Factors governing nitrification in an activated sludge system treating a pharmaceutical wastewater

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By

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Dedicated to all of my family for their support and understanding.

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Abstract

A pharmaceutical activated sludge wastewater treatment plant had an effluent ammonia limit of 10mg/l. Compliance with this was achieved through nitrification, however this was unreliable, leading to licence breaches, with a mean percentage nitrification rate of 69%. Analysis of data from the full scale wastewater treatment plant ruled out temperature, dissolved oxygen, phosphorus and sludge age as causes of nitrification failure. Pilot plant studies were undertaken. It was found that a free ammonia concentration >1.5mg/l resulted in 50% nitrification failure. Nitrification was demonstrated over a pH of 7.5 to 8.5 when the influent COD was < 2,000mg/l however at >4,000mg/l nitrification was more disrupted at pH 7.5 than pH 8.5. The addition of a synthetic nutrient had only a marginal benefit however the removal of the inorganic fraction from the wastewater resulted in nitrification failure under shock COD loadings. To avoid nitrifier starvation, the C/N ratio needed to be <40 or the influent ammonia above 50mg/l NH_4-N. At an influent COD concentration >6,000mg/l significant nitrification failure occurred. Modification of the system, through altering the sludge recycle rate and the provision of a two stage system, was of no benefit to nitrification. However, the encouragement of fixed film nitrification, through the addition of activated carbon and a buoyant plastic media, enhanced nitrification. A key finding was the important role heterotrophs played in degrading inhibitory substances. Heterotrophic bioaugumentation with *Pseudomonas pudia* CP1 showed short term benefits. The F/M was a better control indicator than COD. Modification of the system increased the tolerance for F/M from 0.25 to 0.52. Recommendations from this study were implemented on the full scale WWTP and resulted in 100% nitrification with no licence breach.

Abbreviations

- **AMO:** Ammonia mono oxygenase
- **API:** Active pharmaceutical ingredient
- **ATP:** Adenosine triphosphate
- **BOD:** Biological oxygen demand
- **BPM:** Buoyant plastic media
- **CI:** Confidence Interval
- **COD:** Chemical oxygen demand
- **DNA:** Deoxyribonucleic acid
- **EPA:** Environmental Protection Agency
- **F/M:** Food to mass ratio
- **FA:** Free ammonia
- **FAME:** Fatty acid methyl ester
- **FBR:** Fluidised bed reactor
- **FNA:** Free nitrous acid
- **HRT:** Hydraulic retention time (Days)
- **OUR:** Oxygen uptake rate
- **RBC:** Rotating Biological Contractor
- **RNA:** Ribo nucleic acid
- **SBR:** Sequencing batch reactor
- **SND:** Simultaneous nitrification and denitrification
- **SRT:** Sludge retention time (days)
- **TKN:** Total Kjeldhal Nitrogen
- **VSS :** Volatile suspended solids
- **WWTP:** Waste water treatment plant

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Chapter 1

Introduction

1.0 Introduction

With increasing globalisation actions must be taken at all levels to prevent pollution and to minimise environmental damage. The 1960's and 1970's represented a period of global awakening to the negative influence human activity was having on the environment. This change has resulted in a shift in policy and public expectations such that sustainable development is now an integral part of the European Unions philosophy of improved quality of life without compromising natural resources (Sheerin,1997).

Natural waterways contain indigenous populations of micro-organisms that biodegrade organic substances released into these systems. Provided these organics are sufficiently diluted and the rate at which the water system naturally re-aerates itself, there will be no adverse environmental impact. However, if excess levels are discharged then rapid microbial growth can result, causing dissolved oxygen levels to fall, leading to stagnant and anaerobic waters. Under these conditions the rate of biodegradation decreases leading to a build up of organic compounds and sediment. The overall effect is to pollute the river system leading to the death of various organisms especially sensitive species such as fish. Pollution can also arise from chemical pollutants including organics, acids and alkalis. To prevent environmental impacts, industrial discharges must be treated in a waste water treatment plant. In a biological treatment process the polluting materials are brought into contact with microbes where under suitable and enhanced conditions they are broken down and metabolised into less harmful or polluting forms. As the nature and the composition of waste water varies so much there is no single universal treatment process (Scragg,2005).

Despite its lower level of industrial activity, compared to the rest of Europe, Ireland still faces a significant challenge to achieve "*good water status"* for all waters by 2015 as set out in the European Water Framework Directive. Not all waters in Ireland currently meet this objective;70.2% of Irish rivers have a satisfactory water quality status and although the level of serious pollution in rivers is less than 0.6%, significant efforts are still required to improve the remaining 29.2% of rivers. The main threat to surface water quality is eutrophication, which is the over-abundant growth of plant and algae arising from excess nutrients in the water. The nutrients of concern are phosphorus and nitrogen (Irish EPA,2007).

1.1 Wastewater

Wastewater is spent or used water from homes, communities, farms and businesses that contains enough harmful material to damage water quality. Metals, organic pollutants, sediment, harmless as well as pathogenic microbes may all be found in wastewater. As a result, untreated wastewater can cause serious harm to the environment and threaten human life. There are two primary types of waste water; domestic waste water and industrial waste water. The former originates predominantly from human metabolism and from household activities. Industrial waste water on the other hand is any waste water which is discharged from premises used for carrying out a trade and excludes domestic waste water and run-off from rainfall. Municipal waste water is a mixture of both domestic waste water and industrial waste water (EU, Environment Commission,2008; Scragg,2005)

1.1.1 Waste Water Characteristics

A typical composition of domestic waste water is outlined in Table.1.

Table 1: Typical composition of domestic sewage (Scragg,2005)

Component	Concentration
Total Solids	300-1,200 mg/l
Suspended Solids	100-350 mg/l
Total Organic Compounds	80-290 mg/l
Biological Organic Compounds	110-400 mg/l
Chemical Organic Compounds	250-1,000 mg/l
Total Nitrogen	20-85 mg/l
Ammonia	12-50 mg/l
Nitrates	0 mg/l
Phosphorus	4-15 mg/l

Compared to domestic waste water, there is significant variability and extreme operating conditions in the treatment of chemical/pharmaceutical waste waters. To ensure compliance with stringent effluent permits, waste water treatment plants must react and adapt to significant changes in the physical and chemical composition of this waste water. The flow rate is equally variable which can fluctuate by up to 50% and unlike municipal plants does not follow any regular pattern. Industrial waste water can be rich in nutrients like ammonia and phosphorus requiring nutrient removal systems or equally they can be low in nutrients requiring a nutrient dosing regime for biological based treatment systems (Bury *et al.*, 2002).

Pharmaceutical waste waters not only vary considerably from plant to plant but also vary within the plants themselves depending on the products being manufactured and the processes being used (Munirathinam and Lankford,1988). In treating industrial wastewater, additional challenges face the process designer and operator due to the substantially higher concentrations of organic carbon compounds compared to a municipal waste water (Keller *et al.*, 1997). Consequently, industrial waste waters tend to need large equalisation systems (balance tanks) to dampen the fluctuations and in turn to help achieve stable process operations (Eckendfelder *et al.*,1992). Table.2 lists some of the typical chemical components found in the influent of pharmaceutical waste water.

Table.2 Components of an untreated pharmaceutical waste water (Lankford,1999).

Parameter	**Average mg/l**	**Maximum mg/l**
Methanol	175	703
Ethanol	49	305
Acetone	107	482
Isopropyl alcohol	151	666
Methylene Chloride	68	329
Isopropyl ether	3	132
Ethyl Acetate	74	250
Tetrahydrofuran	48	318
Methyl isobutyl ketone	162	910
Toluene	39	181

Although nitrification has been studied extensively, investigations have largely focused on municipal waste water treatment plants. The average ammonia concentration entering a domestic waste water treatment plant is 25mg/l NH_4-N (Scragg, 2005). Whereas industrial waste water, for example from the fertiliser industry, have nitrogen (ammonia and urea) levels in excess of 1,130mg/l NH_4-N (Krogulska and Mycielski,1981) and even higher levels have been reported from landfills where ammonia in the leachate can exceed 2,000mg/l (Aktas *et al.*,2001).

1.1.2 Legislation covering waste water discharges

The prevention of water pollution in Ireland can be first dated to section 19 of the Public Health (Ireland) Act, 1878. This prohibited sanitary authorities from discharging *"sewage or filthy water"* into any natural stream or watercourse. The protection of rivers against pollution was further refined and strengthened by the Fisheries Act, 1959. This set about managing and maintaining water quality for the protection or improvement of inland fish habitats and stocks. It prohibited the release of any deleterious matter unless it was done in accordance with the terms of a licence. The importance of environmental protection was also considered in relation to new developments through the Local Government (Planning and Development) Act of 1963 and subsequent amendments. This allowed for the refusal or granting of permission subject to conditions for any development which had the potential to cause water pollution. It was not however until 1977 that dedicated water protection legislation was passed through the Local Government (Water Pollution) Acts, 1977 and amendments. Local authorities were now given primary, but not exclusive responsibility, for ensuring the preservation, protection and improvement of water quality. Section 3(1) of the Act provided that *"a person shall not cause or permit any polluting mater to enter waters"*. The act defines polluting matter as "*any poisonous and noxious matter and any substance (including explosive, liquid and gas) the entry or discharge of which into any waters is liable to render those or any other waters poisonous or injurious to fish, spawning grounds or the food of any fish, to injure fish in their value as human food, or to impair the usefulness of the bed and soil of any waters as spawning grounds or their capacity to produce the food of fish or to render such waters harmful or detrimental to public health or to domestic, commercial, industrial, agricultural or recreational uses"*. The Act required a licence for any sewage or trade effluent discharges. Since these early

pieces of legislation, the greatest single influence on the development of Irish Legislation as it relates to pollution control has been the activity of the European Union (Scannell,1995).

Water as a priceless resource that cannot be taken for granted and access to clean water is a basic and essential right for all. Human activities can have a huge influence on water quality and quantity. As a result, the EU has implemented a range of legislation to ensure that drinking water, bathing water, surface waters and groundwater across Europe reaches a high level of cleanliness (Stavros, 2007). The following legislation are key instruments in achieving this objective:

- Council Directive 91/271/EEC concerning urban waste water treatment was adopted on 21 May 1991. Its objective is to protect the environment from the adverse effects of urban waste water discharges and discharges from certain industrial sectors. Since 2005 all local authorities are obliged to provide a minimum of secondary (biological) treatment for urban waste water for population areas less than 15,000 and more stringent tertiary treatment facilities by the year 2008. EC Directive 78/659/EEC on the quality of fresh waters needing protection in order to support fish life categorised receiving fresh waters into salmonid waters or cyprinid waters. The directive also required member states to establish programmes in order to reduce pollution and to achieve water quality values for various parameters including temperature, dissolved oxygen, pH, suspended solids, BOD, phosphorous, nitrates, phenolic compounds, petroleum hydrocarbons, ammonia, ammonium, chlorine, zinc & copper. This Directive was brought into Irish law through the European Communities (quality of salmonid waters) Regulations, 1988 SI 293 of 1998. EC Directives 75/440/EEC, 79/869/EEC, 90/656/EEC and 91/692/EEC legislated the quality required of surface water intended for the abstraction of drinking water after appropriate treatment. The Directives set the minimum quality requirements to be met by surface fresh water. These Directive were brought into Irish law through SI No.439 of 2000; ammonium for example must not exceed 0.3mg/l in any water deemed suitable for human consumption (Office of the Attorney General,2008; EU Environment Commission,2008).

- EC Directive 2000/60/EC established a framework for community action in the field of water policy and this replaced most existing Directives on the prevention of water pollution. By 2013 member states must ensure that all discharges into surface waters from both point and diffuse sources are controlled taking into account emission controls based on best available techniques, relevant emission limit values and best environmental practices. Certain provisions of this directive and the Dangerous Substances Directive EU Directive 76/464/EC were implemented into Irish law through the Water Quality (Dangerous Substances) Regulations, 2001 SI 12 of 2001. This prescribes standards in relation to certain substances in surface waters. These substances are more commonly known as the List I and List II or the Black List and the Grey List. List I contains certain individual substances selected mainly on the basis of their toxicity, persistence and bioaccumulation. These include amongst others; organohalogen, organophosphorus compounds, heavy metals, persistent mineral oils and hydrocarbons of petroleum origin. The standards for water quality detailed in these regulations must be used by the local or sanitary authorities when issuing new effluent discharge licences and by the Irish EPA when issuing new licences. List II contains substances which have a deleterious effect on the aquatic environment and on the characteristics and location of the water into which such substances are discharged. For the relevant pollutants of list II, member states must establish pollution reduction programmes. List II substances include amongst others: a number of metals, biocides, toxic or persistent organic compounds of silicon, inorganic compounds of phosphorus and elemental phosphorus, cyanides, fluorides and substances which have an adverse effect on the oxygen balance, particularly ammonia and nitrites (Scannell,1995; EU Environment Commission,2008).

- Industrial production processes account for a considerable share of the overall pollution in Europe. The European Union has a set of common rules for permitting and controlling industrial installations through the Integrated Pollution Prevention Control (IPPC) Directive of 1996. The purpose of this Directive is to achieve an integrated approach to the prevention and control of pollution arising from certain high risk industrial activities including chemical and pharmaceutical manufacture. It provides for a system of permits for

installations undertaking any of the listed activities. These permits lay down the preventive measures against pollution that must be implemented and set minimum requirements for emission limits of certain substances into the air, water and land. Best Available Techniques (BAT) must be used. This Directive was implemented into Irish law through the Protection of the Environment Act 2003. In 2007 the Irish EPA published its BAT guidance emission limit values from waste water treatment plants for the pharmaceutical and the chemical sector. These are listed in table 3 (Irish EPA,2008; EU Environment Commission,2008).

Table.3 BAT associated discharges for emissions to water.

Constituent Group or Parameter	Emission Levels	Percentage Reduction
pH	6 –9	-
Toxicity	5-10 TU	-
BOD_5	20mg/l	>91-99%
COD	30-250mg/l	>75%
Suspended Solids	10-35mg/l	-
Total Ammonia (as N)	10mg/l	-
Total Nitrogen (as N)	5-25mg/l	>80%
Total Phosphorus (as P)	2mg/l	>80%
Oils Fats and Greases	10mg/l	-
Mineral Oil (from interceptor)	20mg/l	-
Mineral Oil (from biological treatment)	1.0mg/l	-

- European Communities (European Pollutant Release and Transfer Register) Regulations 2007 SI 123 of 2007 provides for enforcement of European Regulation (EC) No. 166/2006 concerning the establishment of a European Pollutant Release and Transfer Register. This regulation establishes an integrated pollutant release and transfer register at Community level (the European PRTR) in the form of a publicly accessible electronic database. Subject to certain thresholds for emissions to air, land and water, sites licenced under the IPPC & waste licence regimes must report on emissions of the specified pollutants for inclusion on the PRTR. Waste water plants discharging more than 5 tonnes of Phosphorus or more than 50 tonnes of nitrogen or COD in any year will have to report this emission and be subject to more focused public attention. This creates an incentive for waste water plants to reduce its emissions below the reporting thresholds (Irish EPA,2008; EU Environment Commission, 2008).

1.2 Waste Water Treatment Systems

1.2.1 Physical/Chemical treatment systems

The disposal of waste wasters as a by-product of production represents a significant challenge while still preventing environmental damage. One of the greatest problems in the treatment of chemical waste water is that many of the compounds present in the waste water are either toxic at low concentrations or they are recalcitrant. Physical or chemical treatment may need to be employed however this choice of treatment can in themselves generate toxic intermediates and the operating costs for such processes are generally high (Arnot *et al.*,1996).

There is a wide range of non biological processes which are applicable to water and to waste water treatment. A summary of these is listed in table 4.

Table.4. Principal physical-chemical waste water treatment processes (Gray 2005)

Process	Removal Function
Filtration	Suspended solids
Air stripping/breakpoint chlorination	Ammonia
Ion exchange	Nitrates, dissolved inorganic salts
Chemical precipitation	Phosphorus, dissolved inorganic salts
Carbon adsorption	Toxic compounds, refractory organics
Chemical oxidation	Toxic compounds, refractory organics
Ultra filtration	Dissolved inorganic salts
Reverse osmosis	Dissolved inorganic salts
Electrodialysis	Dissolved inorganic salts
Volatilisation and gas stripping	Volatile organic compounds

1.2.2 Biological treatment systems

In a biological waste water treatment system, it is predominantly bacteria that are responsible for the oxidation of organic compounds although fungi, algae, protozoa and other higher organisms can have a secondary role. The microbes in a waste water system are classified as either heterotrophic or autotrophs depending on their source of energy.

The former require organic carbon for both energy and cell synthesis whereas autotrophs use inorganic compounds to obtain energy and generally utilise carbon dioxide as the source of carbon. Microbes can be further subdivided by their dependence for dissolved oxygen. Aerobes dominate in most waste water systems requiring free levels of available dissolved oxygen. Aerobic processes are bio-chemically efficient and usually produce carbon dioxide and water as the main by-products. Conversely anaerobes oxidise organics in the absence of oxygen, reaction rates are generally lower and complex by-products can be generated. Facultative anaerobes use oxygen when available but can equally be active in the absence of dissolved oxygen. The degradation of waste water under aerobic conditions can be summarised by Fig.1 (Gray 2005).

$$\text{Biomass + organic matter + } O_2 + NH_4^{2+} + P \longrightarrow \text{New Cells + } CO_2 + H_20$$

Fig.1. bio-chemical reaction of a waste water treatment plant (Gray 2005)

Although anaerobic treatment of waste water is advantageous in terms of lower sludge production and operating costs, in practice aerobic systems tend to dominate largely due to the slow growth rates of anaerobic bacteria (Mersmann,1995). However a combination of integrated aerobic, anoxic and anaerobic systems can be effectively used to remove carbon, nitrogen and phosphorus. In addition this variation in treatment steps promotes improved floc formation, microbial diversity and a higher quality settling sludge (Louzerio *et al.*, 2002; Ahn *et al.*, 2007; Vaiopoulou *et al.*, 2007).

Phosphorus is found in waste water in three different forms; orthophosphate, polyphosphates and organic phosphate. As the biological demand for phosphorus is comparably low a significant excess can be found in certain effluents leading to eutrophication concerns. Coagulation and settlement with lime, aluminium and iron salts are effective means of removing phosphorus from a waste water (Gray 2005). In a process known as “luxury uptake” aerobic polyphosphate accumulating bacteria can be enriched and used to remove phosphorus through accumulation within their cells by alternating aerobic and anaerobic conditions (Pauli,1997; Wang *et al.*,2001).

Biological waste water systems are designed to operate under steady state conditions but in reality most physical and chemical parameters fluctuate significantly for various reasons. Any changes in these conditions can have an adverse effect on the systems

performance; for example a sudden temperature change can cause a shift in the bacterial community and in its function resulting in higher effluent solids and a fall in COD removal efficiency (Nadarajah *et al.*,2007). The activated sludge process operates effectively over a pH range of 6.5 to 8.5 and a temperature range of 4^{o}C to 39^{o}C. Dissolved oxygen levels are normally kept above 5mg/l to ensure good mass transfer. Solids retention and return of these solids to the aeration stage are an important consideration, therefore a good settling sludge and a properly sized and designed clarifier are essential (Eckenfelder *et al.*,1992).

The activated sludge process relies on a dense microbial population being mixed in suspension with the waste water generally under aerobic conditions. The activated sludge process at its simplest operation consists of two stages, aeration and settlement. In the aeration phase the waste water and the activated sludge are mixed together in a tank. Aeration is supplied by surface agitation or by diffusers. This not only provides oxygen to the system but also keeps the mixture in suspension. This is commonly known as the Mixed Liquor Suspended Solids (MLSS). In the second stage the activated sludge is separated from the treated waste water normally by sedimentation. The resulting clarifier effluent is then discharged and most of the activated sludge is returned to the first stage through a sludge return system. The MLSS is a crude measure of the biomass within the aeration tank. The sludge age or sludge residence time affects the characteristics and condition of the activated sludge flocs. It is calculated in days as the total amount of biomass within the system divided by rate of loss of biomass from the system. Young flocs contain actively growing heterotrophic bacteria with a high rate of metabolism. Older flocs, in contrast, have a lower proportion of viable cells being composed mainly of dead cells surrounded by viable bacterial layers. While the majority of these cells are no longer viable they still retain active enzyme systems. The organic loading to a plant divided by the mass of biomass in the system is referred to as the food to mass ratio (F/M). With excess food the rate of cell metabolism is high resulting in high organic carbon removal. However, this results in a poorly settling sludge and a breakthrough of organic carbon to the effluent. Broadly speaking a lower F/M is preferable as there is a limitation in food resulting in a higher quality effluent and a better settling sludge (Gray,1990).

The most important microorganisms in the activated sludge process are bacteria, fungi and protozoa. The most commonly found bacterial genera are *Arthrobacter, Achromobacter, Alcaligenes, Bacillis, Citromonas, Chromobacterium, Flavobacterium, Flexibacter, Micrococcus, and Pseudomonas* (Eckenfelder *et al.*,1992).

Sludge flocs tend to be negatively charged; consequently waste water high in cations can have a critical role in floc formation. Cations such as sodium and potassium can disrupt the binding effect between flocs leading to disintegration resulting in a turbid effluent, a poorly settling sludge and greater difficulty in dewatering. Some of the adverse effects of high cation concentrations can be off-set if aluminium and iron are present in the waste water as these act as coagulating agents (Bruss *et al.*,1992; Park *et al.*, 2006). The charge of the cations is also important; if the ratio of monovalent to divalent cations is >2 then a weak floc structure can result (Higgins and Novak, 1997).

Granular activated sludge is a gradual process whereby conventional activated sludge floc matures into a granule like structure that can be up to 5mm in diameter. In recent years the formation of a granular activated sludge has gained much interest as it offers improved settleability due to their large density and compact shape. They also have a lower water content over that of normal sludge flocs (Etterer and Wilderer, 2001; Wang *et al.*, 2006). The mechanism of granule formation is still not well understood (Diez *et al.*, 2007) however they are most often encountered in a sequencing batch reactor (SBR) (McSwain *et al.*, 2004). Consequently the SBR can be considered as an attractive technology for cultivation of granular activated sludge (Zima *et al.*, 2007).

The SBR process has proven to be a simple, flexible and reliable waste water treatment system. SBRs have the ability to easily modify process parameters without any physical modifications. SBR works on a batch fill and draw basis where a volume of waste water is added to a tank in the presence of an activated sludge. This mixture is aerated for a period of time; the air is reduced if an anaerobic or anoxic period of the treatment cycle is desired or the air is turned off allowing the sludge to settle and after a period of time the supernatant or treated effluent is decanted and the process starts over again (Rim *et al.*, 1997; Keller *et al.*, 1997; Hudson *et al.*, 2001). The time necessary for the sedimentation of the activated sludge and the extraction of the clear water phase can take up a major part of the entire cycle period within the SBR process. If a sludge is not

settling well the time available for carbonaceous removal can become compromised (Krampe and Krauth,2001).

Another variation on the activated sludge process is fluidised bed reactors (FBR). These are also compact treatment systems that can achieve effluent quality comparable to that of conventional activated sludge however at much shorter hydraulic retentions time. FBR's tend to be mostly up flow systems where waste water and aeration are supplied at the bottom of a column. The biological sludge in this column forms around a particle or media such as sand, activated carbon or polypropylene. This support media is kept buoyant part of the way up the column but below the point where it could overflow. The waste water flows from the bottom of the column up through the suspended sludge and overflows from the top of the column as treated effluent. As the dissolved oxygen levels fall through the column it means COD, solids removal and nitrification/denitrification can all take place in the same reactor. FBR's tend to be rich in microorganisms and can operate at high MLSS levels. Good pellet formation of the sludge is an important consideration to ensure good settleability. The abrasive characteristics of the carrier material as well as carry over to the effluent can however lead to suspended solids issues (Tessele *et al.*, 2002; Harri and Bosander,2004; Wang *et al.*, 2007).

Activated sludge systems such as aeration bays, SBR's and FBR's all rely on retaining and separating the biological biomass from the waste water generally through clarification steps. Fixed film systems, also commonly referred to as biotowers or trickling filters, on the other hand rely on the adherence of the microbial biomass i.e. the biofilm onto a permanent media across which the waste water flows or trickles. The formation of biofilm, depending on the thickness and oxygen mass transfer, results in the formation of aerobic, anoxic and anaerobic layers. Aerated biological filters require a small foot print and achieve high performance at high organic loadings more so than that of conventional activated sludge processes. As a result, the operation of downstream treatment stages is also stabilized. Fixed film systems can also be submerged and are ideal for the pre-treatment of industrial waste waters. The submerged fixed-film reactor has proven to be an extremely reliable process, requiring little maintenance that can cope with load fluctuations (Allan *et al.*, 1998; Schlegel and Teichgräber, 2000; Takács *et al.*, 2007).

The rotating biological contactor (RBC) process offers the specific advantages of a fixed film system in the treatment of wastewater for removal of soluble organic substances and nitrogen compounds. Being a unique adaptation of the moving-medium biofilm system, it facilitates easy and effective oxygen transfer. However, process optimisation and adaptability under different conditions remain challenging tasks for the efficient use of this technology (Dutta *et al.*,2007).

Membrane bioreactors offer some advantages over conventional treatment processes including the ability to operate at much higher cell densities leading to improved kinetic performances. The waste water and the biological zone are separated by a synthetic membrane where the organic compounds diffuse across the membrane into the biological zone. However diffusion rates can become a limiting step and the membranes are prone to fouling (Arnot *et al.*,1996).

1.2.3 Microbial Nutritional Requirements

To reproduce and for cells to function properly, micro-organisms require a source of energy, carbon for the synthesis of new cellular material and various inorganic nutrients. The conversion of inorganic carbon to organic cell tissue requires a greater input of energy and explains the slower growth rates of autotrophs compared to heterotrophs. Energy is either obtained through the use of light (phototrophs) or by a chemical oxidation reaction (chemotrophs). Most waste water systems use heterotrophs that obtain their energy from the oxidation of organic compounds; these are known as chemoheterotrophs. Autotrophs that obtain their energy from inorganic chemical reactions such as the oxidation of ammonia are termed chemoautotrophs. The principal inorganic elements required for microbial cell functions are N, S, P, K, Mg, Ca, Fe, Na and Cl. All micro-organisms require nitrogen in some form for cell components, sulphur is essential for certain amino acids while phosphorus is a key component of nucleotides and nucleic acids. Zn, Mn, Mo, Se, Co, Cu and Ni are important especially in enzyme formation but are only required at trace levels. Organic nutrients such as amino acids and vitamins can be important for cell synthesis (Pelczar,1986; Tchobanoglous *et al.*,1991).

Microbes exhibit a considerable diversity in the compounds that they use for growth. All microorganisms are composed of carbon, hydrogen, oxygen, nitrogen, phosphorus, sulphur and a variety of trace elements. These nutrients must be obtained from the environmental medium within which they grow. The absence or insufficient levels of one or more of these components will inhibit their growth. The availability of nutrients and the on going supply of these determine the growth pattern of microbial populations; four distinct phases are evident (Fig.2). The lag phase represents a period of low reproduction where adaption of a microbial community to the environment takes place. In the logarithmic phase the biomass population experiences rapid growth where cell numbers double at regular intervals. Once a nutrient becomes limiting growth slows down and ceases but the cells still remain viable. The final phase of growth is the decline phase where the cellular death rate is greater than the growth rate resulting in a fall in the number of viable cells. In a waste water treatment plant the population needs to be maintained in the logarithmic phase (Scragg,2005).

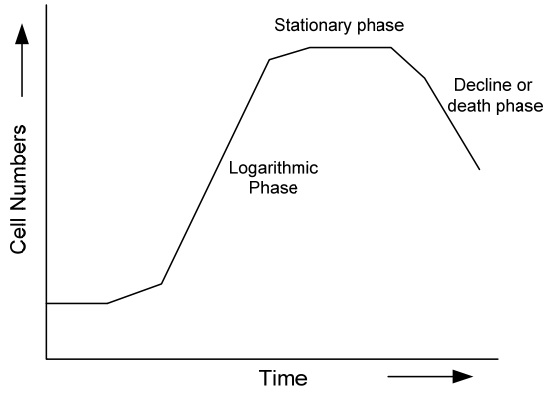


Fig.2. The growth phases of micro-organisms (Scragg,2005).

The derivatives of nitrogen in a pharmaceutical waste water are typically organic nitrogen and more commonly ammonia. Ammonia tends to be liberated by most heterotrophic bacteria through de-amination of nitrogen containing compounds. Ammonia is the preferred nutrient form for cell growth; microbial biomass is on average 17% nitrogen (Wiesmann, 1994). Typical BOD to nitrogen to phosphorus ratios of 100:5:1 are commonly used, that is for every 100kg of BOD that must be removed 5 kg of nitrogen and 1kg of phosphorus is required. However, treatment plants can operate above or below this (Moebius, 1991;). Expressed as COD then the removal of 100kg of COD typically requires 3.4kg of nitrogen and 0.6kg of phosphorus (Prendl and Nikolavic,2000).

1.3 Effect of Ammonia on the environment

Following biological treatment one component commonly found in a waste water effluent is nitrogen. This exists in the form of three main derivatives i.e. ammonia, nitrate and nitrite. Unless limited, only a part of the nitrogen load to an activated sludge plant will be removed by conventional heterotrophic activity, where it is incorporated into microbial biomass. Any excess will end up in the final effluent usually as ammonia (Gray, 1990). Depending on the receiving environment and quantity released these compounds can have a significant adverse effect on the quality and the ecology of a water system. Ammonia and especially nitrite are highly toxic to fish populations whereas nitrate may promote eutrophication. Once released to water, ammonia can react with humic substances, metals and metal complexes. It will also bind to sediments and suspended particles. In the aquatic environment ammonia will either be converted to nitrates which are in turn taken up by aquatic plants or will be used directly by some algae and phytoplankton. In drinking water supplies nitrite and nitrate are toxic to humans causing the oxidation of haemoglobin and in turn diminishing the oxygen transport capacity of blood. The presence of ammonia in drinking water is therefore considered to be a potential health risk (Lawuyi and Fingas,1993, Van der Aa *et al.*, 2002).

Ammonia can also influence the oxygen balance of a river. A waste water effluent with an ammonia concentration of 20mg/l NH_4-N will have an oxygen demand of over 90mg/l O_2 or 4.5 times the ammonia concentration. Therefore apart from toxicity or eutrophication issues it is therefore just as important to oxidise ammonia in waste water as it is to oxidise the carbonaceous demand (Kowalchuk *et al.*, 2001).

1.4 Nitrification

Air stripping and breakpoint chlorination are well developed means of removing ammonia from water. Ammonia oxidation by platinum catalyst to produce nitrogen oxide has also been used for many years as is precipitation with magnesium-ammonium-phosphate. Ion exchange of low levels of ammonia in water can be achieved using zeolites such as Na-mordenite (Wang *et al.*,2007). Although effective in clean water there is no reported long term application on pharmaceutical waste water.

Biological nitrification therefore still remains the most cost effective way to treat ammonia waste waters (Huang *et al.*,2001; Fux *et al.*,2002). Nitrification is the microbial conversion of ammonia into nitrate, whereas denitrification is the reduction of nitrate into nitrogen gas (Gerardi,2002; Gray, 2005).

Nitrification was first observed in 1877 by Schlesinger and Muntz. In 1890, Winogradsky realised that the oxidation of ammonia could supply energy and that the bacteria affecting this transformation would not need reduced carbon compounds but could synthesize cellular material through the reduction of carbon dioxide or bicarbonate. Thus the first concept of chemoautrophic growth was realised (Focht and Chang, 1976). As autotrophic ammonia oxidising bacteria are generally characterised by low growth rates and cell yields, the nitrification step is generally the rate limiting step in a biological treatment process. Maintaining an adequate population of nitrifiers can be difficult (Wang, 2005). For each molecule of carbon assimilated by the nitrifying bacteria over 3 molecules of ammonia or 100 molecules of nitrite must be oxidised. This large ratio explains why nitrifiers have such low growth rates. The generation time of most heterotrophs is 15-30minutes whereas under favourable conditions it is 48-72hrs for nitrifiers (Gerardi, 2002). The growth of autotrophic bacteria is only $^{1}/_{10}$ that of heterotrophs (Sharma and Gupta, 2004).

1.4.1 Biochemistry of nitrification/denitrification

Nitrification is the oxidation of ammonia to nitrate (Fig.3.) by two groups of chemolitotrophic bacteria, the ammonia oxidisers; typified by the genus *Nitrosomonas* in particular *Nitrosomonas europaea* and the nitrite oxidisers i.e. *Nitrobacter* especially *Nitrobacter agilis* and *Nitrobacter winogradskyi* (Poughon, 2000). *Nitrosomonas* is coccus shaped with a diameter of 0.5-1.5µm while *Nitrobacter* is bacillus (elongated) shaped and is 0.5-1.0µm in diameter. Both are Gram negative, *Nitrosomonas* is mobile and reproduces by binary fission whereas *Nitrobacter* is non mobile and reproduces by budding (Gerardi, 2002).

$NH_4^+ + {}^3/_2\, O_2 \rightarrow NO_2^- + 2H^+ + H_2O$ ammonia oxidation

$NO_2^- + {}^1/_2\, O_2 \rightarrow NO_3^-$ nitrite oxidization

Fig.3. Two step nitrification pathway from ammonia to nitrite (Campos *et al.*,1999).

The oxidation of ammonia is however more complicated with intermediates, such as hydroxylamine (NH_2OH) (Kowalchuk *et al.*,2001) as well as end products other than nitrite or nitrate such as nitrogen oxide (NO) and dinitrogen oxide (N_2O) (Shrestha *et al.*,2001; Hwang *et al.*, 2006). No single bacterium is capable of the oxidation of ammonia to nitrate in one single step. The main enzymes involved in the nitrification process are ammonia monooxygenease, hydroxylamine oxidoreducase and nitrite dehydrogenase (Abeliovich, 1993).

Nitrifying bacteria assimilate CO_2 or bicarbonate via the Calvin-Benson cycle (Denecke and Liebig,2003). Nitrification is not just limited to chemoautotrophic bacteria. Under aerobic conditions numerous bacteria and fungi have the ability to oxidise a variety of nitrogenous compounds; this is known as heterotrophic nitrification (Castignetti and Hollocher,1984; Sakai *et al.*,1996). Some of the main genera that have demonstrated heterotrophic nitrification include *Staphylococcus, Micrococcus, Streptococcus, Pseudomonas and Bacillus.* Heterotrophic nitrification generally has limited application as their growth rates are significantly slower, by one or two orders of magnitude, than those of autotrophs (Stevens *et al.*,2002). Heterotrophic nitrification has also been demonstrated under anaerobic conditions where bacteria such as *Pseudomonas aeruginosa* have also been shown to be capable of removing over 150mg/l of ammonia, nitrate and nitrite in the presence of glucose (Young *et al.*, 2007).

Under low oxygen tensions numerous heterotrophic bacteria including; *Achromobacter, Aerobacter, Bacillus, Flavobacterium, Micococcus* and *Pseudomonas* will reduce nitrate to nitrogen gas in a process called denitrification (Fig.4). This is the second step of the nitrification denitrification process. Denitrification itself involves a number of different steps; nitrate is firstly converted to nitrite followed by the production of nitric oxide, nitrous oxide and nitrogen gas. In denitrifying systems the dissolved oxygen levels must be kept at anoxic levels and the optimum pH lies between pH 7 and pH 8 (Tchobanoglous *et al.*,1991).

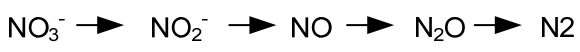


Fig.4. denitrification pathan (Tchobanoglous *et al.*,1991).

As denitrification is a heterotrophic process a source of organic carbon is therefore required. Complete denitrification occurs when a BOD to nitrate ratio of 3:1 exists. Decreasing this ratio to 3:2 causes breakthrough of nitrate to the final effluent. To be successful a carbon source may need to be added. Methanol has gained acceptance as a cheap and reliable carbon source for denitrification where 2.5mg/l of methanol is recommended for every 1mg/l of nitrate (Gerardi,2002). Above a methanol concentration of 8mg/l the rate of denitrification will not improve (Louzerio *et al.*,2002). Acetic acid is also a good source of organic carbon for denitrification, however ethanol is one of the best options. In this case a C/N ratio of 6:1 is optimum. (Kesser *et al.*,2001). Denitrification rates using ethanol are up to 6 times faster than using methanol (Carrera,2003). Although less efficient and less common denitrification is however not just confined to organic carbon sources, elemental sulphate can be used as the electron source and bicarbonate as the carbon source (Soares,2001)

Denitrification can be performed starting not only from nitrate but also from nitrite (Tokutomi *et al.*,2006). This fact can be exploited in a process of partial nitrification or simultaneous nitrification denitrification (SND) (Fig.5). This has proven to be an economic way for the treatment of industrial nitrogen rich effluents by reducing the aeration and external COD requirements. SND is based on the fact that nitrite is an intermediate in both nitrification and in denitrification. SND relies on concurrent aerobic oxidation of ammonia and subsequent anaerobic denitrification by heterotrophic bacteria. To achieve SND, nitrification can only be allowed to take place through the suppression of the aerobic nitrite oxidisers without effecting the ammonia oxidizers (Ciudad *et al.*, 2005; Pambrun *et al.*, 2006; Su *et al.*, 2006).

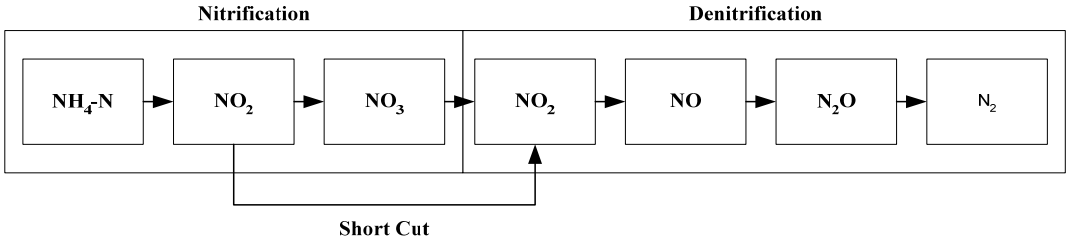


Fig.5. Simultaneous Nitrification Denitrification (Ciudad *et al.*,2005)

There are a several factors that influence the success of the SND process. The carbon to nitrogen ratio, dissolved oxygen and pH are particularly important. (Wang *et al.*, 2005). The optimum operational conditions to achieve SND are pH 8.0, a dissolved oxygen of 1.0mg/l, a hydraulic retention time of 16 hours and a temperature of 35^{o}C (Gang,2006).

There are a number of ways to suppress aerobic nitrite oxidation thereby allowing a build up of nitrite for SND to commence. Free ammonia can be used as nitrite oxidisers are more sensitive to free ammonia than ammonia oxidisers (Turk and Mavinic,1990; Bae *et al.*,2001). Temperatures above 30^{o}C will also inhibit nitrite oxidation (Fux *et al.*,2002). By shorting the settling time in an SBR ammonia oxidisers can be selectively enriched and granulated resulting in wash out of the nitrite oxidisers (Kim *et al* 2006a). Dissolved oxygen is the most important control for successful SND. This can be achieved by operating a low oxygen level as ammonia oxidation can take place at lower dissolved oxygen levels than nitrite oxidation (Shrestha *et al.*,2001. Canziani *et al.*, 2006). Nitrite accumulation occurs at a dissolved oxygen level of 1.4-1.5mg/l and will reach a maximum at 0.7mg/l where 65%-75% of the ammonia load is converted and maintained as nitrite (Ruiz *et al.*, 2003). SND can be achieved in a biofilm system where oxygen levels within the air can be controlled to less than 1% oxygen for nitrite accumulation to occur (Chuang *et al.*,2007). SND is particularly successful for waste waters with high ammonia and a low C/N ratio. This tends to rule out most pharmaceutical waste waters (Peng and Zhu,2006).

Another ammonia removal process is Anammox, this is carried out by a group of bacteria known as the *Planctomycetes*. As the doubling time is very low up to 3 weeks a reactor with very efficient biomass retention is needed (Jetten *et al.*, 2001). Both SND and Anammox can be successfully combined into a single process called Canon (Fig.6). Nitrite must firstly be produced as stable partial nitrification is essential for the subsequent anammox step (Sliekers *et al.*, 2002; Fux *et al.*, 2002).

Ammonia $\rightarrow$hydroxylamine$\rightarrow$ nitrite $\rightarrow$ nitrogen oxide$\rightarrow$dinitrogen Oxide$\rightarrow$ nitrogen gas

Fig.6. Anaerobic/anoxic oxidation of ammonia / Simultaneous Nitrification/ Denitrification and associated by-products (Sliekers, 2002; Fux, 2002).

Some methane oxidising bacteria have also demonstrated ammonia oxidation (Kowalchuk *et al.*, 2001; Lee *et al.*, 2001). Although some ammonia oxidisers can assimilate organic compounds under mixotrophic conditions, heterotrophic growth has not been observed. Many nitrite oxidisers on the other hand are able to grow heterotrophically and these may play a role in reducing the inhibitory effect of organic compounds in a waste water (Poughon *et al.*, 2000).

1.4.2 Nitrification Kinetics

A hydraulic retention time (HRT) of greater than 15 hours is normally sufficient for nitrification for an influent ammonium concentration of 100mg/l (Hanaki *et al.*, 1990). At higher COD loadings the HRT may need to be increased to compensate for any reduction in nitrifier activity (Dincer and Kargi,2000b). In general a HRT time of 2 days is optimum for treating a concentrated waste water of greater than 1,000mg/l total nitrogen (Gupta and Sharma,1996).

Although on a biofilm a lower HRT may be preferable for nitrification. Competition can result in the faster growing heterotrophs growing on the outer layers of a biofilm, where both substrate concentrations and detachment rates are high, while the slow growing nitrifying bacteria stay deeper inside the biofilm. Thus a heterotrophic population can form above the nitrifiers creating a disadvantage in terms of oxygen transfer. This effect can be compounded by extending the HRT allowing for a greater build up of heterotrophic biofilm; increasing the HRT to improve nitrification is not always the best option (Nogueria *et al.*, 2002).

Methods to quantify the growth rate of nitrifiers from that of the dominant heterotrophic bacteria are important. Although microbial probes can be used to identify the proportion of bacteria that are nitrifiers they do not give any information on the growth rates. This can be accurately determined by measuring the rate at which a radioactive label is incorporated into the DNA of a dividing nitrifier bacterial cell in the presence and absence of a known inhibitor (Pollard, 2006). The growth rate of ammonia oxidisers is normally higher than that of nitrite oxidisers. Although the growth rate of nitrifiers is well defined, the decay rate is still uncertain. The decay rate of a pure culture is significantly lower (up to 10 times) than nitrifiers in an activated sludge system. The rate also varies depending on the oxygen conditions; it is higher in an aerated system

compared to an anaerobic system (Canziani *et al.*, 2006; Salem *et al.*, 2006). Typical decay rates range from a low of $0.021d^{-1}$ (Dincer and Kargi, 2000a) to a high of $0.44d^{-1}$ (Pambrun *et al.*, 2006) but typically range from $0.06\text{-}0.11d^{-1}$ (Katehis *et al.*, 2002).

The biomass yield co-efficient of nitrification of ammonia and nitrite ranges from 0.11-0.15g biomass/g N (Wiesmann,1994) to 0.34g biomass/g N (Dincer and Kargi ,2000a). The rate at which this nitrification takes place varies depending on various factors. A list of nitrification rates is presented in Table 5.

Table 5: Typical reported nitrification rates

Nitrification Rate	Reference
9 to 12 mg N/g of SS/Hr	Jonsson *et al.*, 2000
0.991mg TKN /mg VSS/day	Gupta and Sharma, 1996
0.37g NH_4-N g VSS d^{-1}	Carrera, 2003
25mg N/litre/Hr	Dincer and Kargi , 2000a
1.8kg ammonium /m^3/d	Han D *et al.*, 2001
35mg N/g VSS/hr	Jetten *et al.*, 2001
4.3 mg N/g VSS/hr for ammonia oxidisers 2.4mg N/g VSS/hr for nitrite oxidiser	Moussa *et al.*, 2006b
0.7kg NH_4-N/m^3/d or 650-1,300mg NH_4-N/m^2/d	Rodgers *et al.*,2005
1.46kg NH4-N /m^3/D	Chuang *et al.*, 2007

**Abbreviations:- SS: suspended solids; TKN: Total Kjeldahl Nitrogen; VSS: Volatile Suspended Solids*

1.4.3 Nitrifier species

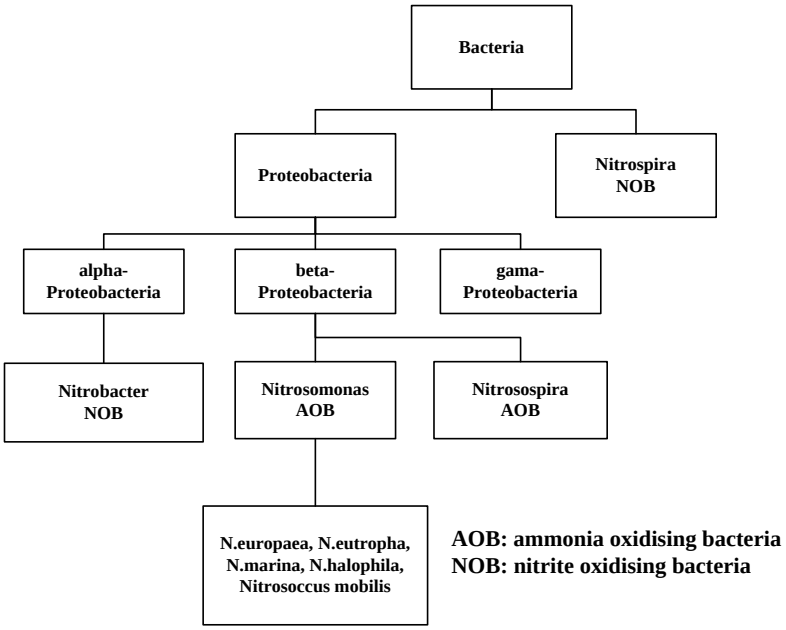
One of the mostly widely used tools for molecular identification of nitrifiers is the 16S rRNA targeted approach. The 16S rRNA gene contains phylogenetic information that is extracted and amplified using a procedure called the polymerase chain reaction. In addition to the 16S gene Ammonia Mono Oxygenase (AMO) is a key enzyme for ammonia oxidizing bacteria and using a process called restriction fragment length polymorphism comparisons of the DNA sequences can be made. RNA targeted oligonucleotide probes can be used to monitor populations numbers and viability in situ (Ballinger *et al.*, 1998; Smith, 2004). The main species responsible for the nitrification of ammonia are *Nitrosomonas, Nitrosococcus, Nitrosospira ,Nitrosolobus* and *Nitrosovibrio* while *Nitrobacter, Nitrococcus* and *Nitrospira* complete the oxidation of nitrite (Waston *et al.*, 1986). Of these *Nitrosomonas* and *Nitrobacter* are regarded as the

primary nitrifiers within a nitrifying waste water environment (Ballinger *et al.*, 1998; Kim *et al.*,2006b). Some specific species of nitrifiers isolated and identified are listed in table 6.

Table 6: list of nitrifier species.

Nitrifier Species	Reference
Nitrosomonas europaea	Ballinger (1998);Wanger (1995) ; Nogueira,2002 ; Wittenbolle (2005)
Nitrosomonas communis	Ballinger (1998);Wanger (1995) ; Nogueira,2002
Nitrosomonas eutropha	Ballinger (1998);Wanger (1995) ; Nogueira,2002 ;Wittenbolle (2005)
Nitrosomonas ureae	Ballinger (1998);Wanger (1995) ; Nogueira,2002
Nitrosomonas marina	Ballinger (1998);Wanger (1995) ; Nogueira,2002
Nitrosomonas nitrosa	Wanger (1995) ; Nogueira,2002
Nitrosomonas oligotropha	Wanger (1995) ; Nogueira,2002 ;Wittenbolle (2005)
Nitrosomonas halophila	Wanger (1995) ; Nogueira,2002
Nitrosococcus mobilis	Ballinger (1998); Nogueira,2002
Nitrospia briensis	Ballinger (1998)
Nitrosovibrio multiformis	Ballinger (1998); Nogueira,2002
Nitrosovibrio tenuis	Ballinger (1998); Nogueira,2002
Nitrobacter winogradskyi ,	Bock (1987); Teske,1994.
Nitrobacter hamburgensis	Bock (1987);Teske,1994.

Nitrite and ammonia oxidisers are widely distributed within the proteobacteria (Fig.7). Although phylogenetically diverse there are common themes. Ammonia oxidisers are restricted to two evolutionary lineages of the class Proteobacteria, furthermore all strains isolated from terrestrial and freshwater environments belonged to a single monophyletic evolutionary group within the beta subclass of the class Proteobacteria. This provides evidence of their descent from a single chemolitotrpophic ammonia oxidising ancestor. Notably most of the nitrifiers are affiliated with phototrophs suggesting a close evolutionary link between photosynthesis and nitrite/ammonia oxidation. Thus nitrifiers are not only derived from an ancestral nitrifying phenotype but appear to have arisen independently multiple times possibly from different photosynthetic ancestors (Teske *et al.*,1994; Kowalchuk *et al.*, 2001).



Fig,7. Evolution pathway of nitrifiers (Kowalchuk *et al.*, 2001)

Differences in plant design and operating conditions will result in different nitrifier ecology even on the same waste water composition. In a three stage pilot plant fed with synthetic waste water containing 500mg/l ammonium, different kinetic conditions were created where the first stage had a high ammonia saturation constant compared to the second and the final stage. By applying a sequential system rather than single mixed tank varying influents were produced both in ammonia and metabolite content. This allowed different populations to become established and therefore promoted nitrifier diversity (Noto *et al.*, 1998).

Nitrifiers in an activated sludge waste water system typically consist of about 78% *Nitrosomonas* and 22% *Nitrobacter* whereas those on a biofilm tend to predominantly belong to the genus *Nitrosomonas* with a small amount of *Nitrospira* (Gee *et al.*, 1990a; Persson *et al.*, 2002). Equally in a combined recirculating activated sludge/biofilm reactor *Nitrosomonas* and *Nitrospira* were the main genera present with little or no *Nitrobacter*. This is explained by the fact that *Nitrobacter* is generally only found under high nitrite conditions whereas *Nitrospira* thrive on low nitrite concentrations. Genus *Nitrospira* can exploit low amounts of nitrite more efficiently than *Nitrobacter.* In contrast *Nitrobacter* prefers high nitrite levels and grows faster than *Nitrospira*. The specific nitrite oxidiation activity for *Nitrobacter* is 9 times higher than *Nitrospira*. Many species of *Nitrobacter* are able to grow mixotrophically giving them a

competitive advantage over *Nitrospira*. The ecology of nitrite oxidizing bacteria can therefore be dependent on the concentration of nitrite (Nogueira *et al.*, 2002; Kim *et al.*, 2006b). Nitrite oxidizing bacteria tend to be more stable and are less prone to population shifts than ammonia oxidisers (Horsch *et al.*, 2004).

The activity of *Nitrobacter* is strongly dependent on the population of *Nitrosomonas* but not vice versa. Nitrite oxidation in the absence of ammonia results in an unstable system. It was observed that *Nitrobacter* activity was highly dependent on the ratio between *Nitrobacter* and *Nitrosomonas*. When this ratio was at a minimum with only ammonia as the substrate the specific activity of *Nitrobacter* was the highest. As the ratio of *Nitrobacter* to *Nitrosomonas* increased the specific activity of *Nitrobacter* decreased. However *Nitrosomonas* was not affected by the ratio at all. There may be some enzymatic commensalism or biochemical energy transfer phenomenon between the two genera. If ammonia levels fall this could cause *Nitrobacter* activity to decrease thereby leading to a build up of nitrite which in-turn could inhibit *Nitrosomonas* (Gee *et al.*, 1990b). More recently study indicates that the kinetics of nitrite oxidation with or without complete inhibition of ammonia oxidation are not significantly different (Chandran and Smets,2000). On a higher ecological level there are two protozoa *Epistylis* and *Vorticella* that are often seen in a nitrifying sludge. They can be used as indicators as conditions favourable for nitrification. Ciliated protozoa help nitrifiers to flocculate through the coating action of secretions; nematodes and other multicellular organism also play a role (Gerardi, 2002).

1.4.4 Inhibition of Nitrification

Within a waste water environment nitrifiers are particularly sensitive to inhibition. Even when there is an abundant population of nitrifiers and when growth conditions are close to optimum nitrification is easily disrupted at low concentrations of inhibitory substances (Svenson *et al.*, 2000). As inhibition problems often occur for limited time and are caused by different substances it is often difficult to identify those compounds that are responsible (Kroiss *et al.*, 1992).

Understanding the influence of compounds that are inhibitory is further complicated by the fact that certain inhibitors can interact with each other thereby reducing the combined effect. Where the effect of an inhibitor is large the addition of another inhibitor doesn't make an appreciable difference. Conversely where two inhibitors are only moderately inhibitory their addition together can be greater than the individual effect (Tomlinson, 1966). An inhibitor can be specific to only one or both of the nitrification steps (Suthersan and Ganczarczyk, 1986b; Nowak and Svardal, 1993).

The degree of inhibition depends on a number of factors (Tyagi and Couillard,1988; Polanco *et al.*, 1994), these include:

- Concentration of inhibitor
- Species of organism present
- Suspended solids concentration
- Sludge age
- pH
- Solubility of inhibitor
- Presence of other cations/anions
- Reactor geometry

An inverse relationship can exist between the degree of inhibition and the concentration of nitrifiers i.e. as the number of nitrifiers increases the rate of inhibition decreases at the same concentration (Suthersan and Ganczarczyk, 1986a). *Nitrosomonas* is an order of magnitude more sensitive than heterotrophs to a range of chemicals. Therefore aerobic heterotrophs stand out as the best group of bacteria for ranking toxicity to other organisms. If aerobic heterotrophs are inhibited the probability that nitrification will be inhibited is also very high (Blum and Speece, 1991).

Nitrifiers have a broad susceptibility to inhibitory agents. Nitrification is adversely effected by six general classes of industrial toxins; i.e. electrophilic organic solvents (1-chloro-2,4-dinitronbenzene); heavy metals (cadmium); hydrophobic chemicals (1-octanol); uncoupling agents (2,4-dinitrophenol); alkaline pH and cyanides. As chemical inhibitors impact ammonia oxidisers and the nitrite oxidisers differently this suggests there are different inhibitory mechanisms in operation (Kelly *et al.*, 2004). At low concentrations the chemical structures of inhibitors seem to be the predominant factor whereas at higher concentrations the hydrophobicity of the chemical plays an important role. The hydrophobic nature of certain chemicals leads to an accumulation on the lipid based membranes (Zepeda *et al.*,2006).

A broad range of sulphur containing compounds also inhibit nitrification including thiosulphates, thiocarbamates, xanthates and sulphur containing ammonia acids. Specific compounds include; carbon disulphide, thiourea, allylthiourea, guanylthiourea, mercaptobenzothiazole, sodium diethyldithiocarbamate. Other compounds most noted for inhibition include heavy metals, cyanides, halogenated compounds, phenols, mercaptans, thiourea, amines and nitrogenous organsulphur compounds (McCarty,1999). In broad terms lower rates of nitrification are seen with increasing chlorine atoms (Ely *et al.*, 1997). The presence of an anaerobic stage in a treatment system can result in a drop in the nitrifying population (Nowak *et al.*,1999a; Krogulska and Mycielski,1981 and 1984a). For example volatile fatty acids produced as byproducts from anaerobic digestion are inhibitory to nitrification. Formic acid is the most inhibitory and acetic acid the least inhibitory. Propionic and n-butyric acid all inhibit nitrite oxidation but not ammonia. Isobuteryic, n-valeric and n-capronic inhibit both ammonia and nitrite oxidation (Eilersen ,1994). A septic wastewater containing a sulphide concentration of greater than 0.5mg/l can result in a 30-40% reduction in the rate of nitrification (Esoy *et al.*,1998).

