitored in the unstirred tank and stirred tank during the experiment and the difference in concentrations was less than anticipated from visual observation (Figure 6.40).

From the observations made during this run it was confirmed that the raised temperature alone was not sufficient to promote nitrification in the reactor. The conditions in the stirred tank were necessary to achieve the high nitrification rate observed.

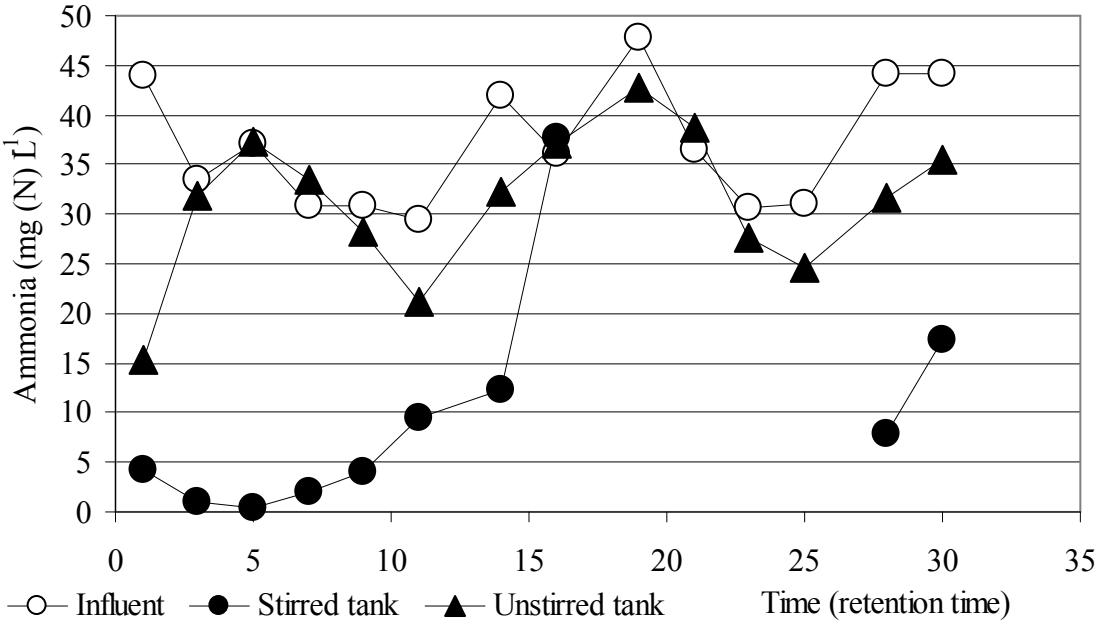

Figure 6.39 Run 6, stirred tank and unstirred tank ammonia concentration plotted against time.

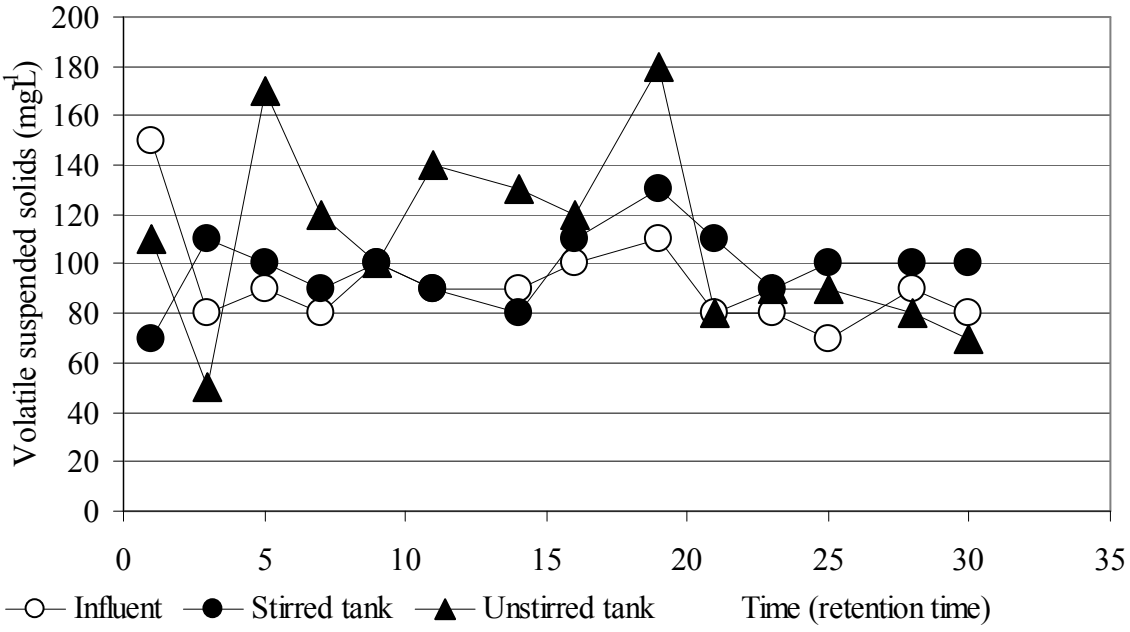

Figure 6.40 Run 6, Volatile suspended solids plotted against time for influent, stirred and unstirred tanks.

6.6 TREATMENT RELATIONSHIPS

The ammonia removal and removal rate graphs revealed a peak at stirrer speed 15 s^{-1} 12 h retention time (Figure 6.26 and Figure 6.28 respectively) which corresponded to a trough on the various carbonaceous load removal graphs (Figure 6.11, Figure 6.13, Figure 6.15 and Figure 6.16). The trend appeared to be an observed decrease in carbonaceous load removal driven by an increase in nitrification; this pattern was observed for overall run conditions (Figure 6.41). It appeared that a shift occurred with changing reactor conditions, from nitrification coexisting with carbonaceous load removal to excluding carbonaceous load removal at the conditions of maximum nitrification.

There are two possible mechanisms allowing nitrification rate to increase while the carbonaceous removal rate decreases. The carbonaceous bacteria may generally impede the growth of nitrifying bacteria due to their rapid growth rate and tendency to form flocs. Heterotrophs tend to grow on the outside of flocs and biofilms; therefore

restricting the access of nitrifying bacteria to oxygen and ammonia. If the conditions in the reactor were unfavourable to the heterotrophic bacteria the nitrifying bacteria may use the opportunity to grow and nitrify at a greater rate than is usually seen. The nitrifiying bacteria are highly oxygen and temperature dependant the conditions of good mass transfer and temperatures close to the optimum may allow them to grow more rapidly.

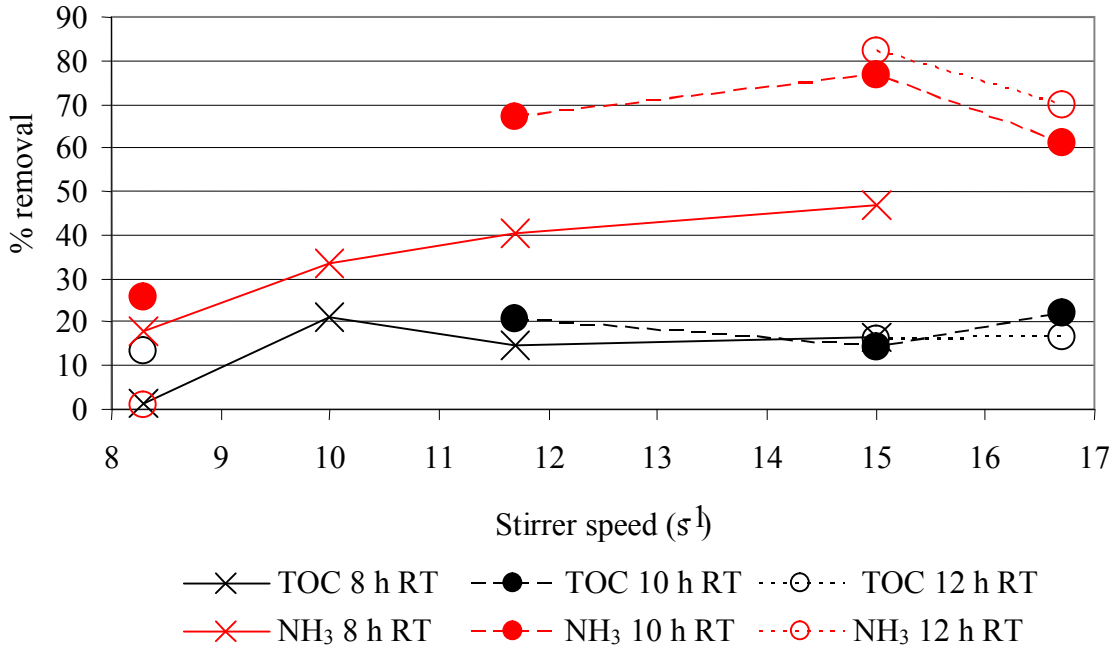

Figure 6.41 Mean ammonia and TOC percentage removal plotted for each retention time against stirrer speed.

6.7 Stirring, Mixing and Shear

The effects of shear and mixing need to be separated to allow an understanding of the mechanisms causing the increased nitrification, accompanied by decreased carbonaceous load removal presented above. Impeller type affects the amount of shear, mixing and power input to a system. During run 8 2 alternative impeller types were tested to attempt to differentiate between shear and mixing or pumping effects. The impeller tip speeds were matched to those of the Disk turbine at 11.7 s^{-1} and 8.3 s^{-1}.

From retention time 59 there was a sharp decrease in ammonia load and nitrite was found in the influent (Figure 6.42 and Figure 6.43). The change in influent composition was a result of flow to an online analyser being turned off in the pilot hall, this increased the retention time in the header tank for approximately 2 days from a retention time of a few hours. This experiment was conducted in July when the weather was warm and these factors presumably allowed bacterial growth in the holding tank. The key parameters of COD, ammonia and nitrite were measured 5 more times when the cause of the problem had been identified and remedied.

The HSD appeared to almost stop nitrification at both stirrer speeds (Figure 6.42, Figure 6.43 and Figure 6.44). When the HSD was replaced by the LE20 some ammonia removal occurred and nitrite appeared in the stirred tank sample. At the LE20 higher stirrer speed the highest ammonia removal occurred, unfortunately it was during this part of the experiment that the feed problems occurred, 5 further samples were taken to this section shows the best nitrification results.

