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AN INVESTIGATION INTO THE USE OF ANAEROBIC
DIGESTION FOR THE TREATMENT OF TANNERY WASTEWATERS

Thesis

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"Knowledge keeps Man in harmony with the Universe"

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ABSTRACT

The anaerobic digestion of tannery wastewaters was investigated with a view to using this form of treatment in the tanning industry. As these wastewaters are extremely complex and contain high concentrations of both inorganic and organic compounds, the effect of these individual compounds on the anaerobic digestion process was investigated in detail, in order to ascertain the fate of these compounds during the digestion process. The experiments comprising the initial toxicity study were carried out as adaptation experiments using a synthetic wastewater. It was found that the heavy metals such as chrome, aluminium and iron precipitated and accumulated in the sludge bed of the digesters. The soluble ions such as sodium and chloride were not retained and passed through the digesters. Approximately 20 % of the calcium ions were removed through precipitation, with the remainder being present in the digester effluent. Under the anaerobic conditions, ammonification of the organic nitrogen occurred, and influent sulphates were reduced to sulphides. These sulphides were present as either H_2S , HS^- or insoluble sulphides. As these compounds under investigation caused no inhibition of the anaerobic digestion process at the concentrations found in tannery wastewaters, the anaerobic treatment of these wastewaters appeared to be possible, provided the bacteria were given sufficient time to adapt to the potentially toxic compounds. However, despite the findings of the synthetic study, the successful anaerobic digestion of the tannery effluents could not be achieved. Although the use of acid was found to be essential in order to control the digester pH in the optimum range, the metabolism of the methanogenic bacteria was inhibited by the presence or absence of unknown compounds. Neither the addition of essential trace nutrients, nor the prevention of the competition between the methanogens and the sulphate-reducing bacteria were able to reverse this inhibition. As tannery effluents contain very low concentrations of phosphorous, it is possible that the methanogens were inhibited by a lack of phosphorous, which is essential during methanogenesis. In contrast to the results obtained from the effluent experiments, the anaerobic digestion of tannery sludge was found to be possible. Of the organic solids present in the sludge, 60 % were degraded and converted into biogas, which had a methane content greater than 70 %. The degradation of the organic solids ensured that COD and PV reductions of greater than 90 % were achieved, and the fate of the compounds in the digesters were in agreement with the findings of the

synthetic study. Efforts to improve the efficiency of the digestion process through the addition of trace nutrients and the use of a two-stage process were only successful in bringing about a minor improvement in digester performance. The overall results of this investigation show, therefore, that although the anaerobic treatment of the tannery effluent was not achieved, the successful anaerobic digestion of tannery sludge is possible at low loading rates. As many difficulties still need to be solved, a great deal of further research is necessary if anaerobic digestion is to be used on an industrial scale for the treatment and disposal of tannery wastewaters.

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LIST OF ABBREVIATIONS

mS/m	-	Milli Siemens Per Metre
ppm	-	Parts Per Million
mg/ℓ	-	Milligrams Per Litre
COD	-	Chemical Oxygen Demand
PV	-	Permanganate Value
BOD	-	Biochemical (Biological) Oxygen Demand
DAF	-	Dissolved Air Flotation
IAF	-	Induced Air Flotation
HRBF	-	High Rate Biofiltration
%	-	Percentage
nm	-	Nanometre
mm	-	Millimetre
cm	-	Centimetre
m	-	Metre
m ³	-	Cubic Metre
RO	-	Reverse Osmosis
SAR	-	Sodium Absorption Ratio
NAD	-	Nicotinamide Adenine Dinucleotide
NADH ₂	-	Reduced Nicotinamide Adenine Dinucleotide
ADP	-	Adenosine Diphosphate
ATP	-	Adenosine Triphosphate
VFA	-	Volatile Fatty Acids
K _a	-	Ionization Constant for an Acid
HRT	-	Hydraulic Retention Time
SRT	-	Solids Retention Time
ADUF	-	Anaerobic Digester Ultrafiltration
CoA	-	Coenzyme A
ΔG°	-	Gibbs Free Energy Change
MFR	-	Carbon Dioxide Reduction Factor
FAF	-	Formaldehyde Activating Factor
H ₄ MPT	-	Tetrahydromethanopterin
CoM	-	Coenzyme M
UASB	-	Upflow Anaerobic Sludge Blanket
MARS	-	Membrane Anaerobic Reactor System
TA	-	Total Alkalinity
N	-	Normality

kPa	-	Kilopascal
SG	-	Specific Gravity
MM	-	Molecular Mass
°C	-	Degrees Celsius
TKN	-	Total Kjeldahl Nitrogen
pK_w	-	Dissociation Constant of Water
$\mu\ell$	-	Microlitre
ml	-	Millilitre
ℓ	-	Litre
TS	-	Total Solids
VS	-	Volatile Solids
TIS	-	Total Inorganic Solids
SS	-	Suspended Solids
TVS	-	Total Volatile Solids
TDS	-	Total Dissolved Solids
TDVS	-	Total Dissolved Volatile Solids
VSS	-	Volatile Suspended Solids
TDIS	-	Total Dissolved Inorganic Solids
Ksp	-	Solubility Product
F:M	-	Food to Microorganism Ratio
kcal	-	Kilocalorie
kJ	-	Kilojoule
MJ	-	Megajoule
G + C	-	Guanine + Cytosine Content
mV	-	Millivolt
STP	-	Standard Temperature and Pressure
PVC	-	Polyvinyl Chloride
i.d.	-	Internal Diameter
μmhos	-	Micrometre Ohm
α	-	Absorption Coefficient for H_2S
f	-	Fraction of Free Ammonia
K	-	Degrees Kelvin
mA	-	Milliampere
FID	-	Flame Ionization Detector
TCD	-	Thermal Conductivity Detector
d_4^{20}	-	Density
g	-	Gram
kg	-	Kilogram
atm	-	Atmosphere

CHAPTER ONE

INTRODUCTION

CHAPTER ONE

INTRODUCTION

The pollution and contamination of the environment is an inevitable consequence of the activities of man. The deposition by both industrial and agricultural societies of waste material into the environment, has been both historically and socially a characteristic of man's activities. Historically, throughout the development of the industrial age, waste products of manufacturing processes have been returned to the earth, and therefore, the maintenance of man's material wealth and personal comfort has produced an undesirable consequence, namely that of ever increasing pollution. Because of man's communal nature, the by-products of his activities have also been concentrated, resulting in his surrounding habitats being polluted to such a degree, that natural purification systems are becoming overloaded (Hattingh et al, 1985).

The pollution of water resources by industrial wastes had its beginnings at the time of the Industrial Revolution, during the latter half of the eighteenth century. Although the industrial waste problem had a small beginning, it, like industry, did not remain small for long. As the number of process industries increased, as the number of factories multiplied within each industry, and as these factories expanded and enlarged their plants, so there was a corresponding increase in the discharge of pollutorial wastes. As most polluting materials eventually find their way into some stage of the hydrological cycle, these materials have some impact on the aquatic environment. It is imperative that the effect of these materials on the environment is minimized, and therefore good industrial and water management practice dictates that waste materials be discharged into a receiving water in such a way that nature's ability to assimilate these wastes is utilized without any deleterious effects on water quality. Obviously, the ability of surface waters to accept waste materials without causing harmful effects has to be considered as a beneficial use of water, because when overloaded with excess waste, these waters become grossly polluted, offensive to the eye and nose, unsuitable for fish life, unsafe for recreational purposes, and expensive to downstream communities and industries that must re-purify the water for their own consumption. Improving the quality of water resources is, therefore, no longer a luxury measure, it is one which in the long run, will

generate immeasurable benefits in terms of protecting public health and other natural resources, and indirectly contributing to economic growth.

In South Africa, water is the most important natural resource limiting growth and development. The annual rainfall increases dramatically from West to East, from 125 mm on the arid West Coast to 1000 mm on the East Coast. During any year only 10 % of the landmass is likely to receive more than 750 mm (Department of Water Affairs Monograph, 1986), and it has been estimated that at the present rate of population growth, local demand for water will exceed the available supply within the next 20 to 30 years. As this is a generalized projection, the geographical distribution of water resources across the country, as determined by rainfall distribution in the major catchment areas and planned storage facilities, means that localised water shortages will be experienced long before the year 2000.

The rapid population growth rate in South Africa has necessitated an unprecedented degree of industrial expansion over the past two decades. Such industrial growth and development has, however, resulted in an ever increasing demand for water, together with an intensification of water pollution problems. Because of the lack of dilution available as a result of the low rainfall, pollutant substances such as inorganic salts, heavy metals, harmful organics, etc. accumulate, and this has led to a steadily deteriorating quality of existing water supplies. The need to protect and conserve the precious water resources is therefore of prime importance, and as water is a public commodity that is affected by the actions of users and polluters, only central government is able to exercise overall control.

The use of water and the control of effluents are governed by the Water Act (No. 54 of 1956) as amended by the Amendment Act No. 96 of 1984. The administration and enforcement of the Act is the responsibility of the State and the Minister of Water Affairs. The Minister, after consultation with the South African Bureau of Standards, is empowered to prescribe quality standards for the purification of all industrial and domestic sewage effluents before discharge to the aquatic environment. The Sections of the Act most applicable to such discharges are 21 to 24, 26, 30 and 170 which relate to the disposal of treated industrial and sewage effluents (Hattingh et al, 1985).

Quality standards for discharge of industrial effluents to public streams are

separated into (a) a General Standard and (b) a Special Standard for specified streams, areas and industrial processes. These discharge regulations, as updated by Government Notice No. 991 of 18 May 1984, are shown in Appendix I.

Where it is considered impractical for an industry to treat its effluent to the prescribed standard, the Minister may issue a permit exempting such industry from compliance. The degree of exemption must be specified as well as the point of discharge, which must be such that dilution will render the effluent harmless to flora and fauna.

Where a municipality agrees to accept the discharge of industrial effluents into its sewage system, the municipality is classified under the provisions of the Water Act as a "user of water for industrial purposes", and must therefore purify its eventual discharge to meet the consent conditions for this discharge, and/or the relevant standards given in Appendix I, and/or to those standards governing marine disposal. To cover the cost of treatment in the municipal sewage system, municipalities charge tariffs for accepting industrial effluents. The tariffs are generally calculated on a strength and volume formula, and reflect the costs and degree of difficulty involved in treating industrial effluents (Hart et al, 1987).

As stricter regulations have been laid down by governments and local authorities on the permitted levels of pollutants in effluents, it has become increasingly important economically for industries to treat effluents prior to discharge to local authorities sewage works. The very survival of several industries has become dependent on whether these industries are able to purify or dispose of their effluents in a manner acceptable to the authorities, and has compelled these industries to re-evaluate their policies towards environmental pollution. What has become clearly evident is that there is a need for the development of more effective, yet economical, industrial wastewater treatment processes.

In the context of the ever-tightening water budget, it is clear that any large, single group of consumers, i.e. industry, can best look after its own long-term interests by actively participating and co-operating in water-saving exercises. In a country such as South Africa, which has a critical water shortage, the implementation of water reclamation and reuse schemes, including recycling of water in industrial processes, reclamation of purified industrial

effluent for reuse, and reclamation of purified domestic sewage for reuse in industry, agriculture and for domestic purposes will be vital.

The tanning industry is a major consumer of water. As the water usage in the tanning process is essentially non-consumptive, the industry is also a major producer of large volumes of effluent. Although the modern trend towards shorter processing times, lower water usage, and more intensive chemical usage has reduced the volume of the effluents produced, this has resulted in the production of far more concentrated effluents. To enable the local tanning industry to participate and co-operate even more in the national water-saving exercise, the development of more advanced scientific and technological methods for handling its solid wastes and effluents is required. This is essential if the treated effluents are to be reused directly, or be discharged back to the aquatic environment without a serious and/or unacceptable water quality deterioration.

The study described in this thesis offers one possible solution to the pollution problem being faced by the tanning industry. However, in order to understand more fully the complexity of the problem, it is important to understand the tanning operations and processes in greater detail.

CHAPTER TWO

THE ORIGIN OF TANNERY WASTEWATERS AND EXISTING METHODS OF TREATMENT

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THE ORIGIN OF TANNERY WASTEWATERS AND EXISTING METHODS OF TREATMENT

2.1 Introduction

Tanneries are by definition industrial processing operations in which raw or semi-processed animal hides and skins are converted into a stable, usable end-product known as leather (Hart et al, 1987).

The tanning of hides, which developed as an art form for many centuries, was radically modified at the beginning of the twentieth century by the application of scientific principles, and by the emergence in particular of the chemistry of chrome tanning and of dyeing. The growth of the chemical industry resulted in chemicals replacing the traditional tanning agents such as vegetable tannins and animal and fish oils, although for certain types of leather vegetable tanning is still used. Thus apart from the mechanical operations and manipulations necessary, the tanning process as such is basically chemical (Murray, 1987).

In order to understand the complexity and the nature of the resulting wastewater arising from the operation of a tannery, it is necessary to have a clear understanding of the chemical transformations of the hide or skin into leather.

2.2 The Process of Leather Manufacture

The principle hides and skins used in the manufacture of leather are cattle hides, calf skins, goat and kid skins and sheep and lamb skins. While these account for over 90 % of the world's leather production, other "exotic" hides and skins are also processed commercially. These include horse, pig, kangaroo, deer, aligator, lizard, snake, seal, walrus, fish, crocodile, ostrich, elephant and antelope of various types.

These hides and skins arrive at tanneries as either cured or "green" hides. "Green" hides have a high water content (approximately 75 %) and are putrescible. They therefore have to be processed immediately, as without

treatment, they rapidly become the target for bacterial putrefaction. Cured hides are hides which have been processed to eliminate the danger of bacterial decay. Curing is carried out either by salting, drying or a combination of both. These curing methods serve to dehydrate the hides in order to prevent the development of bacterial activity.

Irrespective of whether hides are "green" or cured, there are three principal stages in their transformation into leather:-

- (a) beamhouse processes
- (b) tanning processes
- (c) dressing and finishing

2.2.1 Beamhouse Processes

The beamhouse processes prepare the hide for tanning, and consist of a number of operations which eliminate those parts of the hide which are not to be transformed into leather. The epidermis, the hair and the subcutaneous tissue are removed, leaving only the dermis of the hide which is transformed into leather.

2.2.1.1 Soaking

Soaking is the first operation carried out in the tannery. Its main purpose is to restore moisture which was lost during preservation and storage, and to restore the hides and skins as closely as possible to the fresh or "green" state. This restoration of moisture is essential to render the hides soft and flexible so that they will not be physically damaged by the action of washing under agitation, and subsequent mechanical processes such as green fleshing. It is also necessary to ensure that sufficient moisture is present to carry out the action of the unhairing chemicals used in the subsequent liming processes. Soaking also removes any dirt, manure, urine, blood and non-fibrous proteins that may have adhered to the hide.

The soaking of hides and skins is carried out in drums or paddle wheel vats filled with water. Soaking must be achieved as quickly as possible, since the hide, even partially rehydrated, once again becomes a highly putrescible material. Chemical additions are thus frequently made to accelerate

rehydration, and soaking efficiency is increased if the liquors are alkaline. The addition of a surface active agent (detergent or wetting agent) which has an emulsifying action on natural grease is also advantageous.

Although the duration of soaking is variable depending on the manner in which the hides were preserved, it is normally completed within 24 - 36 hours. The soak liquors often contain a 0,1 % solution of sodium chlorite, sodium hypochlorite, sodium trichlorophenate or sodium pentachlorophenate to ensure a sterile soak.

2.2.1.2 Unhairing and Liming

The unhairing and liming operations are carried out simultaneously. The hides are placed in paddle vats containing water and the unhairing chemicals. The concentration of the chemicals, the water temperature and the degree of agitation directly affect the rate at which unhairing proceeds.

The removal of hair usually employs calcium hydroxide and unhairing agents such as sodium sulphide. These chemicals produce a concentrated alkaline solution which destroys the hair or attacks the hair roots, loosens the epidermis and removes certain soluble skin proteins. They also cause the hide fibres to absorb large amounts of moisture. This results in the hide swelling to about twice its normal thickness. This opening up of the fibre structures facilitates the penetration of subsequent tan liquors by dissolving out non-fibrous proteins.

2.2.1.3 Dehairing

Dehairing is a mechanical operation which eliminates the epidermis and hairs loosened by liming. When the hides have been treated by a weak lime solution, the hairs are more or less intact and it is necessary to use a machine which, by rubbing, separates the hairs from the hide.

2.2.1.4 Fleshing

Fleshing is another mechanical operation which removes excess flesh, fat and muscle from the hides. The fleshing machine passes the hide through rollers and across rotating spiral blades which remove the flesh from the hide. The

fleshings are normally recovered and sold to plants for rendering or conversion to glue.

2.2.1.5 Splitting

Splitting adjusts the thickness of the hide to the requirements of the finished product. Hide thickness varies depending partly on the age of the animal, and different parts of the skin also have various thicknesses. Splitting yields a grain portion of uniform thickness and a split (or fleshed) side.

The operation is carried out using a machine which brings the hide against a sharp metal blade which divides the hide into two parts. A constant jet of water is used to ensure the hide remains elastic and to cool the cutting blade at the same time.

Splitting at this stage results in a more uniform tannage. However, it can be delayed until after tanning when it is more accurate.

2.2.1.6 Deliming and Bating

After the removal of the hair and surplus flesh, the hides contain fairly high concentrations of residual alkaline chemicals which have to be removed prior to tanning. During deliming, salts of ammonium sulphate, ammonium chloride, sodium bisulphite or weak acids such as acetic, lactic or butyric are used in various combinations to convert the residual lime into soluble salts. As this step progresses, the excessive alkaline swelling begins to disappear and the hides return to their normal thickness. Deliming is completed by rinsing to eliminate any soluble salts which may have formed.

Bating is the biochemical action produced by proteolytic enzymes. These natural catalysts facilitate separation of the collagen protein fibres through hydrolytic destruction of peptide bonds, which cross-link the chains of amino acids. Bating also attacks and destroys most of the remaining undesirable constituents of the skin, such as hair roots and pigments. Removal of these materials softens the grain surface and gives it a cleaner appearance. For the action of the enzymes to be efficient, the operation must be carried out in a drum at a temperature of 30 - 37°C.

At the end of the beamhouse processing of the original hide, only the dermis remains, in an almost pure state. It is now called a pelt or pelt leather. It is extremely putrescible which is difficult, if not impossible, to preserve. It is therefore necessary to pass to the tanning stage as quickly as possible.

2.2.2 Tanning Processes

The tanning processes convert the raw, putrescible skin into material which is resistant to biological attack.

2.2.2.1 Pickling

Pickling prepares the hides to accept the tanning agents by providing an acid environment, an essential step in chrome tanning. Chrome tanning agents have minimum solubility under alkaline conditions; the acid environment thus prevents precipitation of chromium salts. Pickling most commonly uses sulphuric acid and sodium chloride. If acid alone was used, excessive swelling would occur, either producing inferior leather or converting the hide into an untannable gelatinous mass.

Controlled acid swelling helps separate collagen protein fibres, which facilitates further complexing of the hide by the tanning agent. This results in more complete stabilization.

The time of pickling varies from a few minutes to several hours, depending on the type of leather being produced.

2.2.2.2 Tanning

Tanning agents convert the raw collagen fibres of the hide into a stable product which is no longer susceptible to putrefaction. They also significantly improve many of the mechanical properties of the raw material including dimensional stability, resistance to abrasion, chemical resistance, heat resistance, flexibility and the ability to endure repeated cycles of wetting and drying.

The predominant tanning agents used are trivalent chromium, and vegetable

tannins extracted primarily from specific tree barks. Other principle tanning agents include alum and syntans.

Vegetable tanning was the first method employed by man to render hides non-putrescible whilst preserving its suppleness. It is carried out in a solution containing plant extracts, and is applicable for the production of heavy leathers such as sole leather, mechanical leather and saddle leather. The process usually takes place in pits because of the longer reaction times involved.

Chrome tanning is the most widely used method of tanning and has replaced other methods, particularly vegetable tanning, for a large number of leather articles. Its principle advantages lie in the rapidity of the operation and the fact that it produces leather that best combines most of the chemical and physical properties preferred for the majority of leather uses.

The ability of the chromium salts to combine permanently with the collagen fibres depends upon the chemical state of the tanning agents as well as the condition of the hides to be tanned. For good results, the tanning agent must penetrate as completely as possible into the thickness of the hide and fix itself permanently to the collagen fibres. The addition of sodium bicarbonate and formic acid increases the affinity of the separated collagen protein fibres for the chrome.

Chrome tanning is usually carried out in rotating drums. Basic chromium sulphate containing 25 - 30 % chromium oxide is used. The water used must be absolutely free from suspended solids and iron and must have a low hardness. The absorption and fixing of chrome by the hides depends on the pH of the tanning bath. The tanning cycle starts at a low pH of approximately 3,1 - 3,2 due to the sulphuric acid from the pickling phase. After the hides have rotated in the drum for about three-quarters of the duration of the tanning, the acidity of the bath is neutralized with sodium carbonate in order to fix the basic chromium salts to the fibres of the hide. The hides are then rinsed to get rid of excess residues of the tanning agents.

At this stage the leather is non-putrescible and may be kept, under certain conditions, for several months. It is commercialized under the name of wet-blue for chrome tanned leather, and crust leather for vegetable tanned

hides and skins. If for any reason, the leather is dried at this stage, it will no longer possess the qualities necessary for its final use. It will therefore need to undergo a series of operations before and after drying which will give it the properties for its ultimate use. This is the object of the third phase of tannery operations, dressing and finishing.

2.2.3 Dressing and Finishing

Dressing and finishing transforms wet-blue into finished leather. The operations are many and varied depending on the type of leather to be produced. It should be noted that from the same basic wet-blue, different finished leathers can be produced according to the dressing and finishing techniques used.

2.2.3.1 Sammying

Sammying eliminates mechanically a high proportion of the water content of the tanned hide. The hide is passed between rollers which apply pressure to it. This operation is necessary in order to carry out the operations which follow.

2.2.3.2 Splitting and Shaving

Splitting can be carried out at this stage or prior to the deliming and bating processes (Section 2.2.1.5).

Shaving removes any remaining fleshy matter from the grain portion of the hide, where the thickness was insufficient for contact with the splitting knife.

2.2.3.3 Dyeing

The aim of dyeing is to colour the leather without hiding the natural attributes of the grain surface. This is achieved by the chemical fixation of the colouring agents (dyes) onto the leather fibres.

Different dyes are used for chrome tanned leather as opposed to vegetable tanned leather. Acidic or anionic dyes fix themselves particularly well onto chrome tanned leather and less easily onto vegetable tanned leather, whilst

the opposite is true for basic or cationic dyes.

Shades and degrees of penetration can be varied by controlling pH, which affects the affinity of the dye for the leather fibres.

2.2.3.4 Fatliquoring

The fatliquoring process lubricates the fibres so that they can slide over one another. Oils and related fatty substances in fatliquors replace the natural oils lost in the beamhouse and tanning processes. Chemical emulsifiers added to the fatliquor ingredients permit their dispersion in water. Use of differing amounts of oil permits the tanner to vary the suppleness of the final product. Fatliquors typically and predominantly are either of animal or vegetable origin, or are synthetics made from modified mineral-based oils.

2.2.3.5 Drying

The drying of leather is of great importance in the manufacturing process since this operation has not only a technical influence on the quality of the leather, but equally an economic effect upon manufacturing costs, since it affects the surface yield.

As a general rule it is not recommended to dry leather too rapidly or at too high a temperature. However, the operation must not be too slow either, otherwise the cost of the plant would be excessively high.

2.2.3.6 Staking

Staking stretches and flexes the leather on automatic equipment to make it soft and pliable.

2.2.3.7 Buffing

Buffing smooths or corrects irregularities in the grain surface by mechanical abrasion.

2.2.3.8 Finishing

Finishes are applied to the leather depending on the end use and the type of hide. The four main groups of finishes are water finishes, cellulosic finishes, finishes based on synthetic polymers and lacquers. Therefore, although they vary widely in their composition, there are certain properties which they should have in common. They must be opaque to hide defects and to colour the leather, they must have pliability and elasticity, they must adhere well, they must be reasonably waterproof, and the finished film should not harden or become brittle on ageing.

2.2.3.9 Plating and Embossing

Plating involves passing the leather between a roller and a heated metal plate. This flattens the leather and consolidates and improves the appearance of the finish. The warm plate often softens the finish, which, under pressure, flows to a smooth even film with a considerably improved gloss.

Embossing is carried out on the same type of machine used for plating, but instead of a smooth metal plate, the leather is pressed up against an etched or engraved plate which transfers the desired imprint to the leather. In this way, any pattern may be embossed onto the leather surface e.g. crocodile, lizard, snake, etc.

After finishing, the surface area of the hide is determined, and the product is graded for temper, uniformity of colour and thickness, and the extent of any surface defects.

The objectives thus achieved by the complex, multistep tanning process are:-

- (a) to render hides and skins resistant to decomposition or bacterial decay, particularly when wet
- (b) to achieve specified physical properties in the tanned hides and skins such as tensile strength, flexibility, resilience and abrasion resistance
- (c) to render leather insoluble in water at relatively high temperatures.

2.3 The Origin of Tannery Wastewaters

From the preceding section, it is evident that water is used in many of the leather processing operations (Table 1). This water is generally added on a proportional mass : mass basis according to the quantity of hide material, and its usage is generally non-consumptive, apart from relatively minor losses due to evaporation, steam generation, moisture held up in the final product, etc. It is estimated that between 86 and 95 % of the water used is recovered in the form of effluent (Table 1). The South African national effluent volume generated is thus of the same order as the water intake into the tanneries, namely 500 000 m³ per annum (Hart et al, 1987).

TABLE 1
THE VOLUME OF WATER ADDED AND RECOVERED IN THE FORM OF
EFFLUENT DURING THE TANNING PROCESS IN SOUTH AFRICA
(Boast et al, 1986)

Processing Stage	Wet Salted Hide		Green Hide	
	Water Added (ℓ)	Effluent Produced (ℓ)	Water Added (ℓ)	Effluent Produced (ℓ)
Presoak (1st Wash)	116	81	117	90
Soak (2nd Wash)	116	100	117	113
Unhair	77	70	78	62
Lime Wash	77	75	78	73,5
Pre Delime Wash	116	101	117	127
Delime	41,3	39,1	48	58,5
Delime Wash	83	74	95	96,5
Pickle/Tan	70,4	52,6	66,5	74
Chrome Wash	83	81,5	95	83
Total	780	674	811,5	777,5
% Water Recovered	86,4 %		95,3 %	

Note: The wet salted hide was 77,2 kg and the green hide 78 kg.

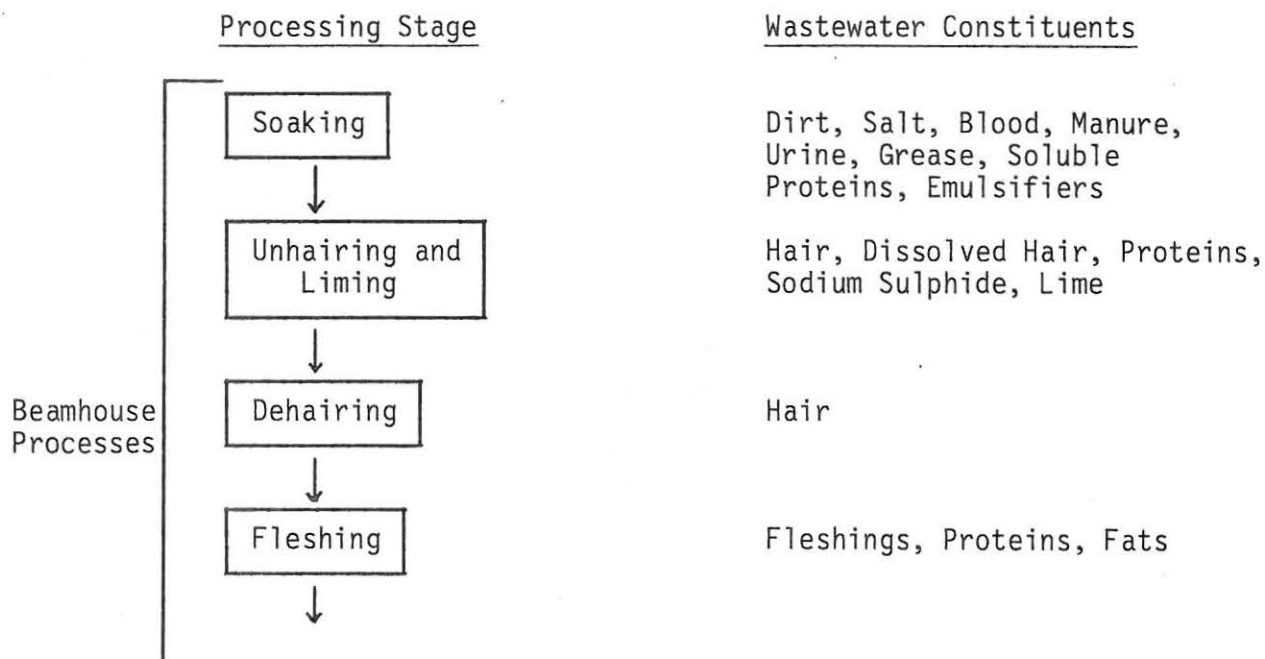
As leather manufacture is carried out batchwise and involves many stages of processing, it results in an effluent whose rate of discharge, pH and composition vary considerably throughout the day (Table 2).

TABLE 2

VARIATIONS IN EFFLUENT COMPOSITION DURING A TYPICAL WORKING DAY AT A SOUTH AFRICAN TANNERY (SILVERTON TANNERY, PRETORIA) (Murray, 1987)

Parameter	Time (Hours)		
	0700 - 1045	1200 - 1830	1830 - 0600
PV (mg/l)	2360	2460	118
COD (mg/l)	6800	5600	500
pH	8,3	8,4	8,2
TDS (mg/l)	10546	4879	2359
SS (mg/l)	5378	3510	236
Cl ⁻ (mg/l)	2400	900	520
Sulphide (mg/l)	90	78	10
Cr ₂ O ₃ (mg/l)	256,5	99,6	35,8
Conductivity (mS/m)	1066	697	275

More significantly, however, due to the widely varying chemical processes applied at the various stages of hide and skin processing, the resultant effluent fractions differ widely in terms of their pollutant characteristics (Figure 1). The majority of the pollution load arises from the wastes discharged from the beamhouse and tanyard sections of the tannery. Although the beamhouse wastes comprise only 30 % of the total volume of wastewater, they produce about 80 % of the pollution load (Cooper *et al*, 1983).



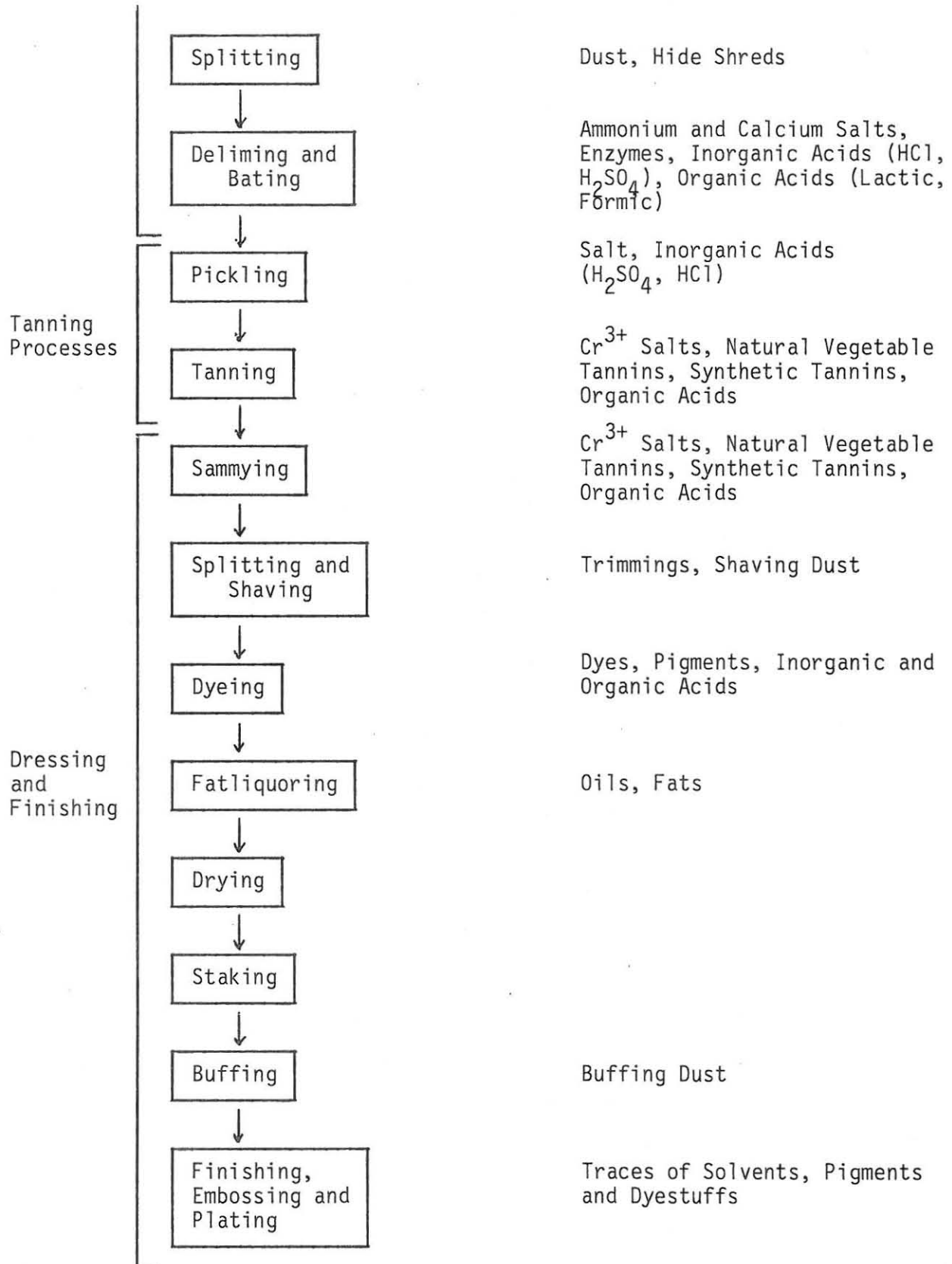


FIGURE 1 - Flowchart of the tanning process and the wastes produced (Costle et al, 1979; Bailey, 1977; Tsotsos, 1986; Carre et al, 1976 ; Aloy et al, 1976)

Although the flow rate and composition of the individual waste streams are variable, tanneries normally combine all the individual waste streams to produce an effluent known as a total mixed effluent. This total mixed effluent, although considerably different to the more potent individual waste streams, is still highly polluted. It is characterized by high concentrations of organic material (proteins and fats), suspended solids and dissolved solids (organic and inorganic, consisting respectively of soluble/colloidal proteinaceous material and of excess salts from process chemical additives). Table 3 gives a breakdown of typical gross pollutant characteristics of raw effluents from South African tanneries.

TABLE 3
TYPICAL GROSS POLLUTANT CHARACTERISTICS OF
RAW EFFLUENTS FROM SOUTH AFRICAN TANNERIES
(Hart *et al*, 1987)

Parameter	Chrome Tannery Full Finishing	Wet Blue Tannery	Chrome and Vegetable Tannery, Full Finishing
pH	9 - 11	9 - 11	9 - 11
COD (mg/l)	7000	10000 - 20000	6000
SS (mg/l)	2000 - 4000	5000 - 10000	2000 - 5000
TDS (mg/l)	15000 - 20000	20000	10000 - 15000
Sulphide (mg/l)	100	500	300
Chromium (mg/l)	100	500	50

From the above it is clear that tannery wastewaters are highly pollutive and require proper treatment before discharge. Expanding water demands and the drive towards clean rivers have necessitated the constant revision and upgrading of tannery effluent disposal systems. This has resulted in the development of a number of different treatment methods to reduce the impact of these wastewaters on the environment.

2.4 Existing Treatment Methods for Tannery Wastewaters

The degree of on-site effluent treatment to be carried out at a tannery is determined by whether the treated effluent is recycled or discharged to a municipal sewer, evaporation ponds or land (Hart *et al*, 1987).

Tannery wastewater is not readily accepted into the domestic wastewater treatment plants of local municipal authorities without some form of treatment, the extent of this being governed by costs and the local situation. In deciding how much treatment a particular tannery should have, it has to balance the capital and running costs of its own treatment to certain stages of purity, against the ability of the local municipal authority to accept this wastewater, and the relative charges for local authority acceptance at the various possible levels of treatment. It should be remembered that local authority charges can only rise in the future (Cooper and Rowsell, 1989).

On site effluent treatment can be divided into primary treatment and secondary treatment. In primary treatment, gross pollutants are removed by relatively simple and inexpensive processes. In secondary treatment, a higher treated effluent quality is aimed at, and the processes involved are consequently more technically complex and costly. Secondary treatment processes are generally preceded by primary treatment, which is usually justifiable both in terms of overall treatment costs and in ensuring a more consistent quality of influent to the secondary treatment stage (Hart et al, 1987).

Although there are many different primary and secondary effluent treatment systems available, on closer examination it may be found that from a tanner's viewpoint, the actual choice is very limited, as external factors outside a tanner's control may effectively narrow down the systems which could possibly be installed (Winters, 1986a).

In addition to discharge standards imposed by the local authority, the following factors dictate to a large extent the choice of effluent treatment systems:-

(a) Location

In rural areas the possibility exists to install lagooning systems employing large areas of land with low capital cost. On the other hand, in heavily built-up areas more compact treatment units, with high capital costs, may be necessary due to land scarcity. The presence of nearby residential accommodation may necessitate upgraded treatment systems to prevent air pollution. The location will also affect the dilution available to the tannery effluent (Winters, 1986a).

(b) Availability of Municipal Treatment Plants

From a capital investment viewpoint, the presence of such existing municipal plants may be critical. Where they exist the tanner has the choice of treating his effluent sufficiently to be acceptable to the municipal plant which will carry out the completion of the treatment, or the tanner may decide to carry out the complete treatment (Winters, 1986a).

(c) Presence of Other Tanneries in the Area

Where a number of tanneries exist in relative close proximity, the possibilities of a centralised co-operative tannery effluent treatment plant offers appreciable capital savings. They have proven economically and technically advantageous in a number of areas and have permitted more sophisticated and efficient treatment plants than could have been supported by individual tanneries (Winters, 1986a).

(d) Cost of Treatment Plants

In a number of countries where governments have recently introduced strict tannery effluent discharge standards, they have arranged for tanners to receive low interest loans for "equipment". In such circumstances, treatment plants have tended to be "over installed" with regards to equipment, with minimal civil works for which no loan was available. Contrasting strategies can be found in a number of developing countries where effluent treatment equipment is not manufactured and must be imported at high cost. In such circumstances one may see treatment plants with maximum local civil engineering work with only minimal essential equipment being installed e.g. facultative and other lagoon systems predominating (Winters, 1986a).

(e) Available Technology

This may have a great influence on the choice of treatment systems. It would obviously not be sensible to install sophisticated automatically controlled plants in locations where expertise in the electronic servicing field was not available. Similarly it would not be opportune to install a sophisticated activated sludge system without assurance that competent personnel were available for day-to-day control (Winters, 1986a).

Given the importance of the above factors, coupled with the varied tanning technologies employed, and the tremendous variation in scale of production within tanneries, it is obvious that any discussion of effluent treatment systems must be somewhat generalized. It should be noted that although an attempt will be made to mention all types of treatment currently applicable to tannery effluents, it should not be taken as implying that all of these forms of treatment would necessarily be applied to one particular effluent. Rather, the available options must be individually assessed in each case to arrive at the best practical solution.

2.4.1 Pretreatment

The use of pretreatment prior to the major effluent treatment can result in the installation of more simplified, more efficient and lower cost treatment plants. The three most important forms of pretreatment are:-

- (a) the removal of sulphide
- (b) the recovery and recycling of chrome
- (c) grease removal

2.4.1.1 Removal of Sulphide

One of the major components of the effluent from the tanning industry is sulphide. These sulphides present a serious disposal problem. When discharged into a river or stream, they cause a drastic reduction in dissolved oxygen, and the formation of dark-coloured precipitates with iron and other minerals present in the water. If these sulphides come into contact with acidic wastes, hydrogen sulphide (H_2S) is formed. H_2S causes severe corrosion of iron pipes in sewers. This occurs when the H_2S redissolves in condensation on sewer walls and is oxidized by bacteria to sulphuric acid. More importantly, H_2S is extremely toxic to man, wildlife and aquatic organisms. Concentrations as low as 10 mg/l are detrimental to fish (Sayers and Langlais, 1977; Bailey, 1978).

Although low concentrations of sulphide are toxic to fish and aquatic organisms, higher concentrations can be tolerated by biological treatment processes. It has been shown that activated sludge plants and oxidation ditches can tolerate sulphide concentrations of 50 and 100 mg/l respectively

(Van Vlimmeren, 1972). Despite this, the sulphide concentrations present in unhairing and liming liquors are still much higher, and therefore, they need to be reduced to an acceptable level before discharge. In order to prevent the formation of H_2S , any treatment carried out on unhairing and liming liquors is done so before they are mixed with other effluents.

Chemical treatment by ferrous sulphate is the oldest technique used to eliminate sulphide from tannery wastewater (Foster, 1950). The sulphides precipitated as FeS and a reduction of 90 % was noted. $Fe(OH)_2$ was also precipitated. The precipitation of the hydroxide caused a lowering of the pH and partial precipitation of proteins present. This resulted in a 50 % reduction in the biochemical oxygen demand (BOD) of the wastewater. This technique has two major disadvantages. The volume of sludge that is produced is considerable and the sulphides are merely precipitated but not destroyed.

Sulphides can also be chemically oxidized by hydrogen peroxide. This oxidizes sulphides to sulphate and completely destroys the sulphides within one to two hours. This reaction also reduces the chemical oxygen demand (COD) by approximately 20 %, but its use is limited by the cost of the treatment which is very high (Aloy et al, 1976).

The most economical and at present the most widely used technique is the catalytic oxidation of sulphides by air. It consists of aerating the lime liquors in the presence of a suitable catalyst. The Na_2S present is oxidized to thiosulphate and sulphate, and the thiosulphate then decomposes into sulphur and sulphite. The pioneering work was carried out at the British Leather Manufacturers Research Association (Bailey and Humphreys, 1967). It was shown that the addition of Mn^{2+} in the form of the sulphate or chloride together with good aeration was effective in reducing the sulphide concentrations to approximately 10 - 20 mg/l. When subsequently diluted 4 or 5 fold with non-sulphide bearing wastes, a sulphide level acceptable to the local authorities was obtained. Large scale experience has shown that vigorous action is needed to prevent the manganese being removed from the system by fine hairs and slurry, because the manganese is in the form of hydroxide at the high pH of the lime liquors (Berg et al, 1967; Van Vlimmeren, 1972).

Although manganese catalyzed aeration is widely used in many countries, it has

been shown that the activated sludge process oxidizes sulphides without the catalyst (Cooper et al, 1976) and large scale practice in several countries has proved that sulphide and pH reduction prior to the use of extended aeration or activated sludge can conveniently be carried out without catalyst addition by using a surface aerator in the balancing tank (Rawlings et al, 1975).

The recovery and reuse of sulphides from tannery wastes has also been successfully tested on a pilot scale plant (Sayers and Langlais, 1977). The sulphide-bearing wastes were pumped on a continuous basis to a degasifier in which acidification to pH 5,0 - 5,5 using sulphuric acid was effected. The H_2S liberated was carried by an air stream to an absorption tower where it was absorbed in recirculating NaOH until a desired Na_2S concentration was achieved. The Na_2S was then reused in the tannery's unhairing process.

2.4.1.2 Chrome Recovery and Recycling

Trivalent chromium (Cr^{3+}) is used in the tanning industry as the tanning agent. As tanners are not prepared to run the risk of untanned spots in the leather, the tanning liquors always contain an excess of Cr^{3+} . This excess Cr^{3+} finds its way into the wastewater and ultimately ends up in the wastewater sludge, which then has to be disposed of (Feikes, 1986). Most of these wastewater sludges are disposed of on land or in landfill sites.

Cr^{3+} is relatively non-toxic. It has little effect on soils, plants, animals and man (Gauglhofer, 1986; Shivas, 1978b; Langerwerf, 1985). The toxicity of Cr^{3+} is low because of its ability to react and form stable complexes, its insolubility and its poor membrane permeability. In contrast to this, hexavalent chromium is very toxic (Gauglhofer, 1986).

The major concern over Cr^{3+} is that if the Cr^{3+} somehow percolates into the groundwater, this groundwater may be used as a drinking water supply downstream. When the water is chlorinated at the drinking water plant to kill bacteria, the chlorine can potentially oxidize the Cr^{3+} to the toxic Cr^{6+} (Lee, 1986). In view of this a great deal of research has been carried out to reduce the Cr^{3+} discharged in tannery wastewaters. This has resulted in the development of recovery and recycling techniques.

The chrome bearing liquors are collected separately and treated with an alkali to precipitate the chromium in the hydroxide form. The hydroxide sludge is then passed to a filter press or belt and the cake so formed is then redissolved with sulphuric acid and reused. Alternately, the liquor can be left undisturbed overnight. Virtually chrome-free supernatant can then be drawn off and discharged to the effluent system while the remaining settled hydroxide sludge is redissolved with acid and subsequently reused (Lee, 1986; Langerwerf, 1985).

The alkali used for precipitating the chrome in spent liquors depends on the subsequent recycle system. Hydrated lime is an effective precipitating agent, the precipitated chromium hydroxide reacting favourably with the flocculent to form a large, sturdy, settleable floc that is easily passed to a filter press. If sludge redissolution is employed, a variety of alkalis can be used, including sodium hydroxide and magnesium oxide. Kaolin may be used to clarify the solution prior to redissolution. It has been found that a certain amount of trial work is needed to find the optimum mixture of alkalis (Winters, 1986a).

The recovery and recycling of chrome from waste liquors has been successfully operated on full scale plants (Boast, 1988; Daigle and Bennett, 1986). In addition to the substantial environmental benefits, considerable cost savings are achieved due to the use of less chromium sulphate in the tannery. The wastewater sludges that are produced are also suitable for use as fertilizers in agriculture, as they do not contain high concentrations of chromium.

2.4.1.3 Grease Removal

The fats and greases in tannery wastewaters form floating fat layers in effluent balancing tanks under quiescent conditions. This grease has detrimental effects on subsequent treatment stages. It can cause poor sedimentation in settling tanks, can reduce the oxygen transfer in aeration tanks and can reduce the evaporation rate in ponds and lagoons. As most of the fats and greases are contained in the alkaline unhairing and subsequent wash liquors, grease removal should be carried out on these liquors prior to them being mixed with other waste streams in the balancing tanks (Hart et al, 1987).

The simplest form of grease removal trap is a tank with submerged weir or piped outlets below the level of the accumulated floating grease or oil layer. The volume and surface area of the grease traps should be large enough to ensure that the retention time and downflow rate in the system are adequate for grease entrapment (Hart et al, 1987). The grease and oil that is removed can be converted to tallow by steam coagulation at a low pH, usually obtained by the addition of sulphuric acid.

2.4.2 Primary Treatment

Primary treatment refers to the physico-chemical separation of the suspended solids and some of the organic pollution, allowing these materials to be removed from the effluent. Depending on the type of primary treatment carried out, approximately 95 % of the suspended solids and 40 - 70 % of the BOD can be removed.

The four major types of primary treatment are:-

- (a) screening
- (b) balancing and equalization
- (c) sedimentation
- (d) air flotation

2.4.2.1 Screening

Screening is essential to ensure that the large solids and skin fragments which inevitably appear in tannery effluent are not allowed to block pipes and pumps. It also ensures the removal of solid matter which can reduce the efficiency of the wastewater treatment plant (Aloy et al, 1976). However, while reducing undesirable wastewater constituents, screening contributes to the volume of solid waste which has to be disposed of.

A wide variety of screens are used in tanneries. Where low capital investment is necessary, a series of bar screens can prove efficient. Such cheap screens would, however, require regular manual cleaning. A variety of "self-cleaning" screens are also available. When correctly used, such units are most economic and efficient, but it should be noted that if installed incorrectly, they still require regular manual cleaning. In practice, the "brushed screens"

appear to be the most efficient, with very low maintenance costs, although such units require greater floor space and a power supply. When correctly dimensioned, they guarantee freedom from pump or pipe blockages and require minimum labour for removal of solids (Winters, 1986a; Shivas, 1981).

Screens must be dimensioned to cope with peak flow. This simple process is often incorrectly dimensioned and plants which cannot cope are frequently installed. This results in continuing operational problems in subsequent treatment (Costle et al, 1979).

2.4.2.2 Balancing and Equalization

The balancing and equalization of waste streams is an important aspect of primary treatment. The batch nature of tannery operations creates wide fluctuations in waste flow and strength. Such variations can be difficult to handle and may result in over- or under-design of the secondary treatment units. The volume and strength of waste streams vary depending on process formulations and scheduling of tannery operations. Alkaline wastes are associated with beamhouse operations, while acid discharges arise from the tanning process. In order to produce optimum results in subsequent treatment operations, the equalization of flow, strength and pH of the waste streams is essential (Costle et al, 1979; Hart et al, 1987).

To ensure the necessary mixing to achieve equalization and to avoid settlement of suspended solids, mechanical stirring or aeration is essential. Today the majority of equalization tanks employ surface aerators or diffused air as the means of agitation. Technically, air injected into the bottom of the equalization tank may be preferable as in addition to avoiding settlement of suspended solids, the oxygen present will inhibit anaerobic conditions and encourage flocculation. However, it has been found that some types of diffusers become clogged in time, and at present floating aerators are more popular for this process (Winters, 1986a; Aloy et al, 1976).

2.4.2.3 Sedimentation

Sedimentation is the most widely practised form of effluent treatment in tanneries. It allows a proportion of the solids present to be removed from the effluent prior to discharge, thus reducing the discharge costs,

particularly as some of the particulate matter contributes to the COD of the waste.

The basic theory of sedimentation is very simple. The effluent is retained in a tank or basin, quiescent or flowing, for sufficient time to allow the required proportion of the suspended matter to accumulate as an underlying sludge, leaving a clarified liquid layer known as the supernatant. The speed at which particles of suspended matter settle depends primarily on their size, the difference in density between the solid and liquid phases and the viscosity of the liquid (Aloy et al, 1976).

There are three major types of settling tanks used in tanneries. Horizontal flow tanks are the simplest of these. They are often used in low cost installations, but unless they are mechanically scraped, they soon become clogged and often operate inefficiently, with the tank full of sludge and the effluent rapidly passing over the sludge without complete sedimentation occurring. A spare tank should be installed to allow for one unit to be taken out of operation for cleaning at regular intervals (Winters, 1986a).

Vertical sedimentation tanks are the most cost effective sedimentation tanks. The sides of the conical sections have an angle of slope of at least 60°. The solids settle and concentrate at the base of the cone from where they can be drawn off by gravity (Winters, 1986a).

Radial flow tanks are the most sophisticated and potentially the most effective of the types of settling tanks found in tanneries. They are circular in plan with a relatively high ratio of diameter to depth. The base of the tank has a slight fall from the periphery inwards, with a sludge well at the centre. The effluent enters centrally and flows first downwards and then outwards and upwards to the periphery. The sludge accumulates at the base of the tank and is encouraged to move towards the central well by the action of a slowly moving scraper mechanism (Aloy et al, 1976).

Because the suspended solids in tannery effluents tend to settle readily, simple gravity sedimentation results in removal rates of 70 - 80 % for suspended solids (Costle et al, 1979; Hart et al, 1987). Should an increased degree of solids removal be required, the use of flocculents and coagulants would be necessary.

The main coagulants used in tanneries are ferrous sulphate, ferric chloride, aluminium sulphate and lime (Aloy et al, 1976). The actual chemical dosage required is dependent on the type and strength of effluent, the degree of pretreatment carried out before coagulation, the initial pH and degree of alkaline buffering capacity, and the final pH required. This is typically in the range pH 6 to 7,5 (Hart et al, 1987).

Improved coagulation and floc formation can be achieved by the use of flocculents. The most widely used flocculents are polyelectrolytes, with the best results being obtained with anionic polyacrylamide types. The cost of these polyelectrolytes, of which there is a wide range commercially available, is high but low dosages are usually required. In many applications, care has to be taken to optimise the pH and mixing conditions within narrow operating ranges to achieve efficient use of these chemical agents (Hart et al, 1987). The best chemical agents to be used and the required chemical dosages for a particular effluent have to be determined by laboratory tests in order to ascertain the settling speed and the density of the floc formed.

Coagulants and flocculents have been used successfully in treating tannery effluents (Pastore et al, 1984; Poole, 1982; Cooper et al, 1975; Bitcover et al, 1977; Shivas, 1978a). However, despite the improved effluent quality that is achieved, their use does have some disadvantages. Iron salts produce a considerable volume of sludge which is black in colour due to the presence of fine FeS particles. The use of aluminium sulphate also results in the formation of considerable quantities of sludge which are difficult to dewater. The disposal of this sludge is a major problem due to the presence of high concentrations of heavy metals. The concentration of certain inorganic ions i.e. chlorides and sulphates is also increased in the resulting effluent through the use of flocculents and coagulants, thereby increasing the total dissolved solids (TDS) of the effluent (Shuttleworth, 1978).

2.4.2.4 Air Flotation

The principle of flotation involves encouraging, by introducing bubbles into the effluent, the small or light particles which do not settle readily to separate from the liquid phase in an upward manner and form a floating sludge blanket instead of an underlying sludge sediment. The raw effluent is introduced towards the base of a tank where air is also introduced under

pressure. When this pressure is subsequently lowered in the treatment vessel, small air bubbles are released. The small particles collect on the surface of the bubbles and the buoyancy takes the aggregate to the surface where the solids form a floating blanket. This floating sludge blanket is then removed by a scraper and the clarified liquor is removed as a separate overflow (Winters, 1986a; Arnoldi et al, 1980).

There are two main methods for introducing the air bubbles:-

- (a) dissolved air flotation (DAF) where dissolved air under pressure in a portion of the effluent is released into the unit, or
- (b) induced air flotation (IAF) where a venturi introduces the air in the form of a foam.

Both of these methods have been used successfully on tannery effluent. It has been shown that DAF can reduce the COD and suspended solids (SS) of tannery effluent by 67 and 86 % respectively (Arnoldi et al, 1980). In another trial on aerated, partially pre-settled combined chrome tannery effluent, DAF reduced the COD and SS by 56 and 88 % respectively (Hart et al, 1987; Cooper et al, 1983).

IAF has proved to be more successful than DAF. A full-scale IAF plant operating on pretreated combined effluent from a mixed chrome and vegetable tannery has been found to give an average performance efficiency of 85 % COD removal and 90 % SS removal (Hart et al, 1987; Roets and Heunis, 1984).

To operate flotation, relatively high levels of coagulants and polyelectrolytes have to be used. However, the cost of such chemicals can be justified, as flotation has certain advantages over sedimentation:-

- (a) the system, when well operated, yields high levels of pollution removal
- (b) short retention times of the order of 1 hour or less have been used, thus drastically reducing the size of the plant required
- (c) the sludge is readily accessible on the surface of the liquor
- (d) the sludge floated off has a high solids content which is much easier to handle than that obtained from simple sedimentation
- (e) the sludge generated is well aerated and is less prone to anaerobic decomposition

The main disadvantage of flotation is that it is not easy to construct the plant on site. They normally have to be purchased from specialist manufacturers at a high cost (Winters, 1986a).

2.4.3 Secondary Treatment

Tannery effluent, when treated efficiently by primary, physico-chemical treatment, can be suitable for discharge to a municipal treatment plant where significant dilution is available. However, in circumstances where there is no municipal facility, or when suitable dilution is not available, further treatment of tannery effluent may be necessary. This is normally effected by secondary biological treatment (Winters, 1986b).

In secondary biological treatment, bacterial respiration is the primary method of oxidizing organic substances present in the wastewater. This results in a substantial reduction of the effluent BOD and COD.

There are four major types of secondary treatment:-

- (a) lagoons
- (b) biofiltration
- (c) oxidation ditches
- (d) activated sludge

In practice, some treatment plants are based on combinations of several systems.

2.4.3.1 Lagoons

Lagoons can be used to treat tannery wastewaters where land is available and where land values are low enough to make lagoon systems an economic alternative. There are three types of lagoons based on the biological environment that exists for the stabilization of organic wastes:-

- (a) Aerobic lagoons: Biological stabilization takes place in the presence of oxygen. These lagoons use aeration methods to increase the dissolved oxygen content in the lagoon. The aeration has to be sufficient to prevent sedimentation of the suspended solids which would then become

anaerobic (Aloy et al, 1976).

- (b) Anaerobic lagoons: Biological stabilization takes place in the absence of free oxygen. The anaerobic metabolic pathways will be discussed in further detail in Chapter 3.
- (c) Anoxic lagoons: These lagoons operate with several distinct layers, the surface layer aerobic and the lower layers anaerobic. Such anoxic systems should employ natural photosynthesis to keep the surface layer aerobic. However, in practice this makes the system subject to changes in the climate. In order to overcome this, a partially mechanically aerated lagoon which receives sufficient oxygen to allow biological purification activity, but insufficient stirring capacity to maintain the solids in suspension, has been developed. This creates areas of complete aeration around the surface aerators, while other areas, especially at the base of the lagoon, will have anaerobic activity present. Such systems have been shown to reduce the BOD of tannery effluent by 85 % at a retention time of 9 days (Aloy et al, 1976). Anoxic lagoons have also been used to treat vegetable tannery wastewater. BOD and COD reductions of 97 and 76 % respectively were noted for lagoons operating at a retention time of 36 days. If the retention time was reduced, a significant reduction in efficiency was noted, particularly with respect to the COD. The colour of the wastewater was not improved during lagooning (Parker, 1971).

Containment anoxic lagoons are used in countries with high evaporation rates, the size of the lagoons being designed on the basis of net evaporation data. These lagoons provide complete protection of adjacent river systems, and reuse of the purified effluent in the beamhouse is possible (Shuttleworth, 1978). The main problem found with these lagoons, however, is that after a period of time, the concentration of the inorganic salts increase to such an extent that reuse of the effluent becomes impossible (Rowswell et al, 1984).

The capital and running costs of lagoon systems are very low, although this will depend on the land requirement and the energy input for aeration (if any). The main disadvantages of using lagoons to treat tannery wastes are the problems caused by odour if no mechanical aeration is supplied, and the build

up of sludge. Normally no facilities exist to control and dispose of the sludge. It is therefore necessary to drain and desludge every 5 - 10 years. This requires the installation of additional lagoons which can remain operational while others are being cleaned. This would, however, require even greater land area (Winters, 1986b). Another major disadvantage is the decrease in efficiency during the winter months. A four-fold difference in efficiency between summer and winter has been noted (Costle et al, 1979).

Thus, under proper biological conditions i.e. pH control, sufficient nutrients, etc., and with long retention times usually requiring large amounts of land in relation to plant size, lagoons can provide consistent effluent quality in warmer climates. Land values and availability dictate whether lagoons are more economical than alternative treatment systems. In colder climates, winter operation can never approach summer treatment efficiency, unless the system has sufficient storage capacity to hold several months discharge (Costle et al, 1979).

2.4.3.2 Biofiltration

Biofiltration is a process whereby effluent is sprayed over and percolates through a bed of material, typically graded stone. Aeration occurs by surface renewal as the liquid passes through the filter medium. Oxygen transfer therefore takes place from the liquid film surface, through the liquid film, and into the bacterial film which develops on the surface of the packing material. Organic material from the liquid is absorbed onto this biological film, where it is degraded biologically (Hart et al, 1987).

As the biological film grows in thickness, the depth to which oxygen and organic material can penetrate decreases. Depending on the relative rates of oxygen supply and transfer, and the rate at which organic material is absorbed and degraded, different biological ecosystems are established throughout the filter. An aerobic zone is generally established at the outer portion of the slime layer, while at the surface of the media, the environment may be anaerobic if the diffused oxygen is consumed before it reaches this level of the slime layer. Similarly, if the organic material is degraded before it reaches the deepest level of the slime layer, the microorganisms will lose their viability and ability to cling to the media surface, and will be sloughed off (Hart et al, 1987).

In high rate biofiltration (HRBF), the traditional stone media is replaced by artificial media, usually plastic media. This plastic media has a high specific surface area, a low specific mass and a high void space. The size of the filters can therefore be significantly reduced, and due to the light weight of the media, it is possible to build towers up to 12 m in height whereas the limit for traditional materials is 3 to 4 m. Due to the high void space, the passage of the effluent through the filter is very rapid and consequently the overall purification is quite low. It is therefore necessary to recycle the effluent several times through the biofilter. This recycling not only improves the degree of purification, but also reduces the strength of the incoming effluent and adjusts the hydraulic versus organic loading rate. The microbial biomass which is sloughed off is settled in a clarifier (Hart et al, 1987; Aloy et al, 1976).

HRBF have been used successfully for the treatment of tannery effluents. BOD reductions of 40 % have been achieved on leather dressing wastes which contain high concentrations of vegetable tannins (Bailey et al, 1972). A COD reduction of 57 % was achieved for combined chrome tannery effluent which was pretreated by balancing, aeration, chemical dosing and DAF before being fed to the biofilter (Hart et al, 1987). However, adequate pretreatment is required in order to prevent clogging of the filters. Operational experience has also shown that lime deposition on the filters reduces their biological activity, and temperature is critical. Low temperatures in winter yield very low efficiencies (Costle et al, 1979).

2.4.3.3 Oxidation Ditches

The oxidation ditch or carrousel system is a process that incorporates simplicity, ease of operation, ease of maintenance, low cost of operation, a great deal of flexibility and a low initial capital cost. It consists of a circular channel ditch equipped with one or several rotating metal brushes. The metal brushes serve to entrain the necessary oxygen in the liquid to sustain microbial life. They also keep the contents of the ditch mixed and moving to ensure ready contact of all microorganisms with the incoming waste or food supply. The ends of the ditch are well rounded to prevent eddying or dead areas, and the velocity of the liquid should be sufficient to prevent settling of the solids (Berk, 1982).

The oxidation ditch operates in the extended aeration mode (one day hydraulic retention time or greater) and at high mixed liquor solids. The resulting food-to-microorganism (F:M) ratio is very low. At these low F:M ratios, long retention times and high sludge age, the microorganisms have to change to endogenous respiration i.e. oxidation of reserve substances. As a result, the volume of secondary sludge is considerably reduced and has a completely different composition. It is highly mineralized so that very little, if any, fermentation or putrefaction will occur (Costle et al, 1979; Van Vlimmeren, 1972).

BOD removal and the conversion of nitrogenous material to nitrate is accomplished in the aerobic zones. The purpose of the anoxic zone is to provide for bacterial breakdown of nitrate to nitrogen, which is released to the atmosphere. Odour problems do not develop because of the short period of time in the anoxic zone (Barber, 1979).

The treated waste and microorganisms flow to the final clarifier for separation. The solids are allowed to settle and the clarified liquid passes over the effluent weir and is discharged. The sludge which settles at the base of the clarifier is returned to the ditch. As the oxidation ditch is a closed system, it is necessary to remove excess sludge at periodic intervals (Berk, 1982).

It has been demonstrated that wastes from a chrome tannery could be purified, after primary settling, by means of an oxidation ditch. The BOD of the effluent was reduced by 98 % (Eggink and Kagei, 1971). The efficiency of this plant did not decrease during the winter months. This non-sensitivity to winter temperatures in removing BOD has been shown in further studies (Costle et al, 1979). In another study using the oxidation ditch in combination with primary treatment using lime, aluminium sulphate and polyelectrolytes, the overall removal of pollutants was 95 % or better (Barber, 1979).

2.4.3.4 Activated Sludge

The activated sludge process is one of the most controllable and flexible of all secondary treatment systems. It is applicable to almost all treatment situations, and is being used increasingly by tanneries where high discharge standards are required (Costle et al, 1979).

Activated sludge systems all operate on the same basic principle. Organically polluted effluent is brought into contact with a very large concentration of aerobic bacteria and other microorganisms present in the form of a flocculent biological agglomerate known as "activated sludge". The activated sludge or floc is kept in contact with the effluent by aerators. Aeration was originally achieved by compressed air, but in recent years the trend has been towards the surface aerators. Organic constituents in the effluent, together with minerals, are used as a food source for the microorganisms, and are converted to microbial cell mass, carbon dioxide, metabolic by-products and water. In this way, the organic strength of the effluent is reduced. As aerobic microbial activity is the essential effluent treatment mechanism, the conditions and rate at which this activity can be maintained are the basic design criteria (Winters, 1986b; Hart et al, 1987).

Activated sludge systems are characterised by the effluent retention time and the levels of floc maintained in the system. To retain this level of floc, it is necessary that the treated effluent passes through a sedimentation tank, with the floc being returned to the activated sludge tank (Winters, 1986b).

The most common activated sludge systems used for tannery effluent treatment are known as "extended aeration" systems where the effluent retention time is approximately two days. The longer retention time and consequently lower organic load has enabled these systems to withstand the shock loads and other irregular conditions associated with tannery effluents. Additionally, "extended aeration" systems yield substantially lower levels of sludge (Winters, 1986b).

Successful operation of the activated sludge treatment system depends on a number of factors such as:

- (a) continuity and uniformity of the feed by means of equalization
- (b) maintenance of nutrient balance and high mixed liquor solids in the aeration basin
- (c) final clarification designed for very low overflow rates of suspended solids

In addition to this, activated sludge is normally preceded by some form of primary treatment, especially in tannery applications (Costle et al, 1979).

Tannery effluents have been successfully treated by the activated sludge process. A system which involves a lime holding tank, a 30 hour retention balancing tank, a 2 hour retention primary sedimentation tank and a 24 hour retention in an activated sludge tank was shown to reduce the BOD and COD of the waste by 93 and 88 % respectively (Berg, 1973). Similar removal efficiencies have been reported for various other activated sludge plants treating tannery wastewaters, whether this is a completely mixed chrome tannery effluent, a lime-sulphide unhairing effluent or a wet-blue tannery effluent (Cooper et al, 1983; Hart et al, 1987; Kashiwaya and Yoshimoto, 1980; Cooper et al, 1976).

From the foregoing discussion it is evident that a number of different wastewater treatment methods are available to the tanner. In most cases these will enable him to meet the discharge requirements, with the exception of conductivity and total dissolved inorganic solids (TDIS) (Rowswell et al, 1989). However, although these treatment methods may solve many of the problems associated with tannery wastewaters, in many instances they create additional problems.

2.5 The Need for Improved Technology

One of the major problems in effluent treatment is the treatment and disposal of the sludge resulting from the effluent purification processes. The biochemical oxidation and purification processes used in effluent treatment, convert the soluble polluting matter and concentrate the suspended solids into a sludge that requires efficient processing and disposal. The treatment and disposal of this sludge is such a serious problem that it has been suggested that the design of effluent treatment plants should be centred around the sludge disposal part of the plant (Gale, 1972).

Wastewater sludge arises from both the primary and secondary treatment stages (Thomas, 1974). The techniques which are currently available for its treatment and disposal are outlined in Table 4.

TABLE 4
METHODS AVAILABLE FOR THE TREATMENT
AND DISPOSAL OF TANNERY SLUDGES
(Everett, 1977)

Treatment Methods	Disposal Methods
Thickening	Land Disposal for Agricultural Use
Conditioning	Incineration
Dewatering	Landfill

Sludge treatment processes have the ultimate common objective of concentrating the sludge into smaller, more manageable volumes for ultimate disposal. The three main treatment methods include:

- (a) thickening
- (b) conditioning
- (c) dewatering

Sludge thickening is a process in which the solids concentration of settled sludge is increased, thus reducing the volume of sludge to be further treated, dewatered or disposed of. As tannery wastewater sludges tend to thicken naturally under gravity at the initial point of collection to concentrations in the range similar to thickened sludge, a separate sludge thickening stage need not be considered for these sludges (Hart et al, 1987).

Sludge conditioning refers to treatment methods to improve the dewaterability of the sludge. The main sludge conditioning methods used are:-

- (a) Chemical conditioning, where chemicals such as lime, ferric chloride, aluminium sulphate and organic polyelectrolytes are applied to sludges in order to coagulate the solids and release the absorbed water.
- (b) Heat treatment to coagulate the solids, break down water-retaining gel structures, and reduce the water affinity of the sludge solids. This requires high temperatures and pressures (Hart et al, 1987).

- (c) Additional conditioning methods include freezing, irradiation and elutriation.

All of these sludge conditioning methods have distinct disadvantages. The cost of chemicals is high and the addition of further heavy metals to the sludge reduces its potential uses. Heat treatment systems are expensive and are usually not economically justifiable on the scale of most tanning operations. Elutriation is a specialist application which is also very expensive. It also uses vast quantities of water, and because the elutriate contains the fine material from the sludge, it has a high pollution load and requires biological treatment (Hart et al, 1987; Everett, 1977).

Sludge dewatering reduces the moisture content of the sludge to a level where it is generally spadeable. Sludges are dewatered in order to reduce transport costs if the sludge is disposed of at remote dump sites, to improve the calorific value prior to incineration, to reduce odour and putrescibility in odour-sensitive areas, or to reduce leachate production at landfill sites (Hart et al, 1987).

Sludges can be dewatered by using natural air-drying i.e. sludge-drying beds or sludge lagoons, or by using mechanical equipment such as vacuum filters, centrifuges, filter presses, etc. Except where land space is at an absolute premium, or where even a minor odour problem is a major consideration, the extremely high cost of all mechanical dewatering equipment makes the primary choices for dewatering of most tannery sludges either sludge drying beds or sludge lagoons. However, these dewatering methods also have their disadvantages. The efficiency of drying depends on the climate. In wet weather or during winter, this efficiency is markedly reduced. Odour is also a tremendous problem, particularly when loading or cleaning the beds or lagoons. This is particularly applicable to sludge lagoons, where the sludge remains in the wet state for longer periods of time (Hart et al, 1987; Everett, 1977; Aloy et al, 1976).

After treatment, the dried, dewatered sludges have to be disposed of. The main disposal routes are:

- (a) land disposal for agricultural use
- (b) incineration

(c) landfill

Because tannery sludges contain high concentrations of nitrogen, lime and protein, they have been used successfully as fertilizers in agriculture (Paoletti, 1987; Bilyk *et al*, 1988; Silva, 1987; Massantini, 1987). In some instances, tannery effluents have been used for agricultural purposes (Martin and Ritter, 1989; Chaturvedi, 1986). Because of the lime content, these sludges are most useful as soil conditioners in areas with acid soils. The main concern over the use of tannery sludges in agriculture is the high concentration of chrome present in the sludges. Although this chrome has the potential of being leached into the groundwater, studies have shown that it is bound to the soil or precipitated in a stable form (Martin and Parkin, 1986; Simoncini, 1987). The chrome in the sludge also has the effect of producing a slow release fertilizer (Hughes and Girdlestone, 1989). Other minor concerns include the presence of synthetic organics, dyestuffs, lacquers, etc., and the effect of these sludges on the Sodium Absorption Ratio (SAR) of the soil. Therefore, in some countries, tannery sludges are only spread on non-food crops or grassed areas (Martin and Ritter, 1989), and their continued use in the future is uncertain.

Incineration of tannery sludge is used when it is impossible to use or discharge the sludge because of economic considerations, legislation problems, or technical limitations. Before the sludge can be incinerated, it has to be substantially dewatered. Because of the cost of the plant and the fuel that is used, this disposal method is extremely expensive. Careful control is also necessary to prevent air pollution (Thomas, 1975). A more serious problem is the oxidation of the trivalent chromium to hexavalent chrome during the incineration process (Simoncini, 1987; Aloy *et al*, 1976). The residual ash consequently has to be handled and disposed of in a sensible and environmentally acceptable manner.

The disposal of tannery sludges to landfills is carried out in numerous countries. The main problems with this type of disposal are the difficulty of obtaining suitable sites and the reluctance of local authorities to accept landfill sites in their areas. This implies that transport distances and costs will increase in the future (Calcutt and Frost, 1987). In addition, there has been concern over the presence of chromium in the leachate from these landfills (Department of the Environment Monograph, 1978).

Although methods are available for the treatment and disposal of tannery sludge, future treatment and disposal will have to respond to pressures to reduce costs and to minimize the environmental impact. Research is therefore necessary to investigate and develop alternative wastewater treatment technology, which can not only be used to reduce the pollution load of tannery effluents, but at the same time reduce the volume of sludge produced. One possible method is anaerobic digestion, which has gained popularity recently as a treatment method for many industrial wastewaters.

CHAPTER THREE

ANAEROBIC DIGESTION - A BRIEF REVIEW

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ANAEROBIC DIGESTION - A BRIEF REVIEW

3.1 Introduction

Anaerobic digestion is the transformation of complex organic matter to methane (CH_4) and carbon dioxide (CO_2) by the concerted action of a wide variety of microorganisms in the absence of oxygen and exogenous electron acceptors (Britz, 1990).

Anaerobic bacteria mark the first signs of life on earth, ca $3,5 \times 10^9$ years ago. Aerobic metabolism evolved later, c 3×10^8 years ago, once phototrophy was established. The evidence supporting this theory comes mainly from geochemistry and to a lesser degree from astrophysics (Hughes, 1980). The arguments for the early appearance of anaerobic microbes is supported by the presence of fossil bacteria in South African and Canadian cherts dated approximately $3,1$ to $3,4 \times 10^9$ years ago. That these were anaerobes is supported by evidence that free molecular oxygen, if present, was extremely low in the earth's atmosphere despite its continuous abiological photo production. The atmospheric gases that were predominant at about the time that life first appeared were nitrogen, carbon dioxide, argon and water vapour. This strongly reducing atmosphere ensured obligate anaerobic metabolism (Hughes, 1980).

The depletion of available fermentable substrates led to the development of alternative inorganic substrates for energy production (chemolithotrophy) and the utilization of photons as an energy source (photolithotrophy). The oxygen released by these processes had two effects. Aerobic metabolism was fully developed by about 3×10^8 years ago, but more importantly, it restricted the number of niches for anaerobic activity. Consequently, anaerobic metabolism is restricted to those habitats where there is continually or intermittently, a total absence of oxygen (Hughes, 1980). Some of the anaerobic habitats, such as the depths of the oceans, support little metabolic activity. However, many anaerobic habitats are rich in organic matter; in fact, it is often this abundance of organic matter that is responsible for the anaerobic conditions. In swamps, ponds, estuaries, and other bodies of water that contain decaying vegetation, large microbial populations develop. The anaerobic decay of this

organic matter produces approximately 80 % of the atmospheric methane (Ehhalt and Schmidt, 1978). The anaerobic microorganisms are therefore a necessary link in the recycling of carbon from the anaerobic niches of the biosphere, and form an integral part of the methane cycle (Figure 2).

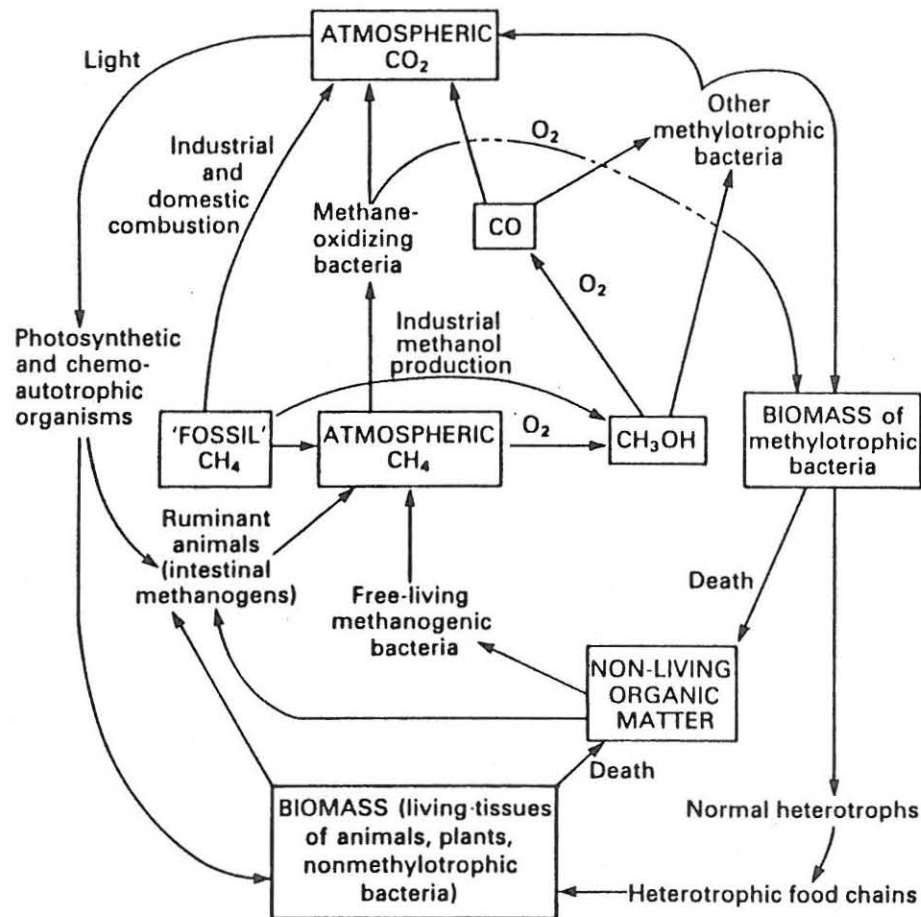


FIGURE 2 - The methane cycle (Large, 1983)

The discovery by Italian physicist Alessandro Volta in 1776 that "combustible air" was being formed in the sediments of streams, bogs and lakes rich in decaying vegetation led to subsequent discoveries by Béchamp, Popoff, Tappeneiner, Hoppe-Seyler, Söhnngen and Omelianski that methane gas has a microbial origin (Balch et al, 1979).

Research on the commercial application of anaerobic digestion commenced as early as 1814 when Davy, in a study of the fertilizer value of raw and digested cattle manure, collected biogas from the digesting manure (Colleran et al, 1982).

The first applications of anaerobic digestion were for the removal of suspended solids from domestic sewage. "Mouras' Automatic Scavenger", developed in 1881, was an air-tight chamber in which suspended organic matter was liquified by anaerobic bacterial action (Callander and Barford, 1983a).

