# ADDIS ABABA UNIVERSITY
# SCHOOL OF GRADUATE STUDIES

# BIOLOGICAL NITROGEN REMOVAL FROM TANNERY WASTEWATER USING ALKALIPHILIC SLUDGE

**By**

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# Abbreviations

| | |
|---|---|
| AOB | Ammonia Oxidizing Bacteria |
| APHA | America Public Health Association |
| ATP | Adenosine Triphosphate |
| AUR | Ammonia Uptake Rate |
| $BOD_5$ | Biological Oxygen Demand |
| C:N | Carbon to Nitrogen ratio |
| COD | Chemical Oxygen Demand |
| COD:N | Chemical Oxygen Demand to Nitrogen ratio |
| DO | Dissolved Oxygen |
| GOV | Government of Ethiopia |
| EPA | Environmental Protection Authority |
| FISH | Fluorescent in Situ Hybridization |
| HRT | Hydraulic Retention Time |
| MLSS | Mixed Liquor Suspended Solids |
| MLVSS | Mixed Liquor Volatile suspended Solids |
| NEQS | National Environmental Quality Standards |
| NOB | Nitrite Oxidizing Bacteria |
| NUR | Nitrate Uptake Rate |
| OLR | Organic Load Rates |
| OUR | Oxygen Uptake Rate |
| rRNA | ribosomal Ribonucleic Acid |
| pH | Potential Hydrogenation |
| SNNPRG | Southern Nation Nationality People of Regional Government |
| SRT | Sludge Retention Time |
| TKN | Total Kjeldhal Nitrogen |
| TOC | Total Organic Carbon |
| UNEP | United Nation Environmental Protection |

# Abstract

Untreated tannery wastewaters contain high levels of organic materials and nitrogen. The principal forms of nitrogen in tannery wastewater are organic nitrogen (mainly proteins) and ammonia obtained from hides and skins. The presence of nitrogen in wastewater discharge can be undesirable because it has ecological impacts and can affect public health. Methemoglobin, eutrophication and depletion of dissolved oxygen in aquatic ecosystems are some of the major problems related to release of nitrogenous wastewater to the environment. Because of these pollution problems, nitrogenous compounds must be eliminated or reduced to the acceptable limits together with the organic carbon during wastewater treatment. The nitrogen in wastewater will be converted to the harmless nitrogen gas by microbial processes mainly through ammonification, nitrification and denitrification.

The objective of this study was to evaluate sludge biomass activity obtained from alkaliphilic environment for removal of nitrogen and organic matter from tannery effluent using lab-scale predenitrification/nitrification activated sludge system. This was fed with simulated and actual tannery wastewater. The raw tannery wastewater was obtained from the Modjo Tannery. The system was inoculated with sludge biomass prepared using sediment slurry from alkaline soda lake and artificial wastewater in batch reactor. The potential of sediment sludge to remove nitrogen and organic matter was analyzed using COD, BOD, TN/TKN, $NH^{+}_{4}$-N, $NO^{-}_{3}$-N, $S^{2-}$ and $SO_4^{2-}$ concentrations. The sludge activity was also tested for the occurrence of denitrifiaction and nitrification at higher pH using Nitrate and Ammonia Uptake Rates (NUR and AUR). The system was operated at three different organic loading rates (OLR) (10, 5 and 2.5 gm $l^{-1}$ $d^{-1}$).

The influent had an average concentrations of 8193.33, 2355, 645.67, 686, 222.33 and 564.67 mg $l^{-1}$ COD, BOD, TKN, $NH_4^{+}$-N, $S^{-2}$ and $SO_4^{2-}$, respectively, at 10 gm $l^{-1}$ $d^{-1}$ OLR. Average effluent concentrations of the aforementioned parameters at 5 gm $l^{-1}d^{-1}$ OLR were 4209.33, 1409.33, 834, 578, 256.67 and 458.67 mg $l^{-1}$ while at 2.5 $gml^{-1}d^{-1}$ OLR were 1886.67, 598.33, 672.33, 280.33, 241.67 and 401.33 mg $l^{-1}$.

The sediment sludge biomass was able to achieve 65.3, 89.2 and 97.1 % removal of COD and 59.7, 87.8 and 97.3 % removal of BOD at 10, 5 and 2.5 gm $l^{-1}$ $d^{-1}$ of OLRs, respectively. In addition, TKN removal efficiencies of 44.2, 86.7 and 96.6 % and $NH_4^+$-N removal efficiencies of 62.2, 74.5 and 94.8 % were achieved at 10, 5 and 2.5 gm $l^{-1}$ $d^{-1}$ of OLRs, respectively. The removal efficiency of sulphide was 38.2, 89.7 and 92.3 % at respective OLRs. Their potential to remove nitrate and ammonia nitrogen during NUR and AUR Test were 69.5 and 82.8 % at range pH values of 10.2 and 10.21 and 9.50 and 9.75, respectively. Maximum removal efficiencies (97.1, 97.3, 96.6, 94.8 and 92.3 %) of COD, BOD, TKN, $NH_4^+$-N and $S^{2-}$ were obtained at 2.5 gm $l^{-1}d^{-1}$ of OLR. At this OLR, the final COD, BOD, TKN, $NH_4^+$-N, $NO_3^-$-N and $S^{-2}$ were 55.33, 14.67, 22, 14.6, 4.53 and 18.67 mg $l^{-1}$, respectively. This is in line with the effluent discharge limit values in Ethiopia. Thus, alkaliphiles could be a good alternative to be used as inoculums for nitrogen and organic matter removal from industrial wastewaters.

**Key words/phrases**: *Activated sludge, alkaliphiles, ammonification, nitrification, denitrification.*

# 1. Introduction

## 1.1 Sources of Pollution

Pollution is unfavorable alteration of our environment largely as a result of harmful materials or excess of permitable limits of foreign substances. Several industries e.g. tanneries, slaughterhouses, fertilizer industries etc. discharge significant amounts of pollutant into their surroundings. Thus, the environment is under increasing pressure from wastes emanating from such different industrial activities. As compared to other industries, leather tanning is one of the most polluting activities. Tanning industry also has one of the highest toxic intensity per unit of output (Khan *et al.,* 1999). Because converting hides into leather involves several chemical intensive processes, the processing stages are pre-tanning (soaking, unhairing and liming, fleshing, deliming, washing, bating and de-greasing), tanning (pickling, chrome tanning, wet-blue storage, sorting, splitting and shaving), wet finishing (neutralization, re-tanning, washing, fat liquoring, dyeing and washing), dry machine process (setting, drying, shaving and pressing), and finishing (spraying /coating, drying and polishing) (UNEP,1991).

## 1.2 Nitrogen Sources in Leather Processing

Raw leather is made up of three main layers. The middle layer (corium) is the one to react with the tanning agent and to constitute the leather product, while others are removed during processing. The bottom layer is removed by mostly mechanical means. The upper layer (epidermis) has a complex structure and contains hair, glands, muscles, etc. Removal of this layer is usually done by chemical means, often through liming and bating processes.

A soaking process is employed for the rehydration of skins before the liming. Liming is the process where hair is removed from the skin. The reductive process is the most common method of liming. Two important processes take place during liming: hydrolysis and chemical reduction of keratin. Hydrolysis occurs in alkaline medium and lime being a base of limited solubility provides the most suitable conditions for the process by bringing and buffering the pH around 12.5. Globular proteins can easily be solubilized and removed out of the skins. Keratin and

collagen have comparable resistance to hydrolysis. To prevent hydrolysis of collagen, which is the main fibrous protein of corium, reduction of keratin is realized by the use of alkali sulfide salts. Elastin, another strong fibrous protein is not soluble under the conditions of liming. Bating is the removal of all unwanted proteins that remain in the liming step. This is carried out by solubilization of proteins using enzymes. In this step solubilized proteins are a mixture of epidermis proteins and their degradation products. Some chemically resistant fibrous proteins such as elastin are also removed. In the pickling process some globular proteins can be removed by the aid of pretanning agents (Thorstensen, 1985).

Leather tanneries produce all three categories of waste: wastewater, solid waste and gaseous emissions. However, wastewater is by far the most important environmental challenge being faced by tanneries. The wastewater comes from two main processes: beamhouse and tanning. In the beamhouse process highly pollutant loaded effluents are generated. The pollutant includes organic matter, suspended solids (as a result of unhairing and skinning), sulphides (used in the unhairing) and chlorides (from the salt present in the hides). The wastewater also contains bactericides (that is added to prevent degradation of the hide) and surfactants (to improve soaking). In the tanning process, the main pollutant is chromium, while other compounds may be used in the retanning process, such as tannins.

In the course of processing of hides into leather, roughly 50-150 liters of water is used per kilogram of converted leather (Khan *et al.,* 1999). Thus, effluents discharged from tanneries are voluminous, highly colored, contain a heavy sediment load including toxic metallic compounds, chemicals, biologically degradable materials and large quantities of putrefying suspended mater. Many authors have done a thorough characterization of different streams generated by tanneries (Tünay *et al.*, 1995; Orhon *et al.*, 1999; Rivela *et al.*, 2002; Seyoum Leta *et al.*, 2004). A detailed source characterization study conducted by Zengin *et al*. (2002) and Seyoum Leta *et al.* (2004) have also shown nitrogen speciation and distribution for bovine and sheep and goatskins leather processing plant, respectively. The amount and composition of total nitrogen in wastewater varies with the type of leather processed, amount of chemicals used in the processing (particularly ammonium sulfate), water use and several other factors such as duration of process.

In Ethiopia, leather tanning is one of the large income generating industries. Currently there are more than 20 factories with annual production capacity of around 1.7 million tones of hides. These industries create 2.0 to 2.5 million $m^3$ amount of wastewater (EPA, 2003). Tannery wastewater is highly polluted and its discharge contains higher pollutants than the limits set by the National Environmental Quality Standards (NEQS) for all important wastewater parameters (Seyoum Leta *et al.,* 2004).

For most part, the current practice is to discharge this water into the local environment without any treatment, resulting in contamination of surface as well as sub- surface water. The lack of effective rules and regulations as well as poor treatment practices have further aggravated the pollution problem caused by the tanning industry in developing counties (Sedlack, 1991).

## 1.3 Environmental Effects of Nitrogen

The principal forms of nitrogen in tannery wastewater are organic substances mainly proteins and amines from hides and skins, a volatile irritating, offensive odorous ammonia gas ($NH_3$) and a highly pollutant and carcinogenic compound of nitrate ($NO_3^-$). The presence of nitrogen in wastewater discharge can be undesirable because it has ecological impacts and can affect public health (Reed, 1984). Thus, nitrogen is becoming increasingly important in wastewater management (Halling-Sorensen and Jorgensen, 1993).

Nitrogen oxides are the major pollutants in the atmosphere, being a precursor to acid rain. Kelter *et al.* (1997) reported that, the oxides, mainly nitric oxide (NO), nitrous oxide ($N_2O$) and nitrogen dioxide ($NO_2$) are corrosive and hazardous to health. Ammonia is extremely toxic to fish and many other aquatic organisms. It is also an oxygen-consuming compound, which can deplete the dissolved oxygen (DO) in water. This is a problem since maintenance of a high oxygen concentration is crucial for the survival of the higher life forms found in aquatic ecosystem (Reed, 1984).

Nitrate can produce a serious problem in fish and causes what is known as brown blood disease (Halling-Sorensen and Jorgensen, 1993). Its conversion to nitrite is also a concern to public

health. Nitrite is a potential public health hazard in water consumed by infants (Sedlak, 1991). In the body, nitrite can oxidize the iron (II) and form methemoglobin, which binds and transports oxygen less effectively than normal hemoglobin. The resulting decrease in oxygen levels in young children leads to shortness of breathe, diarrhea, vomiting and in extreme cases even death (Kelter *et al.,* 1997).