Metals are potent inhibitors of nitrification. Inhibition is not a just a function of the metal concentration in solution but rather the intracellular concentration. Different metals will reach intracellular equilibrium at different rates. Zinc, nickel and cadmium will continue to increase in concentration twenty four hours after exposure whereas copper will reach intercellular equilibrium within 4 hours. Metal inhibition is also not a function of the total metal concentration but rather the free cation or free metal concentration. Most metal inhibition seems to interfere with protein structures and functions within the cell (Hu *et al.*, 2002 and 2004). Inhibition to nitrification by heavy metals is also attributable to adsorption of the metallic ion onto the biological floc thereby partially or completely blocking the enzyme mechanism (Martin and Richard, 1982).

A table summarising inhibitory concentrations for metals is present in Table 7.

Table 7: Inhibitory thresholds for common metals

Metal	Extent of Inhibition	Concentration	Reference
Aluminium	Substantial or complete inhibition	270mg/l	Meiklejohn,1954
Arsenic	100% inhibition of nitrification	943mg/l	Beg and Hassan,1987
	80% Inhibition *Nitrosomonas*	125mg/l	Beg and Hassan,1987
Barium	Substantial or complete inhibition	27400mg/l	Meiklejohn,1954
Cadmium	Inhibitory 42%	14.3mg/g VSS	Martin and Richard,1982
Calcium	Substantial or complete inhibition	8000mg/l	Meiklejohn,1954
Chromium	80-90% inhibition	100mg/l	Jonsson, 2001
	20-40% inhibition	10mg/l	Jonsson,2001
	80% Inhibition *Nitrosomonas*	20mg/l	Beg and Hassan,1987
	100% inhibition of nitrification	164mg/l	Beg and Hassan,1987
Cobalt	Inhibits *Nitrosomonas*	0.08-0.5mg/l	Loveless and Painter,1968
	Substantial or complete inhibition	59mg/l	Meiklejohn,1954
Copper	Threshold Inhibitory to *Nitrosomonas*	0.4mg/l	Martin and Richard,1982
	Inhibitory concentration at 50%	187mg/l	Koenig A et al 1999
	Inhibits *Nitrosomonas*	0.05-0.56mg/l	Loveless and Painter,1968
Chromium	Threshold Inhibitory to *Nitrosomonas*	1mg/l	Martin and Richard,1982
Fluoride	60% Inhibition *Nitrosomonas*	800mg/l	Beg and Hassan
	100% inhibition of nitrification	2565mg/l	Beg and Hassan
Iron	Substantial or complete inhibition	560mg/l	Meiklejohn,1954
Lead	Threshold Inhibitory to *Nitrosomonas*	0.5-1mg/l	Martin and Richard,1982
	Substantial or complete inhibition	2080mg/l	Meiklejohn,1954
Magnesium	Inhibits *Nitrosomonas*	50-100mg/l	Loveless and Painter,1968
	Substantial or complete inhibition	12000mg/l	Meiklejohn,1954
Manganese	Substantial or complete inhibition	550mg/l	Meiklejohn,1954
Mercury	Substantial or complete inhibition	2mg/l	Meiklejohn,1954
Nickel	Threshold Inhibitory to *Nitrosomonas*	0.1mg/l	Martin and Richard,1982
	Partial Inhibition	100mg/l	Lee 1997
	Severe Inhibition	250mg/l	Lee 1997
Potassium	Substantial or complete inhibition	19500mg/l	Meiklejohn,1954
Silver	Substantial or complete inhibition	0.25mg/l	Meiklejohn,1954
Sodium	Substantial or complete inhibition	11500mg/l	Meiklejohn,1954
Zinc	20-40% inhibition; 90-100% inhibition	5-10mg/l;40mg/l	Jonsson,2001

Chemical waste waters can have significant levels of salinity; the rate of nitrification decreases linearly with increasing salinity. At 60,000mg/l NaCl ammonia oxidation was reduced by 71% or by 50% at 30,000mg/l. The effects were more pronounced on nitrite where rates fell by 83% at 60,000mg/l NaCl. Nitrite oxidation was more sensitive to salinity. The effect is however readily reversible (Sanchez *et al.*,2004).

Other studies have shown that salinity can be inhibitory at much lower concentrations. At 5,000mg/l salinity ammonia oxidation was inhibited by 20%; this increased to 40% at 10,000mg/l and was over 80% at 20,000mg/l. Nitrite oxidizers, although also inhibited, were slightly less effected by salt stress compared to ammonia oxidizers. Above 40,000mg/l Cl^{-1} there is a complete inhibition to nitrification. Under elevated salt levels, up to 30,000mg/l, only *N.europaea* and *Nitrosococcus mobilis* were present. Above 30,000mg/l only *N.europaea* survived. These results show that *N.europaea* is more resistant to a salinity increase than *N.mobilis* even though both species are believed to be halotolerant. An indicator of high salinity levels is an absence of higher organisms above 5,000mg/l Cl^{-1}. Only nematodes are capable of surviving high salinity levels. The specific activity of nitrifiers is 10-15 times higher under salt free conditions (Moussa *et al.*,2006a).

A change in salinity will result in a shift in population. A salinity concentration below 4,120mg/l favours nitrification however when this increases nitrification rates fall. There is a shift in population from non saline resistant species such as *Nitrosomonas europea* and *N.eutropha* to saline resistant species such as *Nitroscoccus mobilis* (Chen *et al.*, 2003). Nitrification can adapt to salinity. A rotating biological contractor was slowly adapted to treating an ammonia rich saline waste water. A salinity of 6,000mg/l had no impact on nitrification. When this reached 10,000mg/l an initial disruption was seen but nitrification quickly adapted with nitrification occurring up to a salinity of 30,000mg/l (Windey *et al.*, 2005).

There are several references on the inhibition levels of a wide range of organic chemical compounds. A table summarising those more relevant to a pharmaceutical waste water is presented in Table 8.

Table 8: Inhibitory thresholds for common industrial chemicals used in pharmaceutical manufacture

Compound	Extent of Inhbition	Concentration	Reference
1 Octanol	IC50 *Nitrosomonas*	67mg/l	Blum and Speece,1991
1 Propanol	IC50 *Nitrosomonas*	980mg/l	Blum and Speece,1991
1,1 Dichloroethane	IC50 *Nitrosomonas*	0.91mg/l	Blum and Speece,1991
1,1,1 Trichloroethane	IC50 *Nitrosomonas*	8.5mg/l	Blum and Speece,1991
1,2 Dichlorobenzene	IC50 *Nitrosomonas*	47mg/l	Blum and Speece,1991
Acetone	75% Inhibition of IC50 *Nitrosomonas*	840mg/l; 2000mg/l	Downing,1965;Tomlinson,1966
	Nitification inhibited	8100mg/l	Hockenbury,1977
	100% Inhibition	0.14mole	Hooper,1973
Acetonitrile	Inhibitory concentration at 50%	352mg/l	Koenig A,1999
	IC50 *Nitrosomonas*	73mg/l	Blum and Speece,1991
Aniline	EC-50 *Nitrosomonas*	4.9mg/l	Ficara and Rozzi,2001
	75% Inhibition of nitrosomonas	7.7mg/l	Tomlinson,1966
	Nitification inhibited by 50%	1mg/l; 8.2mg/l	Hockenbury,1977; Koenig A,1999
Benzene	IC50 *Nitrosomonas* 50%	13mg/l ;28.4mg/l	Blum and Speece,1991; Koenig A,1999
	20 % inhibition	165mg/l	Wood,1981
Blum & Speece,1991	IC50 *Nitrosomonas*	0.71mg/l	Blum and Speece,1991
Chloroform	75% Inhibition of *Nitrosomonas*	18mg/l	Tomlinson,1966
	IC50 *Nitrosomonas*	0.48mg/l	Blum and Speece,1991
Ethanol	75% Inhibition of *Nitrosomonas*	2400mg/l	Tomlinson,1966
	Nitification inhibited	4100mg/l	Hockenbury,1977
	IC50 *Nitrosomonas*	3900mg/l	Blum and Speece,1991
Ethyl acetate	Nitification inhibited	18000mg/l	Hockenbury,1977
Ethylbenzene	IC50 *Nitrosomonas*	96mg/l 190mg/l	Blum & Speece,1991;Koenig,1999
Methanol	100% inhibition	129mg/l; 300-500mg/l	Oslislo and Lewandowski,1985;Jonsson,2001
	IC50 *Nitrosomonas*	880mg/l	Blum and Speece
Methylene Chloride	IC50 *Nitrosomonas*	1.2mg/l	Blum and Speece
N-Butanol	Nitification inhibited	8200mg/l	Hockenbury 1977
Nitrobenzene	IC50 *Nitrosomonas*	0.92mg/l	Blum,1991
N-Propanol	Nitification inhibited	20000mg/l	Hockenbury,1977
Phenol	EC-50 pure culture	1.3-5.7mg/l	Halling-Sorensen,2001
Toluene	IC50 *Nitrosomonas*	84mg/l	Blum and Speece,1991
Triethyamine	Nitification inhibited by 50%	127mg/l	Hockenbury,1977
Trimethylamine	75% Inhibition *Nitrosomonas*	118mg/l	Tomlinson, 1966
	Nitification inhibited	590mg/l	Hockenbury,1977
Xylene	IC50 *Nitrosomonas*	100mg/l	Blum and Speece,1991
	Inhibitory concentration at 50%	37.5mg/l	Koenig A,1999

1.4.5 Inhibition test methods

The importance of inhibition testing is an essential component to the effective management of a waste water treatment plant that is receiving a variable and often unknown stream. The benefit of an inhibition test method is to allow the operator to screen a new waste water and to assess the potential for this to disrupt nitrification. It can also give early warning that nitrification is stressed or likely to fail allowing for some early remedial action to be taken.

Identifying an inhibitory chemical in a mixed aqueous waste water is difficult. The responsible compound can however be narrowed down by establishing the inhibition rates for a waste water after various stages of clean up. For example a raw waste water gives a total percentage inhibition; by stripping the volatiles the % inhibition falls by a certain percentage, by passing through carbon or resins it then falls by a further percentage. This process of cleaning up a sample and analysis of the sample with greatest inhibition makes identification easier as the number of analyte components are falling (Svenson *et al.*, 2000).

The international ISO Standard 8192 (1986) specifies a method for assessing the potential toxicity of substances, mixtures or waste water to activated sludge. Activated sludge in the presence of a suitable easily biodegradable substrate will consume oxygen rapidly at a rate depending on among other factors the concentration of micro-organisms. Addition of a toxic concentration of a test material can result in a decrease in the oxygen consumption rate. The percentage inhibition of the oxygen consumption is estimated by comparison with a control mixture containing no test material. ISO Standard 9509 (1989) specifically assesses the short term inhibitory effects of test substances on nitrifying bacteria in activated sludge. The principle of the test is at a constant temperature of 20-25^{o}C parallel aeration of a nitrifying sludge in the presence and absence of a test material and assessment of the difference in concentration of nitrate produced gives an indication of the degree of inhibition (International organisation for standardisation, 1986 and 1989).

Inhibition assessments can be done using the population naturally present in a waste water plant or by using pure cultures of *Nitrosomonas* and *Nitrobacter*. The inhibition of ammonia or nitrite oxidation can measured over a four hour period in test tubes. By

comparing to a reference the percentage inhibition can be calculated (Grunditz and Dalhammar, 2001). The activity of a nitrifying sludge can be established over that of general heterotrophic activity by adding sodium chlorate to selectively inhibit *Nitrobacter* and allylthiourea to inhibit *Nitrosomonas* (Surmacz *et al.*, 1995; Lee *et al.*, 2000).

To maintain an actively nitrifying population, efficient process control requires monitoring of the toxicity and the ammonium concentration. On-line probes for measuring ammonium and nitrate continuously within a treatment system can be used. The probes are accurate and generally require little maintenance. These can be used as part of a control strategy to maintain nitrification and to give early warning of an potential problem (Rieger *et al.*, 2002). However they are limited to the point that they are not measuring the kinetics of nitrification directly, an increase in effluent ammonia may be too late to initiate corrective action (Massone *et al.*, 1998).

Hydrogen ions are a by product of the nitrification process; the number of hydrogen ions is related to amount of ammonia oxidised. Titrimetric inhibition methods are based on pH; a set point is set and the amount of caustic added to maintain the pH is a measure of the amount of protons produced due to nitrification. A side sample is pumped to the test chamber with a caustic/acid controller. Several processes determine pH: CO_2 production by heterotrophic respiration; CO_2 stripping from the aeration basin and the nitrification rate. To avoid any buffer effect it is necessary to take a sample to a side chamber and allow this buffer to be consumed. Once a pH decline occurs this is attributed to nitrification. The slope of this line is a measure of the nitrification rate. This test can be considered to be a good, reliable and cost effective measurement for nitrifier inhibitors with a +/- 10% error. This method is not affected by fouling problems or chemical interference; it does not require expensive equipment or sample pre-treatment and it is specific to ammonia oxidisers unlike oxygen uptake measurement (Massone *et al.*, 1998; Ficara and Rozzi, 2001).

Information from a titrimetric sensor was used to detect and divert streams that are inhibitory to buffer tanks for release in a safer manner. This resulted in a significant process improvement at a WWTP (Melidis and Aivasidis, 2005). A combination of the oxygen uptake rate (OUR) and a titrimetirc sensor allowed the calculation of the amount of nitrogen nitrified and the short term BOD of the sample. Operation of these two

measurements together gives a more accurate representation. OUR is limited by the fact that it relies on a number of kinetic assumptions whereas titrimetiric sensors encounter problems from falling pH due to carbon dioxide formation, also they are complicated by organic nitrogen that breaks down to release ammonia. Operation of OUR and titrimetric sensors in parallel help overcome some of these difficulties (Yuan and Bogaert, 2001). The use of allylthiourea and sodium chlorate to selectively inhibit ammonia oxidation and nitrite oxidation respectively can therefore be used to separate the nitrifier OUR from heterotrophic OUR (Kim *et al.*, 2001).

Nitrogen oxide and nitrogen dioxide are produced under oxygen stressed conditions when levels of nitrite build up. These gases can be captured and sent to an online gas analyser. The concentration of N_2O formed is directly related to the build up of nitrite and in turn to degree of inhibition (Stephenson, 2001). Measuring N_2O in the off gas from an aeration basin gave an early warning detection of nitrification failure. A strong correlation was found for shock ammonia loads, oxygen depletion and a chemical nitrifier inhibitor i.e. allylthiourea. The latter gave 6 hours advance warning before ammonia/nitrite was seen in the effluent. (Burgess *et al.*, 2002). Although N_2O formation can be observed immediately after an activated sludge was shock loaded with ammonia and when it is exposed to deprived oxygen conditions this does not always lead to increased effluent ammonia thereby questioning the benefit of this inhibition test method (Butler *et al.*,2005).

Adenosine triphospahte (ATP) is a fundamental part of cellular metabolic processes. It is therefore an attractive parameter that can be used to study the response of cells to their environment. ATP is present in relatively constant amounts in viable cells depending on their physiological states and thus is related to viable biomass and metabolic activity (Tyagi and Couillard, 1998). A dehydrogenase test can be used to measure biomass activity where a compound called INT reagent (2-p-iodophenyl-3-p-nitrophenyl-5-5phenyl chloride) turns colourless to red when it is reduced by the electron transport system. The intensity of this is read by a spectrophotometer and percentage viable biomass can be calculated that distinguishes nitrifiers from heterotrophs by using selective inhibitors (Lee *et al.*, 2000).

Fatty acid methyl ester (FAME) analysis can be used to estimate presence of nitrifying organisms, their abundance and act as an early, easy and rapid means of detecting any inhibition. FAME analysis is based on the principal that many bacteria have unique so called signature fatty acids in their membrane. Through centrifugation, extraction into solutions of KOH/Methanol, acetic acid and hexane and through GC analysis a profile of fatty acids can be compared against a library of cultures. There is no fatty acid that is unique to *Nitrosomonas* but there is one that is unique to *Nitrobacter*; 19:0 cyclo w11c fatty acid. Monitoring this fatty acid can therefore be useful in monitoring the performance and condition of a nitrifying population (Perey,2001).

1.5 Aims of the project

Schering-Plough (Avondale) Company (Fig.8) manufactures pharmaceutical active ingredients (API's) at a production plant in Co. Wicklow, Ireland. Unlike large chemical manufacturing, pharmaceutical plants tend to be smaller and are involved in more diverse chemistry with a variable and wide range of processes steps. The manufacture of finished products at this plant typically involves operations such as reactions, refluxing, crystallisations, centrifuging and drying. Over >160,000m^3/yr of an aqueous waste water is generated; this is treated at an on site Waste Water Treatment Plant (WWTP) which in turn discharges to a fresh water river.

On an annual basis over 200 different raw materials may be used, many of which end up at the WWTP. These include a wide range of complex molecules that are mostly carbon-hydrogen based but also include chlorinated, fluorinated, sulphur and nitrogen based compounds.

Fig.8. Aerial overview of Schering-Plough (Avondale) Co.

Given the site's rural location, it has no access to a municipal sewer system and consequently must discharge its treated trade effluent to the Avonmore river. This river originates from a national park in a mountainous location and falls rapidly to the sea. The river is consequently a soft water lacking calcium and it can occasionally become naturally acid. This coupled with the fast flowing conditions of the water means fish population densities are generally low. Stoneloach, Stickleback, Minnow are the main fish present. Although not a designated salmon river, salmon have been observed. The river flows through areas of special conservation, natural heritage areas and nature reserves. Management and protection of the river is therefore of national importance (Narin and Crowley,1998).

Given the importance of this river, the company is required to produce a high quality final effluent that is low in ammonia. To achieve this the Company operates its own on-site WWTP using fixed film (biotowers) and activated sludge (aeration bay) in a two stage biological process. The management strategy and design of this plant was towards carbonaceous removal and suspended solids; to this extent the WWTP has performed very well. However, the influent waste water into the WWTP contains significant levels of ammonia. This arises either directly from production operations or is a by-product of organic nitrogen compound breakdown after microbial treatment in the biotowers.

Typical influent ammonia levels ranged from 30-50mg/l NH_4-N however peaks of over 200mg/l NH_4-N have been recorded. These levels have to be biologically reduced by nitrification to less than 10mg/l NH_4-N. The Company has experienced on going difficulties in achieving this emission limit value.

The aim of this project was to study nitrification at a pharmaceutical WWTP with a view to improving the performance of this microbial process. The project was conducted in three stages:

Stage 1: Data from the full scale WWTP for the period 2000 to 2003 was studied in order to identify any relationships between nitrification and various environmental and operational conditions.

Stage 2: Significant findings of the data review were confirmed in pilot plant investigations in order to establish the critical tolerances for nitrification failure at the full scale WWTP. Options to enhance nitrification were also studied.

Stage 3: Data analysis of the full scale WWTP together with the results of the pilot plant studies were applied to the full scale WWTP in order to assess if the performance in nitrification was improved.

Chapter 2

Materials and Methods

2.0 MATERIALS/METHODS

2.1 Materials

2.1.1 Microbial Cultures

The activated sludge for the pilot plant studies was obtained from the aeration bay of the full scale waste water treatment plant at Schering-Plough (Avondale) Co., Wicklow, Ireland (Fig.9, section 2.2.1).

Pseudomonas pudia CP1 was supplied by Dr. Brid Quilty, School of Biotechnology, Dublin City University, Dublin 9.

2.1.2 Chemicals

Chemicals used in the pilot plant system are presented in Table 9.

Table 9: Chemicals used in the pilot plant

	Chemical	Chemical Formula	Supplier
pH Neutralisation	1M hydrochloric acid, sodium hydroxide	1M HCl or NaOH	Reagecon, Clare, Ireland
Electrolytes	Friscol B	n/a	Mason Technology, Dublin, Ireland
Polyelectrolyte's	Cationic polyacrylamide dispersed in a light mineral oil; Zetac 7888	(2.5-6% Alcohol, C11-14-iso-, C13-rich, ethoxylated propoxylated. <3% Naphta, <3% adipic acid).	Industrial water management, Dublin, Ireland
Antifoam	Foam Block 695	n/a	Enva, Dublin, Ireland

All other chemicals were supplied by Sigma-Aldrich Ireland Ltd. (Dublin, Ireland) unless otherwise stated.

2.2 Waste Water Treatment Systems

2.2.1 Full Scale Waste Water Treatment Plant (WWTP)

Schering-Plough (Avondale) Co. operates an on-site WWTP to treat aqueous wastes from pharmaceutical operations (Fig.9a and b). Two types of waste water streams are produced; a concentrated waste water and a dilute waste water.

The concentrated waste water, approximately 20-30m^3/d, originates directly from process reactions and is balanced in a 2,000m^3 strong waste tank. This tank has a hydraulic retention time (HRT) of 66-100 days. The dilute waste water, approximately 500m^3/d, originates from scrubbers, vacuum pumps and general cleaning. This passes through a 20mm screen to remove large solids and is subsequently balanced in one of two 1,000m^3 dilute waste tank with a HRT of 1.4-2.0 days.

After daily analysis of both the strong waste tank and the dilute waste tanks, predetermined volumes are withdrawn, pH adjusted and combined to give a target influent COD concentration of less than 7,000mg/l or less than 4.5 tonnes of COD/day. The influent ammonia concentration to the WWTP is typically <100mg/l NH_4-N. Each day 1-5 litres of phosphoric acidic is routinely added to the WWTP to ensure that the system is not deficient for phosphorus.

The first biological treatment stage takes place in two biotowers. These are packed with hanging sessile strips and operate in parallel to one another. The biotowers typically remove 50-70% of the influent COD loading. These are heated during the winter to over 15^oC. Any solids that shed from these biotowers are carried through to the aeration bay. The biotowers are fully enclosed and are aerated with a 7:1 air recycle ratio; the extracted air is sent to an odour control scrubber.

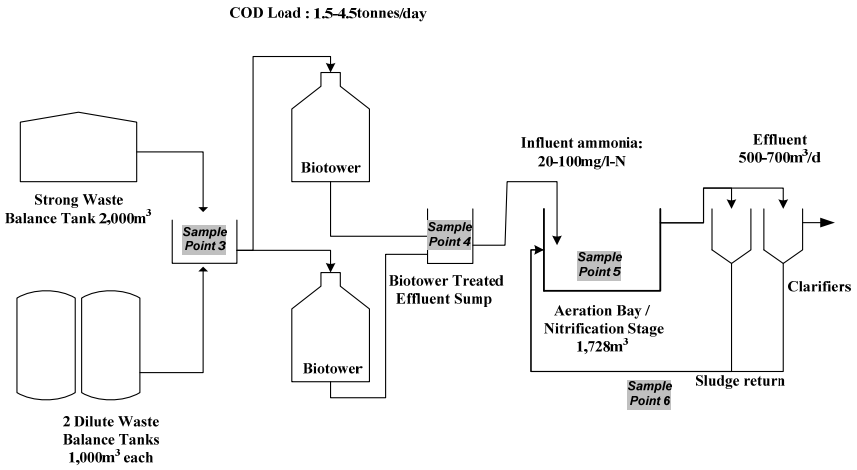
The aeration bay is an activated sludge stage; this has two parallel rows each with 4 bays giving a total volume of 1,728m^3. This is aerated by submerged fine bubble blowers that automatically ramp up and down to maintain a minimum of 3mg/l dissolved oxygen. Influent is split equally into each row and moves in a mixed/plug flow manner. The aeration bay treats 1.5-2.5 tonnes/day of COD.

The MLSS is typically 3,000-4,500mg/l; the hydraulic retention time is 3-4 days and the food to mass (F/M) as COD is normally <0.25. After clarification in one of two clarifiers, sludge is returned to the head of the aeration bay. The plant typically operates to a sludge retention time (SRT) or sludge age of 20-50 days. After biological treatment the main nitrogen derivatives in the effluent are organic nitrogen, ammonia and nitrate. The plant is operated 24 hours a day, 7 days a week, 365 days a year. The plant has a dedicated on-site laboratory. Each week day a number of points along the treatment process are sampled and tested as listed in Table 10.

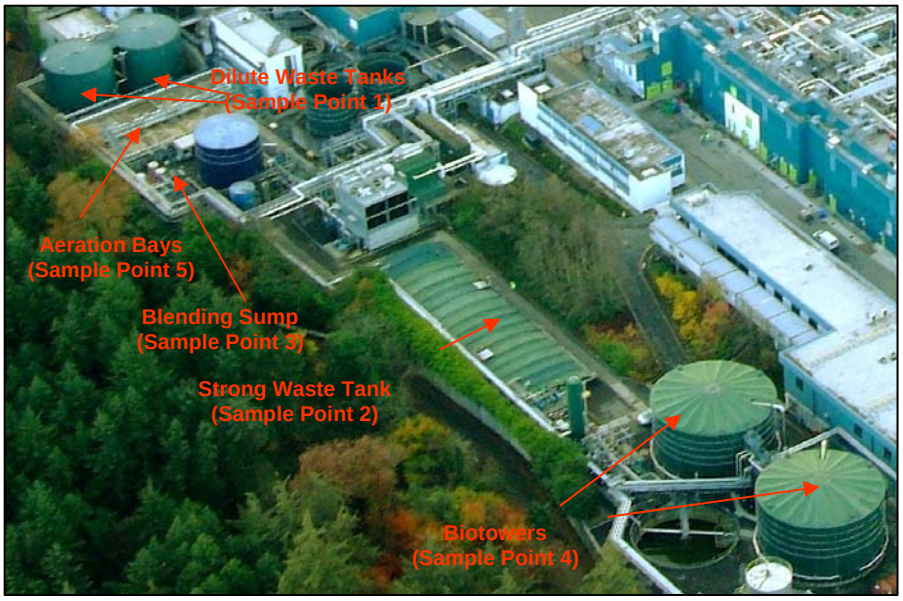
The same laboratory and the same test methods (section 2.3) were used to analyse samples from the full scale WWTP and also from the pilot plant studies.

Table 10: Sample and Testing Regime for the main WWTP

Sample Point		Parameters "✓" Yes "×" No												
		pH	Temp	O_2	MLSS / SS	SSV	BOD	COD	NH_4	NO_3	P	CL^{-1}	SO_4	F^{-1}
(1)	Dilute Balance Tanks	✓	×	×	×	×	×	✓	✓	×	×	×	×	×
(2)	Strong Waste Balance Tank	✓	×	×	×	×	×	✓	✓	×	✓	×	×	×
(3)	Blending sump	✓	×	×	×	×	×	✓	✓	×	✓	✓	✓	×
(4)	Post Biotower Sump	✓	✓	✓	✓	×	×	✓	✓	×	✓	×	×	×
(5)	Aeration Bay	✓	✓	✓	✓	✓	×	✓	✓	✓	✓	×	×	×
(6)	Return Sludge	×	×	×	✓	×	×	×	×	×	×	×	×	×
(7)	Final Effluent	✓	✓	×	✓	×	✓	✓	✓	✓	✓	✓	✓	✓



(a)



(b)

Fig. 9. Full scale Waste Water Treatment Plant at Schering-Plough (Avondale) Co. (a) schematic of WWTP and (b) aerial overview of WWTP

2.2.2 Pilot plant System

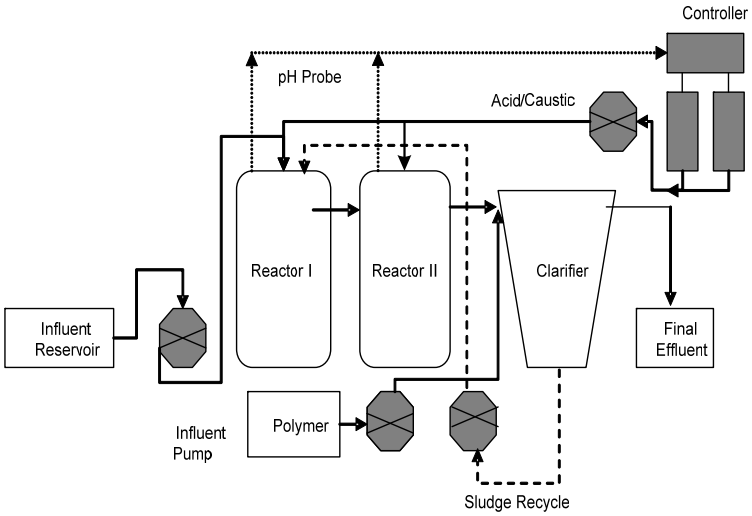
In all studies the pilot plants were first operated for at least one sludge age, typically 20 days, to establish steady state conditions. The standard operational controls used in all pilot studies, unless otherwise stated, are presented in Table 11. These were chosen as being representative when scaled up to that of the full scale WWTP.

Table 11: Standard environmental conditions for the pilot plant system

Parameter	Fixed Operational Range	Units	Test Frequency
Air flow rate	0.75-1.5	l/min/reactor	Weekly
Dissolved Oxygen	>3mg/l	mg/l	Daily
Temperature	18-22^{o}C	^{o}C	Daily
pH	7.5	-	Continuous/Daily
$^{(1)}$Sludge return (continuous)	4.8	litres/day	Daily
$^{(1)}$Sample Flow rate	5.52	litres/day	Daily
$^{(1)}$Hydraulic Retention Time (HRT)	3.6	days	Daily
Antifoam	0.012-0.024	litres/day	Daily
Polymer	0.048-0.050	litres/day	Daily
Sludge Retention Time	20-50	days	Daily
Mixed Liquor Suspended Solids (MLSS)	3,000-6,000	mg/l	Daily

Note (1) for continuous feed mode only.

Two identical pilot plants were commissioned. Each system consisted of two 10 litre cylindrical reactors with an inlet/outlet and inter-connecting over flow (Fig.10 a and b). After the second reactor there was a 10 litre conical clarifier with sludge recycle back to the first reactor. Submerged aeration from a compressed air line was used to aerate both reactors. No direct temperature control was applied other than ambient heating from the room. The temperature of the liquor was maintained between 18-22^{o}C. The pH was automatically controlled using on line pH probes linked to an acid or caustic controller. Antifoam and polymer were continuously dosed into the clarifier.



(a)

(b)

Fig.10. Schematic and photograph of the pilot plant system

2.2.2.1 Operational control

Prior to the commencement of any study the full system was drained down, washed with water and a fresh nitrifying activated sludge was added. This was obtained from the full scale WWTP where a 40 litre grab sample was taken from the last cell of the aeration bay (sample point 5, Fig.9a). This point of the aeration bay was fully aerated and well mixed to ensure a homogenous sample was taken. The sample was split equally into two clean 25 litre plastic containers upon which they were taken to the pilot plant system within 30 minutes. The contents were re-agitated for 5 minutes by shaking/inverting the container and 10 litres was poured into each of the four reactors. Aeration was immediately applied.

The pilot plants were operated in either a batch mode or in a continuous feed mode.

Batch Mode

In batch mode operation the overflow between each reactor and the clarifier was sealed to prevent any intermixing. Each reactor was therefore isolated and was considered to be a stand alone unit. The starting volume of liquid was 9 litres, the level of which was marked on the outside with indelible ink. Prior to sampling accumulated sludge on the side walls was dislodged by scraping back into the reactor and any water losses through evaporation was replaced to the indelible ink mark with de-ionised water. A 100mls sample was taken each day and the new liquid level was marked. There was no feed into the reactors or sludge wasted other than what was removed for testing.

Continuous Feed Mode

In the continuous feed mode, waste water feed was pumped into the first reactor of each pilot plant using a peristaltic pump at a rate of 230mls/hr or 5.52litres per day. This in turn overflowed to the second reactor and in turn to the clarifier from where settled sludge was returned to the first reactor at a recycle rate of 200mls/hr. The overall hydraulic retention time equated to 3.6 days.

To achieve a minimum dissolved oxygen level of 3mg/l, submerged aeration from a compressed air line was used to aerate both reactors at a rate of 0.75 litres air/min/reactor. This was increased to a maximum of 1.50 litres/min/reactor under high COD loadings. The air flow rates were calculated by connecting the compressed air line to a 10 litre teflon gas sampling bag; the length of time taken to fill this bag was recorded. Using a calibrated gas sampling pump the contents of the bag were pumped down and the volume recorded. The volume of air per unit time was therefore established.

2.2.2.2 Waste Water Feed

The waste water feed for the pilot plant systems was the waste water generated by Schering Plough (Avondale) Co. Wicklow, Ireland. This waste water was balanced in the strong waste tank (sample point 3, Fig.9, section 2.2.1). The typical chemical composition of this waste water is listed in Table 12.

Table.12. Typical composition of the waste water feed in the strong waste tank

Component	Typical Range	Component	Typical Range
Organics		**Inorganics**	
Acetone	5,000-10,000mg/l	Ammonia	5-200mg/l
Dimethyl Foramide (DMF)	100-1,000mg/l	Calcium	500-1,000mg/l
Methylene Chloride	100-500mg/l	Carbonate ($CaCO_3$)	500-1,000mg/l
Ethanol	500-5,000mg/l	Chloride	500-1,000mg/l
Hexane	500-1,000mg/l	Fluoride	250-750mg/l
Isopropanol	3,000-10,000mg/l	Magnesium	10-50mg/l
Methanol	5,000-10,000mg/l	Potassium	10-25mg/l
Tetrahydrafuran (THF)	500-1,500mg/l	Phosphates	1-10mg/l
Toluene	500-1,000mg/l	Sodium	10-25mg/l
Xylene	100-500mg/l	Sulphate	500-2,000mg/l

An 800 litre sample was taken from this tank and stored in a 1,000litre intermediate bulk container (IBC), this represented the primary stock solution. For each study a secondary stock solution was created by diluting the primary stock $^1/_5$ with deionised water. This was stored in 200litre steel drums. The secondary stock feed solution was analysed ten times for ammonia, nitrate and COD. As illustrated by Fig.11 the mean ammonia level was 172mg/l NH_4-N with a minimum of 168mg/l NH_4-N and a maximum of 184mg/l NH_4-N.The 95% confidence interval (CI) for the mean was 168mg/l to 177mg/l NH_4-N. The mean nitrate level was 5mg/l NO_3-N with a minimum of 0mg/l NO_3-N and a maximum of 10mg/l NO_3-N. The 95% CI for the mean was 2.3mg/l to 7.7mg/l NO_3-N. The mean COD concentration level was 19,956mg/l with a minimum of 19,100mg/l and a maximum of 21,500mg/l. The 95% CI for the mean was 19,369mg/l to 20,515mg/l.

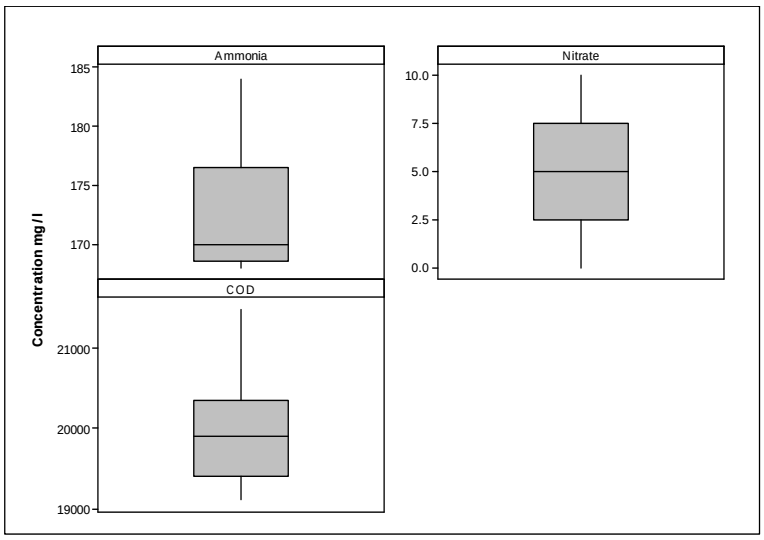


Fig.11. Box plot of 10 samples of the secondary stock feed solution for ammonia, nitrate and COD taken from the strong waste tank at the full scale WWTP.

2.2.2.3 Modification of the Waste Water Feed

Varying the Influent Chemical Oxygen Demand (COD)

For each pilot plant study the secondary stock feed solution was diluted with de-ionised water to a target COD level which was measured as outlined in Section 2.3.4. Each time this dilution was performed a sample was taken and analysed in duplicate. If this was more than 10% from the target COD concentration the diluted feed was re-made until it was within specification.

Varying the influent carbon to nitrogen (C/N) ratio

The waste water feed was taken after the biotower at the full scale WWTP (sample point 4, Fig.9, section 2.2.1). A 12 litre sample was taken each day and a 48 litre sample was taken each Friday to cover the weekend period. This was tested for COD and ammonia as per section 2.3.4 and section 2.3.5. The C/N ratio was calculated by dividing the measured influent COD concentration (mg/l) by the measured influent ammonia concentration (mg/l). This waste water feed was used to represent the daily variation and fluctuation of ammonia in the waste water under typical circumstances.

Synthetic Nutrient Medium

The synthetic nutrient medium was based on a modification of the medium by Love (1999). The chemical composition of this nutrient mix is presented in Table 13. This was added directly as a solid into a 10 litre container into which the 5.5 litres of waste water was added. The container was capped and shaken a number of times to ensure that the nutrients were fully dissolved. The media was made fresh each day.

Table 13: Compounds for the synthetic nutrient. A modification of the medium by Love *et al.* (1999).

Compound	Key Element	mg added each day to 5.52 litre feed
K_2HPO_4	P	154.56
$NaHCO_3$	CO3	3,864.00
KH_2PO4	K	408.48
$MgCL_2$-$6H_20$	Mg	469.20
$MgSO_4$	S	82.80
$CaCL_2$-$2H_20$	Ca	132.48
$FeCL_3$	Fe	44.71
$MnSO_4$-H_2O	Mn	22.63
$CoCL_2$-$6H_2O$	Co	7.73
$ZnCL_2$	Zn	7.73
$CuCL_2$-$2H_20$	Cu	2.48
$NaMoO_4$-$2H_2O$	Mo	2.48
HB_3O_3	B	0.77

Preparation of the feed without the inorganic fraction

The inorganic fraction was removed from the waste water feed by taking 10 litres of the primary stock solution and adjusting to pH 14 with 50mls of 40% v/v NaOH. This solution was allowed to stand for 3 hours to precipitate any solids. The supernatant was decanted and 9 litres of supernatant was recovered. The pH adjusted supernatant was analysed in duplicate for solids, COD and ammonia and compared with the primary stock solution (Fig.12).

Adjusting the waste water to pH 14 precipitated over 77% of the total suspended solids out of solution. These solids were mostly inorganic as evident from a marginal 4% drop in COD levels. There was only a slight reduction in ammonia levels by 8%.

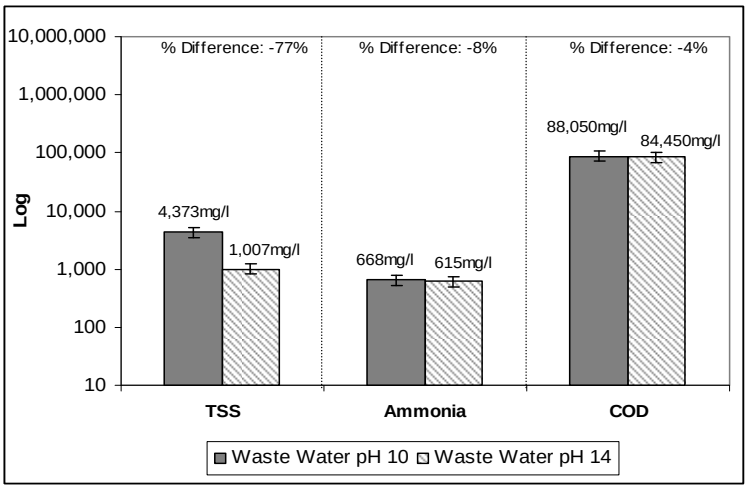


Fig. 12. **Comparison of the waste water for total suspended solids, ammonia and chemical oxygen demand (COD) from the strong waste water balance tank of the full scale waste water treatment plant before and after pH adjustment from pH 10 to pH 14 and removal of the resulting inorganic fraction by settlement.**

2.2.2.4 Pilot Plant System Modifications

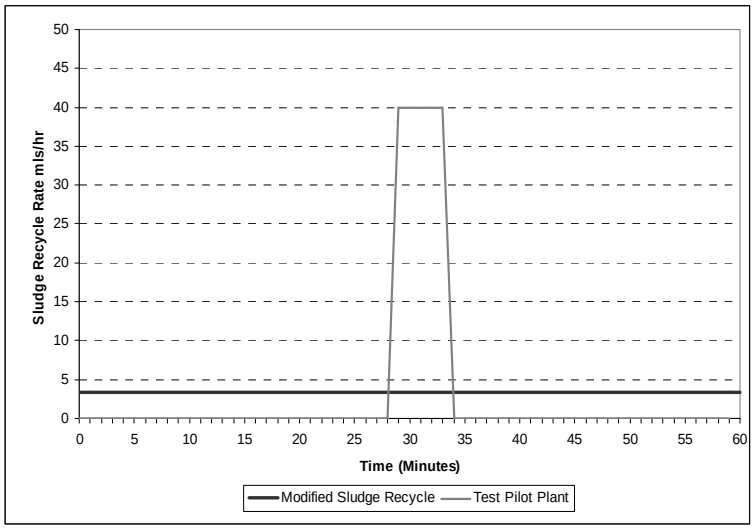
For certain studies the pilot plant system was modified from the standard configuration.

Modification of the sludge recycle rate

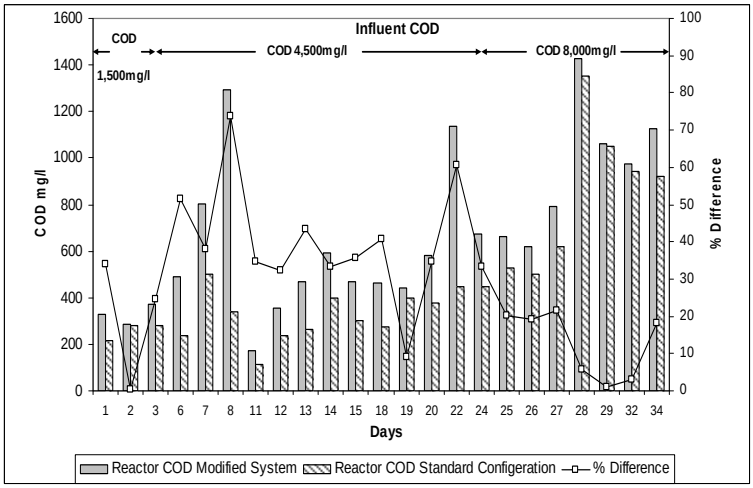
The sludge return ratio to the influent flow on the pilot plant system was normally set at a continuous rate of 1.15. The sludge recycle was modified by maintaining this ratio at 1.15 however the pumping profile was altered. This was configured such that the sludge recycle pumped each hour over a 5 minute period with no flow for the remaining 55 minutes. This pumping profile is illustrated in Fig.13a.

Although the overall volume recycled in any one hour was the same this intermittent pattern created higher levels of COD within the first reactor of the modified system compared to the standard configuration. The first stage of the modified system had a mean reactor COD value of 679mg/l with a 95% CI of 524mg/l to 826mg/l compared to that of the standard configuration with a mean of 479mg/l and a 95% CI of 346mg/l to 612mg/l.

A two sample T-Test confirmed the means were statistically different with a P value of 0.046 thereby passing the 0.05 hypothesis acceptance criteria. Overall COD levels were on average 30% higher in the first reactor of the modified system (Fig.13b).



(a)



(b)

Fig.13. Influence on nitrification in the pilot plant system by varying the sludge recycle profile under varying chemical oxygen demand (COD) loadings; (a) sludge recycle profile and (b) COD levels in the first reactor of the modified system compared to the standard configuration

Two stage system

The pilot plant configuration was modified by placing an Eckenfelder reactor (Fig.14) of 10 litre capacity before the standard pilot plant arrangement. The first reactor of the standard pilot plant configuration was isolated to keep the overall capacity i.e. 20 litres and therefore the HRT the same as the control system.

The modified system had two clarifiers and two separate independent sludge recycle systems; the sludge recycle from the Eckenfelder reactor returned sludge only to this reactor. Equally the second stage sludge recycle returned sludge only to this section. Effluent from the first stage was collected in a reservoir and this was then pumped to the second stage.

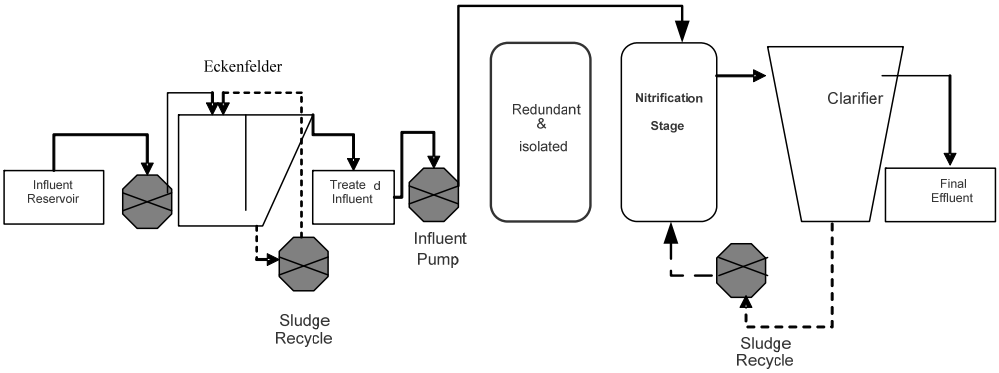


Fig.14. Test pilot plant configuration for two stage trial

Addition of Activated Carbon

Determination of potential contaminants from clean unused activated carbon was studied by adding 2g of activated carbon into one litre of deionised water. This deionised water was pre-tested for ammonia, nitrates and COD. The 1 litre carbon water slurry was mixed for one hour after which it was allowed to settle for 30 minutes. 200mls of the supernatant was decanted and filtered; the filtrate was tested for ammonia, nitrate and COD.

The chemical adsorption affinity of the activated carbon was determined; the concentration of COD, ammonia and nitrate in the stock feed solution was measured before and after contact for 60 minutes with various concentrations of activated carbon. The secondary stock feed solution was diluted to a target COD of 5,000mg/l. As nitrate levels in the stock solution were low, 300mls of a 1,000mg/l NO_3-N standard stock solution was added to 3,700mls of the diluted stock feed solution. This stock feed was then analysed in triplicate for ammonia, nitrate and COD. 1g, 2g and 3g's of activated

carbon was added each to 1 litre glass jars. The volume was made up to the 1 litre mark with the stock feed solution. Each jar now had an activated carbon concentration of 1,000mg/l, 2,000mg/l and 3,000mg/l respectively. A control was also provided; this had 75mls of the 1,000mg/l NO_3-N standard stock solution and 925mls of the stock feed solution. There was no activated carbon added to the control. The pH of the four jars was adjusted to pH 7.5. The content of each jar was stirred for 60 minutes; after which it was allowed to settle for 30 minutes. 100mls of supernatant was decanted and filtered. The solution was analysed in triplicate for COD, ammonia and nitrate.

In the experimental method, activated carbon was weighed and mixed with 25mls of the stock feed solution for 1 hour. This slurry was then added to the influent feed. The measured MLSS concentration (section 2.3.7) for the test system was adjusted for the quantity of activated carbon added by noting how much was added each day minus what was removed through sludge wastage.

Addition of buoyant plastic media (BPM:- Kaldnes®)

Four litres of a BPM was added to the first reactor of the standard pilot plant configuration (Fig.15). The BPM used was Kaldnes® K1 (European parent no.0,575,314; US Patent no. 5,458,779). Each item of BPM was 10mm in diameter with a surface area of 0.0005m^2/media or 500m^2/m^3. This media was obtained from Enpure (WORCS,UK). After 24 days of operation the sludge recycle was retuned to the second reactor only.

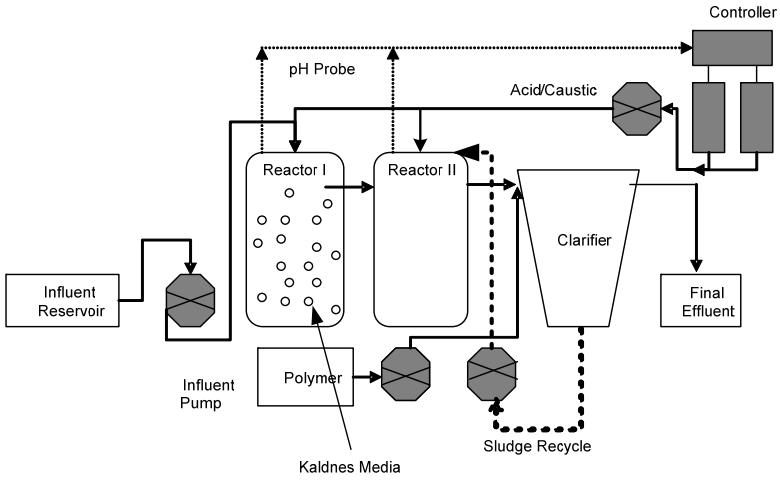


Fig.15. Schematic of the operational set up of the Test Pilot plant. The sludge recycle was only moved to the second reactor after Day 24.

Calculation of the number of BPM:- A clean 100ml glass beaker was oven dried (4 hours at 110^{o}C). This was allowed to cool for 1 hour in a dessicator. This was then placed on a balance and zeroed; 20 individual BPM were carefully counted and placed into the beaker. This beaker was placed in an oven for 4 hours at 110^{o}C upon which it was removed and placed in a desiccator for one hour to cool. The beaker was re-weighed; the weight divided by 20 represented the average weight of one clean BPM.

A clean/dry (4 hours at 110^{o}C) glass beaker was weighed and loosely filled with BPM to the 1 litre level. This was placed in an oven for 4 hours at 110^{o}C upon which it was removed and placed in a desiccator for one hour to cool. It was then reweighed; the number of BPM in the pilot plant reactor was therefore calculated by Equation 1.

Equation 1: Calculation of the number of BPM in the pilot plant reactor

$$\textbf{Number of BPM} = \left(\left(\frac{\text{Net Weight of 1 litre of BPM}}{\text{Weight of 1 BPM}} \right) \text{ X } 4 \right)$$

Determination of biomass on BPM:- The quantity of biomass adhered to the BPM was determined by taking twenty of the unused clean BPM and twenty BPM from test pilot plant after 5-6 weeks in the reactor. These were placed into individual clean dried and zeroed glass beakers (4 hours at 110^{o}C). The two beakers were in turn placed in an oven for 4 hours at 110^{o}C. 20 clean BPM was added to the test pilot plant to compensate for those removed and thereby maintaining the same number through out the study. After drying, both beakers were removed and placed in a desiccator for one hour to cool upon which they were re-weighed. The amount of biomass adhered to one BPM and the total biomass expressed as a MLSS equivalent was calculated by Equation 2 and 3 respectively.

Equation 2: Calculation of biomass adhered to one BPM

$$\textbf{Biomass in mg on 1 BPM} = \frac{\text{Weight (mg) of 20 BPM from Pilot Plant - Weight (mg) of 20 clean BPM}}{20}$$

Equation 3: Calculation of the MLSS equivalent

$$\text{MLSS equivalent mg/l of suspended MLSS and BPM biomass} = \frac{\text{Biomass (mg) of 1 BPM x Total number BPM}}{10}$$

On average, there was 3,736 individual BPM in the test pilot plant giving an additional surface area of 1.87m^2. In total 17,878mg of biomass was adhered to the BPM. If this was all suspended in the 10 litre reactor it would have had an MLSS equivalent of 1,788mg/l (Table 14).

Table 14: Calculation of number of individual BPM media and resultant Biomass growth in the Test Pilot plant

	Day Sampled			
	Day 36	**Day 44**	**Day 46**	**Average**
Net Weight of 20 clean/dry BPM	3.3170	3.3670	3.3300	3.3380
Net Weight/ 20 = average weight of one BPM	0.1658	0.1683	0.1665	0.1669
Weight of 1 litre of BPM	155.852	155.677	155.844	155.791
Number of BPM media in I litre	940	925	936	934
Number of BPM media in Test Pilot plant (x 4)	3,760	3,703	3,744	3,736
Surface area m^2	1.88	1.85	1.87	1.87
Weight of 20 used BPM from the Test Pilot plant	3.4084	3.4566	3.2769	3.3806
Minus weight of 20 clean BPM	0.0914	0.0896	0.1061	0.0957
Difference / 20 BPM by total number of BPM	17,183mg	16,589mg	19,861mg	17,878mg
Divided by 10(litres) equals MLSS equivalent	1,718mg/l	1,660mg/l	1,986mg/l	1,788mg/l

Bioaugumentation with *Pseudomonas pudia CP1*

Pseudomonas pudia CP1 was cultivated aerobically overnight at 30^{o}C in nutrient broth (Oxoid). The biomass was concentrated by centrifugation (5,000 x g, 10mins) and 250mls containing 20g of biomass was added to the test system.

2.3 Analytical Methods and Calculations

2.3.1 Calibration of Automatic Pipettes

The 100µl, 1,000µl and 10ml automatic pipettes were checked by setting the pipette to its maximum dispensing volume and pipetting this volume of de-ionised water into a dry clean and zeroed flask. This was placed on a certified balance, after stabilising the weight was recorded to four decimal places. Using 10 separate measurements the mean and standard deviation were calculated for the pipette at the volume dispensed. Using the results obtained the percentage accuracy were calculated as per equation 4.

Equation 4: Calculation of the percentage accuracy of the Automatic Pipettes

$$\text{\% Accuracy} = \frac{\text{Standard Deviation} \times 100}{\text{Mean}}$$

The procedure was repeated with the pipette set to its minimum volume i.e. 10% of the maximum volume. The acceptance criteria for accepting a calibrated pipette is outlined in Table 15.

Table 15: Acceptance criteria for the pipette calibration checks

Pipette Model	Minimum Volume Dispensed	% Accuracy	Maximum Volume Dispensed	% Accuracy
10ml	1ml	≤3%	10ml	≤0.8%
1000µl	100µl	≤3%	1000µl	≤0.8%
100µl	10µl	≤3%	100µl	≤0.8%

2.3.2 Measurement of Temperature

Temperature was measured using the hand held portable MP 120 Toledo pH meter. There was no calibration capability for this; it was however checked monthly against a certified laboratory glass thermometer. Only if it read within +/-1^{o}C was it deemed to be suitable for use.

2.3.3 Measurement of pH

pH 4, 7 and 10 buffers supplied by Radiometer Analytical (Dublin, Ireland) were used to calibrate the pH meters. These were calibrated as follows; the pH probe was removed from the reactor. At the control junction box *"CAL"* was selected. The pH probe was rinsed in water, dried with clean tissue and placed in pH buffer 10, until the meter displayed "*Complete"*. The probe was rinsed in water, dried again and placed in pH buffer 4; the meter should then read *"Successful"* for a valid calibration. If unsuccessful the process was repeated and or new pH probes were attached. The probe was then placed in pH 7; this was used as a test to ensure the calibration was carried out correctly. Only if this read pH 7.0 +/- 0.1 was the instrument accepted for use.

The pH was also cross checked using a hand held portable MP 120 Toledo pH meter. This was calibrated as follows; the instrument was switched on by pressing "*read*". The *"CAL"* button was pressed and the probe placed into a pH 4 buffer solution. After "*Autoread"* was reached the probe was rinsed and placed in a pH 10 buffer solution. The *"CAL"* button was pressed again. The meter automatically came to an end point and gave a slope expressed as a percentage. Successful calibration was achieved if the reading gave a slope of greater than 97% otherwise the calibration was repeated. The probe was then rinsed with de-ionised water, dried with clean tissue and placed in pH 7 buffer solution as a test reading. Only if this read pH 7.0 +/- 0.1 was the instrument accepted for use.

2.3.4 Measurement of Chemical Oxygen demand (COD)

COD was measured using HACH COD Vials (0-1,500ppm) containing the sample digested for 120 minutes in a HACH Digester Block preheated to 150^{o}C. Results were determined by colorimetric analysis using HACH Spectrophotometer D/2500.

