ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

EVALUATION OF NITROGEN REMOVAL RATES FROM ABATTOIR WASTEWATER IN A PILOT PREDENITRIFICATION-NITRIFICATION ACTIVATED SLUDGE WASTEWATER TREATMENT PLANT

By

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A Thesis Presented to the School of Graduate Studies of the Addis Ababa University in Partial Fulfillment of the Degree of Master of Science in Biology (Applied Microbiology)

July, 2005

ACKNOWLEDGEMENTS

I am grateful to Dr. Fassil Assefa, my advisor, for allowing me to work on this research problem. I highly appreciate his myriad benevolence, his endless encouragement and assistance, his confidence on me, and the freedom I enjoyed while I was working under his advice. His lectures in the courses and his orientations during the research work immersed my soul in an interest to study Environmental Microbiology. I am really lucky to have him as an advisor because I decided to work with him after leaving another research, I have had started, for some reason.

I would like to thank Ato Zeleke Teferi at the Addis Ababa Water and Sewerage Authority (AAWSA) for his encouragement and material support. I am also thankful to the Addis Ababa Abattoirs Enterprise for allowing me to take samples from the abattoir effluent in the whole study period. I especially acknowledge the help and technical support from Ato Asfaw, the chemist, at Addis Ababa Abattoirs Enterprise.

I would like to thank Dr. Kifle Dagne at the department of Biology, AAU, for his concern during the difficult times before this study. I also thank Prof. Mogessie Ashenafi, Dr. Teshome Gebremichel and Ato Meshesha Balkew at the Institute of Pathobiology, AAU, for their appreciation and high opinion while I was working in their laboratories, which in one way or the other contributed for my success.

I would like to acknowledge SNNP education bureau for granting me the study leave to AAU. I would like to thank Ato Zewge Gedlu for receiving and sending my salary at the right time for the whole study leave. I am grateful to W/t Yenenesh Gebeyehu and Ato Endeshaw for their accountability when I leave from my work place to join the university.

I am deeply indebted to my brother Ato Debebe Woldesenbet for his endless encouragement and support throughout the study period. It was by his support that I realized my dreams. I am thankful to my sister Meseret Melkamu (Enu) for the untold care and support she provided me in the whole study period. It would have been very

difficult for me to succeed in my studies if she was not helping me in every step I walked. I am very thankful to my family for the continuous encouragement, as well as moral support they provided me. I would also like to express my deepest thanks to W/t Netsanet Kebede, my friend, for her continuous encouragement and support through out the study period.

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ABBREVIATIONS

AUR	Ammonium Uptake Rate
BOD	Biochemical Oxygen Demand
CFU	Colony Forming Unit
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
EPA	Environmental Protection Authority/Agency
GOE	Government Of Ethiopia
MCRT	Mean Cell Retention Time
MLSS	Mixed Liquor Suspended Solids
MLVSS	Mixed Liquor Volatile Suspended Solids
NUR	Nitrate Uptake Rate
R	Ratio to the Inflow Rate
SRT	Sludge Retention Time
SVI	Sludge Volume Index
TDS	Total Dissolved Solids

ABSTRACT

Abattoir wastewater has a complex composition and is very harmful to the environment. It causes eutrophication, suffocation of aquatic life and contamination of ground water. Nitrogen is the main component of the abattoir wastewater. Because of environmental and health problems they cause, nitrogen compounds should be reduced to the acceptable discharge limit level before the wastewater is released to water bodies and the environment. Biological methods of nitrogen removal are considered to be cost effective and environmentally friendly alternatives than chemical treatment. The objectives of this study were therefore: to evaluate the treatment processes of a predenitrification-nitrification activated sludge system for abattoir wastewater treatment; Characterize the activated sludge involved in the treatment process through Ammonium uptake rate and Nitrate uptake rate, and its settlability by sludge volume index.

Abattoir wastewater was sampled from Addis Ababa Abattoirs Enterprise, analyzed for selected parameters to check its biodegradability and fed into a predenitrification nitrification activated sludge wastewater treatment system at varying operational parameters. The removal efficiency for the selected parameters and the characteristics of the sludge at each operational condition was evaluated. A removal efficiency of 94-98% for COD, 95-99% for BOD_5, 85-97% for total nitrogen, 58-95% for Ammonium-Nitrogen and 46-53% reduction in phosphate was obtained in the treatment processes. The predenitrification-nitrification rates measured as Ammonium Uptake Rate and Nitrate Uptake Rate ranged from 5.9-9.8 mg NH_4-N/gVSSh and 8.2-12.1 mg NO_3-N/gVSSh respectively. The sludge volume index (SVI) ranged from 51.5 ml/g to 87.6 ml/g. The abattoir wastewater was found to be suitable for biological wastewater treatment. It is also showed that the predenitrification-nitrification system was efficient in organic matter and nitrogen removal from abattoir wastewater.

1. INTRODUCTION

In the present day highly urbanized and industrialized society with a rapidly expanding world population, the problems related to the management of wastewater have become a formidable task. Because of urbanization and large consumption of a variety of products, there is an increase in the discharge of more neutral and toxic wastes in the form of municipal refuse, sewage sludge, agricultural residues, toxic and chemically reactive byproducts of manufacturing processes to the environment (Manahan, 1990; Harrison, 1995).

Domestic and industrial effluents are the principal sources of environmental pollution. However, industrial wastes are by far the major contributors by producing huge tonnage of xenobiotic and other anthropogenic compounds, with pollution effects much higher than municipal sewage and other sanitary and domestic wastes (Gurnham, 1965; Hamer *et al.*, 1985). Uncontrolled (improper) waste disposal deteriorates the quality of surface water (streams, rivers, reservoirs) by changing the chemical, physical and organoleptic properties of water.

Environmental pollution derived from domestic and industrial activities is the main threat to the surface and ground water qualities in Ethiopia (EPA, 2003). It is reported that the majority of industries in the country discharge their wastewaters into nearby water bodies and open land with out any form of treatment (EPA, 2003). Due to the absence of controlled waste management strategies and waste treatment plants, untreated wastes are dumped into water bodies and the surrounding environments. The streams and rivers into which waste is dumped are used for various purposes such as horticulture, drinking, washing and other domestic activities by down stream inhabitants. Ground water from boreholes or springs is also the source of drinking water for the dwellers. This definitely leads to serious health and ecological problems.

Slaughterhouses are among the industries which produce/release wastewater of high pollution loads. High chemical and biological oxygen demand, nitrogen, phosphorus and coliforms are the main pollutants in abattoir wastewater. The wastewater is usually composed of dissolved solids, blood, gut contents, urine and water (Adeyemo *et al.*, 2002). Blood, one of the major dissolved pollutants in slaughterhouse wastewater, has a chemical oxygen demand (COD) of 375,000 mg/l (Tritt and Schuchart, 1992).

Slaughterhouse wastewater may also contain high concentrations of suspended solids including pieces of fat, grease, hair, feathers, flesh, manure, grit and undigested feed (Bull *et al.*, 1982). Effluent discharges from slaughterhouses can cause the deoxygenation of surface waters, introduce enteric pathogens and excess nutrients in to them (Quinn and Farlane, 1989; Meadows, 1995) and contaminate ground water (Sangodoyin and Agbawhe, 1992). Discharge of nutrients (nitrogen and phosphorus) in to water bodies is identified as a particular concern.

The general concern for natural water resources and the environment is growing. This has resulted in an increasing interest and development of methods and systems by which wastewater can be recycled. The need for technologies for environmentally friendly treatment of wastewater is therefore obvious.

Nitrogen is the main nutrient which has a variety of ecological and environmental effects. Thus there is a lot of interest to control the nitrogen content of wastewaters released in to water bodies and the environment. Because removal of nitrogen compounds from wastewater has very few alternatives, is difficult and costly, there is a need for a relatively cost effective and environmentally friendly wastewater treatment systems. In a wastewater treatment plant, nitrogen can mainly be removed by biological means. Biological nitrification-denitrification is the most widely practiced and economical approach for nitrogen removal/control in wastewater treatment (Gupta and Gupta, 2001).

The consequences of uncontrolled waste disposal are now being realized in many parts of Ethiopia. This has forced the government to formulate regulations and standards for discharge limits (GOE, 2002). Any industrial wastewater should meet the standards for discharge before it is released into the environment. Thus there is an urgent need to find effective and at the same time economically attractive solutions. Previously industrial wastewater treatment has not been given due attention in the country. Currently due to environmental legislation industries are obliged to introduce wastewater treatment systems. Recently, apart from introducing high technology treatment system, low cost treatment systems are tried on tannery wastewater at pilot plant project levels (Issayas Tadesse, 2003; Seyoum Leta, 2004).

The objectives of the present work were therefore:

1. To evaluate the performance (efficiency) of the predenitrification nitrification pilot wastewater treatment plant for nitrogen, phosphorus and organic matter removal from abattoir wastewater at varying (different) operational parameters.
2. To evaluate the nitrification and denitrification potentials of the system using Ammonium Uptake Rate (AUR) and Nitrate Uptake Rate (NUR) of the activated sludge.
3. To characterize the activated sludge formed in terms of its quality and quantity.

2. LITERATURE REVIEW

2.1. Characteristics of Major Industrial Wastes

Industrial effluents are wastewaters discharged from different processing firms. They contain huge tonnage of organic and inorganic materials that pollute the air, land and water environment. Industrial wastes differ from one another in the nature of chemicals they contain. Food, dairy, canneries, leather tanning, paper and pulp, and sugarcane industries are characterized by easily degradable wastes, where as petrochemical and pesticide industries are dominated by highly xenobiotic chemicals such as polychlorinated bipheniles and alkyl benzene sulfonates that are resistant to biodegradation (Harrison, 1995).

Industrial wastes can be categorized into two major classes on the basis of their discharge, BOD content i.e. low BOD and high BOD wastes. Low BOD wastes have effluents as dissolved mineral content and/or recalcitrant hazardous chemicals and heavy metals. Industries known to discharge hazardous and recalcitrant heavy metals into water bodies and aquifers include Plastic, Paint, and Metal industries (Harrison, 1995; Young and Cerniglia, 1995). Petrochemical, Cosmetic, Pharmaceutical, and Pesticide industries release low BOD wastewater with recalcitrant materials (Finstein and Morris, 1975; Kennedy *et al.*, 1990; Smolinske, 1995).

Wastes from sugarcane and sugar beet refineries, fruit and vegetable canneries, dairy, pulp and paper, textile, leather tanning industries and abattoirs/slaughterhouses are known for the production of high organic load (BOD) wastes (Grunham, 1965; Henze *et al.*, 1997).