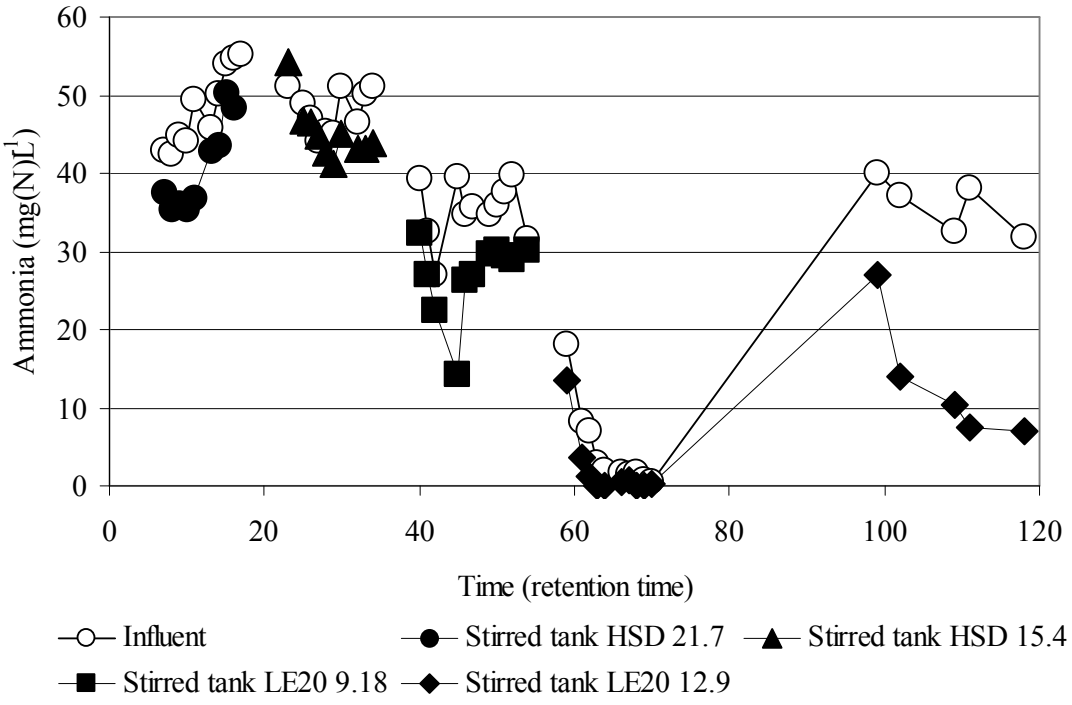

Figure 6.42 Run 8, ammonia concentration plotted against retention time, for the High Shear Disk and LE20 impellers (stirrer speed (s^{-1}) given after impeller in legend)

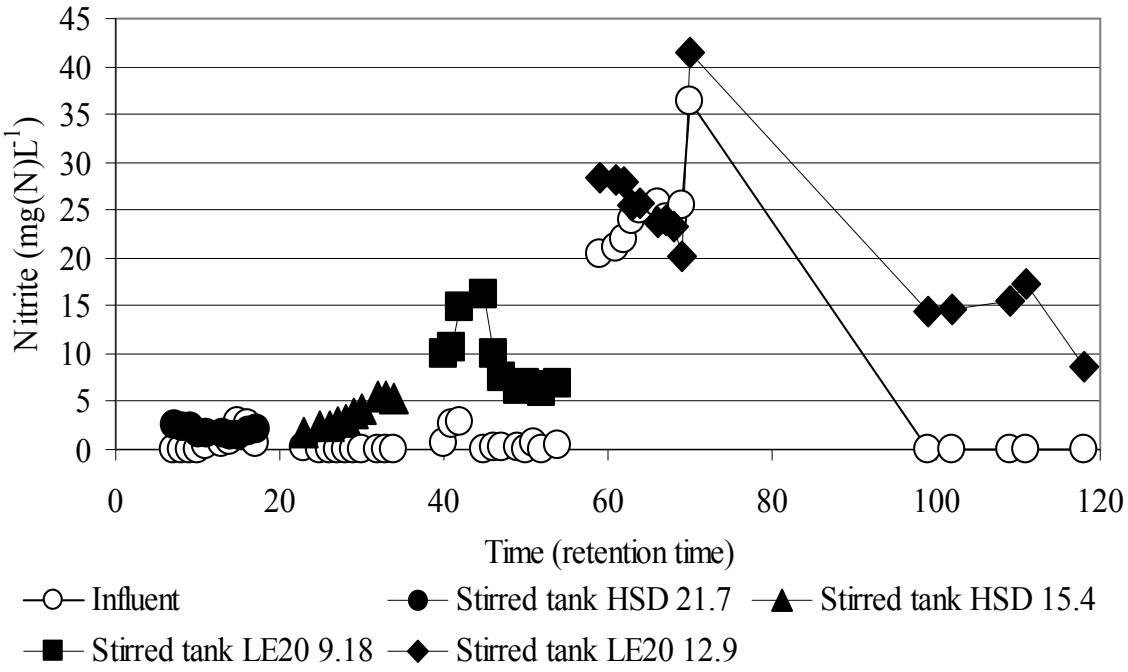

Figure 6.43 Run 8, nitrite concentration plotted against retention time for the HighShear Disk and LE20 impellers (stirrer speed (s^{-1}) given after impeller in legend)

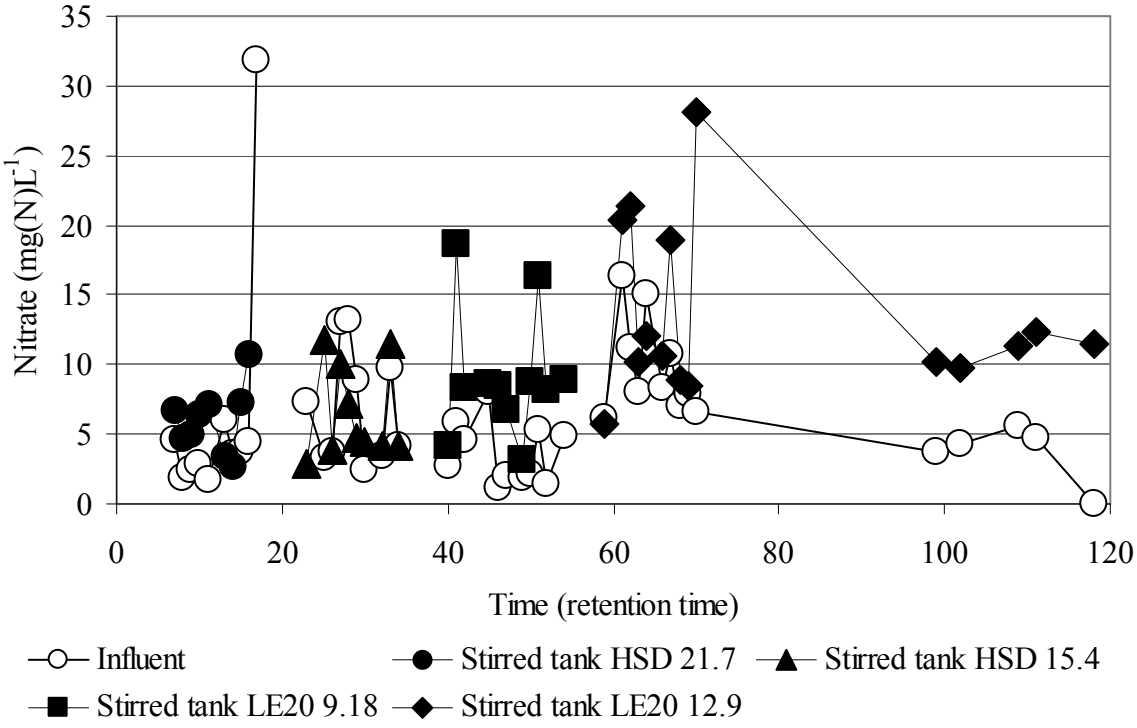

Figure 6.44 Run 8, nitrate concentration plotted against retention time for High Shear Disk and LE20 impellers (stirrer speed (s-1) given for impeller in legend)

The $NBOD_5$ results show that nitrifying bacteria built up in the system and were causing the stored influent system to nitrify, during the problems the $NBOD_5$ of the influent was over 20 mgL^{-1} (Figure 6.45).

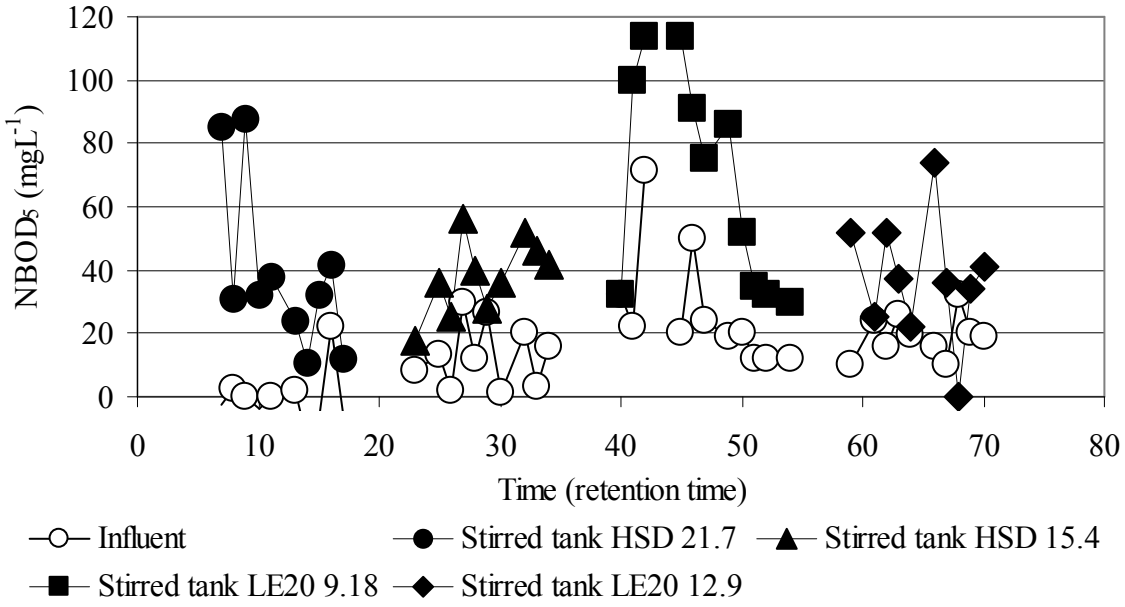

Figure 6.45 Run 8, Nitrifying BOD plotted against time for High Shear Disk and LE20 impellers (stirrer speed (s^{-1}) written for impeller in legend)

Carbonaceous BOD_5 removal results showed very good removal running the high shear disk at the higher stirrer speed (21.7 s^{-1}) with 73 % removal achieved (Figure 6.46). The removal of $CBOD_5$ with the high shear disk running at 15.4 s^{-1} remained high but was close to good removal using the Disk turbine (54 %). The LE20 performed poorly with only 3 % removal and negative removal at the higher stirrer speed, this may have been due to the problems with the influent that caused problems to the ammonia removal during Run 8(4) (Figure 6.46). The soluble COD and TOC followed a similar removal pattern while the total COD appears very different from these measures with little removal until the final high speed LE20 run during the additional run time (Figure 6.48, Figure 6.49 and Figure 6.47 respectively).