A 500mg/l and 5,000mg/l COD standard was prepared. 0.25g of potassium hydrogen phthalate (KHP) was dried to a constant weight at 120°C. Following this, 0.2125g of the dried KHP was dissolved in deionised water and diluted to 500mls. This was now equivalent to 500mg/l COD. 2.5g of potassium hydrogen phthalate (KHP) was dried to a constant weight at 120°C. Following this, 2.125g of the dried KHP was dissolved in de-ionised water and diluted to 500mls. This was now equivalent to 5,000mg/l COD. Both solutions were placed into a 500ml amber bottle and stored in the fridge at 4^{o}C for

up to three months after which a fresh solution was prepared. All COD vials were from the same batch. Where more than one batch of vials was used a blank and standard was prepared for each batch. A blank was prepared by holding the vial at a 45 degree angle and pipetting 2ml of de-ionised water into the vial.

To prepare the low range standard the vial was held at a 45 degree angle and 2ml of 500mg/l COD Standard was pipetted into the vials. The test sample was prepared by pipetting 2ml of well mixed sample into the vial at a 45 degree angle. The caps were securely tightened and the contents mixed thoroughly. The vials were placed in the digestion block for 120 minutes. After digestion the vials were left in the block for twenty minutes to cool. The vials were next removed and inverted several times and allowed to cool to room temperature. The program 435 COD HR/HR Plus was selected on the DR 2500 spectrophotometer. The outside of each vial was cleaned with a damp tissue, followed by a dry tissue to remove any marks or fingerprints. The blank vial was inserted into the cell holder and *"zero"* was pressed. The display read 0mg/l COD. The standard vial was inserted into the cell holder and *"read"* was pressed. The COD result in mg/l was displayed. Where a dilution was used, the COD value in mg/l was calculated as per equation 5;

Equation 5: Calculation of the COD value

Sample COD mg/l = COD reading in mg/l x Dilution Factor

Where a high range standard was required, the procedure was repeated however in this instance 200µl of the 5,000mg/l COD standard was pipetted into the vial and the calculated COD value in mg/l was as per Equation 6.

Equation 6: Calculation of the COD value allowing for dilution

Sample COD mg/l = COD reading in mg/l x 10 x Dilution Factor

Based on the measured COD values the waste water stock feed solution was diluted with water to achieve the target influent COD concentration as per equation 7.

Equation 7: Calculation of dilution factor of stock solutions to give target COD influent concentration

$$\text{Dilution Factor (DF)} = \frac{\text{CODmg/l Stock Solution}}{\text{Target CODmg/l}}$$

$$\text{Volume to add to influent reservoir (litres)} = \left(\frac{1}{\text{DF}}\right) \text{ x Volume of Resevoir (litres)}$$

The COD removal rates per weight of the activated sludge biomass was calculated as per equation 8**.**

Equation 8: Calculation of the COD removal rates

$$\text{COD Removal Rate (mg COD/mg Biomass)} = \frac{\text{Influent COD mg/l} - \text{Effluent COD mg/l}}{\text{MLSS concentration mg/l}}$$

2.3.5 Measurement of Ammonia

Ammonia was measured using an Orion 95-12 Ammonia Electrode and an Orion 920A Meter. Standards of 1mg/l, 2.5mg/l, 7.5mg/l and 10mg/l NH_4-N were prepared using diluents of a commercially purchased and certified 1,000mg/l ammonia stock solution. A separate check solution containing 5mg/l NH_4-N using a $^{1}/_{200}$ dilution of the 1,000mg/l NH_4-N standard was also prepared: all stock solutions were discarded 6 months after opening.

The Orion meter was set to read in concentration (CON) mode. The *calibrate* function was pressed and at the prompt the number of standards to be used was entered. The electrode was placed in 50mls of the most dilute standard together with a magnetic flea. While stirring the solution at a constant rate, 0.1ml of 12.5N NaOH was added to ensure a pH of >11. When the prompt *Ready Enter Steady* appeared, the concentration value for the standard was entered in mg/l. These steps were repeated for each standard; with rinsing and drying of the electrode with de-ionised water/tissue between measurements. The electrode slope should be in the range of -54 to -60mV/decade. If the calculated slope value fell outside the –54 to -60mV/decade range, the electrode calibration was repeated.

The calibration was verified by placing the electrode in the 5mg/l NH_4-N check solution and the returned value was recorded on a quality control (QC) chart. If the electrode was continuously used over a period of time the calibration was verified once every two hours using fresh check solution and by recording the result on a QC chart. If this value had drifted outside the chart limits, the electrode calibration was repeated using fresh standards. With the meter in *measure* mode, the electrode was placed in the sample and a magnetic flea added together 0.1ml of NaOH. When the meter display reads *ready*, the measured concentration was recorded. If the measured concentration of the sample was greater than 10mg/l, the sample was diluted in a volumetric flask using de-ionised water until brought within the calibration range of the electrode.

The measured concentration was then multiplied by the dilution factor to provide the result for the sample. In investigations where a target influent ammonia concentration was required, either 50mg/l NH_4-N or 100mg/l NH_4-N, then the influent concentration of ammonia was first measured and on the basis of this ammonium chloride was added as per equation 9.

Equation 9: Calculation of quantity of ammonia to add to give desired target influent Concentration.

MC = Target NH_4-N mg/l - Measured Influent NH_4-N mg/l

Weight of ammonium chloride (mg) to add to 5.56 litres of influent = (MC/0.26) x 5.56.

Where;

MC= missing concentration mg/l
0.26= percentage nitrogen in ammonia chloride
5.56= Volume of daily influent feed in litres.

On the basis of the measured total ammonia, pH and the temperature the free ammonia levels were calculated as per Equation 10.

Equation 10: Calculation of Free Ammonia (FA) levels (Ford and Chrchwell, 1981).

$$\textbf{Free Ammonia mg/l} = \frac{17}{14} \frac{(\text{Total } NH_4\text{-N mg/l}) \times (10^{pH})}{(Kb/Kw) + 10^{pH}}$$

Kb= Ionisation constant of the ammonium equilibrium
Kw= Ionisation constant of water

The ratio has the following relationship;

$Kb/Kw = e^{(6334/273) + T)}$
T= temperature (^{o}c)

2.3.6 Measurement of Nitrate

Nitrate was measured colorimetrically using a HACH Spectrophotometer D/2500 and Nitraver 5 Pillows. A standard was prepared by pipetting 500µl of a commercially purchased and certified 100mg/l Nitrate standard into a labelled 25ml volumetric flask and making up to the mark using deionised water. 500µl of each sample was pipetted into a labelled 25ml volumetric flask and made up to the mark using deionised water. A spiked sample was prepared by pipetting 500µl of one of the samples into a labelled 25ml volumetric flask and made up to the mark with deionised water. 500µl of 100mg/l Nitrate standard was pipetted into the neck of each flask and this was mixed thoroughly. A blank sample was prepared by filling a labelled 25ml volumetric flask to the mark with deionised water.

Method number 353 was selected on the DR/2500 spectrophotometer. For each blank, standard, sample and spiked sample prepared the contents of one Nitraver 5 pillow was emptied into a labelled 30ml vial. The contents of each volumetric flask was next added to the vials, the cap secured and the first timer started (1 minute) on the DR/2500. The sample vials were shaken vigorously until the timer beeped.

The vials were then placed on a level surface and the second timer started on the DR/2500 to begin the five minute reaction period. After the five minute period, the outside of each vial was polished with a dry tissue. Zero was selected and the vial containing the blank inserted into the spectrophotometer and the instrument zeroed. The standard was inserted into the spectrophotometer. The displayed value should read 2.0mg/l +/-0.5. If outside of this range the calibration process was repeated. The

concentration of nitrate in the standard was recorded by multiplying the displayed value by 50.

As per Hooper (1973) nitrifier growth rates were calculated by plotting the natural log of the concentration of nitrate in the reactor (y-axis) against time in days (x-axis). The slope of the fitted line was equivalent to the nitrifier growth rate.

2.3.7 Calculation of the Percentage Nitrification and the Nitrification Rate

The Percentage nitrification and the nitrification rates per gram of MLSS were calculated as per Equation 11 and 12.

Equation 11 : Calculation of Percentage Nitrification

$$\textbf{\% Nitrification} = \left(\frac{\text{Effluent Nitrate mg/l}}{\text{Effluent Ammonia mg/l + Nitrate mg/l + Nitrite mg/l}} \right) \text{ x } 100$$

Equation 12: Calculation of the nitrification rate

(1)

Nitrification Rate mg NH_4-N/litre/hr =

$$\left(\frac{\text{mg/l of } NO_3\text{-N (Time x) - mg/l of } NO_3\text{-N (Time 0)}}{\text{Time x}} \right) /24$$

Time 0 = concentration of nitrate at start of test
Time x-= concentration of nitrate at end of test (days)

(2)

Nitrification Rate Relative to MLSS
mg NH_4-N/litre/hr/g MLSS = $\dfrac{\text{Nitrification Rate}}{\text{MLSS (mg/l)/1000}}$

2.3.8 Measurement of Mixed Liquor Suspended Solids (MLSS)

A 25ml sample was taken from each reactor and the MLSS was measured gravimetrically in duplicate. The balance was allowed to warm up for five minutes prior to use. The accuracy of the balance was checked each day by using a certified 1g check weight.

A cool clean and oven dried beaker was placed on the balance and this was zeroed. Labeled Whatman GF/C 90mm filter papers as supplied by Reagecon, Clare, Ireland, were placed into beakers and heated in the oven for more than 4 hours at 110^{o}C. They were allowed to cool for half an hour and were re-weighed and the weights were recorded. A 25mls sample was filtered onto the labelled Whatman glass fibre filter. This sample and filter were dried in an oven at 110^{o}C for 4 hours and then allowed to cool in a desiccator for 24 hours after which the filters was removed and weighed and this weight recorded. The MLSS was calculated as per Equation 13.

Equation 13: Calculation for MLSS

$$\textbf{Suspended Solids/MLSS mg/l} = \frac{(A - B) \times 1x10^{-6}}{\text{Sample Volume (mls)}}$$

Where:

A= weight of filter paper, beaker and dried residue (g)

B= weight of filter paper and beaker (g)

2.3.9 Measurement Suspended Solids

Suspended solids were measured as per the MLSS method (Section 2.2.3.8) except the sample volume filtered was 100mls.

2.3.10 Measurement of dissolved oxygen and the oxygen uptake rate (OUR)

Dissolved oxygen was measured using a hand held portable Metler Toledo MP 120 portable DO Meter. This was calibrated by selecting the *"% Oxygen Mode"*. A one point calibration was carried out daily by placing the probe in a sealed bottle with a water wet sponge for 30 minutes to allow a 100% saturation environment to develop. After the auto end point was reached *"Read*" was pressed. Pressing "*Mode"* converted

the reading to mg/l O_2. A two point calibration was carried out weekly by placing the probe in a zero oxygen standard. A quality control check was carried out daily by vigorously aerating water for 30 minutes with an air pump and fritted head diffuser to its oxygen saturation point. If this read <97%, the instrument was recalibrated until a valid calibration was achieved.

Oxygen Uptake Rate (OUR) of the Mixed Liquor Suspended Solids (MLSS)

A 500ml sample was removed from the last reactor of each of the pilot plants and this was vigorously aerated for 5 minutes until the dissolved oxygen was above 7mg/l. To distinguish the OUR associated with heterotrophic activity with that associated with ammonia oxidation the starting ammonia concentration in the test liquor was measured. 400mls of this sample was poured into a biological oxygen demand (BOD) bottle, a magnetic flea was added and placed on a magnetic stirrer. A dissolved oxygen (DO) probe was promptly inserted, ensuring there was no headspace in the BOD bottle and after allowing the DO reading to settle for 2-3 minutes the first DO value was recorded as time 0mins. Subsequent readings were taken every 2 minutes over a 20 minute period or until the DO levels fell below 1mg/l. The sample was retested for ammonia and the original 500ml solution was returned to the pilot plant. The OUR was calculated as per Equation 14.

Oxygen Uptake Rate (OUR) of the Buoyant Plastic Media (BPM)

A one litre sample was taken from the reactor containing the BPM and the activated sludge was allowed to settle for 30 minutes. 600mls of supernatant was recovered and retained in a 1 litre glass beaker. The settled sludge and the BPM were returned to the pilot plant. Using a glass beaker a 160mls sample was taken (40% V/V of 400ml BOD Bottle) from the first reactor containing the MLSS and BPM.

The liquor was discarded and the BPM retained in the 100ml beaker. This was washed gently with the 100mls of supernatant to ensure that all of the activated sludge was removed. This liquor was discarded and the BPM added to the remaining 500mls of collected supernatant. The solution was aerated for 10 minutes and all the BPM and 100mls of supernatant were added to a BOD bottle and the OUR was undertaken as normal.

Equation 14: Calculation for the Oxygen Uptake Rate (OUR)

$$\text{Total OUR g } O_2\text{/Hr} = A = \left(\left(\frac{\text{DO (0 mins)} - \text{DO (20 mins)}}{20} \right) \text{X } 60 \right) / 1{,}000$$

$$\text{Nitrification OUR g } O_2\text{/Hr} = B = \left(NH_4\text{-N (0 mins)} - NH_4\text{-N (20mins)} \right) \text{X } 4.5$$

$$\text{Heterotrophic OUR g } O_2\text{/Hr} = C = A - B$$

$$\text{Heterotrophic OURg } O_2\text{Hr/g MLSS} = C / (\text{MLSS (mg/l)} /1000)$$

2.3.11 Measurement of the Total Phosphorus and Orto-Phosphate.

A 10mg/l phosphorous standard solution was prepared by dissolving 0.0439g of anhydrous potassium dihydrogen phosphate (KH_2PO_4) into 1 litre of deionised water. Using this solution a 2mg/l P standard was prepared by pipetting 5ml of the 10mg/l P standard into a labelled 25ml volumetric flask and this was made up to the mark with deionised water. A 8mg/l P standard was prepared by pipetting 20ml of 10mg/l P standard into a labelled 25ml volumetric flask and this was made up to the mark with de-ionised water.

Each sample was filtered and placed into a labelled 50ml conical flask for acid digestion. To each sample the contents of one potassium persulfate powder pillow was added together with 2ml of 5.25N sulphuric acid, the contents were mixed gently. Each flask was placed onto a pre-heated hotplate and boiled gently for 30 minutes concentrating the sample to less than 20mls but not allowing the solution to boil dry. After concentrating the sample the volume was made up to 20mls by adding de-ionised water. The conical flasks were removed from the hotplate and the samples allowed to cool to room temperature. Once cooled, 2mls of 5N sodium hydroxide was added into each conical flask and mixed gently.

Programme "*485 P react. amino acid"* was selected on the HACH DR2500 Spectrophotometer. "*Start*" was pressed and the units selected so that the total phosphorous results reported was as mg/l P. Each sample was transferred into a

labelled sample cell. 1ml of molybdate reagent and 1ml of amino acid reagent was added into each cell and the caps secured and mixed thoroughly. The timer on the DR 2500 was pressed to start the ten minute reaction period. A blue colour appeared if phosphorous was present. A blank vial containing de-ionised water was cleaned with a damp tissue, followed by a dry one to remove any marks or fingerprints. The vial was inserted into the cell holder and "*zero*" pressed. The same procedure was repeated for the 2mg/l and 8mg/l standard, by placing each vial into the cell holder and pressing "*Cal*". The instrument was now calibrated and ready to read the samples. These were inserted and "*Read*" was pressed. The result of the sample was calculated as per equation 15.

Equation 15: Calculation of Phosphorous

$$\text{Result, mg/l P} = \text{Reading, mg/l P} \times \text{Dilution Factor}$$

To determine the concentration of Ortho-Phosphate (reactive phosphorous) the procedure was repeated as above with a fresh sample however no acid digestion was applied.

2.3.12 Measurement of Inorganic Carbon (Alkalinity)

The pH meter was calibrated as per section 2.3.3. 50mls of a filtered sample was placed into a 100ml beaker containing a magnetic flea and this was placed on a stirring plate. The pH electrode was inserted into the sample and the pH reading recorded.

Using an automated pipette in "*titrate"* mode, 0.02N sulphuric acid was slowly added to the sample. When the pH reached a stable value of 4.5, the total volume of sulphuric acid added was recorded.

The alkalinity of the sample was calculated as per equation 16.

Equation 16. Calculation of the alkalinity of a waste water sample

$$\text{Alkalinity, mg } CaCO_3/l = \frac{A \times N \times 50{,}000}{\text{ml sample}}$$

Where:

A = ml standard acid used

N = normality of standard acid

2.3.13 Nitrifier gene probe stain & microscopy

Viable nitrifiers were viewed using a Nitri-VIT kit as supplied by Vermicon AG (Munich, Germany). The stained slides were viewed using a Hund Wetzlar (Hesse, Germany) fluorescent microscope with attached red and green filters. A glass slide, provided with the commercial Vermicon test kit, was mounted into the cap of a VIT reactor ensuring that the clear end of the slide (where the Verimcon logo is positioned) is securely fixed into the cap and that the wells were facing upwards. The slide has three wells marked "+", "-" and "VIT" (Fig.16.)

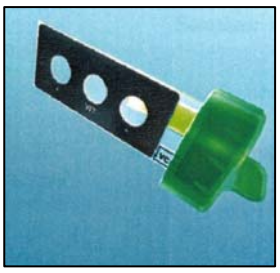


Fig.16. Vermicon glass slide

Distribution of nitrifiers within the MLSS was determined by taking a 50ml sample from the aeration bay of the full scale WWTP and from one of the reactors of the pilot plant system. Each sample was stirred and using a plastic pipette 1-2 drops were placed onto each of the three wells of a Vermicom glass slide. The 50 ml samples were then allowed to stand for 30 minutes after which 1-2 drops of the supernatant was placed onto each of the three wells of a Vermicom glass slide. The slides were allowed to dry in an incubator at 46^{o}C for 20 minutes or until it was completely dry. These slides were then stained for viable nitrifiers.

One drop of Solution A was placed into each well and the slide was returned to the incubator for 15 minutes or until it was completely dry. One drop of Solution B2 was placed into each well and the slides were returned to the incubator for 15 minutes or until it was completely dry. The slide was now fixed. 30ml of the washing solution was next prepared using a $^{1}/_{10}$ dilution of Solution D1 and Sterile Water (3ml D1 + 27ml H_2O). For more than one slide the volume of washing solution was increased proportionately. The washing solution was placed in a sealable container, and allowed to heat up to 46^{o}C in the incubator for approximately 90 minutes. The kit tank was inserted into the VIT reactor and 25 drops of Solution C1 was added.

The three staining solutions were removed from the kit: Positive Control (Red Cap), VIT (Green Cap) and Negative Control (Brown Cap). One drop of each was placed onto the corresponding well on the slide (Positive Control → +, Negative Control → -, VIT → VIT). While holding the slide by the cap it was carefully inserted into the reactor and incubated at 46^{o}C for 90 minutes. The reactor was removed from the incubator and the cap and the slide was carefully removed. The reactor was placed vertically and filled to the mark with the pre-heated washing solution. The cap was replaced such that the slide is returned to the same position and it was incubated upside down with respect to its previous position for 15 minutes. The reactor was removed from the incubator and the slide was taken out, dipped in sterile water and allowed to dry at 46^{o}C for 15 minutes or until completely dry. The slide was finally finished by placing a drop of Finisher Solution in the space between the "+" and "VIT" wells and in the space between the "VIT" and "-" wells. A cover slip was gently applied to ensure diffusion of the finisher over each of the three wells. The slide was now ready to view using a fluorescent microscope.

The microscope fluorescent light source was allowed to warm up for 15 minutes prior to use. The various magnification lenses were cleaned with microscope tissue. The stained slides were placed and viewed by 500 magnification. Prior to an assessment of the sludge the positive and negative control was checked. The positive control fluoresced all cells present, including heterotrophs. This was an indication that the staining process was successful. The negative control displayed only the background interference of the test. This interference was discounted when viewing the VIT well. Switching to the VIT well; and applying the red filter any ammonia oxidisers were highlighted while the green filter highlighted any nitrite oxidisers.

2.3.14 Calculation of the Hydraulic Retention Time (HRT)

The HRT is a measure of the time in days taken for the influent to pass through the treatment system. The aeration volume of the pilot plant (20litres) divided by the influent feed (5.52 litres per day) equated to the HRT (3.62 days).

2.3.15 Calculation of the Sludge Retention Time (SRT) or the Sludge Age

The age of the activated sludge is calculated by the SRT in days. Each day a volume of the mixed liquor was removed from the reactors and discarded, this volume varied as it was dependant on achieving the targeted MLSS levels. The total volume removed including that taken for sampling was recorded. The SRT was calculated as per equation 17. Any sludge in the clarifier or in the sludge return was not included in this mass balance; however the sludge volume in the clarifier was minimized by keeping the blanket in the clarifier below 1inch from the conical base.

Equation 17: Calculation of the Sludge Retention Time

$$\textbf{SRT (Days)} = \frac{V \times M}{(Qw \times Mw) + (Qe \times Me)}$$

where:

V=Volume of pilot plant (litres)

M=MLSS mg/l

Q_w =Sludge Wastage Rate (litres/d)

M_w = Wasted Sludge MLSS mg/l

Q_e = Volume of effluent/day (litres)

M_e = Effluent Suspended Solids (mg/l)

2.3.16 Calculation of the Food to Mass (F/M) Ratio

The F/M ratio is a measure of the influent COD, i.e. food, to the proportion of activated sludge in the system i.e. the mass. On the basis of the measured MLSS levels and the measured influent COD the F/M of the system was calculated as per equation 18.

Equation 18: Calculation of the Food to Mass (F/M) Ratio

$$F/M = \frac{\text{Influent COD mg/l X Flow Rate litres/day}}{\text{(MLSS mg/l X 20)}}$$

2.3.17 Calculation and calibration of pilot plant system pumps

Master Flex L/S digital standard drives were used to automatically adjust the pH and Master Flex console drives were used for the influent feed, sludge return, anti foam and polymer dosing. The pumps were calibrated daily as per Equation 19 by measuring the time taken to fill a 25ml graduated cylinder. The pump speed was adjusted accordingly until the desired rate was achieved.

Equation 19: Calibration of Pilot plant Pumps

$$\textbf{Pump Rate mls/hr} = \left(\frac{25}{\text{Time (mins) to fill 25ml Cylinder}} \right) \text{x } 60$$

2.4 Data Analysis

2.4.1 Statistical Analysis

Minitab 14 was used for statistical analysis of data for the full scale plant and for the pilot plant studies. The following statistical tests were carried out:

Pearson's Correlation

This measured the degree of the linear relationship between two selected variables. The correlation coefficient assumed a value between -1 and +1. This was expressed as a percentage.

Regression Co-efficient

Regression analysis was used to investigate and model the relationship between a response variable and one or more predictors. The co-efficient of determination measured the proportion of the variation in the dependant variable that is shared or explained by the independent variable.

Hypothetical Testing: P value

The hypothesis test was used to decide whether to reject or accept a null hypothesis. The p-value is the probability of obtaining a test statistic that is at least as extreme as the actual calculated value, if the null hypothesis is true. That is, the p-value represents the probability of rejecting the null hypothesis when it is actually true. The smaller the p-value, the smaller the probability of making a mistake by rejecting the null hypothesis. The cut-off value for accepting or rejecting a hypothesis was 0.05.

Mean/median

The mean describes an entire set of observations with a single value represents the centre of the data set. The mean is the sum of all the observations divided by the number of observations. The median is the middle observation in the data set. It is determined by ranking the data and finding observation number [N + 1] / 2

Standard deviation

The sample standard deviation provided a measure of the spread of the data from the mean or the median.

Confidence Interval Testing

A 95% confidence interval estimate was interpreted to the mean, that is all possible samples of the same size *n* are taken , 95% of them include the true population mean are somewhere within the interval around their sample means, and only 5% of them do not.

2.4.2 Presentation of results

Results were presented in table format or in graphical format. A data distribution plot was a direct output from minitab used to summarise the distribution, range, normality, mean and medium for a single variable. A scatter plot was used for the regression analysis of two dependant variables. A three dimensional scatter plot was used in the regression analysis of three dependant variables. A time series plot was used to present changing trends over time for one or more different parameters.

Chapter 3

Results

3.0 Results

The study to investigate factors influencing nitrification at the full scale Waste Water Treatment Plant (WWTP) at Schering Plough (Avondale) Co. was conducted in three stages as outlined in Fig.17. In the first stage (Section 3.1), data from the period 2000 to 2003 were studied to identify historical causes of nitrification failure at the full scale WWTP. In the second stage of the study (Section 3.2), a pilot plant study was carried out to further investigate the factors influencing nitrification in the WWTP and in the final stage of the study (Section 3.3), the operation of the WWTP was modified and the influence of these modifications on nitrification was observed.

3.1 Study of full scale WWTP

Data from the period 2000 to 2003 was chosen as a representative time when the full scale WWTP suffered several events of nitrification disruption and or complete failure. Final effluent ammonia and nitrates fluctuated significantly during this time (Fig.18a). A data distribution plot over the same period showed the mean effluent ammonia was 10.88mg/l NH_4-N and the highest concentration recorded was 95mg/l NH_4-N (Fig.18b). A data distribution plot of the percentage nitrification showed that the mean percentage nitrification was 69.3% with a 95% CI of 64.99% to 73.69% and a higher 95% CI for the median of 93.98% to 96.31% (Fig 18b). The significant difference between the mean and median is a result of non-normally distributed data associated with a high percentage of results at 0% nitrification.

The influence of key environmental and operational factors on nitrification was investigated. The factors studied were; temperature, dissolved oxygen, sludge age, phosphorus, inorganic carbon, pH, free ammonia, influent COD, food to mass ratio (F/M) and the carbon to nitrogen ratio (C/N).

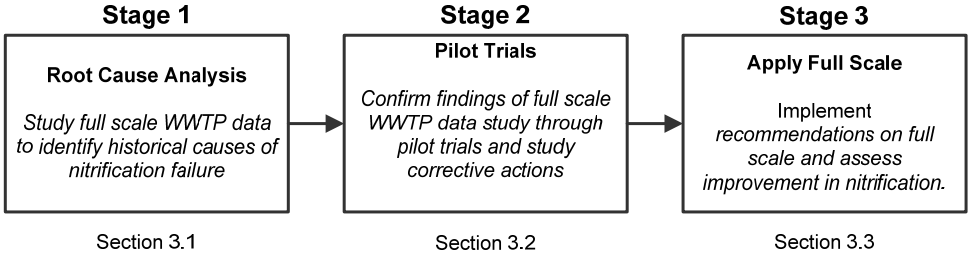
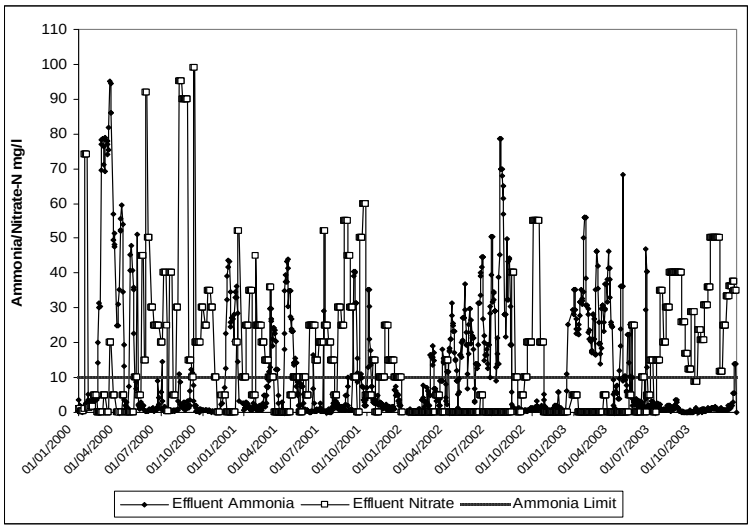
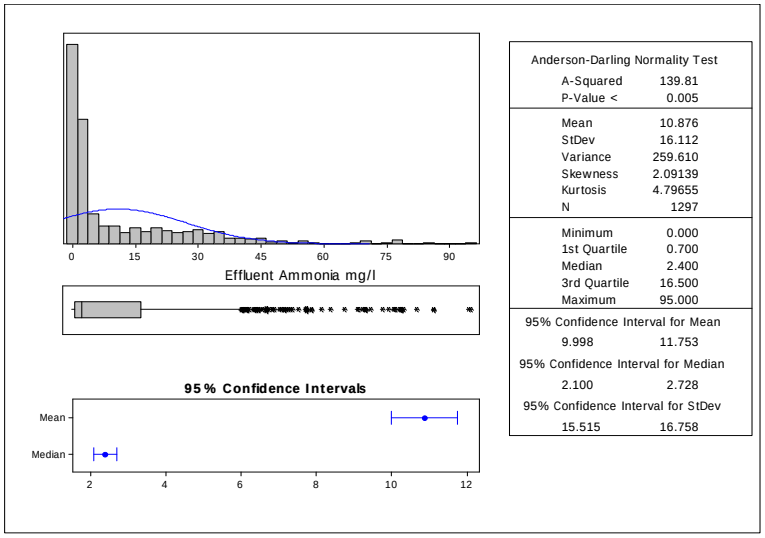


Fig.17. Stages of the study plan undertaken to investigate the causes of nitrification failure at the full scale waste water treatment plant.



(a)



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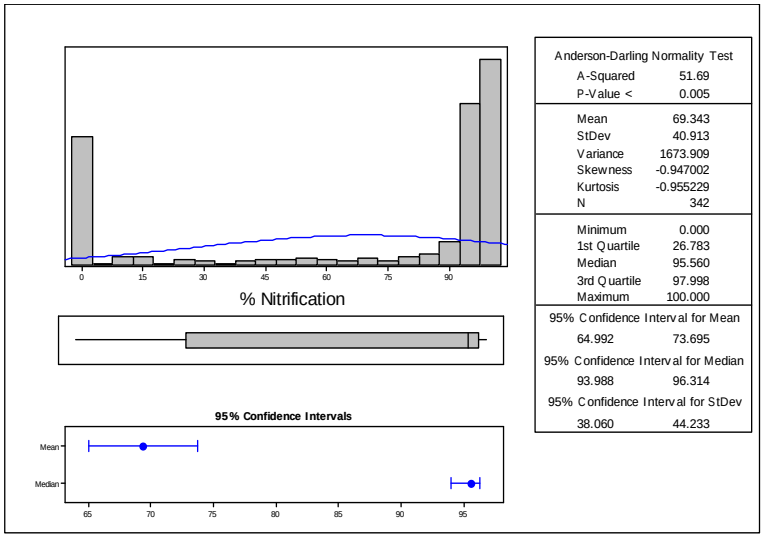


(c)

Fig.18. Nitrification at the full scale waste water treatment plant from 2000 to 2003; (a) time series plot of final effluent ammonia and nitrates and (b) data distribution plot of effluent ammonia, (c) data distribution plot of the percentage nitrification.

3.1.1 Temperature

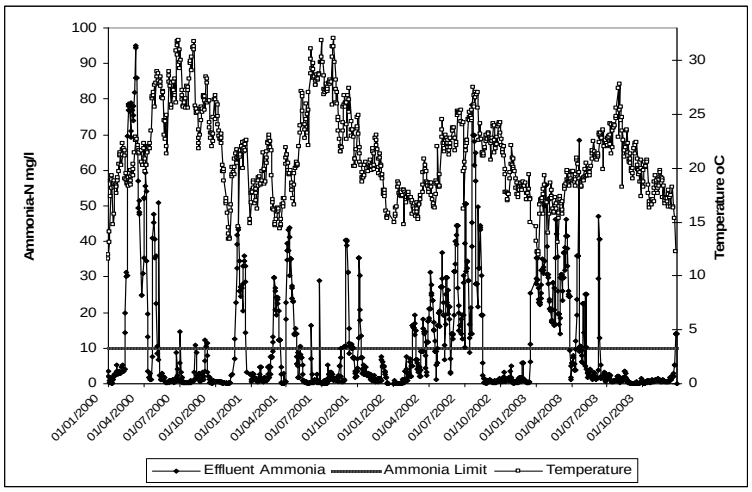
From 2000 to 2003 the aeration bay temperature followed a seasonal pattern in line with the ambient air temperature and ranged from 11.6^{o}C to 32^{o}C. The levels of effluent ammonia fluctuated considerably during this time and exceeded the ammonia limit when the temperature was at both high and low values (Fig.19a). The mean temperature of the aeration bay liquor was 21.23^{o}C with a 95% CI of 20.98^{o}C to 21.48^{o}C. The range for the median was also similar (Fig 19b). There were only 30 days when the temperature was less than 15^{o}C.

A scatter plot of temperature between 11^{o}C and 21^{o}C was undertaken against the percentage nitrification. This showed no significant relationship. This was confirmed by a regression co-efficient of 2.5%, a Pearson's correlation of only 16.4% and a P value of 0.545 which failed the hypothesis test criteria of 0.05 (Fig.19c).

3.1.2 Sludge Age

A time series plot of the sludge age showed no apparent relationship between the sludge age and final effluent ammonia levels either when the sludge age was high or when it was low (Fig.20a). The mean sludge age was 48 days with a 95% CI of 46 to 50 days with a lower median of 37 to 40 days due to a number of outliers (Fig.20b). The maximum sludge age was 285 days and the minimum was 0 days, although this was only for 11 occasions over the four year period.

A scatter plot of sludge age between 0 and 48 days and the resulting percentage nitrification returned no significant relationship (Fig.20c). The regression co-efficient was 0.3%; the Pearson's correlation 6.2% and the P value of 0.086 failed the hypothesis test criteria of 0.05. Sludge age was discounted as a causative factor for the failure of nitrification at the full scale WWTP.



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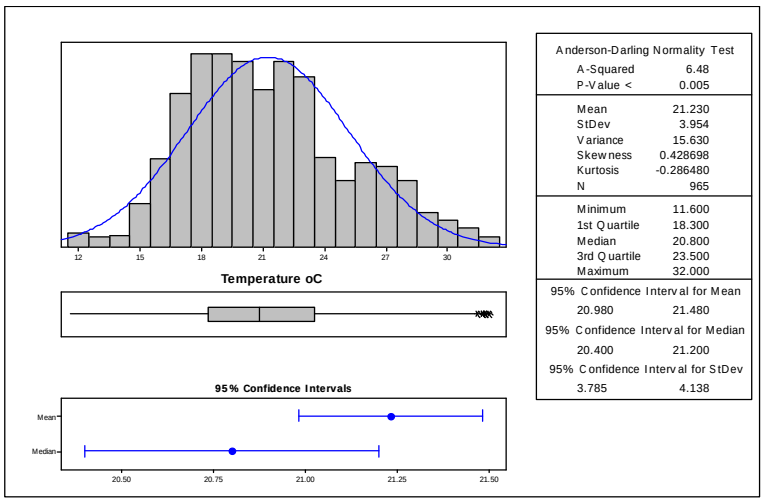

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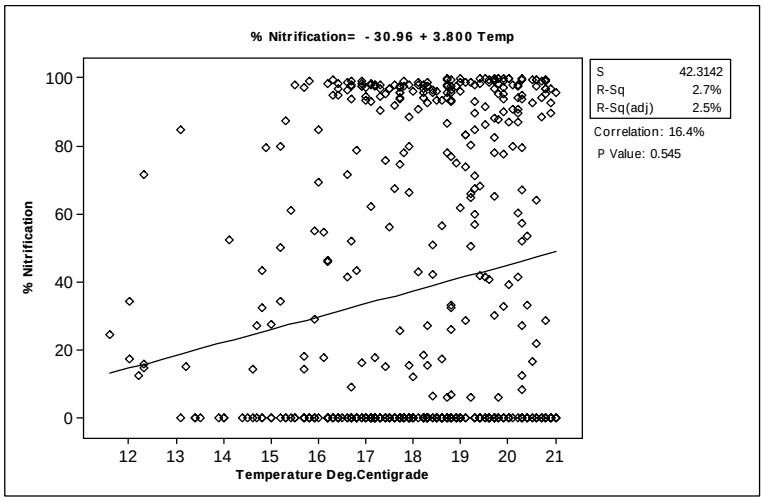
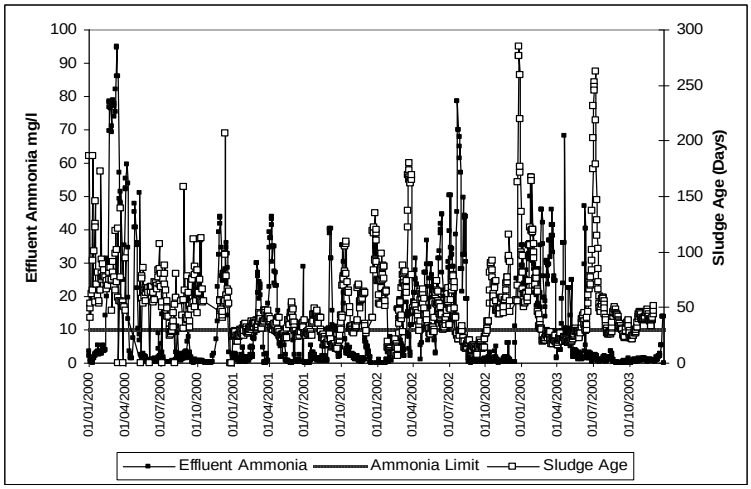
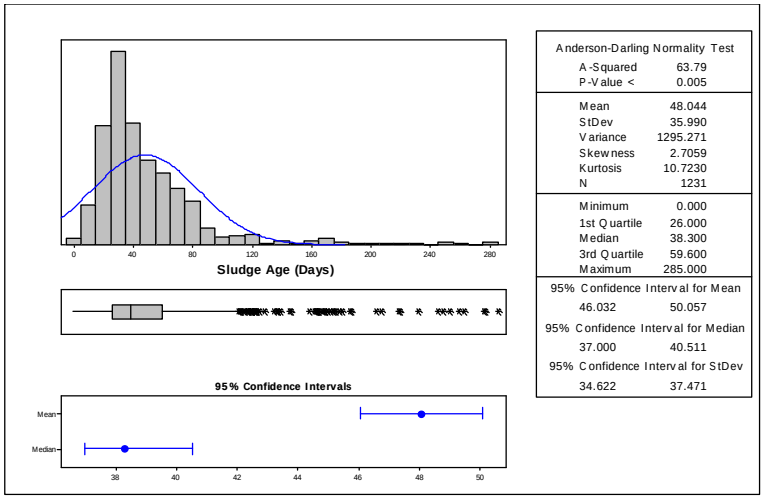


(C)

Fig.19. Aeration bay temperature at the full scale waste water treatment plant from 2000 to 2003; (a) time series plot of the aeration bay temperature and final effluent ammonia, (b) data distribution plot of the aeration bay temperature and (c) scatter plot of the aeration bay temperature below 21°C and the corresponding percentage nitrification.



(a)



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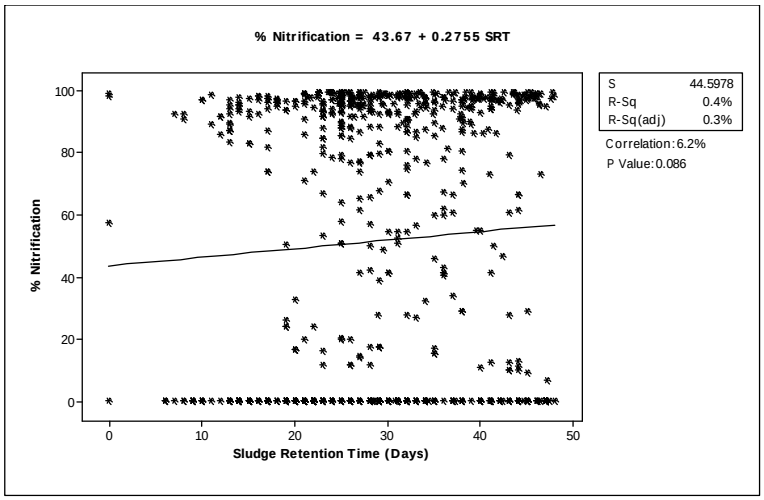


(c)

Fig.20. Sludge age at the full scale waste water treatment plant from 2000 to 2003; (a) time series plot of the sludge age and effluent ammonia, (b) data distribution plot of the sludge age and (c) scatter plot of the sludge age below 48 days and the corresponding percentage nitrification.

3.1.3 Dissolved Oxygen

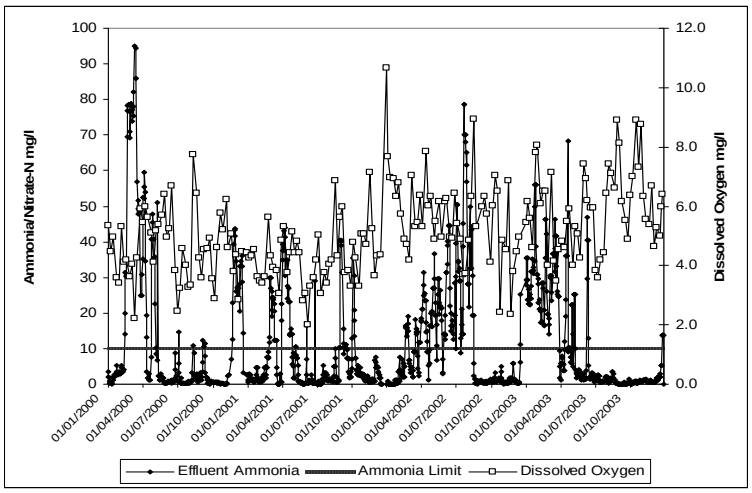
As evident from a time series plot, the concentration of dissolved oxygen was generally between 2mg/l and 10mg/l (Fig.21a). There was no apparent relationship with the levels of final effluent ammonia as elevated levels were observed when the dissolved oxygen level was low as well as when it was high.

The levels of dissolved oxygen in the aeration bay were normally distributed around a mean of 5.06mg/l with a 95% CI of 4.95mg/l to 5.16mg/l (Fig.21b). The distribution for the median was also close to this range. The lowest recorded dissolved oxygen level was 0.6mg/l. There was only 11 days over the four year period when the dissolved oxygen levels were less than 1.25mg/l. A scatter plot of dissolved oxygen levels between 1.25mg/l and 5mg/l relative to the percentage nitrification was plotted (Fig.21c). Although the P value of 0.008 passed the hypothesis acceptance criteria being less than 0.05 the result indicated that as dissolved oxygen levels increased the percentage nitrification decreased. As the statistical strength of the regression co-efficient was only 5.6% and given the weak Pearson's correlation of -25%, dissolved oxygen was therefore discounted as a causative factor for nitrification failure.

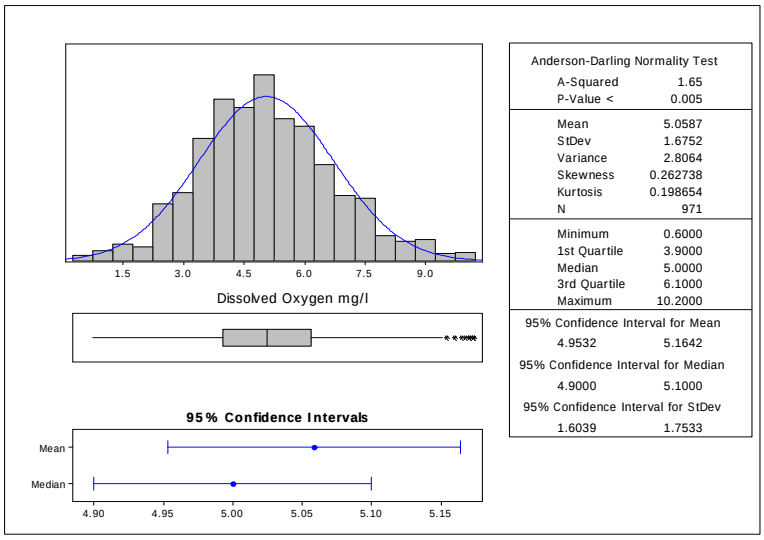
3.1.4 Phosphorus

A time series plot of the effluent phosphorus levels and ammonia levels showed that phosphorus levels varied significantly with notable peaks and troughs. High effluent ammonia was evident at both high and low levels of phosphorus (Fig.22a). A data distribution plot showed the minimum phosphorus level recorded was 0.03mg/l and the maximum was 24mg/l (Fig.22b). The resultant mean was 2.5mg/l P with a 95% CI of 2.05mg/l to 6.31mg/l, with a lower median of 2.60mg/l to 2.91mg/l due to a number of outliers. A scatter plot of the percentage nitrification and the phosphorus levels between 0mg/l and 2.5mg/l P indicated a slight positive relationship (Fig.22c). Although the P value passed the hypothesis acceptance criteria with a value of 0.006 the Pearson's correlation was nevertheless weak at 21% and the regression co-efficient was only 4.1%.

Any relationship between the percentage nitrification and the effluent phosphorus levels at the full scale WWTP from 2000 to 2003 was not statistically significant



(a)



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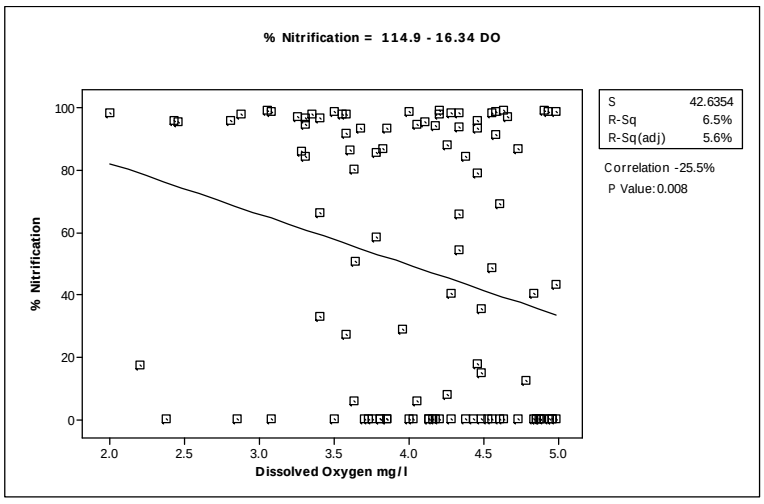
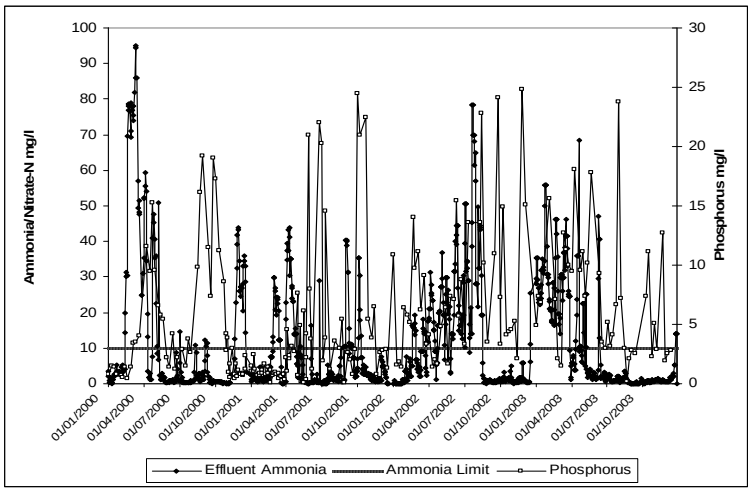
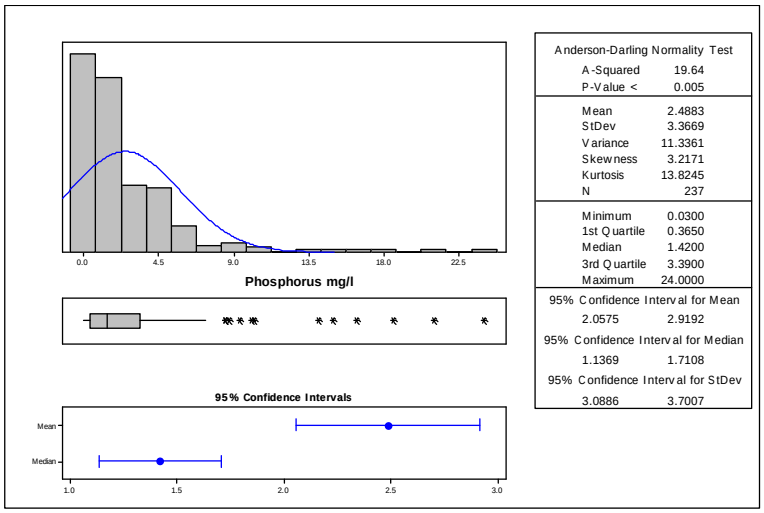


(c)

Fig.21. Dissolved oxygen levels in the aeration bay of the full scale WWTP from 2000 to 2003; (a) time series plot of the aeration bay dissolved oxygen levels and effluent ammonia, (b) data distribution plot of the aeration bay dissolved oxygen levels and (c) scatter plot of the aeration bay dissolved oxygen levels below 5mg/l and the corresponding percentage nitrification.



(a)



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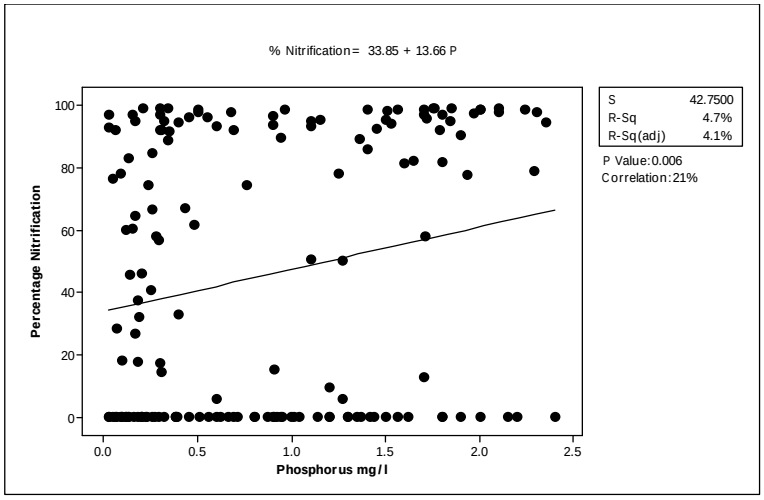


(c)

Fig. 22. Final effluent phosphorus levels at the full scale waste water treatment plant from 2000 to 2003; (a) time series plot of the effluent phosphorus levels and effluent ammonia, (b) data distribution plot of the effluent phosphorus and (c) scatter plot of the effluent phosphorus below 2.5mg/l and the corresponding percentage nitrification.

3.1.5 Inorganic carbon (Alkalinity)

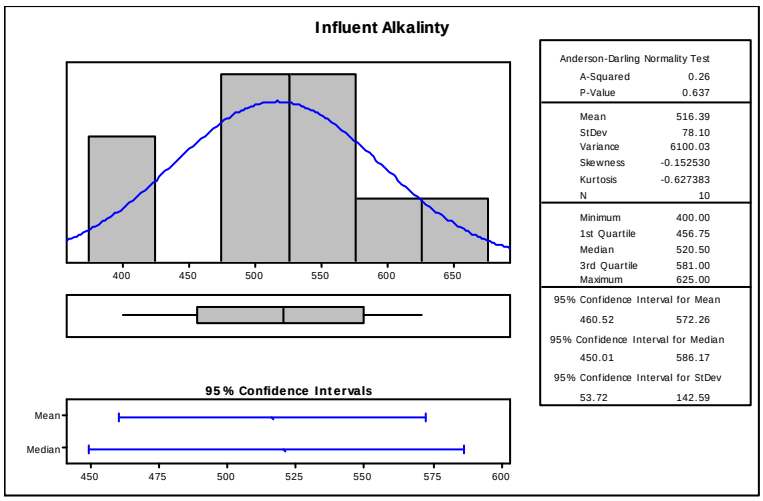
Inorganic carbon reported as alkalinity ($CaCO_3$) was only occasionally measured at the full scale WWTP. The mean influent alkalinity to the WWTP was 516mg/l $CaCO_3$ with a 95% confidence interval of 460mg/l$CaCO_3$ to 573mg/l $CaCO_3$ (Fig.23a). The minimum alkalinity was 400mg/l $CaCO_3$ and the maximum was 625mg/l $CaCO_3$. Upon passage through the WWTP there was a mean 50% drop in alkalinity levels with a final effluent mean of 237mg/l $CaCO_3$; the 95% confidence interval for the mean was 178mg/l $CaCO_3$ to 296mg/l $CaCO_3$. The minimum recorded level in the effluent was 125mg/l $CaCO_3$ and the maximum was 421mg/l $CaCO_3$ (Fig.23b).

A scatter plot of alkalinity levels and the percentage nitrification showed no relationship. This was confirmed by a regression co-efficient of 0%, a Pearson's correlation of 0% and a P value of 0.997 which failed the hypothesis test criteria of 0.05 (Fig.23c).

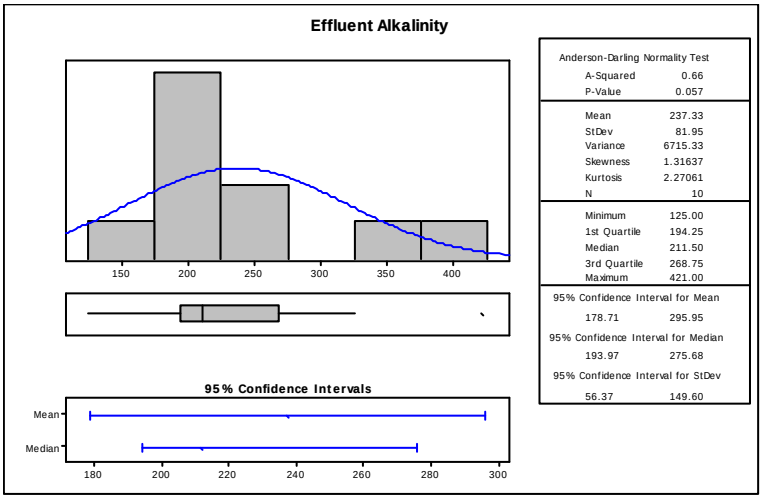
3.1.6 pH

High peaks of effluent ammonia were evident both when the pH was above and below pH 8.0 (Fig.24a). The overall pH of the aeration bay ranged from 5.5 to 8.7 with a mean of 7.93. The 95% CI was between 7.96 and 8.02; the median was marginally higher at 8.10 with a 95% CI of 8.0 to 8.1. Overall, the pH was normally distributed and was in general less than pH 8.5 with less than 9 outliers below pH 7.0 (Fig.24b).

From 01/01/00 until 29/03/00 there was a notable, sustained and gradual fall in the pH from pH 8.4 to pH 6.3. A scatter plot of this pH data and the corresponding percentage nitrification at this time returned a significant regression co-efficient of 60.6% and a strong Pearson correlation of 78% (Fig.24c). As the P value for this data was 0.000, this confirmed a statistical relationship between the fall in pH and the fall in the percentage nitrification. It was concluded that the aeration pH may have had an influence on nitrification and warranted further investigation in the pilot plant study.