2.2. Abattoir Wastewater

Abattoirs and slaughterhouses are interchangeable words and are essentially synonymous with each other. These are plants, which slaughter livestock and dress carcass only, with limited or no processing of by products (COWI Consulting Engineers and Planners AS, 2001).

As compared to other industries slaughterhouses are fairly efficient in terms of waste recovery and management. Most discarded animal parts are sent for further transformation. For example, blood is dried and transformed into an animal protein feed. Slaughterhouses, however, consume significant amounts of water in animal processing operations for hygienic reasons, and thus produce large volumes of wastewater that must be treated (Masse and Masse, 2000).

The important steps in slaughtering and beef processing are, stunning and bleeding, dressing and hide removal, evisceration, cutting and boning, inspection and cleaning. Cleaning and other hygiene-related requirements are by far the daily processes that require the greatest amount of water, and also those that contribute the highest contaminant loads in the wastewater effluent streams (COWI Consulting Engineers and Planners AS, 2001).

Due to the nature of the waste materials, including blood, paunch, and stomach contents, cleaning effluents from abattoirs contain not only high organic loads, but also high concentrations of chemicals from the detergents and disinfectants used. Nitrogen, Phosphorus and phosphorus–containing compounds are primarily found in blood and manure, as well as in undigested stomach contents, which are removed during the evisceration phase. Phosphorus can also be found as trisodium phosphate, which is a common component of cleaning and sanitizing compounds and detergents (EPA, 2002). Fats, oils and grease (FOG) also contribute to the over all strength of the wastewater streams from abattoirs.

Due to the presence of manure, total and fecal coliforms, and fecal streptococci are present in large quantities in abattoir wastewaters. These bacteria are usually present in quantities of several million colony-forming units (CFU) per 100ml, but are not usually pathogenic. However, they may indicate the possible presence of pathogens such as *Salmonella spp*, *Campylobacter jejuni*, and gastrointestinal parasites, including *Ascaris sp.*, *Giardia lamblia*, and *Cryptosporidium parvum*.

According to Tritt and Schuchardt (1992) and Johns (1995) slaughterhouse wastewater quality depends on a number of factors, such as: blood capture, water usage, type of animal slaughtered, and amount of rendering or meat processing activities. The efficiency in blood retention during animal bleeding is the most important factor that greatly affects the level of chemical or biological oxygen demand (COD or BOD) of the wastewater. In abattoirs water economy usually leads to increased pollutant concentration although total COD or BOD mass will remain constant. COD or BOD is higher in wastewater from beef than hog slaughterhouses. Plants that only slaughter animals produce a stronger wastewater than those that are also involved in rendering or meat processing activities.

2.3. Pollution Effects of Organic Matter, Nitrogen and Phosphorus

Natural waterways contain a population of microorganisms that utilize dissolved organic compounds that in turn are part of the food chain for protozoa, insects, worms and fish. Under normal conditions in a waterway, this population of organisms forms a balanced ecosystem. The balanced system can be destabilized by the addition of excess metabolizable organic material, such as high levels of organic wastewater.

The addition of metabolizable organic compounds causes a considerable increase in the growth and metabolism of the aerobic microbial population of the waterway, which will use up all the available oxygen dissolved in the water. The increased microbial metabolism creates anaerobic conditions in the waterway which leads to the death of other aquatic organisms such as fish and crustacea.

Nitrogen is a key component in wastewater streams. It is becoming increasingly important in wastewater management because nitrogen can have many effects on the environment (Halling-Sorensen and Jorgensen, 1993). The presence of nitrogen in wastewater discharge is undesirable because it has ecological impacts and can affect public health. The presence of nitrogenous wastes in receiving waters may cause dissolved oxygen depletion, toxicity, eutrophication, and methemoglobinemia.

Nitrogen can exist in different forms because of its various oxidation states, and it can readily change from one to another depending on the oxidation state present at the time. Nitrogen may be found in four forms: Organic nitrogen (urea, amines, proteins and fecal materials), Ammonia nitrogen (NH_3-N or NH_4-N), Nitrate nitrogen (NO_3-N) and Nitrite nitrogen (NO_2-N) (Reed, 1984). In the environment, living organisms can accomplish changes from one oxidation state to another.

Ammonia nitrogen discharged into a receiving stream will undergo the process of oxidation in the presence of dissolved oxygen and nitrifying bacteria. This will cause the depletion of dissolved oxygen in receiving water. More over, although ammonium ions are the preferred nitrogen nutrient for most organisms, they are converted to free ammonia with increasing pH. Free ammonia is extremely toxic to fish and many other aquatic organisms.

Another ecological impact caused by nitrogen is eutrophication of aquatic ecosystems. Since the different forms of nitrogen (NH_4^+, NO_2^- and NO_3^-) serve as nitrogen nutrients for the growth of aquatic plants, especially algae, they will grow excessively. This in turn causes dissolved oxygen depletion from the receiving water by microbial activity to decompose the dead plants.

Consumption of drinking water with high nitrate levels is associated with methemoglobinemia and gastric cancer due to endogenous formation of genotoxic *N*-nitroso compounds by bacteria in the gastrointestinal tract (Van Maanen *et al.*, 1996). Although nitrate itself is not toxic, its conversion to nitrite is a concern to public health (Sedlak, 1991). In the body nitrite can oxidize the iron (II) in hemoglobin and form methemoglobin, which binds oxygen less effectively than normal hemoglobin. The resulting decrease in oxygen in young children leads to shortness of breath, diarrhea, vomiting, and in extreme cases even death (Kelter *et al.*, 1997).

Phosphorus is an essential element for the growth of algae and other organisms. It can also cause eutrophication in surface waters. Thus the amount of organic matter, nitrogen and phosphorus compounds that enter surface waters in domestic and industrial waste discharges and natural runoff should be controlled.

2.4. Classification of Wastewater Treatment Methods

Wastewater treatment methods can be categorized into unit operations and processes. Methods of treatment in which the application of physical forces predominates are known as unit operations while methods of treatment in which the removal of contaminants is brought about by chemical or biological reactions are known as unit processes.

Unit operations and processes are grouped together to provide what is known as primary, secondary and advanced (or tertiary) treatment. In primary treatment, physical operations such as screening and sedimentation are used to remove the floating and settleable solids found in wastewater. In secondary treatment, biological and chemical processes are used to remove most of the organic matter. In advanced treatment, additional combinations of unit operations and processes are used to remove other constituents, such as nitrogen and phosphorus that are not reduced significantly by secondary treatment (Metcalf and Eddy, 1991).

2. 5. Biological Methods for the Treatment of Wastewaters

The major biological processes used for wastewater treatment are five major groups: aerobic processes, anoxic processes, anaerobic processes, combined aerobic, anoxic and anaerobic processes, and pond processes. The individual processes are further subdivided, depending on whether treatment is accomplished in suspended growth systems, attached growth systems or combinations thereof (Metcalf and Eddy, 1991).

2.5.1. Anaerobic Digestion Processes

Anaerobic digestion process is one of the oldest means of wastewater treatment. The primary use of anaerobic digestion is in the stabilization of suspended organic matter (Grady and Lim, 1980). It has got a wide application and acceptance for less energy requirement, less sludge yield as a byproduct, precipitate toxic heavy metal ions, production of methane gas as energy source (Torphy, 1989). From an energy consumption viewpoint anaerobic digestion seems to be by far the most economic wastewater treatment process.

2.5.2. Lagoon Systems

Lagoon systems are shallow basins, which hold the wastewater for several months to allow for the natural degradation of sewage. These systems take advantage of natural aeration and microorganisms in the wastewater to renovate sewage. They rely on the interaction of sunlight, algae, microorganisms, and oxygen. Lagoons are slow, cheep, and relatively inefficient, but can be used for various types of wastewater (Ouano, 1983).

2.5.3. Fixed Film Systems

Fixed film systems grow microorganisms on substrates such as rocks, sand or plastic. The wastewater is spread over the substrate, allowing the wastewater to flow past the film of microorganisms fixed to the substrate. As organic matter and nutrients are absorbed from the wastewater, the film of microorganisms grows and thickens. Trickling filters, rotating biological contactors, and sand filters are examples of fixed film systems (Metcalf and Eddy, 1991).

2.5.4. Suspended Film Systems

Suspended film systems stir and suspend microorganisms in wastewater. As the microorganisms absorb organic matter and nutrients from the wastewater they grow in size and number. After the microorganisms have been suspended in the wastewater for several hours, they are settled out as sludge. Some of the sludge is pumped back into the incoming wastewater to provide "seed" microorganisms. The remainder is wasted and sent on to a sludge treatment process. Activated sludge, extended aeration, oxidation ditch, and sequential batch reactor systems are all examples of suspended film systems.

2.6. The Activated Sludge Process

Activated sludge is at present the most widely used biological treatment process for both domestic and industrial wastewaters. The activated sludge process refers to a continuous or semi-continuous (fill-and-draw) aerobic method for biological wastewater treatment, including carbonaceous oxidation and nitrification. The process relies on a dense microbial populations being mixed in suspension with the wastewater under aerobic conditions.

In the presence of adequate nutrients and oxygen a high rate of microbial growth and respiration is achieved. This results in the utilization of the organic matter present in the production of oxidized end products such as CO_2, NO_3^-, SO_4^{2-} and PO_4^{3-}, and/or the biosynthesis of more microorganisms. Activated sludge treatment removes from the wastewater the biodegradable organics as well as the unsettleable suspended solids and other constituents, which can be adsorbed on, or entrapped by, the activated sludge floc (Cloete and Muyima, 1997).

Part of the settled sludge is drawn off as waste; the rest is recycled to the aeration basin to maintain a high concentration of bacteria (Ecknfelder *et al.*, 1985). Unless the cell tissue produced from the organic matter is removed from the solution, complete treatment has not been accomplished because the cell tissue, which itself is organic, will be measured as BOD in the effluent. If the cell tissue is not removed, the only treatment that has been achieved is that associated with the bacterial conversion of a portion of the organic matter originally present to various gaseous end products. According to Metcalf and Eddy (1991) obtaining good results from an activated sludge is accomplished by producing a sludge, which will meet the requirements of the process, and maintaining the proper environment so that the sludge can do its work.

