Nitrification Inhibition Assessment of Industrial Effluents and Influent to Amanzimtoti Wastewater Treatment Plant

By

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Abstract

The aim of process industries is to produce products and intermediates from raw materials and other intermediates. Inevitably, there are waste products to be disposed of and if these are of no use, they must be returned to the air, water or land environments. Such returns should be carried out in such a way as to minimise any adverse effects on the environment, otherwise the waste is bound to cause pollution to the environment. Wastewater is one such product that has to be returned to the environment. A weakness in the current practice of wastewater treatment is that the potential toxicity of the effluent is only addressed through the prevention of specific types of waste being discharged to the sewer. The discharge of effluents containing toxic or inhibitory substances is currently not directly addressed or controlled by many industries and local authorities. While cost recovery is important, due consideration must be given to the possible effect on the receiving environment. The magnitude of the problem of toxic components in the inflow to wastewater treatment plants in South Africa is largely unknown. However, it is thought by some treatment authorities to be relatively serious. In addition, there has been no attempt to quantify the effect of individual toxicants on the performance of the treatment processes and thus put a monetary value to individual discharges. Nitrification is one of the important biological processes that takes place in wastewater treatment plants, which may be affected by toxicants from wastewater. The toxicants may inhibit the nitrification process and create problems in the treatment plant.

The aim of this study was to determine if the Amanzimtoti Wastewater Treatment Plant is experiencing inhibition of nitrification, and if so, determine whether large industries discharging into the plant contribute to this problem. The study site used in this research was the Amanzimtoti Wastewater Treatment Plant, located at Isipingo, in Durban, together with some selected industries that discharge their effluents into this treatment plant. In this study, the Amanzimtoti Wastewater Treatment Plant together with 10 industries that discharge effluent into it, were surveyed for inhibition of nitrification. A screening method for estimation of inhibition of nitrification at municipal wastewater treatment plants described by Jönsson (2001) was used in the investigations. This involved testing inhibition of nitrification at various dilutions of wastewater effluent from 20% to 80% dilution.

An investigation was conducted of inhibitory substances within influent wastewaters to the Amanzimtoti Wastewater Treatment Plant, and inhibitory substances were detected in all four sampling weeks. The level of inhibition was in general up to 29%, with the greatest inhibition being observed at 20% and the least at 80% dilution.

In order to investigate the source of inhibition, inhibition of nitrification was measured in the sewage influent during times when industries are open and when they are closed. Inhibition was significantly lower during December when industries close, supporting the hypothesis that industrial effluent contributes to inhibition of nitrification.

Comparison of wastewater from different industries showed that of 10 surveyed industries, 9 generated wastewaters that were found to be inhibitory, with Industry D showing the highest inhibition of approximately 30% over the 4 dilutions. The least inhibitory effluent was from Industry C with an average of 10%. Industry A was found to stimulate nitrification. There was no correlation found between the daily volume contribution of the industries to the treatment plant, and the inhibition of nitrification. There was also no correlation found between the inhibition of nitrification and the chemical oxygen demand and settleable solids concentration of wastewater from each of the industries. . At 80% dilution, the nitrification inhibition results obtained for all nine industries were similar and it was difficult to distinguish between them, whereas at 20% dilution, the differences among the industrial effluents on nitrification could be clearly evaluated. Industries B, D, E, G and J were found to have higher inhibition than the other four surveyed industries. Results obtained at the 20% dilution could therefore be used as a decision making tool by wastewater pollution officers to identify industries requiring close monitoring.

From the study, it was clear that the inhibition of nitrification that resulted from mixtures of industrial wastewaters cannot be readily predicted from nitrification inhibition by the individual wastewaters. New compounds may be formed during mixing in the sewer network that are more or less inhibitory than if the wastewaters are not mixed.

Preface

The experimental work described in this dissertation was carried out in the School of Life and Environmental Sciences, University of KwaZulu Natal, Durban, from November 2003 to January 2004.

Declaration

I declare that the work submitted in this dissertation is all my own, except where specifically indicated in the text, that all sources have been acknowledged and referenced in the text, and that this work has not previously been submitted, either in the whole or any part to any other university for degree purposes.

Signed: T O'Reilly **Date:** 22/03/05

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Glossary

Activated sludge: Accumulated microbial biomass that effects to the treatment of wastewater in the presence of dissolved molecular oxygen (O_2).

Activated sludge suspension: A mixture of activated sludge, substrates and tap water.

Adaptation: A change in the microbial community that increases the rate of transformation of a test compound as a result of prior exposure to that test compound.

Allosteric: The conformational changes occurring in some proteins, especially enzymes, in which a compound combines with a site on the protein other than the active site.

Anoxic: An environment where molecular oxygen (O_2) is absent.

Autotrophs: Organisms able to utilize CO_2 to produce carbohydrate in the presence of water and energy source such as light.

Biofilm: A thin layer of living cells, usually micro-organisms, coating a surface.

Chemical Oxygen Demand (COD): A measure of the total amount of organic material in the waste stream.

Denitrification: Conversion of nitrate into nitrogen gases under anaerobic conditions, resulting in loss of nitrogen from ecosystems.

Dissimilatory process: Nitrate rather than oxygen is used to oxidise organic matter

Effluent: A stream flowing from a sewage tank or industrial process.

Enzyme: A protein functioning as a metabolic catalyst in living organisms, each of which promotes a specific reaction or groups of reactions.

Eutrophication: A process of nutrient enrichment in bodies of water. It may occur naturally with the gradual input of nutrients, but typically occurs through anthropogenic inputs

Feedback inhibition: Inhibition by an end product of the biosynthetic pathway involved in its synthesis.

Heterotrophs: Organisms obtaining energy and carbon from organic compounds.

Inhibition: An impairment of bacterial function.

Inhibition of nitrification:	Some link in conversions from ammonium nitrogen to nitrite or nitrate nitrogen doe not work properly.
Inhibition pattern:	A general trend of inhibition of microbial breakdown of waste products
Kinetics:	Explanation of the observed characteristics of chemical reactions.
Lithotroph:	An organism that can obtain its energy from oxidation of inorganic compounds
Metabolism:	The physiochemical transformations through which foodstuffs are synthesised into complex substances or where complex substances are broken down into simple ones and energy is made available for use by the organism.
Methanogenenis:	The production of CH_4 and CO_2 by biological processes that are carried out by a group of organisms known as methanogens.
Nitrification:	The conversion of ammonia to nitrate.
Nitrification rate:	Biological oxidation of ammonium per unit of time expressed as mg N/ (g VSS.h), mg N / (g SS.h) or mg (N / (1.h) where N is NH_4-N or NO_x-N.
Nitrobacter:	Genus name for a group of organisms that oxidises nitrite to nitrate.
Nitrosomonas	Genus name for a group of organisms that oxidises ammonia to nitrite.
Oxidation:	A process by which a compound gives up electrons, acting as an electron donor.
Oxidised nitrogen:	A soluble form of nitrogen ($NO_2 + NO_3 = NO_x$)
Pollution:	The introduction of foreign substances into the environment.
Retention time:	Average period of time that incoming sludge is retained in the digester for completion of biological reactions.
Sludge:	The general term applied to the accumulated solids separated from wastewater.
Sludge bulking:	Long strands of bacteria called filamentous bacteria can grow too large an form mats that prevent the flocs from settling
Substrate	The molecule undergoing reaction with an enzyme.
Total suspended solids:	The concentration of particulate material present in water, expressed as grams of dry matter per litre, which is retained at a

	filter of specified pore size when a known volume of suspension is filtered.
Toxic:	An adverse effect (not a substance that has necessarily lethal) on biochemical metabolism.
Toxicity:	An adverse effect (not necessarily lethal) on bacterial metabolism.
Toxins:	Any substance that can adversely affect metabolism.
Trade effluent:	Any effluent (liquid waste) that is discharged from any premises being used for carrying on a trade or industry.
Volatile suspended solids:	Volatile solids are those solids lost on ignition (heating to 550 degrees C.). The total concentration of volatile suspended solids is the organic fraction of suspended solids.
Wastewater:	Liquid effluent discharged to a sewage treatment facility.

Abbreviations

BOD:	Biochemical oxygen demand
DO:	Dissolved oxygen
DWAF:	Department of Water Affairs and Forestry
EPA:	Environmental Protection Agency
ISO:	International Organisation for Standardisation
IWA:	International Water Association
KL/day:	Kilolitre per day
Ks:	Saturation values
NO_x:	Oxidised nitrogen (NO_3 and NO_2)
SS:	Settleable solids
VSS:	Volatile suspended solids
WWTP:	Wastewater Treatment Plant

Chapter 1

Introduction

1.1 Background

The aim of processes industries is to produce products and intermediates from raw materials and other intermediates. Inevitably, there are waste products to be disposed of and if these are of no use, they must be returned to the air, water and/or land environment. Returns should be carried out in such a way as to minimise any adverse effects on the environment, otherwise the waste will cause pollution to the environment.

Public awareness and concern of the impact that industry and business in general are having on the environment has increased. The entrenchment of an environmental constitutional right and the reintroduction of South Africa to the global community have resulted in a public ethos which now increasingly perceives pollution to be a crime (Durban Metro, 1999). This has not been the traditional public position, where, save for interest in nature reserves and wilderness areas, the general public has had little concern for the threat to the environment of pollution and had little knowledge as to what constitutes an environmental offence. The growing awareness of pollution has resulted in the courts treating pollution crimes more seriously.

Biological wastewater treatment systems are a critical component of pollution control and abatement worldwide. These systems must be designed and operated properly in order to effectively prevent contamination of natural surface waters and to avoid downstream problems in water reuse. Due to increasing concern over the issue of water resources protection, more rigorous legal restrictions on nutrient concentrations in final effluents that are discharged to the environment have been implemented in recent years, making instruments for nitrification control a fundamental tool for wastewater treatment plant (WWTP) operation.

In many industrialized countries, allowable concentrations of ammonium-nitrogen in effluents from municipal and industrial WWTPs are being reduced and strictly regulated. In most cases, biological processes are considered the only economically feasible technology for nitrogen removal (Barnes and Bliss, 1983).

In most developing countries, sewage treatment plants have been designed to treat domestic sewage. However, due to financial restrictions, sewage treatment plants are often used for treatment of both domestic sewage and industrial effluent. The introduction of industrial effluent into a sewage treatment plant can interfere with the efficient functioning of the plant because in most cases, the biological process that are applied to domestic sewage primarily remove chemical oxygen demand and cannot treat high strength or toxic organic effluent. Therefore, the rate of growth of microorganisms in the biological processes of wastewater treatment decreases.

Trade effluent regulations and tariffs have evolved to protect the sewers, the treatment processes and the receiving environment, and to recover the capital and treatment costs from the polluter. In order that the sewer system can be protected and that the treatment plants can function biologically, effluent by-laws are compiled by the Water Services Authority stipulating the hydraulic and concentration limits of different pollutants and parameters of significance that can be discharged into the sewer.

1.2 Wastewater treatment in South Africa

A weakness in the current practice of wastewater treatment in South Africa is that the potential toxicity of the effluent is only addressed through the prevention of specific types of waste being discharged to sewer. The discharge of effluents containing toxic substances or those that inhibit the efficient treatment of wastewater is not directly addressed or controlled. While cost recovery is important, more consideration should be given to the possible effect on the receiving environment in the treatment plant or the environment to which the wastewater is ultimately discharged. The magnitude of the problem of toxic components in the inflow to wastewater treatment plants in South Africa is largely unknown. However, it is thought by some treatment authorities to be relatively serious as it inhibits efficient wastewater treatment. Furthermore, there has been no attempt to quantify the effect of individual toxicants on the performance of the treatment processes and thus put a monetary value to the individual discharges. Thus, the cost of treatment is equally borne by society at large, and there is little incentive to control biologically harmful effluent at source.

A method of ensuring that the polluter pays is to apply a tariff that relates to the toxicity of the effluent to encourage reduction of toxicants and inhibitors at source Preventing the discharge of toxic components into sewers enables sewage treatment plants to operate to their full potential, leading to either enhanced effluent quality and/or the delay of new capital expansion. It should also lead to an improved environment, a reduction of the operating costs and the delay of capital works for wastewater treatment plant authorities, and a general reduction in wastewater treatment tariffs.

Alternatively, the effluent from each factory into the sewer should be measured in terms of toxicants that might be harmful to the nitrification or any other biological process in the treatment plant and a tariff should be charged for additional costs to the treatment plant for ensuring adequate wastewater treatment. The measurement of the presence and the concentrations of toxicants present in the inflow to a sewage works thus leads to a more equitable recovery of treatment costs and guides policy and decision makers regarding pollution control.

This project is part of a larger study in which a method for determining toxicity of the inflow to the wastewater treatment plants will be established and used as a basis for charging industries for the full cost of effluent treatment.

The study site used in this research is the Amanzimtoti Wastewater Treatment Plant, located at Isipingo, in Durban, together with some selected industries that discharge their effluents into this treatment works. Most of the industries are located at the Prospecton Industrial Area, which is within the South Durban Metropolitan Area (Figure 1). Amanzimtoti Wastewater Treatment Plant receives an average of 19 ML/day of wastewater from different industries. Most of the wastewater received is likely to contain nitrogen that has to be removed from the wastewater through biological nitrification; otherwise, it would be released directly into the environment untreated. This would mean that if biological nitrification is inhibited by industrial effluents, the treatment plant is unable to accomplish one of its primary tasks, which is to remove nitrogen.

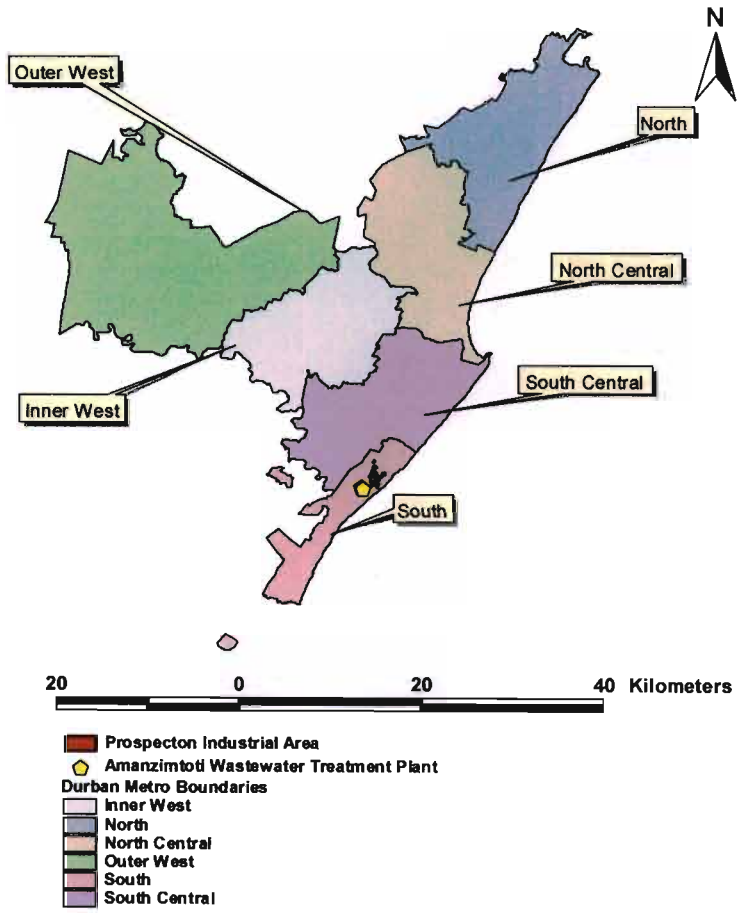

Figure 1 The Durban Metropolitan Area

1.3 Aims and objectives

The aim of this study was to determine if the Amanzimtoti Wastewater Treatment Plant is experiencing inhibition of nitrification, and if so, determine whether large industries discharging into the plant contribute to this problem. To achieve the aim, the following objectives were set:

- To determine the general temporal weekly pattern of nitrification inhibition at the Amanzimtoti Wastewater Treatment Plant.
- To establish the general nature of the effluent that industries discharge to sewer.
- To determine nitrification inhibition of effluent from individual industries
- To determine the effect of combining individual effluents on nitrification inhibition.
- To determine whether a link exists between industrial activity and nitrification inhibition at the associated treatment facility.

1.4 Limitations

The purpose of the study was to look at the toxicity from the perspective of a wastewater treatment plant, not to judge the effect of toxicity on the environment in general. This means that the investigation was based on the degree of toxicity to nitrifying bacteria in activated sludge. Furthermore, the study was restricted to inhibition by toxic compounds that might be present in wastewater. Other types of inhibition were not considered.

Chapter 2

Literature review

2.1 Nitrification and denitrification processes

Most of the discussion in the literature review, is devoted to understanding biological nitrification and denitrification, and to identifying factors known to affect these processes and which therefore may result in inhibition of nitrification. Nitrification is achieved in an aerobic environment in aerated tanks, while denitrification is achieved in anaerobic environment in anaerobic tanks (Figure 2.1). These two stages may be separated temporally in a single reactor in which case oxygen may be supplied and then withdrawn. Alternatively, two reactors are used, with the first being aerated. Once nitrification is complete, the sludge is transferred to an anaerobic digestor.

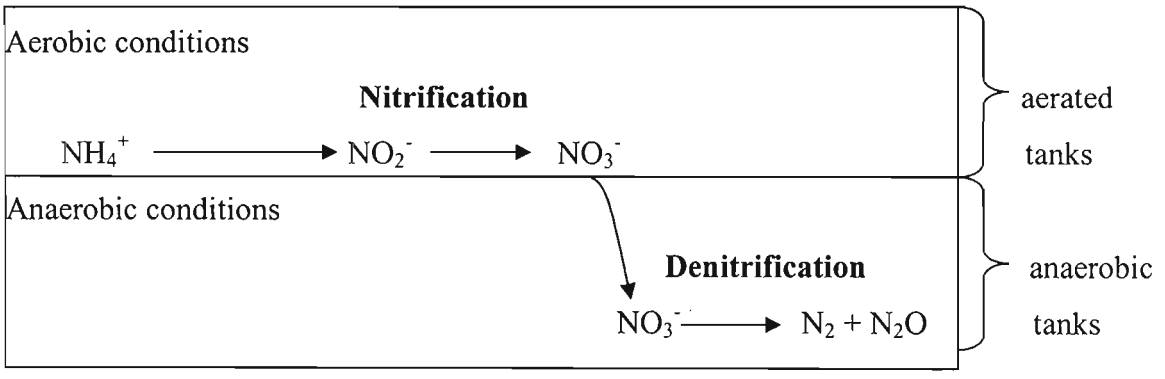

Figure 2.1 Nitrification and denitrification processes

2.1.1 Biological nitrification and denitrification

Nitrogen exists in many forms because of the high number of oxidation states it can take. The state for nitrogen is –III in its most reduced form, such as in ammonia and organic nitrogen compounds (those closely associated with plants, animals and domestic wastewaters). At the other extreme is the most oxidated state of nitrogen, such as, nitrate where the oxidation is +V (Brock and Madigan, 1991). Nitrogen in untreated wastewater is present in the form of ammonia and organic nitrogen, both in soluble and particulate forms, with most particulate organic nitrogen being transformed to ammonium and other organic forms during biological treatment. The major forms of nitrogen in nature are proteins and amino acids, which yield

ammonia upon biodegradation. Nitrate and nitrite typically exist in untreated wastewaters (Metcalf and Eddy, 1991a). Additionally, especially in industrial wastewaters, there may be any of a variety of organic nitrogen-containing compounds that are often not produced biologically, but which can be inhibitory to either heterotrophic organisms or cells involved in biological wastewater treatment processes. In the wastewaters, some of the ammonia-nitrogen may be incorporated into the microbial cell mass. The majority of organic- and ammonia-nitrogen is oxidised by dissimilatory processes, ultimately to nitrate (NO_3^-). It is this energy-producing, dissimilatory process that is referred to as biological nitrification. Nitrification involves several biochemical reactions, which may be summarised by equation 1 and 2 below;

$$NH_4^+ \longrightarrow NH_2OH \longrightarrow NO_2^- \qquad \text{(eq. 1)}$$

$$NO_2^- \longrightarrow NO_3^- \qquad \text{(eq. 2)}$$

Similarly, some nitrate-nitrogen may be reduced to ammonia-nitrogen and assimilated into cell mass, but the bulk of the nitrate-nitrogen is removed from wastewater via dissimilatory metabolism, ultimately producing nitrogen gas (N_2). It is this dissimilatory process which is referred to as biological denitrification (Figure 2.1).

2.1.2 The importance of nitrification

It is well known that the presence of nitrogen compounds in wastewater is generally considered a serious danger for the environment and for human health. These compounds may cause the stimulation of excessive algal growth, fish toxicity, and dissolved oxygen depletion. In order to protect the environment and human health, a greater number of stricter discharge limits are being introduced. Thus, wastewater treatment plants should increasingly deal with the often-critical issue of achieving a stable nitrification process.

Nitrification exerts an oxygen demand, thus, it is important to minimise or eliminate the amount of ammonia being discharged to the environment (Barnes and Bliss, 1983). However, it is not only ammonia-nitrogen, that can lead to eutrophication in receiving bodies of water. Because other forms of nitrogen, such as nitrate, are plant nutrients, high loads of nitrogen in nearly any form are likely to lead to algal blooms or the extensive growth of higher aquatic plants, creating a heavy oxygen demand and possibly odour problems (Barnes and Bliss, 1983; Metcalf and Eddy, 1991b). Thus, it is apparent that not only is the nitrification of

wastewater desired, but the denitrification is also necessary in order to minimise the likelihood of eutrophication.

2.1.3 The importance of denitrification

The main reason to nitrify a wastewater without denitrification is to remove ammonia-nitrogen. Such a goal is desired to minimise ammonia toxicity to aquatic organisms (Metcalf and Eddy, 1991b). It has been shown that ammonia (NH_3) is toxic at concentrations as low as 0.5mg/L, especially to higher aquatic organisms such as fish (Barnes and Bliss, 1983). Other studies based on combined nitrification / denitrification have also shown that the yield from nitrate respiration of denitrification sludges is lower than conventional aerobic carbonaceous oxidation, resulting in less sludge production and handling costs than for similar carbonaceous discharges, but with the added bonus of far superior effluent quality in terms of nitrogen concentrations (Barnes and Bliss, 1983).

There are other reasons why nitrification or combined nitrification /denitrification of a wastewater is desired. If the receiving water is to be used as a drinking water supply, high nitrogen concentrations create a greater chlorine demand, and high nitrates can make the source a drinking hazard, causing methemoglobinemia, which in infants is known as blue baby syndrome (Barnes and Bliss, 1983). Nitrification in receiving water can also result in corrosion of cement structures and natural stones.

Aside from preventing negative effects, the biological nitrification/ denitrification process can provide benefits over a process which does not nitrify at all, or which nitrifies without denitrifying. For instance, denitrification reduces aeration costs because organic compounds are oxidised with nitrates as the terminal electron acceptor, requiring less oxygen to remove any remaining chemical oxygen demand. Biological denitrification occurring in a basin results in better settling sludge. A denitrifying sludge tends to be less susceptible to bulking. Finally, biological denitrification results in the production of alkalinity, helping maintain a stable environment for a nitrifying sludge while saving on the cost of chemicals required for pH control (Gray, 1990).

To summarise, the three major reasons that at least nitrification, and ideally, denitrification of effluents is done, are:

- the prevention of eutrophication of receiving bodies of water,

- reduction of sludge production; especially in the case of nitrifying/denitrifying sludges,
- the reduction in handling costs.

2.1.4 Organisms responsible for nitrification and denitrification

Determination of which organisms are present or responsible for treatment was not conducted in this study, but it is important to have a basic understanding of which organisms are known to carry out nitrification and denitrification, how it is possible for them to achieve it, and what conditions are suitable for maintaining high levels of activity. All such factors are important in understanding what conditions must be maintained and monitored, and how best to do so.

For the most part, wastewater nitrification is commonly regarded as a two-step process. The first step is the conversion of ammonia to nitrite, generally considered to be achieved by *Nitrosomonas*, and the second step is the further oxidation of nitrite to nitrate, which is commonly achieved by *Nitrobacter* (Brock and Madigan, 1991). Both of these genera are autotrophic, meaning that inorganic carbon such as carbon dioxide and its aqueous form act as the carbon supply for the synthesis of new cells, while the oxidation of inorganic nitrogen serves as the energy source (Brock and Madigan, 1991). However, *Nitrobacte*r is not an obligate autrotroph, and can grow using organic carbon as an energy source, although this occurs at a slower rate than either its own autotrophic growth on nitrite or the growth rate of other heterotrophs (Brock and Madigan, 1991).

2.2 The achievement of biological nitrification and denitrification

The following discussion is based upon autrotrophic nitrification, which is commonly accepted as the means by which wastewater is nitrified, and will be assumed to be the main means of nitrification for the wastewater, although it is recognized that different organisms and biochemistries may be involved.

2.2.1 Biological description

Nitrification is defined as the oxidation of reduced nitrogen compounds (from the –III oxidation state) to oxidized nitrogen compounds (to the +V oxidation state), through chemical combination with oxygen. Therefore, it does not simply involve the oxidation of ammonium (Prosser, 1989). In general, nitrification occurs at a detectable rate only in the presence of a select group of chemoautotrophs and heterotrophic bacteria (Prosser, 1989). It is commonly accepted to be a two-step process. The first step of nitrification, oxidising ammonia-N (-III) to nitrite -N (+III), is attributed primarily to the autotrophic *Nitrosomonas europaea*, while the second step, oxidising nitrite –N (+III) to nitrate –N (+V), is considered to be dominated by the species *Nitrobacter winogradskyi* (Metcalf and Eddy, 1991b).

The complete biochemistry of ammonia or nitrite oxidation is still not fully understood. There is still no solid evidence demonstrating whether it is the ammonium ion or free ammonia (or both) which are transported into the cells of ammonia oxidisers, nor is it known how the transport occurs (Prosser, 1989). It is generally believed, however, that unionised ammonia (NH_3), as opposed to the ammonium ion (NH_4^+), forms the substrate for oxidation by *Nitrosomonas*, and that free nitrous acid (HNO_2) appears to be the substrate of *Nitrobacter*. The forms of the substrates are functions of pH and temperature equilibria.

Nitrogen removal, as opposed to mere nitrogen oxidation, is achieved either by assimilation or by the conversion of nitrate, ultimately to nitrogen gas (Metcalf and Eddy, 1991). This is what is meant by biological denitrification, and is achieved by denitrifying organisms in the absence of molecular oxygen, although an organic carbon source is required. Like nitrification, denitrification is also regarded as a two-step process, with step 1 being the reduction of nitrate to nitrite. This step is followed by the production of nitric oxide (N_20), and nitrogen gas (N_2). Both of these products are gases and can be released into the atmosphere. Unlike nitrification however, it is not clear whether there are specialised organisms responsible for the whole process, or whether the two steps are necessarily performed by two separate groups of organisms.

