DEGRADATION OF NITROGEN AND CARBON BY BACTERIA ISOLATED FROM THE TANNERY EFFLUENT.

BY

SHIJI. M. G.

Reg. No. 93 PLS 07

A THESIS SUBMITTED TO THE AVINASHILINGAM INSTITUTE FOR HOME SCIENCE AND HIGHER EDUCATION FOR WOMEN (DEEMED UNIVERSITY) COIMBATORE - 641 043

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE IN LIFE SCIENCES

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CERTIFIED AS BONAFIDE RESEARCH WORK

9/5/95	9/5/95	9-5-95
Signature of the Head of the Department	**Signature of the Dean of Faculty**	**Signature of the Guide**

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Contents

CONTENTS

LIST OF TABLES

LIST OF FIGURES

Introduction

INTRODUCTION

Industrial development has increased the volume of wastewater to be disposed off, whereas the capacity of receiving water to accept and neutralise increasing loads of organic and inorganic pollution is unlimited. This results in the deterioration of the environmental quality (Kiestra and Eggers, 1986).

Pollution of water resources is a significant and rapidly increasing threat to health, well-being and prosperity of the people and to the quality of the environment (Somers,1977).

In Jhingran's view (1982) water requirement rapidly approached the limits of available supply in most cases with increasing industrialisation. The entire ecosystem may be thrown out of gear and may head towards a severe biological imbalance if the water quality of rivers and streams get degraded beyond certain limits.

Environmental pollutants from industries are substances having detrimental effect on man and his environment Environmental pollutants has been defined as 'substances' which enter the environment through human activity and which can appear in such concentrations that they become hazardous

to living things, in particular man himself'. Many of these organic compounds are difficult to degrade and persist in the environment. They are taken up by the organisms, move up in the food chains leading to bioaccumulation and magnification in higher organisms exhibiting deleterious action (Shukla, 1981).

Rapid development of industries has led to the continuous inflow of ever increasing amounts of various synthetic compounds, many of which has toxic and mutagenic properties. Some of these pollutants are rather persistent in the environment and yield slowly to microbial conversion. Accumulation of these pollutants in the biosphere may create ecological stress which is frequently the cause of regional or global contamination (Golovleva et al ., 1992).

Various kinds of industries generate pollutants of different kinds. The tanning industry like many other industry, is finding it necessary to give increasing attentions to the problems associated with the disposal of effluents (Gates and Linn, 1988).

Tanning industry in India is one of the oldest industries. It stands among five top export oriented industries (Dhaneswar, 1990). As India has largest population of cow and goat in the world, it supports major share in leather production.

There are a number of tanneries scattered all over the country but the main areas of their concentrations are Tamilnadu, Uttarpradesh and West Bengal (Arora, 1981). In Tamilnadu these units are mainly located at Vaniambadi, Dindugal, Ambur, Ranipet, Erode and Vellore.

As the tanning industry requires water, most of the industry have been located near water source or inhabituated location. Dhaneswar(1990) calculated that 30 litres of water is necessary for the production of 1 kg of leather. 314,000 tones of skin are processed and 9,420,000 m^3 effluent is discharged annually from tanning industry.

According to Arora (1981) the tannery effluents are considered to be more dangerous than all other industrial wastes. A high degree of pollution by tannery effluent contamination was recorded by Rao and Nandakumar, 1981 in Chennasamudram reservoir of Chennasamudram Village.

The effluents from tanneries have high pollution potential. The main polluting constituents of a final composited effluent are high pH value, high concentration of chlorides and sulphides, high concentration of chromium and tannins, high concentrations of oxidisable organic matter (COD and BOD) (Arora, 1981).

Tannery wastes when discharged into streams have a marked de-oxygenating effect. They greatly affect the aquatic life thereby posing a great threat to aquatic ecosystem (Eye and Lawrence, 1971). Because of discharge of untreated waste water into surface water bodies, sewers on land, these polluting components create problems and cause environmental disruption. It is therefore necessary to emphasise that these waste water need adequate treatment before they are discharged (Dhaneswar, 1990).

Besides the problem of pollution, there is another important point to be looked into. Tannery is one of the industries that require a large quantity of water daily. Such a large number of tanneries tap the ground water sources daily for their water requirement and discharge the effluents which pollute the ground water sources. This process of excessive use of ground water and simultaneous pollution of it may lead to the situation one day when such town is left with little ground water potential and whatever left is polluted so heavily, unfit for any kind of use (Rao et al., 1991).

In recent years, considerable work has been done on the characteristics of waste water from different tanneries and from different operations in a tannery (Sastry et al., 1972 and Prasad et al., 1980). The treatment of tannery waste by chemicals like alum, ferric chloride, lime and carbon-di-oxide (Bose et al., 1960) and biological treatments such as anaerobic digestion, trickling filters (Chakraborty, 1972) activated sludge process, oxidation ditch are available. These methods may not have wide application in tanneries because of the cost involved. In such a situation simple biological treatment may be adopted before final disposal of effluent (Govindan, 1985).

Bioremediation using microbes to cleanup environmental pollution is becoming the technology of choice for many application. Bioremediation is often the most cost-effective means of cleaning up a polluted or hazardous waste site (Genetic Engineering and Biotechnological monitor, 1994).

Bacteria and other microorganisms exhibit a number of metabolism dependent and independent processes for the uptake and accumulation of heavy metals and other organic pollutants. The removal of such harmful substances from effluents and waste water by microbe based technologies may provide an alternative method of waste water treatment and environmental protection (Gadd, 1990).

Many scientists and research workers (Gurujeyalakshmi and Oriel 1989 and Brunsbach and Reineke, 1990) believed that biological agents are potentially cheaper, safer and more effective for degradation of organic pollutants. In principle, strategy of selectively breeding microorganism to destroy pollutants is fairly simple. The bacteria might be deprived of their normal nutrient culture, confronting them with alternatives of developing a taste for pollutants or dying of starvation (Henis, 1987).

The energy and chemical demands of the microbial processes are less, compared to the activated sludge processes and this process is amenable to easy and faster start-up and shut down (Jayaraman, 1991).

Biological treatments has so far been carried out with the help of naturally occurring microorganisms which come into contact with the waste water. There are a number of cultures each containing several strains of bacteria having a unique contribution to offer to biological process, giving inter-related biological action (Gibson and Subramanian, 1984). It could be effectively and economically used in any conventional biological treatment. Laboratory and on site pilot plant data showed that the bacteria removed around 90% of the dissolved organic carbon (Doc) and chemical oxygen demand (COD) from the wastes (Genetic engineering and biotechnological monitor, 1994).

At present the efforts of a number of researchers promoted the establishment in this country of a collection of microorganisms able to degrade volatile toxic pollutants, widely distributed xenobiotic and ecologically hazardous pollutants.

The active strains and destructors are mainly representatives of the genera pseudomonas and Rhodococcus. Research into their physiological characteristics, key enzymes, genetic mechanisms determining the degradation of these foreign compounds and behavior of the strains in a real environment made it possible to develop the theoretical principles of using these microbial cultures to purify real industrial wastes and remediate polluted areas of soil and water (Golovleva et al., 1992).

In recent years, great attention has been paid in this country to the development of waste-free technologies, use of microorganisms for de-toxication of various pollutants and working out the microbial methods of clean-up for various plants.

Hence the present study aims at degrading the nitrogen and carbon of tannery effluent by bacteria isolated from the same effluent.

Review of Literature

REVIEW OF LITERATURE

The disposal of solid and liquid waste products resulting from industrial processes is receiving increasing attention on a global basis. The rapidly increasing demand for water for beneficial purposes has made men to assess and examine water reuse technology more seriously than ever (Singh et al., 1992). Tanning industry is a major water consuming industry and generates huge quantities of waste water which are relatively difficult to treat (Daryapurker et al.,1993). Hence the present study attempts to depollute tannery waste water using bacteria and to observe the change in values of carbon and nitrogen.

For the present study, the available literature was reviewed as follows:

Studies on tannery effluents:

Moore (1953) reported cases on record where anthraz was transmitted to human beings through water course receiving tannery waste discharge.

The toxic effects of tannery effluents on aquatic microorganisms and fishes were noted by Ray (1960).

The physicochemical characteristics of tannery wastes and their impact on water bodies were analysed by Arora et al. (1972) in Kanpur and by Kothandaraman et al. (1972) in Madras.

Miakhan and Raman (1972) reported that discharge of tannery wastes into surface water like channels and tanks have increased their salinity to objectionable levels.

Corning (1976) reported that the biological treatment of tannery effluents is feasible and can be used in any situation.

Problem of pollution of groundwater used for drinking purposes in villages situated near tanneries and textiles has been reported by Singh (1978).

Arora (1981) studied the biodegradation of effluents from tanning industry. Aerobic treatment was carried out for waste water with low concentrations of organic matter. The organic matter was broken down into final products like CO_2, H_2O, NO_2 etc.

Preliminary studies on treatment of chromium tannery waste sludge by anaerobic digestion using bacteria obtained from waste water treatment system of leather factory was studied by Haeda et al. (1984).

Srinivas et al. (1984) reported that the effluents discharged from tanneries located in North Arcot district have caused serious deterioration in the ground water Quality and consequent reduction of Quantity and Quality of agricultural products.

Govindan (1985) studied the possibility of treating tannery waste water in admixture with domestic sewage in waste stabilisation pond using acclimatised algal cultures.

The impacts of tannery effluents on the water quality and on behaviour and respiration of Macrones keletius was studied by Shahul Hameed (1985).

Saxena et al. (1986) studied the impact of tannery effluents on some pulse crops. Tannery effluent retarded the germination of pulse crops considerably.

Effect of tannery effluent on the respiratory parameters and biochemical constituents in air breathing fish Channa punctatus was studied by Jayachandran and Chockalingam (1987).

Narendar (1988) reported on the control of pollution of tannery effluents by fungi. Breakdown utilisation of tannin by fungi enhanced in the presence of additional carbon source.

Imhoffs et al. (1989) revealed that the tanning of leather produces waste water that are very strong in organic and inorganic substances. They contain biodegradable organics mainly from spent solution and inorganic toxic compounds.

The characterisation of chrome tannery waste water from goat skin production was studied by Qureshi et al (1989).

Aparna et al.(1990) studied the pollution of water by tanneries in Dindigul. The level of various physicochemical factors indicative of pollution were found to exceed the quality standards and affect the water utility pattern.

Beg et al. (1990) reported on the pollution due to tannery effluents in the korangi industrial area, Karachi. The study revealed that the uncontrolled discharge of heavily polluted tannery effluents has contaminated underground water and caused adverse effects on the immediate environment.

Dhanapal et al. (1990) reported on the toxicity of tannery effluent on the fish, Sarotherodon mossambicus. The survival time of the fish and the effluent concentrations were found to be increasingly proportional to each other.

Determination of chromium (VI) in tannery waste by the chelation - extraction method was studied by Menden et al.(1990).

The effect of tannery effluents on the respiratory metabolism of odonate nymphs has been studied by Thilagavathy and utharah (1990).

A study on the extent of pollution of ground water sources in and around the tannery units of Dindigul revealed that the amount of total solids, hardness and chlorides in all ground water sources located in and around the tannery units were several times higher than the tolerance limits (Rao et al., 1991).

Somanath (1991) studied the protein, carbohydrate and lipid levels in the tissues of the fish Labeo rohita, exposed to sublethal concentrations of tannic acid an important constituent of tannery effluents.

In vitro utilisation of chromium by the bacterial strains isolated from tannery effluent was studied by Khan et al. (1991). Treatment of tannery waste water by upflow anaerobic sludge blanket reactor was studied by Khusheed and Siddiqi, 1991.