The activated sludge process has undergone various modifications able to meet most wastewater treatment needs (Toerien *et al.*, 1990). These changes relate to the flow regime in the reactor, the size, number and configuration of the reactors, recycled flow, influent flow and others (Ekama and Marais, 1984). Although the process has undergone various adjustments, the basic principles stay the same: the removal of soluble biodegradable organic compounds from wastewater by a flocculant slurry of microorganisms, the removal of microorganisms by sedimentation, the recycling of a small amount of return sludge from the clarifier underflow to the reactor and the dependence of the systems' high performance on the mean cell residence time (MCRT) of microorganisms (Grady and Lim, 1980). One modification for nitrogen removal from wastewater has aerobic basin for nitrification and anoxic basin mainly for denitrification and carbonaceous matter removal (Seyoum Leta, 2004). While modifications for phosphorus removal include anaerobic reactor(s) before the aerated tank (Metcalf and Eddy, 1991).

The activated sludge process has been renowned for producing an effluent of high quality at reasonable cost. It is capable of achieving equal reductions in soluble substrate in reactors of much smaller volume while producing an effluent relatively free of suspended solids (Grady and Lim, 1980). The activated sludge process removes certain priority pollutants with an efficiency of 95% or more (Eckenfelder *et al.*, 1985). Moreover, the activated sludge process is controllable, because through adjustment of the amount of sludge wasted, the operator is able to regulate the mean cell retention time (MCRT) to obtain the desired effluent quality. Activated sludge reactors are relatively resistant to shock loads and can achieve acceptable effluent in spite of dynamic inputs, although they do perform better under more stable conditions (Seyoum Leta, 2004).

2.6.1. Microflora of Activated Sludge

Microorganisms play an important role in wastewater treatment processes. Activated sludge contains a range of different types of microorganisms comparable with that of soils. The ecological relationships between the various components of the population are quite complex. It consists of bacteria, both heterotrophic and autotrophic, fungi, algae, protozoa, metazoa, and viruses. The bacteria are probably the most important group of microorganisms in wastewater treatment (Wesley, 1966).

The concentration of bacteria in an activated sludge is 10^{7}-10^{9} cells/ml (Henze *et al.*, 1997). Fungi appear to play a small part in treatment (Horan, 1990). Algae are mainly found in the surface of biofilters. Protozoa and metazoa are also common in biofilters: in activated sludge they occur in varying numbers depending on the loading of the plant (low load-many protozoa and metazoa). The protozoa graze on the bacteria; eat fungi, algae and suspended organic matter. Hardly any thing is known about the role of viruses in wastewater treatment (Henze *et al.*, 1997).

The organisms, which do have significant roles in the process, can be divided according to their roles as floc-forming organisms, saprophytes, predators, and nuisance organisms. Any particular organism may fit into more than one of these roles at a time or may change roles with a change in the condition in the plant.

Since the activated sludge process is continuously inoculated with a large variety of microorganisms, the population, which is able to maintain itself in the process, is a function of the environment and the ecological relation ships between the organisms present. The environmental conditions in the system will also influence the form of growth of the organisms and their metabolic activities (Wesley, 1966).

2.6.2 Biological Requirements for the Activated Sludge Process

According to Wesley (1966) there are three essential biological requirements for the activated sludge process:

(1) A mixed population of aerobic microorganisms must be able to degrade the noxious components of the waste.

(2) The required population must be able to grow in the environment of the aeration tank.

(3) The organisms must grow in such a form that they will settle out in the clarifier (settling tank).

The first of these three requirements corresponds to a part of the question of biological treatability of the waste. If the major components of the organic matter in the waste are compounds, which can be assimilated and oxidized by some microorganisms, if adequate amounts of nitrogen, phosphorus and other nutrients are present so that the organic matter can be metabolized, and if no toxicity problems occur, then some microorganisms will be able to grow in the waste. If microorganisms can grow in the waste, they separate the organic matter from the waste and, in most instances, separate it so rapidly enough that the activated sludge process is a feasible method of treating the waste. After the organic matter is separated from the waste, it is either oxidized or synthesized in to fairly stable cellular components.

In some cases microorganisms will grow in the waste, but they grow in a form such that they do not separate from the waste by sedimentation. All of the operating problems, which are the product of deficiencies in the design, maintenance, or operation of the activated sludge process, are manifested as sedimentation problems; i.e., activated sludge appears in the effluent from the process. When a biologically treatable waste is introduced into an activated sludge process, any one of many different types of sludge may form. Presumably, there is an optimum type of activated sludge to treat each type of waste and an activated sludge process could be designed and operated to produce the optimum type of sludge (Pipes, 1967).

2.6.3. Predenitrification-Nitrification Activated Sludge System

A common design for an activated sludge process performing nitrogen removal has two separate anoxic and aerated basins, respectively. The separate reaction zones are used for carbon oxidation, nitrification and anoxic denitrification. The wastewater initially enters an anoxic denitrification zone to which nitrified mixed liquor is recycled from a subsequent combined carbon oxidation-nitrification compartment. The ammonium is oxidized via nitrite to nitrate by nitrifying bacteria in the aerated part of the basin. The nitrate is recirculated to the first part of the basin where the conditions are optimized for denitrification with no aeration and a large supply of organic substances from the incoming wastewater (Grunditz, 1999).

Consequently the wastewater to be denitrified in biological nitrification/denitrification systems must contain sufficient carbon (organic matter) to provide the energy source for the conversion of nitrate to nitrogen gas by the bacteria. The carbon requirements may be provided by internal sources, such as wastewater and cell material (endogenous decay), or by an external source e.g. methanol (Metcalf and Eddy, 1991). Thus the nitrate is reduced to nitrogen gas by denitrifying bacteria.

Denitrification can also be performed after the aerated basin (Post-denitrification). In this case, an external carbon source is needed. In this type of design the ammonia in the wastewater passes unchanged through the first anoxic basin to be nitrified in the first aeration basin (Grunditz, 1999).

The major and more permanent removal mechanism of organic nitrogen in treatment plants is the sequential processes of ammonification, nitrification and denitrification. As part of the nitrogen cycle, the various forms of nitrogen are converted into gaseous components that are expelled into the atmosphere as nitrogen gas (N_2) or nitrous oxide (N_2O).

2.6.3.1. Ammonification

Proteolysis and degradation of amino acids leads to the liberation of ammonia by the different mechanisms of ammonification (Gallert and Winter, 1999), including hydrolytic, oxidative, reductive, and desaturative deamination (eqs. a-d), respectively.

$$R\text{-}NH_2 + H_2O \longrightarrow R\text{-}OH + NH_3 \qquad (a)$$

$$R\text{-}CHNH_2COOH + H_2O \rightleftarrows R\text{-}CO\text{-}COOH + 2(H) + NH_3 \qquad (b)$$

$$R\text{-}CHNH_2\text{-}COOH + 2(H) \longrightarrow R\text{-}CH_2\text{-}COOH + NH_3 \qquad (c)$$

$$R\text{-}CH_2\text{-}CHNH_2\text{-}COOH \rightleftarrows R\text{-}CH{=}CH\text{-}COOH + NH_3 \qquad (d)$$

Many microorganisms, as well as plants and animals, are capable of converting organic nitrogen into ammonia, which then can be assimilated by a number of organisms (Atlas, 1988), or nitrified. A significant amount of ammonia from urea cleavage or from ammonification of amino acids is assimilated in aerobic treatment processes for growth of bacteria (surplus sludge formation). For elimination of ammonia that is not used for cell growth during wastewater treatment, it must first be nitrified and then denitrified to molecular nitrogen (Gallert and Winter, 1999).

2.6.3.2. Nitrification

Nitrification is essentially carried out by two distinct groups of bacteria (ammonium and nitrite-oxidizers (eqs. e and f) respectively) belonging to the family *Nitrobacteriaceae*. Strictly chemolithotrophic species oxidizing ammonium belong to the genera *Nitrosospira*, *Nitrosovibrio*, *Nitrosolobus*, *Nitrosococcus*, *Nitrosomonas*, and *Nitrosocystis*. Those oxidizing nitrite to nitrate (facultative chemolithotrophs) are grouped under *Nitrobacter*, *Nitrococcus*, *Nitrospira* and *Nitrospina* (Prosser, 1989; Wagner *et al.*, 1996; Gerardi, 2002; Madigan *et al.*, 2002). Albeit nitrification is widely believed to be an oxic process, investigations have shown that at least ammonia oxidizers are able to oxidize ammonia under anoxic conditions (Schmidt and Bock, 1997).

$$\text{Nitritation:}\quad 2NH_4^+ + 3O_2 \longrightarrow 4H^+ + 2H_2O + 2NO_2^- \qquad \text{(e)}$$

$$\text{Nitratation:}\quad 2NO_2^- + O_2 \longrightarrow 2NO_3^- \qquad \text{(f)}$$

$$NH_4^+ + 4O_2 \longrightarrow 4H^+ + 2H_2O + 2NO_3^- \qquad \text{(g)}$$

Various heterotrophic and lithotrophic micro-organisms, including some bacteria of the genera *Arthrobacter*, *Flavobacterium*, or *Thiosphaera* (actinomycetes and planctomycetes), algae and fungi are able to catalyse heterotrophic nitrification of nitrogen-containing organic substances (eq. h) (Focht and Verstraete, 1977; Robertson and Kuenen, 1992; Strous *et al.*, 1997; Jetten *et al.*, 1998; Jetten *et al.*, 2001; Thamdrup and Dalsgaard, 2002).

$$R\text{-}NH_2 \longrightarrow R\text{-}NHOH \longrightarrow R\text{-}NO^- \longrightarrow R\text{-}NO \longrightarrow NO_3^- \qquad \text{(h)}$$

Heterotrophic nitrifiers oxidize reduced nitrogen compounds such as hydroxylamine, aliphatic and aromatic nitrogen containing compounds, but in contrast to autotrophic nitrification no energy is gained by nitrate formation. For this reason an organic substrate must be respired to satisfy the energy metabolism. Some of the heterotrophic nitrifiers are able to denitrify nitrate or nitrite under aerobic growth conditions (Gallert and Winter, 1999). Since autotrophic nitrification usually occurs at higher rates than heterotrophic nitrification it is believed to play a more important role in nature (Focht and Verstraete, 1977; Winkler, 1984; Robertson and Kuenen, 1992).

2.6.3.2. 1. Environmental Factors Influencing Nitrification

Since there are relatively few microbial genera able to nitrify, nitrification is particularly sensitive to environmental stress. The occurrence of nitrification is significantly influenced by temperature, pH, alkalinity, inorganic carbon source, the microbial population and concentration of NH_4–N, dissolved oxygen and inimical pollutant compounds (Grunditz *et al.*,1998; Dangcong *et al.*,2000; Jonsson *et al.*,2000; Gerardi, 2002; Hu *et al.*, 2002; Hu *et al.*, 2004).