2.2.2 Heterotrophic versus autotrophic nitrification

Both *Nitrosomonas* and *Nitrobacter* are autotrophic, using inorganic carbon (carbon dioxide, bicarbonate, or carbonate) for synthesis instead of organic carbon, and ammonia or nitrite to derive energy. The whole process of nitrification and growth lies in a very delicate balance, as both groups of nitrifiers are inhibited by high concentrations of their own substrates. The majority of energy derived from their respective inorganic nitrogen oxidation reactions must be used to produce reducing power for the fixation of carbon for growth via the Calvin cycle (Barnes and Bliss, 1983). It has been estimated that autotrophic nitrifiers must convert approximately ten times their own cell weight of ammonia-N or nitrite -N to double in their mass (Prosser, 1989).

Unlike autotrophic nitrification, where nitrification is required in order to generate energy necessary for growth, it is generally accepted that heterotrophic nitrification is not linked to cellular growth as such, but the majority of nitrification appears to occur in heterotrophs in the stationary phase of growth (Focht and Verstraete, 1977). No heterotrophic nitrification has been demonstrated to be associated with energy production or growth, but rather, growth and energy production are considered to be via endogenous or secondary metabolism (Prosser, 1989). This would help explain why nitrification occurring at significant rates is generally attributed to autotrophic nitrification. Unlike autotrophic nitrification, which is associated with ammonia, substrates for heterotrophic nitrification include nitrites, hydroxylamine, hydroxamic acids, amino or oxine nitrogen, and aliphatic and aromatic nitrogen compounds. Products include NO_2^-, NO_3^-, and nitrogenous compounds (Prosser, 1989).

2.2.3 Electron donors required for denitrification

Biological reduction of nitrate to nitrite and nitrogen gas requires a suitable electron donor, which is usually an organic compound such as acetic acid, citric acid, and methanol, with methanol being the most preferred compound commonly used at wastewater treatment plants because it is inexpensive, easy to get, and very effective (Barnes and Bliss, 1983). Industrial and agricultural waste can be an inexpensive alternative option, provided they are easy to obtain consistently and contain no compounds that are inhibitory to the denitrification process. Barnes and Bliss (1983) have reported that methane and elemental sulphur are acceptable electron donors for denitrification, and wastewater itself may provide a sufficient

carbon source. Endogenous respiration may also supply a source of carbon, but reaction rates are much reduced.

The rate of denitrification depends primarily upon the nature and concentration of organic matter present. If there are excess electron donors present for denitrification requirements, denitrification will occur at a rate independent of the electron donor concentration. Using readily biodegradable electron donors such as methanol can yield denitrification rates ten times those obtained from sludges, from wastewater or endogenous respiration (Barnes and Bliss, 1983) indicating that denitrification in treatment plants is generally substrate limited.

2.2.4 Electron acceptors that may interfere with denitrification

An alternative electron acceptor is required under conditions where little or no dissolved oxygen is present. Inorganic anions such as nitrate, phosphate, and sulphate generally serve this purpose. Under aerobic conditions, oxygen is the preferred electron acceptor, and aerobic oxidation will be the predominant reaction. Nitrate is the next-most favoured electron acceptor, offering much more energy gain than anaerobic pathways (Barnes and Bliss, 1983). Since less energy is gained using nitrate as the electron acceptor, oxygen is used preferentially, and denitrification will occur only at low to zero dissolved oxygen concentrations (Barnes and Bliss, 1983). Although other inorganic anions are known to serve as electron acceptors in the absence of dissolved oxygen, in a wastewater which has undergone nitrification, the concentration of nitrate ions is likely to be present in much greater concentrations than phosphate or sulphate ions. Thus, under conditions of low dissolved oxygen, denitrification can be expected to occur (Barnes and Bliss, 1983). The rate of denitrification depends primarily upon the nature and concentration of the organic matter present. Thus, it is commonly accepted that denitrification is zero-order with respect to nitrate concentration down to very low concentrations.

2.2.5 Consortia dynamics

A consortium refers to a bacterial culture of two or more species in which each organism benefits from the other. The role of consortia cannot be stressed enough in the activated sludge process, especially when treating industrial wastewaters. A consortium of various species may lead to difficulty in achieving nitrification, but more likely, toxic or inhibitory

effects will be buffered, as it is possible that species not known for nitrification will be induced to nitrification. The proportion of a given microbial species present in a mixed culture will depend upon the relative abundance and type of electron donor (generally an organic substance), electron acceptor (oxygen, nitrate, phosphate, etc.), and amount of energy gained by using the particular electron acceptor (Barnes and Bliss, 1983). Generally, nitrifiers are only a small percent of the biomass, which is typically mostly heterotrophic (Barnes and Bliss, 1983). Because of this fact, the sludge age must be carefully controlled. If the biomass growth rate (and therefore the wastage rate) is greater than the nitrifier growth rate, the percentage of nitrifiers in the biomass will taper off until nitrification is lost (Jönsson, 2001). Reducing the organic loading rate per unit of biomass can reduce the net biomass growth rate.

2.2.6 Single sludge versus multiple sludge systems

A separate sludge system is one where each sludge has its own settling and recycling properties (Barnes and Bliss, 1983). In other words, an anoxic reactor has its own anoxic clarifier, and an aerobic reactor has its own aerobic clarifier. Thus, for a process that includes nitrification, nitrifiers usually grow along sideside aerobic heterotrophs. Nitrifiers will predominate only if there is no carbonaceous material to remove. In a single-sludge system, only one clarifier is used for the entire treatment process. In single-sludge systems, the mixed liquor contains both heterotrophs and autotrophs. Autotrophs will grow only in the aerobic basin, while heterotrophs will grow in the aerobic, anoxic, or anaerobic basins as long as metabolisable organic matter is present (Sedlak, 1991). While this may result in a lower percent of nitrifying biomass, there are many economic benefits to using a single-sludge process instead of a separate-sludge process. For instance, only one clarification step is used, saving on size and pumping costs. Furthermore, an external carbon source is less likely to be required, pH control chemical requirements are lower, and oxygen requirements are lower as well (Sedlak, 1991). Additionally, the problem of poor-settling nitrifiers is greatly reduced, and a more stable sludge resistant to shock loads is likely to be produced (Barnes and Bliss, 1983; Prosser, 1989). Denitrification rates in single-sludge systems are approximately half the rates of separate-sludge systems (Metcalf and Eddy, 1991b), but since nitrification is generally the rate-limiting step, such a fact will likely be of little concern, although it should be kept in mind.

2.2.7 Physical and chemical processes for nitrogen removal

Physical and chemical processes have been used for nitrogen removal, such as air stripping, breakpoint chlorination, and ion exchange. Only very few treatment facilities use such techniques, however, due to cost, inconsistent performance, and operation and maintenance problems (Metcalf and Eddy, 1991b; Sedlak, 1991). Biological nitrification-denitrification is generally best because of high potential removal efficiency, high process stability and reliability, relatively easy process control, low land area requirements, and moderate costs. However, physical/chemical means of removal may be viable options in certain circumstances (Metcalf and Eddy, 1991b; Sedlak, 1991).

2.2.8 Other environmental factors affecting nitrification and denitrification

Sludge may be affected by other prevailing conditions in the plant. Therefore it is important to monitor all conditions appropriately.

2.2.8.1 Temperature effects

Temperature affects wastewater treatment in many ways, both directly and indirectly affecting the biomass. There is an optimal temperature or range of temperatures for any organism, and the optimal temperature for growth will not necessarily be the same as the optimal temperature for substrate oxidation/reduction (Charley *et al.,* 1980). What defines the optimal temperature is not necessarily dependent upon the organism of interest itself, but a wide array of factors which are affected by temperature, such as electron donor or acceptor availability, the chemical form of the substrate at given temperatures and pH, sensitivities to inhibitors at different concentrations, and the efficiency of enzymes involved. Numerous studies have shown various ranges for optimal temperatures as there are many factors involved (Charley *et al.,* 1980). For instance, in pure culture versus activated sludge studies, the biomass concentration, substrate concentration, dissolved oxygen concentration, sludge age, pH, short term *versus* long term effects, growth conditions prior to testing, and difference between growth and test conditions, all affect performance (Charley *et al.,* 1980). For the most part, however, nitrifier growth and activity tends to increase with temperature up to a threshold value, as is the case for any microorganism.

2.2.8.2 The effect of pH

The pH will obviously affect several different factors, which in turn will affect the growth and activity of organisms involved. In brief, the pH is known to affect enzyme activity, affinity for the substrate, substrate availability, effects of inhibitory compounds, and substrate or product inhibition (Antoniou *et al.,* 1990; Groeneweg *et al.,* 1994; Prosser, 1989). Most nitrifiers have an optimum pH at approximately 7.5-8.0 (Brock and Madigan, 1991) and will grow within a pH range of approximately 2 pH units (Painter and Loveless, 1983; Prosser, 1989). Previous studies have shown that the growth for *Nitrosomonas europaea* occurs in the pH range of 5.8 to 8.5, suggesting there is a diversity of strains within the species. Optimal pH ranges are similar for mixed cultures and pure cultures (Prosser, 1989). Thus, similar optimal pH ranges can be expected for activated sludge processes. As with temperature, the optimum pH for growth will not necessarily be the same as the optimum pH for activity (Barnes and Bliss, 1983 and Painter and Loveless, 1983). For nitrification activity, the optimum pH range is commonly accepted to be 7.5 to 8.5 (Metcalf and Eddy, 1991a; Painter and Loveless, 1983 citing Downing *et al.*, 1964; Sedlak, 1991), with little to no nitrification occurring below approximately pH 6 to 6.5 or above 10 (Groeneweg *et al,* 1994; Painter and Loveless, 1983; Painter and Loveless, 1983). More specifically, the optimum pH range for *Nitrosomonas* activity tends to occur within the range of 6.7 to 9.2, while the optimum range for *Nitrobacter* tends to be approximately 8.0 to 9.5 (Prosser, 1989) .

2.2.8.3 Aeration effects on nitrification and denitrification

Barnes and Bliss (1983) have reported dissolved oxygen as an absolute requirement for growth of both *Nitrosomonas* and *Nitrobacter.* While *Nitrosomonas* and *Nitrobacter* are obligate aerobes for growth, prolonged lack of oxygen is not lethal (Barnes and Bliss, 1983). A critical value for dissolved oxygen, below which nitrification does not occur, has been reported at 0.2 mg/L for pure cultures of both *Nitrosomonas* and *Nitrobacter* (Barnes and Bliss, 1983). In general, nitrite oxidisers appear to be more sensitive to low dissolved oxygen concentrations than ammonia oxidisers (Prosser, 1989). While dissolved oxygen of at least 1.0 mg/L appears to be a requirement to prevent oxygen from becoming limiting in nitrification (Metcalf and Eddy, 1991a), 2 mg/L of dissolved oxygen is typically considered the cutoff value to fully ensure nitrification is not oxygen-limited (Wild *et al.,* 1971). Saturation constants for oxygen for pure cultures of ammonia and nitrite oxidisers are in the

range of 0.25 to 2.5 mg/L of dissolved oxygen (Prosser, 1989). Similar oxygen saturation constant values are reported for a suite of 9 mixed cultures. Saturation values for nitrifiers are generally higher than for heterotrophs, meaning the nitrifiers are likely to be out-competed by the heterotrophs at low dissolved oxygen concentrations (Prosser, 1989).

2.2.8.4 Sludge age

Sludges with a long sludge age can be expected to have a smaller fraction of viable biomass than sludges with a short sludge age (Jönsson et al., 2000). However, a longer sludge age is generally required in order to have a sustainable nitrifier population (Metcalf and Eddy, 1991b). As a sludge age increases, the nitrifier population increases to reach an optimum, following which the viable biomass decreases. Although nitrifiers are generally regarded as the rate limiters in the nitrification/denitrification process, a careful balance must be achieved, since several studies have shown that rates of denitrification are lower at higher sludge age (Barnes and Bliss, 1983, citing various sources).

Activated sludge can become adapted by either resistance on the nitrifying organism itself (adaptation in the biological and ecological sense) or by the change in bacterial community structure as a whole, developing an increased ability to resist inhibiting substances (Jönsson, 2001).

2.2.8.5 Inhibitory trends

This study focuses on inhibition of nitrification as opposed to inhibition of denitrification. Therefore, the following discussion focuses mainly on inhibition of nitrification.

Nitrifying autotrophs are commonly accepted as being more sensitive to toxins than the wide range of heterotrophs responsible for denitrification (Barnes and Bliss, 1983). The oxidation of ammonia to nitrite by *Nitrosomonas* is considered to be the more sensitive of the two steps in nitrification, as *Nitrosomonas* is generally more susceptible to inhibition from other compounds than is *Nitrobacter* (Barnes and Bliss, 1983; Blum and Speece, 1991). Most inhibitors stop ammonia oxidation but they may inhibit nitrite oxidation as well at high enough concentrations (Prosser, 1989). Most compounds inhibitory toward nitrification

inhibit ammonia oxidisers, with only a small percentage of compounds tested being more toxic to *Nitrobacter* than *Nitrosomonas* (Tomlinson *et al.*, 1966). Excessive chemical oxygen demand/biological oxygen demand loading tends to cause inhibition of nitrification. More commonly, however, nitrification inhibition by the presence of organic matter can be attributed to dissolved oxygen depletion caused by heterotrophic organisms utilising the organics present (Barnes and Bliss, 1983). Although biochemical oxygen demand levels up to 40-50 mg/L can be tolerated in nitrifying reactors (Wild *et al.*, 1971), it has been shown that biochemical oxygen demand levels over 40 mg/L can lead to a reduction in the rate of nitrification of greater than 50%. Such a phenomenon was postulated to be due to low levels of dissolved oxygen due to out-competition by heterotrophs, even though dissolved oxygen values indicated sufficient levels of oxygen were present in the bulk solution (Azevedo *et al.*, 1995). Such problems may be indicative of consortia dynamics and the physics of flocs or biofilms, where microenvironments will develop. The out-competition for oxygen by heterotrophs is one key reason that aerobic nitrifying reactors are often placed last in a sequence, in order to minimise the amount of organic substrate present.

2.2.8.6 Acclimation

Long-term effects of inhibitors are often different from the immediate effects observed in activated sludge, with one of the main reasons for this fact being that sludges can often acclimate to inhibitory conditions and compounds. For instance, in long-term studies, evidence for acclimation to thiourea concentrations of 0.76 mg/L was observed, a level that would have been completely inhibitory to unadapted *Nitrosomonas* species (Tomlinson *et al.*, 1966). In their study, Tomlinson and co-workers (1966) determined that short-term effects of inhibitors were not good estimates of long term effects of a continuous activated sludge operating under steady conditions. One reason for a difference in short- and long- term effects is that, for instance, other organisms in activated sludge can develop the ability to decompose an inhibitor (Tomlinson *et al.*, 1966). In the case of acclimation in pure culture studies, Tomlinson *et al.* (1966) postulated that either there are strains of *Nitrosomonas* which are resistant to certain inhibitors and are gradually selected under inhibitory conditions, or that normally susceptible strains can develop resistance. Occasionally, long-term effects can be more inhibitory than short-term effects. This is perhaps the result of compounds that produce no measurable effects during the duration of short tests, but which do suppress growth or yield to a measurable degree over longer periods (Tomlinson *et al.*, 1966). What is important

about either acclimation or greater inhibition resulting from prolonged exposure to compounds is the understanding that long-term studies and not merely batch tests are important to determine the effect on nitrification inhibition.

2.3 Nitrification inhibition

Nitrification involves several biochemical reactions, all of them essential for a successful total reaction. The first step of the nitrification process is the conversion from ammonium to nitrite. The final step of nitrification is the conversion of nitrite to nitrate.

2.3.1 Rate limiting steps in nitrification inhibition

Inhibition of nitrification means that some link in the chain of conversions from ammonium nitrogen to nitrite or nitrate nitrogen does not work properly (Jönsson, 2001). In most practical applications, the rate-limiting step is the first reaction, the oxidation of ammonia, since it is generally appreciably slower than the oxidation of nitrite. Therefore, usually, nitrite is overlooked as an intermediate reaction product, and the overall nitrification kinetics are more simply described as the direct oxidation of ammonia to nitrate, using the kinetic parameters of the first reaction. Several authors follow this approach. This approximation is acceptable if, as it occurs in most practical applications, the rate-limiting step is the first reaction, making accumulation of nitrite negligible. However, there are cases in which the kinetics related to the second step are slower than those related to ammonia oxidation and appreciable nitrite concentrations build up (Alleman, 1985). Environmental conditions which slow down nitrite oxidation include high temperature, low dissolved oxygen concentrations associated with high pH values (Alleman, 1985), high free ammonia concentrations and the presence of specific inhibitors to nitrite oxidisers (Hynes and Knowles, 1993; Alleman, 1985).

2.3.2 Modes of action of nitrification inhibition

Nitification plays an important role in biological functions involved in the wastewater treatment plant, and there is a need to understand the influence of substances within the treatment plant. Municipal wastewater can contain almost every chemical used in different factories. These substances might interact with microorganisms in activated sludge, and create great complexity. The following discussion of inhibition of nitrification is describing

inhibition at an enzyme level, rather than at a whole organism level. This is because many of the most powerful inhibitors act directly on the enzymes that catalyse the biological reaction. This way of inhibition is very effective, as the enzymes that catalyse catalytic effect often play a key role for speeding up the reaction rates. A decrease in the enzyme effectiveness will be observed as a decrease in the reaction rate.

2.3.2.1 Reversible and irreversible inhibition

By definition, inhibitors are substances which, when added to a mixture of enzyme and substrate solution, reduce the rate of reaction. They do this by combining with the enzyme molecules to form enzyme-inhibitor complexes that cannot combine with substrate molecules. Reversible inhibition is inhibition that disappears when the inhibitor disappears from the activated sludge, while the effect of irreversible inhibition implies that one or more vital cellular functions are permanently damaged by the inhibitor. Basically reversible inhibitors bind to the enzyme, but not permanently, so that inhibition can be transient. However, irreversible inhibitors bind permanently, so that inhibition is complete (Symbolism and Terminology in Enzyme Kinetics, 1981). Thus, in wastewater treatment, when an inhibitor persists in the system and causes reversible inhibition, nitrification rate will be affected until the particular inhibitor is washed out of the system. On the other hand, irreversible inhibition results in prolonged impaired process performance, as full nitrification capacity has to await the growth of new nitrifiers (Jönsson, 2001). There are three principal forms of reversible inhibition: competitive, non-competitive and uncompetitive. The two common forms, competitive and non-competitive inhibition, will be discussed.

2.3.2.2 Competitive and non- competitive inhibition

Competitive inhibitors combine with the active site of an enzyme molecule, thereby stopping substrate molecules from attaching to the enzyme. Competitive inhibitors normally strongly resemble the substrate for the enzyme in shape and size, and therefore compete with the substrate for the substrate-binding site on the enzyme (Symbolism and Terminology in Enzyme Kinetics, 1981). Therefore, competitive inhibitors reduce the rate of reaction by lowering the proportion of enzyme molecules that have bound substrate. Increasing the

concentration of substrate can usually reduce the effect of a competitive inhibitor, since this decreases the likelihood of an enzyme-inhibitor collision (Symbolism and Terminology in Enzyme Kinetics, 1981).

A non-competitive inhibitor does not combine with the active site of the enzyme but with another part of the enzyme molecule. In doing so it changes the shape of the enzyme molecule (conformational change), so that the catalytic ability of the enzyme is reduced or abolished. Usually the place of attachment on the enzyme is far away from the active site. This changes the shape of the enzyme and therefore prevents it from functioning normally. The substrate level does not affect non-competitive inhibition, as the substrate and the inhibitor do not compete for the same binding sites. Some enzyme molecules have another specific site, positioned well away from their active site, which can combine with substances other than the substrate. These sites are called allosteric sites and enzymes possessing them are called allosteric enzymes. By combining with an enzyme allosteric site, a substance causes a reversible change in the structure of the enzyme active site. Allosteric inhibitors reversibly combine with an enzyme allosteric site and slow down the rate of reaction of the active site. (Jönsson, 2001).

2.3.2.3 Feedback inhibition

The activity of almost every enzyme in a cell is regulated by feedback inhibition. Feedback inhibition is an example of a common biological control mechanism called negative feedback. The end product of a reaction pathway can inhibit the first reaction in the sequence. This form of inhibition is more likely to be allosteric than competitive. When the pathway end-product is in abundance, it binds with its enzyme active site (Symbolism and Terminology in Enzyme Kinetics, 1981) . As the end-product is used up, inhibition is reduced and more enzyme products can be produced. In this way the concentration of the enzyme reaction product is always controlled within a certain range which is compatible with the rate of the metabolic pathway as a whole (Symbolism and Terminology in Enzyme Kinetics, 1981).

In interactions between inhibitors, when inhibitors are mixed in a solution, the resulting reaction rates may be due either to the interactions between the inhibitors, or to the mean of the individual inhibitors on their own.

2.4 Problems caused by inhibition of nitrification

The effective management of activated sludge from a biological treatment plant is of critical importance in order to minimise treatment costs and to avoid pollution of receiving waters by discharged effluents. Plants that treat industrial wastewater may allow toxic or inhibitory chemicals to pass through treatment systems with little effective removal. Even in well-managed treatment systems, it is not uncommon for influent characteristics to change rapidly and unexpectedly as a result of changes in upstream discharges. Storm flows can introduce toxins from leachates and other urban runoff. The effects of toxicity on the activated sludge bacteria can therefore result in unacceptable levels of effluent toxicity.

Toxic chemicals or chemicals inhibitory to the nitrifiers directly affect the health of the activated sludge bacteria. When microorganisms encounter toxic chemicals, nitrification is affected and ammonia levels may increase. Toxicity produces a reduction in the respiration rate, in the rate of growth, and in the rate of breakdown of biochemical oxygen demand. Inhibition therefore gives rise to failed discharge treatment efficiencies, often in the absence of visible operational problems.

Once the wastewater treatment plant experiences inhibition of nitrification, it will be unable to accomplish one of its primary tasks, which is to remove nitrogen from the wastewater. Inhibition of nitrification may result in increased costs in the form of lost nitrification capacity, and in the end it may lead to expansion or reconstruction of the treatment plant.

2.5 Effect of inhibitory wastewaters on municipal wastewater treatment plant

The effect of a discharge of inhibitory substances may lead to shorter or long-term inhibition, if it affects the nitrification process at all.

2.5.1 Factors affecting inhibition

Toxic substances in the inlet to a wastewater treatment plant may affect the nitrification process in a wide variety of ways. The effects can be acute disruption of the function of the nitrification process, a general reduction of the nitrification capacity or, in some cases the

nitrifying bacteria will adapt to a toxicant to some extent. Partial inhibition of the nitrification rate does not necessarily imply deteriorated operational results, but it will entail an increased sensitivity to peak loads of ammonium. The effects of partial inhibition will only be detected at the outlet of the nitrification stream if the wastewater treatment plant is run almost to capacity. Measurements during a prolonged period are thus a prerequisite for the detection of inhibition phenomena (Jönsson, 2001). The effect of inhibitory discharges on a wastewater treatment plant depends on many different factors, which can be divided up into four principal areas: the properties of the inhibitor, the pre-treatment of the wastewater, the arrangement of nitrification within the treatment plant, and the discharge pattern of the inhibitor.

The properties of an inhibitor determine whether the substance is likely to be removed in the pre-treatment process or not. In cases where volatile, foaming or particulate inhibitors are present in an influent, the inhibitor will perhaps never reach the nitrification process. If they have not been removed previously in the sewer system, volatile substances may be stripped off if an aerated grit chamber precedes the biological process or if the wastewater is aerated in other ways. Foaming substances may also be reduced in the water phase by these kinds of pre-treatment. Particulate inhibitors may be removed by pre-treatment comprising preliminary settling. Some substances may also be removed by pre-precipitation. Substances removed by preliminary sedimentation or pre-precipitation may be converted in the sludge treatment processes and returned to the inlet together with the internal flow from the sludge handling system (Jönsson, 2001).

It is seldom possible to assess the effect of pre-treatment on any inhibitory substances, as it is seldom known exactly what toxicants are affecting the nitrification process. Some clues can be extracted from the industrial connection to the sewer system, especially if the catchment area is small and dominated by a few industrial types. If the industrial connection is substantial and embraces a variety of industrial branches, it will be impractical to find out what toxic substances are present in the wastewater. Hence, it will also be impossible to estimate the effect of pre-treatment.

The properties of the inhibitor also include the toxicity of the substance. The concept of toxicity comprises several aspects, such as whether the acute toxicity is high or low, whether the toxic substance is entrapped in the bio-culture or not, and whether the nitrifying bacteria are destroyed or just temporarily inhibited as long as the substance is present.

The position of the nitrification process within the wastewater treatment plant is also a determinant of the effect of discharge of a toxic substance. The more processes preceding the nitrification, the larger the possibility that nitrification is protected against inhibitory substances. In addition to an extended pre-treatment, the order of the biological processes themselves is also a factor of importance. A separate biological step for Chemical oxygen demand removal that precedes the nitrification process may degrade or capture inhibitory substances (Jönsson, 2001). An anoxic zone in a pre-denitrification system might also protect the subsequent nitrification process. Properties such as degradability and reaction rate for the degradation of the inhibitor, hydrophobicity and electrical charge play a role in effecting the degree of degradation or entrapment.

Moreover, the type of nitrification process may influence the degree of nitrification inhibition caused by a toxic or inhibitory substance. Nickel *et al.* (1985), using a culture of *Pseudomonas aeruginosa,* showed that bacteria growing in a biofilm were considerably less sensitive to toxic substances in the bulk liquid than planktonic bacteria. This phenomenon is probably related to the diffusion of the toxic substances into the biofilm and to the biodegradability of the toxicants. The penetration depth of a substance into a biofilm is proportional to the diffusion coefficient, which in its turn is in inverse proportion to the radius of the substances according to Strokes-Einstein equation. Accordingly, the molecular size of the toxicant may influence its inhibitory effect. If the molecules of the inhibitor are large, diffusion may be hindered so that a protected layer of nitrifiers can exist in the interior of a biofilm. This can also be the case if the inhibitor is degraded in the outer part of the biofilm. On the other hand, the concentration of a peak-load of a toxicant will be higher in a trickling filter than in an activated sludge system, owing to dilution resulting from recirculation in the activated sludge system.

The flow pattern in an activated sludge process leads to different concentrations of pollutants in the treatment tanks for the same discharge peak. The toxic effect of an inhibiting substance depends on the concentration of the inhibitor. The flow pattern also has an impact on the exposure time, which might have an effect on the inhibition obtained. Furthermore, the flow pattern on a micro-level might also influence the extant of inhibition, as the probability of contact between a toxic molecule and a bacterium increases with rising turbulence.