(a)



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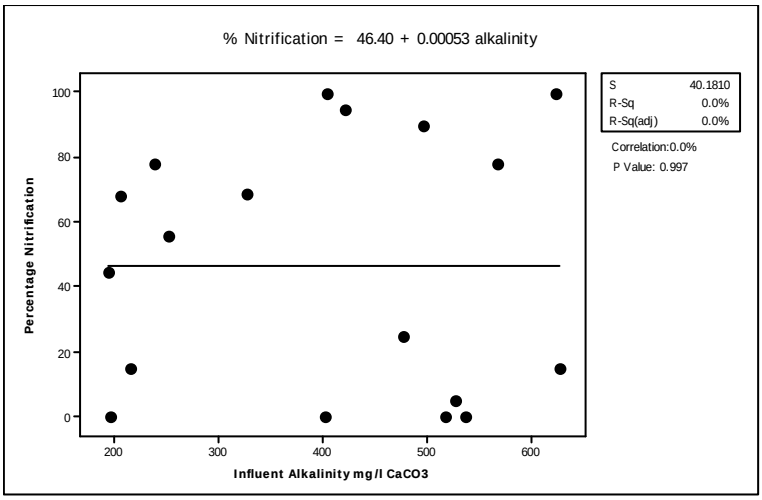
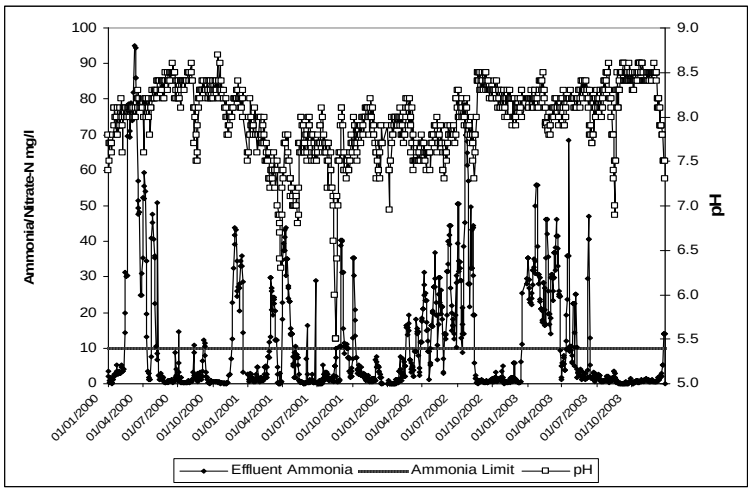
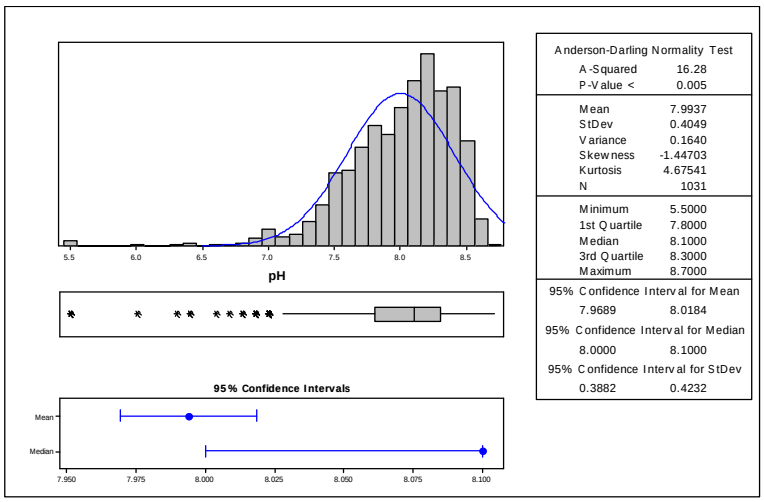


(c)]

Fig.23. Alkalinity at the full scale waste water treatment plant from 2000 to 2003; (a) data distribution plot of the influent alkalinity, (b) data distribution plot of the effluent alkalinity and (c) scatter plot of alkalinity and the corresponding percentage nitrification.



(a)



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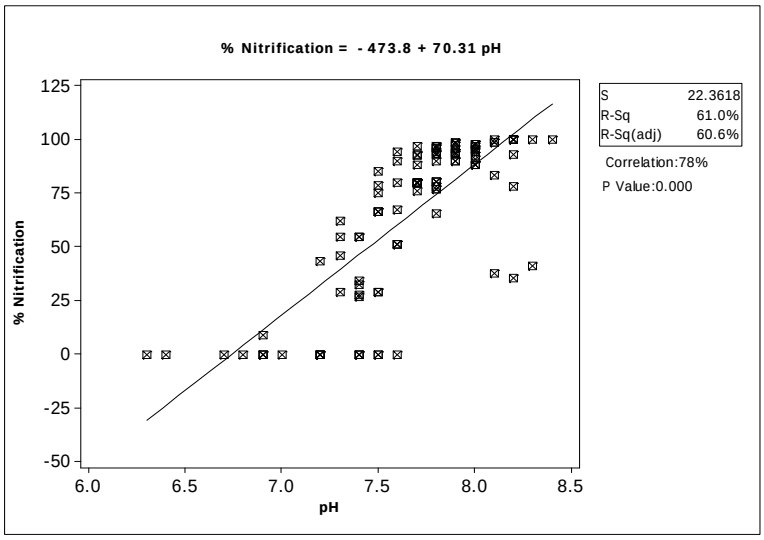


(c)

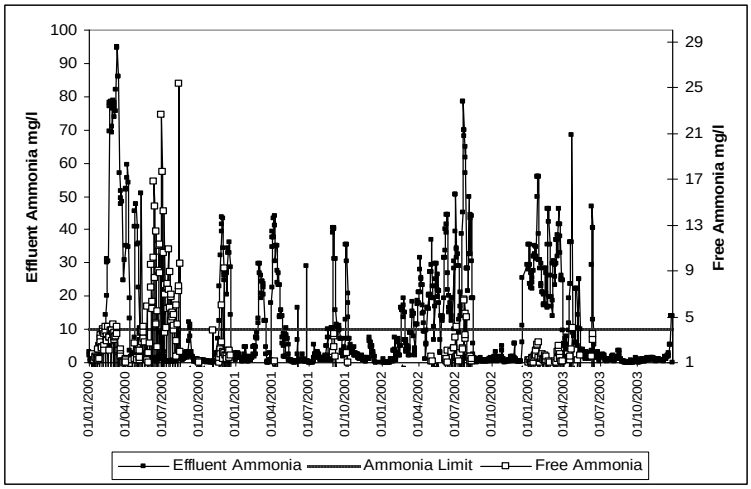
Fig.24. Aeration bay pH at the full scale waste water treatment plant from 2000 to 2003; (a) time series plot of the aeration bay pH and effluent ammonia, (b) data distribution plot of the aeration bay pH, (c) scatter plot of the aeration bay pH from 01/01/00 until 29/03/00 and the corresponding percentage nitrification.

3.1.7 Free Ammonia

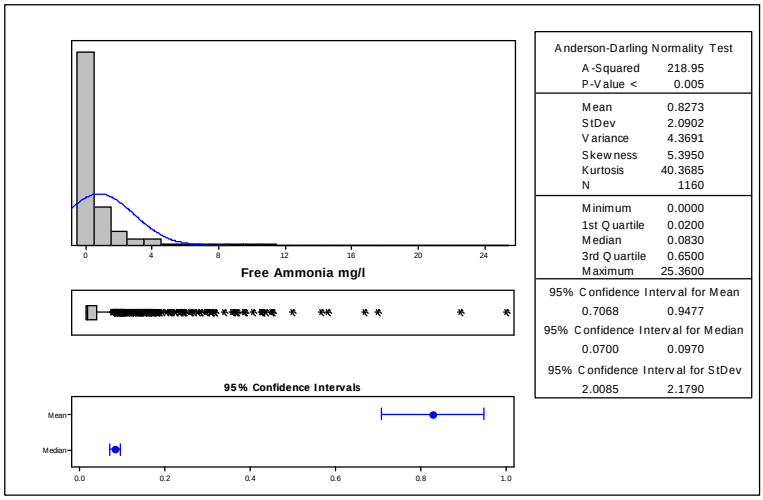
The levels of free ammonia in the aeration bay fluctuated during the period 2000 to 2003 (Fig.25a). Overall, free ammonia levels ranged from 0mg/l to 25.36mg/l. A data distribution plot of the free ammonia levels returned a mean of 0.83mg/l with a 95% CI of 0.71mg/l to 0.95mg/l (Fig.25b). The median was substantially lower with a 95% CI of 0.07mg/l to 0.10mg/l. This was due to a high number of outliers above 2mg/l.

From May 2000 to August 2000 there was a notable and sustained period of high levels of free ammonia (up to 25mg/l) which appeared to coincide with elevated effluent ammonia. A scatter plot of these data (Fig.25c) returned a significant regression co-efficient of 82.3% and a P value of 0.000. Based on a fitted curve, free ammonia levels in excess of 1mg/l resulted in a 50% failure in nitrification and at 4mg/l free ammonia there was 0% nitrification observed at the full scale WWTP showing that free ammonia levels had an influence on nitrification.

A scatter plot of the relationship between pH and free ammonia is plotted in Fig.26. Although a P value of 0.000 passed the hypothesis test criteria of 0.05, this relationship was weak with a Pearson's correlation of 19.6% and a regression co-efficient of only 3.8%. There was a poor relationship between free ammonia levels and the pH of the system.



(a)



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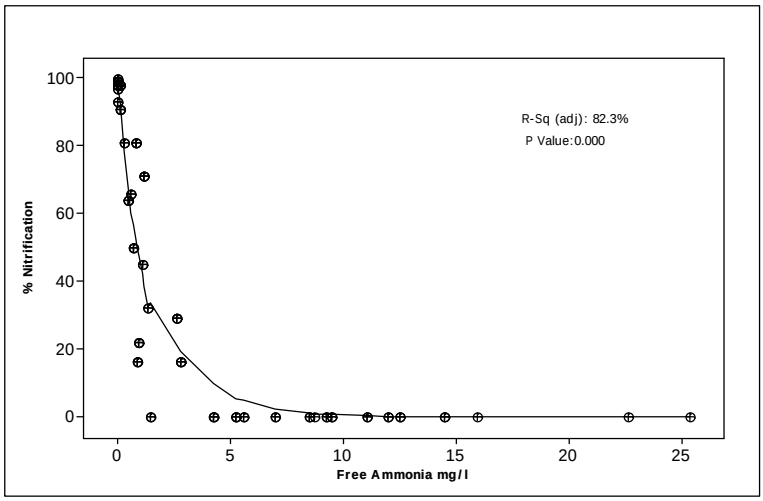
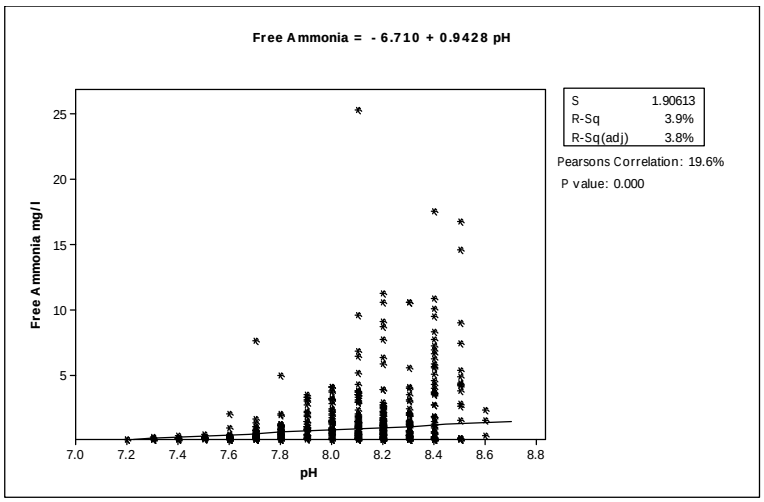


(c)

Fig.25. Free ammonia levels at the full scale waste water treatment plant from 2000 to 2003; (a) time series plot of the aeration bay free ammonia levels and effluent ammonia, (b) data distribution plot of the aeration bay free ammonia levels and (c) scatter plot of the aeration bay free ammonia levels from 01/05/00 to 14/08/00 and the corresponding percentage nitrification.

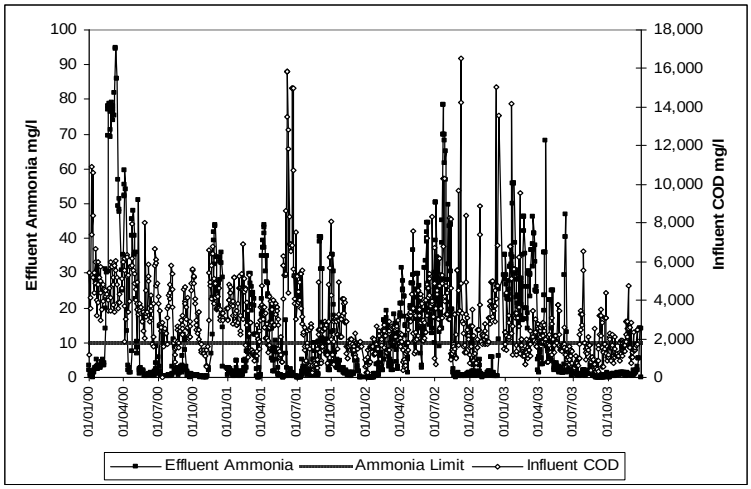


(d)

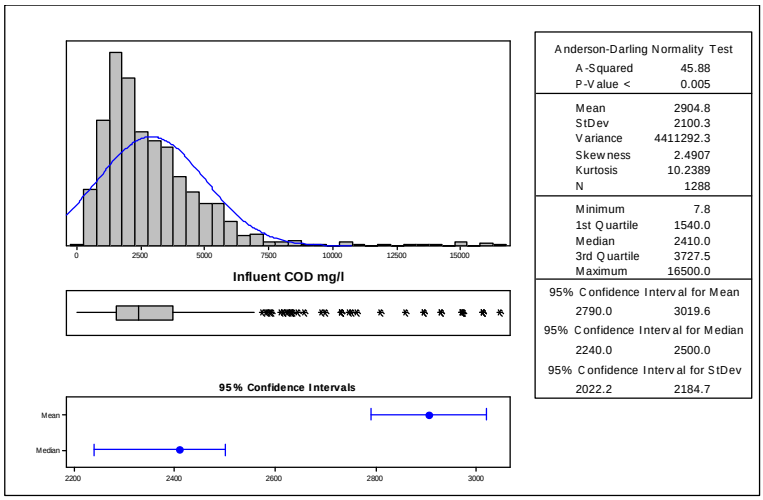
Fig.26. Free ammonia levels at the full scale waste water treatment plant from 2000 to 2003. Scatter plot of the relationship between the aeration bay pH and the free ammonia.

3.1.8 Influent COD

The influent COD to the full scale WWTP varied considerably from 2000 to 2003 with several periods of peaks and troughs (Fig.27a). There were some occasions where concentrations above 10,000mg/l COD were recorded. Given the number of data points it was difficult to relate any peaks and troughs to the ammonia concentration but periods of rising effluent ammonia and rising influent COD were evident from 29/12/01 until 19/08/02. A data distribution analysis showed the influent COD from 2000 to 2003 ranged from a low of 7.8mg/l to a maximum of 16,500mg/l. The mean was 2,905mg/l with a 95% CI between 2,790mg/l and 3,019mg/l. There were a number of outliers above 7,500mg/l; as a result the 95% CI for the median was lower at 2,240mg/l to 2,500mg/l (Fig.27b). A scatter plot of data between an influent COD of 1,500mg/l and 5,000mg/l which was the typical range targeted by the WWTP operators, and the corresponding percentage nitrification showed a significant fall in the percentage nitrification with a rising influent COD. The regression co-efficient was 74.8%, the Pearson's correlation 83% and the P value 0.000 (Fig.27c). There was a predicted 50% failure in nitrification above an influent COD concentration of 4,227mg/l.



(a)



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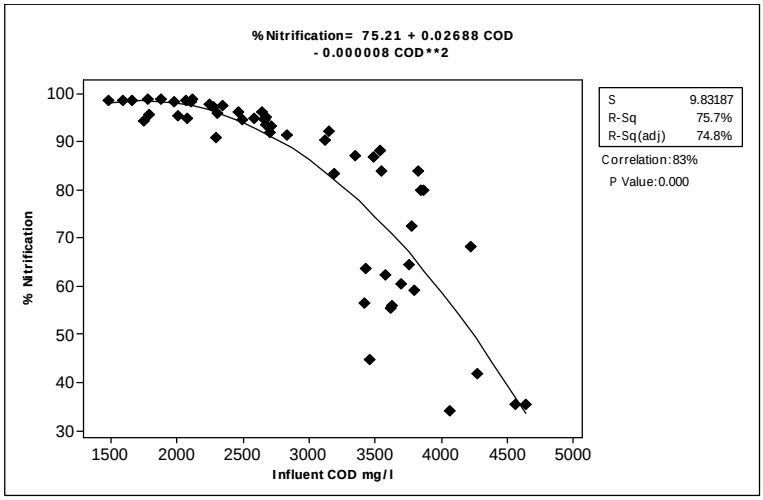


(c)

Fig.27. Influent COD to the full scale waste water treatment plant from 2000 to 2003; (a) time series plot of the influent COD and effluent ammonia, (b) data distribution plot of the influent COD and (c) scatter plot of the influent COD between 1,500mg/l and 5,000mg/l and the corresponding percentage nitrification.

A period of data was noted from March to April 2001 when the influent COD was within this 1,500mg/l and 5,000mg/l range and when the aeration bay pH was also low. A 3D dimensional scatter plot of these data and the percentage nitrification indicated a statistically significant multi linear relationship between the influent COD, the aeration bay pH and the corresponding percentage nitrification with a regression co-efficient of 69.2% and a P value of 0.000 (Fig.28).

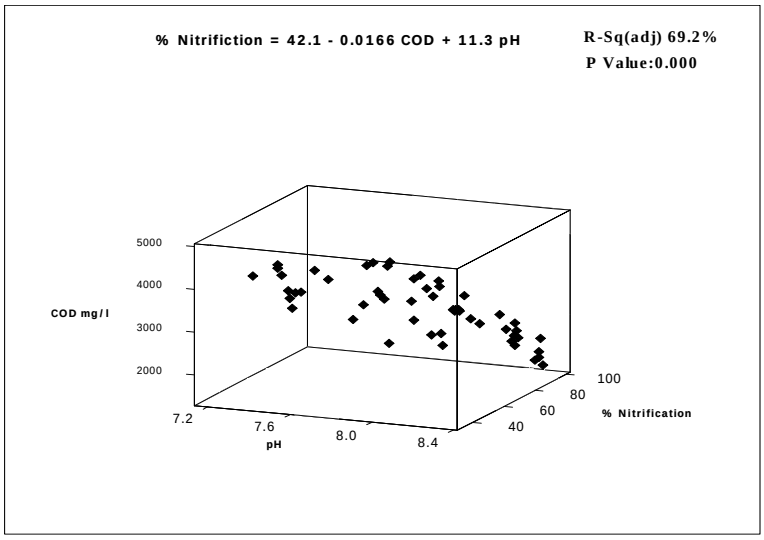


Fig. 28. Three dimensional scatter plot of the relationship between the influent COD (between 1,500mg/l and 5,000mg/l), the aeration bay pH and the resulting percentage nitrification at the full scale waste water treatment plant from March to April 2001.

3.1.9 Food to mass ratio (F/M)

A time series plot of the F/M during 2000 to 2003 showed that this parameter varied significantly (Fig.29a). Given the number of data points it was difficult to observe any relationship between this and the effluent ammonia. The F/M ranged from 0 to 0.68. The mean F/M was 0.196 with a 95% CI of 0.18 to 0.20.

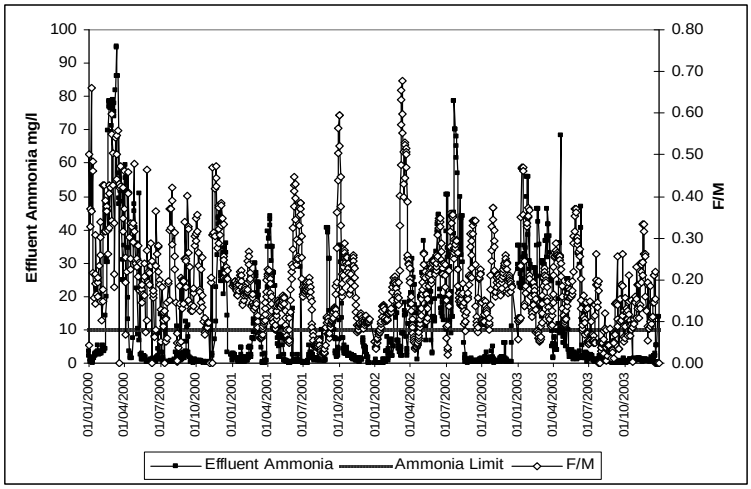
The median was slightly lower with a 95% CI of 0.17 to 0.19 (Fig.29b). A scatter plot of F/M's above 0.20 and the percentage nitrification showed a weak relationship between increasing F/M and falling nitrification (Fig.29c). The regression co-efficient was 20.3%, the Pearson's correlation was stronger at -45.5% and a P value of 0.000 confirmed a statistically positive relationship.

Given the stronger correlation returned by the influent COD and nitrification, the weaker relationship for the F/M was unexpected. This result suggested that the biomass played a significant role in the nitrification performance of the system.

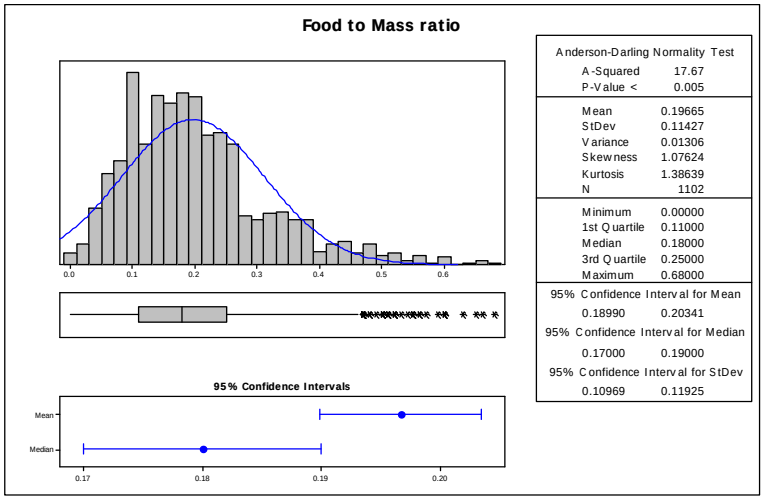
3.1.10 Carbon to Nitrogen ratio (C/N)

The C/N ratio in the full scale WWTP varied considerably during 2000 to 2003 (Fig.30a). The mean C/N ratio was 130 with a 95% CI of 124 to 136. The median was lower at 94 to 107. There were several data points where the C/N was >200 (Fig.30b). From the period 02/09/02 to 16/1/03, data were noted where there was a high C/N ratio followed by a prolonged period of a low C/N ratio. There was also at this time an extended period when both the effluent ammonia and in particular the effluent nitrates were <1mg/l. From day 1 to 45 the mean C/N ratio was less than 135 during which there were low levels of effluent ammonia and significant levels of nitrates. From day 45 to 70 the mean C/N ratio was over 200 with a peak at 480, largely due to a fall in the influent ammonia to <15mg/l NH_4-N. Although effluent ammonia was < 1mg/l NH_4-N nitrates also fell to under 1mg/l NO_3-N. In response to a rise in the influent ammonia, from day 65, effluent ammonia broke through to the final effluent exceeding 20mg/l NH_4-N with no corresponding rise in nitrates (Fig.30c).

A scatter plot of this data returned a Pearson's correlation of -78%, a regression co-efficient of 57% and a P value of 0.002. A linear fit of these data predicted a 50% failure of nitrification at a C/N ratio of 230 (Fig.31).



(a)



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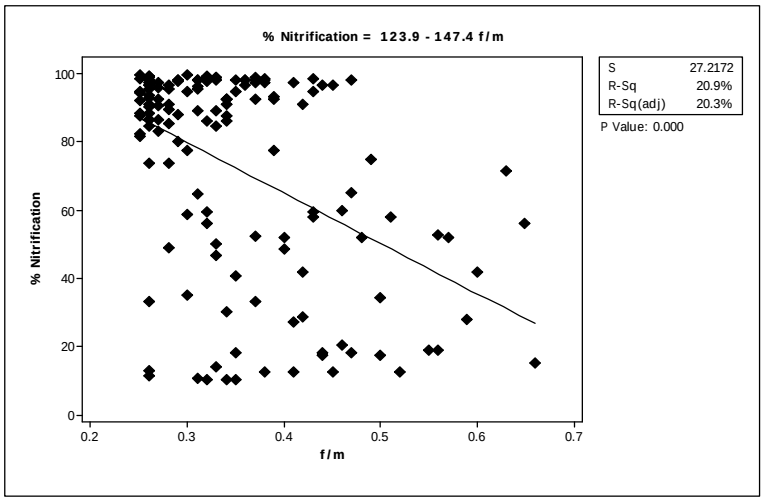
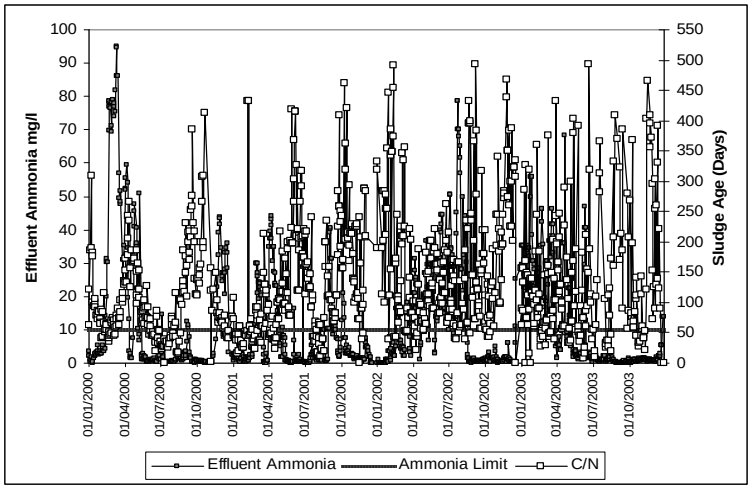
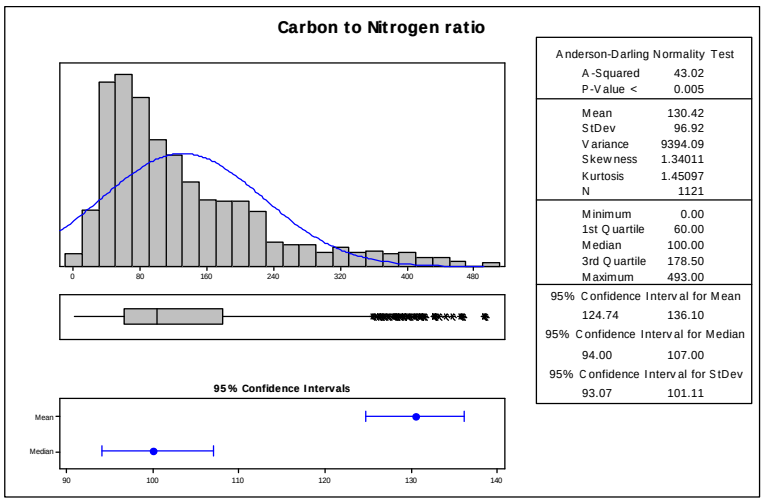


(c)

Fig.29. The food to mass (F/M) ratio at the aeration bay of the full scale waste water treatment plant from 2000 to 2003; (a) time series plot of the aeration bay F/M and effluent ammonia, (b) data distribution plot of the aeration bay F/M and (c) scatter plot of the aeration bay F/M above 0.20 and the corresponding percentage nitrification.



(a)



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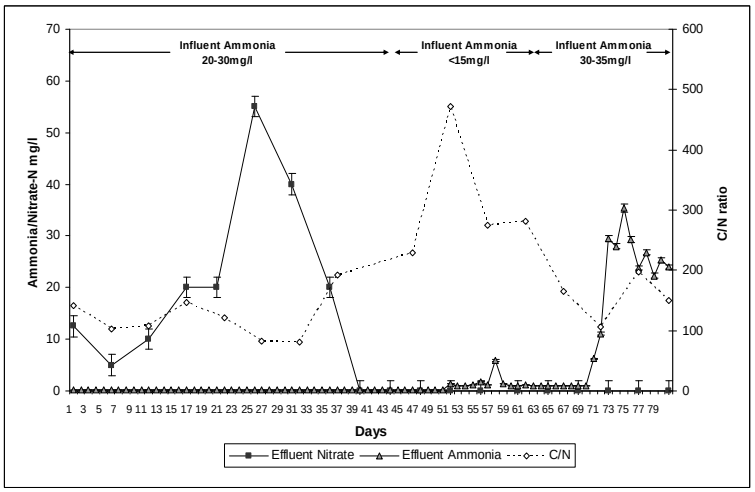
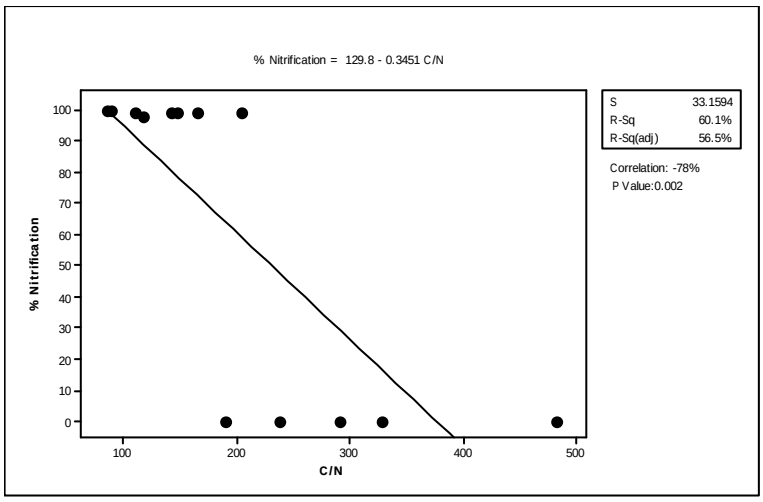


(c)

Fig.30. Carbon to nitrogen (C/N) ratio to the aeration bay of the full scale waste water treatment plant from 2000 to 2003; (a) time series plot of the C/N and effluent ammonia, (b) data distribution plot of the C/N and (c) time series plot from 02/09/02 to 16/01/03 for the C/N ratio, the influent ammonia and effluent ammonia/nitrates.



(d)

Fig.31. Scatter plot of the relationship between the carbon to nitrogen (C/N) to the aeration bay and the percentage nitrification at the full scale waste water treatment plant from 02/09/02 to 16/1/03.

3.1.11 Summary of the findings of Part 1 of the study.

A summary of the findings of Part 1 of the study is presented in Table 16. A number of the parameters investigated were found to have no significant effect on nitrification. These included:

- Temperature of the aeration bay
- Sludge age of the nitrifying activated sludge
- Dissolved oxygen levels in the aeration bay
- Phosphorus levels in the final effluent
- Inorganic carbon

In contrast there was evidence that some of the operating parameters played a role in nitrification failure at the full scale WWTP. These parameters were:

- The pH of the aeration bay
- Free ammonia levels in the aeration bay
- Influent COD to the aeration bay
- F/M of the activated sludge system
- Low influent ammonia to the aeration bay measured as the C/N ratio

To confirm these relationships, the influence on nitrification of these factors was studied in the pilot plant.

Table 16: Summary of factors studied at the full scale WWTP and their relationship to nitrification.

Factor	Positive relationship to nitrification (Yes/No)	Data Range Analysed	Main finding
Temperature	No	11°C-21°C	No impact within this range
Sludge Age	No	0-48days	No impact within this range
Dissolved oxygen	No	2.0mg/l-5.0mg/l	No impact within this range
Phosphorus	No	0mg/l-2.5mg/l	No impact within this range
Inorganic Carbon	No	200-626mg/l	No impact within this range
pH	Yes	6.3-8.4.	Disruption below pH 7.5
Free ammonia	Yes	0-25mg/l	50% failure >1mg/l
Influent COD	Yes	1,500-5,000mg/l	50% failure > 4,000mg/l
Food to mass ratio	Yes	0.20–0.70	50% failure > 0.50
Carbon to nitrogen ratio	Yes	100-500	50% failure >230

3.2 Pilot Plant Studies

In Stage 2 of the study plan, pilot plant studies were undertaken to confirm the findings of the data study (Stage 1) of the full scale WWTP and to investigate remedial options to improve nitrification performance. The pilot plant studies comprised of three main parts as outlined in Fig.32.

In the first part the influence of environmental factors on nitrification was studied. The influence of the system design was investigated in part 2 and in part 3 the role of the MLSS and heterotrophic population was studied.

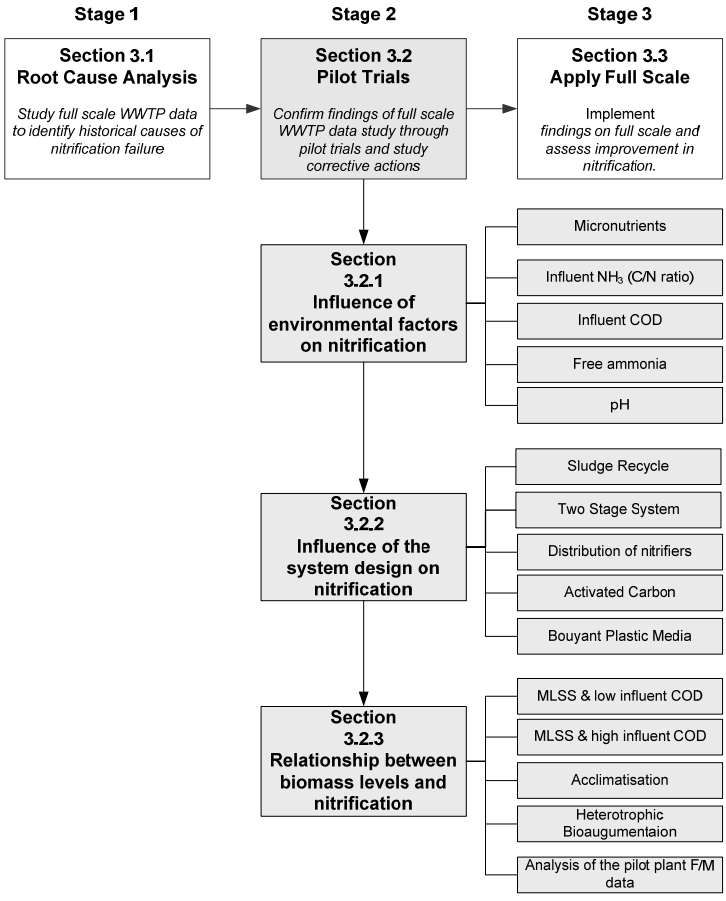


Fig.32. Outline of Stage 2 of the nitrification study plan summarising the factors studied in the pilot plant system.

3.2.1 Environmental Factors

The data study of the full scale WWTP from 2000 to 2003 indicated nitrification was affected by a number of environmental factors. The influence of these factors on nitrification was therefore studied in the pilot plant.

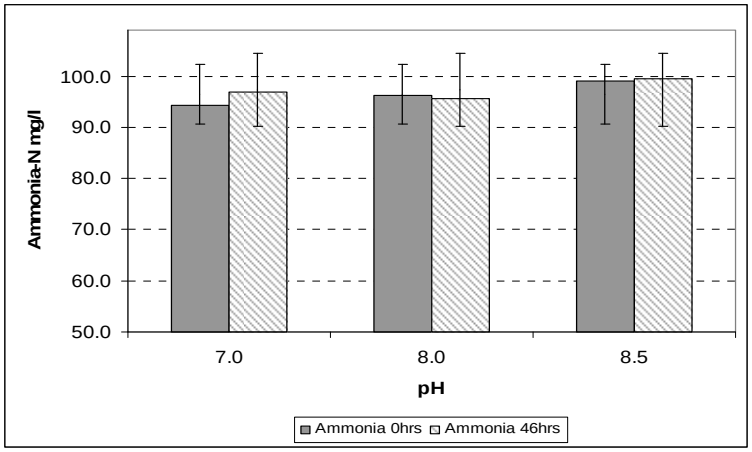
The factors studied included the availability within the waste water of nutrients, in particular the need for adequate micronutrients and the effect on nitrification of the inorganic fraction. In addition sufficient levels of influent ammonia and the relationship of the carbon to nitrogen (C/N) ratio were also examined. Finally the effect of the influent COD, the operating pH and the levels of free ammonia were studied.

Prior to these studies it was first necessary to demonstrate that any ammonia loss from the pilot plant was due to microbial activity and not physical air stripping.

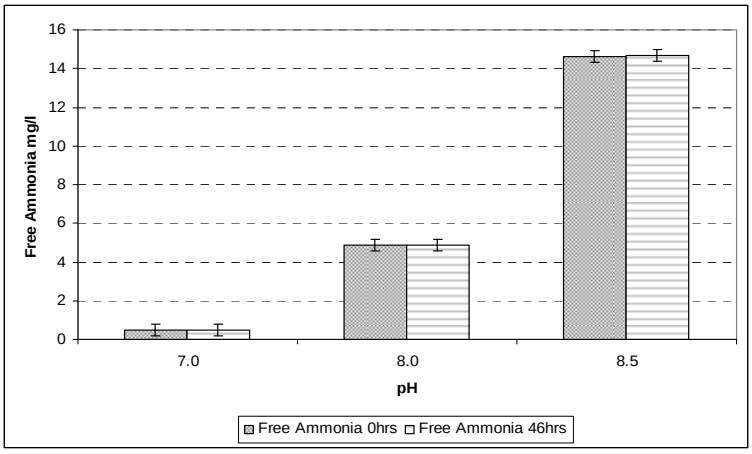
3.2.1.1 Ammonia Stripping Assessment

The pilot plants were operated in batch mode. The reactors were filled with 9 litres of distilled water and 3,461.5mg of NH_4Cl to give a residual ammonia concentration of 100mg/l NH_4-N. The air flow rate was set at its maximum of 1.5litres/min/reactor. There were no nitrifiers or activated sludge in the reactor. The pH of Reactor I was adjusted to 7.0; Reactor II to pH 8.0 and Reactor III to pH 8.5. The contents were agitated for 30 minutes after which a sample was taken and analysed in duplicate for ammonia. A further sample was taken after 46 hours. This was repeated as above for pH's 7.0, 8.0 and a further three times for pH 8.5 as stripping is more prone the more alkaline the pH.

Ammonia loss by air stripping from the pilot plant system was not a significant factor (Fig.33a). There was no notable difference in total ammonia levels or the calculated free ammonia levels at any of the pH's tested before and after 46 hours of aeration (Fig.33b).



(a)



(b)

Fig. 33. Assessment of ammonia loss from the pilot plant reactors by air stripping at various pH's: (a) total ammonia and (b) calculated free ammonia.

3.2.1.2 The role of micronutrients in nitrification

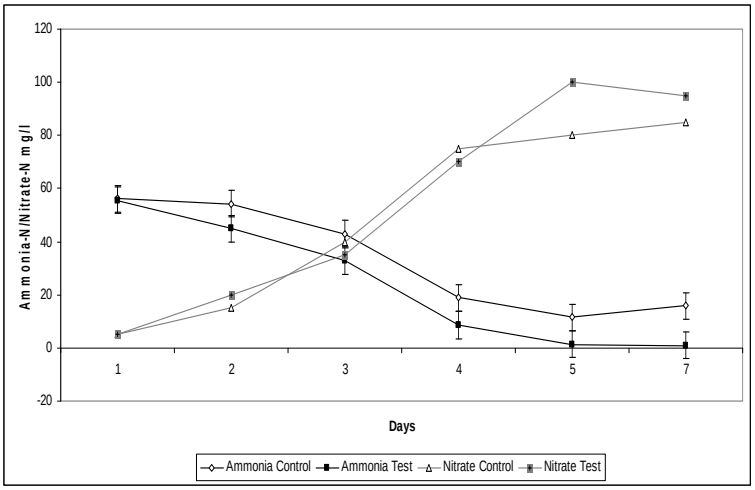
Nutrients are present in the wastewater feed to the WWTP; as there was no routine monitoring data available it was of interest to determine whether the supply of micronutrients normally present in the wastewater feed was sufficient to sustain nitrification. The feed is stored in a large balance tank the pH of which is normally between pH 10-12. If the pH of this tank is allowed to become too high inorganic nutrients will precipitate out of solution. Two experiments were carried out; in one experiment the influence of additional micronutrients was investigated and in the second, the influence of removing the inorganic fraction was studied.

Addition of micronutrients

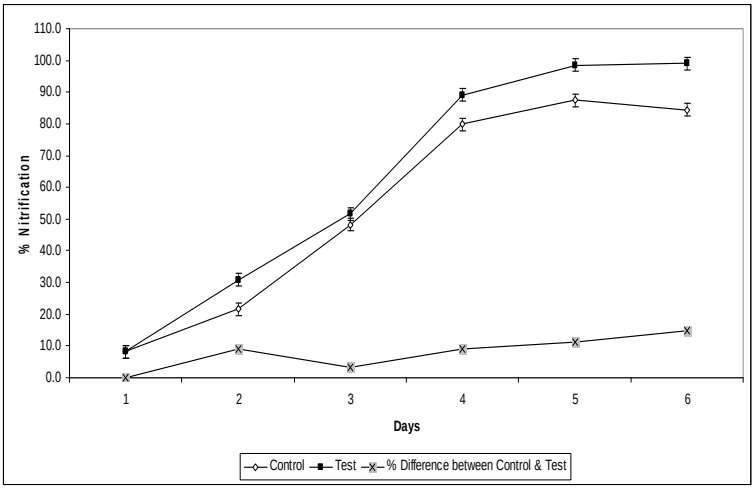
The influence of micronutrients on nitrification was studied in the pilot plant. The preparation and selection of the nutrient mix is outlined in section 2.2.2.3. Both the control and test systems were operated in continuous feed mode at pH 7.5 with an MLSS of 4,000mg/l and an influent COD of 1,000mg/l. A mixture of micronutrients was added to the test system feed. Both systems demonstrated good nitrification with falling ammonia and rising effluent nitrates when monitored for a seven day period (Fig.34a).

The start up of nitrification in the test pilot plant was marginally quicker and by the end of the trial on day 7 slightly lower effluent ammonia and higher nitrates were recorded compared to the control. The test pilot plant reached a maximum of 100% nitrification compared to 88% in the control. Overall the percentage nitrification was on average 8.3% better than the control (Fig.34b).

However, on examination of the nitrifier growth rates based on the amount of nitrate produced per day, this returned a growth rate of $1.00d^{-1}$ for the test system compared to $0.97d^{-1}$ for the control. This represented only 3% of a difference (Fig.34c) showing that the addition of a synthetic nutrient to this nitrifying sludge had no significant effect on the rate of nitrification.



(a)



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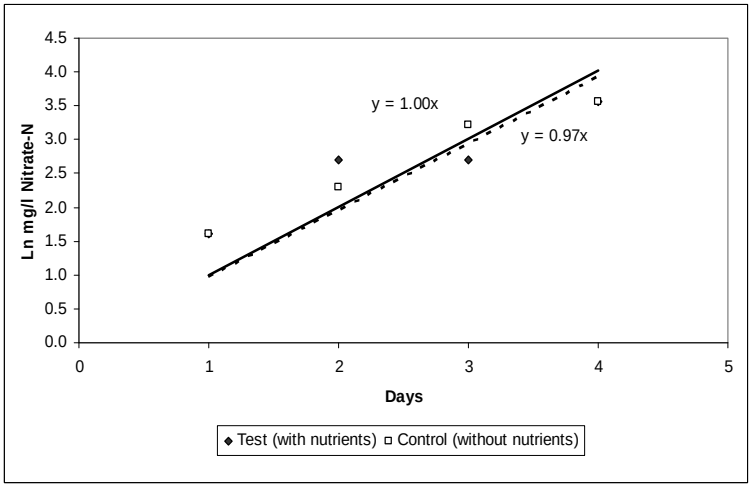


(c)

Fig. 34. Nitrification performance in the pilot plant system with micronutrients (test pilot plant) and without any micronutrient addition (control pilot plant); (a) effluent ammonia & nitrates, (b) percentage nitrification and (c) plot of natural log of nitrate formed per day.

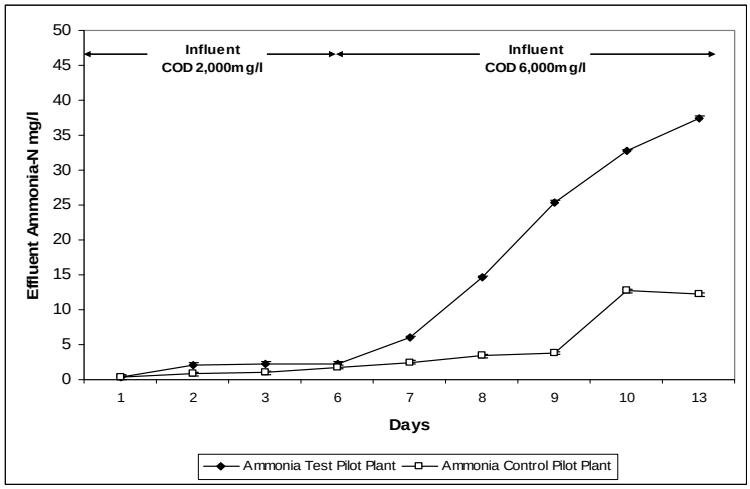
Removal of the inorganic fraction

Pilot plant systems were operated in continuous feed mode at pH 7.5 with a mean MLSS of 4,000mg/l. The feed to the test pilot system had been modified to remove 70% of the inorganic fraction (section 2.2.2.3). The influent COD was 2,000mg/l and on day 5 the COD was increased to 6,000mg/l in both systems and monitored for a further 8 days.

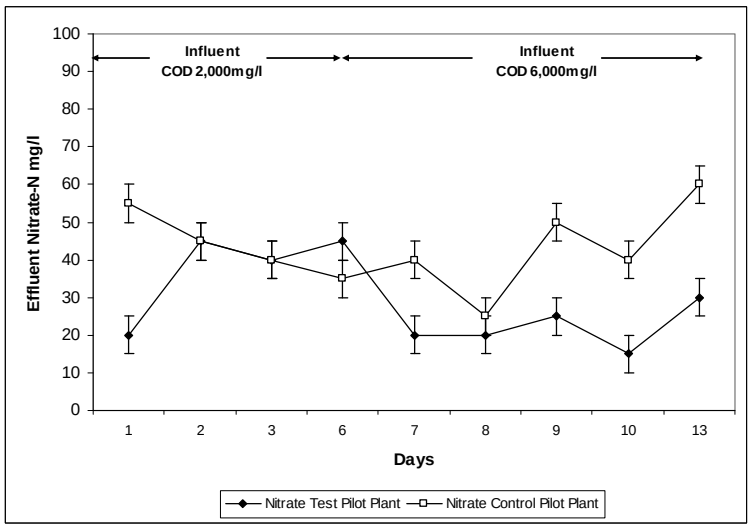
Full nitrification was achieved on both waste waters with an influent COD of 2,000mg/l. Effluent ammonia levels less than 1mg/l NH_4-N in the control and slightly higher levels of 2.1mg/l to 2.3mg/l NH_4-N in the test pilot plant system were recorded. Following an increase in the influent COD to >6,000mg/l, effluent ammonia levels reached a peak of 37.5mg/l NH_4-N in the test pilot plant compared to only 12.7mg/l NH_4-N in the control pilot plant (Fig.35a).

A similar pattern was observed for the effluent nitrates where at the end of the study there was less than 30mg/l NO_3-N in the test pilot plant compared to 60mg/l NO_3-N in the control system (Fig.35b). The corresponding lowest percentage nitrification recorded was 31% in the test pilot plant and 76% in the control (Fig.35c).

The results indicated that removing 70% of the inorganic fraction had no effect on nitrification at a low influent COD however it had a deleterious effect on nitrification when the system was shocked loaded to 6,000mg/l COD.



(a)



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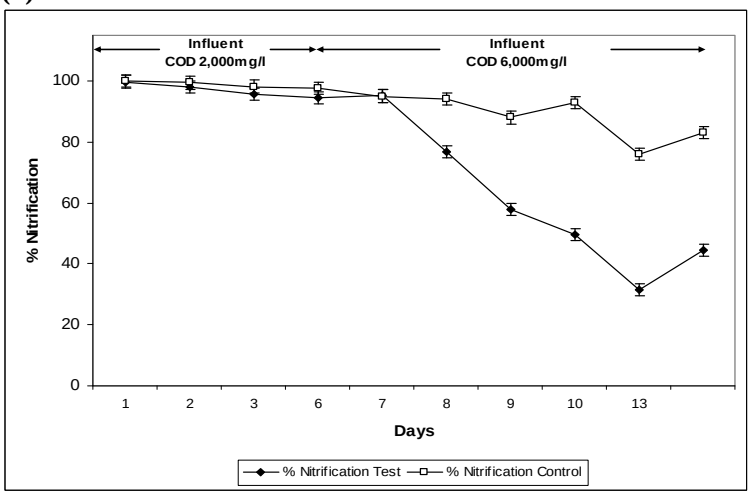


(c)

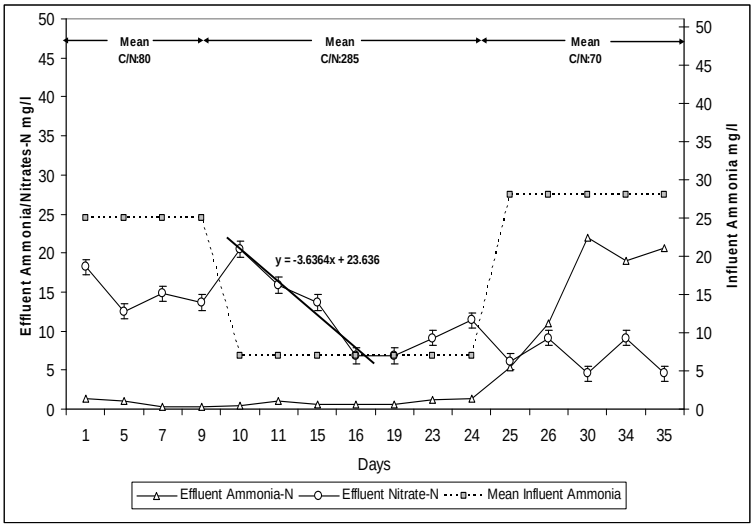
Fig. 35. Nitrification in the pilot plant systems with the inorganic fraction of the waste water (control system) and with 70% of the inorganic fraction removed from the waste water (test system); (a) effluent ammonia, (b) effluent nitrates and (c) percentage nitrification.

3.2.1.3 The effect of influent ammonia concentration and the carbon to nitrogen ratio (C/N) on nitrification.

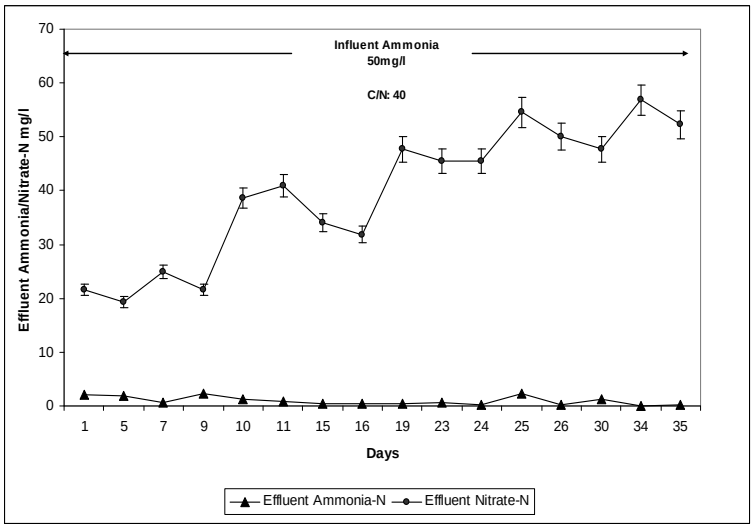
The carbon to nitrogen (C/N) ratio is the concentration of influent COD divided by the concentration of influent ammonia. The pilot plant systems were operated in continuous feed mode at pH 7.5 and an MLSS of 3,500mg/l. The influent COD was 2,000mg/l through out the 40 day trial. The feed for the test system had additional ammonia added to give a constant influent ammonia concentration of 50mg/l NH_4-N (section 2.2.2.3).

From day 1 to day 9 the mean influent ammonia and the C/N ratio to the control pilot plant was 25mg/l NH_4-N and 80 respectively (Fig.36a). During this time effluent ammonia was less than 1mg/l NH_4-N and nitrates were between 12mg/l and 21mg/l NO_3-N. From day 10 to day 24 there was a step change in the C/N ratio to a mean of 285; this was a result of the influent ammonia falling to 7mg/l NH_4N on average. In response nitrates started to fall at a rate of 3.6mg/l NO_3-N per day reaching a low of 6.82mg/l NO_3-N by day 16. On day 25 the influent ammonia rose to 28mg/l NH_4-N; in response effluent ammonia also increased reaching a peak of 21.9mg/l NH_4-N. There was no significant change in nitrates over this same period and by the end of the study effluent nitrates were only 4.55mg/l NO_3-N.

In comparison the test pilot plant (Fig.36b) with a fixed influent ammonia concentration of 50mg/l NH_4-N and a constant C/N ratio of 40 maintained full nitrification with low effluent ammonia and relatively stable effluent nitrates that reached over 50mg/l NO_3-N by the end of the study. Expressed in terms of percentage nitrification it was evident that the test pilot plant maintained 100% nitrification on a C/N of 40, in comparison the control system although achieving 89% nitrification at a C/N of 250 this fell to a low of 18% due to its inability to respond to a step increase in the influent ammonia (Fig.36c).



(a)



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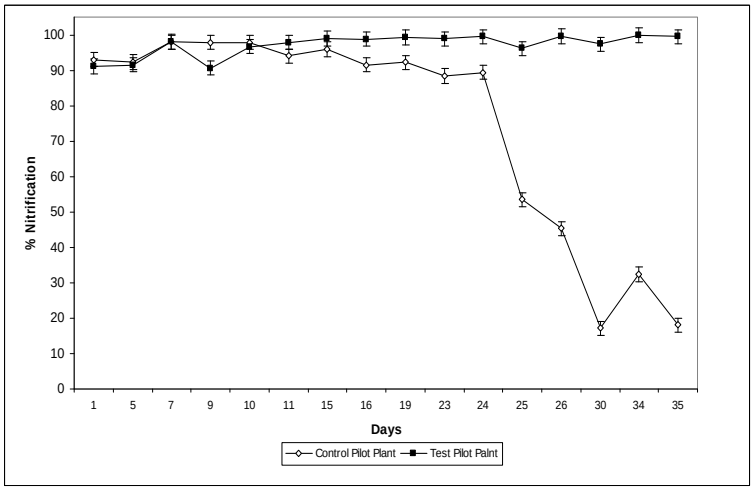
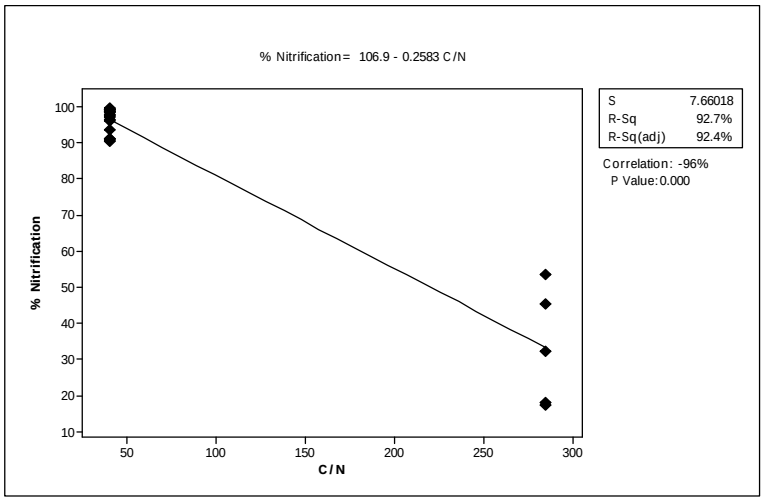


(c)

Fig. 36. **Performance of nitrification in the pilot plant system on an influent COD concentration of 2,000mg/l with a fixed influent ammonia concentration (test system) and without any ammonia addition to the waste water; (a) influent ammonia, effluent ammonia and nitrates for the control pilot plant, (b) influent ammonia, effluent ammonia and nitrates for the test pilot plant and (c) percentage nitrification for both systems.**

A scatter plot of the C/N ratio and the corresponding percentage nitrification at an MLSS of 3,500mg/l is presented in Fig.37. To allow for the retention times and the effect of the step increase in influent ammonia, only the C/N ratios for the last 5 days of the control system were plotted. This returned a strong Pearson's correlation of -96% and an equally strong regression co-efficient of 94%. As the P value was 0.000 this confirmed that with an increase in the C/N ratio the percentage nitrification decreased. The results of this study indicated that nitrification will fail at an influent ammonia concentration of <6mg/l NH_4-N or expressed as a C/N then a 50% failure in nitrification will occur at a C/N of >220.

