Developing and evaluating the performance of sequencing batch reactor for the treatment of sulfur rich Modjo tannery effluent

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By: Hanna Habtemariam

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Developing and evaluating the performance of sequencing batch reactor for the treatment of sulfur rich modjo tannery effluent

BY

Hanna Habtemariam

Approval of the Board of Examiners

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Examiner
DECLARATION

This thesis is my original work, has not been presented for a degree in any university and all sources of materials used for the thesis has been gratefully acknowledged.

Hanna Habtemariam

Signature __________________________

Date ______________________________

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<td>Cr</td>
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ROP  Redox Potential
S²⁻  Sulfide ion
SBR  Sequencing Batch Reactor
SO₄²⁻  Sulfate ion
SOB  Sulfur Oxidizing Bacteria
SNR  Sulfide oxidizing nitrate reducing bacteria
TDS  Total Dissolved Solid
TKN  Total kjeldahl nitrogen
TN  Total Nitrogen
TP  Total Phosphorous
Tₚ  Total Reaction Phase
TS  Total solid
TBM  Thiosulfate Basal Medium
Tₚ  Reaction time
TSS  Total Suspended Solid
U.S. EPA  United States Environmental Protection Authority
UNIDO  United Nations Industrial Development Organization
UNEP  United Nations Environmental Programme
Vₚ  Fill volume
WHO  World Health Organization
ABSTRACT

Ethiopia gets vast amount of foreign exchange from the leather industry sector by exporting semi processed or finished leather product. Most tanneries in Ethiopia partially treat or do not treat their effluent before discharging it into the receiving water bodies therefore untreated leather effluent responsible for tremendous pollution of water resources in the country. Therefore the main objective of the study was to developing and evaluating the performance of sequencing batch reactor (SBR) for the treatment of sulfur rich tannery effluent in a case study of Modjo tannery. Sequencing batch reactor was used because it is a technology with high removal efficiency, easily expandable, simple operation and process which has the capability to receive shock organic loads and low capital costs. The treatment performance of SBR was assessed for selected parameters such as, BOD$_5$, COD, TSS, TN, TP, NO$_3^-$, NH$_4^+$, SO$_4^{2-}$, TDS, TS and electrical conductivity based on APHA manual. The working condition of SBR was HRT, 48hr cycle, sludge age of 15 days temperature 20°C and with optimum pH, EC and DO. The system showed high removal efficiency for sulfide( S$^2$), which was 99.73% and good removal efficiency for BOD$_5$ (86.77%), COD (95.7%), TSS (90.7%), TN (86.7%), TP (66.1%), NO$_3^-$ (83.1%), SO$_4^{2-}$ (64.5%), TDS (58.7%), TS (68.4%) the achievement of high removal efficiency could be related to suitable working condition for the consortia of bacteria in each reactor while the concentration of NH$_4^+$ showed increment from influent by 25%. Nitrate reducing sulphite oxidizing bacteria (NR-SOB) bacteria were isolated from anoxic reactor and their removal efficiency of nitrate and sulfide was evaluated. Out of 27 isolates of consortia two potential isolates were obtained with nitrate removal efficiency of 90% and 81.4% for isolate 22 & 26 respectively and sulfide removal efficiency of 98.4% for isolate 26 within an hour. Generally the SBR system showed significant removal of pollutants especially sulfide which could be related to the presence of high efficient sulfide oxidizer and nitrate reducer bacteria in anoxic condition. Therefore, it can be concluded that SBR is very effective secondary tannery effluent treatment method and it is recommended to apply it at large scale to overcome pollution problem of tannery industries by including other tertiary treatment method.

Keywords: Sequencing batch reactor, wastewater treatment, NR-SOB consortia, Tannery effluent
CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Leather industry is one of the fast growing economic sectors in Ethiopia which play a significant role in the generation of foreign currency, which is ultimately utilized for developing all the other economical sectors.

Ethiopia has a major comparative advantage in the raw materials needed for the leather industry because it has the largest livestock production in Africa and the 10th largest in the world (Kiruthu, 2002). This makes it an ideal place to leather production and making leather products to local use and export.

Leather tanning has been ranked as one of the most polluting activities because of the highest toxic intensive per unit of output (Lofrano et al., 2006; UNEP, 1991; Khan et al., 1999). The environmental impacts of tannery originate from liquid, solid and gaseous waste streams due to consumption of raw materials such as raw hides and skins, chemicals and water. The transformation of raw or semi-pickled skins into commercial products requires high water consumption roughly 50-150 liters of water and about 130 chemicals are used per one kilogram of converted leather (Infogate/GTZ, 2002; World Bank, 1998).

The main chemicals used in the various processing stages include sodium sulfide, lime powder, ammonium sulfate, sodium chloride, sulfuric acid, chromium sulfate, sulphonated and sulfated oils, formaldehyde, pigments, dyes and anti-fungal agents (ETPI, 1998; IFC, 2007).

The solid and liquid wastes emanating from the leather industry are inevitable by-products of the leather manufacturing process and cause significant pollution when discharged direct in to streams and rivers without treatment.

1.2 Statement of the Research problem

In developing countries like Ethiopia most tanning industries partially or do not treated their effluent before discharging to receiving water bodies (Zinabu Gebremariam & Zerihun Desta,
Untreated tannery effluent causes different problem due to high toxicity levels which accelerates water quality changes to occur. In order to assess the effect of leather effluent to the receiving environment different studies has been conducted in some of these tanneries throughout the world bodies (Zinabu Gebremariam & Zerihun Desta, 2002).

The study conducted at Modjo Tannery indicates that environmental pollution problem arises from the discharge of untreated tannery effluents with high toxicity of chromium and sulfides (Seyoum Leta et al., 2004).

Sulfide ($S^{2-}$) in untreated tannery effluent under alkaline conditions, and lower pH undergo higher rate of evolution, characterized by a smell of rotten eggs, a severe odor of hydrogen sulfide gas which is fairly soluble in liquid (Roth, 1993). When absorbed, weak sulfuric or sulfite acids can be formed; this causes corrosion and weakens metal roofing, griders and building supports.

In sewers major problems can arise for metal fittings structural reinforcements and pipe works. If the sulfide contained wastewater discharged into surface water, even at low concentrations poses toxicological hazards because in aquatic environment sulfides reduced from sulfate eventually depletes the oxygen level in receiving water bodies (Berna et al., 2007).

Wastewater from leather tanning process affects land surface, surface water and ground water table (UNEP, 1991). Effects on surface water include rapid deterioration of physical, chemical and biological qualities (oxygen depletion and eutrophication). Destruction on soil structure and acceleration of soil erosion are impacts on land surface whereas, seepage of wastewater chemicals is a principal effect on the ground water table (UNEP, 1991).

1.3 Objectives of the Study

1.3.1 General Objective

The goal of this study was to evaluate the performance of sequencing batch reactor for treatment of sulfur rich tannery effluent from Modjo Tannery.

1.3.2 Specific Objectives

I) To characterize wastewater for selected parameters.
II) To study the removal efficiencies of sequencing batch reactor for parameters such as sulfur compound (S²⁻, SO₄²⁻), nitrogen compound (TN, NH₄ and NO₃⁻, total phosphate, Organic matter (BOD₅, COD) and solid substances (TSS, TDS, and TS).

III) To Isolate and characterize nitrate reducer and sulfide oxidizing (NR –SOB) consortia from sequencing batch reactor.

IV) To evaluate sulfide and nitrate removal efficiency of selected NR-SOB isolates.

1.4 Justification

The environmental protection law of Ethiopia seems to offer a guarantee for environmental safety, but the standards set are too high for tannery treated effluents which in turn leads to environmental pollution. Although to attain WHO standards is above real technical and financial capability of developing countries; it is important to safeguard the environment because postponing the solution to prevailing problem will bring serious problems to the management of the environment to the extent of the impossibility to live in the area (Andrea, 2002).

Various physico-chemical techniques have been studied for their applicability to the treatment of tannery wastewater but they are expensive and produce a byproduct which again poses environmental problems. As solution bioremediation techniques like sequencing batch reactor has been recommended by different scholars for assimilating pollutants especially in municipal and industrial wastewater (Andualem Mekonnen, 2008).

SBR as a developed activated sludge process was used for treatment of many types of industrial wastewater such as tannery, fiber, slaughterhouse, dyes, dairy and phenol wastewater (Arrojo et al., 2004; Farabegoli et al., 2007; Ganjидoust and Avati, 2004; Vaigan et al., 2009; Cassidy and belia, 2005; Sirianuntapiboon et al., 2006 ).

The reason that attracts the public attention about sequencing batch reactor is a technology with high removal efficiency, easily expandable, simple operation, process has the capability to receive shock organic loads and low capital costs. Also it can meet the interest of tanneries to produce as much tanned leather as possible at lowest effects of pollution, hence increases the commercial success of the tannery and acceptability by the surrounding communities (Bicudo et al., 1999; Kim et al., 2002).
According to the studies which have been conducted on bioremediation of tannery effluents regarding water quality standards due to high organics, chromium, nitrogen and sulfur, no conclusive studies have been conducted on the potential of SBR for the treatment of sulfur rich tannery effluents. Hence, this study specifically was concerned with biological sulfur transformation (Sulfide and Sulfate) and removal of other parameters like; Chemical Oxygen Demand, Biological Oxygen Demand, Ammonium, Nitrates and Phosphates by microorganisms.

1.5 Significance of the Study
The outcome obtained from the study can be used by environmental scientists, Environmental Protection Authority of Ethiopia (EEPA), tannery industries, and other stakeholders for environmental pollution control. Also researchers will use the results for further investigation to overcome the pollution effect of tanneries. The selected isolates can be further used in bioagumentation for industrial wastewater treatment of high toxicity.

1.6 Scope and Limitations
The scope of the study was to develop and evaluate the performance of SBR at the laboratory scale and based on the result obtained to give a recommendation which will be applicable at large scale in tannery industries.

However identification those potent bacteria isolates from wastewater of anoxic reactor in SBR was done based on morphological, biochemical and physiological test only because of limitation in enzymes and other chemical needed for molecular techniques. Thus further molecular techniques are required to develop the method fully.

In addition to that during characterization of wastewater some essential parameters like NO₃, TKN which have effect on nitrification rate were not analyzed due to the unavailability of chemicals.
CHAPTER TWO

LITERATURE REVIEW

2.1 Tannery industry

Leather industry performs an environmentally important activity by giving a new life to the leftover of the meat industry. The transformation of this by-product is, however, potentially pollution intensive. Tanning is widely perceived as a consumer of natural resources like water and other minerals which in turn generate wastewater containing heavy metals, toxic chemicals, chloride, lime with high dissolved and suspended salts and other pollutants (Uberai, 2003).