Nitrogen compounds act as nutrients for algae, weeds and other aquatic plants in water bodies. Discharging wastewater that contains high amount of nitrogen into water body results in excessive growth of algae, weeds and other aquatic plants. The excessive growth and death of plants in water bodies results in the problem of eutrophication. In eutrophication, increased concentrations of the limiting nutrients for growth are added to the ecosystem. The excessive growth of these organisms as well as decomposition of dead organic matter by microbes contributes to the reduction of the oxygen level in water. Thus, the water bodies start to stink (Reed, 1984).

All such ecological and health problems due to discharging tannery pollutants into the environment arise through time since the level of inputs of nitrogenous wastes become beyond the natural capacity of the microorganisms in the ecosystem. As a result of environmental concern, the Ethiopian government urges to formulate strict legislation on effluent discharge (GOE, 2002). Interest has also grown recently for the development of methods, which allows recycling and sustainable using of wastewater to achieve standards for discharge limits.

## 1.4 Wastewater Treatment Methods

Treatment methods used to remove contaminants from wastewater are broadly classified into unit operations and unit processes. In order to achieve acceptable level of contaminant removal, wastewater treatment procedures are further classified as primary, secondary, and tertiary wastewater treatment systems (Metcalf and Eddy, 2003).

Unit operations (physical processes) are treatment methods where physical forces are applied in order to remove floating and settleable solids found in wastewater. Today, they still form the

basis of most process flow systems for wastewater treatment. Screening, comminution, flow equalization, sedimentation, flotation and granular-medium filtration are common physical wastewater treatment methods.

Unit processes (chemical and biological processes) are those treatment methods in which removal of contaminant is brought about by means of chemical or biological reactions. Chemical processes used in wastewater treatment are designed to bring about some form of change by means of chemical reactions. They are always used in conjunction with physical unit operations and biological processes. In general, chemical unit processes have an inherent disadvantage compared to physical operations in that they are additive processes. That is to say, there is usually a net increase in the dissolved constituents of the wastewater (Ecknfelder *et al.,* 1985). This can be a significant factor if the wastewater is to be reused. The main chemical unit processes include chemical precipitation, adsorption, disinfection and dechlorination.

Physio-chemical units based on chemical precipitation have been commonly used to treat effluents from tannery industry. As these methods are viewed from the standpoint of industrial wastewater treatment, some of them seem to be inapplicable, or at least very difficult or costly to apply. For instance, Gallert and Winter (2005) explained that, unlike phosphates, all nitrogen compounds with the exception of magnesium ammonium phosphates are easily soluble in water and cannot be removed by precipitation.

## 1.5 Biological Unit Processes

During the last few decades processes based on biological treatments have been increasingly studied and applied. They are simple, accessible, affordable, cost effective and environmentally friendly for the treatment of nitrogenous rich tannery wastewaters to meet the discharge limits (Han *et al.,* 2001; Gerardi, 2002; Issayas Tadesse, 2003; Seyoum Leta, 2004; Kasia *et al.,* 2005). In these processes, microorganisms, particularly bacteria, convert the colloidal and dissolved carbonaceous organic matter into various gases and into cell tissue, which is then removed in sedimentation tanks (Grady *et al.,* 1999). Biological processes are usually used in conjunction with physical and chemical processes, with the main objective of reducing the organic content

(measured as BOD, TOC or COD) and nutrient content (notably nitrogen and phosphorus) of wastewater.

Many types of biological nitrogen removal systems have been developed. Biological processes used for wastewater treatment may be classified under five major headings: aerobic processes, anoxic processes, anaerobic processes, combined processes and pond processes. These processes are further subdivided, depending on whether the treatment takes place in a suspended-growth system, an attached-growth system or a combination of both. Trickling filter, rotating biological contactors and submerged or expanded bed aerobic filters are examples of attached growth system. Activated sludge, aerated lagoons and oxidation ditch are grouped under suspended growth systems (Metcalf and Eddy, 2003).

## 1.6 Activated-Sludge Process

Most of the research on nitrogen removal has involved using an activated sludge system. The activated-sludge process is the most widely used biological treatment method at present for carboneous oxidation and nitrification of both industrial and domestic wastewaters. It is a continuous- or semi-continuous-flow system containing a mass of activated microorganisms that are capable of stabilizing organic matter. An active mass of microorganisms, mainly bacteria and protozoa aerobically degrade organic matter into $CO_2$, $H_2O$, new cells and other end products. The unsettleable suspended solids and other constituents adsorbed on or entrapped by the activated sludge floc (Cloete and Muyima, 1997).

The bacteria involved in activated sludge systems are carbon oxidizers, nitrogen oxidizers, floc formers and non-floc formers, and aerobes and facultative anaerobes. The protozoa, for their part, include flagellates, amoebas and ciliates. Algae, metazoan and viruses are also common in activated sludge process (Wesley, 1966).

For effective functioning of the system, it should fulfill the following biological requirements (Wesley, 1966):

a. The required population must be able to degrade the components of the wastewaters and grow

in the environment of the aeration tank.

b. The organisms must grow in such a form that they will settle out in the clarifier.

Control of the activated-sludge process is important to maintain a high treatment performance level under a wide range of operating conditions. According to Gerardi (2002), the principal factors in process control are the following:

(a) Maintenance of dissolved oxygen levels in the aeration tanks;

(b) Control of the waste and amount of returning activated sludge

The main operational problem encountered in a system of this kind is sludge bulking, which can be caused by the absence of phosphorus, nitrogen and trace elements and wide fluctuations in pH, temperature and DO. Bulky sludge has poor settleability and compactibility due to the excessive growth of filamentous microorganisms (Liu and Liptak, 1999; Metcalf and Eddy, 2003). While many parameters such as solid retention time and hydraulic retention time are known to affect the operation of wastewater treatment systems. Some other conditions particularly the carbon to nitrogen ratio of the wastewater, temperature and pH in the reactor, and inhibition of nitrification are important to biological nitrogen removal systems

The activated-sludge process has undergone different modification based on the flow system in the reactor, the size, number and configuration of the reactors, recycled flow, influent flow and others to achieve discharge limits during wastewater treatment (Ekama and Marais, 1984). Modifications to the conventional activated sludge process include step and complete aeration, contact stabilization, plug flow and other systems.

## 1.7 Predenitrification-Nitrification Activated Sludge Process

Some treatment schemes conduct nitrification and denitrification in separate systems, and are known as a dual sludge process. One disadvantage to this approach is the involvement of more equipment, such as additional clarifiers and piping. Single sludge processes are also used, where nitrification and denitrification occur in one system but in different zones. These can involve any number of tanks. Some of the first single sludge treatment processes developed for nitrogen

removal were the Modified Ludzack-Ettinger (MLE) and the Bardenpho (Figures 1 and 2 respectively) (Metcalf and Eddy, 2003). These processes work by operating separate aerated and non-aerated tanks in a series.

In the MLE, a mixed liquor recycle runs from the aerobic reactor back to the anoxic reactor. An aerobic environment is maintained in the basin by means of diffused or mechanical aeration, which also serves to keep the contents of the reactor (or mixed liquor) completely mixed. After a specific retention time, the mixed liquor passes into the clarifier, where the sludge is allowed to settle and a clarified effluent is produced for discharge. The process recycles a portion of the settled sludge back to the aeration basin to maintain the required activated sludge concentration (Ecknfelder ***et al.***, 1985). The process also intentionally removes a portion of the settled sludge to maintain the required solids retention time (SRT) for effective organic removal. The Bardenpho adds two additional reactors (one anoxic and the other aerobic) after the first anoxic and aerobic reactors that allow more denitrification to occur in the second anoxic reactor by using endogenous and slowly degradable substrate as a carbon source for denitrification.

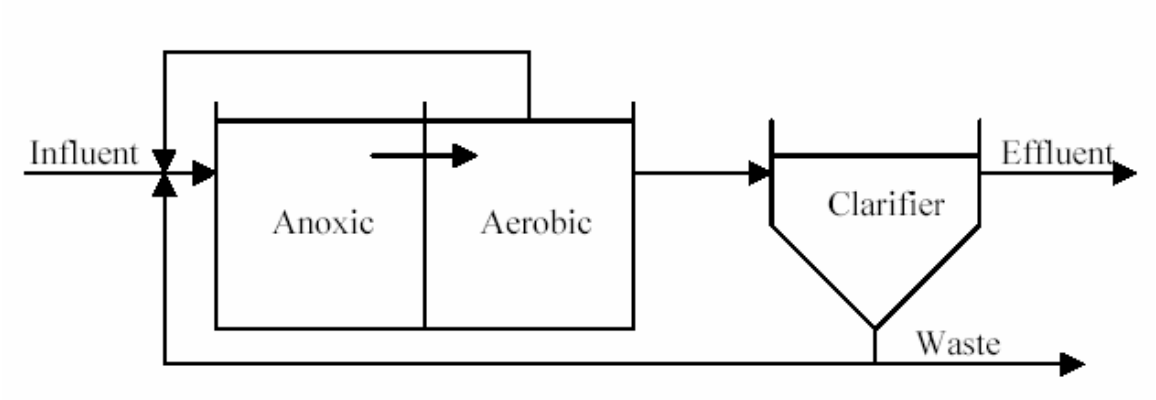


Figure 1. Modified Ludzack-Ettinger (MLE) process for biological nitrogen removal (Metcalf and Eddy, 2003).

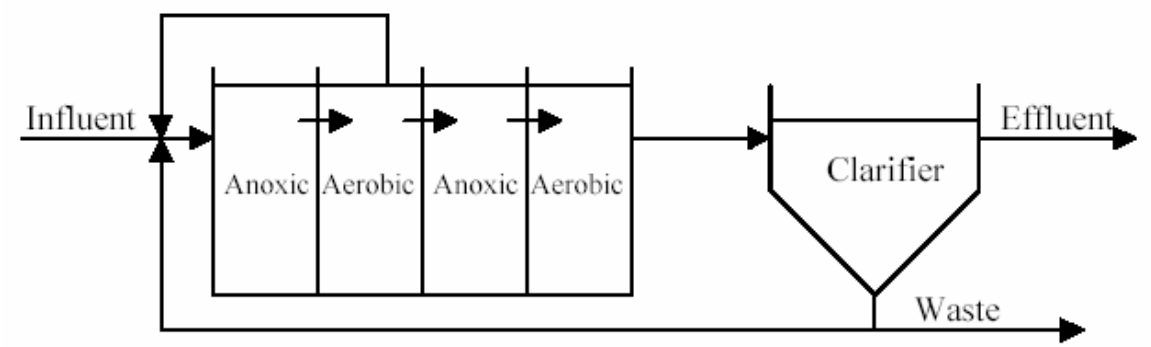


Figure 2. Bardenpho process for biological nitrogen removal (Metcalf and Eddy, 2003).

The advantage of using pre-denitrification nitrification process is that the original carbon source in the influent can be used for denitrification to substitute an additional carbon source and also avoid sludge disposal problems. This is because, returning of a mixed liquor which contain nitrate to anoxic basin reduced to elemental nitrogen using carbon source from incoming wastewater (Grunditz, 1999). The nitrogen in wastewater will be converted to the harmless nitrogen gas through three major biological transformations during removal of nitrogen in microbial treatment systems. The three processes are ammonification, nitrification and denitrification.

