SPECIES COMPOSITION OF SULFUR OXIDIZING AND DENITRIFYING BACTERIA FROM THE TANNERY WASTEWATER TREATMENT PLANT, MODJO, ETHIOPIA

A THESIS PRESENTED TO THE

SCHOOL OF GRADUATE STUDIES

ADDIS ABABA UNIVERSITY

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS OF

THE DEGREE OF MASTER OF SCIENCE IN BIOLOGY

(APPLIED MICROBIOLOGY)

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March, 2000

ACKNOWLEDGMENTS

I wish to express my deepest gratitude and appreciation to my advisor Dr. Fassil Assefa with out whose initiatives, encouragement and support, this work would not have been possibly completed.

I am also deeply grateful to Ato Issayas Tadesse, head of the research on the low cost Tannery Effluent Treatment plant at Modjo. His unreserved support during sample collection from Modjo Tannery plant was highly appreciated. Special thanks would also go to Professor Berhanu Abegaz Gashe for sending me important and relevant literature of the work from Gaborone, Botswana. I am greatly indebted to Dr. Masresha Fetene and His Wife Solome Bekele for their pertinent help and encouragement throughout the study.

My greatest appreciation and full thanks must go to my mother W/ro Abebech Kefelegn, family members W/ro Taytu Yirdaw, Ato Cheru Affessa, Anteneh Tesfaye, Mamo Kibret, my sisters: W/t Shewareged Bekele, Hirut Bekele, Woinshet Bekele and others for their encouragement to enroll in this program.

I am highly indebted to W/ro Desta Mekonnen, Lt/Col Teshome Ayallew, W/ro Yigardu G/Michaeal, W/t Mulu G/ micheal and W/t Sindu G/ Michaeal for their unreserved support to my family throughout the study.

Special thanks must deserve to my friends indeed, Ato Getachew Mammed, Dr. Teshome Yizengaw, Ato Fikremariam W/Yohannes, Ato seid Ahmed, Ato Getahun Molla, Ato Leul Teka, Ato Berhanu Gebre W/ro Wolella Berhanu, Ato Ali Abate and the staff members of Hottie comp Secondary School. My brothers Birhanu Askenachew and Seifu G/ Yes are highly appreciated for their courage, help and advice till the completion of the study. I am also much greatful to W/t Elizabeth Bekele for typing the manuscript, W/ro Belaynesh, W/o Mulualem, W/ro Almaz W/t Banchie and Yemisirach for their encouragement and help in their part for the completion of the program.

I have to Acknowledge greatly my graduate friends, and their family: Ahmed Idris and his wife, W/o Yalemzerf Yimenu for their unreserved courage and hospitality; Driba Muleta, Bekele Bahiru, and Azage Hassen and others for their advice, courage and sharing of experiences and special thanks must also be given to Ato Nigussie and Ato Admassu for material supply and advice while working in the microbiology laboratory.

My great appreciation and special thanks must go to Ato Tilahun T/Haymanot, Ato Amsalu Ayana, and Ato Gashaw Mammo for their unreserved help and advice in every aspect of the work.

Finally, I extend special thanks to the Biotechnology Research Project in the Biology Department for the financial support for this work

Above all, I thank God for helping me endure the rigours of every day life and to overcome the challenges of graduate studies.

DEDICATION

This work is dedicated:

To my wife ***Tsigereda G/ Micheal***

To my children ***Friezer, Bisrat and Biruktawit Sissay***

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ABSTRACT

The discharge of tannery effluents to different water bodies increase pollution because of heavy accumulation of toxic compounds such as sulfur and nitrogen containing organic wastes, chromium, arsenic etc. Thirty-six strains of Sulfur Oxidizing Bacteria (SOB) belonging to sixteen genera were isolated from the three tannery ponds of a Tannery Waste Treatment system at Modjo. They were characterized on the basis of their cultural, morphological, and physiological characters. The genera were *Acidiphilium, Acromatium, Macromonas, Sulfolobus, Thermothrix, Thiobacillus, Thiobacterium, Thiomicrospira, Thiosphaera, Thiospira, Thiovulum Rhodobactor, Rhodocyclus, Rhodomicrobium, Rhodopseudomonas,* and *Rhodosprillum.* Out of these isolates, twenty-one strains falling into twelve genera were capable of denitrification. Bacterial enumeration based on MPN technique showed a population number between 1.2 x 10^3 and 1.1x10^6. The number of strains recovered from the different ponds varies such that twenty, sixteen, and nine strains were isolated from AFP, SFP, and MP, respectively. The dominant strains isolated from different pond systems were the genera *Thiobacillus* (8 strains), *Rhodopseudomonas*, (4 strains), and *Rhodomicrobium* (3 strains). The most frequently isolated strains in the three different pond systems were *Thiobacillus* (AU59) and *Rhodomicrobium* (AU142). Isolated strains showed variation in growth on different media and some such as *Acidiphilium* (AU8); *Sulfolobus* (AU12); *Thiobacillus* (AU59) *Thiomicrospira* (AU62); *Thiosphaera* (AU57) *Rhodomicrobium* (AU142, AU164, AU176) failed to grow on denitrifying medium but were capable of growing on nitrogen free (nif) medium. Most of the isolates were found to be slow growers reaching to their exponential stage between 48-72 hours. In their spatial distribution, the anaerobic strains in AFP system and the aerobic isolates in the SFP and MP systems were found to be dominant.

1. INTRODUCTION

In the present day society, because of urbanization and large consumption of a variety of products, there is an increase in the discharge of more neutral and toxic wastes in the form of municipal refuse, sewage sludge, agricultural residues, toxic and chemically reactive byproducts of manufacturing processes to the environment (Manahan, 1990; Harrison, 1995).

Industrial wastes are the major causes of environmental pollution by producing huge tonnage of xenobiotic and other anthropogenic compounds, with pollutional effect much higher than municipal sewage and other sanitary and domestic wastes (Gurnham, 1965 and Hamer *et al.*, 1985). The tannery industry is one of the manufacturing units that utilizes chemicals mixed with sulfides, sulfhydrates, amines, chrome etc. for processing of hides and skins into finished leather (O'flaherty, 1965; Harrison, 1995). The effluent from the tanning process is also rich in nitrogen-containing organic substances, and is disposed off as discharge to sewer, river, estuary, sea and underground acquifers.

The combined tannery effluent, discharged to the surrounding environment contains compounds such as hydrogen sulfide (H_2S), a toxic volatile reduced sulfur compound, ammonia (NH_3^-), a volatile, irritating (offensive) odorous gas, and Nitrate (NO_3^-), a highly pollutant and carcinogenic compound. These pollutants are highly toxic and may induce health hazards. (Alexander, 1981; Hamer *et al.,* 1985 and Harrison, 1995).

In order to do away with the dangers of these toxic chemicals, different methods are employed to treat waste effluents. The application of conventional technologies such as chemical precipitation treatment, balancing tank and isolation of drainage lines, however, are found to be poor for waste water treatment of the tannery effluent (Petruzzelli *et al.,* 1994 and Harrison, 1995). This problem, therefore, necessitates the search for an acceptable method of treating tannery effluent using a treatment pond system of wastewater involving microorganisms (Hamer *et al.*, 1985).

An integrated activity of various microorganisms such as aerobic and anaerobic bacteria of phototrophic sulfur oxidizing, sulfate reducing, denitrifying groups are responsible for the degradation of tannery wastes into simple and inert chemical compounds (Vishniac and Santer, 1957; Silverman and Ehrlich, 1964; Alexander, 1974; Paul and Clark, 1989; Atlas and Bartha, 1993).

In Ethiopia, the biological industrial wastewater treatment has not been given due attention and leather industries are established near rivers to discharge the effluent at will. Recently, because of environmental concerns, researches on wastewater treatments have been started. Preliminary observations on one tannery effluent of the country have shown that a large spectrum of microorganisms and macroorganisms are involved in the break down processes in a low cost tannery effluent waste treatment plant (Issayas Taddesse, 1999).

Although the screening has achieved to enumerate some of the micro and macroorganisms, an understanding of the biodiversity of sulfur oxidizing and denitrifying bacteria that are responsible

for the detoxification of sulfur and nitrogen containing tannery effluent has not been attempted. In order to get the true picture of the nature of the degradation processes of any treatment plant, it is essential to have information of the microorganisms and their efficiency in the detoxification process of a system.

In general, the knowledge of the spectrum of microorganisms of these industrial wastes is also important in the integrated management of waste treatment and the production of useful products such as biofertilizer and biogas. These products can be supplementary sources of fertilizers and energy in view of the ever-increasing consumption of non-renewable fossil fuels and the steep rise in the cost of chemical fertilizers.

The objective of the present work, therefore, is;

OBJECTIVES

1 To investigate the physiological diversity of sulfur oxidizing (SOB) and denitrifying (DEN) bacteria in a Tannery Waste Treatment Plant.

2 To estimate the number and evaluate the spatial distribution of these microorganisms in the different ponds of the Treatment Plant.

2. LITERATURE REVIEW

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

The discharge of high organic matter wastes with out any treatment in waterbodies, results in a high rate of biodegradation with the consumption of high dissolved oxygen (DO) that creates anoxic conditions with the concomitant formation of bad odor and toxic products. This affects the aquatic organisms and stimulates high primary productivity with algal bloom. The condition, together with the high biomass of aerobic microorganisms and the production of organic acids by anaerobic organisms, brings asphyxation and fish kill in the waterbodies (Holsen *et al*, 1991 and Henze *et al.*, 1995).

Synthetic and xenobiotic wastes on the other hand contain highly recalcitrant chemicals which are resistant to biodegradation. These wastes settle as sludge in the water-bed and affect the

methanogenic and other anaerobic bacteria that are sensitive to slight environmental changes in the benthic region (Klute and Han, 1994). Inorganic wastes that include hazardous heavy metals and salts may exist in the effluent as dissolved and suspended particulate matter and settlable bottom deposits. These substances are difficult to eliminate and persist for a long time to have a long-lasting environmental effect on the ecosystem and are a source of carcinogenic materials to human beings (Taber, 1976).

2.1. CHARACTERISTICS OF MAJOR INDUSTRIAL WASTES

Industrial wastes can be categorized into two major classes on the basis of their discharge, BOD content i.e. low BOD and high BOD wastes (Table 1).

Table 1. The BOD and major waste products of different industries

Industry	BOD mg/l	Major waste products	Environmental effect	References
Pulp and paper	600	- organic wastes, fibers, cellulose, lignin rich in carbon - suspended solids	- algal bloom, fish kill, toxic, sludge deposition &leaching	(Gurnham, 1965)
Sugar cane and sugar beet refineries	8157	-organic waste rich in carbon	- algal bloom fish kill	(Gurnham ,1965)
Fruit and vegetable canneries	800 - 5000	"	"	(Henze *et al.*, 1995)
Dairy and Slaughterhouses industries	2000	" "	- strong smell - aesthetic inconveniences	" "
Textile Industry	1500	- fibers & high concentration. of soluble in organic & organic wastes, dyes, bleaching agents	- corrosive & - aesthetic inconveniences	"
Leather Tanning Industry	100 - 10000	- sulfur containing nitrogen wastes; chromium, arsenic salts	- corrosive - toxic, carcinogenic	(Harrison, 1995) (Henze *et al.*, 1995)
Petrochemical Industry	> 100	- oil spills, chlorinated H-carbons - PCB - Heavy metals	- in water body protect light entry, recalcitrant, and carcinogenic/toxic	(Gurnham, 1965)
Plastic Industry	about 6	- antioxidants,reagents, vinyl chlorides; heavy metals; polyethylene, dyes.	- carcinogenic	(Young & Cerilegia 1995)
Pesticide Industry	?	- recalcitrant and toxic	Toxic effect aesthetic inconvenience and bio accumulation	(Henze *et al.*, 1995)
Paint Industry	?	- heavy metals, - dyes - chlorinated hydrocarbons	- toxic/ carcinogenic -leached to under ground water bodies	"
Metal Industry	?	-Heavy metals	- toxic/ carcinogenic -leached to under ground water bodies	(Harrison, 1995)

2.1.1. Low BOD wastes

Industries under this category discharge their wastes in the effluent as dissolved mineral content and/ or recalicitrant hazardous chemicals and heavy metals. These wastes are discharged in a continuous flow to the water bodies and soil. Heavy metals and other hazardous chemicals markedly affect the toxicity of salts to fish and other living organisms in the aquatic ecosystem (Young and Cerniglia, 1995). The toxicity of the heavy metals such as arsenic (As), Cadmium (Cd), chromium (Cr.), lead (Pb), Mercury (Hg), Nickel (Ni), Copper (Cu) and Zinc (Zn) is severe when leached and get into water bodies or acquifers Lester and Sterritt, (1985). Polychlorinated Bipheniles (PCBs), tri and tetra chloroethylene etc. are recalcitrant compounds that are released through petrochemical waste sludge to the environment (D'Itri and Kamrin, 1983; Safe, 1995 and Eastaldi and Ford, 1995).