The discharge pattern of toxic substances can also affect inhibition. If the discharges have high concentration of toxic substances, then they are more harmful. If inhibition accidents come very often, the nitrifying bacteria might be prevented from recovering between the inhibition events. Therefore frequent and prolonged discharges are usually more harmful than rare discharges of short duration.

The effect of discharge of inhibitory substances could also differ depending on where in the catchment area the source of the toxicants is situated. If the point of discharge is very close to the treatment plant, the mitigatory effects of dispersion and dilution will be less.

2.5.2 Transformations of wastewater in sewers

The approach of analysing interactions between potential toxicants or inhibitors in different parts of the sewerage system is well recognised as far as the hydraulics and transport phenomena are concerned. However, the concept has seldom been applied when considering the biological processes. The sewer is a reactor, leading to microbial changes of the wastewater during transport that affect the quality of the wastewater, and thereby the successive treatment processes. The traditionally narrow distinction between collection and treatment of wastewater must be reviewed and extended: "wastewater treatment is starting at the kitchen sink" (Talib *et al*, 2002).

Scientific and technological methods for design and management of the urban wastewater system can now be applied as far as aerobic and anaerobic microbial processes in sewers are concerned. Under aerobic conditions, substantial changes take place in wastewater during transport in sewers. Readily biodegradable organic matter is removed and solubilisation of particulate chemical oxygen demand takes place (Talib *et al*, 2002). Such aerobic transformations can be managed to comply with primary and secondary treatment of wastewater. However, under anaerobic conditions in the sewer, readily biodegradable substrate is preserved and even produced, and it is thus made available for potential biological nitrogen and phosphorus removal in subsequent advanced wastewater treatment processes (Talib *et al.*, 2002).

The microbial transformation of organic compounds thus starts in the sewer network. The dominating process will be the availability and the type of electron acceptors. The electron

acceptors are utilised in a fixed sequence: oxygen for aerobic respiration, nitrate for denitrification, organic compounds for fermentation, sulphate for sulphate reduction and carbon dioxide for methanogenesis (Talib *et al*, 2002).

2.6 Examples of studies conducted on nitrification inhibition

A study of nitrification inhibition was initiated at Esundsverket Treatment Plant (Sweden) when it became obvious that the wastewater in this Helsingborg Treatment Plant contained considerable amounts of substances inhibitory to nitrification. At that time, no knowledge existed regarding the situation in Sweden in general. Therefore, an investigation of influent wastewater in 109 Swedish municipal wastewater treatment plants was conducted. A rapid screening method was used for estimation of inhibition of nitrification at municipal wastewater treatment plants. Inhibitory substances were detected in about 60% of the wastewaters (Jönsson, 2001). The level of inhibition was generally low, but at 4% of the plants, considerable inhibition was found.

The present study is based on research conducted by Karin Jönsson (Lund Institute of Technology, Lund University; Sweden) at the Oresundsverket Wastewater Treatment site in Helsinborg in 1996. Industries in that catchment were discharging toxins that were inhibiting the nitrification process in domestic sewage treatment plants. The six most important industries exhibited up to 60% inhibition in 20% solution (Jönsson *et al.*, 2001).

Another study on nitrification inhibition was conducted in the city of Copenhagen, Denmark. A pilot study in the industrial regions showed that the wastewater of Copenhagen inhibited nitrifiers and that the major contributor of inhibitory substances was the industrial wastewater. Among 21 studied catchments of two wastewater treatment plants, five were identified as generating inhibitory effects, with inhibition of more than 50% commonly measured in these areas. Furthermore, a high degree of inhibition was typically detected in areas with industrial activity, whereas no or minor inhibition was observed in residential or office areas. The compounds causing inhibition were not identified (Harremoës *et al.*, 1998).

Kroiss *et al.* (1991) conducted a study on inhibition of nitrification in Linz, Austria, where a new amendment of the Water Act resulted in stricter requirements for wastewater treatment efficiency. Based on this Act, the city of Linz, with large industrial effluent discharges to the sewer system, was forced to extend its wastewater treatment plant to achieve the required

nutrient removal. During pilot plant investigations, complete inhibition of nitrification was observed. Three of the fifteen industrial effluent samples were found to be inhibitory. One sample demonstrated 35% inhibition while two samples, originating from a steel industry and chemical works, were found to be completely inhibitory. The other twelve samples showed no inhibition. This study enabled the decision-makers of the city of Linz to define the number and sources of inhibitory wastewaters and provided a first estimation of their inhibitory effects, guiding identification of solutions.

In South Africa, nitrification inhibition has been reported in Durban by Tolond (2003), where industrialeffluent from four industries discharging to the Amanzimtoti Wastewater Treatment Plant was found to be inhibitory, compared to domestic influent from Kingsburgh Treatment Plant. No study has, as yet, investigated fully the effects of industrial effluent on nitrification of domestic sewage, or isolated and identified the specific toxins causing inhibition. This lack of published research and information was the motivating factor behind the present study. It attempted to evaluate the effects of industrial toxins on nitrification in activated sludge at Amanzimtoti Wastewater Treatment Plant, the same plant investigated in lesser depth by Tolond (2003).

2.7 Other methods for estimating nitrification inhibition

There are numerous other methods for estimating nitrification inhibition including ISO 9509, the use of pure cultures and Microtox®. The following discussion reviews a comparison between these methods and justifies the choice method for the current study.

2.7.1 Comparison of the screening method with ISO 9509 (1989)

Studies of nitrification inhibition conducted in Helsinborg, Sweden (Jönsson, 2001), using different industrial effluent types, were conducted using both the ISO method and the screening method (Table 4.1). The basic principle of the ISO 9509 method is virtually the same as the screening method, although the volumes, type of reactor, initial ammonia concentration, and incubation times differ slightly. It was concluded by the Swedish EPA (Jönsson, 2001) that there was no significant difference between the results obtained by the two methods.

Table 2.1 A comparison between the screening and ISO 9509 method as measures of nitrification inhibition (results reported by Jönsson, 2001).

	Screening Method	**ISO 9509**
Total liquid volume in the reactor (ml)	10	250
Type of reactor	Capped test tubes	Open reactors
Minimum permissible oxygen concentration (mg O_2 L^{-1})	4	2
Initial ammonium concentration in the reference reactors (mg NH_4–N L^{-1})	25	50
Incubation time (min)	80 - 120	240
Test organisms	Activated sludge	Activated sludge
What was measured?	Increase in NO_X–N (Decrease in NH_4–N)	Increase in NO_X–N or Decrease in NH_4–N
Single or duplicate determination	Duplicate	Single
Number of reference determinations per test	9	2
Prescribed pH adjustment of the test samples	Yes	No

2.7.2 Comparison of the screening method with pure culture

Investigations into the mechanisms of nitrification inhibition caused by toxins, on a detailed microbiological level, almost always utilises pure cultures of nitrifying bacteria (Tolond, 2003). Furthermore, these investigations usually consider the effect of only one toxin. The situation at municipal wastewater treatment plants differs considerably from such experiments as municipal wastewater may contain almost every chemical used in contemporary society, and the activated sludge from a municipal wastewater treatment plant comprises a wide spectrum of species of microorganisms in a complex matrix. There is a significant difference in the degree of sensitivity between the tests in which individual toxins are tested on either activated sludge or pure cultures of nitrifying bacteria. As a result, inhibition studies involving chemicals would benefit from using the application of pure culture assays, whereas investigations involving specific inhibition problems at wastewater treatment plants would benefit from using the screening method based on activated sludge (Jönsson, 2001).

2.7.3 Comparison of the screening method with Microtox®

The basic technology of the Microtox® Test System is based upon the use of luminescent bacteria, specifically the strain *Vibrio fischeri* NRRL B-11177, to measure toxicity from environmental samples. According to Pedersen and Peterson (1996), when properly grown, luminescent bacteria produce light as a by-product of their cellular respiration. Cell respiration is fundamental to cellular metabolism and all associated life processes. Bacterial bioluminescence is linked directly to cell respiration, and any inhibition of cellular activity (toxicity) results in a decreased rate of respiration and a corresponding decrease in the rate of luminescence (Pedersen and Peterson, 1996). The more toxic the sample, the greater the percent of light loss from the test suspension of luminescent bacteria. Bacterial bioluminescence has proved to be a convenient measure of cellular metabolism and consequently, a reliable sensor for measuring the presence of toxic chemicals in aquatic samples. Several studies have suggested that investigations performed by Microtox® cannot be expected to reflect the effect on nitrifying organisms since the principle of the Microtox® method is distinctly different from the principle of the screening method (Jönsson, 2001). When comparing samples of the same wastewater type, no correlation has been found between the Microtox® method and the screening method used by the Swedish EPA (Jönsson, 2001).

2.8 Background to pollution management

The introduction in 1999, by the Durban Metropolitan Council, Department of Wastewater Management, of amended sewage disposal bylaws, has seen the metropolitan area take a further step towards achieving a stringent environmental management system. The impact on the day-to-day operations of companies within the metropolitan area is not trivial.

South African companies are now at a crossroads where they are faced with having to make decisions regarding how to conduct their business in terms of the contemporary pollution liability regime (Durban Metro Economic Development, Department of Corporate Services, 2003). They are concerned with issues such as the standards that need to be achieved to ensure compliance, the effect that new laws have on business costs and personal liability for directors and officers. On the other hand, South African companies have an opportunity to

compare the standards and liability faced by companies overseas, and in a proactive manner adjust their policies and procedures during the periods it takes enforcement agencies and courts to catch up with global pollution control trends. Thus, they are able to position themselves favourably to avoid potential negative implications of enforcement.

The environmental ethos of a company forms part of its corporate responsibility, whereby it selects particular production methods, processes and waste streams that will have the least impact on the environment. Corporate accountability, on the other hand, relates to legal compliance in terms of which companies must ensure that their products and operations do not violate prescribed environmental norms and standards (Durban Metro Economic Development, Department of Corporate Services, 2003). Many companies have used the excuse that environmental responsibility and accountability cost too much in a developing economy. However, environmental non-compliance in business terms is simply a decision by a company to transfer or externalise its pollution-related costs of production to the taxpayers and other natural resources.

Globalisation or world shrinkage is not only a trend, but it has become a way of life over the past decade. South Africa has not been isolated from this process. International environmental law is a good example of the way in which globalisation has occurred. Globalisation results in the adoption of international environmental principles by South Africa. The principles often involve a paradigm shift away from the traditional manner in which companies have thought. For instance, the wise use of non-renewable resources, the preservation of biodiversity, and the *'polluter pays principle'*, were not issues companies in South Africa considered in the past.

2.8.1 Water quality standards

Maintaining water quality depends on the combined use of a number of regulatory mechanisms. These include resource directed measures, which specify the desired level of protection for a water resource, and on that basis sets clear goals for the quality of the resource (Palmer Development Group, 2000). Resource-directed measures control the impacts on the water resource through statutory requirements for meeting effluent discharge standards and the use of regulatory measures such as permits and registration. Monitoring the status of water resources on a continual basis ensures that standards are being met.

2.8.2 Water quality and effluent legislation

If an industry is to dispose of its effluent to a local water body, that effluent must meet certain legislated standards. The original standards covering effluent quality were set in The General and Special Effluent Standards (SA Government Gazette, 1984). They have since been superseded by the National Water Act (SA Act No. 36, 1998). Under the National Water Act, new standards were developed based on the quality of receiving water bodies. These standards, described in Government Gazette No.20526 (SA DWAF, 1999), allow for a set quantity and quality of effluent to be released, depending on the status given to the receiving aquatic system. Table 2.1 shows the discharge limit values applicable to discharge of wastewater into a water body (Water Amendment Act, 1999). The table shows discharge limits for different elements including ammonia and nitrate, on which this study focuses. As it was mentioned before, ammonia can become highly toxic to aquatic organisms when levels increase beyond the natural eutrophication level. Nitrite released into treated wastewater causes a different yet equally disturbing outcome. It is for this reason that DWAF special standards for ammonia are 2.0 mg/l and1.5 mg/l for nitrate/nitrite (Table 2.1).

Table 2.2 Discharge limit values applicable to discharge of wastewater into a water body from National Water Act (Sa Act No.36, 1998)

Characteristic	General limit	Special limit
pH	5.5-9.5	1 (median) and 2.5 (max)
Ortho-Phosphate (mg/l)	2.5	0
Soap, oils, greases, waxes (m/l)	2.5	0
Free Chlorine (m/g)	1 000	500
Fluoride (m/g)	1	1
Dissolved Cyanides (m/g)	0.02	0.01
Suspended Solids (m/g)	25	10
Electrical Conductivity (mS/m)	70 mS/m above intake to a max. of 150 mS/m	50 mS/m above background receiving water to a max. of 100 mS/m
Ammonia (mg/l)	3	2
Nitrate/Nitrite (mg/l)	15	1.5
Fecal Coliforms (per 100 ml)	1000	0
COD (mg/l)	75*	30*
Dissolved Copper ((mg/l)	0.01	0.002
Dissolved Zinc (mg/l)	0.01	0.04
Boron (mg/l)	1	0.5
Dissolved Selenium (mg/l)	0.02	0.02
Dissolved Manganese (mg/l)	0.1	0.1
Dissolved Lead (mg/l)	0.01	0.006
Dissolved Cadmium (mg/l)	0.005	0.001
Mercury and its compounds (mg/l)	0.005	0.001
Dissolved Chromium (mg/l)	0.05	0.02
Dissolved Arsenic (mg/l)	0.02	0.01
Dissolved Iron (mg/l)	0.3	0.3

Note:* After the removal of algae

Special limit is used when effluent arising in the catchment area is draining water to any river or tributary at any place between the source and the river.

General limit is used for effluent arising in any area other than an area in which the special standard is applicable. It is these standards that are applied to the Prospecton trade effluent and effluent from Amanzimtoti Wastewater Treatment Plant.

2.8.3 Impact of pollutants on design and costs of treatment works

The general parameters that govern the design of wastewater treatment plants are carbon and nitrogen loadings (Herold *et al.*, 1998). The effluent must comply with chemical oxygen demand concentrations of < 75 mg/l and a nitrite/nitrate concentration of 15mg/l (Table 2.1). There may be specific restraints on other pollutants such as sodium, colour from dye material, total dissolved solids, conductivity, or phosphate concentrations. If these pollutants, either individually or collectively, require reduction to environmentally acceptable concentrations, then advanced tertiary treatment will be required. In the case of industrial effluent, such treatment may increase operating costs at the local treatment plant. The local authority or service provider may need to implement tariffs for industrial effluent such that the costs associated with the pollution load from industry are fully recovered.

2.8.4 Water pollution and effluent quality criteria

Pollution is a relative effect and water may be considered polluted by one user while it may be accepted as quite satisfactory by another. The parameters that are considered to have a large impact on tariffs charged by most service providers and billed to each industry are COD, settleable solids and volume. The underlying principle governing tariffs for effluent discharges is that industry and other users of the sewerage and treatment system must pay for the services rendered by the local authority. When discharges of industrial effluent are made into a municipal sewer and conveyed to the treatment plant for purification, the local authority becomes the polluter of the receiving water.

The Department of Water Affairs and Forestry (DWAF) has always adopted the principle that the polluter must pay for the abatement of its own pollution. This approach has been reinforced in the National Water Act (Act 36 of 1998), where compliance with prescribed standards and water management practice policies are dealt with through a tariff system. Legislation on pollution control requires that effluent purification be an integral part of industrial processes. The underlying philosophy of the *polluter pays principle* is for the tariff system to reflect the true costs of the service provided, including all activities involved in delivering the service (Herold *et al.*, 1998). The discharge of industrial effluent into the sewer is subject to the requirements of the National Water Act, 1998, and the Water Services Act, 1997. The local authority assumes responsibility for the purification and disposal of effluent in accordance with the requirements of the Act. In the process of fulfilling this responsibility,

the local authority may incur expenditure to provide a sewerage network and treatment/purification facilities to meet the additional hydraulic and COD load contribution to the system, and to ensure that the standards prescribed by DWAF are attained. The local authority uses a tariff structure designed to ensure that the costs incurred will be proportionately recovered from industry for the services provided. If the water resource is treated as a free good, then it leads to abuse and exploitation of the natural resource (Business Partners for Development, 2001). One method of reducing the above externality is to force the polluter to internalise the cost by making the polluter pay.

2.8.5 Who is the polluter?

In a strictly legal sense, the prime polluter of the receiving water is the local authority. Although DWAF stipulates the pollutant limits in the effluent discharge permit, the effluent discharged from wastewater treatments will still impose an additional load on the water resource, and this could have a detrimental impact on the water resource and the ecosystem. The DWAF pricing strategy promotes charging for point and diffuse sources of pollution. Any charges over and above the direct operating expenses of the sewerage and treatment systems will have to be sustained partially by industry. Since domestic householders also contribute to the final pollution load discharged into the water environment, they will also have to bear their share on a proportionate basis. Before any effluent discharges are accepted into the sewer system for treatment, specific binding legal agreements and documentation must be completed between industry and the local authority (Kerdachi, 2002. By taking on this responsibility, the local authority may have to acquire capital to sustain all purification costs, and pay for water resource and catchment management charges (Kerdachi, 2002) .

Most companies pay for services whereby their wastewater is treated to reduce pollution impacts. These payments are done monthly, sometimes without a proper understanding by the companies of the factors that have contributed to the charges. Some companies have a clear understanding of the contributing factors of the charges that they face monthly, but do not have means or do not know how to reduce these factors. The National Water Act, 1998, promotes the *polluter must pay principle,* and also implies that the polluter must pay for any damages to the environment and for any clean-up operation resulting from illegal discharges. If different contributing factors to the charges are made clear to the companies, industry is

likely to devote more attention to wastewater management, and financial benefits may accrue to them.

2.8.6 Environmental impact of industrial wastewater

Without doubt companies and their waste products may negatively impact on the environment. Liquid wastes produced by companies have to be given special attention because most of them can end up in water bodies. These water bodies are important to humans, especially in South Africa, where water is a critically limited resource and may ultimately limit prosperity of the economy. Rapid population growth in South Africa has not only resulted in an increased water demand, but also in accelerated degradation of water systems through increased agricultural activity, urbanization and industrialization. Apart from these monetary values, rivers have an intrinsic environmental, scientific, educational and cultural value, which cannot be measured in monetary terms.

It is therefore imperative that a continuous monitoring and evaluation system be put in place to assess and monitor the status of wastewater from companies, such that appropriate mitigatory frameworks can be adopted and ultimately sustainable management of wastewater can be achieved.

2.9 Conclusion

Substances that are inhibitory to nitrification are also environmentally hazardous in a broader perspective. If they are not degraded by the treatment processes, they will end up either in the residual sludge, or in the air if they are volatile, or in the receiving environment. To sum up, inhibition of nitrification presents numerous environmental, economic and practical aspects to consider. Therefore, it is of vital importance to test and monitor the effects of certain substances, such as trade effluents and their associated toxins, on the nitrification process at wastewater treatment plants.

Chapter 3

The study area

Durban is the second largest industrial centre in South Africa. It has a wide range of industries including large petrochemical refineries, a paper mill, a sugar refinery, textile and clothing factories, general manufacturing, metal processing and numerous other chemical and smaller industries.

3.1 Background information

The Durban Metropolitan Area covers an area of 1370 km^2, and stretches 72 km along the Indian Ocean and 52 km inland (Figure 1, Chapter 1). The population is approximately three million (Durban Metro Water, 2001). This area is also one of the fastest growing industrial hubs and urban centres in the country. eThekwini Water Services is the municipal department that deals with provision of affordable and acceptable water services within the Durban Metropolitan Area. It is also responsible for sewage and wastewater disposal and treatment where necessary.

3.2 Climate

Prospecton Industrial Area is subtropical with summer maximum daily temperatures of approximately 30 °C. Because of the typically high humidity, conditions get sultry. In winter days are mild, with minimum daily temperatures occasionally dropping below 10 °C (Durban Metro, 1999) .

Precipitation is dominant mainly from October to March, particularly in the months of December, January and February, when tropical thunderstorms occur frequently. Mean annual rainfall is approximately 85mm with 65 % occurring in the summer months from October to March (Durban Metro, 1999).

Air quality in the area is considered to be generally poor. A range of industries emit pollutants to the atmosphere. There are a number of isolated industrial sources of air pollution close to the area, including paper and petrochemical industries that result in poor air quality.

Sugar cane burning from nearby sugar cane belts also contributes to air pollution within this area.

3.3 Drainage

Amanzimtoti Wastewater Treatment Plant discharges its final return flow directly into the Amanzimtoti River. This has resulted in instances of bacterial contamination and eutrophication in the Amanzimtoti and the Little Amanzimtoti Rivers. The area naturally has a high water table due to its low elevation and proximity to the coast, but artificial drainage has lowered this in order to promote development.

3.4 Geology

The area is mostly reworked marine sediments, with alluvium (mud) occurring in the harbour and the mouths of streams flowing into the harbour and river as well as those drained directly into sea.

3.5 Land use

Before the 1900s, the entire Prospecton area was a sugar cane belt. Not long thereafter, the sugar belt was bought and controlled by the Platt family. Sugar cane cultivation in Prospecton continued into the mid 1900s. Today, much of Prospecton is an industrial area, although Durban International Airport is situated close by, and several residential areas were established nearby in order to provide labour for industries.

3.6 Socio-economic circumstances

Prospecton is a large and developing industrial area about 10 kilometres south of Durban. It has undergone much technological development due to the existence of some large international and local manufacturers as well as the Durban International Airport. This area is of importance to the residents of Amanzimtoti and Isipingo, as well as Durban as a whole, as it provides employment. To the south of Amanzimtoti the coast becomes more rural where agricultural development has taken place. Local businesses are small and rely on local residents for trade.

3.7 Industries and pollution management in the Prospecton Industrial Area

eThekwini Water Services is faced with the task of pollution management. This requires an analysis of environmental management activities at industries within the urban boundary in promoting a healthy environment. Responsibilities of minimising environmental impacts within the Metropolitan Area lie both with the public and private sectors (Durban Water and Waste, 1996). The basic task of environmental management with respect to industrial wastewater is to ensure that overall environmental damage is reduced to a point where the social benefits and environmental costs of regulation are balanced against each other.

3.8 Characteristics of Industries surveyed

In carrying out this study, some industries within the Prospecton Industrial Area in Durban were surveyed. The Prospecton Industrial Area constitutes an area of major industrial activity such as car manufacturing, food processing, textile and clothing manufacturing, rubber manufacturing and metal finishing. Figure 3.1 is an aerial photograph showing the location of the factories that were surveyed within the Prospecton Industrial Area as part of this study. Wastewater samples from these industries were used in this study to investigate the inhibition of nitrification at the Amanzimtoti Wastewater Treatment Plant. In order to be able to collect and analyse samples, an agreement was established between each factory concerned and the eThekwini Water Services, ensuring that the name of the industry would not be made public. As a result of this confidentiality clause, the ten industries are referred to as industry A, B, C, D, E, F, G, H, I and J in this dissertation.

Figure 3.1 An aerial photograph of the surveyed industries and location of Amanzimtoti Wastewater Treatment Plant

Industry A is a manufacturer of alkyd resins (Table 3.1). Effluent comes mainly from esterification and kettle washings. Some styrenes / acrylate monomers are also used. Solvents used are white spirits, mainly xylene. Industry B is involved in the cleaning of wool using dilute sulphuric acid. This acidic effluent is neutralised by adding lime and precipitated calcium sulphate is removed. Industry C manufactures beer. Effluent comes from the washing of filters after the brewing process as well as the bottle washing plant. The effluent is treated prior to discharge to sewer in a "bio plant", which includes solids removal. Industry D is a vehicle assembly plant. Effluent comes mainly from washing panels and some from the paint shop. The effluent foams, so a flocculant is added and oils are skimmed off prior to discharge to sewer. Industry E manufactures rubber products such as lines of power transmission belts. The effluent contains high strength organic matter and sulphates. The effluent also contains

high amounts of hydrogen sulphide, which occurs in the anaerobic treatment when there are significant amounts of sulphate in the feed to the anaerobic digestion process. Industry F recycles glass, rubber, plastic, paper, cardboard, ferrous scrap metal and non-ferrous scrap metal. The effluent has a high metal content. Industry G manufactures automotive filters and filtration systems. The effluent has a high metal content. Industry H is a car assembly plant. It is mostly involved in metal finishing, therefore, the effluent contains some traces of metals. Industry I produces pop rivets and the effluent also has traces of metals. Industry J manufactures vehicles. Its effluent also has some traces of metals. Quantities of effluent discharged to sewer vary from 10 KL/day to 3000 KL/day, which contributes between 0.05 and 15.8 percent of the total discharge to the sewage treatment plant (Table 3.1).

Table 3.1 Background information on the 10 industries surveyed in this study.

Industry	Type of industry	Major constituents of effluent	Contribution to Amanzimtoti load (19M L/day)	% contribution
A	Alkyd resin manufacturer	Water of esterification, little vegetable oil, xylene	10 ($KL.d^{-1}$)	0.05
B	Textile washing	Dilute sulphuric acid, lime, $CaSO_4$, lanolin oil	100 ($KL.d^{-1}$)	0.5
C	Beverage	SO_4, some sugars,	3000 ($KL.d^{-1}$)	15.8
D	Vehicle assembly	Low % of metals (Zn, Ni)	1000 ($KL.d^{-1}$)	5.3
E	Rubber manufacturer	Cd, Cr, Cu, mineral oils, Ni, high strength organic matter and sulphate content	30 ($KL.d^{-1}$)	0.16
F	Recycling company	Oil, high % of metals (Fe, Ni, Zn)	30 ($KL.d^{-1}$)	0.16
G	Automotive filters manufacturer	High % of metals (Cu, Ni, Zn)	10 ($KL.d^{-1}$)	0.05
H	Car assembly	Low % of Cd, Cr., Cu, Ni, Zn	30 ($KL.d^{-1}$)	0.16
I	Vehicle assembly	Low % of Cd, Cr, Cu, Ni	40 (KL/day)	0.2
J	Car parts manufacturer	Low % Cd, Cr, Cu, Pb, Ni	50 (KL/day)	0.26

3.9 The Amanzimtoti Wastewater Treatment Plant

The layout of Amanzimtoti Wastewater Treatment Plant is shown schematically in Figure 3.2. The layout represents stages where the treatment plant achieves its objectives by a variety of methods. These include: removing the solid waste by screening and settling the solids before they are removed, degrading ammonia via the biological and aerobic procedure of nitrification, and in most cases, converting nitrate to nitrogen gas by the process of denitrification (Grüttner *et al.*, 1994). Among all the stages, the aeration tanks are most susceptible to nitrification inhibition impacts.