The environmental compatibility of chromium containing tannery and other leather product wastes at land disposal sites was studied by Rutland,1991.

The treatability of chromium tannery wastes was studied by Kabdasli et al. (1993). The studies showed that activated sludge process provided limited nitrification and almost complete BOD removal while COD could only be reduced to around 500 mg/litre.

Studies on microbial degradation:

Studies on the biological denitrification of municipal and agricultural waste water were carried out by Francis and Callahan (1975). This process was found to be effective only for waste water containing relatively low concentrations of nitrate.

Studies conducted on the microbial degradation of organic components in oil shale retort water showed that the organic acids which constituted approximately 12% of the dissolved organic carbon was degraded by bacteria mainly to CO_2 (Rogers et al., 1981).

Savage et al. (1985) studied the biological treatment of organic toxic wastes using microbial degradation. The microbial breakdown of the substances was found to be brought about by the enzymes secreted by the microbes.

Randall (1986) experimented on the use of mutant bacterial cultures in waste water treatment plants. Mutant bacterial cultures were used in treatment plants and a reduction in COD values, oil and grease were observed.

Studies on the various methods of treatment of industrial waste water was carried out by Klestra and Eggers, (1986). In terms of COD and BOD reduction, energy demand etc., the biological treatment was found to be less sensitive and allowed nitrification in the same reactor.

The removal of various metals like arsenic, cadmium, lead, mercury etc. in waste water by bacteria was studied by Belliveau et al. (1987).

Gupta (1988) studied the microbial denitrification of a highly oxidised nitrogenous waste water obtained from a biological nitrification unit. A pH of 7 was found to be optimum for maximum denitrification rate.

Bengtsson et al. (1989) studied the nitrate reduction by indigenous bacteria on microcosms. Nitrate was completely reduced in the microcosm. Of this nitrate, 80-90% was converted by aerobic denitrification to N_2 whereas only 35% was denitrified in the anaerobic microcosm where more than 50% of NO_3 was reduced to ammonia.

Cheremisinoff (1990) suggested that biological treatment is typically applicable to aqueous stream with organic contaminants. In biological treatment, the microorganisms decompose the simple and complete organics to CO_2 and H_2O.

Gadd (1990) reported that bacteria and other microorganisms are capable of uptake and accumulation of heavy metals. Such bacteria can be used for the removal of harmful substances from effluents and waste water.

The degradation of organic fractions in municipal sludge by a mixture of eleven strains of thermophilic bacilli was studied by Kume and Fujio (1990).

Kasan (1993) has focussed on the development of low cost biological method for the bioremediation of industrial effluents.

A new anaerobic process which uses naturally occurring bacteria such as Pseudomonas to reduce the nitrate in the water was reported in science service (1993)

Genetic Engineering and biotechnological monitor (1994) reported that researchers at Monsanto have developed a unique process that uses immobilised bacteria to treat complex industrial waste water. The bacteria removed about 90% of the dissolved organic carbon and chemical oxygen demand from the waste.

Methodology

MATERIALS AND METHODS

Tannery is one of the major industries in Tamilnadu creating water pollution problem. The untreated tannery wastes corrode sewerlines, cause ground and surface water pollution and odour problems. These adverse effects emphasise the need to treat the wastewater before discharging them into the receiving bodies and to safe guard the limited surface and ground water sources of Tamilnadu. The treatment of wastewater is considered as a part of the total effort towards conservation of the quality of natural environment. Hence the present study is concerned with

1.Aerobic treatment of tannery effluent.

2.Biological treatment of tannery effluent with bacteria isolated from the effluent. Treatment were studied with special reference to the aerobic and biological degradation of carbon and nitrogen components of tannery wastewater.

3.Study of the survival time of the fish Oreochromis mossambicus introduced into the treated tannery effluent.

For the present study, tannery effluent was collected from a leather industry at Erode and stored in plastic cans.

The following parameters were studied for the raw tannery effluent.

Colour: The colour of the effluent was visually observed.

Odour : The odour of the effluent was observed by smelling it.

Dissolved oxygen: The dissolved oxygen in the tannery effluent was estimated by winkler's method.

Biochemical oxygen demand: The BOD in the tannery effluent was estimated as per Indian standard 1966, Part I

Chemical oxygen demand : The COD in the tannery effluent was estimated as per Indian standard, 1976, Part V

Hexavalent chromium : The hexavalent chromium in the effluent was estimated as per Indian standard, 1968, part II

Nitrate nitrogen: The nitrate nitrogen in the tannery effluent was estimated by colorimetric method.

500 ml of the effluent was taken in an ammonia distillation apparatus. 50ml of 10% . Sodium hydroxide was added and evaporated to about 200ml.

The solution was cooled. Then 3 gms of finely ground Devarda's alloy and 30ml of 10%. Sodium hydroxide were added to it. The flask was connected with a vertical condenser immediately. The outlet of the condenser was dipped into a receiver containing 200 ml of 0.2% ammonium sulphate. The solution was distilled at 50 - 80 ^{o}C for one hour. The receiver was disconnected and the volume of the solution in the receiver was made up to 250 ml.

5-10 ml of the aliquot was taken in a 50 ml volumetric flask and neutralised to pH 4.5 using 0.01N Hcl. Then 2 ml of Nessler's reagent was added and the absorbance was estimated at 424 nm in a spectrophotometer.

This method is valid for Nitrate concentrations greater than 0.5 ppm, while for concentrations exceeding 5 ppm, the distillate is directly back titrated with standard alkali (0.2N NaoH) using methyl red as indicator.

Nitrite nitrogen

The nitrite nitrogen in the tannery effluent was determined as per Indian standards 1968, Part II.

40 ml of the sample was taken in a volumetric flask and pH was adjusted to 7.0 using 0.01N Hcl or 0.01N NaoH. 2ml of sulphanilamide solution was added to the sample, shaken well and allowed to stand for 10 minutes. 2ml of 1-naphtylamine-7-sulphonic acid solution was added, diluted to 50ml, mixed thoroughly and within two hours the resulting purple azo dye was measured at 543nm.

Ammoniacal nitrogen

The ammoniacal nitrogen in the tannery effluent was estimated by alkaline permanganate method.

20ml of the sample was taken in a distillation flask. Few glass beads were added to the distillation flask to avoid frothing and bumping. 100ml of 0.32% potassium permanganate and 100ml of 2% sodium hydroxide were added to the sample. The sample was distilled and the distillate was collected in 20ml of 2% boric acid with the double indicator (Methyl red/methyl blue). 30 ml of the distillate was collected.

The collected ammonia in boric acid was titrated with N/50 sulphuric acid.

Ammoniacal nitrogen was calculated using the formula

$$\% \text{ of Ammoniacal nitrogen} = \frac{\text{titre value} \times 0.00028 \times 100}{\text{Volume of sample}}$$

where, 1ml of N/50 H_2SO_4 = 0.00028 gN.

Total Nitrogen

The total nitrogen in the tannery effluent was estimated by the Kjeldahl method.

100 ml of the sample was taken in a Kjeldahl flask. 10 ml of conc. Sulphuric acid and 1 ml of 10 % copper sulphate solution were added to the flask. A few glass beads were added and boiled until the solution was clear. The solution was digested for additional 30 mts and allowed to cool. The contents of the flask were transferred to a distillation flask and diluted to 300 ml. The solution in the flask was made alkaline with 50 % Sodium hydroxide using Phenolphthalein indicator. The solution was distilled after immersing the tip of the condenser in 50 ml of 2% boric acid solution taken in a conical flask. About 200 ml of the distillate was collected.

0.5 ml of mixed indicator (methyl red/ methyl blue) was added into the distillate. It was titrated against 0.02 /n

sulphuric acid. At the endpoint, the colour changed from pale green to lavender. The same experiment was conducted with a blank. The total nitrogen was calculated using the formula:

$$\text{mg/l total nitrogen} = \frac{(\text{ml } 0.02\text{ N. } H_2SO_4 \text{ for sample} - \text{ml } 0.02\text{ N } H_2SO_4 \text{ for blank}) \times 0.28 \times 1000}{\text{ml of sample taken for determination}}$$

Organic carbon

The organic carbon present in the effluent was estimated by walkley and Black method

About 10 to 15ml of the effluent was added into a 500ml conical flask. 10ml of 1N potassium dichromate solution and 20ml of concentrated sulphuric acid were added into the flask and mixed by gentle swirling. the flask was left to stand on a sheet of asbestos for 30 minutes. Then 200ml of distilled water and 10ml of phosphoric acid were added to it. 1ml of diphenylamine was added as an indicator. The colour changed to bluish purple. The contents of the flask was titrated against ferrous ammonium sulphate. At the end point, the blue colour disappeared and the colour changed to brilliant green. The organic carbon was estimated using the formula

$$\% \text{ of organic carbon} = \frac{V_1 - V_2 \times 0.003 \times 100}{\text{Volume of sample}}$$

Where,

V_1 - Volume of potassium dichromate

V_2 - Volume of ferrous ammonium sulphate.

Tap water was used to dilute the tannery effluent. The following parameters DO , BOD , COD , alkalinity, salinity, nitrogen and carbon were determined for the tap water.

Preparation of diluted effluent

The tannery effluent was diluted with tap water in the ratio 1:10. To 4.5 litres of tap water 500ml of the effluent was added.

The parameters studied for the raw effluent were also studied for the diluted effluent and diluted effluent aerated for 8 hrs, 16hrs and 24 hrs.

Bacterial culture

Isolation of bacteria from the tannery effluent

Bacteria was isolated from the tannery effluent by Winogradsky's enrichment culture technique.

Enrichment culture technique

1. 100gms of garden soil was taken in a beaker.
2. The effluent was added to the soil till it was soaked with the effluent.
3. The beaker was incubated for 7 days at 30 ^{o}C or at room temperature.
4. The effluent was added whenever the soil becomes dry.

5. After the 7th day, 5gms of the soil sample was taken and transferred to a 100ml distilled water blank, stirred and shaken well and plated 1ml of the suprnatent onto a rich medium like yeast extract glucose agar medium or nutrient glucose agar medium. While preparing the medium filtered effluent was used in place of water.

6. The particulates were incubated for 3-5 days and carefully isolated the bacterial colonies on the petriplates.

Bacterial colonies on petriplates

After 3-5 days bacterial colonies were found in petriplates (Fig.1). viable bacterial colonies were counter and noted.

Preparation of agar slant

With the help of sterilised inoculation loop bacterial colonies from petrilates were transfered into test tube containing same agar medium. After 3-5 days bacterial colonies were found in the agar slant (Fig.2).

Bacterial slide preparation

From this agar slant one unit of bacterial colonies was taken using sterilised, inoculation loop and introduced into the test tubes containing 10ml sterile distilled water. From this 2ml of sterile distilled water containing bacteria were taken and mixed with 5ml sterile distilled water ~~in other~~ in other test tube. One drop of this was spread on the glass slide and fixation of the slide was done. Few drops of

ammonium oxalate,crystal violet or saffranin was used to stain the bacterial colony. After two minutes, it was washed with tap water and examined under microscope (Fig.3).

Transfered the bacteria from agar slant to conical flask

From the agar slant, bacteria were transfered with the help of sterilised inoculation loop into 250 ml conical flask containing some agar medium (Fig.4).

Transfer of bacteria into tannery effluent

100ml of sterile distilled water was added into the flask where the microorganisms were growing. After stirring with a needle, the cells were collected in a 500ml flask. The collected cells were diluted to 500ml and this constituted the innoculum.