Whereas nitrification occurs over a wide temperature range of 4–40^{0}C, the optimum temperature in pure cultures ranges from 25–30^{0}C in water bodies, and 30–40^{0}C in soils (Bitton, 1999; Gerardi, 2002). A narrow optimum pH (7.2–8.6) exists (Bitton, 1999; Im *et al.*, 2001; Gerardi, 2002) but acclimatized systems can be operated to nitrify at a much lower pH value (Kadlec *et al.*, 2000).

Nitrification is obligatorily coupled to oxygen consumption and has an effect on the decrease in wastewater alkalinity. Such a decrease in wastewater alkalinity might cause a decrease in its pH when the alkalinity of the wastewater is low or when its ammonia content is relatively high (Kadlec *et al.*, 2000). During ammonium oxidation, the wastewater alkalinity increases slightly due to CO_2 consumption for autotrophic growth whereas acidic nitrite formation results in a drop in wastewater pH. Thus if the buffer capacity of the system wastewater is weak, the pH might drop well below 6.7 preventing further autotrophic nitrification (Gerardi, 2002).

Although effective nitrification has been reported in systems with residual oxygen as low as 0.5 mg/lL (Cloete and Muyima, 1997), DO concentrations below 1.5 mg/L are reported to limit the nitrification process (Hammer and Knight, 1994; Lee *et al.*, 1999; Gerardi, 2002). Of the many operational requirements known to affect nitrifying bacteria, dissolved oxygen (DO) concentration is one of the most important requirements. Because nitrifying bacteria are strict aerobes, they can only nitrify in the presence of dissolved oxygen. As they reduce CO_2 for growth and obtain energy by oxidizing ammonia and nitrite through aerobic metabolism, there is a competition for DO with organotrophs in the activated sludge system.

At DO concentrations less than 2 mg/l, the nitrification rate of the treatment plant would be low, and at DO less than 0.5 mg/l, almost no nitrification occurs (Gerardi, 2002). Therefore, the DO level within the aeration tank should be carefully monitored and kept above 1.5 mg/l (Cheremisinoff, 1996; Gerardi, 2002). However numerous reports have indicated that in order to ensure that DO is not a limiting factor in the nitrification reaction, the level not less than 2.0 mg/l must be maintained (Cao *et al.*, 2002). Both longer Sludge Retention Time (SRT) and higher dissolved oxygen (DO) conditions are prerequisites for improving nitrification (Cloete and Muyima, 1997).

Nitrification, like most bacterial processes, is also affected by pH and temperature. Studies show that the optimal range of pH and temperature for nitrification is 6.5-8.6 and 5-30^{0}C, respectively (Cheremisinoff, 1996; Grunditz *et al.*, 1998; Im *et al.*, 2001).

Nitrifying bacteria are sensitive organisms that are extremely susceptible to a wide range of inhibitors present in wastewaters. Such inhibitory pollutants include phenolic compounds, cyanide, thiourea, anilines and heavy metals primarily originating from industrial processes. Extremely high concentrations of ammonical nitrogen and nitrous acid are reported to be inhibitory (substrate inhibition) to the nitrification process

(Grunditz *et al.*, 1998; Bitton, 1999; Gerardi, 2002). Similarly, high organic loading inhibits nitrification by promoting heterotrophic growth and activity which culminate in limited nitrifier growth and activity as a result of strong competition for the available oxygen and ammonia (Focht and Verstraete, 1977; Hanaki *et al.*, 1990; Van Niel *et al.*, 1993; Tijhuis *et al.*, 1994; Ohashi *et al.*, 1995; Van Benthum *et al.*, 1997).

The fast growing heterotrophs tend to occupy the outer layers of the biofilm, where both substrate concentration and detachment rate are high; whereas the slow growing nitrifying bacteria stay deeper inside the biofilm. Thus the heterotrophic layer forming above the nitrifiers in the biofilm negatively influences the nitrification process through limited oxygen availability to autotrophic nitrificants as a result of consumption and resistance to mass transfer within the heterotrophic layer (Nogueira *et al.*, 2002).

2.6.3.3. Denitrification

Denitrification is a stepwise enzymatic anoxic reduction process (eqs. j and k) in which nitrite and nitrate are reduced to molecular nitrogen or nitrogen gases by chemoorganotrophic, lithoautotrophic, and phototrophic bacteria (Bitton, 1999; Kadlec *et al.*, 2000). In this microbial process, the nitrogen oxides (in ionic and gaseous form) irreversibly serve as terminal electron acceptors in the electron transport chain. The electrons are usually but not exclusively transferred from organic compounds through a series of carrier systems to a more oxidized nitrogen form (eq. k). The resultant free energy conserved as ATP is used by the denitrifying organisms to support respiration (Kadlec *et al.*, 2000).

Denitrification is a significant mechanism in treatment plants for the permanent removal of N from wastewater (Hammer and Knight, 1994). In treatment plants, the nitrification rate is usually much slower than the denitrification rate, and thus the first process affects the latter (Verhoeven and Meuleman, 1999). The supply of NO_3–N which limits the denitrification process has often been identified as a problematic issue (Hseih and

Coultas, 1989; Busnardo *et al.*, 1992; Newman *et al.*, 2000) and remains a challenge in treatment plants.

$$6(CH_2O) + 4NO_3^- \longrightarrow 6CO_2 + 2N_2 + 6H_2O \qquad (j)$$

$$2NO_3^- \xrightarrow{a} 2NO_2^- \xrightarrow{b} 2NO \xrightarrow{c} N_2O \xrightarrow{d} N_2 \qquad (k)$$

a/ Nitrate reductase

b/ Nitrite reductase

c/ Nitric oxide reductase

d/ Nitrous oxide reductase

Denitrifying bacteria are defined solely by the ability to reduce nitrate (NO_3) or nitrite (NO_2) to N_2O or N_2 in the absence of oxygen. During denitrification, the nitrogen compounds act as terminal electron acceptors instead of oxygen during respiration. Thus, denitrification occurs when oxygen becomes limited for aerobic respiration (Hochstein and Tomlinson, 1988). Although denitrification is generally assumed to occur under anaerobic conditions (Atlas, 1988), it has been shown that it can take place in the presence of oxygen (Robertson and Kuenen, 1984; Simpkin and Boyle, 1988; Bonin and Gilewicz, 1991; Patureau *et al.*, 1998; Plessis *et al.*, 1998).

Most activated sludge microorganisms have the ability to use nitrate nitrogen as the final electron acceptor in biochemical reactions. Most denitrifiers are heterotroph/organotrophs and thus need organic compounds as carbon and energy sources. Many denitrifying bacteria can also grow by fermentation. Thus, denitrifying bacteria are quite metabolically diverse in terms of alternative energy-generating mechanisms. The common denitrifiers in wastewater treatment plants are chemoorganotrophic bacteria belonging to several genera such as *Pseudomonas, Paracoccus, Alcaligenes, Micrococcus, Achromobacter, Bacillus, Brevibacterium, Flavobacterium* and *Moraxella* (Metcalf and Eddy, 1991; Cloete and Muyima, 1997; Magnusson *et al.*, 1998). Moreover denitrification is not specific to any one phylogenetic group; the trait is found in about 50 genera, mostly in the *Proteobacteria* species (Zumft, 1997).

Some denitrifiers are obligate or facultative chemolithotrophs that utilize hydrogen or reduced sulfur as sources of energy (electron donors). For instance, *Thiobacillus denitrificans* and *Ferrobacillus ferroxidans* are facultative and obligate chemolithotrophs respectively. Chemolithotrophic denitrification involves the oxidation of a reduced inorganic substrate such as pyrite (eq. I) (Mc Eldowney *et al.*, 1993; Sengelton, 1995; Bridge and Jonson, 1998).

$$2FeS_2 + 6NO_3^- + 2H^+ \longrightarrow 3N_2 + 4SO_4^{2-} + 2Fe^{2+} + 2H_2O \qquad \text{(I)}$$

2.6.3.3. 1. Environmental Factors Influencing Denitrification

Several factors including anoxic conditions, pH, temperature, presence of denitrifiers, growth requirements, organic matter (energy source) and inhibitory substances are known to influence denitrification rates.

The presence of oxygen suppresses the synthesis of the enzyme needed for the substitution of nitrogen for oxygen as the terminal electron acceptor (Cooper *et al.*, 1996). Moreover, the optimum pH range is reported to lie between 7.0 and 8.5 (Metcalf and Eddy, 1991; Bitton, 1999; Kadlec *et al.*, 2000; Gerardi, 2002). However, alkalinity produced during the denitrification process can result in increased pH. This is essential for nitrification process in maintaining pH.

Denitrification is also highly temperature dependent, with reaction rates significantly decreased at temperatures below 5^0C (Gerardi, 2002). Growth requirements such as nitrate/nitrite concentrations that serve as an electron acceptor in denitrification and presence of organic carbon (energy source) that donates electrons for denitrification to proceed. Denitrifiers are less sensitive to toxic chemicals than are nitrifiers (Delwiche and Bryan, 1976; Gumaelius *et al.*, 1996; Grunditz *et al*, 1998; Seyoum Leta, 2004).

2.7. Characterization of Activated Sludge

The biomass of the activated sludge is the active agent in biological wastewater treatment. To describe and control the processes occurring in wastewater treatment plants, this biomass is often characterized in terms of: (1) characterization and quantification according to metabolic activities, (2) identification and classification of microorganisms (Seyoum Leta, 2004), and (3) activated sludge quality.

The most common methods of characterization and quantification of activated metabolic activities are indirect, i.e. the rate of consumption of components entering the biochemical reactions or the rate of formation of reaction products is measured instead of quantifying specific metabolic groups (Cloete and Muyima, 1997). Tests on the ammonium utilization rate (AUR), nitrate utilization rate (NUR) and oxygen utilization rate (OUR) can be applied to measure different biomass activities (Henze *et al.*, 1986; Niekerk *et al.*, 1987; Jansen *et al.*, 1992). Through these simple characterization procedures, functional groups in biomass can be quantified (nitrifiers, denitrifiers and *heterotrophs*). The procedures can also be applied for characterization of wastewater (Kristensen *et al.*, 1992). The nitrifying and denitrifying capacities of activated sludge are determined by nitrification rate (measured as Ammonium Uptake Rate (AUR) or nitrate production rate), and denitrification rate (Nitrate Uptake Rate, NUR) respectively.

Some of the important applications of NUR as a tool for wastewater characterization include: (1) establishment of the denitrification activity for a given sludge, and (2) determination of the sludge capacity for anoxic degradation of wastewater or a specific carbon source for denitrification (Kristensen *et al.*, 1992).