Primary treatment consists only of physical separation. Wastewater entering the treatment plant is passed through a series of grates and screens that remove large, heavy solids including stones, rags, bottles, cans and plastic items. This process is important because it removes the solid material from the raw wastewater that would otherwise accumulate in the digester, thereby reducing its working volume. The wastewater is then passed to primary sedimentation tanks and left for a number of hours to allow suspended solids to settle, giving two fractions: sludge and supernatant. The sludge is treated in the secondary treatment process in anaerobic digesters.

The liquid fraction from the primary sedimentation tanks is mixed and aerated in a large tank containing activated sludge. Pure oxygen is normally forced through activated sludge tanks to increase the levels of dissolved oxygen, thereby increasing the rate of biodegradation. The complete mineralization of organic matter to oxidised products is expected to occur at this stage of the treatment process. Slime-forming bacteria grow and form flocs, and these flocs form the substratum to which protozoa and other animals attach (Brock and Madigan, 1991). The effluent containing flocs is then pumped into a clarifier where the flocs settle. Some of the floc material is then returned to the aerator to serve as innoculum while the rest is discharged to the next stage. The final step of the treatment is a physiochemical process employing chlorination to reduce the levels of inorganic nutrients.

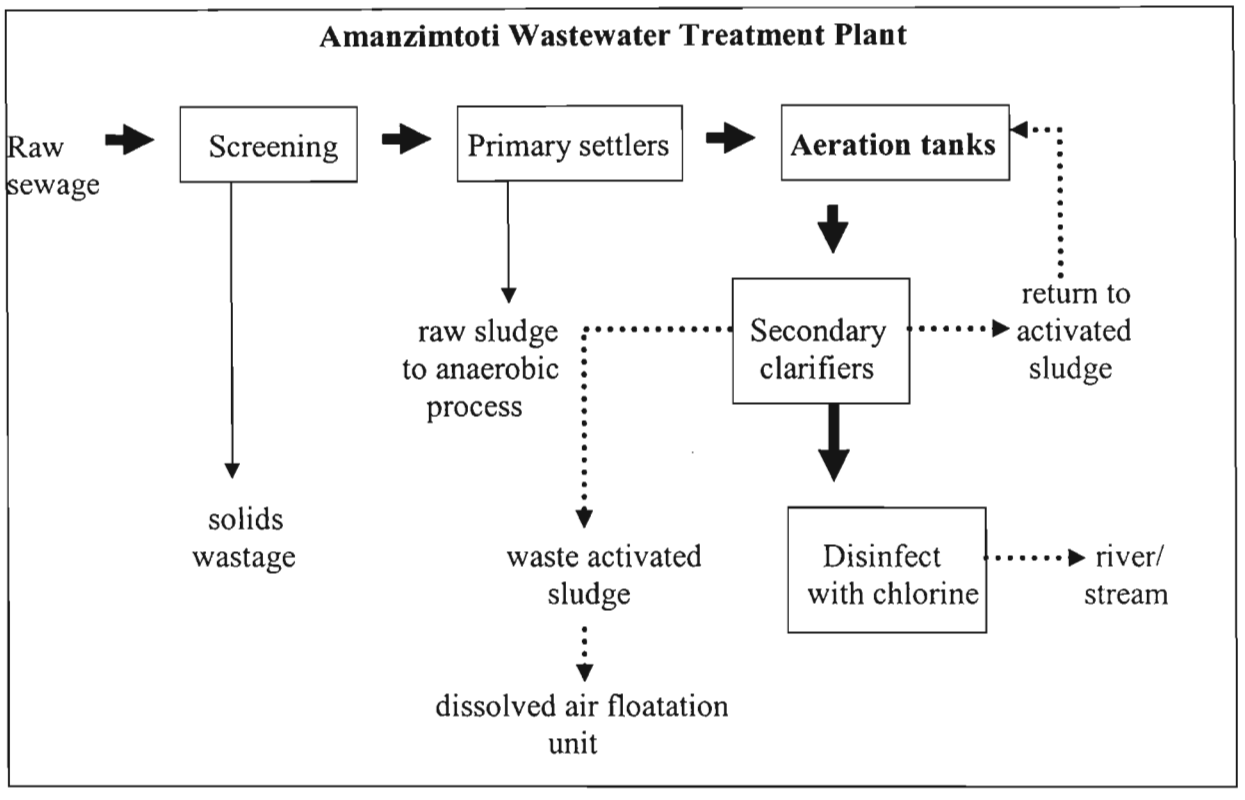

Figure 3.2 A diagram illustrating the layout of treatment processes of Amanzimtoti Wastewater Treatment Plant

Chapter 4

Methodology

4.1 Background to methodology

4.1.1 Nitrification inhibition measurements in the sewer network

The more acute severe the inhibition of nitrification by effluent, the more important it is to determine the source of the inhibition. If the retention time in the sewer net is several hours, measurements directly at the industries or at junctions in the sewer system can enable rapid determination of source. However, this approach demands that the location of the possible sources of toxicants is known. If an industry discharges toxicants discontinuously, which often is the case, continuous measurements or knowledge about the inhibition pattern is necessary for the successful location of the source of inhibition.

Sampling to prevent inhibition accidents (an event where a pulse of toxic or inhibitory substance in the influent to the WWTP causes inhibition of nitrification resulting in washout of nitrifiers, and/or elevated concentrations of ammonia in the effluent and/or disruption of the denitrification process) is difficult. Measurements close to the source of the discharge give the advantage that the toxicant is not diluted or mixed with other toxicants, thus making it possible to detect the source of inhibition. However, dilution and mixing of inhibitors in the sewer network produces effects on microorganisms that are not predictable based on samples from individual sources of inhibition. Thus one would wish to sample for inhibition as close to the treatment plant as possible, but such sampling makes it difficult to determine the source of inhibition.

Unless a sampling system in the sewer network is extremely comprehensive, a number of questions may be left unanswered for the pollution control officers. These include: how will dilution affect the inhibitory effect found later at the treatment plant, how will other substances that enter the sewer system act together with or counteract such inhibition? Therefore, sampling strategies typically involve a compromise between sensitivity and the detection of inhibitors: If the method is too sensitive it will give rise to many false alarms, resulting in uncertainties for the operator about how to react on an indication of inhibition. If

the method is not sensitive enough, inhibition accidents are unlikely to be prevented. The detection method should exhibit similar sensitivity to inhibiting substances as the processes that are going to be protected. If it is a nitrification process that is going to be protected, nitrification-inhibition measurements should be used. It is often advantageous if nitrifying bacteria from the treatment plant itself can be used in the measurements. Pure cultures of bacteria have a response pattern that differs from the response pattern of the bacteria found in the sludge matrix in the treatment plant. However it has also been shown that even if activated sludge from the treatment plant in question is used in the test method, the response of the treatment plant itself may differ from the response found by the test method. It is thus important to check the accuracy of the method. One reason for such a discrepancy could be that a temporary inhibition is not noticed at the treatment plant if it is not run almost to capacity.

4.1.2 The screening method for estimating inhibition of nitrification

The screening method for estimation of inhibition of nitrification at municipal wastewater treatment plants is based on the principle of the ISO method for assessing the inhibition of nitrification of activated sludge microorganisms by chemicals and wastewater (ISO 9509, 1989). However, the screening method is less costly than the ISO method. Jönsson (2001) describes the method that has been used in this study with some minor procedural modifications.

The principle of the screening method is that nitrifying activated sludge is mixed with nutrient solution (containing ammonia and bicarbonate). The suspension is mixed with tap water and the wastewater under consideration in similar proportions to the dilution of the wastewater. The mixture is shaken for 120 minutes, and then nitrification is stopped by filtration and cooling of the samples. A physical illustration of this procedure is shown in Figure 4.1. Nitrification inhibition is found by comparing the nitrate production in the samples containing wastewater with reference samples without wastewater (Appendix A). This comparison implies that, for samples without inhibitory substances, the measured inhibition will be zero. If the measured inhibition for a sample is negative, the wastewater is stimulating rather than inhibiting the activity of the nitrifying bacteria. However, if the measured inhibition is positive, inhibitory substances are present. As a standard procedure, the examination is

performed with a fixed dilution of the wastewater in question. A dilution of 50% is used for municipal wastewater. However, often the

examination is performed with a series of dilutions in order to establish a dose-response relationship. The test method can be applied for pure substances as well, and in such cases, the concentration replaces the dilution.

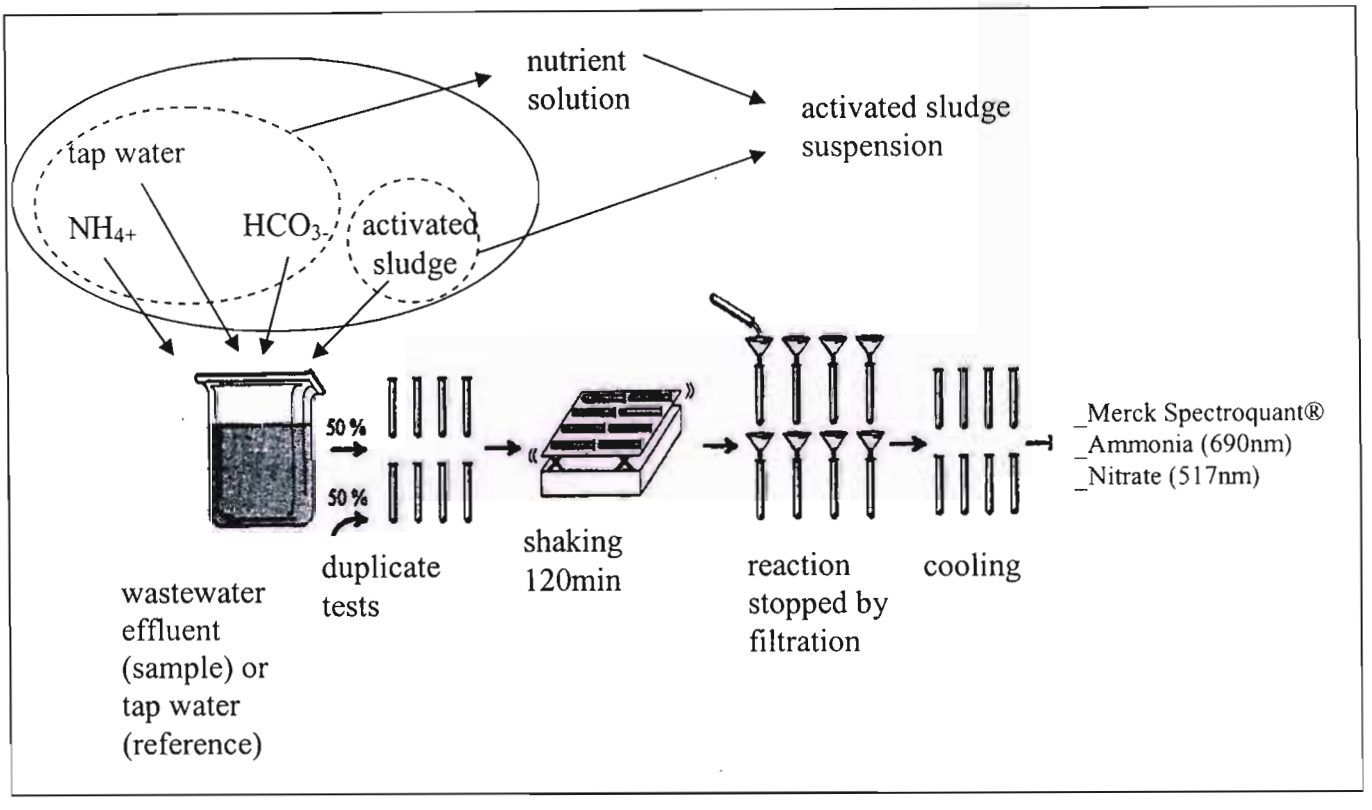

Figure 4.1 A schematic description of the screening method for detection of nitrification inhibition (adapted from Jönsson, 2001)

4.1.3 The screening method over the other methods

From the discussion on comparison of other methods with the screening method (Chapter 2), it can be concluded that the ISO 9509 (ISO 9509, 1989) method yields results closest to that of the screening methods, followed by the pure culture method. The Microtox® method appears to yield results significantly different to the screening method (Jönsson, 2001). The method selected for use in this study was the screening method of Jönsson (2001). The screening method was chosen over the ISO method because of the following:

- The minimum permissible oxygen concentration in the reactors is 2 mg O_2 /l for the ISO method while the screening method prescribes 4 mg O_2/l. Low oxygen concentrations are inhibitory to nitrification and the minimum permissible oxygen concentration of the ISO method is questionable since such low oxygen levels will affect measured

inhibition to great extent. At this low level, small variations in the oxygen concentration will result in different inhibition results.

- The added amount of ammonium in the reactors is twice as high as for the ISO method as it is for the screening method. Thus, the ISO method will return lower inhibition values for competitive inhibitors than the screening method. The screening method prescribes duplicate determinations, but nevertheless five times as many test samples can be analysed per day.

4.2 Selection of water quality variables

Wastewater is susceptible to contamination by a large number of solutes, making it almost impossible to test all potential contaminants. The selection of variables for the assessment of water quality depends on the purpose of such an assessment (Helmer and Hespanol, 1997). Wastewater assessments are normally carried out to examine their effects on specific activities in the wastewater treatment plant and tariffs are set accordingly. The first step in such a study is to identify variables that are likely to affect the tariff paid by polluters. In this case, volume of effluent, COD and settleable solids concentrations were considered important as they affect the performance of the operation of the treatment plant and provide an estimate of the risks associated with their presence in the wastewater. No laboratory tests on these components were conducted in this study, but the records from 2002-2003 from eThekweni Water Services, Wastewater Department were analysed.

4.2.1 Volume

Volume is the amount of liquid waste (which may contain matter in solution or suspension) discharged as a result of any industrial activity. This parameter governs the overall amount that the industry will have to pay the service provider for accepting and treating the wastewater.

4.2.2 Chemical oxygen demand

Chemical oxygen demand (COD) is the amount of oxygen required to completely oxidise the organic compounds of wastewater. The higher the COD value of wastewater, the more oxygen the discharge demands from the receiving water body. COD is a vital test for assessing the quality of effluents and wastewater prior to discharge. The COD test predicts the oxygen requirement of the effluent and is used for the monitoring and control of discharges,

and for assessing treatment plant performance. COD does not reflect the biologically oxidisable portion, and therefore is not a true reflection of the oxygen demand load going to a receiving water, but it has the true advantage of being: quick, reproducible and easy.

4.2.3 Settleable Solids

Settleable solids (SS) are solids that will settle out in a prescribed period of time when a sample of wastewater is allowed to stand undisturbed. Sedimentation tanks facilitate the separating out of settleable solids from liquid wastewater. The layer of settleable solids that forms at the bottom of the sedimentation tank is called sludge solid. Settleable solids are that portion of the suspended solids that are of sufficient size and weight to settle in one hour. The results are reported as millilitres of settled solids per litre of wastewater. Settleable solids are typically 75 percent organic and 25 percent inorganic (Walkers and Wink, 1981).

4.3 Test for nitrification inhibition

4.3.1 The methodology to test for nitrification inhibition

Measurements of nitrite and nitrate produced during the test period were made in activated sludge in the presence (test sample) or absence (control sample) of wastewater. The tests were performed using activated sludge from the Amanzimtoti Wastewater Treatment Plant, incubated with influent to the Amanzimtoti Wastewater Treatment Plant or wastewater samples at different dilutions. The inhibition of nitrification in activated sludge was calculated as the difference between nitrification rates in the reference sample and in the test tube with wastewater. The difference was expressed as a percentage of the nitrification rate of the reference (Appendix A) (Harremoës *et al.*, 1998). The procedure of the screening method is outlined in Figure 4.1 and a full explanation of this procedure appears in Appendix A.

The concentrations of ammonia and nitrate (mg/l) were analysed using the Merck Spectroquant® Ammonium Test (1.14752) and the Merck Spectroquant® Nitrate Test (1.14773.001), respectively. The ammonia test was based on the colorimetry of blue 2.2-isopropyl-5.5-methyl-indophenol (Berthelot's Reaction). Following adjustment to pH 13 (with reagent NH_4-1B), ammonia was reacted with hypochlorite (Reagent NH_4-2B) to form monochloramine, which in turn formed a blue indophenol dye in a catalysed 2-stage reaction

with thymol (Reagent NH_4-3B).The concentration of this was determined spectrophotochemically at a wavelength of 690 nm (Merck, 2002a). Figure 4.2 shows the ammonia standard curve that was used for analysis.

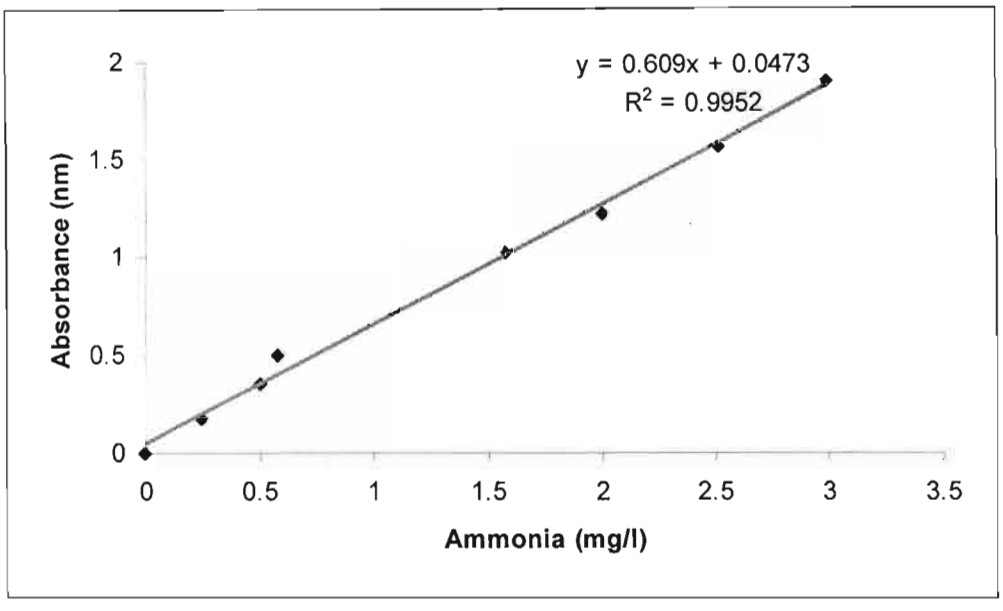

Figure 4.2 Standard curve of response of the ammonia test at a range of ammonia concentrations

The nitrate test was based on the biochemical principle that in concentrated sulphuric acid, nitrate ions reacted with benzoic acid to form a red nitro compound, the concentration of which was determined specrophotochemically at a wavelength of 517nm (Merck, 2002b). The nitrate standard curve that was used for this analysis is shown in Figure 4.3.

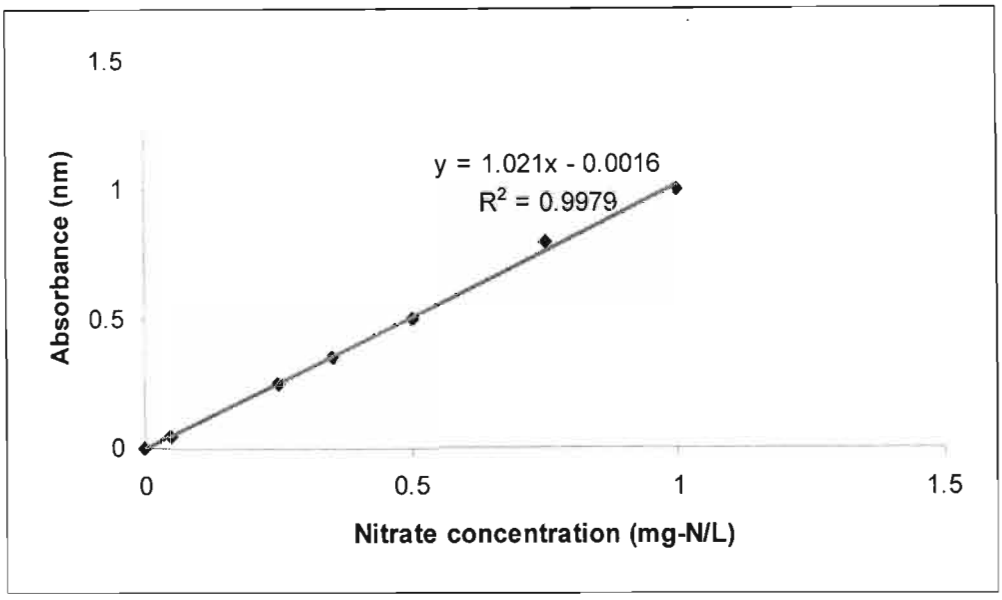

Figure 4.3 Standard curve of response of the nitrate test at a range of nitrate concentrations

4.3.2 Use of analysis of variance

ANOVA (analysis of variance) is a procedure designed to determine if the manipulation of one or more independent variables in an experiment has a statistically significant influence on the value of the dependent variable. It is assumed that each independent variable is categorical and independent variables are called factors and their values are called levels.

If only two means are being compared, a simple t-test will test if they differ significantly. However, when there are more than two means the problem of comparing means becomes more complicated. If there are more than two means and analysis of variance has concluded that the means are not all the same, ANOVA can be used to test differences between sample means in the following ways:

- test whether any number of means differ
- investigate the interacting effects of two or more variables
- Compare variability within and between experimental groups to test differences between means.

The technique used to differentiate between these possibilities is called a multiple range test. Multiple range tests can be placed into two categories.

- Constant Least Significant Difference (LSD). In these a single LSD is found and used to compare all means in a pair wise manner. Tests differ in the algorithm used to calculate the LSD. Examples include Fisher's LSD, Tukey's HSD, Sheffe's LSD and Waller-Duncan's LSD.
- Variable LSD. In these tests the means are ranked and the magnitude of the LSD is determined by the number of intervening means between the two being compared. Examples are Newman-Keul's test and Duncan's multiple range tests. Thus, samples can be grouped according to whether means differ or not.

4.3.3 The experimental approach

In order to achieve the objectives of the study, a number of questions were asked and the experimental work was based on trying to come up with answers to the questions. The following questions were asked;

1. ***Does Amanzimtoti Wastewater Treatment Plant experience inhibition of nitrification?***
2. ***Does industrial effluent contribute to inhibition of nitrification?***
3. ***Do some industries have a greater inhibitory effect than others?***
4. ***What patterns of short-term (daily) variability need to be considered in developing an appropriate tariff?***
5. ***Is there evidence of acclimation?***
6. ***Are there combined effects that are not predicatable?***
7. ***Are there other water quality variables that can be used in developing a tariff structure?***

Inhibition of nitrification was tested using Amanzimtoti influent that comprises a mixture of all effluent entering the Amanzimtoti Wastewater Treatment Plant, as well as discharges from the individual industries listed in Table 3.1. A variety of dilutions was used in the experimental work as described in the following sections in order to establish a dose-response relationship. It should be noted that for the experimental samples, the percentage dilution referred to is the activated sludge suspension, and that 100 minus this percentage comprises wastewater effluent (Figure 4.1). According to the Swedish EPA (Jönsson, 2001), when testing the influent of a wastewater treatment plant for nitrification inhibition, the

recommended dilution is 50%. In this study, a number of dilutions were used as Swedish conditions might be different to those in South Africa.

4.3.3.1. Does Amanzimtoti Wastewater Treatment Plant experience inhibition of nitrification?

Influent to the Amanzimtoti Wastewater Treatment Plant was sampled weekly over the period of 4 weeks. A single sample of activated sludge was collected from the Amanzimtoti Wastewater Treatment Plant and mixed with nutrient solution to form an activated sludge suspension. This was mixed with influent at the start of the experiment to make up 3 experimental replicates. In addition 3 reference replicates were also set up. For the following 3 weeks, activated sludge from the previous experimental replicates was used, and mixed with nutrient solution and newly collected wastewater influent. Inhibition was determined using the screening method based on nitrate production as described in Section 4.3.1 and Appendix A. To determine whether inhibition occurred or not, SPSS was used to compare nitrate production in the experimental replicates with the reference replicates based upon a t-test. The student t-test is designed to determine the significance of the difference between two mean values, each calculated from 3 replicates. Inhibition would be indicated by a significantly ($p<0.05$) lower nitrate production rate percentage in the experimental compared to the control, which is indicated by positive values for the values of nitrification inhibition as calculated in Appendix A.

4.3.3.2. Does industrial effluent contribute to inhibition of nitrification?

It is difficult to establish whether or not industries contribute directly to nitrification inhibition, and an indirect approach was devised. It involved establishing temporal variation in inhibition at the Amanzimtoti Wastewater Treatment Plant over a period when industries typically close down. This happens in summer over the period from the middle of December to early in January. Therefore, if industries are a source of inhibition, it may be expected that with all other things being equal, inhibition of nitrification would be greater in November and January than in December.

The measurements of inhibition of nitrification were made weekly over a period of three months (November, December and January (2003 / 2004)) using Amanzimtoti influent, in order to quantify the inhibition pattern over this time period. During each week, activated

sludge and influent wastewater were collected. Activated sludge was mixed with nutrient solution and then with wastewater influent in triplicate, and references were also set up in triplicate. Inhibition was determined using the screening method based on nitrate production as described in section 4.3.1 and Appendix A. To determine whether industries had an effect, SPSS was used to compare the means for each month based on a multiple range test. Significantly lower ($p<0.05$) values in December, compared to November and January would suggest that industrial effluent is having an effect on nitrification inhibition.

4.3.3.3. Do some industries have a greater inhibitory effect than others?

Individual industrial wastewaters were tested at a range of dilutions to compare the degree of inhibition of industries and to establish the best dilution for detection of possible inhibitory effects. Effluents from industries A-J were sampled weekly at dilutions of 20, 40, 60 and 80% and mixed with activated sludge suspension collected and made up weekly. Triplicate samples were set up over the 4 week period. Triplicate references were established each week. Inhibition of nitrification was determined using the screening method based on both nitrate production and ammonia degradation described in Section 4.3.1 and Appendix A. To determine whether effluent from each industry was inhibitory or not, SPSS was used to compare nitrate production in the experimental replicates with the reference replicates based upon a t-test. To determine whether industries differed from each other in their toxicity, a multiple range test was used at $p<0.05$.

4.3.3.4. What patterns of short-term (daily) variability need to be considered in developing an appropriate tariff?

The 5 most important sources of inhibition (greatest inhibition) were analysed every Monday, Wednesday and Friday over a 2 week period. This was of interest because these industries were considered to have a serious negative effect on the treatment plant and tariffs need to incorporate temporal variability in the inhibitory effects of wastewater effluent. The other 5 least inhibitory samples were not investigated further. Wastewater effluents from the 5 most inhibitory industries were collected on each day the experiment was run. A single activated sludge suspension was prepared at the beginning of the week. Wastewater effluent was mixed with activated sludge suspension at a dilution of 20% on each day of the experiment. Inhibition of nitrification was determined using the screening method based on nitrate production described in Section 4.3.1 and Appendix A. This was exploratory work that was

intended to draw attention to daily variation in nitrification inhibition for purposes of developing an appropriate tariff structure.