To five litres of the diluted tannery effluent, 500ml of the innoculum containing the bacterial cells were added and taken in an experimental set-up. Three experimental set-up were prepared and were aerated for 8 hrs, 16hrs and 24 hrs respectively (Fig.5). The physicochemical parameters - D.O, BOD, COD, hexavalent chromium, nitrogen and carbon were determined for the treated effluent.

The fish Oreochromis mossambicus (Tilapia) of fingerling size were brought from singanallur pond and acclimatised to the laboratory conditions for one week. The fish Tilapia was selected because it is sturdy and gets easily acclimatised to laboratory conditions.

In order to study the effect of aerobically and biologically treated effluent on the survival of the fish, 6 fishes were introduced into each experimental set up. Simultaneously 6 fishes were introduced into the untreated effluent. A control was also maintained. The survival time of the fishes were observed.

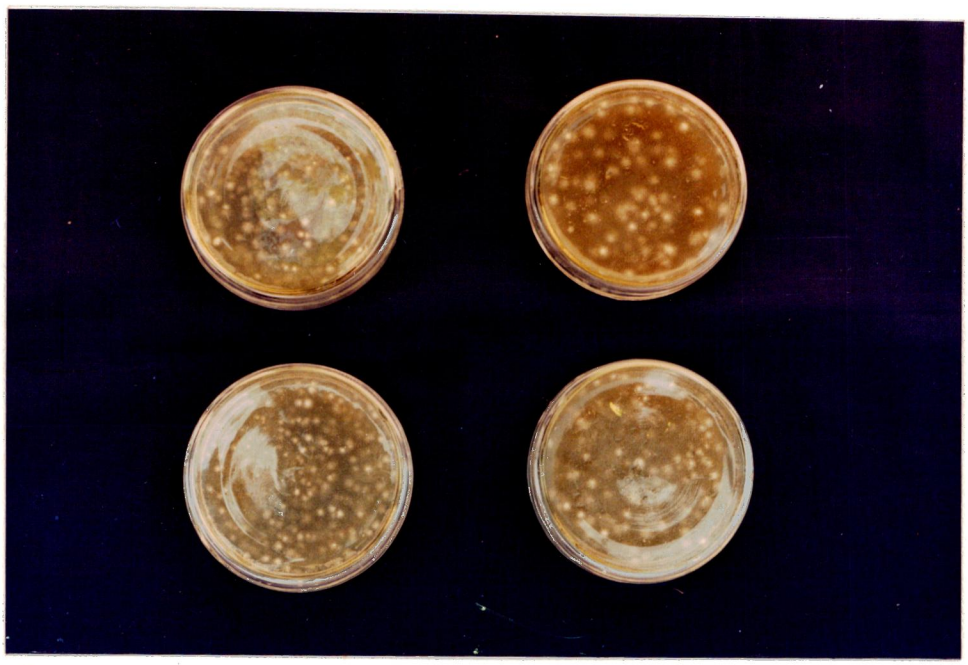


Fig. 1. Petriplates containing viable bacterial colonies.

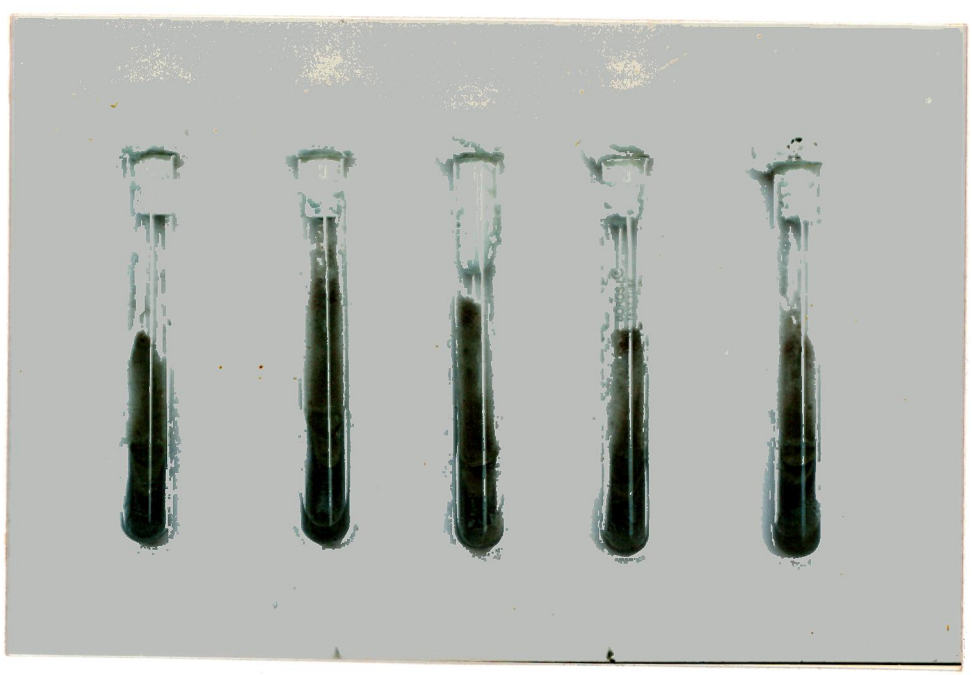


Fig. 2. Bacterial colonies in agar slants.

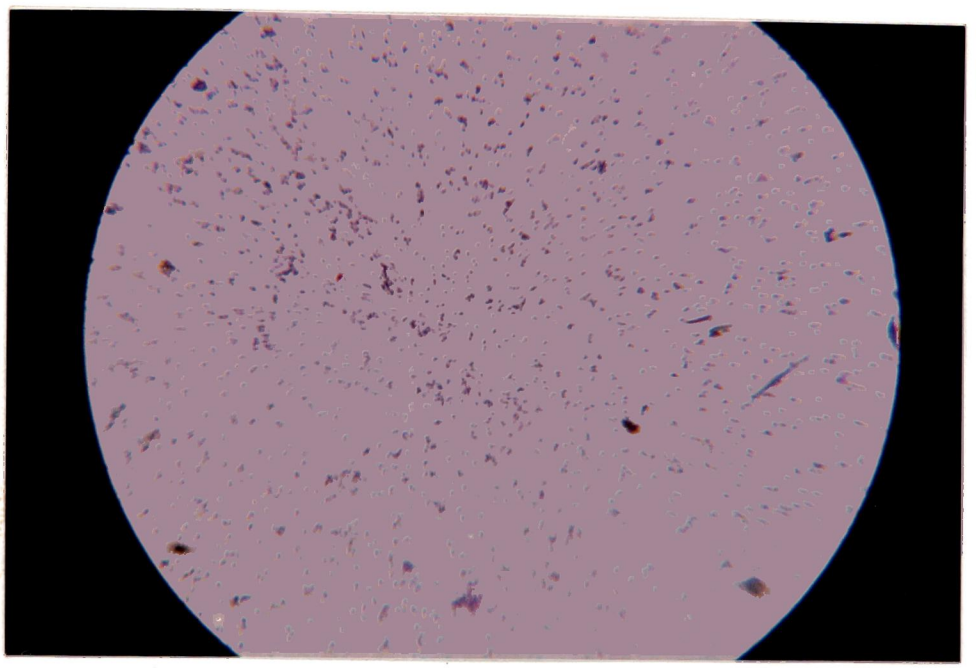


Fig. 3. Different types of bacteria examined under the microscope.

Fig. 4. Conical flask with agar medium containing one unit of bacteria.

Fig. 5. Tannery effluent treated with bacteria for 8 Hrs, 16 Hrs and 24 Hrs.

Results and Discussion

RESULTS AND DISCUSSION

The leather tanning industry is one of the most significant polluters in terms of both toxic and conventional parameters. The effluents from tanneries at Erode, on the banks of river cauvery is discharged into a common effluent canal and mixed with the river near Erode (Dhanapal et al.,1990) (Fig 6,7and 8).

For the present study, tannery effluent was collected from Erode in plastic containers. The physical parameters of the tannery effluent like colour and odour were noted. The tannery effluent was found to be olive green in colour and had an offensive foul smell. The chemical parameters like Total nitrogen, ammoniacal nitrogen, nitrate and nitrite nitrogen, organic carbon, dissolved oxygen, biochemical oxygen demand, chemical oxygen demand and hexavalent chromium were studied for the raw effluent. The results were given in Table I.

The raw tannery effluent was found to contain high amounts of the chemical parameters studied. This high level was due to the various chemicals used during the processing and finishing of leather.

Similar observations were made by Mahadevan and Muthukumar (1980). They reported that tannery effluent was

brownish in colour and had highly disagreeable foul odour. The effluent contained high amounts of organic and inorganic materials. High concentrations of chromium was also observed.

Barnhart (1978) reported that tannery effluents contain sodium sulphide, chromium and other tanning agents that remove oxygen from the receiving water and give it an unpleasant odour.

According to sengul and Gurel (1993) the composition of wastewater from the tanning industry contains pollutants from the hides, products formed from their decomposition and chemicals and various solutions used for the preparation of the hides and during tanning process.

Aparna et al. (1990) reported that the levels of various physicochemical factors indicative of pollution in the tannery effluent were found to exceed the quality standards.

Dhanapal et al. (1990) reported high levels of Sulphate, chlorides, total chromium, total suspended solids in the tannery effluent.

The characteristics of chromium tannery waste was studied by Kabdasli et al. (1993). Their results showed high values of sulphide, chromium, BOD, COD, oil and grease

in the untreated effluent. The waste water was also found to contain nitrogen which partly originates from the chemical used in delimising and dyeing processes.

Though pollution potential was based on many physiochemical and toxic parameters the priority given in this study is for organic carbon and nitrogen.

In the present study the raw effluent was diluted with tap water in the ratio 1:10. The chemical parameters of the tap water were studied and given in table III. The chemical parameters like total nitrogen, ammoniacal nitrogen, nitrate, nitrite nitrogen and organic carbon were studied for the diluted effluent and the results were given in Table II.

The chemical parameters analysed for the diluted effluent were compared with the raw effluent and given in table IV and fig.9.

The total Nitrogen in the diluted effluent decreased by 12.33%. The ammoniacal nitrogen in the diluted effluent decreased by 29.50%. The organic carbon decreased by 57.37%. The decrease in the values indicate that dilution brings about a reduction in the pollution potential to a certain extent.

Studies on the fish survival in industrial effluents by Alam et al. (1991) showed 100% mortality in fishes exposed to raw effluent while fishes suffered less mortality in effluents diluted by 50%. This study shows that the growth of the fish in diluted effluent was due to low concentrations of chemicals in the diluted effluent.

The diluted effluent was aerated for 8hrs, 16 hrs and 24 hrs respectively. After aeration the chemical parameters analysed for the diluted effluent were analysed for the aerated effluent and the results were given in table V and fig. 10,11 and 12.

The chemical parameters analysed for the aerated effluent were compared with the parameters for diluted effluent and the comparison was given in table VI and fig.13.

As compared to the diluted effluent, the level of total nitrogen decreased by 66.18% in 8 hrs aerated effluent and by 86.39% and 88.39% in 16 and 24 hrs aerated effluents.

The level of ammoniacal nitrogen decreased by 60.59% in 8 hrs aerated effluent and by 87.5% and 90.21% in the 16 hrs and 24 hrs aerated effluent respectively.

The reduction in the level of nitrate was by 58.88% in 8 hrs aerated effluent, 53.39% in 16 hrs and 49.01% in 24 hrs aerated effluent respectively.

The decrease in the level of total nitrogen, ammoniacal nitrogen and nitrate nitrogen in the aerated effluent shows that nitrogen in the waste water is removed during aeration.

The nitrite nitrogen was found to be absent in the raw, diluted and aerated effluents. This is because nitrite is unstable and soon gets converted to nitrate.