2.8. Methods for Abattoir Wastewater Treatment

2.8.1. Land application

Land application of slaughterhouse wastewater by spray irrigation has been mainly used in the USA (Bull et al. 1982). The advantages of the system are its simplicity and low cost. The disadvantages include possible surface and ground water contamination, odour problems, greenhouse gas emission, and soil pore clogging from excessive fat loads. Application on constructed wetlands could also be used as a polishing treatment for biologically treated wastewater (John, 1995).

2.8.2. Physico-chemical treatments

Grit chambers, screens, settling tanks, and dissolved air flotation (DAF) units are widely used for the removal of SS, colloidals and fats from slaughterhouse wastewater. In DAF units, air bubbles injected at the bottom of the tank transport light solids and hydrophobic material, such as fat and grease, to the surface where scum is periodically skimmed off.

Blood coagulants (e.g. aluminium sulphate and ferric chloride) and/or flocculents (e.g. polymers) are sometimes added to the wastewater in the DAF unit to increase protein flocculation and precipitation as well as fat flotation.

2.8.3. Aerobic treatment

In aerobic digestion, microorganisms degrade organics in the presence of oxygen. Besides lagoons, extended aeration systems and trickling filters have been the most popular aerobic processes for the treatment of meat packing and slaughterhouse wastewater (Bull et al. 1982).

2.8.4. Anaerobic treatment

During anaerobic digestion, organics are degraded by a diversity of bacteria into methane in the absence of oxygen. Anaerobic treatment can be divided into two main categories, low-rate (lagoons) and high-rate systems.

3. MATERIALS AND METHODS

3.1. The Wastewater Sampling Site

Addis Ababa Abattoirs slaughter pigs, sheep, goats and cattle. The animals slaughtered in large number are cattle. The number of slaughters is in hundreds during fasting months of the Orthodox Church and goes up to 3000 heads a week in non-fasting seasons. Friday is the date of maximum slaughter in the week.

3.2. Sample Collection at Addis Ababa Abattoirs Enterprise

Composite samples of raw abattoir wastewater were taken from the effluent of Addis Ababa abattoirs enterprise each Friday after 5:00 pm for five months filtered with 1 mm sieve and settled. The wastewater was then analyzed for selected parameters and fed to the system.

3.3. Analyses

The wastewater samples were analyzed for the following parameters: Temperature, pH, Chemical oxygen demand (COD), Biological oxygen demand (BOD_5), Total Nitrogen, Ammonium-N (NH_4-N), Nitrate-N (NO_3-N), Phosphate (PO_4^{3-}) and Total dissolved solids (TDS). Temperature and pH were measured by a pH meter (sens*ion1*). COD, Total Nitrogen, NH_4-N, NO_3-N and Phosphate (PO_4^{3-}) were all measured colorometrically using a spectrophotometer (DR/2010 HACH, Loveland, USA) according to HACH instructions. BOD_5, Total Dissolved Solids (TDS) were measured according to Standard methods (APHA, 1998).

Mixed Liquor Suspended Solids (MLSS), and Mixed Liquor Volatile Suspended Solids (MLVSS) were also measured according to Standard methods (APHA, 1998).The sludge volume index (SVI) was calculated as SVI = settled sludge volume (ml/l) x

1000/suspended solids (mg/l), after having measured the MLSS and settled sludge volume (SSV) (APHA, 1998). Dissolved oxygen (DO) measurement in the aerated tank was made using a DO probe (YSI 5905Yellow Springs Instruments, Yellow Spring, Ohio).

3.4. The Pilot Plant

The research study was undertaken on a predenitrification-nitrification wastewater pilot plant based on an activated sludge model designed by Seyoum *et al* (2004a). The plant had an influent feeding tank, a denitrification reactor of 100l e quipped with a vertical stirrer, an aerobic activated sludge unit of 200l and a 50l sedimentation (clarifier) tank. (Figure 2).

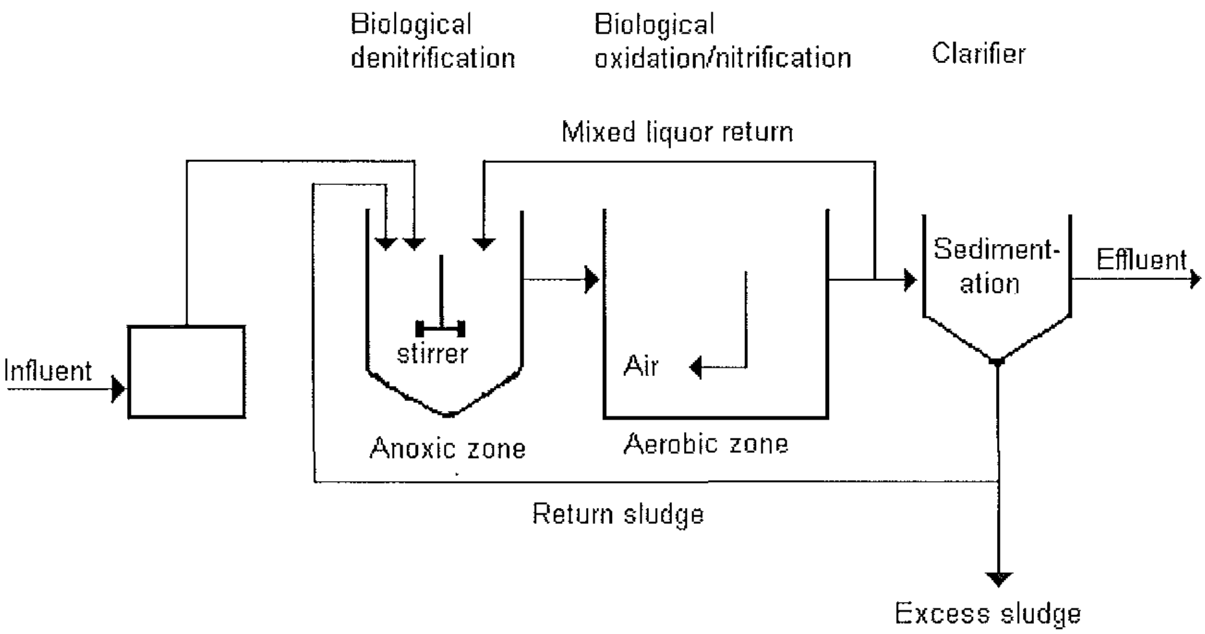

Figure 1. Design of the pilot activated sludge wastewater treatment plant for organic matter and nitrogen removal (Seyoum *et al.*, 2004a).

3.5. The Process Parameters

The pilot wastewater treatment plant was operated in such a way that the wastewater (influent) from the feeding tank was pumped to the anoxic tank at a rate of 35 ml/min to make the hydraulic retention time (HRT) of the anoxic tank 47.62 hours and that of the oxic tank 95.24 hours. The mixed liquor from the activated sludge, and sludge from the effluent were recycled to the head of the denitrification reactor at rates of 100-200% and 100-150% in that order. The treatment process in the pilot plant was monitored for 5 months from June to October 2004. The experiments were run in four successive feeds in 28, 25, 26 and 29 days, respectively. The process parameters for the four runs are listed in table 1.

Table 1. The pilot plant operational parameters.

Parameter	Feeds			
	I	II	III	IV
COD loading rate (kg/m^3d)	1.70	1.39	1.25	1.05
BOD_5 loading rate (kg/m^3d)	0.81	0.73	0.67	0.61
Mixed liquor recycle, (R)	1R	1R	2R	2R
Sludge return, (R)	1R	1R	1.5R	1.5R
MLSS (g/l)	2.6	2.9	3.2	3.7
MLVSS (g/l)	1.8	2.3	2.4	2.6
SVI (ml/g)	51.5	66.4	79.3	87.6
DO aerated (mg/l)	2.1	2.5	3.1	4.5
DO anoxic (mg/l)	0.03	0.06	0.01	0.04

concentration of 20-30 mg N/l. 20ml samples of mixed liquor were withdrawn at intervals of 15-60 minutes for 3-4 hours. The samples were immediately filtrated, and the filtrate was collected for analysis. AUR was calculated from the slope of the ammonia utilization curve and as a control also from the nitrate plus nitrite production curve. Nitrification inhibition test was not made as abattoir wastewater has no much toxic substances in it.

3.6.2. NUR Test

To determine NUR, a completely mixed, closed atmosphere, three liter reactor was used. Sludge was withdrawn from the anoxic tank and stirred in the closed reactor for 30 minutes before starting the tests. Nitrate was added to a start concentration of 20-30 mgN/l. In the experiments, carbon was in excess, and acetate was added to a start concentration of 120-160 mg/l COD. Sampling procedures were just like those for the AUR test but included COD analysis. NUR was calculated from the slope of the resulting nitrate plus nitrite utilization curve. The same test was made using the wastewater as a carbon source instead of acetate.

4. RESULTS

The result of the physico-chemical analysis of the influent and effluent wastewaters is presented in Table 3. The influent wastewater had high concentrations of pollutants. On average COD ranged from 4164 mg/l to 6762 mg/l, BOD_5 from 2434 mg/l to 3210 mg/l, total nitrogen from 694 mg/l to 938 mg/l, ammonium nitrogen from 153 mg/l to 206 mg/l. Nitrate-nitrogen was below detection limit and phosphate concentration in the range 80 mg/l to 98 mg/l. The influent abattoir wastewater physico-chemical characteristics as compared to other industrial wastewaters is presented in table 2.

While the effluent COD ranged from 63 mg/l to 406 mg/l, BOD_5 from 36 mg/l to 161 mg/l, total-Nitrogen from 20 mg/l to 140 mg/l, ammonium-nitrogen from 6 mg/l to 86 mg/l, nitrate-nitrogen from 8 mg/l to 42 mg/l and phosphate concentration varied from 38 mg/l to 53 mg/l.

Table 2. The influent abattoir wastewater physico-chemical characteristics as compared to other industrial wastewaters.

Industry	pH	COD (mg/l)	BOD_5 (mg/l)	N (mg/l)	P (mg/l)	Heavy metals and others (mg/l)	Adapted from:
Abattoir	7.2-8.3	3840-7200	1980-3560	560-1124	21-37		This study
Tannery	10.5-11.3	7950-5240	1920-5840	680-2640	4-27.2	Total Cr: 12.3-64 SO_4^{2-}: 280-1180 S^{2-}: 325-932.5	Seyoum Leta, 2004
Distillery *-wine* *-grain*	6-9 5-9	10000-9000 71000	6000-25000 32000	240-450 280	0.044-0.092 0.19	-	Rosenwinkel *et al.*, 2001
Brewery	6-9	18000-30000	11000-15000	0.03-0.1	10-30	-	Rosenwinkel *et al.*, 2001
Sugar factory	6-11	6000-30000	4000-18000	50-180	-	-	Rosenwinkel *et al.*, 2001
Mineral water & Beverages	6-7	200-1600	110-800	2-35	0-18	-	Rosenwinkel *et al.*, 2001

Table 3. Average characteristics of the influent and effluent wastewaters for the four experimental feeds.