4.3.3.5 Is there evidence of acclimation?

The same experiment that is described in Section 4.3.3.1 was used to seek evidence of acclimation. Use of a single sludge suspension for the four week period meant that if inhibition remained constant, no acclimation could be inferred, whereas a consistent decline in inhibition over the four week period would indicate acclimation. A multiple range test using SPSS for each dilution compared mean values for each week of the experiment. Significantly lower ($p<0.05$) levels of inhibition over time would suggest acclimation.

It is important to note that the inhibition characteristics of the sample may change during storage. Vaporisation may lead to inhibition diminishing with time (Tomlinson *et al.*, 1966). Precipitates formed during freezing of a sample may also lead to reduced inhibition. On the other hand, substances entrapped in particles may be released as a consequence of the same processes, and this may in turn lead to increased inhibition.

4.3.3.6 Are there combined effects that are not predictable?

When two inhibiting wastewaters are mixed, it is expected that the resulting inhibition will be intermediate between the effects of individual wastewater on their own. If this is not observed, then effluents do not act independently of each other. In order to appreciate the effect of wastewater mixing, inhibition of nitrification was investigated using mixtures of a 1:1 ratio of wastewaters from 2 different industries across 4 dilutions (20, 40, 60 and 80%), with 4 replicates per mixture per dilution. Samples were prepared by mixing effluent from industries as follows: A and D, J and C, B and I, G and H, and E and F. Wastewater effluent from each industry was collected, and appropriate effluent mixtures were prepared. Inhibition of nitrification was determined using the screening method based on nitrate production described in Section 4.3.1 and Appendix A. A multiple range test using SPSS compared mean values for each industry and for mixtures described above, was used to test for significant differences ($p<0.05$) between each industry as well as combined effluents.

4.3.3.7 Are there other water quality variables that can be used in developing a tariff?

Wastewater quality and quantity information (summarised in Table B.2, Appendix B) was obtained from eThekwini Water Services for volume (quantity), COD and SS (quality). A correlation coefficient quantifies systematic variation between two variables. It can take a value between positive one and negative one. A positive value means the two variables vary together in a similar way, which means that when one variable increases the other increases as well. A negative value means that two variables vary such that when one variable increases the other decreases. A zero value, or a near zero value, shows that the two variables are unrelated. The relationships between the effluent characteristics and the measured inhibition, using Spearman's correlation coefficient, were determined, including the significance of correlations.

Chapter 5

Results

Effluent is discharged to the sewer by industry as well as from suburban homes in Amanzimtoti. In the case of the Amanzimtoti Wastewater Treatment Plant, 80% of the volume is from industry while only 20% is domestic sewage. Mixing of industrial and domestic effluents takes place in the sewer network. Depending upon the chemical characteristics of the mixture, it may inhibit breakdown of waste.

5.1 Analysis of wastewater characteristics (volume, settleable solids and chemical oxygen demand)

Table 5.1 shows the effluent quantities and the chemical oxygen demand (COD) and settlable solid concentration discharged by each industry over the period from 2002-2003. Industry C has the largest discharge. This industry produces beer and other beverages, which use large volumes of water in the manufacturing processes. Industry D produces the second largest volume, while the third largest volume is produced by Industry B. The remaining industries discharge much lower volumes.

The average concentration of COD is extremely high for Industry A (over 15000 mg/l). The remaining industries produce wastewater with less than 1000 mg/l COD. Industry J has a COD close to 1000mg/l while the remaining industries have values less than 400mg/l.

The concentration of settleable solids was highest for Industry A (37 mg/l, Table 5.1). The remaining industries had concentrations below 5mg/l.

The volumes discharged were compared with the concentrations of COD and settleable solids, and no relationship was found (Table 5.2). Furthermore, there was no relationship between the concentration of COD and settleable solids in wastewater discharge. All regression coefficients (R^2 values) were very low.

These results suggest that industries A and C are experiencing problems with their effluent. Industry A has a problem of high COD and settleable solids and Industry C has a problem of high volumes. These results do not reveal whether the identified industries contribute significantly to the inhibition of nitrification at the treatment plant, but they give an indication of industries for which increased tariffs may be justified.

Table 5.1 Effluent profile for industries A-J

Industry	Average volume (kl/month)	Average COD (mg. l^{-1})	Average SS (ml. l^{-1})
A	356	15104	37
B	3500	336	1.2
C	92532	250	3.3
D	27829	320	3.3
E	907	302	2.3
F	939	303	0.1
G	300	372	0.15
H	953	219	1.0
I	1200	151	1.5
J	1835	903	1.7

Table 5.2 Correlation matrix for average volume, COD and SS showing R^2 values

	COD	SS
SS	$R^2 = 0.033$	
Volume	$R^2 = 0.026$	$R^2 = 0.007$

5.2 Inhibition of nitrification

Most of the results in this section are based on nitrate production because of the difficulties in getting some of the ammonia degradation results.

5.2.1 The effect of effluent dilution on nitrification inhibition (nitrate production)

There was evidence of inhibition by effluent entering the Amanzimtoti Wastewater Treatment Plant at all dilutions and for each week of the 4 week study period as all results differed significantly ($p<0.05$) from the control tests (Table 5.3 and Appendix C1). The lowest inhibition of the Amanzimtoti influent was observed in all weeks at 80% dilution and the highest inhibition was observed at 20% dilution for each week of the four week study period. There was a general decline in inhibition with increasing dilution for all of the weeks.

Based on a multiple range test, mean values for each dilution showed a fairly systematic decline in inhibition over time, although not all means were significantly different over the four week study period (Table 5.3 and Appendix C2). At 20% dilution, the results of inhibition of the Amanzimtoti influent during week 4 differed significantly ($p<0.05$) from all the other weeks (Appendix C2). The means of weeks 3 and 2 were not significantly different ($p<0.05$) from each other while weeks 2 and 1 were not significantly different ($p<0.05$) from each other. However, weeks 1 and 3 were significantly different ($p<0.05$). At 40% dilution all of the inhibition means at each week were significantly different from each other ($p<0.05$). At 60% dilution, only the inhibition mean from week 1 was significantly different ($p<0.05$) from the other weeks. At 80% dilution, only the inhibition mean from week 1 can be considered significantly different ($p<0.05$) from the others. These results suggest that the nitrifying microorganisms were acclimating to the composition of the effluent.

Table 5.3 Mean values and standard deviation for nitrification inhibition by Amanzimtoti influent at 4 influent dilutions measured weekly over a 4 week period. Results are based on nitrate production.

Dilution	Week	N	Mean (%)	Std. Deviation
20	1	4	38.50	1.29
20	2	4	35.00	0.81
20	3	4	31.75	5.12
20	4	4	23.10	2.22
40	1	4	30.32	1.51
40	2	4	25.22	0.79
40	3	4	20.50	2.38
40	4	4	15.12	0.85
60	1	4	22.12	2.01
60	2	4	18.17	0.62
60	3	4	17.70	1.36
60	4	4	17.32	1.03
80	1	4	17.50	1.29
80	2	4	16.27	0.49
80	3	4	13.75	1.14
80	4	4	15.02	0.18

5.2.2 Effects of individual industrial effluents on inhibition

Table 5.4 shows the percentage inhibition of nitrification in Amanzimtoti activated sludge at different dilutions by effluent from each of the 10 surveyed industries discharging effluent to Amanzimtoti Wastewater Treatment Plant based on nitrate production. Results represent a mean of 3 samples per industry per dilution, tested weekly over a month-long period. The results showing nitrification inhibition of the 10 different industries based on ammonia degradation are presented in Table 5.5. These results represent a mean of 3 samples per industry at a dilution of 20%.

All results for nitrate production differ significantly from the control (0% inhibition) at $p<0.10$ (Table 5.4 and Appendix C3). Industry D produced the most inhibitory effluent, averaging 30% inhibition over the 4 dilutions when calculating nitrification inhibition from nitrate production, and 24% when calculating inhibition from ammonia degradation at a dilution of 20% (Tables 5.4 and 5.5). Industry J had the next most inhibitory effluent, averaging 28% and 22% based on nitrate production and ammonia degradation respectively. This industry was followed by Industry G with an inhibition of 23% when calculated from nitrate production and 18% when calculated from ammonia degradation. The fourth most

inhibitory effluent was from Industry E with an average of 22% inhibition when calculated from nitrate production and 20% inhibition when calculated from ammonia degradation. This was followed by Industry B with an average of 19% inhibition when calculated from nitrate production and an average inhibition of 17% when calculated from ammonia degradation. Industry I was the next most important source of inhibition averaging, 17% and 16 % for nitrate production and ammonia degradation respectively, followed by Industry F averaging 15% and 14% respectively. The second least inhibitory effluent was from Industry H, averaging 12% and 10% inhibition for nitrate production and ammonia degradation respectively. The least source of inhibition was Industry C with an average of 10% inhibition when calculated from nitrate production and 8% from ammonia degradation. In contrast to these industries, Industry A appeared to stimulate the nitrifying bacteria present in activated sludge at Amanzimtoti Wastewater Treatment Plant by 24% based on nitrate production. No results could be determined for Industry A when calculating inhibition from ammonia degradation.

Table 5.4 Mean values and standard deviations for nitrification inhibition by 10 surveyed industries at 4 dilutions measured weekly over a 4 week period. Results are based on nitrate production.

Industry	Dilutions (%)	N	Mean inhibition (%)	Std deviation
A	20	4	-44.6	8.05
	40	4	-24.6	4.92
	60	4	-21.3	10.8
	80	4	-10.6	2.49
B	20	4	36.3	6.94
	40	4	19.0	2.44
	60	4	13.0	2.94
	80	4	7.30	2.05
C	20	4	23.3	4.49
	40	4	12.3	2.05
	60	4	8.30	1.24
	80	4	6.10	1.63
D	20	4	59.9	4.38
	40	4	31.6	3.29
	60	4	18.6	2.05
	80	4	14.0	2.44
E	20	4	42.0	4.32
	40	4	21.3	2.86
	60	4	15.3	2.05
	80	4	14.3	2.62
F	20	4	30.3	2.86
	40	4	19.3	1.69
	60	4	11.6	1.69
	80	4	9.30	3.39
G	20	4	44.0	5.09
	40	4	20.6	3.29
	60	4	12.6	2.05
	80	4	13.0	0.81
H	20	4	24.6	3.68
	40	4	8.60	1.69
	60	4	7.30	2.05
	80	4	6.60	2.49
I	20	4	32.6	5.24
	40	4	16.6	2.49
	60	4	8.30	3.09
	80	4	9.00	3.55
J	20	4	54.0	4.89
	40	4	27.3	2.05
	60	4	19.0	2.44
	80	4	12.3	2.05

Table 5.5 Mean inhibition of nitrification for 10 surveyed industries at 20% dilution over a 4 week period based on ammonia degradation

Industry	Dilutions (%)	N	Mean inhibition (%)	Std deviation
A	20	3	N/R	N/R
B	20	3	17	3.51
C	20	3	8	7.09
D	20	3	24	5.13
E	20	3	20	5.29
F	20	3	14	3.51
G	20	3	18	5.69
H	20	3	10	4.51
I	20	3	16	6.43
J	20	3	22	6.00

Note: N/R= No results

Multiple range tests indicated that there were significant differences between the mean nitrification inhibition by different industries at 20% dilution and these depended upon dilution. Results of the multiple range tests are presented in Appendix C4. Industry A differed from all others (Appendix C4). Industries C, H, F, I and B formed a second group with means that could not be distinguished from each other, while industries H, F, I, B and E formed a further distinct group. The additional groups were identified as follows; F, I, B, E and G; B, E, G and I; and E, G, J and D.

At the 40% dilution, Industry A differed from all others (Appendix C4). Industries H, C and I formed a second group with means that could not be distinguished from each other. The third group was formed by industries C, I, B, F, G and E with means that could not be distinguished, while industries B, F, G, E and J formed a further distinct group. The additional group was identified as industries J and D.

At dilutions 60 and 80%, Industry A differed from all other industries and the rest of the industries were not significantly different from each other and it became difficult to distinguish between them.

5.3 Temporal variation in inhibition

5.3.1 Long-term (monthly) patterns of variation

Variation in inhibition over a 3 month period, from November 2003 to January 2004, of influent to Amanzimtoti Wastewater Treatment Plant is shown in Figure 5.1 (see also Table B1, Appendix B). During December 2003, inhibition at the Amanzimtoti Wastewater Treatment Plant was lower than in November 2003 and January 2004. The differences between the means in November and December are significantly different ($p<0.05$) while those of December and January are significantly different at $p<0.01$ (Appendix C5). The reason for this is likely to be that most of the industries were closed for about 2 weeks in December, before and after Christmas, which resulted in lower industrial effluent loads being discharged to sewer and entering the treatment plant at this time. These results suggest that nitrification inhibition is caused, at least to some extent, by industrial effluent.

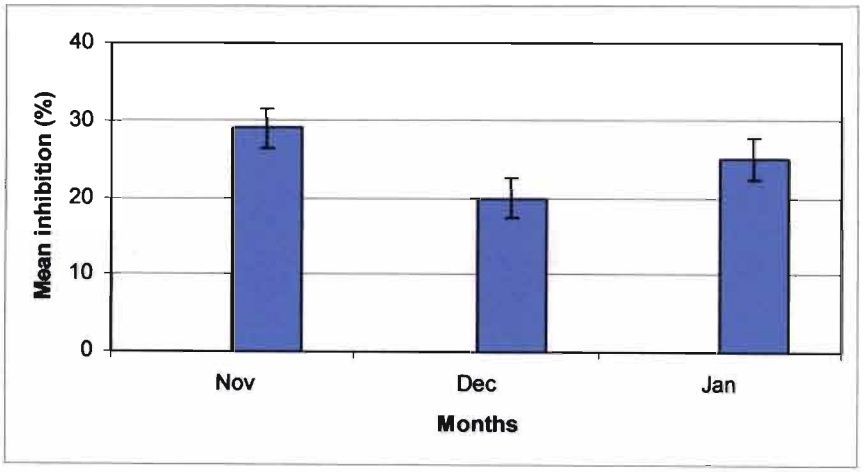

Figure 5.1 Variation of inhibition of Amanzimtoti influent during the months November, December and January 2003/2004 based on nitrate production

5.3.2 Short-term (daily) patterns of variation

Based on the results for the 20% dilution of wastewater for the industries investigated in this study, industries B, D, E, G and J were found to be most inhibitory to nitrification (Tables 5.4 and 5.5). Closer analysis of these industries over a two-week period revealed that effluent varied in its effect on nitrification, and also that effluent from different industries showed different inhibition patterns (Figure 5.2). Industry G had relatively constant inhibition on different days, with lowest inhibition on Fridays. Industry E showed highest inhibition on

Wednesdays and lowest inhibition on Fridays. Both industries D and J showed the highest inhibition, with Mondays and Wednesdays showing the highest values and Fridays showing lower inhibition. The general trends for the five industries were that they experienced highest inhibition on Mondays and Wednesdays, while on Fridays inhibition was lowest. Thus, there is short-term (daily) and longer-term (monthly) variation in inhibition of nitrification that may be caused by variation in effluent discharge. Such variation needs to be incorporated into a tariff structure.

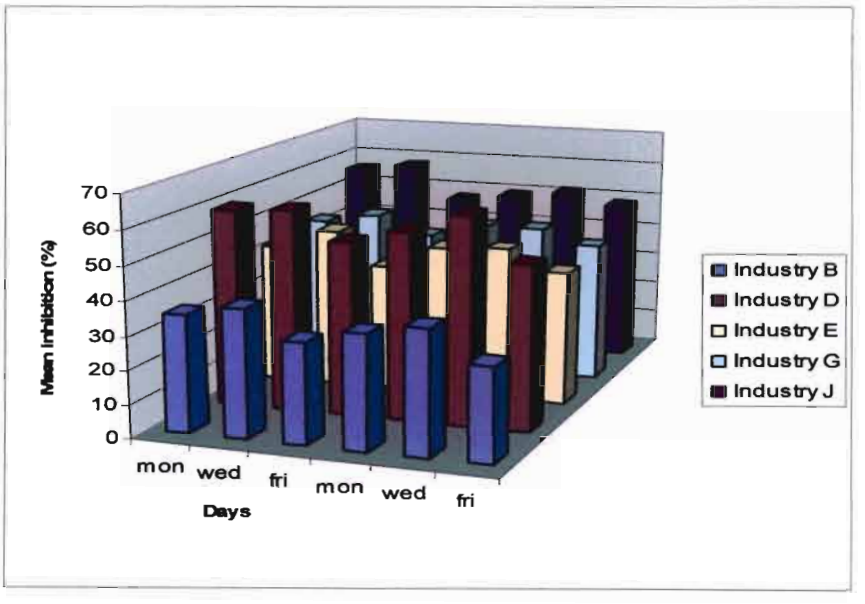

Figure 5.2 Inhibition of nitrification using 20% wastewater dilution over the period of 2 weeks for five selected industries

5.4 Combined effects of mixing effluents from different industries

Effluents from individual industries are mixed in the sewer before they reach the treatment plant. In an attempt to understand the effects of mixing, it was necessary to determine the effect of combining individual effluents on nitrification inhibition. When two inhibiting wastewaters are mixed, it is expected that the resulting inhibition should be intermediate between the individual inhibition values. If this pattern is not observed, then the effluents do not act independently of each other, making planning and compiling tariffs very complex. All the results are based on nitrate production.

Effluent from Industry A stimulated nitrification over the entire range of dilutions (Table 5.4 and Fig. 5.3). However, effluent from Industry D was inhibiting. The effluent mixture (D+A) and effluent from Industry D showed similar patterns of inhibition, with the greatest inhibition at a dilution of 20%. Mixture (D+A) is more inhibitory than either of the individual effluents on their own. This seemed true at all dilutions (Fig.5.3). The mixture (D+A) had an average of 36% nitrification inhibition over the 4 dilutions while Industry D had an average of 30% and Industry A stimulated nitrification by an average of 24%. Based on the multiple range test there was no significant difference ($p<0.05$) between the inhibition mean obtained from effluent for Industry D and the mixture (D+A) across all dilutions (Appendix C6). Inhibition from Industry A, however had an inhibition value which differed significantly from the inhibition means of the effluents from industries D and (D+A) for all the dilutions.

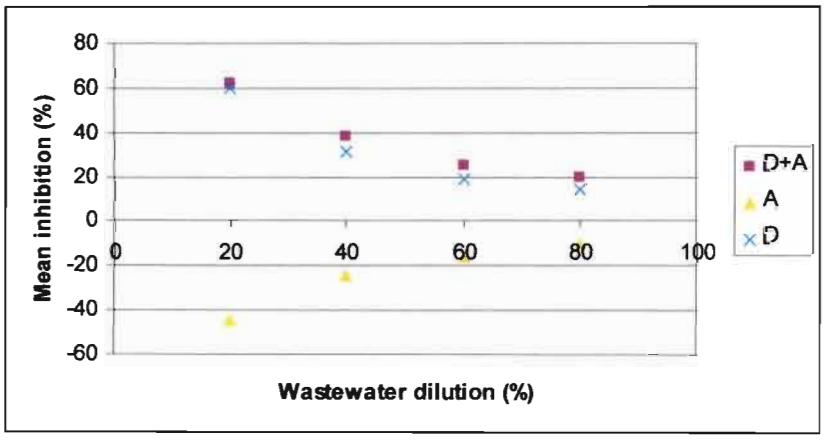

Figure 5.3 Inhibition of nitrification caused by effluent from industries, A, D and a mixture of the two

Results for industries J, C and a mixture (J+C) show that all 4 dilutions showed the same pattern of inhibition for effluent from industries J and C and the effluent mixture (J+C), with the 20% dilution category showing the highest inhibition for all tests, whereas the 80% dilution category showed the lowest inhibition across all the tests (Fig. 5.4). The effluent mixture from industries J and C (J+C), when mixed at a ratio of 1:1 exhibited a higher nitrification inhibition at all dilutions than the two effluents analysed separately (Fig. 5.4). The mixture (J+C) had an average of 32% nitrification inhibition over the 4 dilutions while Industry J had an average of 28% and Industry C inhibited nitrification by an average of 10%.

In general there was no significant difference ($p<0.05$) between the inhibition mean obtained from effluent for Industry J and the mixture (J+C); (Appendix C6) across all dilutions. Industry C, however had an inhibition value which differed significantly from the inhibition means of the effluents from industries J and J+C for all the dilutions.

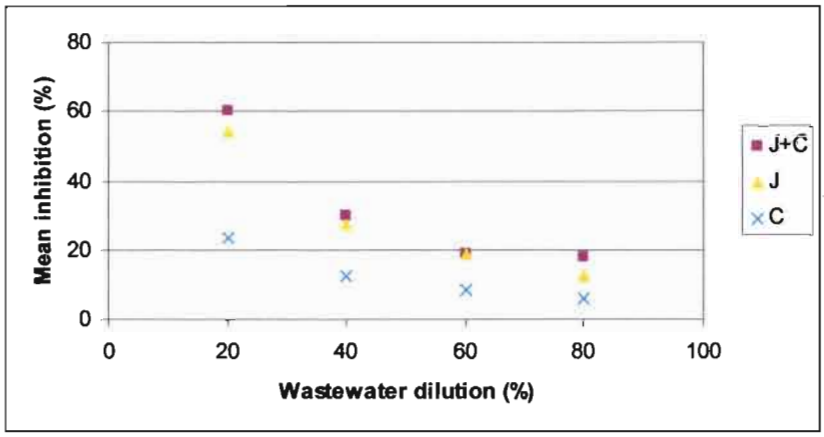

Figure 5.4 Inhibition of nitrification caused by effluent from industries, C, J and a mixture of the two

Effluent from industries B and I inhibited nitrification over the entire range of dilutions (Table 5.4 and Fig. 5.5). The effluent mixture (B+I) and effluents from industries B and I showed similar patterns of inhibition with the greatest inhibition at 20%. The effluent mixture from industries B and I (B+I), when mixed at a ratio of 1:1, showed higher nitrification inhibition at all dilutions than the two effluents analysed separately (Fig. 5.5). The mixture (B+I) had an average of 28% nitrification inhibition over the 4 dilutions while Industry B had an average of 19% and Industry I inhibited nitrification by an average of 17%. Based on the multiple range test there was no significant difference ($p<0.05$) between the inhibition mean obtained from effluents from industries B and Industry I across all dilutions (Appendix C6). At 20% dilution, there was no significant difference ($p<0.05$) between the inhibition means obtained from all the effluents, B, I and B+I.

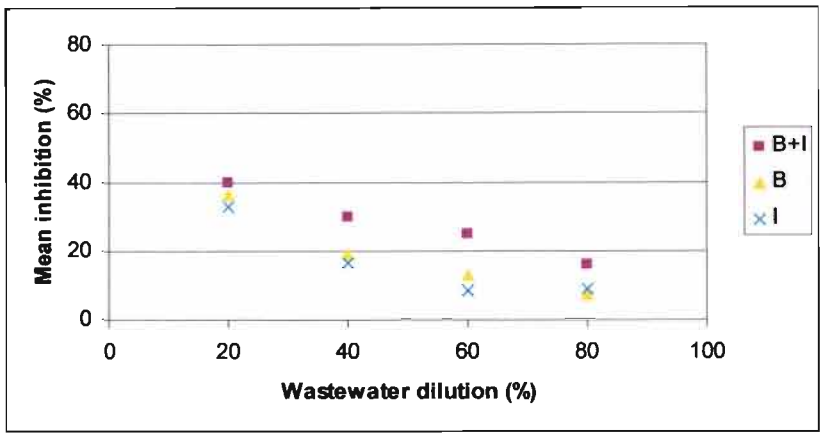

Figure 5.5 Inhibition of nitrification caused by effluent from industries, B, I and a mixture of the two

Effluent from industries G and H inhibited nitrification over the entire range of dilutions (Table 5.4 and Fig.5.6). The effluent mixture (G+H) showed similar pattern of inhibition with the greatest inhibition at dilution 20%. The effluent mixture from industries G and H when mixed at a ratio of 1:1 showed less inhibition than the effluent from Industry G, but greater inhibition than effluent from Industry H. The mixture (G+H) had an average of 16% nitrification inhibition over the 4 dilutions while Industry G inhibited nitrification by 22% and Industry H had inhibition of 12%. At dilutions 60 and 80% there was no significant difference ($p<0.05$) between the inhibition mean obtained from effluent for industry H and the mixture (G+H) (Appendix C6).

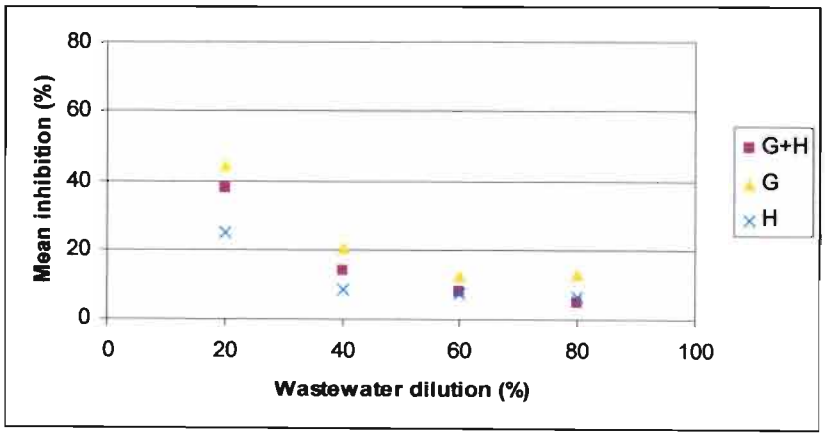

Figure 5.6 Inhibition of nitrification caused by effluent from industries, G, H and a mixture of the two

Effluent from industries E and F inhibited nitrification over the entire range of dilutions (Table 5.4 and Fig. 5.7). The effluent mixture (E+F) showed similar pattern of inhibition with the greatest inhibition at a dilution of 20%. The effluent mixture from industries E and F (E+F), when mixed at a ratio of 1:1 showed higher nitrification inhibition than the effluent from Industry F but lower than the inhibition from Industry E (Fig. 5.7). The mixture (E+F) had an average of 18% inhibition over the 4 dilutions while industries E and F had an average of 23 and 17% respectively. In general, there was no significant difference ($p<0.05$) between the inhibition means of all the effluents; E, F and mixture E+F (Appendix C6) across all dilutions.