The organic carbon decreased by 37.44%, 49.76% and 63.50% in 8hrs, 16 hrs and 24 hrs aerated effluent. During aeration the organic carbon is oxidised to CO_2 and water. This accounts for the decrease in carbon with increase in hours of aeration.

Similar studies on the removal of organic carbon and nitrogen during aeration were reported by earlier workers.

Sin and Chiu (1987) observed that aeration can remove forms of inorganic nitrogen from sewage effluents.

Arora (1981) observed that aerobic treatment of the tannery effluents brings about oxidation of organic matter into final products like CO_2, water and NO_2.

Similar results for other parameters like sulphides, chromium and BOD were also reported.

Studies on the treatability of leather industry waste water by Sengul and Gurel (1993) revealed that sulphides can be removed by aeration in the presence of a catalyst.

Dhaneswar (1990) reported that the BOD in the tannery effluent reduced from 821 mg/lit to 80 mg/lit after 24 hrs aeration. Sulphides were completely eliminated with 12 hrs aeration and complete removal of chromium was achieved with 6 hrs aeration.

Bacteria isolated from the chrome tannery effluent were used in the treatment process. They belong to three different genera namely Pseudomonas, Bacillus and Escherichia. The bacteria were obtained from the effluent by Enrichment culture technique.

A number of researchers used the bacteria isolated by enrichment culture technique to treat waste water. Singh and seth (1989) studied the degradation of malathion from industrial effluents by bacteria isolated by enrichment culture technique. Francis et al. (1976) isolated a species of Pseudomonas from sewage capable of utilising DDT analogues by the enrichment culture technique.

The bacteria belonging to the genus Bacillus and Pseudomonas are strict aerobes while Escherichia consists of aerobes as well as facultative anaerobes.

The diluted effluent was treated with bacteria isolated from the effluent and aerated for 8hrs, 16 hrs and 24 hrs respectively. After treatment the chemical parameters like

ammoniacal nitrogen, nitrate nitrogen, nitrite nitrogen and organic carbon were analysed and the results were given in Table VII and fig.14,15 and 16. The table shows a gradual decrease in the level of ammoniacal nitrogen. From the table it is also evident that as the level of ammonia decreases, there is a corresponding increase in the level of nitrate. The decrease in the level of ammonia and the increase in the level of nitrate are negatively correlated (fig.17). There is a high degree of negative correlation between the two parameters.

Nitrogen in the raw wastewater is usually in the ammonia form. Virtually all organic nitrogen compounds entering the environment from the industries are degraded to ammonia. Ammonium is the major source of nitrogenous waste released into streams, lakes and estuaries (Goering,1970). This accounts for the high concentration of ammoniacal nitrogen in the raw effluent.

The presence of ammonia in fresh water represents a problem, because it is the primary cause of eutrophication, is toxic to animals and gives an offensive smell. Sin and Chiu (1987) found that fish kills in commercial ponds in Hong Kong were mainly caused by excessive manuring and the collapse of algal populations, resulting in oxygen depletion and ammonia concentration.

A major environmental problem is the removal of nitrogen from waste water. In the present study during treatment with bacteria for 8,16 and 24 hrs the level of ammoniacal nitrogen gradually decreases and there is a corresponding increase in the level of nitrate nitrogen. The transformation of ammonia to nitrate results from nitrification which occurs in the aerated water treated with bacteria.

In well aerated water nitrifying bacteria rapidly oxidises ammonia to nitrate, a process that is beneficial in waste water treatment plants. The process of nitrification and denitrification provides the basis for the safe disposal of sewage and the supply of re-usable water. Microorganisms are essential for both the formation and interconversion of nitrogen compounds in the environment. Ammonia is rapidly oxidised in any well aerated environment by nitrifying bacteria which convert it first to nitrite and ultimately to nitrate (Cole, 1993).

Randall (1986) has introduced a nitrifying culture of mutant bacteria in waste water treatment plants to promote refinery nitrification. He found the system nitrifying at better than 95% efficiency within 30 days after the addition of nitrifying culture.

Landreth (1989) also reported the importance of cultured bacteria in improving the performance of biological treatment plant in the removal of ammonia. He reported that ammonia is degraded to nitrates in an aerobic system.

Nitrification potentially use large quantities of oxygen and at times it produces oxygen-depleted water in streams, lakes and estuaries subjected to large amounts of high nitrogen wastes (Goering, 1970).

Yet another process employed in wastewater treatment plant for the removal of nitrogen is denitrification. The term denitrification refers to the reduction of nitrates to gaseous end products like NO, N_2O and N_2 in which nitrates serve as the essential hydrogen acceptor enabling bacterial growth.

The denitrification reaction in aquatic environment occurs in two separate steps. In step-I nitrate disappears and nitrite accumulates until nitrate concentrations are low or nitrate is depleted. In step-II nitrite is further degraded to gaseous nitrogen.

Oxygen is known to be a potent inhibitor of the denitrification process, by virtue of its effective competition with nitrate as an electron acceptor in the

energy metabolism of cells (Delwiche, 1956). Rae and Skerman (1957) also observed that with low oxygen concentration, localised anoxic zones exist which are the active sites of denitrification.

Since oxygen is the potent inhibitor of denitrification we consider that the reduction in the level of ammonia observed during treatment with bacteria under aerobic condition is most probably as a result of nitrification which occurs under oxic conditions in well aerated water.

In the present study observations made on the level of organic carbon in the treated effluent showed a decrease from 15 mg/l in 8 hrs treated effluent to 12.8 mg/l in 16 hrs treated effluent and 10 mg/l in 24 hrs treated effluent. The decrease in the level of carbon with increase in hours of treatment indicates that carbon has been degraded by bacteria.

Similar studies on the degradation of organic carbon by microorganisms were carried out by a number of researchers.

Cheremisinoff (1990) reported that microorganisms rely on enzymes for organic decomposition. In aerobic biological treatment, simple and complex organics were eventually decomposed to CO_2 and water.

Researchers at Monsanto (genetic Engineering and Biotechnology Monitor,1994) developed a strain of bacteria to treat industrial wastewater. The bacteria removed around 90% of the dissolved organic carbon and COD from the wastes.

Gauger and williams (1987) found out that micro organisms were cultivated to yield a specific product (eg.antibiotic) which will remove certain undesired constitutents like organic carbon in waste water.

When aerobic microorganisms are brought into contact with degradable organic matter, under suitable physical and chemical conditions, part of the organic matter is oxidised to form CO_2 and H_2O while the remainder serves as raw material for the synthesis of new cell protoplasm (Robinson,1974,1977).

Studies on the biodegradation of tannery effluents by Arora (1981) showed that bacteria like Bacillus, Pseudomonas, Nitrosomonas, Flavobacterium etc. were capable of aerobic breakdown of organic matter present in the waste water.

Studies on the microbial degradation of organic components in oil shale retort water by Rogers et al. (1981) showed that 25-30% of DOC of retort water was aerobically degraded to CO_2.

Studies by Kume and Fujio (1990) revealed that organic fractions in the sewage sludge was digested by thermophilic Bacilli. The bacteria was found to secrete some lytic enzymes which digested the organic fractions.

Bacteria are important in waste water treatment because bacterial cultures can be used to remove organic material and some undesirable minerals from waste water (Schroeder, 1977).

The survival time of the fish Oreochromis mossabicus in the 8hrs, 16 hrs and 24 hrs treated effluent was observed and given in table VIII and fig.18.

The observations showed that fishes survived for a maximum of 12 days in 24 hrs treated effluent. The fishes introduced into 16 hrs treated effluent survived for 8 days and the fish in 8 hrs treated effluent survived for about 6 days. This shows that prolonged treatment decreases the toxicity of the effluent, resulting in an increase in the fish survival time.

Similar studies on the survivability of fishes in different concentrations of the effluent were studied by Dhanapal et al. (1990). The results showed that the survival time of the fish and the effluent concentrations were inversely proportional to each other.

The culture of silver carp, big head, grass carp and common carp in oxidation ponds treating secondary effluents of a pilot sewage treatment plant was studied by Sin and Chiu (1987). The study revealed that the maintenance of a good quality of effluent would help to minimize fish mortality and maximise fish survival and growth and production of stocked fish.

Table I

Physico Chemical Parameters of Raw Tannery Effluent

No.	Parameters	Observations
1.	Colour	Olive Green
2.	Odour	Offensive
3.	Total Nitrogen mg/l	546.5
4.	Ammoniacal Nitrogen mg/l	522.0
5.	Nitrate Nitrogen mg/l	16.0
6.	Nitrite Nitrogen mg/l	Absent
7.	Organic Carbon mg/l	49.5
8.	Dissolved Oxygen mg/l	Absent
9.	Biochemical oxygen demand mg/l	887.0
10.	Chemical oxygen demand mg/l	937.9
11.	Hexavalent Chromium mg/l	12.5

Table II

Chemical Parameters analysed in the diluted tannery Effuent

No.	Parameters	Observations in mg/l
1.	Total Nitrogen	497.13
2.	Ammoniacal Nitrogen	368.0
3.	Nitrate Nitrogen	36.48
4.	Nitrite Nitrogen	Absent
5.	Organic Carbon	21.1

Table III

Chemical Parameters of Tap Water Used for the dilution of raw tannery effluent

No.	Parameters	Observations in mg/l
1.	Total Nitrogen	35.0
2.	Ammoniacal Nitrogen	30.0
3.	Nitrate Nitrogen	2.0
4.	Nitrite Nitrogen	Absent
5.	Organic Carbon	1.5
6.	Dissolved Oxygen	7.5
7.	BOD	40.0
8.	COD	210.0
9.	Hexavalent Chromium	Absent

Table IV

Comparison of chemical parameters analysed in the diluted effluent with that of raw tannery effluent

No.	Parameters	Observation in mg/l Raw tannery effluent	Diluted tannery effluent	Percentage Increase/Decrease
1.	Total nitrogen	546.5	479.13	12.33
2.	Ammoniacal Nitrogen	522.0	368.0	29.50
3.	Nitrate Nitrogen	16	36.48	56.14
4.	Nitrite Nitrogen	--	--	--
5.	Organic Carbon	49.5	21.1	57.37

Table V

Chemical Parameters analysed in the aerated tannery effluent

No	Parameters	8 Hrs aerated effluent	16Hrs aerated effluent	24 Hrs aerated effluent
1.	Total Nitrogen	162.0	65.2	55.6
2.	Ammoniacal Nitrogen	145.0	46.0	36.0
3.	Nitrate Nitrogen	15	14	18.6
4.	Nitrite Nitrogen	--	--	--
5.	Organic Carbon	13.2	10.6	7.7

Table VI
Comparison of chemical parameters analysed in aerated effluent with that of diluted effluent

No.	Parameters	Observations In mg/l				Percentage Increase/Decrease		
		Diluted effluent	8 Hrs aerated effluent	16 Hrs aerated effluent	24 Hrs aerated effluent	In 8 Hrs aerated effluent	In 16 Hrs aerated effluent	In 24 Hrs aerated effluent
1.	Total Nitrogen	497.13	162.0	65.2	55.6	66.18	86.39	88.39
2.	Ammoniacal Nitrogen	368.0	145.0	46.0	36.0	60.59	87.5	90.21
3.	Nitrate Nitrogen	36.48	15.0	17.0	18.6	58.88	53.39	49.01
4.	Nitrite Nitrogen	--	--	--	--	--	--	--
5.	Organic Carbon	21.1	13.2	10.6	7.7	37.44	49.76	63.50

Table VII

Chemical Parameters analysed in the treated tannery effluent

No.	Parameters	Observations in mg/litre		
		8 Hrs treated effluent	16 Hrs treated effluent	24 Hrs treated effluent
1.	Ammoniacal Nitrogen	92.0	64.0	46.0
2.	Nitrate Nitrogen	22.0	50.4	72.0
3.	Nitrite Nitrogen	---	--	---
4.	Organic Carbon	15	12.8	10