The following are lists of industries that are known to discharge hazardous and recalcitrant heavy metals in to water bodies and acquifers.

i. Plastic Industry

It is one of the consumers of petrochemical industry products such as antioxidants, plasticizers, reagents and other bulk raw materials: Styrene, vinylchloride, aniline and solvents like toluene, benzene and methanol (Young and Cerniglia, 1995). The plastic industry wastewater contains

much of recalcitrant pollutants, hazardous chemicals, dyes and heavy metals and is characterized by low BOD content (Table 1).

ii. Paint Industry

Chemicals used in paint formulation such as solvents (xylene, toluene methyl, ethyl ketone, isobutyl ketone) and preservatives (Mercury-containing compounds, chlorinated phenolies and formaldehyde releasing chemicals) are very important wastewater compositions. They are biocides with low BOD in water bodies (Young and Cerniglia 1995).

iii. Metal Industry

The source of wastes in metal processing industry are numerous and also extremely variable both in quantity and quality. Discharges of metals to the aquatic environment have been a major cause of concern (Mattsby-Baltzer *et al.* 1989). The heavy metals; Lead, mercury, copper, zinc etc. are toxic and pollute underground waters and aquifers (Harrison, 1995).

iv. Petrochemical Industry: -

Petrochemical is a source of almost all of the chemical production as well as the largest producer of waste by volume. It produces basic chemicals such as vinyl chlorides, benzene, aniline,

naphthalene, phthalates etc, which serve as precursor in a number of other industries that produce solvents, pesticides, aluminum, synthetic rubber and plastics (Finstein & Morris, 1975). Wastewaters from petrochemical industry contain oils, chemicals including acids, alkalis, sulfides, mercaptans, ammonia, phenols, and suspended solids (Gurnham, 1965). The BOD of these wastes is below 100g O_{2/m^3}

V. Cosmetic Industry and Medical/ Pharmaceutical Industry

A large number of active ingredients are used in the general sector of cosmetics and drug industries. Thus, it is difficult to classify compounds as they are numerous and variable in nature. The active ingredients, preservative in cosmetic products and/ or drugs are the most important classes of compounds, which are rich source of pollution (Smolinske, 1995).

vi. Pesticide Industry

It is the most important consumer of petrochemical products and producer of organic wastes that include, reactants, solvents, intermediate and final products such as DDT, malathion, methyl-parathion, aldrin, dinoseb, dichlone etc. The pesticide Industry wastewater contains very commonly organophosphrous compounds (organo-phosphate, urea, carbamates etc.) that cause algal bloom and fish kill. The chlorinated pesticides are resistant to microbial attack and are

recalcitrant toxic compounds that may be biomagnified in food-chain relationship in the ecosystem, (Wilson *et al.*, 1985 and Kennedy *et al.*, 1990).

2.1.2. High BOD Wastes

Wastes from sugar cane and sugar beet refineries, fruit and vegetable canneries, dairy industries, slaughter house wastewater, pulp and paper, textile, leather tanning industries are known for the production of high organic load (BOD) wastes (Gurnham, 1965; Henze *et al.,* 1995).

i. Sugar cane and Sugar Beet Refineries

Sugar is extracted from two principal raw materials: Sugar cane and sugar beets. The principal uses are in soft drinks, bakery, cereal products; canned and frozen foods; confectionery; ice cream and dairy products. Industrial processing of sugar cane/ beet produces solid and liquid wastes. These wastes are characterized by one of the highest BOD value that is associated with carbon rich nature of the wastewater. The liquid waste also contains the soda and acid wastes such as NaOH, NaCl and HCl, which are toxic to living organisms in the system.

ii. Fruit and Vegetable Canneries

Waste materials that originate in fruit and vegetable canning operations fall into two general classes, solid materials, such as trimmings, pits peels, leaves, stems, and defective whole pieces, that are discarded within the plant; and liquid materials consisting of sugars, starches, and other carbohydrates leached from fruits and vegetables. Wastewater leaving the canning plant contains organic matter with the highest BOD next to that of the sugar processing waste. However, BOD contents are variable depending upon individual canning operations.

iii. Dairy and slaughterhouse industries

The processing and manufacturing of dairy products is perhaps the most wide spread of all industries in the world. It is processed into fluid products such as homogenized pasteurized milk for immediate consumption or is manufactured into dry milk powder, cheese, and other less perishable products that may be stored. Wastewaters from slaughterhouses vary depending on facilities, type of animal slaughtered and degree of mechanization of operation. Wastewater is generated in the slaughter area during production and during the clean up operations following production. Both dairy and slaughterhouse wastes are of equal magnitude in BOD but differ in their effect when they are discharged in to water bodies.

iv. Pulp and Textile industries.

The major stream pollution problems arising from the discharge of pulp, paper and textile processing wastewaters contain suspended matter and dissolved organic substances. The discharge of highly dispersed solids such as fiber debris, filler, and coating materials can render a stream opalescent which retards self purification by limiting light penetration (Gurnham, 1965). Dissolved solids such as wood and cotton sugars, deplete the dissolved oxygen and stimulate the growth of slime organisms, causing biological imbalance to occur. Table 1 shows the BOD of the pulp and textile industry wastes in comparison with other industrial wastes. The application of bleaching chemicals containing sulfur compounds in the pulp and paper industries and the incorporation of inorganic salts, heavy metals (Cr, Zn) and dyes in the textile industries are also incriminated with an increase in the pollution load of the effluent (Henze *et al.,* 1995).

v. Leather Tanning Industry

The tannery wastes are usually highly colored and alkaline water borne effluents formed by the continuous operations and discharge into water bodies. The effluent contains small bits of skin, tissues, soluble proteins, hair, blood dirt or filth, manure, salts of many kinds all mixed with sulfides, sulfhydrates, amines, chrome, arsenic and vegetable tanning agents such as sugars, starches, oils and fats, acids, alkalis, dyes, and pigments that considerably increase its pollutional character (O'flaherty, 1965; Harrison, 1995).

Tannery wastes like any other wastes are evaluated for their pollutional characters by the following parameters: BOD; total, dissolved, settlable and suspended solids; pH; free mineral acidity; color and turbidity; chlorides; sulfides; kjeldahl nitrogen; lipids; and tastes and odor. Biological Oxygen Demand (BOD) is usually the highest of all industrial wastes.

Poorly treated effluents cause sludge deposits in the receiving stream or sewer and their settlable solids vary from 7-12% (Gurnham, 1965; Henze *et al.,* 1995; Harrison, 1995). The pH of the effluent varies between high acidity to high alkalinity. This pH is adjusted by the addition of alkali, which is usually in excess, and the resultant pH is alkaline in the range from 8 to 11. The pickle liquor (Sulfuric acid and sodium chloride) has a pH of 2 or less. Chrome liquors are of pH 3-5, vegetable liquors vary from 3 to 6, and the lime liquor, which contains sulfide and amines, may be as high as a pH of 13 (Gurnham, 1965).

The mixing of all tannery discharges into a single system is the most practical and economic method for the control of BOD, solids, and pH. Turbidity, in tannery waste is caused by many of the constituents in the effluent and indirectly associated with the color, which varies from a dirty brown to green, blue, and a milky appearance. The most serious aspect of turbidity is the attenuation of sunlight and its effect on aquatic life.

Toxicity of the tannery waste

The toxicity of tannery waste is very closely associated with the presence of arsenic, chromium, sulfides with their reducing action, high alkalinity, amines, sodium chloride, soda, ash (McEldowney *et al.,* 1993; klute and Hahn, 1994). These wastes cause mal-odors and are toxic to aquatic and terrestrial organisms. The total mal-odors are also contributed by the decomposition of organic matter and formation of hydrogen sulfide from a mixture of sulfides and acids. The H_2S gas is a colorless gas toxic to aquatic and land organisms living around. Inhalation of H_2S (1000 ppm) kills faster than even HCN, in air. The H_2S emission into the atmosphere reacts with O_2 and forms SO_2, a green house gas that may bring global warming effect (Harrison, 1995). The SO_2 and NO_2 gases, by means of oxidation reacting with H_2O produce sulfuric and nitric acid respectively that create acid rain problem. Acid rain kills fish and any other aquatic life by acidifying lake waters and leach minerals deep into the soil (Paul and Clark, 1989; Brombacher *et al.,* 1998). The SO_2 produced is also known to cause damage to respiratory function of human and other organisms.

Chromium Cr (III) is a common contaminant at hazardous waste sites. In acidic aqueous solution it exists as soluble dichromate, $Cr_2O_7^{2-}$, and in more basic solution as chromate anion, CrO_4^{2-}. The basic chromium (III) sulfate, that is used for some leather tanning is transformed to chromium (VI) by means of photo oxidation upon discharge in the environment and forms compounds *carcinogenic* to humans, (Manahan, 1991).

The nitrogenous organic compounds increase the BOD of the effluent and of its toxicity by favoring the rapid growth of pathogenic microorganisms. The decomposition of nitrogen containing organic compounds produce nitrate (NO_3^-), which is the heavy pollutant of water bodies, soil, and air upon conversion to nitrite (NO_2^-) by the enzyme nitrate reductase (Glidwell, 1990). Nitrite potentially poses carcinogenic and *methaemoglobinaemia* (blue baby syndrome) health hazards (McEldowney *et al.,* 1993; Harrison, 1995).

2.2. Microbiology of Sulphur oxidation, Desulphurication and Denitrification in the Tannery Waste Treatment System.

The tannery wastes are usually highly colored and alkaline containing high nitrogenous organic matter with an increased BOD and other compounds. The heterogeneous microbial consortium of bacteria, fungi, algae, protozoa, rotifiers etc. decomposes (mineralize) the organic nutrient and other inorganic substances in the pond system to reduce the BOD of the effluent and of its pollutional character. Of all microorganisms, the bacteria are the most versatile (functionally diversified) group capable of autotrophic, heterotrophic and facultative mode of nutrition.

The sulfur oxidizing and denitrifying bacteria are the dominant groups that detoxify and mineralize the toxic reduced sulfur (H_2S), nitrate (NO_3^-) and decompose organic matter in the effluent (Henze, *et al.*, 1995). The functional group of sulfur oxidizing bacteria comprises of:

1. The anoxygenic phototrophic and the oxygenic chemolithotropic groups that oxidize reduced sulfur to elemental and sulfate (SO_4^{2-}) forms, respectively (Staley *et al.*, 1989). The anoxygenic phototrophic bacteria include the bacteria *Chromatium, Thiocystis* (Paul and Clark, 1989; Atlas and Bartha, 1993); The sulfur bacteria: *Chlorobcum, Pelidictyon* (Staley *et al.*, 1989) and the non - sulfur bacteria: *Rhodospirillum, Rhodopseudomonas* (Singleton, 1995) that inhabit the anaerobic environment (raw sewage, ocean sediments, pads) that contains reduced sulfur compounds and the hydrogen sulfide which are subjected to phototrophic oxidation in anaerobic environments to elemental sulfur deposition.

2. The oxygenic chemolithotrophic group comprises the aerobic chemolithotrophic groups (*Thiobacillus, Thiomicrospira*), *Acromatium, Macromonas, Thiobacterium, Thiovulum* and the non photosynthetic gliding bacteria (*Beggiatoa, Theothrix* and *Thioploca* which are merely heterotrophic groups that depend on the organo sulfur decomposition in soils and sediment to mercaptans and H_2S (Staley *et al*; 1989) the process is analogues to desulfuration (Singleton, 1995). These bacteria utilize reduced and /or elemental sulfur as a sole source of electron in the system (Staley *et al.*, 1989; Atlas and Bartha, 1993). The common characteristics of Sulfur Oxidizing Bacteria (SOB) is their ability to oxidize reduced sulfur compounds by using oxygen or nitrate as terminal electron acceptor (Kelly and Kuenen, 1984; Staley *et al.*, 1989).