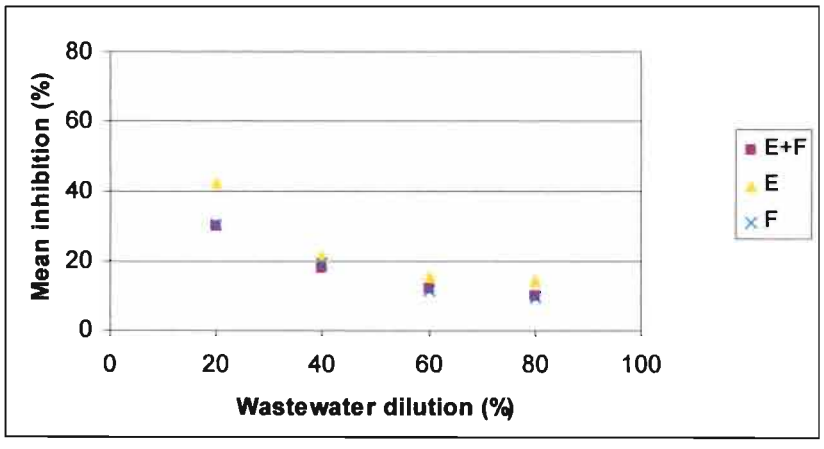

Figure 5.7 Inhibition of nitrification caused by effluent from industries, E, F and a mixture of the two

5.5 Correlation of inhibition with characteristics of industrial wastewater

Wastewater records of industrial effluent characteristics (COD, SS and volume) summarised in Table 5.1 were obtained from eThekwini Wastewater and were analysed for relationships with measured inhibition, using Spearman's correlation coefficient. There are no significant relationships ($p<0.05$) between nitrification inhibition and percentage volume of effluent, or between nitrification inhibition and COD, or between nitrification inhibition and SS. Therefore, it appears that nitrification inhibition cannot be predicted on the basis of commonly monitored wastewater characteristics.

Table 5.6 Correlation coefficients between inhibition and three effluent characteristics (COD, SS and volume)

		Inhibition
COD	Correlation Coefficient	0.236
	Sig. (2-tailed)	0.511
	N	10
SS	Correlation Coefficient	0.116
	Sig. (2-tailed)	0.751
	N	10
Volume	Correlation Coefficient	0.127
	Sig. (2-tailed)	0.726
	N	10

Chapter 6

Discussion

Overall inhibition of nitrification at a treatment plant can be caused by different effluents discharged into the sewer network. Interactions that occur in the sewer network can be expected to affect the effluent characteristics in complex ways before it gets to the treatment plant.

6.1 Effect of dilution

6.1.1 Effect of dilution on inhibition

Rates of microbial activity are inhibited by addition of industrial effluent to domestic sewage, suggesting that industrial wastewater is toxic to microbes that are found in the treatment plant. It is seen in all the inhibition results (Chapter 5) that inhibition declines with increasing dilution. This decline in inhibition can be expected as inhibition is likely to decrease with decreased toxin concentration.

6.1.2 Useful dilution for the nitrification inhibition tests

The greatest significant difference in the combination of screening tests for nitrification inhibition was at 40% dilution (Table 5.3), which seems appropriate to use for purposes of identifying problems of wastewater inhibition. The value of 40% is similar to that of Swedish researchers who showed that 50% is the most appropriate dilution when testing the influent to a wastewater treatment plant with respect to nitrification inhibition (Jönsson, 2001). Hence, a 40% to 50% dilution should be used to test the effect of influent on the Amanzimtoti Wastewater Treatment Plant on nitrification inhibition. The suggestion here is that this can be used as an *early warning tool*. Thus a 40% dilution should be used to monitor potential nitrification inhibition events by wastewater pollution control officers as well as by the superintendents of Amanzimtoti Wastewater Treatment Plant.

6.2 Acclimation

The screening tests at Amanzimtoti Wastewater Treatment Plant revealed that there was inhibition of nitrification at the treatment plant. Inhibition varies temporally in a way that suggests that nitrifying microorganisms may be acclimating to the influent. This reinforces the fact that long-term effects are different from short-term effects observed. The fact that this investigation which was done over a period of 4 weeks, revealed that sludges can often acclimate to inhibitory conditions, is remarkable.

In the investigation of Swedish municipal wastewater (Jönsson, 2001), described in Chapter 2, all samples from plants where inhibition by industrial effluent was detected were tested using the sludge of the local plant if a nitrifying activated sludge was available. Ten different wastewaters were tested using activated sludge from the corresponding treatment plant and the results were compared with the inhibition on a single reference sludge. In all cases it was found that the inhibition was less for the local sludge than for the reference sludge, suggesting microbial acclimation with respect to local industrial effluents.

Tolond (2003) compared inhibition of nitrification between the Kingsburgh treatment plant (which is 100% domestic) and the Amanzimtoti Wastewater Treatment Plant. The results showed that Amanzimtoti influent was very inhibitory to the Kingsburgh sludge with an average inhibition of 43.47% at dilution 20%. This was concluded to be a result of the non acclimated-nature of Kingsburgh activated sludge to industrial effluent, as it only receives domestic effluent that is assumed to be less toxic than the industrially-loaded wastewater at Amanzimtoti. The study of Tolond (2003) also showed that inhibition occured when Kingsburgh influent was tested on the Amanzimtoti activated sludge compared to when Amanzimtoti influent was tested in the Amanzimtoti sludge. This was probably because the Kingsburgh influent contained no industrial effluent, only domestic effluent, and the microbial biomass in the Amanzimtoti sludge was acclimated to the presence of toxins in industrial effluent. Activated sludge can become adapted by either an elevated resistance on the part of the nitrifying organism itself (physiological or structural adaptation in the biological sense) or by a change in bacterial community structure as a whole where different microbial species are present that are able to resist inhibiting substances (Jönsson, 2001).

Therefore, it can be concluded that activated sludge becomes adapted, at least to some extent, to toxic substances often present in the wastewater.

6.3 Nitrification inhibition in Amanzimtoti activated sludge exhibited by the 10 surveyed industries

Temporal variability in inhibition over time scales of months is consistent with levels of industrial activity over these same time scales, suggesting that industrial effluent is a major cause of inhibition at the treatment plant. Microorganisms are able to acclimate to these circumstances, but this is likely to happen over long time periods. In order to fully understand the difference in effects of Amanzimtoti influent on activated sludge, more thorough investigations of nitrification inhibition over an extended period are required. In the case of the Amanzimtoti Wastewater Treatment Plant, less inhibition was observed during the month of December than during both November and January. Inhibition during January was also less than during November. This could be due to the fact that most of the industries were closed for about 2 weeks in December and early January before and after Christmas, which resulted in lower industrial effluent loads being discharged to sewer and entering the sewage works at this time. At this time the discharge of domestic sewers is not likely to decline, but it should either remain constant or increase due to large numbers of holiday-makers at the coast. This supports the hypothesis that inhibition at the treatment plants is mainly caused by industrial effluent, and justifies the special focus on solving this problem.

6.3.1 Industrial effluent stimulating the activity of the nitrifying bacteria

In the search for important contributors of toxic substances in municipal wastewater, 10 different industrial effluents were examined. Industry A is an alkyl resin manufacturer. The effluent from this industry was found to be the only one that stimulated the nitrifying bacteria present in Amanzimtoti activated sludge and consequently promoted nitrification (Table 5.4). The explanation for this behaviour is not known, but analysis of records from the eThekweni Water Services, Wastewater Department, revealed that Industry A had an effluent with a high COD content. From the literature, it would be expected that this effluent inhibits nitrification (Jönsson, 2001). The average COD content in the effluent was over 15000 mg/L, which is very high compared to other industries and in relation to the DWAF standard of 75mg/l COD. The stimulation of nitrification by effluent from Industry A may be due to the presence of a substrate in the effluent that stimulates nitrification. This may be direct or through inhibition of competing microorganisms. Tolond (2003) also found this result by looking at the test tube contents under light microscope and found most visible organisms to be dead, thereby supporting the hypothesis that competitor were inhibited.

6.3.2 Effluent of 10 surveyed industries inhibiting the activity of the nitrifying bacteria

Among the 10 surveyed industries, effluent from industries B, D, E, G and J showed higher nitrification inhibition than effluent from the other five industries, with effluent from Industry D having the highest nitrification inhibition. Nitrification inhibition for Industry D across all the dilutions for both ammonia degradation and nitrate production was approximately 30%. Industry D had effluent which comprised a low concentration of metals, but, these levels differed little from industries J, I, H and F. Additionally, Industry H was one of the industries that was least inhibitory, but the effluent had a high metal concentration. Therefore, it cannot be concluded that the high percentage of inhibition in the case of effluent from Industry D was due to its metal content. Based on this study it is impossible to determine the pollutant causing inhibition.

Overall, industries B, D, E, G and J produced the most inhibitory industrial effluents. Although these industries contribute only 0.5, 5.3, 0.16, 0.05, 0.26%, respectively to the daily load of Amanzimtoti Wastewater Treatment Plant (Table 3. 1) and are therefore considered "small industries", these results should not be ignored.

At the 80% dilution, the nitrification inhibition results obtained for all the industries except Industry A are similar, and it is difficult to distinguish amongst them. At the 20% dilution, the differences among the effects of each industrial effluent on nitrification at Amanzimtoti Wastewater Treatment Plant can be clearly evaluated. Results obtained at the 20% dilution could therefore be used as a *decision making tool* by the wastewater pollution officers to identify industries requiring close monitoring. Thus, industries with high inhibition values at the 20% dilution should be monitored closely by pollution control officers.

6.3.3 Controlling inhibition of nitrification at Amanzimtoti Wastewater Treatment Plant

As briefly mentioned previously, Table 3. 1 shows that the contribution of each industry to Amanzimtoti daily load is less than 20%. This means that the results that most closely resemble operational conditions are the data obtained at 80% dilution. Consequently, wastewater pollution control officers can use the results obtained at 80% dilution as a *monitoring tool*. These results are able to provide pollution control officers with a crude idea of the possible nitrification inhibition attributable to 10 industries at their current contribution to Amanzimtoti daily load. Based on these results, pollution control officers can evaluate

whether it would be beneficial for any of the 10 industries either to change the composition of their effluent in order to lower nitrification inhibition, or to reduce their contribution to the daily load at the plant.

Jönsson (2001) suggests that industrial effluent demonstrating nitrification inhibition of more than 20% but less than 50%, should not be permitted unless it can be confirmed that the discharge will not harm the processes at the wastewater treatment plant. Accordingly, the nature of the toxicity found in the highly inhibitory effluent (Industry D) should be further investigated. If the constituents of industrial effluent can be identified, the toxicity can be judged based on the known degradability and toxicity of separate substances. If the composition of the effluent is complex or if the constituents are unidentified, the efficiency of detoxification techniques can be shown and verified before and after the treatment, by measurement of inhibition. This method has been implemented in the studies in Sweden for biological detoxification of tar–water. Jönsson (2001) further recommends that if the inhibition is greater than 50%, a permit to discharge should not be issued. In this study, if one examines the results obtained at 80% dilution, which is closest to the situation in reality, all industries are below this 50% inhibition level and therefore, based on the Swedish study (Jönsson 2001), they would be permitted to discharge their effluent into the public sewer system. However, it should be noted that South Africa should not blindly adopt Swedish recommendations.

6.4 Combined effects of more than one effluent samples

The effluent mixtures, (D+A, J+C and B+I) showed an increase in nitrification inhibition when mixed (Fig. 5.3-5.4), compared to the individual samples measured separately. This could mean that when the different wastewaters are mixed, there could be formation of a new substance that is inhibitory to nitrification. An alternative explanation for these results could be that the inhibition results from these mixtures are additive in some way. However the mechanism is not simply additive as the mixtures show less inhibition than the sum of the inhibitions when tested separately.

Effluent mixture G+H showed lower inhibition than effluent from Industry G but higher than Industry H. A similar pattern was shown by effluent mixture E+F, with effluent from Industry E producing higher inhibition and effluent from Industry F producing lower inhibition than

the mixture of the two. The explanation for these results could be that the toxic substances in the effluents are diluted in the mixtures or they form some new compounds that are less inhibitory.

Effluents are mixed in complex ways before entering the wastewater treatment plant, and the effects of combined effluent discharges on inhibition is not predictable from the effects of individual effluents alone. It is therefore difficult to predict the inhibitory effects of bulk effluent discharge from the individual industries on their own (Jönsson 2001).

The findings from this study revealed that volume, SS and COD do not correlate with inhibition of nitrification and therefore cannot be used predictively in the management of inhibition of nitrification.

6.5 Comparison with previous studies

The aim of this study was to determine whether the Amanzimtoti Wastewater Treatment Plant is experiencing inhibition of nitrification and whether the industries discharging into the treatment plant contribute to this problem. The results of this study suggest that inhibition is occurring and that industrial effluent may contribute to inhibition. The inhibition calculated for the nine inhibiting industrial effluents was significantly lower than the inhibition exhibited by industries in the Swedish studies conducted by Jönsson (2001). Jönsson *et al.* (2000) reported high levels of acute inhibition (up to 90% inhibition) several times for a 20% dilution of incoming industrial effluent.

The study conducted by Harremoës *et al.* (1998) in the city of Copenhagen (Denmark) concluded that the nitrification inhibition detected at two wastewater treatment plants was as a result of toxins present in industrial effluent. Inhibition of higher than 50% was found in 24% of the effluents tested.

After pilot studies provided substantial evidence that the wastewater influent at the Copenhagen Treatment Plant was inhibiting nitrification, further studies were conducted and co-operation programmes between wastewater treatment plants and the dominating industries in the catchment area were established. This co-operation led to a steady decrease in the rate of nitrification inhibition. Source control and a general concept of cleaner technology made

industries improve their operations with respect to discharge of nitrification inhibitors to the sewer system (Harremoës *et al.*, 1998).

A study carried out in the city of Linz (Austria) recorded 20% of all industrial effluents sampled having an inhibitory effect (Kroiss *et al.* 1992), which is again lower than found in the current study. However, two of the wastewaters in Linz were completely inhibitory, which was not observed in the present study. Generally, both the literature and the current study show that industrial effluent may have an inhibitory effect on nitrification in activated sludge in municipal sewage, but to differing degrees, depending on the composition of the industrial effluent, the dilution effects of the sewer system, and the efficiency of the wastewater treatment plant.

The five most important sources of inhibition exhibited more than 20% inhibition at 20% dilution on all days, with Mondays and Wednesdays showing the highest inhibition and Fridays the lowest inhibition. Results from six of the most important sources of inhibition from a study done in Stockholm, Sweden showed different trends from this study (Jönsson 2001). A printing industry exhibited constant inhibition of less than 10% on all days when samples were taken, while another printing industry showed an inhibition pattern typical for industries that practice batch production: two days of high inhibition and five days of no inhibition at all. The inhibition found in car-wash wastewater was higher during week-ends than during weekdays. Therefore, the pattern of nitrification inhibition is likely to depend on the type of industry and the discharge pattern.

6.6 Practical applications of nitrification inhibition studies

The practical applications of investigations of nitrification inhibition by industrial effluents are illustrated by the situation in the City of Copenhagen, described in Chapter 2. After pilot studies provided substantial evidence that the wastewater influent at the Copenhagen treatment plant was inhibiting nitrification, further studies were conducted and co–operation programmes between wastewater treatment plants and the dominating industries in the catchment area were established. This co-operation led to a steady increase in the rate of nitrification. Source control and a general concept of cleaner technology made industries clean up their operations with respect to discharge of nitrification inhibitors to the sewer system (Harremoës *et al.*, 1998).

However, accidents may occur, and acute situations must be handled. As a result of the inhibition accident at Helsinborg when nitrification process was brought to a standstill, a plan of action for acute situations was drawn up. This plan proved to be useful on later occasions, when large quantities of acid were spilled by an industry. Furthermore, ambitious co-operation programmes agreed between the sewage works and major industries in Helsinborg have been realized since the results from Jönsson's study (2001) and numerous others revealed complete inhibition for several of the industries sampled. "The common awareness of environmental issues and the inconsistency of presenting a good corporate image while at the same time permitting hazardous discharges are probably also contributory causes to a remarkable general decrease of toxic substances in the wastewater in Helsinborg" (Jönsson, 2001).

6.7 Controlling inhibition of nitrification at wastewater treatment plants

Measuring nitrification inhibition at wastewater treatment plants is still new in most developing countries. In order to control inhibition of nitrification, there has to be awareness so that action can be taken. Jönsson, (2001) defined four main areas of interest as:

- Actions against inhibition of nitrification when it has been established that inhibitory substances exist in a catchment area.
- Taking the possibility of toxic wastewater into account when designing treatment plants that include nitrification.
- Outlet requirements for industries for the prevention of future nitrification inhibition.
- Controlled inhibition of nitrification as a way to create new process alternatives.

As it was established in this study that the Amanzimtoti Wastewater Treatment Plant experiences inhibition of nitrification, a strategy should be designed that can be used in order to minimise the existing problem. A plan of action for acute situations should be established, and causes for incidents investigated. Screening tests for nitrification inhibition are a logical route for monitoring the effluent. In cases where it is not possible to perform the tests, an alert system can be put in place to monitor the effluent as it was suggested that the test is meant as a quick, simple and practical test for pollution monitoring and treatment plant management. It is not meant for, nor is it suitable for, studies of kinetics of nitrification (Jönsson, 2001).

6.7.1 Alert criteria

The alert criteria proposed here are a modified version of air pollution alert criteria proposed by Diab and Scott (2000).

In cases where the possibility of an acute inhibition incident exists, there should be an early warning system in place. Automatic measurements of inhibition of nitrification at the inlet of the wastewater treatment plant, at the junctions in the sewer network, or at individual industries, may provide this warning. However, there should be sufficient research and knowledge of toxic substances which can be correlated to inhibition of nitrification. There should also be enough information concerning all the industries discharging to a particular wastewater treatment plant, including the chemical profile of the different effluents. The periods over which the effluents are likely to contain toxic substances and the discharge pattern should also be known. This is important because some of the industries have more than one different processes and the effluent composition therefore varies. Furthermore, some industries practice batch production where inhibitory substances are released in large quantities over a very short period of time. Therefore, toxic substances may be released during certain days and not during other days.

Once enough information is available, the alert criteria can be devised. The first alert signal would advise the wastewater treatment plant of toxic substances to be released. At this stage, the officials at the treatment plant would not have to take any action except that sufficient methods to enhance the nitrification process would have to be on hand. The first alert level can be upgraded to a second alert level when the industries have analysed the effluent and found the toxic substances which are known to cause inhibition. The screening tests would also have to be conducted when possible, to confirm nitrification inhibition. If the effluent quality is improved, the alert level will be downgraded to an all clear system. A third alert level would require wastewater treatment plant officials to take action within a short period so as to overcome inhibition of nitrification as toxic substances will be present in the plant. These measures would have to be continued until an all-clear alert level is given. In the December holidays, the all- clear alert system would be expected as most industries close for the Christmas holidays. A summary of the proposed alert criteria is given in Table 6.1.

Table 6.1 A summary of the proposed alert criteria

Alert stage	Description
1st alert system	Industries expect the effluent to contain toxic substances known to cause inhibition of nitrification, due to processes that have been carried out recently.
2nd alert system	Effluent has been analysed and found to contain toxic substances known to cause inhibition of nitrification. Screening tests have been conducted (where possible) and results show inhibition of nitrification.
3rd alert system	Toxic substances which cause inhibition of nitrification are present in the treatment plant.
All clear alert system	No discharges likely to inhibit nitrification anticipated.

6.7.2 Finding causes and explanations of incidents

The Amanzimtoti Wastewater Treatment Plant is a small plant to which many industries discharge their effluent. The question of finding the cause of an inhibition incident could in principle be straight-forward if all the industries were well investigated. For large treatment plants which are connected to a large number of industries, the task is likely to be more complex. Jönsson (2001) suggests that there are two different approaches that can be applied in order to identify the sources of inhibiting substances in a catchment area:

- Investigations in the sewer network in order to identify industries that discharge toxic substances.
- On-site investigations of effluents from industries, since knowledge about the individual industrial discharges can assist in explaining incidents, especially in small catchment areas.

In addition to external sources of inhibition, internal sources at wastewater treatment plants should not be neglected.

Small industries should not be overlooked as sources of inhibition, because there is no connection between the size of an industry and the toxicity of its wastewater. Industry C is one of the biggest industries among the 10 surveyed industries, but the inhibition was lower than for most of the other industries. It is possible that a few cubic meters of highly

concentrated toxic discharge might disrupt the whole nitrification process at a wastewater treatment plant.

Grüttner *et al.* (1994) found that the sources of nitrification inhibiting substances are more likely to be found in industrial than in domestic areas. Therefore, for wastewater treatment plants serving small catchment areas, there is little point in searching for sources of inhibiting substances in the sewer system. Instead it may be worthwhile to get into direct contact with the few existing industries.

6.7.2.1 Applications of the screening method in management and control of a WWTP

The purpose of this study was to provide eThelwini Wastewater Services with a tool for making informed decisions in order to control nitrification inhibition at wastewater treatment plants in the future. Figure 6.1 illustrates a modification of a possible strategy of control of nitrification inhibition proposed by Grüttner *et al.* (1994) that could be utilised by the wastewater pollution control officers in the Prospecton Industrial Area. The diagram shows sampling and control strategies for sources of toxic substances in the catchment area, where three investigation levels are defined; inlet to treatment plants, junctions representing selected parts of the catchment areas, and connections for individual industries. Depending on the degree of inhibition found at each level, actions according to Figure 6.1 may be taken and the following important tools are discussed:

- early warning tool
- monitoring tool
- decision making tool

In a treatment plant, a program for continuous assessment and control of plant is appropriate so that there may be a detection of irregularities or interruptions in the plant. For treatment plants that are experiencing inhibition in the inlet, there should be control of the load discharged according to general guidelines. However, if a treatment plant is not experiencing any inhibition, the program for continuous assessment should always be in place. Figure 6.1 illustrates three important tools that can be used to reduce inhibition of nitrification. In most cases, inhibition of nitrification is detected at the inlet of the treatment plant and this can be considered as an *early warning tool*, which should be used to initiate monitoring of the effect of industrial effluent on microbial activity.

If a program of continuous assessment does not operate to full capacity, inhibition of nitrification may exist in the treatment plant without being noticed. Therefore it is important that there is a monitoring program at the junctions. The screening method that was used in this study is one such an example that may be used for monitoring at the junctions. As soon as inhibition is detected at the junctions, the load that goes into the treatment plant should be controlled according to general guidelines. The monitoring may be used to determine the relationship between effluent and inhibition, since effluent is likely to vary in quality and quantity over time. The *monitoring system* consisting of screening test methodologies, people, laboratories and a plan of action should be able to trace the source of inhibition in order to find out how the sources of inhibition vary from each other. This will assist in setting individual requirements for each industry.

The discharges from individual industries should be monitored. The industries that discharge wastewater of a persisting inhibitory nature should also introduce a program of toxic effluent reduction by eliminating inhibitory chemicals. This may assist in setting standards for each individual industry. A *decision making tool* which will form the basis for charging industries tariffs if the inhibition of nitrification measured at each industry is above the general criteria is also necessary.

High concentrations of inhibitory substances originating from industries should be reduced to lower concentrations in the junctions because of dilution of the inhibiting substances. Thus, most of the toxic substances are expected to be more or less eliminated at the inlet to the wastewater treatment plant. Inhibition of nitrification measured at the inlet to a wastewater treatment plant is, however, a function of discharges of inhibitory substances from all industries that discharge into the treatment plant. Apart from the fact that inhibition patterns of different industries will be superimposed when observed through a sample taken at the inlet of a wastewater treatment plant, synergistic or antagonistic effects of different inhibitory substances might also blur the picture. This makes prediction of nitrification inhibition at the treatment plant complex.

Level of sampling, responsibility and control

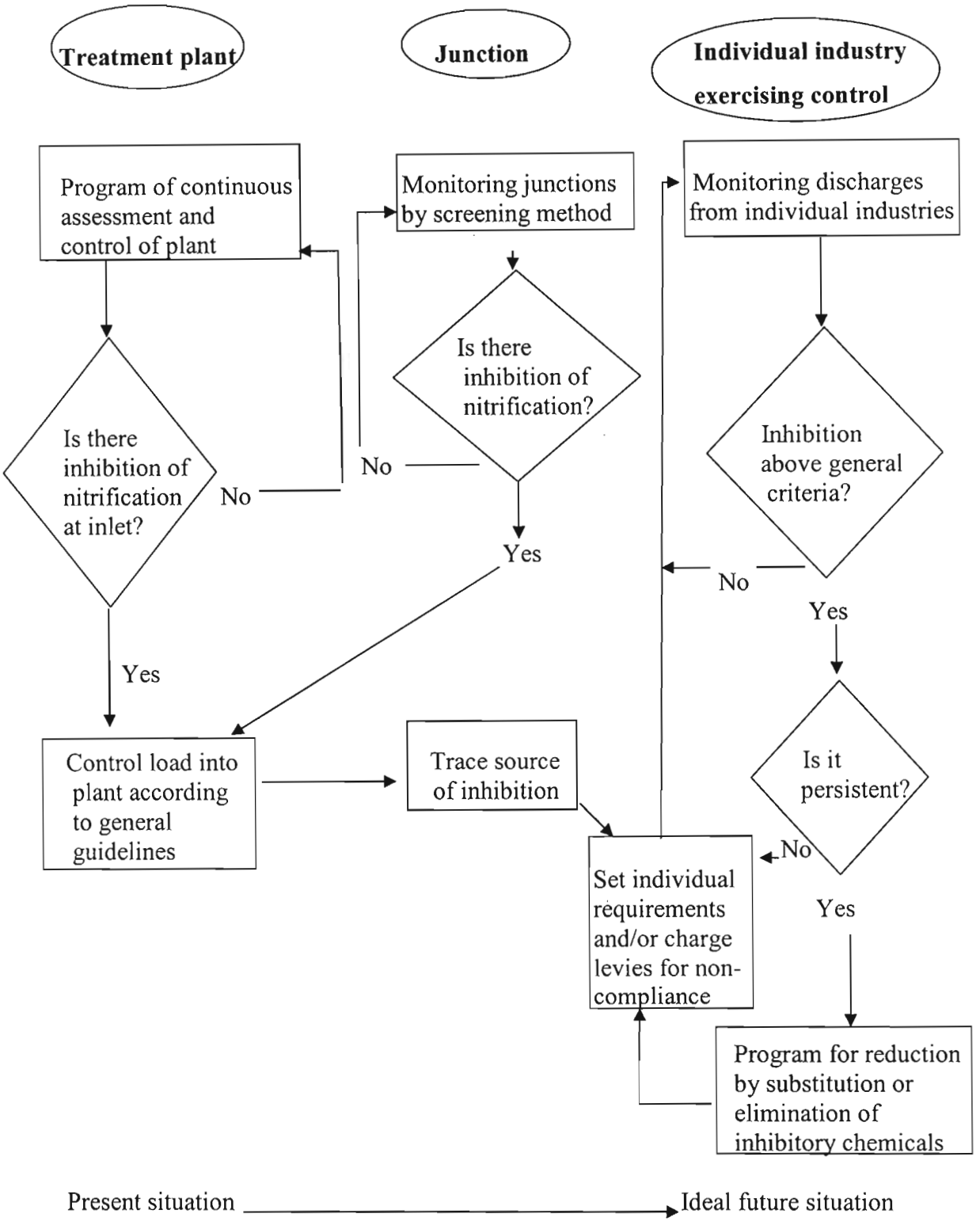

Figure 6.1 Suggested strategy for reducing nitrification inhibition. Modified from Grüttner et al. (1994).