### 1.7.1 Ammonification

The main organic compounds in tannery wastewater are proteins from hides and skins. Ammonification occurs when organic nitrogen is converted to ammonia. It is an important mechanism that ultimately allows organic nitrogen to be removed from wastewaters through hydrolysis to amino acids. Degradation of amino acids leads to liberation of ammonia by the various mechanisms of ammonification (Rheinheimer ***et al.***, 1988). These include hydrolytic, oxidative, reductive and desaturative deamination, which are presented from Eqn. 1 to 4, respectively.

$R\text{-}NH_2 + H_2O \longrightarrow R\text{-}OH + NH_3$ ……..Eqn. 1

$R\text{-}CHNH_2COOH \longrightarrow R\text{-}CO\text{-}COOH + 2H^+ + NH_3$ ……..Eqn. 2

$R\text{-}CHNH_2\text{-}COOH + 2H^+ \longrightarrow R\text{-}CH_2\text{-}COOH + NH_3$…….Eqn. 3

$R\text{-}CH_2\text{-}CHNH_2\text{-}COOH \longrightarrow R\text{-}CH{=}CH\text{-}COOH + NH_3$ ….Eqn. 4

Under aerobic treatment process, bacteria assimilate nitrogen as ammonia or nitrite and form cellular mass. Although this assimilation does result in a net loss of nitrogen from the soluble phase, it is not one of the major transformations of nitrogen that leads to removal. In most high strength wastewaters high levels remain even after the bacteria use what they need for growth. To eliminate ammonia that is not used for cell growth during treatment, it must be nitrified and then denitrified to molecular nitrogen (Gallert and Winter, 2005).

### 1.7.2 Nitrification

Nitrification is the biological oxidation of ammonium nitrogen in to nitrite or nitrate through the action of group of bacteria called nitrifiers. In nitrification, there are two distinct groups of bacteria (ammonia and nitrite oxidizing bacteria), which belongs to the *Nitrobacteriaceae* family. Ammonium nitrogen is oxidized to nitrite ($NO_2^-$-N) by ammonia oxidizing bacteria (AOB) and then to nitrate ($NO_3^-$-N) by nitrite oxidizing bacteria (NOB). The first reaction is nitritation (Eqn. 5) and the later one is nitratation (Eqn. 6). Nitrifiers use $NH^+_4$ or $NH_3$ as an electron donor, $O_2$ as electron acceptor and $CO_2$ as a carbon source for the building of their biomass. Hence, many of AOBs and NOBs are autotrophic and few heterotrophic bacteria are also known to function as nitrifiers (Painter, 1977; Prosser, 1989; Gerardi, 2002).

$$NH^+_4 + 1.5\ O_2 \longrightarrow NO^-_2 + H_2O + 2H^+ \text{ .....…Eqn. 5}$$

$$NO^-_2 + 0.5\ O_2 \longrightarrow NO^-_3 \text{ …… Eqn. 6}$$

The overall reaction is:

$$NH^+_4 + 2\ O_2 \longrightarrow NO^-_3 + H_2O + 2\ H^+$$

Autotrophic nitrifiers are aerobic microbes and include strictly chemolithotrophic species that catalyze nitritation and facultative chemolitotrophs which catalyze nitration. The strictly chemolithotrophs belong to the genera of *Nitrosomonas, Nitrosococcus, Nitrosolobus, Nitrosospira, Nitrosovibrio and Nitrocystis,* whereas the facultative chemolithotrophs are members of the genera *Nitrobacter, Nitrococcus, Nitrospira and Nitrospina* (Prosser, 1989; Wagner *et al.,* 1996; Gerardi, 2002). Due to their slow growth, autotrophic nitrifiers cannot compete well with heterotrophs for oxygen. In a highly loaded activated sludge system, the

heterotrophic sludge flora, which consumes oxygen, overgrows the autotrophic nitrifiers (Wild *et al.,* 1971).

Nitrification also proceeds by heterotrophs. Heterotrophic nitrifiers oxidize reduced nitrogen containing compounds like hydroxylamine, aliphatic and aromatic nitrogen containing compounds (Eqn. 7). Some bacteria from the genera of *Flavobacterium, Arthrobacter* and *Thiosphaera* are known to be heterotrophic nitrifiers (Robertson and Kuenen, 1992; Schmidt and Bock, 1997; Jetten *et al.,* 1998; Thamdrup and Dalsgaard, 2002). Unlike autotrophic nitrifiers, in heterotrophic nitrification no energy is gained by nitrate formation, as a result there is an absolute request for organic metabolism (Strous *et al.,* 1997).

$$R\text{-}NH_2 \longrightarrow R\text{-}NHOH \longrightarrow R\text{-}NO^- \longrightarrow R\text{-}NO \longrightarrow NO^-_3 \ldots\ldots \text{Eqn. 7}$$

Because of higher rates of autotrophic nitrification than heterotrophic nitrification, in nature it is believed that autotrophic nitrifiers play a more important role than the heterotrophs (Robertson and Kuenen, 1992). In most situations, very little nitrite exists in a system at any one time because the conversion of ammonia to nitrite by AOBs is generally the rate-limiting step (Antoniou *et al.,* 1990) and the nitrite oxidation follows quickly. The nitrate formed can then be used as a nitrogen source or as an electron acceptor under anaerobic conditions by denitrifiers.

**Environmental Factors Affecting Nitrification**

Nitrifiers are highly susceptible to a wide range of environmental factors like dissolved oxygen (DO), pH, temperature, concentration of $NH_4^+$-N, presence of inhibitors and the microbial population (Gerardi, 2002).

Since autotrophic nitrifiers are obligate aerobes, DO concentration in wastewater treatment is one of the most important requirements for optimum function. At lower DO levels (i.e. < 2 mg/l) the nitrification rate will be significantly reduced. At DO levels below 0.5 mg/l there is no nitrification at all (Gerardi, 2002). Numerous reports indicated that in order to ensure that DO is not a limiting factor in the nitrification reaction, a level not less than 2.0 mg/l must be maintained (Wuhrmann, 1973; Cloete and Muyima, 1997; Gerardi, 2002).

Nitrification, like most bacterial processes, is affected by pH conditions. Generally, optimum conditions have been found to exist between 6.5 and 8.6 (Antoniou ***et al.,*** 1990). Variations in pH optima results shock effects in adjusting culture conditions or improper acclimatization (Im ***et al.,*** 2001). There are also some studies that indicate the occurrence of nitrification at pH value about 10. For instance, Sorokin ***et al.*** (1998) isolates five strains of lithotrophic, nitrite-oxidizing bacteria from sediments of three soda lakes (Kunkur Steppe, Siberia; Crater lake and Lake Nakuru, Kenya) and from a soda soil (Kunkur Steppe, Siberia) after enrichment at pH 10 with nitrate as sole electron source.

Nitrification is a hydrogen ion producing reaction (Eqn. 5). This hydrogen ion reacts with the bicarbonate in the wastewater, resulting in an increase in $CO_2$ concentration that ultimately causes a decrease in pH (Eqn. 8). Acidic nitrate formation also results in a drop in wastewater pH. A pH value of less than 6.7 could likely reduce or even stop further autotrophic nitrification (Gerardi, 2002), the alkalinity of wastewater is an important consideration for the treatment process. However, $CO_2$ consumption for autotrophic growth slightly increases wastewater alkalinity and preceding the process together with denitrification maintains the pH.

$$H^+ + HCO^-_3 \longrightarrow CO_2 + H_2O \text{ ……. Eqn. 8}$$

Nitrification occurs over a range of temperature approximately between 4 and 40 $^oC$. The optimum temperature for pure cultures ranges from 25-30 $^oC$ in water bodies (Gerardi, 2002). In a suspended and supported growth nitrification system differences in the reported temperature sensitivity may be due to differences in reactor solid retention time (SRT). Increasing the SRT results in a decrease in the temperature sensitivity (Cloete and Muyima, 1997).

Nitrifiers are highly susceptible to a number of inhibitors that may be present in industrial and municipal wastewaters. The inhibitors include phenolic compounds, halogenated solvents, heavy metals and extremely high concentration of ammonia nitrogen and nitrous acid (Bitton, 1999). Since addition of high organic load promotes hetertrophic growth due to strong competition for the available $O_2$ and $NH_3$ over nitrifiers leads to reduction of nitrification rates (van Benthum ***et al.,*** 1997). In addition, fast growing heterotrophs occupy the outer layer of the biofilm and nitrifiers inside it, limits $O_2$ availability, it affect nitrification rates (Nogueira ***et al.,*** 2002).

### 1.7.3 Denitrification

Denitrification is the key process involved in nitrogen removal from wastewater. It is the reduction of oxidized nitrogen compounds (nitrate and nitrite) into elemental nitrogen using microorganisms. This is known as dissimilatory denitrification. It occurs when the oxygen concentration in the wastewater becomes low enough that the bacteria begin to utilize nitrate as an electron acceptor under anoxic conditions (Oswald *et al.,* 1996). The process of denitrification involves the following enzyme catalyzed sequence of reactions (Eqn.9).

$$NO^-_3 \xrightarrow{\text{nitrate reductase}} NO^-_2 \xrightarrow{\text{nitrite reductase}} NO\ (g) \xrightarrow{\text{nitric oxide reductase}} 0.5\ N_2O\ (g) \xrightarrow{\text{nitrous oxide reductase}} 0.5\ N_2\ (g) \text{ …..... Eqn. 9}$$

During denitrification, the organic compounds serve as electron donor and the nitrates act as electron acceptor. The electron moves via a series of electron acceptor from donor to final acceptor in the electron transport system. On the other hand, in the assimilatory denitrification, many aerobic and anaerobic bacteria reduce nitrate to supply the cells with ammonia for synthesis of cell substance (Eqn. 10). However, the enzymes for nitrate assimilation are expressed only at concentration of ammonia less than 1 mM (Gallert and Winter, 2005).

$$NO^-_3 \xrightarrow{a} NO^-_2 \xrightarrow{b} HNO \xrightarrow{b} NH_2OH \xrightarrow{b} NH_3 \text{ ….....Eqn. 10}$$

Here, nitrate reductase B (enzyme a in Eqn.10) reduces nitrate to nitrite, which is then reduced to ammonia by the nitrite reductase complex (enzyme b in Eqn.10).

In wastewater treatment plants, the nitrification rate is usually much slower than the denitrification rate. Thus, the supply of $NO_3^-$-N, which limits the denitrification process, has often been identified as a problematic issue and remains a challenge in treatment plants (Busnardo *et al.,* 1992; Newman *et al.,* 2000).

A wide variety of microorganisms in activated sludge have the ability to use nitrate as final electron acceptor. Most denitrifiers need organic substrates as carbon and energy sources i.e. they are heterotrophs (organotrophs). Some of them are obligate or facultative chemolithotrophs that

utilize hydrogen or reduced sulfur as sources of energy (electron donors) and $CO_2$ as carbon source (Eqn. 11). ***Thiobacillus denitrificans*** and ***Ferrobacillus ferroxidans*** are examples of the first and second groups, respectively (Bridge and Jonson, 1998). However, for wastewater treatment technology, only the hetertrophic nitrate dissimilation, i.e., denitrification is important (Plessis ***et al.,*** 1998).

$$FeS_2 + 6\ NO^-_3 + 2\ H^+ \longrightarrow 3\ N_2 + 4\ SO_4^{2-} + 2\ Fe^{2+} + 2\ H_2O \quad \text{……Eqn. 11}$$

The common denitrifying bacteria in wastewater treatment plants are ***Paracoccus, Brachymonas, Micrococcus, Achromobacter, Spirilium, Bacillus, Alcaligenes, Brevibacterium, Flavobacterium*** and ***Moraxella*** (Delwiche, 1976; Cloete and Muyima, 1997; Metcalf and Eddy, 2003; Seyoum Leta, 2004). Certain yeast species such as ***Trichosphoron cutaneum, Fellomyces fuzhovensis*** and ***Candida sp***. have also been screened for their incomplete denitrifing activities (i.e., reducing nitrite to nitrous oxide (Cloete and Muyima, 1997).