2.2.1. The steocheometry of Reduced sulfur oxidation

Inorganic sulfur exists primarily as sulfate SO_4^{2-} or sulfide (S^{2-}), in gypsum, aluminum sulfate, or various metal sulfides, which predominate in the metal ores at mining areas. In addition to this, sulfur occurs as organic forms of sulfur containing aminoacids in biological materials (cysteine, cystine, methionine etc.). The functional groups of bacteria make use of inorganic sulfur compounds for dissimilation and thereby establish the sulfur cycle in nature. Reduced inorganic sulfur is oxidized by aerobic bacteria (mainly *Thiobacillus*) to yield sulfuric acid.

Reduced inorganic sulfur compounds contain sufficient energy to support growth of the members unified in the functional group of strictly aerobic sulfur oxidizing bacteria. The following reaction enthalpies depict the amount of energy produced during each reaction (Fig 1. adopted form Kellogg *et al.*, 1972).

$$H_2S + 1/2O_2 \rightarrow S^o + H_2O \text{ (-210Kj)}$$

$$S^o + 1/2O_2 + H_2O \rightarrow H_2SO_4 \text{ (-496Kj)}$$

$$HS^- + 2O^{2-} \rightarrow SO^{2-}{}_4 + H^+ \text{ (-716Kj)}$$

$$S_2O^{2-} + 2O_2 + H_2O^- \rightarrow 2SO^{2-}{}_4 + 2H^+ \text{ (-936Kj)}$$

Reduced, sulfur is oxidized aerobically through a variety of intermediates by microbial and /or chemical pathways (Fig.1-). The oxidation state range form + 6 in SO_4^{2-} to +4, +2, O, and -2 in

H_2S and its derivatives (Atlas and Bartha, 1993). Oxidation of sulfur in soils generates acidity (H_2SO_4), and depending on the type of soil, this may have either a positive or negative effect on the whole soil environment (Paul and Clark, 1989).

In the bacterial oxidation of iron sulfides, ferric iron is produced by oxidation of ferrous minerals (Merson, 1992; Brombacher *et al.*, 1998). Soluble ferric iron acts as an electron acceptor for the chemical oxidation of sulfide minerals. The role of the iron oxidizing thiobacilli is then to reoxidize the reduced election carrier. The sulfuric acid increases solubility of irons, thereby enhancing the rate of metal solubilization. (Hallberg *et al.*, 1996).

The use of acidophilic thiobacilli and sulfolobus in the mining industry (microbial leaching) to improve the yield of various metals from low grade sulfide ores (e.g. sulfides of Fe, Zn, Ni, Co, U, Cu, Sn) (Silverman and Ehrlich, 1964; Hallberg *et al.*, 1996) and the desulfurization of coal (Merson, 1992) is well known. The sulfur deposits that are mined today are biogenic which are formed by sulfate reduction involving sulfur bacteria. However, the magnitude of biogenic H_2S emissions is presently not well known, (Paul and Clark, 1989; Atlas and Bartha, 1993).

In all habitats containing sulfur compounds, the thiobacilli are found to be the most important genera in waste degradation, stabilization and metal recovery through bioleaching activity by which, with only few exceptions, obligately chemolithoautotrophic bacteria get the energy from sulfur oxidation (organic or inorganic) and assimilate CO_2 employing the Calvin cycle (Paul and

Clark, 1989). Their methabolism is respiratory and depend on the availability of oxygen. *Thiobacillus ferroxidan* can gain energy from the oxidation of Fe^{3+} (Merson, 1992) and in the absence of O_2 some thiobacilli can denitrify using nitrate as terminal electron acceptor (Singleton, 1995). The archaebacteria groups, which are the thermophilic, acidophilic groups of the genus sulfolobus and or acidanus, oxidizes elemental sulfur in hot acidic habitats to generate their required energy.

Consequently, the genera *Thiobacillus* and *Sulfolobus* are the two most important groups of the SOB that have attracted interest in the industrial and environmental application for metal recovery and sulfur wastes stabilization. They facilitate this through the oxidation of reduced sulfur to sulphate and the removal of sulfur from extraction mining sites through bioleaching activity (Merson, 1992, Fowler and Crandwell, 1998; Gehrke *et al.,* 1998).

2.2.2. Denitrification

Denitrification is a process of reducing nitrate to the molecular atmospheric nitrogen. The process of denitrificaion involves the following sequence of reactions.

NO_3^- ----[a]----> NO_2- ----[b]---> NO (gas) ----[c]---> N_2O (gas) ----[d]---N_2 (gas)

a. nitrate reductase (membrane bound enzyme)

b. nitrite reductase (membrane bound and cytoplasmic enzyme)

c. nitric oxide reductase

d. Nitrous oxide (N_2O) reductase) (cytoplasmic enzyme)

During denitrification, the nitrogen compounds acts as terminal electron acceptors instead of oxygen during respiration. Thus, denitrification occurs when oxygen becomes limited for aerobic respiration (Hotchstein and Tomlinson, 1988). The following are the group of microorganisms with respect to their function in the process of denitrification.

2.2.2.1. Hetrotrophic denitrification

The majority of denitrifying bacteria are heterotrophs. Denitrification by heterotrophic bacteria involve the oxidation of organic compounds and the reduction of nitrate to nitrogen. The organic substrate serves as electron donors, energy source during respiration (Mc Eldowney *et al.*, 1993).

$$2\ 1/2\ CH_2O + 2NO^-_3 + 2H^+ \text{----> } N_2 + 2\ 1/2\ CO_2 + 3\ 1/2\ H_2O$$

2.2.2.2. Chemolithotrophic denitrifiers

Some denitrifiers are obligate facultative chemolithotrophs that utilize hydrogen or reduced sulfur as sources of energy, i.e. electron donors. Eg. *Thiobacillus denitrificans* (a facultative chemolithotrophs) and *Ferrobacillus ferrooxidans* (an obligate chemolithotrophs). Chemolithotrophic denitrification involves the oxidation of a reduced inorganic substrate such as pyrite (McEldowney *et al.*, 1993; Sengleton, 1995; Bridge and Jonson, 1998).

$$2\ 1/2\ FeS_2 + 7NO_3^- + 2H^+ \text{------>} 3\ 1/2\ N_2 + 5SO_4^{2-} + 2\ 1/2\ Fe^{2+} + H_2O$$

2.3. Waste Water treatment Strategies

Modern urbanization and collectivization activities are characterized by the production and accumulation of more municipal, industrial, and agricultural wastes per unit area of land than the era of pre-civilization. Consequently, human society needs sustainable, robust, and economically feasible solutions for the removal of waste to protect the environment from pollution with the realization of the need for control of environmental pollution and remediation of polluted environment (Hamer *et al.*, 1985), biofuel (biogas) generation (Becari *et al.*, 1996), and recycling of resources such as metal (Brombacher *et al.*, 1998).

The pattern of industrial wastewater treatment varies from country to country, depending on prevailing economic and environmental policies that suit the particular characteristics of the aqueous effluents produced and factors pertinent to the sink into which effluents are discharged.

2.3.1. Physical Treatment

Physical treatment of waste involve the separation or removal of coarse, fine, settlable, low specific gravity solids and liquids; concentration of liquids, sludge, trace impurities and recovery of valuable materials involving physical screening process such as centrifuging, filtration, sedimentation, floatation, freezing, solvent extraction, ion exchange, reverse osmosis and adsorption. In order to manage the physical separation of waste compounds, the knowledge about the physical and biochemical properties of the compound as solids, liquid or gases as well as in mixtures of these states of matter is important (Manahan, 1990).

2.3.2. Chemical Treatment

Chemical wastewater treatment involves the application of chemicals to neutralize, precipitate and solid sorption of hazardous wastes in the effluent. The application of chemical treatment to wastes depends upon the chemical properties of the waste constituents. These properties include

acid base oxidation-reduction, precipitation and complexion behavior, reactivity, flammability/ combustibility, corrosivity, and compatibility with other wastes (McEldowney *et al.*, 1993).

2.3.3. Biological Treatment

Wastewater is material derived from domestic sewage or/industrial processes, which for reasons of public health and for recreational, economic and aesthetic considerations, can not be disposed off untreated into convenient lakes, streams or rivers. The undesirable and toxic materials in the water must first be either removed or rendered harmless (Henze *et al.*, 1995). Treatment practice or strategy depends on the type of effluent and of its composition. Inorganic materials such as clay, silt, and other debris are removed by mechanical and chemical methods (primary treatment) and microbes participate only causally or not at all (Brock, 1991; Harrison, 1995). Successful, secondary treatment on wastewater with high BOD (organic matter) involves consorted activities of microorganisms (aerobic, anaerobic bacteria, fungi, algae etc.). Biological process for treating wastewater therefore can be divided into aerobic and anaerobic treatment processes.

The aerobic waste treatment is well adopted to the use of an activated sludge process applied to remediate hazardous wastes of chemical processes and land fill leachets. Anaerobic waste treatment process has got a wide application and acceptance for less energy requirement, less sludge yield as a byproduct, generate sulfides (HS^-), precipitate toxic heavy metal ions. All the more, production of methane gas (CH_4), as energy source (Torphy, 1989) and of its advantages as

a means to stabilize waste are worth mentioning (Becari *et al.*, 1996). In the anaerobic fermentation of wastes, the biogas generated made up of mainly the inflammable methane, (50-65%), carbondioxide (36-45%), and traces of hydrogen sulfide as a result of the conversion of up to 90% of the combustible part of the degradable organic matter. The gas is used for heating and electricity production.

2. 4. Waste Stabilization Ponds

Waste stabilization ponds refer to treatment of wastewater in pond systems. It is an old method of wastewater treatment known to man. Some studies indicate that the wisdom of impounding wastewater in basins for natural purification is over 3000 years old (Middlebrooks, 1987). Ponds are widely used in both developed and developing countries. In developed countries, there is an increasing use of ponds for the treatment of wastewaters from small communities, tourist resort areas and game sites, besides application in big urban centers. In developing countries waste stabilization ponds offer the best option for total treatment of sewage and industrial effluents, as climate in most of these countries is favorable and land is inexpensive (Mara *et al.*, 1983).

Typical layouts of waste stabilization ponds are consisting of primary facultative pond followed by one or more maturation ponds. Facultative pond preceded by anaerobic pond as deep basins within the primary pond system followed by two or more maturation ponds is recommended for treatment of strong wastewater to reduce the high organic load. The final pond effluent can

therefore be used for unrestricted irrigation because of their undisputed benefits interms of high organic load reduction (Harrison, 1995).

2.5. Tannery Industries in Ethiopia :

There are about 15 tannery firms in the country. Most of them are accumulated in and around the capital city, Addis Ababa (Netseha and Tequam, 1999). Previously, the tanneries were established near river sites to discharge the effluent at will. Table 2 gives some of the contents of a few tannery effluents in the country. However, recently due the environmental concerns, strict legislation on effluent discharge is being formulated. Consequently, some researches on waste treatment are being conducted in collaboration with UNIDO and other donor organizations.

Table 2. Chemical and physical analysis of some tannery wastes in Ethiopia

Tannery factory	Parameters						
	DS mg/l	SS mg/l	TDS mg/l	BOD	COD	H_2S	pH
A.A Koda	-	0-500	151-1192	-	35-707	-	6.8-8.4
Awash Koda	-	0-5586	597-23641	-	96-3052	-	6.8-7.6
Walya Koda	447-9300	336-1871	-	54-7856	151-1376	0.1-14.0	7.7-12.5
Dire Koda	32-7950	400-440	-	11-3753	39-4510	0-12.5	7.2-9.4
Ethiopia Leather Factory	-	0-2008	472-67698	-	120-6375	-	7.6-11.6

(Courtesy of Agajie M., 1993)

3. MATERIAL AND METHODS

3.1 The sampling site and Description of the Modjo Tannery Effluent Treatment Research Facility

The sampling site, Modjo Tannery Waste Treatment research pond system is located at Modjo town 79 km to the east of the capital city, Addis Ababa. It is a pilot-scale biological treatment system consisting of three reactors arranged in series. The first reactor in the series is a primary facultative pond known as Advanced Facultative pond (AFP) with an integrated fermentation pit. The second reactor is known as Secondary Facultative Pond (SFP) and the third reactor is known as Maturation Pond (MP).