An influent ammonia concentration of 50mg/l NH_4-N or a C/N ratio of less than 40 will ensure that excess ammonia is available to sustain a population of nitrifiers and maintain a 100% nitrification rate. Therefore for all further pilot studies where a low COD loading was applied an influent ammonia concentration of 50mg/l was sufficient. However, for any trial where a high COD loading was applied, leading in turn to a high C/N ratio, an influent ammonia concentration of 100mg/l NH_4-N was adopted. This also represented the peak level likely to be seen at the full scale WWTP.



(d)

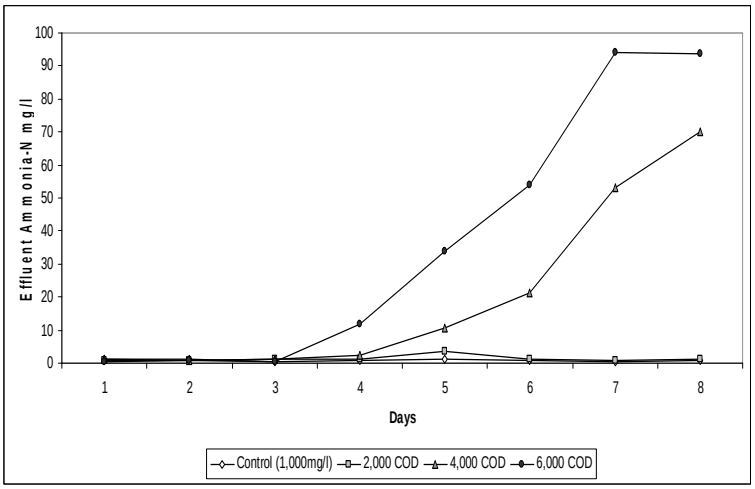
Fig. 37. Scatter plot of the relationship between percentage nitrification and the carbon to nitrogen (C/N) ratio in the pilot plant system systems.

3.2.1.4 Influence of the influent chemical oxygen demand (COD) on nitrification

Although the mean influent COD levels in the full scale WWTP were less than 3,000mg/l there were several occasions when it exceeded 5,000-7,000mg/l. A pilot plant investigation was carried out to study the influence of the influent COD concentration on nitrification. Modification of the feed to create different levels of COD is detailed in section 2.2.2.3. The pilot plant systems were operated in continuous feed mode at pH 7.5 and an MLSS of 3,500mg/l. From days 1 to 3 an influent COD of 1,000mg/l was applied to both pilot plant systems. On day 4 the influent COD was increased to 2,000mg/l for the first run; 4,000mg/l for the second run and 6,000mg/l for the third run. The control system remained on an influent COD of 1,000mg/l for all three runs.

Both the control and the pilot plant on an influent COD of 1,000mg/l and 2,000mg/l maintained full nitrification with low effluent ammonia and nitrates in excess of 90mg/l NO_3-N. However at an influent COD of 4,000mg/l nitrification failure occurred with effluent ammonia reaching a peak of 70mg/l NH_4-N and nitrates a low of 45mg/l NO_3-N. This disruption to nitrification was more pronounced at an influent COD of 6,000mg/l where effluent ammonia reached 94mg/l NH_4-N and nitrates fell to 10mg/l NO_3-N (Fig.38a) and (Fig 38b).

When expressed as percentage nitrification; 100% nitrification was achieved at an influent COD of 1,000mg/l and 2,000mg/l. However at an influent COD of 4,000mg/l this fell to 39% nitrification and only 9.6% nitrification for an influent COD of 6,000mg/l (Fig.38c). All pilot plants had similar and stable MLSS concentrations but the variation in COD therefore created different food to mass ratio (F/M's). The average F/M for the control was 0.08; for the pilot plant on an influent COD of 2,000mg/l it was 0.16; for the pilot plant on an influent COD of 4,000mg/l the F/M was 0.31 and the F/M was 0.45 for the pilot plant on an influent COD of 6,000mg/l (Fig.39). There was a strong statistical relationship between the influent COD and the percentage nitrification using the results from day 8; this data point was chosen to allow for the effect of a three day hydraulic retention time. The Pearson's correlation was -98%, the regression co-efficient was 87% and the P value was 0.023. On the basis of the linear fit; a 20% failure in nitrification occurred at an influent COD of >2,300mg/l. A 50% failure in nitrification was likely to occur at an influent COD of >3,800mg/l (Fig.40).



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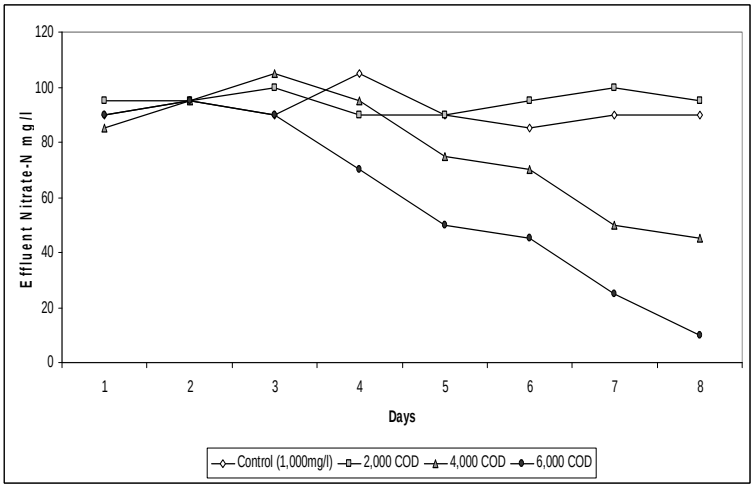

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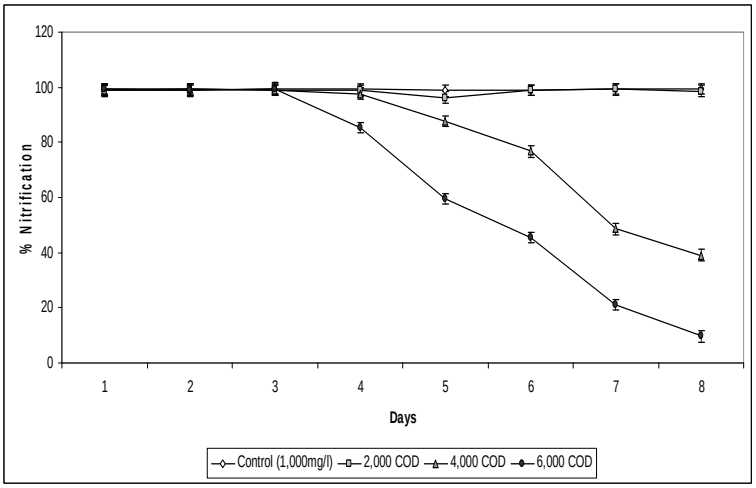


(c)

Fig.38. Influence of the influent chemical oxygen demand (COD) on nitrification in a pilot plant system; (a) effluent ammonia, (b) effluent nitrates and (c) percentage nitrification.

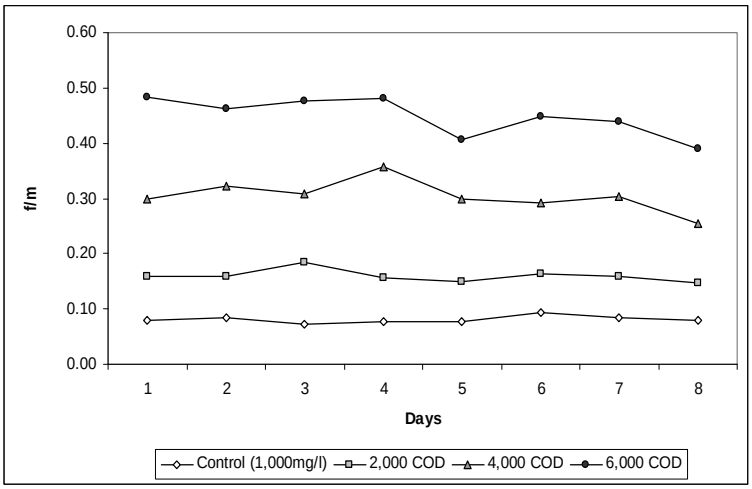


Fig.39. Food to mass (F/M) ratios in the pilot plant system that studied the influence of varying influent chemical oxygen demands (COD) on nitrification.

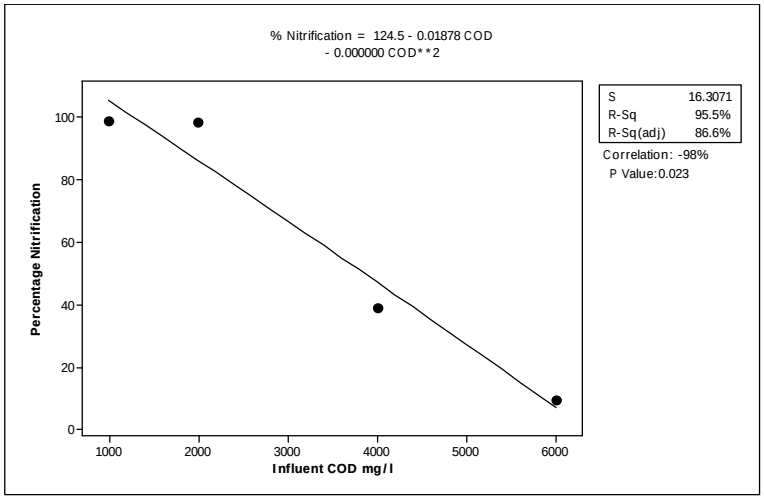


Fig.40. Scatter plot of the relationship between the influent chemical oxygen demand (COD) and the corresponding percentage nitrification in the pilot plant system.

3.2.1.5 Investigation into the influence of free ammonia on nitrification.

Two investigations were carried out; in the first investigation the pilot plants were operated in batch mode to determine the threshold levels of free ammonia inhibition. Three reactors were operated at pH 7.5, 8.0 and 8.5 to create different levels of free ammonia at the same concentration of total ammonia. The MLSS was 3,500mg/l and the COD within the reactors was less than 500mg/l. There was no influent feed. In the second investigation the ability of the system to recover from high free ammonia levels was studied. The pilot plants were operated in a continuous feed mode at an MLSS of 3,500mg/l. The influent COD was 1,000mg/l. The pH in both systems was initially uncontrolled and this ranged from pH 8.2 to 8.5, on day 5 it was reduced and maintained at pH 7.5 in the test system only.

The percentage nitrification for the first investigation in all reactors was approximately 20% at the start of the trial by virtue of some residual nitrates and a starting total ammonia concentration of 50mg/l NH_4-N. The reactor at a free ammonia level of 5.5mg/l FA failed to nitrify as evident by a fall in the percentage nitrification to 0% by day 1 until the end of the study on day 9. On the other hand, the reactors with a free ammonia concentration of less 1mg/l FA and less than 3mg/l FA respectively demonstrated full nitrification with the percentage nitrification reaching over 90% by day 9 (Fig.41).

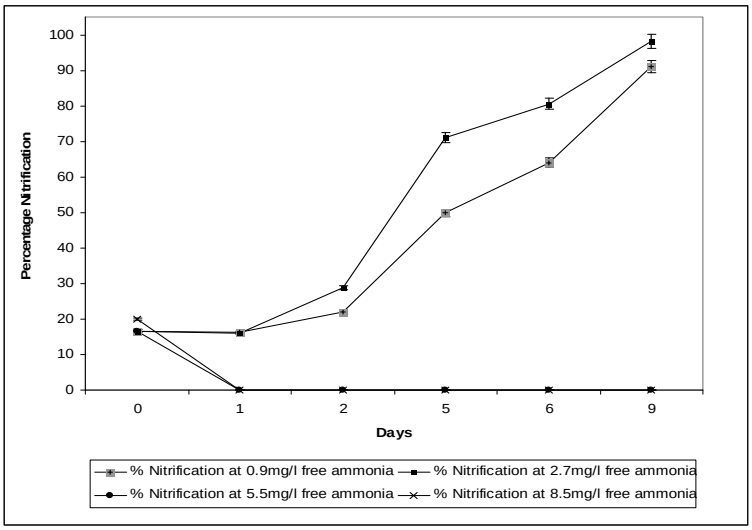
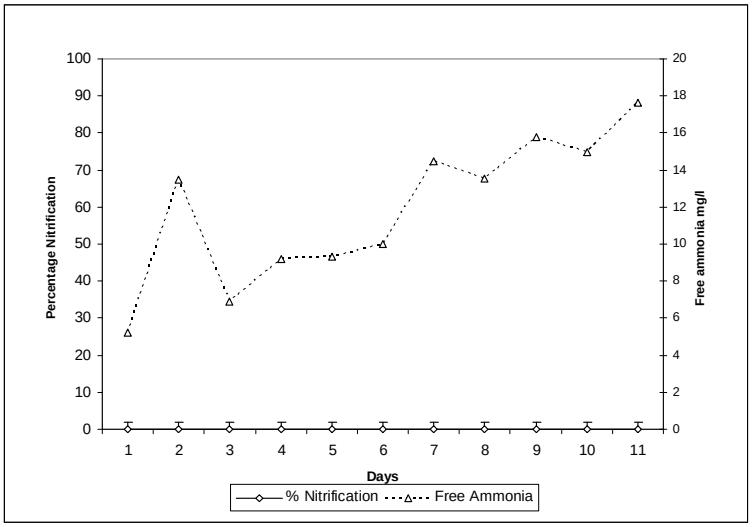


Fig. 41. Influence of free ammonia on the start up of nitrification in three pilot plant reactors.

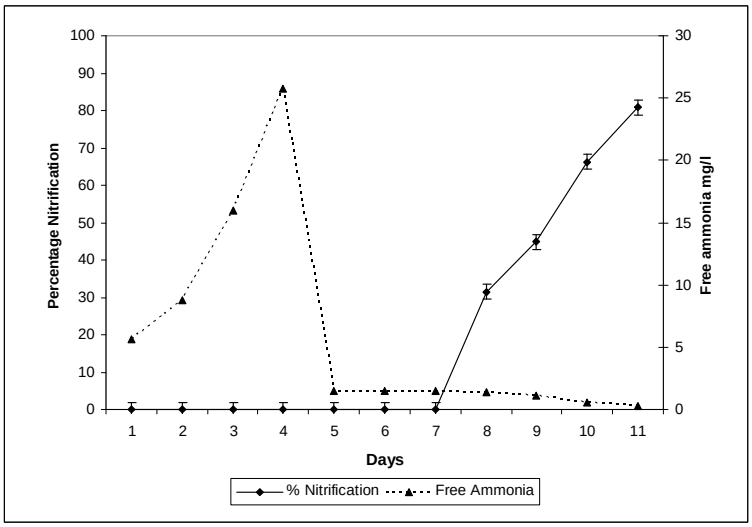
In the second investigation the starting free ammonia level was greater than 5mg/l FA in both systems (Fig.42a) and (Fig.42b). After 6 days there was no evidence of nitrification in either pilot plant. A reduction in the pH of the test pilot plant to pH 7.5 in turn reduced the free ammonia to <2mg/l FA and nitrification commenced within 1 day reaching a percentage nitrification of over 98% by day 9. Over the same period the control reactor failed to nitrify where free ammonia levels continued to increase due to rising total ammonia within the reactor, reaching 18mg/l FA by the end of the trial.

A regression plot of the levels of free ammonia relative to the percentage nitrification demonstrated a positive relationship for a cubic fitted line with a Pearson's correlation of -69.9% and a stronger regression co-efficient of 84.4%. A P value of 0.000 confirmed this statistical relationship (Fig.43).

The results of this study indicated that free ammonia is inhibitory to nitrification and that 50% nitrification failure will occur at a free ammonia level greater than 1.5mg/l FA. All further pilot plant trials calculated and recorded the free ammonia levels; these were kept below <1.0mg/l FA by operating the pilot plant systems at pH 7.5 unless otherwise stated.



(a)



(b)

Fig. 42. Influence of free ammonia on nitrification in a pilot plant system; (a) free ammonia levels and the percentage nitrification in the control pilot plant, (b) free ammonia levels and the percentage nitrification in the test pilot plant

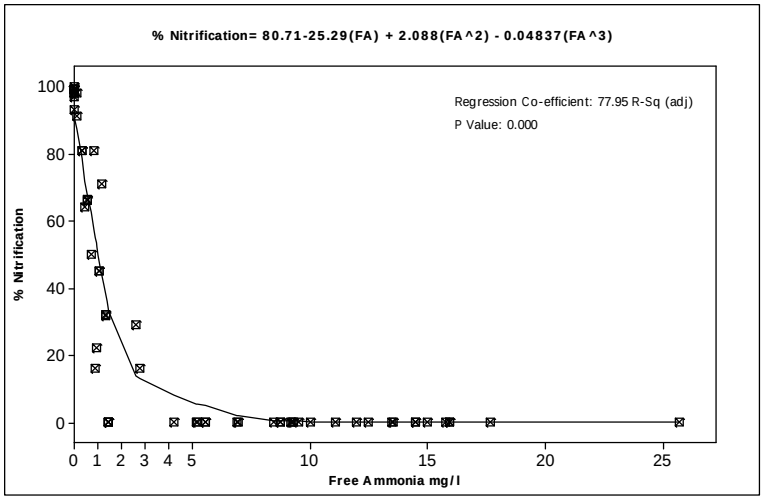


Fig.43. Scatter plot of the relationship between free ammonia and the percentage nitrification in the pilot plant system.

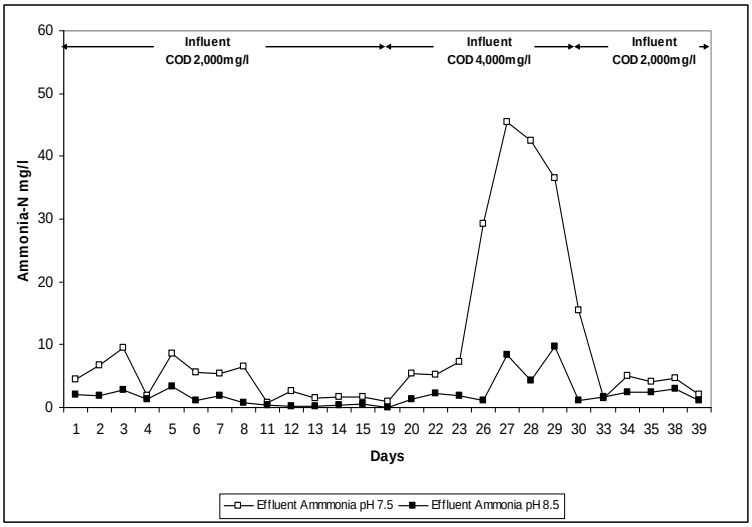
3.2.1.6 Influence of the pH on nitrification

The influence of pH on nitrification was studied at pH values of 7.5 and 8.5. The data study of the full scale WWTP had observed a combined relationship between the pH of the system and the influent COD. The influent COD was 2,000mg/l and the systems were operated in a continuous feed mode. The effect of a shock COD load of 4,000mg/l was also studied at both pH values. The mean MLSS in both systems was 4,000mg/l.

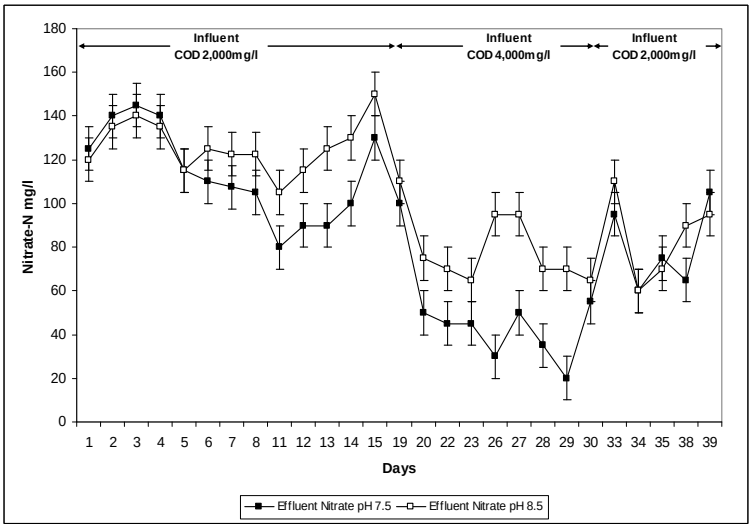
Low effluent ammonia was observed in both pilot plants at an influent COD concentration of 2,000mg/l from day 1 to day 15. The levels of effluent ammonia in the pilot plant at pH 7.5 were slightly higher with a mean of 4.3mg/l NH_4-N compared to the pilot plant at pH 8.5 with a mean of 1.7mg/l NH_4-N (Fig.44a). This difference was more pronounced when on day 16 the influent COD was increased to 4,000mg/l. Effluent ammonia in both plants increased and reached a peak of 45.5mg/l NH_4-N in the pilot plant at pH 7.5 compared to a peak of only 9.6mg/l NH_4-N in the pilot plant at pH 8.5. Both systems fully recovered following a reduction in the influent COD back to 2,000mg/l from day 33 until the end of the study.

A similar profile was observed for the effluent nitrates (Fig.44b). Overall, higher nitrates were recorded in the effluent at pH 8.5 than for the pilot plant at pH 7.5. As with the effluent ammonia the difference in nitrate levels was also more evident when the influent COD was increased to 4,000mg/l. The pilot plant at pH 7.5 recorded a minimum nitrate level of 20mg/l NO_3-N compared to 65mg/l NO_3-N for the pilot plant at pH 8.5.

When the experimental systems were shock loaded the percentage nitrification in the pilot plant at pH 7.5 fell to a minimum of 35% compared to 88% for the pilot plant at pH 8.5 (Fig.44c). Both systems recovered and supported 100% nitrification once the shock load of COD was removed.



(a)



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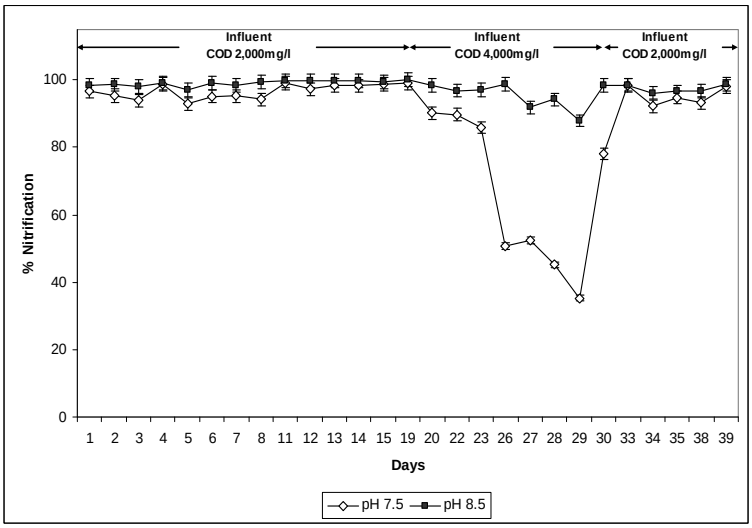


(c)

Fig. 44. Influence of the operating pH on nitrification in a pilot plant system in response to a shock chemical oxygen demand (COD) load; (a) effluent ammonia, (b) effluent nitrates and (c) percentage nitrification.

There was a statistical relationship, as presented in Table 17, between the influent COD and the percentage nitrification; this was stronger at pH 7.5 than at pH 8.5. The Pearson's correlation was -75.6% and the regression co-efficient was 55.5% at pH 7.5; this compared to -61.4% and 35.2% respectively at pH 8.5. In both instances the P value was 0.000. Based on the difference of the regression co-efficient, the nitrifying sludge at pH 7.5 was 20.3% more sensitive to a rise in influent COD than the pilot plant at pH 8.5. The maximum level of free ammonia was less than 1.4mg/l FA (Fig.45). As the highest levels of free ammonia were in the pilot plant at pH 8.5, this can be ruled out as the source of inhibition. The results of this study suggested that nitrification was more sensitive to a shock COD load at a pH of 7.5 than at a pH of 8.5.

Table 17: **Statistical analysis between the influent COD and the percentage nitrification for the pilot plant systems at pH 7.5 and pH 8.5.**

Test	pH 7.5	pH 8.5
Pearson correlation	-75.6%	-61.4%
Regression R-Sq(adj)	55.5%	35.2%
Hypothesis test P-value	0.000	0.000

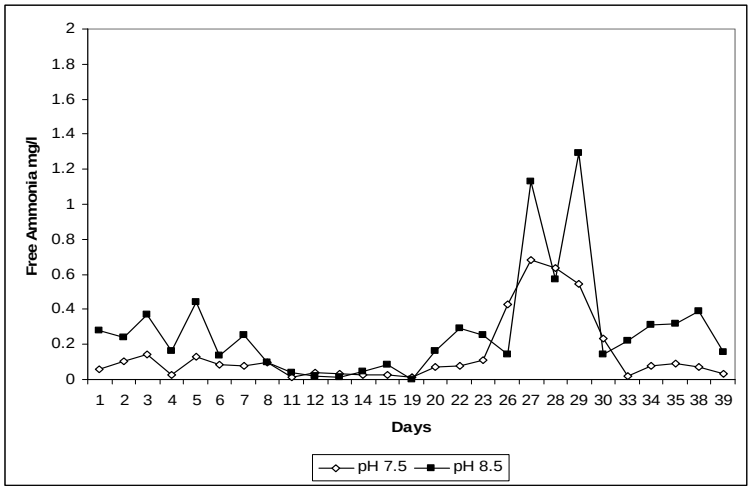


Fig. 45. Comparison of the free ammonia levels in the pilot plant systems studying the influence of the operating pH on nitrification in response to a shock chemical oxygen demand (COD) loading.

3.2.1.7 Summary of findings

The findings from part 1 of the pilot plant study were:

- The addition of micronutrients had no significant benefit to nitrification however the removal of the inorganic fraction from the waste water had a significant deleterious effect under the conditions of a high COD loading.

- An influent ammonia concentration of at least 50mg/l NH_4-N or a C/N ratio of less than 40 sustained a nitrifier population. Below 6mg/l NH_4-N or above a C/N of 220 the nitrifier population was starved from the system within 2 weeks.

- The influent COD needed to be kept below 3,800mg/l to avoid nitrification inhibition.

- A 50% failure in nitrification is likely to occur at a free ammonia level of greater 1.5mg/l.

- On a high COD loading nitrification was more inhibited at pH 7.5 than at pH 8.5.

3.2.2 Modification of the system design

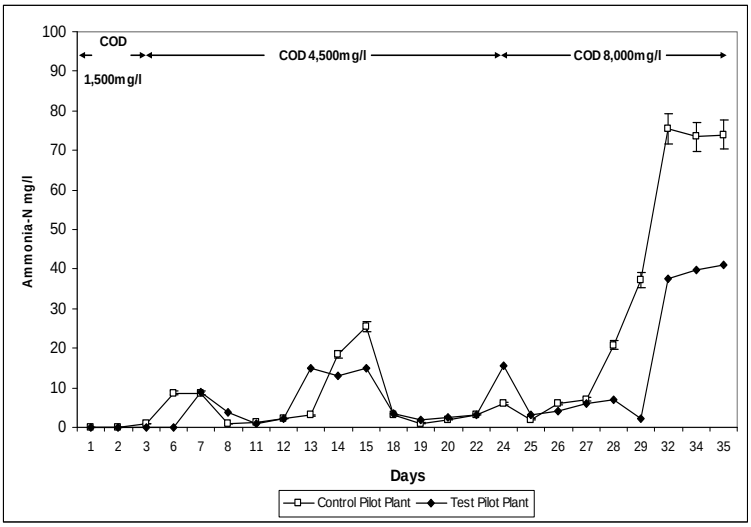
Modification of the pilot plant system was investigated to determine if nitrification could be enhanced by:- modification of the sludge recycle; the benefits of a two stage system and addition of a fixed film support; (a) activated carbon and (b) buoyant plastic media.

3.2.2.1 Modification of the sludge recycle

The sludge recycle rate in the test system was altered as described in Section 2.2.2.4. The pilot plants were operated in a continuous feed mode using the stock feed solution. The influent ammonia concentration was 100mg/l NH_4-N. The pH was 7.5 and a mean MLSS level of 6,000mg/l was maintained. The influent COD was 1,500mg/l from day 1-3; 4,500mg/l from day 4-20 and 8,000mg/l from day 21 until the end of the trial on day 35.

When the effluent ammonia levels were monitored, the levels initially were similar in both systems. The levels rose following a COD shock load of 4,500mg/l, recovered and rose again more dramatically following an increase in influent COD from 4,500mg/l to 8,000mg/l. The levels of effluent ammonia increased in both systems but the increase was less in the test pilot plant with a peak value of 41mg/l NH_4-N compared to the 75mg/l NH_4-N in the control (Fig.46a). The levels of nitrate in both systems mirrored the levels of effluent ammonia. The levels fell following the initial COD shock load of 4,500mg/l, recovered and fell sharply following the shock load of 8,000mg/l COD (Fig.46b). Throughout the run and in particular during the initial COD shock load, the levels of nitrate were lower in the test system.

When the percentage nitrification was studied (Fig.46c), the results were similar for both systems however the fall in nitrification following the second and greater COD shock load was more pronounced in the control system. While the percentage nitrification in the test system was slightly greater than that in the control system, the difference between the systems was less marked than that observed when the levels of effluent ammonia were measured. This suggested that the metabolic activity of the heterotrophic population in the test system was greater than the control system.



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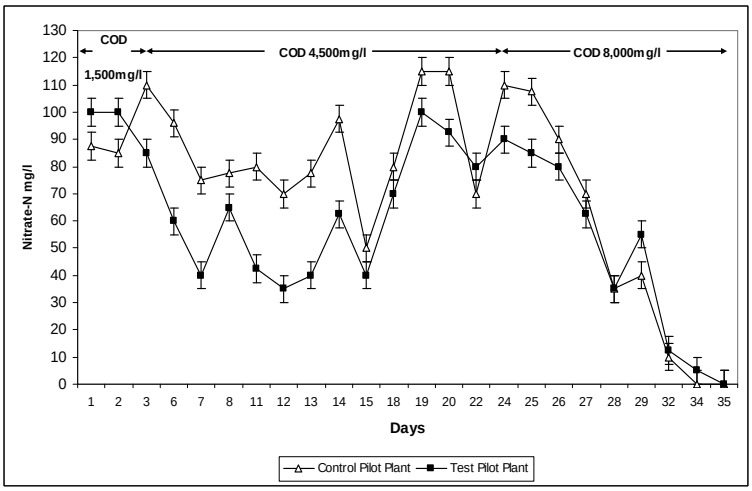

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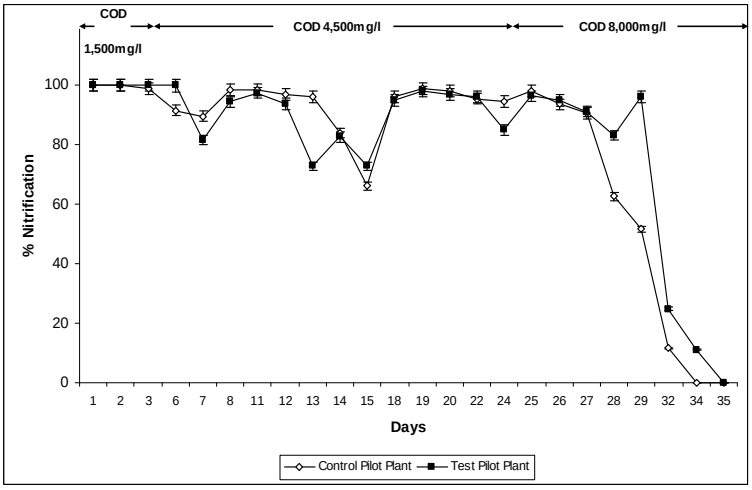


(c)

Fig.46. Influence on nitrification in the pilot plant system by varying the sludge recycle profile under shock chemical oxygen demand (COD) loadings; (a) effluent ammonia, (b) effluent nitrates and (c) the percentage nitrification.

When the COD removal efficiency in both systems was measured, the removal efficiency was slightly greater for the test system following the initial shock load and was significantly greater following the second shock load. The test pilot plant removed a mean 0.22mg of COD/mg biomass compared to 0.18 mg COD per mg of biomass for the control pilot plant (Fig.47).

The results showed that the system modification had little effect on nitrification however the performance of the heterotrophic population was enhanced.

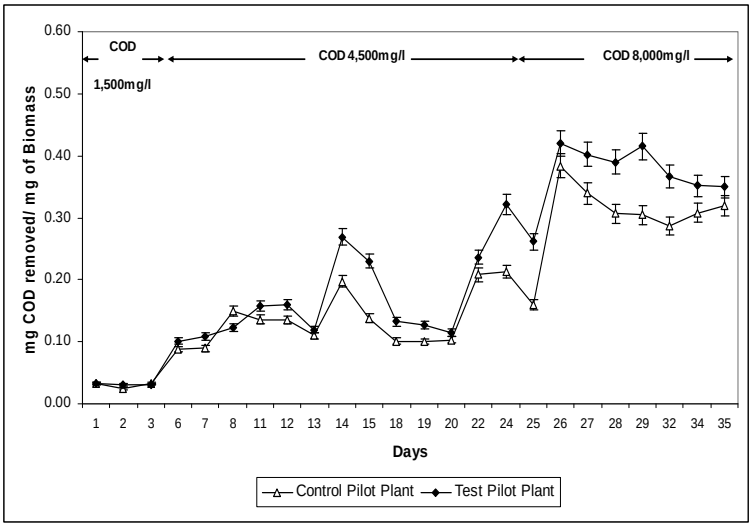


Fig.47. Chemical oxygen demand (COD) removal rates in two pilot plant systems studying the influence on nitrification by varying the sludge recycle profile under shock chemical oxygen demand (COD) loadings.

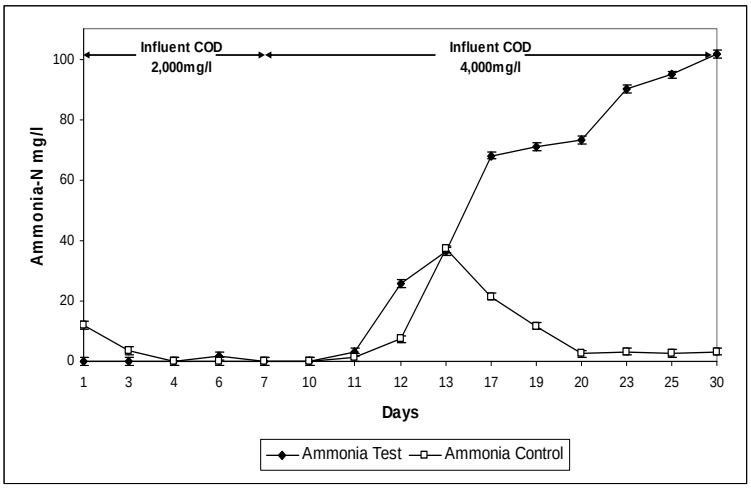
3.2.2.2 Two stage system

The variation of the sludge recycle rate was unsuccessful in significantly enhancing nitrification therefore a true two stage system was studied. An Eckendfelder reactor was placed in front of the test system as described in section 2.2.2.4. Both pilot plant systems were operated in continuous feed mode. The pH of both systems was 7.5, the mean MLSS at the start of the study was 3,500mg/l. An influent COD of 2,000mg/l was applied to both systems from day 1-6 and this was increased to 4,000mg/l from day 7 until the end of the trial on day 30. The influent ammonia to the second stage of both systems was maintained at 100mg/l NH_4-N.

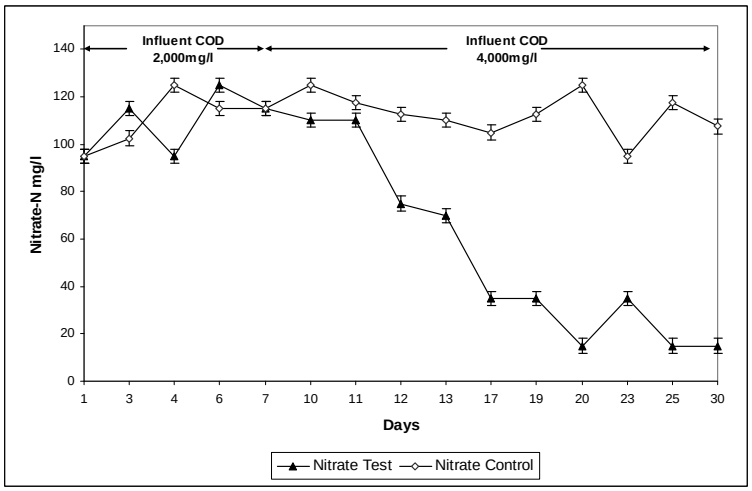
Nitrification

Both systems achieved full nitrification with effluent ammonia levels less than 1mg/l NH_4-N and nitrates in excess of 100mg/l NO_3-N when a COD of 2,000mg/l was applied (Fig.48a,b,c). On day 7 both systems were shocked loaded with a COD of 4,000mg/l. In response the effluent ammonia levels increased in both pilot plants. In the control, a peak of 37mg/l NH_4-N was detected on day 13 after which the levels fell back to less than 3mg/l NH_4-N. Effluent ammonia levels in the test pilot plant also increased but showed no evidence of recovery and by day 30 levels were in excess of 100mg/l NH_4-N (Fig.48a). The levels of effluent nitrates in the control system remained above 100mg/l NO_3-N, while in the test system the nitrates fell to a low of 15mg/l NO_3-N (Fig. 48b). The percentage nitrification in the control system, while it dipped slightly following the shock load, quickly recovered to 100%. However, in the test system the percentage nitrification fell consistently following the shock load to a final low of 11% on day 30 (Fig.48c).

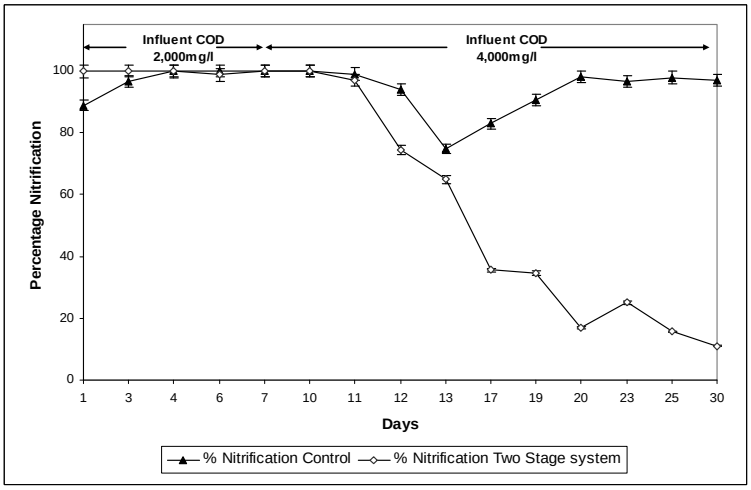
A closer examination of nitrates in each of the reactors of both pilot plant systems showed there was no nitrification taking place in the Eckenfelder reactor. Nitrates were evident in the second stage of this system until day 11 after which they fell steadily to <15mg/l NO_3-N by the end of the study. In comparison, equal and sustained levels of nitrates were recorded in both reactors of the control system. This showed nitrification was equally split between both reactors of the control system but was confined to the second stage only of the test system (Fig.49). The results of this study suggested that a two stage system was not beneficial to nitrification under high COD loadings.



(a)



(b)



(c)

Fig.48. Influence on nitrification of a two stage pilot plant (test system) compared to a single stage system (control pilot plant) in response to a shock chemical oxygen demand (COD) load; (a) effluent ammonia, (b) effluent nitrate and (c) percentage nitrification.

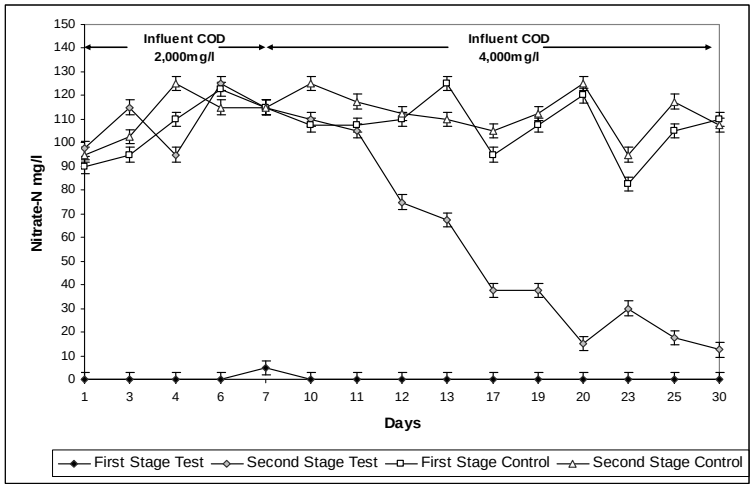


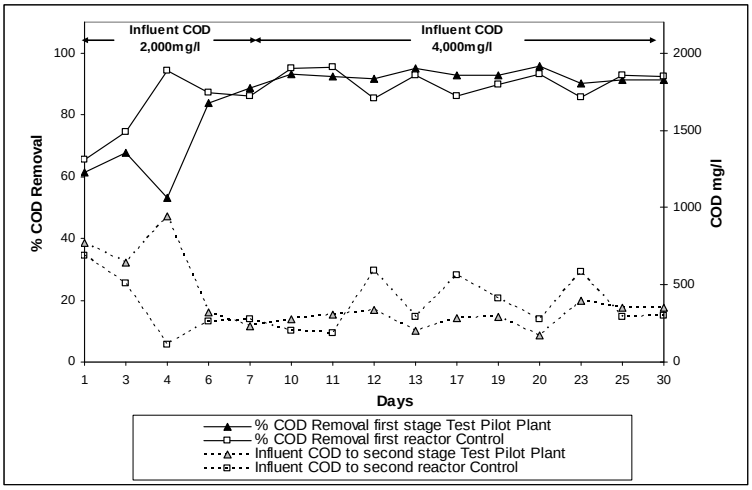
Fig.49. Levels of nitrates in each reactor of the test and the control pilot plant

COD removal

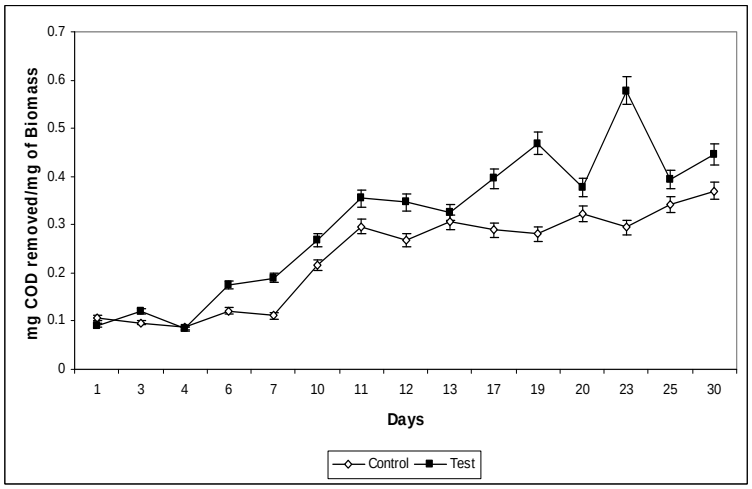
When the percentage COD removal was monitored it was found that 90% of the COD was removed in the first stage of both systems leaving a low level of COD of less than 500mg/l in the second stage of both systems (Fig.50a). However, when the removal of COD was expressed in terms of the biomass, the removal efficiency was greater in the test system (Fig.50b). The test system removed 0.40mg COD/mg biomass while the control system removed 0.30mg COD/mg biomass. The two stage test system was more effective in removing the influent COD than the control system.

MLSS

The high COD removal efficiency of Eckenfelder reactor had a significant effect on MLSS levels in the second stage of this system. The MLSS levels in the Eckenfelder reactor remained relatively steady with a mean MLSS of 2,900mg/l. However the MLSS levels in the second stage of the test system fell steadily from over 3,000mg/l to 955mg/l at the end of the trial (Fig.51a). In comparison the MLSS levels in both reactors of the control system were stable with an overall mean MLSS of 3,363mg/l (Fig.51b). A nitrifier stain of the MLSS in the second stage of each system on day 30 showed no significant levels of nitrifiers in the test system compared to the control (Fig.52a and b).

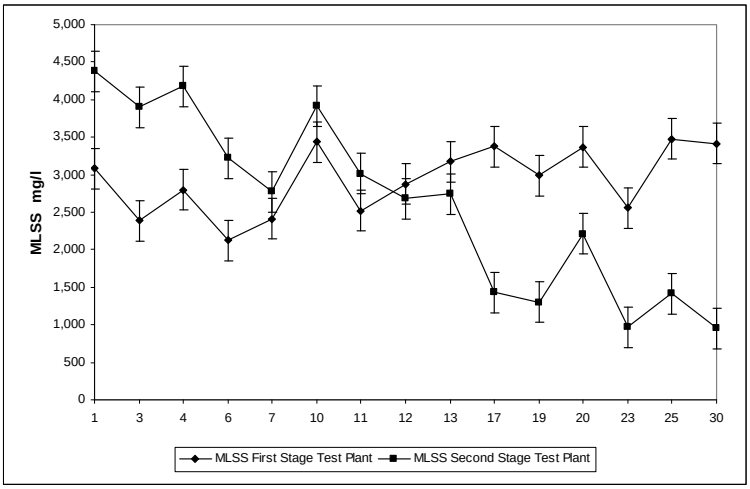


(a)

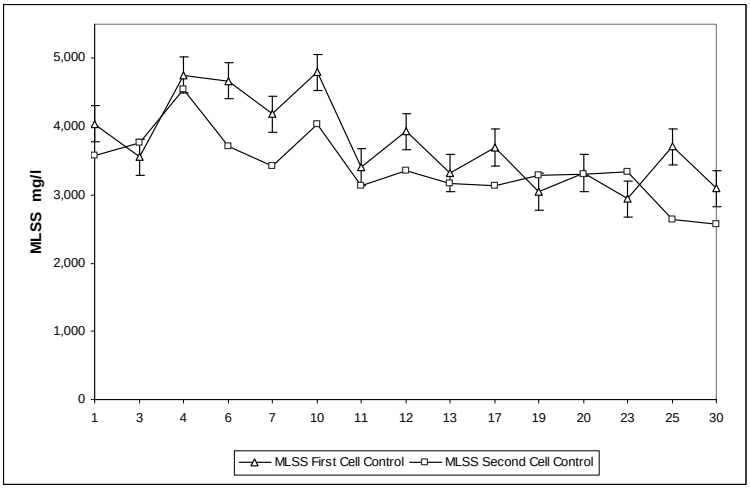


(b)

Fig.50. Chemical oxygen demand (COD) removal rates of a two stage system pilot plant system compared to a single mixed system; (a) COD removal efficiency and (b) standardised COD removal rates per unit biomass for both systems.

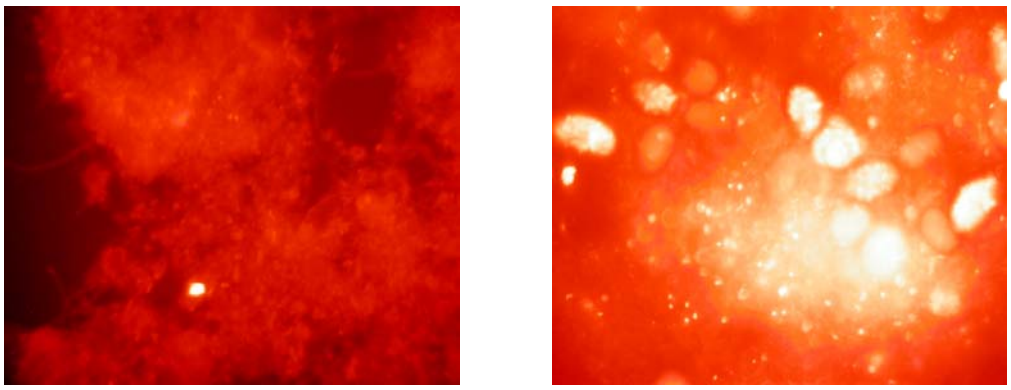


(a)



(b)

Fig.51. Influence on nitrification of a two stage pilot plant (test) compared to a single mixed stage system (control) in response to a shock chemical oxygen demand (COD) load; (a) mixed liquor suspended solids (MLSS) levels in the mixed system (control) and (b) MLSS levels in the two stage system (test).



(a) **(b)**

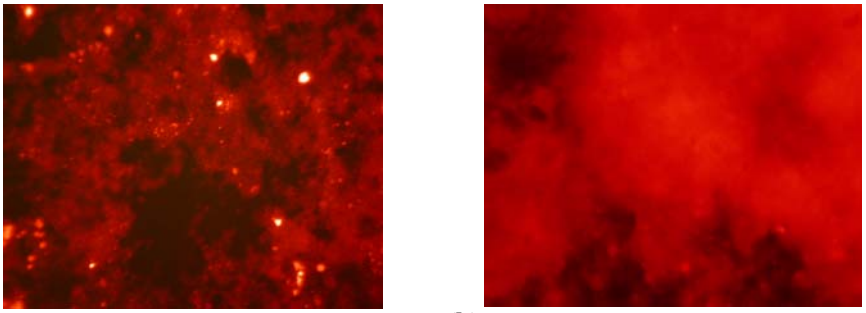
Fig.52. Nitrifier gene probe stain at the end of the study assessing the effect on nitrification of a two stage pilot plant (test system) compared to a single mixed stage system (control system) in response to a shock chemical oxygen demand (COD) load; (a) nitrifier stain of test pilot plant second stage and (b) nitrifier stain of the control system.

3.2.2.3 Distribution of nitrifiers within the MLSS.

A nitrifier targeted gene probe stain was used to study the location of nitrifiers in both the main WWTP and the pilot plant systems. The nitrifiers in the full scale WWTP were found to be attached to the sludge floc with no evidence of any nitrifiers in the supernatant (Fig.53a) and (Fig.53b).

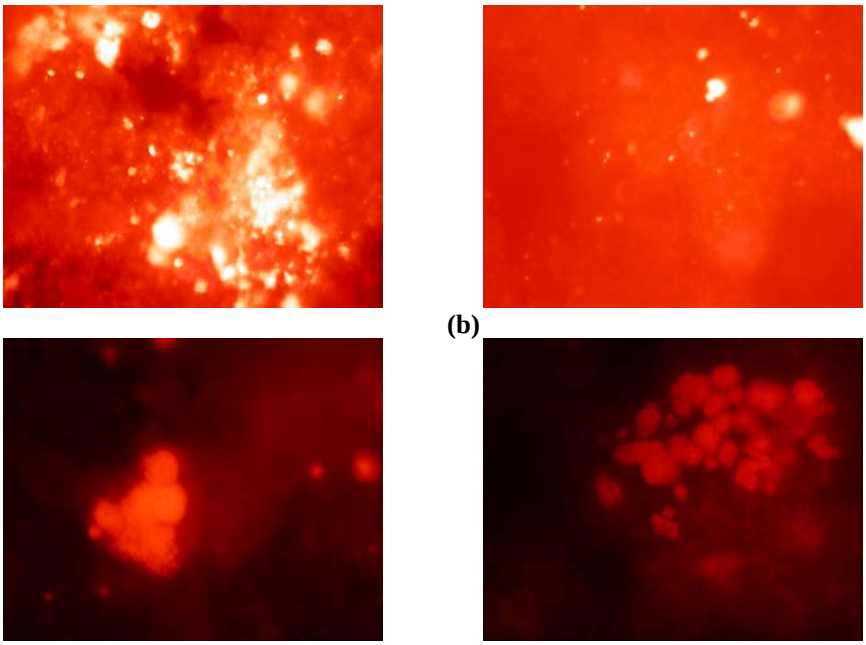
A gene probe stain on the pilot plant system showed that similarly to the full scale WWTP, the nitrifier population was also predominantly attached to the activated sludge floc (Fig.54a) with very few cells in suspension (Fig.54b). A closer examination of a single point of fluorescence from the pilot plant system showed that the individual nitrifier cells were clustered together to form a ball like structure (Fig.54c and Fig.54d).

The results showed that the majority of the nitrifiers were associated with the suspended solids. The addition of a solid matrix which might enhance the growth of nitrifiers was therefore investigated. Activated carbon and a buoyant plastic medium (BPM) were the materials selected for the study.



(a) (b)

Fig.53. Nitrification gene probe stain of the activated sludge from the full scale WWTP achieving 100% nitrification on an influent ammonia of 20-25mg/l NH_4-N; (a) stain of sludge floc x 500 magnification, (b) stain of the supernatant x 500 magnification after sludge was allowed to settle for 30 minutes.



(a) (b)

(c) (d)

Fig.54. Nitrification gene probe stain of the activated sludge from the pilot plant achieving 100% nitrification on an influent ammonia of 100mg/l NH_4-N; (a) stain of sludge floc x 500 magnification, (b) stain of the supernatant x 500 magnification after sludge was allowed to settle for 30minutes, (c&d) cluster of nitrifier cells x 1000 magnification.

3.2.2.4 The influence of activated carbon on nitrification

Prior to using activated carbon in the study, the potential of activated carbon to leach contaminants and adsorb chemicals from the waste water feed was examined (section 2.2.2.4).

There was no rise in COD or nitrate after 1 hour contact with 2,000mg/l of activated carbon and de-ionised water. There was a negligible increase in ammonia levels of less 0.02mg/l (Table 18).

Table 18 COD, ammonia or nitrate levels in water without and without activated carbon.

	De-ionised Water without any activated carbon mg/l		De-ionised water after mixing for 1 hour with 2g activated carbon mg/l	
	Sample A	**Sample B**	**Sample A**	**Sample B**
COD	0	0	0	0
Ammonia	0	0	0.02	0.02
Nitrate	0	0	0	0

The COD concentration in the waste water feed decreased with an increasing concentration of activated carbon. This relationship had a Pearson's correlation of -92.2% and a regression co-efficient of 99.7% Given the best fit for COD was a quadratic line then the rate of adsorption of COD was not linear but decreased with an increasing activated carbon concentration. As the P value is 0.078 and given that this is just above the 0.05 hypothesis acceptance criteria then any relationship between increasing COD adsorption and increasing activated carbon is rejected.

Ammonia levels appeared to have increased slightly with a Pearson correlation of 94.2% and a regression co-efficient of 83.2%. As the strongest regression relationship was linear for ammonia then 1 mg of activated carbon will potentially desorb 0.008mg of ammonia. However, as the P value was 0.058 and as this was above the 0.05 hypothesis acceptance criteria then any relationship was also rejected. There was no significant relationship between the activated carbon concentration and the adsorption of nitrate with a Pearson's correlation of -36.3%, a regression co-efficient of 0.0% and a P value of 0.637 (Fig.55 and Table 19).

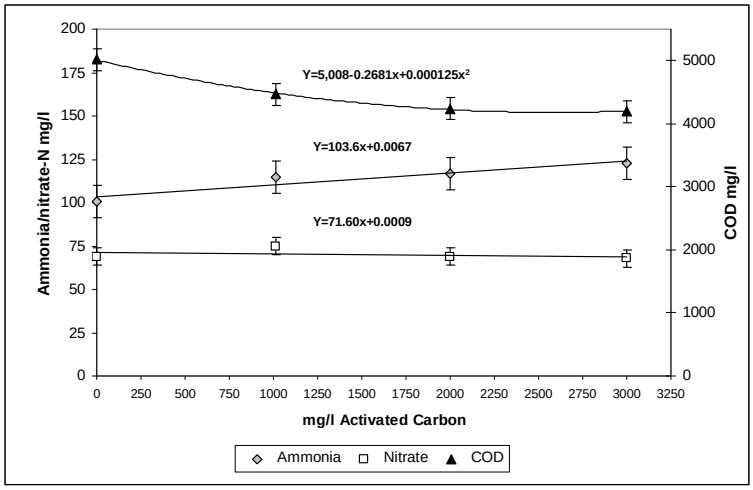


Fig. 55. Relationship between the concentration of activated carbon mixed with waste water and the resulting adsorption of (a) chemical oxygen demand (COD), (b) ammonia and (c) nitrate.