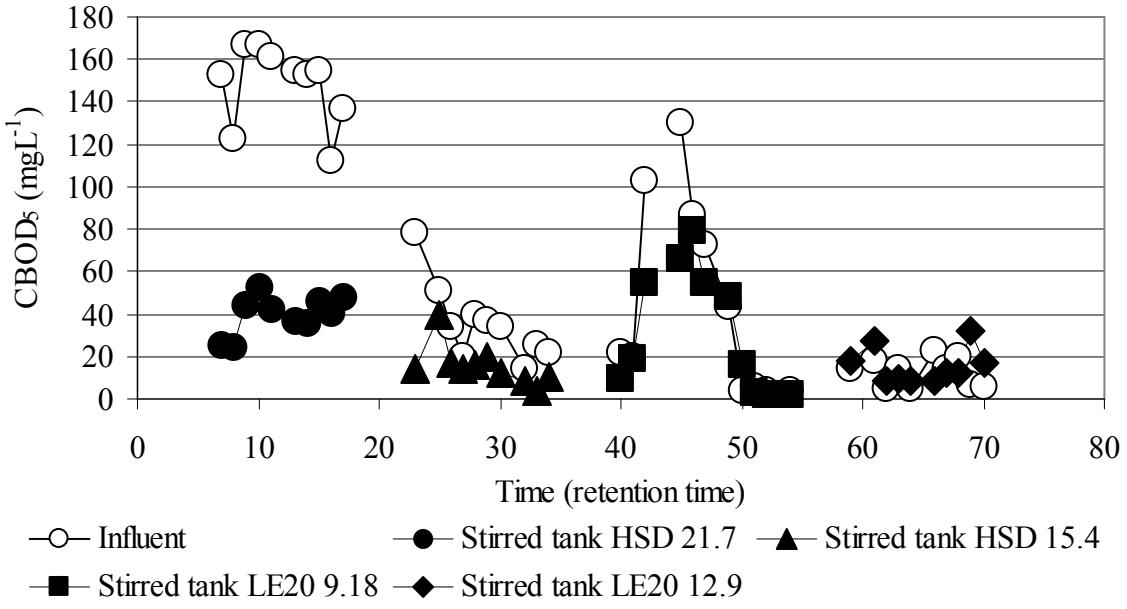

Figure 6.46 Run 8, $CBOD_5$ plotted against time for High Shear Disk and LE20 impellers, (stirrer speed (s^{-1}) given in legend).

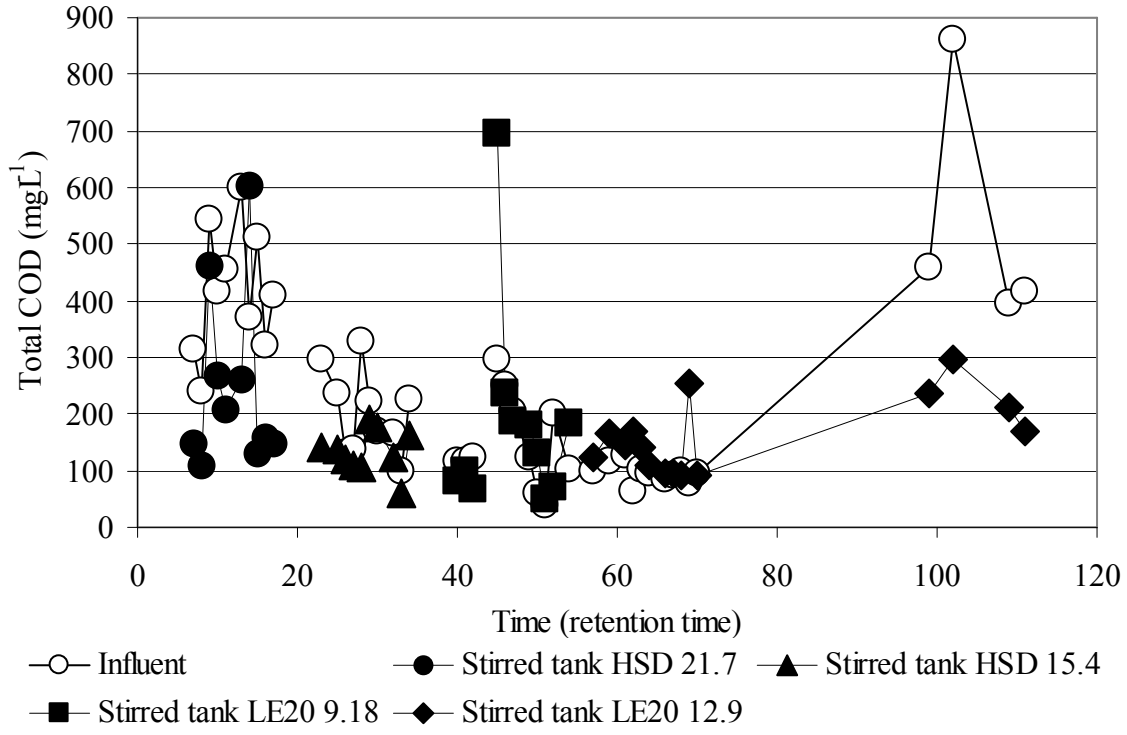

Figure 6.47 Run 8, total COD plotted against retention time for High Shear Disk and LE20 impellers (stirrer speed (s^{-1}) given in legend).

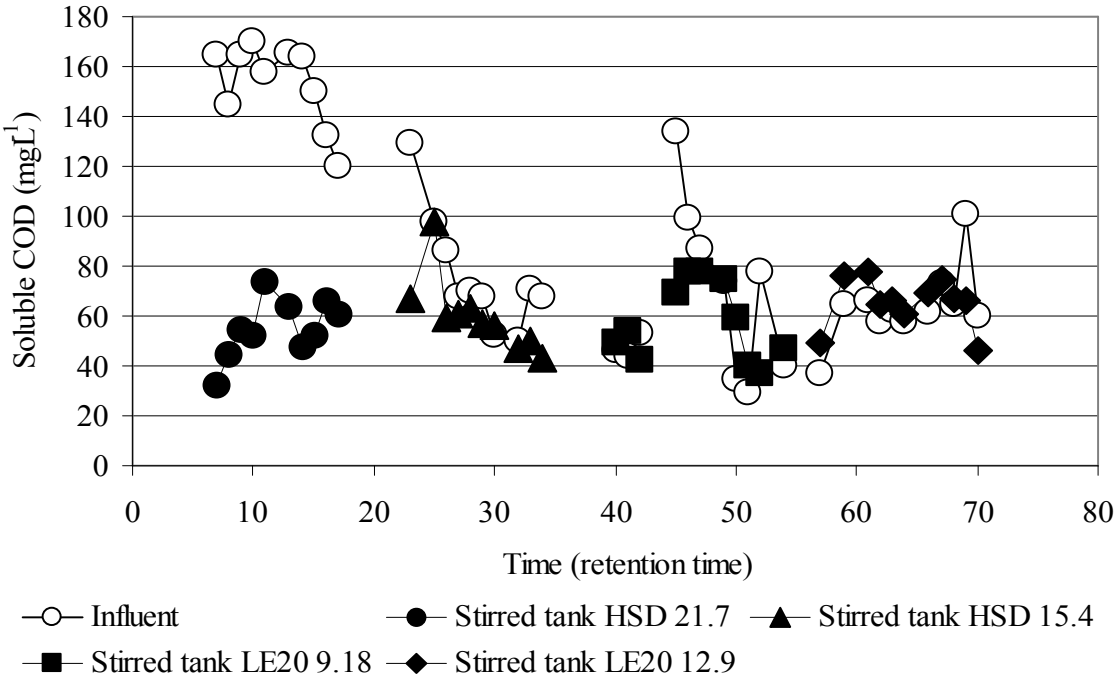

Figure 6.48 Run 8, soluble COD plotted against time for the High Shear Disk and LE20 impellers (stirrer speed (s^{-1}) is marked for each part of the run.

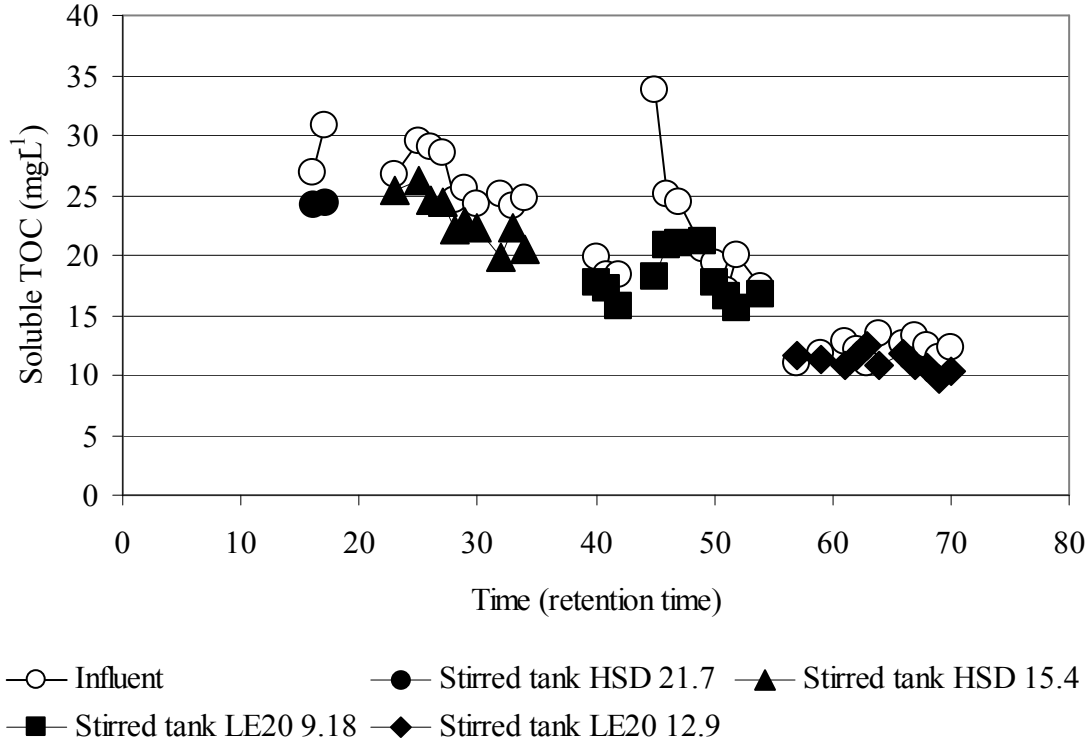

Figure 6.49 Run 8 Influent and stirred tank TOC concentration plotted against retention time. The impeller and stirrer speed (s^{-1}) for each part of the run is marked.