Table VIII

Percentage of survivability of fish in the treated effluent

Days	Percentage of survivability In the treated effluent		
	8 Hrs	16 Hrs	24 Hrs
1.	100	100	100
2.	100	100	100
3.	83	100	100
4.	83	83	100
5.	83	83	100
6.	83	83	100
7.	0	83	100
8.	0	83	67
9.	0	0	67
10	0	0	67
11.	0	0	67
12.	0	0	67
13.	0	0	0

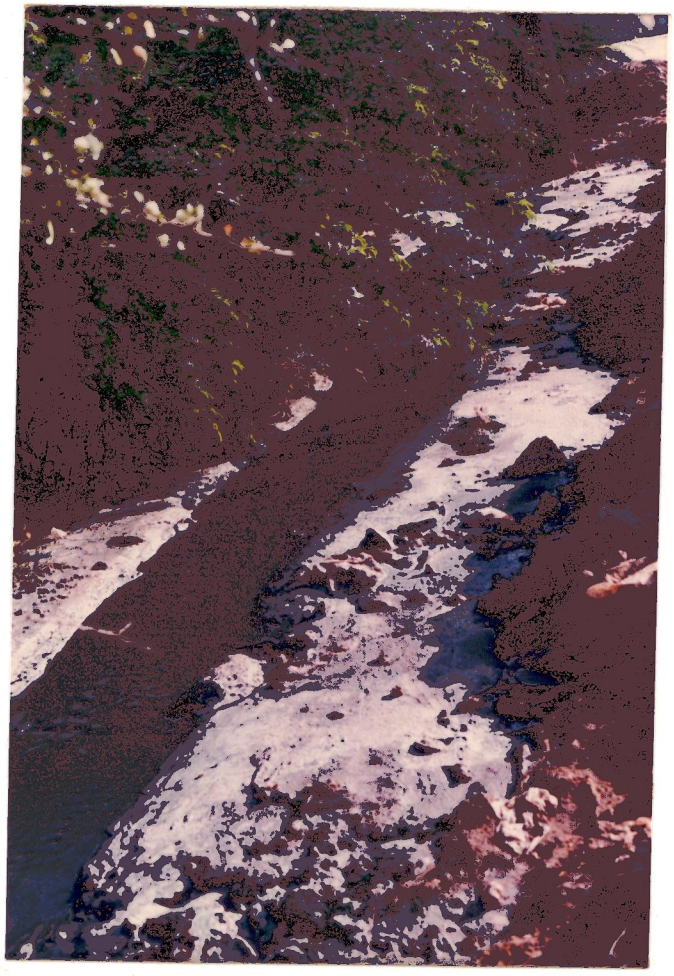


Fig. 6. Common effluent canal carrying effluent from tanneries.

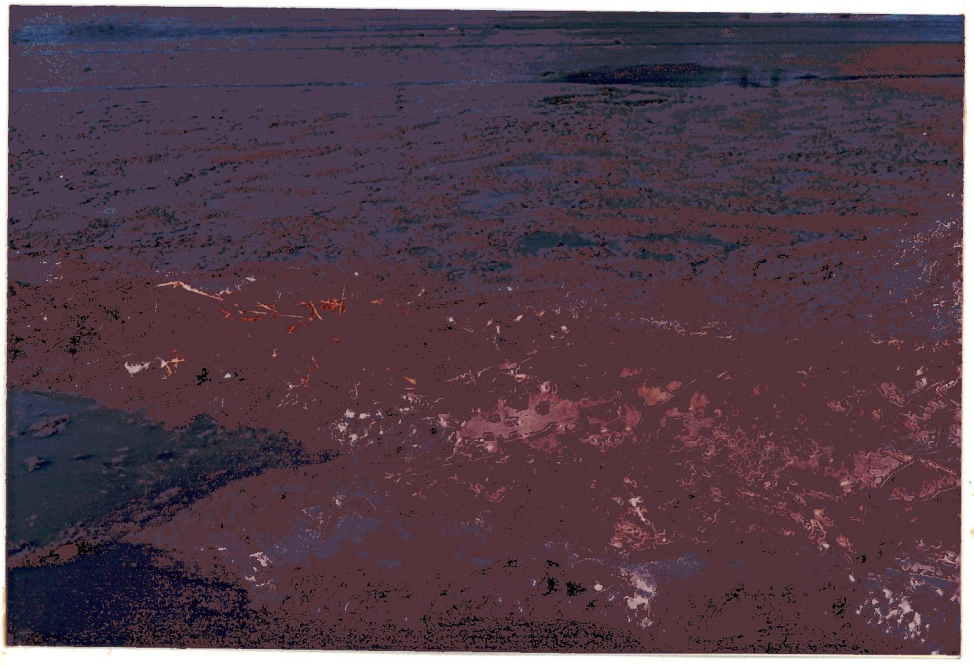


Fig. 7. Mixing of tannery effluent with river water.

Fig. 8. Water bodies polluted by tannery effluent.

Fig. 9. Comparison of total nitrogen, ammoniacal nitrogen and organic carbon in the raw and diluted effluent.

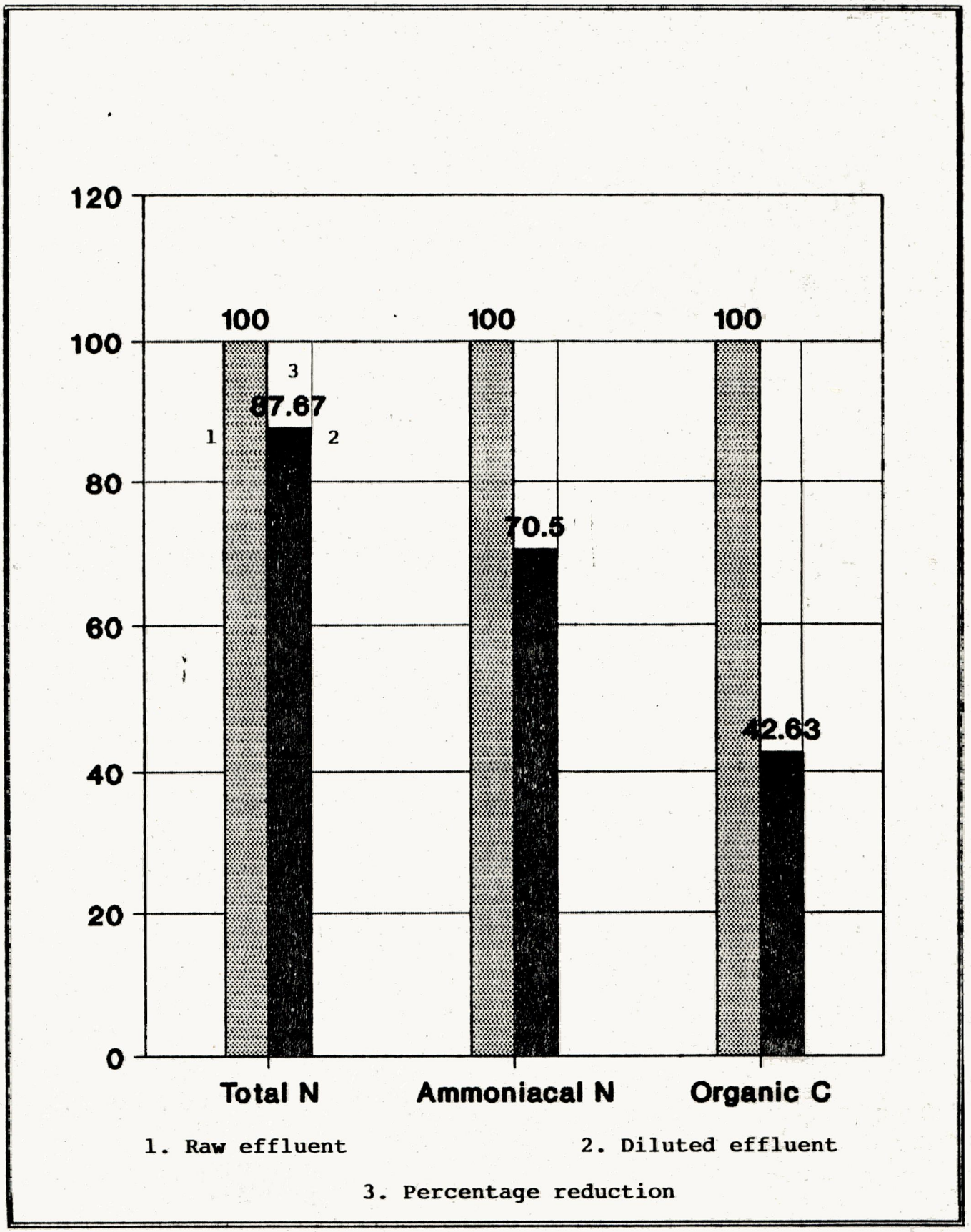


Fig. 10. Decrease in total nitrogen during aeration for 8, 16 and 24 hours.

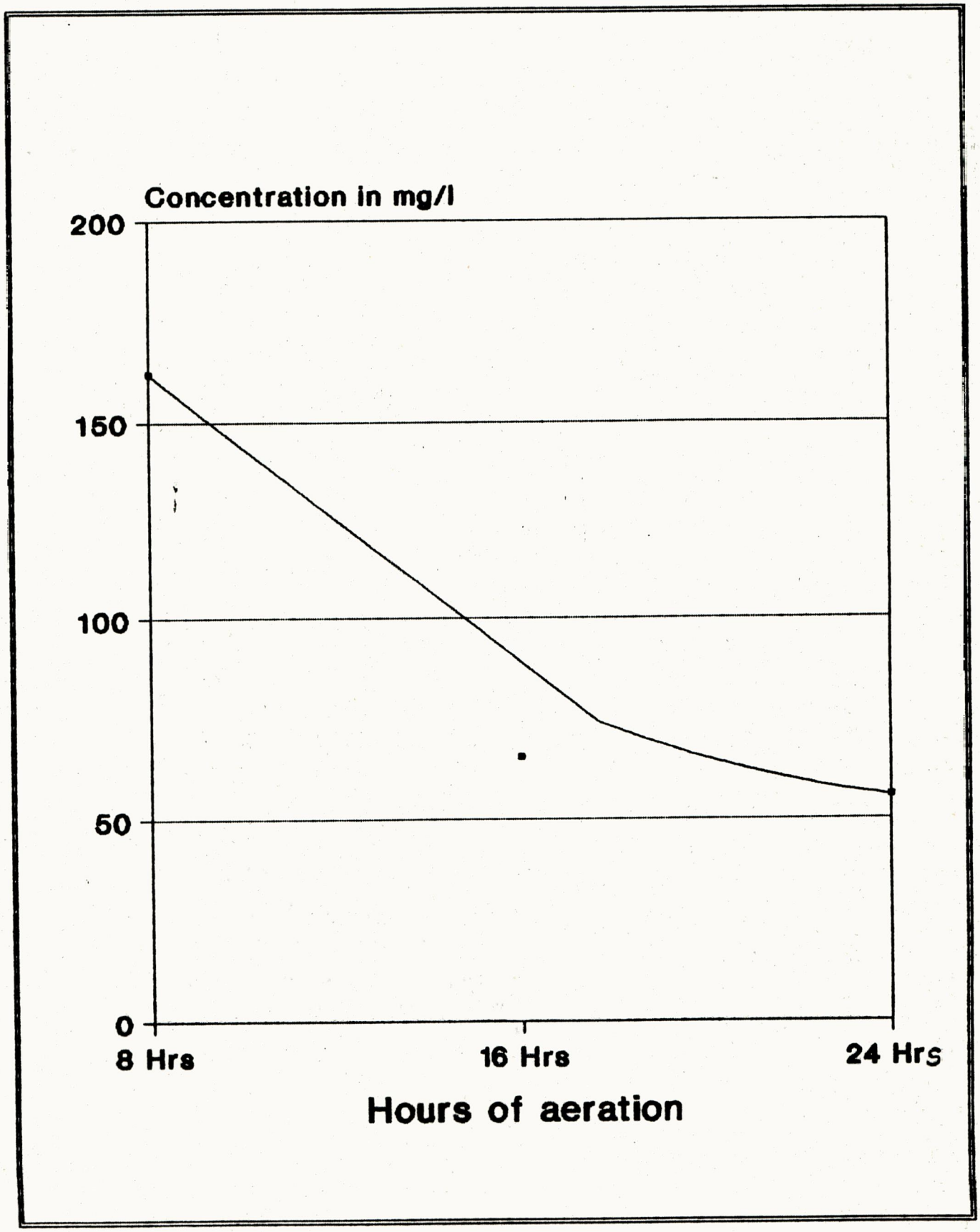


Fig. 11. Decrease in ammoniacal nitrogen during aeration for 8, 16 and 24 hours.