Table 19. Statistical testing of the trend lines in Fig.55 of the adsorption/desorption of ammonia, nitrate and COD at different activated carbon concentrations

Parameter	R-Sq(adj)	Pearson's Correlation	P value
Ammonia	83.2%	94.2%	0.058
Nitrate	0.0%	-36.3%	0.637
COD	99.7%	-92.2%	0.078

The pilot plant systems were operated in a continuous feed mode at a pH of 7.5 and a mean MLSS of 5,000mg/l The systems initially received a low COD loading of less than 1,500mg/l until day 11 and then both systems were shocked loaded with an influent COD of 8,000mg/l until the end of the study at day 27. On day 11, 500mg/l of activated carbon was added to the influent feed to the test pilot plant (section 2.2.2.4). The addition of the activated carbon was repeated daily until the end of the study.

Nitrification

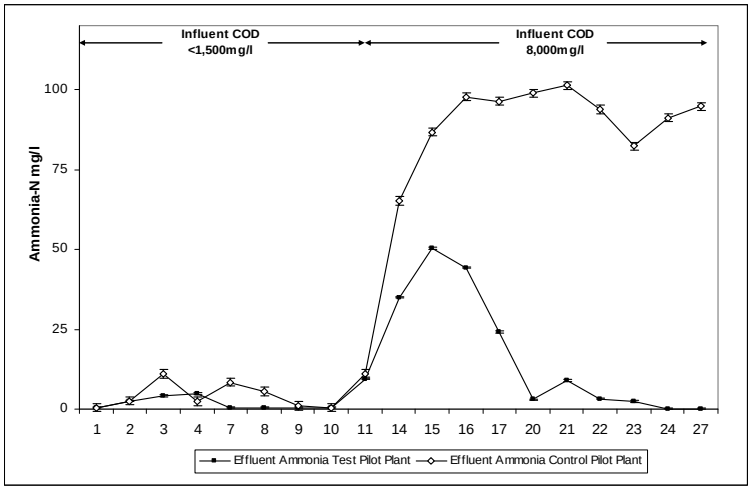
There was full nitrification in both systems for the first 11 days (Fig.56a). The levels of ammonia were less than 1mg/l NH_4-N in the effluent at an influent COD load of 1,500mg/l. Following the shock COD load on day 11 (COD 8,000mg/l) there was a loss of nitrification in both systems. Significant effluent ammonia breakthrough was recorded in the control pilot plant with a peak effluent ammonia of 101.3mg/l NH_4-N.

The levels of effluent ammonia remained above 80mg/l NH_4-N for the remainder of the run. In the test pilot plant, effluent ammonia levels peeked at 50.4mg/l NH_4-N on day 15, representing half the levels detected in the control system, and then declined reaching the original low levels on day 20.

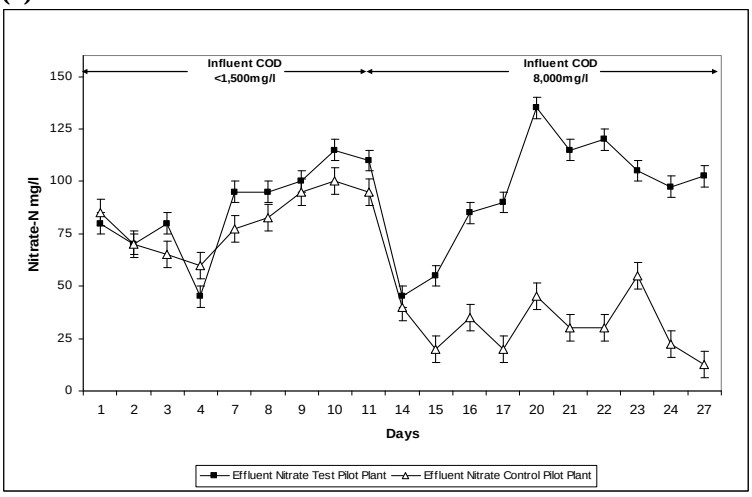
The levels of effluent nitrates closely complimented the effluent ammonia profiles in both pilot plants. During the first 11 day period when nitrification was present in both systems, the levels of effluent nitrates were similar in both pilot plants and fluctuated between 45mg/l and 115mg/l NO_3-N. Following the shock load on day 11, the levels of effluent nitrates fell sharply in both systems. In the control system, the levels fell to 20mg/lNO_3-N and remained below 55mg/l NO_3-N for the remainder of the run. In the test system, the levels fell initially to 45mg/l NO_3-N and then rose reaching the original levels by the end of the run (Fig.56b).

The percentage nitrification was similar and complete in both systems for the first 11 days. Following the COD shock load, nitrification fell in both systems. While the test pilot plant recovered within 6 days following the shock load, nitrification in the control system fell more dramatically and did not recover. Overall, the lowest percentage nitrification recorded in the control was 11.7% compared to 52.18% in the test pilot plant (Fig.56c).

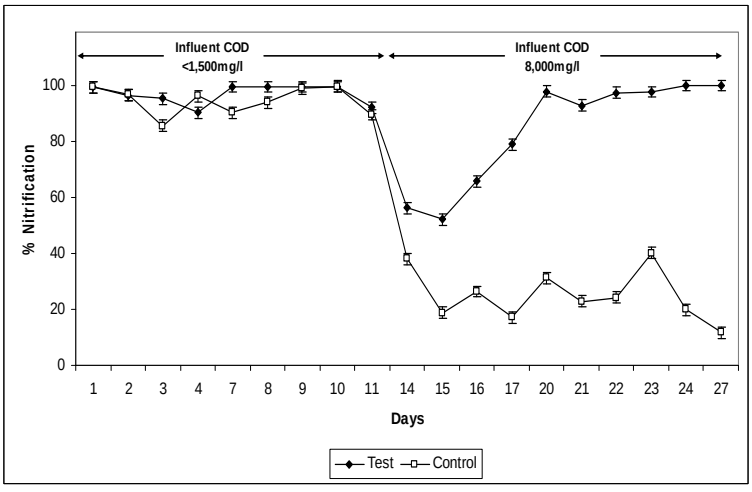
The recovery in nitrification in the test system correlated with the rise in concentration of activated carbon within the reactor. A scatter plot of the activated carbon concentration and the percentage nitrification returned a Pearson's correlation of 97%, a regression co-efficient of 94% and the P value of 0.000 passed the hypothesis acceptance criteria of 0.05 (Fig.57). At an activated carbon concentration of 1,000mg/l the nitrification rate was over 80% on an influent COD of 8,000mg/l.



(a)



(b)



(C)

Fig.56. Influence on nitrification of adding 500mg/l of activated carbon from day 11 onwards in a pilot plant system under shock chemical oxygen demand (COD) loading; (a) effluent ammonia, (b) effluent nitrates and (c) percentage nitrification.

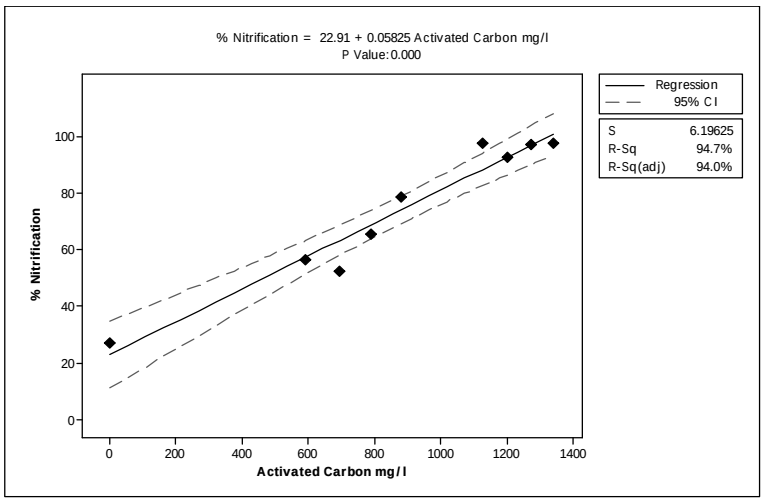


Fig.57. Scatter plot of the relationship between the concentration of activated carbon in the pilot plant reactor and the resulting percentage nitrification on an influent chemical oxygen demand (COD) of 8,000mg/l for a waste water from the full scale waste water treatment plant.

COD removal

A plot of the mg of COD removed per mg of biomass showed that the test pilot plant performed better than the control (Fig.58a). The control pilot plant, following the shock COD load, removed on average 0.48mg COD/mg of biomass whereas the test pilot plant removed 0.54mg COD/mg of biomass.

Oxygen Uptake Rate (OUR)

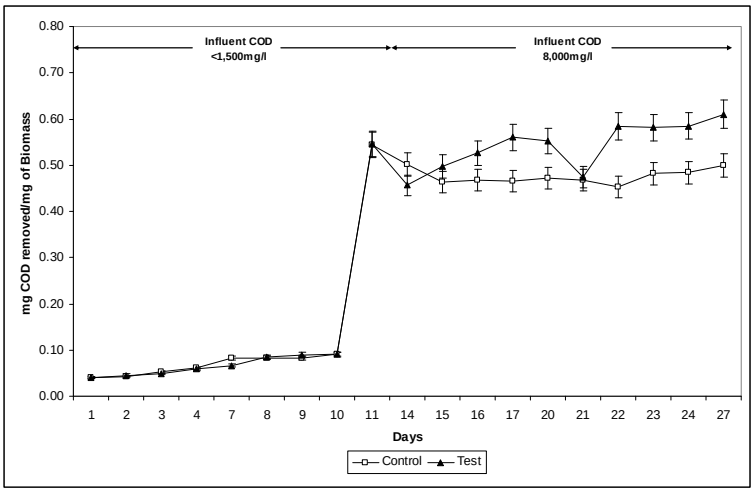
When the oxygen uptake rate for both systems was monitored (Fig.58b), the test system showed a higher level of oxygen uptake following the COD shock load indicating a higher metabolic activity in that system. The mean OUR following the shock COD load for the control and the test pilot plant was 6.73g O_2/hr/g MLSS and 7.34g O_2/hr/g MLSS respectively. The higher rate of oxygen uptake correlated with the higher rate of COD removal suggesting enhanced metabolic activity of the heterotrophic population in test system.

Effluent suspended solids

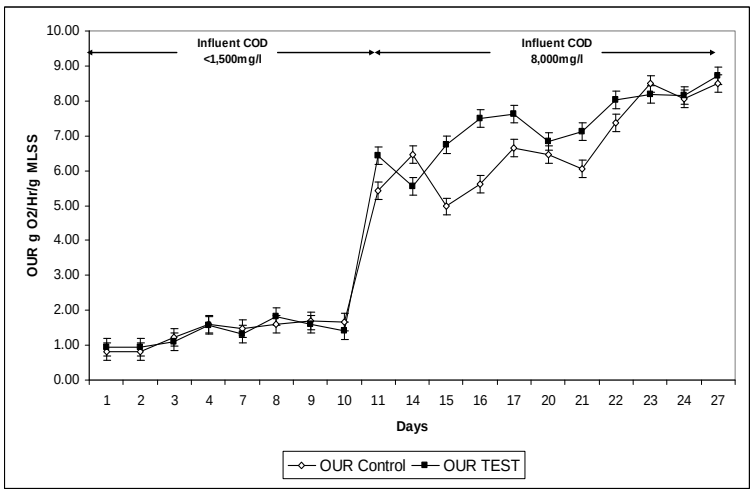
Final effluent suspended solids were less than 50mg/l in both systems prior to the increase in COD loading (Fig.58c). Following the shock COD loading effluent suspended solids increased in both pilot plants however overall lower levels were noted in the effluent of the test pilot plant. The mean effluent suspended solids were 176mg/l for the control compared to 118mg/l for the test pilot plant.

A sample of the floc from the test pilot plant was examined microscopically and showed that the activated carbon was associated with the floc. There was no evidence of free activated carbon in the liquor (Fig.59).

The results of this study indicated that the addition of activated carbon reduced the inhibition of nitrification under high COD loadings. The presence of the activated carbon also enhanced the metabolic activity of the heterotrophic population and led to a reduction in the levels of suspended solids in the final effluent.



(a)



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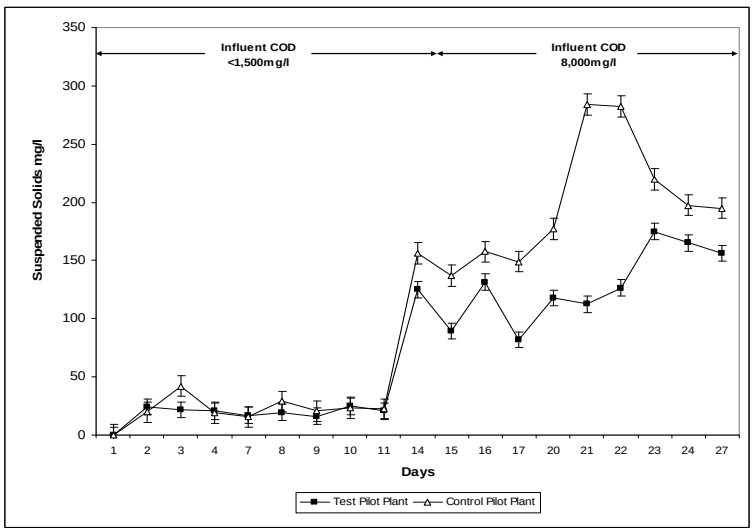
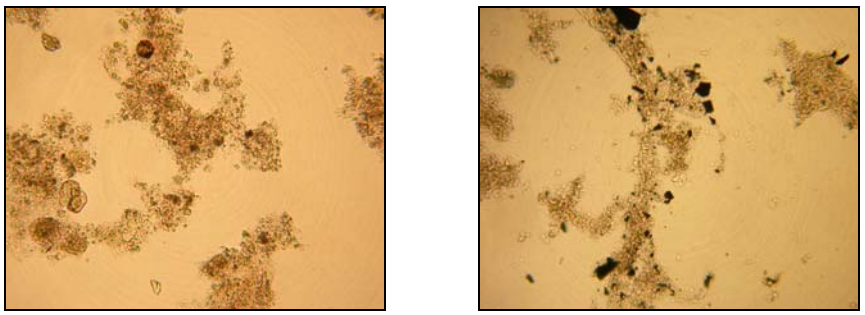


(c)

Fig.58. Influence on nitrification of adding 500mg/l of activated carbon from day 11 onwards in a pilot plant system under shock chemical oxygen demand (COD) loading; (a) COD removal rates, (b) oxygen uptake rates and (c) final effluent suspended solids.



(a) (b)

Fig. 59. Microscopic examination of an activated sludge floc from the pilot plant system following the addition of an activated carbon compared to a pilot plant without any activated carbon addition; (a) photograph by 10 magnification control pilot plant floc and (b) photograph by 10 magnification of the test pilot plant floc.

3.2.2.5 The role of a buoyant plastic media (BPM:- Kaldnes®) in an activated sludge reactor and its influence on nitrification.

The addition of a Buoyant Plastic Media (BPM) as a permanent fixed film system was investigated to assess the tolerance of a nitrifying sludge under inhibitory COD levels compared to conventional activated sludge.

The pilot plant systems were operated in continuous feed mode at pH 7.5 and a starting MLSS of 5,000-6,000mg/l. From day 1 until day 12 an influent COD load of 2,000mg/l was applied to both systems. This was increased to 3,500mg/l from day 13 until the end of the trial at Day 45. Four litres of Kaldnes® was added to the first reactor of the test system and operated as described in section 2.2.2.4

Nitrification

Good nitrification was evident in both pilot plants until day 23. During this time, the effluent ammonia levels in both systems were under 1mg/l NH_4-N (Fig.60a). From day 24-30 there was a temporary peak of effluent ammonia in the test pilot plant. This coincided with the relocation of the sludge recycle to the second reactor. On day 33 effluent ammonia levels increased again, this time in both pilot plants. This was unexpected as the influent COD was still only 3,500mg/l. The mean F/M in the control at this time was 0.27 compared to the test where the mean F/M was higher at 0.34 by virtue of a lower biomass.

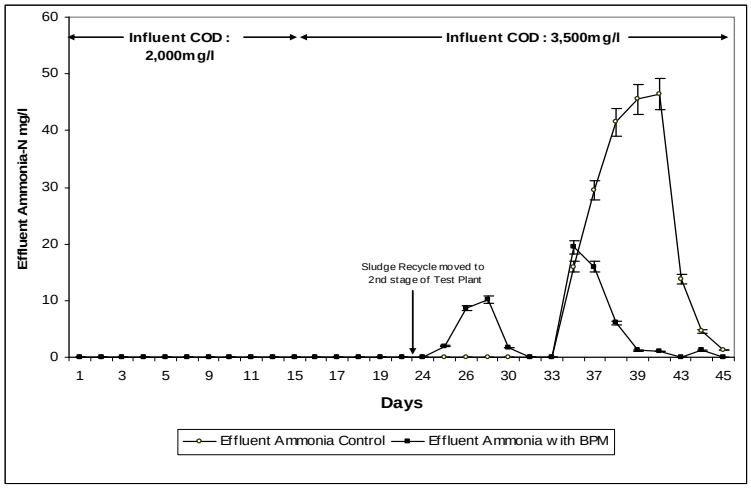
Effluent ammonia reached a peak of 19.5mg/l NH_4-N in the test pilot plant on day 35 and 46.5mg/l NH_4-N in the control pilot plant on day 42. Having reached these peak values, the levels of effluent ammonia in both systems then dropped. A similar profile was evident from day 33 for the effluent nitrates (Fig.60b); a minimum nitrate level of 67.5mg/l NO_3-N was recorded in the control compared to over 100mg/l NO_3-N in the test pilot plant. The percentage nitrification following day 33 showed that the lowest percentage nitrification recorded in the control was 60% compared to 83% in the test system (Fig.60c). While both systems recovered nitrification, the rate of recovery was greater when the BPM was present. Full nitrification was restored in the test pilot plant within 7 days compared to 12 days for the control.

Biomass in the systems

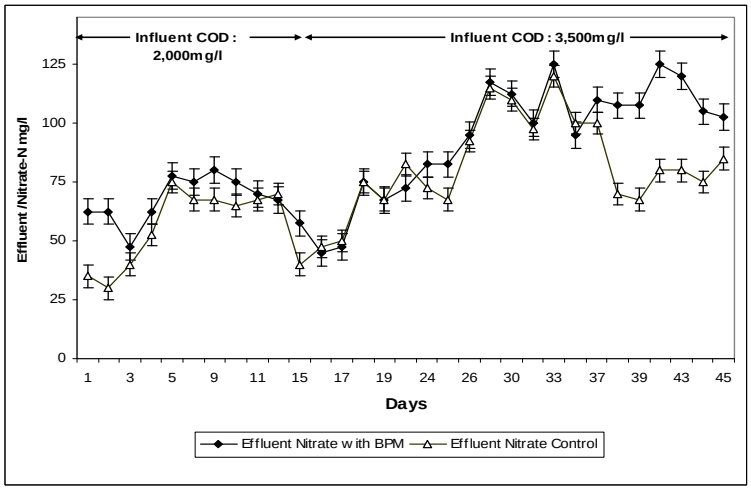
The MLSS in both plants fell at a gradual rate throughout the experiment. The levels were similar in both reactors of the control system and in the second reactor of the test system. However, towards the end of the experiment, the MLSS in the first reactor of the test system was lower than the other reactors. On day 23 the sludge recycle was moved to the second stage of the test pilot plant to encourage biofilm growth on the BPM. This resulted in a significant drop in the MLSS of the first stage of the test pilot plant (Fig.61a). By day 45 there was only 410mg/l MLSS in the first stage of the test pilot plant compared to over 3,400mg/l in the first stage of the control.

At the end of the study the level of solids in the control system was 3,600mg/l and was 2,500mg/l for the test pilot plant (Fig.61b). Overall, there were lower biomass levels in the test system than the control. The figure for the test system included the biomass growing on the BPM. The growth of a biofilm on the BPM was evident when visually compared to some unused BPM (Fig.62a).

A nitrifier gene probe stain showed that nitrifiers were located on the sludge floc in both systems and that significant numbers of nitrifiers were associated with the BPM. In the test system, the nitrifiers were growing predominantly on the BPM with a much smaller distribution suspended in the liquor in the first stage of the test pilot plant (Fig. 62b,c and d).



(a)



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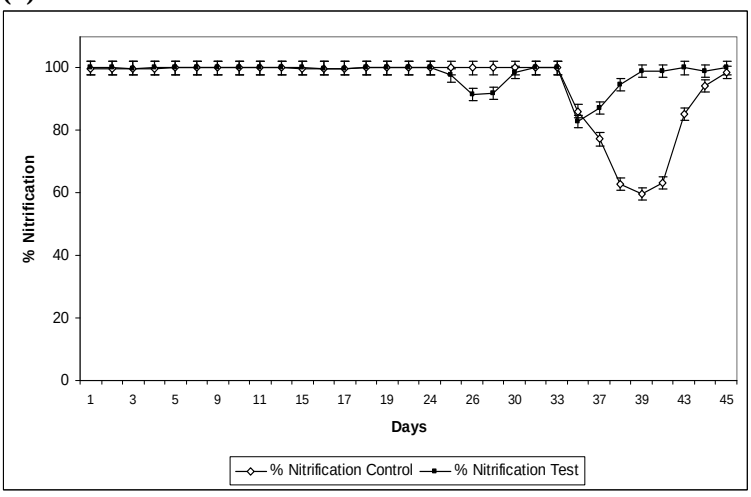
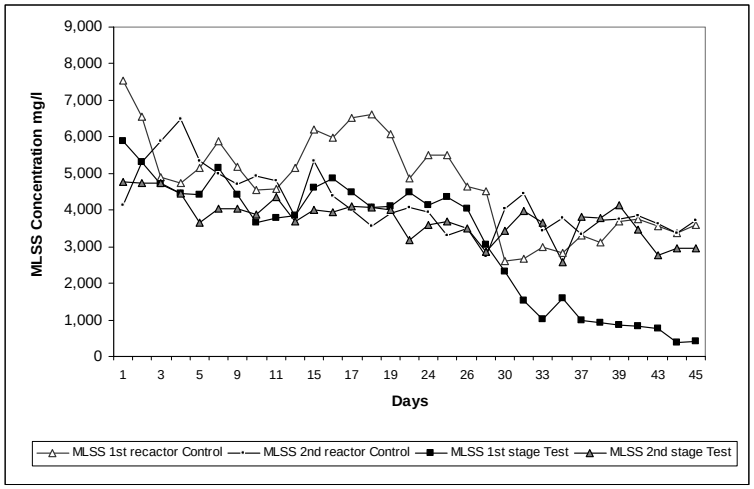


(c)

Fig.60. Influence on nitrification in two pilot plant systems. The test pilot plant had 40% v/v of a buoyant plastic media (BPM) added to the first reactor; (a) effluent ammonia,(b) effluent nitrates and (c) percentage nitrification.



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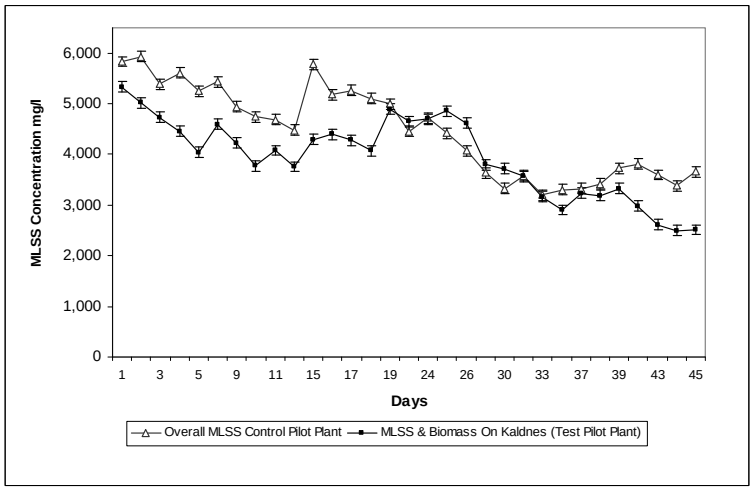


(b)

Fig.61. Comparison of the performance in nitrification in two pilot plant systems. The test pilot plant had 40% v/v of a buoyant plastic media (BPM) added to the first reactor; (a) MLSS levels in each reactor and (b) overall biomass levels in both pilot plant systems.

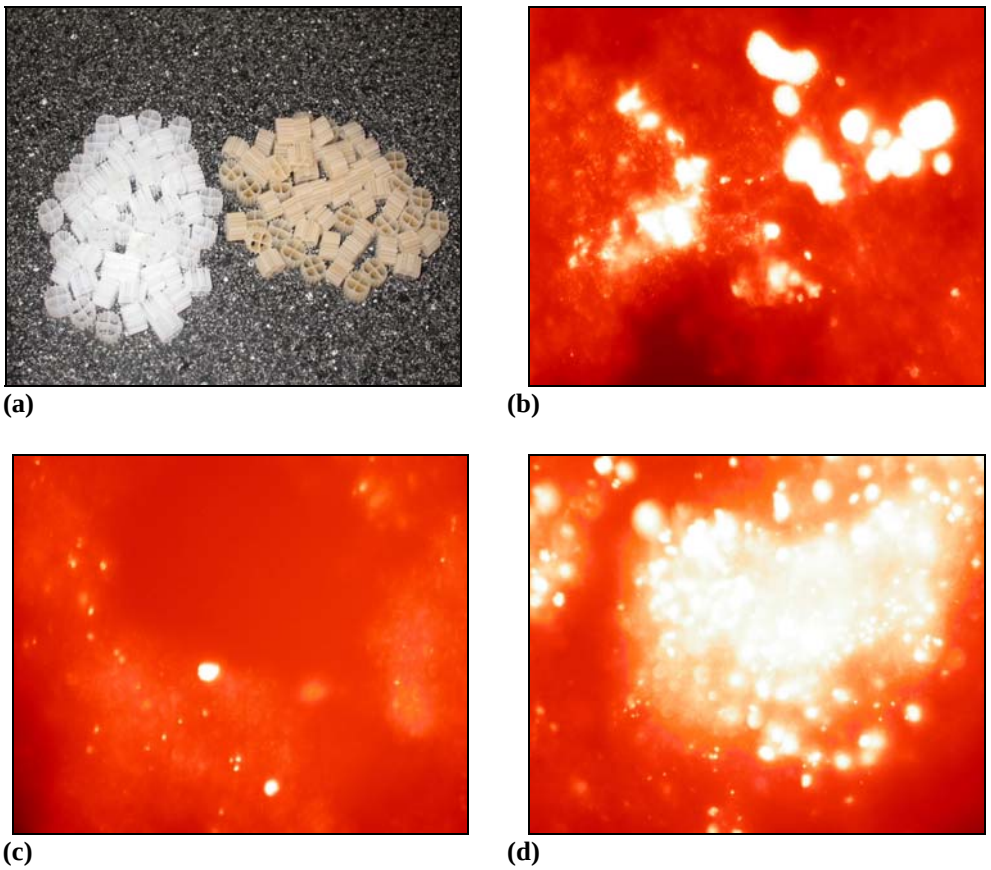


Fig. 62. Comparison of biofilm growth and the distribution of nitrifiers in a pilot plant system with 40% v/v of a buoyant a plastic media (BPM) added to the activated sludge compared to a conventional pilot plant activated sludge without any BPM addition ; (a) photograph of unused BPM with BPM from the pilot plant after 6 weeks; nitrifier gene probe stains of (b) sludge floc from the control pilot plant, (c) sludge floc from the test reactor with BPM and (d) a scraping of biofilm from the BPM.

COD removal and oxygen uptake rate (OUR)

When the removal of COD per mg of biomass was monitored the test system performed better especially towards the end of the study when the removal of COD was 1.3-1.4mg COD/mg biomass for the test system compared to 0.8-0.9mg COD/mg biomass for the control system (Fig.63). The mean heterotrophic oxygen uptake rates (OUR) of the two pilot plant systems showed that the respiratory activity in the test system was significantly greater than that in the control system. The control system had a mean heterotrophic OUR of 0.18g O_2/hr/g MLSS in the first reactor and 0.10g O_2/hr/g MLSS in the second reactor. This compared to a mean heterotrophic OUR of 1.23g O_2/hr/g MLSS in the first reactor of the test pilot plant, of which 0.79g O_2/hr/g MLSS or 64% of the OUR was attributable to the biofilm on the BPM. The heterotrophic OUR in the second reactor was comparable to the control with a value of 0.11g O_2/hr/g MLSS (Fig.64).

The results of this study indicated that there was a positive benefit to nitrification through the addition of a buoyant plastic medium under inhibitory COD loads. When the medium was present the nitrifiers were predominantly associated with the solid surface and the metabolic activity of the heterotrophs was enhanced.

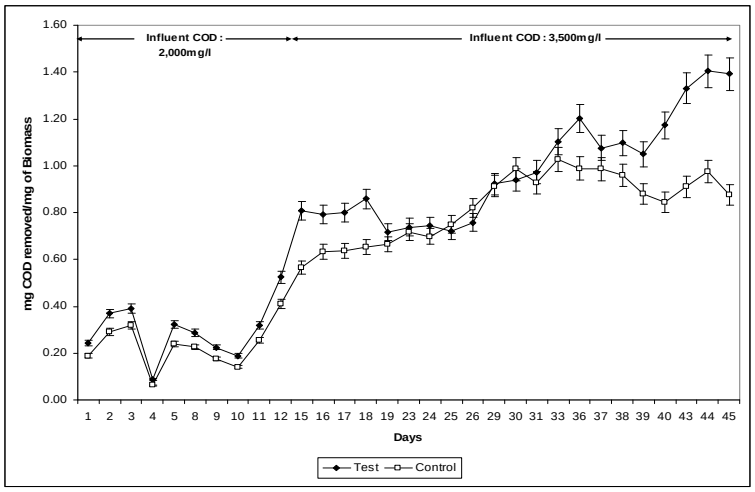


Fig.63. Influence on the chemical oxygen demand (COD) removal rates following the addition of a 40% V/V buoyant plastic media (BPM) to the test pilot plant system.

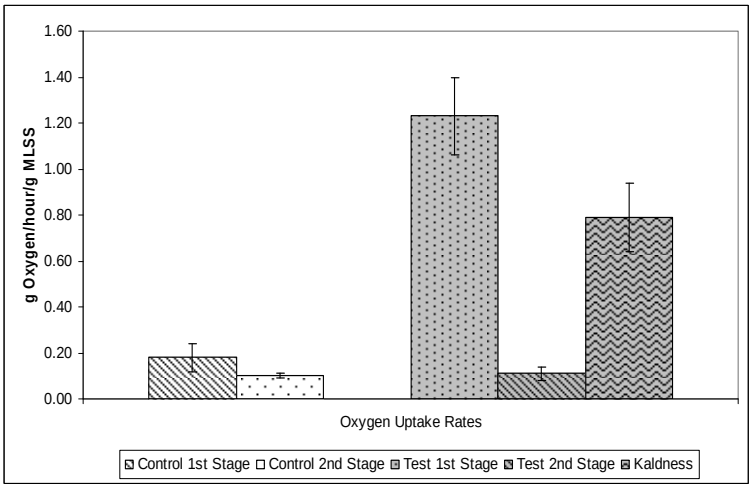


Fig.64. Comparison of the mean oxygen uptake rates of two pilot plant systems. The test pilot plant had 40% v/v buoyant plastic media (BPM) added to the first reactor only.

3.2.2.6 Summary of findings

The findings from part 2 of the pilot plant study were:

- Modification of the sludge recycle rate was of no benefit to nitrification under a shock COD load of 8,000mg/l, however better COD removal rates and a higher uptake of ammonia was shown by the heterotrophic population.

- The addition of an Eckenfelder reactor to create a true two stage system did not improve nitrification. The Eckenfelder reactor removed 90% of the 4,000mg/l influent COD. This reduced the COD load to the second stage to less than 500mg/l which in turn lead to a fall in the MLSS in this stage where nitrification was taking place.

- A gene probe stain of the MLSS and supernatant of the full scale WWTP and of the pilot plant showed the majority of the nitrifiers were associated with the suspended solids.

- The addition of 500mg/l of activated carbon as a support structure protected nitrification under a shock COD loading of 8,000mg/l. Higher heterotrophic oxygen uptake rates, better COD removal rates and lower effluent suspended solids were recorded for the system with activated carbon.

- The addition of a 40% V/V of buoyant plastic media (BPM) resulted in better nitrification. Higher heterotrophic oxygen uptake rates and COD removal rates were recorded for the system with the BPM. The highest OUR was associated with the biofilm adhered to the BPM.

3.2.3 Relationship between biomass levels and nitrification

The enhancement of nitrification by a modification in system design also led to an enhancement of the heterotrophic population. In order to investigate the relationship between the heterotrophic biomass population and nitrification, a number of factors were investigated including, the role of the MLSS, the importance of acclimatisation of the activated sludge to high COD loadings and the bioaugumentation of the system with a heterotroph *Pseudomonas pudia* CPI.

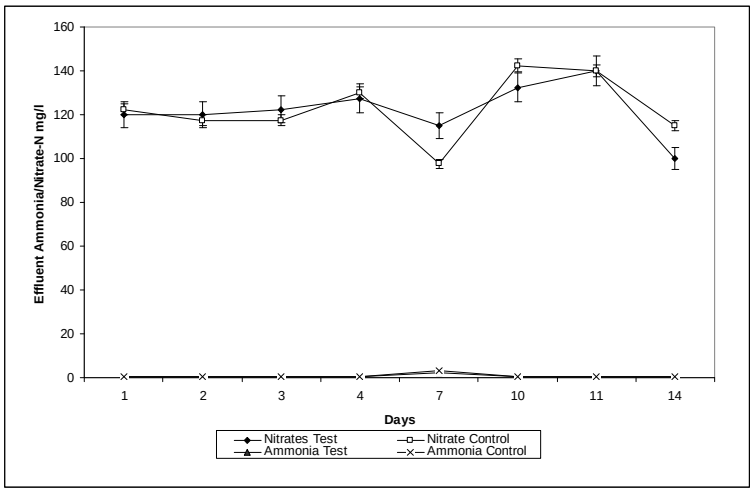
3.2.3.1 The role of MLSS levels in nitrification on a low and a high COD loading

The relationship between MLSS and nitrification was investigated at low and high influent COD levels.

Low COD

The relationship between MLSS and nitrification at low influent COD levels was investigated at two concentrations of MLSS. A mean MLSS concentration of 4,000mg/l was established in the control pilot plant and a mean MLSS of 8,000mg/l was applied in the test pilot plant. The pH of the pilot plant systems was maintained at pH 7.5 and both systems were operated in a continuous feed mode. The influent COD concentration was 2,000mg/l for the 14 day trial. As both systems had the same influent COD concentration but different MLSS levels the F/M in both plants was also therefore different. The mean F/M in the control system was 0.06 and was 0.03 in the test system.

Comparable levels of nitrates and ammonia were evident in the effluent (Fig.65a) and both systems maintained 100% nitrification (Fig.65b). The final effluent COD values fell over the duration of the experiment, however the values were similar with a mean of 121mg/l in both systems (Fig.65c).



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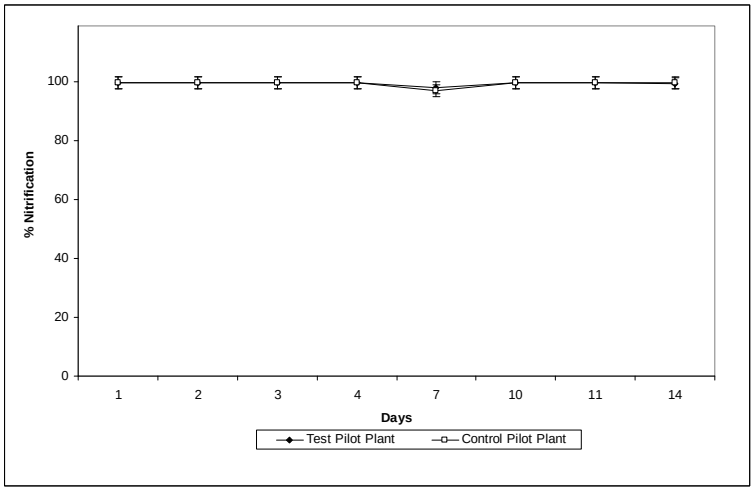

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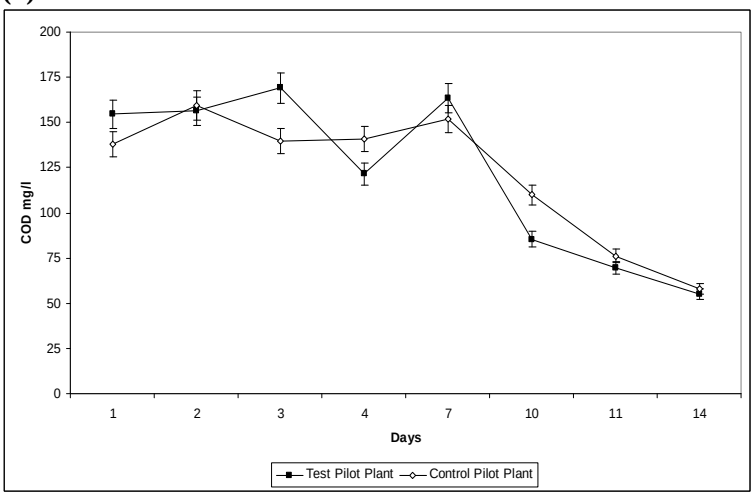


(c)

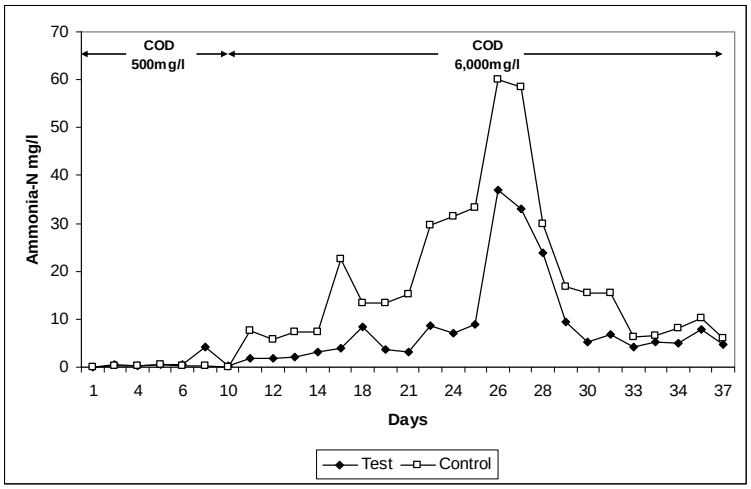
Fig.65. Nitrification performance in a pilot plant system on a low COD loading with an MLSS of 4,000mg/l (control pilot plant) and with an MLSS of 8,000mg/l (test pilot plant); (a) effluent ammonia and nitrate, (b) percentage nitrification and (c) effluent COD's.

High COD

To assess if a higher MLSS level would protect nitrification under a high COD loading an investigation was carried out using the two pilot plant systems. A mean MLSS concentration of 4,700mg/l was established in the control pilot plant and a mean MLSS of 8,100mg/l in the test pilot plant. The pH was maintained at pH 7.5. From day 1 to day 9 an influent COD of less than 500mg/l was applied. The systems were then shock loaded on day 10 to 6,000mg/l for the remainder of the study until day 37. The mean F/M in the control was 0.36; this was higher than the test by virtue of a lower MLSS level. The mean F/M of the test system was 0.20.

Low effluent ammonias were initially recorded in both systems on an influent COD of 500mg/l (Fig.66a). Following the application of a shock COD load of 6,000mg/l effluent ammonia increased in both pilot plants and by day 26 reached a peak of 60mg/l NH_4-N in the control pilot plant compared to only 37mg/l NH_4-N in the test pilot plant. This observation was reflected in the levels of effluent nitrates. In line with rising effluent ammonia, effluent nitrate levels fell over the same period reaching a minimum of 27mg/l NO_3-N in the control and 63 mg/l NO_3-N in test pilot plant (Fig.66b). The COD shock load caused a drop in the percentage nitrification in both systems. The percentage nitrification reached a low of 31% in the control pilot plant and 68% in the test pilot plant. However, while nitrification in both systems recovered, nitrification in the test system with the higher concentration of MLSS recovered more quickly (Fig.66c).

The levels of effluent COD was similar in both systems at low COD loading. However, following the COD shock load, the test pilot plant produced lower effluent COD's with a mean of 533mg/l compared to the control system with a mean COD of 732mg/l (Fig.67).



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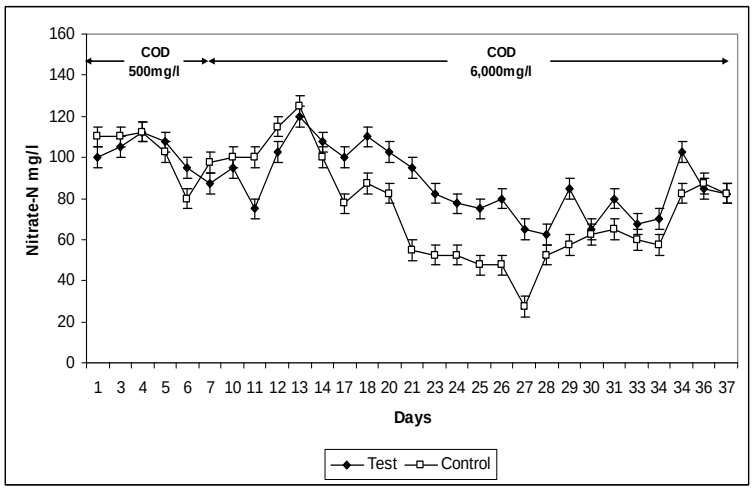

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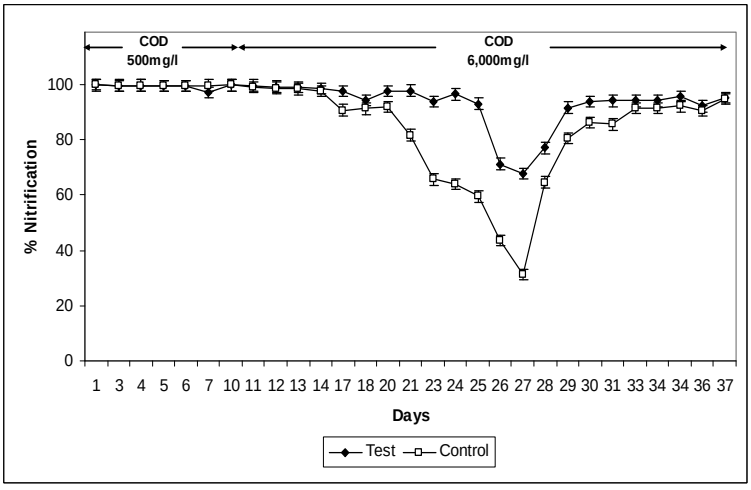


(c)

Fig.66. Nitrification performance in a pilot plant system in response to a shock COD loading with an MLSS of 4,700mg/l (control pilot plant) and with an MLSS of 8,100mg/l (test pilot plant); (a) effluent ammonia and nitrate, (b) percentage nitrification and (c) effluent COD's.

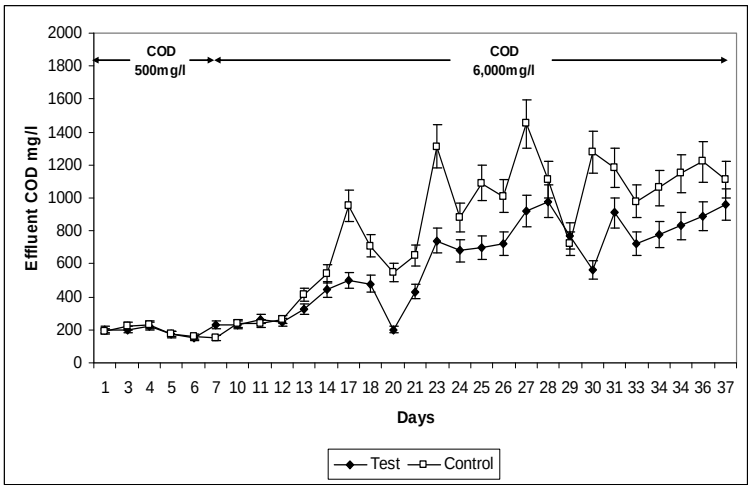


Fig.67. Final effluent chemical oxygen demand (COD) following a shock COD loading in a pilot plant system with a mixed liquor suspended solids (MLSS) of 4,700mg/l (control pilot plant) and with an MLSS of 8,100mg/l (test pilot plant).

3.2.3.2 Comparison of an acclimatised and non-acclimatised activated nitrifying sludge to a shock COD load.

Nitrifying activated sludge was acclimated to the waste water feed and its nitrifying ability was compared with the same but non-acclimated sludge. Both systems were operated in continuous feed mode and the pH was maintained at 7.5. The mean MLSS in the control system was 6,100mg/l and was 5,000mg/l in the test system. The influent COD concentration to both pilot plants was 1,500mg/l from day 1-3. The influent COD was maintained at this level in the control until day 13. However, the influent COD to the test pilot plant was gradually increased in increments as follows: 2,000mg/l day 4-8; 2,500mg/l day 9-11 and 3,000mg/l day 12-14. On day 15 the influent COD to both pilot plants was shock loaded to 6,000mg/l until the end of the study at day 30.

From day 1 to day 13 both pilot plants had equally low levels of effluent ammonia, reflected in 100% nitrification (Fig.68a). After applying an influent COD load of 6,000mg/l final effluent ammonia increased in the control pilot plant to reach a peak of 58mg/l NH_4-N on day 22. In comparison the test pilot plant showed no evidence of any ammonia breakthrough to the final effluent. Nitrification quickly recovered in the control system with effluent ammonia levels falling back to less than 1mg/l NH_4-N by day 24.

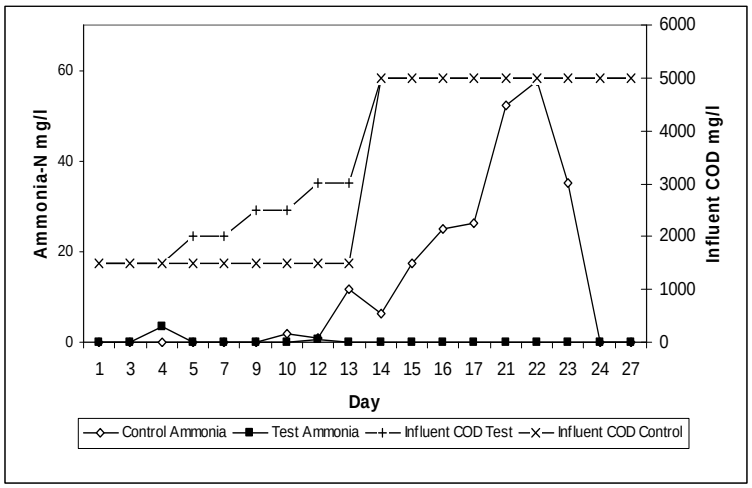
The levels of nitrates were similar in both systems during the first 13 days (Fig 68b). Upon application of the shock COD loading to 6,000mg/l nitrates quickly fell in the control pilot plant reaching a minimum level of 15mg/l NO_3-N on day 22. In line with the drop in effluent ammonia levels, the levels of nitrate rose sharply and reached 105mg/l NO_3-N by the end of the study. Over this same period, nitrates in the test pilot plant remained relatively steady at 70mg/l NO_3-N before reaching 127mg/l NO_3-N at the end of the study.

When the results were expressed as percentage nitrification, both systems showed 100% nitrification for the first 13 days (Fig.68c). The percentage nitrification in the control system fell dramatically following the COD shock load reaching a minimum of 20% on day 22 before recovering completely on day 24. Nitrification in the test system however remained at 100% despite the COD shock load.

The incremental increase in influent COD to the test pilot plant resulted in the F/M rising by approximately $0.02d^{-1}$ until day 13 (Fig.69a). This compared to the control pilot plant, where the F/M over the same period, was steadily maintained close to an F/M of 0.08 to 0.09. When both systems were shock loaded to 6,000mg/l COD the F/M in the control pilot plant reached 0.21-0.26. This was lower than the test pilot plant by virtue of having a slightly higher level of MLSS. The F/M of the test system ranged from 0.26-0.32.

When the COD removal rate per mg of biomass was monitored, the control pilot plant removed a maximum of 0.21mg of COD /mg of biomass compared to 0.27mg COD/mg biomass for the test pilot plant (Fig.69b).

The results of this investigation suggested that an activated sludge that was acclimatised to the influent COD, leading in turn to a gradual increase in the F/M, was more resistant to a shock COD load resulting in better nitrification than an activated sludge that had to react to a large step change in the COD or the F/M.



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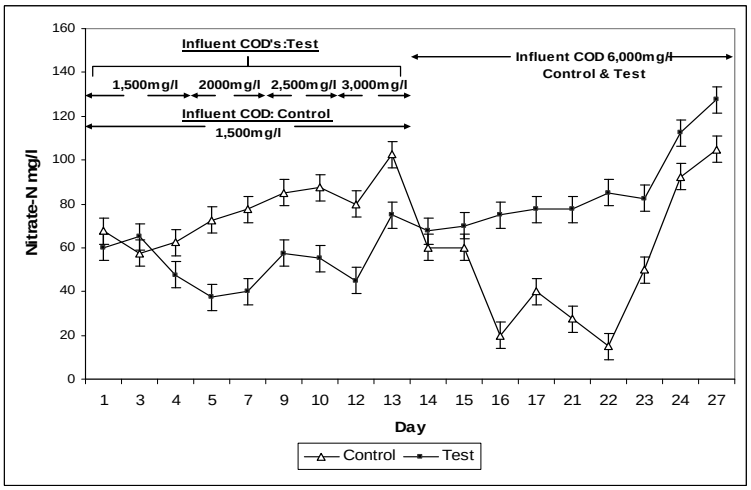

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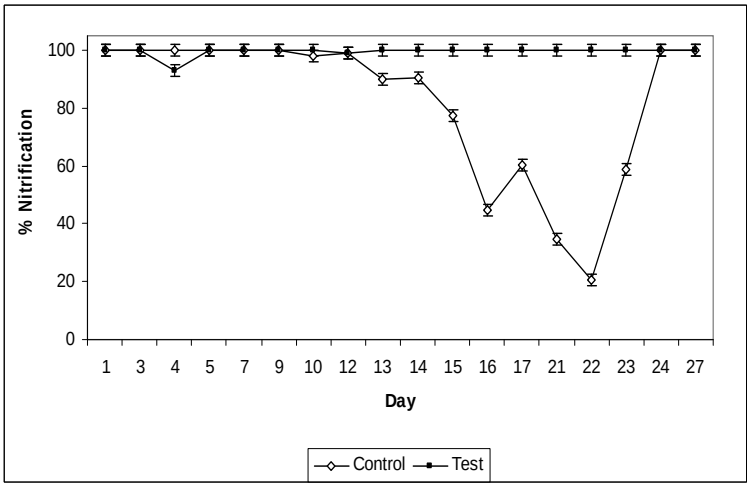
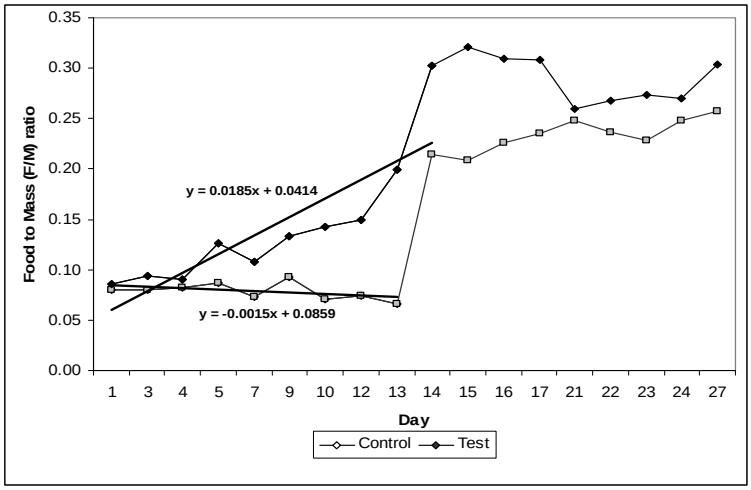


(c)

Fig.68. Comparison of the performance in nitrification of two pilot plant systems to a shock COD load where the activated sludge was acclimated by gradually increasing the influent COD (test system) compared to a step change in the influent COD (control system); (a) effluent ammonia, (b) effluent nitrates and (c) percentage nitrification.



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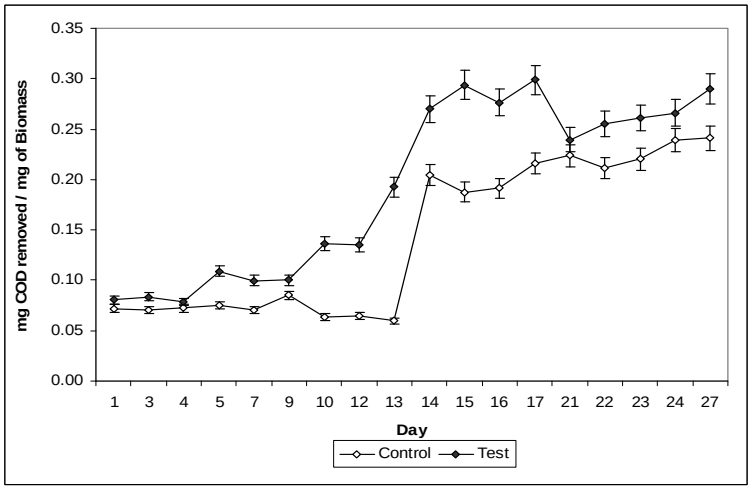


(b)

Fig.69. Food to mass ratio's and COD removal for two pilot plants systems where the activated sludge was acclimated by gradually increasing the influent COD (test system) compared to a step change in the influent COD (control system); (a) F/M's and (b) COD removal rates.

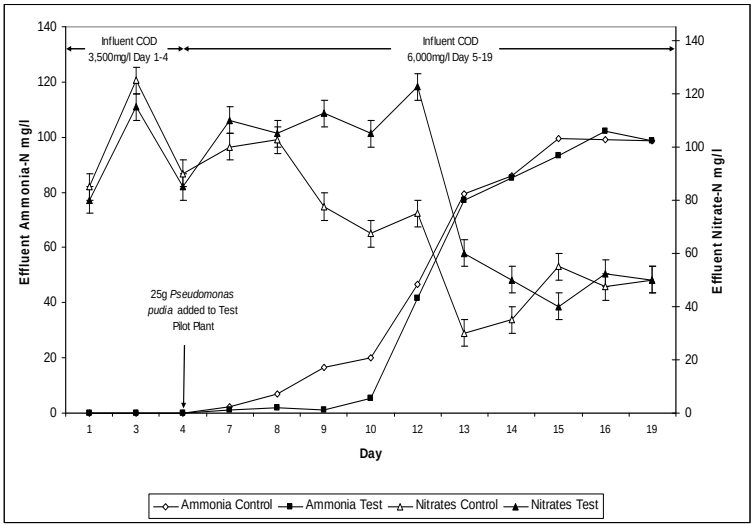
3.2.3.3 Bio-augmentation of a nitrifying sludge with *Pseudomonas pudia* CP1 and its influence on nitrification under high COD loadings

The pilot plant systems were operated in continuous feed mode at pH 7.5. The mean MLSS concentration in the control pilot plant was 5,000mg/l and was slightly lower at 4,000mg/l in the test pilot plant (section 2.2.2.4). On Day 4, 20g of *Pseudomonas pudia* CP1 was added to the test pilot plant; this increased the mean MLSS to 5,000mg/l. Both systems therefore had the same F/M ranging from 0.38-0.41. From day 1 to day 4 the influent COD was 3,500mg/l. This was shocked loaded to 6,000mg/l on day 5 until the end of the trial on day 19.

By day 10 effluent ammonia in the control pilot plant had reached 20mg/l NH_4-N compared to only 5mg/l NH_4-N for the test pilot plant. Although by day 19 the levels of effluent ammonia in both systems were similar at 98mg/l NH_4-N. The effluent nitrates followed a similar profile as the effluent ammonia where by on day 10 there was 67mg/l NO_3-N in the control pilot plant effluent compared to 105mg/l NO_3-N in the test pilot plant. Nitrates also reached equal levels in both pilot plants by the end of the study (Fig.70a).

While the percentage nitrification fell to 30% in both systems on day 15, the system which had been bioaugmented did sustain nitrification for a longer period of time (Fig.70b). From day 7 until day 15 the COD removal of the bioaugmented system was slightly higher than the control system (Fig.70c).