A tank with an empty space below a bed of stones was constructed by W D Scott-Moncrief in 1890. Wastewater passed upwards through the stones in an early version of an anaerobic filter, and a significant reduction in the volume of suspended organic matter was achieved (Callander and Barford, 1983a).

In 1895, Donald Cameron in Exeter, England, modified Mouras' Automatic Scavenger and named it the septic tank (Figure 3). By 1897, the entire waste water of Exeter was treated by this system. It was discovered that the liquefaction of sewage solids produced a combustible gas containing methane, and this gas was therefore collected and used for heating and lighting at the treatment works (Callander and Barford, 1983a).

As the effluent from septic tanks was often dark and offensive and contained undigested suspended material which blocked downstream filters, new designs resulted in the solids separating from the wastewater and remaining in the digesters for longer periods of time. The first of these, the Travis tank (Figure 3), was further improved by Imhoff (Figure 3), and was widely used in the USA (Callander and Barford, 1983a).

Full scale practical anaerobic digestion of sewage sludge commenced in 1911 when Watson and O'Shaughnessy introduced the process at the Birmingham Sewage Works to stabilize the sewage sludge produced by a population of one million (Summers and Bousfield, 1976).

In 1927 it was shown that heating can markedly improve the efficiency of digestion and, although the early digesters were then subsequently heated, they were not mechanically mixed. Their efficiency was reduced by the formation of a thick scum layer and the settling of the sludge solids,

reducing their effective capacity (Figure 3). To prevent this scum layer from forming, and to ensure better contact between bacteria and solids, various mixing devices were introduced, ranging from gas recirculation to propeller action (Figure 3). This is the design that is widely used today for the digestion of sewage sludges. Raw waste is added and digester contents removed one or more times per day (Callander and Barford, 1983a).

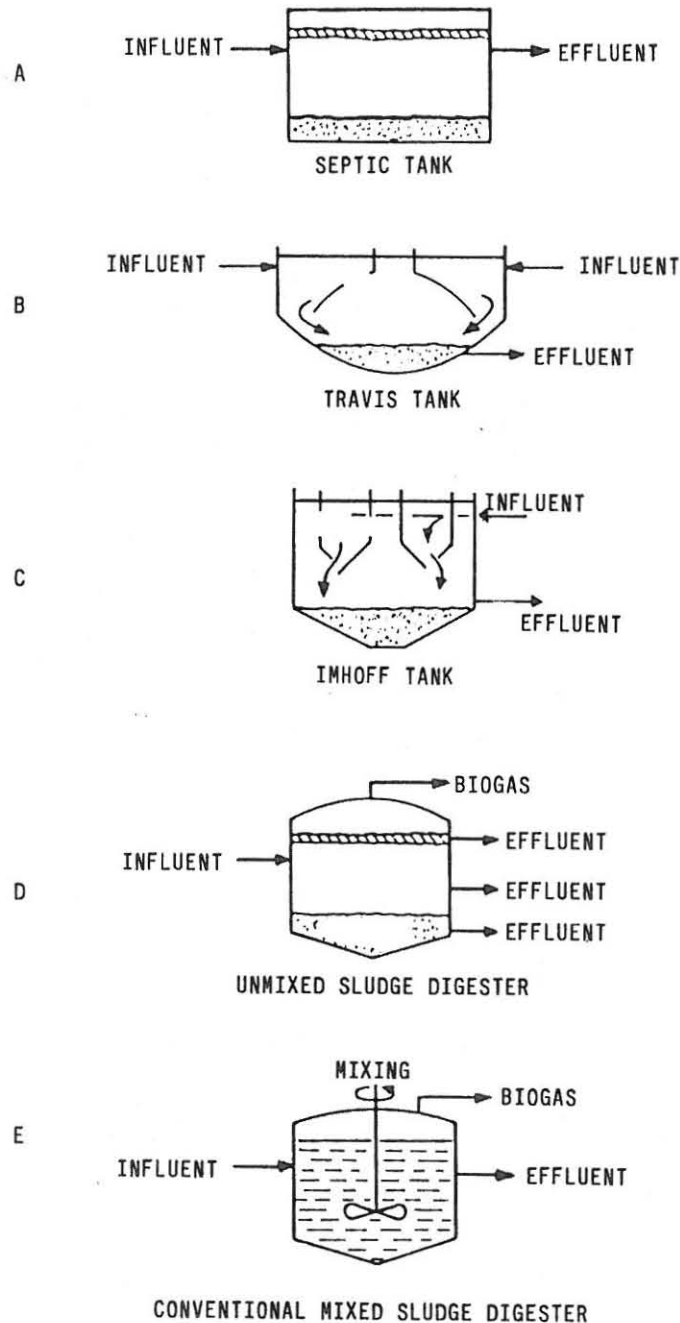


FIGURE 3 - The stages of development of the anaerobic digestion process (Callander and Barford, 1983a)

After World War II, low energy prices and the better efficiency of aerobic processes drew attention away from anaerobic digestion and even the anaerobic treatment of sludge became less popular. However, the onset of spiralling energy costs in the early 1970's, coupled with an acute awareness of environmental pollution emphasized in the late 1960's, provided the incentive for intensive research and development in the field of anaerobic digestion. This research culminated in the development of a range of digester designs suitable for the treatment of high and low strength soluble wastewaters of industrial origin (Section 3.5). Anaerobic processes were thus developed specifically to provide the industrialist with a cost-effective, reliable and flexible method of effluent treatment (Colleran et al, 1982; Anderson et al, 1982).

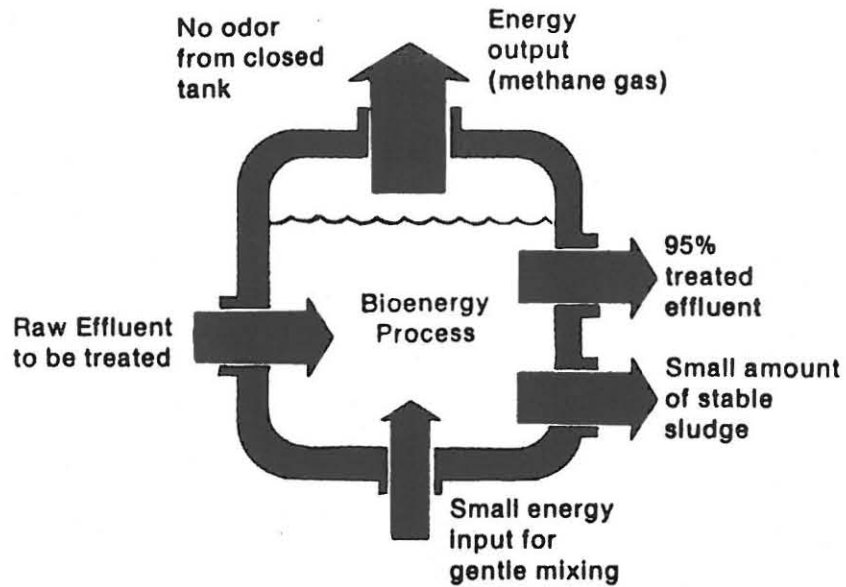
Today, many industries use anaerobic biological treatment as an economical method of waste disposal and/or a financially attractive method of recovering a useful and valuable by-product. Whereas previously anaerobic digestion was mainly used for sludge treatment and disposal, it is now being used increasingly as a pretreatment method for a variety of industrial wastewaters (Olthof et al, 1984; Anderson et al, 1984; Fannin et al, 1987; Maat and Habets, 1987; Ng et al, 1985; Maree et al, 1986; Britz et al, 1983; Brune et al, 1982; Guiot and Van den Berg, 1985).

An obvious attraction of the anaerobic process is the production of a valuable fuel from wastewater. In addition, there are many other aspects of the process which, although less obvious, are no less attractive to the industrialist.

3.2 The Advantages and Disadvantages of Anaerobic Digestion

The anaerobic method of wastewater treatment offers, under the present circumstances, a number of significant advantages over other treatment methods. These advantages are best illustrated by comparing anaerobic and aerobic treatment processes (Figure 4).

Anaerobic



Aerobic

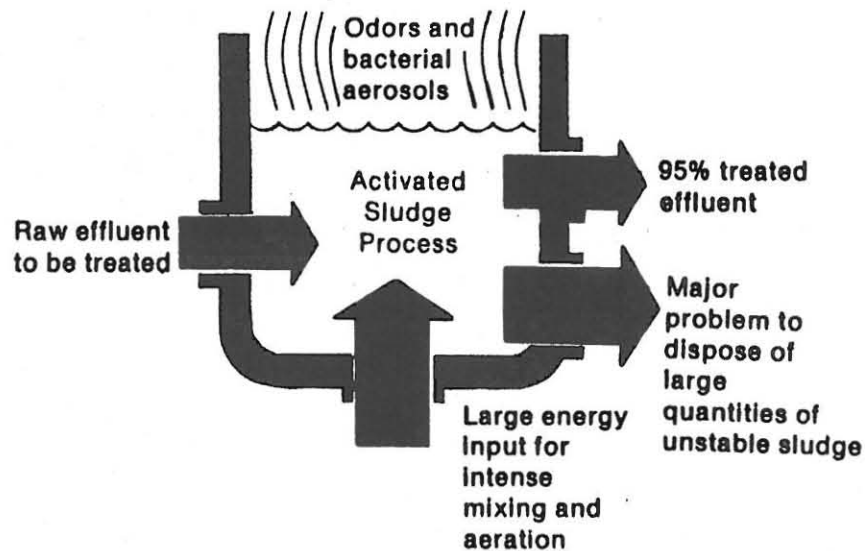


FIGURE 4 - A comparison of anaerobic and aerobic wastewater treatment processes (Ditchfield, 1986)

In more detail, these advantages are:-

- (a) In aerobic treatment i.e. the activated sludge or biofiltration processes, the waste is mixed with large quantities of microorganisms and air. Microorganisms use the organic waste as a food source and use the oxygen in the air to burn a portion of this food to CO_2 and H_2O for

energy. Since these microorganisms obtain substantial quantities of energy from this oxidation, their growth is rapid and a large proportion of the organic waste is converted into new cells. The organic matter converted to cells is not actually stabilized, but is simply changed in form. The pollution problem is therefore merely transferred from a soluble to an insoluble form as biomass or sludge. This biological sludge presents a significant disposal problem (Section 2.5). In anaerobic treatment, the waste is also mixed with large quantities of microorganisms, but here air is excluded. Under these conditions, bacteria grow which are capable of converting the organic matter into CO_2 and CH_4 gas. Unlike aerobic oxidation, the anaerobic metabolism yields little energy to the microorganisms. Consequently, their rate of growth is slow and only a small portion of the waste is converted to new cells, the major portion of the degradable waste being converted to CH_4 gas. Such conversion to CH_4 gas represents waste stabilization, since the gas is insoluble and escapes from the waste stream where it can be collected and burned to CO_2 and H_2O for heat. It has been shown that anaerobic digestion produces 0,04 - 0,14 kg excess biological sludge/kg COD as compared to 0,3 - 0,5 kg/kg COD for aerobic processes (Forday and Greenfield, 1983; Rippon, 1983; McCarty, 1964a).

- (b) As only a small proportion of the waste is converted to cells, the problem of disposal of excess sludge is greatly reduced. The disposal costs, such as transport or tipping, as well as the dewatering costs, are significantly reduced (Ditchfield, 1986; Aloy et al, 1987).
- (c) The sludge produced by the anaerobic process is more attractive than aerobic sludge, as it will not undergo further rapid decomposition, and therefore it is more stable and less odorous. It is easier to dewater, being non-colloidal, and can be used in agriculture for its unaltered content of assimilable nitrogen (Scammell, 1975).
- (d) As there is less cell synthesis in the anaerobic process, the nutrient requirements are correspondingly less than in the aerobic system. This is particularly important in the treatment of industrial wastes, which often lack nutrients such as nitrogen and phosphorous (McCarty, 1964a; Eckenfelder, 1966).

- (e) Anaerobic digestion has a favourable energy balance. In aerobic treatment, it is necessary to provide O_2 , which requires power and therefore energy. With anaerobic treatment, it is possible to produce CH_4 gas, which represents an available energy source. This methane can be utilized for the production of electric power to be used for the wastewater treatment plant and/or for the production of hot water to heat the digesters, thus reducing operational costs (McCarty, 1964b; Olthof et al, 1984).
- (f) The anaerobic conditions ensure a high kill rate of pathogenic organisms (Ghosh et al, 1975).
- (g) For very high strength wastewaters, the anaerobic process is the only practicable means of treatment. This is because the aerobic process encounters severe difficulties in obtaining an adequate rate of transfer of oxygen. With the recent development of new digester designs (Section 3.5), it is now also possible to treat low-strength wastewaters anaerobically. For certain types of wastes, such as starch and carbohydrate wastewaters, anaerobic treatment is more successful, as it overcomes the problem of sludge bulking which is experienced in aerobic treatment (Ellis, 1979; Forday and Greenfield, 1983).
- (h) Higher organic loading rates are possible in anaerobic treatment as compared to aerobic treatment (Lettinga et al, 1980b).
- (i) The anaerobic biomass has the ability to lie dormant for several months and then to be fully operational within days. This is of particular importance when seasonal wastewaters are to be treated (Anderson et al, 1984).
- (j) Anaerobic reactions can be carried out in depth, without the need for large surfaces or interfacial areas. The limiting factor for most aerobic treatment processes is contact area between the liquid and air in order to maintain the aerobic state of the liquid. In anaerobic treatment, there is no such requirement, and the entire process is carried out in closed tanks. The space requirement for anaerobic treatment is therefore less than that for aerobic treatment, and the smell and fly nuisance is completely eliminated (Gurnham, 1955; Scammell,

1975).

The anaerobic treatment process does have some disadvantages which may limit the use of this process for certain industrial wastes.

- (a) The main disadvantage of anaerobic treatment is related to the slow rate of growth of the methane-producing bacteria. The obligate methanogenic bacteria, which carry out the final stage of the process, have a minimum doubling time of the order of days. Because of this, longer periods of time are required for starting up the process as it is essential that the rate of loss of the active microorganisms does not exceed their rate of synthesis. This slow rate of growth also limits the rate at which the process can adjust to changes in waste load, temperature, and other environmental conditions (McCarty, 1964a; Rockey and Forster, 1985).
- (b) High process efficiency requires elevated temperatures (35°C) and the use of heated reaction tanks. The methane gas produced by the reaction can be used to provide this heat. However, dilute wastes may not produce sufficient CH_4 , and therefore additional heating may be required (McCarty, 1964b; Eckenfelder, 1966).
- (c) Anaerobic treatment seldom produces the high quality treated effluent which is attainable from aerobic processes. The final effluent usually has a high residual COD which has to be further reduced by aerobic treatment. For this reason, anaerobic digestion is used as a pretreatment method for industrial wastewaters (Lettinga et al, 1980b; Gurnham, 1955).
- (d) Anaerobic digestion is a rather sensitive process for certain compounds such as CHCl_3 , CCl_4 and CN^- (Lettinga et al, 1980b). However, adaptation and acclimatization can help prevent this problem.

The advantages of anaerobic treatment are quite significant, while the disadvantages are relatively few. More recently, advances in the basic understanding of the microbiology and biochemistry, along with advances in digester technology, have helped to overcome many of the problems associated with anaerobic digestion. Consequently, the advantages normally far outweigh the disadvantages, and anaerobic digestion has therefore become a viable

alternative industrial wastewater treatment method.

3.3 The Biochemistry and Microbiology of Anaerobic Digestion

Early studies of anaerobic digestion led to the postulation of three distinct stages in the breakdown of high molecular weight organic molecules to methane. These were the hydrolysis of insoluble polymers, the fermentation of the breakdown products and the generation of methane using fermentative end-products as substrates. The later observation that methanogenic bacteria can use only a limited range of substrates led to the discovery of a fourth stage, intermediate between fermentation and methanogenesis, involving the formation of acetate and hydrogen from higher volatile acids.

The four stages of anaerobic digestion form a food chain with the final products being CO_2 and CH_4 . However, the interactions are far more complex than a simple end product-substrate interchange. Volatile and long chain fatty acids, hydrogen ions, hydrogen gas and carbon dioxide may inhibit growth or change the metabolism and end products of other organisms. Competition for substrates also occurs between these bacteria. Consequently, a complex anaerobic ecosystem exists in which the efficient metabolism of each group of microorganisms is dependent on the others (Forday and Greenfield, 1983). The formation of methane is therefore a result of the combined and coordinated metabolic activities of the digester population. For the sake of clarity, however, each stage of the anaerobic process will be discussed separately.

3.3.1 Hydrolysis

The initial phases of hydrolysis of a substrate occur extracellularly since the large, insoluble polymers cannot be transported into the cell. In general, only a few bacteria are able to produce the enzymes necessary for the degradation of the very large molecules. These bacteria are obligate or facultative anaerobes and have another important function in that they remove small amounts of oxygen introduced for example by feeding the digester. The hydrolysis of complex compounds such as lipids, proteins and polysaccharides is often the rate-limiting step in anaerobic digestion (Hawkes and Hawkes, 1987).

Little is known about lipid hydrolysis in anaerobic digestion. Clostridium

and micrococci appear to produce most of the extracellular lipases in anaerobic digesters. These enzymes attack triglycerides to produce fatty acids and glycerol as reaction products (Sterritt and Lester, 1988).

A wide variety of extracellular proteases are produced in anaerobic digesters. Some of these enzymes are very specific, while others will degrade a range of proteins and peptides. The proteases also operate in a fairly broad pH range of 5 - 11 (Sterritt and Lester, 1988).

Of the polysaccharides degraded in anaerobic digesters, cellulose, hemicellulose and starch are the most important. Pectin and dextran can, however, also be degraded. These polysaccharides are solubilized by extracellular cellulases and amylases produced by a variety of different bacterial genera (Zeikus, 1980b).

Hydrolysis therefore ensures that proteins are degraded to amino acids, polysaccharides to sugar monomers and fats and oils to polyols and long chain fatty acids.

3.3.2 Acidogenesis

Most of the available information on acidogenesis is based on the fermentation of hexoses e.g. glucose.

Hexoses generated from biopolymer hydrolysis are converted into C_2 and C_3 intermediates and reduced electron carriers by a few common pathways. Most anaerobic bacteria utilize the Embden-Meyerhof pathway for hexose metabolism (i.e. glycolysis). In this pathway, hexoses are converted into 2 moles of pyruvate and 2 moles of reduced pyridine nucleotide (Figure 5). This pyruvate is then fermented to a number of different metabolic compounds (Figure 6) (Zeikus, 1980a).

The reactions (for glucose) which take place in acidogenesis are outlined in Table 5. The formation of these products is energetically favourable due to their continuous removal by the acetogenic bacteria. More detailed information regarding the anaerobic metabolism of glucose is obtainable from recent reviews (Zeikus, 1980a; Morris, 1985).

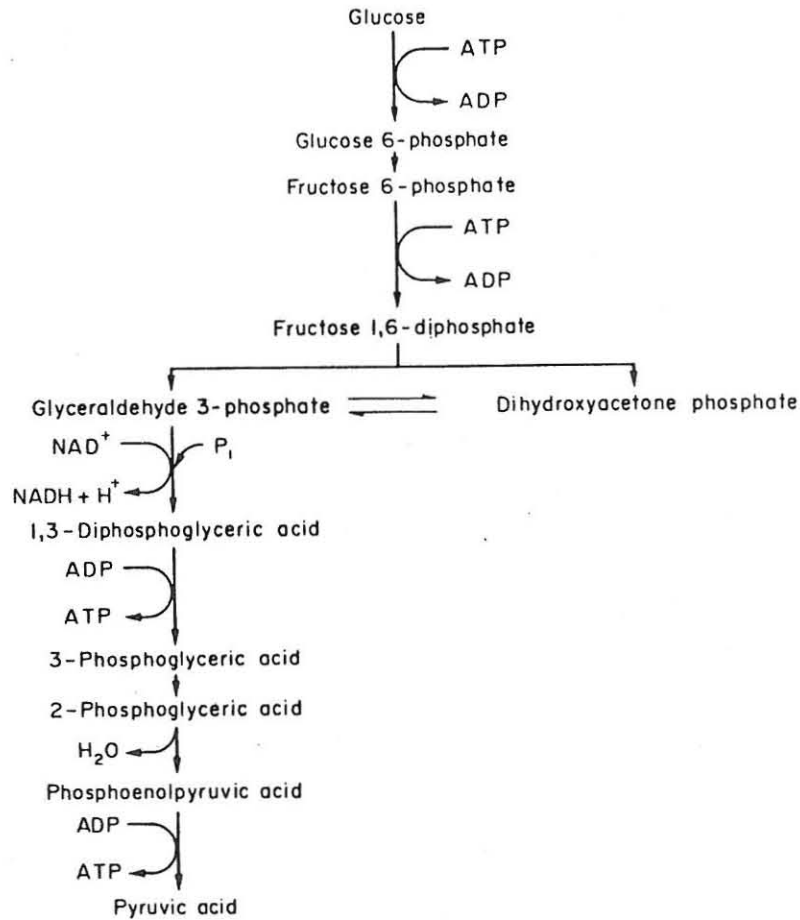


FIGURE 5 - The Embden-Meyerhof pathway of glycolysis (Morris, 1985)

Proteinaceous materials are fermented to the products indicated in Figure 6, and in addition, products including isobutyrate, isovalerate, 2-methylbutyrate, n-valerate and various aromatic acids such as phenylacetate and indoleacetate are produced (Bryant, 1979).

Acetate is also generated from the breakdown of long chain alkanolic acids by β -oxidation, a cyclical process which removes two-carbon fragments from the end of the molecule during each turn of the cycle. Anaerobic β -oxidation also results in the formation of large quantities of NADH_2 (Sterritt and Lester, 1988).

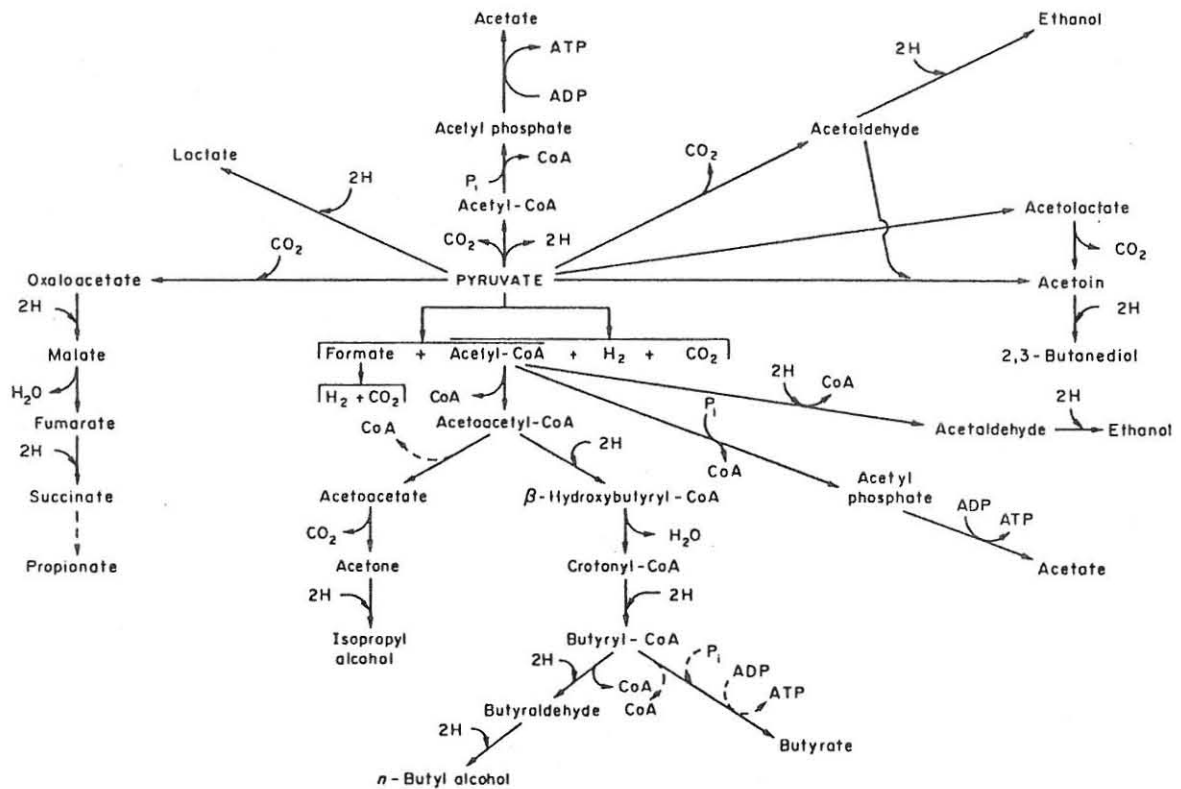


FIGURE 6 - The major fates of pyruvate during various anaerobic bacterial fermentations of glucose (Morris, 1985)

TABLE 5

REACTIONS OCCURRING DURING THE ACIDOGENESIS OF GLUCOSE
(Van Andel and Breure, 1984)

Reaction	ΔG° (kJ)
$C_6H_{12}O_6 + 4H_2O \longrightarrow 2CH_3COO^- + 2HCO_3^- + 4H^+ + 4H_2$	- 206
$C_6H_{12}O_6 + H_2O \longrightarrow CH_3COO^- + CH_3CH_2COO^- + HCO_3^- + 3H^+ + H_2$	- 283
$C_6H_{12}O_6 + 2H_2O \longrightarrow CH_3CH_2CH_2COO^- + 2HCO_3^- + 3H^+ + 2H_2$	- 255
$C_6H_{12}O_6 + 2H_2O \longrightarrow 2CH_3CH_2OH + 2HCO_3^- + 2H^+$	- 226

The concentration of H_2 in the digester plays an important role in controlling the proportions of the various products produced by the acidogenic bacteria. This is due to NAD-linked H_2 formation.



The oxidation of NADH with H_2 production is essential for the degradation of organic matter to proceed, yet the equilibrium of the reaction is strongly in the direction of NADH formation unless the partial pressure of H_2 is maintained at a very low level, as it is in the presence of efficiently metabolizing H_2 -utilizing methanogenic bacteria. The production of H_2 during pyruvate degradation is energetically much more favourable and proceeds even with relatively high concentrations of H_2 in the digester. When the H_2 concentration increases as, for example, when stress is put on the methanogenic bacteria, there is an increasing tendency of the electrons of NADH generated to be utilized in the increased catabolism of pyruvate to the reduced products especially propionate rather than acetate, CO_2 and H_2 (Figure 6). Butyric, valeric and caproic acid production may also increase and in more extreme cases it is possible to find significant formation of other reduced products such as ethanol and lactate (Bryant, 1979). Since the methanogens cannot utilize these end-products as substrates directly, their accumulation ensues, which leads to a decrease in the pH of the anaerobic digester. In an efficiently operating methanogenic digester, i.e. one in which the partial pressure of H_2 is maintained at very low levels, most of the organic matter is fermented via acetate, CO_2 and H_2 formation without the major production of other fatty acids (Bryant, 1979).

Thermodynamic considerations of the reactions involved in the conversion of glucose to methane suggest that if hydrogen is maintained at sufficiently low levels ($< 10^{-4}$ atm), the production of propionic acid need never occur. In practice, however, the nature of wastewater substrates is rarely as well defined or as simple as glucose. Moreover, an anaerobic digester treating organic wastewater is also likely to receive a variable input. These inevitabilities serve to ensure the appearance of propionic and butyric acids in some proportion during the course of wastewater treatment (Harper and Pohland, 1986).

The acidogenic bacteria form by far the largest of the trophic groups,

comprising approximately 90 % of the total digester population (Zeikus, 1980b). The principle intermediates produced by these bacteria are the short chain volatile fatty acids. Depending on the original substrate, the acidogens include members of the genera Bacillus, Clostridium, Streptococcus, Pseudomonas, Lactobacillus, Klebsiella, Escherichia, Acinetobacter, Bacteroides, Butyrivibrio, Propionibacterium and Bifidobacterium (Britz, 1990).

3.3.3 Acetogenesis

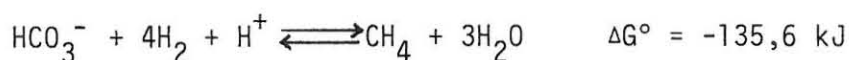
The products formed during acidogenesis i.e. the volatile fatty acids and alcohols, are anaerobically oxidized to acetate, CO_2 and H_2 (Table 6) during acetogenesis.

TABLE 6
THERMODYNAMICS OF REACTIONS OCCURRING DURING ACETOGENESIS
(Harper and Pohland, 1986)

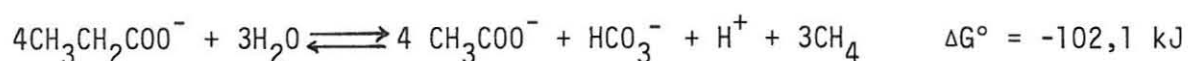
Reaction	ΔG° (kJ)
$\text{CH}_3\text{CH}_2\text{OH} + \text{H}_2\text{O} \longrightarrow \text{CH}_3\text{COO}^- + \text{H}^+ + 2\text{H}_2$	+ 9,6
$\text{CH}_3\text{CH}_2\text{COO}^- + 3\text{H}_2\text{O} \longrightarrow \text{CH}_3\text{COO}^- + \text{H}^+ + \text{HCO}_3^-$	+ 76,1
$\text{CH}_3\text{CH}_2\text{CH}_2\text{COO}^- + 2\text{H}_2\text{O} \longrightarrow 2\text{CH}_3\text{COO}^- + \text{H}^+$	+ 48,1

Longer chain even-numbered-carbon fatty acids (caproate) are converted to acetate and H_2 directly. However, long chain odd-numbered-carbon fatty acids (valerate) are first converted to acetate, propionate and H_2 . The propionate is then further degraded as shown in Table 6. This is accomplished through a syntrophic association of different bacterial species (McInerney et al, 1980). The conversion of these higher volatile fatty acids, caproate, valerate, butyrate and propionate to the methanogenic substrates, hydrogen, CO_2 and acetate, is an important step in the anaerobic digestion process, as the un-ionized form of these acids are particularly toxic to the methanogenic bacteria (Forday and Greenfield, 1983). The maintenance of an extremely low partial pressure of H_2 in the digester by the methanogens is essential for the growth and catabolism of the fatty acids by the obligate hydrogen-producing

acetogenic bacteria. This can be illustrated by the catabolism of propionate. The free-energy change (ΔG°) for the reaction is highly positive and therefore the reaction would not proceed alone. However, the use of hydrogen by methanogenic bacteria according to the reaction



is highly favourable. If the two species of bacteria are placed in syntrophic association, the combined reactions then become energetically favourable enough to proceed, i.e.



Consequently, the ability of the acetogenic bacteria to function depends on the rapid removal of hydrogen from the digester. If conditions cause the inhibition or death of the methanogenic bacteria, the H_2 concentration will increase and the pH of the digester will decrease due to an accumulation of volatile fatty acids (Bryant, 1979; Daniels, 1984).

The acetogenic bacteria are the least understood of all the trophic groups involved in anaerobic digestion. This is due to their extreme sensitivity to hydrogen, difficulty in isolation and cultivation and their slow growth characteristics. However, research has shown that members of the genera Syntrophomonas, Clostridium, Syntrophococcus, Syntrophus and Acetomaculum form part of the acetogenic population (Britz, 1990).

Another group of bacteria that play an important role in anaerobic digestion are the homoacetogenic bacteria. They convert CO_2 and H_2 into acetate, and therefore remove H_2 as quickly as it is formed, helping to maintain the low hydrogen partial pressure required for successful digestion. Although acetate is the major end-product, other metabolites such as carbon dioxide, ethanol, propionate, butyrate and valerate may be formed depending on the oxidation state of available substrates. The genera that form the homoacetogenic trophic group include Acetobacterium, Acetogenium, Clostridium, Butyribacterium and Sporomusa (Britz, 1990).

Interspecies hydrogen transfer therefore plays a very important role during anaerobic digestion. Recent reviews provide more detailed information on this

interspecies hydrogen transfer as well as acetate synthesis by acetogenic bacteria (Wolin and Miller, 1982; Schlegel and Schneider, 1985; Ljungdahl, 1986).

3.3.4 Methanogenesis

The combined action of hydrolysis, acidogenesis and acetogenesis ensure the conversion of large complex molecules into the three major substrates, namely acetate, CO_2 and H_2 from which the methanogenic bacteria can produce methane. As methanogens are limited to simple growth substrates, they do not have an energy-rich metabolism. A comparison of the free energies of hydrolysis of ATP (-31,8 kJ) and those of methane formation from the substrates H_2 and CO_2 (-135,6 kJ/mol CH_4), and acetate (-31,0 kJ) leads to the conclusion that only small amounts of energy are available to these organisms (Jones et al, 1987).

There are two major groups within the methanogens, namely the hydrogen-utilizing bacteria which reduce carbon dioxide with hydrogen to produce methane, and the acetoclastic organisms which produce methane by the decarboxylation of acetate. An in depth explanation of metabolism and methanogenesis in methanogenic bacteria is beyond the scope of this thesis. Detailed information on the biochemistry of methanogenesis can be obtained from numerous recent reviews (Jones et al, 1987; Hauska, 1988; Escalante-Semerena et al, 1984; Zeikus, 1983; Zeikus et al, 1985; Fuchs and Stupperich, 1984; Mah et al, 1977; Vogels and Visser, 1983; Vogels et al, 1984; Nagle and Wolfe, 1985). However a brief discussion has been included in order to illustrate the two major mechanisms of methane production.

The bacterial biosynthesis of CH_4 from H_2 and CO_2 is not well understood. The extreme sensitivity to oxygen of some of the coenzymes and enzymes has hampered progress on the understanding of the biochemical pathways leading to the formation of CH_4 . However, the advent of techniques suitable for the fractionation of cell-free systems under strictly anaerobic conditions has greatly facilitated work in this field, and several coenzymes, which are unique to methanogens, have been shown to be involved in methanogenesis. These include coenzyme M, coenzyme F_{420} , N-7-mercaptoheptanoyl-O-phosphothreonine (formerly component B of the methylreductase system), the carbon dioxide reduction factor (MFR), methanopterin, and a methanopterin-like compound known as formaldehyde activating factor (FAF) (Escalante-Semerena et

al, 1984).

The pathway of CO_2 reduction to CH_4 as it is now understood is shown in Figure 7.

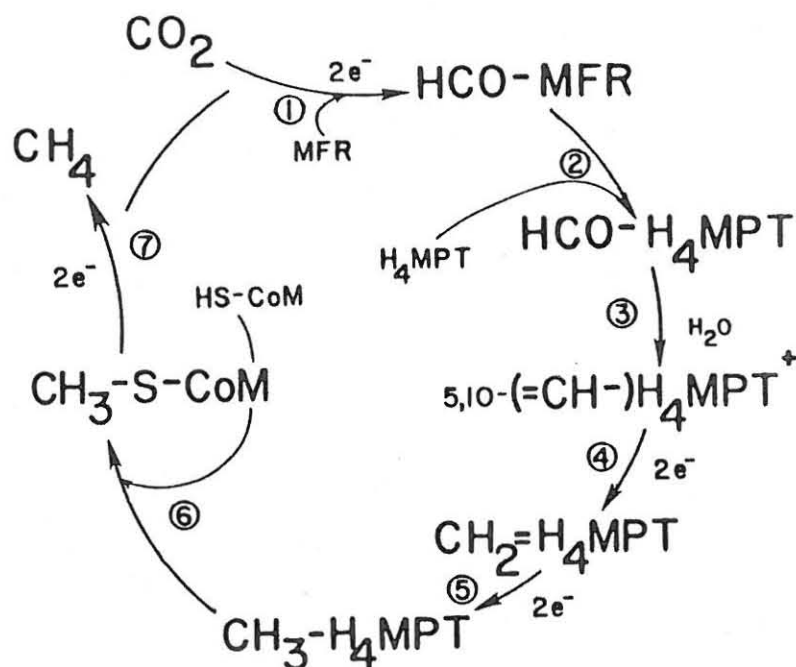
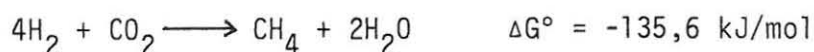


FIGURE 7 - Pathway for the reduction of CO_2 to CH_4 (CoM = coenzyme M; MFR = carbon dioxide reduction factor; H_4MPT = tetrahydromethanopterin) (Jones et al, 1987)

The initial reduction of CO_2 produces the product formyl-MFR (step 1). The transfer of the formyl equivalent from formyl-MFR to tetrahydromethanopterin (H_4MPT) yields 5-formyl- H_4MPT (step 2). Step 3 of the pathway is catalyzed by the cyclohydrolase enzyme which converts 5-formyl- H_4MPT to 5,10-methenyl- H_4MPT . This compound is then converted to methylene- H_4MPT (step 4) by the enzyme 5,10-methenyl- H_4MPT dehydrogenase. Coenzyme F_{420} is the specific electron carrier in this reversible reaction and is the first use of this deazaflavin cofactor in methanogenesis. Step 5, catalyzed by methylene- H_4MPT reductase, and step 6, the transfer of the methyl equivalent from $\text{CH}_3\text{-H}_4\text{MPT}$ to HS-CoM have not been directly demonstrated but merely postulated. The terminal step of the reaction (step 7) is catalyzed by the enzyme methyl coenzyme M methylreductase. CH_4 is released and HS-CoM is regenerated in

order to accept another CH_3 group from $\text{CH}_3\text{-H}_4\text{MPT}$ (Jones et al, 1987). The overall reaction can thus be written as follows:-



Acetoclastic methanogenesis is dependent upon the ability of the cell to cleave the acetate molecule, reduce the methyl equivalent, and to oxidize the carboxyl equivalent. The standard free energy of this reaction (-31 kJ/mol of methane) is nearly equal to that required for synthesis of a molecule of ATP from ADP and inorganic phosphate (+31,8 kJ/mol). Consequently, acetoclastic methanogenesis is not an energy-rich metabolism compared with methanogenesis from one-carbon substrates. If better growth substrates are available, the cell will utilize acetate for biosynthesis and form methane via the energetically more favourable pathways. However, the control of cellular switching from the use of one substrate to another is not yet understood (Jones et al, 1987).

The mechanism of methanogenesis from acetate has begun to be elucidated. The current model is shown in Figure 8. Acetate is transformed into an acetyl compound which is then cleaved to yield a carbonyl [CO] and a methyl group; [CO] is oxidized to CO_2 by CO dehydrogenase. A soluble electron and proton carrier (e_1) is coupled to CO dehydrogenase and interacts with a membrane-bound electron and proton carrier (e_2). The proton carrier e_2 interacts with another membrane-bound component (e_3) which only carries electrons, and thus protons are released on the outside of the membrane. Reduced e_3 then transfers electrons to a soluble proton and electron carrier (e_4) which reduces $\text{CH}_3\text{-CoM}$ to methane via methylreductase. Protons for methyl CoM reduction are drawn from the cytoplasmic pool. The resultant proton gradient is used to drive ATP synthesis by membrane-bound adenosine triphosphatase (Zeikus et al, 1985).

The present model accounts for energy conservation and the function of CO dehydrogenase during growth of methanogens on acetate. CO dehydrogenase plays an important role, as during growth of Methanosarcina barkeri on acetate, the specific activity of the enzyme increases fivefold compared to when M. barkeri is grown on CO_2 and H_2 . The enzyme also accounts for 5 % of soluble protein in acetate-grown cells (Zeikus et al, 1985).

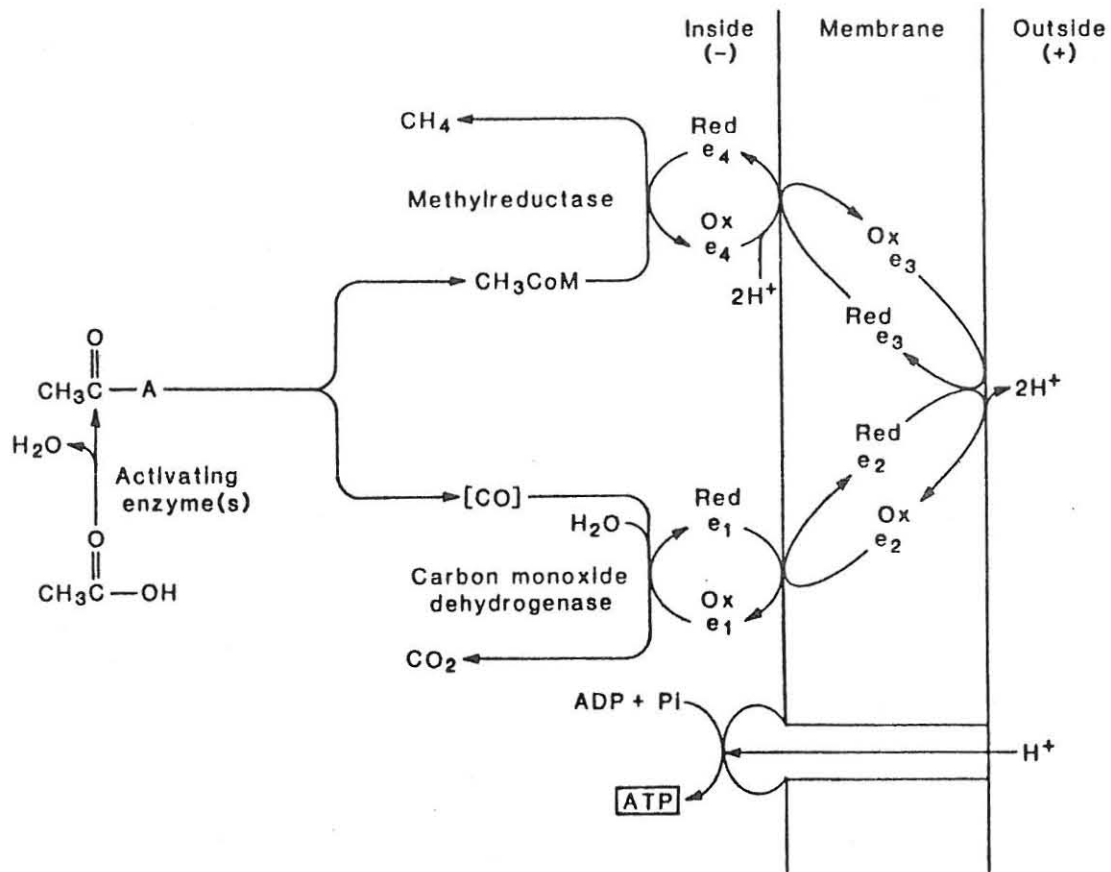
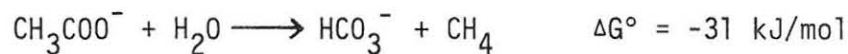


FIGURE 8 - Proposed model of carbon and electron flow for methanogenic bacteria grown on acetate (ADP = adenosine diphosphate; ATP = adenosine triphosphate; e_1 = soluble electron and proton carrier; e_2 = membrane-bound electron and proton carrier; e_3 = membrane-bound electron carrier; e_4 = soluble proton and electron carrier) (Zeikus *et al*, 1985)

The overall reaction of acetoclastic methanogenesis can thus be written as follows:-



Approximately 70 % of the methane produced in an anaerobic digester arises from the acetoclastic pathway, but the importance of the H_2 -utilizing methanogens should not be underestimated. They remove hydrogen from the system and this removal promotes the conversion of propionic and butyric acids to acetate. An accumulation of these higher acids is thereby averted and the

digestion process proceeds under stable, steady state conditions (Ditchfield, 1986).

Methanogens are members of a newly defined kingdom of prokaryotes, the Archaeobacteria (Fox et al, 1980). The non-methanogenic members of the Archaeobacteria are either thermophilic or halophilic and are found in hot or high-salt environments. Early classifications dispersed methanogens among various morphological groups as they may be Gram-positive or Gram-negative and have most of the possible cell shapes i.e. cocci, rods, spirilla and sarcina (Nagle and Wolfe, 1985). The methanogens are now separated into three orders, Methanobacteriales, Methanococcales and Methanomicrobiales (Table 7). These divisions were made because analysis of sequences of 16s ribosomal RNA showed that there is a wide diversity among the methanogens; this is consistent with their diverse morphology and G + C ratios in the range 27 - 52 % (Holland et al, 1987).

TABLE 7

TAXONOMIC CLASSIFICATION OF METHANOGENS
(Jones et al, 1987; Wolin and Miller, 1985; Dubach and Bachofen, 1985)

Order	Family	Genus
Methanobacteriales	Methanobacteriaceae	Methanobacterium Methanobrevibacter Methanosphaera
	Methanothermaceae	Methanothermus
Methanococcales	Methanococcaceae	Methanococcus
Methanomicrobiales	Methanomicrobiaceae	Methanomicrobium Methanogenium Methanospirillum
	Methanosarcinaceae	Methanosarcina Methanotherix Methanococcoides Methanlobus Halomethanococcus
	Methanoplanaceae	Methanoplanus

Normal peptidoglycan is absent from all of the Gram-positive methanogens. Instead they contain a heteropolysaccharide which does not contain any of the usual cell wall aminosaccharide components. The Gram-negative methanogens have protein cell walls (Holland et al, 1987). The lipids of the cell membranes are also unlike other prokaryotic lipids and consist of isoprenoid hydrocarbon chains of 25 - 30 carbon atoms (mainly squalene) of different degrees of unsaturation, together with C₂₀ (phytanyl) or C₄₀ (biphytanyl glycerol) 1,2 diethers. There are therefore no saponifiable lipids such as are found in other living cells (De Rosa et al, 1986).

All of the methanogens are obligate anaerobes requiring a redox potential of lower than -300 mV, and all produce methane as a metabolic end-product. As a result of their unique metabolism, they control the rate of organic degradation and direct the flow of carbon and electrons by removing toxic intermediary metabolites and by enhancing thermodynamic efficiency of interspecies metabolism. They are thus responsible for the terminal reactions in anaerobic digesters and can therefore be considered to be the key organisms in anaerobic digestion (Britz, 1990). Recent reviews provide detailed information on the taxonomy and diversity of the methanogens (Jones et al, 1987; Balch et al, 1979; Wolin and Miller, 1985; Dubach and Bachofen, 1985).

Methanogenesis is the most important step in the anaerobic digestion process. The CH₄ gas that is produced is insoluble in water. Therefore it does not contribute to the COD or BOD of the final effluent. In this way a COD or BOD reduction of the waste is achieved. If methanogenesis is inhibited, high energy intermediates i.e. volatile fatty acids, accumulate and since there is no loss of organic carbon, no reduction in the pollutant load of the waste occurs.

Although the anaerobic digestion process consists of four stages, the success of the process depends on the interaction of several microbial populations. The overall anaerobic digestion pathway is shown in Figure 9.

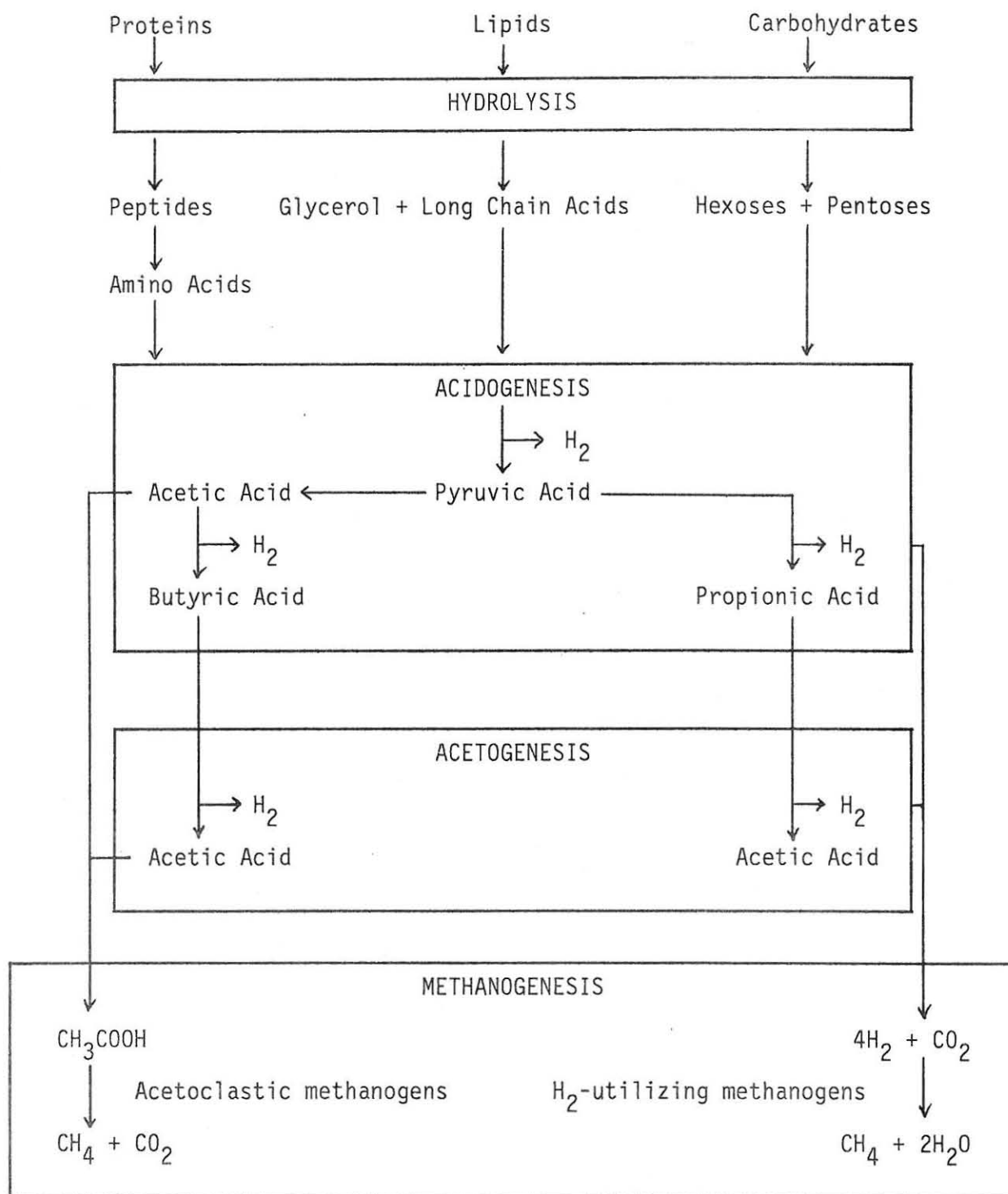


FIGURE 9 - An outline of anaerobic digestion at the molecular level (Ditchfield, 1986)

3.4 Process Control and Monitoring of Anaerobic Wastewater Treatment

Anaerobic treatment of industrial wastes is often regarded as an unstable process especially when compared with aerobic treatment processes. However, it need not be unreliable if properly controlled.

Process control can be defined as actions taken to achieve a desired result (Frostell, 1985). In anaerobic treatment a desired result usually means:-

- (a) consistent achievement of a high degree of waste stabilization
- (b) an as high as possible conversion of waste to methane
- (c) an as low as possible production of excess solids at the highest possible loading rate

To achieve the desired result, the environmental requirements of the microorganisms must be fulfilled. Even under optimal conditions, the rate of anaerobic digestion is slow; therefore near optimal environmental conditions have to be maintained in order for satisfactory efficiency and rate of digestion to occur.

In order to optimize the operation of an anaerobic digester, the temperature, pH and alkalinity should be controlled, toxic substrates should be eliminated, essential growth minerals and nutrients should be supplied, shock loads should be prevented and the digester design should allow for the adequate retention of the active biomass. These requirements necessitate a level of expertise and sophistication in the design, monitoring and control of anaerobic digesters. In general, an increased rate of treatment requires more frequent monitoring since parameter values change more rapidly (Frostell, 1985).

Parameters which require control include the following:-

3.4.1 Temperature

The three optimum temperature ranges at which anaerobic digestion and other biological reactions occur are:- psychrophilic ($< 20^{\circ}\text{C}$), mesophilic ($20 - 45^{\circ}\text{C}$) and thermophilic ($50 - 65^{\circ}\text{C}$). As indicated, the psychrophilic and mesophilic temperature ranges form a continuous group while the thermophilic range is separate. Gas production falls off rapidly at the limits of these

ranges and the effects of a short (usually accidental) period of raised temperature from the mesophilic to the thermophilic range may take many weeks to overcome (Forday and Greenfield, 1983; Hawkes and Hawkes, 1987).

In all microbial systems, a temperature increase leads to increased microbial activity. However, changes in overall process efficiency due to increased metabolic activity are balanced by a corresponding increase in the rate of microbial inactivation. Hence the optimal process temperature represents the best compromise between these two factors. Above this optimum temperature, process efficiency decreases. During anaerobic treatment, a relatively constant temperature is desirable, and should not fluctuate by more than 2°C per day. The need for a constant temperature is explained by the different kinetic growth rates of acidogenic and methanogenic microorganisms. Owing to their faster growth rates, the acidogens acclimatize more rapidly to changed conditions, causing an accumulation of volatile fatty acids. The result is an overall imbalance (Frostell, 1985).

Low temperature waste digestion is slower than mesophilic digestion. The former process is, however, less energy demanding and hence suitable for small scale waste treatment. Thermophilic digestion processes offer a number of advantages. The rapid metabolic activity of the digester population results in shorter retention times, higher loading rates and therefore smaller digesters. Bacterial pathogens are also eliminated. However, thermophilic operation requires high energy inputs and the high rate of gas formation causes excessive agitation and poor separation of digested sludge. Maintenance costs are also higher with thermophilic digesters (Forday and Greenfield, 1983; Gurnham, 1955). Most anaerobic treatment systems are therefore designed to operate in the mesophilic range.

3.4.2 pH and Alkalinity

The optimum pH range for anaerobic digestion is 6,8 - 8,0 (Balch et al, 1979). Maintenance of this near neutral pH range is due to the conversion of acid end products to methane by the combined activities of the acetogenic and methanogenic bacteria.

The sensitivity of anaerobic digestion to pH changes is based on the pH sensitivity of the methanogenic population. Acidogenic bacteria have a better

tolerance for low pH values, and acid production may continue relatively undisturbed despite methanogenesis being inhibited. The ultimate result if pH is not controlled is complete inhibition of methanogenesis and digester failure. Control of pH therefore has the aim of overcoming pH-inhibition of the methanogenic bacteria. For most wastewaters, this is only necessary during start-up and overload conditions, but sometimes continuous pH control is required (Frostell, 1985).

Problems with high pH are seldom experienced, although they may occur when large amounts of protein are present in the wastewater (Bryant, 1979). The anaerobic digestion of animal wastes results in a high digester pH, and care has to be taken to prevent ammonia toxicity (Section 5.8).

A high level of alkalinity is desirable in anaerobic digestion since it provides a good safety margin against sudden increases in the concentration of volatile fatty acids. The major acid-base systems encountered in anaerobic treatment are listed in Table 8. Since a high buffer capacity in a given acid-base system is achieved only near its pKa value, the bicarbonate, the sulphide and the phosphate systems are the most important at the near-neutral pH values. As the last two are usually present in only very low concentrations compared with bicarbonate, the major chemical system controlling pH is the carbon dioxide-bicarbonate system (Frostell, 1985).

TABLE 8
ACID-BASE SYSTEMS PROVIDING BUFFER CAPACITY
DURING ANAEROBIC TREATMENT
(Capri and Marais, 1975)

Acid-Base System	pKa (25°C)
$\text{H}_2\text{CO}_3 / \text{HCO}_3^-$	6,4
$\text{NH}_4^+ / \text{NH}_3$	9,3
$\text{H}_2\text{S} / \text{HS}^-$	6,9
$\text{H}_2\text{PO}_4^{2-} / \text{HPO}_4^-$	7,2
Acetate-Propionate-Butyrate	4,8 - 4,9

The carbon dioxide-bicarbonate system is related to pH according to the following equation:-

$$[H^+] = \frac{K_1 [H_2CO_3]}{[HCO_3^-]}$$

The carbonic acid concentration $[H_2CO_3]$ is related to the percentage of CO_2 in the digester gas, K_1 is the ionization constant for carbonic acid, and the bicarbonate ion concentration $[HCO_3^-]$ forms a part of the total alkalinity of the system (McCarty, 1964b). Figure 10 shows the relationship between these factors for anaerobic treatment at 35°C.

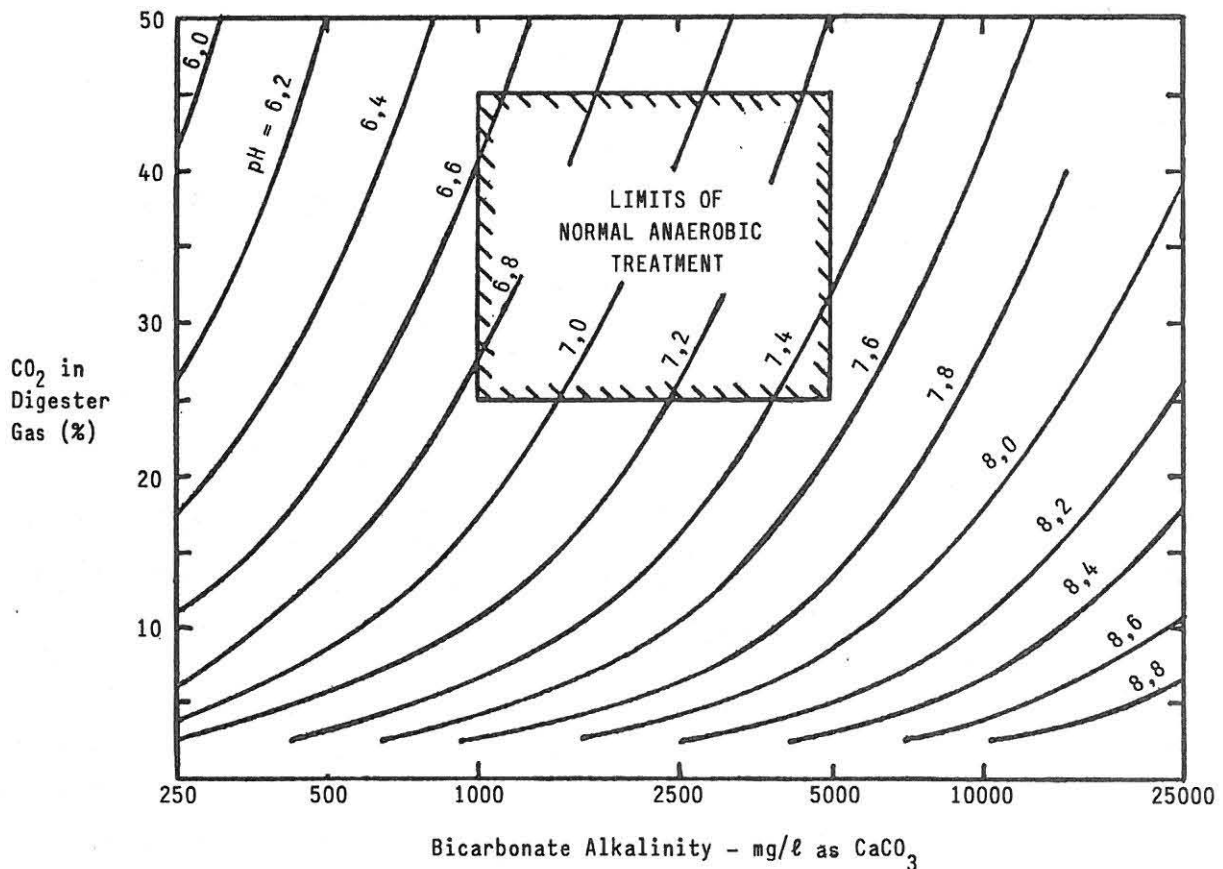


FIGURE 10 - The relationship between pH and bicarbonate concentration at 35°C (McCarty, 1964b)

The bicarbonate ion concentration or bicarbonate alkalinity is approximately equivalent to the total alkalinity for most wastes when the volatile acid concentration is very low. When the volatile acids begin to increase in

concentration, they are neutralized by the bicarbonate alkalinity, and in its place form volatile acid alkalinity. Under these conditions, the total alkalinity is composed of both bicarbonate alkalinity and volatile acid alkalinity. An increase in volatile acids over and above the available cation component of the alkalinity results in the formation of free volatile acids and partial destruction of buffering potential, accompanied by a pH decrease and decreased gas production (Ross and Louw, 1987).

A low digester bicarbonate alkalinity i.e. 1 000 mg/l, does not provide much of a safety margin for anaerobic treatment, as a small increase in volatile acids will result in a significant decrease in bicarbonate alkalinity and digester pH. Conversely, a bicarbonate alkalinity in the more desirable range of 2 500 - 5 000 mg/l provides greater buffer capacity so that a much larger increase in volatile acids can be handled with a minimum decrease in pH. This gives a good margin of safety and allows time for control if an imbalance occurs. If an increase in volatile acid concentration significantly decreases the bicarbonate concentration, and a serious drop in pH is imminent, then the bicarbonate alkalinity should be controlled. This can be carried out by reducing the feed rate to allow the volatile acids to be utilized and decrease in concentration, or it may require the addition of alkaline materials such as lime or sodium bicarbonate (McCarty, 1964b).

3.4.3 Retention Time and Substrate Loading

The hydraulic retention time (HRT), i.e. the length of time liquid added remains in the digester, is determined as follows:-

$$\text{HRT} = \frac{\text{Digester Volume}}{\text{Volume of waste fed daily}}$$

The solids retention time will be longer than this if particles are retained in the digester. HRT can vary from hours to months, depending on the type of digester in use. The most important aspect in controlling the HRT is to prevent washout of the microbes, especially the slow-growing methanogens (Hawkes and Hawkes, 1987).

The loading rate or rate at which solids are added to the digester is a function of both the volume added daily and the solids content of this feed

material. The loading rate is expressed as weight of organic matter (usually kg VS or COD) per m^3 of digester per day, and the maximum loading rate which can be applied to a digester is a function of the digester design and the type of waste (Hawkes and Hawkes, 1987).

Digester loading can also be expressed in terms of sludge load rate (kg COD/kg VSS.d). This parameter provides a measure of biomass activity and is dependent on the sludge concentration (Ross and Louw, 1987).

3.4.4 Nutritional Requirements

The bacteria responsible for anaerobic digestion require nitrogen, phosphorous and other nutrients in trace quantities for optimum growth. Municipal waste sludge normally contains a variety of these nutrients, and therefore usually provides an ideal environment for growth. However, industrial wastes are more specific in composition and biological nutrients often have to be added to achieve optimum operation (McCarty, 1964a).

The wastewater composition thus influences the growth rate of the microorganisms. In a wastewater with a balanced composition regarding carbon source as well as macro- and micro-nutrients, the growth rate of the microorganisms may be several fold higher than that on an unbalanced wastewater. This can have a great influence on whether anaerobic treatment is used or not, since with an unbalanced wastewater the digester size would have to be much larger.

The most important nutrients are nitrogen and phosphorous. The required amounts of these elements can be estimated from biomass yield coefficients which vary with the energy source (Table 9). In this table a biomass composition of $\text{C}_5\text{H}_7\text{O}_2\text{N}$ (McCarty, 1964a) and a phosphorous requirement of 15 % of the nitrogen requirement (Speece and McCarty, 1964) has been assumed.

The estimated optimum COD : N : P ratios shown in Table 9 are related to the amount of COD consumed. This means that the requirement in relation to influent COD will be lower, especially for wastewaters with a high COD : BOD ratio. Another important factor to consider is microorganism decay. It varies with temperature and growth rate and results in lower net solids yields than are predicted from yield coefficients (Frostell, 1985).

TABLE 9

THEORETICAL BIOMASS YIELD COEFFICIENTS AND CORRESPONDING OPTIMUM
COD : N : P RATIOS FOR DIFFERENT SUBSTRATES DURING ANAEROBIC TREATMENT
(Frostell, 1985)

Substrate	Overall Yield		Optimum COD : N : P
	g/g substrate	g/g COD	
Hydrogen	1,04	0,13	416 : 6,7 : 1
Acetate	0,04	0,038	1420 : 6,7 : 1
Propionate	0,18	0,12	451 : 6,7 : 1
Butyrate	0,29	0,16	338 : 6,7 : 1
Long Chain Fatty Acids	0,40	0,14	386 : 6,7 : 1
Carbohydrates	0,23	0,22	246 : 6,7 : 1
Fats	0,42	0,14	386 : 6,7 : 1
Proteins	0,13	-	-

If decay is neglected, the estimates in Table 9 suggest that the ratio between biodegradable COD and available nitrogen and phosphorous should be kept at 300 : 6,7 : 1 to 500 : 6,7 : 1. If nutrients have to be added to maintain sufficient nutrition, addition may usually be lowered once the system is in a steady-state. This is particularly so if a long solids retention time is used (Frostell, 1985).

Besides nitrogen and phosphorous, a large number of trace nutrients have been shown to be necessary for optimum anaerobic treatment. These will be discussed in more detail in Chapter 6 and 7.

3.4.5 Toxic Compounds

A toxic compound is any substance that causes a disturbance of the biological processes (Frostell, 1985). Since almost all substances suppress biological activity when present at sufficiently high concentrations, toxicity should be discussed in terms of toxic levels rather than of toxic materials. The toxic level varies from one substance to another and may differ by several orders of magnitude.

Substances that are obligatory constituents of biological cells, or which can be converted to these, are usually required at low concentrations, are stimulatory at intermediate concentrations and inhibitory at high

concentrations (McCarty, 1964c). Most other substances are tolerated only in low concentrations.

Bearing in mind the huge quantities of chemicals in current use and the large number known to be toxic at extremely low levels, it is impossible to list all substances that may cause toxicity in anaerobic treatment. Generally with municipal wastes, the major problem results from heavy metals. Industrial wastes, on the other hand, may have inhibitory concentrations of various common salts such as those containing sodium, potassium, magnesium, calcium, ammonium or sulphide. Heavy metals may also be a problem (McCarty, 1964c).

An understanding of the nature of the toxicity caused by compounds, and their control, is therefore important in evaluating the potential of the anaerobic process for the treatment of industrial wastes. This will be discussed in more detail in Chapter 5.

The monitoring of anaerobic digesters is also very important in order to determine the efficiency of the digestion process. Parameters which require monitoring include the following:-

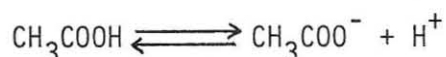
3.4.6 Volatile Fatty Acids

During anaerobic digestion, volatile fatty acids (VFA) are both produced and consumed. Under steady-state conditions, the rate of production of VFA is balanced by an equal rate of consumption. Under conditions of stress or shock loading, however, the rate of production of acids can outstrip the rate of consumption. This is as a result of the slow growth rate of the methanogenic bacteria. If there was a sudden influx of waste, the hydrolytic and acidogenic bacteria would metabolize it, leading to a rapid increase in digester acetate, butyrate and propionate concentrations. The more slowly responding methanogens would be unable to metabolize the increased concentration of their substrate. Consequently, the concentration of the VFA would increase, causing the digester pH to decrease. This in turn would further inhibit the methanogens, and butyric and propionic acids would accumulate because hydrogen would not be removed from the system (Ditchfield, 1986).

A sudden increase in the VFA concentration is frequently one of the first indicators of digester imbalance, and will often indicate the onset of adverse conditions long before any of the other parameters are affected.

It should be noted that a high VFA concentration is the result of unbalanced treatment and not the cause (McCarty, 1964b). Thus a high VFA concentration in itself is not harmful, but indicates that some other factor is affecting the methanogenic bacteria.

The accumulation of VFA results in a decrease in the pH of the digester. A pH-dependent equilibrium exists between the ionized and un-ionized volatile acid fractions in accordance with the equation:



A decrease in pH results in a leftward displacement of this equilibrium and a rise in the un-ionized volatile acid concentration (Ross and Louw, 1987). The un-ionized i.e. the protonated forms of the VFA are highly toxic. This is because the bacterial cell wall is far more permeable to undissociated molecules when compared to their ionized state. Therefore more VFA will be taken up by the microorganisms at low pH values. Once taken up, the VFA dissociates within the cell and cause a decrease of the intracellular pH, which adversely affects the general activity of cellular enzymes.

The degree of inhibition of methanogenesis by VFA is therefore linked to digester pH and is also dependent on the distribution of acids present. The concentration of un-ionized acids is greater at lower pH values, and the ratio of un-ionized acids : ionized acids at a given pH increases as the average chain length of the VFA increases. This is shown as follows: The ratio of un-ionized VFA (HA) to ionized acid (A^-) in an aqueous solution can be calculated using the equation

$$[\text{HA}]/[\text{A}^-] = [\text{H}^+]/K^1_a$$

where K^1_a is the dissociation constant of a given acid. The K^1_a values of acetic, propionic and butyric acids are:-

acetic acid (25°C) $K^1_a = 1,74 \times 10^{-4} \text{ mol/l}$

	pKa = 3,76
butyric acid (25°C)	$K_a^1 = 1,54 \times 10^{-4} \text{ mol/l}$
	pKa = 3,81
propionic acid (25°C)	$K_a^1 = 1,35 \times 10^{-4} \text{ mol/l}$
	pKa = 3,87

Hence at a given pH, the ratio $[HA]/[A^-]$ for acetic acid is less than that for butyric and propionic acids. A further influence of the distribution of VFA on the extent of inhibition is that the toxicity of the un-ionized VFA increases with the carbon length of the acid. Consequently, as the concentration of the higher volatile acids (carbon length > 2) increases, the degree of inhibition at a given pH will also increase (Forday and Greenfield, 1983).

Since the pH directly affects the toxicity of the VFA, the alkalinity or buffering capacity of the system therefore also plays an important role in VFA toxicity. The VFA to alkalinity ratio is a better indicator of the stability of an anaerobic system than their absolute individual values. A ratio of total volatile acids (as acetic) to total alkalinity (as calcium carbonate) of less than 0,1 is desirable (Kaspar and Wuhrmann, 1978).

3.4.7 Biogas Yield

The biogas produced from the digestion of organic mixtures is composed primarily of CO_2 and CH_4 with small quantities of NH_3 , H_2S , H_2 , N_2 and O_2 being present (Eckenfelder and O'Connor, 1961). The volume of biogas produced and its composition are sensitive parameters for anaerobic treatment, and have the advantage of being easily measured. Since they are the result of the microbial activity in the treatment system, they will immediately respond to changed conditions, and can therefore be used as a fairly sensitive monitoring parameter.

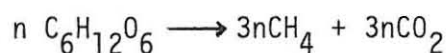
If pure organic substrates are completely broken down to CO_2 and CH_4 , then the following gas yields can be calculated per kg of substance added:

starch/cellulose/glucose	0,8 m ³ /kg
fatty acids (based on stearate)	1,5 m ³ /kg
protein (based on $C_5H_7NO_2$)	0,9 m ³ /kg

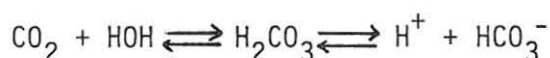
Gas yields as high as this are rarely reached in practice, since the conversion of organic matter to gas is generally incomplete (Hawkes and Hawkes, 1987). In addition, the gas yield is affected by factors such as the digester operating temperature, the retention time, the presence of inhibitors, and the mass of biodegradable solids supplied.

Since the methane formed is one desired end product in anaerobic treatment, it is an attractive parameter to monitor. It also instantly reflects the activity of the methanogenic bacteria which are the most sensitive. If conversion to methane is stable, it is a good indication of process stability. The methane yield, which is expressed as $\text{m}^3 \text{CH}_4/\text{kg COD removed}$, normally lies in the range of 0,34 - 0,36 $\text{m}^3 \text{CH}_4/\text{kg COD removed}$ at 35°C for wastewaters with a BOD : COD ratio greater than 0,5. This ratio indicates the presence of substantial amounts of biodegradable material (Anderson et al, 1982).

In the conversion of carbohydrates to CO_2 and CH_4 , equal volumes of each gas are produced.



However, all of the CO_2 is not released as a gas but also enters into reactions with water and hydroxide ions.



The CO_2 incorporated in the bicarbonate ion is removed from the digester in the liquid phase rather than the gas phase. Methane, however, is insoluble in water and escapes from the digester.

The biogas thus produced has a methane content of 60 - 75 % and an energy content of approximately 26 MJ/m^3 (Hawkes and Hawkes, 1987). It is therefore common practice to use the biogas as a fuel, both for heating and lighting the wastewater treatment plant and for maintaining temperature in the digesters.

3.4.8 Treatment Efficiency

BOD or COD can be removed in anaerobic treatment by conversion of organic matter to methane gas. This methane produced can provide a quick estimate of

the treatment efficiency, since it indicates the COD stabilization rate. Care should be taken, however, if oxidants such as O_2 , NO_3^- , H_2O_2 and SO_4^{2-} are present, as these compounds can be toxic to the anaerobic microorganisms. Figure 11 shows the flow of COD in an anaerobic treatment process.

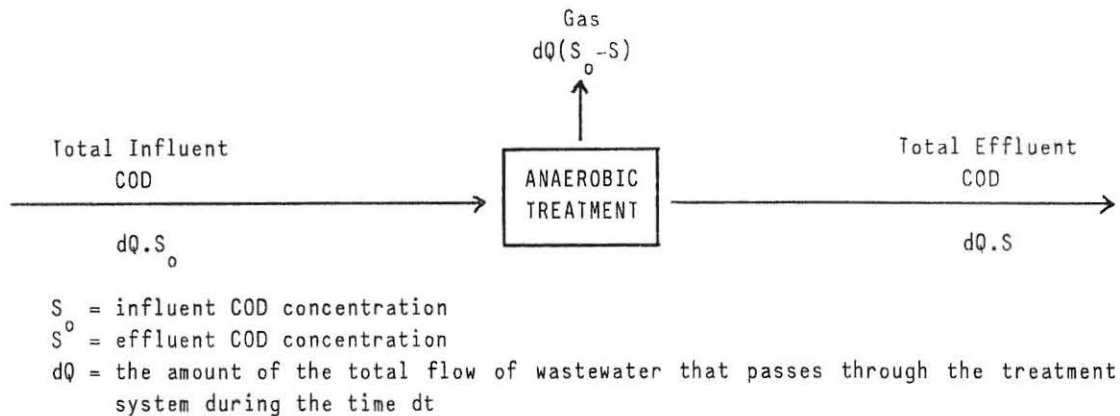
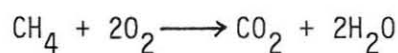


FIGURE 11 - The flow of COD through the anaerobic treatment system (Frostell, 1985)

If no exogenous oxidants are present, the amount of COD leaving the system is the same as the amount entering. This means that the COD of the gas stream is equivalent to the COD of the influent stream minus that of the effluent stream. In the gas stream, the carbon dioxide is a fully oxidized compound and consequently the amount of CH_4 produced is directly related to the COD. A complete oxidation of CH_4 to CO_2 requires two moles of oxygen per mole of CH_4 :



From this equation, the COD-equivalent of $1 \text{ m}^3 \text{ CH}_4$ at STP can be calculated. It has been shown that $1 \text{ m}^3 \text{ CH}_4 = 2,86 \text{ kg COD}$ (Frostell, 1985).

COD reduction can thus be calculated as follows:-

$$E = \frac{2,86 \cdot G \cdot c}{Q \cdot S_o}$$

where : E = efficiency (% COD removed)
 $2,86$ = gas to COD conversion factor
 G = gas production (m^3/d at STP)

- c = % methane in gas
 Q = influent flow rate (m^3/d)
 S_0 = influent COD concentration ($\text{kg COD}/\text{m}^3$)

This equation is quite useful for rapid assessment of the process treatment efficiency. However, it is more common to use COD, TOC or BOD reduction as an indicator of treatment efficiency. A reduction in the treatment efficiency is an indication of inhibition of methanogenesis, as it is only through CH_4 production that a reduction in the COD of the waste can be achieved (Kugelman and McCarty, 1965). Low COD removal rates can be obtained with wastes which have a large component of non-biodegradable though chemically oxidizable material.

3.4.9 New Developments in Process Control and Monitoring

Hydrogen plays an important role in the anaerobic digestion process and its concentration affects the rate of degradation of many organic compounds (Section 3.3). Continuous on-line monitoring of H_2 in the biogas could therefore provide a constant monitoring system to control the rate of supply of substrates to the digester. Such monitoring systems have been used successfully in laboratory scale (Whitmore and Lloyd, 1986) and pilot scale (Archer et al, 1986) anaerobic digesters.

Methanogenic bacteria contain a unique electron carrier, coenzyme F_{420} , which has fluorescent properties (Cheeseman et al, 1972). The concentration of coenzyme F_{420} is directly related to the metabolic activity of the methanogenic bacteria, and therefore it can be used as a sensitive measure of the degree of methanogenesis occurring in an anaerobic digester (Delafontaine et al, 1979; Binot et al, 1981; Gorris et al, 1988). The fluorescent properties of coenzyme F_{420} also enables identification of methanogenic bacteria by fluorescence microscopy (Mink and Dugan, 1977).

The advantage of these two monitoring systems is that they provide "real-time" monitoring of the critical metabolic pathways occurring during anaerobic digestion. Changes in loading rate, retention time, etc. can therefore be carried out immediately, without the delay that usually occurs before an increase in the concentration of VFA or a decrease in the biogas volume becomes evident. The other advantage of these two methods is that both are

very sensitive techniques.

Today, the amount of monitoring considered necessary in anaerobic treatment is ever-increasing. This reflects the overall complexity and better understanding of the process. However, there are still limited possibilities for real control of the process. Therefore the development of improved monitoring methods will, in all probability, further increase the popularity, versatility and stability of anaerobic wastewater treatment.

3.5 Anaerobic Digester Designs

The early development of the anaerobic digestion process (Figure 3) was slow, due to a lack of understanding of the biochemistry and microbiology of the process. Up until the 1950's, the anaerobic digesters commonly used on an industrial scale were the so-called conventional anaerobic digesters (Figure 12). The conventional sludge digester consists essentially of a completely mixed, one step process operating on a continuous basis without cell recycle. Due to the low specific growth rate of the methanogenic bacteria, effective digestion of the waste can only be obtained at long retention times. In practice, liquid retention times of 20 days or longer are essential for efficient waste stabilization, with the result that conventional digesters tend to be of a large size, with a corresponding high initial capital cost. They are also extremely susceptible to shock loads of organic matter and toxic compounds (Colleran et al, 1982; Switzenbaum, 1983).