In tanneries hide and skins passes through many liquors, each quite different in chemical composition and playing its part in the conversion of unstable fibrous nature protein into a relatively stable non-putrescible leather. Annually, the recent global processing capacity of tanneries is $9 \times 10^9$ kg of hides and skins and approximately $30-40$ m$^3$ of water are used per ton of hide processed (Suthanthararajan et al., 2004). Hence it is estimated that $30-40 \times 10^{10}$ liters of liquid effluent is generated from tanneries (Thanikaivelan et al., 2004). This gives rise to two major problems for the leather industry: the availability of good quality water and the treatment of such large quantities of effluent.

The tanning of hides and skins to convert them into leather has been an important activity since ancient times. An animal hide consists of water (61%), fibrous proteins (34%), globular proteins (1%), lipids (2%), mineral salts (1%) and other components, such as pigments.

A hide can be divided into three structurally different parts, the epidermis, the dermis and the hypodermis. The epidermis contains hair and mainly consists of keratin. The dermis is the part that is normally considered to be skin and which is later transformed into leather; the main fraction of the dermis is collagen. The structure of the hypodermis is formed by horizontal fibers linked with blood vessels, muscles, fat, nerves, etc; and the dominating fraction is flesh.

For the transformation of a hide into leather, the epidermis, hair and flesh have to be eliminated, which is established in a production process of several different sub-processes where every tannery follows the same scheme. The only variations are based on different combinations of sub
processes or use of different chemicals. The first phase of the process, called beam house, which serves to clean the hide from hair, remaining flesh and prepare it for the actual tanning process.

Tannery wastewater is also characterized by being strongly alkaline with a high salt content, variable pH and high concentrations of suspended solids, BOD$_5$, COD, and chemicals including tannins which are extractant from trees used before tanning process which currently is replaced by chromium (Nandy et al., 1999). Excess amounts of chromium uptake are very dangerous due to its carcinogenic effect. Chromium in soils affects plant growth, it is non-essential for microorganisms and other life forms and when in excess it exerts toxic effect on them after cellular uptake (Singanan et al., 2007).

2.1.1 Tannery production processes
The production of leather from hides and skins involves the treatment of raw materials, i.e. the conversion of the raw hide or skin, a putrefiable material, into leather, a stable material. This material is obtained after passing through different treatment and processing steps. The tanning process can be partitioned in three main categories; beam house operation, tanyard operation, and finishing operation including dyeing and surface treatment (Lefebvre et al., 2006; ETPI, 1998; UNIDO, 2000; Infogate/GTZ, 2002).

2.1.1.1 Hides and skins storage and beam-house operations.
Upon delivery, hides and skins can be sorted, trimmed, cured and stored. The operations in the beam house are: soaking, de-haring, liming, fleshing and splitting, bating and pickling. Hides and skins have the ability to absorb tannic acid and other chemical substances that prevent them from decaying, make them resistant to wetting, and keep them supple and durable. The majority of the skins are treated simultaneously with enzymes during the bating step where skin gets softened, relaxed, cleaned and ready for pickling and tanning (Oke et al., 2005; UNIDO, 2000; ETPI, 1998; Infogate/GTZ, 2002).

2.1.1.2 Tanning Operations
In the tanning process the collagen fiber is stabilized by the tanning agents so that the hide (the raw material) is no longer susceptible to putrefaction by being converted to a non-putrescible material (leather) which can be tradable as intermediate products (wet blue). However, if leather is to be used to manufacture consumer products, it needs further processing and finishing. The
two main categories of tanning agent are mineral (trivalent chromium salts) and vegetable tannins (quebracho and mimosa) (Ockermann and Hansen, 1988). Now days, chrome tanning is favored by the majority of the leather industry because of the speed of processing, color of the leather and greater stability of the resulting product. However; in the chrome tanning practice, only 50 - 60% of chromium applied is taken by the leather and the remaining is discharged as waste (Rajamanickam, 2000).

2.1.1.3 Post-Tanning Operations
Post-tanning generally involves washing out the excess applied chrome to the leather after the required being absorbed during tanning process. Depending on the desired leather type to be produced, the leather is re-tanned (to improve the feel and handle of leathers), dyed with water-soluble dyestuffs (to produce even colours over the whole surface of each hide and skin), fat liquored (leathers must be lubricated to achieve product-specific characteristics and to re-establish the fat content lost in the previous procedures) and finally dried. After drying, the leather may be referred to as crust, which is a tradable intermediate product (Ockermann and Hansen, 1988).

2.1.1.4 Finishing Operations
The art of finishing is to give the leather as thin finish as possible without harming the known characteristics of leather, such as its look and its ability to breathe. The aim of this process is to treat the upper (grain) surface to give it the desired final look such as: grounding (applying a base coat to leather to block pores before applying the true finish coats), coating, seasoning, decoration (to create a raised design upon a leather surface by pressure from a heated stamped plate or roller) and ironing (to pass a heated iron over the grain surface of the leather to smooth it and/or to give it a glossy appearance)(Ozgunay et al., 2007)
The overall objective of finishing is to enhance the appearance of the leather and to provide the appropriate performance characteristics in terms of color, gloss, and handling, among others. Further, leather will have, as desired by fashion, a shiny or matt, single or multi-colored, smooth or clearly grained surface (Gerhard, 1996).
The final step in the finishing operations include leveling the color, covering grain defects, controlling the gloss and providing a protective surface with good resistance to water, chemical attack and abrasion (Ockermann and Hansen, 1988; Infogate/GTZ, 2002; UNIDO, 2000).
Through these processes and complex stages, animal hides and skins are transformed into leather by consuming high quantities of water and substantial quantities of chemicals like lime, sodium sulfide, ammonium sulfate, sodium chloride, bactericides, vegetable tannins, and chromium salts applied at different stages are released in the effluent (Cooman et al., 2003).

2.2 Environmental impact of sulfur compounds in tannery wastewater

Among various environmental pollutants of wastewater released from different industries, tannery waste is the major challenging and devastating pollutant. Leather industry is one of the most harmful to the environment for being responsible for extreme pollution of water resources. Environmental pollution problem arises from discharges of untreated tannery effluents which causes high level pollution due to high toxicity of chromium and sulfides (Seyoum Leta et al., 2004).

The sulfide content of tannery effluent results from the use of sodium sulfide and sodium hydrosulfide for breakdown hair in the dehairing process. When the pH of the effluent falls below 9.5 hydrogen sulfide is evolved from the effluent and the lower the pH the greater the fate of evolution. This creates unpleasant smell (even in small quantity), smell of rotten egg, and cause toxicity for many forms of life (Buljan et al., 2000).

Comparable in toxicity to hydrogen cyanide, even a low level of exposure to the gas induces headaches and nausea, as well as possible damage to the eye like eye irritation. However, the biggest problem is due to the release of hydrogen sulfide gas to the atmosphere and the oxygen depletion of water caused by sulfide oxidation. At higher levels, death can rapidly set in and countless deaths attributable to the build-up of sulfide in sewage systems and while opening drums during the deliming process, through cleaning operations/ sludge removal in gullies and pits, and bulk deliveries of acid or chrome liquors being pumped to containers holding solutions of sodium sulfide.

When absorbed, weak acids can form and cause corrosion. This weakens metal roofing, girders and building supports. In sewers, major problems can arise as metal fittings, structural reinforcements and pipe work corrode (Berna et al., 2007). Hydrogen sulfide is a colorless gas and has a strong odor of rotten eggs (HSDB, 1999). Hydrogen sulfide is soluble in certain polar
organic solvents, notably methanol, acetone, propylene carbonate, sulfolane, tributyl phosphate, various glycols, and glycol ethers.

It also, dissolved by condensation to form weak acids which can cause structural problem of corrosion (HSDB, 1999). Hydrogen sulfide is soluble in glycerol, gasoline, kerosene, carbon disulfide, and crude oil. Aqueous solutions of H$_2$S are not stable; absorbed oxygen causes the formation of elemental sulfur and the solutions become turbid rapidly (HSDB, 1999).

Sulfide-containing waste streams are generated by a number of industries such as petrochemical plants, tanneries, viscose rayon manufactures, the gasification of coal for electricity production, or by the anaerobic treatment of sulfate containing wastewaters (Kuenen and Robertson, 1992; Rinzema and Lettinga, 1988).

It is emitted into the environment as dissolved sulfide (S$^{2-}$ and HS$^-$) in wastewaters and as H$_2$S in waste gases. Three forms of sulfide are present within wastewater and wastewater collection systems. Much depends on the pH of the solution in determining the relative concentration of the three forms. As the pH drops below 7, most of the sulfide is present as hydrogen sulfide as either the non-volatile ionic species of hydrogen sulfide (HS-) or as hydrogen sulfide gas (H$_2$S) (Brimblecombe et al., 1989).

Hydrogen sulfide is generated in relatively stagnant wastewater systems as a result of the biological breakdown of sulfates (SO$_4^{2-}$) in anaerobic wastewater environments as shown in the following biochemical reaction (Elizabeth, 2005).

SO$_4^{2-}$ + organic matter (anaerobic bacteria) $\rightarrow$ S$^{2-}$+H$_2$O+CO$_2$

Sulfate is a component of tannery effluent, emanating from the use of sulfuric acid or products with a high (sodium) sulfate content. Many auxiliary chemicals contain sodium sulfate as a by-product of their manufacture, like chrome tanning powders contain high levels of sodium sulfate, as do many synthetic re-tanning agents.

In addition, the source is created by removing the sulfide component from effluent by aeration since the oxidation process creates a whole range of substances, including sodium sulfate. These sulfates can be precipitated by calcium-containing compounds to form calcium sulfate which has a low level of solubility. Problems arise with soluble sulfates, however, for two main reasons (Bosnic et al., 2000).

First, Sulfates cannot be removed completely from a solution by chemical means. Under certain biological conditions, it is possible to remove the sulfate from a solution and bind the sulfur into
microorganisms. Generally, however, the sulfate either remains as sulfate or is broken down by anaerobic sulfur-reducing bacteria to produce malodorous hydrogen sulfide (Berna et al., 2007). This process occurs very rapidly in effluent treatment plants, sewage systems and water courses, if effluents remain static. This bacterial conversion to hydrogen sulfide in sewage systems results in the corrosion of metal parts unless sulfate-resistant concrete were used. Secondly, if no breakdown occurs, the risk of increasing the total concentration of salts in the surface water and groundwater runs is incurred (Bosnic et al., 2000).

2.3 Biological Sulfur compound transformations:
Sulfur compound in its occurrence is similar to nitrogen because it has significant atmospheric component (but atmospheric S is a far smaller pool than atmospheric N) and similar to phosphorus S is primarily rock derived. Bacteria convert sulfur compound under anaerobic, aerobic and anoxic condition to different forms.