Parameter	Feed I			Feed II			Feed III			Feed IV		
	Influent	Effluent	Removal Efficiency (%)	Influent	Effluent	Removal Efficiency (%)	Influent	Effluent	Removal Efficiency (%)	Influent	Effluent	Removal Efficiency (%)
PH	7.5	7.3	-	7.6	7.4	-	7.6	7.3	-	7.8	7.4	-
Temp. (^{0}C)	16.8	18.8	-	17.9	20.1	-	18.6	19.4	-	21.1	20.0	-
COD	6762	406	94	5520	221	96	4950	144	97	4164	63	98
BOD_5	3210	161	95	2905	87	97	2647	69	97	2434	36	99
Total N	938	140	85	910	100	89	728	48	93	694	20	97
PO_4^{3-}	98	53	46	94	46	49	87	44	51	80	38	53
NH_4-N	206	86	58	191	58	69	164	15	91	153	6	96
NO_3-N		42			49			20			8	
TDS	3640	583	84	3100	279	91	2845	143	95	2514	101	96

The removal efficiencies of the pilot plant are given in table 3. The nutrient removal efficiencies increased from feed one to feed four. The COD removal efficiency ranged from 94% to 98%, the BOD_5 removal efficiency from 95% to 99%. It was in the fourth experimental feed that the maximum COD and BOD_5 removal efficiencies were observed in the treatment process. In all the four experimental feeds almost all of the COD and BOD_5 were removed. The total-nitrogen removal efficiency increased from 85% in the first experimental feed to 97% in the fourth feed.

The Ammonium-nitrogen removal efficiency increased from 58% in the first feed to 96% in the fourth experimental feed. Ammonium removal efficiency of the system slowly increased from 58% to 96% and was highest at a COD loading rate of 1.05 kg/m^3d (Figure 3).

The concentration of dissolved oxygen in the oxic tank was shown to be proportional to the ammonium removal efficiency of the pilot plant (figure 4). As the DO was increased from 2.1 mg/l to 4.5 mg/l, the NH_4-N removal efficiency increased from 58% to 96%.

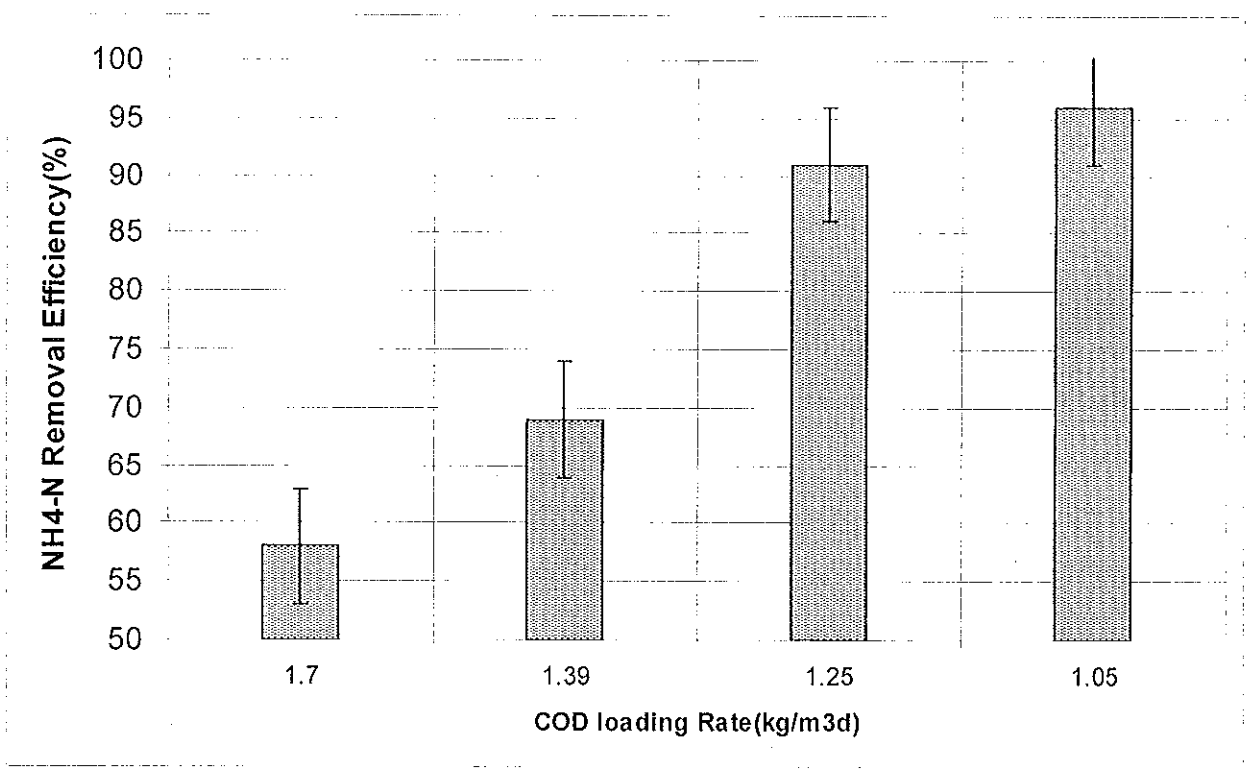

Figure 3. The effect of organic loading rate on the ammonium removal efficiency of the pilot plant.

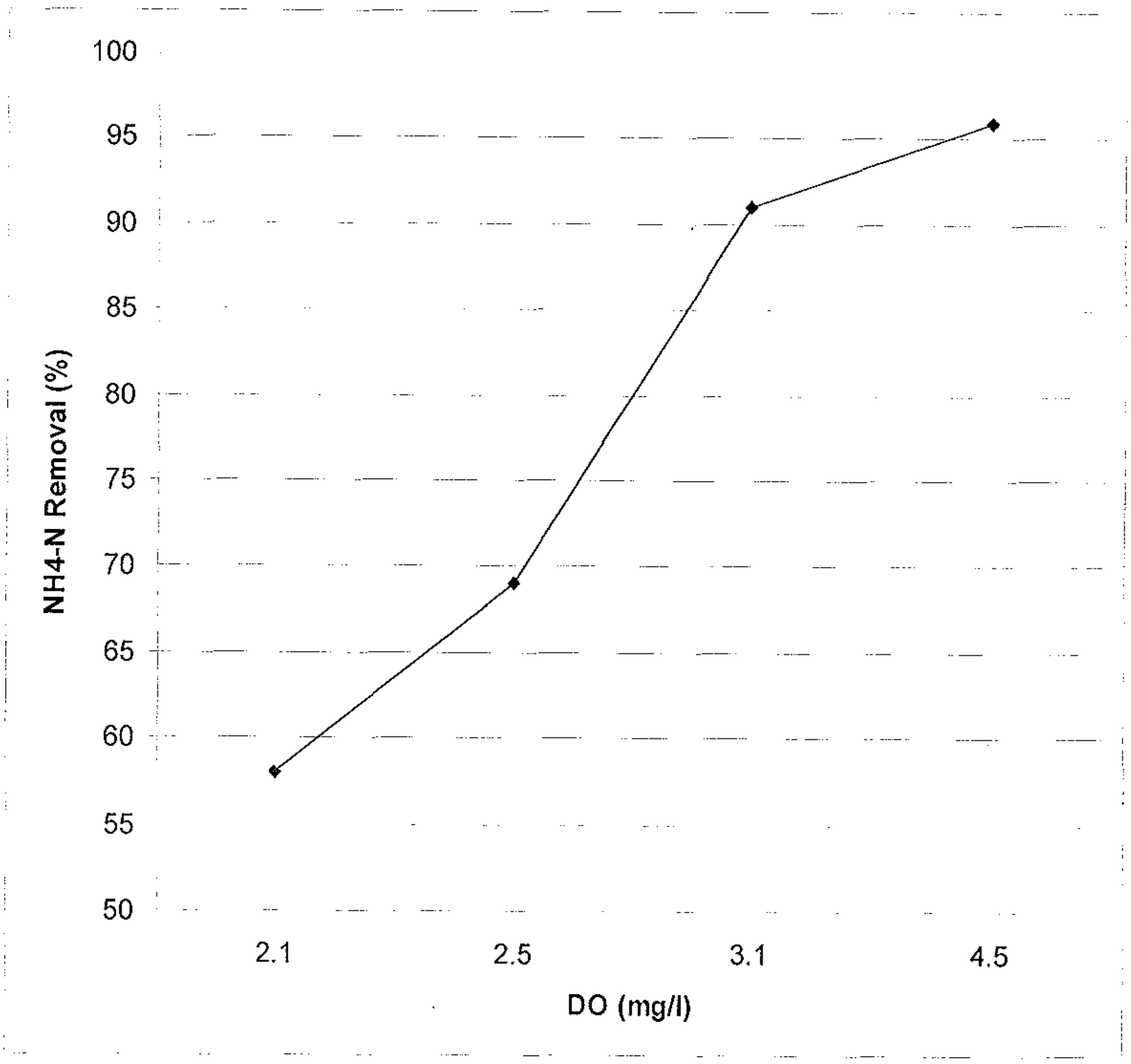

Figure 4. The effect of Dissolved Oxygen (DO) concentrations on ammonium removal efficiency of the pilot plant system

The phosphate removal efficiency of the treatment plant varied from 46% to 53%. It increased with an increase in MLSS concentration of the oxic tank. The maximum phosphate removal efficiency observed in the processes was 53% (Figure 5).