6.8 DENITRIFICATION

The anoxic reactor was set up for run 7 and seeded with activated sludge. The liquid volume was assumed to remain at 2.5 L throughout the experiment. The hydraulic retention time of the anxoic tank was calculated as reactor volume over influent flow rate, and did not relate to the stirred tank, because the stirred tank volume varied with reactor conditions. As the anoxic reactor was a form of down flow fixed film reactor, the solids were attached and there was growth and build up within the reactor and a pipe balancing the effluent, where solids settled causing a decrease in the effluent solids concentration (Figure 6.50). The retention time values used to identify time on the graphs continue to relate the stirred tank to remain consistent with previous results.

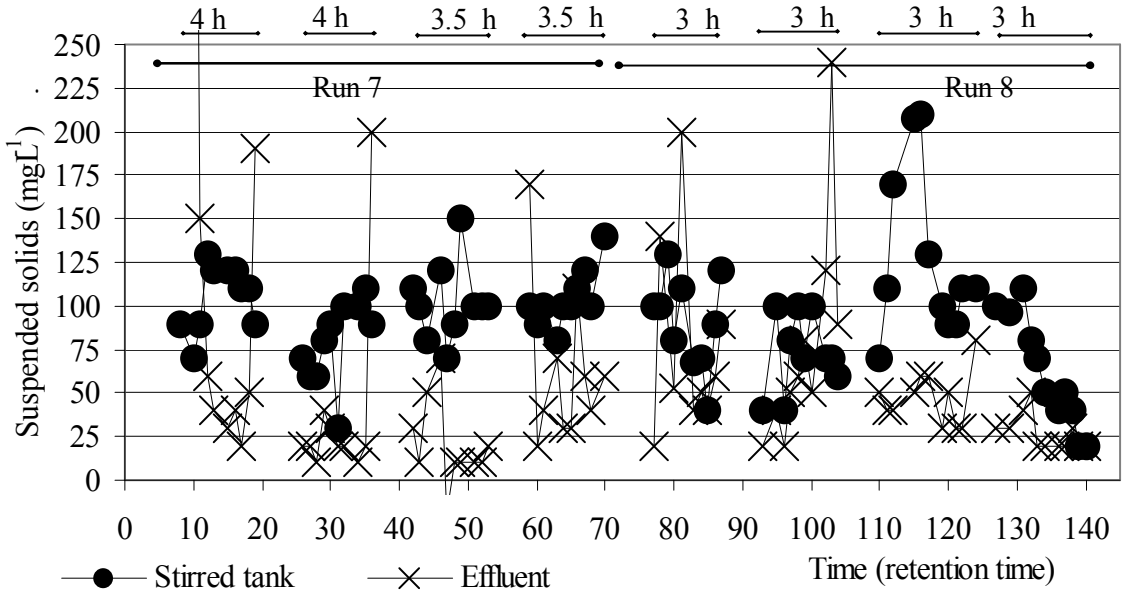

Figure 6.50 Run 7 and 8, stirred tank and effluent suspended solids concentration plotted against retention time. The anoxic reactor hydraulic retention time, given above the data points.

Nitrate and nitrite removal were observed within 11 retention times (stirred tank). During run 7 there was some denitrification with less nitrogen emerging from the anoxic reactor than from the stirred tank or the influent (Figure 6.51). There was also some $CBOD_5$ removal during run 7 (Figure 6.52). During run 8 poor denitrification results were obtained due to little nitrate or nitrite entering the anoxic reactor caused by poor nitrification in the stirred tank (Figure 6.51, Figure 6.43, Figure 6.44).

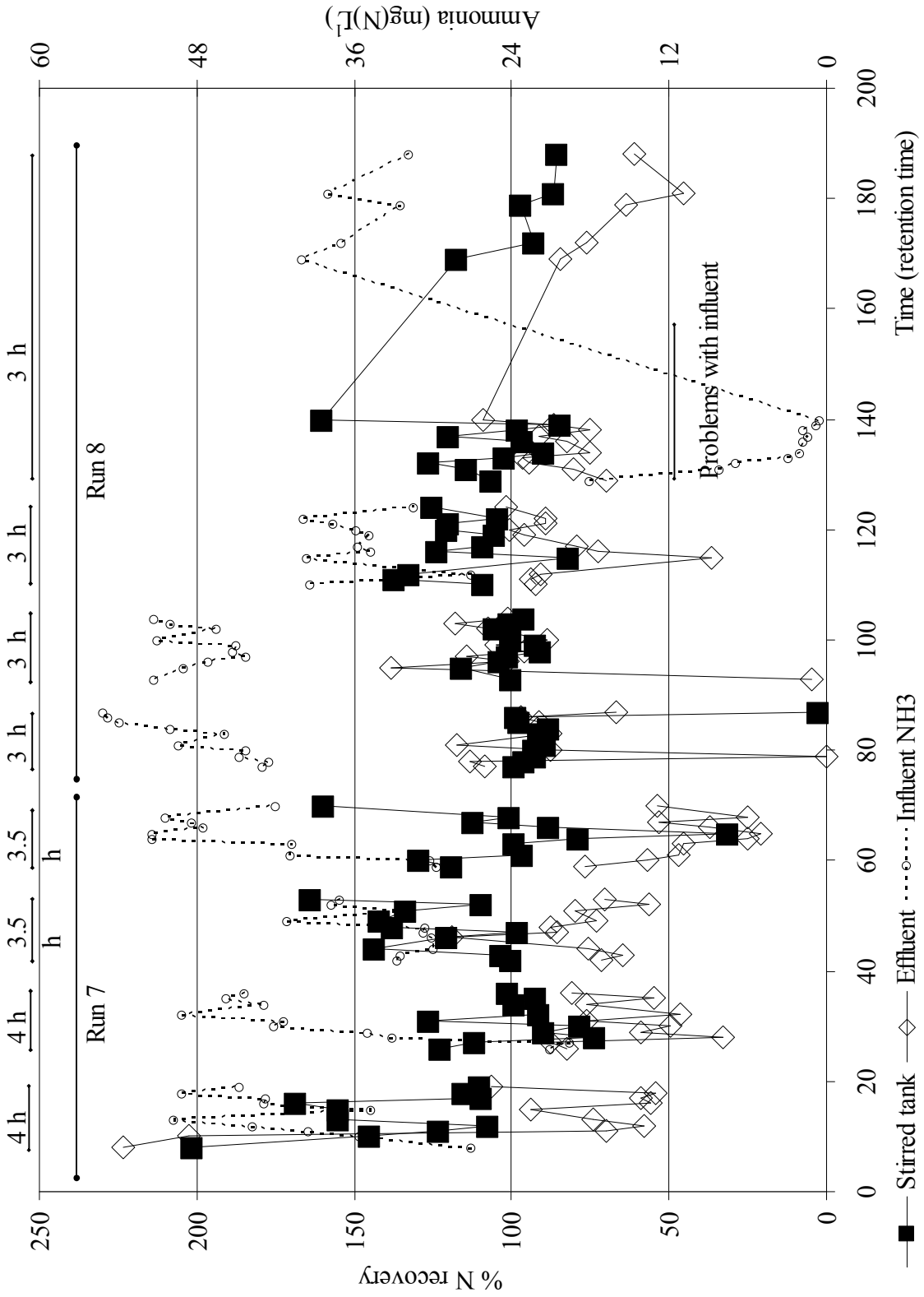

Figure 6.51 Run 7 and 8, inorganic nitrogen species % of measured influent in stirred tank and effluent plotted with influent ammonia concentration against time. The hydraulic retention time is give above the data points.

The nitrogen recovery was calculated as the nitrogen balance (Table 6.13) from the total nitrogen in compared to the total nitrogen measured in the stirred tank and the effluent. Ideally to demonstrate nitrification occurring in the stirred tank the nitrogen concentration should continue to be at 100 %. Denitrification in the anoxic tank should mean that less than 100 % of the influent nitrogen is measured in the effluent. This may not be the case because a large quantity of sludge was added that may have a significant nitrogen component. The concentration of the effluent did remain below the concentration of the stirred tank throughout the experiment.

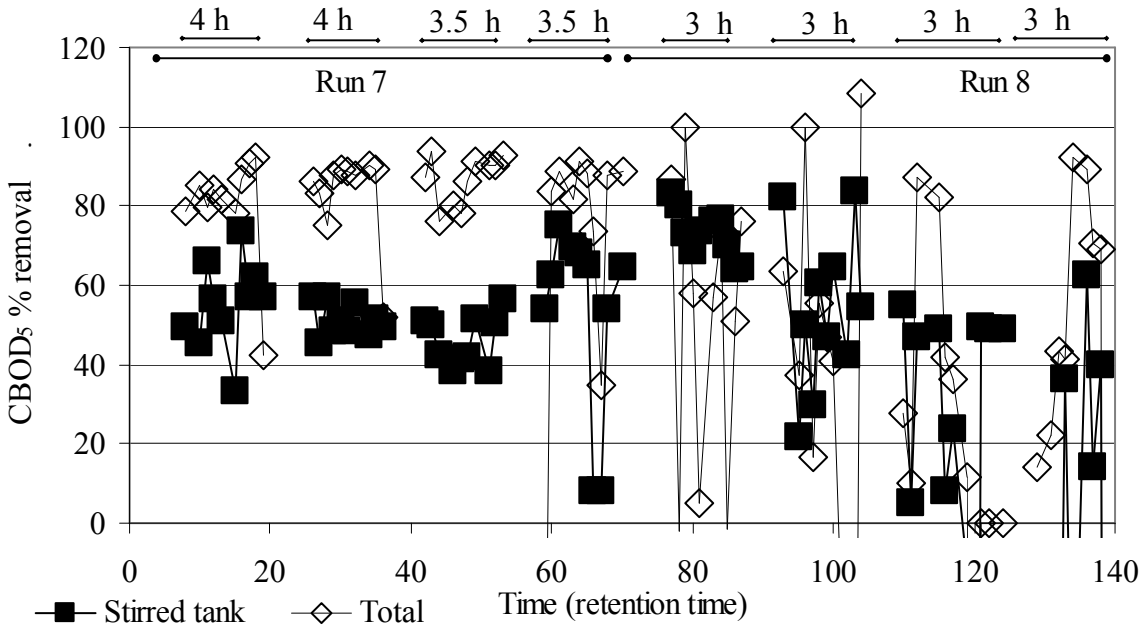

Figure 6.52 Run 7 and 8, percentage $CBOD_5$ removal plotted against time for stirred tank and effluent. The hydraulic retention time of the anoxic rig is given above the data points.

7 DISCUSSION

The stirred tank mixing characteristics were excellent. The dispersion and tanks in series model showed the mixing regime tending towards a totally mixed system, with D/uL tending towards infinity and the number of tanks in series tending towards 1. There did appear to be some level of short circuiting with the High Shear Disk impeller as the average retention time here was lower than the predicted value (Levenspiel 1999).