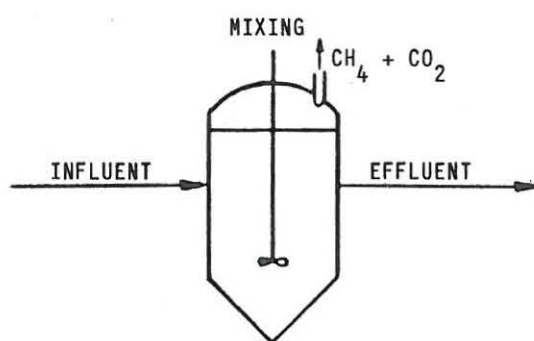


FIGURE 12 - The conventional anaerobic digester (Switzenbaum, 1983)

A rapid escalation in energy costs and a heightened environmental awareness in the 1960's provided an incentive to improve the anaerobic digestion process and to extend its application to industrial wastewater treatment. However, as most industrial wastewaters are soluble, low-strength wastes that are produced in large volumes, the long retention times used in conventional digesters were economically unacceptable.

The capital cost of an anaerobic treatment plant is dependent on the size of the digestion tank. Smaller, cheaper tanks result in shorter waste retention times. However, for a conventional digester, short retention times are restricted by:

- (a) the relatively slow rate of conversion of organic matter by anaerobic bacteria, so that the degree of waste conversion is reduced significantly as its retention time is decreased below 10 days
- (b) the relatively slow growth rates of anaerobic bacteria, leading to their washout and process failure if waste retention times are reduced below about 5 days
- (c) the possibility of process failure at short retention times through the accumulation of gaseous hydrogen or volatile fatty acids, which inhibit further waste breakdown either directly or through reduction of the digester pH

One method of overcoming these limitations has been to increase the digester biomass concentration by retaining biomass within the digester for periods longer than the waste undergoing digestion. This requires a means of solid/liquid separation. Two mechanisms for achieving this separation and maintaining elevated biomass densities in digesters are:-

- (a) providing conditions within digesters which enhance the natural tendency of biomass to form aggregates which are large enough to be separated from the wastewater by settling
- (b) providing surfaces within digesters to which bacteria can attach in films; the bacterial support materials are themselves retained in digesters by gravity or by tight packing

In this way the hydraulic retention time (HRT) and the solids retention time (SRT) can be varied independently (Callander and Barford, 1983a; Christensen

et al, 1984). In retained biomass digesters, the SRT is usually maintained at greater than 15 days, while the HRT can be reduced to a few hours or a few days.

The five most widely studied and applied high-rate retained biomass digesters have all been developed over the last twenty five years (Van den Berg, 1986). These include:-

- (a) the anaerobic contact digester
- (b) the anaerobic filter
- (c) the upflow anaerobic sludge blanket digester
- (d) the anaerobic fluidized or expanded bed digester
- (e) ultrafiltration and membrane digesters

3.5.1 The Anaerobic Contact Digester

This was the first retained biomass digester to be studied and developed. The principle involved is the same as the activated sludge process. The effluent from the digester is pumped to a settling unit, from where a portion of the settled sludge is returned to the digester, enabling the contact unit to maintain a high concentration of active biomass (Figure 13). The solids concentration can therefore be maintained independently of waste flow using the method of biomass solids recycle (Callander and Barford, 1983a; Van den Berg, 1986).

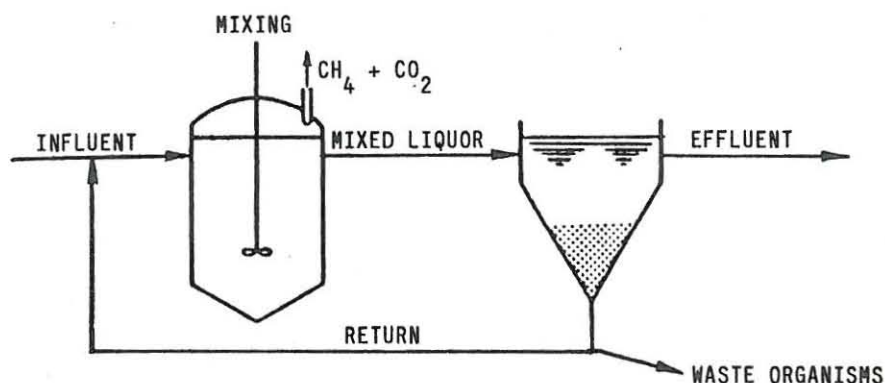


FIGURE 13 - The anaerobic contact digester (Switzenbaum, 1983)

The major problem encountered in the contact process is the separation and concentration of biomass flocs prior to their return to the digester. This is due to the continued production of methane in the settler, which results in the formation of buoyant biomass. Several methods have been used to overcome this problem i.e. sedimentation, settling combined with chemical flocculation, vacuum degassification, flotation or centrifugation (Lettinga *et al*, 1980b).

COD loading rates of up to $10 \text{ kg/m}^3 \cdot \text{d}$ are attainable, although rates of $2 - 5 \text{ kg/m}^3 \cdot \text{d}$ are more typical. The process appears to operate well with high wastewater COD's of typically $2 - 10 \text{ g/l}$, especially if the waste contains a certain amount of hard-to-digest solids which settle readily or attach themselves readily to settleable solids. Hydraulic retention times as low as 0,5 days with moderate strength wastes can be achieved, although HRT of a few days are more typical (Van den Berg, 1986; Sterritt and Lester, 1988).

3.5.2 The Anaerobic Filter

The anaerobic filter is composed of one or more vertical filter beds containing an inert material such as gravel, commercially available PVC supports, glass beads, baked clay or needle-punched polyester. This packing material acts as a stationary support surface for microbial film attachment. Wastewaters are pumped upwards through the support media, allowing contact between attached microorganisms and the wastewater (Figure 14).

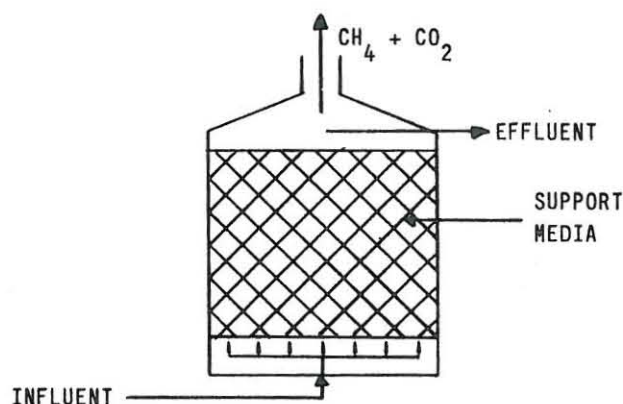


FIGURE 14 - The upflow anaerobic filter (Young and Yang, 1989)

It has been observed that a major portion of the biomass is not attached to the support material, but lies loosely in flocs in the interstitial spaces.

It appears that when bacterial flocs are buoyed by attached gas bubbles, these separate when the rising floc hits the overlying support. The floc then returns to its original position, and the gas bubble continues to rise. In this manner, a long SRT can be maintained at short HRT (Callander and Barford, 1983a; Switzenbaum, 1983).

Anaerobic filters can also be operated in a downflow mode. The support material is, however, arranged differently to that used in the upflow filter (Figure 15) in order to prevent compacting of the anaerobic sludge at the base of the digester.

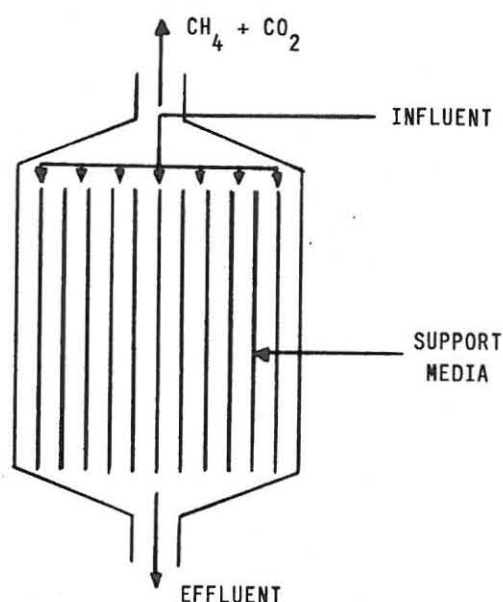


FIGURE 15 - The downflow anaerobic filter (Young and Yang, 1989)

The main disadvantage of anaerobic filters is the accumulation of solids in the packing material which may plug the digester. These solids can be waste suspended solids, material precipitated from the waste, or suspended growth. In addition, hard-to-digest suspended solids that settle readily interfere with the operation of the digester. In large digesters, an inadequate liquid distribution system may cause channelling and very short retention times (Van den Berg, 1986).

Because the anaerobic filter is capable of producing long SRT, it is particularly suitable for treating dilute soluble wastes, soluble wastes which can be made dilute by recirculating effluent or wastes with easily degradable suspended material (Van den Berg, 1986; Young and Yang, 1989).

3.5.3 The Upflow Anaerobic Sludge Blanket Digester

The upflow anaerobic sludge blanket (UASB) digester was developed in the early 1970's by a Dutch group investigating the anaerobic filter as a means of processing potato and sugar-beet wastes (Lettinga et al, 1980a). An unpacked region was created at the bottom of the digester to encourage the accumulation of biomass. This region was further extended until the digester was completely devoid of packing. A gas-collecting device was then inserted at the top of the digester (Figure 16).

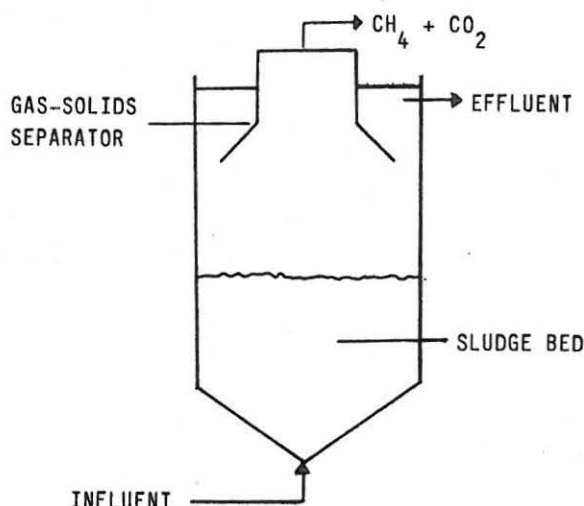


FIGURE 16 - The upflow anaerobic sludge blanket (UASB) digester (Switzenbaum, 1983)

In the UASB process, the wastewater is introduced at the base of the digester and flows upwards, through an accumulation of retained solids, and then through a baffle arrangement at the top of the digester before being discharged. This baffle arrangement separates the gas which is generated in the digester from the solids and the liquids. The treated effluent flows out of the digester while most of the solids are retained (Christensen et al, 1984; Maat and Habets, 1987).

Because the UASB digester uses no packing material to support and concentrate biological growth, the efficiency of the process depends on the development within the digester of highly settleable biomass, either as flocs or as dense granules 1 - 5 mm in size. The biological mechanism of granule formation is as yet unknown, although certain theories have been proposed (Sam-Soon et al,

1987; Dolfig et al, 1985; Hulshoff et al, 1984). It would appear that granulation is encouraged by no or minimal mechanical agitation of the sludge bed. The type of wastewater also influences sludge pelletization (Sam-Soon et al, 1989; Ross, 1984; Moosbrugger et al, 1989). The excellent settling properties of these granules ensure the formation of a dense bed of granular sludge at the base of the digester, and permit very high loading rates i.e. greater than $30 \text{ kg COD/m}^3\text{.d}$. Even if granulation does not occur, good removal efficiencies can still be achieved at loadings of up to $5 \text{ kg COD/m}^3\text{.d}$ (Maat and Habets, 1987).

The UASB process has the potential advantages of simplicity and low cost. New UASB digesters can also be seeded with granular sludge adapted from other full-scale plants. Thus the full organic load can be introduced from the start. Start-up periods of several months can therefore be reduced to less than a day. Consequently, it is possible to achieve a high removal efficiency within a much shorter time than was believed possible (Maat and Habets, 1987).

Problems with UASB digesters are usually associated with the development of the granular sludge. While certain wastes produce a granular sludge quite readily, other wastes develop this sludge very slowly or not at all. Inoculation with a large amount of granular sludge can help, but the sludge does not always maintain its characteristics when changing from one waste to another. The presence of inert suspended solids entering the digester with the wastewater can also interfere with granule formation, and hence the UASB process is only really suited to wastewaters with low suspended solids concentration. Inefficient operation can also occur if the influent wastewater forces channels through the sludge blanket instead of passing uniformly through the whole volume of the bed (Van den Berg, 1986; Hawkes and Hawkes, 1987; Sterritt and Lester, 1988).

3.5.4 The Anaerobic Fluidized or Expanded Bed Digester

Expanded and fluidized bed systems employ very small diameter support media with high specific surface areas which permit the growth of thin biological films maintaining high biomass concentrations within the digester. Clogging is avoided by introducing the influent wastewater through a distribution system at the base of the digester at a sufficient upflow velocity to impart an expansion of the bed particles (Figure 17).

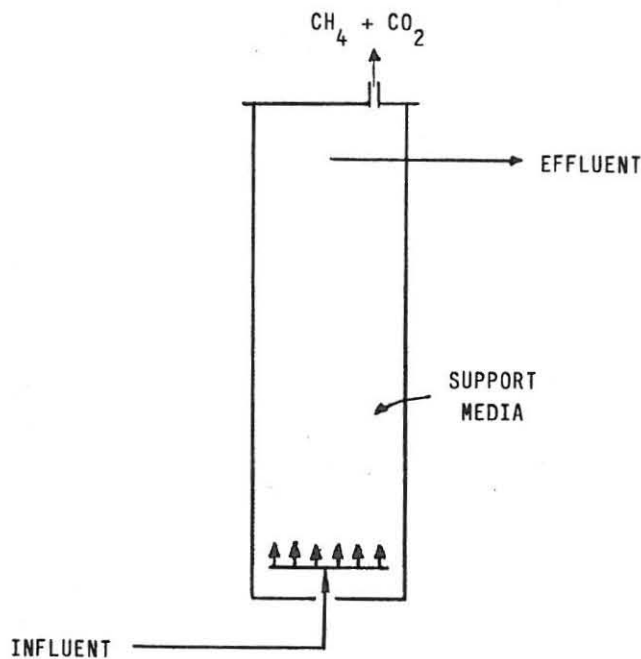


FIGURE 17 - The anaerobic expanded or fluidized bed digester (Switzenbaum, 1983)

In order to maintain the upflow velocity, effluent recycle is often necessary. In an expanded bed system, the particles remain in fairly close contact and the bed expansion is 5 - 20 %, whereas in the fluidized bed system, the particles are continuously moving and bed expansion is 30 - 100 %. An optimum support medium has as yet not been found, although sand, PVC and alumina granules have been used (Hawkes and Hawkes, 1987; Sterritt and Lester, 1988).

Fluidized or expanded bed digesters are capable of treating both very low strength (150 - 600 mg/l COD) and very high strength wastewaters. However, these wastewaters should be soluble or at least the suspended material should be easily degradable. Very high loading rates i.e. 20 - 30 kg COD/m³.d are possible (Van den Berg, 1986; Callander and Barford, 1983a).

The main disadvantages of these digesters are the very long start-up periods, and the cost and power consumption of the recycle pumps. The net energy yield is therefore lower than that for other digesters. Reactor performance also depends very much on the evenness of the flow of the liquid, and as a result the system of liquid distribution is critical (Van den Berg, 1986; Mosey, 1982).

3.5.5 Ultrafiltration and Membrane Digesters

The essential prerequisites for successful high-rate anaerobic digestion are biomass concentration and biomass retention. Various anaerobic digesters have been designed to increase the SRT while at the same time reducing the HRT i.e. the anaerobic filter, the UASB digester, the anaerobic contact digester and the fluidized or expanded bed digester. However, at high loading rates, a high concentration of suspended solids is found in the digester effluent (Ross *et al*, 1989a).

The growing interest in anaerobic digestion has led to a demand for a more efficient process. This has directed attention towards the use of membranes for biomass separation and retention in anaerobic digesters, and has led to the development of two processes, the Membrane Anaerobic Reactor System (MARS) and the anaerobic digestion ultrafiltration (ADUF) process. These two processes are essentially identical, with the only significant difference being that the MARS anaerobic digester contains a support material, whereas the ADUF digester has no internal packing material (Li *et al*, 1984; Ross *et al*, 1989a).

The ADUF and MARS processes incorporate two main units viz. an anaerobic digester and an external ultrafiltration unit (Figures 18 and 19).

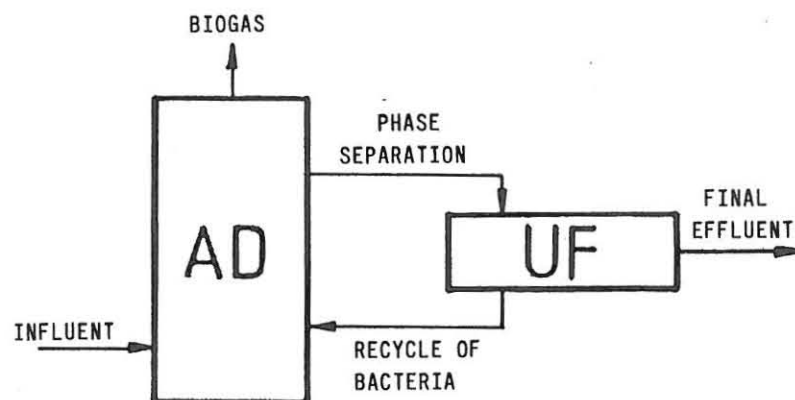


FIGURE 18 - The anaerobic digestion ultrafiltration (ADUF) process (AD = anaerobic digester; UF = ultrafiltration unit) (Ross *et al*, 1989a)

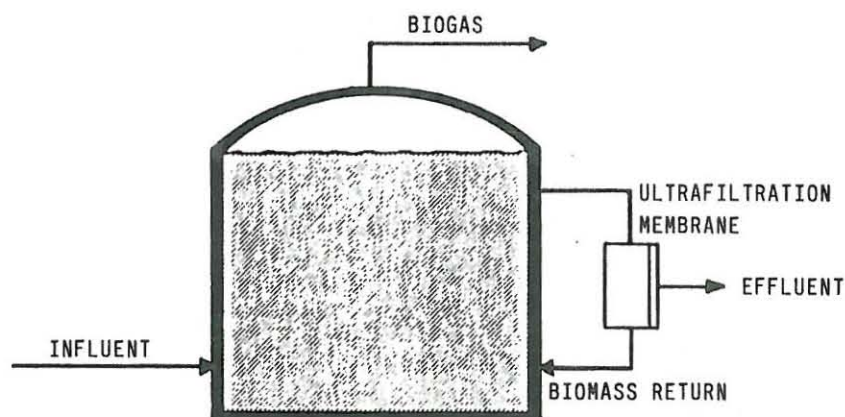


FIGURE 19 - The MARS process (Li et al, 1984)

The wastewater is introduced at the base of the digester where it is evenly distributed. The digester output is withdrawn at the top of the digester and pumped to the ultrafiltration unit. In contrast to conventional filtration, in which flow direction is perpendicular to the filter medium, flow direction in an ultrafiltration system is parallel to the membrane surface, greatly reducing the problems of clogging. The permeate from the membrane is the final effluent, while the sludge concentrate containing the bacteria, nutrients and undigested compounds are recycled back to the digester with minimal activity loss and temperature decrease. This recycling provides sufficient mixing in the digester to enhance its performance (Ross et al, 1989a).

Ultrafiltration digesters have several advantages over other high-rate digesters (Ross et al, 1989a; Ross et al, 1989b; Li et al, 1984):-

- (a) the biological solids can be completely retained in the digester resulting in a high digester biomass concentration
- (b) a shorter start-up time is possible since the seed bacteria will be retained in the digester

- (c) influent particulate organics can be degraded since they will also be held in the digester by the ultrafiltration process
- (d) a high quality effluent is achieved since no particulate material will be present
- (e) high loading rates are possible
- (f) both low and high strength wastes can be treated in these digesters

3.5.6 Two-Stage Phase-Separated Digesters

Since the reactions involved in anaerobic digestion occur sequentially, with the volatile fatty acids representing the most important intermediates, physical separation of the acidogenic phase from the methanogenic phase is feasible. Each phase can then be controlled separately for independent optimization. The use of two-stage phase-separated digesters for wastewater treatment will be discussed in more detail in Section 7.3.

Although a number of different digester designs are available, UASB digesters were used in this investigation. The reasoning behind the use of these digesters is explained in detail in Section 4.1.4.1.

3.6 The Application of Anaerobic Digestion in the Tanning Industry

Tannery wastewaters are extremely difficult to treat due to extreme pH values, the presence of high concentrations of slowly biodegradable proteins, fats and vegetable tannins, and high concentrations of inorganic compounds. The combination of high concentrations of organic and inorganic compounds often creates problems for on-site treatment and for discharge of untreated effluents to municipal sewage works.

Numerous aerobic treatment methods have been used to treat tannery wastewaters, with varying degrees of success (Section 2.4). However, more stringent legislation and heightened environmental awareness have served to stimulate research into investigating more effective and economical methods of treatment. The success obtained with the anaerobic treatment of many industrial wastes inevitably focused attention on anaerobic digestion as a possible treatment method for tannery wastewaters. Despite this, however, only a limited number of studies have been carried out to investigate the anaerobic treatability of these wastes (Arora and Chattopadhyaya, 1980; Gates

and Lin, 1966; Friedman et al, 1982; Bailey et al, 1984; Bajwa and Forster, 1988; Eye and Ficker, 1982; Tunick et al; 1985; Yong, 1988; Aloy et al, 1989). The results of these studies will be discussed in more detail in Chapter 6 and 7.

Compared to other industrial wastes, the information available on the anaerobic digestion of tannery wastewaters is extremely limited. Very little is known regarding the fate of the compounds present in these wastewaters and the long-term effects of these compounds on the digestion process. In addition, very few studies have been carried out using actual tannery effluents and sludges. In the few instances in which these wastewaters have been used, the studies were usually of a short duration. Detailed studies are, therefore, still necessary if anaerobic digestion is to be used to successfully treat tannery wastewaters on an industrial scale, and the study described in this thesis was an endeavour at such an investigation.

The aim of this project was therefore:-

- (a) to determine the fate and the potential toxicity of the individual tannery wastewater compounds on the anaerobic digestion process
- (b) to investigate the feasibility of the anaerobic digestion of tannery effluents
- (c) to investigate the feasibility of the anaerobic digestion of tannery sludges
- (d) to improve the efficiency of the process if the anaerobic digestion of tannery wastewaters was found to be possible.

CHAPTER FOUR

MATERIALS AND METHODS

CHAPTER FOUR

MATERIALS AND METHODS

4.1 Materials

4.1.1 Reagents

Reverse osmosis (RO) purified water (Millipore Systems) was used throughout this investigation with the exception of the atomic absorption spectrophotometric determinations, where deionized water (Elgastat) was used. Chemical reagents were purchased from commercial sources (Merck, BDH, Fluka, Carlo Erba, Riedel-de Haen, May and Baker and Oxoid) and were analytical grade unless otherwise stated. The analytical grade gases used in the gas chromatographic and atomic absorption spectrophotometric determinations were purchased from Afrox Ltd and Fedgas (Pty) Ltd.

4.1.2 Anaerobic Sludge Inocula

The anaerobic sludge inocula, which were used as a source of anaerobic bacteria, were obtained from a completely mixed anaerobic digester at the Grahamstown Municipal Sewage Works, which treats mainly domestic sewage.

4.1.3 Wastewater

Three types of wastewater were used in this investigation:-

4.1.3.1 Synthetic Wastewater

A synthetic wastewater was used in the initial investigations to determine the effects of individual tannery wastewater compounds on the anaerobic digestion process (Chapter 5). At the start of this research project, a decision had to be made on whether to use a synthetic waste or tannery wastewater for the initial toxicity experiments. Because of the inconsistency and variability from tannery to tannery of the latter, and the fact that tannery wastewaters already contain high concentrations of the toxic compounds to be investigated, it was decided to make use of a synthetic waste. The use of this synthetic waste ensured that the concentration of the different toxic compounds could be

varied, and because of its uniform composition, a comparison of the relative toxicity of each of the tannery compounds could be made.

As tannery wastewaters are very complex, it was extremely difficult to design a synthetic waste that corresponded exactly with the industrial wastewater. However, in this investigation, every effort was made to maintain the important parameters, such as pH, alkalinity, chemical oxygen demand, permanganate value and total Kjeldahl nitrogen as close as possible to those of tannery effluents.

Protein accounts for 35 % of the hides and skins. Collagen fibres constitute 85 - 90 % of the proteins, with the remainder made up of elastin, keratin and other non-fibrous proteins (Venkatesan, 1979). These proteins are therefore found in substantial concentrations in tannery wastewaters. However, there are several reasons why they were not used as substrates in the synthetic waste. Firstly, they are not soluble at neutral pH and therefore, if they had been used, the pH of the waste would have had to have been acidic i.e. 2,5 - 3,0 (Miller and Rhodes, 1982). As the tannery effluents and sludges to be used in future experiments (Chapter 6 and 7) had an initial pH of 7,0 - 8,0, the results of toxicity experiments using an acidic waste (pH 2,5 - 3,0) would have been meaningless, as pH affects not only the solubility of the heavy metals, but also the distribution of sulphide and ammonia species (Gadd and Griffiths, 1978; Lawrence *et al*, 1966; Koster, 1986). Methanogenic bacteria also have a pH optimum of 6,8 to 8,0 (Balch *et al*, 1979). Secondly, very few bacteria produce collagenolytic and proteolytic enzymes (Seifter and Harper, 1970), and thus it would have been difficult to obtain a healthy and reproducing methanogenic population using these proteins as the substrate. In view of the above, an alternative substrate had to be used.

A literature survey indicated that in previous studies using a synthetic waste to elucidate the effects of tannery compounds on anaerobic digestion, either acetate (Speece and Parkin, 1986; Bajwa and Forster, 1988), glucose, urea and yeast extract (Hurlow, 1986), or a combination of glucose, gelatine and yeast extract (Maeda *et al*, 1984) were used to supply the carbon and nitrogen requirements of the waste.

As proteins could not be used in this investigation for the reasons stated previously, a combination of yeast extract (which contains amino acids) and

urea (which is an intermediate product in the degradation of organic matter) were used to supply the nitrogen content of the media at the same concentration as that present in tannery effluents i.e. a total Kjeldahl nitrogen (TKN) of approximately 300 mg/ℓ. Thus having decided on the nitrogen in the waste, a decision on the carbon source had to be made bearing in mind that the C : N ratio of tannery wastewaters is approximately 6 : 1 (Maeda *et al*, 1984; Carre *et al*, 1976), and that the chemical oxygen demand (COD) of the effluents to be used in future experiments were approximately 3000 mg/ℓ. Glucose was used to increase the carbon content of the waste to satisfy these requirements, with the result that the final synthetic waste obtained had a similar pH, COD, PV, TKN and alkalinity as the actual tannery effluents (Table 10).

TABLE 10
CHARACTERISTICS OF THE SYNTHETIC WASTEWATER

pH	6,90 - 7,10
Bicarbonate Alkalinity (mg/ℓ CaCO ₃)	1200 - 1400
Chemical Oxygen Demand (mg/ℓ)	2500 - 3000
Permanganate Value (mg/ℓ)	600 - 700
Total Kjeldahl Nitrogen (mg/ℓ)	290 - 310

The composition of the synthetic waste is shown in Table 11. The trace nutrients, which are essential for the growth and metabolism of methanogenic bacteria (Wolin and Miller, 1985; Speece and Parkin, 1986), were added in the form of a trace nutrient solution (5 ml). The effect of these trace nutrients on the anaerobic digestion of tannery wastewaters will be discussed in more detail in Chapters 6 and 7.

After the addition of the individual tannery compound under investigation to the synthetic waste, it was neutralized with either 6 N HCl or NaOH pellets. The waste was not autoclaved, and therefore some partial pre-acidification took place, a process that occurs naturally in tannery wastewaters as evidenced by the high concentration of volatile fatty acids in these wastewaters.

TABLE 11
COMPOSITION OF THE SYNTHETIC WASTEWATER

Constituent		Concentration (mg/l)
<u>Macronutrients</u>	Glucose	2500
	Urea	500
	Yeast Extract	500
	K_2HPO_4	500
	$MgSO_4 \cdot 7H_2O$	500
<u>Trace Nutrients</u>	$CaCl_2$	100
	NaCl	100
	$FeCl_3$	10
	$MnSO_4 \cdot 4H_2O$	1
	$CoCl_2 \cdot 6H_2O$	0,5
	$CuSO_4 \cdot 5H_2O$	0,5
	$Na_2B_4O_7 \cdot 10H_2O$	0,1
	$ZnSO_4 \cdot 7H_2O$	0,5
	$NiSO_4 \cdot 6H_2O$	0,5
	$Na_2MoO_4 \cdot 2H_2O$	0,2

4.1.3.2 Tannery Effluent

The tannery effluent used in this investigation was obtained from King Tanning Company (King William's Town, South Africa), a tannery which produces full aniline leathers, corrected grain and nappa leather, suede and dressed splits. Currently, approximately 8500 hides per week are being finished with some 5000 hides per week being processed to wet-blue. Chrome recycling is carried out at the tannery.

The effluent is collected in one large reservoir and is then pumped via an 8,6 km pipeline to a settling tank on a farm owned by the Company. The sludge is de-watered by drying on drying-beds. The settled effluent is mixed with other local, domestic effluent and is used to irrigate 150 acres of grassland under

kikuyu.

The effluent that was used in this investigation was collected from the outlet of the pipeline prior to it entering the settling tank, and thus contained all the settleable matter. After collection, it was maintained at 4°C until it was used. It was allowed to equilibrate to room temperature before being pumped into the digesters.

4.1.3.3 Low-Sulphate Tannery Effluent

The low-sulphate effluent was prepared using the tannery effluent obtained from King Tanning Company. A sample was centrifuged at 3000 x g to precipitate the suspended matter. The supernatant was acidified to a pH of 4.5 using 6 N HCl, and then boiled. While stirring gently, warm 10 % barium chloride was added, ensuring, however, that the SO_4^{2-} concentration was stoichiometrically greater than the concentration of Ba^{2+} ions to prevent the presence of free Ba^{2+} ions in solution. The precipitated barium sulphate was removed by filtering through Whatman 42 filter paper. The filtrate was collected and added to the insoluble matter from the initial centrifugation stage to constitute the low-sulphate tannery effluent. This low-sulphate effluent was then stored at 4°C until it was used. It was allowed to equilibrate to room temperature before being pumped into the digester.

4.1.3.4 Tannery Sludge

The tannery sludge used in this investigation was also obtained from King Tanning Company. The sludge was collected from the base of the settling tank mentioned previously (Section 4.1.3.2) and was therefore in a "wet" form i.e. it had not been de-watered. After collection, it was maintained at 4°C until it was used. It was allowed to equilibrate to room temperature prior to being pumped into the digesters.

4.1.4 Experimental System

Two different experimental systems were used in this investigation:-

4.1.4.1 Synthetic Wastewater and Tannery Effluent Experimental System

Upflow anaerobic sludge blanket (UASB) digesters were used throughout these investigations. The choice of these digesters was dictated by the highly particulate nature of tannery wastewaters. Anaerobic filters are prone to clogging and the capital cost of the packing material is high. Similarly, fluidized or expanded bed digesters were ruled out due to the fact that it would have been virtually impossible to remove any precipitated inorganic solids from the digester without removing substantial quantities of the fine support material. The capital and operational costs of these digesters are also very high. As the anaerobic contact digester has not proved viable due to operational difficulties, the obvious choice was the UASB digester. Thus, although UASB digesters should be used for treating essentially soluble wastewaters with no particulate matter, they appeared to be the best choice available at the commencement of this research project. They have also been more widely used for the treatment of industrial wastewaters than the other high-rate digesters (Maat and Habets, 1987).

The synthetic wastewater and tannery effluent experiments were performed in 9 l UASB digesters (Figures 20 and 21). These digesters, which were constructed from colourless perspex, had a total height of 66 cm, an internal diameter of 14 cm, and an operating volume of 9 l (Table 12). The inlet and outlets were located 3 cm and 59 cm from the base of the digester respectively. Sampling ports were located at intervals between the inlet and outlet viz. 16 cm, 29 cm and 42 cm from the base. The gas-solids separator consisted of a circular ring of plastic of triangular cross-section positioned below an inverted plastic funnel. Rising gas bubbles were therefore displaced inwards towards the collection funnel.

The temperatures of the digesters were maintained at $35 \pm 1^\circ\text{C}$ by pumping water (Iwaki MD-20R Magnetic Pump) from a constant temperature waterbath (Julabo EM Thermostat) through internal heating coils. The digesters were covered in tinfoil in order to help maintain mesophilic temperatures, as well as to prevent the growth of photosynthetic anaerobes which could have influenced the results. As industrial scale anaerobic digesters would be constructed out of steel, photosynthetic anaerobes would not be present, and consequently, for this reason, they were not permitted to grow in the laboratory scale digesters.

Biogas was evolved from the top of the digester and, after passing through a sampling tube, it was collected in a sealed 5 l aspirator bottle containing a 20 % NaCl - 5 % H_2SO_4 solution. This solution was used in order to minimize CO_2 solubility (Mackie and Bryant, 1981). The volume of gas was measured by fluid volume displacement and was corrected to 760 mm Hg pressure and 0°C (STP).

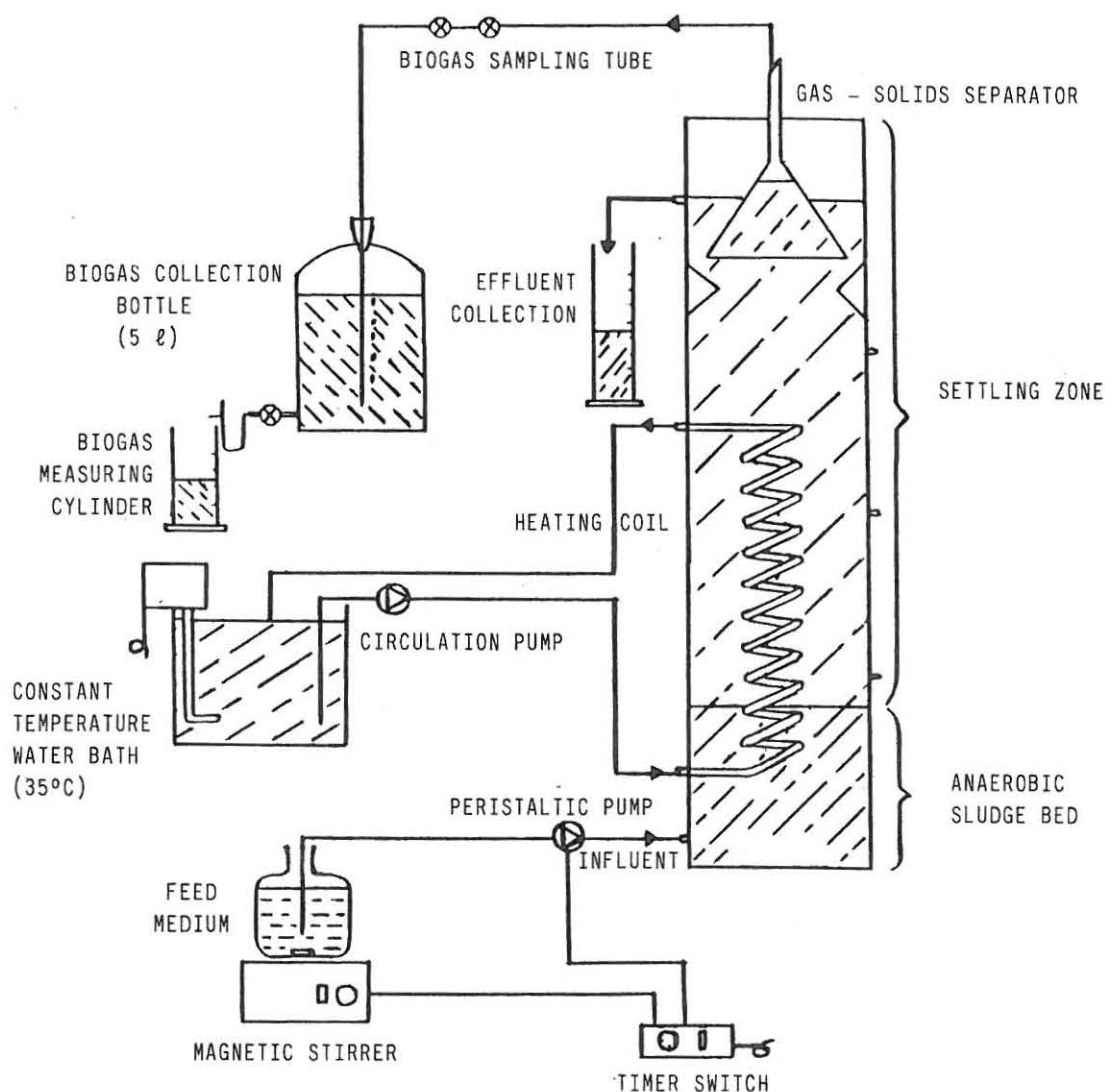


FIGURE 20 - Schematic diagram of the synthetic wastewater and tannery effluent experimental system (Hurlow, 1986)

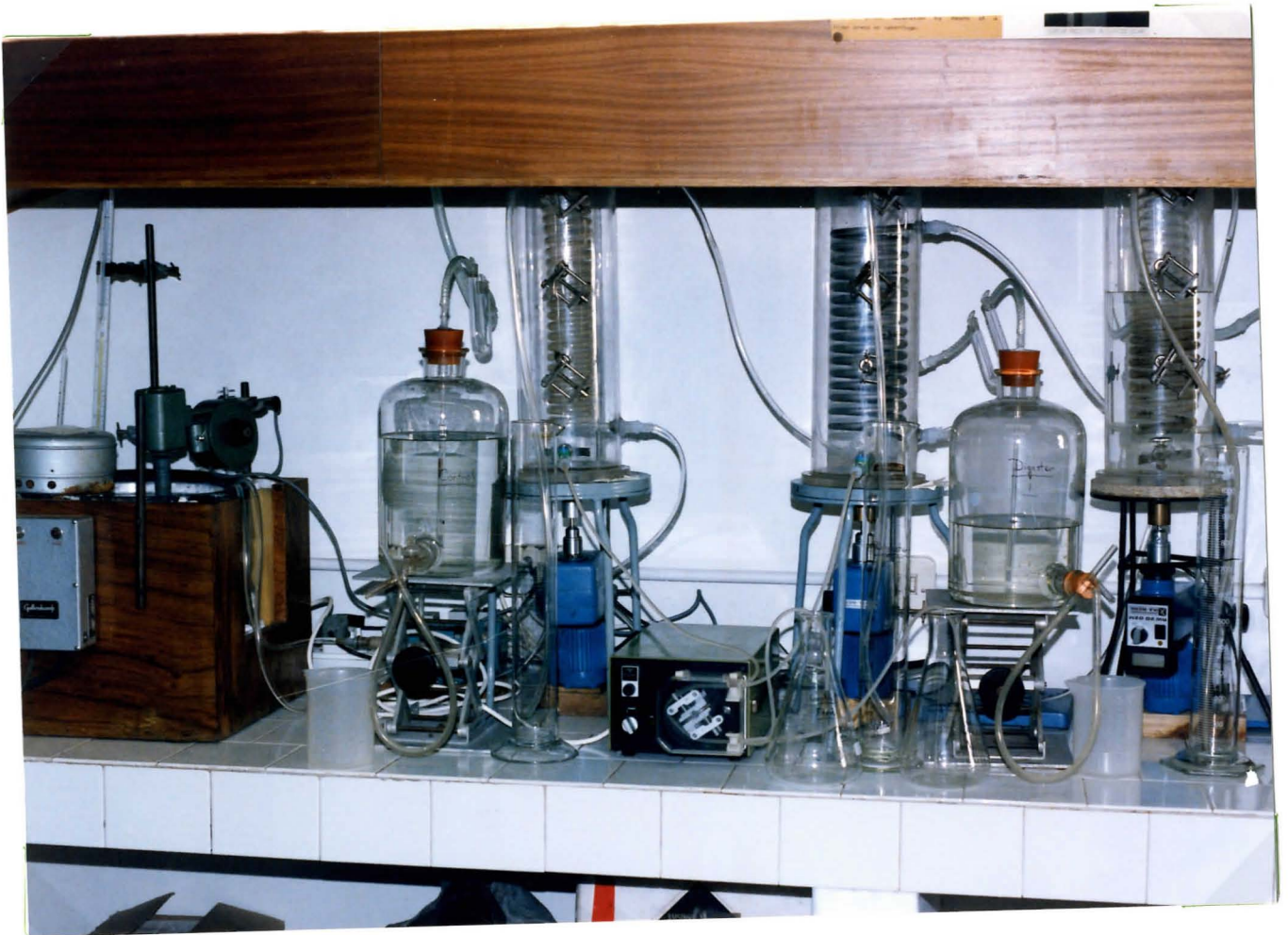


FIGURE 21 - Photograph of the synthetic wastewater and tannery effluent experimental system

Wastewater was supplied to the digesters by means of a Watson-Marlow 501 peristaltic pump using 5 mm diameter (3 mm i.d.) silicone tubing. During additions to the digester, the wastewater was stirred with a T + C 010 magnetic stirrer in order to obtain a uniform distribution of precipitated material. The supply of wastewater to the digesters was semi-continuous and was controlled by means of an Electromatic SC 185 timer system.

The complete experimental system was constructed under a fume hood in order to prevent an accumulation of methane gas in the laboratory.

TABLE 12
CHARACTERISTICS OF THE UASB DIGESTERS USED
IN THE SYNTHETIC WASTEWATER AND TANNERY
EFFLUENT EXPERIMENTS

Working Volume (ℓ)	9
Total Height (cm)	66
Internal Diameter (cm)	14
Inlet from the Base (cm)	3
Outlet from the Top (cm)	59
Temperature (°C)	35
Construction Material	Perspex

4.1.4.2 Tannery Sludge Experimental System

Before the commencement of the tannery sludge experiments, it was evident that the equipment that had been used for the synthetic wastewater and tannery effluent experiments would not be suitable for the sludge experiments. The sludges used in this investigation had a very high solids content which made it impossible for them to be pumped by means of the peristaltic pump. It was realized that specialized sludge pumps would be required and in view of their size and pumping rates, this in turn necessitated the need for larger digesters.

The sludge experiments were performed in 50 ℓ UASB digesters (Figures 22 and

23). These digesters, which were constructed from PVC, had a total height of 143 cm, an internal diameter of 22 cm and an operating volume of 50 l (Table 13). The inlet and outlets were located 7 cm and 132 cm from the base of the digester respectively. Sampling ports were located at intervals between the inlet and outlets viz. 40 cm, 73 cm and 106 cm from the base. The gas-solids separator consisted of a circular ring of plastic of triangular cross-section positioned below an inverted plastic funnel.

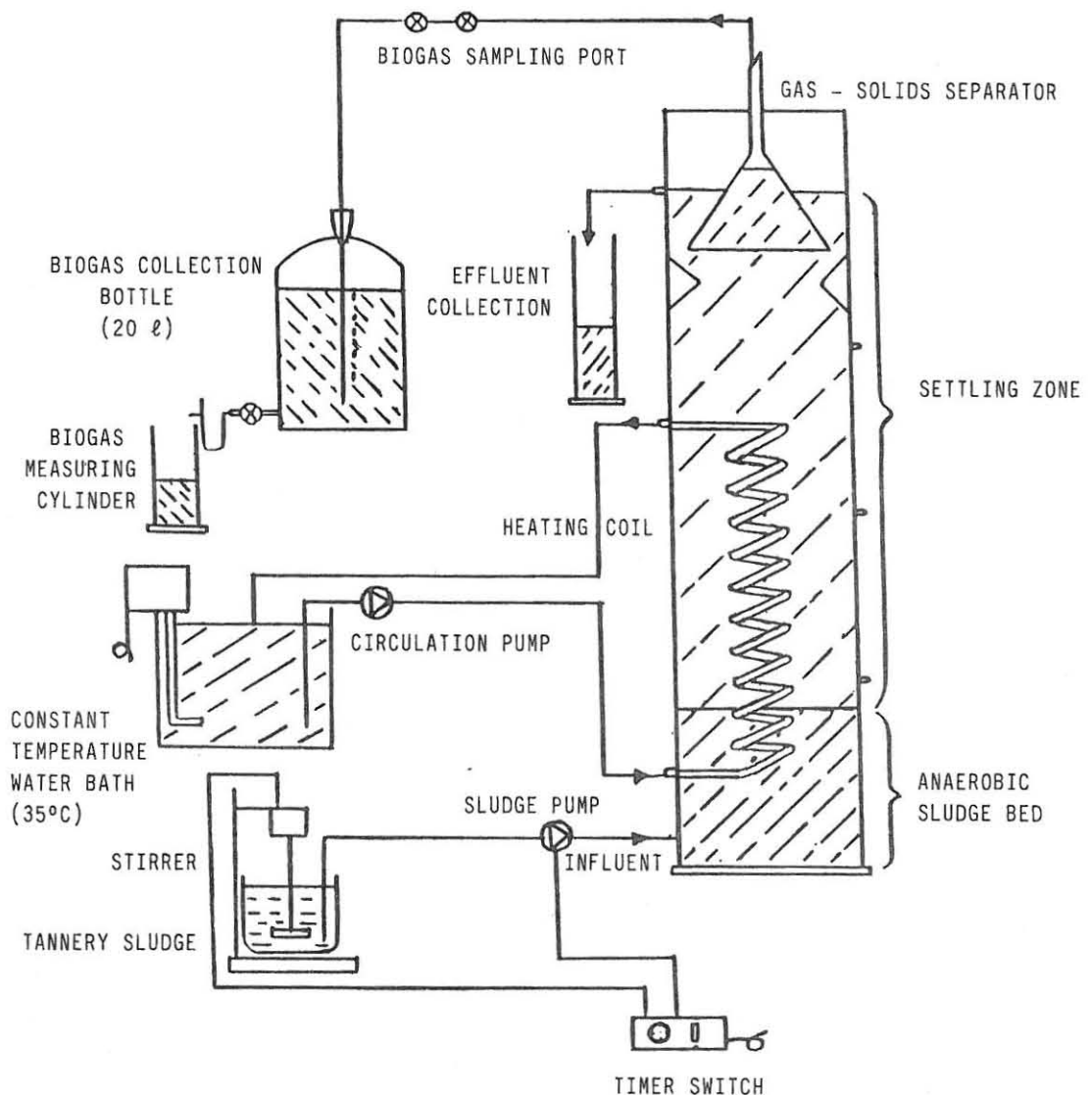


FIGURE 22 - Schematic diagram of the tannery sludge experimental system (Hurlow, 1986)



FIGURE 23 - Photograph of the tannery sludge experimental system

The temperatures of the digesters were maintained at $35 \pm 1^\circ\text{C}$ by pumping water (Iwaki MD-20R Magnetic Pump) from a constant temperature water bath (Colora Ultra Thermostat) through internal heating coils. The digesters were covered in tinfoil in order to help maintain mesophilic temperatures. Biogas was evolved from the top of the digester and, after passing through a sampling tube, it was collected in a sealed 20 ℓ aspirator bottle containing a 20 % NaCl - 5 % H_2SO_4 solution (Mackie and Bryant, 1981). The volume of gas was measured by fluid volume displacement and was corrected to 760 mm Hg and 0°C (STP).

Tannery sludge was supplied to the digester by means of a Mono GB sludge pump using 25 mm PVC tubing. A 25 mm non-return valve was situated between the pump and the digester inlet in order to prevent the flow-back of sludge through the pump. During additions to the digester, the sludge was stirred with a Janke and Kunkel Ika-Werk variable speed stirrer in order to obtain a uniform distribution of the precipitated material. The supply of sludge to the digesters was semi-continuous and was controlled by means of a Rhomberg-Bräsler ST-203 timer system.

The complete experimental system was constructed under a fume hood in order to prevent an accumulation of methane gas in the laboratory.

TABLE 13

CHARACTERISTICS OF THE UASB DIGESTERS USED
IN THE TANNERY SLUDGE EXPERIMENTS

Working Volume (ℓ)	50
Total Height (cm)	143
Internal Diameter (cm)	22
Inlet from the Base (cm)	7
Outlet from the Top (cm)	132
Temperature ($^\circ\text{C}$)	35
Construction Material	PVC

4.2 Analytical Methods

4.2.1 pH

pH determinations were carried out daily using a Digital Data Systems 300 pH meter standardized at pH 4,0 and 7,0 with standard buffer solutions (BDH).

4.2.2 Conductivity

Conductivity determinations were carried out weekly using a Digital Data Systems 200 combined conductivity, temperature, pH and mV meter standardized with a 1413 $\mu\text{mhos/cm}$ standard solution of KCl. Measurements were expressed as mS/m.

4.2.3 Alkalinity

4.2.3.1 Total Alkalinity

A sample was centrifuged at 3000 x g for 5 minutes in order to precipitate the suspended matter. The supernatant (25 ml) was then titrated with 0,2 N HCl to a pH of 4,0 (APHA, 1980).

Calculation:

$$\text{Total Alkalinity (mg/l CaCO}_3\text{)} = \frac{A \times N \times 50000}{\text{sample volume (ml)}}$$

where: A = volume (ml) of 0,2 N HCl used

N = normality of HCl used

Total alkalinity determinations were carried out daily.

4.2.3.2 Bicarbonate Alkalinity

Bicarbonate alkalinity can be determined by calculation when the volatile fatty acids and total alkalinity are known (McCarty, 1964b).

Calculation:

$$\text{Bicarbonate alkalinity} = \text{TA} - (0,85) (0,833) [\text{VFA}]$$

(mg/ℓ CaCO_3)

where: TA = total alkalinity (mg/ℓ CaCO_3)
 [VFA] = volatile fatty acids concentration (mg/ℓ as acetic acid)
 0,85 = only 85 % of the volatile acid alkalinity is measured by titration of the total alkalinity to pH 4,0
 0,833 = converts volatile acid units to equivalent alkalinity units

Bicarbonate alkalinity determinations were carried out three times a week.

4.2.4 Chemical Oxygen Demand (COD)

4.2.4.1 Total Chemical Oxygen Demand

A sample of uniform composition (2 ml or 5 ml diluted to 20 ml) was pipetted into a 250 ml refluxing flask containing mercuric sulphate CP Grade (1,0 g) and several glass beads (Appendix II). Standard 0,25 N potassium dichromate (10 ml) and concentrated sulphuric acid containing 0,3 g of dissolved silver sulphate CP Grade (30 ml) were then added.

After refluxing for two hours, the mixture was cooled and water (80 ml) was used to wash down the inside of the condenser. After cooling to room temperature, the excess of potassium dichromate was titrated with standard 0,1 N ferrous ammonium sulphate using ferroin indicator (3 drops). The end point was reached when the colour changed from blue-green to reddish-brown. A blank determination was carried out using water (20 ml) (APHA, 1980).

Calculation:

$$\text{Total COD} = \frac{(A - B) \times N \times 8000}{\text{sample volume (ml)}}$$

(mg/ℓ)

where: A = volume (ml) of ferrous ammonium sulphate used for the blank
 B = volume (ml) of ferrous ammonium sulphate used for the sample
 N = normality of the ferrous ammonium sulphate

COD determinations were carried out three times a week.

4.2.4.2 Soluble Chemical Oxygen Demand

Soluble COD's were carried out as described previously with the exception that the sample was first filtered through Whatman 42 filter paper prior to the COD determination.

4.2.5 Permanganate Value (PV)

4.2.5.1 Total Permanganate Value

A sample of uniform composition (2 ml or 5 ml diluted to 100 ml) was pipetted into a 250 ml glass-stoppered bottle to which 9 N sulphuric acid (25 ml) and 0,0125 N potassium permanganate (50 ml) had been added. The bottle was then maintained at 27°C in a constant-temperature water bath for 4 hours. Thereafter, potassium iodide (0,5 g) was added and the sample was titrated with 0,0125 N sodium thiosulphate to a pale straw colour. Starch indicator (1 ml) was then added and the titration continued to the first disappearance of the blue colour. A blank determination was carried out using water (100 ml) (Republic of South Africa Government Gazette, 1969).

Calculation:

$$\text{Total PV} = \frac{(A - B) \times N \times 8000}{\text{sample volume (ml)}} \quad (\text{mg/l})$$

where: A = volume (ml) of sodium thiosulphate used for the blank
 B = volume (ml) of sodium thiosulphate used for the sample
 N = normality of the sodium thiosulphate

PV determinations were carried out three times a week.

4.2.5.2 Soluble Permanganate Value

Soluble PV's were carried out as described previously with the exception that the sample was first filtered through Whatman 42 filter paper prior to the PV determination.

4.2.6 Volatile Fatty Acids

Volatile fatty acids (VFA) were analyzed three times a week using a Hewlett-Packard 5830A gas chromatograph equipped with a flame ionization detector. A 2 m glass column (2 mm i.d.) was packed with Chromosorb W AW, 100 - 120 mesh, coated with 10 % SP-1000 and 3 % H_3PO_4 (Supelco). The flow rate of the carrier gas nitrogen was 35 ml/minute. The temperature of the injection port, column and detector were 170°C, 150°C and 180°C respectively. The inlet pressures of hydrogen, nitrogen and air were 276 kPa, 276 kPa and 360 kPa respectively (Hurlow, 1986).

A sample (2 ml) was acidified with 6 N HCl (3 drops) to a pH of less than 1,0 and centrifuged at 3000 x g for 5 minutes (Mackie and Bryant, 1981). The supernatant (0,5 µl) was then analyzed for volatile fatty acids, and individual fatty acids were identified and concentrations determined by comparison with a mixture of standards of known concentration (0,1 % in water) (Appendix III).

Calculation:

$$\text{VFA (mg/l)} = \frac{A_{\text{sample}} \times V_{\text{sample}} \times \text{SG} \times 1000}{A_{\text{standard}} \times V_{\text{standard}}}$$

where: A = integrated area of the standard or sample peak

V = volume (ml) of the standard or sample

SG = specific gravity of the individual VFA (Appendix IV)

Individual volatile fatty acids were expressed as acetic acid (Ross, personal communication).

Calculation:

$$\text{VFA acetic (mg/l)} = [\text{VFA}] \times \frac{\text{MM VFA}}{\text{MM acetic acid}}$$

where: VFA acetic = concentration (mg/l) of the individual volatile fatty acid expressed as acetic acid

[VFA] = concentration (mg/l) of the individual volatile fatty acid
 MM VFA = molecular mass of the individual volatile fatty acid
 MM acetic acid = molecular mass of acetic acid

Using the formula above, it was possible to calculate the total concentration of volatile fatty acids expressed as acetic acid (Ross, personal communication).

Calculation:

$$\text{Total VFA as acetic (mg/l)} = [\text{acetic acid}] + [\text{propionic acid}] \frac{\text{MM propionic acid}}{\text{MM acetic acid}} +$$

$$[\text{butyric acid}] \frac{\text{MM butyric acid}}{\text{MM acetic acid}} + [\text{valeric acid}]$$

$$\frac{\text{MM valeric acid}}{\text{MM acetic acid}} + [\text{caproic acid}] \frac{\text{MM caproic acid}}{\text{MM acetic acid}}$$

$$= [\text{acetic acid}] + 1,23 [\text{propionic acid}] + 1,47 [\text{butyric acid}] \\ + 1,70 [\text{valeric acid}] + 1,93 [\text{caproic acid}]$$

where: [acetic acid] = concentration (mg/l) of acetic acid
 MM acetic acid = molecular mass of acetic acid
 [propionic acid] = concentration (mg/l) of propionic acid
 MM propionic acid = molecular mass of propionic acid
 [butyric acid] = concentration (mg/l) of butyric acid
 MM butyric acid = molecular mass of butyric acid
 [valeric acid] = concentration (mg/l) of valeric acid
 MM valeric acid = molecular mass of valeric acid
 [caproic acid] = concentration (mg/l) of caproic acid
 MM caproic acid = molecular mass of caproic acid

4.2.7 Biogas Composition

Biogas was analyzed 3 times a week for methane (CH₄) and carbon dioxide (CO₂)

using a Hewlett-Packard 5830A gas chromatograph equipped with a thermal conductivity detector. A 2 m glass column (2 mm i.d.) was packed with Poropak Type N (Waters Associates). Helium was used as the carrier gas at a flow rate of 35 ml/minute. The temperature of injection port, column and detector were 80°C, 60°C and 80°C respectively. The inlet pressures of helium and air were 276 kPa and 360 kPa respectively. A sample (0,2 ml) was assayed for percentage composition.

The CH₄ and CO₂ peaks were identified using a standard of known composition (Supelco) (Appendix V) and the relative percentages of each gas were calculated using a determined thermal conductivity relationship between CH₄ and CO₂ (Appendix VI).

Calculation:

$$\% \text{CH}_4 = \frac{A \text{CH}_4}{A \text{CH}_4 + (0,57 \times A \text{CO}_2)} \times 100$$

where: A CH₄ = integrated area of the CH₄ peak in the sample

A CO₂ = integrated area of the CO₂ peak in the sample

$$\% \text{CO}_2 = 100 - \% \text{CH}_4$$

The above calculations were based on the assumption that biogas consists entirely of CH₄ and CO₂, which is universally acceptable for laboratory experiments.

4.2.8 Sulphate

A sample, which was first centrifuged at 3000 x g to remove any suspended matter, was diluted with water to contain approximately 50 mg of sulphate (SO₄²⁻) ions in a final volume of 250 ml. The pH was adjusted to less than 4,5 using 6 N HCl, and the sample was then boiled. While stirring gently, warm 10 % barium chloride was added and when precipitation was complete, excess barium chloride (2 ml) was added. The precipitated barium sulphate was then filtered through Whatman 42 filter paper, and washed several times with warm water to remove any interfering chloride ions. The filter paper and precipitate were dried and placed in a crucible of known weight. After

ignition of the precipitate in a furnace at 600°C for 6 hours, the crucible was cooled in a desiccator and reweighed (APHA, 1980).

Calculation:

$$\text{SO}_4^{2-} \text{ (mg/l)} = \frac{\text{mg BaSO}_4 \times 411,6}{\text{sample volume (ml)}}$$

where: mg BaSO₄ = increase in weight (mg) of the crucible after ignition of the precipitate at 600°C

Sulphate determinations were carried out weekly.

4.2.9 Sulphide

A sample (5 ml), which was removed directly from the digester in order to avoid loss of sulphide as hydrogen sulphide, was immediately pipetted into a 300 ml Erlenmeyer flask containing 0,025 N iodine solution (10 ml), 6 N HCl (2 ml) and water (10 ml). The sample was discharged under the surface of the solution. The excess iodine was immediately back-titrated with 0,025 N sodium thiosulphate, using starch solution (1 ml) as the indicator. The end point was reached at the first disappearance of the blue colour (APHA, 1980).

Calculation:

$$\text{Sulphide -S (mg/l)} = \frac{[(A \times B) - (C \times D)] \times 16000}{\text{sample volume (ml)}}$$

where: A = volume (ml) of the iodine solution used

B = normality of the iodine solution

C = volume (ml) of sodium thiosulphate solution used

D = normality of the sodium thiosulphate solution

Sulphide determinations were carried out weekly.

4.2.10 Hydrogen Sulphide

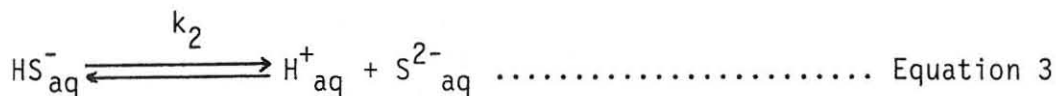
In anaerobic digesters, sulphide is distributed between hydrogen sulphide

(H₂S) in the gas phase, H₂S, HS⁻, and S²⁻ in solution, and insoluble metallic sulphides. The equilibrium between H₂S in the gas phase and free H₂S in solution is governed by Henry's Law (Lawrence et al, 1966):

$$[H_2S]_{aq} = \alpha [H_2S]_g \dots\dots\dots \text{Equation 1}$$

where: α (absorption coefficient) = 1,83 at 35°C

The H₂S in solution is a weak acid and dissociates as follows:-



At the neutral pH required for anaerobic treatment, only the first dissociation of H₂S is of importance (Lawrence et al, 1966). The equilibrium equation for the dissociation is:

$$\frac{[H^+_{aq}] [HS^-_{aq}]}{[H_2S_{aq}]} = K_1 \dots\dots\dots \text{Equation 4}$$

where: $K_1 = 1,49 \times 10^{-7}$ at 35°C

A consideration of the relationship between pH and the different forms of soluble sulphide, as well as the solubility of hydrogen sulphide gas itself, allows prediction of the distribution of the sulphides between the gas and aqueous phase as follows, where the total concentration of soluble sulphides is [TSS].

$$[TSS] = [HS^-] + [H_2S_{aq}] \dots\dots\dots \text{Equation 5}$$

Substituting for [HS⁻] from equation 4 yields:-

$$[TSS] = \frac{K_1 [H_2S_{aq}]}{[H^+]} + [H_2S_{aq}] \dots\dots\dots \text{Equation 6}$$

Substituting for $[H_2S_{aq}]$ from equation 1 then yields:-

$$\frac{[TSS]}{[H_2S_g]} = \alpha \left[\frac{1 + K_1}{[H^+]} \right] \dots\dots\dots \text{Equation 7}$$

Calculation:

$$H_2S_g \text{ (mg/l)} = \frac{TSS}{\alpha \left[\frac{1 + K_1}{[H^+]} \right]}$$

where: TSS = concentration (mg/l) of total soluble sulphides

α = 1,83 at 35°C

K_1 = $1,49 \times 10^{-7}$ at 35°C

$[H^+]$ = hydrogen ion concentration

H_2S determinations were carried out weekly.

4.2.11 Total Kjeldahl Nitrogen (TKN)

A sample (50 ml of effluent or 2 g of sludge) was placed in a 500 ml Kjeldahl flask containing a few glass beads, and digested with digestion mixture (100 ml) using a Kjeldahl apparatus for the suitable removal of the SO_3 fumes. After the complete oxidation of the sample i.e. the cessation of SO_3 production, the sample was boiled for a further 30 minutes. After allowing to cool, water (200 ml), a small quantity of medium grit Carborundum and dechlorinating agent at a ratio of 1 ml per 100 mg Cl^- ions, were added to the flask. The solution was again cooled and 12 N NaOH (100 ml) was then added. The sample was immediately distilled and the distillate (200 ml) was collected below the surface of indicating boric acid (50 ml). The ammonia in the distillate was titrated with 0,2 N H_2SO_4 . The end point was reached when the solution turned from green to lavender (APHA, 1980).

Calculation:

$$TKN \text{ (mg/l)} = \frac{A \times N \times 14000}{\text{sample volume (ml)}}$$

where: A = volume (ml) of 0,2 N H_2SO_4 used for the sample
 N = normality of the H_2SO_4

TKN determinations were carried out weekly.

4.2.12 Ammonia-Nitrogen ($\text{NH}_3\text{-N}$)

Ammonia-nitrogen was determined as described previously (Section 4.2.11) with the exception that the sample was not digested with the digestion mixture (APHA, 1980).

Calculation:

$$\text{NH}_3\text{-N} = \frac{A \times N \times 14000}{\text{sample volume (ml)}} \quad (\text{mg/l})$$

where: A = volume (ml) of 0,2 N H_2SO_4 used for the sample
 N = normality of the H_2SO_4

$\text{NH}_3\text{-N}$ determinations were carried out weekly.

4.2.13 Organic Nitrogen

Organic nitrogen was calculated as the difference between the total Kjeldahl nitrogen and the ammonia-nitrogen (APHA, 1980).

Calculation:

$$\text{Organic Nitrogen} = \text{Total Kjeldahl Nitrogen} - \text{Ammonia-Nitrogen}$$

(mg/l) (mg/l) (mg/l)

Organic nitrogen was determined weekly.

4.2.14 Free Ammonia-Nitrogen

The un-ionized fraction of the total amount of ammonia-nitrogen was calculated using the following equation (Shafai et al, 1986):

Calculation:

$$f = \frac{1}{10^{(pKa - pH)} + 1} \times 100$$

where: f = % of total ammonia in the un-ionized state

pKa = dissociation constant for ammonia

$$= 0,0901821 + \frac{2729,92}{T}$$

T = temperature (K)

$$\text{Free } NH_3\text{-N} = f_{NH_3} \times NH_3\text{-N}$$

(mg/l)

where: f_{NH_3} = fraction of un-ionized ammonia-nitrogen

$NH_3\text{-N}$ = total ammonia-nitrogen concentration (mg/l)

Free ammonia-nitrogen was determined weekly.

4.2.15 Chloride (Cl^-)

A sample (1 ml) was added to a 300 ml Erlenmeyer flask containing atomic absorption grade water (100 ml), K_2CrO_4 indicator (1 ml) and 30 % hydrogen peroxide (1 ml). After adjusting the pH to 7,0 using dilute HCl or NaOH, the sample was titrated with 0,0141 N $AgNO_3$. The end point was reached when the solution turned from yellow to orange-red (APHA, 1980).

Calculation:

$$Cl^- = \frac{A \times N \times 35450}{\text{sample volume (ml)}}$$

(mg/l)

where: A = volume (ml) of 0,0141 N $AgNO_3$ used for the sample

N = normality of the $AgNO_3$

Chloride determinations were carried out weekly.

4.2.16 Phosphorous

In order to release the phosphorous bound to the organic matter, a sample (10 ml of effluent or 0,5 g of sludge) was digested with 1 : 1 HCl-HNO₃ (20 ml). HCl-HNO₃ acids were used as this is a mild digestion technique. Stronger digestion methods result in the oxidation of the Cr³⁺ present in the tannery wastewaters to Cr⁶⁺ which interferes with the vanadomolybdophosphoric acid colourimetric method (Appendix VII). After the preliminary acid digestion, the sample was diluted to 50 ml with water.

The phosphorous present in the sample was determined by means of the vanadomolybdophosphoric acid colourimetric method (APHA, 1980). The acid-digested sample (35 ml) was placed in a 50 ml volumetric flask. 7 N HNO₃ (3 ml), 0,021 N ammonium vanadate (5 ml) and 0,04 N ammonium molybdate (5 ml) were then added and the solution was diluted to 50 ml with water. A blank solution was prepared in the same manner with the exception that water was used in place of the sample. After allowing to stand for 10 minutes in order for the colour to develop, the absorbance of the solution was measured relative to the blank on a Beckman Acta CII spectrophotometer at a wavelength of 400 nm. The concentration of phosphorous was determined from the absorbance by comparison with a standard curve (Appendix VIII).

Phosphorous determinations were carried out weekly.

4.2.17 Sodium

Due to the high solubility of sodium ions, a preliminary acid digestion was not carried out. A sample (1 ml) was diluted to 1 litre using atomic absorption grade water. The concentration of sodium was determined by means of a Varian 1275 atomic absorption spectrophotometer (Appendix IX). The wavelength, slit width and lamp current were 589 nm, 0,5 nm and 5,0 mA respectively. Air-acetylene was used as the gas mixture (Appendix X).

Sodium determinations were carried out weekly.

4.2.18 Calcium

Because calcium is very resistant to acid digestion, the volume of the samples

used were kept very small. A sample (1 ml of effluent or 0,5 g of sludge) was placed in a 300 ml Kjeldahl digestion flask and digested with 1 : 1 HCl-HNO₃ (20 ml). The digested sample was then diluted to 100 ml with atomic absorption grade water. A HCl-HNO₃ acid mixture was used as this results in the most efficient recovery of calcium from tannery samples. Sulphuric acid cannot be used for the digestion as it reacts with calcium to form insoluble calcium sulphate (Kallenberger, 1983). The concentration of calcium was determined by means of a Varian 1275 atomic absorption spectrophotometer (Appendix IX). The wavelength, slit width and lamp current were 422,7 nm, 0,5 nm and 3,5 mA respectively. Nitrous oxide-acetylene was used as the gas mixture (Appendix X).

Calcium determinations were carried out weekly.

4.2.19 Chrome

A sample (20 ml of effluent or 1 g of sludge) was placed in a 300 ml Kjeldahl digestion flask and digested with 3 : 1 of 60 % perchloric : sulphuric acid (15 ml) and nitric acid (10 ml). Digestion was continued until oxidation of the sample was complete, as indicated by the appearance of white HClO₄ fumes. After diluting to 100 ml with atomic absorption grade water, the concentration of chrome was determined by means of a Varian 1275 atomic absorption spectrophotometer (Appendix IX). The wavelength, slit width and lamp current were 357,9 nm, 0,2 nm and 7 mA respectively. Air-acetylene was used as the gas mixture (Appendix X).

Chrome determinations were carried out weekly.

4.2.20 Iron

A sample (20 ml of effluent or 1 g of sludge) was placed in a 300 ml Kjeldahl digestion flask and digested with 3 : 1 of 60 % perchloric : sulphuric acid (15 ml) and nitric acid (10 ml). Digestion was continued until oxidation of the sample was complete, as indicated by the appearance of white HClO₄ fumes. After diluting to 100 ml with atomic absorption grade water, the concentration of iron was determined by means of a Varian 1275 atomic absorption spectrophotometer (Appendix IX). The wavelength, slit width and lamp current were 248,3 nm, 0,2 nm and 5,0 mA respectively. Air-acetylene was used as the gas mixture (Appendix X).

4.2.21 Aluminium

A sample (20 ml of effluent or 1 g of sludge) was placed in a 300 ml Kjeldahl digestion flask and digested with 3 : 1 of 60 % perchloric : sulphuric acid (15 ml) and nitric acid (10 ml). Digestion was continued until oxidation of the sample was complete, as indicated by the appearance of white HClO_4 fumes. After diluting to 100 ml with atomic absorption grade water, the concentration of aluminium was determined by means of a Varian 1275 atomic absorption spectrophotometer (Appendix IX). The wavelength, slit width and lamp current were 309,3 nm, 0,5 nm and 10 mA respectively. Nitrous oxide-acetylene was used as the gas mixture (Appendix X).

Aluminium determinations were carried out weekly.

4.2.22 Total Solids (TS)

A sample (20 ml of effluent or 5 g of sludge) was placed in a crucible of known weight and evaporated to dryness on a steam bath. The crucible was then dried in an oven at 80°C and, after cooling to room temperature in a desiccator, it was reweighed (APHA, 1980).

Calculation:

$$\text{Total Solids (TS)} = \frac{(A - B) \times 1000}{\text{sample volume (ml)}} \quad (\text{mg/l})$$

where: A = weight (mg) of the dried sample + crucible
B = weight (mg) of the crucible

Total solids determinations were carried out weekly.

4.2.23 Suspended Solids (SS)

A sample (50 ml) was centrifuged at 3000 x g in a MSE Super Minor centrifuge for 15 minutes. The supernatant was discarded and the pellet was resuspended in water and placed in a crucible of known weight. After evaporating to dryness on a steam bath and drying in an oven at 80°C, the crucible was cooled in a desiccator and reweighed.

Calculation:

$$\text{Suspended Solids (SS)} = \frac{(A - B) \times 1000}{\text{sample volume (ml)}} \quad (\text{mg/l})$$

where: A = weight (mg) of the sample + crucible

B = weight (mg) of the crucible

Alternately, suspended solids were determined by calculation.

$$\text{Suspended Solids (SS)} = \text{Total Solids} - \text{Total Dissolved Solids} \quad (\text{mg/l}) \quad (\text{mg/l}) \quad (\text{mg/l})$$

Suspended solids determinations were carried out weekly.

4.2.24 Total Dissolved Solids (TDS)

After first filtering through Whatman 42 filter paper, the sample (20 ml) was placed in a crucible of known weight and evaporated to dryness on a steam bath. The crucible was then dried in an oven at 80°C, cooled in a desiccator and reweighed.

Calculation:

$$\text{Total Dissolved Solids (TDS)} = \frac{(A - B) \times 1000}{\text{sample volume (ml)}} \quad (\text{mg/l})$$

where: A = weight (mg) of the sample + crucible

B = weight (mg) of the crucible

Total dissolved solids determinations were carried out weekly.

4.2.25 Total Volatile Solids (TVS)

The crucible from the total solids determination (Section 4.2.22) containing the dried sample was placed in a furnace at 600°C for 6 hours. After cooling to room temperature in a desiccator, the crucible was reweighed.

Calculation:

$$\text{Total Volatile Solids (TVS)} = \frac{(A - B) \times 1000}{\text{sample volume (ml)}} \quad (\text{mg/l})$$

where: A = weight (mg) of the crucible before ignition

B = weight (mg) of the crucible after ignition

Total volatile solids determinations were carried out weekly.

4.2.26 Total Inorganic Solids (TIS)

Total inorganic solids (TIS) were determined by calculation.

Calculation:

$$\text{Total Inorganic Solids (TIS)} = \text{Total Solids} - \text{Total Volatile Solids} \quad (\text{mg/l}) \quad (\text{mg/l}) \quad (\text{mg/l})$$

Total inorganic solids determinations were carried out weekly.

4.2.27 Total Dissolved Volatile Solids (TDVS)

The crucible from the total dissolved solids (TDS) determination (Section 4.2.24) containing the dried sample was placed in a furnace at 600°C for 6 hours. After cooling to room temperature in a desiccator, the crucible was reweighed.

Calculation:

$$\text{Total Dissolved Volatile Solids (TDVS)} = \frac{(A - B) \times 1000}{\text{sample volume (ml)}} \quad (\text{mg/l})$$

where: A = weight (mg) of the crucible before ignition

B = weight (mg) of the crucible after ignition

Total dissolved volatile solids determinations were carried out weekly.

4.2.28 Total Dissolved Inorganic Solids (TDIS)

Total dissolved inorganic solids (TDIS) were determined by calculation.

Calculation:

$$\text{Total Dissolved Inorganic Solids (TDIS)} = \frac{\text{Total Dissolved Solids (mg/ℓ)}}{\text{(mg/ℓ)}} - \frac{\text{Total Dissolved Volatile Solids}}{\text{(mg/ℓ)}}$$

Total dissolved inorganic solids determinations were carried out weekly.

4.2.29 Volatile Suspended Solids (VSS)

The crucible from the suspended solids (SS) determination (Section 4.2.23) containing the dried sample was placed in a furnace at 600°C for 6 hours. After cooling to room temperature in a desiccator, the crucible was reweighed.

Calculation:

$$\text{Volatile Suspended Solids (VSS)} = \frac{(A - B) \times 1000}{\text{sample volume (mℓ)}} \text{ (mg/ℓ)}$$

where: A = weight (mg) of the crucible before ignition

B = weight (mg) of the crucible after ignition

Volatile suspended solids determinations were carried out weekly.

CHAPTER FIVE

THE EFFECT OF INDIVIDUAL TANNERY WASTEWATER COMPOUNDS ON ANAEROBIC DIGESTION

CHAPTER FIVE

THE EFFECT OF INDIVIDUAL TANNERY WASTEWATER COMPOUNDS ON ANAEROBIC DIGESTION

Every industrial wastewater exhibits its own specific profile of chemical and biological oxygen demand, solids concentration, pH, and concentrations of anions and cations. Effluent analysis is therefore essential in obtaining an initial assessment of the potential of the anaerobic process to degrade the wastewater organics and to allow a suitable experimental design to be constructed. It is also important to establish the presence and concentration of essential nutrients and possible toxic compounds.

Tannery wastewaters are characterized by high concentrations of both organic and inorganic compounds. Anaerobic digestion is an ideal process of reducing the organic content of these wastewaters. However, the presence of high concentrations of inorganic compounds poses a potential inhibitory threat to the anaerobic digestion process. A survey of South African tannery wastewater analyses revealed that several compounds are present in sufficiently high concentrations to cause digester toxicity. These compounds include trivalent chromium, sulphates and sulphides, sodium chloride, calcium, iron, aluminium and ammonia. Most of these compounds are waste products of the tanning process. However, a few, e.g. iron and aluminium, are added to improve existing wastewater treatment processes, and are therefore also present in the final effluents and sludges. If anaerobic digestion is to be considered as a possible treatment method for tannery wastewaters, the long term effects and fate of these compounds requires detailed investigation.

Toxicity studies can be carried out either as shock load or acclimatization studies. In this investigation, the studies were carried out as acclimatization experiments. The reasoning behind this is that during the startup phase of an anaerobic digester, the loading rate is initially kept low, and over a period of time, it is slowly increased. The anaerobic bacteria are therefore exposed to increasing concentrations of toxic compounds if they are present, and are therefore able to adapt to the presence of these compounds as the loading rate is increased. The tolerance to toxic compounds through adaptation is usually far greater than that achieved for shock load experiments. Adaptation experiments therefore provide more meaningful results

on the ability of bacteria to withstand toxic compounds.

As mentioned previously (Section 4.1.3.1), all of the toxicity experiments were carried out using a synthetic wastewater, and all of the compounds under investigation were added as chloride salts.

5.1. Anaerobic Digester Startup on the Synthetic Wastewater

5.1.1 Introduction

The ultimate stabilization of a given type of waste is dependent on the growth and metabolism of non-methanogenic and methanogenic bacteria. These two groups of microorganisms are very different from each other in terms of physiology, nutritional requirements, growth kinetics and sensitivity to environmental stresses. Efficient operation of the anaerobic process requires that the acid-forming microorganisms grow in harmony with the methanogenic microorganisms, because any loss in the biochemical balance that exists between these two groups, particularly in favour of the relatively fast growing acid formers, leads to an upset in the digestion process (Ghosh et al, 1975).

If a digester is accidentally supplied with a high concentration of organic matter, the hydrolytic and acid-forming bacteria will respond quickly and will metabolize it. This will result in a rapid increase in digester acetate, butyrate and propionate concentrations. The more slowly responding methanogens will be unable to metabolize the increased concentration of their substrate, acetate. Consequently, the concentrations of the intermediate acids will increase, causing the digester pH to decrease. This in turn will inhibit the methanogens, and butyric and propionic acids will accumulate because hydrogen will not be removed from the system.