![Redox process and prokaryotes in the sulfur cycle](Source: Bartholomeus, 1966)

2.4 Occurrences of sulfur compound
Sulfur in nature occurs in various forms. It can be obtained from gas stream to aerosol, aqueous, soil mineral and with in biological system
Table 1: Naturally occurring sulfur compounds and their oxidation state

<table>
<thead>
<tr>
<th>Oxidation state</th>
<th>Gas</th>
<th>Aerosol</th>
<th>Aqueous</th>
<th>Soil</th>
<th>Mineral</th>
<th>Biological</th>
</tr>
</thead>
<tbody>
<tr>
<td>-II</td>
<td>H₂S, RSH, DMS</td>
<td>H₂S, HS⁻, S₂⁻, RS</td>
<td>S₂⁻, RS</td>
<td>S²⁻</td>
<td>Methionine, cysteine</td>
<td></td>
</tr>
<tr>
<td>-I</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>FeS₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
<td></td>
<td>H₂SO₃</td>
<td>S⁰</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+II</td>
<td></td>
<td>S₂O₃</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+IV</td>
<td>SO₂</td>
<td>HSO₃</td>
<td>HSO₃, SO₃²⁻</td>
<td>SO₃²⁻</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+VI</td>
<td>SO₃</td>
<td>HSO₄⁻, SO₄²⁻</td>
<td>HSO₄⁻, SO₄²⁻</td>
<td>CaSO₄, CaSO₄</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Source: (Lens and Hulshoff, 2000)

2.5 Biological sulfide removals

Biological H₂S (g) removal is widely used due to their efficiency and economy in these processes (Janssenn et al., 2002). The gaseous pollutant is solublized into an aqueous solution where it is oxidized by microorganisms to non-volatile compounds such as elemental sulfur and sulfate. The pH conditions in these biological systems are usually mildly or extremely acidic (Kraakman, 2003; Gabriel and Deshusses, 2003). Chemolithoautotrophic bacteria from the genera *Thiobacillae* and *Acidithiobacillae* have been reported to be the main organisms in the sulfide oxidation. At alkaline conditions (pH > 9) in biological treatment processes, H₂S (g) may be desirable to increase transfer and possibly the reaction rate due to its higher concentration. This is due to genera of bacteria develop as generally grouped as alkaliphilic sulfoxidizing bacteria (ASB) which are obligate or facultative chemolithoautotrophic. These bacteria can grow at pH between 9 and 11 and their main function is to oxidize reduced sulfur compounds such as thiosulfate, sulfide (Sorokin et al., 2001).
2.6 Sulfide Bacteria

Several groups of micro-organisms are involved in sulfide oxidation. \( \text{H}_2\text{S} \) is oxidized to elemental sulfur under aerobic by \( \text{Thiobacillus thioparus} \) through the following reaction:

\[
\text{S}^{2-} + \text{O}_2 + \text{H}^+ \rightarrow \text{S}^0 + \text{H}_2\text{O} \quad \text{(Chung et al., 1996)}
\]

Under anaerobic conditions, oxidation of \( \text{S}^{2-} \) is carried out by photoautotrophs and a chemoautotroph, \( \text{Thiobacillus denitrificans} \).

There are several pathways for the biological removal of the sulphur as presented below.

\( \text{H}_2\text{S} \) (Hydrogen sulfide…reduced form of sulfur) is an energy source for some autotrophs such as Phototrophs (green and purple bacteria groups) and chemoautotroph (\( \text{Beggiatoa} \) and \( \text{Thiobacillus} \)). Under anaerobic conditions phototrophic purple sulfur bacteria and green sulfur bacteria convert \( \text{S}^{2-} \) to \( \text{S}^0 \), \( \text{S}_2\text{O}_3^{2-} \) and \( \text{SO}_4 \). Methane and \( \text{CO}_2 \) are the main products in anaerobic environments where sulfate is absent but sulfide and \( \text{CO}_2 \) are the product sulfide oxidation often occurs in steps with elemental sulfur as an intermediate product (Yang, 1992).

**Oxidation** of sulfur

Under oxygen limited environment oxidation may proceed only to elemental sulfur producing less energy cells can either deposit sulfur inside or outside their cell membranes other reduced sulfur compounds such as thiosulfate can also be oxidized for energy.

Sulfide is used as electron donor for cell synthesis in the presence of light by green and purple sulfur bacteria during a process called an oxygenic photolithoautrophic growth (Zehnder, 1988) during the process sulfate is produced as final product. Significant amount of sulfur are also produced as intermediate .green sulfur bacteria and ectothiorhodospira species, cholorobiaceae species form extracellular sulfur where as chromatium species form intracellular sulfur globules (Zehnder, 1988).

\[
\text{H}_2\text{S} \rightarrow \text{elemental sulfur (S)} \rightarrow \text{SO}_4
\]

Sulfate (\( \text{SO}_4 \)) is assimilated by plants and bacteria into proteins. Decomposition by microbes releases sulfur (dissimulation) as \( \text{H}_2\text{S} \) to reenter the cycle. Photosynthetic bacteria use \( \text{H}_2\text{S} \) as an electron donor and oxidize \( \text{H}_2\text{S} \) to \( \text{S}^0 \), which is stored within the cells of chromatiaceae (purple...
sulfur bacteria) or outside cells of cholorobiaceae (green sulfur bacteria). Also, Filamentous sulfur bacteria (*Beggiatoa thiothrix*), carries out H$_2$S oxidation to S, which is deposited in S granules (Castenholz, 1977; Jorgensen *et al.*, 1979; Oren and Padan, 1978; Oren and Shilo, 1979).

Table 2: Energy source for representative chemotrophs

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Electron donor</th>
<th>Electron acceptor</th>
<th>Carbon source</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiobacillus sp. (general)</td>
<td>S$^0$, H$_2$S, S$_2$O$_3^{2-}$</td>
<td>O$_2$</td>
<td>CO$_2$</td>
<td>SO$_4^{2-}$</td>
</tr>
<tr>
<td>Thiobacillus denitrificans</td>
<td>S$^0$, H$_2$S, S$_2$O$_3^{2-}$</td>
<td>O$_2$, NO$_3^-$</td>
<td>CO$_2$</td>
<td>N$_2$, SO$_4^{2-}$</td>
</tr>
<tr>
<td>Thiobacillus ferrooxidans</td>
<td>Fe$^{2+}$, S$^0$, H$_2$S</td>
<td>O$_2$</td>
<td>CO$_2$</td>
<td>SO$_4^{2-}$, Fe$^{3+}$</td>
</tr>
</tbody>
</table>

Source: (Prescott *et al.*, 2003)

2.7 Sulfate bacteria

Sulfate occurs widely in seawater, sediment, or water rich in decaying organic material. Sulfate-reducing bacteria are common in anaerobic environments where they aid in the degradation of organic materials (Dexter, 2003). In these anaerobic environments, fermenting bacteria extract energy from large organic molecules; the resulting smaller compounds such as organic acids and alcohols are further oxidized by acetogens and methanogens and the competing sulfate-reducing bacteria (Larry, 1995).

Sulfate reducing bacteria are bacteria and archaea which obtain their energy by oxidizing organic compounds or molecular hydrogen H$_2$ while reducing sulfates to sulfides, especially to hydrogen sulfide (Ernst *et al.*, 1993). In a sense, they "breathe" sulfate rather than oxygen. Sulfate-reducing bacteria can be traced back to 3 billion years ago and are considered to be among the oldest forms of bacteria (Ernst *et al.*, 1993).

Many bacteria reduce small amounts of sulfates in order to synthesize sulfur-containing cell components; this is known as *assimilatory sulfate reduction*. By contrast, the sulfate-reducing bacteria reduce sulfate in large amounts to obtain energy and expel the resulting sulfides as
waste; this is known as *dissimilatory sulfate reduction*. They are anaerobes which use sulfate as the terminal electron acceptor of their electron transport chain (Larry, 1995).

Most sulfate-reducing bacteria can also reduce other oxidized inorganic sulfur compounds, such as sulfite, thiosulfate, or elemental sulfur.

The toxic hydrogen sulfide is a waste product of sulfate-reducing bacteria; its rotten egg odor is often a marker for the presence of sulfate-reducing bacteria in nature (Dexter, 2003). Sulfate-reducing bacteria are responsible for the sulfurous odors of salt marshes and mud flats. Much of the hydrogen sulfide will react with metal ions in the water to produce metal sulfides. These insoluble metal sulfides, such as ferrous sulfide FeS, are often black or brown, leading to the dark color of sludge (Ernst *et al.*, 1993).

The sulfate-reducing bacteria have been treated as phenotypic group, together with the other sulfur-reducing bacteria, for identification purposes. They are found in several different phylogenetic lines (Pfennig and Biebel, 1986), three lines are included among the Proteobacteria, all in the delta subgroup:

Desulfo bacterales, Desulfovibrionales, Syntrophobacterales a fourth group including thermophiles is given its own phylum, the Thermodesulfobacteria. The remaining sulfate-reducers are included with other bacteria among the Nitrospirae and the gram-positive Peptococcaceae - for instance *Thermodesulfovibrio* and *Desulfotomaculum*, respectively. There is also a genus of Archaea known to be capable of sulfate reduction, *Archaeoglobus*. The overall sulfate reduction process can be represented by the following generalized equation:

Fermentative end products + S\(_{0}^{2-}\) --> acetate + HS\(^{-}\) + HCO\(_{3}^{-}\)
The end products of biomass fermentation and sulfate are converted by this reaction to acetate, bisulfide or hydrogen sulfide, and bicarbonate. Application of this process to acidic mine effluents may improve water quality in five respects

2.8 Nitrate reducer and sulfide oxidizer consortia

Consortia of bacteria that can oxidize sulfide and reduced nitrate are included under the genera of *Thiobacillus denitrificans*, *Thioploca*, *Thiosphaera*, *Thiomicrospira denitrificans*, *Thermothrix* (Stanier *et al.*, 1986).

Sulfur pollution can be controlled by microbial oxidation of sulfide to elemental sulfur (Eckford and Fedora, 2002). Consortia of chemolithotrophic microorganism in anoxic environments that oxidizes sulfide to elemental sulfur are of two major types of nitrate reducing bacteria (NRB): one is the chemooorganotrophic (heterotrophic) NRB that use organic compound as electron donors and the second type is the chemolithotrophic nitrate reducing - sulfide oxidizing bacteria (NR-SOB) (Eckford and Fedora, 2002). Several studies have been conducted to remove sulfide from different sulfide waste environment by adding nitrate (Jenneman *et al.*, 1986; Londry and Suflita, 1999; Davidova *et al.*, 2001).

2.9 Tannery Wastewater Treatment method

The pollution of water by industrial effluents especially process industries is a serious problem in most countries. This problem requires a greater attention for the scientific community. The treatment of contaminated wastewater by means of biological and chemical processes has been widely implemented from classical urban to industrial wastewater. From economic and operational points of view, biological treatment has proved to be a healthy and more energy efficient for treating biodegradable wastewater only if good process control could be maintained (Grady *et al.*, 1999).