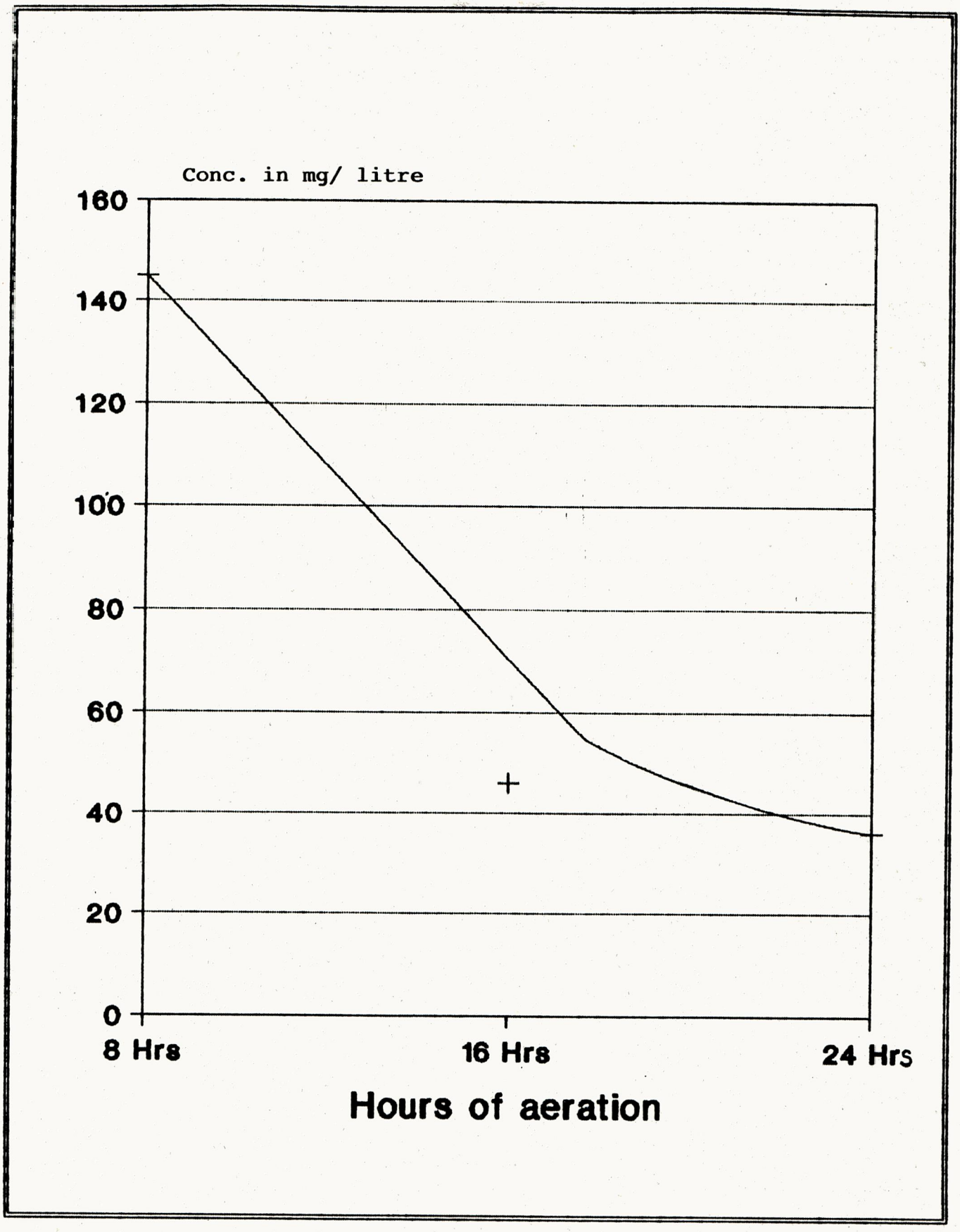


Fig. 12. Decrease in organic carbon during aeration for 8, 16 and 24 hours.

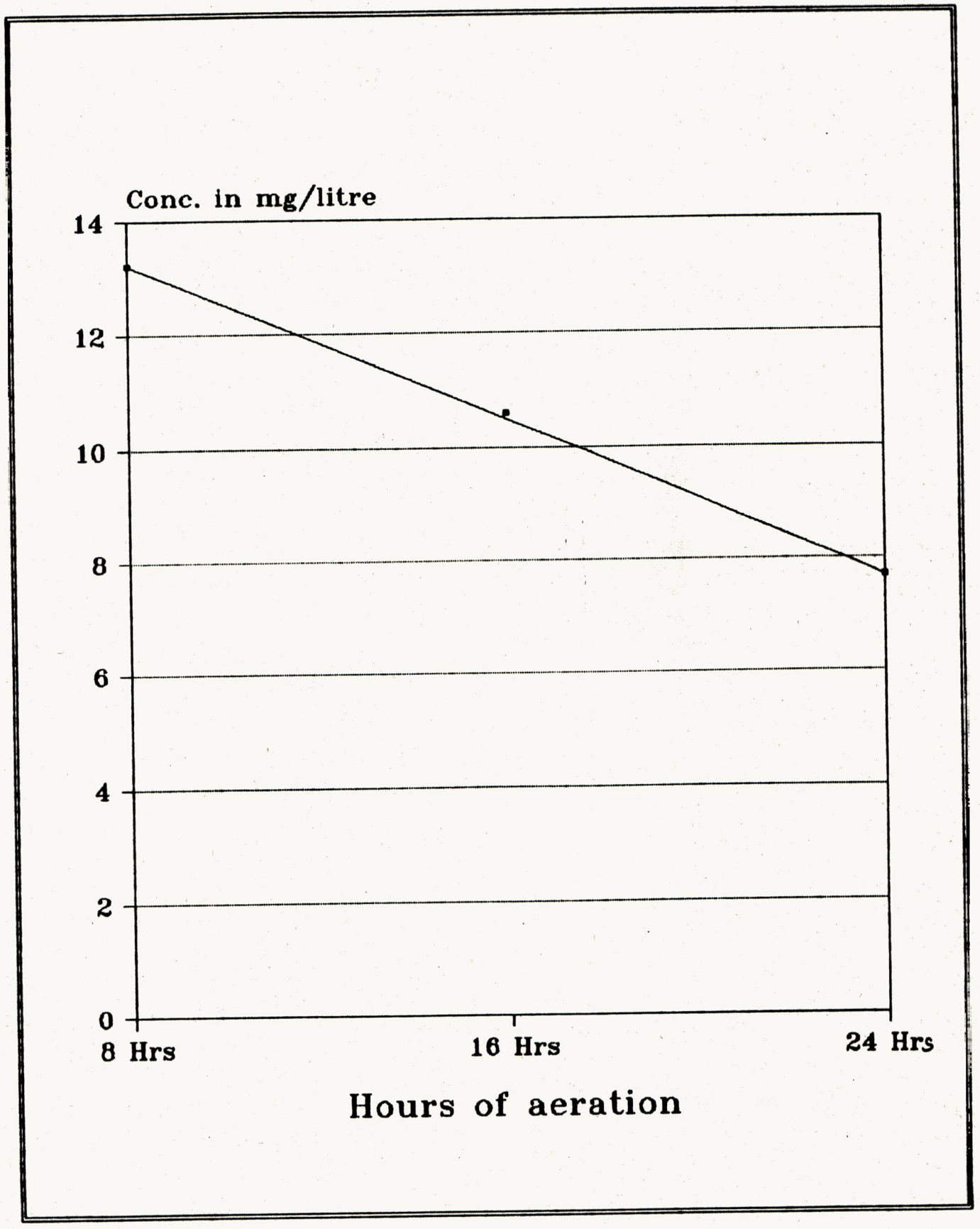


Fig. 13. Comparison of total nitrogen, ammoniacal nitrogen and organic carbon in the diluted and aerated effluent.

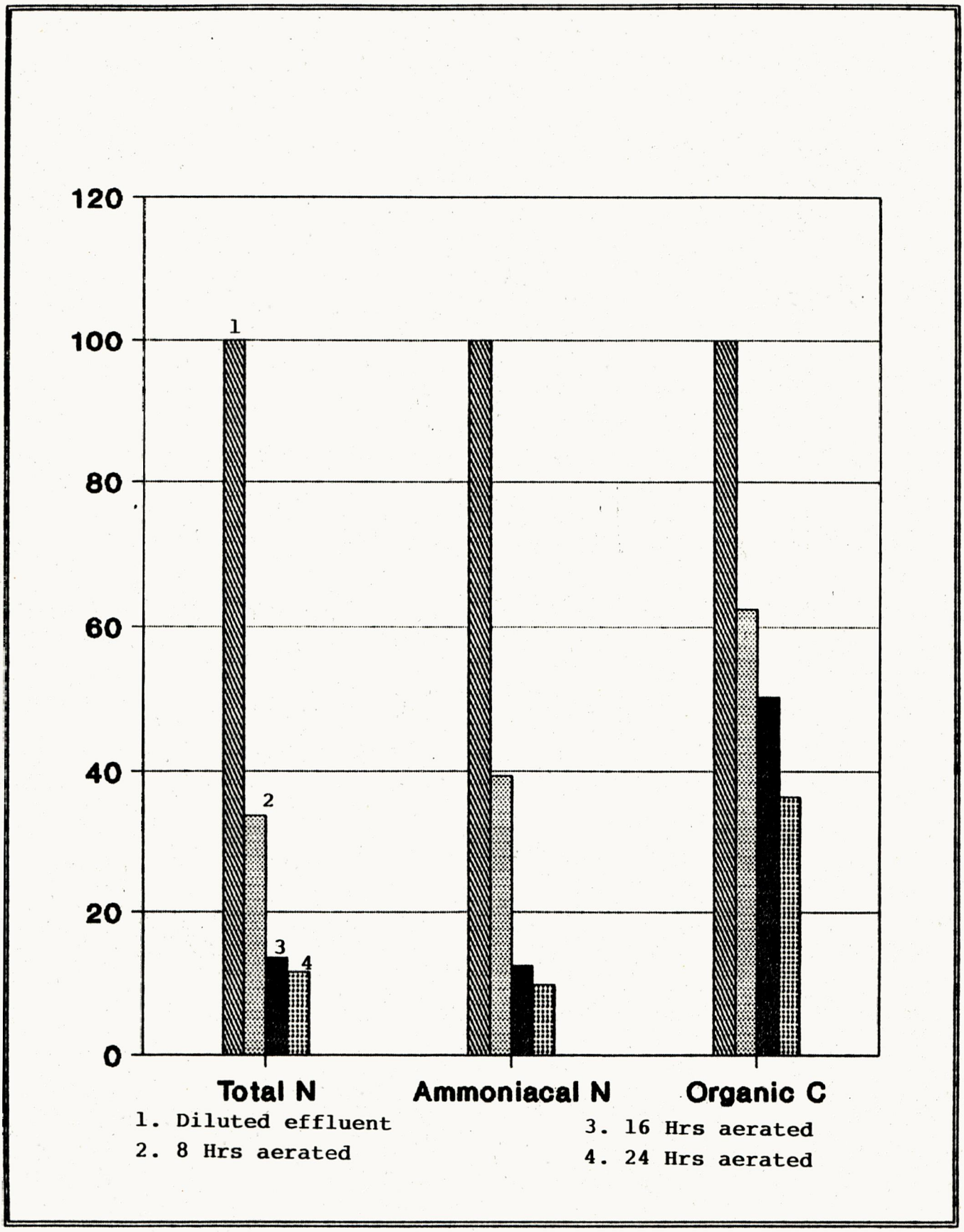


Fig. 14. Decrease in ammoniacal nitrogen in the 8, 16 and 24 hours treated effluent.