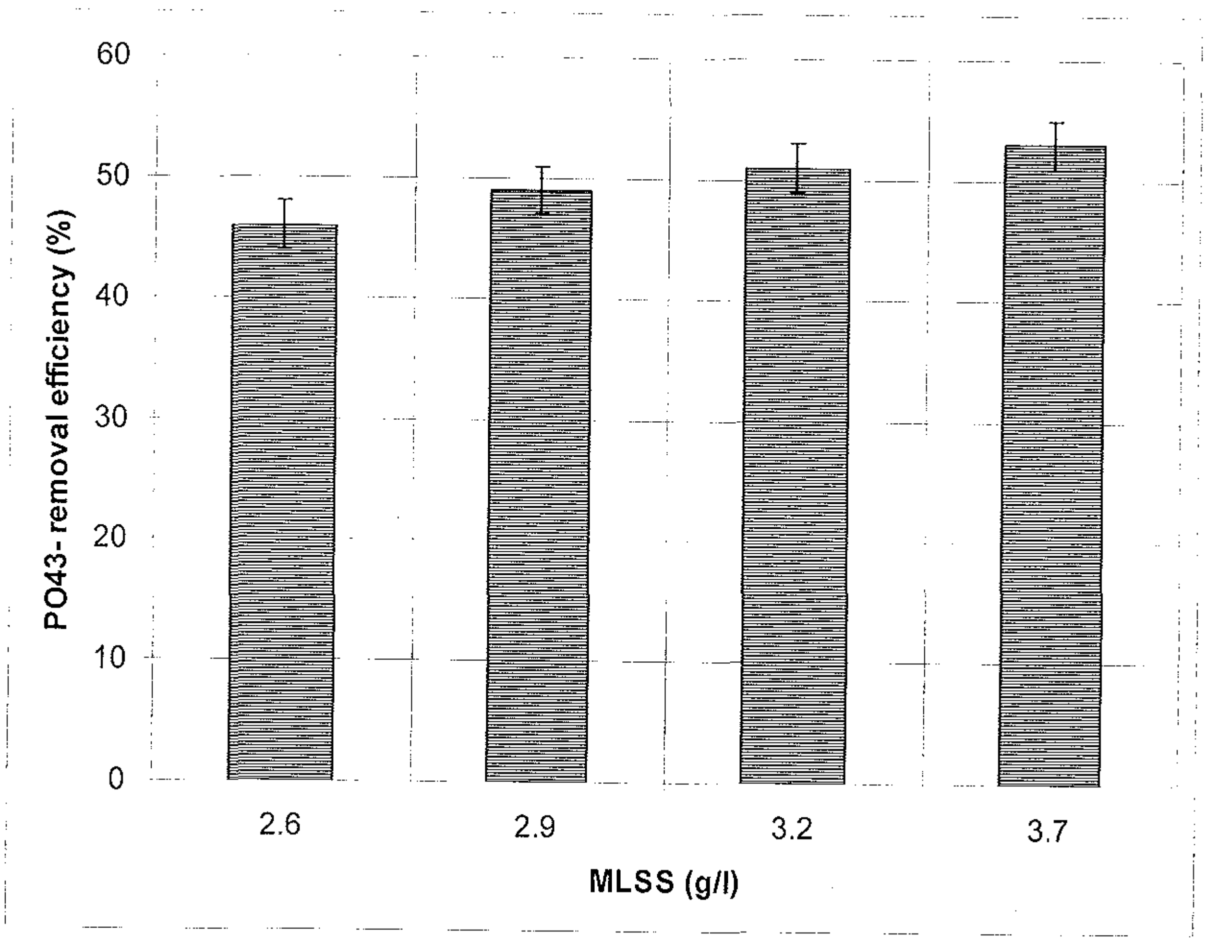

Figure 5. The effect of mixed liquor concentration on phosphate removal efficiency of the pilot plant

The nitrification capacity of the sludge measured as ammonia uptake rates of the pilot plant varied from 5.9 to 9.8 mg NH_4-N g /VSS h (Figure 6). The rate increased through the successive feeds. The fourth feed was characterized by the highest nitrification rate in the process.

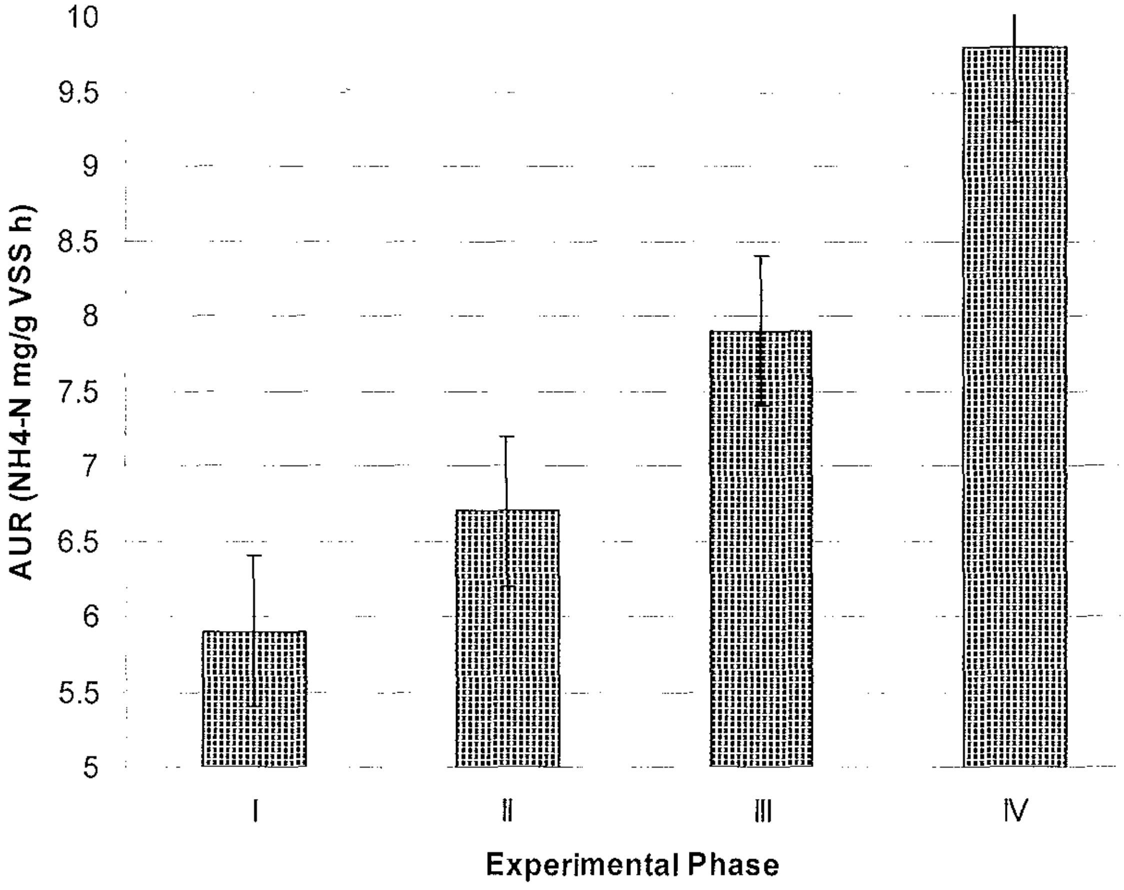

Figure 6 Sludge activity showing the nitrification potential of the pilot plant (AUR)

The denitrification capacity of the sludge measured as nitrate uptake rate varied from 7.6 to 11.0 NO_3-N g/VSS h with acetate serving as carbon source and 8.2 to 12.1 NO_3-N g/VSS h using the abattoir wastewater as carbon source. The denitrification capacity was higher with the wastewater than the acetate in all experiments. The highest average nitrification rate was 12.1 NO_3-N g/VSS h in the fourth feed.

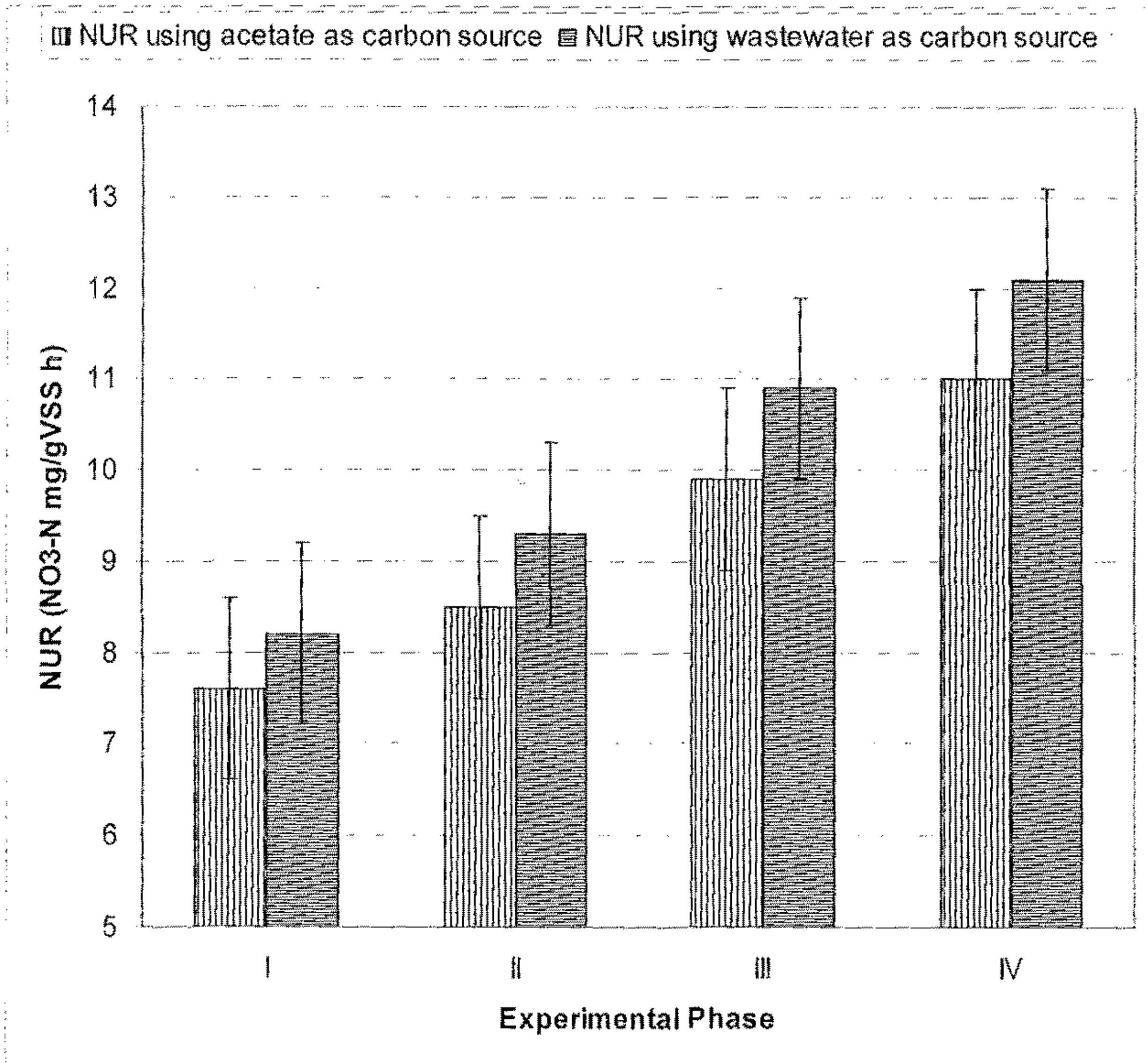

Figure 7. Sludge activity showing the denitrification potential of the pilot plant (NUR)

5. DISCUSSION

This study examined the characteristics of the filtered and settled abattoir wastewater for selected physico-chemical parameters, its biological treatability in a pilot plant operation, the activity of the sludge and the effect of varying the different operational parameters on the treatment efficiency. The wastewater was found to have enough nutrients to be treated biologically and a high pollution load of the selected chemical parameters (Table 2 and 3). The sludge activity and the whole performance of the system increased from the first to the last feeds.