2.9.1 Physico-chemical processes

Physico-chemical treatment of tannery effluents consist of coagulation, flocculation, sedimentation, filtration, air stripping, chemical precipitation, adsorption, ion exchange, electrochemical (electro-oxidation), and chemical oxidation (Ramesh *et al.*, 2007; Kyung- Sok *et al.*, 2004; Lofrano *et al.*, 2006; Metes *et al.*, 2004; USEPA, 2004; Linda and Peter, 1999).
Coagulation and flocculation are important treatments given to the industrial effluent before discharging them into receiving waters to remove toxic waste.

2.9.2 Biological Processes

Treatment methods in which the removal of contaminants is brought about by biological activity are known as biological unit processes. Biological treatment is used primarily to remove the biodegradable organic substances (colloidal or dissolved) from wastewater. Basically, these substances are converted into gases that can escape to the atmosphere and into biological cell tissue that can be removed by settling. Biological treatment is also used to remove nutrients (nitrogen & phosphorus) from wastewater (Metcalf and Eddy, 2003). With proper environmental control, wastewater can be treated biologically in most cases. Biological treatment methods use microorganisms, mostly bacteria, in the biochemical decomposition of wastewaters to stable end products. More microorganisms, or sludge, are formed and a portion of the waste is converted to carbon dioxide, water and other end products (Nicholas, 1996).


There has several study conducted on Modjo tannery on biological treatment of tannery effluent. Seyoum Leta et al., 2004 and Yemisirach Mulugeta use activated sludge system, 2008, Andualem Mekonnen, 2008 use sequencing batch reactor while Asaye Ketema, 2009 use constructed wetland to treat the tannery effluent.

2.10 Sequencing batch reactor technology

The sequencing batch reactor (SBR) is widely and commonly used in biological wastewater treatment in its most basic form, it is a set of tanks that operate on a fill-and draw basis (Mace and Mata, 2002). SBRs technology is considered as an alternative to the activated-sludge process for the removal of nutrient from wastewater. This configuration has a higher flexibility and controllability allowing more rapid adjustment to changing influent characteristics (Baetens, 2000).
Unlike activated sludge system lower investment and recurrent cost is necessary because secondary settling tanks and sludge return systems are not required (Nowak and Lindtner, 2004). According to USEPA (1999) report, an SBR is no more an activated sludge plant that operates in time rather than space. Further, it is especially appropriate for places where there is significant flow, load variability and where space problems become a restriction (Matcalf and Eddy, 2003). The technology has been successfully applied in WWTPs treating urban (Lee et al., 2004) and industrial (Keller et al., 1997; Vives et al., 2003).

The SBR technology was first used in 1914. (Arden and Lockett, 1914) During the 20th century this technology has gained popularity mainly due to its operation advantages and flexibility. In addition the computerization of SBR operation has made their implementation much easier and has definitely contributed to the development of this technology, research began in the 1970s (Irvine and Davis, 1971).

The sequencing batch reactor system is a modern version of fill and draw system. Sequencing Batch Reactors consists of four steps: feeding, reaction, settling and treated effluent withdrawal consisting of one or more tanks, each capable of waste stabilization and solids separation. The number of tanks may be varied, depending on the sophistication of the control system.

The treatment cycle can be adjusted to undergo aerobic, anaerobic, and anoxic conditions in order to achieve biological nutrient removal, including nitrification, denitrification, and some phosphorus removal. For biochemical oxygen demand (BOD) Irvine and Ketchum (1988) demonstrated that the use of static and mixed fill periods could help to achieve nitrogen and phosphorus removal. The application of SBR technology is a more appropriate alternative to treat wastewater than conventional continuous systems but it requires a higher level of control and automation the instrumentation, control and automation of the process is a key factor when the process must be operates to achieve restricted discharge levels.

Due to wastewater discharge permits becoming more stringent, the SBRs offer a cost-effective way to achieve lower effluent limits. However, the discharge limits that require a greater degree of treatment may necessitate the addition of a tertiary filtration unit following the SBR treatment phase. This consideration should be an important part of the design process Ketchum (1997).
CHAPTER THREE

MATERIALS AND METHODS

3.1 Description of the Study Area

Modjo is a town in the central rift valley of Ethiopia, named after the nearby Modjo River. It is located in East Shewa Zone of the Oromia Regional State, this town has a latitude and of longitude of 8°39′N 39°5′E / 8.65°N 39.083°E / 8.65; 39.083 with climatic condition of tropical rainfall (Richard, 1968). Modjo tannery is found in Modjo town and has an annual processing capacity of 844, 000 Sheepskins and 1,656,000 Goat skins (Seyoum Leta et al., 2004).

3.2 Sampling

3.2.1 Sampling techniques

Composite samples were taken from the effluent of Modjo tannery every seven days. The samples were collected and brought to laboratory scale treatment plant set in Faculty of Science.
Addis Ababa University at bio-earn treatment plant. The system was designed to treat 5L of effluent per day.

3.3 Experimental set-up

The wastewater characterization was performed to enable the design of lab scale SBR (sequencing batch reactor), for parameters like: hydraulic retention time (HRT), amount of air required, aeration time, mixing time and sludge retention time. The sizes of the containers designed were of volume of 10L for anaerobic SBR and 5L for aerobic SBR.

Figure 3: laboratory scale sequencing batch reactor

1. Influent feeding tank (sedimentation tank)  2. Anaerobic reactor  3. Air compressors
4. Aerobic reactor which include (oxic and anoxic condition)  
5. Vertical stirrer  
6. Clarifier

3.4 Laboratory Analysis

3.4.1 Physico-chemical and physical parameters

Samples from sedimentation tank, anaerobic reactor, aerobic reactor (oxic/anoxic reactor) and clarifier of sequencing batch reactor were analyzed for parameters BOD$_5$, COD, Sulfides (S$^{2-}$), Sulfate (SO$_4^{2-}$) in Azide modification, Reaction digestion HR, Methylene blue and Sulfaver 4 method respectively. TP, NH$_4$, NO$_3$, TN were analyzed in Phosver $^R$ 3 with acid persulfate digestion, Nessler, Cadmium reduction HR and Persulfate digestion method respectively. All parameters were analyzed according to America Public Health Association (APHA) 1995.

Conductivity, pH, level of dissolved oxygen (DO) and temperature were measured using a (CC-401, ELMETRON), (CO-411), pH meter (Model HI 9024 HANNA), ELMETRON and a hand-held thermometer respective.

The removal efficiency of the system was calculated using removal efficiency formula

\[
\text{% Removal Efficiency} = \frac{(C_{\text{inf}} - C_{\text{eff}}) \times 100}{C_{\text{inf}}} 
\]

Equation 3.1

Where, $C_{\text{inf}}$ = Initial parameter concentration and $C_{\text{eff}}$ = Final parameter concentration

The hydraulic retention time for anaerobic, oxic and anoxic reactor were calculated by

\[
V \ (m^3) = Q \ (m^3/day) \times HRT \ (day) 
\]

Equation 3.2

Where V= Volume  Q= Flow rate and HRT = hydraulic retention time

3.5 Operation of Sequencing Batch Reactor

The study was conducted in nine months in two different operational periods: period 1, at start up period where the SBR was operated for four months from October, 2009 to January, 2010, this time was assigned for acclimatization period. Bacterial sludge in the reactor was taken from tannery sludge in Modjo tannery. During this period, the reactors were operated in 96hrs cycle mode. Where, 94hrs was given to the reaction phases ($T_R$) and the remaining 2-hrs for settle, draw and idle phases ($T_S + T_D + T_I = 2$-hrs).
Where: \( T_R = \) Total reaction phase. \( T_S = \) Total time for settle, \( T_D = \) Total time for decant (draw) and \( T_I = \) Total time for idle.

From 96h cycle 47h reaction period was for anaerobic reactor the rest 47h was give for aerobic reactor where 28h for oxic phase and 20h anoxic.

During period 2, the SBR was operated for five months from February, 2010 to June, 2010 with different hydraulic retention time from the first period reaction. The reactor was operated for 48 hrs cycle mode where 46hrs was given to the reaction phases \( T_R \) and the remaining 2-hrs for settle, draw and idle phases \( (T_S + T_D + T_I = 2\text{-hrs}) \). 24hrs reaction period was for anaerobic reactor. The rest 24hrs was allotted for aerobic reactor which include the settling draw and idle phases where 14h for oxic phase and 10h for anoxic phase.

In both periods, the system was adjusted to a total sludge age of 15 days. Experiments were carried out at 20 °C and in a pH range of 8–8.5. A fill volume \( (V_F) \) of 5L was selected while 3.75L were drown the reactors for stationary volume \( (V_O) \) and corresponding to \( V_O/V_F = 0.75 \).

Table 3: Details of sequencing phase variation during cycle operation

<table>
<thead>
<tr>
<th>Phase</th>
<th>Cycle period</th>
<th>Air supply</th>
<th>Recirculation</th>
<th>Anaerobic condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fill (static fill)</td>
<td>20min</td>
<td>Non</td>
<td>Non</td>
<td>_</td>
</tr>
<tr>
<td>React</td>
<td>46hrs</td>
<td>13 &amp; 1/2hrs</td>
<td>91/2 hrs</td>
<td>23hrs</td>
</tr>
<tr>
<td>Settle</td>
<td>1h</td>
<td>Non</td>
<td>Non</td>
<td>_</td>
</tr>
<tr>
<td>Decant</td>
<td>20min</td>
<td>Non</td>
<td>Non</td>
<td>_</td>
</tr>
<tr>
<td>Idle</td>
<td>20min</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
</tbody>
</table>

3.6 Environmental working condition in the reactors

The SBR reactors (anaerobic & anaerobic) work under different environmental conditions. Oxic condition operated at environmental condition of pH 8, EC 12.5ms/cm, temperature 20 °C, hydraulic retention times of 14h, dissolved oxygen of 6.5g/L and sludge age of 15 days while
anaerobic condition was running at environmental operating conditions of pH 8.38, EC, 12 ms/cm, temperature 20 °C, hydraulic retention time of 24hrs and sludge age of 15days finally anoxic environmental working condition was temperature 20 °C, EC 11.9ms/cm, pH 8.67, HRT of 10hrs.

3.7 Isolation, Screening and Enumeration of Sulfide Oxidizing and Nitrate Reducing Consortia

Chemolithotrophic nitrate-reducing and sulfide oxidizing bacteria (NR-SOB) were isolated & cultured from anoxic reactor of tannery effluent sample in the applied microbiology laboratory in Addis Ababa University.