The results of this study suggested that the addition of a viable culture of a heterotroph i.e. *Pseudomonas pudia* CP1 temporarily resulted in better nitrification under a high COD loading. Bioaugumentation with *Pseudomonas pudia* CP1 protected nitrification for 3-4 days longer than the control system when the COD shock load was applied.



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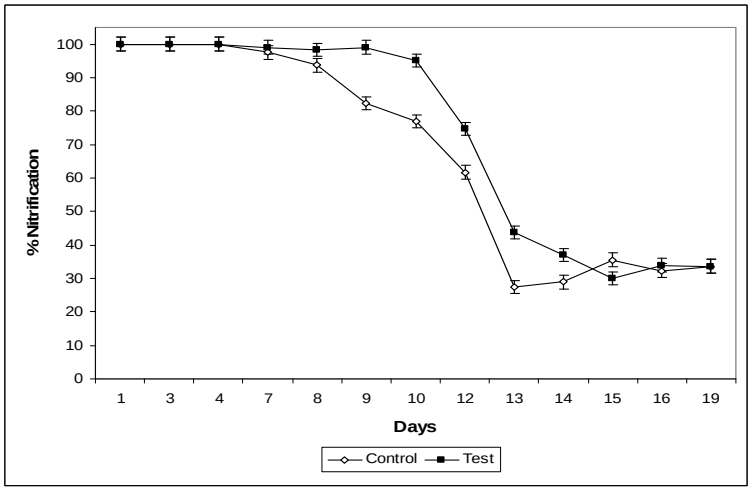

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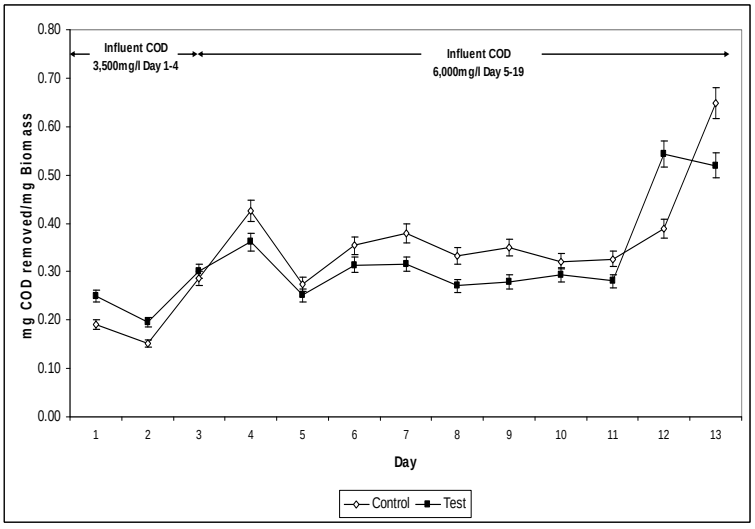


(c)

Fig.70. Influence on nitrification following a shock COD loading in a pilot plant system after the addition of a culture of *Pseudomonas pudia* CP1 to the test pilot plant; (a) effluent ammonia and nitrates, (b) percentage nitrification and (c) COD removal rates

3.2.3.4 Pilot plant data analysis of the food to mass ratio (F/M) and nitrification.

The biomass and the influent COD are key factors that influence nitrification. It was of interest to investigate the relationship between the F/M and percentage nitrification. An analysis was carried out on experimental data from a select number of pilot plant studies where modification of the biomass had a positive effect on nitrification. The systems investigated included the use of high biomass levels, the use of acclimated biomass, the addition of activated carbon and the addition of BPM.

The percentage nitrification was plotted against the F/M values for both the control and the test systems (Fig. 71). At a low F/M the percentage nitrification for the control systems and the modified test systems was similar. As the F/M increased the percentage nitrification fell in both systems but this fall was significantly greater in the control systems compared to the test systems (Fig.71).

Statistical analysis of the data (Table 20) predicted a 50% failure in nitrification in the control systems at an F/M of 0.42 with a 95% CI of 0.40 to 0.45. The same failure point was significantly higher for the test systems where a 50% failure in nitrification was predicted to occur at a F/M of 0.75 with a 95% CI of 0.71 to 0.87.

However, given the low effluent limit of 10mg/l NH_4-N, a percentage nitrification of less than 80% was likely to result in a licence excursion. In the control systems, this was shown to occur at an F/M of greater than 0.25 with a 95% CI of 0.22 to 0.28. While in the test systems, the predicted F/M was 0.52 with a wider 95% CI of 0.46 to 0.59.

There was a good statistical fit for the controls with a regression co-efficient of 59%, a Pearson's correlation of -75% and the P value of 0.000 passes the hypothesis acceptance criteria. Although a similar P value is returned for the test systems, the regression co-efficient and correlation of 22% and -45% respectively are lower than the control. This was due to the greater spread of data points beyond an F/M of 0.25 and because certain tests were more or less successful than others.

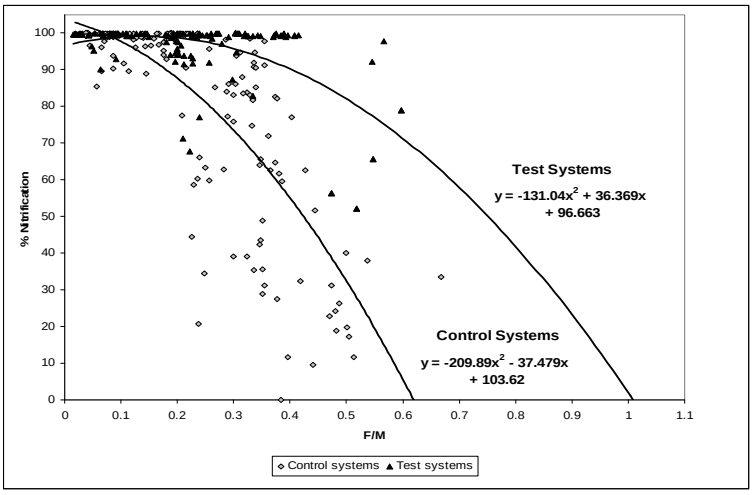


Fig.71. Scatter plot of the relationship between the food to mass (F/M) ratio and the percentage nitrification using all data points for the control and the test pilot plant studies that demonstrated a positive effect on nitrification.

Table 20: Predicted 20% and 50% failure in nitrification relative to the operating F/M.

Criteria	Control Pilot plants F/M	Test Pilot plants F/M
50% Failure Point	0.42	0.75
95% Confidence Interval	0.40-0.45	0.71-0.87
20% Failure Point	0.25	0.52
95% Confidence Interval	0.22-0.28	0.46-59
R-Sq(adj)	59%	22%
Pearson's Correlation	-75%	-45%
P value	0.000	0.000

When the data for the test systems was further analysed and compared, it was found that the acclimatised biomass was the most effective at minimising nitrification failure, followed by the addition of a buoyant plastic media and in turn the systems operating at a higher biomass level. The least effective modification was the addition of activated carbon (Fig. 72).

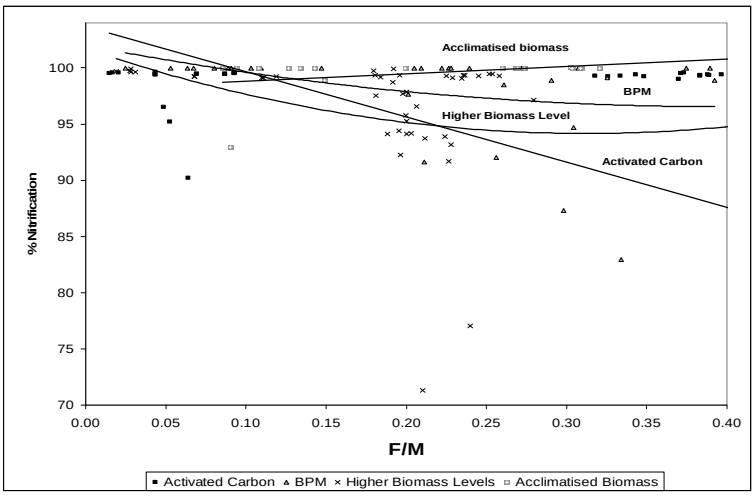


Fig.72. Scatter plot of the relationship between the food to mass (F/M) ratio and the percentage nitrification using all data points from the test pilot plant studies that demonstrated a positive benefit to nitrification.

3.2.3.5 Summary of findings

The findings from part 3 of the pilot plant study were:

- There was no relationship between the MLSS and nitrification at a low COD loading of 500mg/l. However at a shock COD loading of 6,000mg/l, nitrification and COD removal rates in the system with an MLSS of 8,100mg/l performed better than the system on the lower MLSS of 4,700mg/l.

- An activated sludge that was gradually acclimatised to an increasing influent COD up to 6,000mg/l was more resistant to nitrification failure and than a system on a low sustained F/M. Enhanced COD removal rates were also recorded for the acclimatised system.

- Bio-augmentation of a nitrifying sludge with 20g *Pseudomonas pudia* CP1 temporarily resulted in better nitrification and COD removal rates at an influent COD loading of 6,000mg/l.

- Analysis of the pilot plant data showed that the effluent limit of 10mg/l NH_4-N would be exceeded at an F/M of 0.25 for the control systems. This could be increased to an F/M of 0.52 through various modifications; in particular though acclimatisation of the biomass, followed by the addition of BPM and in turn by operating at a higher biomass level. The least effective modification was the addition of activated carbon.

3.3 Performance of main plant in 2006 after implementation of findings.

In response to the findings from Stage 1 and from Stage 2 of the study a number of modified operational measures were implemented on the full scale WWTP (section 3.3.1) and the improvement to nitrification was assessed (section 3.3.2).

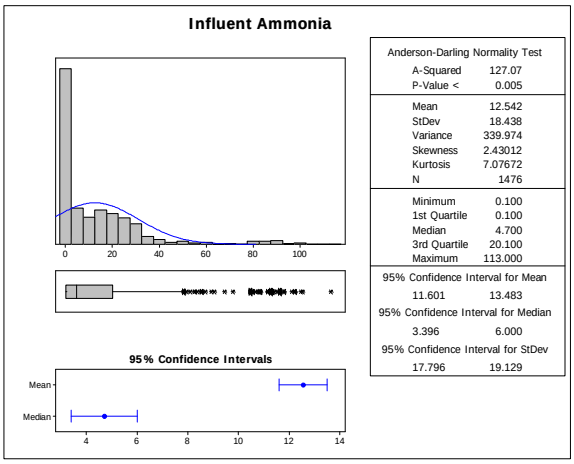
3.3.1 Modifications to the full scale WWTP

The modifications at the full scale WWTP included greater control of the influent ammonia and the carbon to nitrogen ratio (C/N); the influent COD; the aeration bay pH and free ammonia levels and the mixed liquor suspended solids (MLSS) and the food to mass (F/M) ratio.

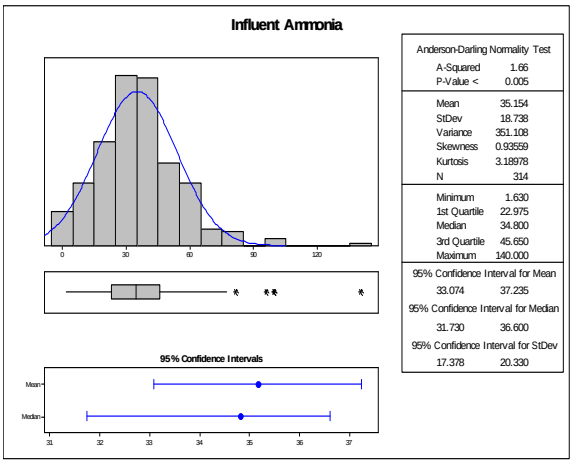
Influent Ammonia and the Carbon to Nitrogen (C/N) ratio

The pilot plant study concluded that an influent ammonia concentration of 50mg/l NH_4-N or a C/N ratio of less than 40 would ensure sufficient ammonia is available to the nitrifiers after heterotrophic uptake. A new 1,000 litre tank containing 40% aqueous ammonia was installed at the biotower blending sump (sample point 3). Ammonia was continuously pumped into this sump. Ammonia levels were tested each day and the dosing rate was adjusted to achieve an influent target level of 40-50mg/l NH_4-N. In the event of elevated influent ammonia arising from production ammonia dosing was discontinued until the peak subsided.
As a result of these changes the influent ammonia levels in 2006 were only slightly below the target value with a mean of 35mg/l and a 95% CI of 33mg/l to 37mg/l NH_4.N. This compares to a mean influent ammonia level of 12.5mg/l NH_4-N from the period 2000 to 2003 (Fig.73a and b).

The improved control of influent ammonia resulted in a mean C/N ratio in 2006 of 41 with a 95% CI of 38-44. This compares favourably to 2000 to 2003 when the mean C/N was 130 (Fig.74a and b).

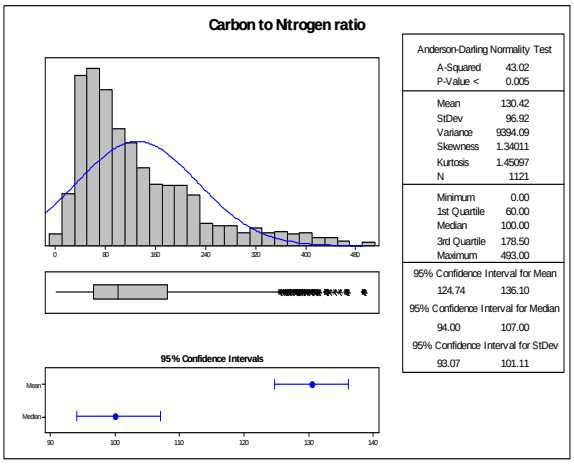


(a)

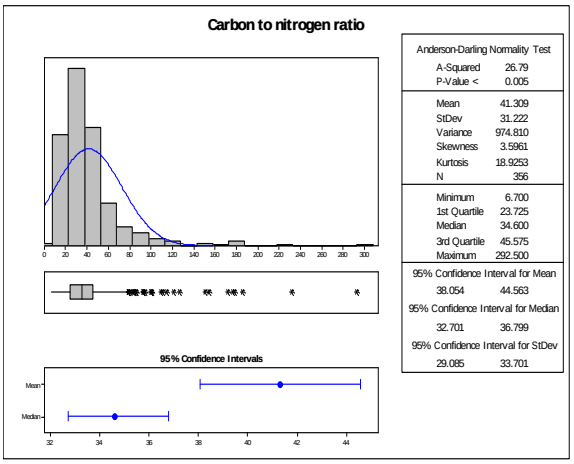


(b)

Fig.73. **Data distribution analysis of the influent ammonia to the full scale waste water treatment plant, (a) from period 2000 to 2003 and (b) after implementation of the findings of stage 1 and stage 2 of the study plan.**



(a)



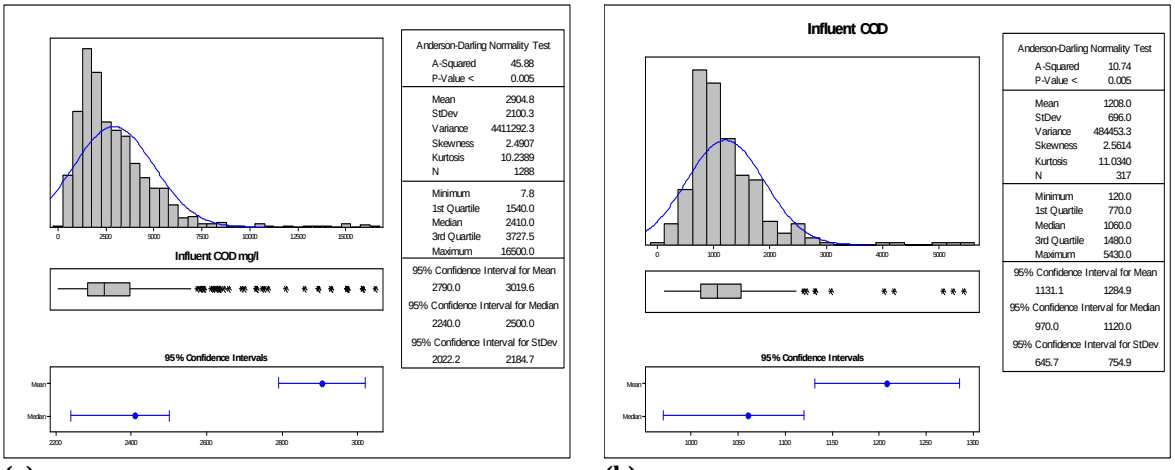
(b)

Fig.74. **Data distribution analysis of the C/N ratio to the full scale waste water treatment plant, (a) from period 2000 to 2003 and (b) after implementation of the findings of stage 1 and stage 2 of the study plan.**

Influent COD

Both the data study of the full scale WWTP and the pilot plant study found that at an influent COD from 3,800-4,000mg/l resulted in a 50% inhibition of nitrification. To enhance plant design into a two stage configuration the random packing media in the biotowers, before the aeration bay, was upgraded with new hanging sessile strips. This was to provide better upfront COD removal. The sloughed solids were however allowed to carry forward to the aeration bay to help prevent starvation and consequently any fall in the MLSS. A number of process steps were identified that were releasing high concentrations of solvents wastes to the WWTP. These were intercepted and shipped off-site to help reduce the COD loading on the plant.

Improved control of the influent COD to the aeration bay is evident with a mean COD of 1,208mg/l, with a 95% CI of 1,131mg/l to 1,285mg/l. This is a significant reduction when compared to 2000 to 2003 when the mean influent COD loading to the aeration bay was 2,905mg/l with several occasions where this exceeded 7,500mg/l (Fig.75a and b).



(a) **(b)**

Fig.75. Data distribution analysis of the influent COD to the full scale waste water treatment plant, (a) from period 2000 to 2003 and (b) after implementation of the findings of stage 1 and stage 2 of the study plan.

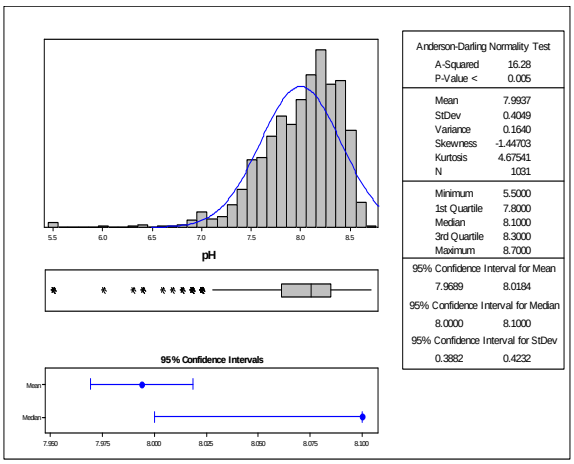
Aeration Bay pH

Improved nitrification was observed in the pilot plant study at pH 8.5 compared to pH 7.5 especially under elevated influent COD conditions. Previously pH could only be adjusted at the head of the WWTP. There was no direct control available at the aeration bay where nitrification takes place. A new pH dosing system was therefore installed thereby allowing tighter pH control. The pH was routinely adjusted to achieve a normal operational pH of 8.0-8.5 within the aeration bay. In the event of a high COD loading a more alkaline pH towards pH 8.5 was adopted. In 2006 the mean pH in the aeration bay of the full scale WWTP was pH 8.33 with a 95% CI of 8.30 to 8.35. The lowest pH recorded was pH 7.7 and the highest was pH 8.8. This distribution of pH was more alkaline and the range was narrower than from 2000 to 2003 when the mean pH was 7.93, with a minimum of pH 5.5 and a maximum of pH 8.7 (Fig.76a and b).

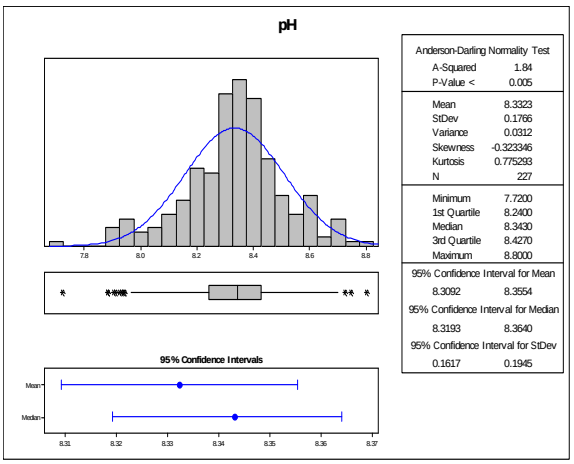
Free Ammonia

The pilot plant study demonstrated that free ammonia levels above 1.5mg/l caused a significant disruption to nitrification. As part of the company's data acquisition and management programme free ammonia levels within the aeration bay were now calculated daily. If the levels exceeded 1mg/l NH_4-N FA then the COD loading was reduced and the aeration bay pH lowered to pH 7.0-7.5.

As a result of this change free ammonia levels in the aeration bay of the full scale WWTP never exceeded 0.05mg/l NH_4-N FA. The mean free ammonia level was 0.009mg/l NH_4-N FA with a 95% CI of 0.0087mg/l to 0.0096mg/l NH_4-N FA. This again compared favourably to the data study from 2000 to 2003 where the mean free ammonia level was 0.83mg/l NH_4-N FA with a number of outliers above 2mg/l and a peak as high as 25mg/l NH_4-N FA (Fig 77a and b).

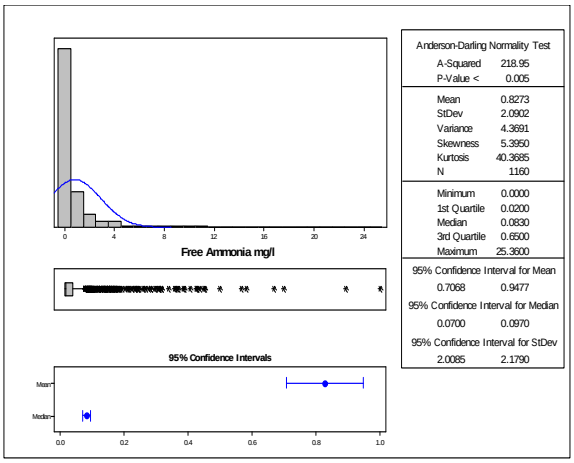


(a)

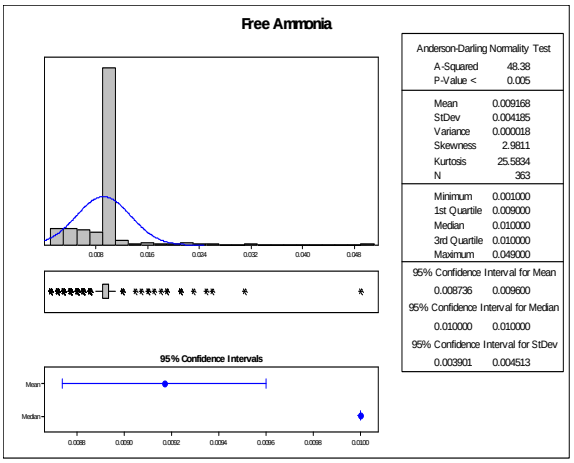


(b)

Fig.76. **Data distribution analysis of the aeration bay pH at the full scale waste water treatment plant, (a) from period 2000 to 2003 and (b) after implementation of the findings of stage 1 and stage 2 of the study plan.**



(a)



(b)

Fig.77. **Data distribution analysis of free ammonia levels in the aeration bay of the full scale waste water treatment plant, (a) from period 2000 to 2003 and (b) after implementation of the findings of stage 1 and stage 2 of the study plan.**

Food to Mass (F/M) ratio

The pilot plant study demonstrated the importance of the MLSS where a higher MLSS concentration protected nitrification than a low MLSS under a high COD loading. The target MLSS level in the aeration bay was therefore increased from 3,000mg/l to 6,000mg/l-8,000mg/l. This required greater monitoring of the sludge blanket level in the clarifiers to avoid overloading and in turn losing any sludge across the weirs to the final effluent. In addition greater liaison with production operations was instituted to have advance warning of predicted high COD loads to the WWTP. In such an event the F/M ratio was gradually increased to increase sludge floc viability.

Operating at the higher MLSS reduced the F/M at the plant. Analysis of data from the pilot plant controls determined that nitrification would fail and result in a licence excursion at an F/M of greater than 0.25. If the F/M exceeded 0.25 the COD loading was reduced or MLSS increased. The F/M at the aeration bay in 2006 was significantly lower than over the period 2000 to 2003. The mean F/M in 2006 was 0.04 with a 95% CI of 0.040 to 0.046; the maximum F/M was 0.19. From 2000 to 2003 the mean F/M was 0.196 with a maximum of 0.68 (Fig.78a and b).

A stock of activated carbon was purchased and is now retained on site for dosing in the event of any observed inhibition. A commercial supplier of a BPM was sourced and a preliminary feasibility study carried out. It was however decided not to implement this option at this stage given the costs involved.

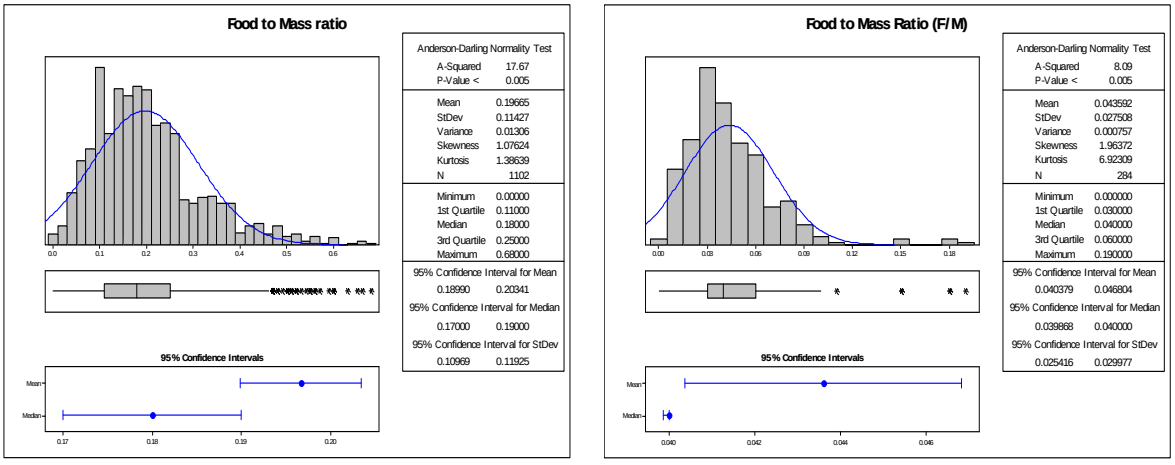
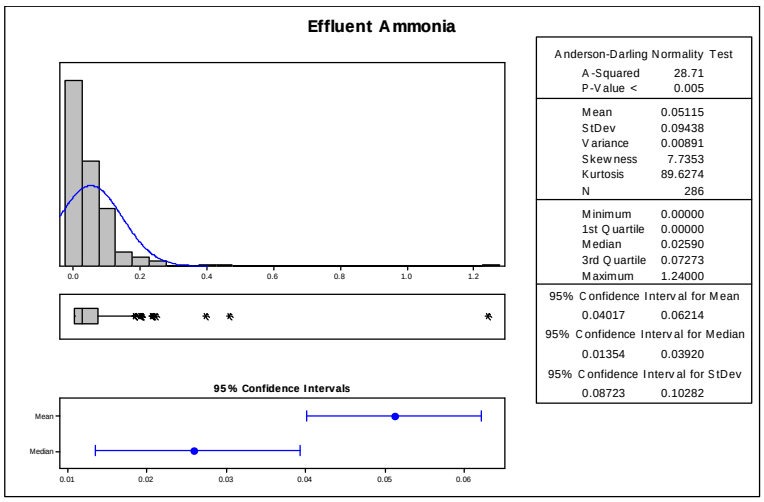


Fig.78. Data distribution analysis of the F/M levels for the aeration bay of the full scale waste water treatment plant, (a) from period 2000 to 2003 and (b) after implementation of the findings of stage 1 and stage 2 of the study plan.

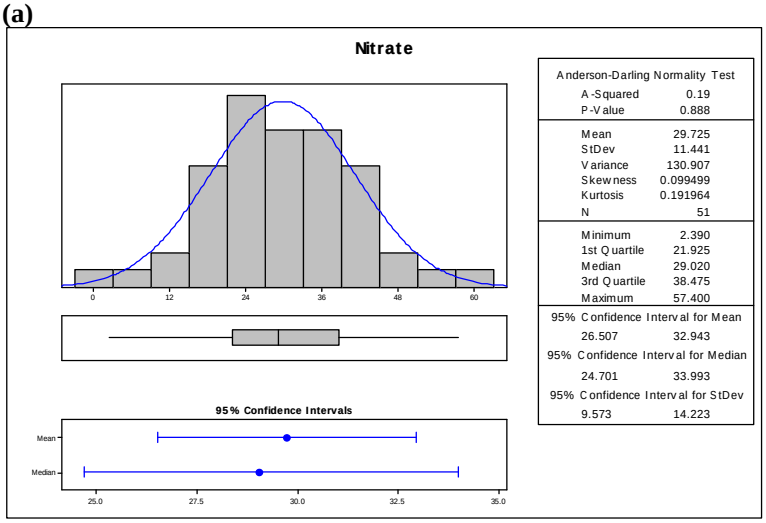
3.3.2 Assessment of the improvement in nitrification after implementation of the modifications.

The full scale WWTP was 100% compliant in 2006 with the 10mg/l effluent ammonia discharge limit. The mean effluent ammonia was 0.05mg/l NH_4-N with a 95% CI of 0.040mg/l to 0.062mg/l NH_4-N. The maximum value recorded was 1.24mg/l NH_4-N (Fig.79a); this coincided with a short term fall in the aeration bay pH to 7.7. Stable nitrification was evident with a mean effluent nitrate level of 29.7mg/l NO_3-N with a 95% CI of 26.5mg/l to 32.9mg/l NO_3-N. The lowest nitrate level recorded was 2.4mg/l NO_3-N and the maximum was 57.4mg/l NO_3-N (Fig.79b). A mean nitrification rate of 99.81% was achieved in 2006 with a 95% CI of 99.74% to 99.87%. The lowest percentage nitrification recorded was 91.1% that also coincided with the maximum effluent ammonia value of 1.24mg/l NH_4-N.

Overall there was a significant improvement in nitrification compared to the period 2000 to 2003 when the mean percentage nitrification achieved was 69.3% with several data points at 0% nitrification (Fig.79c). A time series plot of effluent ammonia and effluent nitrates for 2006 shows that following the implementation of the findings from part 1 and part 2 of the study plan, stable and reliable nitrification was achieved all year round (Fig.80). This represented a major improvement in the performance of the full scale WWTP and demonstrated that nitrification could be successfully implemented and maintained on a full scale system.



(a)



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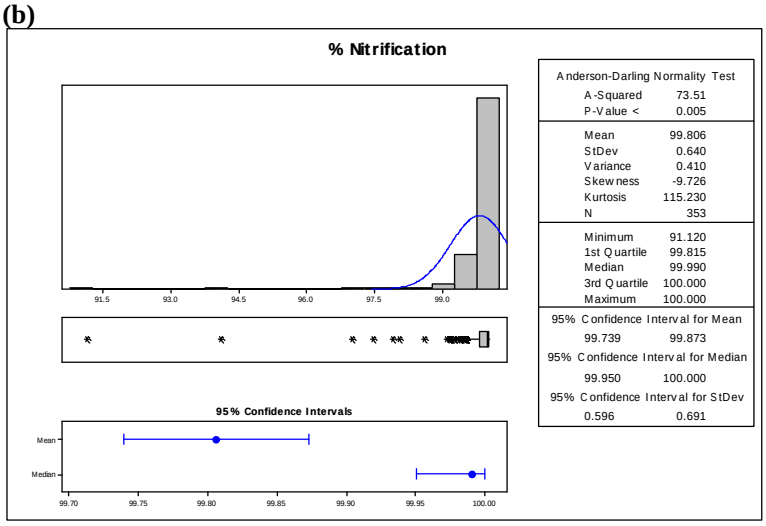

(c)

Fig.79. Data analysis of full scale WWTP plant following implementation of the findings of Stage 1 and Stage 2 of the study plan ; (a) graphical summary of effluent ammonia, (b) graphical summary of effluent nitrates and (c) graphical summary of the percentage nitrification

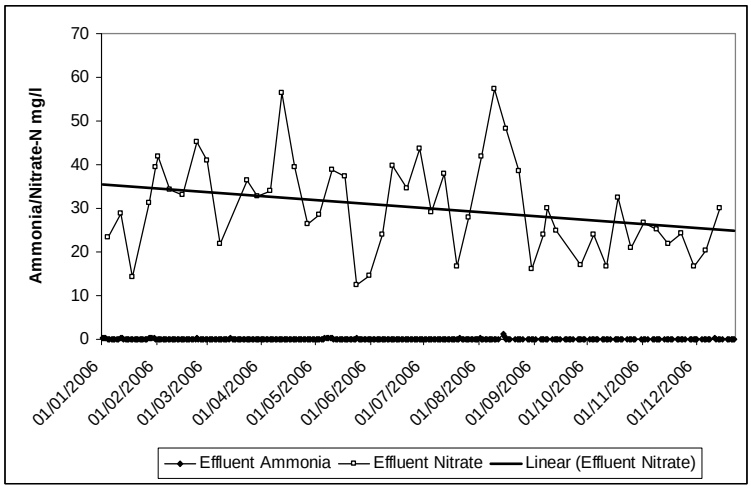

Fig.80 Time series plot final effluent ammonia and nitrates for 2006 from the full scale waste water treatment plant following the implementation of the findings from stage 1 and stage 2 of the study plan.

Chapter 4

Discussion

4.0 DISCUSSION

A study of effluent data showed that the WWTP at Schering Plough was capable of nitrifying for extended periods; however it was equally evident that this specialised microbial process was unstable and unreliable. Although a mean nitrification rate of 70% was recorded over the four year period it is equally noteworthy the proportion of time when there was no evidence of any nitrification within the system. Final effluent ammonia levels of over 95mg/l NH_4-N were recorded. The difficulty in nitrifying at this treatment plant agrees with Nowak *et al* (1994) who reported that nitrification of a pharmaceutical waste water is considerably more challenging as nitrifying bacteria are the most sensitive species within a waste water treatment system.

A new EPA 10mg/l NH_4-N limit was to be applied to the effluent discharge from 2004; based on data from 2000 to 2003 it was predicted that this would be exceeded over 100 times in any calendar year. There was significant concern among plant personnel that the WWTP was incapable of complying with the new limit. The consequence of this was to leave the site open to potential prosecution. This together with negative publicity and a loss of trust with the local community meant the performance of nitrification at this pharmaceutical WWTP needed to be improved.

A three stage study plan was undertaken with the overall aim of improving the nitrification rate to achieve 100% compliance with the new limit. A key consideration in the design of the study plan was to ensure that all learning's were relevant, cost effective and pragmatic so they could be applied to the full scale WWTP. Fortunately, there was extensive operational and effluent monitoring data available for the full scale WWTP. This was a valuable starting point in the study to isolate and correlate potential root causes of nitrification failure. A four year period from 2000 to 2003 was chosen as a representative time when nitrification proceeded well for extended periods but equally when it demonstrated significant failures. This represented Stage 1 of the study plan.

In the second stage of the study plan, two identical pilot plant systems were commissioned. These replicated the conditions of the activated sludge stage of the full scale WWTP. This stage was chosen as nitrification was never observed in the preceding treatment stage i.e. the biotowers. It was also believed that this was the most likely stage at which nitrification could be successfully maintained. The pilot plants were used to test and in turn prove or disprove the findings from Stage 1. They were also used to explore further these findings and to test new hypothesises.

It is readily recognised the limitations associated with any pilot plant trials in particular the reduced scale at which these were operated. Therefore, to confirm the findings of the pilot plant investigations the final stage of the study involved the implementation of these findings and recommendations onto the full scale WWTP and in turn assessing the improvement in nitrification. This improvement would represent a measure of the success of the study plan. It should be noted that the study plan was only interested in nitrification and not in denitrification as the final effluent limit for nitrate was less stringent at 100mg/l NO_3-N.

Biological waste water systems are designed to operate under steady state conditions but in reality most physical and chemical parameters fluctuate significantly for various different reasons. Any changes in these conditions can have an adverse effect on the system. Temperature is one such variable in an activated sludge system where a sudden change in temperature can cause a shift in the bacterial community and in its function resulting in a drop in performance (Nadarajah *et al.*,2007). Nitrifiers follow an Arrhenius type temperature dependency where there is a doubling in metabolic activity for every 10^{o}C rise in temperature between 5-35^{o}C (Hall and Murphy,1985).

Following the data review of the full scale WWTP, temperature was ruled out as a responsible factor for nitrification failure. The mean temperature in the aeration bay was 21^{o}C. Temperature levels were not prone to the same variations in line with the ambient air temperature. This was a benefit of the heating applied to the biotowers during the winter months and heat transfer from the air blowers in the aeration bay. There was less than 30 days when the temperature was below 15^{o}C; the lowest level recorded was 11.6^{o}C and the maximum temperature recorded was 32^{o}C. There were several periods when nitrification was evident during the winter months as well as the summer months.

Nitrification between the mean and the maximum temperature recorded at the full scale WWTP was somewhat below optimum levels. Grunditz and Dalhammar (2001) reported for pure cultures the optimum temperature for *Nitrosomonas* is 35^{o}C and is 38^{o}C for *Nitrobacter*. Polanco *et al* (1994) demonstrated the importance of temperature in a biological filter and cites the optimum temperature for nitrification to between 28-29^{o}C. For every increase by 1^{o}C there was a 2% increase in nitrification rate up to 29^{o}C. In a batch activated sludge reactor with aerobic and anaerobic stages treating a synthetic waste water the optimum temperature for nitrification was between 30-40^{o}C (Bae *et al.*,2001). The mean temperature at the full scale plant was however closer to the range reported by Ramachandran (2003). This author reported the optimum level in a sewage treatment plant for nitrification was between 20-30^{o}C with no growth below 4^{o}C or above 37^{o}C. Also Campos *et al* (1999) achieved nitrification rates in excess of 97-99% for a highly nitrogen loaded activated sludge at a temperature of 20^{o}C.

Temperatures below 21^{o}C within the aeration bay of the full scale WWTP were potentially more of a concern as the maximum growth rate of nitrifiers falls by approximately 65% with a decrease in temperature from 20^{o}C to 10^{o}C (IWA,2000). Head and Oleszkiewicz (2004) also reported a similar reduction with a 58% reduction in nitrification over the same temperature range. This was more significant the greater the range of the fall, for example a 71% reduction in nitrification was recorded from 25^{o}C to 10^{o}C and an 82% reduction from 30^{o}C to 10^{o}C. This decline in nitrification at lower temperatures is attributed to a significant reduction in growth rates.

There was no statistical relationship between nitrification and the temperature between 11^{o}C and 21^{o}C at the full scale WWTP, showing that nitrification was not disrupted within this range. An explanation for this relates to the importance of a sufficiently long sludge age at low temperatures. Sears *et al* (2003) found that the nitrifier growth rate in an activated sludge treating municipal waste water at 12^{o}C was approximately 50% of that at 24^{o}C. However this decline can be counteracted by increasing the sludge age. Sinkjaer *et al* (1994) also reported the importance of sludge age at low temperatures. Nitrification can proceed at temperatures as low as 7-8^{o}C if sludge age is sufficiently long. For every one degree drop in temperature a 10% increase in sludge age is required. At these low temperatures a sludge age of 20 days is required.

As the mean sludge age at the WWTP was significantly higher than these values being over 48 days this points to why nitrification was not adversely influenced on the few occasions when lower temperatures were recorded. A data review of the sludge age also showed no statistical relationship with nitrification between a sludge age of 0 days and 50 days. There were few occasions at the WWTP when the sludge age was less than 10 days and although there were a number of outliers above 120 days, overall the sludge age was within, with a 95% confidence interval for the mean, between of 46 to 50 days. This mean sludge age is slightly higher than recommended by Gupta and Sharma (1996) who reported that an activated sludge age of 30 days was optimum for treating an ammonia rich waste waters (500mg/l NH_4-N) from a urea and a pharmaceutical waste water. Dincer and Kargi (2000b) reported a lower optimum of 12 days using synthetic waste water with an influent ammonia concentration of 100mg/l NH_4-N. When the sludge age fell from 12 day to 3 days ammonia removal efficiency fell from 99% to 79%.

The main concern associated with a low sludge age is population washout may occur. As the sludge age at the WWTP was predominantly above 10 days this was ruled out as being responsible for nitrification failure. This is supported by the work of Azimi and Horan (1991) who maintained steady state nitrification in a plug flow and completely mix activated sludge reactor at a sludge age of only 2 days, however below this most of the nitrifiers were washed out. A similar result was reported by Dionisi *et al* (2002) who demonstrated in an activated sludge reactor treating municipal waste water complete nitrification at a sludge age of 10-20 days with no significant reduction until the sludge age was less than 5 days and only below 2 days did nitrification start to decline.

There was equally no evidence that the mean sludge age at the full scale WWTP was too high. It has been reported that a higher sludge age can be advantageous as it can allow the heterotrophs to degrade inhibitory compounds; the greater the sludge age the more rapidly removed are the inhibitory effects on nitrifying bacteria of compounds commonly found in a chemical waste water (Joel *et al.*, 1977). Bortone *et al* (1995) also recommended a sludge age of over 38 days for troublesome wastes such as from textile and municipal sources with inhibitory compounds and Canziani *et al* (2006) achieved good nitrification at sludge ages greater than 45 days treating a landfill leachate.

The full scale WWTP uses on-line dissolved oxygen probes linked to a controller that automatically ramps up and down air blowers to maintain the desired levels of dissolved oxygen. This gives the WWTP effective control of the dissolved oxygen levels in the aeration bay as was reflected with a mean dissolved oxygen of 5mg/l, a minimum of 2.0mg/l and excluding one outlier a maximum of 10.0mg/l. These levels of dissolved oxygen at the full scale WWTP were well above the minimum recommended levels.

Nitrification can in fact proceed quite well at relatively low levels of dissolved oxygen. This is reflected by the fact that the oxygen half saturation coefficients (Ko_2) for ammonia oxidisers range from 0.3mg/l O_2 for ammonia oxidiser and 1.1mg/l O_2 for nitrite oxidisers (Wiesmann, 1994). A slightly wider range was reported by Weon *et al* (2004) of 0.2mg/l-1.6mg/l O_2 for ammonia oxidiser and 0.9-1.2mg/l O_2 for nitrite oxidisers. Similar levels were also reported by Ciudad *et al* (2006) for an activated sludge with Ko_2 for ammonia oxidisers of 0.99mg/l O_2 and for nitrite oxidisers of 1.4mg/l O_2. In a membrane bioreactor treating ammonia from a landfill leachate greater than 90% ammonia oxidation to nitrite was achieved at a dissolved oxygen level of 0.2-0.5mg/l O_2 (Canziani *et al.*, 2006). The Ko_2 are significantly different for a biofilm compared to an activated sludge i.e. 0.18mg/l O_2 versus 0.79mg/l O_2 for ammonia oxidizers and 0.13mg/l O_2 versus 0.47mg/l O_2 respectively for nitrite oxidizers (Manser *et al.*, 2005).

Low oxygen levels can in fact be advantageous, for example Nowak *et al* (1994), Siegrist *et al* (1999), Hatziconstantinou and Andreadakis (2002) all reported a reduction in the nitrifier decay rate when there was an anoxic zone present in the treatment system. In general as reported by Simm *et al* (2006) ammonia oxidation rates decrease with decreasing oxygen from 2.6 NH_4-N/l/hr at dissolved oxygen levels of 0.5mg/l compared to 16mg NH_4-N/l/hr at dissolved oxygen levels of 3mg/l. However, care needs to be taken in over aerating a system not only for economic reasons but intensive aeration can strip away nitrogen oxide (NO) which may be more important to nitrification than previously thought. By stripping away NO nitrification rates may be suppressed which in turn can reduce the maximum growth rates of nitrifiers (Zart *et al.*, 2000). There was some statistical evidence at the full scale WWTP data that supports this as nitrification rates appear to fall with increasing dissolved oxygen levels. This relationship however was statistically weak and would require further investigation. Overall it was concluded that there was no significant correlation between dissolved oxygen levels at the full scale WWTP and the performance in nitrification.

Prendl and Nikolavic (2000) reported that nutrient dosing is often required for industrial waste waters as the concentration of organics is usually high but the concentration of phosphorus is low. A nutrient dosage strategy must supply phosphorus needed to build up this essential component within a bacteria cell. Dosage strategies must allow for changing COD loads where more phosphorus is added as COD rises and less as it falls. The ability of cells to store phosphorus and release under low loadings needs to be monitored. Therefore, when adopting a dosing control strategy this should not be based on the final effluent alone but must consider the P/COD ratio in the waste water influent. With a stable COD load the phosphorus dosage should be 0.8% of the COD load. In low loads this should be initially reduced to 0.5-0.6% of COD as some phosphorus will be resolubilised from the cells.

The policy at the full scale WWTP was however to add phosphorus as phosphoric acid based not on the influent levels but rather on the residual levels in the final effluent. Residual phosphorus levels in the effluent were taken to indicate an excess that was available if required to the biomass. The mean effluent phosphorus levels at the full scale WWTP was 2.5mg/l P with a 95% CI of 2.05mg/l-6.31mg/l P. The minimum was 0.03mg/l P and a maximum of 24mg/l P. These levels were well above the required concentration for nitrification.

The IWA (2000) reported the half saturation constant (K_p) for phosphorus for nitrification to be a low as 0.01mg/l. Nowak *et al* (1996 and 1999b) also reported a low K_p of 0.01mg/l to 0.05mg/l P but recommended a value of 0.2mg/l P to ensure sufficient levels for nitrite oxidizers who appear to have a higher demand for this compound. Brond and Sund (1995) showed that a rendering plant with an influent ammonium concentration of 355mg/l and a COD of 2,470mg/l, the phosphate concentration was only 0.4mg/l P in the effluent yet the plant showed over 98% nitrification. Mycielski and Blaszczyk (1982) reported a significantly higher optimum effluent phosphorus level of 5mg/l P but this was to guarantee that there was no deficiency for a nitrifying population treating a high influent ammonia concentration for a waste water from a nitrogen fertiliser plant.

As the minimum and in particular the mean phosphorus levels, were well above the recommended concentrations and given there was no statistical relationship evident with the percentage nitrification, phosphorus limitation at the full scale WWTP was therefore discounted as a cause of nitrification failure.

Another important nutrient is carbon. A source of inorganic carbon essential for the lithotrophic nitrifiers (Scagg,2005). Tijhuis *et al* (1995) reported a fall in nitrification when using caustic to control the pH instead of bicarbonate. As soon as a bicarbonate solution was employed nitrification quickly recovered. Analysis of the full scale WWTP indicated a mean inorganic carbon level in the influent of >500mg/l measured as alkalinity or bicarbonate (CaCO3). Based on the mean influent ammonia the average alkalinity/ammonium ratio was therefore >10. The mean inorganic carbon level in the final effluent was lower at 237mg/l $CaCO_3$. A scatter plot also confirmed no relationship between inorganic carbon levels and nitrification.

These levels of inorganic carbon were well above those reported by Lin (2001) who demonstrated that nitrification rates increased proportionally in an activated sludge SBR with an increase in the alkalinity to ammonium ratio. Approximately 3.0mg to 3.5mg of $CaCO_3$ was consumed per mg of Nitrogen. A minimum alkalinity to total nitrogen ratio of 2.4 was required to reach complete nitrogen removal. An alkalinity to ammonium ratio of 2.6 to 3.0 might be optimum or it was also recommended to maintain an effluent alkalinity of >50mg/l CaCO3. Tokutomi *et al* (2006) also reported that high concentrations of inorganic carbon in a pilot plant added as sodium bicarbonate resulted in faster growth rates of ammonia oxidising bacteria but had no significant effect on nitrite oxidation. The highest levels of nitrate formed were when the effluent levels of inorganic carbon exceeded 160mg/l for influent ammonia levels of 350-550mg/l NH_4-N. In a submerged aerated biofilm. Hwang *et al* (2000) achieved nitrification on an influent nitrogen level 340-420mg/l for an alkalinity/ammonium ratio of 4.9. However as the ratio increased to 7.1 some nitrite accumulation was evident in the effluent due to free ammonia inhibition. A high alkalinity to ammonia ratio of 9 may enhance ammonia oxidation but may in turn inhibit *Nitrobacter* if free ammonia levels are not controlled. Both Villaverde *et al* (1997) and Wiesmann (1994) reported higher levels of total inorganic carbon for ammonia oxidation,that would include contributions from carbon dioxide; stoicheometrically the ratio to ammonia oxidiation is 7.14 and 8.6 respectively. This was still lower than the mean influent levels at the full scale WWTP. Nitrification failure due to an insufficient supply of inorganic carbon was therefore rejected.

Although temperature, sludge age, dissolved oxygen , phosphorus and inorganic carbon were not responsible for nitrification failure at the full scale WWTP, there was evidence that pH , free ammonia , COD, F/M and the C/N ratio were all having an adverse

influence. In addition these variables were not always fixed; on some occasions nitrification appeared to be disrupted due to high COD's , while in others high free ammonia, high C/N's or low pH's were responsible. The role of these was complicated further by evidence of a combined interaction of two or more variables at the same time such as COD and pH.

To confirm these relationships and to identify precisely which factors were responsible as well as to establish the tolerance levels, pilot plant studies were undertaken. Pilot plant investigations were necessary not only to control and restrict the many variables of a waste water system but also because the full scale WWTP must remain in compliance at all times. Therefore any proposed changes must be tested in advance so not as to compromise the overall performance of the WWTP.

In all studies, one pilot plant acted as a control and the other acted as the test system. Only one new variable was introduced at any time while maintaining all others close to standard operating conditions that in turn reflected the conditions at the full scale plant. The sludge and the waste water were from the full scale WWTP ensuring the microbial ecology in the pilot plants systems was similar. It was firstly demonstrated that ammonia stripping from the pilot plant system was not a significant factor; therefore any removal of ammonia was associated with microbiological activities. The initial focus of the pilot plant study concentrated on the influence of the waste water on nitrification.

Although there was no evidence of a deficiency in the levels of phosphorus and inorganic carbon at the full scale WWTP, it was nevertheless decided to study the influence of nutrients in general on nitrification. This was to rule out a deficiency of some other element especially a micronutrient. There are few reports on the role of micronutrients for nitrification although Adams *et al* (1997) reported the specific mineral requirements of nitrifiers are similar to most microorganism and these included calcium, copper, magnesium, sodium and iron. These were shown to have a stimulatory effect but at higher concentrations some can become inhibitory. Burgess *et al* (2000) also reported that the addition of a micronutrient to an activated sludge treating an industrial waste water can lead to improved COD removal especially recalcitrant COD. Using the reported concentrations of Love *et al* (1999) with some modifications a mineral nutrient mix was prepared. This had additional phosphorus, bicarbonate and in particular a range of micronutrients including calcium, copper, magnesium, sodium,

cobalt, molybdenum and boron. A comparison in nitrification performance was undertaken whereby one system received only what nutrients were available within the waste water compared to the system with that was supplemented with the nutrient mix. This had an alkalinity/ammonium ratio of 7 and an influent phosphorus concentration of over 30mg/l P and the remaining micronutrients were less than 5mg/l.

The pilot plant study confirmed that the waste water had adequate levels of nutrients as evident by similar start up times and the sustainment of nitrification in the non supplemented system. Although the pilot plant system with additional nutrients did produce slightly lower levels of effluent ammonia and higher nitrates, a more accurate indicator is to compare the rate of nitrate production per day. On this basis it was demonstrated that on a low COD loading the nutrient supplemented system achieved only marginally higher growth rates than the control system without any additional nutrients. The difference in performance was less than 3%. The calculated growth rates were between $1.0d^{-1}$ for the system with nutrients compared to $0.97d^{-1}$ for the system without nutrients (at 20^oC). These growth rates were on the higher side of other reported rates.

Sears *et al* (2003) reported nitrifier growth rates as low as $0.12\text{-}0.15d^{-1}$ at 12^oC to $0.25\text{-}0.30d^{-1}$ at 24^oC for an activated sludge treating a municipal waste water, while Katehis *et al* (2002) reported similar growth rates at 20^oC of $0.32\ d^{-1}$ to $0.35\ d^{-1}$ in a SBR treating a domestic waste water at varying sludge ages. Wiesmann (1994) reported typical nitrifier growth rates of $0.38d^{-1}$ at 20^oC. However Canziani *et al* (2006) achieved somewhat higher rates of $0.45\text{-}0.65d^{-1}$ for ammonia oxidisers with slightly lower growth rates of $0.30\text{-}0.58d^{-1}$ for nitrite oxidiser for a membrane bioreactor and moving bed biofilm treating ammonia from a landfill leachate. Pambrun *et al* (2006) reported the growth rate to be $0.72d^{-1}$ at 20^oC in an SBR treating a synthetic waste water. The growth rates achieved in the pilot study were however close to Jones (2005) who measured nitrifier growth rates in a number of different waste water treatments at high and low F/M's and found that these ranged from $0.8\text{-}1.07d^{-1}$. The highest reported growth rates were by Dincer and Kargi (2000a) and Rozich and Castens (1987) treating a synthetic waste water of $1.15d^{-1}$ and $1.2d^{-1}$ respectively. Nitrifier growth rates are still considerably lower than those of heterotrophs whose growth rates can exceed $6.0d^{-1}$ (IWA,2000) but typically range from $3.4\text{-}6.24d^{-1}$ (Ziglio *et al.*, 2002). On this basis the growth rates for the nitrifier population within this activated sludge appeared to be very good. The waste water appears to have all the essential nutrients to support nitrification;

this was not surprising given the wide range of raw materials used on-site. The influence of nutrients at a high COD loading was not studied as there is no increase in nutrient demand by nitrifiers as the COD increases. The demand for nutrients by the lithotrophic nitrifiers is proportional to their energy source i.e. ammonia which was fixed at a constant level.

Although the waste water may have had adequate levels of nutrients for nitrification it was important that these are bioavailable. The concentrated waste water at the full scale WWTP is stored in a large 2,000m^3 balance tank. This has a typical pH range of pH 9-11. Although this tank is agitated the company has had issues of solids accumulation at the base of this tank. There was also a concern that if the tank's pH became too alkaline that some essential elements may precipitate out of solution, settle at the bottom of the tank and consequently not be fed forward to the nitrification stage.

Results confirmed that at a high pH up to 70% of the inorganic fraction will precipitate out of solution. It was demonstrated in the pilot plants that on a low COD loading of <2,000mg/l the absence of this fraction in the waste water had no effect on nitrification. However under high COD loadings of >6,000mg/l a 45% reduction in nitrification was recorded. The absence of one or more essential inorganic elements, possibly a metal, resulted in a decline in nitrification either directly or indirectly through a fall in the performance of the heterotrophic population. The latter is more likely as the COD has no nutritional influence on nitrification. The removal of the inorganic fraction also suggested that the inhibitor to nitrification was organic and not inorganic.

Braam and Klapwijk (1981) and Tyagi and Couillard (1988) reported that the role of pH is important in relation to its effect on metals, as it is not the total metal concentration that is of interest but rather the free metal concentration. Investigations at different pH's demonstrated a linear correlation between nitrification capacity and the free metal concentration. As the pH falls certain metals become more bioavailable or conversely as the pH increases less bioavailable. Therefore, as an operational management practice the strong waste balance tank must not be allowed to become too alkaline and or the tank must be well mixed so that any precipitate formed is fed forward and re-solubilised at the lower more neutral pH in operation at the aeration bay.