6.7.3 Effluent requirements for industries

As mentioned previously, sources that discharge inhibitory substances are likely to be industries. Therefore, toxic substances should ideally be removed from industrial wastewater prior to its release to sewer. Toxicants which are known to cause harm in the treatment plant should not be discharged into the municipal sewer system at all as preventive measures are preferable to corrective ones. Therefore, the municipality should not accept toxic wastewater unless it is within the discharge limits stipulated.

The proposed alert criteria might be useful, but it means investment in instrumentation in order to obtain early warnings. Nevertheless, the best way of avoiding inhibition accidents at wastewater treatment plants would be to declare that toxic wastewater is not acceptable and therefore to place emphasis mainly on preventing toxic substances from entering the sewer system, not into handling problems when they have already arisen.

The municipality might also want to use penalties or tariffs calculated from the extent of inhibition exerted by a wastewater as a means of encouraging industries to reduce the amount of toxic or inhibitory substance in their discharge. Using this sort of financial incentive to industry, there is double benefit of making the polluter pay the real cost of treatment (extra aeration, and ultimately extension of the treatment facility) and a real incentive for the industry to reduce their toxic emissions.

6.8 Possibilities and limitations: The screening method for estimation of nitrification inhibition

The screening method for estimation of nitrification inhibition is suitable for the first screening of the toxicity of both municipal and industrial effluent to nitrifying bacteria. The following discussion identifies the potential uses and limitations of the screening method for estimation of inhibition of nitrification (Jönsson, 2001). The following factors are discussed:

(1) adaptation of activated sludge to toxins and the role of the choice of activated sludge;

(2) role of analysing samples on different occasions (reproducibility);

(3) role of suspended solids concentration used in the tests (toxin–to–biomass ratio) and

(4) the inhibitory effect of mixtures of toxins compared to the effect of pure toxins.

(1) Adaptation and the role of the chosen activated sludge type

Nitrifying bacteria present in activated sludge populations have been reported to develop resistance to toxins present in wastewater received in small doses over a relatively long duration of time (Koopman and Bitton, 1986). This effect of adaptation has been shown by Tomlinson *et al.* (1966) for heavy metals and by Downing *et al.* (1964), Tomlinson *et al.* (1966) and Knoetze *et al.* (1980) for organic substances.

Hugo (1967) suggests two strategic ways in which an organism can become resistant to a toxin. Firstly, the organism can gain an alternative route for formation of the inhibited end–product. Secondly, the organism may develop enzymes capable of destroying the toxic agent. In addition, the rest of the activated sludge community may have developed an ability to degrade or inactivate the inhibitor (Jönsson, 2001).

In her study (2001), using activated sludge from two different wastewater treatment plants, Jönsson, suggests that activated sludge becomes adopted, at least to toxic substances often present in the influent to a wastewater treatment plant. Jönsson further suggests that at low or moderate inhibition levels the effect on the sludge of the respective plant is less than the reference sludge, while at high inhibition levels the effect is more similar. Therefore at high inhibition levels, the adaptation of the sludge becomes less. Several studies conducted by Jönsson *et al.* (1996), Winther–Nielsen and Jansen (1996) and Jönsson *et al.* (2001) have revealed that different activated sludge types have different degrees of adaptation to certain substances which results in different degrees of inhibition.

(2) Reproducibility

The study conducted by Jönsson (2001) involved duplicating the tests for three of the activated sludge types, over a period of two years. The results were similar even when the interval between the two tests was as long as a year. This seemed to be the case, irrespective of whether the activated sludge was adapted to industrial effluent or not. It would be both useful and interesting, in the current study, to determine whether the results obtained are reproducible over an extended period of time.

(3) The role of the toxin–to– biomass ratio

Inhibition found by the screening method, is calculated as a percentage of a reference nitrification rate, which means that it is not expressed in relation to the biomass concentration (Jönsson, 2001). Nevertheless, some researchers have claimed that inhibition is a function of the toxicant-to-biomass ratio and not simply of the concentration of the toxin itself whilst others have just stated the importance of the biomass concentration (Fitzgerald, 1964). However, usually the activated sludge type is regarded as a more important factor in determining inhibition than the concentration of activated sludge. In this context, inhibition is seen not as a function of the toxin– to–biomass ratio but as a function of the concentration of toxin or percentage of wastewater.

(4) Combined effects of two or more substances

It is not always easy to predict inhibition resulting from the mixtures of two or more substances. Total inhibition may be determined by both a synergistic and antagonistic effect. Therefore, in searching for the cause of inhibition, laboratory experiments that test pure substances may be beneficial. If it is established that a wastewater harbours certain toxic substances, it may help in explaining the toxicity if these substances are tested both individually and in heterogeneous mixtures. Several studies have demonstrated that inhibition results from analyses of a mixture of pure substances are lower than the inhibition resulting from analyses of pure substances alone. Using calculations of TEF (toxic emission factor) values for major industries in the catchment area as a basis, Jönsson (2001) claimed that it was not possible to find the same inhibition level as that measured for samples of the influent of the wastewater treatment plant. In Sweden, in controlled investigation of seven wastewater types by the Swedish EPA, nitrification inhibition was calculated for the separate wastewaters and for a mixture of them (Jönsson, 2001). The investigators had no success predicting the inhibition of the mixture from that by separate wastewaters. Explanations are provided for this; firstly, the investigations do not include each and every industry in the catchment area. Secondly, it is almost impossible to estimate correctly the effects of dilution and the retention of the industrial effluent in the sewer system as the flows vary stochastically.

This is supported by the current study whereby inhibition by mixed wastewaters either higher or lower than the inhibition by each of the ten tested effluents. This type of situation can be seen when comparing Figure 5.3 and Figures; 5.5, 5.6, 5.7. It is clear that the overall inhibition from the effluent of all 10 industries feeding into Amanzimtoti Wastewater

Treatment Plant is significantly higher or lower than the overall inhibition of their mixtures measured as influent at Amanzimtoti Plant.

For more detailed and conclusive results, step–by–step routine sampling and analysis should be performed at the inlet to the wastewater treatment plant, at the industries in the catchment area, and at the junctions in the sewer network (Kroiss *et al.*, 1992).

6.9 Limitations of the study

1. Internal sources of nitrification inhibition in the Amanzimtoti Wastewater Treatment Plant were not taken into consideration. It has been shown that gasification, incineration and drying of organic matter often give rise to wastewater consisting of condensates or scrubber water from the gas-cleaning systems, which have been shown to be strongly inhibitory to nitrifying bacteria (Jönsson 2001).

Studies in Sweden have confirmed that wastewater from the gas-cleaning system of activated sludge incineration plants is highly inhibitory to the nitrification process. Incineration of activated sludge is becoming a routine in Sweden, as municipalities are not able to find a market for activated sludge as a fertilizer as easily as they did in the past. Furthermore, Starberg *et al.* (1999) confirm that new Swedish regulations to be introduced in 2005 will prohibit disposal of organic material such as activated sludge, at refuse dumps. This may further force wastewater treatment plants to incinerate activated sludge, which may lead to considerably altered Swedish wastewater quality (Jönsson, 2001).

In relation to the present study, no attempt at identifying the inhibitory substances in the Amanzimtoti Wastewater Treatment Plant has been made. The Amanzimtoti Wastewater Treatment Plant does not dry or incinerate the activated sludge at the plants, as it is pumped to the Southern Wastewater Treatment Plant for disposal. However, it is strongly proposed that in the future, all internal sources of nitrification inhibition should be thoroughly investigated as this may add valuable information to the issue of nitrification inhibition at wastewater treatment plants.

2. There was no specific or general identification of what components of industrial inhibition caused inhibition, and therefore results from industries cannot be easily extended to similar industries.
3. All of the results and final conclusions are limited by the "grab" nature of samples.

Chapter 7

Conclusion and recommendations

Industrial discharges from a number of industries in the catchment of the Amanzimtoti Wastewater Treatment Plant have been shown to discharge substances that contribute to nitrification inhibition at the Amanzimtoti Wastewater Treatment Plant. In an investigation of influent wastewaters from industries in the Prospecton Industrial Area, inhibitory substances were detected in all four weeks of sampling. The level of inhibition was in general 29%, with the 20% dilution category showing the highest inhibition. Inhibition of nitrification was reduced during December, at a time when industries close, supporting a hypothesis that industrial contributions to the wastewater treatment are the main source of inhibition. While this may be true, internal sources of inhibition should not be neglected. During the period of the survey, there was no general pattern of nitrification inhibition shown in the plant, but more studies should be done. These could probably investigate monthly or annual variations in nitrification inhibition in the plant.

7.1 Inhibition of nitrification by industrial wastewater

Inhibition of nitrification was found in 9 of 10 surveyed industries, with effluent from Industry D showing the highest inhibition of an average of 30% over the 4 dilutions. The least inhibitory effluent was from Industry C with an average of 10%. The industries each contribute significantly different percentages of the daily volume of flow to Amanzimtoti WWTP, and there was no correlation found between the daily volume contribution and the inhibition. There was also no obvious correlation found between inhibition, COD and SS. For all the surveyed industries, the greatest inhibition was observed at 20% and the least at 80% dilution. Industry A was found to stimulate nitrification. Industries B, D, E, G and J were found to have higher inhibition than the other 4 surveyed industries, but there was no similar inhibition pattern for them.

The results of industrial wastewater testing showed that the extent of nitrification inhibition varies substantially between individual industries and at the treatment plant. Therefore, it is

not always possible to draw general conclusions about industrial wastewater regarding nitrification inhibition. The inhibition found for the industrial wastewater cannot be directly related to the measured inhibition of influent water to Amanzimtoti Wastewater Treatment Plant. Even though there were 5 industries identified as being the important sources of inhibitory substances to the Amanzimtoti Wastewater Treatment Plant, the inhibition contributed by other industries have to be known in order to explain all the effects at the Amanzimtoti Wastewater Treatment Plant.

At 80% dilution, the nitrification inhibition results obtained for all the 9 industries are similar and it is difficult to distinguish between them. At the 20% dilution, differences between the effects of each industrial effluent on nitrification were clearly distinguished. Results obtained at 20% dilution could therefore be used as a decision-making tool by the wastewater pollution officers to identify industries requiring close monitoring.

From the study, it became clear that the inhibition potential of mixtures of industrial wastewaters cannot be readily predicted from nitrification inhibition by individual wastewaters. New compounds may be formed that are more inhibitory than when the wastewaters are not mixed.

There are also effects of dilution and the retention of the industrial wastewater in the sewers. These may act positively or negatively to the nitrification processes that occur at the treatment plant. Therefore, this means that when wastewaters are mixed in the sewer, the wastewater characteristics might change. It can be concluded that many mechanisms exist that add to the complexity of inhibition investigations. Additional studies should still be conducted in order to point out any special branch of industry that continuously delivers inhibitory wastewater. Industrial development brings about a continuously changing composition of wastewater and therefore, preparedness for dealing with new problematic substances must be high in a rapidly changing, modern society. The screening test used in this study is a short-term inhibition test. This does not mirror detailed effects on a wastewater treatment plant, but is a useful tool when identifying and solving problems.

7.2 Applications of outcomes of the study

The results of this study may assist wastewater treatment authorities at the Amanzimtoti Wastewater Treatment Plant to determine the sources of inhibition of nitrification, which would be achieved by performing screening tests on individual industries. Further studies should be conducted to determine the nature of the inhibitory substances in the individual effluents. Industries can also use the screening technique to determine whether their effluent is inhibitory. There are more studies still to be done to determine the specific chemicals or process streams containing inhibitory substances, and thus solve the problem at source. By coupling the study with more detailed scientific investigation of nitrification inhibition and computer simulation of the wastewater treatment plant, the authorities will be able to quantify the cost of accepting effluents containing inhibitory substances into the sewer and transfer this cost to the relevant industries. This will ensure that the *"polluter pays principle"* is upheld, and should result in a general decrease in industrial wastewater tariffs to those that discharge effluent without inhibitory substances. The true costing of effluents containing inhibitory substances will result in the postponement of extensions of the wastewater treatment plant (if it causes industries to reduce the toxicity of their effluent), while at the same time maintaining or improving environmental quality.

Similar studies can be conducted at other wastewater treatment plants. For example, The Southern Wastewater Treatment Plant provides 40ML/d of feed water for the Durban Water Recycling Plant. Both of these plants are operated by Vivendi Water Systems and produce high-grade water suitable for industrial use. The recycling of secondary water to industry is a component of the water demand management / water conservation strategy of the Department of Water Affairs and Forestry. Depending on the success of this plant, future plants may be built leading to reduced demand for fresh water.

This study has the potential for positive outcomes at Amanzimtoti Wastewater Treatment Plant similar to those experienced in the city of Copenhagen. Once results have been forwarded to eThekweni Water Services, the authority may decide whether to approach the industries concerned and inform them of the negative effects of toxins on nitrification at treatment plants. Options available are to force local industries to discharge effluent in a more dilute form, or reduce and/or eliminate the nitrifying–inhibiting toxins in the industrial effluent. eThekweni Water Services has the authority and right to charge and fine those

industries that are releasing harmful toxins, as they are impeding the wastewater treatment procedure. It must be borne in mind, however, that this study focuses on nitrification inhibition by separate industrial effluents, and does not test the effects of diluted effluents in the sewer system on nitrification inhibition. Hence, it is recommended that if eThekweni Water Services were to utilise these results, it should be done in conjunction with further tests to establish the effect of the diluted (in the sewer) and mixed industrial effluents on total nitrification inhibition at treatment plants.

7.3 Future directions

Requirements regarding nitrification inhibition should be set and, consequently, applied when outlet permits for industries are to be issued or renewed. The possibilities for a wastewater treatment plant to neutralise the effects of inhibitory wastewater are limited. And therefore, acute inhibition accidents can only be avoided if an early warning system is put in place and if, in addition, possibilities to counteract the effects exist. Consequently, preventive measures are the preferred way to control inhibition.

Possible measures at industries that have been shown to discharge inhibitory wastewater are to change the properties of the wastewater by altered methods of production or by detoxification of the wastewater by internal treatment. Nitrification inhibition measurements on untreated and treated wastewater can be used for judging the detoxification potential of a treatment method. Alternatively, industries can show that the inhibitor is easily degradable or that the biological processes in question easily adapts to the wastewater. One of the ways that eThekwini Water Services could maintain the quality of the wastewater that enters the treatment plant and hence the quality of the water resources, is by identifying all the industries in the Durban Metropolitan Area that are potential dischargers of prohibited wastes or toxic pollutants and the job of protecting the water resource quality begins by eliminating or pre-treating contaminants at their source, before they enter the wastewater stream. The Water Amendment Act (1999) has identified a list of priority pollutants that are either prohibited or strictly limited in discharges to the sewerage system.

In order to apply and enforce discharge limits that protect the collection system, the treatment plant, and the water resources, the eThekwini Water Services could operate an Industrial Wastewater Control Program. The program should be a joint effort or partnership between the eThekwini Water Services, other agencies served by the metropolitan sewerage system, and local industry to control contaminants before they enter the sewer system. In this way, not only the eThekwini Wastewater Services will be responsible for treating wastewater, but industry will also take greater responsibility for their effluents. The program should also issue discharge permits, perform inspections, conduct wastewater monitoring, and enforce sewer discharge standards at industries throughout the entire Durban Metropolitan Area.

In conclusion, this study aimed to further understand the effects of toxins released in industrial effluent on the nitrification process at the Amanzimtoti Wastewater Treatment Plant. Understanding of the toxic compounds and mechanisms that underlie nitrification inhibition is at an early stage. Implementation of controlling measures and plans at the industries and treatment plants are not always easy. Industrial wastewater may undergo interactions in the sewer system because the sewer is a reactor for chemical changes of the wastewater during transport, affecting the quality of the wastewater and thereby the successive treatment process. Therefore, in implementing the control measures for inhibition of nitrification, a full understanding of the wastewater interactions from industry via the sewer system to treatment plant must be developed. This will allow successful nitrification inhibition management and control systems to be gradually improved and implemented.

References

Alleman, J, 1985. **Measurement of Coupled Nitrification-Denitrification in Paddy Fields Affected by Terrazole, a Nitrification Inhibitor.** Departamento de Biología, Universidad Autónoma de Madrid, 28049 Madrid, Spain. 39 (3), 186 - 192 .

Antoniou, P., Hamilton, J., Koopman, B., Jain, R., Holloway, B., Lybertos, G., and Svoronos, S. A., 1990. **Effect of Temperature and pH on the Effective Maximum Specific Growth Rate of Nitrifying Bacteria.** *Wat. Res.* 24 (1), 97-101.

Azevedo, B. D., Mavinic, D. S., and Robinson, H. D, 1995. **The Effect of Ammonia Loading and Operating Temperature on Nitrification and Denitrification of a High Ammonia Landfill Leachate.** *Canadian Journal of Civil Engineering.* 22, 524-534.

Barnes, D. and Bliss, P. J., 1983. **Biological Control of Nitrogen in Wastewater Treatment**. E. & F. N. Spon, New York, NY.

Blum, D. J. W. and Speece, R. E., 1991. **A Database of Chemical Toxicity to Environmental Bacteria and its Use in Interspecies Comparisons and Correlations.** *Research Journal WPCF* 63 (3), 198-207.

Business Partners for Development, 2001. **Water and Sanitation Cluster Focus: Project Synopsis**. Business Partners for Development, Kwazulu Natal, South Africa.

Brock, D. and Madigan, T., 1991. **Biology of Micro-organisms**. 6th Ed Pretoria Hall, Inc.

Charley, R. C., Hooper, D. G., and McLee, A. G., 1980. **Nitrification Kinetics in Activated Sludge at Various Temperatures and Dissolved Oxygen Concentrations** *Wat. Res.* 14 , 1387-1396.

Diab, D. and Scott, M., 2000. **Forecasting Air Pollution Potential: A Synoptic Climatological Approach.** *J. Air and Waste Manage. Assoc.* 50, 1831-1842.

Downing, A., Painter, H. and Knowles, G., 1964. **Nitrification in the Activated Sludge Process**. *J. Proc. Inst. Sew. Purif.* 2, 128-130.

Durban Metro, 1999. Climate of Durban.
http://www.ceroi.net/reports/durban/drivers/Climate/ Accessed 23 July 2003.

Durban Metro Economic Development Department of Corporate Services, 2003. **Fact Sheet1.1**, http://www.Durban.gov.za. Accessed 23 July 2003.

Durban Water and Waste, 1996. **Water for Everyone**. Thomson Press, Durban South Africa.

Durban Metro, 1999. **Durban Metropolitan Area Profile**. Report No1.WEDC Press, Durban, South Africa.

Durban Metro Water, 2001. **Private Sector Partnerships to Serve the Poor**. Thomson Press, Durban South Africa.

Fitzgerald, G.,1964**. Factors in the testing and application of algicides**. *Appl. Microbiol.*, 12(3), 247 – 253.

Focht, D. D. and Verstraete, W., 1977**.** Biochemical Ecology of Nitrification and Denitrification In: **Advances in Microbial Ecology**, Vol 1, Alexander, M. (Ed.), Plenum Press, New York, 135-214.

Gray, N. F., 1990. **Activated Sludge: Theory and Practice**. Oxford University Press, New York, NY.

Groeneweg, J., Sellner B., and Tappe, W., 1994. **Ammonia Oxidation in Nitrosomonas at NH_3 Concentrations Near K_m: Effects of pH and Temperature** .*Wat. Res.* 28 (12), 2561-2566.

Grüttner, H., Winther–Nielsen, M., Jørgensen, L., Bøgebjerg, P. and Sinkjær, O., 1994. **Inhibition of the Nitrification Process in Municipal Wastewater Treatment Plants by Industrial Discharges.** *Wat. Sci. Tech.* 29 (9), 69 – 77.

Harremoës, P., Haarbo, A., Winther–Nielsen, M. and Thirsing, C.,1998. **Six Years of Pilot Plant Studies for Design of Treatment Plants for Nutrient Removal.** *Wat. Sci. Tech.* 38 (1), 219-226.

Helmer, R. and Hespanol, I., 1997. **Water Pollution Control**. E and FN Span, London.

Herold, TH., Zwahlen, F., Bernasconi, S. M., Jordan P., 1998. **Estimated Mean Water Residence Time (d ^{18}o) of Karstic Springs and Karst System Development.** CHYN, University of Neuchâtel, Switzerland.

Hugo, W.,1967. **The mode of action of antibacterial agents.** *J. Appl. Bact.*, 30(1), 17 – 50.

Hynes, R. K., and Knowles, R., 1993. **Inhibition of Chemoautotrophic Nitrification by Sodium Chlorate and Sodium Chlorite: A reexamination**. *Appl. Environ. Microbiol.* 1178-1182.

ISO 9509, 1989. **Water quality – Method for Assessing the Inhibition of Nitrification of Activated Sludge Micro-organisms by Chemicals and Wastewater.** *European Committee for Standardisation (CEN)/ International Organisation for Standardisation (ISO),* Ref. No. EN ISO 9509:1995 E.

Jönsson, K., 2001. **Inhibition of Nitrification in Municipal Wastewater – Sources, Effects, Evaluation and Remedies.** PhD Thesis, Lund University, Sweden.

Jönsson, K., Grunditz, C., Dalhammar, G. and Jansen, J. la C., 2000. **Occurrence of Nitrification Inhibition in Swedish Municipal Wastewaters.** *Wat. Res.* 34 (9), 2455-2462.

Jönsson, K., Aspichueta, E., de la Sota, A. and Jansen, J. la C., 2001. **Evaluation of Nitrification-Inhibition Measurements.** *Wat.Sci. Tech.* 43 (1), 201-208.

Jönsson, K., Magnusson, P., Jönsson, L., Hellstrröm, B., Jansen, J. la C., 1996. **Identifying and Fighting Inhibition of Nitrification at Uresundsverket.** *Wat. Sci Tech.* 33 (12), 29-38.

Kerdachi, D., 2002. **The Review of Industrial Effluent Tariff Structures in South Africa and Guide line on the Formulation of an Equitable Effluent Tariff Structure.** WRC Report No.854/1/02

Knoetze, C., Davies, T. and Wiechers, S., 1980. **Chemical inhibition of biological nutrient removal processes.** *Water SA*, 6: 171 – 180.

Kroiss, H., Schweighofer, P., Frey, W. and Matsche, N., 1992. **Nitrification Inhibition – A Source Identification Method for Combined Municipal and/or Industrial Wastewater Treatment Plants.** *Wat. Sci. Tech.* 26(5-6), 1135 – 1146.

Merck, 2002a. **Instruction Leaflet for Spectroquant Ammonia Test (Art1.14752),** E. Merck, Darmstadt, Germany.

Merck, 2002b. **Instruction Leaflet for Spectroquant Nitrate Test (Art 1.1.23.45),** E. Merck, Darmstadt, Germany.

Metcalf & Eddy, 1991a. Chapter 8: Biological Unit Processes In: **Wastewater Engineering: Treatment, Disposal, and Reuse.** Third Ed. McGraw-Hill, Inc., New York, NY.

Metcalf & Eddy, 1991b. Chapter 11: Advanced Wastewater Treatment In: **Wastewater Engineering: Treatment, Disposal, and Reuse.** Third Ed. McGraw-Hill, Inc., New York, NY.

Nickel, J. C., Ruseska, I, Wright, J.B. and Costerton, J W, 1985. **Tobramycin Resistance of *Psuedomonas aeruginosa* cells Growing as a Biofilm on Urinary Catheter Material.** *Antimicrob. Agents Chemother* 27 (4), 619-624.

Painter, H. A. and Loveless, J. E., 1983. **The Influence of Metal Ions and pH on the Growth of a Nitrosomonas Strain Isolated From Activated Sludge** *J. Gen. Microbiol.* 52, 1-14.

Palmer Development Group, 2000. **Durban South Africa**. WEDC Press. Durban, South Africa.

Pedersen, F. and Peterson, G., 1996. **Variability of Species Sensitivity to Complex Mixtures.** *Wat. Sci. Tech.* 33(6), 109 – 119.

Prosser, J. I., 1989. Autotrophic Nitrification in Bacteria In: **Advances in Microbial Physiology,** Vol 30, A. H. Rose and D. W. Tempest (Eds.), Academic Press Inc., New York, pp 125-181.

SA DWAF, 1999. **Water and Sanitation Program**. South African Department of Water Affairs and Forestry.

SA Government Gazette, 1984. **NO. 9225. Regulation NO, 991. General and Special Standards of Trade Effluent**, South Africa.

SA Government Gazette, 1999. **NO.20706. Act No.45. National Water Amendment Act.** South Africa.

Sedlak, R., Ed., 1991. **Phosphorus and Nitrogen Removal from Municipal Wastewater: Principles and Practice**, Second Ed. Lewis Publishers, New York, NY.

Swedish EPA, 1996. In: Jönsson, K., 2001. **Inhibition of Nitrification in Municipal Wastewater – Sources, Effects, Evaluation and Remedies.** PhD Thesis, Lund University, Sweden.

Symbolism and Terminology in Enzyme Kinetics, 1981. **Enzyme Reactions & Inhibition,** http:// www.chem.qmul.ac.uk/iubmb/kinetics/ek4t6.html#p61. Accessed 20 June 2003.

Talib, S., Jacobsen, T., Vollersten, J., Ujang, Z., 2002 In: **Anoxic Transformations of Wastewater Organic Matter in Sewers- Process Kinetics, Model Concept and Wastewater Treatment Potential.** *Wat. Sci. Tech.* 45(3): 53-60.

Tolond, L., 2003. **Effects of Toxins in Trade effluent on the Nitrification Inhibition in Activated Sludge**. Honours Thesis, School of Life and Environmental Science, University of Natal, Durban, South Africa.

Tomlinson, T. G., Boon, A. G., and Trotman, C. N. A., 1966. **Inhibition of Nitrification in the Activated Sludge Process of Sewage Disposal** *J. Appl. Bact.* 29 (2), 266-291.

Walkers, K. and Wink, A., 1981. **Industrial Effluent Treatment**. Volume 1. Water and Solid Waters. Department of Chemical Engineering, University of Nattingham, UK.