**Environmental Factors Affecting Denitrification**

Several factors such as organic matter (energy sources), anoxic conditions, pH, temperature, presence of inhibitory substances and the diversity denitrifiers are known to affect denitrification rates. The ratio of available organic carbon to nitrate in the treatment plant is the main determinant to affect assimilatory and dissimilatory denitrification. At high ratios, nitrate is reduced to ammonia whereas denitrification is the preferred pathway at low ratios (Whitson ***et al.,*** 1993).

The presence of DO in wastewater treatment plants reduces the denitrification rates. This is because of the effect of oxygen in the synthesis of necessary enzymes or activity of the preformed enzymes required for dissimilatory denitrification (Chang and Morries, 1982). Although denitrification will be observed in systems at low oxygen tension, a completely anoxic reactor will maximize nitrate reduction. However, at the start up of the process a complete anoxic condition does not exist, but subsequent oxidation of organic substances leads to anoxic condition

by depletion of DO in the system (Plessis *et al.,* 1998). Aerobic denitrifiers are also isolated by different researcher (Robertson and Kuenen, 1984; Ludwing *et al.,* 1993).

The optimum pH for denitrification is usually reported to lie between 7.0 and 8.5 (Metcalf and Eddy, 2003). Even though denitrification is a basic reaction which consume hydrogen ion tending to raise the pH, when it occurs together with nitrification maintains the required pH (Kadlec *et al.,* 2000; Gerardi, 2002). One study showed that denitrification also occurs in lakes evidenced from the isolation of three denitrifying bacterial strains from Lake Boney (Ward and Priscu, 1997).

Denitrification is reported to occur over a wide range of temperature (i.e., 5 to 45 $^{o}$C). But if temperature value is less than 5 $^{o}$C and greater than 50 $^{o}$C, the denitrification rates is significantly reduced (Gerardi, 2002). Denitrifiers are less sensitive to toxic substances than nitrifiers and the autotrophic nitrifiers are more sensitive than the heterotrophic denitrifiers (Gumaelius *et al.,* 1996; Seyoum Leta, 2004).

The biomass of the activated sludge is the active agent in biological wastewater treatment and in terms of mixed liquor volatile suspended solid (MLVSS). The processes occurring in wastewater treatment plants are often described in terms of characterization and quantification to metabolic activities. The most common method to characterize activated metabolic activities is done by measuring the rate of consumption of components entering the biochemical reactions or the rate of formation reaction products (Cloete and Muyima, 1997). The nitrifying and denitrifying capacities of activated sludge are determined by nitrification rate (measured as Ammonia Uptake Rate, AUR or nitrate production rate), and denitrification rate (Nitrate Uptake Rate, NUR) (Kristensen *et al.,* 1992).

A modified activated sludge process, usually a pre-denitrification process has often been applied for nitrogen removal in municipal as well as industrial wastewater treatment (Ekama and Marais, 1984; Heijnen *et al.,* 1990; Chen *et al.,* 1991; Cecen and Goenenc, 1995; Collivignarelli and Bertanza, 1999; Gerardi, 2002; Ruiz *et al.,* 2006). The treatment of tannery effluent using different biological wastewater treatment methods have been studied by different researchers. For

instance, Artiga *et al.* (2005) studied the treatment of tannery wastewater using an innovative biofilm suspended biomass hybrid membrane bioreactor and achieved 95 and 97% removal of COD and ammonia-N, respectively.

In Ethiopia the treatment of tannery wastewater in an advanced integrated wastewater pond system was also studied (Issayas Taddesse, 2003). Seyoum Leta (2004) also used pre-denitrification/nitrification system at a pilot plant level to treat a composite tannery wastewater and obtained 98 % removal efficiencies of COD and total nitrogen. This system was also used by Fantahun Woldesenbet (2005) for the treatment of abattoir wastewater and achieved total COD and nitrogen removal efficiencies of 98 % and 97 %, respectively. The seed (inocula) for such treatment were obtained from the pervious tannery effluent treatment plants (Ethiopia tannery wastewater treatment plant and Kaliti wastewater treatment pond system).

Alkaliphiles (alkaline loving) are microorganisms that occupy extreme high pH environments. However, in both naturally and artificially occurring environments, the pH values are often far from constant and are subjected to short term changes in the pH of the environment involved (Ulukanli, 2002). Evolution in alkaliphiles brings unique adaptation and fundamental biological processes for actual or potential applications in various fields of biotechnology (Ivey *et al.*, 1998).

Metabolically diverse groups of microorganisms like organtrophs and autothrophs; aerobic, anaerobic and facultative aerobes; sulfur oxidizing and sulphate reducing; nitrifying and denitrifying bacteria; *cyanobacteria* (mainly *spirulina spp.*), yeasts and filamenteous fungus are found in alkaline environments (soda lakes, deserts, soils, artificially occurring industrial derived wastes) (Horikoshi, 1991; Grant and Horikoshi, 1992; Duckworth *et al.*, 1996; Groth *et al.*, 1997; Ulukanti, 2002; Humayoun *et al.*, 2003).

Alkaline soda lakes are the most studied naturally occurring alkaline environments on earth with pH values generally higher than 10. These lakes are concentrated in particular regions such as in northern (Wadi Natrun) and eastern (Rift Valley) Africa, the western United States and parts of

Asia and Europe (Jones *et al.,* 1998). They support large standing consortia of alkaliphilic microorganisms (Grant, 1992).

Due attention has been given by different researchers to such environment mainly for isolation and characterization of individual organisms with potential industrial applications particularly for extraction of enzymes (Horikoshi, 1996; Amare Gessesse, 1998a) and pharmaceutical compounds (Sato *et al.,* 1983; Tsai *et al.,* 1995), degradation of macromolecules (Crawford, 1986; Kirk and Farrell, 1987; Horikoshi, 1999) and sources of food (Richmond, 1990; Fox, 1997). Their phylogenetic diversity and variations in bacterial community composition with the depths of a lake recently were analyzed in some areas (Duckworth *et al.,* 1996; Humayoun *et al.,* 2003).

A number of alkaline soda lakes are found in Ethiopia. Although the microbial ecology, their enormous potential as a source of new alkaliphiles and applications for various biotechnology purposes are fairly studied (Amare Gessesse, 1998; Azaga Hasana, 2000), there is no published information regarding using alkaliphiles as inocula for biological wastewater treatment technology.

The creator lake Chitu is one of the productive alkaline soda lakes in the Rift Valley system in Ethiopia. It consists of a huge amount of algal population mainly ***Spirulina spp.*** (Elizabeth Kebede *et al.,* 1996). The Spirulina contain about 60-70% of protein and some other nitrogenous compounds (Ulukanli, 2002). The dead algalmate deposits high amounts of partially degraded organic nitrogenous and carbon compounds to the lake. It also receives different nitrogenous source from external environment like human and animal urine, animal dung and human fecal matter from inhabit near by the lake (Elizabeth Kebede *et al.,* 1996).

In the presence of such internal and external waste deposit, the closed basin Lake Chitu is not deteriorated with characteristics of foul odor and color change. This may be due to self-purification activities by the indigenous microbial communities. Given that pH of tannery wastewater is similar to that of alkaline soda lakes and presuppose the presence of specific group of specialized microbes, sediment sludge from Lake Chitu were used as inoculums to evaluate

their potential to remove nitrogen and organic matter from tannery wastewater in this study. The objectives of the present study were, therefore:

1. To evaluate the potential of sediment sludge from alkaliphilic environment to remove nitrogen and organic matter from tannery wastewater using lab-scale predenitrification-nitrification activated sludge system.
2. To test whether dentrification and nitrification can take place at higher pH using NUR and AUR.

# 2. Materials and Methods

## 2.1 Samples and Sampling Sites

### 2.1.1 Lake Water Sample

Sediment alkaline soda lake slurry samples were brought from Lake Chitu in sterile sampling plastic bottles to the Department of Biology, AAU. The crater lake (Lake Chitu) is situated within the Ethiopian Rift Valley at an altitude of 1600 m above sea level. It is the smallest of all known alkaline soda lakes found in the country. It has a moderate maximum depth of 21 m and covers diameter of about 0.8 $km^2$. It is a closed basin with no obvious surface out flow and looks green indicating a high algal biomass. It is the most productive lakes in the country with average chlorophyll-a content of 224 $\mu gl^{-1}$. The lake water is alkaline, having an average alkalinity of 573 $meql^{-1}$ and pH value of 10.2. The alkalinity is attributed to the high content of sodium carbonate (Elizabeth Kebede *et al.,* 1996).

### 2.1.2 Tannery Wastewater

Samples of composite tannery wastewater were brought from Modjo tanning industry, which is located 80 Kms South of Addis Ababa. Modjo tannery is a medium sized leather industry in Ethiopia with annual processing capacity of 844,000 sheepskins and 1,656,000 goat skins (EPA, 2003). The plant is installed near the Modjo River and discharges volumetric effluents which varies between 3,500-5,500 cubic meters per day directly into the river course (Seyoum Leta, 2004). The wastewater samples were collected in sterile plastic sampling bottles and transported to the Department of Biology, AAU for further study.

### 2.1.3 Synthetic Wastewater Composition

Synthetic wastewater similar in composition to tannery wastewater without chromium was simulated using distilled water (Table-1). Sterilization was carried out by autoclaving at 121 $^0C$

for 15 minutes. After sterilization, the pH of the medium was adjusted to 10.3 with sodium carbonate.

Table1: Components of synthetic wastewater (Seyoum Leta, 2004).

| Component | Amount (g/l) |
|---|---|
| Sodium acetate | 0.7 |
| Ammonium sulfate | 1 |
| Potassium phosphate ($K_2HPO_4$) | 0.05 |
| Peptone | 1 |
| casein | 0.4 |
| Sodium thiosulphate | 0.3 |
| Urea | 0.3 |
| Iron chloride | 0.1 |

## 2.2 Experimental Set-up

The laboratory-scale pre-denitrification/nitrification treatment plant used in this study is shown in Figure 3. This system was composed of a series of four reactors. These are ten liter influent feeding tank, two liter anoxic reactor with one liter working volume which has anoxic bacteria for denitrification, four liter aerobic reactor with two liter working volume for organic compounds removal and nitrification and clarifier (sedimentation tank). Aerobic column was aerated using a modular fermenter aerator to supply oxygen. Both aerobic and anaerobic reactors were equipped with a magnetic stirrer for keeping their content and microbes well mixed and distributed throughout the container.