Wastewater sample was taken from an anoxic sequencing batch reactor and serially diluted in 0.85% sterilized saline solution up to $10^{-7}$ then 100µL of the suspension was spread on to thiosulfate basal enrichment medium for growth and enumeration of the bacterial colonies (Smith and Kelly, 1988). The pH of the medium was adjusted to 10 by NaCO₃. Both NaCO₃ and thiosulfate basal medium were sterilized in an autoclave at 121°C for 15 min. The inoculated bacteria were estimated as colony–forming units per ml (C.F.U / mL).

Hence, 26 different pure cultures were isolated and different biochemical and morphological test were done.
Table 4: Component of Thiosulfate Basal Medium (TBM)

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na₂HPO₄</td>
<td>2</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>2</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>1</td>
</tr>
<tr>
<td>(NH₄)₂SO₄</td>
<td>0.4</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>2</td>
</tr>
<tr>
<td>FeCl₃·6H₂O</td>
<td>0.02</td>
</tr>
<tr>
<td>MnSO₄·3H₂O</td>
<td>0.02</td>
</tr>
<tr>
<td>CH₃COONa·3H₂O</td>
<td>2</td>
</tr>
<tr>
<td>Na₂S₂O₃·5H₂O</td>
<td>5</td>
</tr>
<tr>
<td>NaNO₃</td>
<td>2</td>
</tr>
<tr>
<td>Nutrient Agar</td>
<td>28g</td>
</tr>
<tr>
<td>Trace Metal solution</td>
<td>10ml</td>
</tr>
</tbody>
</table>

Table 5: Component of the trace metal solution

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount (g/100mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTA</td>
<td>0.05</td>
</tr>
<tr>
<td>ZnSO₄·7H₂O</td>
<td>0.02</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>0.05</td>
</tr>
<tr>
<td>MnCl₂·2H₂O</td>
<td>0.05</td>
</tr>
<tr>
<td>(NH₄O)₆Mo₇O₂₄·4H₂O</td>
<td>0.01</td>
</tr>
<tr>
<td>CuSO₄·5H₂O</td>
<td>0.015</td>
</tr>
<tr>
<td>CoCl₂·6H₂O</td>
<td>0.02</td>
</tr>
</tbody>
</table>
3.7.1 Bacterial designations and preservation

The isolates were designed as sulfide oxidizing nitrate reducing (SNB) following by their number respectively to mean that they are sulfur oxidizing nitrate reducing bacteria. Individual colonies of bacteria were transferred to fresh agar plates, purified through repeated plating and stored in agar slant at 4 ºC for conservation.

3.7.2 Biochemical test

From anoxic reactor 26 bacterial isolates were isolated and for further identification biochemical and morphological characterization were done.

3.7.2.1 Cytochrome Oxidase test

To determine the presence of cytochrome oxidase system in the isolates, the test was conducted following the method of (Steel, 1961).

Small piece of Whatman filter paper were soaked with 1% (w/v) N, N-dimethyl –p-phenyl di ammonium chloride loop full of 24 hrs old culture were scrapped and rubbed on the filter paper. Appearance of blue color within to 10 to 30 second indicated a positive test for oxidase.

3.7.2.2 Catalase test

The presence of enzyme catalase in the bacterial isolates was checked by flooding a drop of 3% H₂O₂ on pure colonies of 24 hr cultures over the slide. Immediate effervescence of gas bubble was recorded as a positive result (MacFaddin, 1980)

3.7.2.3 Gram reaction

In order to check gram reaction of bacterial isolates small piece of Whatman filter paper were soaked with 3% (w/v) of KOH loop full of 24 hrs old culture were scrapped and rubbed on the filter paper. When the solution is picked up, if the trade like thing formed, it was considered as gram negative if not gram positive (Halebian et al., 1981).

3.7.2.4 Nitrate reduction test

Nitrate reduction tests were performed on overnight culture of the 26 isolates on thiosulfate basal medium (TSB). Nitrate broth with Peptone 5 g/L, Meat extract 3 g/L Zinc (Fluka 93027), Potassium nitrate 1 g/L were prepared and filled in 10ml test tube after adjusting the pH to 10,
then overnight bacteria culture were transferred to the media and incubated at 30°C for 1, 2, 3 days.

Thereafter, 5 drops of Sulfanilic acid (Fluka 86090) = Reagent A and 5 drops of N, N-Dimethyl-1-naphthylamine (Sigma D4011) = Reagent B were put into the tube containing culture to be tested. After shaking the tube well distinct red or pink color, which should develop within a few minutes, indicated nitrate reduction. Suspension which turned pink-red before the addition of Zn powder, the reactions were positive and the test was completed. The suspension which were colorless after the addition of reagents A and B, small amount (“sharp knife point”) of zinc powder were added to the medium and shake vigorously and allowed it to stand at room temperature for 10-15 min. For the medium remains colorless after the addition of Zn powder, the test result was considered positive. The medium which turned pink after the addition of Zn powder, the result was considered negative. The negative control was tested throughout test (Mac Faddin, 1980).

3.7.2 6 Nitrate reductions potential (substrate consumption)
In order to select the potential isolate in denitrification rate activity, 10mg wet biomass of overnight bacteria culture were measured on PCR tube and suspended in 1ml of nutrient broth. 250µL of bacterial suspension were inoculated to microtitre plates in columns of A-H. 200µl NO₂ reagent were added to the rest of wells A₂-H₂ then from 0.75g/100mL of NaNO₂ 5 µL were added to bacterial suspension. Finally, from row 1 of bacterial suspension 5 µL were transfer to detection reagent in row2 of the rest and measured every 30 minutes time interval in spectrophotometer till the red color changed to colorless.

The isolates that change red color to colorless very fast were considered as potential nitrate reducer.

3.7.2 5 Sulfide oxidation potential (substrate consumption)
Potential sulfide oxidizer was identified by inoculating overnight isolate culture in thiosulfate basal broth media by changing the concentration of NaS₂O₃.5H₂O to 50g/L. The media were measured by spectrophotometer at every 30min interval.
3.7.2 Sulfide oxidation and nitrate reduction potential of selected isolates

To estimate the removal efficiency of selected isolates overnight bacteria culture of 100ml broth were prepared. Four hundred milliliter (400mL) of tannery effluent from anoxic reactor was taken and 50mg of NaS$_2$O$_3$ and NaNO$_3$ were added to the effluent. The concentration of S$^{2-}$ and NO$_3$ were measured by using spectrophotometer before the addition of bacterial to the effluent. Then forward the concentration of sulfide and nitrate were measured at the interval of an hour for four hour .parallel to that 1ml of broth were serially diluted and strike to the agar plate in order to know the exact number of bacteria inserted to the effluent.

3.7.2 Estimation of selected bacteria number

In order to estimate the number of colony form unit /mL, overnight broth culture were serially diluted from $10^1$ to $10^7$ and each were strike to thiosulfate basal solid media and the plate count was done after incubation of the bacteria for 24 for serially dilution of $10^4, 10^5, 10^6$.

3.7.3 Morphological and physiological characterization

The ability of the isolates to tolerate different environmental conditions was examined.

3.7.3.1 Temperature tolerance

Overnight broth culture was transfered to thiosulfate basal solid media and incubated at different temperature range of 5ºC- 60ºC at the interval of five. Growth result were recorded qualitatively as + for growth and – for no growth (Tuttle et al., 1972).

3.7.3.2 pH tolerance

In order to check the ability of isolate to grow on acidic and alkaline media, Thiosulfate basal medium (TSB) was adjusted to different pH level of 3 to 12 at interval of one. 1N HCl, and 1N NaOH were used to adjust the different pH then Overnight broth culture were streaked to it and incubated at 30ºC. Growth result were recorded qualitatively as + for growth and – for no growth Collins et al. (1976).

3.7.3.3 Sulfur granule deposition

The inclusion or exclusion of sulfur in or out of the cell or colony was examined according to Bergey’s Manual of Systematic Bacteriology, 2nd edition volume (2005) through epi-fluorescence microscope (Olympus BX51, Japan) attached to a CCD digital camera. The light or deep yellow/ brown/dark coloration was recorded as positive for the test if not negative.
3.7.3.4 Motility test

Motility of isolates was determined during O/F test by observing diffused growth for motile ones and non-diffused growth (growth only at the inoculating regions) for non-motile, based on the methods of Collins et al. (1976).

3.7.3.4 Salt tolerance test

The capacity of isolates in their resistance to salt concentration was examined with NaCl in different percentage for 1% of NaCl to 6% NaCl at interval of one. For isolates that showed growth within a given salt concentration were considered as positive while for non growth negative Collins et al. (1976).

3.7.3.5 Identification of isolates at their species level

In order to identify selected potent isolates at species level further morphological test like: cell shape, cell arrangement and spore staining was done and isolates were classified according to Bergey’s Manual of Bacteriology (2005).

3.8 Statistical Analysis

Statistical analysis was performed with the software of SPSS package release 16.00 to perform: Mean, Standard deviation and Analysis of Variance (ANOVA). The comparison between variables was performed at 5% level of significance.
CHAPTER FOUR

4.1 RESULTS

4.1.1 Modjo tannery wastewater characteristics

Wastewater sample from Modjo tannery was analyzed to characterize the pollutant level of the tannery. Table 4.1 below shows mean results of key parameters intended to evaluate the performance efficiency of the system. The concentration of some parameters in the wastewater were $S^{2-}$ (148.50±6.18mg/L), $SO_4^{2-}$ (2162.50±368.20mg/L), COD (6275.00±1536.97mg/L) and BOD$_5$ (2005.80±579.17mg/L).

Table 6: Characteristic of Modjo Tannery wastewater

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S^{2-}$</td>
<td>148.50±6.18</td>
<td>132-160.70</td>
</tr>
<tr>
<td>$SO_4^{2-}$</td>
<td>2162.50±368.20</td>
<td>1425.9-3133.53</td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>567.00±224.36</td>
<td>142.60-1151.40</td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>261.50±68.51</td>
<td>106-385</td>
</tr>
<tr>
<td>TN</td>
<td>766.00±50.84</td>
<td>715.20-833.80</td>
</tr>
<tr>
<td>BOD$_5$</td>
<td>2005.80±579.17</td>
<td>823.60-3195</td>
</tr>
<tr>
<td>COD</td>
<td>6275.00±1536.97</td>
<td>3100-9200</td>
</tr>
<tr>
<td>TP</td>
<td>13.75±6.12</td>
<td>6.01-32.94</td>
</tr>
<tr>
<td>TSS</td>
<td>2155.00±61.31</td>
<td>1203.69-3216.30</td>
</tr>
<tr>
<td>TDS</td>
<td>7035.00±42.52</td>
<td>6992.47-8077.54</td>
</tr>
<tr>
<td>TS</td>
<td>9175.0±65.00</td>
<td>9110-10240</td>
</tr>
<tr>
<td>EC (ms/cm)</td>
<td>15.50±1.99</td>
<td>10.51-19.39</td>
</tr>
<tr>
<td>pH</td>
<td>8.63±0.56</td>
<td>8.07-9.19</td>
</tr>
<tr>
<td>Temperature °C</td>
<td>20±2</td>
<td>18-22</td>
</tr>
</tbody>
</table>

(Concentration is in mg/L except for pH, conductivity and temperature)
4.1.2 Overall Mean Performance of the System

The combined performance of the whole system was evaluated in order to assess the removal efficiency of the characterized pollutant. The analysis test result of SBR system as shown in Table 4.2 below indicates higher (99.73%) removal of S$^{2-}$ than other analyzed parameters. Besides to that, high removal efficiency of COD (95.5 %) and BOD$_5$ (86.77%) was also observed. The system nutrient removal was recorded as NO$_3^-$ (83.19%), SO$_4^{2-}$ (64.5 %), TN (86.9%) and TP (66.1 %) and removal of solid particles were TSS (90.7%), TS (68.4%) and TDS (57.7%) where, TSS was removed much higher than other solid particles.