Wild, H. E. Jr., Sawyer, C. N., and McMahon, T. C., 1971. **Factors Affecting Nitrification Kinetics.** *Research Journal WPCF* 43 (9), 1845-1854.

Winther-Nielsen, M. and Jansen, J. la C., 1996. **The role of the sludge in nitrification inhibition tests.** *Wat. Sci. Tech.* 30(6): 93 – 100.

Appendix A

Screening Method for Determination of Inhibition of Nitrification of Activated Sludge

1. Introduction

The screening method employed determines short–term inhibitory effects of test substances on the nitrification process performed by the nitrifying bacteria in activated sludge. In this test, the activated sludge was exposed to the test substance for an incubation period of 120 minutes and therefore the degree of nitrification inhibition determined was estimated for a is the reduction in extent of nitrification compared to a control after 120 minutes.

2. The principle of the screening method

The schematic description is described in Chapter 4, Figure 4.1

a) The principle of the screening method was that nitrifying activated sludge was mixed with nutrient solution (containing ammonia and bicarbonate).
b) The suspension was mixed with tap water and the wastewater under consideration in proportions, which secured the proper dilution of the wastewater.
c) The mixture was shaken for 120 minutes, and then the nitrification was stopped by filtration and cooling of the samples.
d) Nitrate and ammonium analysis were conducted using Merck test.
e) Nitrification inhibition was found by comparing the nitrate production in the samples containing wastewater, with reference samples without wastewater.

3. Material and reagents

3.1 Nitrifying activated sludge

Any type of activated sludge can be used provided that it is nitrifying. In this study, sludge collected from Amanzimtoti wastewater treatment plant was used.

3.2 Substrates

Ammonium sulphate ($(NH_4)_2SO_4$)

Sodium bicarbonate ($NaHCO_3$)

Potassium hydrogen phosphate (KH_2PO_4)

3.3 Acid/Base

HCL

3.4 Tap water

3.5 Reagent-grade water

Distilled water was used to prepare dilutions of the test samples

3.6 Activated sludge suspension

Preparation of activated sludge suspension (Jönsson, 2001)

3.6.1 0.236g of ammonium sulphate ($(NH_4)_2SO_4$), 0.672 sodium bicarbonate($NaHCO_3$) and 0.044g KH_2PO_4 were dissolved in tap water in a 1l beaker

3.6.2 Activated sludge with an oxygen concentration of 0.45mgO_2/l was mixed with the substrates.

3.6.3 The volatile suspended solids concentration of the activated sludge suspension was determined (Appendix A, section 8).

4. Test substances

Stock solutions of the test substances were prepared.

5. Equipment

a. Test tubes with caps and capacity of 30 ml
b. Rack for the test tubes
c. Shaker for the test tubes
d. Pipettes (5000µl)
e. pH-meter
f. Thermometer (range of 0-50 °C)
g. Stop watch
h. Magnetic stirrer with stirring bar
i. Beakers
j. Filters and funnels
k. Drying oven, for operation at 105 ± 3 °C

l. Muffle furnace for operation at 550 °C
m. Analytical balance
n. Merck analysis kits for ammonia and nitrate
o. Spectrophotometer

6. Procedure

6.1 Activated sludge suspension was prepared according to 3.6. The suspension was placed in a beaker on a magnetic stirrer and was gently mixed.

6.2 The sludge suspension and test substances were maintained at ambient temperature.

6.3 The pH of the test substances was adjusted to 7.5.

6.4 Two test tubes for each concentration of test substance and five test tubes for references with tap water were prepared.

6.5 Five ml of tap water (3.4) was added to each test tube used as reference and 5 ml of test substance to the test tubes used as samples.

6.6 To each test tube 5 ml of activated sludge suspension (3.6) was added with a wide-bore pipette.

6.7 Two of the test tubes used as reference were incubated for 0 hours and the other for 120 minutes. The same was done for the test samples. The reaction was stopped by filtering the samples.

6.8 The initial concentrations of NH_3-N and NO_3-N of both the reference and sample tubes were analysed, then followed by the final concentration.

7. Calculations of results

The initial concentration was calculated according to the following equation:

$$\mathbf{N_{TT,O} = N_{C,O} + N_{TS} \cdot V_{TS}/V_{TT}} \qquad \textbf{eq A.1}$$

Where

$N_{TT,O}$ = the initial concentration of oxidised nitrogen in the test tubes (mg N/l),

$N_{C,O}$ = average concentration of oxidised nitrogen in the two controls with incubation time 0 hour (mg/l),

N_{TS} = concentration of oxidised nitrogen in the test substance (ml),

V_{TS} = the volume of test substance added to the test tube (ml),

V_{TT} = the total volume of the liquids added to the test tube (=10 ml).

The concentration of volatile suspended solids in the test tubes (VSS_{TT}) was calculated according to the following equation:

$$\mathbf{VSS_{TT} = VSSss.\ V_{SS}/V_{TT}} \qquad \textbf{eq A.2}$$

Where

VSS_{TT} = the concentration of volatile suspended solids in the test tube (gVSS/l),

VSSss = the concentration of suspended solids in the sludge suspension (gVSS/l),

V_{SS} = the volume of sludge suspension added to the test tube (ml),

V_{TT} = the total volume of liquids added to the test tube (= 10ml).

The nitrification rate (R_{NITR}) was calculated as follows:

$$\mathbf{R_{NITR} = \frac{N_{TTend} - N_{TT,O}}{t\ .\ VSS_{TT}}} \qquad \textbf{eq A.3}$$

Where

R_{NITR} = nitrification rate (mg N/(gVSS.h)

N_{TTend} = the final concentration of oxidised nitrogen in the test tubes (mgN/l),

$N_{TT,O}$ = the initial concentration of oxidised nitrogen in the test tubes (mgN/l),

t = the incubation time (h),

VSS_{TT} = the concentration of volatile suspended solids in the test tube (gVSS/l).

The percentage inhibition of production of oxidised nitrogen was calculated as follows:

$$\mathbf{I = \frac{R_{NITR,C} - R_{NITR,S}}{R_{NITR,C}} \cdot 100} \qquad \textbf{eq A.4}$$

Where

I = inhibition of nitrification (%)

$R_{NITR,C}$ = average nitrification rate of the test tubes containing tap water (mgN/(gVSS.h),

$R_{NITR,S}$ = average nitrification rate of the test tubes containing sample (mgN/(gVSS.h)).

Note: For calculation of nitrification rate from ammonia consumption data:

$$\mathbf{R_{NITR.} = \frac{N_{TT,o} - N_{TT,end}}{t.VSS_{TT}}} \qquad \textbf{eq A.5}$$

Where $N_{TT,0}$ and $N_{TT,end}$ are defined as in eq A.3 above

Note: All the other calculations were done in the same way as the nitrate calculations.

8. Volatile suspended solids measurements

This measurement offers an approximation of the amount of the organic matter present in the solid fraction. The Standard Method was applied (APHA,1989). An evaporating dish was placed in muffle furnace (550 ± 50 °C) for 1 h. The dish was cooled in a desiccator, weighed and stored in the desiccator until use. A 20 ml well mixed activated sludge suspension was transferred to the weighed dish and evaporated to dryness in a drying oven. The evaporated sample was then dried at 103 to 105 °C for 1h. The dish was cooled in the desiccator and then re-weighed. The increase in weight represented the total residue.

The total solids (SS) in the sample were calculated from the following equation:

$$\text{mg total residue/l} = \frac{(A-B) * 1000}{\text{sample volume (ml)}} \qquad \text{eq A.6}$$

Where

A = weight of the sample + dish (mg)

B = weight of dish (mg)

The next step was to ignite the residue in a muffle furnace, pre-heated to 550 ± 50 °C, for 20 min. The dish was weighed once completely cooled. The loss of weight on ignition was reported as the total volatile residue.

The total volatile residue (VSS) was calculated as follows:

$$\text{mg volatile solids/l} = \frac{(B-C) * 1000}{\text{sample volume (ml)}} \qquad \text{eq A.7}$$

Where

B = weight of the dish + residue before ignition (mg)

C = weight of dish + residue after ignition (mg)

9. References

American Public Health Association, 1989. **Standard Methods for the Examination of Water and Wastewater.** Washington.

Jönsson, K., 2001. **Inhibition of Nitrification in Municipal Wastewater – Sources, Effects, Evaluation and Remedies.** PhD Thesis, Lund University, Sweden. pp 150-151.

Appendix B

Table B.1 Variation of inhibition during three months at the Amanzimtoti WWTP

Months	Week1	Week2	Week3	Week4	Mean	Std Deviation
Nov	27	30	29	32	29.5	2.081
Dec	18	20	19	23	20	2.160
Jan	25	23	27	25	25	1.632

Table B.2 Wastewater Characteristics and the % inhibition

Industry	% Inhibition at 20% dilution	COD	SS	Volume
A	-44.6	82.72	71.77	0.27
B	36.3	1.84	2.33	2.69
C	23.3	1.37	6.4	70.99
D	59.9	1.75	6.4	21.35
E	42	1.65	4.46	0.7
F	30.3	1.66	0.19	0.72
G	44	2.04	0.29	0.23
H	24.6	1.2	1.94	0.73
I	32.6	0.82	2.91	0.92
J	54	4.95	3.3	1.41

Appendix C

Statistical analyses

Key	
Abbreviation	**Description**
INDUST	**Industry**
Dilu	**Dilution**
W1	**Week 1**
W2	**Week 2**
W3	**Week 3**
W4	**Week 4**
INHIBI	**Inhibition**
COD	**Chemical Oxygen Demand**
SS	**Settleable solid**

Appendix C1: One-sample T-Test comparing the inhibition shown by Amanzimtoti influent and the reference based on nitrate production. These results are summarized as Table 5.3

One-sample test

	Test Value = 0					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
DILU20W1	59.644	3	.000	38.5000	36.4457	40.5543
DILU20W2	85.732	3	.000	35.0000	33.7008	36.2992
DILU20W3	12.394	3	.001	31.7500	23.5974	39.9026
DILU20W4	20.800	3	.000	23.1000	19.5657	26.6343
DILU40W1	40.086	3	.000	30.3250	27.9175	32.7325
DILU40W2	63.603	3	.000	25.2250	23.9628	26.4872
DILU40W3	17.223	3	.000	20.5000	16.7121	24.2879
DILU40W4	35.425	3	.000	15.1250	13.7662	16.4838
DILU60W1	21.954	3	.000	22.1250	18.9178	25.3322
DILU60W2	58.269	3	.000	18.1750	17.1823	19.1677
DILU60W3	25.957	3	.000	17.7000	15.5299	19.8701
DILU60W4	33.406	3	.000	17.3250	15.6745	18.9755
DILU80W1	27.111	3	.000	17.5000	15.4457	19.5543
DILU80W2	65.209	3	.000	16.2750	15.4807	17.0693
DILU80W3	24.027	3	.000	13.7500	11.9288	15.5712
DILU80W4	158.745	3	.000	15.0250	14.7238	15.3262

Appendix C2: A multiple range test showing homogeneous subsets for nitrification inhibition over a 4 week period. Dilutions are shown at the top of each Table.

DILU20

	WEEKS	N	Subset for alpha = .05		
			1	2	3
Tukey HSD(a)	4	4	23.1000		
	3	4		31.7500	
	2	4		35.0000	35.0000
	1	4			38.5000
	Sig.		1.000	.421	.361
Scheffe(a)	4	4	23.1000		
	3	4		31.7500	
	2	4		35.0000	35.0000
	1	4			38.5000
	Sig.		1.000	.498	.437

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 4.000.

DILU40

	WEEKS	N	Subset for alpha = .05			
			1	2	3	4
Tukey HSD(a)	4	4	15.1250			
	3	4		20.5000		
	2	4			25.2250	
	1	4				30.3250
	Sig.		1.000	1.000	1.000	1.000
Scheffe(a)	4	4	15.1250			
	3	4		20.5000		
	2	4			25.2250	
	1	4				30.3250
	Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 4.000.

DILU60

	WEEKS	N	Subset for alpha = .05	
			1	2
Tukey HSD(a)	4	4	17.3250	
	3	4	17.7000	
	2	4	18.1750	
	1	4		22.1250
	Sig.		.813	1.000
Scheffe(a)	4	4	17.3250	
	3	4	17.7000	
	2	4	18.1750	
	1	4		22.1250
	Sig.		.852	1.000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 4.000.

DILU80

	WEEKS	N	Subset for alpha = .05		
			1	2	3
Tukey HSD(a)	3	4	13.7500		
	4	4	15.0250	15.0250	
	2	4		16.2750	16.2750
	1	4			17.5000
	Sig.		.242	.256	.271
Scheffe(a)	3	4	13.7500		
	4	4	15.0250	15.0250	
	2	4		16.2750	16.2750
	1	4			17.5000
	Sig.		.311	.326	.343

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 4.000.

Appendix C3: One-Sample T-Test comparing the inhibition shown by effluents of 10 surveyed industries and the reference based on nitrate production. These results are summarized as Table 5.4.

	Test Value = 0					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
INDST A DILU20	-7.842	2	.016	-44.6667	-69.1746	-20.1587
INDST A DILU40	-7.088	2	.019	-24.6667	-39.6403	-9.6930
INDST A DILU60	-2.874	2	.103	-21.3333	-53.2749	10.6082
INDST A DILU80	-6.047	2	.026	-10.6667	-18.2558	-3.0775
INDST B DILU20	7.399	2	.018	36.3333	15.2060	57.4607
INDST B DILU40	10.970	2	.008	19.0000	11.5476	26.4524
INDST B DILU60	6.245	2	.025	13.0000	4.0433	21.9567
INDST B DILU80	5.047	2	.037	7.3333	1.0817	13.5849
INDST C DILU20	7.338	2	.018	23.3333	9.6518	37.0149
INDST C DILU40	8.488	2	.014	12.3333	6.0817	18.5849
INDST C DILU60	9.449	2	.011	8.3333	4.5388	12.1279
INDST C DILU80	5.196	2	.035	6.0000	1.0317	10.9683
INDST D DILU20	19.339	2	.003	59.9333	46.5990	73.2677
INDST D DILU40	13.571	2	.005	31.6667	21.6271	41.7062
INDST D DILU60	12.847	2	.006	18.6667	12.4151	24.9183
INDST D DILU80	8.083	2	.015	14.0000	6.5476	21.4524
INDST E DILU20	13.748	2	.005	42.0000	28.8552	55.1448
INDST E DILU40	10.522	2	.009	21.3333	12.6093	30.0573
INDST E DILU60	10.553	2	.009	15.3333	9.0817	21.5849
INDST E DILU80	7.723	2	.016	14.3333	6.3479	22.3187
INDST F DILU20	14.960	2	.004	30.3333	21.6093	39.0573
INDST F DILU40	16.086	2	.004	19.3333	14.1622	24.5045
INDST F DILU60	9.707	2	.010	11.6667	6.4955	16.8378
INDST F DILU80	3.883	2	.060	9.3333	-1.0090	19.6756
INDST G DILU20	12.203	2	.007	44.0000	28.4866	59.5134
INDST G DILU40	8.857	2	.013	20.6667	10.6271	30.7062
INDST G DILU60	8.718	2	.013	12.6667	6.4151	18.9183
INDST G DILU80	22.517	2	.002	13.0000	10.5159	15.4841
INDST H DILU20	9.475	2	.011	24.6667	13.4651	35.8683
INDST H DILU40	7.211	2	.019	8.6667	3.4955	13.8378
INDST H DILU60	5.047	2	.037	7.3333	1.0817	13.5849
INDST H DILU80	3.780	2	.063	6.6667	-.9225	14.2558
INDST I DILU20	8.801	2	.013	32.6667	16.6959	48.6374
INDST I DILU40	9.449	2	.011	16.6667	9.0775	24.2558
INDST I DILU60	3.812	2	.062	8.3333	-1.0715	17.7381
INDST I DILU80	3.576	2	.070	9.0000	-1.8281	19.8281
INDST J DILU20	15.588	2	.004	54.0000	39.0952	68.9048
INDST J DILU40	18.812	2	.003	27.3333	21.0817	33.5849
INDST J DILU60	10.970	2	.008	19.0000	11.5476	26.4524
INDST J DILU80	8.488	2	.014	12.3333	6.0817	18.5849

Appendix C4: A multiple range test showing homogeneous subsets for nitrification inhibition by effluents of 10 surveyed industries. Dilutions are shown at the top of each table.

DILU 20

	INDUST	N	Subset for alpha = .05					
			1	2	3	4	5	6
Tukey HSD(a)	A	3	-44.6667					
	C	3		23.3333				
	H	3		24.6667	24.6667			
	F	3		30.3333	30.3333	30.3333		
	I	3		32.6667	32.6667	32.6667		
	B	3		36.3333	36.3333	36.3333	36.3333	
	E	3			42.0000	42.0000	42.0000	42.0000
	G	3				44.0000	44.0000	44.0000
	J	3					54.0000	54.0000
	D	3						59.9333
	Sig.		1.000	.325	.076	.267	.067	.060
Scheffe(a)	A	3	-44.6667					
	C	3		23.3333				
	H	3		24.6667				
	F	3		30.3333	30.3333			
	I	3		32.6667	32.6667			
	B	3		36.3333	36.3333	36.3333		
	E	3		42.0000	42.0000	42.0000		
	G	3		44.0000	44.0000	44.0000		
	J	3			54.0000	54.0000		
	D	3				59.9333		
	Sig.		1.000	.141	.058	.059		

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3.000.

DILU 40

	INDUST	N	Subset for alpha = .05				
			1	2	3	4	5
Tukey HSD(a)	A	3	-24.6667				
	H	3		8.6667			
	C	3		12.3333	12.3333		
	I	3		16.6667	16.6667		
	B	3			19.0000	19.0000	
	F	3			19.3333	19.3333	
	G	3			20.6667	20.6667	
	E	3			21.3333	21.3333	
	J	3				27.3333	27.3333
	D	3					31.6667
	Sig.		1.000	.196	.104	.160	.866
Scheffe(a)	A	3	-24.6667				
	H	3		8.6667			
	C	3		12.3333			
	I	3		16.6667	16.6667		
	B	3		19.0000	19.0000	19.0000	
	F	3		19.3333	19.3333	19.3333	
	G	3		20.6667	20.6667	20.6667	
	E	3		21.3333	21.3333	21.3333	
	J	3			27.3333	27.3333	
	D	3				31.6667	
	Sig.		1.000	.067	.192	.067	

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3.000.

DILU 60

			Subset for alpha = .05	
	INDUST	N	1	2
Tukey HSD(a)	A	3	-21.3333	
	H	3		7.3333
	C	3		8.3333
	I	3		8.3333
	F	3		11.6667
	G	3		12.6667
	B	3		13.0000
	E	3		15.3333
	D	3		18.6667
	J	3		19.0000
	Sig.		1.000	.154
Scheffe(a)	A	3	-21.3333	
	H	3		7.3333
	C	3		8.3333
	I	3		8.3333
	F	3		11.6667
	G	3		12.6667
	B	3		13.0000
	E	3		15.3333
	D	3		18.6667
	J	3		19.0000
	Sig.		1.000	.491

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3.000.

DILU 80

			Subset for alpha = .05	
	INDUST	N	1	2
Tukey HSD(a)	A	3	-10.6667	
	C	3		6.0000
	H	3		6.6667
	B	3		7.3333
	I	3		9.0000
	F	3		9.3333
	J	3		12.3333
	G	3		13.0000
	D	3		14.0000
	E	3		14.3333
	Sig.		1.000	.071
Scheffe(a)	A	3	-10.6667	
	C	3		6.0000
	H	3		6.6667
	B	3		7.3333
	I	3		9.0000
	F	3		9.3333
	J	3		12.3333
	G	3		13.0000
	D	3		14.0000
	E	3		14.3333
	Sig.		1.000	.317

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3.000.

Appendix C5: One-sample t-test comparing the differences between the means of nitrification inhibition in November and December and December and January

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	NOV	29.5000	4	2.08167	1.04083
	DEC	20.0000	4	2.16025	1.08012
Pair 2	DEC	20.0000	4	2.16025	1.08012
	JAN	25.0000	4	1.63299	.81650

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	NOV & DEC	4	.964	.036
Pair 2	DEC & JAN	4	-.189	.811

Paired Samples Test

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
NOV- DEC	9.5000	.57735	.28868	8.5813	10.4187	32.909	3	.000
DEC - JAN	-5.0000	2.94392	1.47196	-9.6844	-.3156	-3.397	3	.043

Appendix C6: A multiple range test showing homogeneous subsets for nitrification inhibition by individual industries and for mixtures of 2 industries based on nitrate production. Dilutions are shown above each table. Results for individual industries are presented in Table 5.4 while the results for combined industries are presented in figures 5.3-5.7.

DILU20

	INDUST	N	Subset for alpha = .05						
			1	2	3	4	5	6	7
Tukey HSD(a)	A	3	-44.6667						
	C	3		23.3333					
	H	3		24.6667	24.6667				
	F	3		30.3333	30.3333	30.3333			
	E+F	3		30.6667	30.6667	30.6667			
	I	3		32.6667	32.6667	32.6667			
	B	3		36.3333	36.3333	36.3333			
	G+H	3		38.0000	38.0000	38.0000	38.0000		
	B+I	3		40.6667	40.6667	40.6667	40.6667		
	E	3			42.0000	42.0000	42.0000		
	G	3				44.0000	44.0000	44.0000	
	J	3					54.0000	54.0000	54.0000
	D	3						59.9333	59.9333
	J+C	3						60.0000	60.0000
	D+A	3							62.0000
	Sig.		1.000	.051	.051	.253	.096	.096	.918
Scheffe (a)	A	3	-44.6667						
	C	3		23.3333					
	H	3		24.6667					
	F	3		30.3333	30.3333				
	E+F	3		30.6667	30.6667				
	I	3		32.6667	32.6667				
	B	3		36.3333	36.3333	36.3333			
	G+H	3		38.0000	38.0000	38.0000	38.0000		
	B+I	3		40.6667	40.6667	40.6667	40.6667		
	E	3		42.0000	42.0000	42.0000	42.0000		
	G	3		44.0000	44.0000	44.0000	44.0000		
	J	3			54.0000	54.0000	54.0000		
	D	3				59.9333	59.9333		
	J+C	3				60.0000	60.0000		
	D+A	3					62.0000		
	Sig.		1.000	.227	.087	.087	.077		

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3.000.

DILU40

	INDUST	N	Subset for alpha = .05						
			1	2	3	4	5	6	7
Tukey HSD(a)	A	3	-24.6667						
	H	3		8.6667					
	C	3		12.3333	12.3333				
	G+H	3		14.0000	14.0000				
	I	3		16.6667	16.6667				
	E+F	3		18.0000	18.0000	18.0000			
	B	3			19.0000	19.0000			
	F	3			19.3333	19.3333			
	G	3			20.6667	20.6667			
	E	3			21.3333	21.3333	21.3333		
	J	3				27.3333	27.3333	27.3333	
	J+C	3					30.6667	30.6667	30.6667
	B+I	3					30.6667	30.6667	30.6667
	D	3						31.6667	31.6667
	D+A	3							38.0000
	Sig.		1.000	.061	.082	.061	.061	.927	.287
Scheffe(a)	A	3	-24.6667						
	H	3		8.6667					
	C	3		12.3333					
	G+H	3		14.0000	14.0000				
	I	3		16.6667	16.6667				
	E+F	3		18.0000	18.0000	18.0000			
	B	3		19.0000	19.0000	19.0000			
	F	3		19.3333	19.3333	19.3333			
	G	3		20.6667	20.6667	20.6667			
	E	3		21.3333	21.3333	21.3333			
	J	3			27.3333	27.3333	27.3333		
	J+C	3				30.6667	30.6667		
	B+I	3				30.6667	30.6667		
	D	3				31.6667	31.6667		
	D+A	3					38.0000		
	Sig.		1.000	.109	.071	.057	.321		

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3.000.

DIUL60

	INDUST	N	Subset for alpha = .05		
			1	2	3
Tukey HSD(a)	A	3	-21.3333		
	H	3		7.3333	
	G+H	3		8.0000	
	C	3		8.3333	
	I	3		8.3333	
	F	3		11.6667	
	G	3		12.6667	12.6667
	E+F	3		12.6667	12.6667
	B	3		13.0000	13.0000
	E	3		15.3333	15.3333
	D	3		18.6667	18.6667
	J	3		19.0000	19.0000
	J+C	3		19.0000	19.0000
	D+A	3			25.0000
	B+I	3			25.0000
	Sig.		1.000	.087	.056
Scheffe(a)	A	3	-21.3333		
	H	3		7.3333	
	G+H	3		8.0000	
	C	3		8.3333	
	I	3		8.3333	
	F	3		11.6667	
	G	3		12.6667	
	E+F	3		12.6667	
	B	3		13.0000	
	E	3		15.3333	
	D	3		18.6667	
	J	3		19.0000	
	J+C	3		19.0000	
	D+A	3		25.0000	
	B+I	3		25.0000	
	Sig.		1.000	.063	

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3.000.

DILU80

	INDUST	N	Subset for alpha = .05							
			1	2	3	4	5	6	7	8
Tukey HSD(a)	A	3	-10.6667							
	G+H	3		5.0000						
	C	3		6.0000	6.0000					
	H	3		6.6667	6.6667	6.6667				
	B	3		7.3333	7.3333	7.3333	7.3333			
	I	3		7.6667	7.6667	7.6667	7.6667			
	F	3		9.3333	9.3333	9.3333	9.3333	9.3333		
	E+F	3		10.6667	10.6667	10.6667	10.6667	10.6667	10.6667	
	J	3		12.3333	12.3333	12.3333	12.3333	12.3333	12.3333	
	G	3			13.0000	13.0000	13.0000	13.0000	13.0000	13.0000
	D	3				14.0000	14.0000	14.0000	14.0000	14.0000
	E	3					14.6667	14.6667	14.6667	14.6667
	B+I	3						16.0000	16.0000	16.0000
	J+C	3							18.0000	18.0000
	D+A	3								20.6667
	Sig.		1.000	.099	.136	.099	.099	.184	.099	.071
Scheffe(a)	A	3	-10.6667							
	G+H	3		5.0000						
	C	3		6.0000						
	H	3		6.6667	6.6667					
	B	3		7.3333	7.3333					
	I	3		7.6667	7.6667					
	F	3		9.3333	9.3333	9.3333				
	E+F	3		10.6667	10.6667	10.6667				
	J	3		12.3333	12.3333	12.3333				
	G	3		13.0000	13.0000	13.0000				
	D	3		14.0000	14.0000	14.0000				
	E	3		14.6667	14.6667	14.6667				
	B+I	3		16.0000	16.0000	16.0000				
	J+C	3			18.0000	18.0000				
	D+A	3				20.6667				
	Sig.		1.000	.080	.062	.062				

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3.000.