A sufficiently low C/N ratio is required to ensure an excess of ammonia is available for nitrifiers after utilisation by the heterotrophic population. Ammonia is not only essential for nitrifiers as a source of energy, it is also an important nutrient for the faster growing and more abundant heterotrophic population where of assimilation of ammonia by heterotrophs is proportional to the consumed carbon measured as COD. Heterotrophs will take up ammonia reducing what is available for nitrification leading to a reduction in nitrifier biomass. Ammonia assimilation by heterotrophs takes precedence over nitrification leaving only the surplus ammonia available to nitrification (Hanaki *et al.*,1990). In a nutrient deficient plant the minimum recommended BOD:N ratio is 100:5, however for a plant that is optimised this can be reduced to 100:3.5 (Moebius, 1991). Expressed as COD a heterotrophic sludge requires 0.11-0.77mg of nitrogen per mg of COD (Ning *et al.*, 1999). For highly loaded industrial waste water the amount of nitrogen to dose can be controlled by ensuring there is an excess of only 0.5mg/l NH_4-N in the effluent (Prendl and Nikolavic, 2000). The half saturation constant of nitrifiers for ammonia ranges from; a low in a biofilm of 0.13-0.14mg/l NH_4-N (Manser *et al.*,2005) to 0.4 to 1.1mg/l NH_4-N for a municipal and industrial waste water (Nowak and Svardal, 1993) and in general 1mg/l NH_4-N for an activated sludge (Gee, 1987; IWA,2000).

Influent ammonia levels to the full scale WWTP tended to fluctuate depending on the production cycles in turn creating varying C/N ratio's. The lower this ratio the more nitrogen is available and conversely the higher the less that is available. In the latter instance this may be to the detriment of the slower growing nitrifiers. The mean C/N ratio at the full scale WWTP from 2000 to 2003 was 130 however there was a four month period where under high C/N ratio's of over 270 that nitrification was lost from the system. The extended periods of low effluent ammonia and in particular low effluent nitrates suggested that any incoming ammonia was being used by the heterotrophic population to the detriment of nitrification leading to population starvation or wash out. A 50% failure in nitrification was predicted at a C/N ratio of >350, however the regression co-efficient was only 45%. To replicate this situation, nitrification was established in the pilot plants prior to a predicted fall in the influent ammonia of the full scale waste water following the completion of a production campaign known to produce an ammonia effluent.

One of the pilot plants received the same waste water taken daily from the full scale plant. The other pilot plant although also receiving this waste water had additional ammonia added to maintain the influent ammonia at 50mg/l NH_4-N. This pilot plant successfully sustained 100% nitrification or expressed as a C/N ratio then 100% nitrification was achieved at a C/N ratio of 40. The importance of applying this ammonia dosing regime was demonstrated in the control system; although also nitrifying up to a C/N ratio of 80 when this ratio increased to 250, due to a sharp fall in the influent ammonia, the levels of nitrate also fell at a rate of nearly 4mg/l NO_3-N per day.

This was a significant rate of decline. For a system operating on a low to medium sludge age, within 20-30 days, the nitrifying population could be completely washed out or severely depleted such that the system is unable respond to a sudden rise in the influent ammonia. This was demonstrated when the influent ammonia peaked with resulting breakthrough to the final effluent. As Horan and Azimi (1992) reported a transient increase in ammonium loading will not be accompanied by a commensurate rise in the nitrifier oxidation rate. The nitrifying fraction of the biomass or the population of nitrifiers must therefore increase significantly before further ammonia oxidation will occur. Esfandi and Kincannon (1981) also reported that ammonia will typically breakthrough to a final effluent upon an application of a two fold ammonia shock load from 50mg/l to 100mg/l NH_4-N. A low population of nitrifiers is therefore incapable of responding fast enough to a sudden increase in the influent ammonia.

Inhibition, nutritional or some adverse environmental factor was the unlikely cause of nitrification failure at this time as the ammonia supplemented pilot plant maintained 100% nitrification. What was responsible was the fall in available ammonia due to heterotrophic uptake resulting in a significant decline in the nitrifier population. This population was already low as evident by only 10-20mg/l NO_3-N in the effluent prior to the increase in the C/N ratio. Full nitrification was therefore sustained at a C/N ratio of 40 for this pharmaceutical waste water but quickly failed at a ratio of 250. The actual failure point lies between these two values for this system. Determination of this was not deemed necessary as this is something that can be effectively monitored and controlled when applied on a full scale.

The importance of maintaining a low COD/N ratio has also been reported by other investigators albeit at lower levels. Cheng and Chen (1995) demonstrated 96-97% nitrification in a fluidised bed reactor treating high ammonia concentrations on synthetic waste water with no influent COD i.e. a COD/N ratio of 0. Nitrification fell to 92% at a COD/N ratio of 0.5 and again to 86% at a COD/N ratio of 1.0 due to increased heterotrophic growth. Sharma and Gupta (2004) reported that the percentage nitrification increased with a decrease in the COD/N ratio for an activated sludge treating pharmaceutical waste water. The maximum concentration of nitrifiers was maintained at a COD/N of 0.68 to 1.23. The nitrifier population doubled when the COD/N ratio fell from 2.2 to 0.7. These ratios are very low and could not be applied at the full scale WWTP as it would require maintenance of the influent ammonia levels in excess of 300mg/l NH_4-N.

Sustaining full nitrification at a C/N of 40 agrees more closely with Young *et al* (2007) who recommended a C/N ratio of less than 47 and Bury *et al* (2002) who described achieving full nitrification on a chemical waste water at ratios as high of 9:1 but advised that complete failure will occur above 40:1. As reported by Prendl and Nikolavic (2000) and as confirmed in this study a minimum nitrification capacity needs to be maintained at all times by means of nitrogen addition during periods of nitrogen deficiency. The importance of an ammonia dosing regime for the nitrifiers is therefore important in a pharmaceutical waste water and must be considered an important management practice.

Given the wide variety of chemicals used on-site, the complex interactions and transformations that these can undergo in the waste water, it is difficult to accurately identify and quantify loadings to a plant based purely on the chemical composition. Instead an encompassing indicator test such as the COD is used. As the COD concentration increases the concentration of chemicals also increases and therefore in turn possible inhibitors to nitrification. The strong waste water tank was identified as the most likely source of inhibition as this contains the aqueous wastes from complex process reactions.

Of all of the parameters tested one of the strongest correlations related to the influent COD. It is generally accepted that as the COD concentration increases as the rate of nitrification decreases; both Esfandi and Kincannon (1981) and Hall and Murphy (1985) reported inhibition to a nitrifying activated sludge following an increase in the COD load. There was statistical evidence of COD inhibition to nitrification at the full scale

WWTP from 2000 to 2003. The mean influent COD to the plant was 2,900mg/l with several occasions where it exceeded 5,000mg/l. A strong Pearson's correlation of 83% and a regression co-efficient of 75% predicted a 50% failure in nitrification at an influent COD of 4,000mg/l and 100% failure at 5,000mg/l. The best fit for a trend line was a quadratic curve and not linear, this indicated that rate of inhibition increased proportionally faster with increasing COD.

The sensitivity of nitrifiers to COD was confirmed in the pilot plant studies where no inhibition was evident at an influent COD of less than 3,500mg/l. Above 4,000mg/l the percentage nitrification started to fall and the rate of this decline increased significantly with almost full failure above an influent COD of 6,000mg/l.This compared very well to the data of the full scale WWTP. Tyagi and Couillard (1988) reported that inhibition generally follows three different patterns i.e., linear, parabolic and hyperbolic. The observed pattern for this pharmaceutical waste water followed that of a parabolic curve as full nitrification was maintained at an influent COD from 500mg/l to 3,500mg/l however when this exceeded 6,000mg/l then the percentage nitrification fell sharply.

The sensitivity of nitrifiers to inorganic and especially organic chemicals is well documented and is largely attributed to a membrane bound enzyme called ammonia monooxygenase (AMO). Three solvents commonly found in chemical waste waters are benzene, toluene and xylene. The AMO enzyme can oxidise benzene to acetate; toluene to benzyl alcohol and in turn to butyrate and xylene can be oxidised to acetate and also to butyrate. This process of non substrate oxidation will result in a reduction in ammonia oxidation (Zepeda *et al.*, 2003 and 2006).

AMO has a low substrate specificity i.e. it will not selectively oxidise ammonia. Inhibitory components in the waste water have an influence on AMO by: direct binding and interaction with AMO; interference with the supply of reductant needed for AMO activity and the oxidation of substrates to give products that are highly reactive with AMO and or other enzymes (Keener and Arp,1993, Mc.Carthy *et al.,* 1999). Once a certain concentration of AMO oxidisable chemicals is reached within the waste water then AMO will also oxidise these chemicals to the detriment of ammonia oxidation as there is no energy return to the nitrifiers. This is known as competitive inhibition where alternative substrate oxidation takes place. In non competitive inhibition, loss of ammonia oxidation is not related to competition with the substrate but results from

interference with cellular or enzymatic functions. Some substances can be both competitive and non competitive inhibitors (Love *et al.*,1999).

Competitive inhibition may also explain the observed parabolic response to the increase in the influent COD. Up to 3,500mg/l the carbonaceous removal process of the system is capable of processing the COD loading under normal operating conditions. However following a sudden increase in COD beyond 3,500mg/l the heterotrophic population that is responsible for removing this carbonaceous fraction is unable to react to the sudden rise in available food as evident by a rise in F/M. This results in a rise in the concentration of un-degraded chemicals within the waste water as evident by rising effluent COD's. This is undesirable for nitrification as it results in AMO competitive inhibition. As there are insufficient levels of the AMO enzyme to oxidise both these chemicals and their actual targeted energy source ammonia. The result is un-oxidised ammonia breakthrough to the final effluent equating to a fall in the percentage nitrification.

Data analysis of full scale WWTP and pilot plant studies demonstrated that the effect and degree of COD inhibition was also influenced by the pH of the nitrifying system. The mean pH at the WWTP was pH 7.93 and excluding some outliners generally ranged from pH 7.0 to 8.5. A significant correlation and regression coefficient of 78% and 60.06% respectively was recorded over a 3 month period when the operating pH steadily fell from pH 8.4 to pH 6.3. The pilot plant studies confirmed that on a low influent COD of <2,000mg/l nitrification proceeded equally well at pH 7.5 as at pH 8.5. However at an influent COD of >4,000mg/l nitrification performance was 53% lower at pH 7.5 compared to the same system at pH 8.5. There is no other known reported investigation that has observed a similar relation between the pH and the disruption of nitrification at high COD loadings. It was also noteworthy again that both systems that were initially disrupted at an influent COD of 4,000mg/l, within 3-4 days fully recovered when the influent COD was reduced. This indicates that the COD is inhibitory but is not toxic (cell death) in the short term. Although full nitrification occurred on low COD loadings at pH 7.5 and pH 8.5; the effect of increasing the COD loading and therefore the concentration of chemicals could be increasing the bioavailable concentration of an inhibitory component that is pH related. The results of the inorganic fraction study would suggest this is not the case. A more likely explanation is that pH 8.5 is closer to the optimum pH and under stressed conditions

better microbial cell kinetics occur at a more alkaline pH. Different authors have reported different optimum pH's and the range varies significantly.

The mean pH of 7.93 at the full scale WWTP was close to recommended levels for nitrification; for example using an isolated nitrifier culture and synthetic feed; Wong-Chong and Loehr (1976) reported an optimum pH of 7.0 to 7.5 whereas Srinath *et al* (1975) reported an optimum pH of 7.4 to 7.9. Balmelle *et al* (1992) reported a higher overall optimum pH for nitrification to be pH 8.5 for an activated sludge in an SBR treating a synthetic waste water. However, the actual optimum pH is in fact different for ammonia oxidisers than for nitrite oxidisers. For pure cultures of *Nitrosomonas* isolated from an activated sludge, the optimum pH was 8.1 compared to pH 7.9 for *Nitrobacter* (Grunditz and Dalhammar, 2001). Ramachandran (2003) reported slightly different optima of pH 8.5 for ammonia oxidiser and pH 7.5 for nitrite oxidisers. More recently Tokutomi *et al* (2006) found that ammonia oxidiser prefer a more alkaline pH i.e.7.5-8.5 whereas nitrite oxidisers prefer a more neutral to slightly acidic pH of 6.5-7.0.

Nitrification is completely inhibited below a pH of 6.3 (Hall and Murphy,1985). However Sears (2003) demonstrated that >90% nitrification can take place a low pH's of 5.0 and 5.8 in a nitrifying activated sludge treating municipal waste waters provided the sludge age was >12 days. Temperature also had a significant influence on the ability of the system to nitrifying at a low pH; nitrifier growth rates at pH 6.0-6.3 doubled as the temperature was increased from 12^{o}C to 24^{o}C. Wett and Rauch (2003) and Guisasola *et al* (2007) both reported that inorganic carbon limitation is often neglected at low pH's. Inorganic carbon exists in equilibrium and depends on the pH. The pH should be kept above pH 7 as below this there is little inorganic carbon in the carbonic acid form. This may be the preferred form for nitrification. Ammonia oxidation is more susceptible to inorganic carbon limitation than nitrite oxidation.

Another explanation as to why the pilot plant at pH 8.5 performed better than the same system at pH 7.5 may be related to the differences in free ammonia levels. Total ammonia will exist as either free ammonia or as ammonium. As the pH and the temperature increase total ammonia will shift towards the free ammonia form i.e. its un ionised form. A similar relationship exists for nitrite; where total nitrite will exist as nitrite or nitrous acid. The ammonia to ammonium ratio is therefore an important consideration. It is the unionised form i.e. ammonia and not ammonium that is the preferred substrate for nitrifiers as less energy is required in this form for its transport

into the cell (Wiesmann,1994). Van Hulle *et al* (2005) and Pambrum (2006) reported a half saturation co-efficient (Ks) for free ammonia of 0.5mg/l and both authors also reported an increase in ammonia oxidiation with an increase in free ammonia levels. The mean free ammonia level in the pilot plant at pH 7.5 was 0.14mg/l compared to 0.28mg/l in the system at pH 8.5. By increasing the pH from 7.5 to 8.5 more free ammonia was liberated allowing higher concentrations within the floc; this may have contributed to the improved performance for the pilot plant under high COD loadings at pH 8.5.

Although free ammonia may be essential as a nutrient it is also a potent and well published inhibitor following a typical Monod and Haldane substrate/inhibition profile; that is as free ammonia levels increase, the nitrifier growth rate increased in tandem. This growth pattern however slows down and reaches a plateau such that any further increase in the free ammonia concentration will not lead to an increase in growth rates. Once the critical concentration is reached, growth rates start to fall and free ammonia which was a substrate now becomes an inhibitor (Van Hulle *et al*, 2005; Pambrum *et al.*,2006).

Over the four year period the mean free ammonia at the full scale WWTP was only 0.83mg/l. However levels regularly exceeded 2mg/l and on occasions reached significantly higher levels up to 25mg/l FA. A specific 3 month period was noted when free ammonia levels increased within the system resulting in a reduction in the percentage nitrification. A regression analysis of this data predicted a 50% failure in nitrification at a free ammonia levels above 1mg/l FA and 100% failure above 4mg/l FA. The inhibitory nature of free ammonia was confirmed in the pilot plant study where nitrification was completely inhibited above a free ammonia level of 5mg/l. Regression analysis of this data predicted a 50% failure in nitrification above a free ammonia level of 1.5mg/l similar to the observed inhibitory concentrations from the full scale WWTP data review. Similar to the COD, it was observed in the pilot plant studies, that free ammonia is inhibitory but it is not toxic in the short term. Once free ammonia levels fall back to non inhibitory levels then nitrification quickly resumes. Nitrification fully recovered despite exposure to free ammonia levels as high as 26mg/l FA. This finding agrees with Liu and Capdeville (1994) who also reported that the nitrifier population rapidly recovered it metabolic activity once free ammonia levels fell below 1mg/l.

The general inhibitory nature of free ammonia supports the findings of other investigators. Brond and Sund (1995) lists inhibition of nitrification by free ammonia as a key reason why nitrification was lost when the free ammonia concentration exceeded 2.2mg/l at a rendering waste water treatment plant. Mauret *et al* (1996) reported that it is nitrite oxidation step of nitrification that is most susceptible to free ammonia inhibition. Hwang *et al* (2000) found that a free ammonia concentration of 0.3-16mg/l inhibited nitrite oxidation in a submerged biofilm but this did not impact ammonia oxidation whereas according to Aktas *et al* (2001) free ammonia concentrations as low as 0.1-2mg/l will contribute to nitrite build up in an activated sludge treating landfill leachate. Bae *et al* (2001) reported no effect from free ammonia inhibition below 1mg/l but nitrite accumulation became significant at a free ammonia of 4mg/l in batch reactors treating a synthetic feed.

Investigations by Simm *et al* (2006) found that free ammonia exposure to a pure culture of nitrifiers treating a synthetic waste water was not as inhibitory as previously reported. No inhibition was evident for *Nitrospira* at free ammonia levels of 10mg/l and up to 15mg/l free ammonia for a mixed culture. Part of the explanation for the wide range in reported inhibition thresholds is that the free ammonia inhibition level is in fact not fixed or constant as a nitrifying sludge can become acclimatised and develop a tolerance. This agrees with Turk and Mavinic (1990) who demonstrated that with a sufficiently long acclimatisation period free ammonia levels as high as 40mg/l free ammonia can be tolerated whereas nitrification is inhibited for a non acclimatised population at only 3.5mg/l FA. Vadivelu *et al* (2007) reported that although nitrification may still be occurring at a low concentration of free ammonia the growth rate of the organisms may have significantly slowed down thereby impacting on the overall kinetics of the system.

A rapid means to reduce free ammonia inhibition is to reduce the pH of the waste water system. However this needs to be done gradually. At low pH's there is only ammonium and almost no free ammonia. Specific ammonium transporters are needed for ammonium to enter the cell. Such transporters have been found in the genome of *N.Europaea.* The *de novo* synthesis of these takes time which explains the lag time for adaptation to occur. A slow shift to acidic conditions is therefore desirable to allow these additional cellular functions to become active (Gieseke *et al.*,2006). A wastewater containing high ammonium and lower free ammonia can in fact be more inhibitory than a lower ammonium and high free ammonia. Ammonia inhibition must be assessed in

conjunction with total ammonia and with free ammonia. However this is at total ammonia levels of over 1,500mg/l NH_4-N (Lee *et al.*, 2000). Suthersan and Ganczarczyk (1986a) investigations found that different pH values gave different inhibitory effects at the same free ammonia concentration. From the pattern of inhibition it may be assumed that the effects of free ammonia and of pH are therefore additive. Therefore the pH of the full scale system must be closely controlled where under high COD loadings a more alkaline pH is desirable however if free ammonia levels are too high then a more neutral pH should be adopted and the COD loading reduced.

Although the C/N ratio, the pH and the free ammonia levels can be effectively controlled at the full scale WWTP the ability to regulate and to control the influent COD is less flexible. Above an influent COD of 4,000mg/l a significant disruption in nitrification was observed in both the full scale WWTP data study and the pilot plant study. This had the potential to limit the WWTP treatment throughput capacity and in turn production volumes. To avoid significant capital expenditure to upgrade the WWTP cost effective means were studied that would allow the nitrification process to tolerate a higher influent COD.

Balancing of the waste water is important. Considering the potential biodegradation of inhibitors, rapid changes in waste water composition needs to be avoided (Nowak *et al.*,1995a,b). Nitrification has been demonstrated for example on waste waters arising from coke oven plants, the effluents of which contain several inhibitory substances including; phenols, cyanides, sulphides and polycyclic aromatic hydrocarbons (PAH's). Sufficient upstream balancing is required to equalise short term spikes of toxic compounds (Brond and Sund,1995). As the strong waste tank has a 60 day retention time adequate balancing at the full scale WWTP is already in place.

The influent flow to the full scale WWTP aeration bay is matched by a 1:1 ratio with the sludge recycle. This ratio was traditionally adopted to keep the sludge blanket level low in the clarifiers. This high ratio had the potential to result in a flow pattern that is closer to a mixed system than a plug flow system and in turn reduces the potential formation of a COD or an ammonia concentration gradient from the inlet to the outlet of the plant. This can be significant, as reported by Fearnside and Booker (1995) the importance of dilution within a plant is to lower the toxic effect. Although a trade effluent may be inhibiting, when diluted down through the WWTP the effect of this is

diminished. The preferred profile along the treatment system should be such that carbonaceous removal takes place where the COD and inhibitors are high and nitrification where the COD and inhibitors are low. This is borne out by the fact that nitrification was only ever observed in the aeration bay and not on the higher COD loaded biotowers. Also in the pilot plant studies under low COD loading, nitrification predominantly occurred in the first reactor but that this would shift to the second reactor in response to a rise in the influent COD. The formation of a plug type flow would allow these conditions to take place. Azimi and Horan (1991) and Daigger and Parker (2000) found that a reactor with plug flow kept the ammonia concentration high and this only became limiting near the end of the reactor. The result was nitrifiers grew at their maximum rate throughout most of the reactor. The onset of nitrification was also more rapid under plug-flow conditions and a higher rate for nitrification was observed compared to a mixed system.

Modification of the sludge recycle rate was therefore investigated to create a COD concentration gradient in the activated sludge system. As the pilot plant system is divided into two inter connected reactors it was the objective of this study that the first reactor would be devoted mostly to COD removal and the second reactor to nitrification. By reducing the sludge recycle ratio on the test system this successfully created a moderate concentration gradient with on average 30% higher COD levels in the first reactor compared to same reactor of the control. Despite this, following a shock COD load, nitrification failure occurred equally in both pilot plants, this failure was however delayed by 1-2 days in the test system compared to the control.

Although the sludge recycle modification resulted in lower effluent ammonia on a high COD loading this was not due to nitrification as reflected by the levels of the nitrates. There was evidence of a higher ammonia uptake by heterotrophs in the test system supported by a 14% higher sludge yield. As both pilot plants had the same starting MLSS (6,000mg/l) and the same sludge wastage rate the difference in sludge yield resulted in the test system having a lower F/M of 0.33 compared to 0.38 for the control and on this basis the control system actually removed more mg of COD per mg of biomass. An intermittent sludge recycle can therefore allow a plant to operate on a higher COD loading while maintaining lower effluent ammonia; this was however not due to nitrification. In the event of nitrification loss this could be considered as a short term means to encourage some additional uptake of ammonia but would be of limited value. This finding agrees somewhat with Horan and Azimi (1992) who reported under

inhibitory conditions the performance of nitrification in a completely mixed and in a plug flow reactor operated under the same conditions behaved identically. There was no difference in the potential for the mixed system to dilute shock loads for the benefit of nitrifying bacteria. There was also a slight increase in solids production in the plug flow reactor, this was a similar finding to the pilot plant study.

Although there may be no significant difference between a mixed and a plug flow system Gerardi (2002) reported that a true two stage system is preferable as the toxicant may be degraded before it reaches the second nitrification stage. Munirathinam and Lankford(1997) investigated a single stage activated sludge system; a two stage activated sludge system; a two stage high rate anaerobic/aerobic and a two stage low rate anaerobic/aerobic treating fermentation and pharmaceutical waste waters. All alternatives demonstrated nitrification however the types with two stages were superior. Some of the difference in performance may be explained by Wittenbolle *et al* (2005) who reported that two pharmaceutical waste water treatment plants, a mixed and a two stage system despite treating the same waste water had significantly different bacterial communities demonstrating the significant effect that the configuration of treatment plant can have on the microbial ecology and diversity.

Given that modification of the sludge recycle would not lead to better nitrification, the effect of placing a full treatment system in front of the test pilot plant system was studied thereby creating a true two stage system. An Eckenfelder reactor was chosen as the first stage. This was a compact treatment system with its own aeration and integrated clarifier. This first stage of the two stage system was highly effective in removing over 90% of the 4,000mg/l influent COD and maintaining a low COD loading of less than 400mg/l to the second nitrification stage. In terms of COD removal the two stage system also removed more mg of COD per mg of biomass than the standard pilot plant arrangement. It had already been demonstrated that an influent COD of 4,000mg/l should have resulted in no more than a 50% failure in nitrification. It was therefore unexpected that nitrification completed failed in the two stage system compared to the standard pilot plant arrangement which maintained over 95% nitrification.

A noteworthy distinction between both systems was the MLSS levels. As the first stage and the second stage of the two stage system had independent sludge recycle system there was no inter change of sludge between both stages. As the same sludge age was applied to both systems, the effect of reducing the COD load to the second stage had an

adverse effect on the MLSS population levels. The heterotrophic population in the second stage was starved of COD and consequently the MLSS fell by over 60% to less than 955mg/l. The aerobic cell yield for heterotrophs is 0.63g/g COD compared to 0.24g/g N for nitrifiers (IWA,2000) although Dincer and Kargi (2000a) reported a high nitrifier yield co-efficient of 0.34g/g N, albeit on a synthetic waste water. On this basis the first stage of the test system would have produced 2,520mg d^{-1} of heterotrophic biomass per day compared to only 600mg $d^{-1}$of heterotrophic biomass in the second stage. The nitrifier biomass production was the same in both pilot plant systems at between 132-187mg d^{-1}.

As a gene probe stain comparison of the MLSS and the liquors supernatant demonstrated that the nitrifiers were predominantly attached to the sludge floc; the MLSS population washout therefore carried out the attached nitrifier population. This was as a result of the fact that nitrifiers are themselves unable to form good flocs (Fang *et al.*,1993). Ochoa *et al* (2002) and Carvalho *et al* (2006) reported that in a mixed floc/biofilm system faster growing bacteria tend to predominate in suspension while slower growing bacteria, such as nitrifiers, tend to attach to biofilms. It is largely recognised that fixed film nitrification offers superior performance over conventional activated sludge**.** Further study is required whereby some of the sludge from the first stage would need to be directed to the second stage or the COD removal efficiency of the first stage needs to be reduced to supply adequate food for MLSS growth in the second stage. It was also noteworthy that the control system fully recovered despite remaining on an influent COD of 4,000mg/l that initially caused the disruption.

This study highlighted the importance of providing and maintaining a support structure for the nitrifiers. Although all biological treatment methods such as trickling filters, activated sludge and rotating biological contractors can support nitrification activated sludge has traditionally received more attention as much closer control is possible (Esfandi and Kincannon, 1981). One of the main benefits of a biofilm system over activated sludge is that lower sludge ages can be used (Hu *et al.*, 2001). Long sludge ages have several draw backs in terms of WWTP design and construction. They can also result in unfavourable effluent quality issues such as the excessive growth of filaments. Fixed film and floating media are effective strategies that allow nitrification at a shorter sludge age (Smith, 2004). Immobilising nitrifiers on a support allows operation at a very short sludge age as nitrifiers are retained within the system and consequently do no suffer as much from wash out problems (Gheewala *et al*, 2004).

You *et al* (2003) assessed the benefit of inserting a Rotating Biological Contractor (RBC) to an aerated activated sludge stage versus an activated sludge stage only. The addition of an RBC resulted in higher and more stable nitrification rates with lower effluent ammonia.

Nitrification will occur in trickling filters if the loading is sufficiently low i.e. at an organic loading of <1kg BOD/m^3/d. However even when a support is provided nitrifiers do not readily form biofilms on their own due to their slow growth rates and a lack of extracellular polysaccharides that facilitate mutual adhesion. It takes a long time to form a nitrifying biofilm particularly when the waste water contains low levels of organic compounds (Tsuneda *et al.*,2003). A concentration gradient may also exist in a biofilter where nitrification will occur near the bottom of the filter as the organic loading is lower here (Daigger *et al.,* 1993). The BOD loading on the biotowers at the full scale WWTP was significantly higher than this ranging between 4kg to 8kg BOD/m^3/d explaining why no nitrification was observed here.

Ammonia oxidising bacteria tend to reside on external regions of particles of the biofilm whereas the nitrite oxidiser tend to grow somewhat internally but still close to ammonia oxidisers (Kowalchuk *et al.*, 2001). Various techniques using micro-organisms entrapped in gels are known and used in many industrial activities. A commercially available process using immobilised nitrifiers entrapped in polyethylene glycol (PEG) pellets was evaluated on a pilot scale. The lifespan of these pellets is over 10 years and have a typical dimension of 3mm. The nitrifiers, due to oxygen limitation proliferate just below the surface of the pellet. 4-5mg/l oxygen was needed with fine bubble aeration which also keeps pellets in suspension (Jonkers *et al.*, 2000).

Gene probe stains of the full scale WWTP and of the pilot plant confirmed the preference of nitrifiers to attach to the activated sludge floc rather than form their own individual flocs, methods to promote enhancement of nitrifier attachment within the activated sludge system was therefore investigated. The benefit of adding activated carbon as a convenient and cost effective medium to support fixed film biomass growth with associated benefits to the nitrifying sludge system was therefore studied. This in effect created a combined activated sludge/fixed film system within the same reactor.

The addition of an activated carbon to the pilot plant reactor system resulted in superior nitrification compared to just the activated sludge system. On a high influent COD of 8,000mg/l there was an 88% failure in nitrification in the system without activated carbon compared to only 48% failure in the test system with activated carbon. Although it should have taken a sludge age of approximately 30 days for the activated carbon additions to reach its maximum concentration, as only 2.7g was added each day, nitrification recovered when the concentration of activated carbon within the reactor system reached 1,000mg/l (or total addition of 27g). This represented the optimum concentration for nitrification on this waste water.

The superior nitrification of the system with activated carbon may have been due to attachment and therefore better retention of the nitrifier population under stressed conditions. A microscopic examination showed that the sludge floc was clearly seen to be attached to the activated carbon granule. Lower suspended solids in the effluent also supported this observation. Activated carbon and other similar substances were successfully used by other investigators. Aktas *et al* (2001) added 500mg/l-1,000mg/l activated carbon for the removal of nitrification inhibitors from landfill leachate and Kochany and Lugowski (1998) also used 1,000mg/l activated carbon to remove inhibitors from contaminated groundwater. Eckenfelder *et al* (1992) reported that the addition of an activated carbon to waste water can reduce or eliminate nitrification inhibition by reducing the concentration of the toxic agent through adsorption and subsequent biodegradation. Diab and Shilo (1998) showed that *Nitrosomonas* and *Nitrobacter* rapidly attached to particles of bentonite, calcium carbonate, and amberlite. The nitrifying activity of the attached bacteria was also greater than the activity of freely suspended cells. Liessens and Pipyn (1996) demonstrated that a population of biomass retained on activated carbon was an effective way of treating refractory organic compounds that were inhibitory to nitrification in a pharmaceutical waste water.

It is important to note that in the pilot plant study the activated carbon was pre-soaked in the waste water prior to addition to the pilot plant. It would therefore have had no chemical adsorption ability and would have only offered a higher surface area for nitrifier attachment. A secondary benefit of the activated carbon included a 33% reduction in effluent suspended solids. This agrees with Yu *et al* (1997) who also found that the biomass in an SBR treating a coke waste water interacted with the activated carbon to form better flocs with improved settleability. Improved COD removal was also observed and the aggregate was more resistant to shock loading.

Of more significance was the 8-10% higher mean oxygen uptake rates (OUR) due to enhanced heterotrophic activity. This is supported by the removal rates of COD per mg of biomass. In the system without activated carbon 0.48 COD/mg of biomass was removed whereas the system with activated carbon plant removed 0.54mg COD/mg of biomass. This represented 11% more COD removal which agreed closely with the higher OUR figure. This indicates that the heterotrophic proportion of the biomass was more active through the provision of an activated carbon support. This finding agrees with Cecen and Aktas (2003) who also reported that the addition of a powdered activated carbon in the range of 2,000mg/l to 5,000mg/l to an activated sludge treating a pharmaceutical waste water resulted in higher oxygen uptake rates.

The addition of activated carbon to the full scale plant to reach a concentration of 1,000mg/l would be manageable, equating to over 500kg of activated carbon per day, and would allow a significant increase in the treatment capacity. However in a practical sense it would only offer a short term solution or something that would occasionally be used in anticipation of shock COD load. A more permanent combined fixed film/activated sludge system was required such as the addition of a buoyant plastic media (BPM).

The addition of a Buoyant Plastic Media (BPM) as a permanent fixed film system was investigated to assess the tolerance of a nitrifying sludge under inhibitory COD levels compared to conventional activated sludge. The BPM used was the Kaldnes® Moving Bed™ bio film process. The Kaldnes® media design maximises the active biofilm surface area within a reactor. Since the depth of full substrate penetration is less than 100μm the ideal biofilm is thin and evenly distributed over the surface of the carrier. Shearing forces are therefore important to keep this biofilm from getting too thick (Rusten *et al.*,2006). The media is engineered in a wheel shape and is slightly positively buoyant, allowing a small amount of water flow (created by adding air to the process) to circulate the media throughout the reactor. As the media circulates within the bio tank, it causes old dead bacteria/bio film on the outside, to be removed making space for new younger bacteria/bio films to colonize. The media maintains both a young biofilm and a maturing biofilm providing a more consistent filter performance. The Kaldnes® process has been used extensively for organic removal and nitrification at municipal waste waters (Marcolini and Johnson,2004).

The addition of 40% Kaldnes® v/v to one of the test reactors took over 8 weeks to form a significant biofilm. This provided an additional 1.87m^2 of available surface area to this pilot plant over that of the control system. The biofilm on the Kaldnes® produced a MLSS equivalent of nearly 2,000mg/l. This compares well with Hegemann (1996) in a pilot plant treating dairy waste water who also added 40% V/V. With a starting activated sludge concentration of 3,000mg/l the overall MLSS equivalent was increased to 5,500mg/l after the addition of Kaldnes®. Improved settlability, sludge dewatering and improved BOD removal were also noted by this investigator.

A scraping and gene probe stain of the biofilm from the Kaldnes® showed that the nitrifiers were predominantly growing on the biofilm on the Kaldnes® with very few cells in suspension. This confirmed the nitrifiers preference to attach to a support structure. As with activated carbon the addition of a Kaldnes® media enhanced nitrification compared to an activated sludge system only. Once inhibitory COD levels were reached a nitrification rate of 83% was maintained in the Kaldnes® system compared to only 60% in the non Kaldnes® system. It was unexpected that nitrification disruption occurred in both systems at a relatively low influent COD of 3,500mg/l when investigations to this stage using the same waste water had established that nitrification would not begin to fail until >4,000mg/l COD and not reach significant failure until over 6,000mg/l COD. This questioned the value of using the influent COD as an indicator to predict the point at which nitrification would fail. In the Kaldnes® a sludge wastage rate greater than cell yield was adopted to gradually reduce the MLSS and therefore encourage biofilm development. Consequently although the influent COD at which nitrification failed was relatively low compared to other studies, the F/M was in fact relatively high at 0.27 in the system without Kaldnes® and 0.34 in the system with Kaldnes®. It is again note worthy that both systems fully recovered to reach 100% nitrification. The recovery in the pilot plant with Kaldnes® was significantly quicker than the control system.

Nevertheless there was a clear benefit to nitrification following the addition of Kaldnes®. Rostron *et al* (2001) compared nitrification at different temperatures, COD loadings and sludge ages of freely suspended activated sludge system with that of a system containing Kaldnes® treating a synthetic waste water. Nitrification failed at a HRT of 1.5days for the activated sludge suspended system but the Kaldnes® system maintained nitrification down to a HRT of <1 day. The Kaldnes® system also performed better when the temperature fell from 25^oC to 16^oC. Nitrification rates of

0.36kg $N/m^3/d$ were recorded for the freely suspended nitrifier activated sludge compared to 4.24 kg $N/m^{-2}/d$ with Kaldnes® media. This rate is higher than conventional biofilm; for example Rodgers (2006) reported the maximum ammonia removal rate on a domestic waste water biofilm was 0.3kg N/m^3/day (1.3g$/m^2$/day) although slightly higher rates were reported by Dempsey *et al* (2005), Chuang *et al* (2007) and Pollard (2006) who reported removal rates of 1.7kg NH_4-N/m^3, 1.46kg $N/m^3/d$ and of 1.2 to 2.0 Kg $N/m^3/d$ respectively. Although this study only achieved 0.14kg NH_4-N/m^3 (0.3g $N/m^2/d$) testing of the maximum ammonia removal of the system was not the intent of the study but rather its ability to sustain nitrification under inhibitory conditions.

Similar to the activated carbon study, significantly higher OUR's were also observed in the system with the Kaldnes® media. The mean OUR in this stage 1.23g O_2/hr/g/MLSS; 64% of which was attributable to the fixed film biomass. This OUR was an order of magnitude greater that the system without Kaldnes® which had a mean OUR of 0.18 O_2/hr/g/MLSS. This higher level of biomass activity is also reflected in higher COD removal rates per mg of biomass indicating enhanced heterotrophic activity. Compared to the activated carbon study COD removal rates were more pronounced where the system with the Kaldnes® removed on average over 35% more COD per mg of biomass.

The superior performance of the Kaldnes® may be attributed to it influence on the sludge age. Traditional practices suggest that aerobic sludge ages as high as possible are necessary to ensure high nitrification performance. However with increasing sludge age the rate of nitrification has been observed to fall. Both Sharma *et al* (2004) and Salem *et al* (2006) warn that at too high of a sludge age then the decay rates are important as nitrifiers may be decaying faster than the growth rates**.** A nitrifying sludge with a high decay rate means a plant operating at a given sludge age may be returning a sludge with no active nitrifiers. This is supported by Katehis *et al* (2002) who compared the growth rate and decay rate at two different sludge ages. It was demonstrated that a sludge age of 8 days had a growth rate of $0.55d^{-1}$ and a decay rate of $0.11d^{-1}$. Whereas at a sludge age of 20 days the growth rate was $0.39d^{-1}$ and the decay rate was $0.06d^{-1}$. It seemed that as the sludge age gets higher the specific decay rate fell as did the specific growth rate. This resulted in the retention of older less active nitrifier cells in the system that would otherwise be washed out. Growth rates fell by 30% going from a sludge age of 8 to 20 days.

A lower sludge age under certain circumstances can result in more prosperous nitrifiers. In the test system there is no sludge return to the stage where the Kaldnes® was located. A young active biofilm was therefore maintained and any old sludge that's sloughed off was washed to the second stage and ultimately from the system. In comparison the control systems sludge recycle was returned to the first stage, therefore older and less active sludge may have been retained within the system. Normal sludge wastage removes fixed volumes of sludge but this is not actively selecting the removal of older biomass.

Another key benefit of the Kaldnes® system is better mass transfer conditions offered by the higher surface area. Manser *et al* (2005) compared the mass transfer kinetics of a biofilm and an activated sludge. Floc size is an important consideration that if too large can result in oxygen limitation to the ammonia oxidizers. Floc size in a biofilm is about 10 times smaller than in activated sludge. Higher oxygen saturation coefficients are noted for activated sludge flocs compared to biofilms. This is attributed to mass transfer limitations in the larger flocs of an activated sludge system. Higher sludge ages also lead to larger floc sizes whereas on a biofilm the sludge ages tend to be younger and fall off the biofilm when they get too old.

The addition of a support be it activated carbon or a BPM such as Kaldnes® resulted in better nitrification either directly through the creation of a fixed film resulting in better sludge ages, improved substrate mass transfer and better solids retention within the system. An indirect benefit was the increased carbonaceous removal activity of the heterotrophic population thereby reducing the inhibitory nature of the influent COD. Although nitrification in all pilot plant studies was successfully inhibited by increasing the COD loading it was observed that the point at which nitrification failed varied despite using the same waste water and therefore the same inhibitory components. In some studies this failure was over 6,000mg/l but in others such as the Kaldnes® study it occurred as low as 3,500mg/l. Consequently the original assumption that nitrifier failure could be predicted by the influent COD concentration was questionable. This suggested that the COD is not the only factor when trying to predict a failure point; the importance of the biomass population, in particular the heterotrophs also have a direct bearing on nitrification. This influence may be greater than previously thought.

The food to mass (F/M) is a measure of the ratio of carbonaceous waste measured as BOD or COD to the total biomass in the system. The efficiency of carbonaceous removal process is a function of the F/M; to ensure low effluent COD's a low ratio is desired (Gray,1990). The mean F/M, measured as COD, at the full scale WWTP was 0.20 but did occasionally exceed 0.4 with a maximum of 0.68. This is somewhat high for an extended aeration plant where the typical F/M would be 0.1 to 0.4 for >90% COD removal efficiency. This falls to 85% at an F/M of 1 and in turn to <80% at an F/M of >2.0 (Hammer,1977). Although a correlation between the F/M and nitrification of 46% was recorded at the full scale WWTP this was significantly lower than the same relationship to the influent COD.

The study at which two pilot plants were operated at two different MLSS levels but at the same concentration of influent COD was an important finding. As the COD loading to both systems was the same the concentration of inhibitory chemicals was also the same. However as the MLSS levels were different the F/M's ratios were also therefore different. In the pilot plant study on a low influent COD loading of 2,000mg/l nitrification proceeded equally on a high MLSS (8,000mg/l) and on a low MLSS (4,000mg/l) level. The resulting F/M's were 0.03 and 0.06 respectively. However on a high influent COD loading of 6,000mg/l, the nitrification rate fell to 31% in the pilot plant system with the lower MLSS levels, with an equivalent F/M of 0.34, whereas nitrification fell to only 68% in the pilot plant with the higher MLSS and an equivalent F/M of 0.2. This was an important finding as it confirmed that the biomass population had a direct influence in reducing the inhibition on nitrification even though both pilot plants received the same COD and more importantly the same concentration of inhibitory agents.

Liao *et al* (2006) reported that the variation in F/M ratio had no significance influence on the floc size or distribution of an activated sludge in an SBR. This was more affected by the sludge age; at a lower sludge age (4-9 days) flocs were more irregular and variable in size than those at higher sludge age (16-20days). As the sludge age on both systems was the same then this did not explain the difference in nitrification performance. There was however a higher MLSS concentration and therefore in turn an overall higher surface area available for attachment by the nitrifiers. A second explanation for the better nitrification in the system with the higher MLSS was following an increase in COD levels there was in turn a rise in available food that promoted heterotrophic growth activity. The pilot plant with the higher MLSS may have

had a higher baseline population of heterotrophs and was therefore able to react faster to the rise in COD.

It has been reported that a higher heterotrophic population can result in adverse competitive factors for the slower growing nitrifiers that are unable to compete for nutrients. For example Kim *et al* (2006c) found that nitrification inhibition increased with increasing MLSS due to competition with heterotrophs for available nutrients and in particular oxygen. Gilmore *et al* (1999) and Li *et al* (2002) reported that an increase in organics in the influent can lead to nitrifier displacement due to competition. Hanaki *et al* (1990) reported in a pilot plant reactor fed with a synthetic waste water that an increase in the COD promoted heterotrophic growth to the detriment of ammonia oxidation. This was attributed to the fact that transportation of ammonia from the waste water to the cell of the ammonia oxidiser is hindered by the crowded cells of heterotrophs. As a result diffusion resistance and consumption by heterotrophs true concentrations of ammonia and oxygen around nitrifier cells becomes lower than the concentration in the water. Esfandi and Kincannon (1981) also demonstrated nitrification inhibition following a two fold increase in COD load in an activated sludge pilot plant. This suggested that the heterotrophic population produced some metabolic by-product that was inhibitory to nitrifiers.

The pilot study on this waste water discounted these findings. Firstly, on a low COD loading it was demonstrated that nitrification rates were unaffected either favourably or negatively in association with a high or low MLSS levels. Nitrification rates of 4.3-5.5mg N/g MLSS/hr were recorded and these compared favourably with other studies; for example 1.5 to 5mg N/g of MLSS/hr (Ficra *et al.*,2001), 1.0-2.8 mg N/g MLSS/hr (Lin *et al.*,2001) and 1.4 mg N/g of MLSS/Hr (Kim *et al.*, 2005). Secondly, although both pilot systems were initially disrupted by the increase in COD both fully recovered even though the influent COD concentration was still at 6,000mg/l. Considering this and the fact that the system with the higher population of heterotrophs performed better than the system with a lower population of heterotrophs this ruled out competition with the heterotrophs for available nutrients. Competition for the available ammonia was also not a factor as there was an excess available as evident by ammonia breakthrough to the final effluent. This finding also agrees with Sharma *et al* (2004) who reported the presence of heterotrophs had no effect on the performance of nitrifiers.

The recovery in nitrification at the same COD concentration that initially resulted in inhibition is again an important observation. This was also observed in the pH/COD study, the two stage study and in the Kaldnes® study. This recovery was either due to the response of the heterotrophs or adaptation by the nitrifiers.

Using fill and draw activated sludge reactors, early work by Tomlinson (1966) showed that nitrification could become adapted to an inhibitor. It was shown that the inhibitory effects from thiourea were significantly different for an unacclimaitised nitrifying sludge compared to a sludge that had been exposed to this substance for some time. Two kinds of mechanism were proposed; one was that some of the microbes (heterotrophs) in the sludge acquired the ability to biodegrade inhibitors due to contact over a long period, the other was that nitrifying bacteria acquired the ability to regenerate new proteins within inactivated cells to recover their activity. This means that the recovery of nitrification activity can proceed even when nitrifying bacteria are inhibited. Consequently an acclimatised mixed culture may more readily gain tolerance to an inhibitory waste water. Xiong *et al* (1998) demonstrated both of these adaption mechanisms for thiourea and aniline in an activated sludge pilot plant receiving a synthetic waste water. Aniline is quick to biodegrade hence the adaption period for nitrification to recover was much quicker than for thiourea. Thiourea on the other hand is difficult to biodegrade therefore the observed nitrifier recovery which was much longer than for aniline was to be due nitrifiers developing a tolerance for this substance.

The adaption and acclimatisation of nitrifiers is evident by the fact that industrial sludges are generally more resistant to toxins than either domestic or synthetic sludges. This can be explained by the fact that industrial sludge's exposed to a greater range of inhibitors develops some resistance (Jonsson *et al.*, 2000 & 2001). The importance of the heterotrophic population in degrading an inhibitor and in turn protecting nitrification was demonstrated by Texier *et al* (2007) who reported that up to 150mg/l of P-Cresol could be degraded without significant inhibition on nitrification. The faster the heterotrophic sludge oxidised the cresol into its intermediates the faster the nitrifying bacteria recovered its activity. As the heterotrophic sludge became more adaptive to the cresols, as evident by an increase in the removal rate, the rate of nitrification also increased.

In this study plan the recovery of the systems may have been due to nitrifier adaptation or more likely it was as a result of an increase in the viability of the MLSS following an increase in available food. As the F/M ratio increases so also does the rate of metabolic activity (Viessman,2004). Although F/M is a better indicator of nitrification inhibition than just COD it still has limitations. Ford and Churchwell (1981) reported that MLSS had no real meaning when evaluating a nitrifying system as it doesn't indicate the percentage that are nitrifiers and the percentage that are heterotrophs. Blok (2001) reported that the percentage of a viable biomass in a floc is related to the food to mass (F/M) ratio. At a F/M of >1 the viability was nearly 70%, at a F/M of 0.5 this fell to 55% and was around 40% at 0.25 F/M. It then fell sharply therefore for an F/M of 0.10 the floc was less than 15% viable and at an F/M of 0.05 was less than 10% viable. Rittman *et al* (1999) also reported a similar relationship although lists higher viabilities at the same F/M. At an F/M of 1.0 the viability of the floc was 100%, it was 80% at an F/M of 0.5 and was over 60% at an F/M of 0.25. This also fell to just under 20% at an F/M of 0.05. Moussa *et al* (2005) found that the fraction of active biomass (nitrifiers and heterotrophs) in an SBR is also influenced by the sludge age. This is 33% at a sludge age of 30 days but this falls to 14% at a sludge age of 100 days. As the sludge age to both pilot plants was the same and as this was kept constant this can be discounted as a relevant factor.

The importance of the viability of the MLSS or more correctly the percentage of viable heterotrophs was demonstrated by operating the two pilot plants this time on the same MLSS levels but with one plant on a low sustained F/M (0.08-0.09) compared to the system where the influent COD and therefore the F/M was gradually increased at a rate of $0.02d^{-1}$ over 2 weeks. As with the MLSS study a similar level of disruption was noted in the system on a low sustained F/M with the percentage nitrification falling to 21% at an influent COD of 6,000mg/l. However 100% nitrification was maintained in the system where the F/M was gradually increased. The gradual increase in COD increased the F/M and in turn increased the viability of the MLSS thereby creating a more responsive heterotrophic population as evident by >20% higher COD removal rates per mg of biomass. The results of this investigation suggested that an activated sludge that was acclimatised to a gradual increase in the influent COD, and in turn the F/M was gradually increased, was more resistant to a shock COD load resulting in better nitrification than an activated sludge that had to react to a large step change in the COD or the F/M.

It is also not practical to sustain a high viable F/M on a long term basis as this can result in a poor quality effluent with higher COD's and suspended solids (Tchobanoglous *et al.*,1991). A means to rapidly increase the viable heterotrophic population is required. The addition of specialised bacteria is known as bioaugmentation, bacterial augmentation, biomass enhancement, inoculum addition or seeding. The purpose of seeding is to improve the nitrogen removal capability of the system than would otherwise exist (Plaza *et al.*, 2001). Following bioaugumentation Kim *et al* (2005) reported that the ammonia oxidation rate was increased from 0.0007mg N/mg MLSS/day to 0.0918 N/mg MLSS/day i.e. a 131 fold increase in a domestic waste water plant.

One of the benefits of nitrifier bioaugumentation is to reduce the start up time. This can be significantly reduced from 7.5 months to 2 months by seeding compared to a system without inoculation (Sinkjaer *et al.*, 1996). Neethling *et al* (1998) and Kos *et al* (2001) both reported that nitrification can be maintained at much lower sludge ages when seeding is used to supplement the nitrifier population. Salem (2003 and 2006) studied the potential of augmenting the endogenous nitrifying population by installing a nitrification reactor on the sludge return line. This focused on the benefit of bio-augmentation with plants that are operating below the minimum sludge age needed for nitrification. Bioaugmentation was beneficial once the sludge age fell below 50% of the minimal critical sludge age needed for nitrification. Head and Oleszkiewicz (2004) reported that there can also be limitations to bioaugumentation when the biomass is grown in one environment and transferred to another. If a substance that is inhibiting nitrification in the first instance is not removed then bioaugumentation is of little benefit (Kroiss *et al.*,1992). Smith (2004) however found that an activated sludge plant bioaugumented with nitrifiers resulted in more stable nitrification and it was less susceptible to inhibition.

There is no reported investigation of any practice where bioaugumentaion of the heterotrophic population with a heterotroph for the purpose of protecting nitrification against inhibition exists in practice. Although Wang *et al* (2006) reported that the general performance of waste water treatment can be improved by seeding. It was found in a paper mill waste water that heterotrophic seeding not only shortened the sludge acclimation time to a new waste water but it also improved the treatment efficiency of the system leading to higher COD removal rates and lower suspended solids. The bioaugumented system was also more tolerant to shock COD loads.

Pseudomonas pudia CP1 was used to bioaugument the pilot plant system. Earlier studies had shown that this heterotrophic bacterium was useful for bioaugumentaion of activated sludge due to its ability to auto-aggregate and become integrated in the sludge floc (Mc Laughlin *et al*, 2006). Upon applying an influent COD of 6,000mg/l the addition of *Pseudomonas pudia* CP1 to the test system initially had a positive effect where after 6 days the percentage nitrification in the non-augmented was 76% but was over 95% in the bioaugumented system. Lower COD levels were also evident at this stage. It was also noteworthy that the bioaugumented system by design had a slightly higher F/M meaning this system was more challenged. The improved performance was not sustained and by the end of the study both systems had failed to 30% nitrification. It appeared that the organism did not survive within the system or was washed out through sludge wasting. The failure seemed to coincide after one HRT i.e. after 4 days. Further studies using this organism are required to assess if this is a viable option.

It was concluded that the F/M ratio was the best indicator for predicting nitrification failure for this pharmaceutical waste water. The role of the heterotrophs is important in the application of a successful nitrification management plan. Analysing the F/M's data from all of the controls studies then a significant failure in nitrification was predicted to begin at an F/M of >0.25 with a 95% confidence interval between 0.22 to 0.28.

There are limited reports citing the critical F/M for nitrification. Munirathinam and Lankford (1998) found that a pilot plant treating pharmaceutical waste water with an influent ammonia concentration of 600mg/l had over 99% nitrification on an average F/M of 0.09; once the F/M exceeds 0.2 there is a significant reduction in ammonia removal in a single aeration stage. Ford and Churchwell (1981) and Donahue (1984) also list a F/M of 0.15 with a maximum of 0.25 as being optimum for nitrification.

Analysing the various studies that enhanced nitrification, then less than 20% nitrification failure can be maintained at an F/M up as high as 0.52 with a 95% confidence interval between 0.46 to 0.49. This difference is substantial as it means that effective COD removal can be maintained without significant nitrification failure at an F/M twice that of the current arrangement at the full scale WWTP. Maintaining a high MLSS viability followed by the addition of Kaldnes® media appeared to offer the best options. On the basis of the findings of this study plan a nitrification management strategy was developed and applied to the full scale WWTP. A fundamental change was

to move away from using the recommended tolerances for carbonaceous removal to using those relevant for nitrification. The nitrification process was now considered as the key limiting factor in the waste water treatment process and plants operation needed to centre on this with COD removal as a secondary process.

In response some key changes were implemented at the full scale WWTP, these included ; the development of nitrifier quality control charts with high and low tolerances for pH, free ammonia, sludge age and F/M. Greater control of pH was implemented in order to respond to free ammonia and rising influent CODs. An ammonia dosing regime was adopted to target a C/N ratio of <50 at all times. The operational MLSS levels were increased from 3,000-4,000mg/l on average to over 6,000mg/l with a target F/M of less than 0.25.

Given the large hydraulic retention capacity in the strong waste tank the company had some flexibility in reducing or increasing the flow and in turn COD loading to the WWTP. Therefore, depending on production schedules, if there was a predicted rise in loading the influent COD was gradually increased to increase the F/M viability. As a reactive measure a supply of activated carbon was retained on stock. The use of Kaldnes® was not pursued due to capital costs however this remains an attractive option in the event of the need to increase the F/M capability of the system.

The results of these changes was the full scale WWTP plant achieved 100% nitrification in 2006 with a maximum effluent ammonia level of 1.2mg/l NH_4-N recorded on only one occasion. More importantly there were no licence breaches against the 10mg/l NH_4-N limit. This represented a major improvement in the performance of the full scale WWTP and demonstrated that nitrification could be successfully implemented and maintained on this pharmaceutical waste water.

Chapter 5

Conclusions

5.0 CONCLUSIONS

- Data analysis of the main wastewater treatment plant showed that temperature, dissolved oxygen, sludge age and phosphorus were not direct causes of nitrification failure in the activated sludge system.

- The wastewater had adequate levels of nutrients; however removal of the inorganic fraction from the wastewater had an adverse effect on nitrification when exposed to a shock COD loading.

- An influent ammonia concentration of at least 50mg/l NH_4-N or a C/N ratio of less than 40 sustained the nitrifier population.

- A 50% failure in nitrification occurred at a free ammonia level greater than 1.5mg/l FA.

- At an influent COD of 3,800mg/l nitrification disruption started to occur. Above 6,000mg/l to 8,000mg/l complete nitrification failure occurred. Nitrification inhibition to COD was related to the pH; on low COD loading nitrification performance was similar at pH of 7.5 and pH of 8.5, however at a high COD loading nitrification was more inhibited at pH 7.5 than at pH 8.5. On a number of occasions nitrification fully recovered following a shock COD load.

- Reducing the sludge recycle rate and the placement of an Eckenfelder reactor before the pilot plant system was of no benefit to nitrification. However, the addition of activated carbon and a buoyant plastic media improved nitrification when exposed to inhibitory levels of COD and improved heterotrophic activity was evident by higher removal rates of COD and by higher oxygen uptake rates.

- On a low COD loading the MLSS levels had no influence on nitrification. On a high COD loading operating at an MLSS of 8,000mg/l resulted in better nitrification than operating at an MLSS of 4,000mg/l. Acclimatising an activated sludge through a gradual increase in the F/M resulted in better nitrification, when exposed to a shock COD load, than a system on a low sustained F/M. The addition of a heterotroph,

Pseudomonas pudia CP1, under shock COD loading, enhanced carbonaceous removal and in turn protected nitrification in the short term.

- A 20% fall in nitrification was likely to lead to a licence excursion at an F/M of 0.25 for an unmodified system. By modifying the system this was increased to an F/M of 0.52. The acclimatised biomass was the most effective at minimising nitrification failure, followed by the addition of a buoyant plastic media and in turn operating at a higher biomass level. The least effective modification that showed a benefit to nitrification was the addition of activated carbon.

Chapter 6

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6. Bibliography

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