The shear rate in the stirred tank (Table 6.1) compared to literature examples of activated sludge shear rates revealed shear sufficient to totally deflocculate activated sludge at the lowest stirrer speeds used with the disk turbine (G 1099 s^{-1}). The lowest stirrer speeds gave very high shear when compared to typical operating parameters (23 to 102 s^{-1}) and average operating shear (140 s^{-1}) of activated sludge (Li and Ganczarkyk 1993; Tuntoolavest *et al.* 1983). The lower stirrer speed used with the LE20 caused shear (G 563 s^{-1}) close to that found by Mikkelsen *et al.* (1996) to cause fine particles to be released when pumped at this rate to a reaction chamber. Biggs and Lant (2000) refloccualted sonicated sludge when agitated up to a shear rate of 346 s^{-1}, at higher shear rates primary particles remained. In this study flocs were not formed; the conditions were unsuitable, high shear rates and the single pass short retention time did not select floc forming bacteria. Beun *et al.* (1999) formed aerobic granules in a Sequencing Batch Reactor (SBR) and they found that short settling times encouraged granule formation. Higher shear rates were required to prevent large nutrient limited granules or floc formation (Tay *et al.* 2001; Beun *et al.* 1999).

The lower tip speed of 3 ms^{-1} was close to the 3.11 ms^{-1} found to be optimal for *Brevibacterium flavum* oxygen uptake and growth in a stirred tank (Toma *et al.*, 1991). The highest tip speed tested for the disk turbine (6.03 ms^{-1}) was close to that found by Toma *et al.* (1991) to cause shear stress to the *Brevibacterium flavum* (6.11 ms^{-1}). It is interesting that between these ranges the nitrifying bacteria appeared to reach an optimum and began to decline at the higher tip speed; this study was conducted at similar scale to the work of Toma *et al.* (1991). The stirrer tip speeds greatly exceeded those used by Arnauld *et al.* (1992) with gel entrapped cells, who found that tip speeds of just 0.518 ms^{-1} caused release from the immobilizing medium. An anaerobic culture of *Clostridium acetobutylicium* was also greatly damaged at much lower tip speeds;

Yerushalmi and Volesky (1995) found that a tip speed of 0.69 ms^{-1} was sufficient to stop gas production. Protozoa growth was found to decline at stirrer speeds of 4.33 s^{-1} in a stirred tank (Broudiscou *et al*. 1997). 80 % of *Tetrahymena pyriformis* died when subjected to stirring with a tip speed of 1.8 ms^{-1} (Midler and Finn 1966); lower stirrer speeds than the range used in this study.

The bulk mixing properties and shear are aspects of importance for the reactor operation, another is the K_La. In this reactor the LE20 gave better mass transfer performance than the disk turbine. The disk turbine was a radial flow impeller well suited to gas dispersion (Doran 1999) indicating that there may have been a problem with measurement of this parameter. The gas hold up was lower for the LE20 therefore the gas interfacial area should be lower leading to a low K_La but this was in fact higher than the disk turbine.

Doran (1999) quoted Equation 7.1 that allows K_La to be calculated for specific conditions. With this equation the K_La must always increase with increasing power input especially when reactor volume decreases.

Equation 7.1 $$K_L a = A\left(\frac{P_T}{V_L}\right)^{\alpha} U_G^{\beta}$$ (Doran, 1999)

Where P_T was the total power input (stirrer and bubble induced) U_G the superficial gas velocity and A, α and β were constants.

Ni *et al*. (1995) plotted K_La increasing in a linear manner with increasing stirrer speed in a stirred tank agitated by a disk turbine. The results in this study did not behave in this manner presumably because because α was not equal to 1. The results of Ni *et al*. (1995) and Doran (1999) suggested that there should have been a sharper increase in K_La due to the decrease in liquid volume rather than the decrease seen for this set up at the highest stirrer speed tested. It must be assumed that accurate measurement was not possible at high stirrer speeds with the disk turbine using the method and equipment applied here.

The measured oxygen transfer coefficient for this reactor compared favourably to typical activated sludge values, being closer to some of the pure culture reactors (Table

7.1). It can be assumed that at high stirrer speeds the K_La should in fact be greater than reported for the disk Turbine impeller.

Table 7.1 Literature K_La values compared to this study for a number of systems.

Reference	Reactor system		K_La (s^{-1})
This study	Tip speeds		3 and 4.23 ms^{-1}
	STR disk turbine (highest measured)		0.013 – 0.011 (0.048)
	STR High Shear Disk		0.008 – 0.011
	STR LE20		0.011 – 0.024
Ozbek and Gayik 2001	STR Rushton Turbine 1.67–8.33 s^{-1}	Water	0.0022 – 0.0879
		Biomass support	0.0009 – 0.0246
		50% glycerol	0.0011 – 0.0431
Doran 1999	STR, KLa for plant cell cultivation		0.014
Dursan 1999	Cocurrent downflow contacting reactor for yeast culture		0.005
Arjunwadkar *et al*. 1998	STR many configurations (optium)		0.06
Ju and Sundararajan 1995	STR (magnetic stirrer) 650 rpm		0.035 (max)
Ni *et al*. 1995	STR (yeast)		0.056 (max)
	Batch pulsed baffled reactor (yeast)		0.139 (max)
Ahmed *et al*. 1994	STR *Candida utilis*		0.021 – 0.107
Fujie *et al*. 1994	Diffusers for activated sludge		0.042 – 0.061
Mueller and Stenstl 1990	Activated sludge		0.007
Mines and Sherrard 1987	Bench scale activated sludge plant	Clean water (dynamic)	0.001 – 0.002
		Mixed liquor (steady state)	0.001 – 0.004
Gibilaro *et al.* 1985	STR Rushton turbine Specific power (0.05 – 5.81 kWm^{-3})		0.015 – 0.225
Chapman *et al*. 1982	STR Rushton Turbine (stirrer speed 2.8 - 3.8 s^{-1})		0.055 – 0.107

The influent to the reactor represented a typical wastewater with characteristics of a weak wastewater compared to those quoted in Metcalf and Eddy Inc. (2003). The $CBOD_5$ appeared to be very weak, but it was settled rather than raw sewage. Assuming typical weak influent $CBOD_5$ of 110 mgL^{-1}, primary sedimentation typically removes 25 – 40 % leaving a settled sewage $CBOD_5$ between 66 and 85.5 mgL^{-1}: the average influent $CBOD_5$ concentration (78.9 mgL^{-1}) falls within this range. The influent TOC (21 mgL^{-1}) was also low compared to the typical values given for a weak sewage (80 mgL^{-1}). Primary sedimentation will remove some TOC, the TOC quoted in the results

section was also for soluble TOC as the instrument used required samples to be filtered before analysis, further decreasing the concentration.

A typical weak wastewater has suspended solids of 120 mgL^{-1}; 50 – 70 % were removed during primary sedimentation (Metcalf and Eddy Inc. 2003). The average influent suspended solids concentration was 91.5 mgL^{-1}, so the influent to the works would be expected to contain a minimum of 180 mgL^{-1}, assuming a low level of settling, this would represent a medium concentration influent.

The nutrient concentrations were slightly elevated compared to a weak sewage, the ammonia concentration was 27.9 $mg(N)L^{-1}$ compared to the 25 $mg(N)L^{-1}$ quoted for medium strength wastewater (Metcalf and Eddy Inc. 2003). There was also the presence of some nitrate and nitrite in the influent that could again be due to the pre-treatment stage and storage. The overall composition was typical for a low strength wastewater with a relatively high ammonia concentration.

Although the feed wastewater used during this study was of typical composition, it was still not always possible to easily compare to other studies. Temporal variation was inherent in wastewater systems, with the additional problem of toxic pulses (Boeije *et al.* 1999). Smith (2002) carried out trials using a synthetic wastewater (adapted from O.E.C.D. 1976) and the Cranfield University wastewater (used for this study), and found that the synthetic wastewater contained a higher percentage of the total COD as soluble COD and $CBOD_5$ (89 – 91 % and 57 – 60% respectively) compared to the Cranfield settled sewage (33 – 45 % and 41 – 44 % respectively) at the same COD concentration. The Cranfield wastewater was also found to contain greater concentrations of ammonia compared to the synthetic wastewater (42 – 52 mgL^{-1} compared to 15 – 20 mgL^{-1} respectively). Synthetic sewage aims for experimental reproducibility and is widely used, it is not however wholly representative, with only a limited proportion of the catabolic enzymes present in a normal activated sludge plant expressed (Boeije *et al.* 1999). Real sewage has the advantage of adding a constant bacterial inoculum and maintaining a diverse community (Boeije *et al.* 1999). There is an argument for use of both real and synthetic wastewaters and both sources have been used for CSTR systems trials.

Comparing the results of this study to carbonaceous load removal in other suspended growth systems for wastewater treatment is possible, although differences in feed are important to note. The peak removal of $CBOD_5$ in the stirred tank occurred at 10 h retention time, 15 s^{-1} stirrer speed. Ghyoot and Verstraete (2000) achieved up to 93 % soluble COD removal in a CSTR. However, this is not a fair comparison as they fed their reactor a high concentration of easily degradable COD, (high percentage $CBOD_5$). Influent $CBOD_5$ concentration was demonstrated in Figure 6.12 to be positively related to percentage removal at 99 % confidence.

Lee and Welander (1996) and Ratsak *et al*. (1994) also found comparatively high percentage removals of carbonaceous load in single pass reactors. The influent used in these studies had a high concentration of easily degradable substrate. Another part of the Lee and Welander (1996) study, using bleached kraft mill effluent, achieved 53 % removal. The work of Chang and Alvarez-Cohen (1997) reported a 54 % removal of substrate; figures that were more comparable to the carbonaceous removal achieved in this investigation. Further examples of carbonaceous load reduction in CSTRs are given in Table 7.2,

Table 7.2 Results of literature for single pass stirred tank reactors

Reference	Wastewater	Retention time (h)	Power input (Wm^3)	Influent C loading (mgL^{-1})	% removal	SS influent (mgL^{-1})	SS reactor (mgL^{-1})
This study	Domestic	10	6500	146 $CBOD_5$	55.1	111	122
		12	1200	176 tCOD	37.6	81.7	53
		8	1200	94.3 s COD	44.7	100	102
Ghyoot and Verstraete (2000)	Synthetic	15- 34		1000 (sCOD)	86 – 93		249 – 533
Chang and Alvarez-Cohen (1997)	Methane	120	500 rpm	1.56 L/h CH_4 (in air)	54		
Lee and Welander (1996)	Bleached kraft mill	10		500 (tCOD)	53	40	160
	Recycled fibre mill	3		1340 (tCOD)	82	170	260
Ratsak *et al*. (1994)	Mineral medium	10	500 rpm	420 $C_6H_80_7.H_2O$	91 (C)		

Typically activated sludge mixed liquor suspended solids concentrations are between 2000 and 3000 mgL^{-1} with solids retention times of 72 – 120 h (Metcalf and Eddy Inc. 2003). Reactor suspended solids did not reach these levels in the single pass systems as there was no recycle and the solids retention time was shorter. This was also the case for the cited literature examples in Table 7.2.