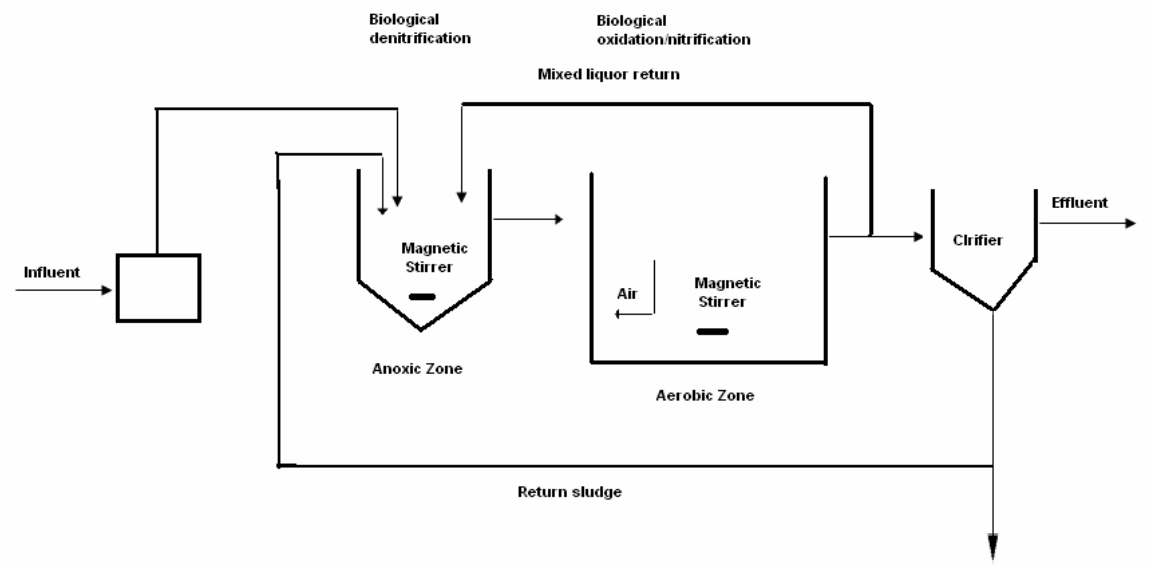


Figure 3: Schematic diagram showing laboratory-scale pre-denitrification/nitrification activated sludge nitrogen and organic matter removal system (Seyoum Leta *et al.*, 2004).

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The influent from feed tank flowed down through the anoxic zone and then to the aerobic zone at rate of 2 ml per minute to make the hydraulic retention time of the anoxic reactor 16.67 hours and that of the oxic reactor 33.34 hours. Concurrently, the mixed liquor from aerobic basin and sludge from effluent were retuned manually to the anoxic one. The treatment plant was installed in the laboratory at room temperature. The synthetic wastewater tested at three different organic loading rates (OLR), i.e. 4.6, 3.1 and 2.6 gm $l^{-1}$ $day^{-1}$, for tannery wastewater at 10, 5 and 2.5 gm $l^{-1}$ $day^{-1}$. Each feed was analyzed three times and each run lasted for 72 hrs monitoring period.

## 2.3 Experimental Procedure

Initially, a one litter alkaline soda lake slurry was mixed with equal amounts of artificial wastewater and poured in to a three litter reactor. The reactor was kept on a magnetic stirrer plate at 8 rpm agitation for 48 hrs. Adaptability of alkaliphiles was determined after measuring and calculating the difference in initial and final amounts of a certain selected parameters (COD, TN, $NH_4^+$-N, $NO_3^-$-N, $S^{2-}$, $SO_4^{2-}$, MLSS and MLVSS).

Before starting the operation, the reactors were filled with the synthetic wastewater, inoculated with sediment slurry and were operated on a batch basin with aeration and mixing for three days to obtain a dense sediment biomass. After sedimentation, the clear supernatant on top was removed and the feed reactor was filled up to 10 liter total volume with the synthetic wastewater. Then, the anoxic and oxic operations were arranged in a sequence and the HRT of each step was kept constant. The first run was left for acclimatization. The second run was used for collection of data. The operational parameters during the three feeds are listed in Table 2.

Table 2: The experimental operational parameters.

| Feed | OLR (gm-COD $l^{-1}$ $d^{-1}$) | MLSS (mg $l^{-1}$) | MLVSS (mg $l^{-1}$) |
|---|---|---|---|
| I | 10 | 1.82-2.23 | 1.47-1.82 |
| II | 5 | 1.86-3.02 | 1.66-2.6007 |
| III | 2.5 | 2.33-4.09 | 1.82-2.84 |

## 2.4. Analyses

The wastewater samples were withdrawn at 0, 24, 48 and 72 hours from the influent, anaerobic, oxic and effluent and centrifuged and/or filtered to remove microorganisms from the liquid. Clear supernatants of synthetic and tannery wastewater were analyzed for the following parameter: pH was measured using a pH meter (Sension-1). Chemical oxygen demand (COD), Total Nitrogen, Ammonium-Nitrogen ($NH_4^+$-N), Nitrate-Nitrogen ($NO_3^-$-N), Sulphides ($S^{2-}$) and Sulphate ($SO_4^{=}$) were measured colorometrically using a spectrophotometer (DR/2010 HACH, Loveland, USA) according to HACH instructions. Biological oxygen demand ($BOD_5$), Mixed Liquor suspended solids (MLSS) and Mixed Liquor Volatile suspended solids (MLVSS) were measured following Standard methods (APHA, 1998). Total Kjeldhal nitrogen (TKN) was also measured with semi-micro Kjeldahal method (APHA, 1998). The diversity and activity of microorganisms as well as the floc shape was observed using epifluorescence microscope.

## 2.5 Measuring the Denitrification and Nitrification Potential of Alkaliphiles

The nitrate and ammonia uptake potential of alkaliphiles was measured according to Kristensen *et al*. (1992)

### 2.5.1 Nitrate Uptake Rate (NUR) Test

The test was performed as a batch test in two liter laboratory reactor with a completely mixed and closed atmosphere. Sludge was withdrawn from the anoxic reactor, aerated and stirred for 30 minutes. Before starting the experiment, sample was taken out for the determination of SS and VSS. Some amounts of $(NH_4)_2\ SO_4$ and $K_2HPO_4$, 1.325 liter sludge, 100 ml containing 59 $mgl^{-1}$ $NO_3^-$-N and 75 ml of tannery wastewater were added to the reactor. Nitrogenous gas was introduced as atmosphere in the reactor. Samples were taken out and filtered after 1, 15, 30, 60, 120 and 180 minutes. NUR was calculated as a change of nitrate-N (mg $NO_3^-$-N $VSS^{-1}$ $hr^{-1}$).

### 2.5.2 Ammonia Uptake Rate (AUR) Test

AUR test was carried out in a two liter lab reactor with an air supply. 1.5 liter activated sludge was poured into the reactor; stirred and aerated for 30 minutes before beginning of the experiment. 50 ml of sample was taken out to measure MLSS and MLVSS. Ammonia was added to an initial concentration of 93 mg-N/l. 20 ml samples were taken out every 15 minutes with the first sample after one minute for three hour. The sample was filtered and later analyzed for nitrogen content. AUR was calculated as a change of $NH_4^+$-N (mg $NH_4^+$-N $VSS^{-1}$ $hr^{-1}$).

## 2.6 Data Analysis

Data that collected on analysis of wastewater were subjected to statistical analysis using Microcal Origin 6.0.

# 3. Results

All the results presented in this study were obtained from the laboratory-scale activated sludge reactors. During the experiments, the most important parameters (COD, BOD, TN or TKN, $NH_4^+$-N, $NO_3^-$-N, $S^{2-}$, $SO_4^{2-}$, MLSS and MLVSS) were determined to evaluate the performance of sediment sludge biomass to remove nitrogen and organic matter. Despite differences in composition and characteristics of the artificial and actual tannery wastewaters, most of the results and conclusions obtained were similar. For this reason most of the results presented and discussed below are focused on the behavior of the system when tannery wastewater was used.

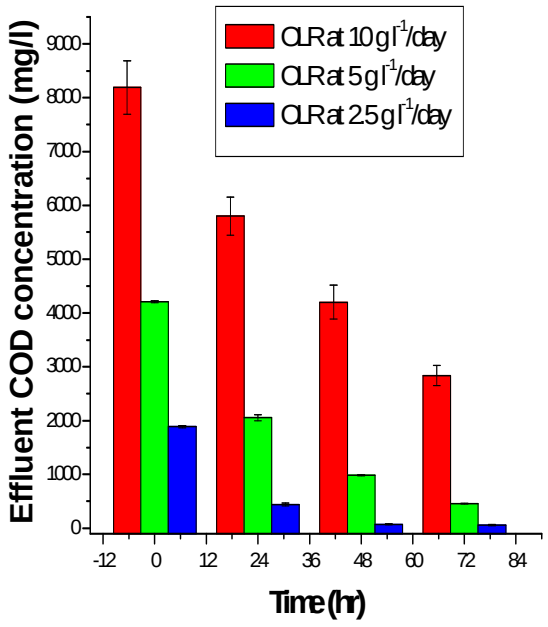
Characteristics of the average influent and effluent of the synthetic and actual tannery wastewater at different organic loading rate are shown in Table 3 and 4 and Figure 4.

Table 3: Average characteristics of the influent and effluent tannery wastewater for the three experimental feeds (concentrations are in $mgl^{-1}$, except for pH).

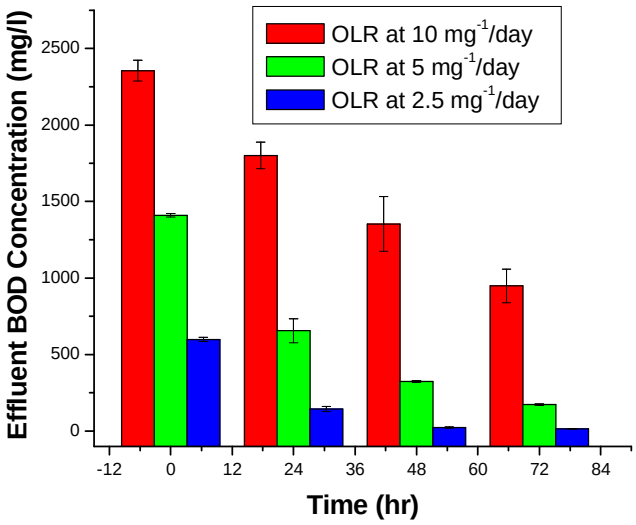
| **Parameters** | Feed I (10 g $l^{-1}day^{-1}$) | | Feed II (5 g $l^{-1}day^{-1}$) | | Feed III (2.5 g $l^{-1}day^{-1}$) | |
|---|---|---|---|---|---|---|
| | **Influent** | **Effluent** | **Influent** | **Effluent** | **Influent** | **Effluent** |
| pH | 10.85 | 9.78 | 9.94 | 10.27 | 9.88 | 10.03 |
| COD | 8193.33 | 2836.67 | 4209.33 | 454.33 | 1886.67 | 55.33 |
| BOD | 2355 | 947.33 | 1409.33 | 172.33 | 598.33 | 14.67 |
| TKN | 645.67 | 358 | 834 | 111.33 | 672.33 | 22 |
| $NH_4^+$-N | 686 | 255.33 | 578 | 147 | 280.33 | 14.6 |
| $NO_3^-$-N | - | 6.33 | - | 7.20 | - | 4.53 |
| $S^{2-}$ | 222.33 | 135 | 256.67 | 26.3 | 241.67 | 18.67 |
| $SO_4^{2-}$ | 564.67 | 1246.33 | 458.67 | 1392.7 | 401.33 | 1572 |

Table 4: Average characteristics of the influent and effluent artificial wastewater for the three experimental feeds (concentrations are in $mgl^{-1}$, except for pH).

| **Parameters** | Feed I (4.6 g $l^{-1}day^{-1}$) | | Feed II (3.1 g $l^{-1}day^{-1}$) | | Feed III (2.6 g $l^{-1}day^{-1}$) | |
|---|---|---|---|---|---|---|
| | **Influent** | **Effluent** | **Influent** | **Effluent** | **Influent** | **Effluent** |
| pH | 10.80 | 10.03 | 10.17 | 10.03 | 10.23 | 10.06 |
| COD | 3830 | 764 | 2356 | 142 | 1896 | 83 |
| Total-N | 662 | 134 | 514 | 46 | 386 | 22 |
| $NH_4^+$-N | 280 | 66 | 261 | 28 | 199 | 13 |
| $NO_3^-$-N | - | 4.2 | - | 2.6 | - | 3.6 |
| $S^{2-}$ | 212 | 8.65 | 168 | 3.6 | 146 | 2.4 |
| $SO_4^{2-}$ | 460 | 1263 | 440 | 1113 | 340 | 1072 |




a)