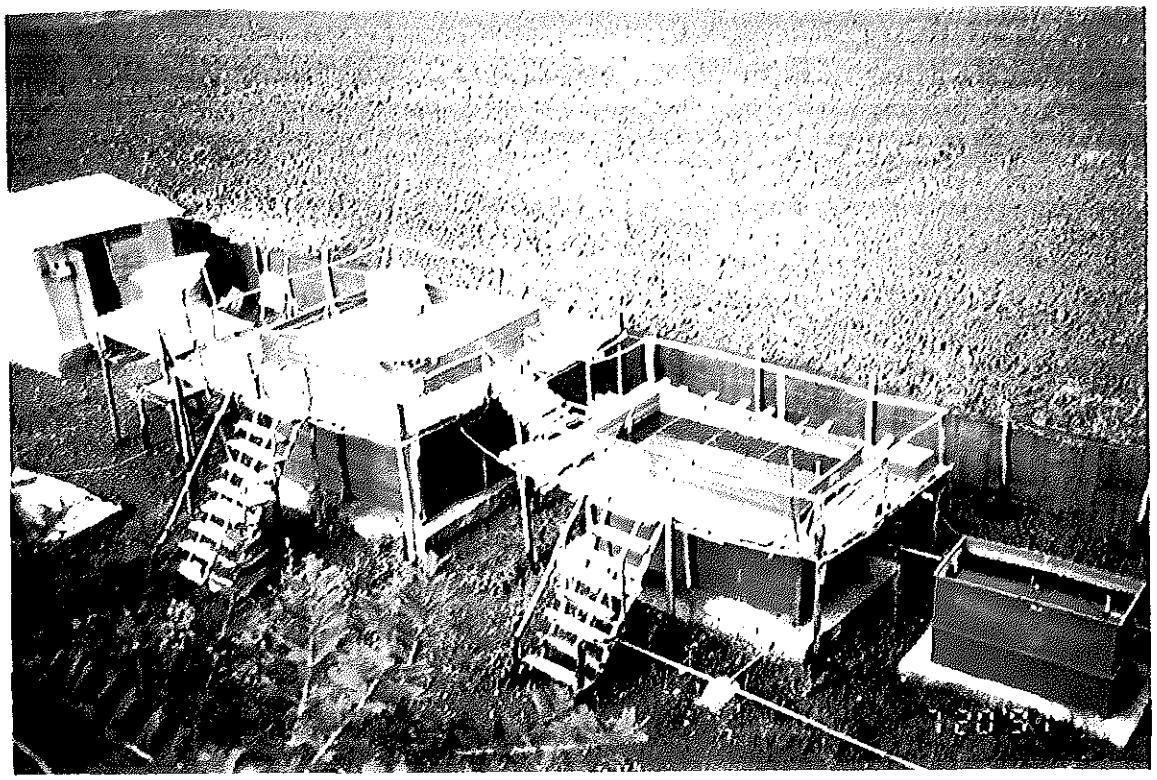

Plate 1. view of the Modjo effluent treatment research center. The reactors from left to right are: Advanced Facultative Pond (AFP), Secondary Facultative Pond (SFP), and Maturation Pond (MP) (Courtesy of I. Tadesse, 1999).

3.2. Sample collection

Thirteen samples at a time: five from AFP, four from each of SFP and MP systems were collected once in a month for three consecutive months. The samples were collected aseptically in plastic bags having a 300 ml capacity using Master flex peristaltic pump (Serial No 561382, Cat. No 7554-20, at 6-600 rpm).

The collection of samples from the three reactors (AFP, SFP, MP) depend on the size and volume of each pond system. Table (3) shows the depth of reactors at which samples were taken including other parameters (temp. and pH) that were recorded during the time of sampling.

Dissolved oxygen and temperature of samples were measured by HACH Dissolved Oxygen Meter (model 16046, 5. No 940, 70000 5496). pH was measured by inventor pH/mV meter (LU 50 S. No 802) with pH measurement 2-12 and redox reading of -500 to +50mv. The ambient temperature of the day was measured by daily maximum and minimum thermometer. Samples were brought to the laboratory, immediately after collection and stored in refrigerator for further work.

3.3 Media Preparation

Solid and liquid media were prepared for growth of sulfur oxidizing (SO) and Denitrifying bacteria (DENB) using the methods described by Smith and Kelly, (1988) and Norris, (1959), respectively.

A) Thiosulfate Medium Composition:

i) Standard basal mineral salts medium (S^o)	*g/l*
Na_2HPO_4	1.2
H_2PO_4	1.8
$MgSO_4$	0.1
$(NH_4)_2 SO_4$	2.0
$CaCl_2$	0.03
$FeCl_3.6H_2O$	0.02
$MnSO_4.4H_2O$	0.02
ii) Trace Metal Solution (TMS)	*10ml*

Composition of trace metal solution:	gm/l
EDTA	50
$ZnSO_4\ 7H_2O$	22
$CaCl_2$	5.54
$MnCl.2H_2O$	5.00
$FeSO4.7H_2O$	4.99
$(NH4)_6Mo_7\ O_{24}.4H_2O$	1.10
$CuO_4.5H_2O$	1.57
$COCl_2.6H_2O$	1.61
Distilled water	1000ml

iii) Sodium acetate (C_2H_3 Na O_2. $3H_2O$)	2.0
iv) Sodium thiosulphate (Na_2 S_2O_3.$5H_2O$)	4.69

The pH of the trace metal solution was adjusted to 6.0 with KOH and/or H_2SO_4 of (0.1M) concentration and the components were mixed in one liter volume of distilled water at final pH adjusted to 7.0-7.2. The medium was brought to one liter volume by distilled water and 15-20 gm of agar was added to prepare a solid media.

B) Denitrifying Bacteria Medium

The denitrifying (DEN) bacteria medium is also thiosulfate medium incorporated with Nitrate with the following constituents as described by (Smith and Kelly 1988 and Wood *et al.*, 1991)

i) Standard basal mineral salt medium (S^o)	***mg/l***
Na_2HPO_4	1.2
$H2PO_4$	1.8
$MgSO_4$	0.1
$(NH_4)_2SO_4$	2.0
$CaCl_2$	0.03
$FeCl_3.6H_2O$	0.02

$MnSO_4.4H_2O$	0.02
(TMS)	10 ml
Distilled water	1000ml
iii) Sodium thiosulfate **($Na_2S_2O_3.5H_2O$)**	***4.69***
iv). Sodium acetate **($C_2H_3NaO_2.3H_2O$)**	***2.0***
v). Ammonium nitrate **(NH_4NO_3)**	***2.0***

The pH was adjusted to about neutral (7.0- 7.2) and was sterilized.

C). Heterotrophic Medium (HET-M)

The heterotrophic medium was constituted as described by (Wood *et al.*, 1991)

Composition	***gm/l.***
Nutrient agar (NA)	13
Yeast extract (0.5%)	5
Distilled Water	1000ml

The pH was adjusted to 7.2 and the culture plates were incubated at 28°C for 48hrs. The presence or absence of growth on the media provided was marked as positive or negative, respectively.

D). Nitrogen free Medium (Nif-M)

The Nitrogen free medium was prepared as described by (Norris, 1959) and used to grow nitrogen-fixing bacteria

(i) Part I	*gm/l*
K_2HPO_4	1
$MgSO_4.7H_2O$	0.2
$CaCO_3$	1
NaCl	0.2
$FeSO_4.7H_2O$	0.1
$NaMoO_4.2H_2O$	0.005
Agar Powder (if required)	15
Distilled water	1000 ml

The pH was adjusted to 7.0 and was sterilized by autoclaving at 15 psi for 15 minutes.

ii). Part II	*gm/l*
D-glucose, anhydrous	10
Distilled water	50 ml

The diluted sugar base composition was sterilized by seitz filtration. Upon cooling to moderate temperature (45°C), both components of part II media and I were mixed aseptically for complete medium formulation and was dispensed into tubes and/or petridish as required.

3.4. Microbial Density measurment

Population density of the different groups of sulfur oxidizing and denitrifying bacteria was estimated using the Most Probable Number (MPN) technique as described by Cochran, (1950) on thiosulfate and denitrifying medium. Serial dilution up to 10^8 was made using 9.9 ml of enumeration media and 0.1 ml of the samples. The initial turbid metric readings of the inoculated tubes were done using Spectrophotometer, 1001. The culture was incubated at 28°C for seven days. Consequently, the MPN tubes considered positive were selected with an increase optical density (OD) reading as opposed to the reference and were analyzed further for microbial identification.

3.5. Isolation and Identification

The respective MPN tubes showed an increase in OD reading were recorded as positive for growth for growth occurrence and were transferred to fresh solid thiosulfate and denitrifying media repeatedly for purification and isolation of colonies. Different colonies (in shape, size,

color) were picked and sub cultured into thiosulfate and denitrifying media as described by Smith and Kelly, (1988) for SOB and Wood *et al.*, (1991) for denitrifying isolates, respectively. The surface colonies appearing on the corresponding plate media as colorless (transparent) to dark brown were picked and characterized further on the basis of morphological and physiological characterization to their respective taxa.

3.5.1. Morphological Characteristics

Cultures were examined for colony appearances such as form, elevation and texture through the reflected (RL) and /or transmitted light (TL) of stereomicroscope model swift instruments international (SA) as described by Starr and Skerman, (1965).

3.5.2. Sulfur Deposition

The sulfur inclusion or exclusion in or out of the cell or colony was examined by Reichert Neovar model light microscope and stereomicroscope model swift instruments international (SA), respectively as described by Staley *et al.*, (1989). The light or deep yellow/brown/dark coloration of the cell /colony was recorded as positive for sulfur deposition and transparent as negative.

3.5.3. Motility

Motility of isolates was observed from 10^8 cells /ml cell concentration of colonies by hanging drop method as described by Bradshaw, (1979). A drop of a suspension was placed on a cover slide. The cover slide was placed on a glass slide cavity made by plasticin in inverted position. Motility of the cells was observed under high power objective of compound microscope.

3.5.4. Biochemical Characteristics

3.5.4.1. Gram reaction

Gram reaction of the isolates was determined by staining reaction technique as described by Collins and Lyne, (1976). Upon examination through light microscope, color retention as blue and /or pink red recorded as gram positive and negative bacteria, respectively. The gram reaction test also assists in the determination of cell morphology and arrangement of the isolates.

3.5.4.2. Oxidase test

Oxidase test was conducted according to the procedure described by Kovacs, (1956). Reagent 'A' (1% α-naphthol in absolute ethanol) and reagent 'B' (1% N, N,-dimethyl p-phenylene diaminonium chloride in distilled water) were mixed in the ratio of 2:3 immediately before use.

Young colonies grown on Nutrient Agar containing 20mM of thiosulfate were flooded with 1-2 drops of the reagent and the formation of blue coloration with in 2 minutes indicates positive results. Very weak or dubious reactions that occurred after two minutes were ignored.

3.5.4.3. Catalase test

Catalase activity was detected as described by Collins and Lyne, (1976) by adding 3% hydrogen peroxide to the surface of pure culture isolates and the observed gas formation (effervescence) was recorded as positive.

3.5.4.4. Carbon source Utilization

Carbon utilization of strains was determined as described by Collins and Lyne, (1976) on four carbohydrate compounds namely: glucose10gm/l, galactose10gm/l, acetate 2gm/l and citrate 2gm/l). Each test carbon source was filter sterilized separately, and complemented with autoclaved basal mineral salt solution, pH adjusted to 7.6. Four ml of 0.2% bromothymol blue was used as indicator and a change in indicator color from blue to yellow was recorded as positive for glucose and/or galactose utilization whereas phenol red with concentration of 0.4% was used as indicator for organic acid utilization and a change in indicator color from yellow to red was recorded as positive.