In contrast to this, in a well run, fully equilibrated digester, the whole metabolic pathway proceeds efficiently with low volatile acid concentrations. In order to achieve a fully equilibrated and balanced digester, the initial startup is extremely important. It is also the most difficult stage of anaerobic treatment and one which causes many problems. Often long periods of time are required for startup, and this is one of the major disadvantages of the anaerobic digestion process.

During digester startup, the loading rate is initially kept low and then slowly increased stepwise after digestion has been satisfactorily achieved at each flow rate. In this way the delicate biochemical balance between the fast-growing non-methanogenic and slow-growing methanogenic bacteria is maintained.

The startup procedure discussed below was carried out for each toxicity experiment prior to the commencement of the actual experiment, and ensured digester stability. Only one variable was therefore changed during the course of each experiment i.e. the concentration of the toxic compound under investigation.

5.1.2 Materials and Methods

The materials and methods used were described in detail in Chapter 4.

5.1.3 Results and Discussion

The anaerobic digester was seeded with 4 l of anaerobic sludge, with a VSS concentration of 28 - 32 g/l, one month prior to the commencement of the startup phase. The loading of the digester commenced at a very low rate (Figure 24) and was gradually increased over the startup period of 25 days to eventually reach the desired rate at which each toxicity experiment was carried out. The biochemical balance between the microorganisms in the digester was maintained as evidenced by the VFA concentration remaining constant throughout the startup phase (Figure 24). The concentration of VFA in the digester effluent on day 1 was significantly higher than that found in the digester effluent during the remainder of the experiment. These VFA were introduced with the anaerobic sludge inoculum, but were successfully metabolized as indicated by their decreased concentration over the 25 day period (Table 14). The metabolism of these VFA ensured that the pH and alkalinity remained constant.

The increasing loading rate resulted in a corresponding increase in the volume of biogas produced (Figure 25). The CH_4 content of the biogas increased marginally over the first few days of the startup, but remained constant thereafter. The high percentage of CH_4 in the biogas can be attributed to the low-strength nature of the waste (Frostell, 1985). The high biogas yield on

day 1 (Table 15) is due to the utilization of organic matter present in the sludge inoculum. However, it is evident that once this organic matter was utilized and the organic matter in the influent became the sole source of substrate, the biogas yield obtained corresponded closely with the theoretical biogas yield (Section 3.4.7).

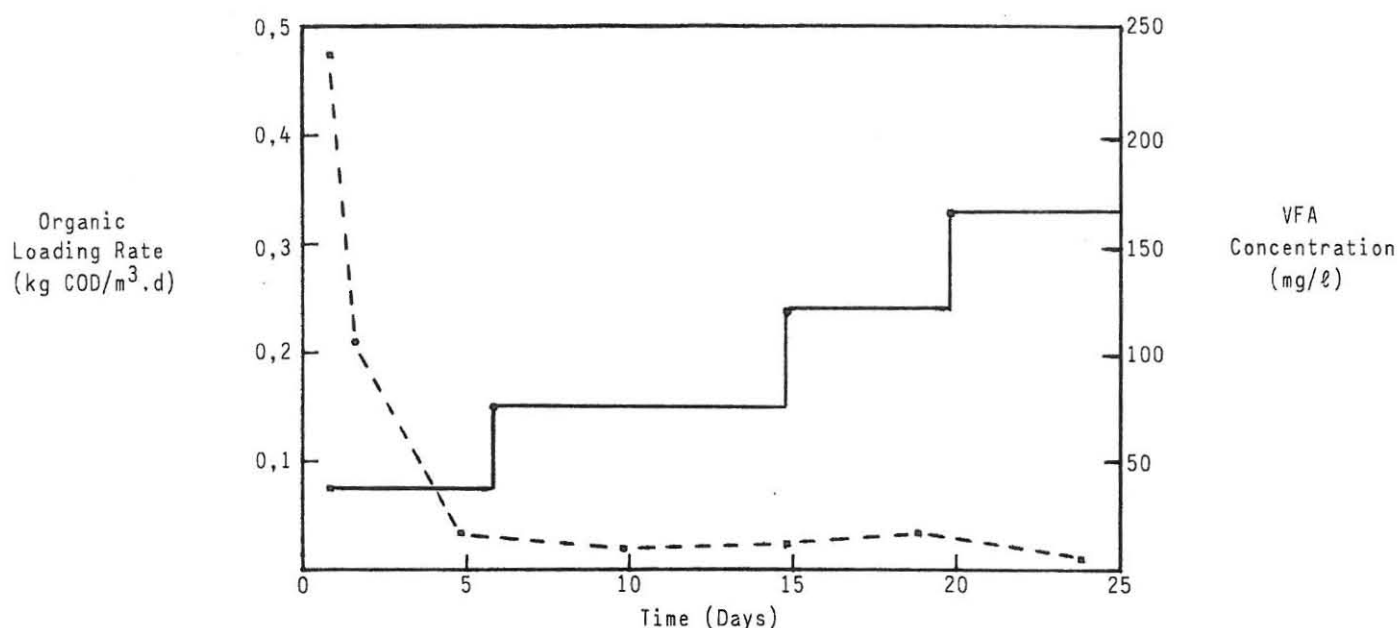


FIGURE 24 - Organic loading rate and effluent volatile fatty acids versus time for the startup of the anaerobic digester on the synthetic wastewater (— organic loading rate; ---- volatile fatty acids as acetic acid)

TABLE 14

CHARACTERISTICS OF THE DIGESTER EFFLUENT DURING
THE STARTUP PHASE ON THE SYNTHETIC WASTEWATER

Parameter	Time (Days)					
	1	5	10	15	20	25
pH	7,36	7,36	7,40	7,42	7,29	7,45
Total Alkalinity (mg/l CaCO_3)	990	924	1034	1254	1188	1232
Bicarbonate Alkalinity (mg/l CaCO_3)	818	908	1023	1241	1172	1224
COD (mg/l)	688	540	340	448	300	284
PV (mg/l)	137	62	48	24	53	34
VFA (mg/l)	242,6	22,9	15,8	17,8	22,9	11,1

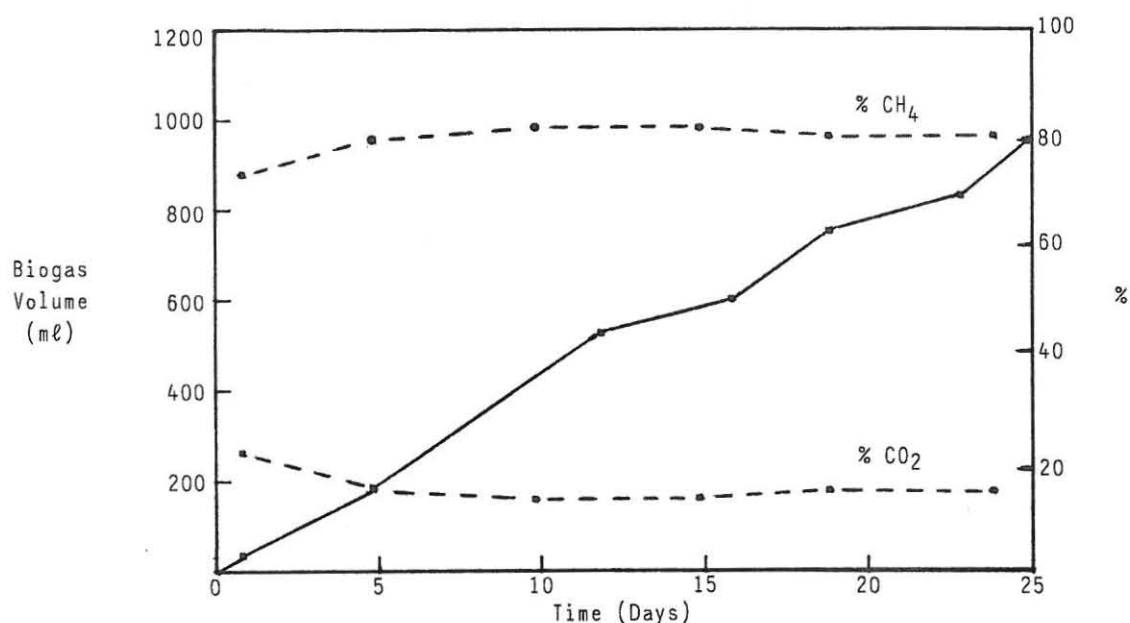


FIGURE 25 - Biogas volume and percentage composition versus time for the startup of the anaerobic digester on the synthetic wastewater (— biogas volume; ---- % CH₄ and CO₂)

Although the percentages of PV and COD removed were low at the start of the experiment, these removal efficiencies increased to remain steady at 95 and 90 % respectively (Table 15).

TABLE 15

OPERATING EFFICIENCY OF THE DIGESTER DURING
THE STARTUP PHASE ON THE SYNTHETIC WASTEWATER

Parameter	Time (Days)					
	1	5	10	15	20	25
Gas Volume (ℓ/d)	0,065	0,210	0,555	0,630	0,780	0,980
Gas Production (m ³ /m ³ .d)	0,007	0,023	0,062	0,070	0,087	0,109
% COD Removed	2,3	30,1	78,4	80,4	86,5	91,0
% PV Removed	41,5	67,2	88,4	95,6	90,8	95,0
Gas Yield (m ³ /kg COD Removed)	3,611	0,890	0,486	0,348	0,401	0,352
CH ₄ Content of Biogas (%)	75,7	82,1	84,3	84,1	82,6	82,7
CH ₄ Yield (m ³ CH ₄ /kg COD Removed)	2,734	0,731	0,410	0,293	0,331	0,291

By day 25, the digester had been successfully stabilized to the synthetic wastewater at the concentration required for all the toxicity experiments. The stepwise increase in the loading rate enabled the biochemical balance in the digester to be maintained. The methanogens were therefore not exposed to high concentrations of VFA and were therefore not inhibited. Following a successful digester startup, each toxicity experiment was then carried out. It should be emphasised that although only one startup has been discussed, each digester was successfully stabilized prior to the start of each toxicity experiment.

5.2 The Effect of Trivalent Chromium on Anaerobic Digestion

5.2.1 Introduction

As anaerobic digestion is a microbiological process, it is influenced by a variety of environmental factors, including the presence of toxic heavy metal ions. During the tanning process, large quantities of chrome (III) sulphate are used to convert the animal hides into chrome leather (Langerwerf, 1985). The tanning liquors always contain an excess of trivalent chromium (Cr^{3+}). This excess Cr^{3+} finds its way into the resultant wastewaters and ultimately ends up in the wastewater sludge.

Numerous studies have been carried out to determine the effects of Cr^{3+} on the anaerobic digestion process. It has been observed that for digesters operating with 10 day retention times, less than 200 mg/l Cr^{3+} was sufficient to cause a 70 % reduction in the volume of biogas evolved (Hayes and Theis, 1978), while a concentration of 450 mg/l Cr^{3+} has been shown to result in digester failure (Mosey and Hughes, 1975). Experiments on the digestion of sewage sludge showed that for digesters with sludge retention times of 21, 28 and 35 days, 300 mg/l Cr^{3+} caused no reduction in biogas volume. However, if the retention time was reduced to 14 days, only 50 mg/l Cr^{3+} could be tolerated without affecting the stability of the digester (Bailey et al, 1970). In contrast to the above, it has been reported that a concentration of 1000 mg/l Cr^{3+} caused no inhibition of anaerobic digestion (Maeda et al, 1984), while in a separate study, a concentration of 3,5 % Cr^{3+} in sludges was found to have no effect on anaerobic digestion (Moore et al, 1961).

In view of the conflicting evidence in the above published data, as well as

the fact that most of these experiments were shock loads of Cr^{3+} to the anaerobic bacteria, the aim of this experiment was to investigate whether an anaerobic bacterial population could be acclimatized to concentrations of Cr^{3+} that were significantly higher than those found in tannery effluents and sludges.

5.2.2 Materials and Methods

The materials and methods used were described in detail in Chapter 4.

5.2.3 Results and Discussion

Although chrome (III) sulphate tanning salts are used as the tanning agent, $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ was used throughout this experiment. The reason for this is that under anaerobic conditions, the sulphate-reducing bacteria reduce sulphates to sulphides, and these sulphides are known to inhibit methanogenic bacteria (Lawrence *et al*, 1966). Any toxicity effects noted could therefore be attributed to either the Cr^{3+} , or the sulphides formed, or both. In order to be able to determine the toxicity effects of only the Cr^{3+} , $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ was chosen as a source of Cr^{3+} . It should be noted that no Cr^{6+} is formed under anaerobic conditions (Shivas, 1980), and consequently all the chrome present in the digester was in the Cr^{3+} oxidation state.

The addition of incremental amounts of Cr^{3+} to the digester (Figure 26) appeared to have no notable effect on digester performance for the first 114 days. The pH, alkalinity and percentage COD and PV removed all remained relatively constant (Table 16 and 17). Although the VFA showed a substantial increase in concentration during the first 35 days, indicating instability in the digester, the concentration decreased substantially thereafter and remained relatively constant until digester failure occurred. It should be noted that at all times their concentration was higher in the digester receiving Cr^{3+} than in the control digester (Table 16).

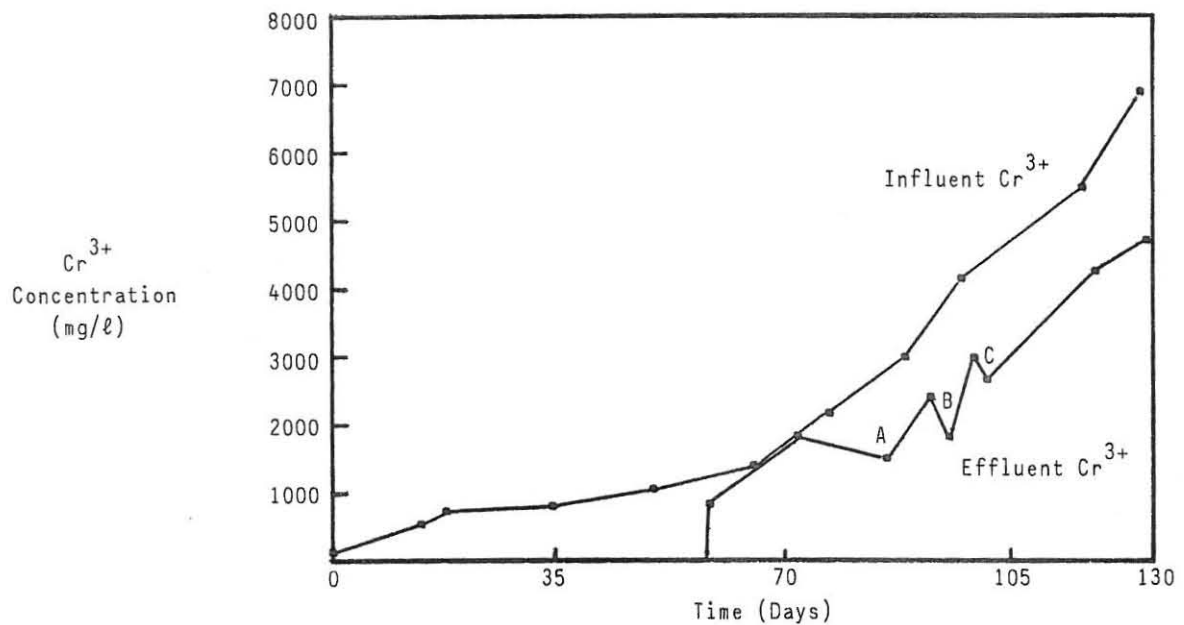


FIGURE 26 - Influent and effluent Cr^{3+} versus time for the anaerobic digester receiving Cr^{3+}
 (Note: A, B and C indicate the removal of $\text{Cr}(\text{OH})_3$ from the digester. The control digester received no Cr^{3+})

TABLE 16

CHARACTERISTICS OF THE DIGESTER EFFLUENT AT VARIOUS STAGES
 DURING THE TRIVALENT CHROMIUM EXPERIMENT

Parameter	Control Digester					Cr^{3+} Digester				
	Time (Days)					Time (Days)				
	1	35	70	105	130	1	35	70	105	130
pH	7,58	7,57	7,46	7,50	7,56	7,30	7,56	7,26	7,38	7,42
Total Alkalinity (mg/l CaCO_3)	1224	1326	1275	1224	1326	1428	1530	1326	816	714
Bicarbonate Alkalinity (mg/l CaCO_3)	1196	1304	1251	1203	1279	1419	1312	1256	750	602
COD (mg/l)	474	468	375	330	348	675	432	549	450	2007
PV (mg/l)	29	48	57	58	45	66	40	42	33	434
VFA (mg/l)	39,7	30,8	34,4	30,3	66,4	13,4	308,0	99,2	92,9	157,8
Cr^{3+} (mg/l)	< 1	< 1	< 1	< 1	< 1	< 1	4,6	2030	2858	4900

The volume of biogas evolved (Figure 27) and the biogas yield (Table 17) were also maintained at a constant level for the first 85 days relative to the control digester. A slight decrease in the biogas volume and the biogas yield was noted for the digester receiving Cr^{3+} between day 85 and 114. During the same period, an increase of 6,6 % in the CH_4 content of the biogas was noted (Figure 28). This increase in the methane content of the biogas as the volume decreases can be explained by the fact that the CO_2 formed during anaerobic digestion is not flushed out at the same rate from the digester with the biogas, due to the reduced biogas yield. This CO_2 is therefore able to dissolve in the liquid inside the digester, resulting in an increased percentage of CH_4 in the biogas. This phenomenon has been noted in previous studies (Lawrence *et al*, 1966; Britz, personal communication).

TABLE 17
THE EFFECT OF TRIVALENT CHROMIUM ON THE OPERATING EFFICIENCY
OF THE DIGESTER AT VARIOUS STAGES DURING THE EXPERIMENT

Parameter	Control Digester					Cr^{3+} Digester				
	Time (Days)					Time (Days)				
	1	35	70	105	130	1	35	70	105	130
Gas Volume (ℓ/d)	1,040	1,060	1,045	1,020	1,095	1,133	1,000	1,025	0,881	0,000
Gas Production ($\text{m}^3/\text{m}^3 \cdot \text{d}$)	0,116	0,118	0,116	0,113	0,122	0,126	0,111	0,114	0,098	0,000
% COD Removed	84,2	84,4	87,5	89,0	88,4	77,5	85,6	81,7	85,0	33,1
% PV Removed	95,5	92,6	91,3	91,1	93,1	89,8	93,9	93,5	94,9	33,2
Gas Yield (m^3/kg COD Removed)	0,412	0,419	0,398	0,382	0,413	0,487	0,389	0,418	0,345	0,000
CH_4 Content of Biogas (%)	75,6	74,3	77,9	76,3	76,1	76,5	77,8	76,4	83,0	89,9
CH_4 Yield ($\text{m}^3 \text{CH}_4/\text{kg}$ COD Removed)	0,311	0,311	0,310	0,291	0,314	0,373	0,303	0,319	0,286	0,000

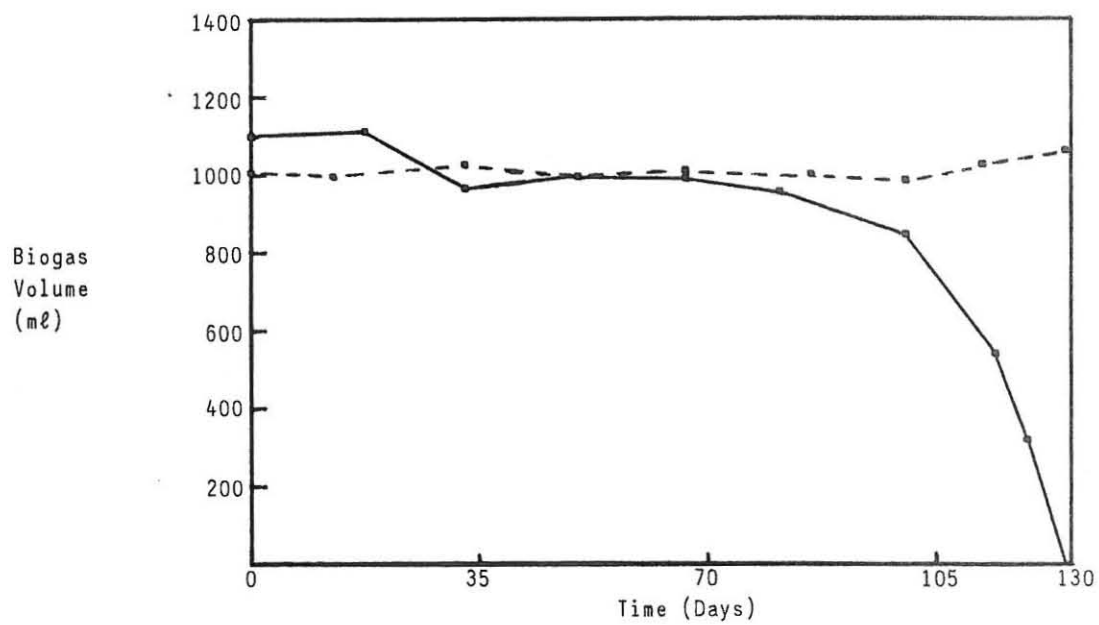


FIGURE 27 - Biogas volume versus time for the anaerobic digester receiving Cr^{3+} (— Cr^{3+} digester; ---- control digester)

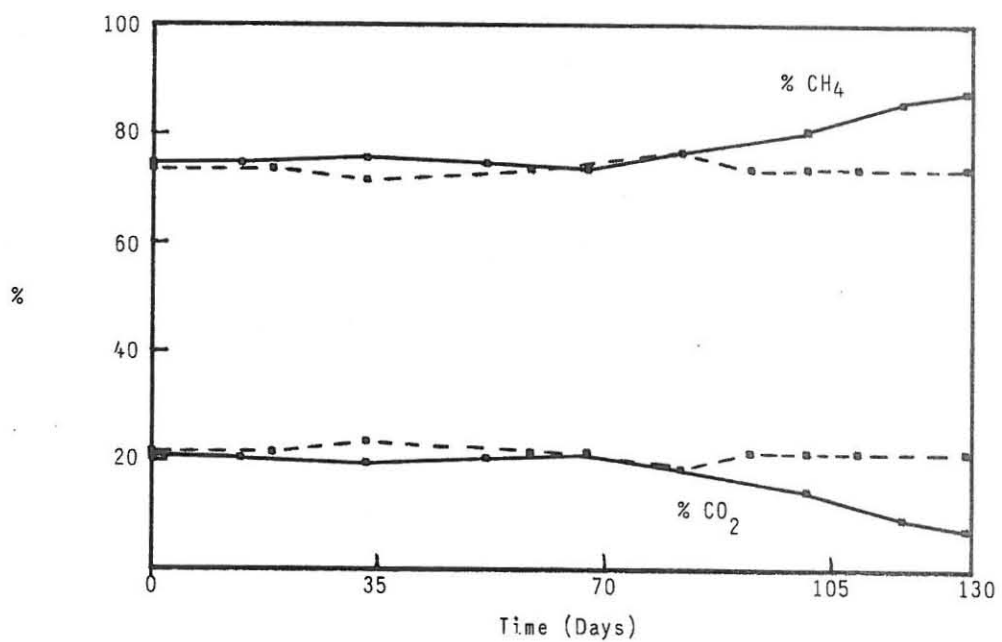


FIGURE 28 - Percentage biogas composition versus time for the anaerobic digester receiving Cr^{3+} (— Cr^{3+} digester; ---- control digester)

When the Cr^{3+} concentration in the feed reached 5025 mg/l (day 115), digester failure occurred. Biogas production ceased (Figure 27) while the percentages of COD and PV removed decreased to 33 and 33,2 % respectively. This decrease in percentage COD removed was a clear indication that the methanogens were inhibited, as it is only through methane production that a reduction in the COD of the waste is achieved (Kugelman and McCarty, 1965). The alkalinity, which determines the buffering capacity of the system, decreased markedly. This was due to the bicarbonate alkalinity being used to neutralize the large accumulation of volatile fatty acids inside the digester. The bicarbonate alkalinity was, however, greater than the VFA concentration, and therefore there were no free VFA in the digester effluent. The neutral pH of the effluent was therefore maintained (Table 16).

The concentration of Cr^{3+} present inside the digester reached 9575 mg/l, which is significantly higher than the Cr^{3+} present in the feed. The reason for this accumulation of Cr^{3+} is that at the neutral pH of the digester, it is likely that the Cr^{3+} precipitated as $\text{Cr}(\text{OH})_3$ as has been shown previously (Shivas, 1978b). The accumulation of Cr^{3+} inside the digester is clearly evident by comparing the stratigraphic concentration of Cr^{3+} at the start and on completion of the experiment (Tables 18 and 19). This precipitation of $\text{Cr}(\text{OH})_3$ also explains the lag-phase prior to the Cr^{3+} emerging in the digester effluent (Figure 26). It should be noted that $\text{Cr}(\text{OH})_3$ was removed from the digester at periodic intervals in order to prevent clogging of the outlet. Hence the frequent decrease in total effluent Cr^{3+} , as shown in Figure 26.

TABLE 18

STRATIGRAPHIC ANALYSIS OF THE DIGESTER CONTENTS
AT THE START OF THE TRIVALENT CHROMIUM EXPERIMENT

Height of Sampling Port Above Inlet (cm)	Control Digester			Cr^{3+} Digester		
	pH	Cr^{3+} (mg/l)	VFA (mg/l)	pH	Cr^{3+} (mg/l)	VFA (mg/l)
59	7,58	< 1	39,7	7,30	< 1	13,4
42	7,41	< 1	18,7	7,66	< 1	23,9
29	7,20	< 1	26,9	7,66	< 1	19,8
16	7,33	5,9	48,9	7,45	8,0	55,3

TABLE 19

STRATIGRAPHIC ANALYSIS OF THE DIGESTER CONTENTS
ON COMPLETION OF THE TRIVALENT CHROMIUM EXPERIMENT

Height of Sampling Port Above Inlet (cm)	Control Digester			Cr ³⁺ Digester		
	pH	Cr ³⁺ (mg/l)	VFA (mg/l)	pH	Cr ³⁺ (mg/l)	VFA (mg/l)
59	7,56	< 1	66,4	7,42	7700	157,8
42	7,16	< 1	60,6	7,28	9575	248,7
29	7,05	< 1	57,3	7,25	9520	353,1
16	7,04	4,8	75,9	6,74	8100	799,1

The results of this experiment are in agreement with the findings of previous studies (Maeda *et al*, 1984; Moore *et al*, 1961). The relative non-toxicity of Cr³⁺, except at very high concentrations, can be attributed to its very low solubility at neutral pH (Shivas, 1978b), as well as its ability to bind to organic matter (Wozniak and Huang, 1982). A few studies have, however, reported low toxicity thresholds for Cr³⁺ (Hayes and Theis, 1978; Mosey and Hughes, 1975; Bailey *et al*, 1970). In most of these studies, Cr₂(SO₄)₃ was used as a source of Cr³⁺. It is therefore possible that the sulphides formed under the anaerobic conditions caused the toxicity rather than the Cr³⁺, as those studies which have used CrCl₃·6H₂O as a source of Cr³⁺ (Maeda *et al*, 1984), have reported very much higher toxicity thresholds.

As tannery effluents and sludges contain Cr³⁺ concentrations significantly lower than that shown in this study to inhibit acclimatized methanogenic bacteria, it can be concluded that the Cr³⁺ present in tannery wastewaters will have no effect on anaerobic digestion, provided the correct operating conditions are maintained. With increased efforts to reduce the chrome in effluent discharges through chrome recycling and recovery (Daigle and Bennett, 1986; Cooper and Boast, 1984), and research into the use of alternative tanning agents (Siegler, 1987; Cooper and Boast, 1986), the concentration of residual chrome will, in time, be reduced substantially. This reduced concentration will result in even less possibility of Cr³⁺ causing anaerobic toxicity.

The findings of this experiment have been previously published (Jackson-Moss et al, 1987a; Jackson-Moss et al, 1988c).

5.3 The Effect of Sulphates and Sulphides on Anaerobic Digestion

5.3.1 Introduction

Sulphur compounds are used at various stages of the tanning process (Section 2.2) and are therefore present in the resultant wastewaters. Sulphides enter the wastewater with the unhairing liquors and can also arise from the degradation of sulphur-containing amino acids viz. cysteine, cystine and methionine. Sulphates are introduced from the deliming and tanning operations through the use of chemicals such as ammonium sulphate and chromium sulphate. Consequently, tannery wastewaters contain high concentrations of sulphates and sulphides. The oxidation state of the sulphur is determined by the sulphur cycle, which is affected by the presence or absence of oxygen.

The sulphur cycle is second in importance only to photosynthesis. It is partly a chemical and partly a biological cycle. Sulphur can exist in many oxidation states, and microorganisms can carry out the conversions between these states (Figure 29). Elemental sulphur, sulphide, sulphur in amino acids and sulphate are the most common forms. Organic sulphur is metabolized during decomposition of protein by many kinds of microbes and can be released as either sulphate or hydrogen sulphide depending on the aeration conditions. Volatile organic sulphur compounds such as dimethyl sulphide are also produced during anaerobic decay. Some of the sulphur is used by microorganisms and this is immobilized, though sulphur is very rarely limiting during decomposition. The hydrogen sulphide produced by anaerobic decay can precipitate metal sulphides, which is the reason for the black colour of many anaerobic muds. Sulphides are oxidized by chemosynthetic bacteria if aerobic conditions occur, or by photosynthetic bacteria under continued anaerobiosis. Many heterotrophic bacteria and fungi can oxidize hydrogen sulphide, but the most important organisms in soil and water are the chemosynthetic Thiobacillus species, which can oxidize elemental sulphur, sulphides, thiosulphate or tetrathionate to sulphate (Ferguson Wood, 1965; Campbell, 1983).

The chief microorganisms present in the sulphur cycle are the anaerobic sulphate-reducing bacteria, the anaerobic purple and green sulphur bacteria

which require light for their oxidations, and *Thiobacillus*, which is aerobic. As the latter two microbial populations are not present in anaerobic digesters, the sulphate-reducing bacteria determine the fate of the sulphur compounds. Recent reviews provide more detailed information on the physiology and taxonomy of these bacteria (Postgate, 1979; Hansen, 1988).

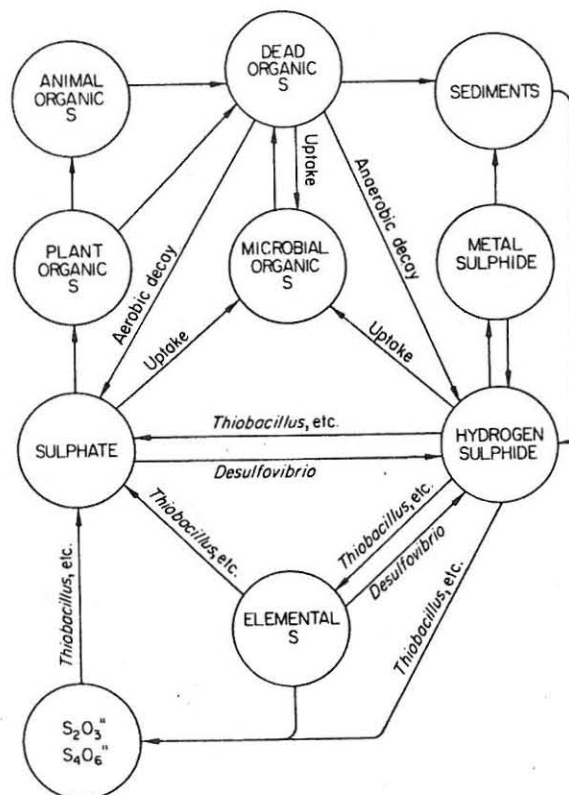
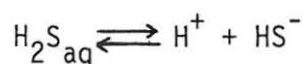


FIGURE 29 - The sulphur cycle (Campbell, 1983)

The main products of the metabolism of the sulphate-reducing bacteria are sulphides. These sulphides can be present as a soluble or insoluble form, depending upon the cations with which they are associated. Several of the common metal sulphides such as those of iron, copper, zinc and nickel are insoluble, and consequently heavy metal cations quantitatively precipitate and remove the sulphides from solution. The remaining soluble sulphide forms a weak acid which ionizes in aqueous solution, the extent depending upon the pH (Figure 30). Thus it is possible to have H_2S , HS^- and S^{2-} in solution. At the neutral pH required for anaerobic treatment, only the first dissociation constant of hydrogen sulphide is of importance i.e.



Because of the limited solubility of H_2S , a certain portion of that formed will escape from the liquid (Lawrence *et al*, 1966).

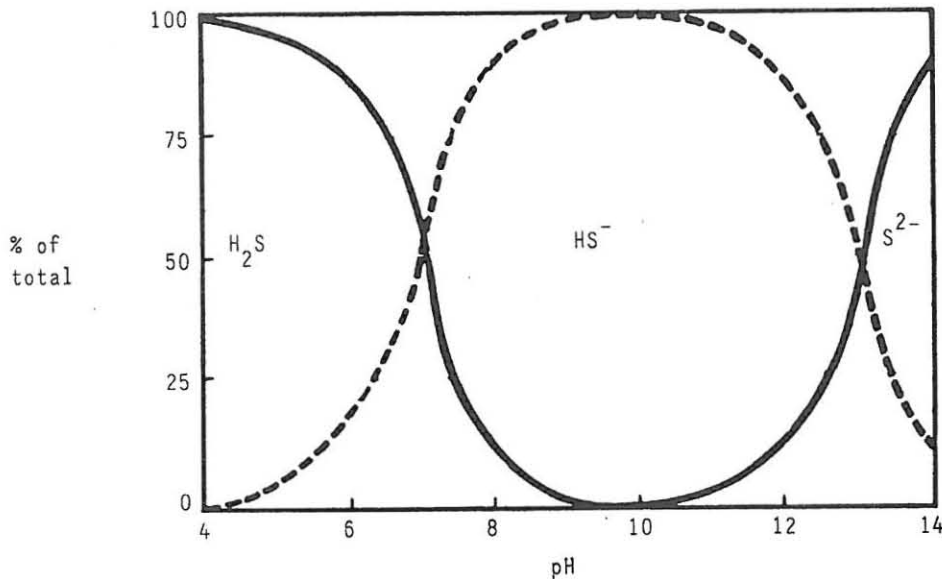


FIGURE 30 - The effect of pH on the sulphide equilibrium in water (Aloy *et al*, 1976)

Sulphides can cause severe corrosion in sewers when evolved H_2S redissolves in the condensation on sewer walls and is oxidized by bacteria to sulphuric acid (Bailey, 1978). H_2S is also extremely toxic. 10 ppm is sufficient to cause fish mortality while 50 ppm has proved fatal for humans (Bailey, 1978). As high concentrations of sulphides will be formed during the anaerobic treatment of tannery wastewaters, the effect of these compounds on the process required investigation.

Methanogenic bacteria require reduced sulphur e.g. sulphide or cysteine for their growth, and methane production is highly dependent on the availability of this sulphur (Jarrell and Kalmokoff, 1988).

Pure culture studies have shown that 5,5 mg/l S (Patel *et al*, 1978), 27,2 mg/l S (Khan and Trottier, 1978), 31,0 mg/l S (Mountfort and Asher, 1979) and 7,9 mg/l S (Rönnow and Gunnarsson, 1981) are the optimum concentrations of sulphur required for the growth of methanogenic bacteria. In all cases poor growth was obtained if sulphur was omitted from the culture medium.

However, as with many other nutrients, sulphates and sulphides are also known to be toxic to methanogens at high concentrations. Shock load experiments have shown that 54 mg/l free H_2S was sufficient to cause a 45 % decrease in biogas production while 155 mg/l free H_2S resulted in a 90 % decrease in biogas production (Kroiss and Wabnegg, 1983). Another study has, however, noted that only a concentration greater than 500 mg/l sulphide could cause inhibition of gas production (Maeda *et al*, 1984). Sulphide toxicity has also been shown to be pH-dependent. At a pH of 6,4, 450 mg/l H_2S was reported to cause total inhibition of biogas formation. However, if the pH was increased to 8,0, 1000 mg/l H_2S could be tolerated without any affects on methane production (Hilton and Oleszkiewicz, 1988).

Adaptation experiments have reported higher toxicity thresholds for sulphates and sulphides. It was found that for an influent concentration of 2400 mg/l SO_4^{2-} , only an 11,1 % decrease in digester biogas volume resulted relative to a control digester. However, if the concentration was increased to 3600 mg/l SO_4^{2-} , digester failure occurred (Lawrence *et al*, 1966). A further acclimatization study (Parkin *et al*, 1983) reported that a concentration of up to 400 mg/l sulphide did not significantly decrease process performance. If the sulphide concentration was increased to 600 mg/l, digester performance became erratic but no inhibition was noted. At 800 mg/l, however, there was a 30 % decrease in gas production, while a concentration of 1000 mg/l resulted in a marked decrease in process performance efficiency.

In contrast to this, industrial wastewaters containing 1600 mg/l SO_4^{2-} (Maree and Hill, 1989), 2480 mg/l SO_4^{2-} (Maree *et al*, 1987) and 3900 mg/l SO_4^{2-} (Brune *et al*, 1982) have been successfully treated by means of anaerobic digestion without any toxicity noted as a result of the high sulphate and sulphide concentrations. There is thus a great deal of conflicting evidence regarding the toxicity of sulphates and sulphides to the anaerobic digestion process. This is particularly so if one compares the results obtained from shock load and adaptation experiments. The aim of this study was therefore to investigate whether an anaerobic bacterial population could be acclimatized to sulphate concentrations higher than those typically found in tannery wastewaters i.e. concentrations in excess of 4000 mg/l SO_4^{2-} .

5.3.2 Materials and Methods

The materials and methods used were described in detail in Chapter 4.

5.3.3 Results and Discussion

The addition of increasing concentrations of sulphates to the digester (Figure 31) appeared to have no detrimental effect on digester performance. The volume of biogas produced (Figure 32) and its composition (Figure 33) remained constant relative to the control digester, and although the concentration of volatile fatty acids in the digester showed a substantial increase during the first 35 days of operation (Table 20), the concentration decreased thereafter to remain at less than 100 mg/l as acetic acid for the remainder of the experiment. The percentage COD and PV removed (Table 21) remained constant over the experimental period.

The soluble sulphides formed as a result of sulphate reduction increased as the sulphate concentration in the feed was increased (Table 20), while the sulphides present as H_2S in the biogas showed a similar trend (Table 21). The presence of H_2S in the biogas can cause problems if the biogas is to be used as an energy source. In order to reduce the corrosive effects of the H_2S , a scrubbing column would be essential (Aloy, personal communication).

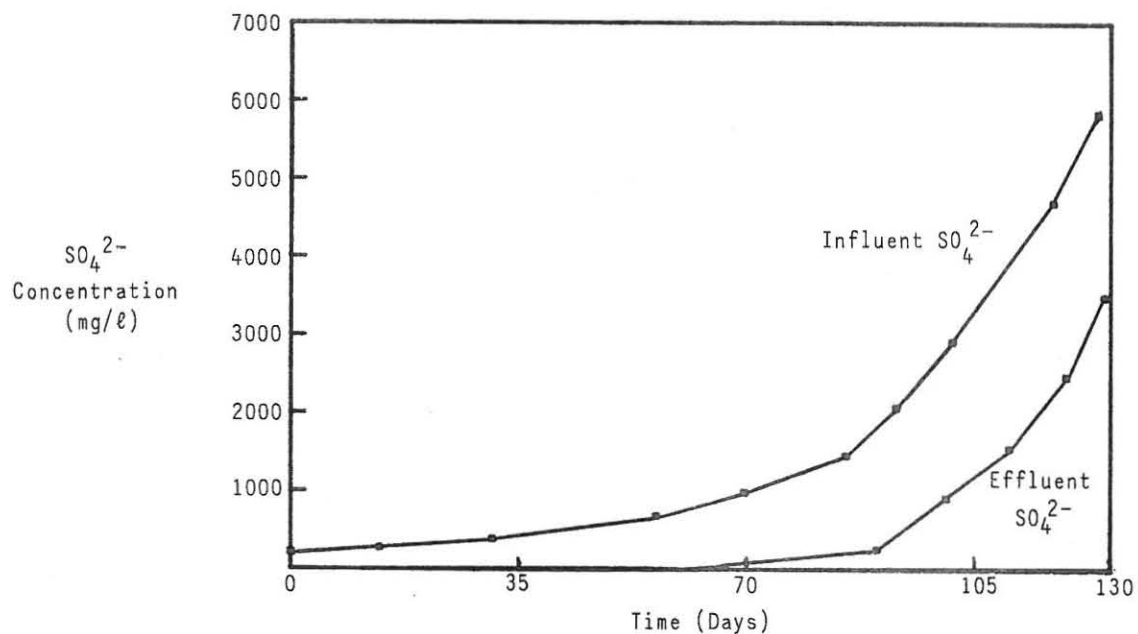


FIGURE 31 - Influent and effluent SO_4^{2-} versus time for the anaerobic digester receiving additional SO_4^{2-} (Note: The control digester had a mean influent and effluent SO_4^{2-} concentration of 200 and 84 mg/l respectively)

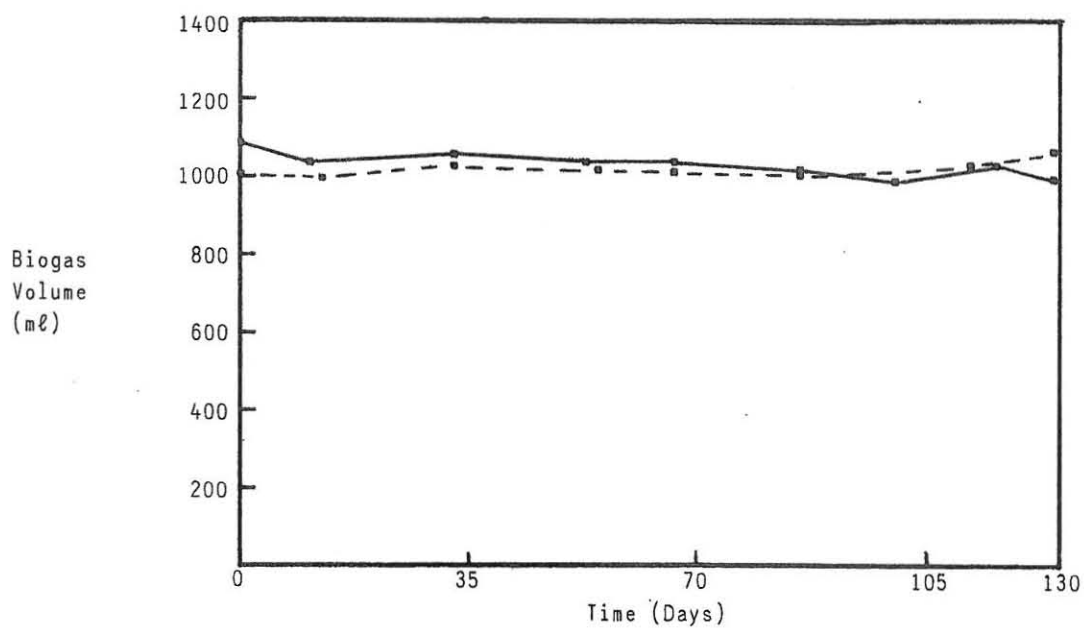


FIGURE 32 - Biogas volume versus time for the anaerobic digester receiving additional SO_4^{2-} (— SO_4^{2-} digester; ---- control digester)

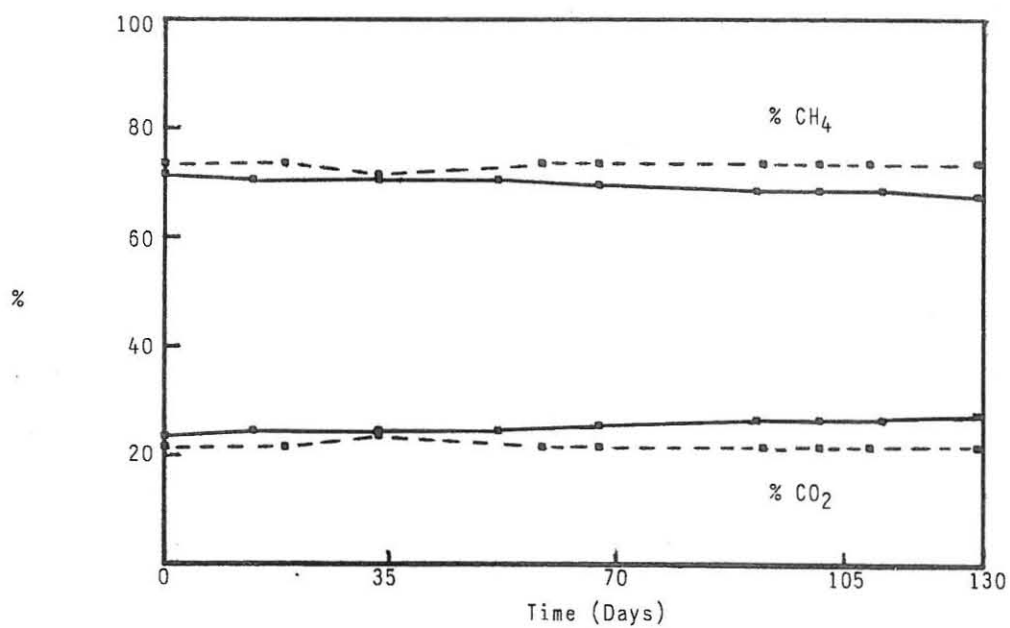


FIGURE 33 - Percentage biogas composition versus time for the anaerobic digester receiving additional SO_4^{2-} (— SO_4^{2-} digester; ---- control digester)

TABLE 20
CHARACTERISTICS OF THE DIGESTER EFFLUENT AT VARIOUS
STAGES DURING THE SULPHATE EXPERIMENT

Parameter	Control Digester					SO ₄ ²⁻ Digester				
	Time (Days)					Time (Days)				
	1	35	70	105	130	1	35	70	105	130
pH	7,58	7,57	7,46	7,50	7,56	7,27	7,66	7,47	7,61	7,81
Total Alkalinity (mg/l CaCO ₃)	1224	1326	1275	1224	1326	1224	1275	1836	2142	2499
Bicarbonate Alkalinity (mg/l CaCO ₃)	1196	1304	1251	1203	1279	1171	998	1796	2116	2466
COD (mg/l)	474	468	375	330	348	654	360	390	429	387
PV (mg/l)	29	48	57	58	45	42	44	38	34	57
VFA (mg/l)	39,7	30,8	34,4	30,3	66,4	74,6	391,1	56,5	36,2	46,5
Sulphate (mg/l)	72	87	95	91	84	97	156	199	1089	3663
Sulphide (mg/l)	12	24	48	24	32	12	24	144	408	432

The pH and alkalinity of the digester receiving additional SO₄²⁻ both increased over the experimental period. The total alkalinity increased from 1224 mg/l CaCO₃ at the start of the experiment to 2499 mg/l CaCO₃ on completion of the experiment. The reason for this is that under anaerobic conditions, sulphates, which are strong acids, are reduced to sulphides which act as weak acids and this results in an increased alkalinity (Lawrence *et al.*, 1966). A stratigraphic analysis of the digester contents at the start and on completion of the experiment (Tables 22 and 23) showed that despite an increase in the concentration of sulphates and sulphides in the digester receiving additional sulphates, the pH and the concentration of volatile fatty acids at each sampling port remained constant relative to the control digester. Consequently even at concentrations as high as 6000 mg/l SO₄²⁻, stable conditions were maintained in the digester.

TABLE 21

THE EFFECT OF SULPHATE ON THE OPERATING EFFICIENCY OF
THE DIGESTER AT VARIOUS STAGES DURING THE EXPERIMENT

Parameter	Control Digester					SO ₄ ²⁻ Digester				
	Time (Days)					Time (Days)				
	1	35	70	105	130	1	35	70	105	130
Gas Volume (ℓ/d)	1,040	1,060	1,045	1,020	1,095	1,120	1,090	1,070	1,020	1,025
Gas Production (m ³ /m.d)	0,116	0,118	0,116	0,113	0,122	0,124	0,121	0,119	0,113	0,114
% COD Removed	84,2	84,4	87,5	89,0	88,4	78,2	88,0	87,0	85,7	87,1
% PV Removed	95,5	92,6	91,3	91,1	93,1	93,6	93,2	94,1	94,8	91,3
Gas Yield (m ³ /kg COD Removed)	0,412	0,419	0,398	0,382	0,413	0,477	0,413	0,410	0,397	0,394
Methane Content of Biogas (%)	75,6	74,3	77,9	76,3	76,1	73,7	72,8	71,5	71,1	70,4
Methane Yield (m ³ CH ₄ /kg COD Removed)	0,311	0,311	0,310	0,291	0,314	0,352	0,301	0,293	0,282	0,277
H ₂ S (mg/ℓ)	< 1	2,0	4,9	2,3	2,5	1,7	1,7	14,6	31,4	22,3

Under anaerobic conditions, an important biological relationship exists between sulphate-reducing and methane-producing bacteria, and in recent years there have been many studies on the terminal anaerobic processes occurring in ecosystems in which sulphate is present. Although numerous researchers have suggested that methanogenesis is inhibited by sulphate-reduction because of competition for precursors such as H₂, acetate or both (Abram and Nedwell, 1978; Kristjansson *et al*, 1982; Schönheit *et al*, 1982; Lovley *et al*, 1982; Robinson and Tiedje, 1984), several investigators have questioned these hypotheses (Szendrey, 1983; Isa *et al*, 1986). This investigation showed that methanogenesis can occur at sulphate concentrations as high as 6000 mg/ℓ SO₄²⁻, and is therefore in agreement with the findings of previous research (Szendrey, 1983; Isa *et al*, 1986). However, it should be noted that for most of the duration of the experiment, the concentration of glucose was greater

than the concentration of sulphate, and consequently competition for the substrate would have been reduced. When the concentration of sulphate was equal to or greater than the substrate concentration, however, a reduction in the methane yield relative to the control digester was evident (Table 21). This could possibly be due to competition for methane precursors by the sulphate-reducing bacteria.

TABLE 22
STRATIGRAPHIC ANALYSIS OF THE DIGESTER CONTENTS AT
THE START OF THE SULPHATE EXPERIMENT

Height of Sampling Port Above Inlet (cm)	Control Digester				SO ₄ ²⁻ Digester			
	pH	SO ₄ ²⁻ (mg/l)	HS ⁻ (mg/l)	VFA (mg/l)	pH	SO ₄ ²⁻ (mg/l)	HS ⁻ (mg/l)	VFA (mg/l)
59	7,58	72	12	39,7	7,27	97	12	74,6
42	7,41	59	32	18,7	7,66	102	16	23,9
29	7,20	53	24	26,9	7,66	126	20	19,8
16	7,33	47	ND	48,9	7,45	111	ND	55,3

ND: not determined as this was in the sludge bed.

TABLE 23
STRATIGRAPHIC ANALYSIS OF THE DIGESTER CONTENTS ON
COMPLETION OF THE SULPHATE EXPERIMENT

Height of Sampling Port Above Inlet (cm)	Control Digester				SO ₄ ²⁻ Digester			
	pH	SO ₄ ²⁻ (mg/l)	HS ⁻ (mg/l)	VFA (mg/l)	pH	SO ₄ ²⁻ (mg/l)	HS ⁻ (mg/l)	VFA (mg/l)
59	7,56	84	32	66,4	7,81	3663	432	46,5
42	7,16	71	64	60,6	7,63	3855	448	48,2
29	7,05	82	80	57,3	7,74	3870	464	47,1
16	7,04	67	ND	75,9	7,74	4233	ND	48,0

ND: not determined as this was in the sludge bed.

The sulphur balance (Table 24) shows that in the digester which received additional SO_4^{2-} , 83,1 % of the sulphur was accounted for on completion of the experiment, while 91,6 % was recovered from the control digester. The reason why a greater proportion of the sulphur is not recovered is that sulphides react with heavy metals under anaerobic conditions to form heavy metal sulphides (Callander and Barford, 1983b). These heavy metal sulphides are highly insoluble, and would therefore have precipitated and accumulated in the sludge bed of the digesters.

TABLE 24
SULPHUR BALANCE FOR THE DIGESTER ON
COMPLETION OF THE SULPHATE EXPERIMENT

Parameter	Control Digester	SO_4^{2-} Digester
Influent SO_4^{2-} -S (mg/l)	67	2000
Influent HS^- -S (mg/l)	0	0
Total Influent S	67	2000
Effluent SO_4^{2-} -S (mg/l)	28	1221
Effluent HS^- -S (mg/l)	31	419
Effluent H_2S -S (mg/l)	2,4	21
Total Effluent S (mg/l)	61,4	1661
% S Recovered	91,6	83,1

The ability of the methanogenic bacteria to adapt to high concentrations of sulphates suggests that the concentration of sulphate present in tannery wastewaters would be insufficient to cause digester failure. However, successful anaerobic digestion is more likely to be achieved by treating those wastewaters that have a high substrate (COD) to sulphate ratio, as this will result in less competition for nutrients between the microbial species present in the anaerobic digester. The use of alternative unhairing methods (Alexander, 1988), the recovery and reuse of sulphides (Sayers and Langlais, 1977), and the use of alternative deliming agents such as carbon dioxide will, in the future, reduce the concentration of sulphur compounds in tannery

wastewaters. This reduced concentration will result in even less possibility of sulphur compounds causing toxicity and inhibition in anaerobic digesters treating these wastewaters.

The findings of this experiment have been previously published (Jackson-Moss et al, 1987b; Jackson-Moss and Duncan, 1990h).

5.4 The Effect of Sodium Chloride on Anaerobic Digestion

5.4.1 Introduction

Sodium chloride (NaCl) is used extensively in the leather industry for the curing of hides and skins, with the result that the soak liquors contain high concentrations of Na^+ and Cl^- ions. In addition to this, these ions are present in the waste streams from numerous other stages in leather manufacture i.e. the unhairing, deliming and bating, pickling and tanning stages (Hart et al, 1987; Aloy et al, 1976). The final tannery wastewater therefore has a high conductivity and total dissolved inorganic solids (TDIS) content.

The effect of NaCl on methanogenesis has been investigated by numerous researchers. A requirement for sodium for both growth and methane production was first reported in Methanobacterium thermoautotrophicum (Perski et al, 1981). Later it was reported that methane production from several different substrates such as CO_2/H_2 , methanol and acetate by methanogens representing several different orders was also dependent on sodium (Perski et al, 1982). It has since been shown that the stimulation of growth and methane production by sodium is due to the formation of a transmembrane electrochemical gradient (Schönheit and Perski, 1983; Müller et al, 1988a; Müller et al, 1988b). It has also been found that one sodium molecule is tightly bound to each molecule of glycolipid present in M. thermoautotrophicum, and this has led to speculation that these glycolipid-metal complexes may be involved in the active transport of Na^+ or K^+ across the cell membrane in this methanogen (Kramer et al, 1988).

Despite the requirement for sodium by methanogens, high concentrations have also been reported to inhibit methanogenesis. Shock loads of Na^+ at concentrations of 3500 - 5500 mg/l have been shown to be moderately inhibitory to methanogenesis (McCarty and McKinney, 1961), while it has been noted that

shock loads of NaCl in excess of 5850 mg/l slowed down the conversion of acetic acid to methane in an enriched methanogenic culture (Van den Berg, et al, 1976). Further experiments investigating Na⁺ toxicity have reported that 10 % and 50 % decreases in the specific activity of the methanogens were caused by shock loads of 4000 mg/l Na⁺ and 8500 mg/l Na⁺ respectively (Rinzema et al, 1988). Na⁺ toxicity also appears to vary from species to species. NaCl concentrations in excess of 890 and 1030 mg/l have been shown to inhibit the growth of pure cultures of Methanobacterium thermoautotrophicum and M. bryanti (formerly M. MOH), while only a NaCl concentration in excess of 5690 mg/l could inhibit the growth of Methanospirillum hungatii GP1 (Patel and Roth, 1977).

From the foregoing discussion, it is evident that the effect of NaCl on methanogenesis is well documented. However, the majority of these studies have involved shock loads of NaCl to the methanogenic microbes. Although shock load experiments are important, studies investigating the ability of methanogens to adapt to toxic chemicals are also necessary. The purpose of this investigation was therefore to determine the effect of NaCl adaptation on anaerobic digestion.

5.4.2 Materials and Methods

The materials and methods used were described in detail in Chapter 4.

5.4.3 Results and Discussion

The incremental addition of NaCl to the digester up to a concentration of 17000 mg/l (Figure 34) had no notable inhibitory effects on the anaerobic microbes. The volume of biogas produced remained constant over the experimental period (Figure 35), while the methane content showed an increase relative to the control digester (Figure 36). The pH (Table 25) and percentage organic matter removed (Table 26) were also maintained at a stable level, indicating that methanogens have the ability to adapt to high concentrations of NaCl.

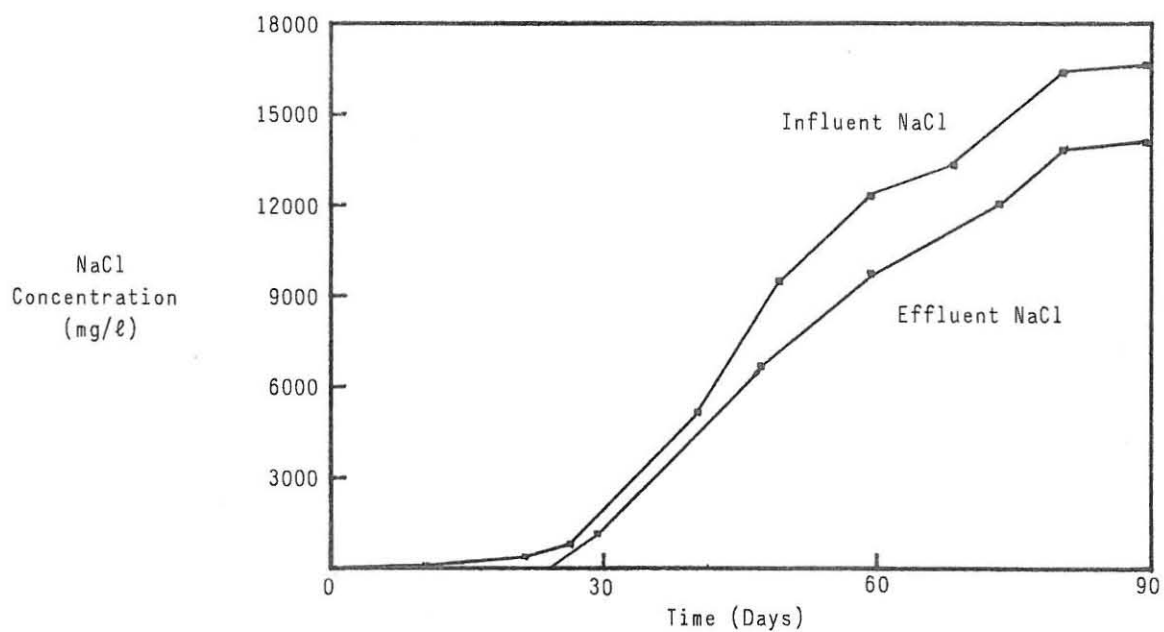


FIGURE 34 - Influent and effluent NaCl versus time for the anaerobic digester receiving additional NaCl (Note: The control digester had an influent and effluent NaCl concentration of 100 mg/l)

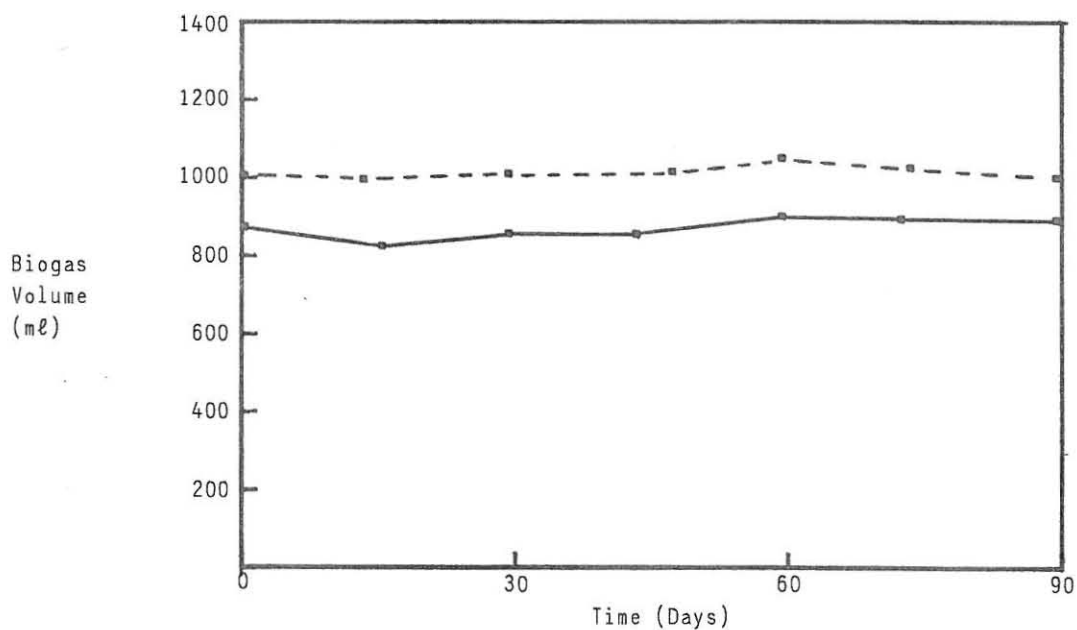


FIGURE 35 - Biogas volume versus time for the anaerobic digester receiving additional NaCl (—— NaCl digester; ---- control digester)

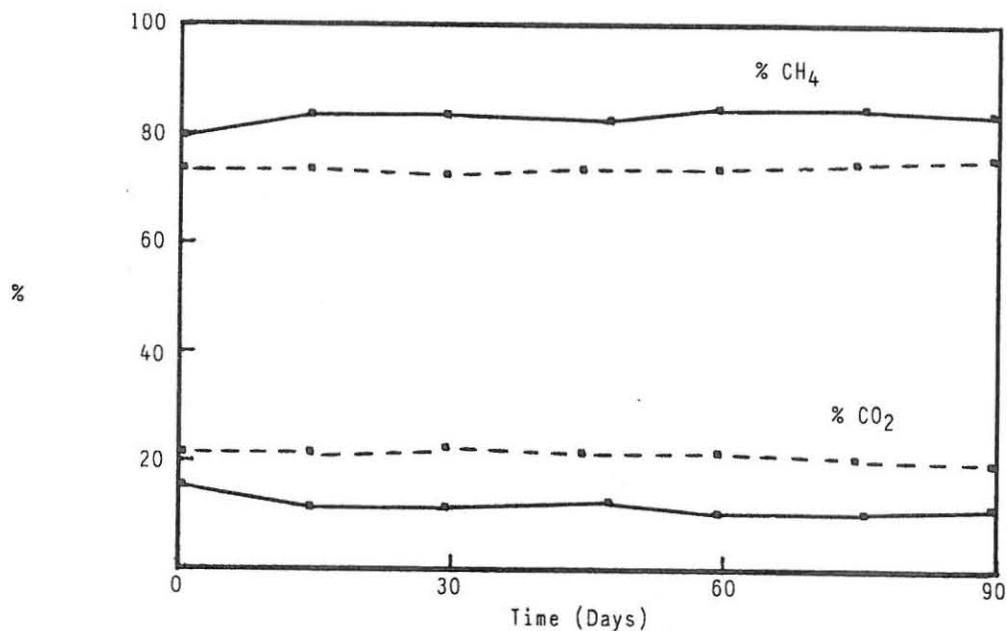


FIGURE 36 - Percentage biogas composition versus time for the anaerobic digester receiving additional NaCl (— NaCl digester; ---- control digester)

The findings of this experiment are in agreement with those of previous studies where it has been shown that it is possible to acclimatize methanogenic populations to concentrations of 22, 65 and 70 g/l of NaCl (Henson *et al*, 1986; De Baere *et al*, 1984; Sowers and Gunsalus, 1988). The isolation of methanogenic bacteria from halophilic environments provides further evidence that methanogens have the ability to survive in high NaCl concentrations (Paterek and Smith, 1985; Zhilina and Kevbrin, 1985; King, 1988; Yu and Kawamura, 1987). High concentrations of NaCl can cause bacterial cells to dehydrate because of high osmotic pressure. Methanogens, however, do not appear to be affected by high salinity. The requirement by these bacteria for sodium has probably enabled them to adapt to high salt concentrations, and to grow in those environments where sodium is abundant.

TABLE 25
CHARACTERISTICS OF THE DIGESTER EFFLUENT AT VARIOUS STAGES
DURING THE SODIUM CHLORIDE EXPERIMENT

Parameter	Control Digester				NaCl Digester			
	Time (Days)				Time (Days)			
	1	30	60	90	1	30	60	90
pH	7,58	7,21	7,30	7,44	7,59	7,20	7,55	7,42
Total Alkalinity (mg/l CaCO_3)	1224	1326	1530	1326	1482	1710	1596	1881
Bicarbonate Alkalinity (mg/l CaCO_3)	1196	1312	1509	1286	1464	1670	1561	1800
COD (mg/l)	474	510	498	513	456	363	474	531
PV (mg/l)	29	31	43	40	54	63	64	55
VFA (mg/l)	39,7	19,6	30,3	56,5	25,4	56,4	48,8	114,8
Na^+ (mg/l)	44	48	46	42	62	630	4000	5700
Cl^- (mg/l)	240	230	240	245	235	850	4950	11100

Sodium is known to enhance the formation of CH_4 from different substrates (Perski *et al*, 1982; Peinemann *et al*, 1988). This could possibly explain the increase in the percentage of CH_4 in the biogas from the digester which received additional NaCl (Table 26). However, despite this increase in the percentage CH_4 in the biogas, there was no noticeable difference in the CH_4 yield between the control digester and the digester which received additional NaCl (Table 26). This is due to the marginally lower gas yield obtained from the digester which received additional NaCl.

A comparison of Tables 27 and 28 illustrates that, unlike heavy metals which tend to precipitate and accumulate in the sludge beds of UASB digesters (Jackson-Moss *et al*, 1988c), there was no stratigraphic accumulation of Na^+ or Cl^- ions, and on completion of the experiment these ions were fairly evenly distributed throughout the contents of the digester (Table 28). This is due to the high solubility of these ions at neutral pH.

TABLE 26

THE EFFECT OF SODIUM CHLORIDE ON THE OPERATING EFFICIENCY
OF THE DIGESTER AT VARIOUS STAGES DURING THE EXPERIMENT

Parameter	Control Digester				NaCl Digester			
	Time (Days)				Time (Days)			
	1	30	60	90	1	30	60	90
Gas Volume (ℓ/d)	1,040	1,044	1,085	1,035	0,909	0,891	0,936	0,927
Gas Production (m ³ /m ³ .d)	0,116	0,116	0,121	0,115	0,101	0,099	0,104	0,103
% COD Removed	84,2	83,0	83,4	82,9	84,8	87,9	84,2	82,3
% PV Removed	95,5	95,2	93,4	93,8	91,7	90,3	90,2	91,5
Gas Yield (m ³ /kg COD Removed)	0,412	0,419	0,434	0,416	0,357	0,338	0,371	0,375
Methane Content of Biogas (%)	75,6	75,3	76,0	77,5	82,2	85,8	86,6	86,4
Methane Yield (m ³ CH ₄ /kg COD Removed)	0,311	0,316	0,330	0,322	0,293	0,290	0,321	0,324

TABLE 27

STRATIGRAPHIC ANALYSIS OF THE DIGESTER CONTENTS AT
THE START OF THE SODIUM CHLORIDE EXPERIMENT

Height of Sampling Port Above Inlet (cm)	Control Digester				NaCl Digester			
	pH	Na ⁺ (mg/ℓ)	Cl ⁻ (mg/ℓ)	VFA (mg/ℓ)	pH	Na ⁺ (mg/ℓ)	Cl ⁻ (mg/ℓ)	VFA (mg/ℓ)
59	7,58	44	240	39,7	7,59	62	235	25,4
42	7,41	39	230	18,7	7,12	41	225	44,7
29	7,20	51	245	26,9	7,10	41	235	30,6
16	7,33	48	245	48,9	7,11	40	230	40,8

TABLE 28
STRATIGRAPHIC ANALYSIS OF THE DIGESTER CONTENTS ON
COMPLETION OF THE SODIUM CHLORIDE EXPERIMENT

Height of Sampling Port Above Inlet (cm)	Control Digester				NaCl Digester			
	pH	Na ⁺ (mg/l)	Cl ⁻ (mg/l)	VFA (mg/l)	pH	Na ⁺ (mg/l)	Cl ⁻ (mg/l)	VFA (mg/l)
59	7,44	42	245	56,5	7,42	5700	11100	114,8
42	7,32	43	235	30,3	6,86	5600	11275	50,4
29	7,10	41	240	48,2	6,83	6500	11500	45,3
16	7,05	41	245	58,8	6,82	6600	11900	40,6

On the basis of this investigation, anaerobic digestion can be used for the successful treatment of wastewaters containing high NaCl concentrations. The Na⁺ and Cl⁻ ions will, however, not be removed during the digestion process, and further specialized treatment such as reverse osmosis would be required to produce an effluent free of these ions.

Presently, considerable attention is being given to reducing the concentration of Na⁺ and Cl⁻ ions in tannery wastewaters. The processing of "green" hides (Vulliermet *et al*, 1988) and the use of antiseptics and biocides (Barrett, 1983) are replacing traditional salt curing, while other changes in leather manufacture such as enzymatic unhairing (Alexander, 1988), have an indirect effect on reducing the Na⁺ concentration in tannery discharges. This reduced concentration will result in even less possibility of the NaCl present in tannery wastewaters being inhibitory to the anaerobic digestion process.

The findings of this experiment have been previously published (Jackson-Moss *et al*, 1988a; Jackson-Moss *et al*, 1989c).

5.5 The Effect of Calcium on Anaerobic Digestion

5.5.1 Introduction

The unhairing stage of leather manufacture makes use of large quantities of calcium hydroxide or lime to bring about a swelling of the hides and skins

(Section 2.2.1.2). Lime is also occasionally used as a flocculent in the wastewater treatment plants at tanneries. Consequently, tannery effluents and sludges contain high concentrations of the calcium cation.

Calcium (Ca^{2+}) is known to be essential for the growth of some strains of methanogenic bacteria (Murray and Zinder, 1985). It is also believed to play an important role in the formation of microbial pellets in anaerobic digesters (Sam-Soon *et al.*, 1987; Schröder and De Haast, 1986; Rozzi *et al.*, 1988). However, very little is known regarding the toxicity of Ca^{2+} to the anaerobic digestion process. One of the few toxicity studies that have been carried out showed that Ca^{2+} is moderately inhibitory at a concentration of 2500 - 4000 mg/ℓ, but is strongly inhibitory at a concentration of 8000 mg/ℓ (Kugelman and McCarty, 1965).

In view of the limited information available regarding the toxic effects of Ca^{2+} on anaerobic digestion, this study was carried out in order to investigate whether an anaerobic bacterial population could be acclimatized to high concentrations of Ca^{2+} .

5.5.2 Materials and Methods

The materials and methods used were described in detail in Chapter 4.

5.5.3 Results and Discussion

The addition of Ca^{2+} ions to the digester up to a concentration of 7000 mg/ℓ (Figure 37) had no inhibitory effect on the methanogenic microbes. The volume of biogas produced remained constant over the experimental period (Figure 38), with the methane content varying between 75 and 80 % (Figure 39). The pH, VFA and percentage COD and PV removed (Tables 29 and 30) were also maintained at a stable level, indicating that methanogens have the ability to adapt to high concentrations of Ca^{2+} and that after acclimatization, the metabolic activity of the microbes was the same as at low concentrations of Ca^{2+} ions (Table 30).

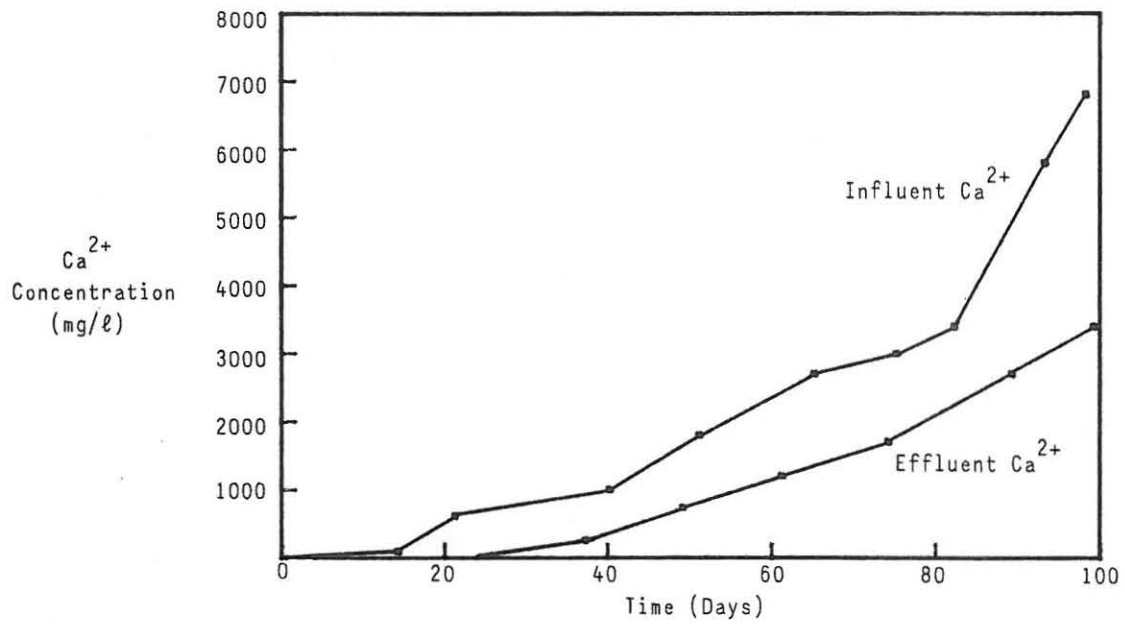


FIGURE 37 - Influent and effluent Ca^{2+} versus time for the anaerobic digester receiving additional Ca^{2+} (Note: The control digester had an influent and effluent Ca^{2+} concentration of 74 and 70 mg/l respectively)

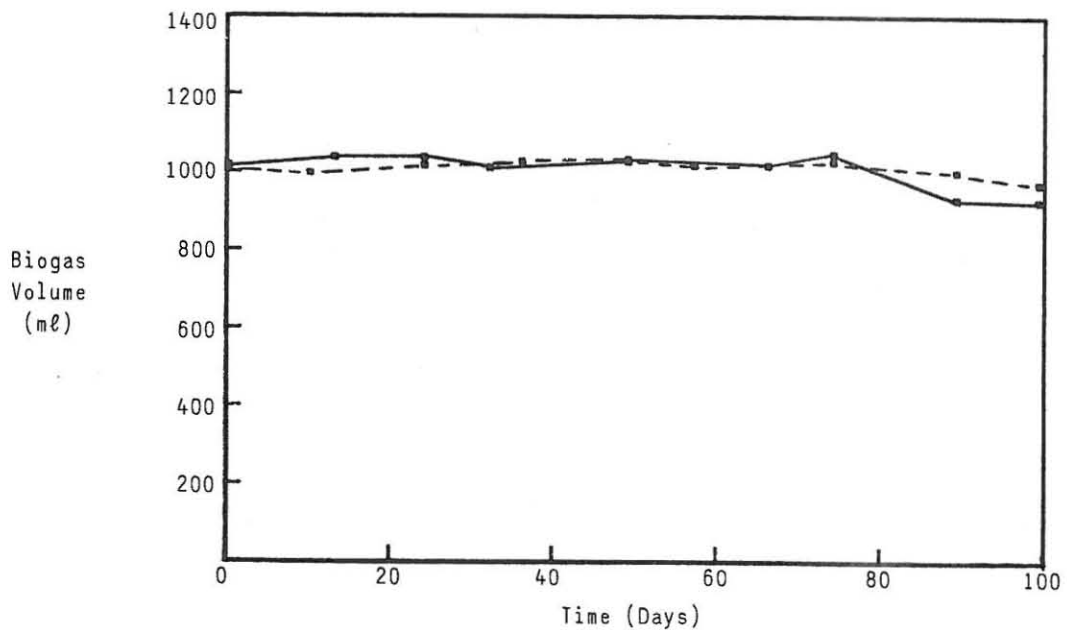


FIGURE 38 - Biogas volume versus time for the anaerobic digester receiving additional Ca^{2+} (— Ca^{2+} digester; ---- control digester)

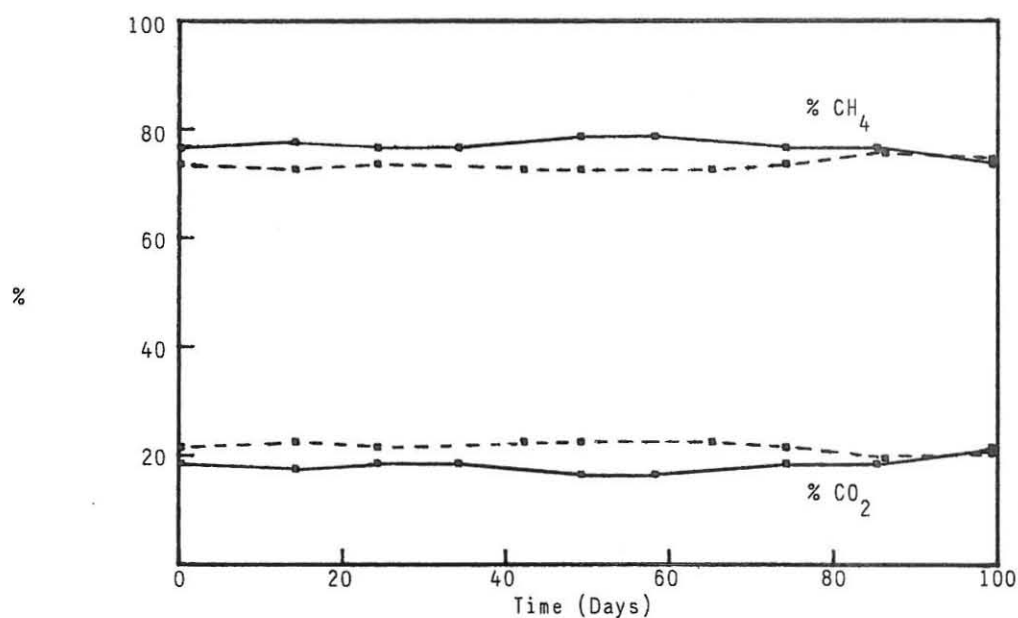


FIGURE 39 - Percentage biogas composition versus time for the anaerobic digester receiving additional Ca^{2+} (— Ca^{2+} digester; ---- control digester)

TABLE 29

CHARACTERISTICS OF THE DIGESTER EFFLUENT AT VARIOUS STAGES DURING THE CALCIUM EXPERIMENT

Parameter	Control Digester					Ca^{2+} Digester				
	Time (Days)					Time (Days)				
	1	25	50	75	100	1	25	50	75	100
pH	7,58	7,57	7,16	7,44	7,44	7,23	7,44	7,43	7,20	7,10
Total Alkalinity (mg/l CaCO_3)	1224	1479	1428	1377	1224	1232	1219	1035	1127	920
Bicarbonate Alkalinity (mg/l CaCO_3)	1196	1447	1406	1349	1177	1177	1157	1000	1077	877
COD (mg/l)	474	441	408	309	441	456	354	288	315	369
PV (mg/l)	29	29	30	38	40	71	68	53	60	63
VFA (mg/l)	39,7	45,9	30,8	39,5	66,3	77,7	87,0	49,2	70,8	61,4
Ca^{2+} (mg/l)	64	72	68	70	74	44	154	934	1894	3594

TABLE 30

THE EFFECT OF CALCIUM ON THE OPERATING EFFICIENCY OF
THE DIGESTER AT VARIOUS STAGES DURING THE EXPERIMENT

Parameter	Control Digester					Ca ²⁺ Digester				
	Time (Days)					Time (Days)				
	1	25	50	75	100	1	25	50	75	100
Gas Volume (l/d)	1,040	1,050	1,060	1,060	1,005	1,050	1,070	1,065	1,080	0,960
Gas Production (m ³ /m.d)	0,116	0,117	0,118	0,118	0,112	0,117	0,119	0,118	0,120	0,107
% COD Removed	84,2	85,3	86,4	89,7	85,3	84,8	88,2	90,4	89,5	87,7
% PV Removed	95,5	95,5	95,4	94,2	93,8	89,1	89,6	91,8	90,7	90,3
Gas Yield (m ³ /kg COD Removed)	0,412	0,410	0,409	0,394	0,393	0,413	0,404	0,393	0,402	0,365
Methane Content of Biogas (%)	75,6	75,9	74,5	76,1	77,4	78,5	78,9	80,7	79,2	76,2
Methane Yield (m ³ CH ₄ /kg COD Removed)	0,311	0,311	0,305	0,300	0,304	0,324	0,319	0,317	0,318	0,278

Unlike heavy metals which tend to precipitate and accumulate in anaerobic digesters (Jackson-Moss *et al*, 1988c), a large proportion of the Ca²⁺ ions passed through the digester and were present in the effluent. This phenomenon has been noted previously (Hunter *et al*, 1987). However, a comparison of Tables 31 and 32 illustrates that there was some accumulation of Ca²⁺ ions at the base of the digester as a result of the precipitation of Ca²⁺ ions as Ca(OH)₂. The solubility product of Ca(OH)₂ at pH 7,0 is 4×10^{-6} , indicating that a proportion of the Ca²⁺ ions will precipitate as the hydroxide at neutral pH (Callander and Barford, 1983b). Calcium can also be precipitated as calcium carbonate ($K_{sp} = 6,9 \times 10^{-9}$) (Maree *et al*, 1989).