The COD and BOD_5 removal efficiencies in the experimental feeds varied from 94 to 99% (Figure 3). An increase in this removal efficiency was observed to match with the increase in the MLVSS (Table 1) and with the increasing denitrification efficiency of the system in the successive feeds (Figure 8). The MLVSS in activated sludge is proportional to the active microbial biomass in the system (Metcalf and Eddy, 1991; (Sidat *et al.*, 1998). The more the active biomass in the system the more will be the activities and the consumption. The heterotrophic bacteria that undergo denitrification require a complex carbon source as an electron source for denitrification (Gallert and Winter, 1999). The increased denitrification rate may have used more and more organic matter (electron source) for the denitrification to proceed at the maximum possible rate under the conditions in the treatment plant.

The result thus shows that almost all of the COD and BOD_5 were removed in the experimental feeds and the system was performing properly for COD and BOD_5 removal. Similar removal efficiencies of 95% to 98% were reported from tannery wastewater treatment in the same system by Seyoum *et al.*, 2004a.

The total Nitrogen removal efficiencies were 85, 89, 93 and 97% in the successive feeds. This increase may be obtained as the nitrification rate, the denitrification rate and the MLVSS increase, resulting in an increased overall removal and consumption of nitrogenous compounds in the system. Removal efficiencies of 82 to 98% was reported earlier by Seyoum *et al.*, (2004a) from tannery wastewater; and 90% to 98% removal efficiency from industrial wastewaters of metal recovery processes by Hirata *et al.*, (2001).

The ammonium nitrogen removal efficiency increased from 58% in the first feed to 96% in the fourth experimental feed (Table 3). This increase may be a result of the increase in the abundance of the nitrifying bacteria which are favored by the decreased organic loading rate, the increased dissolved oxygen level and the increased mixed liquor and sludge return rates (Table 1). The same increasing pattern in ammonium nitrogen removal efficiency was achieved by Seyoum *et al.*, 2004a and Hirata *et al.*, 2001 by acclimatizing microorganisms with increasing the mean cell residence time.

The results obtained in the Ammonium-nitrogen removal efficiency are also reflected in the Ammonium uptake rate lab scale experiments. As can be seen from figure 6 the ammonium uptake rate increased from 5.9 NH_4-N mg/g VSS h to 9.8 NH_4-N mg/g VSS h from the first to the fourth experimental feed. At about the fourth feed the chemolithoautotrophic nitrifiers may have established themselves in the system and perform well making the ammonium removal efficiency appreciably very high. An ammonium uptake rate of 4.3 NH_4-N mg/g VSS h to 6.4 NH_4-N mg/g VSS h was also reported by Seyoum *et al.*, 2004a in a tannery wastewater treatment. The AUR with abattoir wastewater treatment process is higher may be because it is free of toxicant materials like chromium and sulphides that are found in tannery wastewater (Masse and Masse, 2000). Toxic chemicals can block the nitrification process (Atlas, 1988).

Ammonium N removal efficiency was also affected by the influent loading perturbations during the experimental feeding phases. As the COD loading rate decreased from 1.7 to 1.05 kg/m^{3}d, ammonia N removal efficiency of the system increased from 58% to 96% (Figure 3).This illustrates that at higher organic loading rates the heterotrophic (growth rate may exceed) bacteria are favored and dominate the autotrophic nitrifiers lowering their proportion in the activated sludge system and thus their activity (Kristensen *et al.*, 1992; Gupta and Gupta, 2001; Jokela *et al.*, 2002; Seyoum *et al.*, 2004a).

A higher DO level in the aeration tank was observed to increase the ammonium N removal efficiency of the system. As the DO concentration increased from 2.1 mg/l to 4.5 mg/l the ammonium nitrogen removal efficiency raised from 58% - 96% (Figure 4). It is known that in a highly loaded activated sludge system the autotrophic nitrifiers are overgrown by the heterotrophic sludge flora, which consumes the oxygen available in the system (Wild *et al.*, 1971; Focht and Verstraete, 1977; Robertson and Kuenen, 1992). Thus it seems likely that the increased DO level ensured the availability of more oxygen for the slow growing autotrophic nitrifiers that could help them compete with heterotrophic bacteria and maximize their performance.

The NUR experiments gave results varying from 8.2 - 12.1 mg NO_3-N/gVSSh for the test wastewater used as an internal carbon source and 7.6-11.0 mg NO_3-N/gVSSh for acetate (Figure 8). The denitrification rate using the influent abattoir wastewater as internal carbon source was 7.3% - 10% higher than acetate used as an external carbon source. This manifests that abattoir wastewater contains broader and more easily biodegradable organic matter that can maintain more denitrifying consortia to be active in the system. It also shows a complete denitrification capacity of the system with out the need for any external carbon source. The same pattern was obtained by Seyoum *et al.*, 2004 from tannery wastewater.

Although the system was not designed for the removal of phosphate from the wastewater, some reduction in the phosphorus content of the wastewater was obtained. It was observed that the phosphate was reduced by 46% in the first feed and raised to 53% in the fourth feed. The average MLSS concentration also increased from 2.6 g/l to 3.7 g/l in those feeds (Figure 5). Phosphorus removal from wastewater can be accomplished biologically in activated sludge reactors by incorporating an anaerobic stage prior to aerobic basins. The resulting cyclic anaerobic and aerobic conditions favor the growth of microorganisms that utilize intracellular polyphosphate as an energy source during the anaerobic period, which allows them to sequester available carbon for use during the following aerobic stage (Mino *et al.*, 1998). In turn, the aerobic utilization of intracellular stored carbon is accompanied by the uptake of phosphorus and accumulation of phosphorus as polyphosphate. This polyphosphate accumulation results in efficient removal of phosphorus from the wastewater.

Therefore the cause for the reduction in phosphate seems to be utilization by the consertia of microorganisms in the activated sludge. In theory, the higher the MLSS concentration in the aeration tank the greater the efficiency of the process as there will be a greater biomass concentration to utilize available COD or Nutrients (Sidat *et al.*, 1998).

One of the methods to control the activated sludge process is characterization of the sludge in terms of certain characteristics. A well settling activated sludge is characterized by an SVI value less than 100 ml/g, while sludge with bulking problems has an SVI value greater than 200 ml/g (Wesley, 1966). The SVI of the activated sludge in the oxic tank of the system ranged from 51.5 ml/g to 87.6 ml/g in the treatment process (Table 1). Thus the sludge formed in the process had good settling characteristics that allowed it to be completely alienated from the treated wastewater (Gupta and Sharma, 1996; Cloete and Muyima, 1997). An SVI of 38.0 ml/g to 65.5 ml/g was reported by Seyoum *et al.*, 2004a in the same system.

Good performance of activated sludge process depends on the right choice of organic loading rate in order to insure proper floc formation and obtain more than 90% removal efficiencies (Gerardi, 2002; Seyoum *et al.*, 2004a). Thus it seems likely that a COD loading rate of 1.05 kg/m^{3}d is optimum for the maximum removal of organic matter and nitrogen (Table 3) from abattoir wastewater in this system under the operational parameters listed in table 1. Equivalent removal efficiencies were obtained from tannery wastewater treatment in the same system at a COD loading rate of 0.72 kg/m^{3}d (Seyoum *et al.*, 2004a).

The process involved in nitrogen removal from abattoir effluents must be not only technically efficient but also must be able to meet the effluent criteria in an economically viable manner (Cloete and Muyima, 1997). The activated sludge reactors with pre-anoxic tank employed for high strength wastewater in this study were not affected by shock loads and could achieve acceptable effluent quality in spite of dynamic influent feeds. The effluent total Nitrogen, ammonium nitrogen, COD, and BOD_5 concentrations obtained in the last experimental feed (Table 3) were in line with the discharge limit values for effluents into surface water bodies in Ethiopia which are 80 mg/l, 30 mg/l, 250 mg/l, and 80 mg/l respectively (EPA, 2003). This shows that the predenitrification/nitrification system is a good alternative to remove carbonaceous and nitrogenous compounds from abattoir wastewater.

6. CONCLUSIONS AND RECOMMENDATIONS

This study showed that the abattoir wastewater has a high pollution load and has sufficient nutrients for biological treatment. Nitrogen in its various forms can have toxicity effects on aquatic life forms, contributes to eutrophication of the receiving waters and also becomes a concern if the receiving stream is to be used downstream as a source of drinking water. The predenitrification-nitrification process used in this study was found to be efficient for the treatment of abattoir effluents containing high concentrations of degradable organic carbon, nitrogen, phosphate and ammonia. This process enables optimal use of the incoming abattoir effluent as carbon source for denitrification and helps to achieve high degrees of nitrification. High removal efficiencies were obtained for total nitrogen and COD ranging from 85% - 97% and 94% - 98%, respectively. Up to 96% ammonium nitrogen removal efficiency was obtained in the pilot plant. Phosphate reduction of 46% - 53% was also obtained in the process.

A pilot bioprocess plant operated for organic matter and nitrogen removal from abattoir effluents is a good example of an environmental scale bioreactor system. By making use of efficient microorganisms, application of this process with anaerobic modification to suit phosphorus removal to the abattoir industry can alleviate the problems associated with abattoir wastewater.

In order to optimize plant operation as well as to maximize plant efficiency and reliability:

1. Characterization of the nitrifying and denitrifying bacteria efficient in abattoir wastewater treatment should be made, and the isolates' activities should be tested.
2. More research is required in order to refine performance consistency and improve understanding of operational processes and mechanisms in the abattoir wastewater treatment by this type of treatment method. For instance to remove the high amount of phosphate in the abattoir wastewater a modified activated sludge system with anaerobic reactor should be tried.

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