Basal medium	*gm/l*
Magnesium sulfate ($MgSO_4$)	1.0
Sodium chloride ($NaCl_2$)	1.0
Diammonium hydrogen phosphate	2.0
Potassium dihydrogen phosphate	0.5
Distilled water	1000

3.5.4.5. Growth inhibition test medium

Two growth inhibition test media were prepared as described by (Hutchinson *et. al.*, 1967, 1969) as S5 medium mixed with 5% NaCl and the thiosulfate medium mixed with 0.2% chloroamphinicol.

A. (S5 plus 5% NaCl medium)	**mg/l**
Standard basal mineral salt medium (S^o)	10ml
KH_2PO_4	2gm
Agar (BDH)	12gm
Distilled water	1000ml
NaCl	50gm

B. Thiosulfate (S2) medium plus 0.2% Chloroamphinicol

i) Standard basal mineral salt medium (So)	10ml
ii) Trace Metal Solution (TMS)	10ml
iii) Sodium acetate ($C_2H_3NaO_2$. $3H_2O$)	2.0gm
iv) Sodium Thiosulfate ($Na_2S_2O_3$. $5H_2O$)	4.69gm
v) Chloroamphinicol	2.0gm

The thiosulfate medium and the chloroamphinicol added was sterilized by autoclaving and membrane filtration respectively and pH was adjusted to 7 using (0.1) KOH and H_2SO_4.

3.5.4.6. Sulfur Oxidation.

Sulfur oxidation media (Kinsel, 1960; Hutchinson, *et. al.*, 1965) were used with the following composition

Sulfur oxidation medium	*gm/l*
Sulfur	10.0
$(NH_4)_2SO_4$	2.0.
$MgSO_4.7H_2O$	0.5
$CaCl_2$	0.25
$FeSO_4.7H_2O$	0.01

KH_2PO_4	3.0
Distilled Water	1,000ml

The pH of the media was adjusted to 4.0-4.5. The medium was steam sterilized for 30 minutes and the inoculated tubes were incubated at 28°C for 7 days. The decrease in pH of the culture (production of sulfuric acid) was recorded as positive.

3.5.4.7. Iron oxidation

Iron oxidation test was conducted in a medium (Kinsel, 1960) containing the following composition.

Iron oxidation medium	*gm/l*
$FeSO_4.7H_2O$	10.0
$(NH_4)_2 SO_4$	2.0
$MgSO_4.7H_2O$	0.5
$CaCl_2$	0.25
KH_2PO_4	3.0
Distilled Water	1,000ml

The pH of the media was adjusted to 4-4.5 and was steam sterilized for 30 minutes. The culture was inoculated aseptically and kept at 28°C for a week. The presence of oxidized iron (ferric iron) was indicated by the appearance of a red color with the addition of potassium thiocyanate (5g per 100 ml) to 1ml of culture in a spot plate.

3.5.4.8. Gelatin liquification

Gelatin agar medium was used for liquification test as described by Collins and Lyne (1976) and Pfennig (1978). The medium was inoculated with 0.1ml of pure culture isolates and incubated over night at 37°C. Then the plates were flooded with saturated ammonium sulfate solution and the formations of haloes around colonies were recorded as positive for production of gelatinase.

3.5.5. Ecophysiological Characteristics

3.5.5.1. Salt tolerance

Salt tolerance of the isolates was determined following the method described by Hutchinson *et. al.*, (1967). Turbidity of each isolate was observed in sodium chloride broth with 0.1 and 5% sodium chloride concentration.

3.5.5.2. pH Requirement

The acidic growth optimum pH of the isolated colonies was determined on their respective growth media (SOB and /or DEN) media as described by Collins and Lyne (1976). 0.05N concentrations of sodium hydroxide solution and/or sulfuric acid solution were used to adjust the pH of the growth media.

3.5.5.3. Temperature Requirement

Growth temperature requirement test were made as described by Kuenen (1975) on thiosulfate medium. Two temperature ranges: (28°C) and (55°C) were selected for incubation of 48 hrs pure strain colonies. Strains that grow at or above 55°C temperature range were recorded as thermophilic groups while those grown below 45°C (i.e. at 28°C) were recorded as mesophilic groups.

3.5.6. Growth rate Determination

The different isolates standard inocula suspensions were prepared by adjusting the turbidimetric reading to 0.3 at 620NM using (spectrophotometer, 1001). 10ml of inoculate suspension were inoculated to 90 ml of SO and DEN bacteria medium in 250 ml capacity flask. Incubation was made at 28°C with interval shaking for a week. After taking the initial optical density (OD) reading of the culture inoculated, the subsequent measurements were conducted with interval of 24 hrs by withdrawing samples from the flask incubated at 28°C. The corresponding viable counts were made by pour plate technique. 1 ml of serially diluted aliquots was transferred to petridish and 10-12 ml of respective medium of thiosulfate or denitrifying medium were poured.

4. RESULTS

4.1 Physico-chemical Characteristics of the combined tannery effluent

The Modjo Tannery is the pioneer to start a research project on low cost tannery effluent treatment before the waste is discharged to the nearby Modjo River. Data on Table 3 showed measurements on many wastewater characteristics, which are more exhaustive as compared to the hitherto patchy information on the different tannery wastewaters in Ethiopia (Table 2)

Table 3. Physico-chemical charactertics of raw combined effluent from Modjo Tannery

Parameters	Units	Values	
		Range	Typical
Temperature	°C	22 - 26	24
PH	-	6 - 12	9
BOD	Mg/l	2200 - 5800	3000
COD	"	6200 - 15000	9600
Total solids (TS)	"	8000 - 25000	15000
Suspended solids (SS)	"	2100 - 9500	3000
Dissolved solids (DS)	"	5000 - 19000	12000
Volatile solids (VS)	"	800 - 3600	1700
Settleable solids (S)	Mg/l	10 - 100	25
Ammonia - N	"	70 - 200	130
Nitrate - N	"	Nil	Nil
Chromium III	"	9 - 50	45
Sulphate	"	150 - 1500	850
Total sulphide	"	100 - 830	325
H_2S	"	Nil	Nil
Phosphorus (ortho)	"	8 - 60	16
Chloride	"	2500 - 12000	4000
Electrical Conductivity	µs/cm	6200 - 20000	13000

(Courtesy of IssayasTadesse, 1999).

The effluent was found to have high BOD, COD, nitrogen, sulfate, chromium, etc that reflect the different sources of the effluent. The sulfide content and the high pH of the effluent originated from the beam-house where the processing required sulfur containing organic solvents. The organic wastes such as blood and dung came from the soaking process, whereas the low pH and the high chromium emanated from the tanning process. All the three effluent streams used to be emptied combindly to the nearby Modjo River without any treatment.

Table 4: Physical and chemical parameters recorded during the time of sample taking

Sample collected	Depth below or at SWL (cm)	Dissolved oxygen (mg/l)	Temperature (°C)	pH
AFP1	`0'	0.4	18	8.2
AFP2	20	0.0	17	8.2
AFP3	120	0.0	16.5	8.2
AFP4	220	0.0	16	8.2
AFP5	310	0.0	16	8.2
SEP1	`0'	0.8	19	8.4
SEP2	45	0.2	16.2	8.3
SEP3	95	0.0	16	8.2
SEP4	145	0.0	16	8.2
MP1	`0'	>20.0	22.5	8.6
MP2	25	0.4	17.5	8.4
MP3	60	0.4	16.2	8.4
MP4	95	0.4	15.8	8.4

4.2. Bacterial Identification

After enriching the tannery wastewater samples by the thiosulfate medium, more than two hundred SOB colonies were picked and further characterized on the basis of their cultural, morphological, and physiological features to the respective taxa. Isolates displayed different colony characteristics ranging from round, lobate, and irregular colony morphology, with yellow, colorless, dark brown color depending upon the group. The cells were also represented by both motile and non-motile strains with or without sulfur deposition. Most of them were mesophilic with a few thermophilic representatives (Table 5). All the isolates were capable of growing on thiolsulflate (S2) medium with variation in growth on other types of media and different carbon sources.

Based on the above-mentioned characters and growth on different carbon source supply medium, sulfur, inhibition test medium, iron oxidation, gelatin liquification and growth optimum pH, thirty-six Sulfur Oxidizing Bacteria (SOB) isolates falling into sixteen genera were identified. The genera were; *Acidiphilium, Acromatium, Macromonas Sulfolobus, Thermothrix, Thiobacillus, Thiobacterium, Thiomicrospira, Thiosphaera, Thiospira, Thiovulum, Rhodobacter, Rhodocyclus, Rhodomicrobium, Rhodopsudomonas* and *Rhodosprillum* (Table 6, 7, 8). Out of the thirty-six strains, twenty-one (58%) were capable of denitrification in the absence of oxygen.

Table 5. Morphological and Physiological characterization of SulfurOxidizing (SO) and Denitrifying (DEN) Bacteria.

Genus	Colony		Catalase test	Growth temp.(T°) (°C)	Cell		
	Morphology	Color			Shape	Motility	Sulfur deposition
Acidiphilium	Round	Light yellow	+	Mesophilic	Rod	Motile	External
Acromatium	Irregular	Dark brown	+	»	Cocci	»	Internal
Macromonas	Lobate	Golden yellow	+	»	Short rod	Non motile	»
Sulfolobus	Lobate	Colorless	+	Thermophilic	Cocci	Motile/non motile	External
Thermothrix	Round	Goldenyellow	+	»	Short rod	Motile	Internal
Thiobacillus	Round	Colorless	+	Mesophilic	Long rod	Motile	»
	Irregular	Colourless	+	»	Short rod	Non motile	»
Thiobacterium	Lobate	Llight yellow	+	»	»	»	»
Thiomicrospira	Irregular	Colorless	+	»	Spiral	Motile	»
Thiosphaera	Round	Light brown	+	»	Short rod	Non motile	»
Thiospira	Lobate	Colorless	+	»	»	»	»
Thiovulum	Round	Goldenyellow	+	»	»	Motile	»
Rhodobacter	Round	Light yellow	+	»	»	Non motile	Internal
Rhodocyclus	Round	Pinkbrown	_	»	»	»	External
Rhodomicrobium	Round	Colorless	_	»	»	»	»
Rhodopseudomonas	Round	»	_	»	»	Non motile	»
Rhodosprillum	Round	Colorless	+	»	Spiral	Motile	»

The genus *Thiobacillus* was found to be the most dominant represented by eight strains followed by *Rhodopseudomonas* and *Rhodomicrobium* with four and three representative strains, respectively. The two frequently encountered genera in the three pond systems and down the water profile were also *Thiobacillus* and *Rhodomicrobium,* whereas five genera were represented

each by only one strain. *Round/irregular, colorless, colony features with catalase positive, motile and non-motile, long and short rod cellular morphology with intracellular sulfur deposition characterize Thiobacillus. Rhodomicrobium*, however, is exemplified by round colony, catalase negative, short rod, non-motile cells with external sulfur deposition.