TABLE 31

STRATIGRAPHIC ANALYSIS OF THE DIGESTER CONTENTS AT
THE START OF THE CALCIUM EXPERIMENT

Height of Sampling Port Above Inlet (cm)	Control Digester			Ca ²⁺ Digester		
	pH	Ca ²⁺ (mg/l)	VFA (mg/l)	pH	Ca ²⁺ (mg/l)	VFA (mg/l)
59	7,58	64	39,7	7,23	44	77,7
42	7,41	82	18,7	7,25	77	66,4
29	7,20	79	26,9	7,15	112	59,8
16	7,33	97	48,9	6,74	104	76,3

TABLE 32

STRATIGRAPHIC ANALYSIS OF THE DIGESTER CONTENTS ON
COMPLETION OF THE CALCIUM EXPERIMENT

Height of Sampling Port Above Inlet (cm)	Control Digester			Ca ²⁺ Digester		
	pH	Ca ²⁺ (mg/l)	VFA (mg/l)	pH	Ca ²⁺ (mg/l)	VFA (mg/l)
59	7,44	74	66,3	7,10	3594	61,4
42	7,39	76	58,7	6,54	3394	31,0
29	7,19	111	51,7	6,55	3694	11,0
16	7,10	104	48,8	6,57	8994	28,5

From the results of this investigation, it can be concluded that anaerobic digestion can be used for the successful treatment of wastewaters containing high concentrations of calcium ions, provided the microorganisms have sufficient time to adapt to the presence of these ions.

The findings of this experiment have been previously published (Jackson-Moss *et al*, 1988b; Jackson-Moss *et al*, 1989a).

5.6 The Effect of Iron on Anaerobic Digestion

5.6.1 Introduction

Although iron (Fe) is not used in the tanning process, it does arise as a contaminant in the wastewaters from machinery and chemicals. It is also used as a flocculent, namely ferric sulphate, ferrous sulphate, ferric chloride or ferrous chloride, in certain effluent treatment plants (Murray, 1987). This results in high concentrations of iron in the tannery sludges which has the potential to inhibit the anaerobic digestion process.

Iron is essential for the growth of microorganisms as it forms an important component of many of the enzymes involved in the metabolic pathways of the bacteria (Neilands, 1974). Methanogens have a specific growth requirement for iron (Patel *et al*, 1978) and low concentrations, i.e. 2,5 g/l of FeCl_2 , have been shown to markedly increase the conversion of acetic acid to methane (Hoban and Van den Berg, 1979). However, in more than trace amounts, iron is generally considered to be one of the toxic heavy metals. Despite this, very few toxicity studies have been carried out. It has been shown that shock loads of 698 and 1396 mg Fe^{3+} resulted in a decreased biogas production and digester pH, but did not affect the other digestion parameters (Lawrence and McCarty, 1965), while in one of the few adaptation studies carried out (Mosey and Hughes, 1975), concentrations of Fe^{3+} up to 1750 mg/l posed no threat to anaerobic digestion. However, if the concentration was further increased, digester failure occurred.

In view of the limited information available regarding the toxic effects of Fe^{3+} on anaerobic digestion, this study was carried out in order to investigate whether an anaerobic bacterial population could be acclimatized to concentrations of Fe^{3+} greater than those reported to be toxic in previous studies.

5.6.2 Materials and Methods

The materials and methods used were described in detail in Chapter 4.

5.6.3 Results and Discussion

The addition of Fe^{3+} ions to the digester up to a concentration of 5650 mg/l (Figure 40) resulted in a number of different effects on the functioning of the anaerobic digester. The volume of biogas produced was variable. After an initial decrease for the first 10 days, the volume increased for a period of 20 days, whereafter a general decrease was once again noted (Figure 41). In contrast to this the methane content of the biogas increased constantly relative to the control digester (Figure 42) and on completion of the experiment (Day 100), accounted for over 95 % of the biogas produced (Table 33). Despite this overall decrease in the volume of biogas produced, the other digestion parameters such as pH, alkalinity, VFA, percentage COD and PV removed all remained relatively constant over the experimental period (Tables 33 and 34), indicating that the anaerobic digester was able to function normally in the presence of high concentrations of Fe ions. Similar results have been reported in previous experiments investigating the effect of iron on anaerobic digestion (Lawrence and McCarty, 1965).

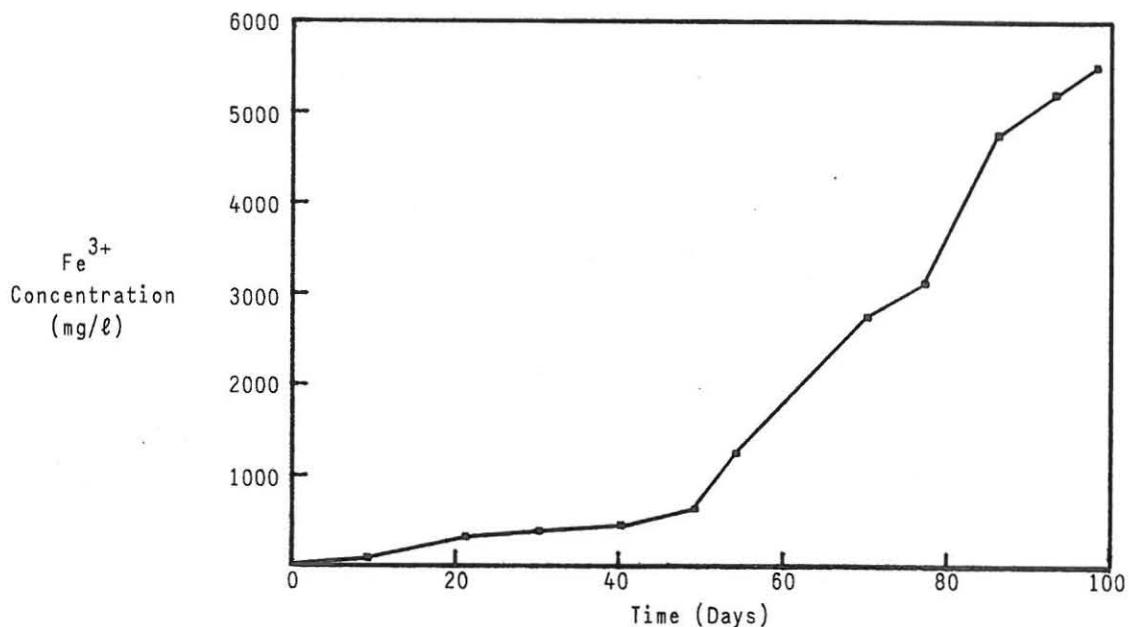


FIGURE 40 - Influent Fe^{3+} versus time for the anaerobic digester receiving additional Fe^{3+} (Note: The effluent Fe concentration was <50 mg/l for the duration of the experiment. The control digester had an influent and effluent Fe concentration of 5 mg/l and 1 mg/l respectively)

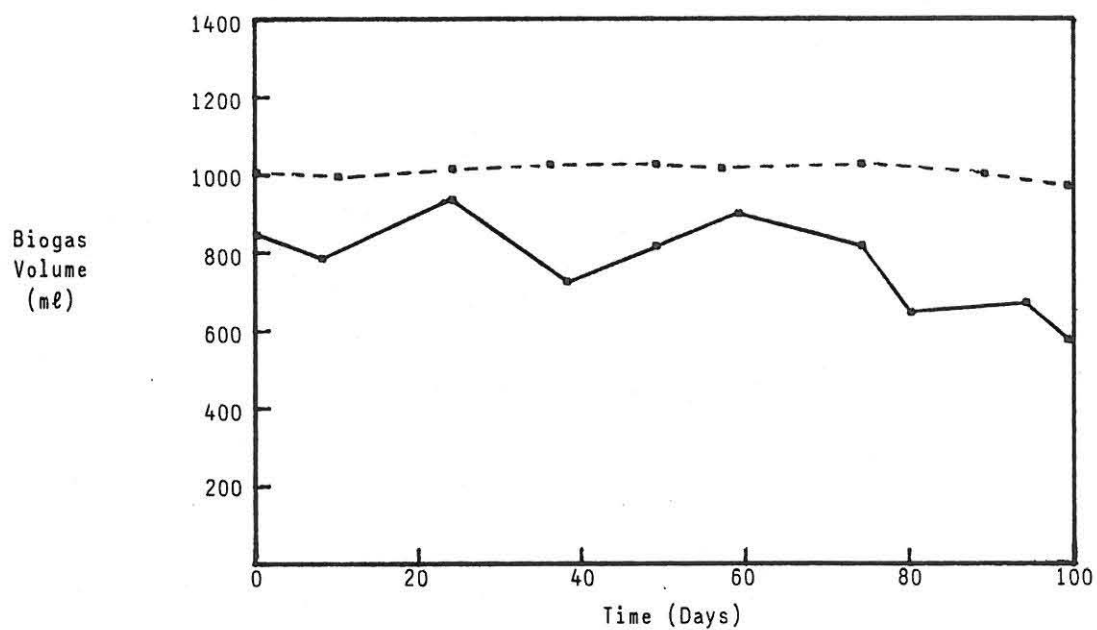


FIGURE 41 - Biogas volume versus time for the anaerobic digester receiving additional Fe^{3+} (— Fe^{3+} digester; ---- control digester)

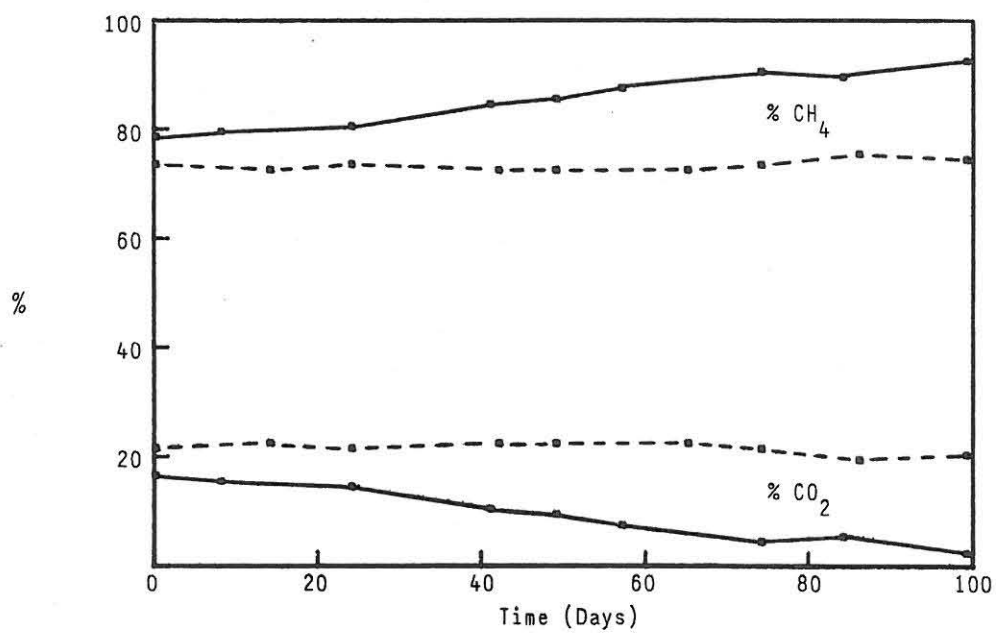


FIGURE 42 - Percentage biogas composition versus time for the anaerobic digester receiving additional Fe^{3+} (— Fe^{3+} digester; ---- control digester)

TABLE 33

THE EFFECT OF IRON ON THE OPERATING EFFICIENCY OF THE DIGESTER
AT VARIOUS STAGES DURING THE EXPERIMENT

Parameter	Control Digester					Fe ³⁺ Digester				
	Time (Days)					Time (Days)				
	1	25	50	75	100	1	25	50	75	100
Gas Volume (ℓ/d)	1,040	1,050	1,060	1,060	1,005	0,880	0,970	0,850	0,850	0,610
Gas Production (m ³ /m ³ .d)	0,116	0,117	0,118	0,118	0,112	0,098	0,108	0,094	0,094	0,068
% COD Removed	84,2	85,3	86,4	89,7	85,3	82,7	89,4	86,8	91,1	86,4
% PV Removed	95,5	95,5	95,4	94,2	93,8	85,0	92,7	93,6	91,4	92,4
Gas Yield (m ³ /kg COD Removed)	0,412	0,410	0,409	0,394	0,393	0,356	0,362	0,325	0,310	0,236
Methane Content of Biogas (%)	75,6	75,9	74,5	76,1	77,4	80,5	83,4	87,7	93,0	95,3
Methane Yield (m ³ CH ₄ /kg COD Removed)	0,311	0,311	0,305	0,300	0,304	0,287	0,302	0,285	0,288	0,225

It is evident from the data that very low concentrations of iron were present in the digester effluent (Table 34). The reason for this is that iron was removed from solution either by chemical precipitation or by absorption to biological floc in the digester. A comparison of Tables 35 and 36 clearly illustrates that the biological sludge at the base of the digester has a tremendous ability to sequester the Fe ions, thus preventing toxicity of this heavy metal. This results in a very high concentration of Fe at the base of the digester i.e. in the sludge bed. Although this accumulation is undesirable as it can lead to a low content of active biomass in the suspended solids, it does have an advantage in that if anaerobic digestion is used as a pretreatment stage for wastewaters, the final effluent will be relatively free of heavy metal ions (Hunter *et al*, 1987).

The precipitation of iron at the neutral pH of the digester contents is another important factor determining the non-toxicity of this heavy metal.

Iron can react chemically with a number of compounds under anaerobic conditions. In this experiment, iron was added to the digester in the form of FeCl_3 . However, under anaerobic conditions, Fe^{3+} is reduced to Fe^{2+} either chemically or biologically (Sorensen, 1982). The resulting Fe^{2+} can react to form precipitates with hydroxides ($K_{sp} \text{Fe}(\text{OH})_2 = 2 \times 10^{-15}$), carbonates ($K_{sp} \text{FeCO}_3 = 2,1 \times 10^{-11}$) or sulphides ($K_{sp} \text{FeS} = 1 \times 10^{-18}$) (Callander and Barford, 1983b). The precipitation of iron as carbonates could explain the decrease in the percentage CO_2 in the biogas and hence the increase in the CH_4 content. Another possible explanation is that as the volume of biogas decreased, the CO_2 was not flushed out of the digester, and therefore dissolved in the digester contents, resulting in an increased CH_4 content.

TABLE 34
CHARACTERISTICS OF THE DIGESTER EFFLUENT AT VARIOUS
STAGES DURING THE IRON EXPERIMENT

Parameter	Control Digester					Fe^{3+} Digester				
	Time (Days)					Time (Days)				
	1	25	50	75	100	1	25	50	75	100
pH	7,58	7,57	7,16	7,44	7,44	7,49	7,57	7,52	7,69	7,87
Total Alkalinity (mg/l CaCO_3)	1224	1479	1428	1377	1224	1634	1600	1680	1606	1320
Bicarbonate Alkalinity (mg/l CaCO_3)	1196	1447	1406	1349	1177	1584	1559	1625	1561	1266
COD (mg/l)	474	441	408	309	441	519	318	396	267	408
PV (mg/l)	29	29	30	38	40	98	46	42	56	49
VFA (mg/l)	39,7	45,9	30,8	39,5	66,3	70,6	57,9	77,9	63,3	75,7
Fe (mg/l)	< 1	< 1	< 1	< 1	< 1	5,2	12,3	36,1	13,9	18,3

In wastewaters that contain very high concentrations of iron it is important that sufficient nutrients are available for the growth of the methanogenic bacteria. It has been shown previously that under low nutrient conditions, iron has the ability to direct the flow of electrons away from methanogens,

resulting in inhibition of methane production (Lovley and Phillips, 1986). However, when nutrients for methanogenic growth were supplied in excess, the iron did not affect the production of methane.

TABLE 35

STRATIGRAPHIC ANALYSIS OF THE DIGESTER CONTENTS AT
THE START OF THE IRON EXPERIMENT

Height of Sampling Port Above Inlet (cm)	Control Digester			Fe ³⁺ Digester		
	pH	Fe (mg/l)	VFA (mg/l)	pH	Fe (mg/l)	VFA (mg/l)
59	7,58	<1	39,7	7,49	5,2	70,6
42	7,41	2,2	18,7	7,45	1,7	68,7
29	7,20	16,4	26,9	7,40	12,6	81,9
16	7,33	850,2	48,9	7,07	793,3	84,5

TABLE 36

STRATIGRAPHIC ANALYSIS OF THE DIGESTER CONTENTS
ON COMPLETION OF THE IRON EXPERIMENT

Height of Sampling Port Above Inlet (cm)	Control Digester			Fe ³⁺ Digester		
	pH	Fe (mg/l)	VFA (mg/l)	pH	Fe (mg/l)	VFA (mg/l)
59	7,44	<1	66,3	7,87	18,3	75,7
42	7,39	6,3	58,7	7,87	50,5	83,2
29	7,19	5,8	51,7	7,84	15300,0	102,1
16	7,10	943,5	48,8	6,99	18300,0	138,8

From the results of this investigation, it can be concluded that anaerobic digestion can be used for the successful treatment of wastewaters containing high concentrations of iron, provided the microorganisms have sufficient time to adapt to the presence of the Fe ions. The concentration of iron found to be non-toxic in this investigation is well in excess of previously reported concentrations.

The findings of this experiment have been previously published (Jackson-Moss et al, 1989b; Jackson-Moss and Duncan, 1990a).

5.7 The Effect of Aluminium on Anaerobic Digestion

5.7.1 Introduction

At present aluminium is only used in small quantities in tanneries for the production of wet-white leather (Cooper and Boast, 1986; Vulliermet et al, 1988). Aluminium sulphate is also used as a flocculent in some wastewater treatment plants. However, growing concern over the use of chromium and its possible effects on the environment has resulted in research and development in the field of alternative tanning agents (Pauckner and Moog, 1989). Aluminium has proven to be one of the best alternatives to chromium, and therefore in the future, the possibility exists that large quantities of aluminium will be discharged and will be present in the final tannery effluents and sludges.

Trace amounts of heavy metals are necessary for specific biological functions in a healthy metabolism. However, excesses can be harmful and cause acute toxicity, or can result in diseases and disorders. Aluminium has recently been implicated in disorders such as Alzheimer's senile dementia (Macdonald and Martin, 1988; Perl and Brody, 1980) and has also been shown to cause severe fish mortality in rivers and streams receiving aluminium-containing effluents (Hunter et al, 1980). No previous studies have, however, been carried out to determine the effects of aluminium on anaerobic digestion.

In view of the lack of information available regarding the toxic effects of aluminium on anaerobic digestion, this study was carried out in order to investigate whether an anaerobic bacterial population could be acclimatized to high concentrations of this heavy metal.

5.7.2 Materials and Methods

The materials and methods used were described in detail in Chapter 4.

5.7.3 Results and Discussion

The addition of incremental amounts of aluminium (Al^{3+}) to the digester (Figure 43) appeared to have no detrimental effects on digester performance for the first 68 days. Despite a decrease in the volume of biogas produced relative to the control digester (Figure 44), an increase in the CH_4 content of the biogas was noted (Figure 45), while the pH, alkalinity and percentage COD and PV removed all remained relatively constant (Tables 37 and 38).

When the Al^{3+} concentration in the feed reached 2500 mg/l (day 68), digester failure occurred. Biogas production gradually decreased to zero (Figure 44) while the percentage of COD and PV removed decreased to 35,9 and 66,7 % respectively. The alkalinity, which determines the buffering capacity of the system, decreased markedly. This was particularly evident for the bicarbonate alkalinity, which was used to neutralize the large accumulation of VFA inside the digester (Table 40).

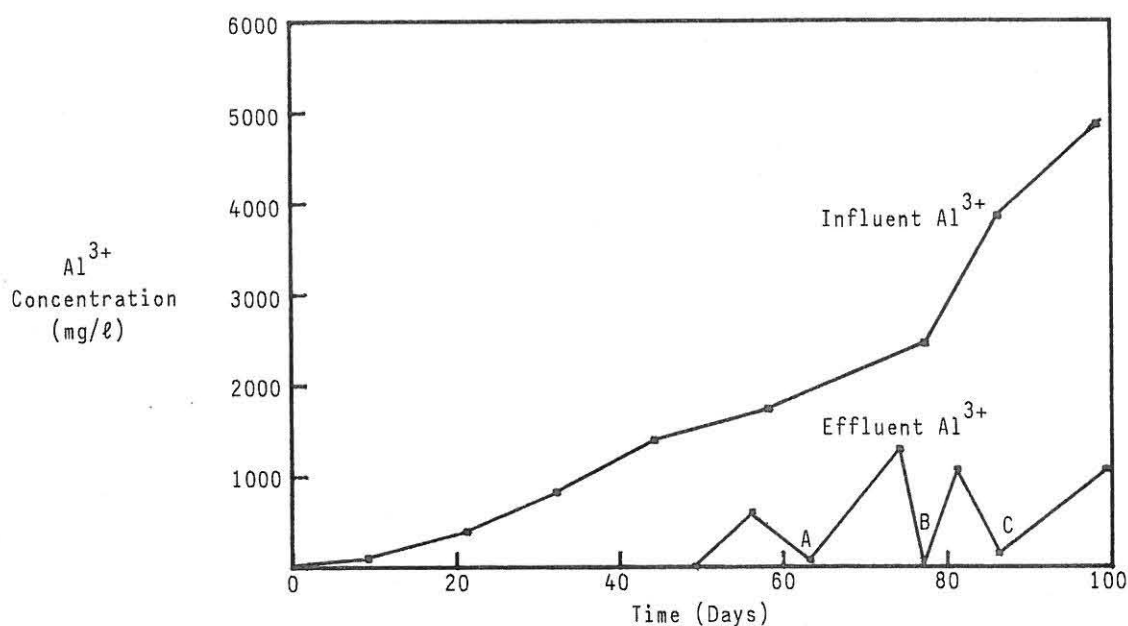


FIGURE 43 - Influent and effluent Al^{3+} versus time for the anaerobic digester receiving Al^{3+} (Note: A, B and C indicate the removal of $\text{Al}(\text{OH})_3$ from the digester. The control digester received no Al^{3+})

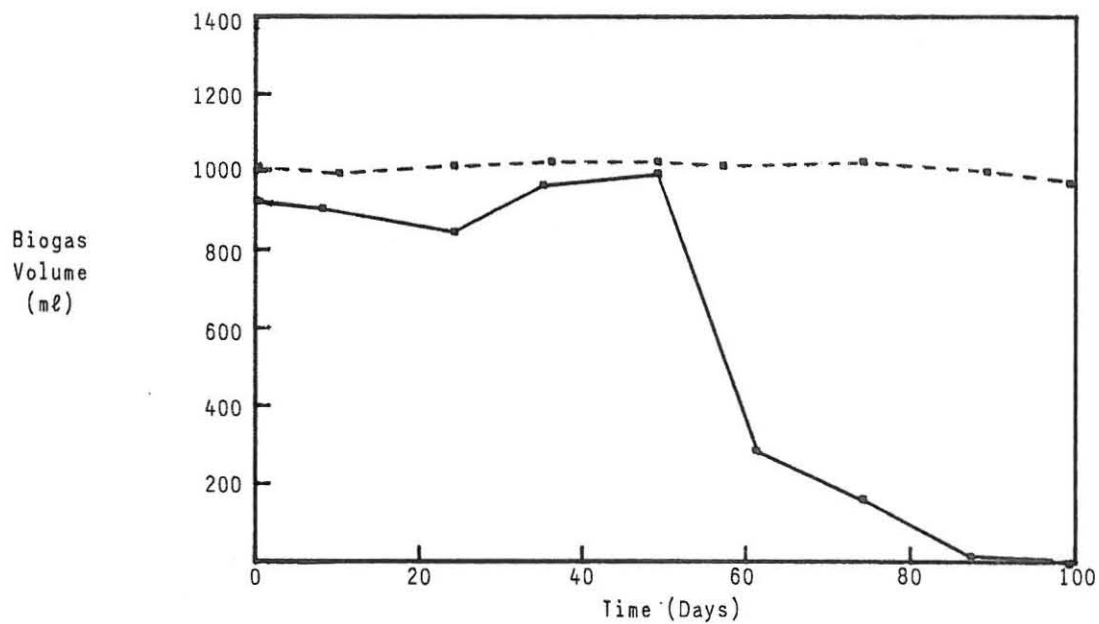


FIGURE 44 - Biogas volume versus time for the anaerobic digester receiving Al^{3+} (— Al^{3+} digester; ---- control digester)

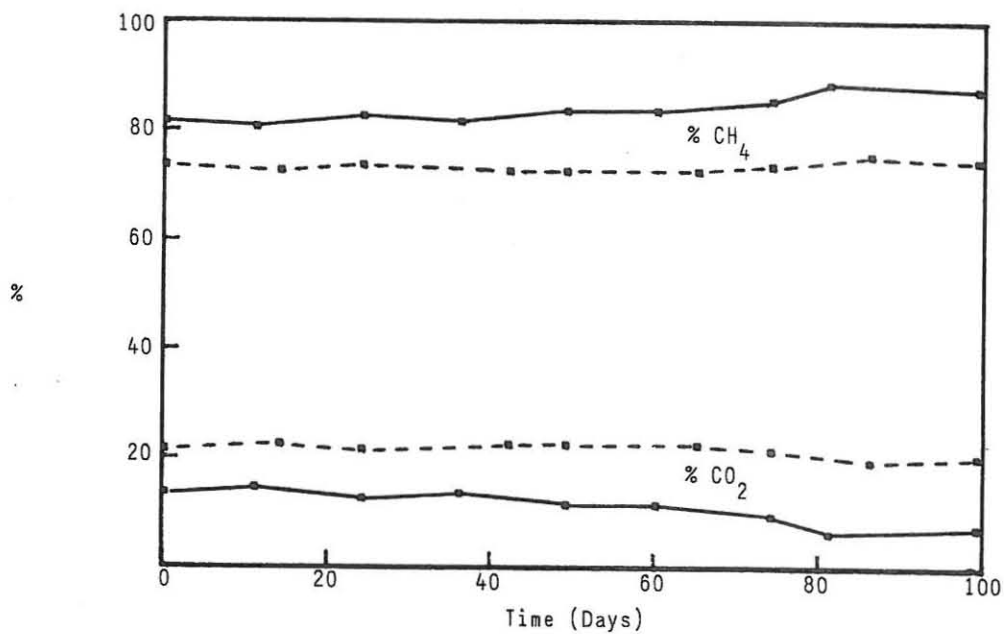


FIGURE 45 - Percentage biogas composition versus time for the anaerobic digester receiving Al^{3+} (— Al^{3+} digester; ---- control digester)

TABLE 37

CHARACTERISTICS OF THE DIGESTER EFFLUENT AT VARIOUS
STAGES DURING THE ALUMINIUM EXPERIMENT

Parameter	Control Digester					Al ³⁺ Digester				
	Time (Days)					Time (Days)				
	1	25	50	75	100	1	25	50	75	100
pH	7,58	7,57	7,16	7,44	7,44	7,63	7,72	7,29	7,65	6,30
Total Alkalinity (mg/l CaCO ₃)	1224	1479	1428	1377	1224	1232	1012	1210	990	638
Bicarbonate Alkalinity (mg/l CaCO ₃)	1196	1447	1406	1349	1177	1182	990	1195	712	71
COD (mg/l)	474	441	408	309	441	510	330	282	567	1923
PV (mg/l)	29	29	30	38	40	40	49	49	64	216
VFA (mg/l)	39,7	45,9	30,8	39,5	66,3	70,2	31,3	21,4	392,0	800,8
Al (mg/l)	< 1	< 1	< 1	< 1	< 1	< 1	< 1	150	1440	1220

The concentration of Al present in the digester reached 140000 mg/l, which is significantly higher than the Al³⁺ present in the feed. The reason for this accumulation of Al is that at the neutral pH of the digester, Al³⁺ precipitates as Al(OH)₃ (Hunter *et al*, 1980). The biological sludge is also able to sequester these heavy metal ions. This is clearly evident by comparing the stratigraphic concentration of Al at the start and on completion of the experiment (Tables 39 and 40); the majority of the Al is present in the sludge bed at the base of the digester. Thus, although the concentration of Al³⁺ in the feed was 2500 mg/l when digester failure occurred, it is likely that the concentration of Al in the sludge bed would have been substantially higher, based on the results given in Table 40.

The precipitation of Al(OH)₃ in the digester also explains the lag-phase prior to the Al emerging in the digester effluent (Figure 43). It should be noted that Al(OH)₃ was removed from the digester at periodic intervals in order to prevent clogging of the outlet. Hence the frequent decrease in the total

effluent Al, as shown in Figure 43.

TABLE 38

THE EFFECT OF ALUMINIUM ON THE OPERATING EFFICIENCY OF THE DIGESTER
AT VARIOUS STAGES DURING THE EXPERIMENT

Parameter	Control Digester					Al ³⁺ Digester				
	Time (Days)					Time (Days)				
	1	25	50	75	100	1	25	50	75	100
Gas Volume (ℓ/d)	1,040	1,050	1,060	1,060	1,005	0,960	0,880	1,030	0,195	0,030
Gas Production (m ³ /m ³ .d)	0,116	0,117	0,118	0,118	0,112	0,107	0,098	0,114	0,022	0,003
% COD Removed	84,2	85,3	86,4	89,7	85,3	83,0	89,0	90,6	81,1	35,9
% PV Removed	95,5	95,5	95,4	94,2	93,8	93,9	92,5	92,4	90,2	66,7
Gas Yield (m ³ /kg COD Removed)	0,412	0,410	0,409	0,394	0,393	0,386	0,330	0,379	0,080	0,028
Methane Content of Biogas (%)	75,6	75,9	74,5	76,1	77,4	84,4	84,6	85,9	87,5	90,0
Methane Yield (m ³ CH ₄ /kg COD Removed)	0,311	0,311	0,305	0,300	0,304	0,326	0,279	0,326	0,070	0,025

TABLE 39

STRATIGRAPHIC ANALYSIS OF THE DIGESTER CONTENTS AT
THE START OF THE ALUMINIUM EXPERIMENT

Height of Sampling Port Above Inlet (cm)	Control Digester			Al ³⁺ Digester		
	pH	Al (mg/ℓ)	VFA (mg/ℓ)	pH	Al (mg/ℓ)	VFA (mg/ℓ)
59	7,58	<1	39,7	7,63	<1	70,2
42	7,41	2,0	18,7	7,59	1,0	68,8
29	7,20	8,0	26,9	7,47	4,0	86,3
16	7,33	400,0	48,9	7,46	300,0	81,7

TABLE 40

STRATIGRAPHIC ANALYSIS OF THE DIGESTER CONTENTS
ON COMPLETION OF THE ALUMINIUM EXPERIMENT

Height of Sampling Port Above Inlet (cm)	Control Digester			Al ³⁺ Digester		
	pH	Al (mg/l)	VFA (mg/l)	pH	Al (mg/l)	VFA (mg/l)
59	7,44	< 1	66,3	6,30	1220	800,8
42	7,39	4,0	58,7	6,54	12250	561,0
29	7,19	8,0	51,7	6,53	120000	405,6
16	7,10	595,0	48,8	6,29	140000	486,3

From the results of this investigation, it can be concluded that both the present and future concentrations of Al³⁺ found in tannery wastewaters will have no detrimental effect on anaerobic digestion provided the digester is operated at a neutral pH. Although an influent concentration of 2500 mg/l Al³⁺ was shown to cause digester failure, the actual concentration present inside the digester would have been significantly higher than this. It is therefore possible to adapt anaerobic microorganisms to high concentrations of aluminium. This is believed to be the first study that has investigated the effect of aluminium on the anaerobic digestion process.

The findings of this experiment have been previously published (Jackson-Moss and Duncan, 1990b).

5.8 The Effect of Ammonia on Anaerobic Digestion

5.8.1 Introduction

Ammonium salts are used extensively during the deliming stage of leather manufacture and are also present in smaller quantities in many of the dyestuffs used in the tannery (Feikes, 1986). Although these represent significant sources of ammonia pollution, the major source is that which arises from the bacterial degradation of the organic matter present in the hides and skins. The resultant tannery wastewaters therefore contain high concentrations of ammonia-nitrogen.

Nitrogen, like sulphur, can exist in a variety of forms, depending on its oxidation state. Changes from one oxidation state to another can be brought about in the environment by living organisms. The relationship between these various nitrogen forms and their transformations are conveniently termed the nitrogen cycle (Figure 46).

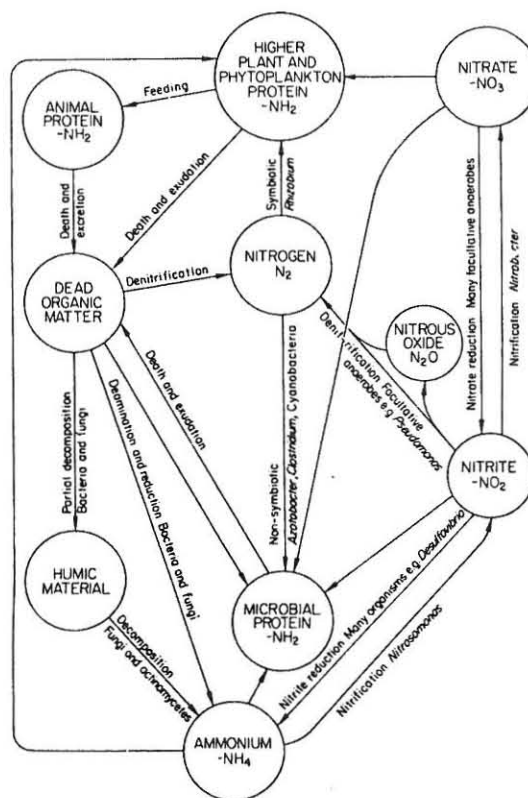
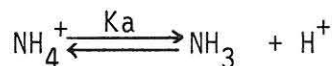


FIGURE 46 - The nitrogen cycle (Campbell, 1983)

The principle transformation reactions which occur during the nitrogen cycle are fixation, ammonification, assimilation, nitrification and denitrification. Each involves a particular set of organisms (usually microorganisms) with a consequent net gain or loss of energy which is an important factor in determining the type of reaction which occurs (Bliss and Barnes, 1981).

In anaerobic digesters, the reducing conditions prevent nitrification and denitrification from taking place and therefore, the most important stage of the nitrogen cycle under anaerobic conditions is the ammonification process. Although a small quantity of ammonia (NH_3) will be assimilated by the anaerobic microorganisms, the NH_4^+ ion and free NH_3 (depending on the pH) represent the terminal nitrogen compounds under anaerobic conditions.

Ammonia is present in wastewaters in equilibrium with the ammonium ion according to the equation



K_a = ionization constant

= $1,13 \times 10^{-9}$ at 35°C (Anderson et al, 1982)

The ammonia species will be distributed such that the free ammonia (NH_3) concentration will represent only 1 % of the total ammonia concentration at pH 7,0 and approximately 10 % at pH 8,0 (Figure 47). This is important as it is the free NH_3 form that is toxic.

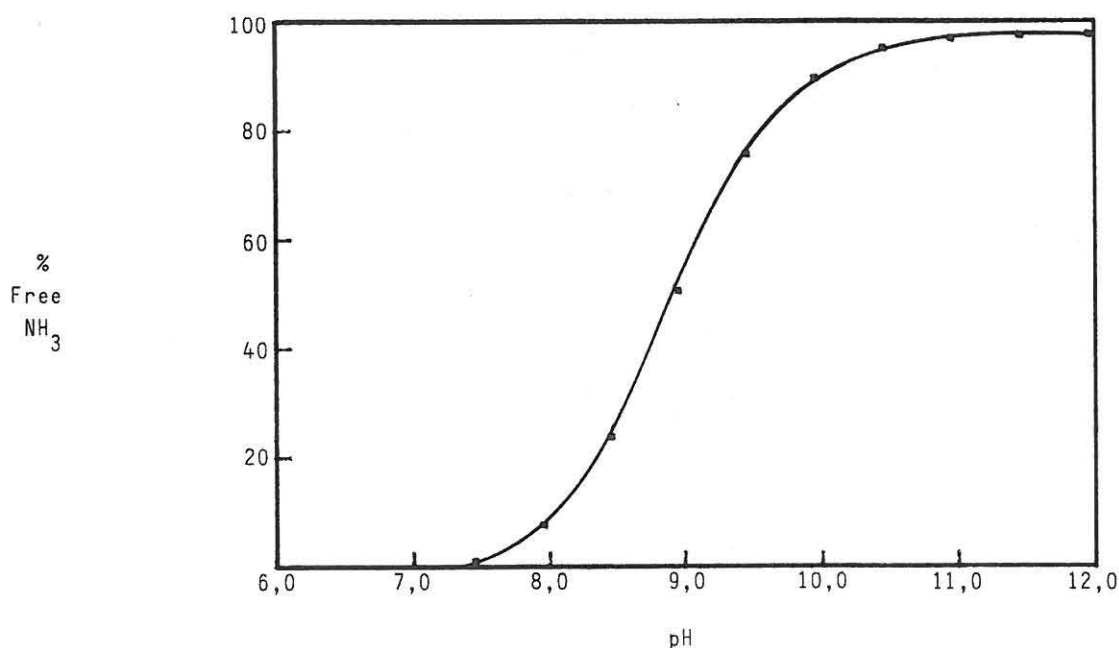


FIGURE 47 - The effect of pH on the free ammonia-nitrogen concentration at 35°C

Methanogens have a specific requirement for ammonia as a source of nitrogen (Jarrell and Kalmokoff, 1988). This ammonia is incorporated into amino acids such as glutamine, glutamate and alanine (Kenealy et al, 1982). At low concentrations, ammonium ions have also been shown to be beneficial to the formation of microbial pellets in UASB digesters (Sam-Soon et al, 1987). However, at high concentrations, ammonia is very toxic and inhibitory to the anaerobic digestion process.

The effect of high concentrations of ammonia on anaerobic digestion has been well studied. Shock load experiments have shown that NH_4^+ concentrations as high as 2700 mg/l (Van den Berg *et al.*, 1976), 4000 mg/l (Maeda *et al.*, 1984) and 5000 mg/l (Parkin *et al.*, 1983) can be tolerated by anaerobic microorganisms, provided a pH of less than 7,5 is maintained. In contrast to this, free ammonia is considerably more inhibitory than the ammonium ion. Shock loads of NH_3 at concentrations as low as 20 mg/l (Kroiss and Wabnegg, 1983) and 150 mg/l (McCarty and McKinney, 1961) have been reported to cause toxicity in anaerobic digesters.

The effects of adaptation to high ammonia concentrations has also been extensively studied. Methane production has been shown to occur at NH_4^+ concentrations as high as 10100 mg/l (De Baere *et al.*, 1984) and 11800 mg/l (Koster and Lettinga, 1988). In both studies, the digester pH was maintained in the range 7,0 - 7,5.

The general conclusions that can be ascertained from previous studies are:-

- (a) the concentration of total ammonia at which inhibition is recorded is pH dependent, and
- (b) the threshold concentration of total ammonia at which inhibition is recorded can be considerably increased by acclimatization of the biomass by means of a gradual increase in the ammonia concentration.

In view of the latter point, the present investigation was carried out in order to investigate whether in the system used in this study, an anaerobic digester could be adapted to high concentrations of ammonium ions.

5.8.2 Materials and Methods

The materials and methods used were described in detail in Chapter 4.

5.8.3 Results and Discussion

The addition of incremental amounts of NH_4^+ to the digester (Figure 48) appeared to have no notable effects on the digester performance. The volume of biogas produced (Figure 49) was maintained at a constant level except during the latter stages of the experiment, while the CH_4 content of the

biogas from the digester which received NH_4^+ was 6 to 7 % higher than that from the control digester (Figure 50). The alkalinity, percentage COD and PV removed, and the VFA concentration all remained constant relative to the control digester (Tables 41 and 42).

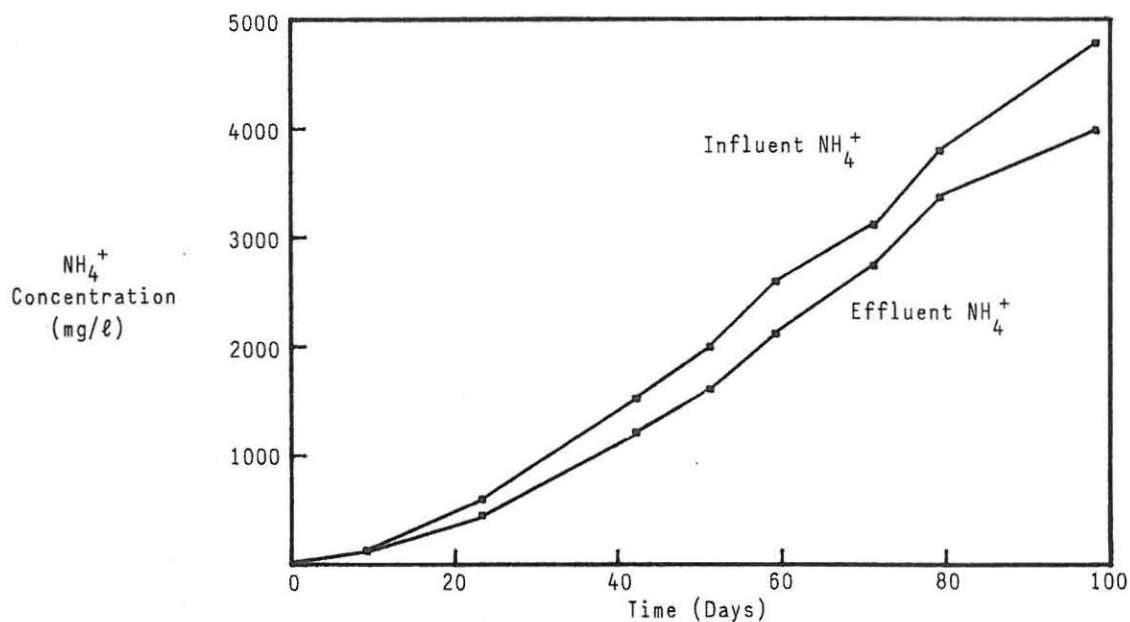


FIGURE 48 - Influent and effluent NH_4^+ versus time for the anaerobic digester receiving NH_4^+ (Note: The control digester had a mean effluent NH_4^+ concentration of 250 mg/l. The influent nitrogen was supplied as urea and yeast extract)

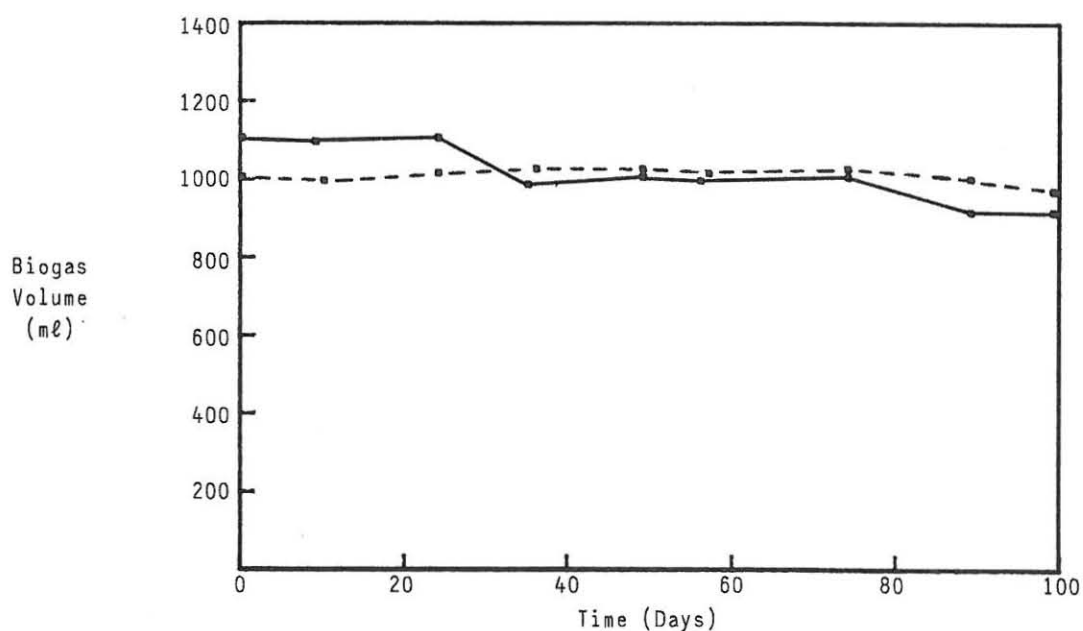


FIGURE 49 - Biogas volume versus time for the anaerobic digester receiving NH_4^+ (— NH_4^+ digester; ---- control digester)

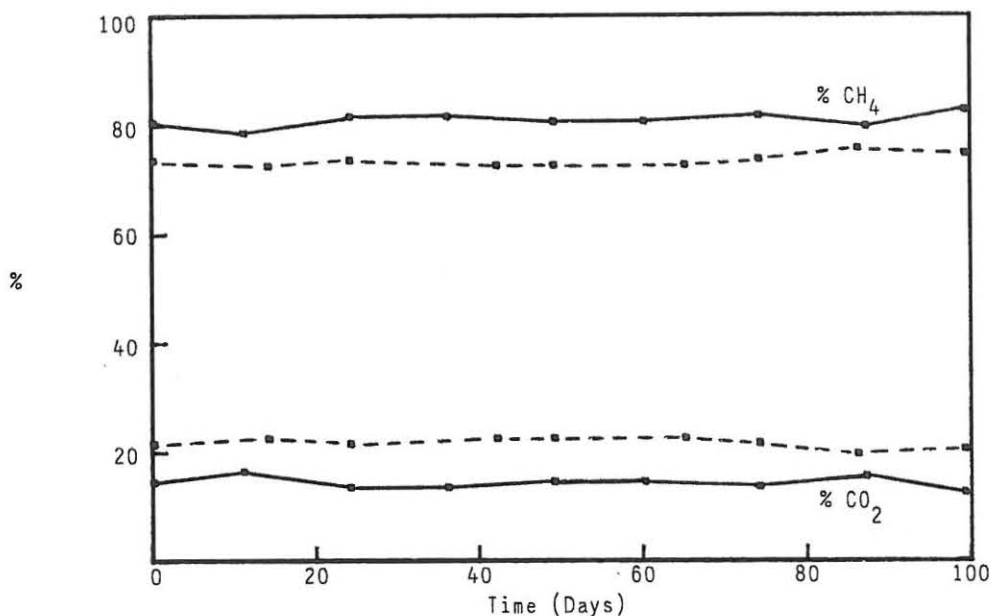


FIGURE 50 - Percentage biogas composition versus time for the anaerobic digester receiving NH_4^+ (— NH_4^+ digester; ---- control digester)

The addition of NH_4Cl to the digester resulted in no increase in the pH or the alkalinity. This is in agreement with previous studies which have been carried out to determine the effect of NH_4Cl on anaerobic digestion (De Baere et al, 1984; Webb and Hawkes, 1985). However, other studies have shown that if the NH_4^+ is formed as a result of the degradation of organic matter, an increase in the pH and alkalinity does occur (Koster and Lettinga, 1988; Webb and Hawkes, 1985). This is due to the formation of ammonium bicarbonate, which acts as a buffering agent inside the digester (McCarty and McKinney, 1961). The fact that this does not occur when NH_4Cl is used as a source of NH_4^+ ions should be noted as NH_3 toxicity is dependent on the pH. Wastewaters such as tannery effluents and sludges contain high concentrations of organic matter, and therefore, an increase in the pH of the digester contents can be expected. This increased pH will result in an increased concentration of free NH_3 which could become inhibitory. It may therefore be necessary to control the pH of digesters treating these wastes in order to maintain the free NH_3 at low concentrations.

TABLE 41

CHARACTERISTICS OF THE DIGESTER EFFLUENT AT VARIOUS
STAGES DURING THE AMMONIA EXPERIMENT

Parameter	Control Digester					NH ₄ ⁺ Digester				
	Time (Days)					Time (Days)				
	1	25	50	75	100	1	25	50	75	100
pH	7,58	7,57	7,16	7,44	7,44	7,62	7,32	7,23	7,06	7,20
Total Alkalinity (mg/l CaCO ₃)	1224	1479	1428	1377	1224	1056	1166	1012	1144	1159
Bicarbonate Alkalinity (mg/l CaCO ₃)	1196	1447	1406	1349	1177	975	1145	990	1130	1128
COD (mg/l)	474	441	408	309	441	462	330	402	429	384
PV (mg/l)	29	29	30	38	40	79	66	55	81	40
VFA (mg/l)	39,7	45,9	30,8	39,5	66,3	114,0	30,3	31,5	20,4	43,2
TKN (mg/l)	242	263	259	236	277	257	567	1689	2833	4469
NH ₄ ⁺ (mg/l)	236	263	252	236	266	251	567	1689	2832	4457
Organic Nitrogen (mg/l)	6	0	7	0	11	6	0	0	1	12

A comparison of Tables 43 and 44 illustrates that there was no stratigraphic accumulation of NH₄⁺ ions in the digester. On completion of the experiment, the NH₄⁺ ions were evenly distributed throughout the contents of the digester and a high concentration was present in the digester effluent. There is therefore no nett removal of nitrogen during anaerobic digestion; the nitrogen is merely converted from one form to another. This has been reported previously (Summers and Bousfield, 1976).

TABLE 42

THE EFFECT OF AMMONIA ON THE OPERATING EFFICIENCY OF THE DIGESTER
AT VARIOUS STAGES DURING THE EXPERIMENT

Parameter	Control Digester					NH ₄ ⁺ Digester				
	Time (Days)					Time (Days)				
	1	25	50	75	100	1	25	50	75	100
Gas Volume (ℓ/d)	1,040	1,050	1,060	1,060	1,005	1,140	1,140	1,040	1,040	0,950
Gas Production (m ³ /m ³ .d)	0,116	0,117	0,118	0,118	0,112	0,127	0,127	0,116	0,116	0,106
% COD Removed	84,2	85,3	86,4	89,7	85,3	84,6	89,0	86,6	85,7	87,2
% PV Removed	95,5	95,5	95,4	94,2	93,8	87,8	89,9	91,5	87,6	93,9
Gas Yield (m ³ /kg COD Removed)	0,412	0,410	0,409	0,394	0,393	0,449	0,427	0,400	0,405	0,363
Methane Content of Biogas (%)	75,6	75,9	74,5	76,1	77,4	82,6	83,9	82,8	83,8	84,6
Methane Yield (m ³ CH ₄ /kg COD Removed)	0,311	0,311	0,305	0,300	0,304	0,371	0,358	0,331	0,339	0,307
Free NH ₃ (mg/ℓ)	9,7	10,5	4,0	7,1	8,0	11,2	13,0	31,6	36,0	78,0

TABLE 43

STRATIGRAPHIC ANALYSIS OF THE DIGESTER CONTENTS AT
THE START OF THE AMMONIA EXPERIMENT

Height of Sampling Port Above Inlet (cm)	Control Digester					NH ₄ ⁺ Digester				
	pH	TKN (mg/ℓ)	NH ₄ ⁺ (mg/ℓ)	Organic N (mg/ℓ)	VFA (mg/ℓ)	pH	TKN (mg/ℓ)	NH ₄ ⁺ (mg/ℓ)	Organic N (mg/ℓ)	VFA (mg/ℓ)
59	7,58	242	236	6	39,7	7,62	257	251	6	114,0
42	7,41	239	236	3	18,7	7,41	266	249	17	122,7
29	7,20	397	318	79	26,9	7,47	519	377	142	109,9
16	7,33	1209	377	832	48,9	7,29	1661	599	1062	117,1

TABLE 44

STRATIGRAPHIC ANALYSIS OF THE DIGESTER CONTENTS
ON COMPLETION OF THE AMMONIA EXPERIMENT

Height of Sampling Port Above Inlet (cm)	Control Digester					NH ₄ ⁺ Digester				
	pH	TKN (mg/l)	NH ₄ ⁺ (mg/l)	Organic N (mg/l)	VFA (mg/l)	pH	TKN (mg/l)	NH ₄ ⁺ (mg/l)	Organic N (mg/l)	VFA (mg/l)
59	7,44	277	266	11	66,3	7,20	4469	4457	12	43,2
42	7,39	277	277	0	58,7	6,91	4575	4469	106	28,9
29	7,19	426	309	117	51,7	6,85	5320	4788	532	22,0
16	7,10	1401	408	993	48,8	6,81	6810	4788	2022	29,0

From the results of this investigation it can be concluded that anaerobic digestion can be carried out at high NH₄⁺ concentrations provided the pH of the digester is maintained neutral. If the pH is allowed to increase, the proportion of the toxic free NH₃ will increase, and digester failure could occur.

Presently, research is being carried out to reduce the concentration of ammonia present in tannery wastewaters. Alternative deliming methods, such as the use of CO₂, as well as ammonia removal from deliming effluents by stripping-reabsorption (O'Brien *et al*, 1986) will, in the future, reduce the concentration of ammonia in tannery wastewaters. This will result in even less possibility of the ammonia causing anaerobic digester toxicity.

The findings of this experiment have been previously published (Jackson-Moss and Duncan, 1990d).

5.9 Conclusion

The results obtained from the synthetic wastewater study clearly illustrate that the anaerobic microorganisms present in anaerobic digesters, particularly the methanogens, have the ability to adapt to high concentrations of potentially toxic compounds. In most cases, not only were the bacteria able to survive, but they were able to continue to grow and metabolize the organic

matter present in the wastewater.

An important factor that is common to all of the experiments carried out, is that of maintaining a neutral pH inside the digester. Proper pH control is important in the operation of any anaerobic digester, as methanogens have a pH optimum of 6,8 to 8,0 (Balch *et al*, 1979). However, when the wastewater being treated contains potentially toxic compounds, this factor becomes even more critical. The pH determines the fate of the toxic compounds and hence also their ability to inhibit the anaerobic microorganisms. At the neutral pH of the digesters, the heavy metals Cr, Al, Fe and Ca precipitated as hydroxides and hence their ability as toxic compounds was markedly reduced. Under acidic conditions these metals would have been more toxic as they would have been freely available in solution and also more permeable to the bacterial cell walls.

Similarly, the toxicity of soluble compounds such as H_2S and NH_3 are also pH-dependent. At pH 7,0, 50 % of the sulphides are present as toxic H_2S . If the pH becomes acidic, this increases the concentration of H_2S available to inhibit the bacteria. Ideally the pH should be maintained at 7,0 or greater to minimize H_2S toxicity. In contrast to this, ammonia toxicity is increased at alkaline pH's, due to the formation of free NH_3 . If a neutral pH is maintained, however, <1 % of the NH_4^+ is available as free NH_3 . For the treatment of a wastewater containing a high concentration of organic matter, this latter point is extremely important.

The precipitation of heavy metals as hydroxides has another advantage in that the effluent from the digesters is relatively free of these ions. However, this precipitation and accumulation does create problems as it results in a high concentration of inorganic compounds in the biological sludge. At some stage the inorganic compounds have to be removed, and this results in the loss of some of the active biomass. Ideally any wastewater to be treated should be free of heavy metal ions.

In view of the success obtained using the synthetic wastewater to determine the effects of individual tannery wastewater compounds on anaerobic digestion, the anaerobic treatment of actual tannery effluents and sludges was subsequently examined.

CHAPTER SIX

THE ANAEROBIC DIGESTION OF TANNERY EFFLUENT

CHAPTER SIX

THE ANAEROBIC DIGESTION OF TANNERY EFFLUENT

Anaerobic digestion was originally used for the treatment of municipal wastewater sludges. These digesters were operated at very long hydraulic retention times of the order of 20 - 50 days. These long hydraulic retention times initially prevented the use of anaerobic digestion for treating industrial effluents, because the large volumes of effluent produced daily would have necessitated the construction of large digesters in order to maintain adequate solids retention times.

Over the last 10 - 20 years, rapid advances in digester technology and a better understanding of the anaerobic process have led to the development of retained biomass digesters, where the hydraulic retention time and solids retention time can be maintained independent of each other. This has drastically reduced the size of digesters required and consequently, the anaerobic process has become favourable as a treatment method for industrial effluents. The use of the anaerobic process in industry has usually been limited to a pretreatment stage, followed by aerobic polishing of the effluent. This is due to the fact that anaerobic digestion very rarely produces an effluent of the same quality as aerobic treatment processes.

In view of the success obtained using anaerobic digestion to treat many other industrial effluents, this study was carried out to investigate the anaerobic pretreatment of tannery effluents. The data obtained in Chapter 5 clearly illustrates that through adaptation, it is possible to acclimatize the anaerobic microorganisms to high concentrations of potentially toxic compounds present in the tannery wastewaters. Thus, by following a similar startup procedure, these compounds should not affect the metabolism of the microorganisms present in the digesters, and therefore the anaerobic treatment of tannery effluents should be possible.

6.1 The Anaerobic Digestion of Tannery Effluent Without pH Control

6.1.1 Introduction

A number of studies have been carried out to investigate the anaerobic

treatment of tannery effluents. The majority of these studies have concentrated on the treatment of vegetable tannery wastewaters (Gates and Lin, 1966; Arora and Chattopadhyaya, 1980; Tunick *et al*, 1985; Hurlow, 1986; Bajwa and Forster, 1988; Yong, 1988) and will therefore not be discussed. Rather, as all the effluents used in this study were collected from a chrome-tannery, only results obtained using this type of wastewater will be considered in detail.

In an earlier study (Friedman *et al*, 1982) it was shown that pretreated tannery beamhouse wastewaters can be treated using an upflow anaerobic filter to routinely remove more than 60 % of the applied COD with a hydraulic retention time of one day and a loading rate of 2,25 kg COD/m³.d. The anaerobic filter proved to be remarkably insensitive to variations in the feed type, loading conditions, shut down periods and the introduction of air into the system. Problems were, however, encountered as a result of mechanical failures and blockages in influent pipelines. A massive accumulation of solids occurred within the digesters. Calcium was found to account for more than a third of the non-volatile matter inside the digesters, with the result that the volatile solids only accounted for 19,8 - 25,2 % of the total solids. In view of the accumulation of solids, it was suggested that where reaction conditions may result in precipitate formation, a downflow mode of operation should be used. This would minimize the potential for clogging, but would also probably reduce the treatment efficiency.

Another investigation (Bailey *et al*, 1984) has also shown that 60 % of the COD of beamhouse wastes can be removed by using a 29 l anaerobic contact reactor operated at a 60 hour hydraulic retention time and a 13,4 day solids retention time. The anaerobic digestion of this waste resulted in an increase in the digester pH, which seriously affected methane production. When the pH reached 8,3, a decrease of 55 % in methane production occurred. However, if the pH was reduced, methane production increased again. High concentrations of sulphides i.e. 650 mg/l were formed in the digester as a result of the degradation of keratin protein. In spite of this, the anaerobic digester continued to function properly.

The information available on the anaerobic digestion of chrome-tannery effluents is very limited. This study was therefore carried out to provide additional knowledge on the anaerobic treatability of these effluents.

6.1.2 Materials and Methods

The materials and methods used were described in detail in Chapter 4. After seeding the anaerobic digester with 4 ℓ of sewage sludge (VSS concentration = 28,0 - 32,0 g/ ℓ), it was started up on a dilute tannery effluent. This was obtained by diluting the effluent (Table 45) with varying proportions of deionized water. The rate of feeding was 1 ℓ per day, with the result that a constant hydraulic retention time of 9 days was maintained throughout the duration of the experiment. The pH of the effluent supplied to the digester was not controlled, and was therefore in the range 7,3 - 7,7 i.e. the pH when collected from the tannery.

6.1.3 Results and Discussion

Although the bacteria were initially exposed to very low concentrations of tannery effluent, any increase in the loading rate resulted in a corresponding increase in the concentration of VFA in the digester (Figure 51). On day 51, the loading rate was reduced in order to lower the VFA concentration. However, as soon as the loading rate was increased again, the VFA concentration once again increased. The methanogenic bacteria were therefore not able to adapt to and utilize the tannery effluent, and in view of this, the experiment was terminated.

The volume of biogas produced decreased rapidly and by day 50 had ceased completely (Figure 52). Similarly, the percentage CH_4 in the biogas also decreased to zero, although this proceeded more slowly (Figure 52). This was once again a clear indication that the methanogens were being inhibited. Because the concentration of tannery effluent which caused digester failure was very low, it is unlikely that any of the compounds present in the effluent were causing the toxicity. It would rather appear that another factor was inhibiting the growth of the methanogenic bacteria. The most feasible factor was the pH. With the exception of the first 25 days, the pH increased steadily over the duration of the experiment and eventually reached a value of 8,30 (Table 46). This is well outside the optimum pH range of the methanogenic bacteria, which lies in the region of 6,8 - 8,0 (Balch *et al*, 1979). The increase in the pH is caused by the ammonification of the organic matter in the tannery effluent (Table 46). The NH_4^+ formed can react to form ammonium bicarbonate, which acts as a buffer inside the digester. Although a

digester effluent pH of 8,0 was only exceeded towards the end of the experiment, the pH surrounding the sludge bed inside the digester probably exceeded this far earlier. Hence the production of no CH_4 gas as early as day 50. This increase in the digester pH during the anaerobic digestion of tannery effluents has been noted previously (Friedman *et al*, 1982; Bailey *et al*, 1984).

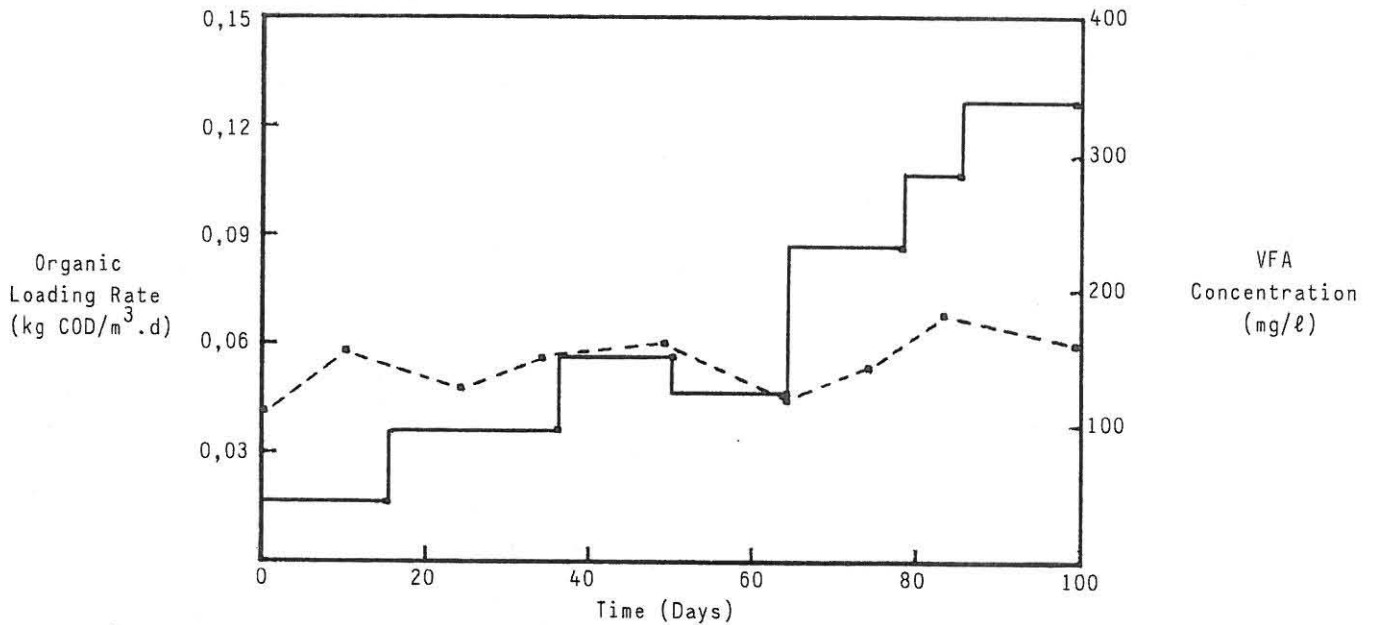


FIGURE 51 - Organic loading rate and effluent volatile fatty acids versus time for the anaerobic digester receiving tannery effluent (— organic loading rate; ---- volatile fatty acids as acetic acid)

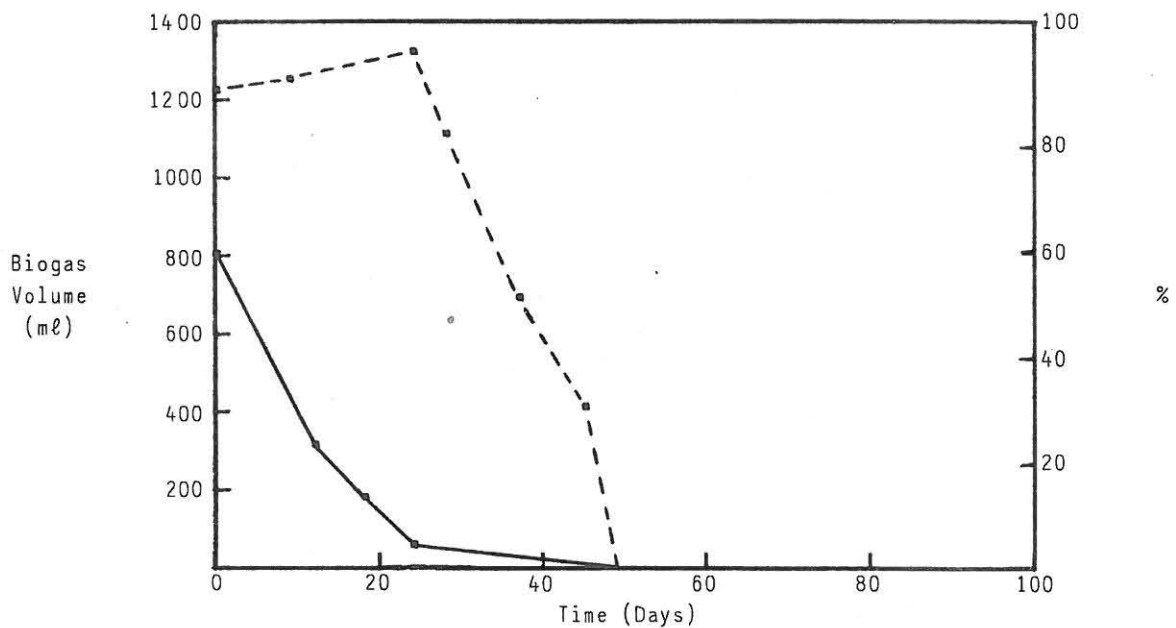


FIGURE 52 - Biogas volume and percentage CH_4 versus time for the anaerobic digester receiving tannery effluent (— biogas volume; ---- % CH_4)

TABLE 45

COMPOSITION OF THE TANNERY EFFLUENT
USED IN THIS INVESTIGATION

Parameter	Concentration
Total COD (mg/l)	4040
Soluble COD (mg/l)	2468
Total PV (mg/l)	786
Soluble PV (mg/l)	437
Total Solids (mg/l)	9400
SS (mg/l)	675
TIS (mg/l)	5215
TDS (mg/l)	8725
IDIS (mg/l)	4805
Conductivity (mS/m)	1255
Sulphate (mg/l)	3726
Sulphide (mg/l)	736
TKN (mg/l)	276
NH ₃ -N (mg/l)	224
Organic N (mg/l)	52
Cl ⁻ (mg/l)	3500
Na (mg/l)	1950
Ca (mg/l)	130
Cr (mg/l)	97
Al (mg/l)	<1
Fe (mg/l)	2,2
P (mg/l)	1,8

The fate of the tannery compounds in the digester is in agreement with the findings of Chapter 5. Sulphates were reduced to sulphides while the organic nitrogen was converted to NH₃-N. As shown previously in Chapter 5, anaerobic digestion does not remove any nitrogen from wastewaters. The soluble ions such as Na⁺ and Cl⁻ passed through the digester (Table 46) with the result that they contributed to the effluent conductivity. In contrast to this, the heavy metals Cr, Fe and Al precipitated and accumulated in the sludge bed. This is clearly evident if one notes the concentration of these heavy metals in the digester effluent. This, however, appears to be contrary to the results suggested by Tables 47 and 48 which show that the concentration of heavy metals at the base of the digester was less on completion of the experiment than at the start of the experiment. However, the reason why lower concentrations of heavy metals were detected at the lowest sampling port on completion of the experiment is due to settling and compacting of the sludge bed. This is evident by comparing the SS, total COD, TKN, organic N and total solids of the lowest sampling port on day 1 and day 100. The sample therefore

withdrawn from the lowest port on completion of the experiment was not directly in the sludge bed, whereas at the start of the experiment it was in the sludge bed.

TABLE 46
CHARACTERISTICS OF THE DIGESTER EFFLUENT AT VARIOUS STAGES
DURING THE EXPERIMENT TREATING TANNERY EFFLUENT

Parameter	Time (Days)				
	1	25	50	75	100
pH	7,64	7,30	7,58	7,87	8,30
Total COD (mg/l)	1429	240	280	492	450
Soluble COD (mg/l)	323	144	208	323	323
Total PV (mg/l)	138	96	55	89	46
Soluble PV (mg/l)	97	92	46	81	43
Total Solids (mg/l)	1545	565	960	1410	2215
TIS (mg/l)	385	225	540	970	1945
SS (mg/l)	700	35	65	65	35
TDS (mg/l)	845	530	895	1345	2180
TDIS (mg/l)	335	210	485	955	1930
Alkalinity (mg/l CaCO ₃)	1044	1332	1196	1242	1380
Bicarbonate Alkalinity (mg/l CaCO ₃)	959	1235	1076	1134	1260
Conductivity (mS/m)	208	120	183	309	419
SO ₄ ²⁻ (mg/l)	120	99	263	422	690
Sulphide (mg/l)	32	40	80	104	136
TKN (mg/l)	142	64	58	97	161
NH ₃ -N (mg/l)	126	64	58	97	155
Organic N (mg/l)	16	0	0	0	6
Cl ⁻ (mg/l)	825	500	700	875	1275
Na (mg/l)	130	90	450	180	310
Ca (mg/l)	113	126	40	70	80
Cr (mg/l)	< 1	< 1	< 1	1,5	< 1
Al (mg/l)	< 1	< 1	< 1	< 1	< 1
Fe (mg/l)	5,0	5,0	4,5	5,0	2,0
P (mg/l)	22,2	3,8	1,8	< 1	< 1
VFA (mg/l)	120,3	136,9	169,9	152,4	169,2

TABLE 47

STRATIGRAPHIC ANALYSIS OF THE DIGESTER CONTENTS
AT THE START OF THE TANNERY EFFLUENT EXPERIMENT

Parameter	Height of Sampling Port Above Inlet (cm)			
	16	29	42	59
pH	7,39	7,60	7,54	7,64
Total COD (mg/l)	2818	1781	1717	1429
Soluble COD (mg/l)	362	377	356	323
Total PV (mg/l)	387	189	163	138
Soluble PV (mg/l)	93	102	92	97
Total Solids (mg/l)	3990	1910	1770	1545
TIS (mg/l)	1360	465	550	385
SS (mg/l)	3120	1020	950	700
TDS (mg/l)	870	890	820	845
TDIS (mg/l)	450	415	260	335
Alkalinity (mg/l CaCO ₃)	1096	1104	1096	1044
Bicarbonate Alkalinity (mg/l CaCO ₃)	998	1024	1018	959
Conductivity (mS/m)	216	222	217	208
SO ₄ ²⁻ (mg/l)	132	118	135	120
Sulphide (mg/l)	36	40	25	32
TKN (mg/l)	420	168	154	142
NH ₃ -N (mg/l)	188	132	126	126
Organic N (mg/l)	232	36	28	16
Cl ⁻ (mg/l)	850	850	800	825
Na (mg/l)	115	120	120	130
Ca (mg/l)	180	120	110	113
Cr (mg/l)	12,5	1,0	1,0	< 1
Al (mg/l)	48,5	4,0	1,0	< 1
Fe (mg/l)	66,5	4,0	3,5	5,0
P (mg/l)	38,8	27,0	29,7	22,2
VFA (mg/l)	137,9	113,6	109,7	120,3

TABLE 48

STRATIGRAPHIC ANALYSIS OF THE DIGESTER CONTENTS
ON COMPLETION OF THE TANNERY EFFLUENT EXPERIMENT

Parameter	Height of Sampling Port Above Inlet (cm)			
	16	29	42	59
pH	8,14	8,01	8,00	8,30
Total COD (mg/l)	445	553	422	450
Soluble COD (mg/l)	292	422	323	323
Total PV (mg/l)	208	221	161	46
Soluble PV (mg/l)	124	140	147	43
Total Solids (mg/l)	2835	2570	2390	2215
TIS (mg/l)	1830	1800	1520	1945
SS (mg/l)	520	270	155	35
TDS (mg/l)	2315	2300	2235	2180
TDIS (mg/l)	1790	1775	1415	1930
Alkalinity (mg/l CaCO ₃)	1150	1150	1196	1380
Bicarbonate Alkalinity (mg/l CaCO ₃)	1100	1124	1163	1260
Conductivity (mS/m)	454	454	439	419
SO ₄ ²⁻ (mg/l)	430	373	380	690
Sulphide (mg/l)	120	120	112	136
TKN (mg/l)	191	179	172	161
NH ₃ -N (mg/l)	172	172	166	155
Organic N (mg/l)	19	7	6	6
Cl ⁻ (mg/l)	1200	1275	1250	1275
Na (mg/l)	320	310	310	310
Ca (mg/l)	80	40	50	80
Cr (mg/l)	6,0	2,0	1,5	<1
Al (mg/l)	10,0	5,0	<1	<1
Fe (mg/l)	9,5	6,5	4,5	2,0
P (mg/l)	6,0	2,5	1,3	<1
VFA (mg/l)	70,9	37,4	46,4	169,2

The percentages of COD and PV removed were between 50 and 60 % (Table 49). As COD was not reduced through CH_4 formation, the most likely explanation for this decrease is that the organic matter was used for the metabolism of the non-methanogenic microbes present in the digester i.e. the sulphate-reducing bacteria, acetogenic bacteria, etc.