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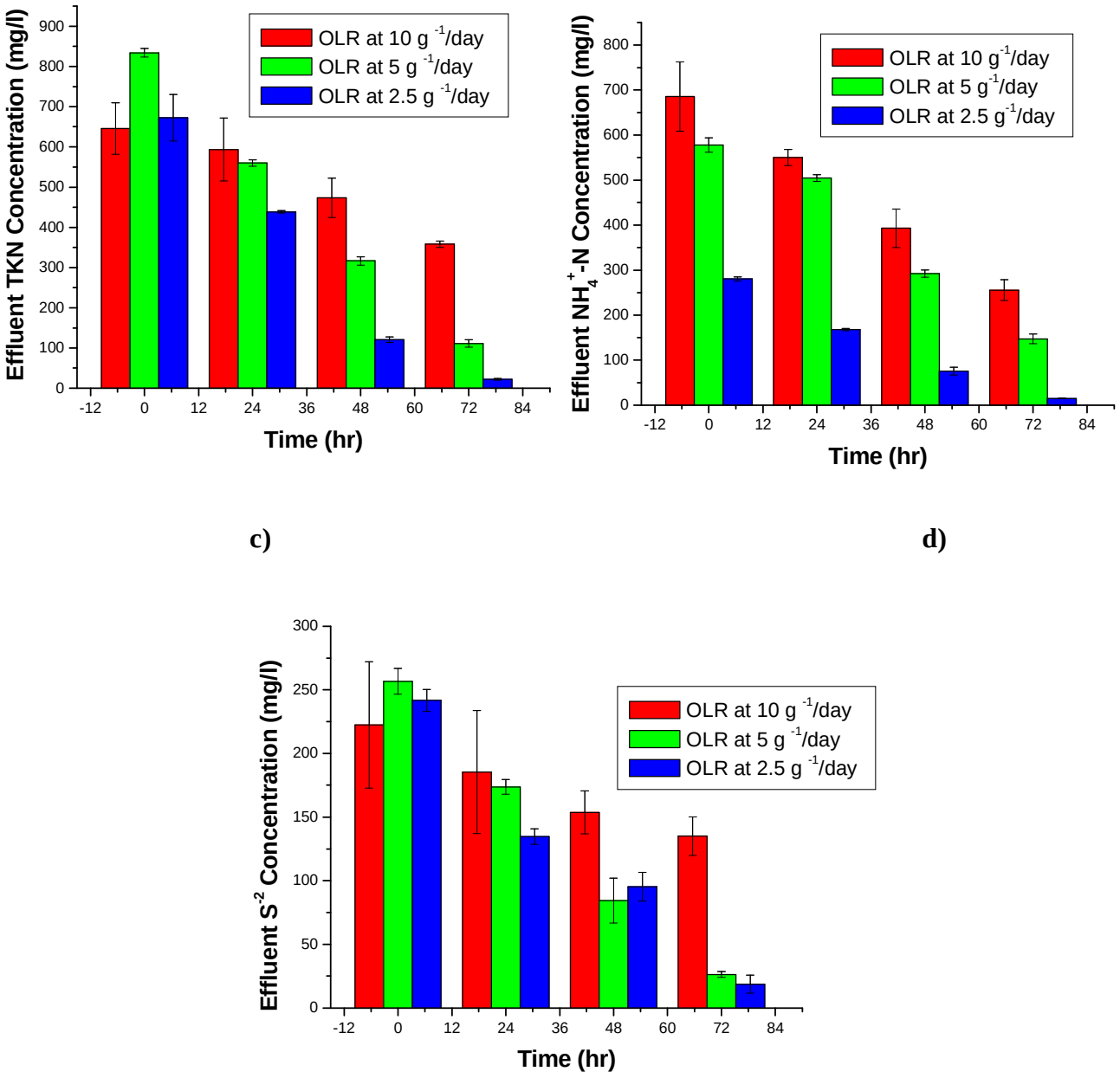



Figure 4: Variations of effluent nutrient concentrations with OLR as a function of time: (a) COD; (b) $BOD_5$; (c) TKN; (d) $NH_4^+$-N; (e) $S^{2-}$.

The influent wastewater was highly alkaline with a pH values around 10.85. It was highly polluted with a $BOD_5$ value ranges from 598.33 to 2355 mg $l^{-1}$. The influent had high total nitrogen (TKN) and COD concentrations ranging from 645.67 to 834 mg $l^{-1}$ and 1886.67 to 8193.33 mg $l^{-1}$, respectively; whereas the ammonium and sulfide had value also ranging from

280.33 to 686 and 222.33 to 256.67 mg $l^{-1}$, respectively. No nitrate was recorded in the influent. Gradually, the total Nitrogen (TKN) and COD in the effluent tank were decreased to values ranging from 22 to 358 and 55.33 to 2836.67 mg $l^{-1}$, respectively. The $BOD_5$ value was also decreased from 14.67 to 947.33 mg $l^{-1}$. Similarly measurement of ammonium-nitrogen and sulphide ranged from 14.6 to 255.33 and 18.67 to 135 mg $l^{-1}$, respectively. Even though, the sulphide concentration in the effluent was substantially decreased the sulphate content increased from influent contents of 401.33 - 564.67 mg $l^{-1}$ to the effluent contents of 1246.33 - 1572 mg $l^{-1}$. The effluent nitrate nitrogen was also increased from 0 to 4.53 and 7.20 mg $l^{-1}$.

## Effects of OLR on Nutrient Removal

The nutrient removal efficiencies are presented in the Table 5 and 6. The removal efficiencies gradually increased from feed I to feed III.

Table 5: The bioreactor removal efficiencies for the selected parameters at different OLR in tannery wastewater.

| **Feed** | **Removal Efficiencies (%)** | | | | |
|---|---|---|---|---|---|
| | **COD** | **$BOD_5$** | **TKN** | **$NH_4^+$-N** | **$S^{-2}$** |
| I | 65.3 | 59.7 | 44.2 | 62.2 | 38.2 |
| II | 89.2 | 87.8 | 86.7 | 74.5 | 89.7 |
| III | 97.1 | 97.3 | 96.6 | 94.8 | 92.3 |

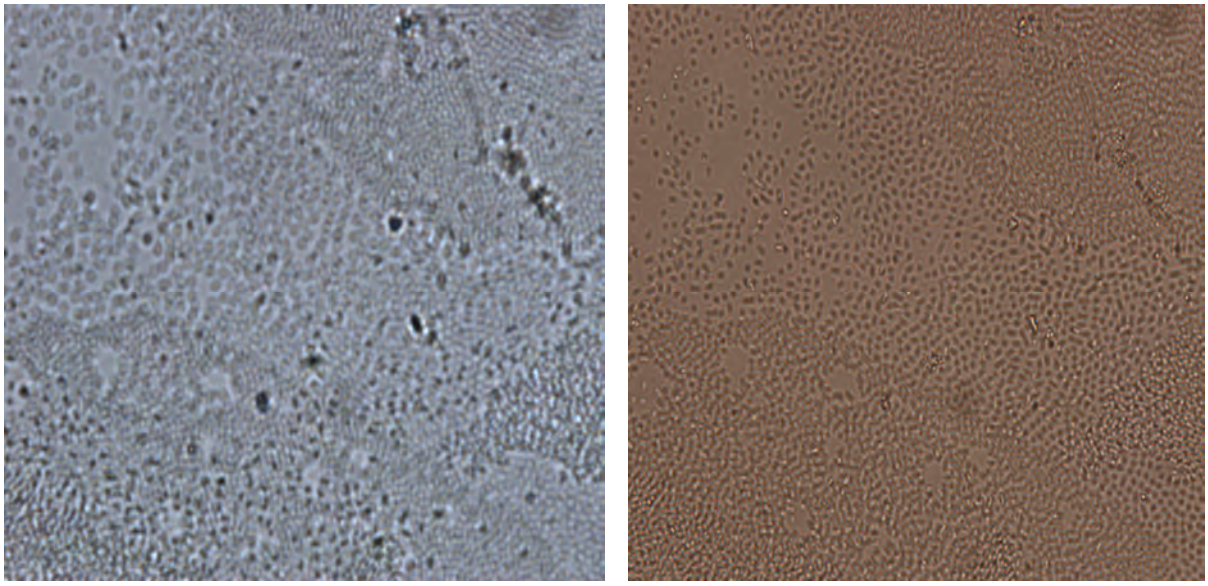
Table 6: Removal efficiencies of the treatment system for the selected parameters at different OLR in artificial wastewater.

| **Feed** | **Removal efficiencies (%)** | | | |
|---|---|---|---|---|
| | **COD** | **Total-N** | **$NH_4^+$-N** | **$S^{2-}$** |
| I | 80.1 | 79.9 | 76.4 | 95.9 |
| II | 94 | 91 | 89.3 | 97.8 |
| III | 95.6 | 94.4 | 93.5 | 98.4 |

Variation of COD removal efficiency with the OLR is presented in Table 5. COD removal efficiencies at three different OLR were tested and obtained 65.3, 89.2, and 97.1 %, respectively. The highest COD removals were obtained at OLR of 2.5 gm $l^{-1}d^{-1}$. The $BOD_5$ removal efficiency is also presented in Table 5. The removal was gradually increased and achieved 97.3 % at 2.5 gm $l^{-1}d^{-1}$ OLR from a value of 59.7 % removal when the OLR was 10 gm $l^{-1}d^{-1}$.

There was also a variation in TKN removal efficiency with OLR. The maximum TKN removal efficiency (96.6 %) was obtained at the OLR of 2.5 gm $l^{-1}d^{-1}$ while the removal at 5 and 10 were 86.7 and 44.2 %, respectively. Ammonium-N removal efficiencies were also affected by OLR (Table 5). Maximum $NH_4^+$-N removal efficiency (94.8 %) was obtained at OLR of 2.5 gm $l^{-1}d^{-1}$. The efficiency obtained at OLR of 5 gm $l^{-1}d^{-1}$ was lower (74.5 %). $NH_4^+$-N removal efficiencies decreased with OLR increased and resulting in 62.2 % removal at 10 gm $l^{-1}d^{-1}$ OLR. At OLR of 10 gm $l^{-1}d^{-1}$ the sulphide removal efficiency of the bioreactor was 38.2 %. But the OLR decreased to 2.5 gm $l^{-1}d^{-1}$ the removal efficiency was increased and achieved 92.3 % at the end of this period.

The diversity and activity of microorganisms as well as the floc shape was observed using epifluorescence microscope. During lower organic loading rate, the bacteria were developed in small chains and /or clump forms (Figure 5). A dispersed growth was present at the start up of the process. They were very active and motile. Ciliates were also observed. As the time goes the clumps were grown and became larger and compact/spherical. However during higher OLR, the bacteria were very inactive and irregular shaped floc was observed.