Nitrification is the oxidation of ammonia to nitrate (NO_3^-) via nitrite (NO_2^-), through a well established pair of reactions represented in Equation 7.2 and Equation 7.3 below (Sharma and Ahlert 1977):

Equation 7.2 $$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + H_2O + 2H^+$$

Equation 7.3 $$NO_2^- + 0.5O_2 \rightarrow NO_3^-$$

Ammonia oxidation is called nitritation (Mauret *et al*. 1996; Sharma and Ahlert 1977) or nitrition (Hagopian and Riley 1998) while nitrite oxidation is called nitratation (Mauret *et al*. 1996, Sharma and Ahlert 1977) or nitration (Hagopian and Riley 1998). The shorter versions (nitrition and nitration) will be used below.

The oxidation reactions are carried out by distinct species of autotrophic bacteria (U.S. E.P.A. 1993). The genera responsible for the oxidation of ammonia to nitrite have been listed as *Nitrosomonas, Nitrosococcus, Nitrosospira, Nitrosolobus, Nitrosovibrio* while those found to be involved in nitration were *Nitrobacter, Nitrococcus, Nitrospira* and *Nitrospina* (Hagopian and Riley 1998). The most common nitrifying bacteria identified in wastewater treatment systems for nitrification are *Nitrosomonas* (nitrition) and *Nitrobacter* (nitration) (U.S. E.P.A. 1993). This has recently been disputed, as new molecular techniques for species identification became increasingly available they have shown that *Nitrobacter* is not commonly found in activated sludge (Burrell *et al*. 1998). Traditional plating techniques had shown *Nitrobacter* to be common, due its facultative heterotrophic ability; truly autotrophic *Nitrospira* species are now believed to be the better indicator of good nitrification in activated sludge.

Stenstrom and Song (1991) found that nitrifying bacteria were more sensitive to oxygen and mass transport limitation than heterotrophic bacteria. Longer solids retention times in suspended growth reactors (e.g. activated sludge) and larger attached growth reactors

are generally required to allow for the lower growth rate of nitrifying bacteria (Metcalf and Eddy Inc. 2003) (Table 7.3).

Table 7.3 Summary of published information on nitrifying bacteria growth rates.

Reference	Bacteria	Doubling time (h)	Specific growth rate (day^{-1})	Retention time (h)
Sharma and Ahlert (1977)	*Nitrosomonas*	8 – 36	0.46 – 0.22	
	Nitrobacter	12 – 59	0.28 – 1.44	
Strotmann and Windecker (1997)	*Nitrosomonas*		0.1 – 0.61	240 – 39
	Nitrobacter		0.1 – 0.45	240 - 53
Hagopian and Riley (1998)	*Nitrosomonas*	7 – 8 max		

The nitrification occurring in this study was due to suspended growth, in contrast to the findings of Lee and Welander (1996) and Ghyoot and Verstraete (2000) who found that nitrification occurring in their systems was due to biofilm formation. The retention times used when nitrification was observed by Lee and Welander (1996) were 3 – 10 h and by Ghyoot and Verstraete (2000) were 3 – 8 h. Ghyoot and Verstratae (2000) noted that the specific growth rate of *Nitrosomonas* was 0.035 h^{-1} at 25 ºC and that this would require a retention time > 29 h to prevent washout. Ghyoot and Verstraete (2000) reported that nitrate and nitrite were both present in the effluent, indicating that both genera responsible for complete nitrification were present in the reactor and appropriate conditions for both must have been met (Table 7.3), reinforcing their finding that nitrification was occurring in the biofilm.

Biofilm growth was suppressed by adding beads to the reactor in Lee and Welander (1996) and Ghyoot and Verstrate (2000). In this study biofilm growth was controlled by an intensive cleaning regime, set out in section 5.2.2, and beads were not added in this case as this would change the system shear and hydrodynamics. Daily cleaning was also used by Hellinga *et al*. (1998) in the lab scale experiments for the Single reactor High activity Ammonia Removal Over Nitrite (SHARON) process. This investigation has demonstrated that nitrition can occur in a dispersed culture with a retention time of less than 12 h. The $NBOD_5$ results confirmed that nitrification was occurring in the reactor liquor rather than as wall growth.

During the different runs of the stirred tank reactor a correlation between nitrification and increased temperature was noticed. Other researchers have long been aware of the

relationship between nitrification rate and temperature. The optimum temperature for nitrification was believed to lie between 28 and 36 ºC (Sharma and Ahlert 1977) with a higher optimum temperature for nitrition than nitration (Hagopian and Riley 1998). The temperature was found not to be the sole factor involved in enabling nitrification with short retention time. A rapid decrease in ammonia removal was observed when the liquor was transferred to the unstirred tank, maintained at the same temperature (Figure 6.39).

Anthonisen *et al.* (1976) investigated substrate inhibition in nitrifying bacteria. Nitritifiying and nitratifying bacteria were found to be affected by high free ammonia concentration at different concentrations. The free ammonia was found to be present in equilibrium with the ammonium ion according to Equation 7.4.

Equation 7.4 $NH_4^+ + OH^- \leftrightarrows NH_3 + H_2O$

The free ammonia concentration can be calculated when the total ammoniacal nitrogen (TAN) concentration (as NH_3), temperature and pH are known, using Equation 7.5.

Equation 7.5 $$NH_3 = \frac{1.214 TAN 10^{pH}}{\left[e^{(\frac{6344}{273+°C})} + 10^{pH} \right]}$$ (Anthonisen *et al.* 1976)

Anthonisen *et al.* (1976) identified a series of conditions where complete nitrification, nitration inhibition and complete inhibition occurred. Free ammonia inhibited nitrition at concentrations above 7 mgL^{-1} (Abeling and Seyfried 1992) or 10 mgL^{-1} (Anthonisen *et al*. 1976) and nitration above 1 – 5 mgL^{-1} (Abeling and Seyfried 1992), 6.6 mgL^{-1} (Mauret *et al*. 1996), while Anthonisen *et al*. (1976), noticed inhibition at a much lower concentration of between 0.1 – 1 mgL^{-1}. The differences could be related to bacterial acclimatization to higher free ammonia concentrations. Turk and Mavinic (1989) reported failure to maintain nitrite build up due to nitrite oxidising bacteria acclimatising to the intermittent 5 mgL^{-1} free ammonia concentration.

In this study the free ammonia concentration reached 15.8 mgL^{-1} on one occasion although generally the free ammonia concentration was below that required to inhibit *Nitrosomonas*. It may have been a factor influencing nitration inhibition as 16 % of samples were had more than 5 mgL^{-1} free ammonia. Nitrite as free nitrous acid was also

found to be inhibitory, but in this study the concentrations never approached 0.22 mgL^{-1}, the concentration found to be inhibitory by Anthonisen *et al*. (1976).

The SHARON process was developed to treat sludge liquor, an ammonia rich waste, by Hellinga *et al.* (1998). The reactor was based on the principle that at 35 ºC the ammonia oxidizing bacteria grow more rapidly than nitrite oxidizing bacteria. A short residence time was used to maintain just the ammonia oxidizers in the reactor, washing out the nitrite oxidizers (Figure 7.1). The SHARON process used a continuously fed aerobic/anoxic sequencing batch reactor to nitrify and denitrify removing much of the nitrogen load in the return sludge liquor. As with the SHARON reactor, in this study a short retention time was maintained 8 to 12 h (Table 7.3). The temperature was also above 20 ˚C during the study

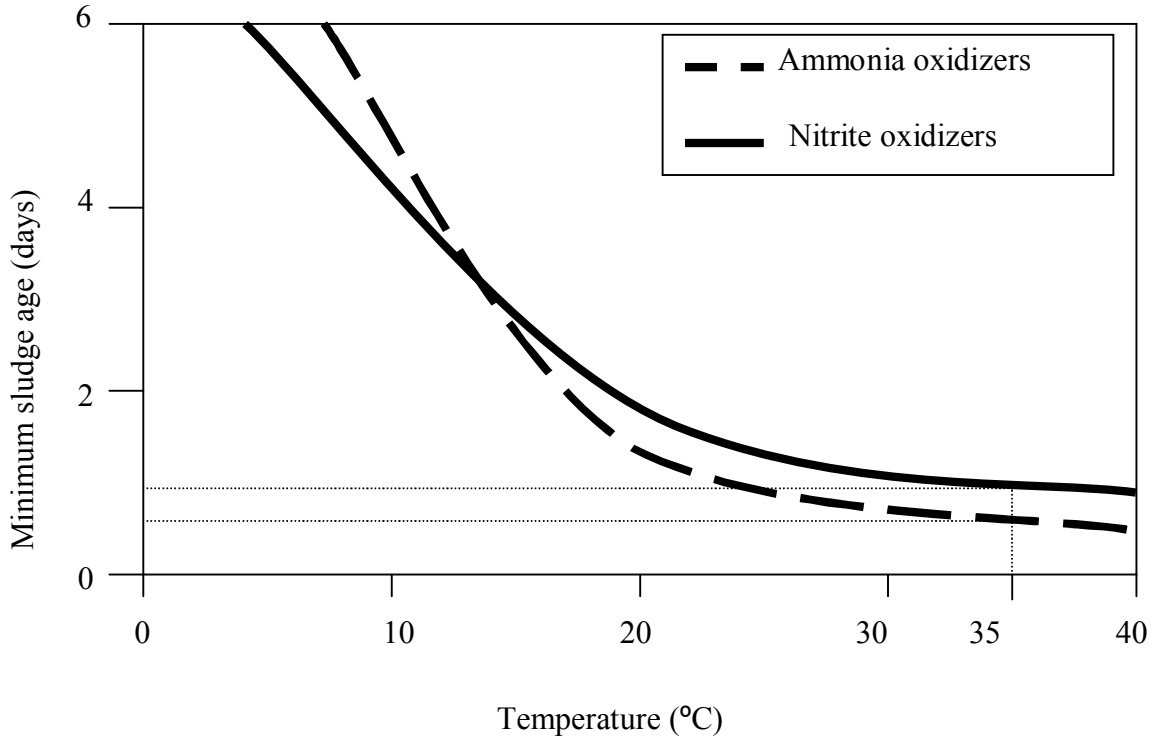

Figure 7.1 Minimum residence times for ammonia and nitrite oxidizers as a function of temperature. (Hellinga *et al*. 1998)

It can be seen from the temperature, retention time and free ammonia results that the accumulation of nitrite would be expected under these conditions. The nitrification rate compared favourably to nitrification in activated sludge, bearing more resemblance to the specific nitrification rate of pure culture nitrifiers (Table 7.4 and Table 7.5). The

results of removal per unit volume were much lower than for the SHARON process and a number of other systems. The ammonia loading rate during the reported run (highest removal rate) was just 0.111 $kgm^{-3}day^{-1}$, this was much lower than for the SHARON (total N loading of 1.2 $kgm^{-3}day^{-1}$, Van Dongen *et al.* 2001) and other high ammonia wastewater sources, making the comparison poor. The reactor performed well compared to standard wastewater treatment plants, where the influent loading would have been more comparable.