TABLE 49
OPERATING EFFICIENCY OF THE DIGESTER AT VARIOUS
STAGES DURING THE EXPERIMENT TREATING TANNERY EFFLUENT

Parameter	Time (Days)				
	1	25	50	75	100
Gas Volume (ℓ/d)	0,840	0,095	0,00	0,00	0,00
Gas Production ($\text{m}^3/\text{m}^3 \cdot \text{d}$)	0,093	0,011	0,00	0,00	0,00
% COD Removed	0,0	33,3	47,0	58,4	58,8
% PV Removed	0,0	15,0	34,9	52,4	54,9
Gas Yield (m^3/kg COD Removed)	-	0,792	0,00	0,00	0,00
CH_4 Content of Biogas (%)	89,6	97,0	0,0	0,0	0,0
CH_4 Yield ($\text{m}^3 \text{CH}_4/\text{kg}$ COD Removed)	-	0,768	0,00	0,00	0,00
Free NH_3 (mg/ℓ)	6,7	1,4	2,4	7,5	28,4
H_2S (mg/ℓ)	13,0	12,5	31,4	47,3	8,8

The solids balance for the anaerobic digestion of tannery effluent is shown in Table 50. If the Total Volatile Solids (TVS) or the organic content of the effluent is concentrated on, then the following is obtained:-

$$\begin{aligned}
 \text{TVS degraded} &= 59,431 \text{ g} - 50,050 \text{ g} \\
 &= 9,381 \text{ g} \\
 &= \underline{15,8 \% \text{ of TVS that entered}} \rightarrow
 \end{aligned}$$

$$\begin{aligned}\text{TVS emerged} &= 50,050 \text{ g} \\ &= \underline{84,2 \% \text{ of TVS that entered}} \rightarrow\end{aligned}$$

In addition to this, the organic content of the sludge bed was reduced by:-

$$\begin{aligned}92,140 \text{ g} - 64,026 \text{ g} &= 28,114 \text{ g} \\ &= \underline{30,5 \%} \rightarrow\end{aligned}$$

The fact that only a low percentage of organic matter that was supplied to the digester was degraded, provides further evidence that the digester was unable to treat the tannery effluent. The finding that the volume of the sludge bed was reduced (Tables 48 and 49) is supported by the results shown in Table 50, where as much as 30,5 % of the organic content of the bed was found to have been reduced. The most likely explanation for this decrease in the organic content of the sludge bed is that the organic matter was probably used as a substrate by the non-methanogenic bacteria.

TABLE 50

SOLIDS BALANCE FOR THE ANAEROBIC
DIGESTION OF TANNERY EFFLUENT

Solids Present in the Digester - Day 1

Total Solids	= 153,320 g
Total Volatile Solids	= 92,140 g
Total Inorganic Solids	= 61,180 g

Solids that Entered the Digester - Day 1 to 100

Total Solids	= 133,480 g
Total Volatile Solids	= 59,431 g
Total Inorganic Solids	= 79,049 g

Solids Present in the Digester - Day 100

Total Solids	= 119,556 g
Total Volatile Solids	= 64,026 g
Total Inorganic Solids	= 55,530 g

Solids that Emerged from the Digester - Day 1 to 100

Total Solids	= 120,880 g
Total Volatile Solids	= 50,050 g
Total Inorganic Solids	= 70,830 g

From the results of this investigation it can be concluded that raw, untreated tannery effluent inhibits the metabolism of the methanogenic bacteria. This inhibition is most probably due to the formation of a high digester pH, as the concentration of the potentially toxic compounds present in the tannery effluent would have been too low to cause digester failure. The importance of pH control was therefore considered and will be discussed in the next section.

6.2 The Importance of pH Control During the Anaerobic Digestion of Tannery Effluent

6.2.1 Introduction

In the previous experiment (Section 6.1), it was found that when tannery effluent was supplied to a healthy, well-functioning anaerobic digester, it caused the total cessation of both biogas and CH_4 production. This toxicity is believed to be due to a high internal digester pH. This high pH is caused by ammonium bicarbonate, which is formed as a result of the ammonification of the organic matter present in the tannery effluent. The high digester pH formed in the previous experiment i.e. 8,30, is well outside the optimum pH range of the methanogens. Therefore, if the pH of the digester is controlled in the optimum range, the possibility exists that this could prevent the inhibition of the methanogens. The aim of this experiment was, therefore, to investigate the importance of pH control during the anaerobic digestion of tannery effluent.

6.2.2 Materials and Methods

The materials and methods used were described in detail in Chapter 4. The anaerobic digester was started up on a dilute tannery effluent in the same manner as described previously (Section 6.1.2). A constant hydraulic retention time of 9 days was also maintained throughout the duration of this experiment. However, in contrast to the previous experiment, the pH of the effluent supplied to the digester was controlled in the range 4,0 - 5,0 by using HCl.

6.2.3 Results and Discussion

As the concentration of tannery effluent supplied to the digester was increased, this resulted in a corresponding increase in the concentration of

VFA in the digester (Figure 53). On day 51, the loading rate was reduced in order to lower the VFA concentration. However, as soon as the loading rate was increased again, the VFA concentration increased. A similar effect was noted in the previous experiment, and hence the controlling of the digester pH did not enhance the ability of the methanogenic bacteria to utilize the VFA produced by the acidogenic and acetogenic bacteria.

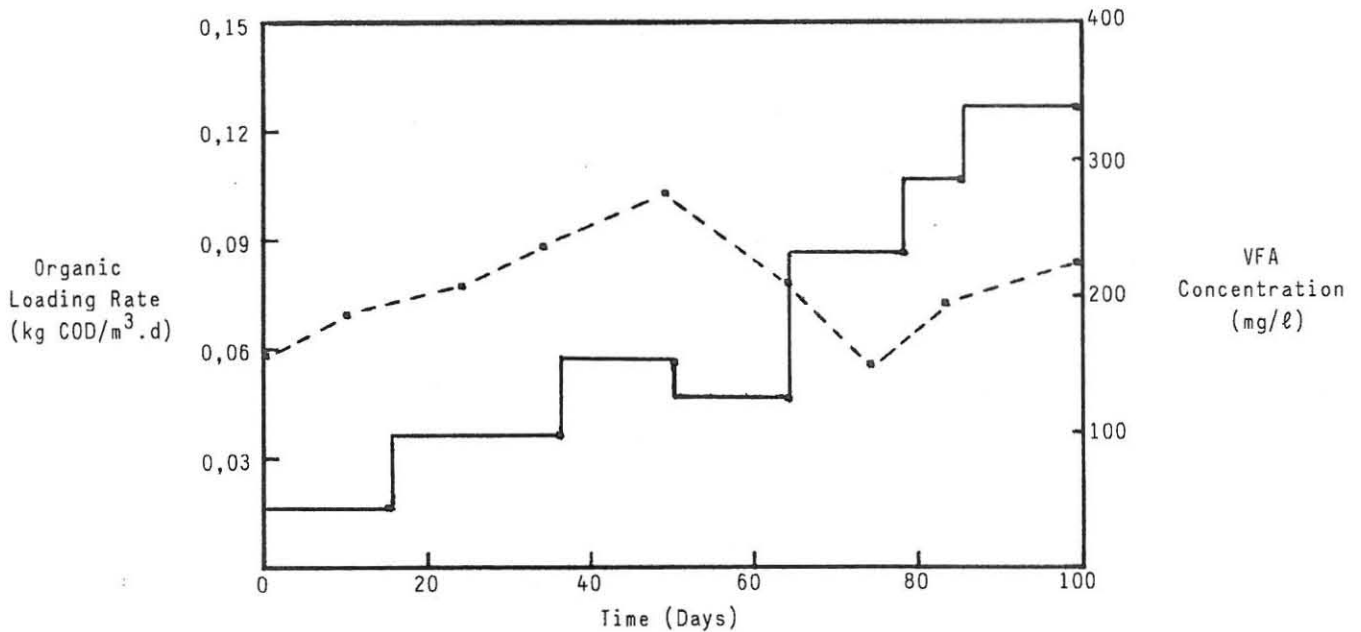


FIGURE 53 - Organic loading rate and effluent volatile fatty acids versus time for the anaerobic digester receiving pH-controlled tannery effluent (— organic loading rate; ---- volatile fatty acids as acetic acid)

As in the previous experiment, the volume of biogas produced decreased rapidly and by day 50 had almost ceased completely (Figure 54). There was no significant increase in the volume of biogas produced compared with the previous experiment. However, a major difference was noted for the composition of the biogas (Figure 55). CH_4 was present in the small quantities of biogas produced throughout the duration of the experiment. The only difference between this experiment and the previous one, is that in this experiment the pH of the digester was controlled in the optimum range (Table 51). This clearly illustrates the importance of pH control in anaerobic digesters treating tannery wastewaters. Unless the pH of the digester is maintained in the range 7.0 - 8.0, the methanogenic bacteria do not function at all.

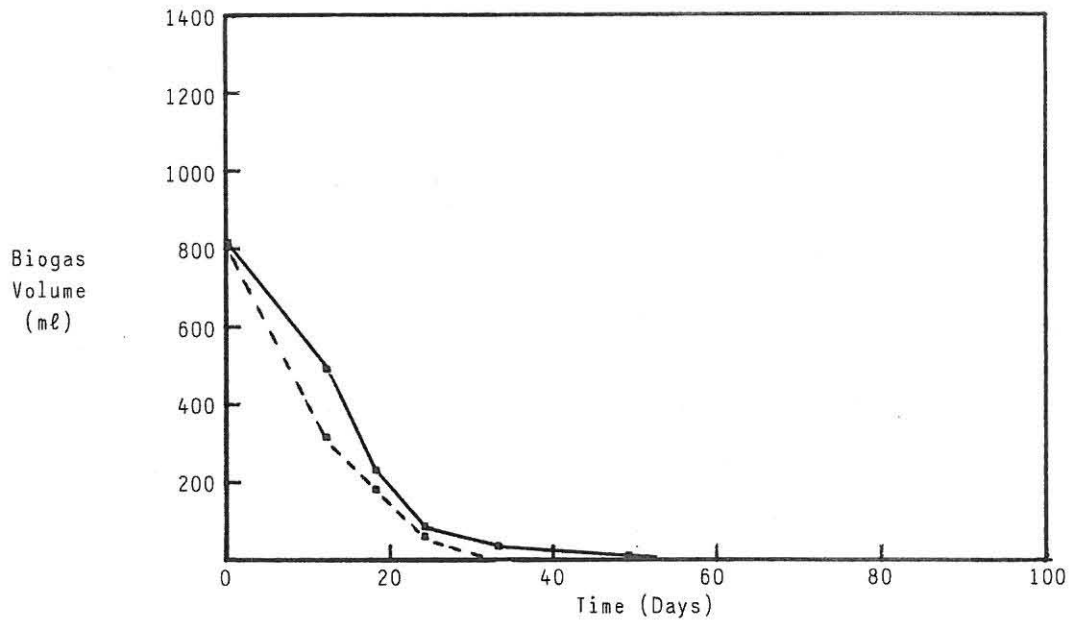


FIGURE 54 - Biogas production versus time for the anaerobic digester receiving pH-controlled tannery effluent (— pH-controlled digester; ---- non pH-controlled digester)

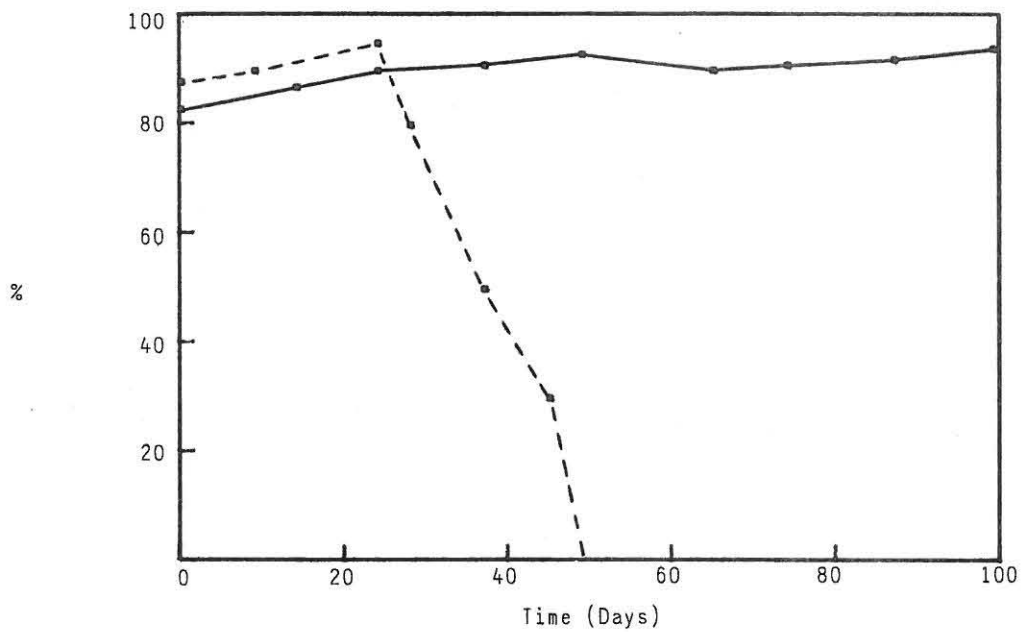


FIGURE 55 - Percentage CH_4 in the biogas versus time for the anaerobic digester receiving pH-controlled tannery effluent (— pH-controlled digester; ---- non pH-controlled digester)

TABLE 51

CHARACTERISTICS OF THE DIGESTER EFFLUENT AT VARIOUS STAGES
DURING THE EXPERIMENT TREATING pH-CONTROLLED TANNERY EFFLUENT

Parameter	Digester + No pH Control					Digester + pH Control				
	Time (Days)					Time (Days)				
	1	25	50	75	100	1	25	50	75	100
pH	7,64	7,30	7,58	7,87	8,30	7,36	7,23	7,38	7,49	7,78
Total COD (mg/l)	1429	240	280	492	450	1723	304	288	476	561
Soluble COD (mg/l)	323	144	208	323	323	350	128	280	411	353
Total PV (mg/l)	138	96	55	89	46	173	93	46	93	55
Soluble PV (mg/l)	97	92	46	81	43	144	81	43	81	46
Total Solids (mg/l)	1545	565	960	1410	2215	2300	605	1165	1380	2670
TIS (mg/l)	385	225	540	970	1945	1165	285	750	975	2005
SS (mg/l)	700	35	65	65	35	620	30	55	20	215
TDS (mg/l)	845	530	895	1345	2180	1680	575	1110	1360	2455
TDIS (mg/l)	335	210	485	955	1930	890	285	740	975	1910
Alkalinity (mg/l CaCO ₃)	1044	1332	1196	1242	1380	1188	1116	1058	1104	828
Bicarbonate Alkalinity (mg/l CaCO ₃)	959	1235	1076	1134	1260	1071	963	857	992	663
Conductivity (mS/m)	208	120	183	309	419	336	112	194	236	469
SO ₄ ²⁻ (mg/l)	120	99	263	422	690	123	108	304	324	775
Sulphide (mg/l)	32	40	80	104	136	32	24	80	168	128
TKN (mg/l)	142	64	58	97	161	142	71	58	90	174
NH ₃ -N (mg/l)	126	64	58	97	155	135	71	58	71	161
Organic N (mg/l)	16	0	0	0	6	7	0	0	19	13
Cl ⁻ (mg/l)	825	500	700	875	1275	1150	575	875	1025	1700
Na (mg/l)	130	90	450	180	310	210	130	130	180	400
Ca (mg/l)	113	126	40	70	80	143	90	60	70	90
Cr (mg/l)	<1	<1	<1	1,5	<1	<1	1,0	<1	1,0	<1
Al (mg/l)	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Fe (mg/l)	5,0	5,0	4,5	5,0	2,0	7,3	5,0	2,5	5,0	2,0
P (mg/l)	22,2	3,8	1,8	<1	<1	11,3	3,8	<1	<1	<1
VFA (mg/l)	120,3	136,9	169,9	152,4	169,2	164,9	215,9	284,4	157,8	232,5

Although the control of digester pH resulted in CH_4 production, there was no significant increase in the volume of biogas produced. Therefore, although the methanogenic bacteria were functional, it appears that they were being inhibited or suppressed by some factor, because although VFA were being produced, these were not being utilized. It therefore appears that a certain unknown factor was limiting the growth of the methanogens.

Although pH control is important for the metabolism of the methanogenic bacteria, only minor changes were noted in the operating efficiency of the pH-controlled digester relative to the digester where the pH was not controlled (Table 52). The volume of biogas produced by the pH-controlled digester was marginally greater than the non pH-controlled digester. Hence, the gas production and gas yield were also slightly greater. There was no significant improvement in the percentage COD and PV removed, although towards the end of the experiment, the percentage PV removed was slightly higher for the pH-controlled digester. The main difference between the two digesters, besides the concentration of CH_4 in the biogas, was the concentration of free NH_3 and H_2S . The concentration of both of these compounds is determined by the pH. It is evident that by controlling the pH, this lowers the concentration of free NH_3 , while the H_2S concentration in the biogas is increased.

Another minor difference between the two digesters is that the concentration of chloride ions is higher in the effluent from the pH-controlled digester (Table 51). This is because HCl was used to reduce the pH of the tannery effluent. These chloride ions also result in the conductivity and the inorganic solids being greater in the pH-controlled digester effluent.

A comparison of Tables 53 and 54 illustrates that the volume of the sludge bed was reduced during the experiment. This is due to compacting of the bed as well as degradation of some of the volatile solids. A similar effect occurred during the previous experiment.

The solids balance for the anaerobic digestion of pH-controlled tannery effluent is shown in Table 55. If the Total Volatile Solids (TVS) or the organic content of the effluent is concentrated on, then the following is obtained:-

$$\text{TVS degraded} = 58,803 \text{ g} - 50,830 \text{ g}$$

$$= 7,973 \text{ g}$$

$$= \underline{13,6 \% \text{ of TVS that entered}} \rightarrow$$

$$\text{TVS emerged} = 50,830 \text{ g}$$

$$= \underline{86,4 \% \text{ of TVS that entered}} \rightarrow$$

In addition to this, the organic content of the sludge bed was reduced by:
 $92,140 \text{ g} - 74,277 \text{ g} = 17,863 \text{ g}$
 $= \underline{19,4 \%} \rightarrow$

These results are similar to those obtained in the previous experiment and illustrate the inability of the digester to treat tannery effluent. A reduction in the volume of the sludge bed also took place.

TABLE 52
 OPERATING EFFICIENCY OF THE DIGESTER AT VARIOUS STAGES
 DURING THE EXPERIMENT TREATING pH-CONTROLLED TANNERY EFFLUENT

Parameter	Digester + No pH Control					Digester + pH Control				
	Time (Days)					Time (Days)				
	1	25	50	75	100	1	25	50	75	100
Gas Volume (ℓ/d)	0,840	0,095	0,00	0,00	0,00	0,850	0,120	0,045	0,025	0,015
Gas Production (m ³ /m ³ .d)	0,093	0,011	0,00	0,00	0,00	0,094	0,013	0,005	0,003	0,002
% COD Removed	0,0	33,3	47,0	58,4	58,8	0,0	0,0	37,9	59,2	57,7
% PV Removed	0,0	15,0	34,9	52,4	54,9	0,0	17,7	22,0	52,6	65,0
Gas Yield (m ³ /kg COD Removed)	-	0,792	0,00	0,00	0,00	-	-	0,256	0,036	0,023
CH ₄ Content of Biogas (%)	89,6	97,0	0,0	0,0	0,0	85,0	91,8	94,9	93,4	95,5
CH ₄ Yield (m ³ CH ₄ /kg COD Removed)	-	0,768	0,00	0,00	0,00	-	-	0,243	0,030	0,022
Free NH ₃ (mg/ℓ)	6,7	1,4	2,4	7,5	28,4	3,4	1,3	1,5	2,4	10,1
H ₂ S (mg/ℓ)	13,0	12,5	31,4	47,3	8,8	10,6	7,0	27,0	62,0	56,1

TABLE 53

STRATIGRAPHIC ANALYSIS OF THE DIGESTER CONTENTS AT THE START
OF THE pH-CONTROLLED TANNERY EFFLUENT EXPERIMENT

Parameter	Digester + No pH Control				Digester + pH Control			
	Height of Sampling Port Above Inlet (cm)				Height of Sampling Port Above Inlet (cm)			
	16	29	42	59	16	29	42	59
pH	7,39	7,60	7,54	7,64	7,46	7,42	7,53	7,36
Total COD (mg/l)	2818	1781	1717	1429	2609	1669	1856	1723
Soluble COD (mg/l)	362	377	356	323	392	387	409	350
Total PV (mg/l)	387	189	163	138	361	199	169	173
Soluble PV (mg/l)	93	102	92	97	141	118	127	144
Total Solids (mg/l)	3990	1910	1770	1545	3775	2100	1950	2300
TIS (mg/l)	1360	465	550	385	1525	920	870	1165
SS (mg/l)	3120	1020	950	700	2855	1000	985	620
TDS (mg/l)	870	890	820	845	920	1100	965	1680
TDIS (mg/l)	450	415	260	335	700	695	640	890
Alkalinity (mg/l CaCO ₃)	1096	1104	1096	1044	1104	1104	1136	1188
Bicarbonate Alkalinity (mg/l CaCO ₃)	998	1024	1018	959	992	1010	1033	1071
Conductivity (mS/m)	216	222	217	208	322	309	318	336
SO ₄ ²⁻ (mg/l)	132	118	135	120	119	108	117	123
Sulphide (mg/l)	36	40	25	32	40	24	32	32
TKN (mg/l)	420	168	154	142	394	178	152	142
NH ₃ -N (mg/l)	188	132	126	126	178	162	140	135
Organic N (mg/l)	232	36	28	16	216	16	12	7
Cl ⁻ (mg/l)	850	850	800	825	1100	1050	1075	1150
Na (mg/l)	115	120	120	130	200	185	200	210
Ca (mg/l)	180	120	110	113	205	130	135	143
Cr (mg/l)	12,5	1,0	1,0	< 1	17,5	1,0	1,0	< 1
Al (mg/l)	48,5	4,0	1,0	< 1	60,0	1,0	1,0	< 1
Fe (mg/l)	66,5	4,0	3,5	5,0	82,5	3,0	4,0	7,3
P (mg/l)	38,8	27,0	29,7	22,2	26,6	13,2	17,5	11,3
VFA (mg/l)	137,9	113,6	109,7	120,3	157,8	132,7	144,9	164,9

TABLE 54

STRATIGRAPHIC ANALYSIS OF THE DIGESTER CONTENTS ON COMPLETION
OF THE pH-CONTROLLED TANNERY EFFLUENT EXPERIMENT

Parameter	Digester + No pH Control				Digester + pH Control			
	Height of Sampling Port Above Inlet (cm)				Height of Sampling Port Above Inlet (cm)			
	16	29	42	59	16	29	42	59
pH	8,14	8,01	8,00	8,30	7,61	7,62	7,60	7,78
Total COD (mg/l)	445	553	422	450	691	499	565	561
Soluble COD (mg/l)	292	422	323	323	384	353	384	353
Total PV (mg/l)	208	221	161	46	214	202	115	55
Soluble PV (mg/l)	124	140	147	43	83	94	82	46
Total Solids (mg/l)	2835	2570	2390	2215	2915	2570	2440	2670
TIS (mg/l)	1830	1800	1520	1945	1750	2140	1855	2005
SS (mg/l)	520	270	155	35	560	110	100	215
TDS (mg/l)	2315	2300	2235	2180	2355	2460	2340	2455
TDIS (mg/l)	1790	1775	1415	1930	1725	2065	1805	1910
Alkalinity (mg/l CaCO ₃)	1150	1150	1196	1380	644	644	644	828
Bicarbonate Alkalinity (mg/l CaCO ₃)	1100	1124	1163	1260	516	531	523	663
Conductivity (mS/m)	454	454	439	419	480	479	460	469
SO ₄ ²⁻ (mg/l)	430	373	380	690	372	469	427	775
Sulphide (mg/l)	120	120	112	136	128	136	132	128
TKN (mg/l)	191	179	172	161	179	154	160	174
NH ₃ -N (mg/l)	172	172	166	155	166	154	160	161
Organic N (mg/l)	19	7	6	6	13	0	0	13
Cl ⁻ (mg/l)	1200	1275	1250	1275	1850	1825	1750	1700
Na (mg/l)	320	310	310	310	470	470	470	400
Ca (mg/l)	80	40	50	80	100	120	120	90
Cr (mg/l)	6,0	2,0	1,5	<1	28,0	3,0	2,5	<1
Al (mg/l)	10,0	5,0	<1	<1	5,0	<1	<1	<1
Fe (mg/l)	9,5	6,5	4,5	2,0	4,5	2,5	3,0	2,0
P (mg/l)	6,0	2,5	1,3	<1	2,5	<1	<1	<1
VFA (mg/l)	70,9	37,4	46,4	169,2	181,2	159,5	171,4	232,5

TABLE 55

SOLIDS BALANCE FOR THE ANAEROBIC DIGESTION
OF pH-CONTROLLED TANNERY EFFLUENT

<u>Solids Present in the Digester - Day 1</u>	
Total Solids	= 153,320 g
Total Volatile Solids	= 92,140 g
Total Inorganic Solids	= 61,180 g
<u>Solids that Entered the Digester - Day 1 to 100</u>	
Total Solids	= 132,070 g
Total Volatile Solids	= 58,803 g
Total Inorganic Solids	= 73,267 g
<u>Solids Present in the Digester - Day 100</u>	
Total Solids	= 128,196 g
Total Volatile Solids	= 74,277 g
Total Inorganic Solids	= 53,919 g
<u>Solids that Emerged from the Digester - Day 1 to 100</u>	
Total Solids	= 137,780 g
Total Volatile Solids	= 50,830 g
Total Inorganic Solids	= 86,950 g

From the results of this investigation, it can be concluded that pH control during the anaerobic digestion of tannery effluent is vital if methanogenesis is to take place. It was found that the influent pH had to be controlled in the range 4,0 - 5,0 in order to maintain the digester pH in the optimum range. This is slightly lower than the values of 5,0 - 5,5 suggested in previous investigations (Friedman et al, 1982; Bailey et al, 1984).

Although the maintenance of an optimum pH in the digester resulted in CH₄ being produced in the biogas, it did not result in any increase in the volume of biogas being produced. It would therefore appear that an unknown factor is limiting or suppressing the metabolism of the methanogens. This suppression could be due to a lack of nutrients i.e. essential nutrients, or due to competition between bacterial species present in the digester for the available substrates. These hypotheses were therefore investigated in more detail in subsequent experiments.

6.3 The Effect of Trace Nutrients on the Anaerobic Digestion of Tannery Effluent

6.3.1 Introduction

In the previous experiment the importance of pH control during the anaerobic digestion of tannery effluent was illustrated (Section 6.2). Although pH control ensured that CH_4 was present in the biogas, it did not result in an increase in the volume of biogas produced. It would therefore appear that the metabolism of the methanogenic bacteria was being suppressed, and it has been postulated that this could be due to a lack of essential trace nutrients present in the tannery effluent.

The effect of trace nutrients on anaerobic digestion is well documented. A large number of studies have shown that the efficiency of anaerobic digestion can be markedly improved through the supplementation of trace nutrients (Jones and Stadtman, 1977; Forsberg, 1978; Schönheit *et al*, 1979; Scherer and Sahm, 1981; Murray and Van den Berg, 1981; Speece *et al*, 1983; Nel *et al*, 1985; Canovas-Diaz and Howell, 1986; Zellner and Winter, 1987). Although the reason for this improved digester efficiency was initially unknown, recent detailed biochemical studies have shown that many of these trace nutrients are required as components of essential cofactors and enzymes involved in the metabolism of the methanogenic bacteria (Diekert *et al*, 1980; Diekert *et al*, 1981; Schauer and Ferry, 1982; Thauer *et al*, 1984; May *et al*, 1988; Cole, 1988; Lin *et al*, 1989; Scherer, 1989). As all methanogens have a specific growth requirement for trace nutrients, their availability is essential for the optimum metabolism of these bacteria.

Tannery effluents contain low concentrations of the nutrients regarded as being essential for the metabolism of the methanogenic bacteria (Table 56). In view of this, an investigation was carried out in order to determine whether it would be possible to successfully treat tannery effluents anaerobically through supplementation with the trace nutrients outlined in Table 56.

TABLE 56

THE CONCENTRATION OF TRACE NUTRIENTS
PRESENT IN TANNERY EFFLUENT

Trace Nutrient	Concentration (mg/l)
Nickel (Ni)	< 10
Cobalt (Co)	< 10
Molybdenum (Mo)	< 10
Tungsten (W)	< 10

6.3.2 Materials and Methods

The materials and methods used were described in detail in Chapter 4. The anaerobic digester was started up on a dilute tannery effluent as described previously (Section 6.1.2). As with the previous experiments, a constant hydraulic retention time of 9 days was maintained throughout the duration of this experiment. The pH of the effluent supplied to the digester was also controlled in the range 4,0 - 5,0 by using HCl. However, in addition to this, it was supplemented with the trace nutrients Ni, Co, Mo and W each at a concentration of 10 mg/l.

6.3.3 Results and Discussion

Despite supplementing the tannery effluent with trace nutrients, no improvement in the operating efficiency of the digester was observed. As in the previous two experiments, any increase in the loading rate resulted in a corresponding increase in the concentration of VFA (Figure 56), and it was also found necessary to reduce the loading rate on day 51, in order to lower the VFA concentration. However, as soon as the loading rate was increased again, the VFA concentration increased. Therefore, the supplementation of tannery effluent with trace nutrients does not enhance the metabolism of the methanogenic bacteria.

As in the previous experiment, the volume of biogas produced decreased rapidly, and almost ceased completely (Figure 57). There was no significant

increase in the volume of biogas produced, or the composition of the biogas (Figure 58) relative to the previous experiment. Thus although the pH was controlled in the optimum range in the digester (Table 57), the addition of trace nutrients did not improve the operating efficiency of the digester treating dilute tannery effluent (Table 58).

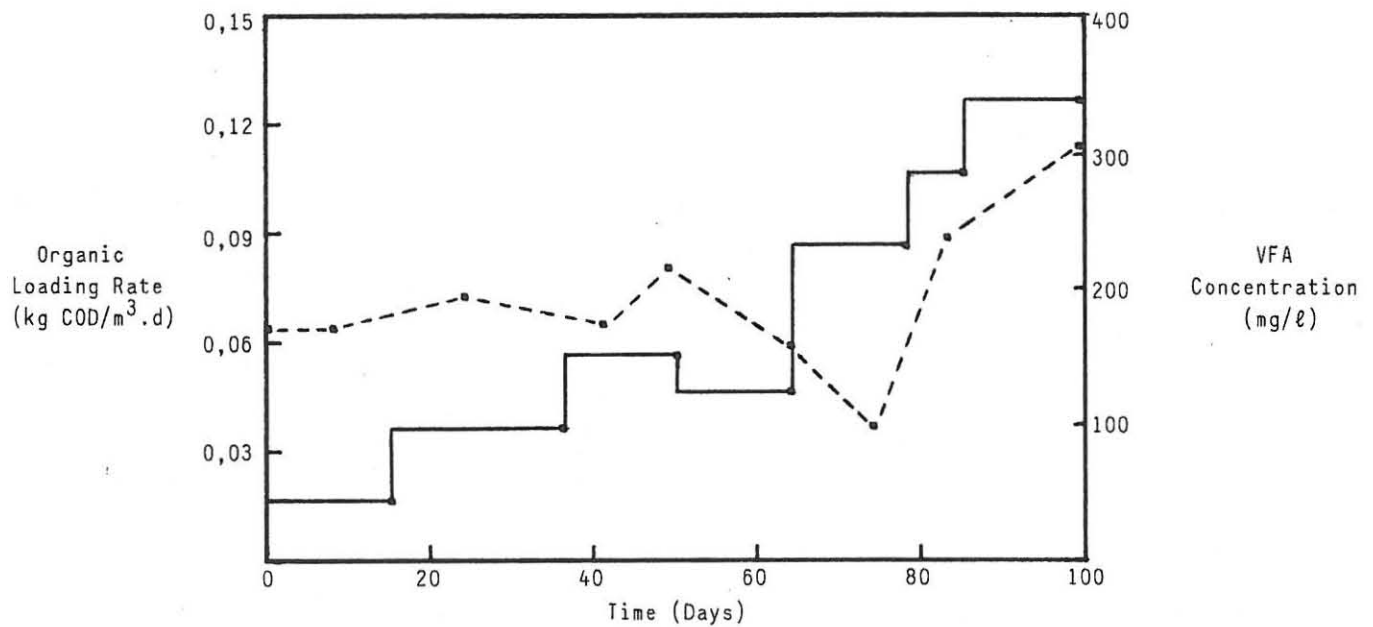


FIGURE 56 - Organic loading rate and effluent volatile fatty acids versus time for the anaerobic digester receiving pH-controlled tannery effluent supplemented with trace nutrients (— organic loading rate; ---- volatile fatty acids as acetic acid)

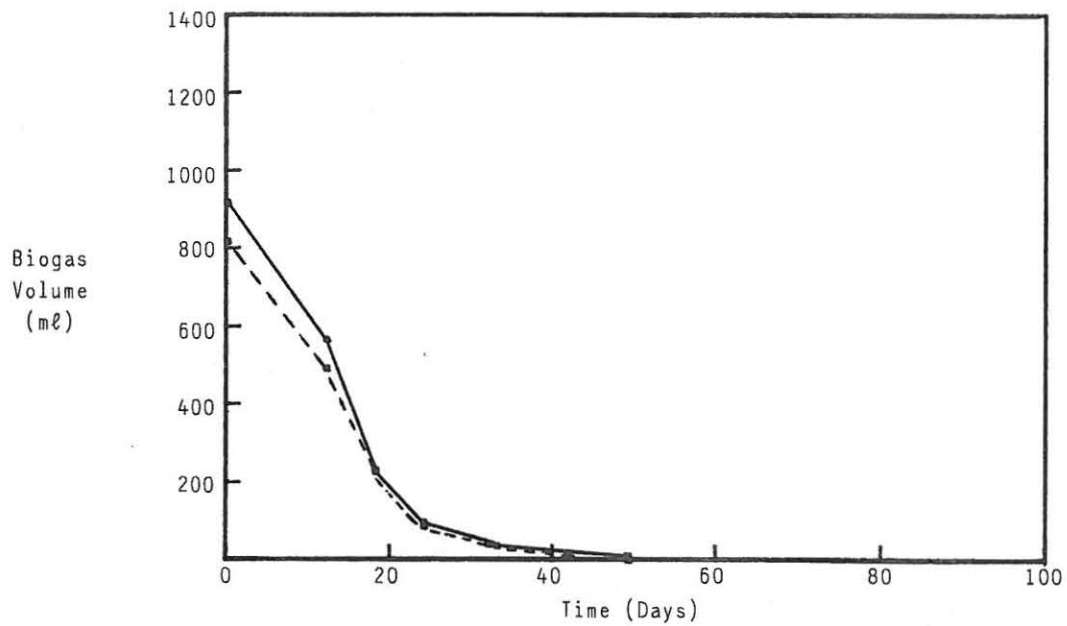


FIGURE 57 - Biogas production versus time for the anaerobic digester receiving pH-controlled tannery effluent supplemented with trace nutrients (— pH-controlled digester + trace nutrients; ---- pH-controlled digester)

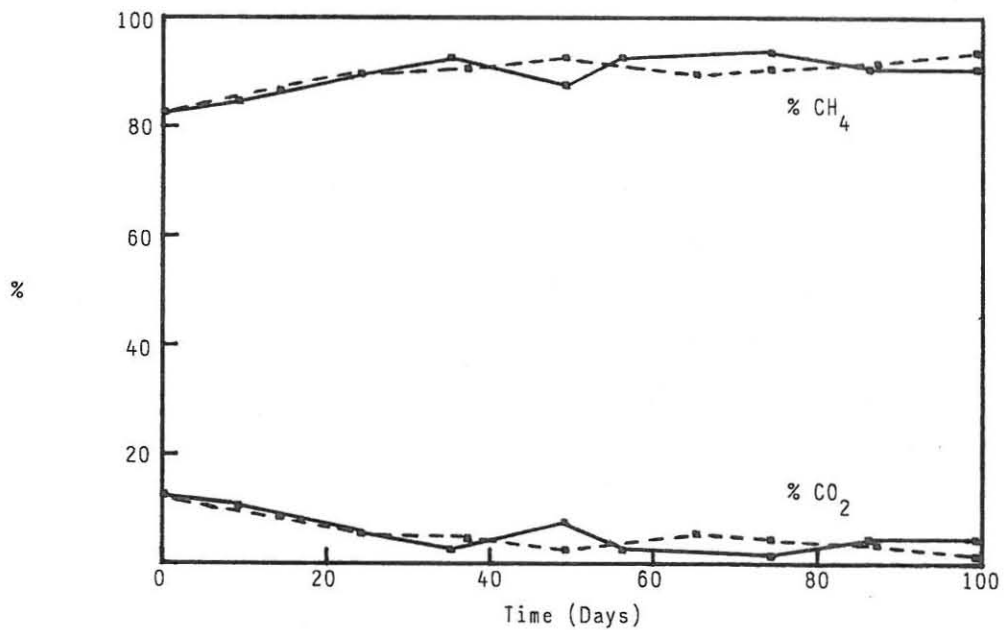


FIGURE 58 - Biogas percentage composition versus time for the anaerobic digester receiving pH-controlled tannery effluent supplemented with trace nutrients (— pH-controlled digester + trace nutrients; ---- pH-controlled digester)

TABLE 57

CHARACTERISTICS OF THE DIGESTER EFFLUENT AT VARIOUS STAGES DURING THE EXPERIMENT
TREATING pH-CONTROLLED TANNERY EFFLUENT SUPPLEMENTED WITH TRACE NUTRIENTS

Parameter	Digester + pH Control					Digester + pH Control + Trace Nutrients				
	Time (Days)					Time (Days)				
	1	25	50	75	100	1	25	50	75	100
pH	7,36	7,23	7,38	7,49	7,78	7,52	7,23	7,15	7,30	7,34
Total COD (mg/l)	1723	304	288	476	561	1986	276	360	430	476
Soluble COD (mg/l)	350	128	280	411	353	387	144	176	388	361
Total PV (mg/l)	173	93	46	93	55	175	90	46	81	100
Soluble PV (mg/l)	144	81	43	81	46	110	81	39	73	83
Total Solids (mg/l)	2300	605	1165	1380	2670	1650	635	1010	1405	2365
TIS (mg/l)	1165	285	750	975	2005	510	345	660	1015	1985
SS (mg/l)	620	30	55	20	215	795	75	40	80	20
TDS (mg/l)	1680	575	1110	1360	2455	855	560	970	1325	2345
TDIS (mg/l)	890	285	740	975	1910	410	290	645	965	1980
Alkalinity (mg/l CaCO ₃)	1188	1116	1058	1104	828	1170	864	966	736	644
Bicarbonate Alkalinity (mg/l CaCO ₃)	1071	963	857	992	663	1042	720	808	660	423
Conductivity (mS/m)	336	112	194	236	469	219	116	194	243	468
SO ₄ ²⁻ (mg/l)	123	108	304	324	775	333	63	364	286	606
Sulphide (mg/l)	32	24	80	168	128	16	24	44	56	120
TKN (mg/l)	142	71	58	90	174	129	52	58	86	154
NH ₃ -N (mg/l)	135	71	58	71	161	116	52	58	80	148
Organic N (mg/l)	7	0	0	19	13	13	0	0	6	6
Cl ⁻ (mg/l)	1150	575	875	1025	1700	950	600	850	1075	1675
Na (mg/l)	210	130	130	180	400	80	200	240	190	410
Ca (mg/l)	143	90	60	70	90	115	70	70	60	80
Cr (mg/l)	<1	1,0	<1	1,0	<1	<1	1,0	<1	1,0	1,0
Al (mg/l)	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Fe (mg/l)	7,3	5,0	2,5	5,0	2,0	8,9	6,0	2,5	6,5	2,0
P (mg/l)	11,3	3,8	<1	<1	<1	15,0	<1	<1	<1	<1
VFA (mg/l)	164,9	215,9	284,4	157,8	232,5	180,4	202,7	223,7	107,6	312,7

TABLE 58

OPERATING EFFICIENCY OF THE DIGESTER AT VARIOUS STAGES DURING THE EXPERIMENT
TREATING pH-CONTROLLED TANNERY EFFLUENT SUPPLEMENTED WITH TRACE NUTRIENTS

Parameter	Digester + pH Control					Digester + pH Control + Trace Nutrients				
	Time (Days)					Time (Days)				
	1	25	50	75	100	1	25	50	75	100
Gas Volume (ℓ/d)	0,850	0,120	0,045	0,025	0,015	0,950	0,130	0,035	0,020	0,030
Gas Production (m ³ /m ³ .d)	0,094	0,013	0,005	0,003	0,002	0,106	0,014	0,004	0,002	0,003
% COD Removed	0,0	0,0	37,9	59,2	57,7	0,0	0,0	29,7	67,3	59,2
% PV Removed	0,0	17,7	22,0	52,6	65,0	0,0	12,6	17,9	64,6	71,5
Gas Yield (m ³ /kg COD Removed)	-	-	0,256	0,036	0,023	-	-	0,230	0,023	0,043
CH ₄ Content of Biogas (%)	85,0	91,8	94,9	93,4	95,5	85,0	91,9	90,2	96,3	92,8
CH ₄ Yield (m ³ CH ₄ / kg COD Removed)	-	-	0,243	0,030	0,022	-	-	0,207	0,022	0,040
Free NH ₃ (mg/ℓ)	3,4	1,3	1,5	2,4	10,1	4,2	1,0	<1	1,8	3,6
H ₂ S (mg/ℓ)	10,6	7,0	27,0	62,0	56,1	6,0	7,0	11,6	17,5	39,1

A comparison of Tables 59 and 60 clearly illustrates that the volume of the sludge bed in the digester was markedly reduced during the course of the experiment. This is evident if one compares the values of the SS, TKN, organic nitrogen, COD and PV obtained from the lowest sampling port on day 1 and 100. A similar effect was found in the previous experiments.

TABLE 59

STRATIGRAPHIC ANALYSIS OF THE DIGESTER CONTENTS AT THE START OF THE EXPERIMENT
TREATING pH-CONTROLLED TANNERY EFFLUENT SUPPLEMENTED WITH TRACE NUTRIENTS

Parameter	Digester + pH Control				Digester + pH Control + Trace Nutrients			
	Height of Sampling Port Above Inlet (cm)				Height of Sampling Port Above Inlet (cm)			
	16	29	42	59	16	29	42	59
pH	7,46	7,42	7,53	7,36	7,43	7,56	7,50	7,52
Total COD (mg/l)	2609	1669	1856	1723	2885	1967	1882	1986
Soluble COD (mg/l)	392	387	409	350	411	392	429	387
Total PV (mg/l)	361	199	169	173	392	181	188	175
Soluble PV (mg/l)	141	118	127	144	152	127	109	110
Total Solids (mg/l)	3775	2100	1950	2300	3880	1925	1875	1650
TIS (mg/l)	1525	920	870	1165	1370	695	620	510
SS (mg/l)	2855	1000	985	620	2970	1130	900	795
TDS (mg/l)	920	1100	965	1680	910	795	975	855
TDIS (mg/l)	700	695	640	890	615	595	550	410
Alkalinity (mg/l CaCO ₃)	1104	1104	1136	1188	1132	1104	1156	1170
Bicarbonate Alkalinity (mg/l CaCO ₃)	992	1010	1033	1071	1025	998	1045	1042
Conductivity (mS/m)	322	309	318	336	233	249	252	219
SO ₄ ²⁻ (mg/l)	119	108	117	123	217	209	242	333
Sulphide (mg/l)	40	24	32	32	32	24	24	16
TKN (mg/l)	394	178	152	142	362	188	152	129
NH ₃ -N (mg/l)	178	162	140	135	172	162	144	116
Organic N (mg/l)	216	16	12	7	190	26	8	13
Cl ⁻ (mg/l)	1100	1050	1075	1150	975	1000	1050	950
Na (mg/l)	200	185	200	210	120	100	110	80
Ca (mg/l)	205	130	135	143	220	155	130	115
Cr (mg/l)	17,5	1,0	1,0	<1	26,5	1,0	1,0	<1
Al (mg/l)	60,0	1,0	1,0	<1	52,0	1,0	1,0	<1
Fe (mg/l)	82,5	3,0	4,0	7,3	78,5	4,5	3,5	8,9
P (mg/l)	26,6	13,2	17,5	11,3	23,3	18,7	13,7	15,0
VFA (mg/l)	157,8	132,7	144,9	164,9	151,8	149,2	156,7	180,4

TABLE 60

STRATIGRAPHIC ANALYSIS OF THE DIGESTER CONTENTS ON COMPLETION OF THE EXPERIMENT
TREATING pH-CONTROLLED TANNERY EFFLUENT SUPPLEMENTED WITH TRACE NUTRIENTS

Parameter	Digester + pH Control				Digester + pH Control + Trace Nutrients			
	Height of Sampling Port Above Inlet (cm)				Height of Sampling Port Above Inlet (cm)			
	16	29	42	59	16	29	42	59
pH	7,61	7,62	7,60	7,78	7,06	7,17	7,13	7,34
Total COD (mg/l)	691	499	565	561	634	461	384	476
Soluble COD (mg/l)	384	353	384	353	292	288	284	361
Total PV (mg/l)	214	202	115	55	204	144	118	100
Soluble PV (mg/l)	83	94	82	46	107	83	96	83
Total Solids (mg/l)	2915	2570	2440	2670	2850	2570	2485	2365
TIS (mg/l)	1750	2140	1855	2005	2005	1870	2035	1985
SS (mg/l)	560	110	100	215	560	140	20	20
TDS (mg/l)	2355	2460	2340	2455	2290	2430	2465	2345
TDIS (mg/l)	1725	2065	1805	1910	1885	1780	2025	1980
Alkalinity (mg/l CaCO ₃)	644	644	644	828	552	598	598	644
Bicarbonate Alkalinity (mg/l CaCO ₃)	516	531	523	663	362	399	400	423
Conductivity (mS/m)	480	479	460	469	496	494	471	468
SO ₄ ²⁻ (mg/l)	372	469	427	775	566	449	473	606
Sulphide (mg/l)	128	136	132	128	112	128	128	120
TKN (mg/l)	179	154	160	174	172	148	166	154
NH ₃ -N (mg/l)	166	154	160	161	160	148	154	148
Organic N (mg/l)	13	0	0	13	12	0	12	6
Cl ⁻ (mg/l)	1850	1825	1750	1700	1775	1825	1750	1675
Na (mg/l)	470	470	470	400	450	400	400	410
Ca (mg/l)	100	120	120	90	100	90	100	80
Cr (mg/l)	28,0	3,0	2,5	<1	14,0	3,0	2,0	1,0
Al (mg/l)	5,0	<1	<1	<1	5	<1	<1	<1
Fe (mg/l)	4,5	2,5	3,0	2,0	5,5	2,5	5,0	2,0
P (mg/l)	2,5	<1	<1	<1	1,3	<1	<1	<1
VFA (mg/l)	181,2	159,5	171,4	232,5	267,8	281,7	279,3	312,7

The solids balance for the anaerobic digestion of pH-controlled tannery effluent supplemented with trace nutrients is shown in Table 61. If the Total Volatile Solids (TVS) or the organic content of the effluent is concentrated on, then the following is obtained:-

$$\begin{aligned}\text{TVS degraded} &= 58,803 \text{ g} - 41,865 \text{ g} \\ &= 16,938 \text{ g} \\ &= \underline{28,8 \% \text{ of TVS that entered}} \rightarrow\end{aligned}$$

$$\begin{aligned}\text{TVS emerged} &= 41,865 \text{ g} \\ &= \underline{71,2 \% \text{ of TVS that entered}} \rightarrow\end{aligned}$$

In addition to this, the organic content of the sludge bed was reduced by:-

$$\begin{aligned}92,140 \text{ g} - 75,227 \text{ g} &= 16,913 \text{ g} \\ &= \underline{18,4 \%} \rightarrow\end{aligned}$$

TABLE 61

SOLIDS BALANCE FOR THE ANAEROBIC DIGESTION OF
pH-CONTROLLED TANNERY EFFLUENT SUPPLEMENTED
WITH TRACE NUTRIENTS

<u>Solids Present in the Digester - Day 1</u>	
Total Solids	= 153,320 g
Total Volatile Solids	= 92,140 g
Total Inorganic Solids	= 61,180 g
<u>Solids that Entered the Digester - Day 1 to 100</u>	
Total Solids	= 132,070 g
Total Volatile Solids	= 58,803 g
Total Inorganic Solids	= 73,267 g
<u>Solids Present in the Digester - Day 100</u>	
Total Solids	= 132,057 g
Total Volatile Solids	= 75,227 g
Total Inorganic Solids	= 56,830 g
<u>Solids that Emerged from the Digester - Day 1 to 100</u>	
Total Solids	= 125,415 g
Total Volatile Solids	= 41,865 g
Total Inorganic Solids	= 83,550 g

These results are similar to those obtained in the previous experiments, with the exception that a slightly higher percentage of the organic matter supplied to the digester was degraded i.e. 28,8 % as opposed to 15,8 and 13,6 %. A reduction in the volume of the sludge bed was, however, still noted.

Although an analysis indicated that tannery effluents could be deficient in essential trace nutrients, supplementation of the tannery effluent being treated with these nutrients did not result in any significant improvement in the functioning of the anaerobic digester. The methanogens were unable to utilize the VFA being produced by the acidogenic and acetogenic bacteria, and therefore, it is obvious that they were unable to function optimally. The results of this investigation clearly illustrate that the suppression of the metabolism of the methanogenic bacteria is not due to a lack of trace nutrients. However, the possibility does still exist that this inhibition could be due to competition between bacterial species present in the digester for available nutrients. In view of the high concentration of sulphur compounds present in tannery wastewaters, the most likely competitors for the methanogens would be the sulphate-reducing bacteria. This hypothesis was therefore investigated in more detail.

6.4 The Effect of Molybdenum on the Anaerobic Digestion of Tannery Effluent

6.4.1 Introduction

High concentrations of sulphate in wastewaters are inhibitory to the generation of methane during the anaerobic digestion of the waste, and several mechanisms have been proposed to explain this inhibitory effect.

The toxicity of sulphide or free H_2S produced by the microbial reduction of sulphate is often suggested as a factor of primary importance (Cappenberg, 1974; Lawrence et al, 1966). The kinetics of competition for the available electron donors between sulphate-reducing bacteria and methane-producing bacteria has also received considerable attention (Abram and Nedwell, 1978; Kristjansson et al, 1982; Schönheit et al, 1982). The sulphate-reducing bacteria apparently have a higher affinity for hydrogen and acetate, which are the major methane precursors, relative to the methanogens. This enables them to maintain the pool of these substrates at concentrations too low for the methanogens when sulphate is present in high concentrations. Thirdly, from a

purely thermodynamical point of view, the reduction of sulphate is energetically more favourable than the reduction of bicarbonate (Khosrovi et al, 1971).

As tannery wastewaters contain high concentrations of sulphur compounds, the sulphate-reducing bacteria would be one of the dominant microbial populations present in anaerobic digesters treating these wastes. It has been estimated that these sulphate-reducing bacteria can utilize between 53 and 92 % of the available substrate electrons (Isa et al, 1986). For this reason, control of the sulphate-reducing bacteria is desirable. The efficacy of potential inhibitors of sulphate-reducing bacteria such as antibiotics, detergents, dyes, mercurials, nitro-compounds, phenolics and sulphate analogues has been tested in pure cultures, sewage sludge and in soils (Saleh et al, 1964; Postgate, 1984). Sodium molybdate has been shown to be a specific inhibitor of the sulphate-reducing bacteria in sediment ecosystems, anaerobic digesters and mixed cultures (Banat et al, 1983; Hilton and Archer, 1988; Yadav and Archer, 1988). Molybdate is a structural analogue of sulphate which interacts with several enzyme systems which use sulphate. It replaces sulphate in the reaction catalyzed by ATP sulphurylase, giving rise to the formation of an unstable adenosine phosphomolybdate compound which breaks down spontaneously. This results in the degradation of ATP in the cell (Taylor and Oremland, 1979).

In view of the inhibitory nature of sodium molybdate on the sulphate-reducing bacteria, the present investigation was carried out to determine whether this inhibitor could play a role in ensuring the successful anaerobic digestion of tannery effluent.

6.4.2 Materials and Methods

The materials and methods used were described in detail in Chapter 4. The anaerobic digester was started up on a dilute tannery effluent as described previously (Section 6.1.2). As with the previous experiments, a constant hydraulic retention time of 9 days was maintained throughout the duration of this experiment. The pH of the effluent supplied to the digester was also controlled in the range 4,0 - 5,0 by using HCl. In addition to this, molybdenum was supplemented at a concentration of 100 mg/l, in order to inhibit the metabolism of the sulphate-reducing bacteria.

6.4.3 Results and Discussion

The addition of molybdenum to the tannery effluent did not result in any improvement in the operating efficiency of the digester. Any increase in the loading rate resulted in a corresponding increase in the concentration of VFA (Figure 59), and it was found necessary to reduce the loading rate twice in order to lower the VFA concentration. Therefore, the supplementation of tannery effluent with molybdenum does not result in the successful anaerobic digestion of this effluent. It appears that the metabolism of the methanogenic bacteria was still being suppressed.

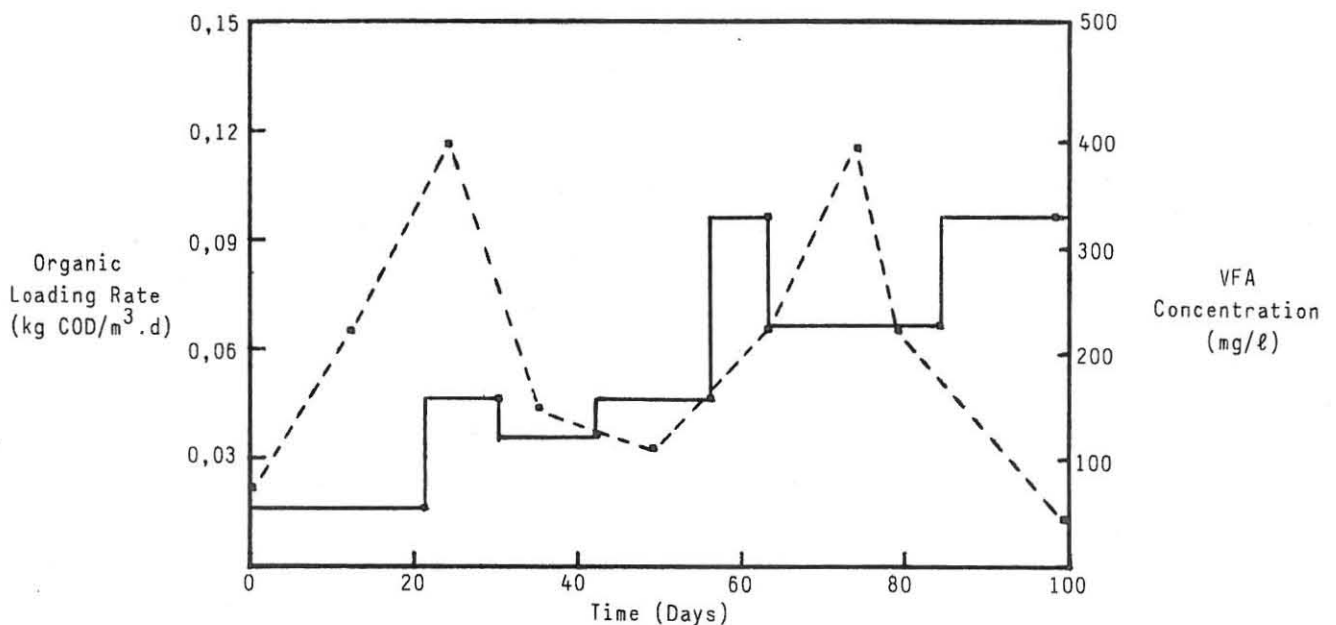


FIGURE 59 - Organic loading rate and effluent volatile fatty acids versus time for the anaerobic digester receiving pH-controlled tannery effluent supplemented with molybdenum (— organic loading rate; ---- volatile fatty acids as acetic acid)

The volume of biogas produced decreased rapidly, and almost ceased completely (Figure 60). There was no increase in the volume of biogas produced, or its composition (Figure 61) relative to the digester receiving pH-controlled tannery effluent (Section 6.2), and therefore there was no improvement in the operating efficiency of the digester (Table 62).

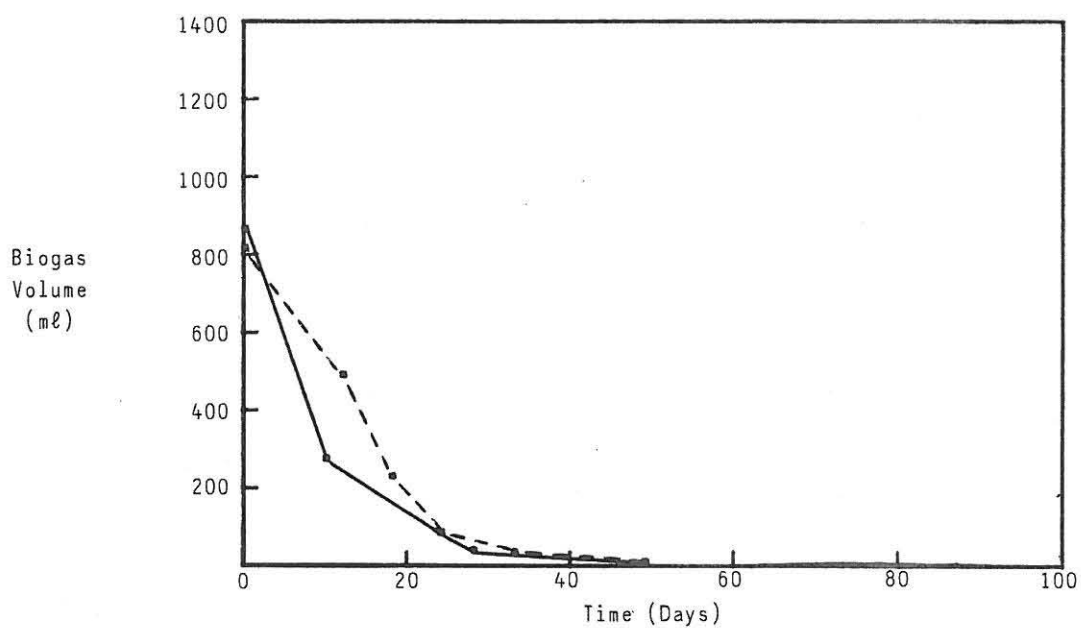


FIGURE 60 - Biogas production versus time for the anaerobic digester receiving pH-controlled tannery effluent supplemented with molybdenum (— pH-controlled digester + molybdenum; ---- pH-controlled digester)

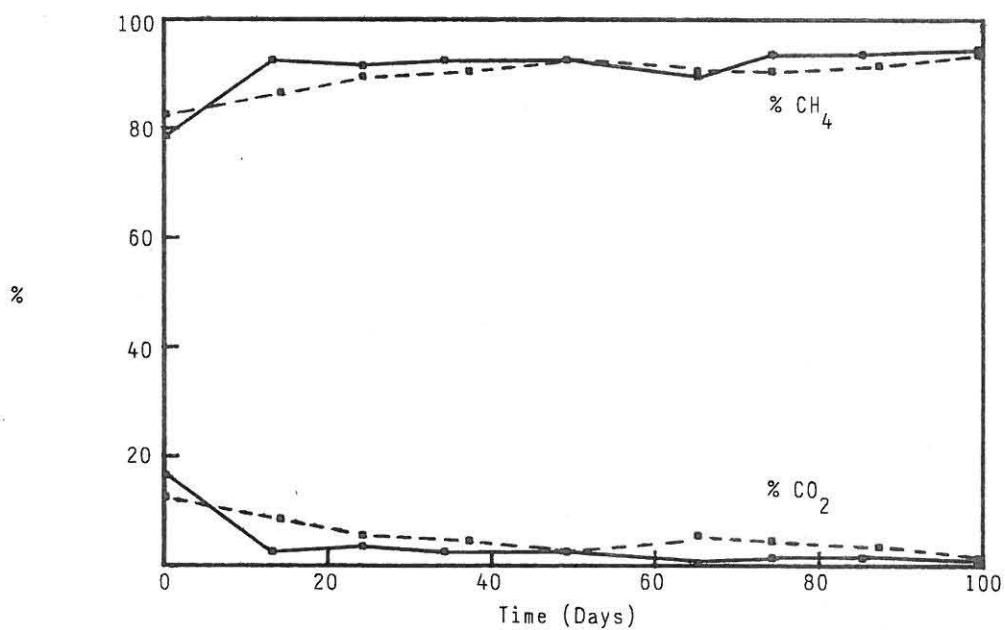


FIGURE 61 - Biogas percentage composition versus time for the anaerobic digester receiving pH-controlled tannery effluent supplemented with molybdenum (— pH-controlled digester + molybdenum; ---- pH-controlled digester)

TABLE 62

OPERATING EFFICIENCY OF THE DIGESTER AT VARIOUS STAGES DURING THE
EXPERIMENT TREATING pH-CONTROLLED TANNERY EFFLUENT SUPPLEMENTED WITH MOLYBDENUM

Parameter	Digester + pH Control					Digester + pH Control + Molybdenum				
	Time (Days)					Time (Days)				
	1	25	50	75	100	1	25	50	75	100
Gas Volume (ℓ/d)	0,850	0,120	0,045	0,025	0,015	0,900	0,120	0,040	0,040	0,025
Gas Production (m ³ /m ³ .d)	0,094	0,013	0,005	0,003	0,002	0,100	0,013	0,004	0,004	0,003
% COD Removed	0,0	0,0	37,9	59,2	57,7	0,0	0,0	32,1	35,6	50,0
% PV Removed	0,0	17,7	22,0	52,6	65,0	0,0	0,0	35,7	54,3	48,3
Gas Yield (m ³ /kg COD Removed)	-	-	0,256	0,036	0,023	-	-	0,261	0,168	0,065
CH ₄ Content of Biogas (%)	85,0	91,8	94,9	93,4	95,5	80,6	93,9	94,9	96,3	96,9
CH ₄ Yield (m ³ CH ₄ / kg COD Removed)	-	-	0,243	0,030	0,022	-	-	0,248	0,162	0,063
Free NH ₃ (mg/ℓ)	3,4	1,3	1,5	2,4	10,1	2,2	<1	<1	<1	<1
H ₂ S (mg/ℓ)	10,6	7,0	27,0	62,0	56,1	32,5	34,6	37,1	44,1	53,0

From Table 63, it is evident that the concentrations of most of the compounds present in the effluent from the digester receiving molybdenum were lower than the concentrations emerging from the digester receiving only pH-controlled effluent. The reason for this is that the loading rate was slightly lower for the digester receiving molybdenum. The large difference between the digester effluents at the start of the experiment (Table 63) could, however, not be attributed to the reduced loading rate. This was rather due to the slightly different composition of the sewage sludge used to seed the anaerobic digesters, and will be noticed in subsequent experiments.

TABLE 63

CHARACTERISTICS OF THE DIGESTER EFFLUENT AT VARIOUS STAGES DURING THE EXPERIMENT
TREATING pH-CONTROLLED TANNERY EFFLUENT SUPPLEMENTED WITH MOLYBDENUM

Parameter	Digester + pH Control					Digester + pH Control + Molybdenum				
	Time (Days)					Time (Days)				
	1	25	50	75	100	1	25	50	75	100
pH	7,36	7,23	7,38	7,49	7,78	7,11	7,07	7,08	7,10	7,22
Total COD (mg/l)	1723	304	288	476	561	672	288	323	430	384
Soluble COD (mg/l)	350	128	280	411	353	416	176	246	292	292
Total PV (mg/l)	173	93	46	93	55	77	41	36	42	62
Soluble PV (mg/l)	144	81	43	81	46	58	18	26	38	52
Total Solids (mg/l)	2300	605	1165	1380	2670	1025	775	1155	1535	1580
TIS (mg/l)	1165	285	750	975	2005	490	540	530	905	1040
SS (mg/l)	620	30	55	20	215	280	110	135	130	60
TDS (mg/l)	1680	575	1110	1360	2455	745	665	1020	1405	1520
TDIS (mg/l)	890	285	740	975	1910	230	450	475	810	1005
Alkalinity (mg/l CaCO ₃)	1188	1116	1058	1104	828	1134	903	756	840	882
Bicarbonate Alkalinity (mg/l CaCO ₃)	1071	963	857	992	663	1074	620	670	560	842
Conductivity (mS/m)	336	112	194	236	469	206	140	186	283	264
SO ₄ ²⁻ (mg/l)	123	108	304	324	775	134	288	367	598	459
Sulphide (mg/l)	32	24	80	168	128	128	144	152	176	184
TKN (mg/l)	142	71	58	90	174	197	80	55	63	59
NH ₃ -N (mg/l)	135	71	58	71	161	160	68	49	58	47
Organic N (mg/l)	7	0	0	19	13	37	12	6	5	12
Cl ⁻ (mg/l)	1150	575	875	1025	1700	700	700	825	1125	950
Na (mg/l)	210	130	130	180	400	80	110	220	240	230
Ca (mg/l)	143	90	60	70	90	90	43	55	73	73
Cr (mg/l)	<1	1,0	<1	1,0	<1	1,0	<1	1,5	1,2	1,3
Al (mg/l)	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Fe (mg/l)	7,3	5,0	2,5	5,0	2,0	5,0	5,2	5,1	4,3	3,8
P (mg/l)	11,3	3,8	<1	<1	<1	6,3	3,8	<1	<1	<1
VFA (mg/l)	164,9	215,9	284,4	157,8	232,5	84,5	400,2	121,3	395,9	56,2

TABLE 64

STRATIGRAPHIC ANALYSIS OF THE DIGESTER CONTENTS AT THE START OF THE EXPERIMENT
TREATING pH-CONTROLLED TANNERY EFFLUENT SUPPLEMENTED WITH MOLYBDENUM

Parameter	Digester + pH Control				Digester + pH Control + Molybdenum			
	Height of Sampling Port Above Inlet (cm)				Height of Sampling Port Above Inlet (cm)			
	16	29	42	59	16	29	42	59
pH	7,46	7,42	7,53	7,36	6,93	6,99	7,07	7,11
Total COD (mg/l)	2609	1669	1856	1723	4320	768	760	672
Soluble COD (mg/l)	392	387	409	350	416	432	448	416
Total PV (mg/l)	361	199	169	173	104	94	85	77
Soluble PV (mg/l)	141	118	127	144	77	64	38	58
Total Solids (mg/l)	3775	2100	1950	2300	3150	1065	1045	1025
TIS (mg/l)	1525	920	870	1165	1015	395	525	490
SS (mg/l)	2855	1000	985	620	2355	280	255	280
TDS (mg/l)	920	1100	965	1680	795	785	790	745
TDIS (mg/l)	700	695	640	890	210	330	355	230
Alkalinity (mg/l CaCO ₃)	1104	1104	1136	1188	1012	1012	966	1134
Bicarbonate Alkalinity (mg/l CaCO ₃)	992	1010	1033	1071	949	921	897	1074
Conductivity (mS/m)	322	309	318	336	236	235	235	206
SO ₄ ²⁻ (mg/l)	119	108	117	123	505	186	119	134
Sulphide (mg/l)	40	24	32	32	144	120	120	128
TKN (mg/l)	394	178	152	142	370	228	216	197
NH ₃ -N (mg/l)	178	162	140	135	209	185	191	160
Organic N (mg/l)	216	16	12	7	161	43	25	37
Cl ⁻ (mg/l)	1100	1050	1075	1150	900	900	825	700
Na (mg/l)	200	185	200	210	60	60	70	80
Ca (mg/l)	205	130	135	143	180	100	90	90
Cr (mg/l)	17,5	1,0	1,0	<1	3,0	1,0	1,5	1,0
Al (mg/l)	60,0	1,0	1,0	<1	60,0	5,0	1,0	<1
Fe (mg/l)	82,5	3,0	4,0	7,3	50,5	4,5	4,0	5,0
P (mg/l)	26,6	13,2	17,5	11,3	47,5	27,5	8,8	6,3
VFA (mg/l)	157,8	132,7	144,9	164,9	89,5	128,5	97,3	84,5

TABLE 65

STRATIGRAPHIC ANALYSIS OF THE DIGESTER CONTENTS ON COMPLETION OF THE EXPERIMENT
TREATING pH-CONTROLLED TANNERY EFFLUENT SUPPLEMENTED WITH MOLYBDENUM

Parameter	Digester + pH Control				Digester + pH Control + Molybdenum			
	Height of Sampling Port Above Inlet (cm)				Height of Sampling Port Above Inlet (cm)			
	16	29	42	59	16	29	42	59
pH	7,61	7,62	7,60	7,78	6,94	6,91	6,84	7,22
Total COD (mg/l)	691	499	565	561	3567	346	353	384
Soluble COD (mg/l)	384	353	384	353	230	215	230	292
Total PV (mg/l)	214	202	115	55	525	93	142	62
Soluble PV (mg/l)	83	94	82	46	86	41	65	52
Total Solids (mg/l)	2915	2570	2440	2670	4355	1655	1685	1580
TIS (mg/l)	1750	2140	1855	2005	2510	1325	1390	1040
SS (mg/l)	560	110	100	215	2765	95	135	60
TDS (mg/l)	2355	2460	2340	2455	1590	1560	1550	1520
TDIS (mg/l)	1725	2065	1805	1910	1460	1275	1375	1005
Alkalinity (mg/l CaCO ₃)	644	644	644	828	640	600	640	882
Bicarbonate Alkalinity (mg/l CaCO ₃)	516	531	523	663	586	529	554	842
Conductivity (mS/m)	480	479	460	469	276	275	274	264
SO ₄ ²⁻ (mg/l)	372	469	427	775	651	509	618	459
Sulphide (mg/l)	128	136	132	128	200	184	192	184
TKN (mg/l)	179	154	160	174	165	65	59	59
NH ₃ -N (mg/l)	166	154	160	161	71	59	59	47
Organic N (mg/l)	13	0	0	13	94	6	0	12
Cl ⁻ (mg/l)	1850	1825	1750	1700	1100	1100	1175	950
Na (mg/l)	470	470	470	400	240	240	230	230
Ca (mg/l)	100	120	120	90	116	58	68	73
Cr (mg/l)	28,0	3,0	2,5	<1	15,7	2,0	2,6	1,3
Al (mg/l)	5,0	<1	<1	<1	15,0	<1	<1	<1
Fe (mg/l)	4,5	2,5	3,0	2,0	16,0	4,4	4,6	3,8
P (mg/l)	2,5	<1	<1	<1	2,5	<1	<1	<1
VFA (mg/l)	181,2	159,5	171,4	232,5	76,6	99,8	120,9	56,2

Despite the addition of 100 mg/l molybdenum to the digester, high concentrations of sulphides were still produced. This is an indication, therefore, that the metabolism of the sulphate-reducing bacteria was not inhibited by the molybdenum. This could be due to the addition of too little molybdenum, or its precipitation as an inorganic complex in the digester, resulting in no free molybdenum available for the inhibition of the sulphate-reducing bacteria. The concentration used in this investigation i.e. 100 mg/l is much lower than that used in a previous study i.e. 1000 mg/l (Hilton and Archer 1988). However, the addition of 1000 mg/l molybdenum to a digester treating sulphate-rich wastewaters was found to not only inhibit the sulphate-reducing bacteria but also the methanogens (Hilton and Archer, 1988). This was therefore the reasoning behind using a concentration of 100 mg/l molybdenum. In pure culture experiments, a concentration of 10 mg/l Mo has been shown to be sufficient to selectively inhibit the sulphate-reducing bacteria (Yadav and Archer, 1988).

A comparison of Tables 64 and 65 illustrates that, as with the previous experiments, the volume of the sludge bed was reduced. However, it appears that this reduction was not as marked as in the previous experiments.

The solids balance for the anaerobic digestion of pH-controlled tannery effluent supplemented with molybdenum is shown in Table 66. If the Total Volatile Solids (TVS) or the organic content of the effluent is concentrated on, then the following is obtained:-

$$\begin{aligned}\text{TVS degraded} &= 52,731 \text{ g} - 46,075 \text{ g} \\ &= 6,656 \text{ g} \\ &= \underline{12,6 \% \text{ of TVS that entered}} \rightarrow\end{aligned}$$

$$\begin{aligned}\text{TVS emerged} &= 46,075 \text{ g} \\ &= \underline{87,4 \% \text{ of TVS that entered}} \rightarrow\end{aligned}$$

In addition to this, the organic content of the sludge bed was reduced by:-

$$\begin{aligned}117,200 \text{ g} - 90,675 \text{ g} &= 26,525 \text{ g} \\ &= \underline{22,6 \%} \rightarrow\end{aligned}$$

The addition of molybdenum was unable to improve the utilization of the

organic matter supplied to the digester. The reduction in the volume of the sludge bed was found to be greater than that in the previous experiments. This result is, however, contradicted by the results obtained from Tables 64 and 65.

TABLE 66

SOLIDS BALANCE FOR THE ANAEROBIC DIGESTION OF
pH-CONTROLLED TANNERY EFFLUENT SUPPLEMENTED
WITH MOLYBDENUM

<u>Solids Present in the Digester - Day 1</u>	
Total Solids	= 178,360 g
Total Volatile Solids	= 117,200 g
Total Inorganic Solids	= 61,160 g
<u>Solids that Entered the Digester - Day 1 to 100</u>	
Total Solids	= 118,440 g
Total Volatile Solids	= 52,731 g
Total Inorganic Solids	= 65,709 g
<u>Solids Present in the Digester - Day 100</u>	
Total Solids	= 150,282 g
Total Volatile Solids	= 90,675 g
Total Inorganic Solids	= 59,607 g
<u>Solids that Emerged from the Digester - Day 1 to 100</u>	
Total Solids	= 123,445 g
Total Volatile Solids	= 46,075 g
Total Inorganic Solids	= 77,370 g

As with the previous experiments, the successful anaerobic digestion of tannery effluent could not be achieved. This was despite the addition of molybdenum to the digester in order to try and inhibit the sulphate-reducing bacteria. However, it appears that these bacteria were not inhibited, as high concentrations of sulphide were formed throughout the duration of the experiment. In view of this, the possibility still exists that these bacteria could be suppressing the metabolism of the methanogenic bacteria. This hypothesis was therefore further investigated.

6.5 The Effect of Acetate Supplementation on the Anaerobic Digestion of Tannery Effluent

6.5.1 Introduction

During the anaerobic digestion of high sulphur-containing wastewaters, the sulphate-reducing bacteria can compete with the methanogens for available substrates (Abram and Nedwell, 1978; Kristjansson *et al*, 1982; Schönheit *et al*, 1982). Molybdenum has been shown to inhibit the metabolism of the sulphate-reducing bacteria, and therefore reduce the competition between the two species. However, in the previous experiment, the molybdenum did not inhibit the metabolism of the sulphate-reducers, as high concentrations of sulphide were still produced. This could be due to the fact that the concentration of molybdenum added was either too low, or it could be due to precipitation of the molybdenum as a complex and hence not being available for the inhibition of the bacteria. The possibility still exists, therefore, that the sulphate-reducers could be competing with the methanogens for the available substrates. In view of this, this experiment was carried out to determine whether the supplementation of additional substrate for the methanogens i.e. acetate, could overcome the competition between these two groups of bacteria, and hence result in the successful anaerobic digestion of the tannery effluent.

6.5.2 Materials and Methods

The materials and methods used were described in detail in Chapter 4. The anaerobic digester was started up on a dilute tannery effluent as described previously (Section 6.1.2). As with the previous experiments, a constant hydraulic retention time of 9 days was maintained throughout the duration of this experiment. Acetate was supplemented at a concentration of 10 ml (S.G. = 1,050) per litre of undiluted tannery effluent to provide additional substrate for the methanogenic bacteria. The influent pH was in the range 3,5 - 4,0.

6.5.3 Results and Discussion

The addition of acetate to the tannery effluent did not result in any improvement in the operating efficiency of the digester. Any increase in the loading rate resulted in a corresponding increase in the concentration of VFA,

especially acetic acid (Figure 62), and it was found necessary to reduce the loading rate twice in order to lower the VFA concentration. The acetate added to the digester was initially utilized. However, after 50 days the concentration began to increase and this was a clear indication that the metabolism of the methanogens was being suppressed. However, it is important to note that this suppression appears not to be due to competition between the sulphate-reducing bacteria and the methanogens. If it was, the excess acetate would have overcome this suppression.

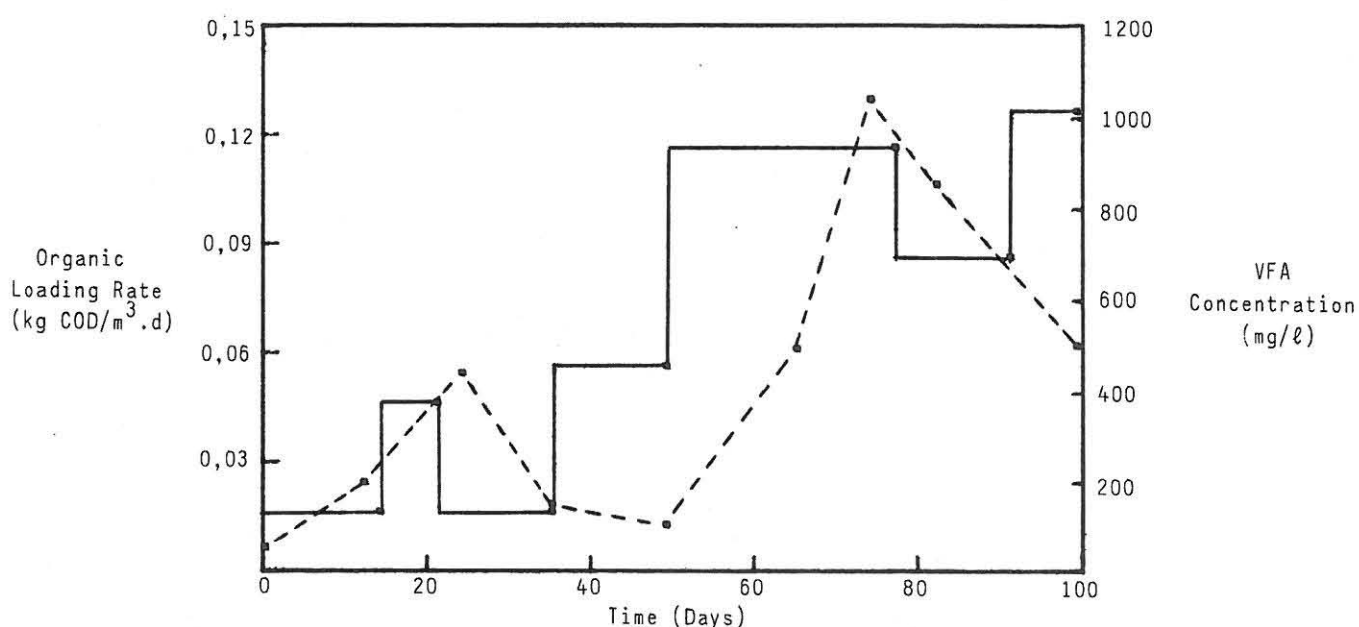


FIGURE 62 - Organic loading rate and effluent volatile fatty acids versus time for the anaerobic digester receiving tannery effluent supplemented with acetate (— organic loading rate; ---- volatile fatty acids as acetic acid)

The volume of biogas produced initially increased (Figure 63). This was due to the additional substrate i.e. acetate being available for conversion to methane by the methanogens. However, within two weeks, it decreased rapidly and almost ceased completely. The percentage methane in the biogas was lower than that obtained for the digester receiving pH-controlled tannery effluent (Figure 64). This is probably due to the high concentration of VFA further inhibiting the methanogens.