**a)** **b)**

Figure 5: Epifluorescence microscope detection of activated sludge at 10 (a) and 2.5 (b) gm $l^{-1}d^{-1}$ OLR

When all the results were examined, OLR of 2.5 gm $l^{-1}d^{-1}$ was the optimal value resulting in nearly maximum removal efficiencies for COD, $BOD_5$, TKN, $NH_4^+$-N and $S^{2-}$ 97.1, 97.3, 96.6, 94.8 and 92.3 %, respectively. Variations of the effluent nutrient concentrations with OLR are depicted in Figure 4. Effluent COD was 454.33 and 2836.67 mg $l^{-1}$ at 5 and 10 OLR, respectively. However, the effluent COD at 2.5 gm $l^{-1}d^{-1}$ OLR was 55.33 mg $l^{-1}$. TKN, ammonium-N, and sulphide concentrations at the end of operations were 22, 14.6 and 18.67mg $l^{-1}$. Higher COD, TKN, $NH_4^+$-N and $S^{2-}$ removal were obtained at this OLR, thus, the 2.5 gm $l^{-1}d^{-1}$ OLR was the optimal value minimizing the effluent nutrient levels.

**Nutrient concentration profiles at the optimal OLR (2.5 gm $l^{-1}d^{-1}$)**

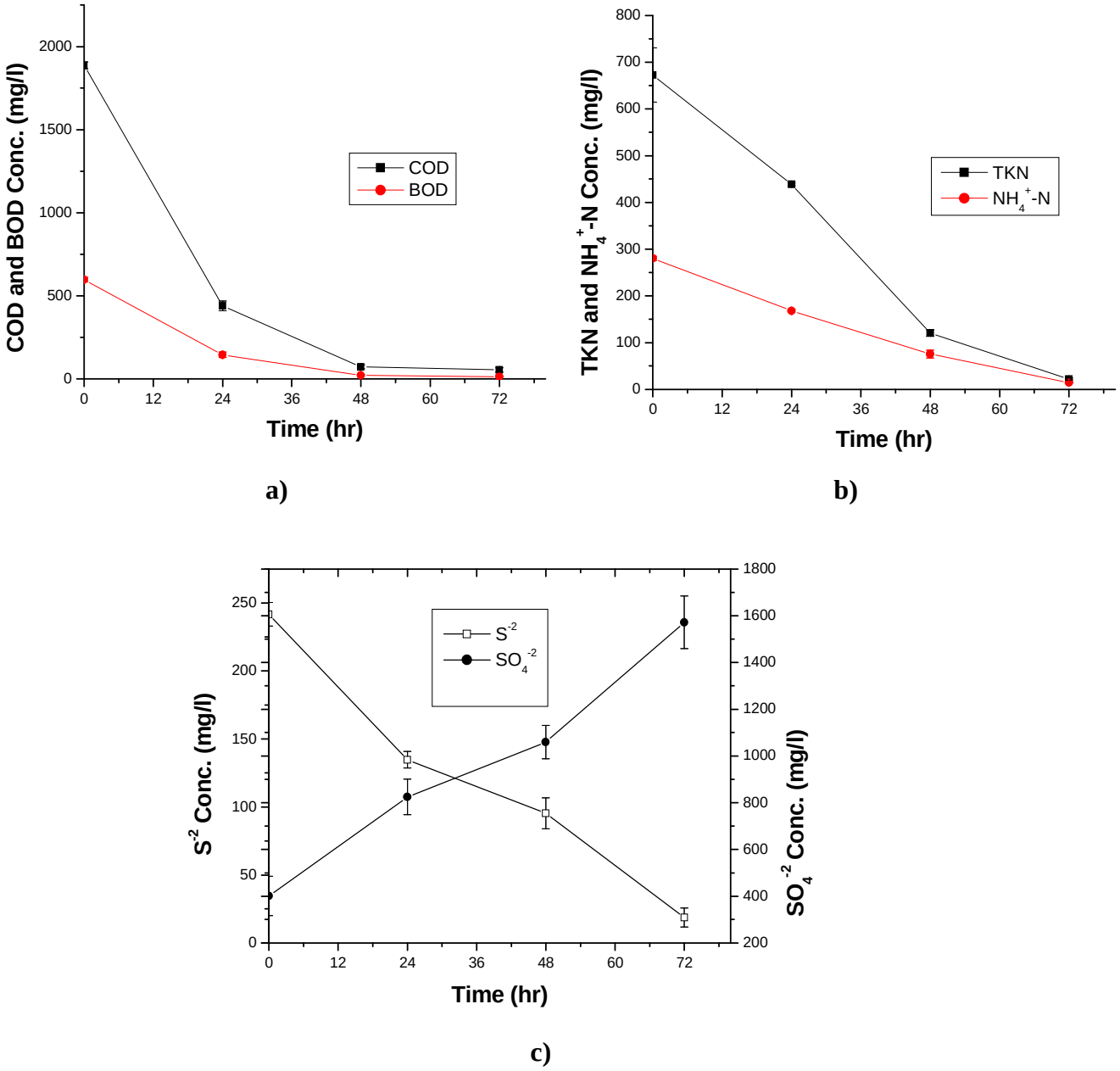


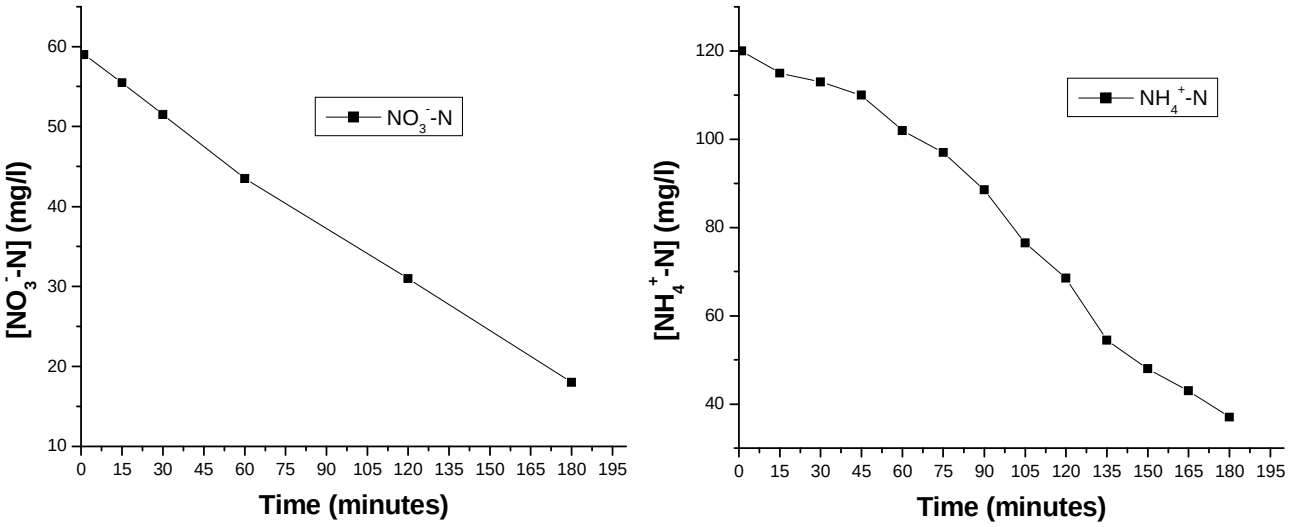

Figure 6: Nutrient concentration profiles for OLR of 2.5 gm $l^{-1}d^{-1}$ in tannery wastewater treatment operation: (a) COD and $BOD_5$; (b) TKN and $NH_4^+$-N; (c) $S^{2-}$ and $SO_4^{2-}$.

Figure 6 shows variations of nutrient (COD, $BOD_5$, TKN, $NH_4^+$-N, $S^{2-}$ and $SO_4^{2-}$) concentrations with time when the system was operated at the optimal OLR of 2.5 gm $l^{-1}d^{-1}$. COD concentration was dropped steadily and reaching a level of about 55.33 mg $l^{-1}$ at the end of 72 hrs of operation. The major fraction of COD was removed mainly in the oxic reactor. At this OLR the $BOD_5$ value

was reduced from 598.33 mg $l^{-1}$ in the influent to 14.67 mg $l^{-1}$ in the effluent at the same time operation. The TKN concentration reduction was significant in anoxic basin and only slight change occurred in oxic ones.

$NH_4^+$-N concentration decreased slightly in anoxic basin. Most of the $NH_4^+$-N was removed in oxic reactor with measured value of 14.6 mg $l^{-1}$ at the end of operation. Initial nitrate-N concentration in the oxic reactor was 5.47 mg $l^{-1}$ and increased to 13.53 and 16.53 mg $l^{-1}$ at the end of 24 and 48 hr operations, respectively, but reduced to the value of 5.37 mg $l^{-1}$ at the end of 72 hr operation. The sulphide concentration was changed from 241.67 to 18.67 mg $l^{-1}$ where as sulphate concentration increased to the value of 1572 mg $l^{-1}$ at the end of operation from 401.33 mg $l^{-1}$ amounts present in the influent.

## NUR and AUR Test




**a)** **b)**

Figure 7: Typical nitrate and ammonia-nitrogen consumption curves during batch experiment at range of pH value10.02-10.21 and 9.50-9.75, respectively: (a) NUR; (b) AUR Test

Typical denitrification profiles deriving from the batch experiment using the tannery wastewater as carbon source and $NaNO_3$ as $NO^-_3$-N source is shown in Figure 7 (a). It is obvious that the figure shows the existence of denitrification at higher pH (between 10.2 and 10.21). Figure 7 (b) depicts nitrification profiles at the pH value between 9.50 and 9.75.

# 4. Discussion

This study examined the characteristics of actual and artificial tannery wastewaters and evaluates the potential of sediment sludge biomass from alkaliphilic environment to remove nitrogen and organic matter when used as a starter to the pre-denitrification/nitrification activated sludge wastewater treatment system. It was done at ambient temperature.

The total nitrogen (TKN) and ammonium nitrogen concentrations in the effluent were decreased with treatment time (Table 3) and the nitrate concentration was low in most of the times. The total and ammonium-nitrogen removal efficiencies were 44.2, 86.7 and 96.6 % and 62.2, 74.5 and 94.8 % for OLR of 10, 5 and 2.5 gm $l^{-1}$ $d^{-1}$, respectively (Table 5). These results suggest that total nitrogen (mainly organic form) hydrolyzed and converted to $NH_4^+$- N in the anaerobic reactor, where as the ammonium nitrogen in wastewater flowed down from anaerobic to aerobic basins where nitrifying bacteria oxidized it to nitrite and nitrate. At the same time, suspended denitrifying bacteria reduced nitrite and nitrate to nitrogen gas using tannery wastewater as carbon source. Nitrification and denitrification therefore occurred simultaneously in the system in aerobic and anaerobic columns, respectively.

Little nitrogen flows to the effluent and the TKN concentration in the effluent was nearly 22 mg $l^{-1}$ at the end of the experiment (Table 3). Actually initial nitrate nitrogen concentration in the influent of all feed batches was 0, which was increased to 6, 7 and 4 mg $l^{-1}$in the effluent of the I, II and III feed reactors, respectively. But it was increased to 16.53 mg $l^{-1}$ at the end of 48 hr in the oxic reactor of feed III (data was not shown) because of nitrification. Since, the mixed liquor from oxic reactor is returned back to the anoxic basin and as a result of denitrification, the $NO_3^-$-N is reduced to 4.53 mg $l^{-1}$ at the end of 72 hrs operations in the effluent.

A similar nitrogen removal efficiency ranged from 90 to 98 % earlier was reported by Hirata ***et al.*** (2001) from metal recovery industrial wastewater. Artiga ***et al.*** (2005) reported 95 % ammonia-N removal efficiency from tannery wastewater using an innovative biofilm suspended biomass hybrid membrane bioreactor. The result coincides with Seyoum Leta ***et al.*** (2004) reported removal efficiencies of 82 to 95 % for total nitrogen and 45 to 95 % for ammonium

nitrogen. The same removal efficiencies (97 % and 96 % of TN and $NH_4^+$- N), respectively were also recorded by the Fantahun Woldesenbet (2005). Similar removal efficiency (96 % of TKN) also reported by another researcher Cao *et al.* (2004).