Table 6. Identification of the *Thiobacillus* and *Rhodomicrobium* strains

Characteristic	*Thiobacillus*								*Rhodomicrobum*		
Carbon source	AU1	AU13	AU59	AU86	AU129	AU46	AU52	AU118	AU 142	AU 164	AU 176
Glucose	-	-	-	-	-		+	+	+	-	-
Lactose	-	-	-	-	-	-	+	-	+	+	-
Acetate	-	+	-	+	-	+	+	-	+	+	+
Kosers citrate	-	+	-	+	+	+	+	+	+	+	+
Growth on											
S2 media	+	+	+	+	+	+	+	+	+	+	+
DEN media	-	+	-	+	+	+	+	+	-	-	-
HET media	-	+	+	+	+	+	+	+	+	+	+
Nif media	+	-	+	-	-	+	+	+	+	+	+
Growth inhibition test											
S5 + 5% NaCl	-	-	-	+	+	-	-	-	-	-	-
Chloroamphinicol	+	-	-	-	-	-	+	+	-	-	-
Iron oxidation	-	-	-	-	+	+	-	-	-	-	-
Growth on sulfur	-	+	-	-	-	+	+	-	-	+	+
Gelatin liqufication test	-	-	-	-	-	+	+	+	-	-	-

Legend: (+) growth present; (-) growth absent; (S2) thiosulfate medium; (DEN) Denitrifying medium; (HET) Hetrotrophic medium; (Nif) Nitrogen free medium

Table 7. Identification of *Acidiphilium, Acromatium, Macromonas, Sulfolobus, Thermothrix, Thiobacterium,.Thiomicrospria, Thiosphaera, Thiospira, and Thiovulum strains*

Characteristic	*Acidiphilium*		*Acromatium*		*Macromonas*		*Sulfolobus*		*Thermothrix*	*Thiobacterium*	*Thiomicrospira*		*Thiosphaera*		*Thiospira*	*Thiovulum*	
	AU8	AU 19	AU67	AU76	AU4	AU16	AU12	AU54	AU33	AU14	AU62	AU91	AU18	AU57	AU94	AU60	AU84
Carbon source																	
Glucose	-	+	-	-	+	+	-	+	+	-	-	-	+	-	+	+	-
Lactose	+	+	-	-	-	+	-	+	+	-	-	-	+	-	+	-	+
Acetate	+	+	-	+	-	+	-	+	+	-	-	+	+	-	+	-	+
Kosers citrate	-	-	+	+	+	+	-	+	+	-	-	+	+	-	+	+	+
Growth on:																	
S2 media	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DEN media	-	+	-	-	-	-	-	+	+	-	-	+	+	-	+	-	+
HET media	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
Nif media	+	-	-	-	-	-	+	-	-	-	+	+	-	+	+	-	-
Salt tolerance:																	
0.01%NaCl	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	-	+
5% NaCl	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	-	+
Growth on sulfur	-	-	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-

Legend: (+) growth present; (-) growth absent; S2 thiosulfate medium; (DEN) Denitrifying medium; (HET) Hetrotrophic medium; (Nif) Nitrogen free medium

Table 8. Identification of *Rhodobacter, Rhodocyclus, Rhodopseudomonas* and *Rhodosprillium* strains

Characterstics	*Rhodobacter*	*Rhodocyclus*	*Rhodopseudomonas*				*Rhodosprillium*	
Carbon source	AU138	AU200	AU152	AU153	AU198	AU217	AU 139	AU147
Glucose	+	-	+	+	+	+	-	+
Lactose	-	-	-	-	+	-	-	-
Acetate	-	+	+	+	+	+	+	+
Kosers citrate	+	-	+	-	-	+	-	-
-Growth on:								
S2 media	+	+	+	+	+	+	+	+
DEN media	+	+	+	+	+	+	+	+
HET media	+	+	+	+	+	+	+	+
Nif media	+	-	+	+	+	+	+	+
Salt tolerance test								
0.01% NaCl	+	+	-	-	-	-	-	-
5% NaCl	+	+	+	+	+	+	+	+
Growth on sulfur	-	+	-	-	-	-	-	-
Gelatin liquification test	-	+	-	-	-	-	-	-
Growth optimum pH	5-8	5-8	5-8	7-7.5	6-7	6.5-7.5	6-8	6-8

Legend: (+) growth present; (-) growth absent; (S2) thiosulfate medium; (DEN) Denitrifying medium; (HET) Hetrotrophic medium; (Nif) Nitrogen free medium

The SOB strains growth response to different carbon sources was found to vary irrespective of their group. One strain from each of the genus *Thiobacillus*(AU52); *Rhodomicrobium* (AU142); *Macromonas* (AU16); *Sulfolobus* (AU54); *Thermothrix* (AU33); *Thiosphaera* (AU18); *Thiospria* (AU94) grew on all test carbon sources, whereas about 15 strains from different genera were capable of growing on the majority of the same. Strains of *Thioibacillus* (AU1, and AU59); *Sulfolobus* (AU12); *Thiobacterium* (AU14), *Thiomicrospira* (AU62) and *Thiosphaera* (AU57) were unable to utilize the provided carbon sources at all.

Twenty-one strains of SOB across the board of the different genera were found to be capable of denitrification. The remaining 15 strains namely; *Thiobacillus* (AU1 and AU59) and *Rhodomicrobium* (AU142, AU164 and AU176) (Table 6); *Acidiphilium* (AU8); *Acromatium* (AU67 and AU76); *Macromonas* (AU4 and AU16); *Sulfolobus* (AU12); *Thiobacterium* (AU14), *Thiomicrospira* (AU62); *Thiosphaera* AU18) and *Thiovulum* (AU60) (Table 7) failed to grow on denitrifying (DEN) medium. In the contrary, eight strains of *Acidiphilium* (AU8); *Sulfolobus* (AU12); *Thiomicrospira* (AU62); *Thiosphaera* (AU57) (Table 7); *Thiobacillus* (AU1 and AU59); *Rhodomicrobium* (AU142, AU164, AU176) (Table 6) were found to grow on nitrogen free (nif) medium.

With regard to growth on different salt concentration, almost all isolates were found to grow on thiosulphate medium containing sodium chloride. However, more isolates (24 strains) were found to grow on 5% than 0.01% sodium chloride. The iron oxidation test on *Thiobacillus* and *Rhodomicrobium* strains showed that most strain fail to oxidize iron as energy source except *Thiobacillus* stains AU129, and AU46.

4.2. 1. Spatial distribution of SOB and DENB in Different Ponds

The strains were identified into three metabolic groups as aerobic and anaerobic types based on their distribution along the water profile in each pond systems (Table 9). The anaerobic isolates and the aerobic isolates were found to be dominant, in AFP, SFP and MP, respectively.

Seven aerobic strains of *Acidiphilium* (AU8); *Macromonas* (AU4); *Sulfolobus* (AU12); *Thiobacillus* (AU1, AU59); *Rhodobacter* (AU138); and *Rhodosprillium* (AU139) were identified from (5-10cm)

depth of AFP, the system where the concentration of dissolved oxygen (DO) was 0.4 mg/l. Fourteen anaerobic strains of *Acidiphilium* (AU19); *Macromonas* (AU16); *Sulfolobus* (AU54); *Thiobacillus* (AU13, AU46, AU52); *Thiobacterium* (AU14); *Thermothrix* AU33); *Thiosphaera* (AU18); *Rhodomicrobium* (AU142, AU164); *Rhodopseudomonas* (AU152, AU153) and *Rhodosprillium* (AU147) were identified from the water sludge (20-310cm) interface of the depth of the pond system where no oxygen was detected

Nine strains of *Thiobacillus* (AU59); *Thiomicrospria* (AU62); *Thiosphaera* (AU57); *Rhodomicrobium* (AU142, AU176); *Rhodosprillium* (AU139); *Acromatium* (AU67); *Thiobacterium* (AU14) and *Thiovulum* (AU60) were identified from aerobic (5-45cm) depth of the SFP system where the concentration of dissolved oxygen was (0.8-0.2mg/l). Eight anaerobic strains of the genera *Thiobacillus* (AU86); *Thiomicrospira* (AU91); *Thiosphaera* (AU18); *Rhodomicrobium* (AU142); *Rhodosprillium* (AU147); *Thiovulum* (AU84); *Thermothrix* (AU33) and *Thiospira* (AU94) were identified from the layer was anaerobic.

Nine strains of *Acromatium* (AU67); *Macromonas* (AU4); *Thiobacillus* (AU59, AU1, and AU118); *Rhodocyclus* (AU200); *Rhodomicrobium* (AU142) and *Rhodopseudomonas* were isolated from the shallow depth of the MP system where the concentration of dissolved oxygen (DO) was 20.0-0.4mg/l.

Of the thirty-six strains identified into sixteen genera, twenty-one strains belonging to eleven genera were found in AFP system, of which many of the strains were anaerobic. The SFP system contains less number of groups (about 10 genera) of the isolates out of which the aerobic metabolic groups were at higher proportion than the anaerobic types (Table 9 and Index1).

Of the sixteen genera, *Thiobacillus* was found to be represented by the highest number of strains (AU1, AU13, AU59, AU86, AU129, AU46, AU52, AU118), followed by *Rhodopsudomonas* (AU152, AU153, AU198 and AU217) and *Rhodomicrobium* (AU145, AU164 and AU176). However, the genera *Thermothrix* (AU33); *Thiobacterium* (AU14); *Thiospira* (AU94); *Rhodobacter* (AU138) and *Rhodocyculus* (AU200) were found to be represented by the lowest number of strains.

Table 9. The distribution of Sulfur Oxidizing (SO) and Denitrifying (DEN) bacteria in the three pond systems (AFP, SFP and MP).

Genera	No of strains	AFP		SFP		MP
		aerobic	anaerobic	aerobic	anaerobic	aerobic
Acidiphilium	2	+(1)	+(1)	-	-	-
Acromatium	2	-	-	+(1)	-	+(1)
Macromonas	2	+(1)	+(1)	-	-	+(1)
Sulfolobus	2	+(1)	+(1)	-	-	-
Thermothrix	1	-	+(1)	-	+(1)	-
Thiobacillus	8	+(2)*	+(3)	+(1)*	+(1)	+(3)*
Thiobacterium	1	-	+(1)*	+(1)*	-	-
Thiomicrospira	2	-	-	+(1)	+(1)	-
Thiosphaera	2	-	+(1)**	+(1)	+(1)**	-
Thiospira	1	-	-	-	+(1)	-
Thiovulum	2	-	-	+(1)	+(1)	-
Rhodobacter	1	+(1)	-	-	-	+
Rhdocyclus	1	-	-	-	-	+(1)
Rhodomicrobium	3		+(2)*	+(2)*	+(1)*	+(1)*
Rhodopseudomonas	4		+(2)	-	-	+(2)
Rhodosprillum	2	+(1)*	+(1)**	+(1)*	+(1)**	-

Legend: +(1) strain found in both aerobic and anaerobic environment;'+'= present; '-' = absent.*= isolates from both aerobic and anaerobic depth of AFP and SFP systems; ** = anaerobic isolate from both AFP and SFP systems.

4.3. Bacterial Enumeration

The MPN value estimation for sulfur oxidizing (SO) and denitrifying (DEN) bacteria ranges between 2.0 x 10^4-1.2 x 10^6. The estimated number of SO and DEN bacteria was found to differ from one pond system to another and along the profile of each pond system (Fig 1, 2 and 3). The AFP system contained the highest number ($1.2x10^6$) of SO and DEN bacteria followed by SFP ($4.0x10^5$) and MP with ($3.0x10^4$), respectively.

In the AFP system, the number of SOB was found to be higher at the surface water (5-10cm) layer than the DENB that dominate the lower depth of the pond system (Fig 1).

In the SFP system, although the number of SO and DEN bacteria was found to decrease in SFP system, the overall distribution was similar to the AFP system (Fig 2).

The MPN estimation for SO and DEN bacteria in MP (Fig 3) showed a decrease in number with out any difference along the depth profile.