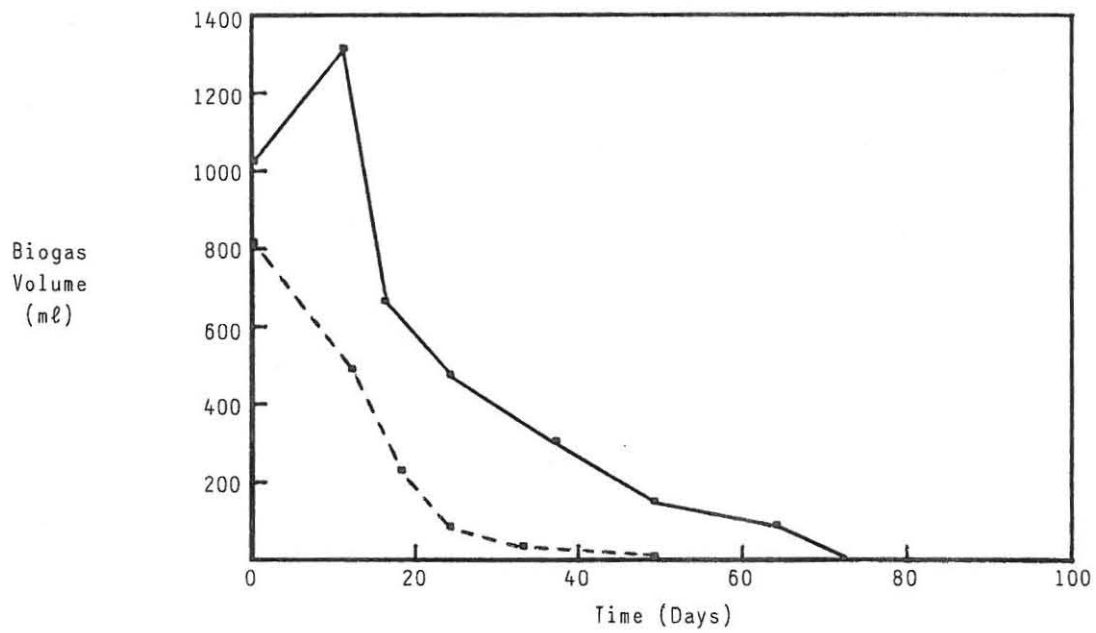


FIGURE 63 - Biogas production versus time for the anaerobic digester receiving tannery effluent supplemented with acetate (— digester + acetate; ---- pH-controlled digester)

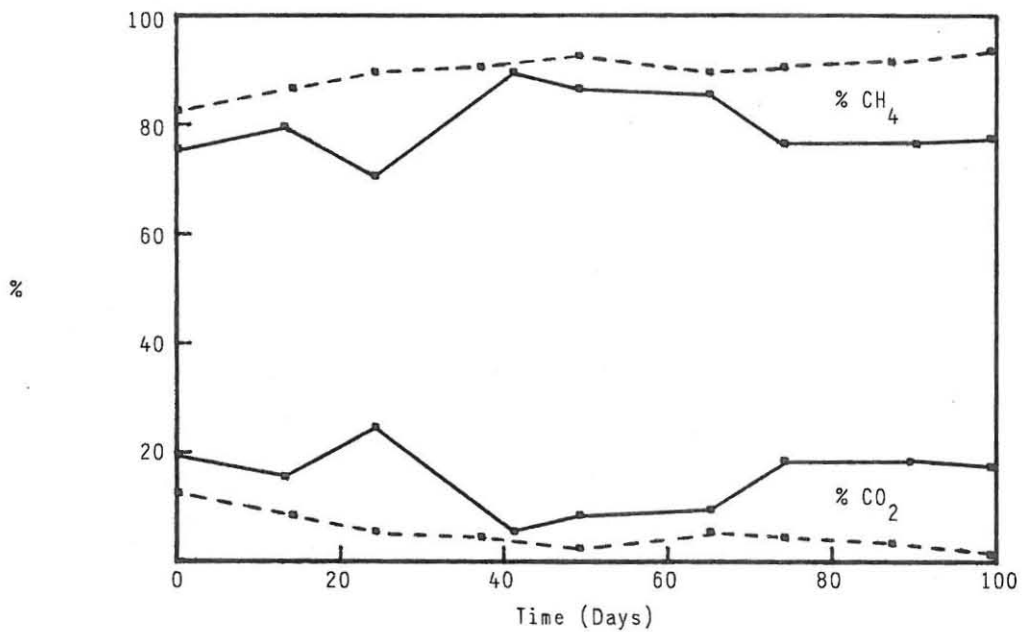


FIGURE 64 - Biogas percentage composition versus time for the anaerobic digester receiving tannery effluent supplemented with acetate (— digester + acetate; ---- pH-controlled digester)

TABLE 67

CHARACTERISTICS OF THE DIGESTER EFFLUENT AT VARIOUS STAGES DURING THE
EXPERIMENT TREATING TANNERY EFFLUENT SUPPLEMENTED WITH ACETATE

Parameter	Digester + pH Control					Digester + Acetate				
	Time (Days)					Time (Days)				
	1	25	50	75	100	1	25	50	75	100
pH	7,36	7,23	7,38	7,49	7,78	7,26	7,15	6,80	6,10	4,56
Total COD (mg/l)	1723	304	288	476	561	528	416	292	845	937
Soluble COD (mg/l)	350	128	280	411	353	396	320	169	737	891
Total PV (mg/l)	173	93	46	93	55	40	49	65	50	50
Soluble PV (mg/l)	144	81	43	81	46	34	39	48	20	48
Total Solids (mg/l)	2300	605	1165	1380	2670	1095	1040	895	1980	1820
TIS (mg/l)	1165	285	750	975	2005	440	470	400	940	1025
SS (mg/l)	620	30	55	20	215	265	315	140	235	185
TDS (mg/l)	1680	575	1110	1360	2455	830	725	755	1745	1635
TDIS (mg/l)	890	285	740	975	1910	345	455	315	800	960
Alkalinity (mg/l CaCO ₃)	1188	1116	1058	1104	828	987	966	798	630	120
Bicarbonate Alkalinity (mg/l CaCO ₃)	1071	963	857	992	663	929	637	705	-	-
Conductivity (mS/m)	336	112	194	236	469	253	154	162	252	260
SO ₄ ²⁻ (mg/l)	123	108	304	324	775	404	209	353	375	630
Sulphide (mg/l)	32	24	80	168	128	136	184	208	176	176
TKN (mg/l)	142	71	58	90	174	277	172	55	58	36
NH ₃ -N (mg/l)	135	71	58	71	161	246	92	49	58	36
Organic N (mg/l)	7	0	0	19	13	31	80	6	0	0
Cl ⁻ (mg/l)	1150	575	875	1025	1700	900	700	625	875	900
Na (mg/l)	210	130	130	180	400	70	80	160	210	210
Ca (mg/l)	143	90	60	70	90	80	86	46	110	96
Cr (mg/l)	<1	1,0	<1	1,0	<1	1,0	1,0	1,2	1,80	1,90
Al (mg/l)	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Fe (mg/l)	7,3	5,0	2,5	5,0	2,0	3,0	5,4	4,1	6,2	11,3
P (mg/l)	11,3	3,8	<1	<1	<1	17,5	6,3	2,5	2,5	<1
VFA (mg/l)	164,9	215,9	284,4	157,8	232,5	82,4	464,2	131,5	1066,4	524,6

The large increase in the concentration of VFA in the digester resulted in a decrease in the digester pH (Table 67). This in turn resulted in a decrease in the alkalinity, and also affected the concentration of free NH_3 and H_2S (Table 68). The percentage COD removed was also significantly reduced towards the end of the experiment. As HCl was not required to reduce the pH of the tannery effluent, the concentration of chloride ions and hence the conductivity were lower in the effluent from the digester receiving acetate than from the digester receiving pH-controlled tannery effluent. The low pH in the digester also appeared to solubilize the heavy metals, as indicated by the concentration of Fe and Cr in the digester effluent (Table 67).

TABLE 68
OPERATING EFFICIENCY OF THE DIGESTER AT VARIOUS STAGES DURING THE
EXPERIMENT TREATING TANNERY EFFLUENT SUPPLEMENTED WITH ACETATE

Parameter	Digester + pH Control					Digester + Acetate				
	Time (Days)					Time (Days)				
	1	25	50	75	100	1	25	50	75	100
Gas Volume (l/d)	0,850	0,120	0,045	0,025	0,015	1,060	0,510	0,185	0,020	0,015
Gas Production ($\text{m}^3/\text{m}^3 \cdot \text{d}$)	0,094	0,013	0,005	0,003	0,002	0,118	0,057	0,021	0,002	0,002
% COD Removed	0,0	0,0	37,9	59,2	57,7	0,0	0,0	47,2	67,2	17,5
% PV Removed	0,0	17,7	22,0	52,6	65,0	0,0	0,0	0,0	35,1	51,0
Gas Yield (m^3/kg COD Removed)	-	-	0,256	0,036	0,023	-	-	0,709	0,012	0,075
CH_4 Content of Biogas (%)	85,0	91,8	94,9	93,4	95,5	78,4	73,1	88,9	79,0	80,0
CH_4 Yield ($\text{m}^3 \text{CH}_4 /$ kg COD Removed)	-	-	0,243	0,030	0,022	-	-	0,630	0,009	0,060
Free NH_3 (mg/l)	3,4	1,3	1,5	2,4	10,1	4,9	1,5	<1	<1	<1
H_2S (mg/l)	10,6	7,0	27,0	62,0	56,1	40,7	48,7	111,0	86,3	96,1

TABLE 69

STRATIGRAPHIC ANALYSIS OF THE DIGESTER CONTENTS AT THE START OF THE
EXPERIMENT TREATING TANNERY EFFLUENT SUPPLEMENTED WITH ACETATE

Parameter	Digester + pH Control				Digester + Acetate			
	Height of Sampling Port Above Inlet (cm)				Height of Sampling Port Above Inlet (cm)			
	16	29	42	59	16	29	42	59
pH	7,46	7,42	7,53	7,36	6,94	7,09	7,04	7,26
Total COD (mg/l)	2609	1669	1856	1723	3080	1228	680	528
Soluble COD (mg/l)	392	387	409	350	628	520	536	396
Total PV (mg/l)	361	199	169	173	704	89	55	40
Soluble PV (mg/l)	141	118	127	144	77	56	43	34
Total Solids (mg/l)	3775	2100	1950	2300	6925	1460	1170	1095
TIS (mg/l)	1525	920	870	1165	2150	385	530	440
SS (mg/l)	2855	1000	985	620	6015	475	340	265
TDS (mg/l)	920	1100	965	1680	910	985	830	830
TDIS (mg/l)	700	695	640	890	625	140	340	345
Alkalinity (mg/l CaCO ₃)	1104	1104	1136	1188	924	1008	1008	987
Bicarbonate Alkalinity (mg/l CaCO ₃)	992	1010	1033	1071	848	961	961	929
Conductivity (mS/m)	322	309	318	336	269	266	267	253
SO ₄ ²⁻ (mg/l)	119	108	117	123	440	377	375	404
Sulphide (mg/l)	40	24	32	32	160	152	112	136
TKN (mg/l)	394	178	152	142	468	271	240	277
NH ₃ -N (mg/l)	178	162	140	135	228	259	228	246
Organic N (mg/l)	216	16	12	7	240	12	12	31
Cl ⁻ (mg/l)	1100	1050	1075	1150	900	875	825	900
Na (mg/l)	200	185	200	210	70	70	60	70
Ca (mg/l)	205	130	135	143	150	90	90	80
Cr (mg/l)	17,5	1,0	1,0	<1	3,0	1,0	<1	1,0
Al (mg/l)	60,0	1,0	1,0	<1	60,0	1,0	1,0	<1
Fe (mg/l)	82,5	3,0	4,0	7,3	52,5	6,5	4,0	3,0
P (mg/l)	26,6	13,2	17,5	11,3	27,5	7,5	6,3	17,5
VFA (mg/l)	157,8	132,7	144,9	164,9	106,8	66,5	66,4	82,4

TABLE 70

STRATIGRAPHIC ANALYSIS OF THE DIGESTER CONTENTS ON COMPLETION OF THE
EXPERIMENT TREATING TANNERY EFFLUENT SUPPLEMENTED WITH ACETATE

Parameter	Digester + pH Control				Digester + Acetate			
	Height of Sampling Port Above Inlet (cm)				Height of Sampling Port Above Inlet (cm)			
	16	29	42	59	16	29	42	59
pH	7,61	7,62	7,60	7,78	4,40	4,39	4,40	4,56
Total COD (mg/l)	691	499	565	561	4301	2006	1428	937
Soluble COD (mg/l)	384	353	384	353	814	860	829	891
Total PV (mg/l)	214	202	115	55	549	175	98	50
Soluble PV (mg/l)	83	94	82	46	37	24	24	48
Total Solids (mg/l)	2915	2570	2440	2670	4140	2805	2290	1820
TIS (mg/l)	1750	2140	1855	2005	2170	1810	1735	1025
SS (mg/l)	560	110	100	215	1895	585	480	185
TDS (mg/l)	2355	2460	2340	2455	2245	2220	1810	1635
TDIS (mg/l)	1725	2065	1805	1910	1485	1550	1550	960
Alkalinity (mg/l CaCO ₃)	644	644	644	828	160	160	120	120
Bicarbonate Alkalinity (mg/l CaCO ₃)	516	531	523	663	-	-	-	-
Conductivity (mS/m)	480	479	460	469	272	272	274	260
SO ₄ ²⁻ (mg/l)	372	469	427	775	620	700	702	630
Sulphide (mg/l)	128	136	132	128	176	176	184	176
TKN (mg/l)	179	154	160	174	194	100	76	36
NH ₃ -N (mg/l)	166	154	160	161	71	53	53	36
Organic N (mg/l)	13	0	0	13	123	47	23	0
Cl ⁻ (mg/l)	1850	1825	1750	1700	1175	1125	1125	900
Na (mg/l)	470	470	470	400	280	270	270	210
Ca (mg/l)	100	120	120	90	96	90	88	96
Cr (mg/l)	28,0	3,0	2,5	<1	58,9	6,1	5,6	1,9
Al (mg/l)	5,0	<1	<1	<1	40	10	<1	<1
Fe (mg/l)	4,5	2,5	3,0	2,0	34,6	21,2	17,8	11,3
P (mg/l)	2,5	<1	<1	<1	2,5	2,2	<1	<1
VFA (mg/l)	181,2	159,5	171,4	232,5	820,1	695,1	758,8	524,6

A comparison of Tables 69 and 70 illustrates that the heavy metals accumulate at the base of the digester i.e. in the sludge bed, although this is not clearly evident as a result of settling and compacting of the sludge bed. However, the low pH in the acetate digester did solubilize some of these metals i.e. the Fe and Cr, and their binding to the organic matter in the sludge bed was not as distinct as with the control digester. Higher concentrations of these metals were therefore present in the effluent from the acetate digester (Table 67).

TABLE 71

SOLIDS BALANCE FOR THE ANAEROBIC DIGESTION OF
TANNERY EFFLUENT SUPPLEMENTED WITH ACETATE

<u>Solids Present in the Digester - Day 1</u>	
Total Solids	= 178,360 g
Total Volatile Solids	= 117,200 g
Total Inorganic Solids	= 61,160 g
<u>Solids that Entered the Digester - Day 1 to 100</u>	
Total Solids	= 261,599 g
Total Volatile Solids	= 205,538 g
Total Inorganic Solids	= 56,061 g
<u>Solids Present in the Digester - Day 100</u>	
Total Solids	= 144,840 g
Total Volatile Solids	= 93,240 g
Total Inorganic Solids	= 51,600 g
<u>Solids that Emerged from the Digester - Day 1 to 100</u>	
Total Solids	= 150,330 g
Total Volatile Solids	= 78,875 g
Total Inorganic Solids	= 71,455 g

The solids balance for the anaerobic digestion of tannery effluent supplemented with acetate is shown in Table 71. If the Total Volatile Solids (TVS) or the organic content of the effluent is concentrated on, then the following is obtained:-

$$\begin{aligned}\text{TVS degraded} &= 205,538 \text{ g} - 78,875 \text{ g} \\ &= 126,663 \text{ g}\end{aligned}$$

$$= \underline{61,6 \% \text{ of TVS that entered}} \rightarrow$$

TVS emerged = 78,875 g

$$= \underline{38,4 \% \text{ of TVS that entered}} \rightarrow$$

In addition to this, the organic content of the sludge bed was reduced by:-

$$\begin{aligned} 117,200 \text{ g} - 93,240 \text{ g} &= 23,960 \text{ g} \\ &= \underline{20,4 \%} \rightarrow \end{aligned}$$

A much greater proportion of the organic matter supplied to the digester was degraded, as compared with previous experiments. The reason for this is that most of this organic matter was in the form of acetate, which was readily utilized by the non-methanogenic bacteria e.g. the sulphate-reducers, etc.

Despite the addition of acetate to the digester, the successful anaerobic digestion of the tannery effluent could not be achieved. A major proportion of the acetate added to the digester was not utilized by the methanogens. It would therefore appear that the methanogens are not being inhibited by the sulphate-reducing bacteria competing for common substrates. If this was the case, then the acetate would have reversed this inhibition. The results of this investigation therefore provide evidence to illustrate that it is not the high concentrations of sulphate present in the tannery effluent that are inhibiting or suppressing the metabolism of the methanogens, but rather some unknown factor. This is in agreement with the findings of the synthetic study (Section 5.3) where it was shown that methanogenesis can take place in the presence of high concentrations of sulphate. The following experiment provides further evidence to support the above finding.

6.6 The Anaerobic Digestion of Low-Sulphate Tannery Effluent

6.6.1 Introduction

Barium salts have been used successfully to remove sulphates from industrial wastewaters (Maree et al, 1989). The sulphates precipitate as barium sulphate, resulting in a relatively sulphate-free effluent. In the previous experiment, it was shown that the suppression of the metabolism of the methanogens is not caused through competition for substrates between these

bacteria and the sulphate-reducing bacteria. In this experiment the anaerobic digestion of a low-sulphate tannery effluent, which was achieved through the use of barium chloride, provides further evidence to support the findings of the previous experiment.

6.6.2 Materials and Methods

The materials and methods used were described in detail in Chapter 4. The anaerobic digester was started up on a dilute low-sulphate tannery effluent as described previously (Section 6.1.2). As with the previous experiments, a constant hydraulic retention time of 9 days was maintained throughout the duration of the experiment. The pH of the low-sulphate tannery effluent was 4,0 - 5,0, which is the optimum pH at which BaSO_4 precipitates (APHA, 1980).

6.6.3 Results and Discussion

Despite the presence of a low concentration of sulphate (100 - 200 mg/l) in the tannery effluent being supplied to the digester, this did not result in any improvement in the operating efficiency of the digester. The loading rate had to be reduced twice in order to lower the VFA that had accumulated in the digester (Figure 65). The metabolism of the methanogens was, therefore, still being suppressed as they were unable to utilize the VFA being produced.

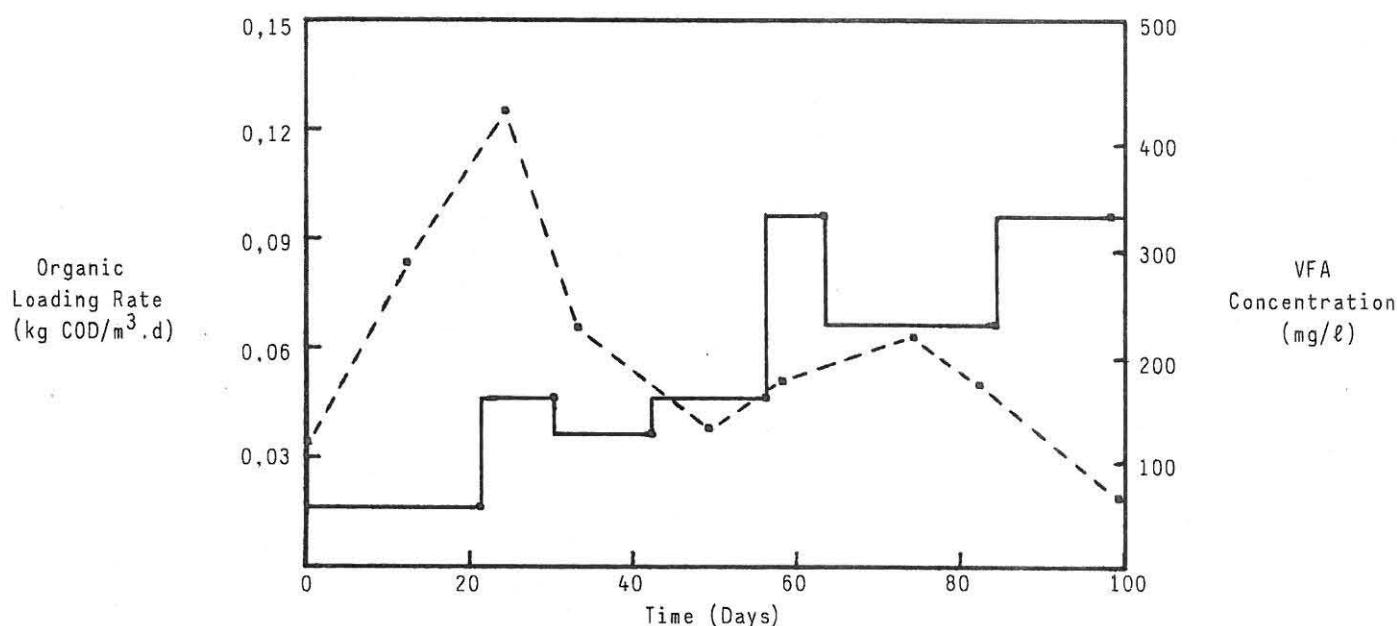


FIGURE 65 - Organic loading rate and effluent volatile fatty acids versus time for the anaerobic digester receiving pH-controlled low-sulphate tannery effluent (— organic loading rate; ---- volatile fatty acids as acetic acid)

Because the concentration of sulphate was low relative to the substrates available, it is unlikely that the sulphate-reducing bacteria could outcompete the methanogens for these substrates. The suppression of the metabolism of the methanogens therefore does not appear to be due to the high concentration of sulphates present in tannery effluents, but rather due to the presence or absence of some unknown compound.

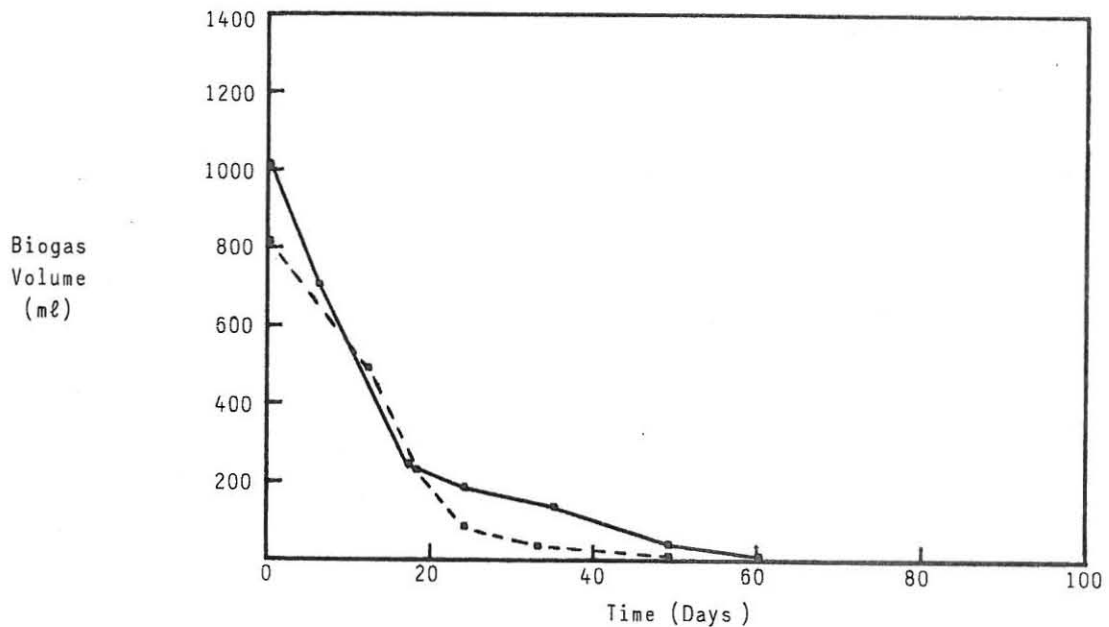


FIGURE 66 - Biogas production versus time for the anaerobic digester receiving pH-controlled low-sulphate tannery effluent (— pH-controlled digester + low SO_4^{2-} effluent; ---- pH-controlled digester)

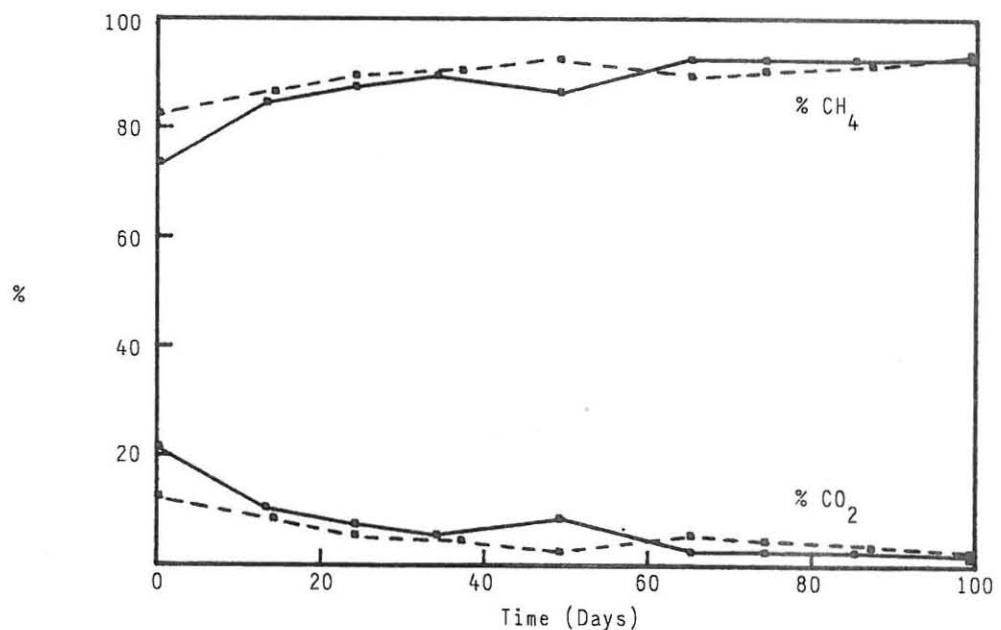


FIGURE 67 - Biogas percentage composition versus time for the anaerobic digester receiving pH-controlled low-sulphate tannery effluent (— % CH_4 ; ---- % CO_2 ; — pH-controlled digester + low SO_4^{2-} effluent; ---- pH-controlled digester)

The volume of biogas decreased rapidly, and almost ceased completely (Figure 66). There was no increase in the volume of biogas produced, or its composition (Figure 67) relative to the digester receiving pH-controlled tannery effluent (Section 6.2) and therefore there was no improvement in the operating efficiency of the digester (Table 72).

TABLE 72
OPERATING EFFICIENCY OF THE DIGESTER AT VARIOUS STAGES DURING THE
EXPERIMENT TREATING pH-CONTROLLED LOW-SULPHATE TANNERY EFFLUENT

Parameter	Digester + pH Control					Digester + pH Control + Low SO ₄ ²⁻ -Effluent				
	Time (Days)					Time (Days)				
	1	25	50	75	100	1	25	50	75	100
Gas Volume (ℓ/d)	0,850	0,120	0,045	0,025	0,015	1,050	0,220	0,075	0,030	0,030
Gas Production (m ³ /m ³ .d)	0,094	0,013	0,005	0,003	0,002	0,117	0,024	0,008	0,003	0,003
% COD Removed	0,0	0,0	37,9	59,2	57,7	0,0	0,0	0,0	41,2	55,4
% PV Removed	0,0	17,7	22,0	52,6	65,0	0,0	0,0	0,0	32,3	59,5
Gas Yield (m ³ /kg COD Removed)	-	-	0,256	0,036	0,023	-	-	-	0,178	0,051
CH ₄ Content of Biogas (%)	85,0	91,8	94,9	93,4	95,5	75,7	89,8	88,5	94,8	95,1
CH ₄ Yield (m ³ CH ₄ / kg COD Removed)	-	-	0,243	0,030	0,022	-	-	-	0,169	0,049
Free NH ₃ (mg/ℓ)	3,4	1,3	1,5	2,4	10,1	5,3	6,8	1,4	< 1	< 1
H ₂ S (mg/ℓ)	10,6	7,0	27,0	62,0	56,1	42,4	65,7	40,7	39,0	93,6

There were no noticeable differences between the effluents obtained from the digester receiving the low-sulphate tannery effluent and that receiving the normal effluent, with the exception of the concentration of sulphates (Table 73). These sulphates were reduced to sulphides, as the concentration of sulphide in the effluent from the digester receiving low-sulphate tannery effluent was no lower than the concentration present in the effluent from the digester receiving normal tannery effluent.

TABLE 73

CHARACTERISTICS OF THE DIGESTER EFFLUENT AT VARIOUS STAGES DURING THE
EXPERIMENT TREATING pH-CONTROLLED LOW-SULPHATE TANNERY EFFLUENT

Parameter	Digester + pH Control					Digester + pH Control + Low SO ₄ ²⁻ Effluent				
	Time (Days)					Time (Days)				
	1	25	50	75	100	1	25	50	75	100
pH	7,36	7,23	7,38	7,49	7,78	7,37	7,65	7,20	7,05	6,73
Total COD (mg/l)	1723	304	288	476	561	368	304	292	307	445
Soluble COD (mg/l)	350	128	280	411	353	360	224	246	184	261
Total PV (mg/l)	173	93	46	93	55	43	55	42	42	30
Soluble PV (mg/l)	144	81	43	81	46	35	25	26	22	25
Total Solids (mg/l)	2300	605	1165	1380	2670	1160	760	1025	1690	1300
TIS (mg/l)	1165	285	750	975	2005	605	480	515	805	460
SS (mg/l)	620	30	55	20	215	250	80	85	115	85
TDS (mg/l)	1680	575	1110	1360	2455	910	680	940	1575	1215
TDIS (mg/l)	890	285	740	975	1910	490	410	450	720	405
Alkalinity (mg/l CaCO ₃)	1188	1116	1058	1104	828	1113	1092	966	945	800
Bicarbonate Alkalinity (mg/l CaCO ₃)	1071	963	857	992	663	1024	788	868	788	746
Conductivity (mS/m)	336	112	194	236	469	254	185	191	294	234
SO ₄ ²⁻ (mg/l)	123	108	304	324	775	537	114	139	81	90
Sulphide (mg/l)	32	24	80	168	128	128	160	144	168	176
TKN (mg/l)	142	71	58	90	174	234	166	80	72	53
NH ₃ -N (mg/l)	135	71	58	71	161	203	142	80	67	53
Organic N (mg/l)	7	0	0	19	13	31	24	0	5	0
Cl ⁻ (mg/l)	1150	575	875	1025	1700	800	625	1125	1525	1025
Na (mg/l)	210	130	130	180	400	60	250	310	220	330
Ca (mg/l)	143	90	60	70	90	140	80	82	112	72
Cr (mg/l)	<1	1,0	<1	1,0	<1	<1	<1	<1	1,0	<1
Al (mg/l)	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Fe (mg/l)	7,3	5,0	2,5	5,0	2,0	3,5	4,1	3,7	4,5	3,7
P (mg/l)	11,3	3,8	<1	<1	<1	7,5	2,0	1,3	<1	<1
VFA (mg/l)	164,9	215,9	284,4	157,8	232,5	126,4	429,1	139,0	221,7	76,0

TABLE 74

STRATIGRAPHIC ANALYSIS OF THE DIGESTER CONTENTS AT THE START OF THE EXPERIMENT
TREATING pH-CONTROLLED LOW-SULPHATE TANNERY EFFLUENT

Parameter	Digester + pH Control				Digester + pH Control + Low SO_4^{2-} -Effluent			
	Height of Sampling Port Above Inlet (cm)				Height of Sampling Port Above Inlet (cm)			
	16	29	42	59	16	29	42	59
pH	7,46	7,42	7,53	7,36	7,18	7,19	7,21	7,37
Total COD (mg/l)	2609	1669	1856	1723	2596	928	516	368
Soluble COD (mg/l)	392	387	409	350	320	464	440	360
Total PV (mg/l)	361	199	169	173	349	113	117	43
Soluble PV (mg/l)	141	118	127	144	54	60	60	35
Total Solids (mg/l)	3775	2100	1950	2300	4650	1275	1220	1160
TIS (mg/l)	1525	920	870	1165	1495	460	560	605
SS (mg/l)	2855	1000	985	620	3785	410	365	250
TDS (mg/l)	920	1100	965	1680	865	865	855	910
TDIS (mg/l)	700	695	640	890	405	405	410	490
Alkalinity (mg/l CaCO_3)	1104	1104	1136	1188	1104	1104	1104	1113
Bicarbonate Alkalinity (mg/l CaCO_3)	992	1010	1033	1071	1017	1002	997	1024
Conductivity (mS/m)	322	309	318	336	255	249	248	254
SO_4^{2-} (mg/l)	119	108	117	123	934	552	521	537
Sulphide (mg/l)	40	24	32	32	128	120	112	128
TKN (mg/l)	394	178	152	142	351	234	234	234
$\text{NH}_3\text{-N}$ (mg/l)	178	162	140	135	228	209	209	203
Organic N (mg/l)	216	16	12	7	123	25	25	31
Cl^- (mg/l)	1100	1050	1075	1150	775	725	775	800
Na (mg/l)	200	185	200	210	70	80	70	60
Ca (mg/l)	205	130	135	143	230	120	130	140
Cr (mg/l)	17,5	1,0	1,0	<1	4,0	1,0	1,0	<1
Al (mg/l)	60,0	1,0	1,0	<1	60,0	5,0	5,0	<1
Fe (mg/l)	82,5	3,0	4,0	7,3	51,0	4,0	4,0	3,5
P (mg/l)	26,6	13,2	17,5	11,3	17,5	17,0	14,0	7,5
VFA (mg/l)	157,8	132,7	144,9	164,9	122,5	144,2	151,3	126,4

TABLE 75

STRATIGRAPHIC ANALYSIS OF THE DIGESTER CONTENTS ON COMPLETION OF THE EXPERIMENT
TREATING pH-CONTROLLED LOW-SULPHATE TANNERY EFFLUENT

Parameter	Digester + pH Control				Digester + pH Control + Low SO_4^{2-} Effluent			
	Height of Sampling Port Above Inlet (cm)				Height of Sampling Port Above Inlet (cm)			
	16	29	42	59	16	29	42	59
pH	7,61	7,62	7,60	7,78	6,49	6,47	6,60	6,73
Total COD (mg/l)	691	499	565	561	2035	653	538	445
Soluble COD (mg/l)	384	353	384	353	246	261	261	261
Total PV (mg/l)	214	202	115	55	261	49	44	30
Soluble PV (mg/l)	83	94	82	46	55	37	38	25
Total Solids (mg/l)	2915	2570	2440	2670	2690	1545	1540	1300
TIS (mg/l)	1750	2140	1855	2005	1090	655	970	460
SS (mg/l)	560	110	100	215	1300	95	140	85
TDS (mg/l)	2355	2460	2340	2455	1390	1450	1400	1215
TDIS (mg/l)	1725	2065	1805	1910	740	575	875	405
Alkalinity (mg/l CaCO_3)	644	644	644	828	736	724	766	800
Bicarbonate Alkalinity (mg/l CaCO_3)	516	531	523	663	695	678	686	746
Conductivity (mS/m)	480	479	460	469	242	243	246	234
SO_4^{2-} (mg/l)	372	469	427	775	109	109	96	90
Sulphide (mg/l)	128	136	132	128	184	184	192	176
TKN (mg/l)	179	154	160	174	112	53	59	53
$\text{NH}_3\text{-N}$ (mg/l)	166	154	160	161	53	53	53	53
Organic N (mg/l)	13	0	0	13	59	0	6	0
Cl^- (mg/l)	1850	1825	1750	1700	1000	1175	1250	1025
Na (mg/l)	470	470	470	400	220	200	230	330
Ca (mg/l)	100	120	120	90	74	76	76	72
Cr (mg/l)	28,0	3,0	2,5	<1	7,5	1,4	<1	<1
Al (mg/l)	5,0	<1	<1	<1	10,0	<1	<1	<1
Fe (mg/l)	4,5	2,5	3,0	2,0	12,9	4,5	4,2	3,7
P (mg/l)	2,5	<1	<1	<1	3,0	1,0	1,3	<1
VFA (mg/l)	181,2	159,5	171,4	232,5	58,6	65,5	112,5	76,0

A comparison of Tables 74 and 75 clearly illustrates that the sulphates in the digester had been substantially reduced on completion of the experiment and also shows that, as in the previous experiments, a reduction in the volume of the sludge bed took place. This is supported by the data obtained from Table 76.

TABLE 76

SOLIDS BALANCE FOR THE ANAEROBIC DIGESTION OF
pH-CONTROLLED LOW-SULPHATE TANNERY EFFLUENT

<u>Solids Present in the Digester - Day 1</u>	
Total Solids	= 178,360 g
Total Volatile Solids	= 117,200 g
Total Inorganic Solids	= 61,160 g
<u>Solids that Entered the Digester - Day 1 to 100</u>	
Total Solids	= 118,100 g
Total Volatile Solids	= 65,770 g
Total Inorganic Solids	= 52,330 g
<u>Solids Present in the Digester - Day 100</u>	
Total Solids	= 134,811 g
Total Volatile Solids	= 82,118 g
Total Inorganic Solids	= 52,693 g
<u>Solids that Emerged from the Digester - Day 1 to 100</u>	
Total Solids	= 111,860 g
Total Volatile Solids	= 49,802 g
Total Inorganic Solids	= 62,058 g

The solids balance for the anaerobic digestion of pH-controlled low-sulphate tannery effluent is shown in Table 76. If the Total Volatile Solids (TVS) or organic content of the effluent is concentrated on, then the following is obtained:-

$$\begin{aligned}
 \text{TVS degraded} &= 65,770 \text{ g} - 49,802 \text{ g} \\
 &= 15,968 \text{ g} \\
 &= \underline{24.3 \% \text{ of TVS that entered}} \rightarrow
 \end{aligned}$$

$$\text{TVS emerged} = 49,802 \text{ g}$$

$$= \underline{75,7 \% \text{ of TVS that entered}} \rightarrow$$

The organic content of the sludge bed was reduced by:-

$$\begin{aligned} 117,200 \text{ g} - 82,118 \text{ g} &= 35,082 \text{ g} \\ &= \underline{29,9 \%} \rightarrow \end{aligned}$$

The removal of the sulphates from the tannery effluent was unable to improve the utilization of the organic matter present in the effluent. The reduction in the volume of the sludge bed was, however, greater than that found in previous experiments.

The results of this investigation provide further evidence to indicate that it is unlikely that the high sulphate concentration in the tannery effluent is the cause of the suppression of the metabolism of the methanogens. Despite the removal of a major proportion of the sulphates through precipitation as BaSO_4 , it was still found that the successful anaerobic digestion of this low-sulphate tannery effluent could not be achieved. The results of this experiment, together with those obtained in the previous experiments, provide conclusive evidence, therefore, that the high sulphate concentrations present in tannery effluents are not the cause of the inability to successfully treat these effluents by means of anaerobic digestion.

6.7 Conclusion

The results obtained from the experiments investigating the anaerobic treatability of tannery effluent clearly illustrate that the successful anaerobic digestion of this effluent was not achieved. Although pH control in the range 4,0 - 5,0 was found to be essential for the maintenance of a neutral pH inside the digester, pH control alone was not sufficient to ensure optimum metabolism of the methanogens. Similarly, the supplementation of trace nutrients was unable to improve the operating efficiency of the digester even though the tannery effluent was found to be deficient in these essential nutrients. In view of the apparent suppression of the metabolism of the methanogens, an attempt was made to try and identify the cause of this suppression.

The high concentration of sulphates in the tannery effluent relative to the

nutrients available indicated that the sulphate-reducers could have been playing an important role in competing with the methanogens for available substrates. However, this was shown not to be the case, as the removal of the sulphate, the addition of inhibitors of the sulphate-reducing bacteria, or the addition of extra substrate in the form of acetate, were found to have no effect on digester performance. The non-involvement of the sulphate-reducers in competing with the methanogens was further substantiated by the fact that when the organic loading rate was increased, this resulted in an increase in the concentration of VFA in the digester. If there was competition for the substrates, these VFA would not have accumulated, because they would have been utilized as soon as they had been formed.

One possible explanation for the inability to anaerobically treat the tannery effluent used in this investigation is the low concentration of phosphorous present in this effluent. The initial concentration was very low (Table 45), and it is evident from the data that this phosphorous was utilized rapidly, as very low concentrations i.e. <1 mg/l were present in the effluent from the digesters for the duration of most of the experiments. It has been shown that methanogens do require phosphorous (Seely and Fahrney, 1984; Archer, 1985; Britz *et al*, 1988) and therefore, as this essential element was lacking, the possibility exists that the low phosphorous concentration could have prevented the optimum metabolism of these bacteria. This would obviously have affected the functioning of the digester. It is therefore proposed that phosphorous supplementation to tannery effluent be attempted in any future experiments on the anaerobic treatment of these effluents.

The fate of the compounds in the digesters is in agreement with the findings of the synthetic study (Chapter 5). The soluble ions such as sodium and chloride were not removed and were present in the digester effluents. In contrast to this, heavy metals such as chromium precipitated and accumulated in the digesters, and therefore the digester effluents contained very low concentrations (i.e. <1 mg/l) of the metals. The sulphates were reduced to sulphides, and in most experiments the ammonification of the organic nitrogen was almost complete. The soluble COD and PV accounted for a major proportion of the total COD and PV present in the digester effluents, and it would therefore appear that the acidification of the wastes was taking place quite successfully. This is further substantiated by the low concentration of suspended solids and organic nitrogen in the digester effluents. This area

requires further investigation as it is possible that anaerobic digesters with short hydraulic retention times could be used to solubilize the solids present in tannery effluents, even though no CH_4 is produced. Once solubilized, the organic matter could be treated by numerous other treatment systems. This could drastically reduce the problems of solid disposal.

The results of this investigation indicate that the anaerobic digestion of tannery effluent was not possible. This is in contrast to the results of previous studies (Friedman et al, 1982; Bailey et al, 1984). As no indication was given of the composition of these effluents, particularly with respect to the concentration of phosphorous, no comparison between this investigation and the previous studies can be made. However, it is possible that through further research, the successful anaerobic digestion of the tannery effluent used in this investigation may be achieved.

CHAPTER SEVEN

THE ANAEROBIC DIGESTION OF TANNERY SLUDGE

CHAPTER SEVEN

THE ANAEROBIC DIGESTION OF TANNERY SLUDGE

In the preceding chapter, it was found that the successful anaerobic digestion of tannery effluents could not be achieved, and therefore it would appear that anaerobic digestion is not suitable as a pretreatment method for these effluents. However, although this may seem to be a serious drawback, methods are available for the treatment of tannery effluents to reasonable degrees of purity (Section 2.4).

In contrast to this, the tannery sludges which arise during the wastewater treatment processes are a major disposal problem. Although methods are available for the disposal of these sludges (Section 2.5), the disposal routes all have their unique problems. As there is a need to minimize the impact of tannery sludges on the environment, research is needed to develop alternative sludge disposal methods that can be used in conjunction with existing wastewater treatment processes. Therefore, despite the results obtained with the tannery effluent experiments, it was still deemed necessary to investigate the anaerobic digestion of tannery sludge.

7.1 The Anaerobic Digestion of Tannery Sludge

7.1.1 Introduction

The information available on the anaerobic digestion of tannery sludge is extremely limited. The results of only one previous study have been published (Aloy et al, 1987; Aloy et al, 1989). In this study, two 65 m³ digesters were run in series treating a mixture of fleshings, trimmings and primary tannery sludge. 8,5 m³ of the mixed sludge was supplied to the digesters daily, and it was found that the COD, total solids and total volatile solids could be reduced by 74,7 %, 43,9 % and 62,1 % respectively. The biogas produced had a CH₄ content of 73,1 %.

In view of the lack of information available, this study was carried out to investigate the anaerobic digestion of tannery sludge. An important aspect of this investigation was to determine if results similar to those obtained in previous studies could be achieved, bearing in mind the results obtained from the experiments investigating the anaerobic digestion of tannery effluents.

7.1.2 Materials and Methods

The materials and methods used were described in detail in Chapter 4. After seeding the digester with 20 l of anaerobic sludge with a VSS concentration of 28,0 - 32,0 g/l, the anaerobic digester was started up on a dilute tannery sludge. This was obtained by diluting the tannery sludge (Table 77) with varying proportions of deionized water. In this way, the loading rate was gradually increased over the experimental period.

TABLE 77
COMPOSITION OF THE TANNERY SLUDGE
USED IN THIS INVESTIGATION

Parameter	Concentration
Total COD (mg/kg)	75890
Soluble COD (mg/l)	5740
Total PV (mg/kg)	4460
Soluble PV (mg/l)	133
Total Solids (%)	6,7
TVS (%)	68,7
TIS (%)	31,3
TDS (ppm)	22870
TDIS (ppm)	12345
Conductivity (mS/m)	2530
Sulphate (mg/l)	783
Sulphide (mg/l)	552
TKN (ppm)	3473
NH ₃ -N (ppm)	1528
Organic N (ppm)	1945
Chloride (mg/l)	10700
Na (mg/l)	6100
Ca (ppm)	3188
Cr (ppm)	2766
Al (ppm)	240
Fe (ppm)	1300
P (ppm)	72,4

7.1.3 Results and Discussion

As the loading rate was increased (Figure 68), the anaerobic bacteria were able to acclimatize to the potential toxic compounds present in the sludge. The fact that adaptation of the anaerobic bacteria to the sludge took place is clearly evident from the concentration of VFA in the digester effluent (Table 78). At the start of the experiment the VFA concentration was very high. However, as the experiment progressed, the concentration decreased to remain at a relatively steady concentration for the remainder of the experiment.

The increasing loading rate resulted in a corresponding increase in the volume of biogas produced (Figure 69). However, in contrast to this, the percentage CH_4 in the biogas decreased from 85,1 % at the start of the experiment to 74,4 % on day 150. This phenomenon of a decreased CH_4 content as the organic loading rate is increased has been previously documented (Frostell, 1985). The methane yield also showed a sharp decrease after the first 50 days (Table 79). However, this levelled off and remained fairly constant for the remaining 100 days. The high rate of methane production and hence the methane yield from the dilute influent COD during the initial stages of the experiment, could be due to the high concentration of VFA in the digester at the start of the experiment. These VFA would be metabolized to CH_4 , and would consequently result in an increased level of CH_4 production.

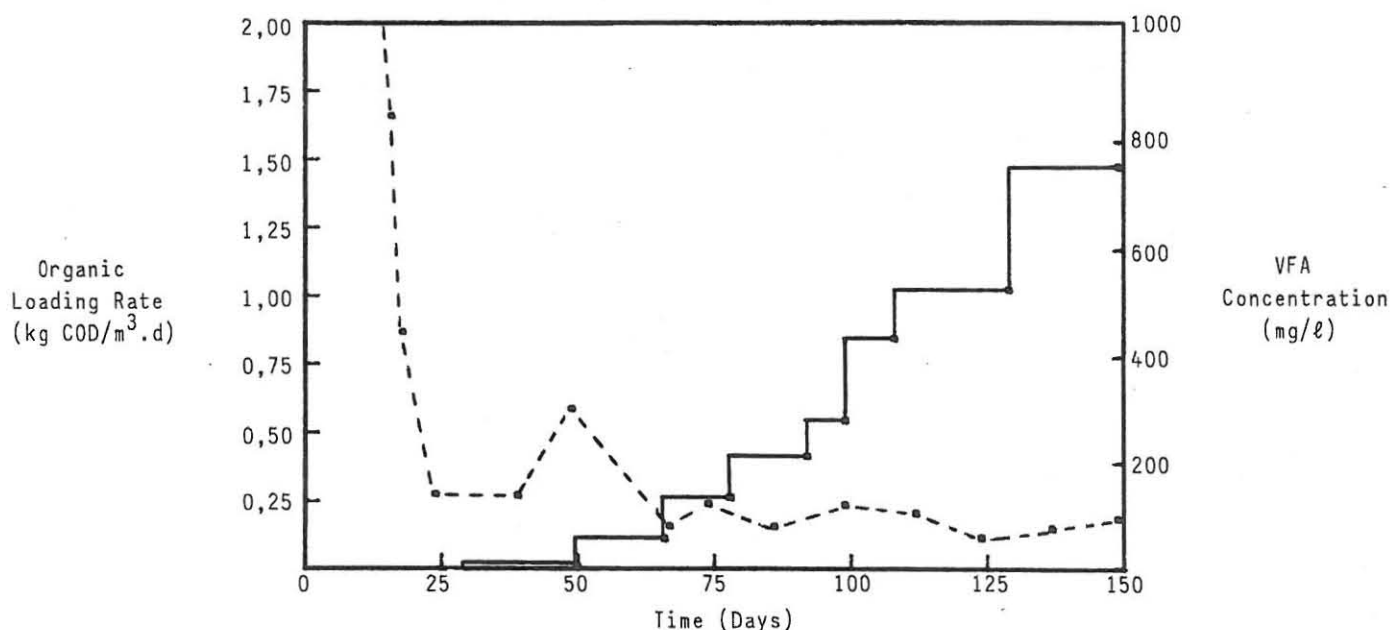


FIGURE 68 - Organic loading rate and effluent volatile fatty acids versus time for the anaerobic digester receiving tannery sludge (— organic loading rate; ---- volatile fatty acids as acetic acid)

TABLE 78

CHARACTERISTICS OF THE DIGESTER EFFLUENT AT VARIOUS
STAGES DURING THE EXPERIMENT TREATING TANNERY SLUDGE

Parameter	Time (Days)						
	1	25	50	75	100	125	150
pH	7,13	7,85	7,74	8,07	8,04	7,56	7,56
Total COD (mg/l)	1299	1736	1656	549	676	1108	1172
Soluble COD (mg/l)	581	828	432	446	636	600	840
Total PV (mg/l)	228	162	268	109	89	185	202
Soluble PV (mg/l)	58	60	157	102	78	146	106
Total Solids (mg/l)	2055	1860	1985	2265	2880	4595	10520
TIS (mg/l)	775	675	775	1325	1410	2725	5165
SS (mg/l)	685	665	705	515	210	585	1965
TDS (mg/l)	1370	1195	1280	1750	2670	4010	8555
TDIS (mg/l)	615	470	550	1220	1380	2455	5100
Alkalinity (mg/l CaCO ₃)	990	1276	1298	1476	1350	1610	1771
Bicarbonate Alkalinity (mg/l CaCO ₃)	-	1162	1074	1375	1250	1552	1688
Conductivity (mS/m)	199	281	337	372	478	689	1214
SO ₄ ²⁻ (mg/l)	303	341	324	126	469	325	222
Sulphide (mg/l)	48	24	16	24	24	48	48
TKN (mg/l)	219	316	316	283	316	386	522
NH ₃ -N (mg/l)	129	232	258	245	283	341	477
Organic N (mg/l)	90	84	58	38	33	45	45
Chloride (mg/l)	625	700	900	1025	1425	2600	4625
Na (mg/l)	240	240	170	190	260	650	1060
Ca (mg/l)	111	91	173	232	292	766	1016
Cr (mg/l)	1,5	1,5	2,1	1,8	2,0	9,6	32,3
Al (mg/l)	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Fe (mg/l)	4,5	4,5	9,3	7,1	3,6	8,5	9,0
P (mg/l)	1,2	2,8	< 1	< 1	< 1	< 1	< 1
VFA (mg/l)	1412,5	160,9	316,6	142,8	140,7	82,2	117,0

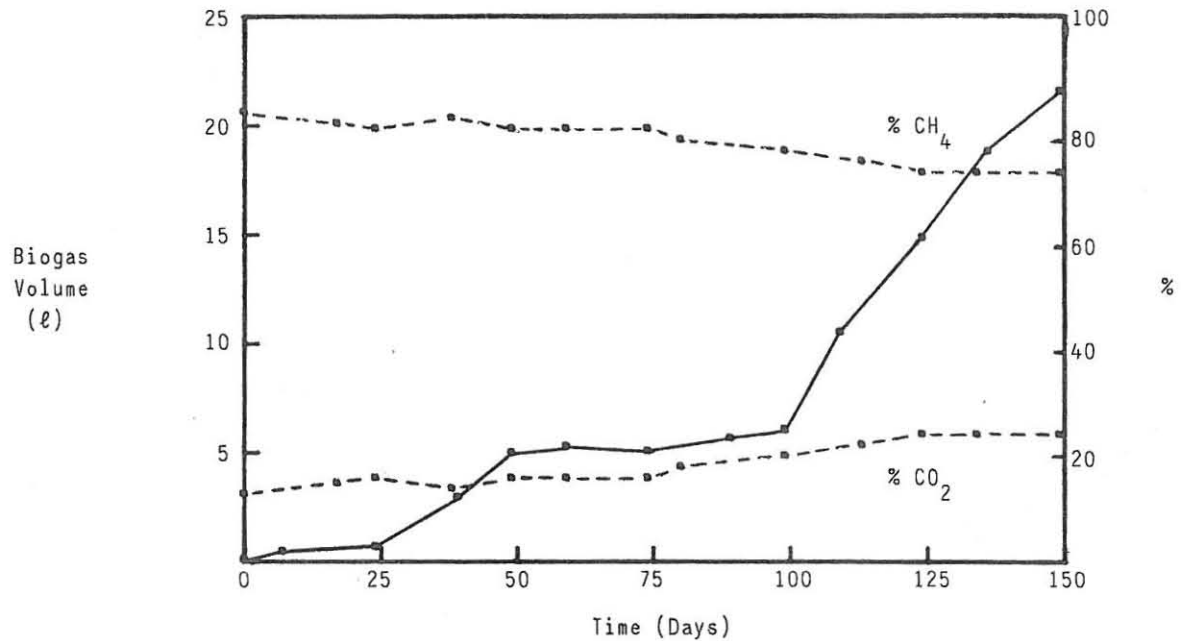


FIGURE 69 - Biogas volume and percentage composition versus time for the anaerobic digester receiving tannery sludge (— biogas volume; ---- % CH₄ and CO₂)

TABLE 79

OPERATING EFFICIENCY OF THE DIGESTER AT VARIOUS STAGES DURING THE EXPERIMENT TREATING TANNERY SLUDGE

Parameter	Time (Days)						
	1	25	50	75	100	125	150
Gas Volume (l/d)	0,750	1,325	5,625	5,700	6,700	15,500	22,225
Gas Production (m ³ /m ³ .d)	0,015	0,027	0,113	0,114	0,134	0,310	0,445
% COD Removed	0,0	37,1	75,7	96,6	98,4	98,0	98,5
% PV Removed	0,0	26,0	51,9	93,0	94,0	95,4	95,5
Gas Yield (m ³ /kg COD Removed)	—	7,528	7,560	0,385	0,162	0,291	0,286
CH ₄ Content of Biogas (%)	85,1	82,4	82,1	81,8	78,4	74,2	74,4
CH ₄ Yield (m ³ CH ₄ /kg COD Removed)	—	6,203	6,207	0,315	0,127	0,216	0,213
Free NH ₃ (mg/l)	1,9	17,2	15,0	28,4	31,1	13,3	18,6
H ₂ S (mg/l)	12,2	10,8	6,9	1,0	< 1	18,6	18,6

The addition of the tannery sludge to the digester resulted in an increase in the digester pH (Figure 70). This was despite the influent pH being initially controlled at 5,5 - 6,0. On day 75, the effluent pH reached the undesirable level of 8,07, and consequently it was deemed necessary to lower the influent pH. This was achieved by the addition of 6 N HCl, and resulted in the pH decreasing to a level more suitable for the optimum metabolism of the methanogenic bacteria i.e. a pH of 7,56. The increase in the internal digester pH is due to the ammonification of the organic matter (Koster, 1986). The influent sludge had a high organic nitrogen content, whereas the nitrogen in the effluent was almost entirely in the $\text{NH}_3\text{-N}$ form (Table 78). This ammonification results in the rather unusual phenomenon in that HCl is required to decrease the pH and thus the buffering capacity of the system. This is in contrast to most anaerobic digestion processes where alkalinity or buffering usually has to be added i.e. in the form of sodium bicarbonate. The addition of HCl does, however, have some disadvantages. On an industrial scale, the cost would be very high. More importantly, however, it increases the concentration of chloride ions in the final effluent, and therefore results in an increased conductivity. In South Africa, which has a critical water shortage, strict regulations exist for the discharge of effluents with high conductivities, as the ability to dilute these effluents is limited. The use of other acids therefore needs to be investigated. However, care has to be taken as some acids e.g. H_2SO_4 , can give rise to toxic compounds viz. sulphides under anaerobic conditions.

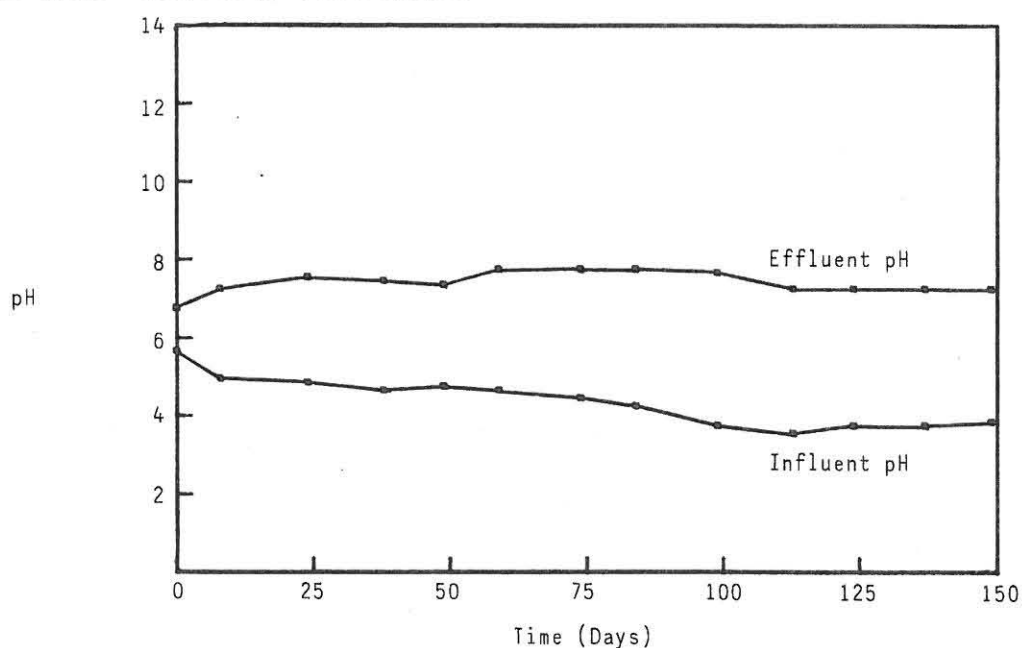


FIGURE 70 - Influent and effluent pH versus time for the anaerobic digester receiving tannery sludge

A comparison of Tables 80 and 81 clearly illustrates that there was a large accumulation of solids in the digester. This accumulation was due to the build up of both organic and inorganic solids. However, although there was an accumulation of organic solids, the anaerobic bacteria present in the digester were able to degrade a large proportion of the total volatile solids present in the tannery sludge. Over 60 % of the volatile solids were converted to biogas (Table 82). However, the total solids present in the digester effluent increased steadily over the experimental period (Table 78). This increase was due to an increasing concentration of TDS and TDIS in the effluent. The TVS remained relatively constant, indicating that the organic matter was being degraded. The low concentrations of TVS in the digester effluent relative to the influent sludge resulted in very high organic removal rates i.e. COD and PV removal rates were maintained at greater than 90 % for the latter half of the experiment (Table 79). The fact that the soluble COD and PV's accounted for the major proportion of the total COD and PV's was further evidence indicating that the degradation and solubilization of the organic matter present in the influent sludge had been achieved.

Despite a high concentration of Cr^{3+} in the tannery sludge (2766 ppm), very low concentrations of Cr^{3+} , i.e. $< 10,0 \text{ mg/l}$, emerged from the digester (Table 78), with the exception of day 150, where $32,3 \text{ mg/l}$ Cr^{3+} was present in the effluent. This is due to the fact that at the neutral to slightly alkaline pH of the digester, it is likely that the Cr^{3+} precipitated as $\text{Cr}(\text{OH})_3$ and consequently accumulated in the digester (Table 81) (Jackson-Moss et al, 1988c). In contrast to this, the more soluble ions such as Na^+ , Ca^{2+} and Cl^- were not removed and were present in the digester effluent, contributing to the high digester effluent conductivity (Table 78). There is therefore no net removal of Na^+ and Cl^- ions during anaerobic digestion (Jackson-Moss et al, 1989c), while a small proportion of the Ca^{2+} ions precipitate as $\text{Ca}(\text{OH})_2$ or CaCO_3 (Table 81) (Jackson-Moss et al, 1989a).

TABLE 80

STRATIGRAPHIC ANALYSIS OF THE DIGESTER CONTENTS
AT THE START OF THE EXPERIMENT TREATING TANNERY SLUDGE

Parameter	Height of Sampling Port Above Inlet (cm)			
	33	66	99	125
pH	7,10	7,08	7,10	7,13
Total COD (mg/l)	5672	1578	1322	1299
Soluble COD (mg/l)	658	636	618	581
Total PV (mg/l)	870	269	233	228
Soluble PV (mg/l)	89	67	61	58
Total Solids (mg/l)	5195	2880	2175	2055
TIS (mg/l)	1465	965	820	775
SS (mg/l)	3770	1465	790	685
TDS (mg/l)	1425	1415	1385	1370
TDIS (mg/l)	720	685	650	615
Alkalinity (mg/l CaCO ₃)	990	1012	966	990
Bicarbonate Alkalinity (mg/l CaCO ₃)	1,0	-	-	-
Conductivity (mS/m)	198	203	201	199
SO ₄ ²⁻ (mg/l)	256	298	347	303
Sulphide (mg/l)	36	40	40	48
TKN (mg/l)	896	280	242	219
NH ₃ -N (mg/l)	146	134	132	129
Organic N (mg/l)	750	146	110	90
Cl ⁻ (mg/l)	630	635	630	625
Na (mg/l)	240	230	220	240
Ca (mg/l)	180	90	100	111
Cr (mg/l)	7,5	1,0	1,0	1,5
Al (mg/l)	190	1,0	< 1	< 1
Fe (mg/l)	190,5	8,0	6,5	4,5
P (mg/l)	85,0	17,9	7,6	1,2
VFA (mg/l)	1397,3	1484,4	1519,0	1412,5

TABLE 81

STRATIGRAPHIC ANALYSIS OF THE DIGESTER CONTENTS
ON COMPLETION OF THE EXPERIMENT TREATING TANNERY SLUDGE

Parameter	Height of Sampling Port Above Inlet (cm)			
	33	66	99	125
pH	7,10	7,08	7,10	7,56
Total COD (mg/l)	43000	35410	37780	1172
Soluble COD (mg/l)	764	661	718	840
Total PV (mg/l)	4800	4420	4280	202
Soluble PV (mg/l)	144	133	102	106
Total Solids (mg/l)	46000	39000	46000	10520
TIS (mg/l)	17020	14740	17110	5165
SS (mg/l)	37315	30595	37520	1965
TDS (mg/l)	8685	8405	8480	8555
TDIS (mg/l)	5795	5880	5560	5100
Alkalinity (mg/l CaCO ₃)	1288	1288	1288	1771
Bicarbonate Alkalinity (mg/l CaCO ₃)	1200	1214	1229	1688
Conductivity (mS/m)	1515	1498	1477	1214
SO ₄ ²⁻ (mg/l)	155	175	152	222
Sulphide (mg/l)	40	32	40	48
TKN (mg/l)	2946	2235	2810	522
NH ₃ -N (mg/l)	956	816	929	477
Organic N (mg/l)	1990	1419	1881	45
Cl ⁻ (mg/l)	6100	5925	6000	4625
Na (mg/l)	3130	2600	1260	1060
Ca (mg/l)	1404	1320	1357	1016
Cr (mg/l)	2074,0	1353,0	1883,4	32,3
Al (mg/l)	366	492	438	< 1
Fe (mg/l)	536,8	516,6	525,6	9,0
P (mg/l)	468	403	520	< 1
VFA (mg/l)	124,3	104,7	83,3	117,0

The solids balance for the anaerobic digestion of tannery sludge is shown in Table 82. If the Total Volatile Solids (TVS) or the organic content of the sludge is concentrated on, then the following is obtained:-

$$\begin{aligned}\text{TVS accumulated} &= 1669,50 \text{ g} - 888,28 \text{ g} \\ &= 781,22 \text{ g} \\ &= \underline{29,5 \% \text{ of TVS that entered}} \rightarrow\end{aligned}$$

$$\begin{aligned}\text{TVS degraded} &= 2652,42 \text{ g} - (781,22 \text{ g} + 280,38 \text{ g}) \\ &= 1590,82 \text{ g} \\ &= \underline{60,0 \% \text{ of TVS that entered}} \rightarrow\end{aligned}$$

$$\begin{aligned}\text{TVS emerged} &= 280,38 \text{ g} \\ &= \underline{10,5 \% \text{ of TVS that entered}} \rightarrow\end{aligned}$$

TABLE 82

SOLIDS BALANCE FOR THE ANAEROBIC
DIGESTION OF TANNERY SLUDGE

<u>Solids Present in the Digester - Day 1</u>	
Total Solids	= 1372,42 g
Total Volatile Solids	= 888,28 g
Total Inorganic Solids	= 484,14 g
<u>Solids that Entered the Digester - Day 1 to 150</u>	
Total Solids	= 3860,88 g
Total Volatile Solids	= 2652,42 g
Total Inorganic Solids	= 1208,45 g
<u>Solids Present in the Digester - Day 150</u>	
Total Solids	= 3064,50 g
Total Volatile Solids	= 1669,50 g
Total Inorganic Solids	= 1395,00 g
<u>Solids that Emerged from the Digester - Day 1 to 150</u>	
Total Solids	= 605,35 g
Total Volatile Solids	= 280,38 g
Total Inorganic Solids	= 324,97 g

The value of 60 % of the TVS degraded is in agreement with the findings of a previous study where it was shown that 62,1 % of the TVS present in tannery

sludge could be degraded during the anaerobic digestion of these wastes (Aloy *et al.*, 1987; Aloy *et al.*, 1989).

The biogas yield from tannery sludge is shown in Table 83. It is clear that for every gram of volatile solids degraded, 0,815 ℓ of biogas are produced. This figure is in close agreement with that obtained in a previous study viz. 0,983 ℓ biogas/g TVS (Aloy *et al.*, 1987; Aloy *et al.*, 1989). The biogas produced can be used as an energy source for heating the digesters, generating electricity, etc., and pilot-plant studies have shown that only 20,8 % of the total energy obtained from the biogas was required for running the anaerobic digesters. The remaining 79,2 % of the energy was available to be used elsewhere in the tannery (Aloy *et al.*, 1987; Aloy *et al.*, 1989).

TABLE 83
BIOGAS YIELD FROM TANNERY SLUDGE

Total biogas produced over the duration of the experiment at STP (Standard Temperature and Pressure)	= 1297,06 ℓ
TVS degraded	= 1590,82 g
1590,82 g TVS	→ 1297,06 ℓ biogas
∴ 1 g TVS	→ 0,815 ℓ biogas
	= 0,611 ℓ CH ₄ (at 75 % CH ₄)
	≈ 5,8 kcal
	≈ 24,3 kJ

The results of this investigation indicate that the anaerobic digestion of tannery sludge is possible despite the high concentration of potentially toxic compounds. Although a large proportion of the organic matter is degraded, the inorganic compounds are not removed, with the exception of the heavy metal Cr³⁺ and a proportion of the Ca²⁺ ions. The accumulation of these heavy metals in the digester is undesirable, as it leads to a low concentration of active biomass in the suspended solids. The contents of the digester would also have to be emptied at some stage in order to remove the accumulated heavy metals. The installation of a chrome recovery and recycling plant would, therefore, eliminate this problem and would also result in the more efficient

anaerobic digestion of tannery sludges. It therefore appears that unless chrome recovery and recycling is carried out, the single-stage anaerobic digestion of tannery sludges is not feasible. A two-stage system could be used, whereby the heavy metals would be removed in the acidogenic stage, leaving the influent to the methanogenic reactor relatively free of heavy metals (Section 7.3). However, this would still result in the accumulation of chrome in the acidogenic reactor which would have to be removed at some stage. It would therefore seem more logical to rather install a recovery and recycling plant.

The findings of this experiment have been previously published (Jackson-Moss and Duncan, 1989; Jackson-Moss and Duncan, 1990c; Jackson-Moss and Duncan 1990e).

7.2 The Effect of Trace Nutrients on the Anaerobic Digestion of Tannery Sludge

7.2.1 Introduction

Although it was found that the anaerobic digestion of tannery effluent was not possible (Chapter 6), the previous experiment showed that it is possible to anaerobically treat tannery sludge. Despite the high concentration of potentially toxic compounds, the methanogens were able to metabolize and convert a large proportion of the organic matter present in the sludge to CH_4 and CO_2 .

Tannery sludges, like tannery effluents, contain very low concentrations of trace nutrients essential for the growth of the methanogens (Table 84).

TABLE 84

THE CONCENTRATION OF TRACE NUTRIENTS
PRESENT IN TANNERY SLUDGE

Nutrient	Concentration (mg/l)
Nickel (Ni)	< 10
Cobalt (Co)	< 10
Molybdenum (Mo)	< 10
Tungsten (W)	760

The possibility therefore exists that through trace nutrient supplementation, the growth of the methanogens could be enhanced and optimized, with a corresponding increase in the rate of digestion of the sludge.

Although the supplementation of trace nutrients to tannery effluents had no significant effect on digester performance (Section 6.3), a large number of studies have shown that trace nutrients do play an important role in stimulating the anaerobic digestion process (Jones and Stadtman, 1977; Forsberg, 1978; Schönheit, *et al*, 1979; Scherer and Sahm, 1981; Murray and Van den Berg, 1981; Speece *et al*, 1983; Nel *et al*, 1985; Canovas-Diaz and Howell, 1986; Zellner and Winter, 1987). This investigation was therefore carried out to determine whether trace nutrients could stimulate and increase the efficiency of digestion of tannery sludge.

7.2.2 Materials and Methods

The materials and methods used were described in detail in Chapter 4. The anaerobic digester was started up on a dilute tannery sludge as described previously (Section 7.1.2). In addition, the trace nutrients outlined in Table 84 were added to the sludge at a concentration of 100 mg/l each. The loading rate was gradually increased over the experimental period.

7.2.3 Results and Discussion

As the loading rate was increased (Figure 71), the anaerobic bacteria were able to adapt to the potential toxic compounds present in the sludge. The concentration of VFA decreased rapidly from the start of the experiment (Figure 71) and remained at a relatively steady concentration for the remainder of the experiment. If one compares the VFA concentration in the digester receiving trace nutrients relative to the control digester (Table 85), then the trace nutrients did not appear to have any effect on the rate of utilization of the VFA.

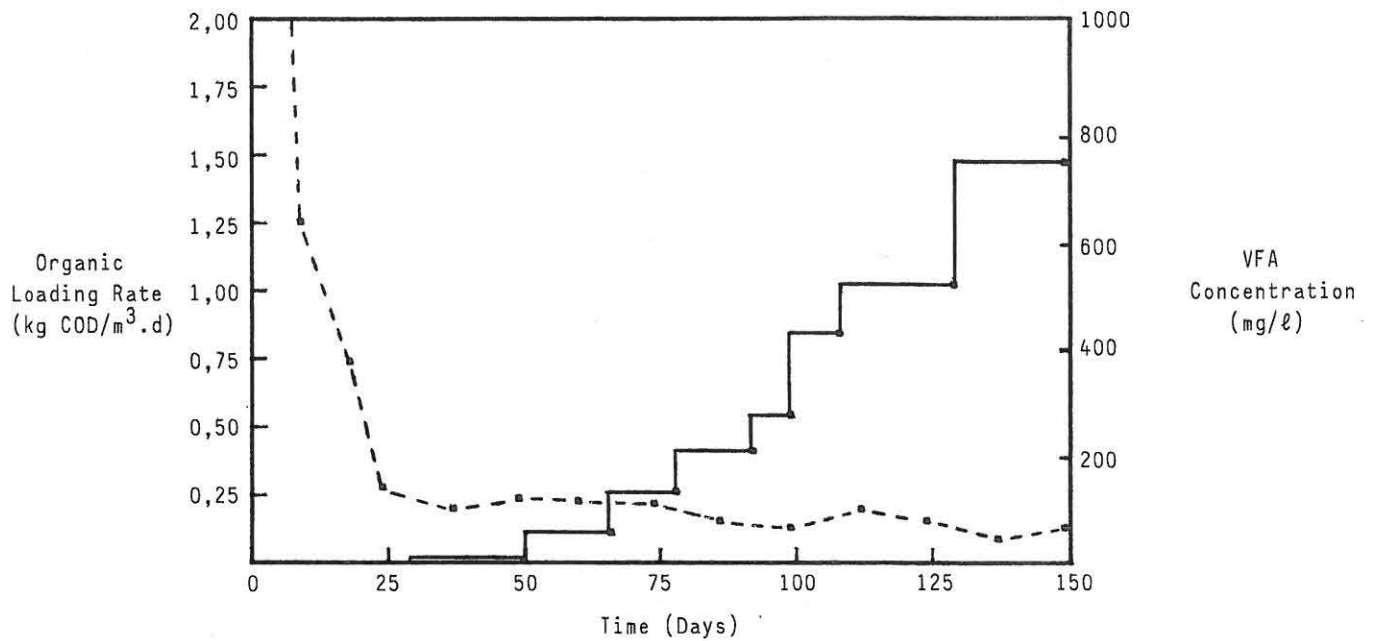


FIGURE 71 - Organic loading rate and effluent volatile fatty acids versus time for the anaerobic digester receiving tannery sludge supplemented with trace nutrients (— organic loading rate; ---- volatile fatty acids as acetic acid)

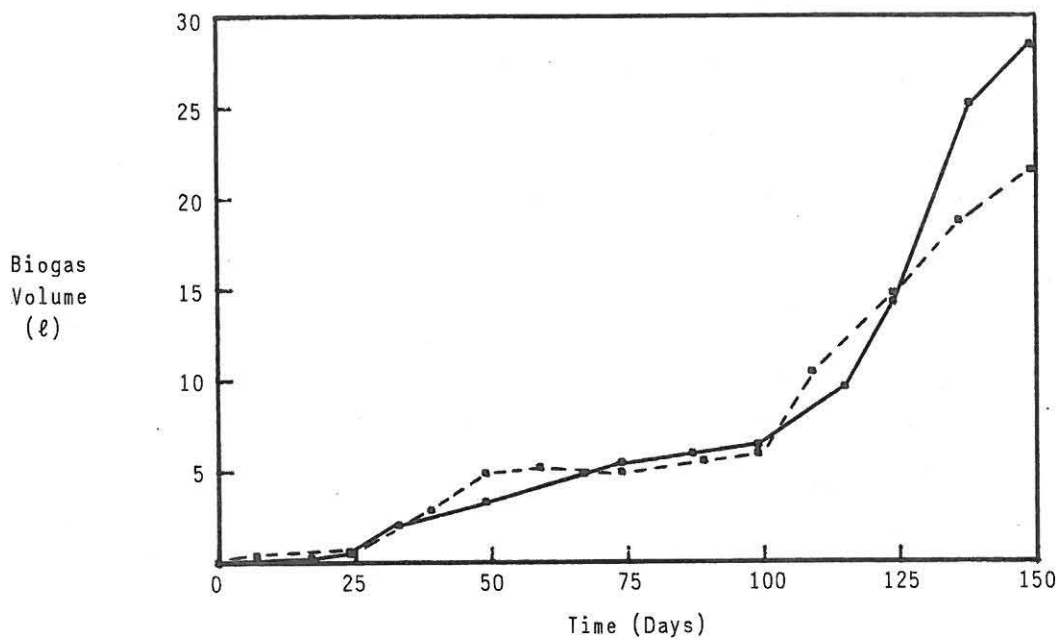


FIGURE 72 - Biogas volume versus time for the anaerobic digester receiving tannery sludge supplemented with trace nutrients (— tannery sludge + trace nutrients digester; ---- tannery sludge digester)

TABLE 85

CHARACTERISTICS OF THE DIGESTER EFFLUENT AT VARIOUS STAGES DURING
THE EXPERIMENT TREATING TANNERY SLUDGE SUPPLEMENTED WITH TRACE NUTRIENTS

Parameter	Tannery Sludge							Tannery Sludge + Trace Nutrients						
	Time (Days)							Time (Days)						
	1	25	50	75	100	125	150	1	25	50	75	100	125	150
pH	7,13	7,85	7,74	8,07	8,04	7,56	7,56	6,58	7,10	7,80	7,96	7,93	7,89	7,57
Total COD (mg/l)	1299	1736	1656	549	676	1108	1172	3012	1436	768	409	688	656	1336
Soluble COD (mg/l)	581	828	432	446	636	600	840	1175	730	272	402	320	304	476
Total PV (mg/l)	228	162	268	109	89	185	202	198	161	141	116	133	124	129
Soluble PV (mg/l)	58	60	157	102	78	146	106	98	62	104	96	98	91	94
Total Solids (mg/l)	2055	1860	1985	2265	2880	4595	10520	2130	1395	1430	1975	3000	4150	9950
TIS (mg/l)	775	675	775	1325	1410	2725	5165	845	600	715	1135	1505	2500	5370
SS (mg/l)	685	665	705	515	210	585	1965	855	345	150	195	200	200	145
TDS (mg/l)	1370	1195	1280	1750	2670	4010	8555	1275	1050	1280	1780	2800	3950	9805
TDIS (mg/l)	615	470	550	1220	1380	2455	5100	745	570	585	1030	1480	2385	5330
Alkalinity (mg/l CaCO ₃)	990	1276	1298	1476	1350	1610	1771	594	880	1320	1152	1152	1380	1058
Bicarbonate Alkalinity (mg/l CaCO ₃)	-	1162	1074	1375	1250	1552	1688	-	765	1219	1058	1089	1309	995
Conductivity (mS/m)	199	281	337	372	478	689	1214	183	243	269	334	458	734	1386
SO ₄ ²⁻ (mg/l)	303	341	324	126	469	325	222	300	168	203	125	468	332	440
Sulphide (mg/l)	48	24	16	24	24	48	48	40	24	16	24	24	32	72
TKN (mg/l)	219	316	316	283	316	386	522	258	206	200	200	251	283	604
NH ₃ -N (mg/l)	129	232	258	245	283	341	477	103	148	180	174	225	264	542
Organic N (mg/l)	90	84	58	38	33	45	45	155	58	20	26	26	19	62
Cl ⁻ (mg/l)	625	700	900	1025	1425	2600	4625	575	900	850	1100	1450	2450	5800
Na (mg/l)	240	240	170	190	260	650	1060	120	170	290	180	250	570	1210
Ca (mg/l)	111	91	173	232	292	766	1016	97	96	139	193	296	360	700
Cr (mg/l)	1,5	1,5	2,1	1,8	2,0	9,6	32,3	<1	<1	1,1	1,5	2,5	4,5	40,5
Al (mg/l)	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Fe (mg/l)	4,5	4,5	9,3	7,1	3,6	8,5	9,0	8,0	4,5	6,5	6,4	3,8	7,5	7,5
P (mg/l)	1,2	2,8	<1	<1	<1	<1	<1	10,0	6,3	6,3	7,5	2,0	1,8	3,8
VFA (mg/l)	1412,5	160,9	316,6	142,8	140,7	82,2	117,0	1145,8	162,8	142,4	132,6	89,0	100,7	88,8

An increase in the volume of biogas produced was noted as the loading rate was increased (Figure 72). However, the trace nutrients did not appear to stimulate the methanogens or result in any increase in biogas production. On the contrary, the digester receiving trace nutrients began to produce less biogas than the control digester. To overcome this, the trace nutrient concentration was reduced from 100 mg/l each to 20 mg/l each on day 130, as the possibility existed that these trace nutrients could have been inhibiting the metabolism of the methanogenic bacteria. The decreased concentration of trace nutrients had an immediate effect on biogas production. The volume produced improved dramatically and exceeded that produced by the control digester. This resulted in an improvement in the operating efficiency of the digester relative to the control digester (Table 86). It would therefore appear that a trace nutrient concentration of 20 mg/l is stimulatory to the methanogenic bacteria, whereas a concentration of 100 mg/l is partially inhibitory to their metabolism.