The oxidizable carbon can approximately be described as COD or $BOD_5$. The COD and $BOD_5$ removal efficiencies during the experimental feeds (feed I, II and III) were 65.3, 89.2 and 97.1 % and 59.7, 87.8 and 97.3 %, respectively (Table 5). This oxidizable carbon removal is due to the utilization by different groups of microorganisms in different reactors for different purposes. In the anoxic reactor of the operation, nitrate and/or nitrite are expected to be denitrified to the nitrogenous gases using organic carbon as the carbon and energy source for the denitrifying bacteria. At this stage, carbon is also consumed by other heterotrophic bacteria (Gallert and Winter, 1999). Organic carbon is also required during the nitrification stage, since it is aerobically degraded in to water and carbon dioxide by heterotrophs, which is used as carbon source for autotrophic bacteria such as the ammonia oxidizers and nitrite oxidizers (Chiou and Ouyang, 2001).

The maximum removal efficiency was coincided with the increase in the MLVSS value (Table 2) and denitrification efficiency of the system. This is because; the MLVSS in activated sludge is proportional to the active microbial biomass in the system (Sidat *et al.,* 1998; Metcalf and Eddy, 2003). The removal efficiencies for such parameters match with the result obtained by Seyoum Leta *et al.* (2004) (98 % removal of both COD and BOD) and Fantahun Woldesenbet (2005) (98 and 99 % removal of COD and BOD, respectively). 97 % removal efficiency of COD was reported by Artiga *et al.* (2005) from tannery wastewater using an innovative biofilm suspended biomass hybrid membrane bioreactor.

Sulphide and sulphate concentrations were measured from all the reactors (anoxic, oxic and effluents, data was not shown except for effluent (Table 3 and 4)). In the anoxic reactor sulphide concentration was reduced with time and sulphate concentration increased slightly. The sulphate concentration was increased rapidly with time in the oxic reactor. This result suggested that, the autotrophic sulpher bacteria like *Thiobacillus denitrificans* oxidize reduced sulphur compounds such as elemental sulphur, hydrogen sulphide and thiosulphate to sulphate while reducing nitrate

and/or nitrite to elemental nitrogen gas (Sorokin *et al.,* 2003). A tremendous increment of sulphate in an oxic reactor with the oxidation of reduced sulphur compounds to the sulphate by chemolithotrophic sulphur- oxidizing bacteria was reported by Milford *et al.* (2000) and Koenig and Liu (2001). Nitrate reduction using sulphide, elemental sulphur and thiosulphate has already been reported by others (Gommers *et al.,* 1988; Dalsgaard and Bak, 1992). The removal efficiency for sulphide coincided with the study conducted by Seyoum Leta *et al.* (2004).

Although several studies showed the optimum pH of nitrification is between 6.5 and 8.6 (Cheremisinoff, 1996; Cloete and Muyima, 1997; Villaverde *et al.,* 1997; Grunditz *et al.,* 1998; Im *et al.,* 2001) and that of denitrification is 7 - 8.5 (Kaldlec *et al.,* 2000; Gerardi, 2002) the present study revealed the occurrence of nitrification and denitrification at pH values between 8.7 and 10.75 (Figure 7). This is similar to the finding of Sorokin *et al.* (1998) who isolated five strains of nitrite-oxidizing bacteria from sediments of three alkaline soda lakes in Siberia and Kenya.

The other interesting observation during the experiment period was variation in the removal efficiencies for most selected parameters with different OLR. Their removal efficiencies of the system decreased when the OLR was increased. For instance, ammonium nitrogen was decreased from 94.8 % removal efficiency during 2.5 gm $l^{-1}$ $d^{-1}$ OLR to 62.2 % at 10 gm $l^{-1}$ $d^{-1}$ OLR. This means that ammonia nitrification rates decreased with the increase of organic loading rates. This phenomenon is generally explained by the competition for oxygen between heterotrophic and nitrifying bacteria. Higher organic loads proliferates the heterotrophic bacteria and decreasing the fraction of nitrifiers present in activated sludge system. Thus, when organic loading rates were increased, the percentages of nitrifying oxidizers in the sludge would certainly decline and this would result in a lower nitrification activity (Hanaki *et al.,* 1990; Kristensen *et al.,* 1992; Wiesmann, 1994; Gupta and Gupta, 2001; Jokela *et al.,* 2002; Seyoum Leta *et al.,* 2004).

The removal efficiencies of COD, TKN, BOD and $S^{2-}$ were also reduced when OLR was increased. This may be due to the result of sludge washed out since at this OLR (10 gm $l^{-1}$ $d^{-1}$) there was a pronounced foam formation. High organic load also contains high amounts of inhibitors such as chromium and sulphide in tannery wastewater, which could shock the microbes

in the system. Thus it reduces their physiological activities. The other possibility to the reduction in removal efficiencies is the decrease in sludge settleability when the organic loading rate (OLR) is increased (Odegaard *et al.,* 2000).

Epifluorescence microscope was used to know the moment working condition at different OLR by observing floc shape and presence or absence of ciliates. During lower organic loading rate, the bacteria were developed in small chains and /or clump forms (Figure 5b). At this stage, adequate amount of food is avilable, thus the bacteria are very active and multiplying rapidly. However, when the sludge age goes, the flocs gets larger and compact and begin to settle. They were very active and motile. Ciliates were also observed. Since ciliates are highly sensitive to toxic and inhibitory substances, their presence generally a sign of stable activated sludge operation (Knoop and Kunst, 1997). However during higher OLR, the bacteria were very inactive and irregular shaped floc with no ciliates were observed (Figure 5a). The complete missing of ciliates is an indication of disturbance or high organic loads (Wiscosin, 2001).

Proper floc formation and higher (greater than 90 %) nutrient removal efficiencies obtained in well stable activated sludge process depends on proper and right choice of organic loading rate (Gerardi, 2002; Seyoum Leta *et al.,* 2004). Using a lab-scale predenitrification/nitrification activated sludge system with in 72 hrs operation, nearly 97.1 % of COD, 97.3 % of BOD, 96.6 % of TKN, 94.8 % $NH_4^+$-N and 92.3 % $S^{-2}$ were removed at the optimal OLR of 2.5 gm $l^{-1}d^{-1}$. The final COD, BOD, TKN, $NH_4^+$-N, $NO_3^-$-N and $S^{-2}$ in the effluent was 55.33, 14.7, 22, 14.6, 4.53 and 18.67 mg $l^{-1}$, respectively. These results are comparable well with the literature values (Seyoum Leta *et al.,* 2004). The value obtained for COD, BOD, TKN and $NH_4^+$-N is in line with the effluent discharge limit values in Ethiopia which is 250 80, 80 and 30 mg $l^{-1}$, respectively (EPA, 2003). Therefore, alkaliphiles could be another source to be used as inocula for nitrogen and organic matter removal from alkaline industrial wastewaters.

# 5. Conclusions and Recommendations

Tanning industry is one of the major industries in Ethiopia. It discharges a large volume of highly polluted wastewater results from several and highly chemical demand processes. The wastewater contains high amounts of different forms of nitrogenous compounds, organic matter, sulphide and chromium. The major nitrogenous substances in tannery wastewater are proteins, amines and ammonia resulted from hides and skins. The presence of nitrogen in wastewater discharge can be undesirable because it has several ecological impacts and can affect public health. As a result, Environmental Protection Authority (EPA) formulates a general rule and regulation in national level to avoid discharge of untreated wastewater to the environment. Thus, finding an alternative means to do that is a must. Now a day, scientists create more efficient and economical wastewater management by blending technology with the natural processes.

So far several types of biological wastewater treatment methods were developed. Among all, biological treatment by denitrification and nitrification is the most widely used process for nitrogen and organic matter removal from wastewaters. In this study therefore, a lab-scale pre-denitrification/nitrification activated sludge system was used to evaluate sediment sludge biomass activity from alkaline soda lake to remove nitrogen and organic matter from a synthetic and actual tannery wastewaters. The sludge biomass contains different groups of microorganisms. In this process, nitrate and nitrite produced by the nitrifying bacteria under aerobic conditions returned to the anoxic reactor and were reduced mainly to molecular nitrogen by denitrifying organisms.

The denitrifying microorganisms are facultative anaerobes able to use nitrogen oxides as final electron acceptors in the absence of oxygen where they used organic carbon as carbon and energy source. It has an advantage of using the original carbon source in the influent for denitrification to substitute an additional carbon source. HRTs were kept constant as 16.67 and 33.34 hrs for the aforementioned reactors and the OLR were decreased from 10 to 2.5 gm $l^{-1}$ $d^{-1}$.

The sludge biomass activities resulted to be very satisfactory and high efficiencies were recorded for the removal of COD (97.1 %), BOD (97.3 %), TKN (96.6 %), $NH_4^+$-N (94.8 %) and $S^{2-}$ (92.3 %) at 2.5 gm $l^{-1}$ $d^{-1}$ OLR. These removal efficiencies, COD and BOD concentrations decreased

from 1886.67 and 598.33 mg $l^{-1}$ to nearly 55.33 and 14.67 mg $l^{-1}$, respectively. TKN, $NH_4^+$-N and $S^{2-}$ concentrations also decreased from initial values of 672.33, 280.33 and 241.67 mg $l^{-1}$ to nearly 22, 14.6 and 18.67 mg $l^{-1}$, respectively at the end of operational time. The treating effluent meets the discharge limits fixed by EPA (2003). Based on such experimental results the following points are recommended:

1. On the basis of the experimental results, the optimal OLR value was found to be 2.5 gm $l^{-1}$ $d^{-1}$, resulting in maximum nutrient removal. However, conditions and requirements of the treatment situation would determine the most convenient alternative. This is because, many studies reported that the performance of activated sludge system is affected by HRT, SRT, influent and recycling flow rates, temperature, etc. Thus, no generalizations are possible; rather in order to get well stable with good performance system several researches based on these operational parameters should be done.

2. The culturing method is the conventional approach to identify the microbial ecosystem. However, this approach has some limitations since most microorganisms in nature are either difficult to isolate (due to unknowing of their nutritional requirements or require a long period of time). Thus, we are not successful in isolating the different groups of microorganisms from the system. In recent years, Fluorescent in situ hybridization (FISH), which is a 16 S rRNA based molecular technology, has been used to detect specific bacteria groups in activated sludge. Thus, for efficient nutrient removal the efficient microbes such as nitrifying, denitrifying, and sulfur reducing and oxidizing bacteria should be isolated and characterized using such recent technology in their functioning sites.

3. The applications of alkaliphiles in biotechnology are vast. This study however, highlights one feature of alkaliphilic microorganisms which emphasis their potential to wastewater treatment biotechnology. Their real potential is still not achieved. Thus, an intensive research should go to these extraordinary microorganisms.

4. Even though in Ethiopia different naturally occurring alkaline environments are found, their microbial diversity (ecology), their enormous potential as a source of new alkaliphiles and their

applications for various biotechnology purposes are poorly studied. This is mainly due to lack of a research program that particularly dedicated to the applications of novel screening. The screening of unexploited and unusual alkaline habitats in Ethiopia will undoubtedly lead to the discovery of a novo species and novel compounds which may be used for biotechnological applications. Therefore, a great deal of research should be done in such unique environments.

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**Declaration**

I, the under signed, declare that this thesis is my original work. It has never been submitted in any institution and that all sources of materials used for thesis have been dully acknowledged.

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