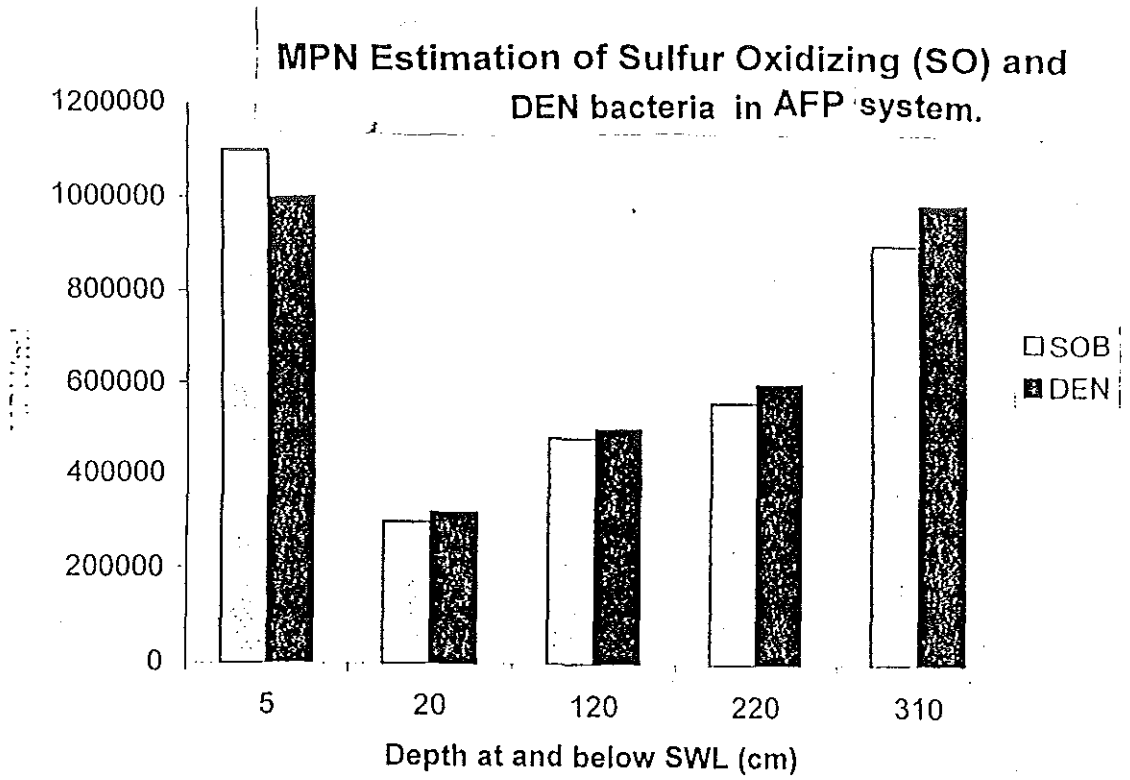

Figure. 1 MPN determination of SO and DEN bacteria in AFP system

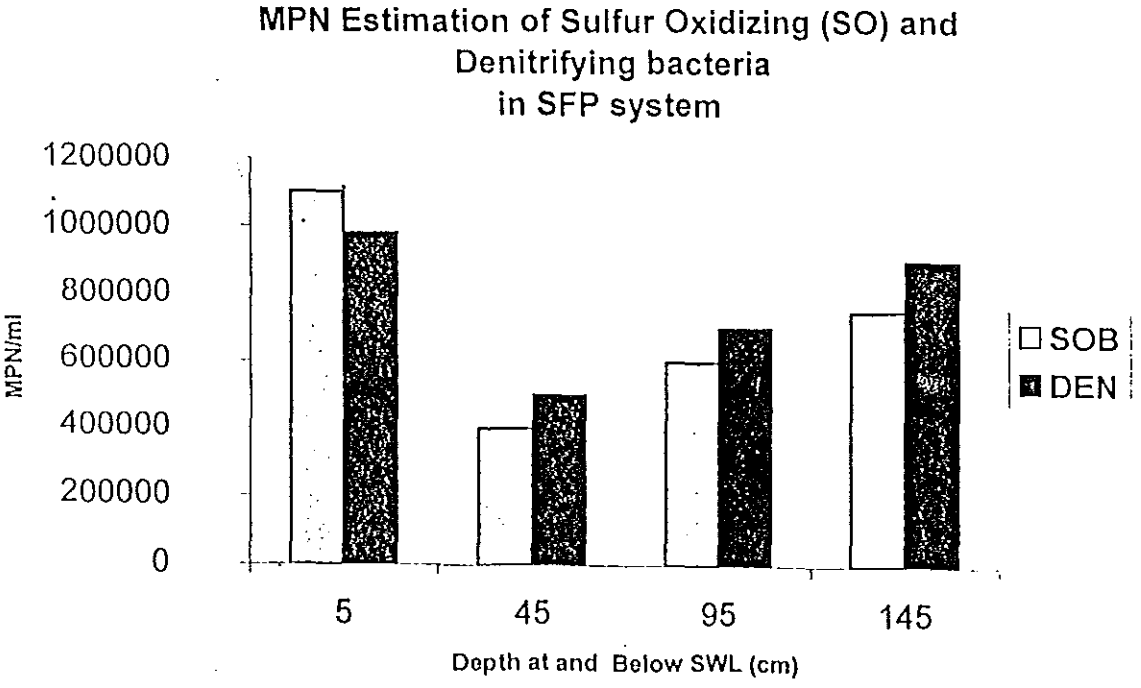

Figure 2. MPN Determination of SO and DEN bacteria in SFP system.

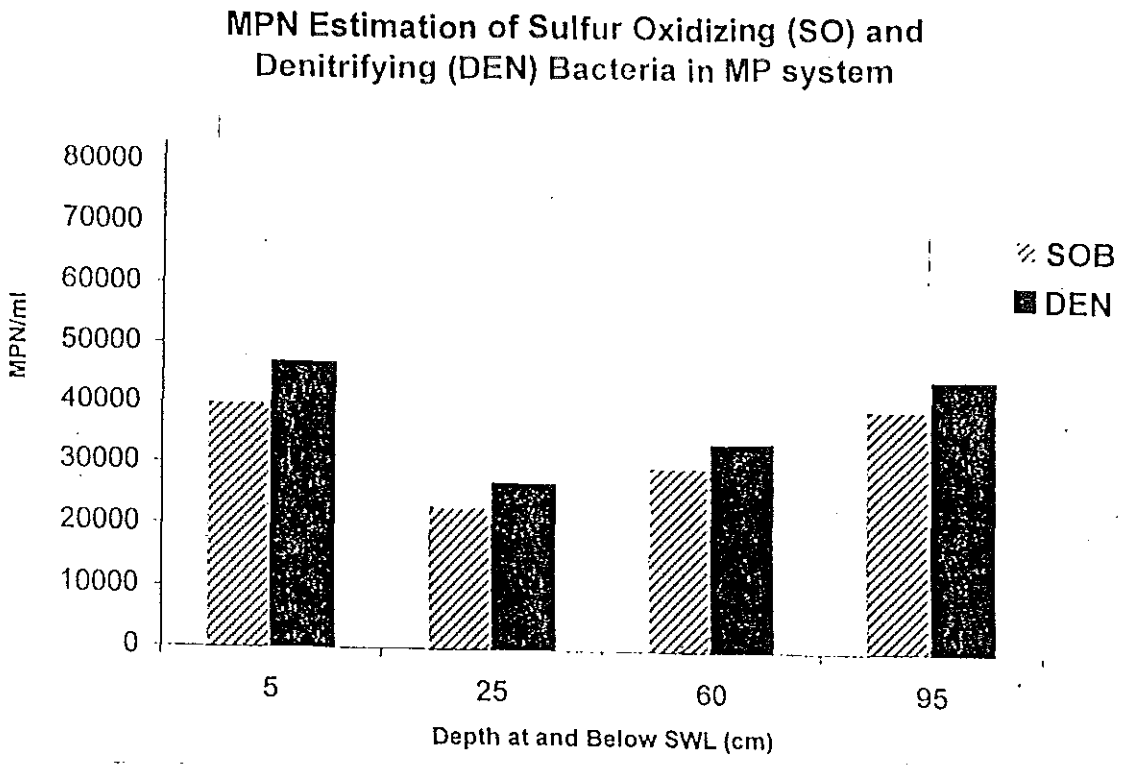

Figure.3. MPN determination of SO and DEN bacteria in MP system

4.4. Growth rate Determination of SOB Strains

The growth rate determination of SOB isolates showed that the majority of the representative strains were slow growers with a population increase of the majority of strains within 48-72 hrs. Stationary phase of all strains was observed after 72 hrs of incubation period. Strains of *Sulfolobous* (AU54), *Thermothrix* (AU33), *Thiobacterium* (AU14) and *Thiosphera* (AU18) however, showed growth within 24 hrs (Table 10).

Table 10. Growth Rate Determination of SOB representatives

Code of Isolates	Time in hours					
	0	24	48	72	96	120
	cfu	cfu	cfu	cfu	cfu	cfu
Thiobacillus (AU1)	$4.7x10^2$	$2.71x10^4$	$2.97x10^7$	$1.89x10^6$	$9.8x10^4$	$8.1x10^3$
Macromonas (AU4)	$3.7x10^2$	$1.49x10^4$	$1.47x10^6$	$1.68x10^5$	$7.1x10^4$	$7.5x10^3$
Acidiphilum (AU19)	$5.4x10^2$	$1.68x10^3$	$1.53x10^6$	$7.9x10^4$	$6.8x10^3$	$3.3x10^3$
Thiovulum (AU60)	$6.8x10^3$	$1.12x10^4$	$1.67x10^5$	$1.02x10^5$	$1.12x10^4$	$6.4x10^3$
Thiobacterium (AU14)	$7.3x10^3$	$1.76x10^5$	$2.18x10^6$	$1.21x10^5$	$9.4x10^3$	$3.4x10^3$
Acromatium (AU67)	$3.2x10^2$	$2.21x10^4$	$1.98x10^6$	$7.20x10^5$	$1.14x10^5$	$9.3x10^3$
Thiosphaera (AU18)	$5.7x10^3$	$1.89x10^7$	$2.71x10^7$	$2.12x10^6$	$1.72x10^6$	$1.17x10^5$
Thermothrix (AU33)	$7.8x10^3$	$1.73x10^5$	$8.3x10^6$	$2.14x10^5$	$1.19x10^5$	$9.7x10^3$
Sulfolobus (AU54)	$1.89x10^3$	$7.2x10^5$	$4.8x10^5$	$1.12x10^5$	$1.01x10^5$	$8.6x10^3$
Thiomicrospira (AU62)	$4.8x10^2$	$1.91x10^4$	$1.77x10^6$	$2.49x10^6$	$1.68x10^5$	$1.04x10^4$
Thiospira (AU94)	$4.3x10^2$	$1.42x10^4$	$2.03x10^6$	$2.67x10^6$	$1.73x10^5$	$7.9x10^3$
Rhodobacter (AU138)	$1.76x10^2$	$1.76x10^2$	$2.1x10^3$	$2.4x10^4$	$3.54x10^5$	$4.3x10^3$
Rhodocyclus (AU200)	$2.13x10^2$	$2.13x10^2$	$3.3x10^3$	$2.44x10^4$	$5.6x10^4$	$3.76x10^3$
Rhodomicrobium (AU176)	$1.98x10^2$	$3.4x10^2$	$2.65x10^4$	$4.5x10^5$	$3.67x10^6$	$2.1x10^4$
Rhodopseudomonas (AU153)	$2.34x10^2$	$2.34x10^2$	$3.4x10^3$	$4.83x10^4$	$2.32x10^5$	$3.45x10^3$
Rhodosprillum (AU147)	$2.12x210^2$	$2.12x10^2$	$4.56x10^3$	$2.34x10^4$	$4.2x10^5$	$1.34x10^3$

Legend: OD Optical Density; (cfu) colony forming unit (Dilution factor x plate count /ml); AU represents the Addis Ababa University isolates with the desigenited number coded as AU1=*Thiobacillus* strain; AU4 *Macromonas* strain; AU19=*Acidiphilium* strain; AU60= *Thiovulum* strain; AU14= *Thiobacterium* strain; AU 67=*Acromatium* strain; AU18=*Thiospaera* strain; AU33= *Thermothrix* strain; AU54= *Sulfolobus* strain; AU62= *Thiomicrospria* strain; AU94= *Thiospira* strain.

Plate 2. The Formation of White Scum on the Surface of the AFP system of the tannery effluent treatment plant, Modjo.

5. DISCUSSION

Different strains of sulfur oxidizing (SO) and denitrifying (DEN) bacteria were identified from the three tannery waste treatment pond systems (AFP, SFP, MP) of Modjo, Ethiopia. The data showed that bacterial density was found to vary from one pond to another. The highest number and diverse group of SO bacteria were isolated from the AFP followed by the SFP and MP systems. The combined effluent stream that was discharged from the tanning process entered into the AFP system at the base of the reactor pit where fermentation took place. This reflects the fact that the AFP was the first recipient of the raw combined effluent with high sulfur and organic waste load, and supports the growth of many a microorganisms.

The MPN count for the denitrifying was found to be higher than the SOB indicating that the data included both the sulfur-oxidizing denitrifying bacteria and the non-sulfur denitrifying ones. The latter were, however, not isolated and characterized in the present work. The distribution of the SOB and DEN bacteria in the three pond systems was found to differ along the water profile. The DEN bacteria were found to dominate the lower strata of the different ponds showing that denitrification is a process with nitrate as a final electron acceptor without the consumption of oxygen (Henze *et al.*, 1995)

With the exception of the shallow MP, the oxygen concentration decreased down the profile in all ponds favoring the denitrification process. Furthermore, the water-sludge interface is characterized by the desulphurication process and the production of reduced sulfur (H_2S, SH) that attracts the denitrifying sulfur oxidizing bacteria (Kuenen and Robertson, 1987). In general, the difference in

the distribution of the non-denitrifying and denitrifying groups of the SOB is associated with the influence of oxygen and substrate availability as described by (McEldowney *et al.,* 1993). .