Table 7.4 Specific nitrification rates for a variety of reactors and culture types

Reference	Reactor	Feed	Nitrification rate
This study	Results (solids)		34.6 $mg(N)g^{-1}h^{-1}$
Ficara *et al*. (2000)	Activated sludge	Synthetic (lab-scale, nitrifier enriched)	5 – 8
Yoo *et al*. (1999)	SBR	Synthetic (lab- scale intermittently aerated,)	25 – 30
Copp and Murphey (1995)	*Nitrosomonas* fed batch fermentor	Synthetic feed pure culture	Maximum 660
Drtil *et al.* (1993)	Activated sludge	Synthetic (respirometer test)	9
Stephenson (1993)	*Nitrosomonas*	Synthetic ammonia solution (Shake flasks)	65
	Activated sludge		2
Stephenson (1993)	Activated Sludge	Real wastewater (full scale)	0.86 0.98
Abeling and Seyfried (1992)	Fixed film reactor	Pre-treated potato starch wastewater	2 - 3
Stenstrom and Song (1991)	Activated sludge	Not reported (Lab scale)	4.5

The growth rate of nitrifying bacteria is well reported as being strongly affected by temperature (Figure 7.1) (Sharma and Ahlert 1977; Hagopian and Riley 1998; Mauret *et al*. 1996; Hellinga *et al*. 1998). Sharma and Ahlert (1977) found nitrifier growth to be temperature dependant with an optimum in the range of between 28 – 36 ˚C. A linear temperature response between 5 and 35 ˚C has been found (Sharma and Ahlert 1977; Hagopian and Riley 1998). During these experiments the temperature rose with stirrer speed making the two independent variables very difficult to ascertain. The multiple linear regression showed that there was a positive correlation for stirrer speed and temperature against ammonia removal. The analysis also found that the retention time

was positively correlated with increasing nitrification occurring at higher retention times. The hypothesis that the stirring had a strong effect on nitrification was proved by using the unstirred reactor under the same operating conditions of the stirred reactor. The nitrification in the unstirred reactor was found decrease rapidly after between 1 and 2 retention times of seeding.

Table 7.5 Volumetric nitrification rate for a variety of reactors and culture types

Reference	Reactor	Feed	Nitrification rate
This study	Results (volume)		0.082 kg(N)$m^{-3}day^{-1}$
Van Dongen *et al*. (2001)	SHARON	Sludge liquor	0.63
Nunez and Martinez (2001)	Activated sludge	Pretreated slaughterhouse waste	0.5
Rostron *et al.* (2001)	CSTR - particle retention	Synthetic (Immobilized nitrifying bacteria in PVA)	0.7
Im *et al.* (2001)	Activated sludge	Pretreated landfill leachate	0.8
	Stirred tank		0.098
Koren *et al*. (2000)	Trickling filter	Synthetic mine effluent	0.25
Gernaey *et al.* (1998)	Activated sludge	Synthetic	0.074
Strotmann and Windecker (1997)	Husmann apparatus	Wastewater supplemented with NH_4Cl	0.88
	SBR		0.45
Mauret *et al*. (1996)	Continuous pilot unit	Synthetic substrate	Nitrition 0.057 Nitration 0.055
Tijhuis *et al.* (1995)	BAS reactor	Synthetic	6

BAS (Biofilm Airlift Suspension)

The occurrence of nitrification under strong mixing and shearing forces exerted in the stirred tank has not been previously reported. Some evidence of nitrifying bacteria resilience under shear has been found. Tijhuis *et al.* (1995) observed nitrifying biofilms were dense and resistant to detachment compared to heterotrophic bacteria. Stenstrom and Song (1991) increased shear rate from 150 to 275 s^{-1} and found the nitrification rate increased from 3.5 to 4.5 $mgg^{-1}h^{-1}$ and then levelled off with no further change up to the maximum rate tested (700 s^{-1}). They considered that this was due to nitrifying bacteria being less competitive against heterotrophic bacteria under mass transfer limited conditions. Hanaki *et al*. (1990) found ammonia assimilation by heterotrophic bacteria occurred in preference to nitrification in a 2 to 10 day retention time CSTR fed with

synthetic medium. This has been found not to be the case in this study at high stirrer speeds, as nitrification appeared to occur to the detriment of carbonaceous load removal.

Nitrite is toxic to fish at much lower quantities than nitrate; 1.8 mgL^{-1} nitrite has been reported to kill rainbow trout within 24 h, while the lethal dose of nitrate for catfish is 6200 mgL^{-1} (Hagopian and Riley 1998). It is therefore not the preferred nitrification product to release into the environment. However, for wastewater treatment nitrition has advantages over full nitrification, as it requires 25 % less oxygen and requires 40 % less electron acceptor to denitrify (Munch *et al*. 1996).

Barnes and Bliss (1983) identified assimilation and denitrification as the ways in which nitrate and nitrite could be removed from wastewater. Assimilation occurred with the growth of bacteria, with nitrate and nitrite reduced to ammonia for cell synthesis (Akunna *et al*. 1992). Denitrification occurred under anoxic conditions when facultative anaerobic bacteria used nitrate or nitrite as an alternative electron acceptor to oxygen for respiration. The electron donor is usually an organic molecule. The reaction happened in two stages; reduction of nitrate to nitrite and reduction of nitrite to dinitrogen gas. The reactions are represented in Equation 7.6 and Equation 7.7 respectively, assuming methane was used as the electron donor (Barnes and Bliss 1983). Raw wastewater has been proved to be an effective electron donor for denitrification (Barnes and Bliss 1983 and Metcalf and Eddy 2003).

Equation 7.6 $$NO_3^- + \frac{1}{3}CH_3OH \rightarrow NO_2^- + \frac{1}{3}CO_2 + \frac{2}{3}H_2O$$

Equation 7.7 $$NO_2^- + \frac{1}{2}CH_3OH \rightarrow N_2 + \frac{1}{2}CO_2 + \frac{1}{2}H_2O + OH^-$$

Equation 7.7 should be expanded to include the intermediate production of nitric and nitrous oxide (Wild *et al*. 1995; Baumann *et al*. 1997). This fact will be neglected for the basis of this work as the denitrification investigation was very limited.

Denitrification from nitrite requires just 60 % of the electron donor compared to denitrification from nitrate (Garrido *et al*. 1997; Akunna *et al*. 1992). Abeling and Seyfried (1992) used the factors of decreased aeration requirement (nitrition) and reduced electron donor need (denitrification) to design a pre-denitrification wastewater

treatment plant with a fixed film nitrition reactor, recycled to the denitrification reactor and a final fixed film nitration reactor to remove the residual nitrite. They found that the reaction rate of denitrification over nitrite was 1.5 – 2 times that of nitrate. Further supporting the use of nitrite as a cost effective route for denitrification, Akunna *et al*. (1992) observed that nitrite caused no more toxicity than nitrate, within the treatment works, up to a concentration of 800 mg(NO_x -N)L^{-1}.

The SHARON process carried out a similar set of reactions to the developed reactor, treating sludge liquor at high temperature and low retention time via nitrite. Logemann *et al*. (1998) investigated the bacteriological makeup of the SHARON sludge using Fluorescent In Situ Hybridization (FISH) and Denaturing Gradient Gel Electrophoresis (DGGE) analysis. They demonstrated that the dominant clones were almost identical to *Nitrosomonas eutropha*. SHARON was operated as a sequencing batch reactor alternately carrying out nitrite oxidation in aerobic conditions followed by denitrification in anoxic conditions. Methanol was added as a carbon source for denitrification (Hellinga *et al*. 1998; Drajer *et al*. 1998). An alternative route for the SHARON process was the substitution of the straight denitrification route for ANaerobic AMMonia OXidation (ANAMMOX) (Jetten *et al*. 1997; Van Dongen *et al*. 2001). ANAMMOX involves conversion of nitrite to dinitrogen gas with ammonium as the electron donor (Equation 7.8).

Equation 7.8 $NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$ (Van de Graaf *et al*. 1995)

In general denitrification takes place as pre or post denitrification. Pre-denitrification in activated sludge makes use of the available electron donor (untreated wastewater CBOD) and recycles between 1 and 4 times the influent flow of nitrified mixed liquor to an anoxic tank before the aerobic zone (U.S. E.P.A. 1993). Post-denitrification can use any nitrification system but requires an additional electron donor, usually methanol (U.S. E.P.A. 1993).

The denitrification reactor used in this study was not expected to be an optimum configuration and the influent to the anoxic reactor was not consistently well nitrified during the test period to allow efficient denitrification. Results from the reactor indicated that the effluent from the stirred tank contained degradable $CBOD_5$ which could be used for denitrification, allowing nitrogen and carbonaceous load removal in

an anoxic stage. The denitrifying reactor requires further work to achieve an efficient process but appears promising.

The power input to the reactor for nitrification using the disk turbine at stirrer speed 15 s^{-1} and with 10 h retention time was 65.9 $kWhm^{-3}$. This was much higher than for activated sludge treatment described in Table 3.1. The LE20 impeller compared favourably to the disk turbine and showed that the power requirement could be significantly reduced with a power input at the higher stirrer speed 12.9 s^{-1} (when the reactor performed well) equivalent to 8.91 $kWhm^{-3}$.

The example plant given in chapter 3 did not denitrify and only returned settled sludge and sludge liquor to the head of the works, assuming denitrification were required using pre-denitrification, additional pumping would be required for the recycle of nitrified activated sludge mixed liquor. U.S. E.P.A. (1993) reported that a generic single anoxic zone activated sludge plant would return from the final sedimentation tank to anoxic zone a volume of Return Activated Sludge (RAS) equivalent to 50 to 100 % of the influent flow and nitrified activated sludge mixed liquor to anoxic basin equivalent to 100 to 400 % of the influent flow. These quantities were used with the calculated pumping power per unit volume from Table 3.1 0.765 $kWhm^{-3}$. Only 3600 m^3day^{-1} of the pumping at the plant was identified as recycle or return, the remaining 5164 m^3day^{-1} was sludge and scrubber liquor return, this pumping would still be required (Table 7.6). The pumping requirement would be greatly increased, no data was available for change in aeration due to the change in regime, presumably the required aeration basin size would be reduced, if the aeration rate were maintained power per unit volume up to 3.3 $kWhm^{-3}$ would be required.

Table 7.6 Projected pumping power requirements if pre-denitrification was added to the existing plant.