Table 7: Removal efficiency of the SBR system

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Influent</th>
<th>Effluent</th>
<th>Removal efficiency µ%</th>
</tr>
</thead>
<tbody>
<tr>
<td>S$^{2-}$</td>
<td>148.50±6.18</td>
<td>0.3893± 0.13</td>
<td>99.73%</td>
</tr>
<tr>
<td>SO$_4^{2-}$</td>
<td>2162.50±368.20</td>
<td>767.67± 202</td>
<td>64.5%</td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>567.00±224.36</td>
<td>95.33± 22.71</td>
<td>83.19%</td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>261.50±68.51</td>
<td>328.33±48.67</td>
<td>0</td>
</tr>
<tr>
<td>TN</td>
<td>766.00±50.84</td>
<td>100.33±17.32</td>
<td>86.9%</td>
</tr>
<tr>
<td>BOD$_5$</td>
<td>2005.80±579.10</td>
<td>266.00±43.47</td>
<td>86.77%</td>
</tr>
<tr>
<td>COD</td>
<td>6275.00±1536.97</td>
<td>276.67±47.02</td>
<td>95.5%</td>
</tr>
<tr>
<td>TP</td>
<td>13.75±6.12</td>
<td>4.66 ± 0.89</td>
<td>66.1%</td>
</tr>
<tr>
<td>TSS</td>
<td>2155.00±61.31</td>
<td>199.67±50.33</td>
<td>90.7%</td>
</tr>
<tr>
<td>TDS</td>
<td>7035.00±42.52</td>
<td>2899.70±288.67</td>
<td>58.7%</td>
</tr>
<tr>
<td>TS</td>
<td>9175.0±65.00</td>
<td>2895.30±577.35</td>
<td>68.4%</td>
</tr>
</tbody>
</table>

(Concentration is in mg/L except for pH, conductivity and temperature)

4.1.3 The Mean Performance of the Treatment Plant in each Reactor

The two sequencing batch reactors (anaerobic & aerobic) were compared to evaluate the removal efficiency of the sample wastewater. As mentioned in annex 1, higher removal of S$^{2-}$ in anoxic condition (83.9%) was observed than oxic and anaerobic reactor 75% & 70% respectively. Largest SO$_4^{2-}$ removal was observed in anaerobic reactor (70%) than anoxic condition (32.4%), however, oxic working condition does not show SO$_4^{2-}$ removal. Higher removal of COD & BOD$_5$ was observed in oxic (76.5% and 56.3%) and anaerobic (60.1% & 55.1%) condition than anoxic condition (32.3% and 24.4%), respectively.
4.1.4 Morphological and Biochemical Characterization of NR-SOB

Biochemical test results as shown in the Table 4.3 below indicated, all isolates were positive for cytochrome oxidase and catalase test. The nitrate reduction test showed that all isolates were able to reduce nitrate except isolate SNB 25 and isolate SNB 6. Specifically, isolate SNB-3, SNB-11, SNB -22, &SNB -26 showed faster nitrate reduction rate than others, hence they were selected as potent bacteria isolates and their nitrate and sulfide removal efficiency rate was examined. All isolates have the efficiency to oxidize thiosulfate. Out of the 26 isolates 50% were gram negative and the rest 50% were gram positive.
Table 8: Summary of biochemical test for isolates

| Test                  | 01 | 02 | 03 | 04 | 05 | 06 | 07 | 08 | 09 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 |
|-----------------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| SNB                   | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| Oxidase               | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| SNB                   | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| Catalase              | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| SNB                   | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| Thiosulfate Denitrificaion | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| SNB                   | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| SNB                   | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| SNB                   | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| SNB                   | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| SNB                   | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| Gm test               | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
As summarized in Table 4.4 physiological and morphological test results showed all bacterial isolates were able to grow under temperate range of $5^\circ$C - $60^\circ$C with optimum growth temperature of $30^\circ$C and slight growth at $5^0$C and $60^0$C.

The tolerable pH limits of all isolates were 4-12, while no growth was recorded at pH of 3 and all were able to grow in salt concentration of 1%-6%. Out of 26 isolates 23% were motile and the rest 77% were non motile. The four potent selected isolates did not store sulfur inside their body except isolates SNB 26.

Table 9: Summary of morphological and physiological test for isolates

<table>
<thead>
<tr>
<th>Isolates (SNB)</th>
<th>Temperature tolerance 5-60</th>
<th>Salt tolerance 1-6%</th>
<th>pH tolerance 4-12</th>
<th>Motility</th>
<th>Sulfur granule deposition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<td>+</td>
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<tr>
<td>18</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>
Sulfur granule deposition test was done for the selected potent isolates SNB 3, SNB 11, SNB 22 and SNB 26

4.1.4.1 Sulfide Oxidation and Nitrate Reduction Potential of Selected Isolates

As indicated in Table 4.5 below, out of the four potent nitrate reducing and sulfide oxidizing isolates, SNB 22 and SNB 26 showed higher substrate uptake of nitrate and sulfide than SNB 3 and SNB 11. SNB 26 reduced the initial concentration of sulfide (27.6 mg/L) to 0.52 mg/L within an hour at removal efficiency of 98.1%, while SNB 22 reduced the initial concentration of nitrate from 70 mg/L to 7 mg/L within two hours at removal efficiency of 90%.

Table 10: Nitrate and sulfide removal efficiency of selected isolates

<table>
<thead>
<tr>
<th>Time in hour</th>
<th>Sulfide concentration mg/L</th>
<th>Nitrate concentration mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Isolate</td>
<td>Isolate</td>
</tr>
<tr>
<td>0</td>
<td>SNB-3</td>
<td>SNB-11</td>
</tr>
<tr>
<td></td>
<td>27.6</td>
<td>27.6</td>
</tr>
<tr>
<td>1h</td>
<td>22.7</td>
<td>23.5</td>
</tr>
<tr>
<td>2hrs</td>
<td>21.1</td>
<td>19.9</td>
</tr>
<tr>
<td>3hrs</td>
<td>16.6</td>
<td>15.8</td>
</tr>
</tbody>
</table>

4.1.4.2 Estimation of selected bacteria number

Bacterial plate count of isolates SNB 3, SNB11, SNB 22, & SNB 26 showed that isolate SNB11 was $10.71 \times 10^8$ cfu/mL, isolate SNB 3 was $6.8 \times 10^7$ cfu/mL, isolate SNB 22 was $3.81 \times 10^9$ cfu/mL.
and isolate SNB 26 was $2.4 \times 10^7$ cfu/ml. Generally, the outcome indicated that the overall abundance of the nitrate reducing sulfide oxidizing bacteria population in the anoxic reactor of SBR showed an estimated range of $2.4 \times 10^7$-$3.81 \times 10^9$ cfu/ml.

### 4.1.4.3 Identification of the potential isolates

After performing biochemical and morphological test for the selected isolates, identification of isolates at the species label was done. Table 4.6 below indicates the identification of potent isolates in their respective species level.

Table 11: Identification of potent bacteria isolates

<table>
<thead>
<tr>
<th>Test</th>
<th>SNB-3</th>
<th>SNB-11</th>
<th>SNB-22</th>
<th>SNB-26</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram reaction</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>_</td>
</tr>
<tr>
<td>Colony color</td>
<td>Light brown</td>
<td>Colorless</td>
<td>Golden yellow</td>
<td>Light yellow</td>
</tr>
<tr>
<td>Cell shape</td>
<td>Short rods</td>
<td>Coccus</td>
<td>Rod</td>
<td>Cocci</td>
</tr>
<tr>
<td>Cell arrangement</td>
<td>Single</td>
<td>Chain</td>
<td>Chain</td>
<td>Single</td>
</tr>
<tr>
<td>Spore staining</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Motility</td>
<td>+</td>
<td>_</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sulfur globules</td>
<td>outside the cell</td>
<td>outside the cell</td>
<td>Outside</td>
<td>Inside the cell</td>
</tr>
<tr>
<td>Catalase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Oxidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Sulfide oxidation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Geneus</td>
<td>Thiobacillus</td>
<td>Sulfbacillus</td>
<td>Thermothrix</td>
<td>Thiosphaera</td>
</tr>
</tbody>
</table>
4.2 DISCUSSION

4.2.1 Performance of anaerobic SBR reactor
Anaerobic SBR reactor showed high removal of organic matter, sulfur compound and nutrients as presented in the preceded results section 4.1.3. The system was running at environmental operating conditions of pH 8.38, EC 12 ms/cm, temperature of 20 °c, hydraulic retention time of 24h and sludge age of 15days.

4.2.1.1 Removal of organic matter in anaerobic reactor
The organic matter considered in tannery wastewater was COD and BOD$_5$. As shown in Figure 4.1 the initial concentration of COD & BOD$_5$ in the influent was 6275mg/L and 2005.8mg/L respectively. In anaerobic reactor their concentration was reduced to 2500mg/L and 900 mg/L with removal efficiency of 60.2 % & 55.1% respectively.

![Organic matter removal in anaerobic reactor](image)

Figure 4: Organic matter removal in anaerobic reactor

The main reason for good removal of COD and BOD$_5$ could be related to maintenance of optimum environmental conditions like temperature and pH required for anaerobic sulfate reducing, acetogenic and methanogenic bacteria. According to Metcalf and Eddy (1991), environmental factors that affect biological organic matter removal are pH and inhibitory substances. pH level less than 6.8 affects biological organic matter removal while pH around neutral makes enables optimum performance to occur. The current PH
Another factor could be related to the uptake of substantial amount of organic matter by methanogenic and sulfate reducing bacteria. Moreover, in anaerobic reactor sulfate, sulfite and nitrate are used as electron acceptor while the organic matter is used as carbon source.

According to Lens and Hulshoff (2000) COD: SO$_4^{2-}$ ratio > 0.67 is necessary for complete COD removal. In the present study, the ratio COD: SO$_4^{2-}$ was 2.90 which favor the uptake organic matter in the system.