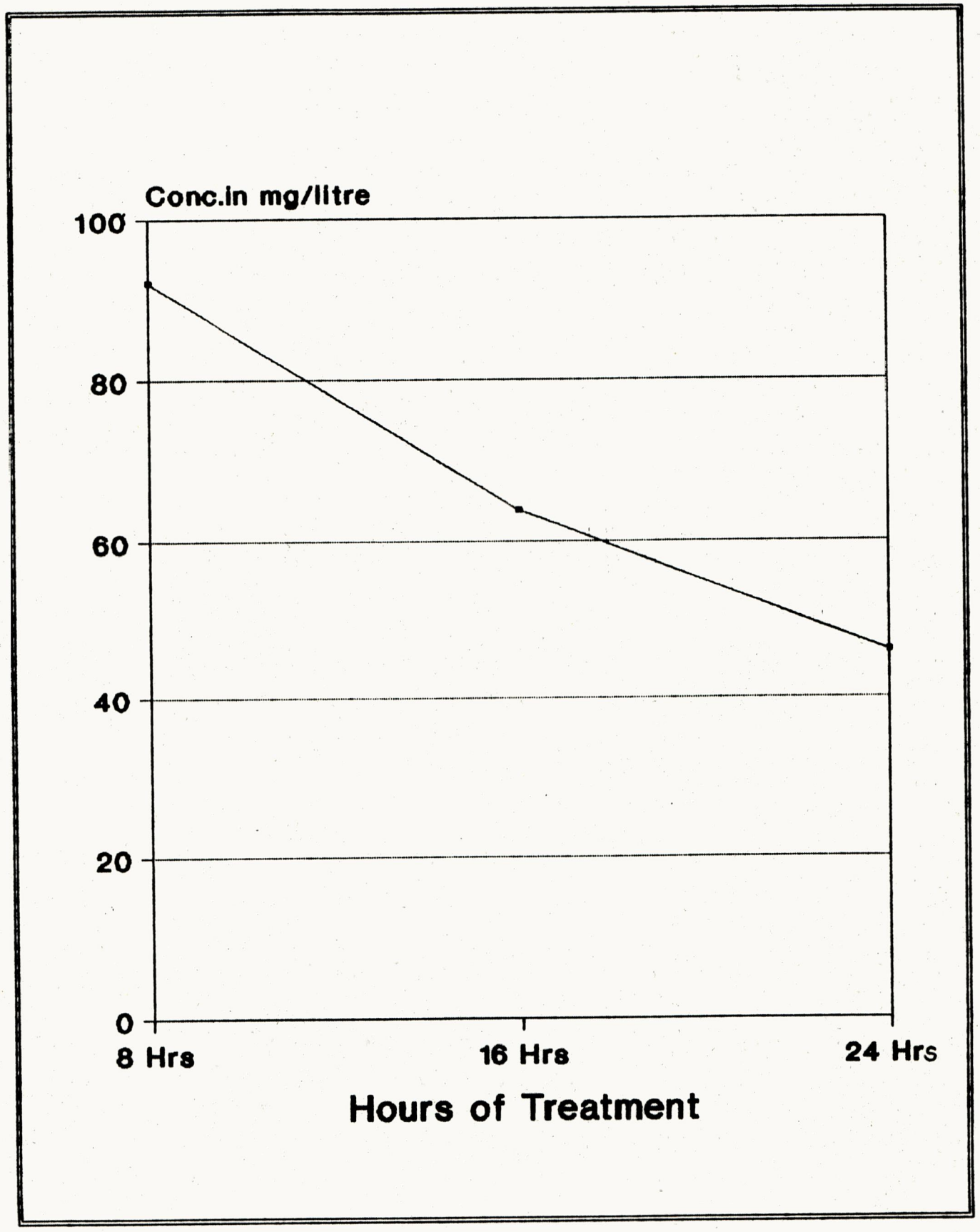


Fig. 15. Increase in nitrate nitrogen in the effluent treated for 8, 16 and 24 hours.

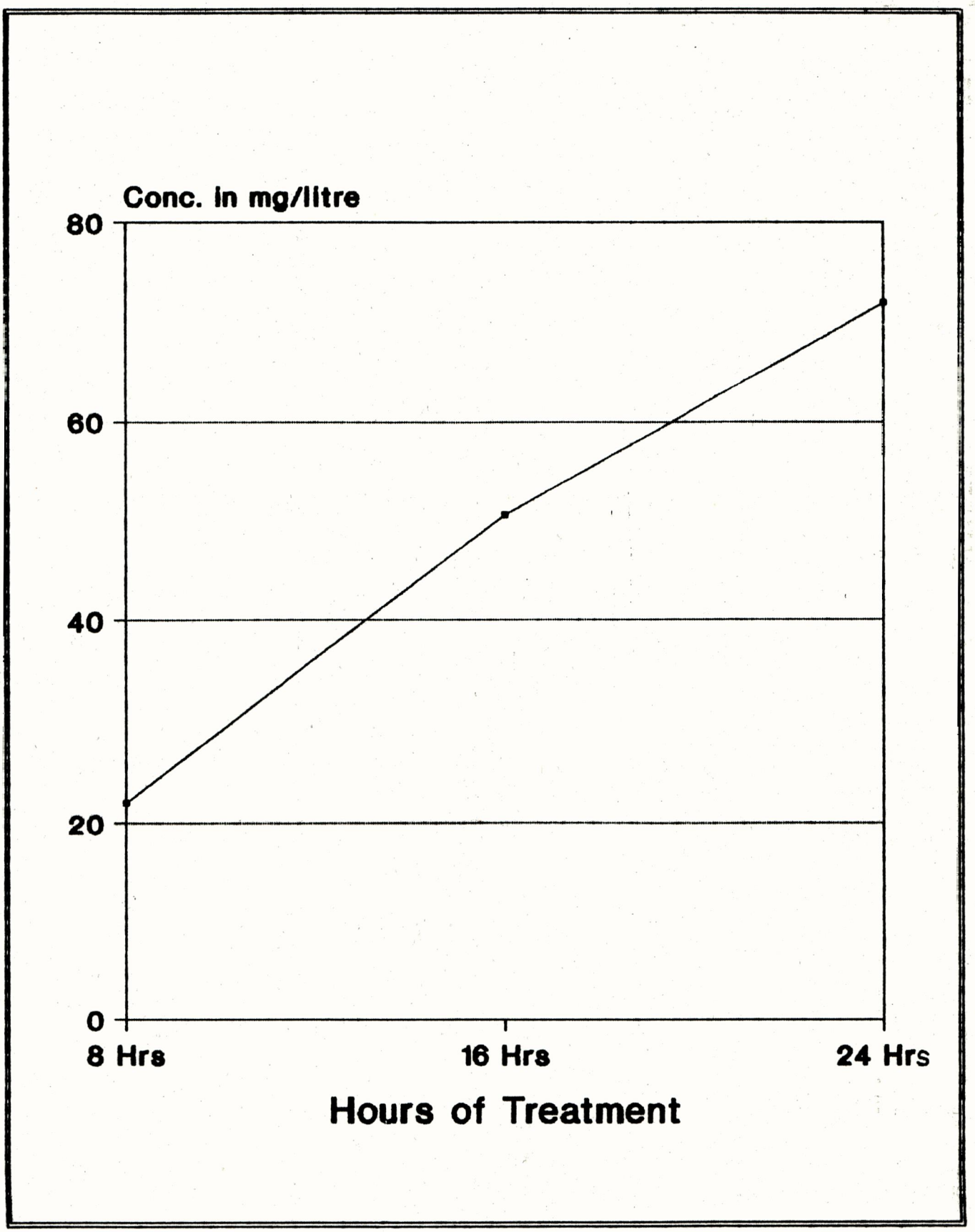


Fig. 16. Decrease in organic carbon in the effluent treated for 8, 16 and 24 hours.

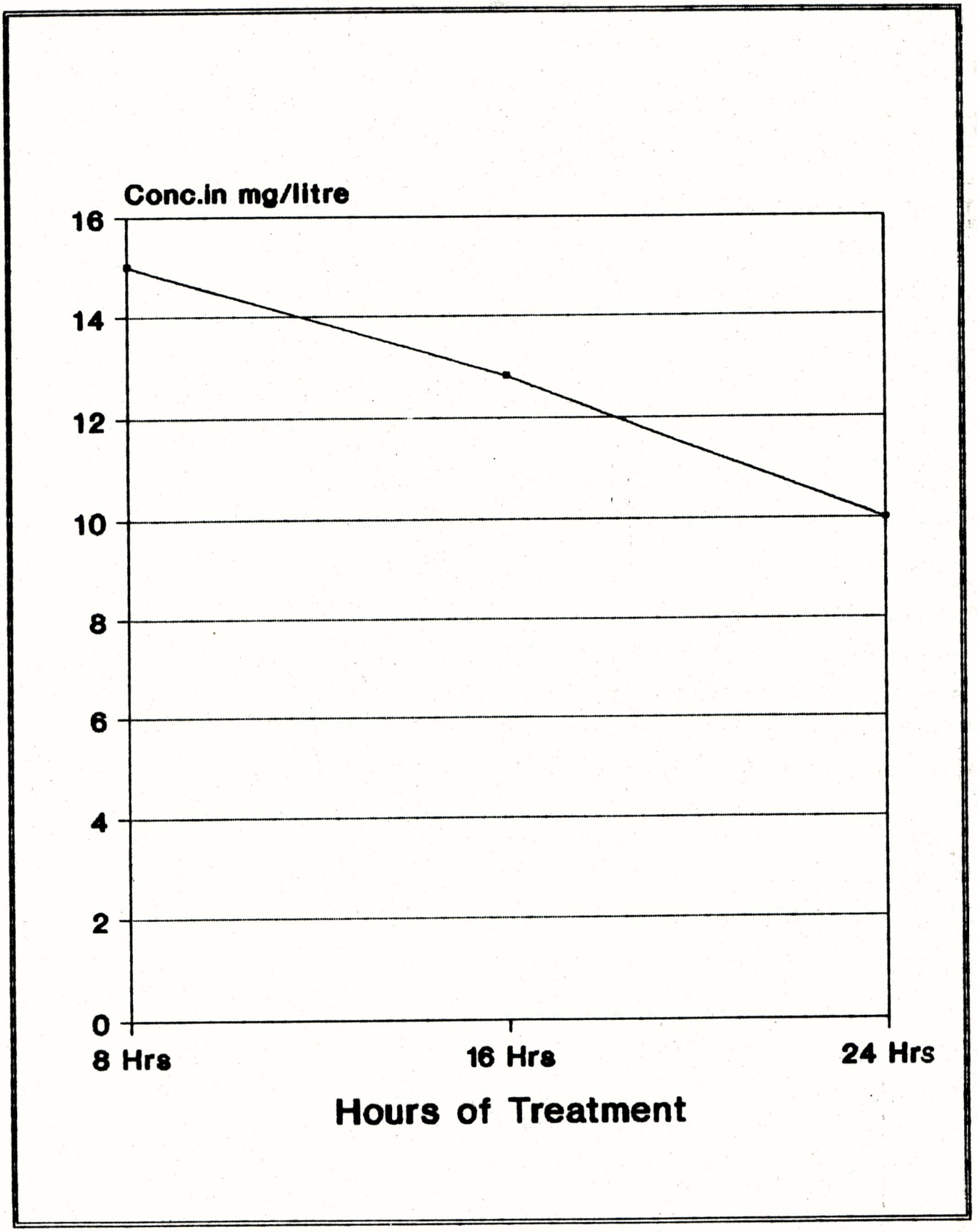


Fig. 17. Scatter diagram showing correlation between decrease in the level of ammonia and increase in the level of nitrate in the treated effluent.