The domination of the *Thiobacillus* and *Rhodomicrobium* strains in the different pond systems showed that the genera are characterized by metabolic diversity and other adaptive capability that enable them to colonize different microhabitats ((Katayama-Fujimura *et al* 1984). Some strains of these genera were also isolated from the aerobic (5-10cm), middle (120cm); and water-sludge interface (220-310cm) of the reactor systems. The distribution of the different strains through out the water profile may indicate the versatile existence of the isolates under aerobic and obligatory anaerobic conditions. This agreed with the work of Whittenbury and Dow (1977). From this result, it can be inferred that aerobic and anaerobic chemolithoautothrophic and chemoorganotrophic representatives of the two genera carry out a considerable amount of decomposition and denitrification processes at the upper and lower layers of the different pond systems.

The carbohydrate utilization tests also showed that a few strains of the genus *Thiobacillus*, *Sulfolobus*, *Thiobacterium, Thiomicdrospria* and *Thiosphaera* were unable to utilize all the carbon sources provided. These strains may represent the chemolithothrophic groups, which may have the ability to fix their carbon source from the atmosphere (Hutchinson *et. Al.*, 1969, Wood, 1991). On the other hand, the SOB that were able to utilize all the test carbohydrates may be considered as chemoorganoheterothrophs that compete and survive by oxidizing reduced sulfur and other organic compounds (Postgate, 1979; Kelly and Kuenen, 1984).

From the result obtained on salt tolerance test, it was evident that almost all isolates were found to grow on the thiosulfate medium containing sodium chloride with the exception of *Thiomicrospira* (AU91) and *Thiovulum* (AU60). The identification of several salt-tolerant strains in the system may have been associated with their physiological adaptation to the effluent that contains high concentration of sodium chloride (4000mg/l) (Issayas T., 1999) used for the soaking of the hide and skins.

The fact that *Thiobacillus* strains AU129 and AU46 isolated from the AFP were found to oxidize iron .may indicate that they may be similar to those strains that utilize iron sulfide and other sulfur conjugate heavy metals as energy source. The most important sulfur bacteria that utilize metal sulfides are *Thiobacillus thiooxidans*, and *Thiobacillus ferrooxidans*, and are used for bioleaching process in hydromethallurgy (Bridge and Johnson, 1998 and Brombacher *et. al*, 1998)

The accumulation of elemental sulfur in the form of white scum deposition (Plate 2) was observed on the surface of AFP system. Although, at present, no data has been generated on the type of sulfur oxidizing bacteria that were supposed to bring about the formation of elemental sulfur from reduced sulfur compounds, the deposition may seem to be made much effectively by chemolithothropic strains of the genus *Acidiphilium* (AU8); *Macromonas* (AU4); *Sulfolobus* (AU12); *Thiobacillus* (AU1, AU59) *Rhodobacter* (AU138); and *Rhodosprillum* (AU139) for energy release and glucose synthesis as it has been explained by Kimball (1968).

The species composition in the MP system was found to be less than the AFP and SFP systems, and dominated by non-denitrifying bacteria. The increase of the non-denitrifying bacteria, and of strains

such as, *Rhodospirillum*, and *Rhodopseudomonas* may be strictly associated with the increase in the oxygen content of the system as a result of the high algal activity. This, together with their nitrogen fixing capability may give them the competitive advantage of colonizing the nitrogen deficient MP.

One of the interesting features of the tannery wastewater at Modjo was the absence of hydrogen sulphide (Table 3) as compared to the different tannery wastes enumerated on Table 2. Hydrogen sulphide is produced in through desulphurication process under anaerobic conditions. It is a very toxic compound giving a pungent odour when released to the open environment. The decomposition activity of the SOB renders hydrogen sulphide harmless and odorless. This, likewise, may have practical application to do away with odorous processes in similar treatment plants such as abattoirs and dairy industries.

The growth rate determination result showed the very slow growth nature of SOB and even more indeed, of the denitrifying bacteria (DENB) which of course, did not exhibit any change at all. The slow growth nature of the species may be associated probably with various factors suggested as media composition, pH, biochemical limitations etc. Of these, however, being providing all the requirements, the biochemical limitations involving the reverse electron transport reaction as described by Madigan *et al.* (1997) may be considered as a possible reason for growth limitation during the incubation time of the isolates in the laboratory.

6. CONCLUSION AND RECOMMENDATION.

Microorganisms play the primary role in the decomposition and mineralization of organic compounds, and recycling of nutrients in the ecosystem. Cleaning the environment is their normal duty in the given time and space. Currently, however, due to urbanization and industrialization, there is a lot of accumulated wastes that should be removed within the limited space and time. This, therefore, necessitates an efficient waste removal system to clean the environment and the production of additional fertilizer and fuel.

Tannery industries are one of the most rapidly developing industries in the country. The industries use different chemicals and organic acids for the processing of skins and hides. Consequently, the effluents comes from these industries are rich in nitrogenous and sulfur wastes that are used to be discharged free in the water bodies and the soils. Nowadays, because of environmental concerns, attempts are being made to treat wastewaters in pond systems to remove as much BOD and COD as possible using microorganisms before they are drained into the aquatic environment. Different groups of aerobic and anaerobic microorganisms are involved in the degradation processes in the waste treatment plants. The Modjo Tannery is the pioneer to start a low cost tannery treatment plant. An integrated management of waste treatment of this plant presupposes a systematic study on the substrate and microbial quality, and type of design of the Plant.

In this study, attempt has been made to isolate and characterize sulfur oxidizing and denitrifying bacteria that are important in the removal of a substantial amount of organic and toxic wastes from the effluent. Thirty-six strains in sixteen genera of sulfur oxidizing and denitrifying bacteria were

identified. Given the limited space of the pond systems in which the effluent is accommodated, the species composition obtained is very rich. Because of the time and material constrains and the limited tests used, no genetic work and exhaustive biochemical works have been attempted.

In order to get a true picture of the SOB and DENB, the following should be covered;

1. Isolation of more of the SOB and DENB from many more tannery effluents and alkaline lakes that are rich in sulfur.

2. More isolates should be subjected to genetic and numerical taxonomy study (dendrogram) to give the true picture of the species diversity of these and other microorganisms in different industrial effluents

3. Selected SOB and DENB so as to prepare inoculum for the efficient wastewater treatment of tannery wastes should target future studies to the study of enzymology and rate of sulfur removal.

4. Attempt should be made to isolate more SOB strains that are capable of oxidizing iron and sulfur to be tested for bioleaching process in hydrometallurgy.

5. *Thiobacilli* strains were found to be the most abundant and diversified genera that require further research and identification of isolates that will use for metal recovery from low grade ore deposits and waste disposal activity. Together with this, the application of isolates for

removal of heavy metals such as chromium should be given due attention.

6. The microbial resource are germ plasms that must be maintained for further study in small or Large scale implementation program at the laboratory or exsitu case study.

7. A consortium action of methanogenic and non-methanogenic bacteria in the production of H_2S, CO_2, alcohol, methane etc. must be studied.

Index 1: Metabolic type Distribution of (SO) and Denitrifying (DENbacteria in the three Pond systems (AFP, SFP, and MP).

Strain	Type of pond	Depth in (cm)	Dissolved(DO) mg/l	Temp.(°C)	pH	Genus name
AU8	AFP	0(SWL)	0.4	18	8.2	*Acidiphilium*
AU19	AFP	20-310	0.0	17	8.2	〃
AU67	SFP	45	0.2	16.2	8.3	*Acromatium*
	MP	0(SWL)	>20.0	22.5	8.6	〃
AU76	SFP	45	0.2	16.2	8.3	〃
AU4	AFP	0(SWL)	0.4	18	8.2	*Macromonas*
	MP	0(SWL)-95	>20-0.4	15.8-22.5	8.2-8.6	*Macromonas*
AU16	AFP	20	0.0	17	8.2	〃
AU12	AFP	0(SWL)	0.4	18	8.2	*Sulfolobus*
AU54	AFP	310	0.0	17	8.2	〃
AU33	AFP	120-310	0.0	16-16.5	8.2	*Thermothrix*
	SFP	145	0.0	16	8.2	〃
AU1	AFP	0(SWL)	0.4	18	8.2	*Thiobacillus*
	MP	60	0.4	16.2	8.4	〃
AU13	AFP	20-310	0.0	16-17	8.2	〃
AU59	SFP	0(SWL)	0.8	16	8.4	〃
	MP	〃	20-0.4	17.5-22.5	8.4-8.6	〃
AU86	SFP	95	0.0	16	8.2	〃
AU129	AFP	20	0.0	17	8.2	〃
	MP	95	0.4	15.8	8.4	〃
AU46	AFP	310	0.0	16	8.2	〃
AU52	AFP	310	0.0	16	8.2	〃
AU118	AFP	20	0.0	17	8.2	〃
	MP	25-95	0.4	15.8-17.5	8.4	〃
AU14	AFP	0(SWL)	0.4	18	8.2	*Thiobactrium*
	SFP	0(SWL)-45	0.8-0.2	16.2-19	8.3-8.4	〃
AU62	SFP	0(SWL)	0.8	19	8.4	*Thiomicrospira*
AU91	SFP	145	0.0	16	8.2	〃
AU18	AFP	20-310	0.0	16-17	8.2	*Thiosphaera*
	SFP	95-145	0.0	15.8-16	8.2-8.4	〃
AU57	SFP	0	0.8	19	8.4	〃
AU94	SFP	145	0.0	16	8.2	*Thiospria*
AU60	SFP	0-45	0.8-0.2	16.2-19	8.3-8.4	*Thiovulum*
AU84	SFP	95	0.0	16	8.2	〃
AU138	AFP	0	0.4	18	8.2	*Rhodobactor*
AU200	MP	0-95	>20-0.4	15.8-22.5	8.4-8.6	*Rhodocyclus*

AU142	AFP SFP SFP MP	20-310 0-45 95-145 0-95	0.0 0.8-0.2 0.0 >20-0.4	16-17 16.2-19 16 15.8-22.5	8.2 8.3-8.4 8.2 8.4-8.6	*Rhodomicrobium* 〃 〃
AU164	AFP	310	0.0	16	8.2	〃
AU176	SFP	0(SWL)	0.8	19	8.4	〃
AU152	AFP	120	0.0	16.5	8.2	*Rhodopseudomonas*
AU153	AFP	120	0.0	16.5	8.2	〃
AU198	MP	0(SWL)	>20.0	22.5	8.6	〃
AU217	MP	95	0.4	15.8	8.4	〃
AU139	AFP SFP	0(SWL) 〃	0.4 0.8	18 19	8.2 8.4	*Rhodosprillum* 〃
AU147	AFP SFP	20 95-145	0.0 0.0	17 16	8.2 8.2	*Rrhodosprillum* 〃

Index 2. Distribution in metabolic type of Sulfur Oxidizing (SO) and Denitrifying (DEN)bacteria in the three pond systems (AFP, SFP and MP).

Genera	No of strains	AFP system	SFP system	MP system	Metabolic types		
					Aerobic	Facultative	Anaerobic
Acidiphilium	2	+	-	-	1	-	1
Achromatium	2	-	+	+	2	-	-
Macromonas	2	+	-	+	2	-	-
Sulfolobus	2	+	-	-	1	-	1
Thermothrix	1	+	+	-	-	-	1
Thiobacillus	8	+	+	+	2	4	2
Thiobacterium	1	+	+	-	1	-	-
Thiomicrospira	2	-	+	-	1	-	1
Thiosphaera	2	+	+	-	1	-	1
Thiospira	1	-	+	-	-	-	1
Thiovulum	2	-	+	-	1	-	1
Rhodobacter	1	+	-	-	1	-	-
Rhodocyclus	1	-	-	+	1	-	-
Rhodomicrobium	3	+	+	+	1	1	1
Rhodopseudomonas	4	+	-	+	2	-	2
Rhodosprillum	2	+	+	-	1	-	1
Total	**36**	**11**	**10**	**6**	**18**	**5**	**13**

Legend: '+'= present; '-' = absent

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