The presence of Sulfide did not have toxicity effect on anaerobic bacteria and organic matter removal because the concentration was much lower than 150mg/l (Rinzema, 1988; Speece, 1983).

According to Brimblecombe et al. (1989) as pH rises above 7, more and more non-volatile sulfide ion (S$^{2-}$) will be present in the reactor. Therefore, at pH (8.39) of anaerobic reactor the available form of sulfide was S$^{2-}$ which does not have toxicity effect. Toxicity by sulphide is related with the undissociated (H$_2$S$_g$) form which can leak into the cell membrane (Rinzema and lettinga, 1988).

**4.2.1.2 Removal of sulfur compound in anaerobic reactor**

Forms of Sulfur in tannery effluent include S$^{2-}$ and SO$_4^{2-}$. As shown in figure 4.2, their initial concentration was 148 mg/L and 2162 mg/L respectively. At the end of anaerobic cycle their concentration reduced to 36 mg/L & 650 mg/L at the removal efficiency of 75.6% and 70% respectively.

Figure 5: Sulfur compound removal in anaerobic reactor
Sulfate reduction in anaerobic system could be related to the use of acetate and hydrogen by sulfate reducers which reduces sulfate to hydrogen sulfide. Like methanogens, some sulfate reducers are able to oxidize H\(_2\) and acetate and thus may compete with methanogens for these substrates (Rinzema and Lettinga, 1988).

Thermodynamic and Monod-kinetic data shown that sulfate reducer generally have higher growth rates and higher affinity for substrates than acetogenic and methanogenic bacteria. Therefore, sulfate reducing bacteria out-competes acetogenic and methanogenic bacteria (Oude Elferink et al., 1994). Factors like COD: SO\(_4^{2-}\) ratio, type of sludge, sludge retention, hydrogen sulphide inhibition, pH and nutrient limitation should be taken into account because it highly influence the outcome of competition between sulfate reducing and methanogenic bacteria (Lens et al., 1998).

It was expected that significant amount of sulfate would be reduced to sulfide in anaerobic environment but excess removal of sulfate couldn’t occur. The reason for this may be due to COD: SO\(_4\) ratio of 2.90 which favors methanogens than sulfate reducer. The presence of 155mg/L nitrate in the wastewater could be another factor because it may have inhibited sulfate to be used as electron donor which intern inhibits effective sulfate removal. According to (Ernst et al., 1993) under anaerobic condition strict anaerobic bacteria in the absence of nitrate, use sulfate as the terminal electron acceptor and low-molecular weight carbon source as, electron donors.

The reason for Sulfide removal in anaerobic reactor could be its use as sulfur source by anaerobic bacteria. Methanogenic bacteria use ammonia and sulfide as nitrogen and sulfur sources respectively. Although un-ionized sulfide is toxic to methanogens at level exceeding 150–200 mg/L (Rinzema and Lettinga, 1988; Speece, 1983), the concentration of sulfide in anaerobic SBR effluent was 36mg/L which was far lower than the limit. Therefore, it favored methanogens to use sulfide as sulfur source to synthesis of new biomass.

Another reason for sulfide reduction could be the conversion of sulfide to sulfur by sulfide oxidation under anaerobic condition. Oxidation is carried out by photoautotrophs and chemoautotrophs. Photosynthetic bacteria (Thiobacillus denitrificans) use H\(_2\)S as electron donor and oxidize H\(_2\)S to S and stored it within the cells of chromatiaceae (purple sulfur bacteria) or outside the cells of chlorobiaceae (green sulfur bacteria). Also filamentous sulfur bacteria (e.g.,
Beggiatoa, Thiothrix) carry out H$_2$S oxidation to S which is deposited in S granules (Ernst et al., 1993).

4.2.1.3 Removal of nutrients in anaerobic reactor

Wastewater must be nutritionally balanced (nitrogen, phosphorus, sulfur, etc.) to maintain adequate anaerobic digestion. Nutrient in tannery includes ($\text{NH}_4^+$, TP and NO$_3^-$) as mentioned in Figure 4.3 their initial concentration at the start of anaerobic reactor was 261.5mg/L, 13.75mg/L, 567mg/L respectively. At the end of anaerobic reaction the concentration of TP & NO$_3^-$ reduced to 10mg/L and 155 mg/l with removal efficiency of 27% &72.6% while the $\text{NH}_4^+$ increase to 616mg/L.

![Nutrient removal in anaerobic reactor](image)

Figure 6: Nutrient removal in anaerobic reactor

The increment of $\text{NH}_4^+$ concentrations was associated to anaerobic conversion of protein containing compounds and organic matter. According to (Gerardi, 2002) suitable temperature and pH range for organic matter degradation is 10-20°C and pH range of 7-8. 5. The anaerobic SBR reactor pH (8.38) and temp (20°C) was in agreement the above fact. NO$_3^-$ may be reduced to other gaseous nitrogen source or may-be used as electron acceptor with SO$_4^{2-}$ and SO$_3^{2-}$. The main reason for phosphorous removal from the reactor could be due to precipitation mechanism even though poly p bacterial adds phosphate source to the reactor phosphorous reduction was observed.
The hydraulic retention time (HRT), which depends on wastewater characteristics and environmental conditions, must be long enough to allow metabolism and nutrient uptake by anaerobic microorganisms in digesters. According to Polprasert (1989) digesters based on attached growth usually anaerobic reactor needs a lower HRT (1–10 days) than those based on dispersed growth usually aerobic reactor (10–60 days). Therefore, the current hydraulic retention time, one day, was in agreement with the above idea.

4.2.2 Oxic SBR condition performance
Oxic SBR reaction condition also showed high removal of sulfide, organic matter (BOD & COD), nutrients like total phosphorous and ammonium. The reactor was operating under environmental condition of pH 8, EC 12.5ms/cm, temperature 20 °C, and hydraulic retention times of 14h, dissolved oxygen of 6.5g/l and sludge age of 15 days.

4.2.2.1 Removal of sulfur compound in oxic condition
As shown in Figure 4.4 below the concentration of sulfide in oxic condition was 36mg/L and at the end of oxic condition the concentration was reduced to 8.7mg/L with removal efficiency of 75%.

Figure 7: Sulfur compound removal in oxic condition
The oxidation of sulfide by sulfide oxidizing bacteria to sulfate under sufficient dissolved oxygen (6.5mg/L) and suitable pH (8) could be related to removal sulfide in the reactor. This result agrees with different literature for good sulfide oxidation condition (Urban, 1981).

The initial concentration of sulfate in oxic condition was 650mg/L and at the end the reaction increased to 1110 mg/L because some amount of sulfide in oxic condition was transformed to elemental sulfur and further oxidized to sulfate. According to Milford et al. (2000) in oxic phase sulfide oxidized sequentially to elemental sulfur and sulfate by chemolithotrophic sulfur-oxidizing bacteria (SOB). Therefore, the increment of sulfate was related to the oxidation of sulfide to sulfate.

**4.2.2.2 Removal of Organic matter in oxic condition**

The initial concentration of BOD₅ & COD as shown in Figure 4.5 below was 900mg/L and 2500mg/L and at the end of oxic condition it was reduced to 393 mg/L & 587mg/L with removal efficiency of 56.3% and 76.5% respectively.

The removal of organic matter in oxic working condition could be related to being used as carbon source for nitrification, nutrient removal and for oxidation of sulfur compound under enough dissolved oxygen and other driven parameter like pH, EC.

![Organic matter removal in oxic condition](image.png)

**Figure 8: Organic matter removal in oxic condition**
The pH and the temperature range of the system enhance the removal efficiency of organic matter. In addition to that DO concentration (6.5mg/L) was sufficient enough for different oxidation process to occur in the reactor like nitrification, sulfide oxidation, organic matter and nutrient removal.

### 4.2.2.3 Removal of Nutrient in oxic condition

The concentration of NH$_4^+$, TP and NO$_3^-$ at the start of reaction was 616 mg/L, 10mg/L and 155 mg /L. At the end of oxic condition the concentration of NH$_4^+$, TP were reduced to 468 mg/L and 6.2mg/L with removal efficiency of 24% and 31% respectively while the concentration of NO$_3^-$ increase to 300mg/L.

![Nutrient removal in oxic condition](image)

Figure 9: Nutrient removal in oxic condition

Since the oxic condition contains poly-6 bacteria, which uptake the soluble phosphorus, the removal of total phosphorous was observed in the system.

In the case of ammonium oxidation the system did not show good nitrification, this could be related to many factors. The COD loading rate is one of basic factor which affect nitrification since heterotrophic bacteria deplete the level of dissolved oxygen when they degrade organic matter which in turn suppress the nitrifying autotrophic bacteria. According to Wild *et al.* (1971), COD levels up to 60-80mg/L can be tolerated by nitrifying bacteria. However, COD level above
60mg/L, can lead to 50% nitrification reduction. In the current study of oxic working condition, the level of COD was 400mg/L which was above the tolerable limit of nitrifying bacteria, hence this may have led to low nitrification process.

Also, the presence of chromium in wastewater affects the nitrification and denitrification at high level (Farabegol et al., 2007). The activity of denitrifying bacteria was not inhibited up to a chromium concentration of 180 mg/l, while inhibition of nitrifying bacteria began at a chromium concentration of 120 mg/l (Farabegol et al., 2007). According to Andualem Mekonnen (2008) the analyzed Cr concentration of Modjo tannery effluent using SBR was 10.2mg/l where it is far lower to affect both nitrification and denitrification.

According to (US EPA, 1975) the optimum pH for *Nitrosomonas* and *Nitrobacter* lies between 7.5 and 8.5 with little to no nitrification occur below approximately pH 6 to 6.5 or above 10 (Painter, 1970; Painter and Loveless, 1983). Generally, in oxic reaction condition of SBR pH, temperature and chromium concentration was within normal conditions that could not affect nitrification, therefore, insufficient HRT, aeration and high organic loading could contribute for less efficiency of nitrification. The concentration of NO$_3^-$ was increased because in oxic condition the ammonium was oxidized to NO$_3^-$.

**4.2.3 Anoxic SBR condition performance**

The performance of anoxic reactor for the transformation of pollutant was evaluated at the working environmental condition of temperature 20°C, EC 11.9ms/cm, pH 8.73, HRT of 10hrs. The reactor showed high removal of organic matter and nutrients. The reduction of nitrate and sulfide was mainly due to the presence of potent microorganism in the system.

**4.2.3.1 Removal of sulfur compounds in anoxic condition**

The reactor showed high removal of sulfide. The sulfide concentration at the start of anoxic condition was 8.7mg/L and at the end of the reaction reduced to 1.4mg/L with removal efficiency of 83.9%.
Figure 10: Sulfide removal in anoxic condition

The reduction in concentration could be related to the conversion of sulfide to elemental sulfur by chemotrophic bacteria that use sulfide as electron donor and nitrate as electron acceptor. The bacteria in anoxic system have high efficiency to remove sulfide and nitrate as mentioned in the result part. *Thiosphaera* sp. (SNB-26) bacterial have sulfide removal efficiency of 98.1% within an hour. Therefore, the highest removal of sulfide could be related to the abundance of this bacterial in the system.