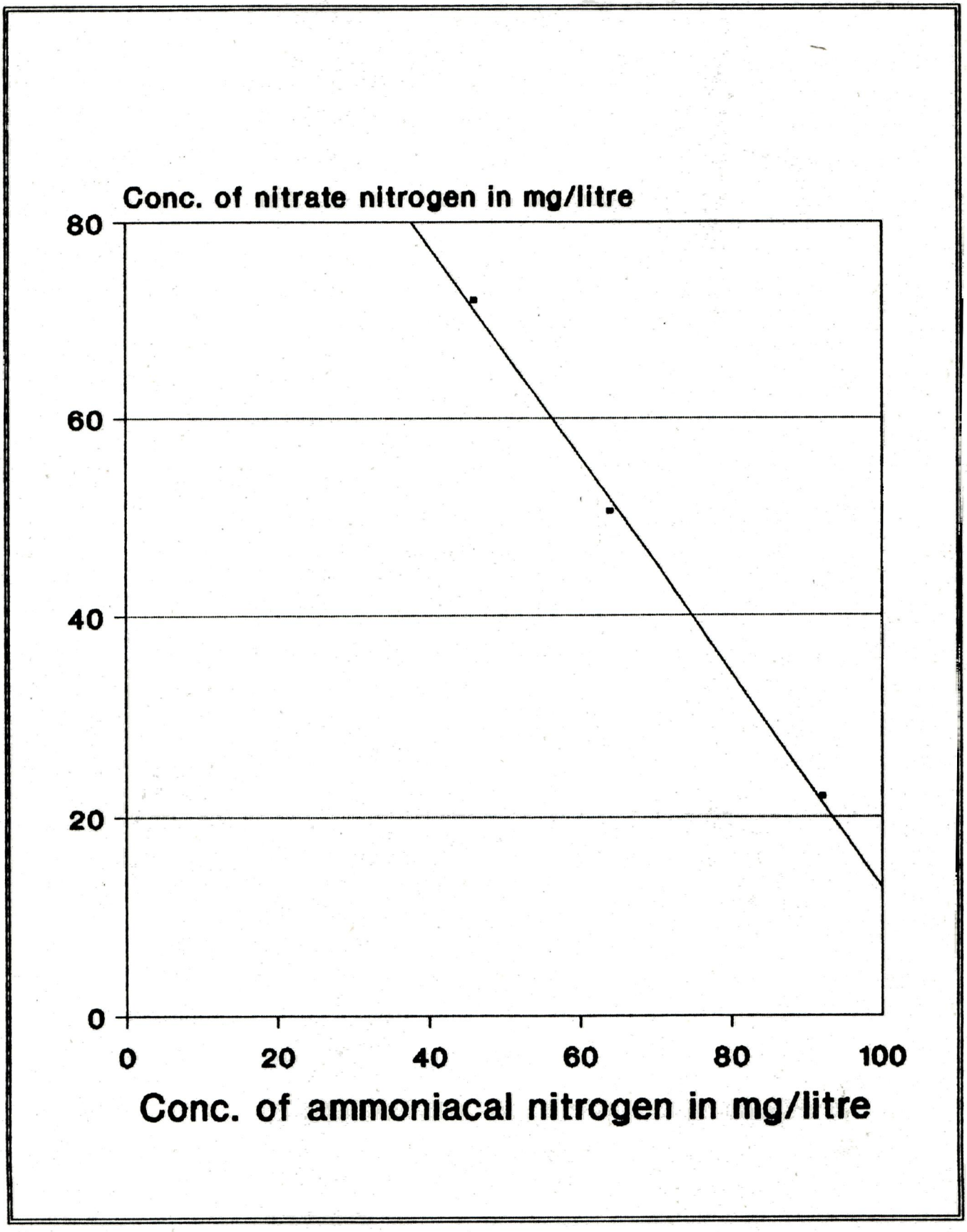
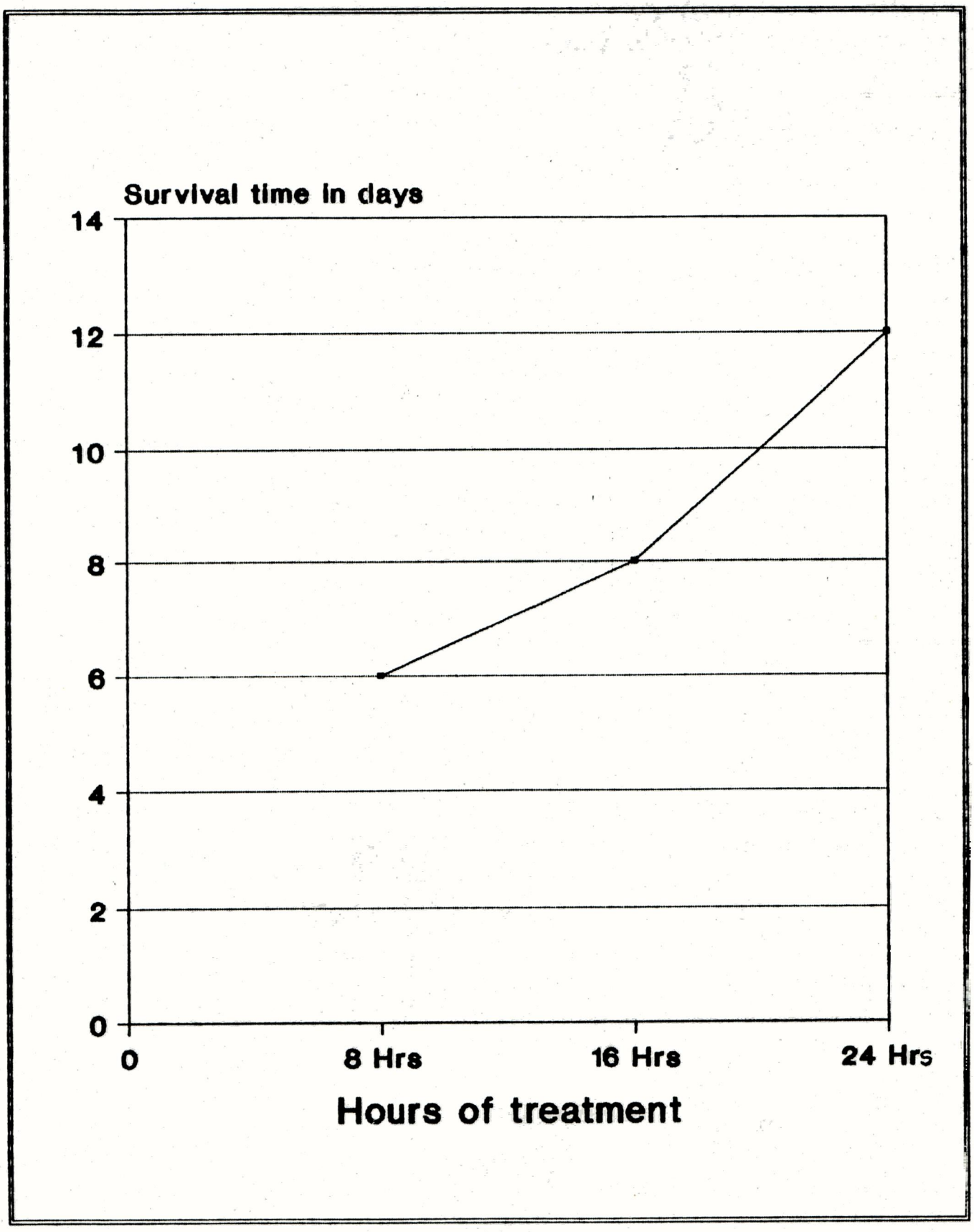


Fig. 18. Decrease in survival time of fish Oreochromis mossambicus during treatment for 8,16 and 24 hours.



Summary and Conclusion

SUMMARY AND CONCLUSION

The outcome of the present study are:

1. The physicochemical parameters analysed in the raw tannery effluent were found to exceed the quality standards.
2. The effluent was found to be olive green in colour and had an offensive foul smell.
3. The chemical parameters analysed were total nitrogen, ammoniacal nitrogen, nitrate and nitrite nitrogen, organic carbon, DO, BOD, COD and hexavalent chromium.
4. Though the pollution potential of the effluent was found to be dependent on several physicochemical parameters, in the present investigation, the treatability of organic carbon and nitrogen was analysed.
5. The effluent was diluted with tap water in the ratio of 1:10. In the diluted effluent, among the chemical parameters analysed the level of total nitrogen, ammoniacal nitrogen and organic carbon showed a decrease bringing down the pollution potential of the effluent to a certain extent.
6. Diluted effluent was aerated for 8,16 and 24 hours and the effect of aeration on pollution of the effluent was analysed.
7. The analysis showed a decrease in the level of total nitrogen, ammoniacal nitrogen and organic carbon with increase in the hours of aeration.

8. The diluted effluent was treated with bacteria isolated from the effluent by enrichment culture technique and aerated for 8,16 and 24 hours respectively.

9. The bacteria isolated belonged to the genera Bacillus, Pseudomonas and Escherichia.

10. In treated effluent the chemical parameters analysed were ammoniacal nitrogen, nitrate, nitrite nitrogen and organic carbon.

11. In the treated effluent there was a gradual decrease in the level of ammoniacal nitrogen with a corresponding increase in level of nitrate nitrogen.

12. Nitrogen in the raw waste water is usually in the ammonia form. The presence of ammonia in aquatic environment causes eutrophication, is toxic to animals and gives an offensive smell.

13. Nitrification was found to be the major cause for ammonia decrease in the treated effluent.

14. Nitrification occurs in well aerated waters in the presence of bacteria.

15. Nitrification oxidises ammonia to nitrite and then to nitrate, a process found beneficial in waste water treatment plants.

16. Denitrification is another process found useful in waste water treatment. Denitrification starts in nitrate reduction and ends in gaseous nitrogen.

17. In the treated effluent these was a gradual decrease in the level of organic carbon. The decrease was due to biodegradation of organic carbon.

18. In aerobic biological treatment, organic carbon was eventually decomposed to CO_2 and H_2O.

19. The studies on fish survivability showed an increase in survival time with increase in the hours of treatment.

From the study, it is concluded that the treatment of waste water with bacteria isolated from the tannery effluent has provided ways for controlling environmental nitrogen and carbon.

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