Parameter	Power requirement $kWhday^{-1}$	Power requirement $kWhm^{-3}$
RAS recycle	76500 – 153000	0.38 – 0.77
Nitrified recycle	153000 – 612000	0.77 – 3.06
Other pumping	3950	0.02
Total for influent	233450 – 627300	1.17 – 3.14

Comparing the values of approximately 3 $kWhm^{-3}$ for a denitrifying activated sludge plant the mixing power requirements of 9 $kWhm^{-3}$ (neglecting aeration requirements)

for the LE20 at a stirrer speed of 12.9 s^{-1} (good but not optimum nitrification) are not as far from power requirements for activated sludge as it first appeared.

The reactor set up has not been optimised in terms of power input and alternative methods of achieving similar results may be found. In this case the current difference in power input per unit volume could be decreased to closer to or preferably less than the current activated sludge power requirements.

Another additional advantage of this system may be the sludge yield, unfortunately data from the anoxic reactor was insufficient to make any claim for this reactor system. However, literature examples of CSTRs for wastewater treatment have aimed for lowered sludge yields than conventional treatment (Lee and Welander 1996; Ghyoot and Verstraete 2000). In this study little suspended solids accumulation occurred in the stirred tank reactor. Lower sludge yields have also been reported for anaerobic treatment (Metcalf and Eddy Inc. 2003; Beaubien *et al*. 1996; Lapart and Alleman 1999). Speculations on power requirement and sludge production would require further investigation for a proper cost benefit analysis to be achieved.

It appears from these preliminary results that an elegant 2 stage wastewater treatment reactor in which ammonia oxidation is achieved for domestic wastewater without nitrite oxidation or substantial carbonaceous load removal. This stage can be followed directly by denitrification without the additional cost of a carbon source for denitrification as is normally required and with post-denitrification reactors (U.S. E.P.A. 1993).

Methods of maintaining the treatment in the reactor but lowering the power requirements would make this an attractive alternative wastewater treatment method. As with the reactor of Abeling and Seyfried (1992) a final nitrite oxidation stage may be advisable for further development to ensure nitrite is not released into the receiving waters.

8 CONCLUSIONS

The main conclusions that were drawn from this work are

- The continuously stirred tank reactor was run for wastewater treatment under conditions of mixing, power input and mass transfer at levels associated with industrial fermentations rather than wastewater treatment applications.
    - Some short circuiting was evident with the high shear disk impeller.
    - The LE20 appeared to give the best oxygen mass transfer.
- A bacterial culture was maintained to treat domestic wastewater in a continuously stirred tank reactor at retention times between 8 and 12 h and stirrer speeds between 8.3 and 16.7 s^{-1}.
    - A combination of stirrer speed and retention time and temperature had an effect on the dominant bacterial community, high stirrer speeds and therefore temperatures with long retention times promoted autotrophic nitrifying bacteria and low stirrer speeds, temperatures and short retention times tended to promote heterotrophic bacteria.
    - Due to a combination of temperature, retention time and free ammonia concentrations the nitrification took place only to nitrite.
    - As nitrification increased in the reactor the carbonaceous load removal was observed to decrease.
    - The maximum specific nitrification rate achieved was close to that of a pure culture.
- Reactor temperature and stirrer speed acted together, but there was evidence to show that temperature was not the sole cause of high nitrification rates.
    - Multi-linear regression showed that the nitrification rate was linked to temperature, stirrer speed and retention time separately.
    - The unstirred tank control experiment showed that when the stirring was removed, with other operating conditions maintained (temperature, influent rate and aeration rate) the nitrification significantly decreased.

- The anoxic reactor showed that the effluent from the stirred tank was suitable for denitrification without an additional carbon source.
- The combination of the stirred tank and anoxic stage would make a simple reactor for wastewater treatment avoiding large costs due to recycle or additional carbon source usually required for activated sludge denitrification.
- The reactor set up as it stands would have power requirements from approximately 3 times those of a denitrifying activated sludge plant.

9 FURTHER WORK

1. To fully understand the reactor and to build a positive relationship between factors the stirred tank needs to be run under various stirrer and temperature conditions using much more stringent control, preferably with very sensitive temperature control, and using synthetic sewage to completely separate the stirrer, temperature and other variations.
2. Further experiments with alternative impeller types would help to quantify which mechanical forces are most significant in achieving the treatment and to aid further optimisation.
3. The relationships between the colonising groups (especially heterotrophic and nitrifying bacteria) should be investigated under a range of conditions to try and better understand the mechanisms by which changes in treatment are occurring. The use of molecular species identification and quantification techniques would be particularly useful.
4. When a good understanding of the factors affecting nitrification in the reactor has been achieved an optimum reactor type should be sought to achieve the best results in a cost effective manner. Investigations should include the effect of intermittent shearing or pumping forces, and incorporation of static mixing devices as measures to attempt to reduce costs.
5. The anoxic stage should be further investigated and an attempt made to optimise the design for complete nitrite removal.
6. The overall power requirements and yield should be carefully compared to the levels typical of activated sludge and other treatment methods to find under what circumstances the reactor would be most suitable.
7. The ability of the reactor to operate with a number of wastewater sources and the susceptibility of the reactor to toxic pulses of influent should be investigated.
8. Another possible application to be investigated would be the use of the stirred tank reactor for seeding and maintaining the nitrifying community within poorly performing wastewater treatment plants. This would rely on the reactor showing resistant to inhibitory effects.

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A GUIDE TO CALCULATIONS

Percentage removal = $\frac{In - ST}{In} \times 100$

Volumetric removal rate ($Kgm^{-3}day^{-1}$) = $\frac{\left(\frac{V \times \left(\frac{24}{RT} \right) \times (In - ST)}{1000000} \right)}{\left(\frac{V}{1000} \right)}$

Specific removal rate ($mgg^{-1}h^{-1}$) = $\frac{In - ST}{VSS} \times v \times 60$

Where

In = influent concentration (mgL^{-1})

ST = stirred tank concentration (mgL^{-1})

V = tank volume (L)

v = liquid flow rate ($Lmin^{-1}$)

RT = retention time

VSS = volatile suspended solids concentration (gL^{-1})

APPENDIX C: ENERGY BALANCE

The temperature of the influent and air was not measured during the experiments. The actual temperature is recorded as the average temperature, for the runs without cooling.

For the energy balance the assumption was made that no potential, kinetic or heat energy in the influent i.e. influent enthalpy = 0. The kinetic energy of the air bubbled into the reactor was also neglected.

Effluent enthalpy= Influent enthalpy+ Impeller power– losses from the system (heat).

To calculate the temperature rise the influent temperature and rate of heat removal from the system must be know. A guide to the temperature rise is calculated below assuming that the liquor has the same specific heat as water (4.18 $Jg^{-1}°C^{-1}$ Manahan S.E. 1993) (Table C).

The calculated energy input rises steadily, while the rise in actual temperature begins to decrease at the higher temperature, this could be due to the decreasing volume of liquid and increased volume of entrained air available to remove the heat. This means that in this case the energy loss from the system is dependant on energy input (Figure C).

Table C Energy balance for a all impellers and reactors tested

Impeller	Stirrer speed (s^{-1})	Retention time (h)	Power input (W)	Volume (L)	Energy input (kJ/L/h)	Energy input (˚C)	Actual temperature (˚C)
DT	8.3	8	9.1	7.5	34.9	1.04	19.13
DT	8.3	10	9.1	7.5	43.7	1.04	20.94
DT	8.3	12	9.1	7.5	52.4	1.04	19.87
DT	10	8	12.7	7	52.3	1.56	26.86
DT	11.5	8	16.9	6	81.1	2.43	36.53
DT	15	8	26.4	4	190.1	5.68	37.71
DT	15	10	26.4	4	237.6	5.68	37.99
DT	15	12	26.4	4	285.12	5.68	40.42
DT	16.7	10	32	3.5	329.1	7.87	36.5
DT	16.7	12	32	3.5	395	7.87	37.87
LE20	9.2	10	2.7	8.5	11.4	0.27	
LE20	12.9	10	7.6	8.5	32.2	0.77	
HSD	15.4	10	0.3	8.5	1.3	0.03	
HSD	21.7	10	0.9	8.5	3.8	0.09	

DT Disk turbine HSD High shear disk

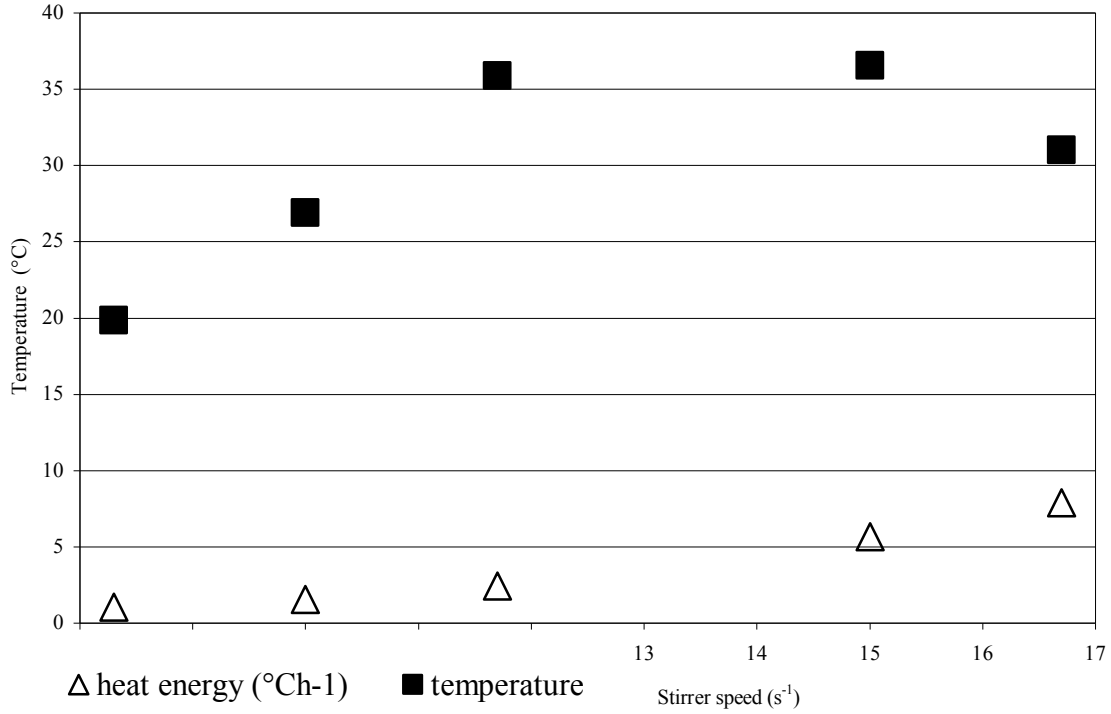

Figure C Possible temperature rise due to impeller power input per hour and average temperature plotted against stirrer speed.