The concentration of sulfate in oxic working condition was 1110mg/L, but during anoxic working condition this concentration reduced to 725mg/L with removal efficiency of 34.6%, therefore, the observed removal efficiency showed that anoxic working condition reduce higher amount of sulfate which increased in oxic condition.

Figure 11: Sulfate removal in anoxic condition
4.2.3.2. Removal of nutrients

The concentration of nutrients (TN, TP and NH$_4^+$) at the end of oxic reactor was 400mg/L, 6.2mg/L, and 468mg/L respectively. In anoxic reaction the above concentration reduced to 303mg/L, 5.1mg/L and 620mg/L respectively. The removal efficiency of TN & TP was 24.3% and 17.7% which indicates further nutrient removal in anoxic condition.

![Nutrient removal in anoxic condition](image)

Figure 12: Nutrient removal in anoxic condition

The removal of phosphorus can be carried out either under anoxic or oxic conditions by phosphorus accumulating organism by utilizing nitrates or oxygen as final electron acceptor (Dae et al., 2001; Janssen et al., 2002). Thus, the observed removal of total phosphorus both in the anoxic and oxic phase of the system might be attributed to uptake by phosphorus removing bacteria and phosphorus precipitation (Jens et al., 1999).

The concentration of NH$_4^+$ was increased by 152mg/L from oxic reactor. The reason for the increment of ammonium could be related to inefficient nitrification in oxic reaction condition. So that, high concentration of ammonium was added to anoxic system where further nitrogen containing organic compound degradation in anoxic condition by proteolytic bacteria abundant in anoxic SBR. Hence SBR system is used as secondary treatment; it needs further polishing systems like constructed wetlands.
4.2.3.2. Removal of nitrate

The concentration of nitrate in anoxic condition was 300mg/L and reduced to 105mg/L at the end of the reaction with removal efficiency of 65%.

![Nitrate removal in anoxic condition](image)

Figure 13: Nitrate removal in anoxic condition

In anoxic system nitrate was converted to gaseous nitrogen by denitrifying bacteria with optimum temperature and other driven parameters. In wastewater, denitrification is most effective at pH values between 7.0 and 8.5 and the optimum is around 7.0 (Metcalf and Eddy, 1991). Denitrification favors a temperature range of 35°C –50°C. It also occur with the temperature range of (5–10°C) at a slower rate. Therefore, the environmental condition of anoxic condition favors the removal of NO₃⁻. The abundance of high efficient denitrifying bacteria in anoxic system could be directly related to the removal efficiency of the system. As mentioned in the result part 4.5 *Thermothrix* sp. (SNB-22) & *Thiosphaera* sp. (SNB 26) have nitrate removal efficiency of 90 and 81.4% within two hours, respectively.

4.2.3.3. Removal of organic matter

The concentrations of organic matter (BOD₅ & COD) in oxic working condition were 393mg/L and 587mg/L, and in anoxic condition the concentration reduced to 300mg/l and 397mg/L respectively with removal efficiency of 24.4% & 32.3%.
The component of organic compound (COD & BOD$_5$) and nutrients that were partially reduced in oxic condition were further degraded in anoxic reactor. The organic matters (BOD$_5$ & COD) in anoxic reactor were used as a carbon source; therefore, the reduction in concentration was related to it.

4.2.4.1 Overall performance of sequencing batch reactors

The overall removal efficiency of SBR was the cumulative effort of biological and physico-chemical reaction undertaken in each reactor. As compared to all analyzed parameters, the SBR system showed high removal efficiency of sulfide (99.73), similarly most parameters also showed significant removal efficiency of pollutant which meets both WHO & EEPA standard of wastewater discharge limit. The test done by ANOVA also indicated that all pollutants showed p value < 0.05 which showed, there is significant difference between each parameters from one reactor to the other.

The removal efficiency of parameters in each reactor were depend on the driven working condition parameter which was pH, ROP, DO, EC, temperature, HRT & Sludge age inoculums concentration, type of organic matter.
The system showed 99.73% removal of sulfide and similar result was also obtained by Andualem Mekonnen (2008), 99.9% at the same working condition except HRT was 24h cycle & number of reactor was one. The result of sulfide removal is almost similar with the results presented in literature using different biological methods. Chung et al. (1997) reported 99.6% sulfide removal efficiencies using biofilters and cha et al. (1999) reported 100% sulfide removal using *Thiobacillus novellus* in biofilter under mix trophic condition.

The overall organic matter removal of BOD$_5$ and COD was 86.77% & 95.5 %, respectively. Farabegoli et al., 2007 obtained 67% COD removal from treatment of tannery wastewater using laboratory scale SBR with different HRT (6-h cycle), one reactor and with larger S$^{2-}$(564mg/l) concentration. Hajiabadi et al., 2009 obtained 94.99% removal of COD with a single reactor at SRT of 5 days. The difference in the above result may be due to the difference in HRT, number of reactor and sulfide concentration.

The overall nutrient removal of SBR was NO$_3^-$ (83.19%), SO$_4^{2-}$ (64.5 %), TN (86.9%) and (TP 66.1 %). The result obtained by Andualem Mekonnen (2008), SBR with one reactor and with similar environmental condition with less HRT which was 24h cycle has lower (49%) & TP (54.3 %) removal efficiency than the current study. Insufficient removal of NH$_4^+$ occurred as the concentration was increased by 22.3% from the influent to effluent the main reason for that could be high COD loading rate and inefficient HRT. Solid particle removal of SBR was TSS (90%), TS (61.3%) and TDS (57.39%) where TSS had larger removal efficiency than the others. This could directly related to the enough settling and decant time of sequencing batch reactor as well as much of the solid part of the tannery effluent were degrade during the reaction.

The control factors such as; pH, EC & temperature were almost similar both reactors but only slight change prevailed from one reactor to the other based on the reaction undertaken in each reactor.
CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

Based on the results obtained, the following points are concluded

Tannery industry is highly pollution intensive industry with high pollution load of many parameters. The pollution load of the selected parameters is above the discharge limit set by environmental protection authority for instant: BOD$_5$ $10\times$, COD $12.5\times$, NH$_3$ $8.7\times$, TN $12.7\times$, SS$\times 43.1$, S$^{2-}$ $148\times$, are above the discharge limit.

Sequencing batch reactor (SBR) is best alternative biological wastewater treatment system to overcome the environment impact of tannery effluent with great sulfide removal efficiency of up to 99.57%.

Isolate 26 and 22 have high sulfide oxidation and nitrate reduction efficiency. When they are bioaugmented to anoxic wastewater, isolate 26 showed removal efficiency of 98.1% and 81.4% removal for sulfide and nitrate. Whereas isolate 22 showed 90% and 57% removal for nitrate and sulfide, respectively.

The Sequencing batch reactor (SBR) does not remove ammonium from the influent because its concentration was increased from the influent while the effluent leaves the SBR.

5.2 RECOMMENDATIONS

Based on the study conducted the following points are recommended

1. Further molecular study required to identify isolate 22 and 26

2. The concentration of ammonium in the effluent was above the influent and the discharge limits set by National Environmental Quality Standard for tannery effluent. Therefore, further studies should be conducted to determine the optimum condition for ammonium removal from tannery wastewater.
3. Leather tanning industries can use SBR at large scale to reduce their pollutant load to the receiving environment.
REFERENCES


Ababa. pp 46.


system for biological nutrient removal on small wastewater treatment plants in Korea.


UNIDO. (2000). Pollutants in Tannery Effluents. Regional Program for Pollution Control in the
Tanning Industry in South-East Asia. UNIDO. pp 362-370.


Annex 1

The mean performance of each reactor (Anaerobic and Aerobic SBRs)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Influent</th>
<th>Treated effluent</th>
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<tr>
<td></td>
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<td>Anoxic</td>
</tr>
<tr>
<td>$\bar{S}^2-$</td>
<td>148.50±6.18</td>
<td>36±4.63</td>
<td>8.7±3.63</td>
</tr>
<tr>
<td>$\text{SO}_4^{2-}$</td>
<td>2162.50±368.20</td>
<td>650±202</td>
<td>1110±230</td>
</tr>
<tr>
<td>$\text{NO}_3^{-}$</td>
<td>567.00±224.36</td>
<td>155±43</td>
<td>300±57.7</td>
</tr>
</tbody>
</table>
| $\text{NH}_4^+$ | 261.50±68.51 | 616±117.8 | 468±96.9 | 627±129. | 328.33± 48.67 | 0%
| TN | 766.00±50.84 | 579±67.4 | 400±33.2 | 303±15.3 | 100.33± 17.32 | 86.9% |
| $\text{BOD}_5$ | 2005.80±579.17 | 900±200 | 393±95.7 | 300±2.72 | 266.00± 43.47 | 86.77% |
| COD | 6275.00±1536.9 | 2500±726. | 587±233. | 397±34.4 | 276.67± 47.02 | 95.5% |
| TP | 13.75±6.12 | 10±.57 | 6.2±.61 | 5.1±.58 | 4.66 ± 0.89 | 66.1% |
| TSS | 2155.00±61.31 | 1330±100 | 453±17.8 | 210±6.84 | 199.67±50.33 | 90.7% |
| TDS | 7035.00±42.52 | 5390±129. | 3677±74 | 2900±37 | 2899.70±288.6 | 58.7% |
| TS | 9175.0±65.00 | 6240±551 | 3045±440 | 2967± | 2895.30 | 68.4% |
Annex 2: EEPA Tanning and leather finishing discharge limit values for receiving water bodies.

<table>
<thead>
<tr>
<th>Parameter</th>
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<tr>
<td>pH</td>
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<td>$\text{BOD}_5$ at 20(^\circ)C</td>
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<td>COD</td>
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<tr>
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</tr>
<tr>
<td>Total ammonia (as N)</td>
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<tr>
<td>Total nitrogen (as N)</td>
<td>80% removal or 60 mg/l, whichever is less</td>
</tr>
<tr>
<td>Total phosphorus (as P)</td>
<td>80% removal or 10 mg/l, whichever is less</td>
</tr>
<tr>
<td>Oils, fats, and grease</td>
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</tr>
<tr>
<td>Mineral oils at oil trap or interceptors</td>
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</tr>
<tr>
<td>Chromium (as total Cr)</td>
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Annex 3: Potential NR-SOB isolates of 3,11,22,26

Isolate 3

Isolate 11

Isolate 26

Isolate22
Annex 4: Biochemical testes of Nitrate reduction test
Annex 5: pH Test
Annex 6: Cytochrome oxidation test
Annex 7: Test result of ANOVA with level of significant of 0.05

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