As the loading rate was increased, the percentage of CH_4 in the biogas decreased (Figure 73). A similar effect was found in the previous experiment. It should be noted that there was no significant increase in the CH_4 content of the biogas from the digester receiving trace nutrients relative to the control digester.

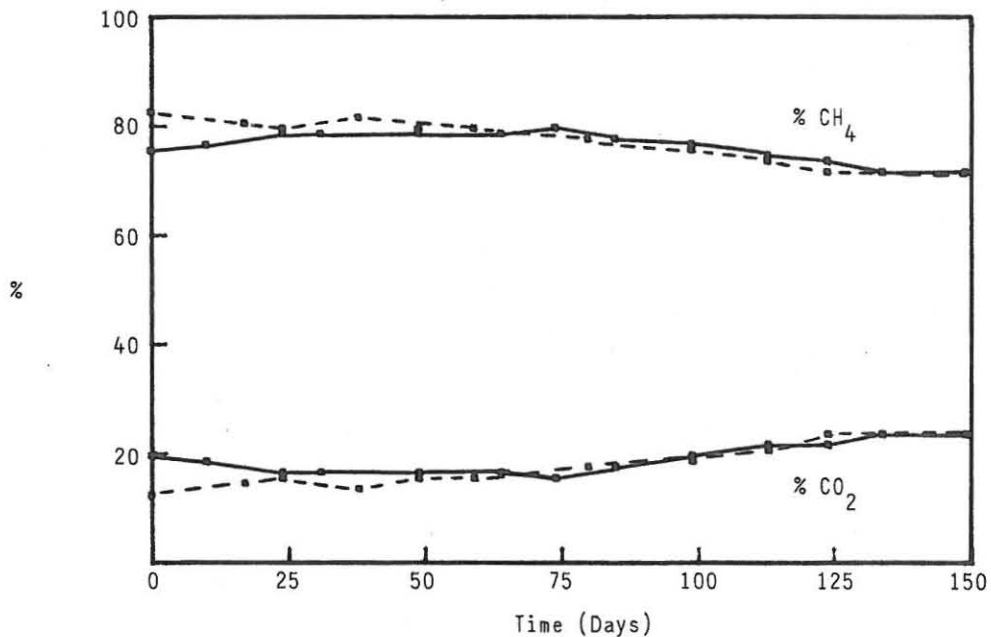


FIGURE 73 - Biogas percentage composition versus time for the anaerobic digester receiving tannery sludge supplemented with trace nutrients (— tannery sludge + trace nutrients digester; ---- tannery sludge digester)

TABLE 86

OPERATING EFFICIENCY OF THE DIGESTER AT VARIOUS STAGES DURING THE
EXPERIMENT TREATING TANNERY SLUDGE SUPPLEMENTED WITH TRACE NUTRIENTS

Parameter	Tannery Sludge							Tannery Sludge + Trace Nutrients						
	Time (Days)							Time (Days)						
	1	25	50	75	100	125	150	1	25	50	75	100	125	150
Gas Volume (ℓ/d)	0,750	1,325	5,625	5,700	6,700	15,500	22,225	0,325	1,225	4,050	6,200	7,175	15,025	29,100
Gas Production (m ³ /m ³ .d)	0,015	0,027	0,113	0,114	0,134	0,310	0,445	0,007	0,025	0,081	0,124	0,144	0,304	0,582
% COD Removed	0,0	37,1	75,7	96,6	98,4	98,0	98,5	0,0	27,0	28,4	95,0	97,5	98,2	98,2
% PV Removed	0,0	26,0	51,9	93,0	94,0	95,4	95,5	0,0	33,5	53,9	89,6	94,8	93,7	97,8
Gas Yield (m ³ /kg COD Removed)	-	7,528	7,560	0,385	0,162	0,291	0,286	-	2,307	13,322	0,798	0,271	0,425	0,391
CH ₄ Content of Biogas (%)	85,1	82,4	82,1	81,8	78,4	74,2	74,4	77,5	80,7	81,4	82,3	79,3	76,4	74,4
CH ₄ Yield (m ³ CH ₄ / kg COD Removed)	-	6,203	6,207	0,315	0,127	0,216	0,213	-	1,862	10,844	0,657	0,215	0,325	0,291
Free NH ₃ (mg/ℓ)	1,9	17,2	15,0	28,4	31,1	13,3	18,6	< 1	2,1	11,9	16,2	19,6	21,1	21,7
H ₂ S (mg/ℓ)	12,2	10,8	6,9	1,0	< 1	18,6	18,6	21,0	6,1	7,1	11,3	11,2	14,7	28,0

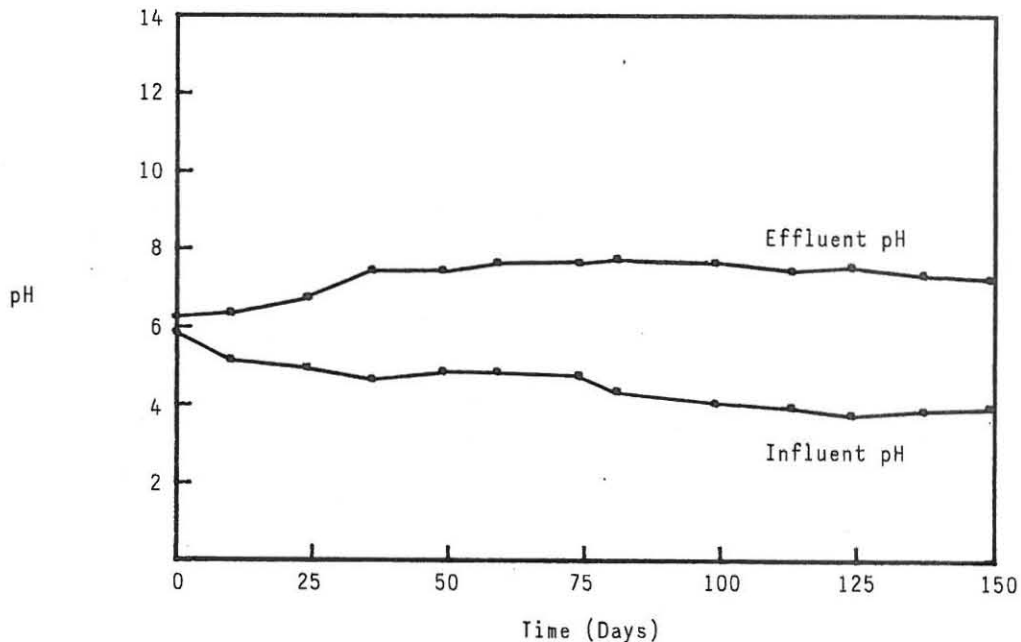


FIGURE 74 - Influent and effluent pH versus time for the anaerobic digester receiving tannery sludge supplemented with trace nutrients

The ammonification of the organic matter present in the sludge (Table 85) resulted in an increase in the pH of the digester. This was despite the pH being initially controlled at 5,5 - 6,0. When the digester pH reached undesirable levels (Day 75), the pH of the influent sludge was further reduced by the addition of 6 N HCl (Figure 74). This resulted in the pH decreasing to a level more suitable for the metabolism of the methanogenic bacteria i.e. a pH of 7,57.

A comparison of Tables 87 and 88 shows that there was a similar accumulation of organic and inorganic solids in the digester as found in the previous experiment. However, one noticeable difference between the two experiments is that there was a reduction in the volume of solids that accumulated in the digester which received trace nutrients. This is supported by comparing the data in Tables 82 and 89 which shows that less solids accumulated in the digester receiving trace nutrients.

TABLE 87

STRATIGRAPHIC ANALYSIS OF THE DIGESTER CONTENTS AT THE START OF THE
EXPERIMENT TREATING TANNERY SLUDGE SUPPLEMENTED WITH TRACE NUTRIENTS

Parameter	Tannery Sludge				Tannery Sludge + Trace Nutrients			
	Height of Sampling Port Above Inlet (cm)				Height of Sampling Port Above Inlet (cm)			
	33	66	99	125	33	66	99	125
pH	7,10	7,08	7,10	7,13	6,62	6,57	6,50	6,58
Total COD (mg/l)	5672	1578	1322	1299	5886	3342	3196	3012
Soluble COD (mg/l)	658	636	618	581	1104	1078	1092	1175
Total PV (mg/l)	870	269	233	228	780	244	210	198
Soluble PV (mg/l)	89	67	61	58	114	108	94	98
Total Solids (mg/l)	5195	2880	2175	2055	4990	2470	2195	2130
TIS (mg/l)	1465	965	820	775	1670	920	815	845
SS (mg/l)	3770	1465	790	685	3670	1105	855	855
TDS (mg/l)	1425	1415	1385	1370	1320	1365	1340	1275
TDIS (mg/l)	720	685	650	615	780	810	770	745
Alkalinity (mg/l CaCO ₃)	990	1012	966	990	632	602	618	594
Bicarbonate Alkalinity (mg/l CaCO ₃)	-	-	-	-	-	-	-	-
Conductivity (mS/m)	198	203	201	199	187	189	192	183
SO ₄ ²⁻ (mg/l)	256	298	347	303	268	257	294	300
Sulphide (mg/l)	36	40	40	48	40	32	40	40
TKN (mg/l)	896	280	242	219	792	286	289	258
NH ₃ -N (mg/l)	146	134	132	129	138	127	109	103
Organic N (mg/l)	750	146	110	90	654	159	180	155
Cl ⁻ (mg/l)	630	635	630	625	585	580	590	575
Na (mg/l)	240	230	220	240	115	120	110	120
Ca (mg/l)	180	90	100	111	206	111	108	97
Cr (mg/l)	7,5	1,0	1,0	1,5	8,8	1,5	1,0	<1
Al (mg/l)	190,0	1,0	<1	<1	150,0	1,0	<1	<1
Fe (mg/l)	190,5	8,0	6,5	4,5	160,5	9,5	6,5	8,0
P (mg/l)	85,0	17,9	7,6	1,2	58,0	27,0	23,5	10,0
VFA (mg/l)	1397,3	1484,4	1519,0	1412,5	1130,1	1167,3	1292,0	1145,8

TABLE 88

STRATIGRAPHIC ANALYSIS OF THE DIGESTER CONTENTS ON COMPLETION OF THE
EXPERIMENT TREATING TANNERY SLUDGE SUPPLEMENTED WITH TRACE NUTRIENTS

Parameter	Tannery Sludge				Tannery Sludge + Trace Nutrients			
	Height of Sampling Port Above Inlet (cm)				Height of Sampling Port Above Inlet (cm)			
	33	66	99	125	33	66	99	125
pH	7,10	7,08	7,10	7,56	7,10	7,10	7,09	7,57
Total COD (mg/l)	43000	35410	37780	1172	36160	26480	14680	1336
Soluble COD (mg/l)	764	661	718	840	556	588	788	476
Total PV (mg/l)	4800	4420	4280	202	3280	2710	950	129
Soluble PV (mg/l)	144	133	102	106	55	50	40	94
Total Solids (mg/l)	46000	39000	46000	10520	47000	37000	17000	9950
TIS (mg/l)	17020	14740	17110	5165	17435	14280	8260	5370
SS (mg/l)	37315	30595	37520	1965	38455	28525	8720	145
TDS (mg/l)	8685	8405	8480	8555	8545	8475	8280	9805
TDIS (mg/l)	5795	5880	5560	5100	5310	4865	4335	5330
Alkalinity (mg/l CaCO ₃)	1288	1288	1288	1771	1150	1242	1150	1058
Bicarbonate Alkalinity (mg/l CaCO ₃)	1200	1214	1229	1688	1079	1169	1088	995
Conductivity (mS/m)	1515	1498	1477	1214	1519	1515	1510	1386
SO ₄ ²⁻ (mg/l)	155	175	152	222	174	191	156	440
Sulphide (mg/l)	40	32	40	48	24	24	24	72
TKN (mg/l)	2946	2235	2810	522	2777	2099	959	604
NH ₃ -N (mg/l)	956	816	929	477	836	817	705	542
Organic N (mg/l)	1990	1419	1881	45	1941	1282	254	62
Cl ⁻ (mg/l)	6100	5925	6000	4625	6325	5950	5975	5800
Na (mg/l)	3130	2600	1260	1060	2750	2540	1780	1210
Ca (mg/l)	1404	1320	1357	1016	1373	770	942	700
Cr (mg/l)	2074,0	1353,0	1883,4	32,3	1668,8	1342,0	304,2	40,5
Al (mg/l)	366,0	492,0	438,0	< 1	596,0	305,0	117,0	< 1
Fe (mg/l)	536,8	516,6	525,6	9,0	685,4	549,0	152,1	7,5
P (mg/l)	468,0	403,0	520,0	< 1	462,0	387,0	122,0	3,8
VFA (mg/l)	124,3	104,7	83,3	117,0	100,9	103,3	88,2	88,8

The solids balance for the anaerobic digestion of tannery sludge supplemented with trace nutrients is shown in Table 89. If the Total Volatile Solids (TVS) or the organic content of the sludge is concentrated on, then the following is obtained:-

$$\begin{aligned}\text{TVS accumulated} &= 1651,65 \text{ g} - 888,28 \text{ g} \\ &= 763,37 \text{ g} \\ &= \underline{28,8 \% \text{ of TVS that entered}} \rightarrow\end{aligned}$$

$$\begin{aligned}\text{TVS degraded} &= 2652,42 \text{ g} - (763,37 \text{ g} + 254,88 \text{ g}) \\ &= 1634,17 \text{ g} \\ &= \underline{61,6 \% \text{ of TVS that entered}} \rightarrow\end{aligned}$$

$$\begin{aligned}\text{TVS emerged} &= 254,88 \text{ g} \\ &= \underline{9,6 \% \text{ of TVS that entered}} \rightarrow\end{aligned}$$

TABLE 89

SOLIDS BALANCE FOR THE ANAEROBIC DIGESTION OF
TANNERY SLUDGE SUPPLEMENTED WITH TRACE NUTRIENTS

Solids Present in the Digester - Day 1

Total Solids	= 1372,42 g
Total Volatile Solids	= 888,28 g
Total Inorganic Solids	= 484,14 g

Solids that Entered the Digester - Day 1 to 150

Total Solids	= 3860,88 g
Total Volatile Solids	= 2652,42 g
Total Inorganic Solids	= 1208,45 g

Solids Present in the Digester - Day 150

Total Solids	= 2960,13 g
Total Volatile Solids	= 1651,65 g
Total Inorganic Solids	= 1308,48 g

Solids that Emerged from the Digester - Day 1 to 150

Total Solids	= 566,02 g
Total Volatile Solids	= 254,88 g
Total Inorganic Solids	= 311,14 g

The value of 61,6 % of the TVS degraded is slightly greater than that obtained in the previous experiment, viz. 60,0 %. This can be attributed to an increase in the metabolism of the methanogenic bacteria as a result of the availability of trace nutrients. As the initial concentration of trace nutrients i.e. 100 mg/l, appeared to partially inhibit the methanogens, this increase in the degradation of volatile solids would most probably have occurred during the latter stages of the experiment i.e. when the trace nutrient concentration had been reduced to 20 mg/l. This correlates with the increase in the volume of biogas produced during the latter stages of the experiment (Figure 72). As with the previous experiment, the percentage organic matter degraded is in agreement with the findings of a previous study (Aloy et al, 1987; Aloy et al, 1989).

The biogas yield from the tannery sludge supplemented with trace nutrients is shown in Table 90. The value of 0,817 l of biogas produced for every gram of volatile solids degraded is almost identical to the value obtained in the previous experiment, and is in close agreement with that obtained from a previous study viz. 0,983 l biogas/g TVS degraded (Aloy et al, 1987; Aloy et al, 1989).

TABLE 90

BIOGAS YIELD FROM TANNERY SLUDGE
SUPPLEMENTED WITH TRACE NUTRIENTS

Total biogas produced over the duration of the experiment at STP (Standard Temperature and Pressure)	= 1335,29 l
TVS degraded	= 1634,17 g
1634,17 g TVS	→ 1335,29 l biogas
∴ 1 g	→ 0,817 l biogas
	= 0,613 l CH ₄ (at 75 % CH ₄)
	≈ 5,8 kcal
	≈ 24,3 kJ

The results of this investigation indicate that the efficiency of the

anaerobic digestion of tannery sludge can be marginally improved through supplementation of the sludge with trace nutrients. However, care should be taken when selecting the concentration of trace nutrients to be used, as in this experiment, a concentration of 100 mg/l of each nutrient appeared to be partially inhibitory to the methanogenic bacteria. However, as soon as the concentration had been reduced to 20 mg/l, this resulted in an improvement in the operating efficiency of the digester over that of the control digester.

The findings of this experiment have been previously published (Jackson-Moss and Duncan, 1990f).

7.3 The Two-Stage Anaerobic Digestion of Tannery Sludge

7.3.1 Introduction

Despite its many theoretical benefits, the application of anaerobic processes to the practice of wastewater treatment is often fraught with difficulties. In particular, the instability of this process makes control difficult and thereby diminishes its performance. Since the anaerobic digestion process is essentially diphasic, this lack of stability may often be due to imbalances occurring between those groups of microorganisms achieving the initial acidification, and those effecting the final conversion of the substrate to methane. This may lead to a subsequent accumulation of intermediary products, with a resultant decrease in the pH (Cohen et al, 1979).

As the two groups of bacteria i.e. the acid-forming and the methane-forming bacteria differ widely with respect to their physiology and nutritional requirements, a separation of the acid-forming phase from the methane-forming phase by employing two separate digesters was proposed (Pohland and Ghosh, 1971). The acid phase would provide the substrate for the methane phase and to a great extent, the performance of the methane phase would depend on the performance of the acid phase. In each of these digesters, optimal environmental conditions for each group of bacteria could be adjusted, which could result in higher conversion rates and increased stability of the overall process (Cohen et al, 1980).

The successful application of the two-phase concept depends on the feasibility of physically separating and maintaining dominant cultures of non-methanogenic

and methanogenic organisms in two separate digesters. Although several methods can be used for phase separation, the most commonly used technique is phase separation through kinetic control (Ghosh and Klass, 1978). For example, the retention time selected for the non-methanogenic or "acid" digester should promote conversion of the substrates to volatile acids at the maximum rate, and at the same time be shorter than the critical retention time of the methanogens to promote the selective enrichment of acidogenic metabolism in a separate environment. This is possible as the growth rates of these acid-forming bacteria are relatively fast, with a doubling time of <5 hours at 37°C , compared with the relatively slow doubling times of days and weeks for methanogenic bacteria (Britz, 1990). An unduly long acid digester retention time is expected to preclude the selective enrichment of the non-methanogenic organisms and to favour the simultaneous growth of both the non-methanogenic and the methanogenic bacteria. The acid-phase digester in this case becomes a conventional mixed-phase digester, and the two-phase system reverts to a "two-stage" digestion process (Ghosh and Klass, 1978).

A number of two-phase anaerobic digestion studies have been carried out (Cohen *et al*, 1979; Cohen *et al*, 1980; Ghosh *et al*, 1975; Datta, 1981; Cohen *et al*, 1982; Ng *et al*, 1985; Tanaka and Matsuo, 1986; Wu *et al*, 1988; Gijzen *et al*, 1988). These have included both laboratory and full-scale investigations, and from these investigations, it has been shown that the two-phase anaerobic digestion process has a number of advantages over the conventional one-phase process:-

- (a) the optimization of environmental conditions for both acidogenesis and methanogenesis in two separate digesters can increase the rates of turnover of substrates, and hence result in a reduction in total plant size.
- (b) the acid reactor can act as a metabolic buffer with respect to rapid changes in the influent composition, thereby reducing adaptive periods of the more sensitive methanogenic phase and hence increasing the stability of the overall process.
- (c) overloading of the methanogenic phase can be prevented by controlling the acid digester influent to the methane digester.

- (d) the disposal of relatively fast growing acid-forming sludge can occur without loss of the methane-producing bacteria.

From the point of view of treating tannery wastewaters, the most important of these advantages is the last one viz. the ability to remove sludge from the digester without losing the methanogenic bacteria. In the previous experiments, it was found that inorganic compounds such as chrome, iron, calcium and aluminium, precipitate and accumulate in the sludge beds of anaerobic digesters. Over long periods of anaerobic digestion of tannery wastewaters, these compounds will accumulate to such an extent that they will eventually have to be removed from the digester. This has to be achieved without removing the methanogenic bacteria, and in view of this, the two-stage anaerobic digestion process was investigated for possible application in the tanning industry. It is important to note that because of the long retention times used, the metabolic phases were not separated. The digesters were merely run in series and consequently two-stage and not two-phase digestion was carried out.

7.3.2 Materials and Methods

The materials and methods used were described in detail in Chapter 4, with the exception that two digesters were run in series. The effluent emerging from the acid digester was pumped into the methanogenic digester. Each digester was operated at a hydraulic retention time of 25 days, and the overall hydraulic retention time was therefore 50 days, the same as that used for the single-stage anaerobic digestion of tannery sludge experiments.

7.3.3 Results and Discussion

7.3.3.1 Acidogenic Digester

After seeding the digester with anaerobic sludge as described previously (Section 7.1.2), it was supplied with tannery sludge at a loading rate of 3 kg COD/m³.d from the start of the experiment. This high initial loading rate had an immediate effect on the metabolic activity of the microorganisms inside the digester. The concentration of VFA increased rapidly (Figure 75) which was a clear indication that the methanogenic bacteria were unable to cope with the high loading rate. The volume of biogas produced decreased markedly (Figure

76) and by day 28 had ceased completely. The percentage CH_4 in the biogas showed a similar decrease and by day 60, no CH_4 could be detected in the biogas (Figure 76). The metabolism of the methanogenic bacteria was therefore successfully inhibited and the digester was able to function as an acidogenic digester.

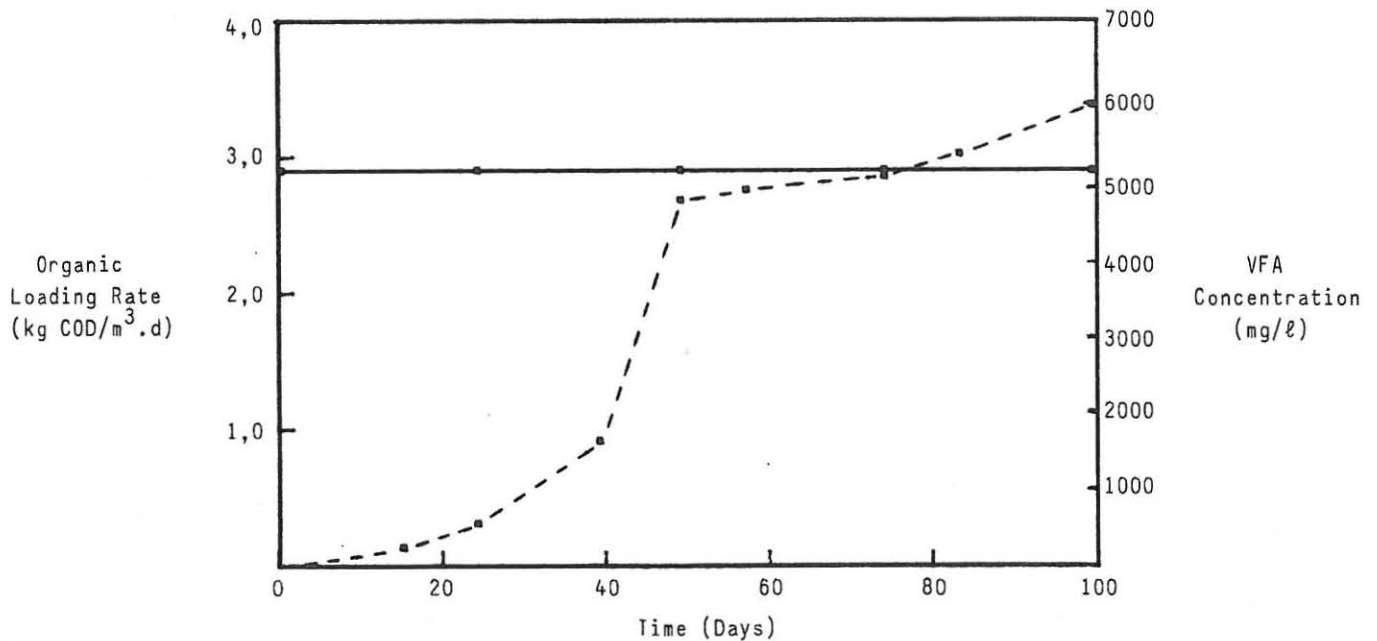


FIGURE 75 - Organic loading rate and effluent volatile fatty acids versus time for the acidogenic digester during the two-stage anaerobic digestion of tannery sludge (— organic loading rate; ---- volatile fatty acids as acetic acid)

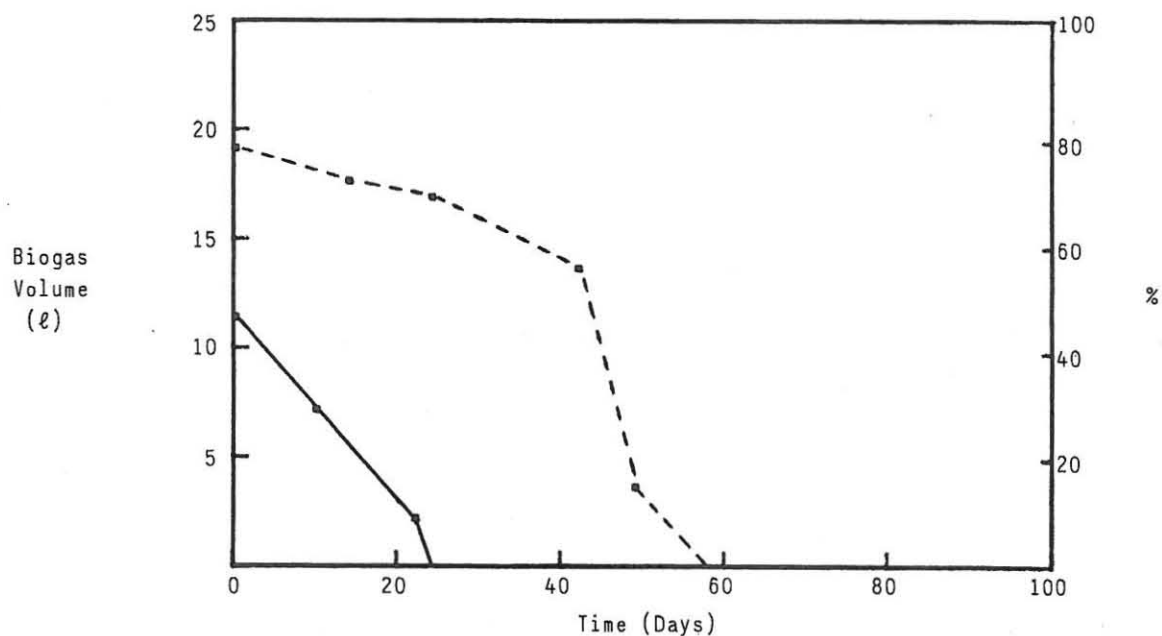


FIGURE 76 - Biogas volume and percentage CH_4 versus time for the acidogenic digester during the two-stage anaerobic digestion of tannery sludge (— biogas volume; ---- % CH_4)

The high concentration of VFA in the digester (Table 91) caused a reduction in the pH of the digester effluent (Figure 77). To prevent the pH from falling too low, less acid (HCl) was used to reduce the influent pH of the sludge. This resulted in the digester pH returning to 6,5 - 6,8 and clearly shows the buffering capacity of ammonium bicarbonate, as this increase in the digester pH occurred despite the high concentration of VFA inside the digester.

The high concentration of COD, PV and SS in the digester effluent (Table 91) ensured that very little, if any, reduction in the COD and PV of the sludge occurred (Table 92). Although there was a high rate of conversion of the organic compounds to VFA, this was not complete, as there was a significantly high concentration of undegraded organic matter in the digester effluent (Table 91). The important aspect to note is that there was also a high concentration of inorganic compounds present in the effluent from the digester i.e. chrome, calcium and iron. These compounds were most probably bound to the organic matter present in the effluent, and would therefore have entered the methanogenic digester. This is an undesirable occurrence as the whole reasoning behind the two-stage digestion process is to prevent the heavy metals from entering the methanogenic digester.

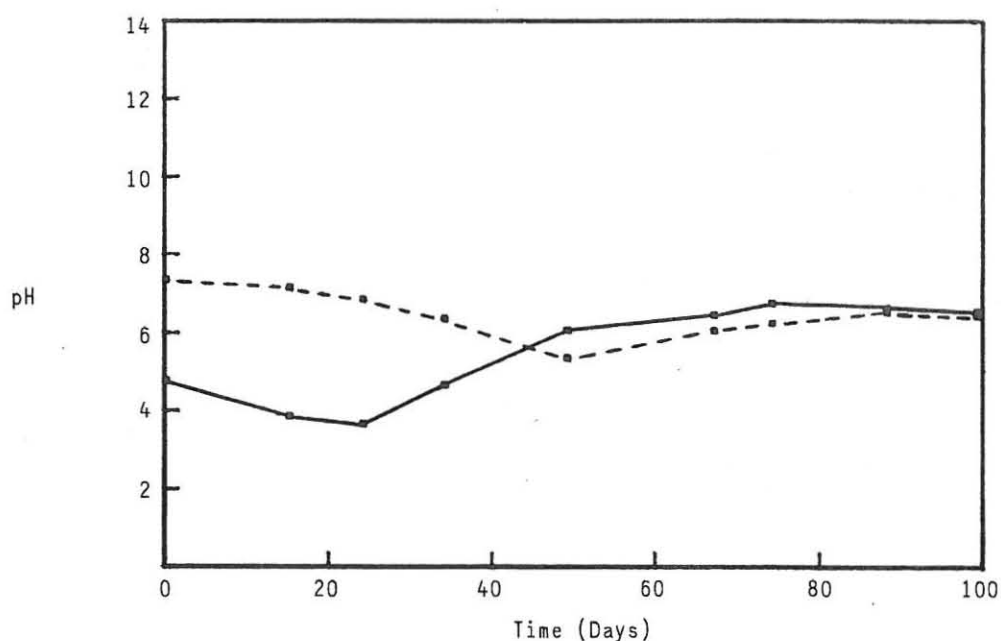


FIGURE 77 - Influent and effluent pH versus time for the acidogenic digester during the two-stage anaerobic digestion of tannery sludge (— influent pH; ---- effluent pH)

TABLE 91

CHARACTERISTICS OF THE ACIDOGENIC DIGESTER EFFLUENT AT
VARIOUS STAGES DURING THE TWO-STAGE ANAEROBIC DIGESTION
OF TANNERY SLUDGE

Parameter	Time (Days)				
	1	25	50	75	100
pH	7,65	7,15	5,74	6,57	6,84
Total COD (mg/l)	3456	3616	17280	58600	61130
Soluble COD (mg/l)	561	762	7560	6874	7488
Total PV (mg/l)	154	168	1270	4290	5280
Soluble PV (mg/l)	124	102	72	88	552
Total Solids (mg/l)	1600	6500	24590	25985	58155
TIS (mg/l)	765	3200	12435	12805	23555
SS (mg/l)	585	1990	7195	11580	43090
TDS (mg/l)	1015	4510	17395	14405	15065
TDIS (mg/l)	570	2500	10250	11060	11300
Alkalinity (mg/l CaCO ₃)	1288	1365	2562	3822	3612
Bicarbonate Alkalinity (mg/l CaCO ₃)	1231	857	—	162	—
Conductivity (mS/m)	249	901	2310	2240	2090
SO ₄ ²⁻ (mg/l)	137	254	722	623	737
Sulphide (mg/l)	40	192	216	672	800
TKN (mg/l)	228	499	1294	2988	2554
NH ₃ -N (mg/l)	191	437	1035	1263	1098
Organic N (mg/l)	37	62	259	1725	1456
Cl ⁻ (mg/l)	700	3125	8175	9575	10050
Na (mg/l)	50	690	1210	4000	6000
Ca (mg/l)	206	414	1120	2740	2760
Cr (mg/l)	1,1	66,0	398,4	444,0	3130,0
Al (mg/l)	<1	<1	<1	<1	<1
Fe (mg/l)	4,0	19,5	75,5	91,5	224,5
P (mg/l)	7,5	10,0	37,5	39,0	70,0
VFA (mg/l)	80,2	716,8	4866,7	5169,3	6090,9

TABLE 92

OPERATING EFFICIENCY OF THE ACIDOGENIC DIGESTER AT VARIOUS STAGES
DURING THE TWO-STAGE ANAEROBIC DIGESTION OF TANNERY SLUDGE

Parameter	Time (Days)				
	1	25	50	75	100
Gas Volume (l/d)	12,000	0,300	0,000	0,000	0,000
Gas Production ($\text{m}^3/\text{m}^3 \cdot \text{d}$)	0,240	0,006	0,000	0,000	0,000
% COD Removed	95,4	95,2	77,2	22,8	19,4
% PV Removed	96,5	96,2	71,5	3,8	0,0
Gas Yield (m^3/kg COD Removed)	0,017	0,004	-	-	-
CH_4 Content of Biogas (%)	78,8	70,3	16,8	0,0	0,0
CH_4 Yield ($\text{m}^3 \text{CH}_4/\text{kg}$ COD Removed)	0,013	0,003	-	-	-
Free NH_3 (mg/l)	9,2	7,0	< 1	5,1	8,8
H_2S (mg/l)	16,4	50,8	117,7	353,0	427,9

It is evident by comparing Tables 93 and 94 that there was a large increase in the solids concentration in the digester. This is supported by the data outlined in Table 95 which shows that 33,5 % of the organic matter supplied to the digester actually accumulated in the digester, while a further 14,0 % emerged in the digester effluent. This large accumulation of solids in the digester, coupled with the high concentration of undigested organic matter in the digester effluent, indicates that acidification of the tannery sludge was not complete. This can possibly be attributed to the nature of the tannery sludge, i.e. the high concentration of hard-to-digest proteins, and the fact that the HRT had been reduced to 25 days. If acidification is to be carried out successfully, with the prevention of the carry over of inorganic compounds such as heavy metals into the methanogenic digester, then a longer HRT may be necessary.

TABLE 93

STRATIGRAPHIC ANALYSIS OF THE ACIDOGENIC DIGESTER CONTENTS AT THE
START OF THE TWO-STAGE ANAEROBIC DIGESTION OF TANNERY SLUDGE

Parameter	Height of Sampling Port Above Inlet (cm)			
	33	66	99	125
pH	7,53	7,51	7,59	7,65
Total COD (mg/l)	3075	3076	2358	3456
Soluble COD (mg/l)	553	549	542	561
Total PV (mg/l)	171	162	148	154
Soluble PV (mg/l)	80	81	112	124
Total Solids (mg/l)	3000	3000	2000	1600
TIS (mg/l)	970	965	780	765
SS (mg/l)	2170	2085	870	585
TDS (mg/l)	830	915	1130	1015
TDIS (mg/l)	590	525	220	570
Alkalinity (mg/l CaCO_3)	1012	966	1012	1288
Bicarbonate Alkalinity (mg/l CaCO_3)	959	917	960	1231
Conductivity (mS/m)	232	245	245	249
SO_4^{2-} (mg/l)	412	333	227	137
Sulphide (mg/l)	24	24	32	40
TKN (mg/l)	246	259	234	228
$\text{NH}_3\text{-N}$ (mg/l)	172	179	172	191
Organic N (mg/l)	74	80	62	37
Cl^- (mg/l)	700	700	675	700
Na (mg/l)	50	50	40	50
Ca (mg/l)	190	190	200	206
Cr (mg/l)	<1	2,0	2,0	1,1
Al (mg/l)	20,0	15,0	<1	<1
Fe (mg/l)	24,5	21,0	17,0	4,0
P (mg/l)	35,0	35,0	25,0	7,5
VFA (mg/l)	74,7	69,7	72,9	80,2

TABLE 94

STRATIGRAPHIC ANALYSIS OF THE ACIDOGENIC DIGESTER CONTENTS ON
COMPLETION OF THE TWO-STAGE ANAEROBIC DIGESTION OF TANNERY SLUDGE

Parameter	Height of Sampling Port Above Inlet (cm)			
	33	66	99	125
pH	6,99	6,94	6,94	6,84
Total COD (mg/l)	154360	125560	84250	61130
Soluble COD (mg/l)	8573	8342	8060	7488
Total PV (mg/l)	16600	11960	6960	5280
Soluble PV (mg/l)	695	600	662	552
Total Solids (mg/l)	93000	94000	77000	58155
TIS (mg/l)	31340	30450	27720	23555
SS (mg/l)	78560	79390	62090	43090
TDS (mg/l)	14440	14610	14910	15065
TDIS (mg/l)	10695	10605	10570	11300
Alkalinity (mg/l CaCO ₃)	4208	4208	3956	3612
Bicarbonate Alkalinity (mg/l CaCO ₃)	-	-	-	-
Conductivity (mS/m)	2320	2270	2230	2090
SO ₄ ²⁻ (mg/l)	641	679	728	737
Sulphide (mg/l)	704	776	710	800
TKN (mg/l)	4105	4286	3287	2554
NH ₃ -N (mg/l)	1622	1580	1390	1098
Organic N (mg/l)	2483	2706	1897	1456
Cl ⁻ (mg/l)	9975	10000	10025	10050
Na (mg/l)	5600	6000	5700	6000
Ca (mg/l)	3241	2933	3080	2760
Cr (mg/l)	3549,0	3080,0	2475,0	3130,0
Al (mg/l)	455,0	308,0	225,0	< 1
Fe (mg/l)	501,0	524,0	473,0	224,5
P (mg/l)	326,4	326,4	180,0	70,0
VFA (mg/l)	6214,6	6288,3	5871,7	6090,9

The solids balance for the acidogenic digester during the two-stage anaerobic digestion of tannery sludge experiment is shown in Table 95. If the Total Volatile Solids (TVS) or the organic content of the sludge is concentrated on, then the following is obtained:-

$$\begin{aligned}\text{TVS accumulated} &= 3976,95 \text{ g} - 897,60 \text{ g} \\ &= 3079,35 \text{ g} \\ &= \underline{33,5 \% \text{ of TVS that entered}} \rightarrow\end{aligned}$$

$$\begin{aligned}\text{TVS degraded} &= 9205,80 \text{ g} - (3079,35 \text{ g} + 1755,60 \text{ g}) \\ &= 4834,95 \text{ g} \\ &= \underline{52,5 \% \text{ of TVS that entered}} \rightarrow\end{aligned}$$

$$\begin{aligned}\text{TVS emerged} &= 1755,60 \text{ g} \\ &= \underline{14,0 \% \text{ of TVS that entered}} \rightarrow\end{aligned}$$

TABLE 95

SOLIDS BALANCE FOR THE ACIDOGENIC DIGESTER
DURING THE TWO-STAGE ANAEROBIC DIGESTION
OF TANNERY SLUDGE

Solids Present in the Digester - Day 1

Total Solids	= 1506,65 g
Total Volatile Solids	= 897,60 g
Total Inorganic Solids	= 609,05 g

Solids that Entered the Digester - Day 1 to 100

Total Solids	= 13400,00 g
Total Volatile Solids	= 9205,80 g
Total Inorganic Solids	= 4194,20 g

Solids Present in the Digester - Day 100

Total Solids	= 6221,75 g
Total Volatile Solids	= 3976,95 g
Total Inorganic Solids	= 2244,80 g

Solids that Emerged from the Digester - Day 1 to 100

Total Solids	= 3222,34 g
Total Volatile Solids	= 1755,60 g
Total Inorganic Solids	= 1466,74 g

7.3.3.2 Methanogenic Digester

After seeding the digester with anaerobic sludge as described previously (Section 7.1.2), the digester was supplied with the effluent that emerged from the acidogenic digester. This effluent was, however, supplemented with the trace nutrients Ni, Co, Mo and W at a concentration of 20 mg/l each, as a result of the findings of the previous experiment (Section 7.2). The loading rate increased as the concentration of COD in the effluent from the acidogenic digester increased (Figure 78). Although there was a slight increase in the concentration of VFA during the first 20 days of operation, their concentration decreased to remain constant for the remainder of the experiment (Figure 78). The increase in the loading rate resulted in a corresponding increase in the volume of biogas produced (Figure 79). The percentage CH_4 in the biogas was maintained at greater than 80 % for most of the duration of the experiment (Figure 79). The influent pH was controlled in the range 4,5 - 5,5 in order to maintain an optimum pH inside the digester (Figure 80). The methanogenic digester was therefore able to successfully treat the effluent received from the acidogenic digester, and low COD and PV concentrations were present in the effluent from the methanogenic digester (Table 96). This resulted in a high operational efficiency for the digester (Table 97).

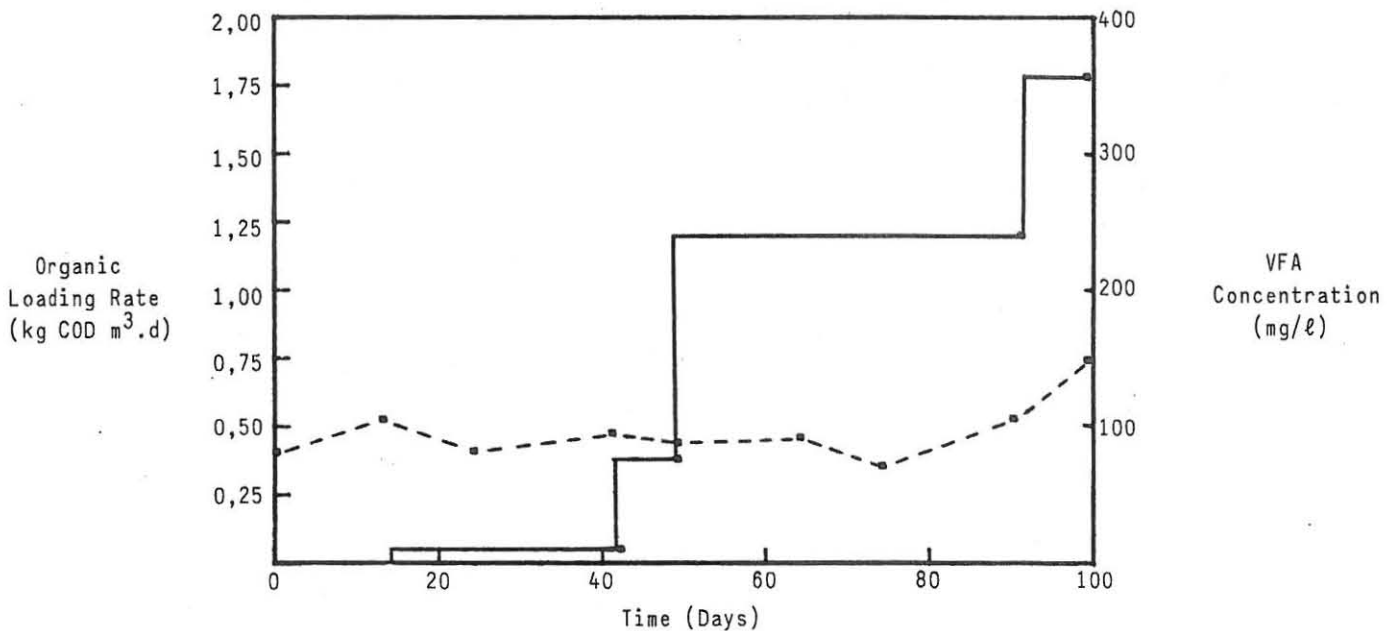


FIGURE 78 - Organic loading rate and effluent volatile fatty acids versus time for the methanogenic digester during the two-stage anaerobic digestion of tannery sludge (— organic loading rate; ---- volatile fatty acids as acetic acid)

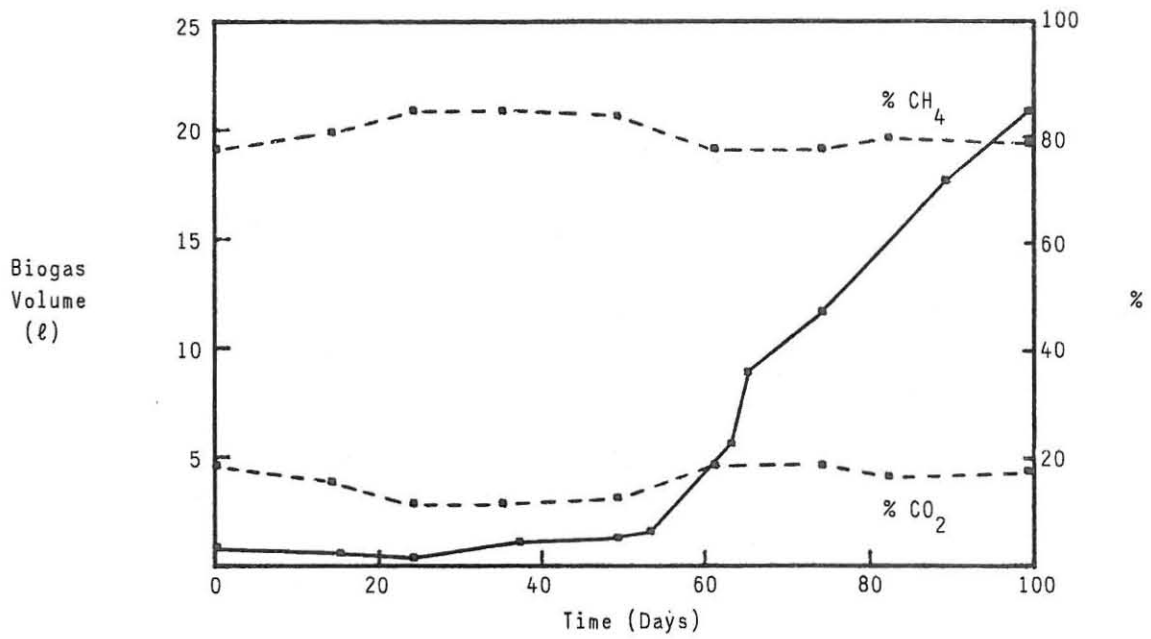


FIGURE 79 - Biogas volume and percentage composition versus time for the methanogenic digester during the two-stage anaerobic digestion of tannery sludge (— biogas volume; ---- % CH₄ and CO₂)

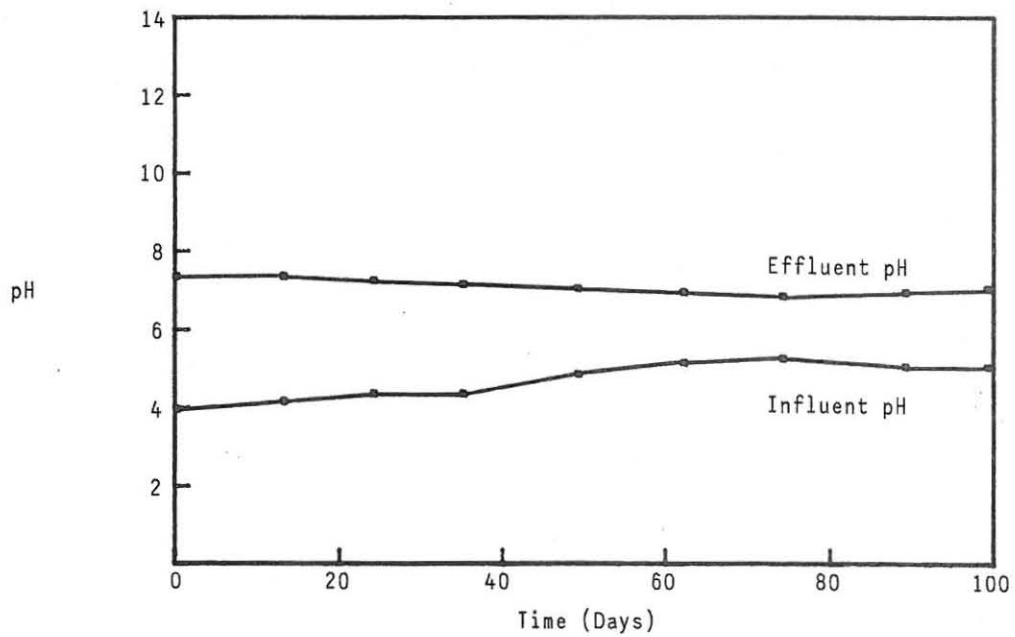


FIGURE 80 - Influent and effluent pH versus time for the methanogenic digester during the two-stage anaerobic digestion of tannery sludge

TABLE 96

CHARACTERISTICS OF THE METHANOGENIC DIGESTER EFFLUENT AT
VARIOUS STAGES DURING THE TWO-STAGE ANAEROBIC DIGESTION
OF TANNERY SLUDGE

Parameter	Time (Days)				
	1	25	50	75	100
pH	7,72	7,55	7,36	7,16	7,38
Total COD (mg/l)	1260	480	600	826	1398
Soluble COD (mg/l)	760	400	496	522	722
Total PV (mg/l)	155	135	106	126	216
Soluble PV (mg/l)	80	90	72	64	115
Total Solids (mg/l)	1905	1425	4470	9725	10640
TIS (mg/l)	700	640	2375	6265	7200
SS (mg/l)	930	115	50	200	470
TDS (mg/l)	975	1310	4420	9525	10170
TDIS (mg/l)	645	535	2360	6105	6850
Alkalinity (mg/l CaCO ₃)	1265	1302	1281	2184	2142
Bicarbonate Alkalinity (mg/l CaCO ₃)	1200	1237	1212	2127	2029
Conductivity (mS/m)	254	299	830	1582	1774
SO ₄ ²⁻ (mg/l)	175	133	240	162	264
Sulphide (mg/l)	32	120	152	168	208
TKN (mg/l)	240	240	407	623	788
NH ₃ -N (mg/l)	203	222	400	609	708
Organic N (mg/l)	37	18	7	14	80
Cl ⁻ (mg/l)	650	950	2875	5850	6300
Na (mg/l)	50	140	320	1360	2800
Ca (mg/l)	82	86	320	872	660
Cr (mg/l)	< 1	< 1	1,3	8,0	25,8
Al (mg/l)	< 1	< 1	< 1	< 1	< 1
Fe (mg/l)	2,5	7,0	7,0	5,5	6,5
P (mg/l)	11,3	6,5	< 1	< 1	< 1
VFA (mg/l)	91,2	91,7	97,8	81,0	159,1

TABLE 97

OPERATING EFFICIENCY OF THE METHANOGENIC DIGESTER AT VARIOUS STAGES
DURING THE TWO-STAGE ANAEROBIC DIGESTION OF TANNERY SLUDGE

Parameter	Time (Days)				
	1	25	50	75	100
Gas Volume (ℓ/d)	1,475	1,000	1,900	12,275	21,475
Gas Production ($\text{m}^3/\text{m}^3 \cdot \text{d}$)	0,030	0,020	0,038	0,246	0,430
% COD Removed	0,0	84,0	94,4	96,5	97,0
% PV Removed	3,1	38,6	92,0	93,3	95,1
Gas Yield (m^3/kg COD Removed)	-	0,398	0,188	0,540	0,483
CH ₄ Content of Biogas (%)	78,5	85,9	85,4	78,5	80,4
CH ₄ Yield (m^3 CH ₄ /kg COD Removed)	-	0,342	0,161	0,430	0,388
Free NH ₃ (mg/ℓ)	11,4	8,4	10,0	9,7	18,4
H ₂ S (mg/ℓ)	13,6	46,0	50,4	44,5	70,1

A comparison of Tables 98 and 99 shows that there was an accumulation of solids inside the digester. This is supported by the data in Table 100. The solids that accumulated were solids that had not been degraded or converted to VFA in the acidogenic digester, and shows the importance of achieving total acidification of the waste. Another compound that accumulated substantially was chrome. Although most of the chrome accumulated in the acidogenic digester, a large quantity was carried over into the methanogenic digester. This is also undesirable as the whole reasoning behind the two-stage system was to prevent an accumulation of both inorganic and organic compounds in the methanogenic digester.

TABLE 98

STRATIGRAPHIC ANALYSIS OF THE METHANOGENIC DIGESTER CONTENTS AT THE
START OF THE TWO-STAGE ANAEROBIC DIGESTION OF TANNERY SLUDGE

Parameter	Height of Sampling Port Above Inlet (cm)			
	33	66	99	125
pH	7,66	7,69	7,74	7,72
Total COD (mg/l)	8950	1755	1552	1260
Soluble COD (mg/l)	746	757	727	760
Total PV (mg/l)	2770	180	186	155
Soluble PV (mg/l)	94	92	126	80
Total Solids (mg/l)	24000	2000	3000	1905
TIS (mg/l)	7775	655	850	700
SS (mg/l)	23270	1215	2330	930
TDS (mg/l)	730	785	670	975
TDIS (mg/l)	450	350	240	645
Alkalinity (mg/l CaCO ₃)	1334	1058	1058	1265
Bicarbonate Alkalinity (mg/l CaCO ₃)	1279	998	1003	1200
Conductivity (mS/m)	227	243	242	254
SO ₄ ²⁻ (mg/l)	142	229	255	175
Sulphide (mg/l)	24	24	16	32
TKN (mg/l)	1177	283	277	240
NH ₃ -N (mg/l)	172	191	191	203
Organic N (mg/l)	1005	92	86	37
Cl ⁻ (mg/l)	775	700	675	650
Na (mg/l)	50	40	40	50
Ca (mg/l)	240	90	80	82
Cr (mg/l)	8,0	2,5	2,5	< 1
Al (mg/l)	240,0	10,0	< 1	< 1
Fe (mg/l)	156,5	29,0	23,5	2,5
P (mg/l)	240,0	75,0	25,0	11,3
VFA (mg/l)	78,1	84,9	77,6	91,2

TABLE 99

STRATIGRAPHIC ANALYSIS OF THE METHANOGENIC DIGESTER CONTENTS ON
COMPLETION OF THE TWO-STAGE ANAEROBIC DIGESTION OF TANNERY SLUDGE

Parameter	Height of Sampling Port Above Inlet (cm)			
	33	66	99	125
pH	6,88	7,08	7,04	7,38
Total COD (mg/l)	78960	41660	37900	1398
Soluble COD (mg/l)	892	874	856	722
Total PV (mg/l)	7860	5960	5130	216
Soluble PV (mg/l)	148	154	130	115
Total Solids (mg/l)	72000	52000	49000	10640
TIS (mg/l)	25775	20330	19160	7200
SS (mg/l)	60305	41815	38875	470
TDS (mg/l)	11695	10185	10125	10170
TDIS (mg/l)	9235	7605	7590	6850
Alkalinity (mg/l CaCO ₃)	2352	2310	2310	2142
Bicarbonate Alkalinity (mg/l CaCO ₃)	494	2119	2051	2029
Conductivity (mS/m)	1940	1820	1830	1774
SO ₄ ²⁻ (mg/l)	141	195	187	264
Sulphide (mg/l)	176	168	176	208
TKN (mg/l)	3268	2954	2783	788
NH ₃ -N (mg/l)	1495	1378	1312	708
Organic N (mg/l)	1773	1576	1471	80
Cl ⁻ (mg/l)	6250	6250	6200	6300
Na (mg/l)	3000	2100	2800	2800
Ca (mg/l)	2862	1632	1285	660
Cr (mg/l)	2627	1584	1404	25,8
Al (mg/l)	309	330	270	<1
Fe (mg/l)	525	594	540	6,5
P (mg/l)	203,2	235,5	208,5	<1
VFA (mg/l)	2624,5	269,2	365,5	159,1

The solids balance for the methanogenic digester during the two-stage anaerobic digestion of tannery sludge experiment is shown in Table 100. If the Total Volatile Solids (TVS) or the organic content of the solids is concentrated on, then the following is obtained:-

$$\begin{aligned}\text{TVS accumulated} &= 1944,00 \text{ g} - 897,60 \text{ g} \\ &= 1046,40 \text{ g} \\ &= \underline{59,6 \% \text{ of TVS that entered}} \rightarrow\end{aligned}$$

$$\begin{aligned}\text{TVS degraded} &= 1755,60 \text{ g} - (1046,40 \text{ g} + 216,93 \text{ g}) \\ &= 492,27 \text{ g} \\ &= \underline{28,0 \% \text{ of TVS that entered}} \rightarrow\end{aligned}$$

$$\begin{aligned}\text{TVS emerged} &= 216,93 \text{ g} \\ &= \underline{12,4 \% \text{ of TVS that entered}} \rightarrow\end{aligned}$$

TABLE 100

SOLIDS BALANCE FOR THE METHANOGENIC DIGESTER
DURING THE TWO-STAGE ANAEROBIC DIGESTION
OF TANNERY SLUDGE

Solids Present in the Digester - Day 1

Total Solids	= 1506,65 g
Total Volatile Solids	= 897,60 g
Total Inorganic Solids	= 609,05 g

Solids that Entered the Digester - Day 1 to 100

Total Solids	= 3222,34 g
Total Volatile Solids	= 1755,60 g
Total Inorganic Solids	= 1466,74 g

Solids Present in the Digester - Day 100

Total Solids	= 3196,40 g
Total Volatile Solids	= 1944,00 g
Total Inorganic Solids	= 1252,40 g

Solids that Emerged from the Digester - Day 1 to 100

Total Solids	= 498,24 g
Total Volatile Solids	= 216,93 g
Total Inorganic Solids	= 281,31 g

In contrast to the previous sludge experiments, a very high concentration of the organic matter accumulated in the methanogenic digester (Table 100) i.e. 59,6 % as opposed to 29,5 and 28,8 % in the previous experiments. This is rather unusual as a large volume of CH_4 gas was still produced. A possible explanation for this is that because of the presence of the acidogenic digester, the population of active acidogenic bacteria in the methanogenic digester may have been reduced. This reduced population may, therefore, not have been able to cope with the undigested organic matter that was being supplied to the methanogenic digester, and hence the accumulation of this organic matter. This once again shows the importance of obtaining the optimum conditions in each digester so that the overall process can function optimally.

Despite the accumulation of solids inside the digester, a large volume of CH_4 gas was produced. For every 1 g TVS degraded, 1,282 l of biogas was evolved (Table 101). This value is significantly higher than that obtained in the previous sludge experiments (Tables 83 and 90). It is possible therefore, that through the use of a two-stage digestion system, the biogas volume and hence the CH_4 yield can be increased.

TABLE 101

BIOGAS YIELD FROM THE METHANOGENIC DIGESTER DURING
THE TWO-STAGE ANAEROBIC DIGESTION OF TANNERY SLUDGE

Total biogas produced over the duration of the experiment at STP (Standard Temperature and Pressure)	= 631,175 l
TVS degraded	= 492,27 g
492,27 g TVS	—————→ 631,175 l biogas
∴ 1 g TVS	—————→ 1,282 l biogas
	= 1,026 l CH_4 (at 80 % CH_4)
	≈ 9,7 kcal
	≈ 40,5 kJ

7.3.3.3 Overall Treatment Efficiency

A comparison of the final effluent obtained from the two-stage anaerobic digestion system with that obtained from the single-stage digestion of tannery sludge supplemented with trace nutrients (Table 102) shows that there was no significant improvement in the final effluent quality. Similarly, if one compares the overall TVS degraded in the two-stage system i.e. 4834,95 g and 492,27 g, this accounts for 57,9 % of the TVS that entered the whole system. This value is comparable with those obtained in the previous sludge experiments i.e. 60,0 and 61,6 %. Therefore it would appear that the two-stage system does not result in any improvement in the ability of the anaerobic process to degrade the organic matter present in tannery sludges.

The only advantage that the two-stage process appears to have had over the single-stage process is an increase in the CH_4 yield (Table 103). However, it should be emphasized that the two-stage anaerobic digestion experiment carried out in this investigation was not run under optimal conditions, as a large concentration of organic solids emerged in the effluent from the acidogenic digester. The total acidification or degradation of the organic matter into VFA was therefore not achieved in the acidogenic digester, and organic solids, but more importantly, heavy metals such as chrome, were present in the effluent from this digester and were supplied to the methanogenic digester. This is highly undesirable, and therefore further research is necessary to determine the optimum operating conditions for each digestion phase. If operated under optimum conditions, it is believed that the two-stage process will be more feasible and less problematic than the single-stage process.

TABLE 102

A COMPARISON OF THE FINAL EFFLUENT QUALITY OBTAINED FROM THE
SINGLE-STAGE AND TWO-STAGE ANAEROBIC DIGESTION OF TANNERY SLUDGE

Parameter	Single-Stage Digestion	Two-Stage Digestion
pH	7,57	7,38
Total COD (mg/l)	1336	1398
Soluble COD (mg/l)	476	722
Total PV (mg/l)	129	216
Soluble PV (mg/l)	94	115
Total Solids (mg/l)	9950	10640
TIS (mg/l)	5370	7200
SS (mg/l)	145	470
TDS (mg/l)	9805	10170
TDIS (mg/l)	5330	6850
Alkalinity (mg/l CaCO_3)	1058	2142
Bicarbonate Alkalinity (mg/l CaCO_3)	995	2029
Conductivity (mS/m)	1386	1774
SO_4^{2-} (mg/l)	440	264
Sulphide (mg/l)	72	208
TKN (mg/l)	604	788
$\text{NH}_3\text{-N}$ (mg/l)	542	708
Organic N (mg/l)	62	80
Cl^- (mg/l)	5800	6300
Na (mg/l)	1210	2800
Ca (mg/l)	700	660
Cr (mg/l)	40,5	25,8
Al (mg/l)	< 1	< 1
Fe (mg/l)	7,5	6,5
P (mg/l)	3,8	< 1
VFA (mg/l)	88,8	159,1

TABLE 103

A COMPARISON OF THE OPERATING EFFICIENCIES OBTAINED FROM THE
SINGLE-STAGE AND TWO-STAGE ANAEROBIC DIGESTION OF TANNERY SLUDGE

Parameter	Single-Stage Digestion	Two-Stage Digestion*
Gas Volume (ℓ/d)	29,100	21,475
Gas Production ($\text{m}^3/\text{m}^3\cdot\text{d}$)	0,582	0,430
% COD Removed	98,2	97,0
% PV Removed	97,8	95,1
Gas Yield (m^3/kg COD Removed)	0,391	0,483
CH ₄ Content of Biogas (%)	74,4	80,4
CH ₄ Yield (m^3 CH ₄ /kg COD Removed)	0,291	0,388
Free NH ₃ (mg/ℓ)	21,7	18,4
H ₂ S (mg/ℓ)	28,0	70,1

* Data obtained from the methanogenic digester

The findings of this experiment have been previously published (Jackson-Moss and Duncan, 1990g).

7.4 Conclusion

The results of the sludge experiments show that the anaerobic digestion of tannery sludge is possible. This is in direct contrast to the results obtained from the tannery effluent experiments, where no successful digestion could be obtained. A high proportion of the organic solids present in the tannery sludge viz. 60 %, were degraded and converted into biogas with a CH₄ content varying between 75 and 80 %. COD and PV reductions of greater than 90 % for all the sludge experiments were found to be possible. As was shown previously (Section 6.2), pH control is very important during the anaerobic digestion of tannery wastewaters, and in these experiments, the sludge was acidified to a pH of 4,0 - 5,0 prior to being pumped into the digesters.

After obtaining the successful anaerobic digestion of tannery sludge (Section 7.1), different methods were investigated with a view to improving the

efficiency of the process. One possible method of improving the digestion process is through the supplementation of trace nutrients to the waste being treated. Although this was investigated (Section 7.2), only a marginal increase in the TVS degraded, the biogas volume produced, and the CH_4 yield were noted. However, it should be mentioned that the initial concentration of trace nutrients used i.e. 100 mg/l of each nutrient, was found to have an inhibitory effect on the metabolism of the methanogenic bacteria, and only when the concentration was reduced to 20 mg/l, was a stimulatory effect noted. It is possible, therefore, that if the correct trace nutrient concentration is used from the start of an experiment, a marked improvement in the operating efficiency of the digester may be obtained. As the optimum concentration of trace nutrients required is not known, this requires further investigation.

Another method of improving the anaerobic digestion process is through the use of a two-stage digestion system. This process was also investigated (Section 7.3) in order to improve upon the results obtained from the initial single-stage sludge experiments. Although there was no improvement in the final effluent quality, or in the quantity of organic solids degraded, the methanogenic digester was found to produce biogas at a very much higher efficiency, i.e. 35 % greater than the single-stage anaerobic digesters. This could possibly be due to a reduced concentration of heavy metals entering the methanogenic digester, as well as the methanogenic bacteria receiving a more uniform composition of waste. Although the methanogenic digester showed an improved operating efficiency, the overall efficiency was no better than that obtained from the single-stage anaerobic digestion process. The reason for this is that the acidogenic digester was not operated under optimum conditions. The total acidification of the waste was not achieved, and therefore large quantities of undigested organic matter together with inorganic compounds bound to this organic matter, were allowed to enter the methanogenic digester. This should be prevented at all costs, and therefore further research is necessary to optimize the operating conditions of each digester. Once the optimum conditions are obtained, the overall efficiency and stability should be far superior to that obtained from the single stage anaerobic digestion of tannery sludge.

The fate of the tannery compounds in the digesters is in agreement with the findings of the synthetic study (Chapter 5). The soluble ions such as sodium and chloride were not removed and were present in the digester effluents. In

contrast to this, heavy metals such as chromium precipitated and accumulated in the digesters, and were only present in the digester effluents when bound to organic matter. The sulphates were reduced to sulphides, and a large proportion of the organic nitrogen was converted into ammonia.

In conclusion the results of this investigation show that the anaerobic digestion of tannery sludge is possible, and this is therefore in agreement with the findings of a previous investigation (Aloy et al, 1987; Aloy et al, 1989). Although the successful digestion of the sludge was achieved, a great deal of research is still necessary in order to optimize the process.

CHAPTER EIGHT

GENERAL DISCUSSION AND CONCLUSION

CHAPTER EIGHT

GENERAL DISCUSSION AND CONCLUSION

As tannery wastewaters are extremely complex and contain high concentrations of both inorganic and organic compounds, the effect of these individual compounds on the anaerobic digestion process was investigated in detail, in order to ascertain the fate and effect of these compounds during the digestion process. It was found that the heavy metals such as chrome, aluminium and iron precipitated and accumulated in the sludge bed of the digesters, while the soluble ions such as sodium and chloride were not removed. These soluble ions merely passed through the digesters and were present in the digester effluent. Approximately 20 % of the calcium ions were removed, with the remainder being present in the digester effluent. Ammonification of the organic nitrogen occurred under the anaerobic conditions, and the sulphates were reduced to sulphides. These sulphides were present as either H_2S , HS^- or insoluble sulphides. The low inhibitory effect of these compounds on the anaerobic digestion process indicated that in theory, there was no obvious reason why tannery effluents and sludges could not be treated by means of anaerobic digestion. These findings were supported by the results obtained during the sludge experiments.

The experiment carried out to elucidate the effects of aluminium on anaerobic digestion is believed to be the first determination of the toxicity of this heavy metal to the anaerobic digestion process.

Despite the complex nature of tannery effluents and sludges, the anaerobic digestion of the sludges was found to be possible. The successful digestion of the effluents could, however, not be achieved. This is rather unusual because both the effluents and sludges used in this study were obtained from the same tannery and at the same time and, therefore, if toxic compounds were present in the effluents, they would also have been present in the sludges. The other unusual feature is that the sludges are far more concentrated than the effluents and therefore contain very much higher concentrations of the potentially toxic compounds. One would therefore have expected the reverse situation to have occurred i.e. the successful digestion of the effluents, with no success being achieved with the sludges. One possible explanation for the inability to treat the tannery effluents could be a lack of essential

nutrients, particularly phosphorous. Phosphorous is essential for methanogenesis to take place (Archer, 1985). If one considers the COD : P ratio of the tannery effluent used, this is 4040 : 1,8 or 2244 : 1. If this is compared with the COD : P ratio of the sludges used i.e. 75890 : 72,4 or 1048 : 1, it can be seen that while both of these ratios are far higher than the suggested optimum ratio of 250 - 400 : 1 (Frostell, 1985), the sludges do contain a significantly higher phosphorous concentration. This could therefore be a possible explanation for the ability to anaerobically treat only the tannery sludges, and not the effluents.

One of the major advantages of anaerobic digestion is that, unlike aerobic treatment processes, it has the ability to degrade organic solids. In the sludge experiments it was found that approximately 60 % of the organic solids that were supplied to the digesters were degraded and converted into biogas, which is comparable with the findings of a previous investigation (Aloy et al, 1987; Aloy et al, 1989). This conversion of organic solids into CH₄ gas has a dual advantage, as not only is the volume of solids reduced, but a potential energy source is produced. The CH₄ gas can be burned to produce heat, which in turn can be used to maintain the anaerobic digesters at their optimum temperature i.e. 35°C. In pilot-plant studies digesting tannery sludge (Aloy et al, 1987; Aloy et al, 1989), it was found that only 20 % of the energy that was produced through burning the CH₄ gas was necessary to heat the anaerobic digesters. The additional 80 % was available to be used elsewhere in the tannery. Thus the anaerobic process does not only have the potential to reduce the pollution load of the wastewaters, but it is also possible that it can be used to reduce the energy demand of the tannery, and hence reduce the energy costs.

Another advantage of the anaerobic digestion process is that it is a biological process and has the potential to eliminate the use of wastewater treatment chemicals. Over the last decade, there has been a definite trend towards using natural biological purification processes. The reason for this is that although chemical processes are simple to operate, the costs of the chemicals are high and these chemicals create additional problems in that they increase the volume of sludge and the concentration of heavy metals to be disposed of. Many tanneries make use of the flocculents FeCl₃, FeSO₄ and Al₂(SO₄)₃ in their wastewater treatment plants to precipitate the insoluble compounds. If anaerobic digestion could be successfully used to treat the

total mixed wastewaters prior to the separation of the solids, then it could provide a large saving in the cost of the treatment. Although in this investigation the anaerobic digestion of these effluents was not found to be possible, it is feasible that through further research, the successful treatment of these effluents may be achieved.

Despite the success obtained with the anaerobic treatment of the tannery sludge, a number of difficulties do exist and therefore a great deal of further research is necessary in order to overcome these problems. A major problem that does arise during the anaerobic treatment of tannery wastewaters is that of pH control. pH control is extremely important during the anaerobic digestion of any wastewater and this is even more important with tannery wastewaters. The reason for this is that tannery wastewaters contain very high concentrations of organic matter. Under anaerobic conditions, this organic matter is converted into ammonia, and this ammonia reacts in the digester to form ammonium bicarbonate. Ammonium bicarbonate is a strong buffer, and its formation leads to an increase in the pH of the digester contents. If left uncontrolled, the pH will eventually be well in excess of 8,5, and because the methanogens have their optimum metabolism at a pH of 6,8 - 8,0 (Balch *et al*, 1979), the anaerobic digestion process will be inhibited. Therefore, if the anaerobic process is to be carried out successfully, the pH of the digester has to be controlled in the range that is optimum for the metabolism of the methanogens. In view of this, acid has to be added to the tannery wastewaters prior to them being pumped into the digesters.

In this investigation it was found that the pH of the sludge had to be reduced to as low as 4,0 - 4,5 in order to maintain a neutral pH in the digester. What this means, therefore, is that substantial quantities of acid will have to be used to reduce the pH of the wastewaters, and this will therefore increase the cost of the treatment process. An alternative to using vast quantities of acid would be to separate the acidic waste streams and to prevent the lime liquors from mixing with them. Although practical problems may exist, this area needs to be looked at carefully as it could have an important effect on the cost of the overall treatment. However, even if the waste streams are separated, it is unlikely that it will be possible to do away with the use of acid altogether.

The choice of acid that is to be used is very important. In this

investigation, HCl was used to maintain the digester pH in the optimum range. This acid was chosen in view of its relatively cheap cost when compared to other acids. However, in a full-scale treatment plant in South Africa, it would not be possible to use HCl. The reason for this is that its use would drastically increase the concentration of chloride ions and hence the TDIS of the final effluent. Because South Africa is a country that is critically short of water, very strict regulations exist for the discharge of soluble ions in effluents (Appendix I), because the dilution factor to dilute out these ions is not available as it would be in a country with a high rainfall. To overcome this problem it means that alternative acids will have to be considered for controlling the digester pH. However, care has to be taken because not all acids are suitable. For example, if sulphuric acid is used, this could lead to a high sulphide concentration in the digester which could inhibit the digestion process. Two possible choices are therefore nitric acid and phosphoric acid. Phosphoric acid could be a convenient choice as it has been postulated that the inability to anaerobically treat the tannery effluents in this investigation, is due to a phosphorous deficiency. The use of this acid could therefore overcome this problem. However, care will have to be taken, as South Africa also has very strict regulations on the discharge of phosphorous in industrial effluents (Appendix I). Its use will therefore have to be strictly controlled. It is possible that a combination of acids may be the best solution. The cost of these acids, their effect on the final effluent quality, and their effect on the anaerobic digestion process will decide the choice of acids used, and because of the importance of pH control, this whole area needs to be investigated in more detail. Obviously in countries with a high rainfall and where strict regulations for the discharge of chloride ions do not exist, HCl could be used, as was done so in this investigation.

Another problem that arises during the anaerobic digestion of tannery wastewaters is that of the accumulation of heavy metals in the digester. These heavy metals e.g. chrome, aluminium and iron precipitate at the neutral pH of the digester and accumulate in the sludge bed. Over a period of time, their concentration will increase to such an extent that eventually they will have to be removed. The only way of doing this would be to remove all of the sludge from the digester. However, this would necessitate that the digester be re-seeded with fresh sludge. This is impractical and therefore the most obvious solution would be to prevent the heavy metals, in particular chrome,

from getting into the tannery sludge in the first place. The only way of achieving this would be to install a chrome recovery and recycling plant (Section 2.4.1.2), which would ensure that very little chrome is discharged in the effluent.

Another solution to this problem is to make use of a two-stage anaerobic process. The heavy metals would then precipitate and accumulate in the acidogenic digester, and would not be permitted to enter the methanogenic digester. When necessary, the contents of the acidogenic digester could be emptied to remove all the heavy metals. The digester could then be seeded with fresh sludge and as the acidogenic bacteria grow very quickly, it would not be long before the digester was fully operational again. In this way the methanogens would be protected from the heavy metals and it would not be necessary to disturb the sensitive metabolism of these bacteria. Care has to be taken, however, to optimize the process. Although a two-stage process was used in this investigation, the digesters were not run under optimum conditions and it was found that large quantities of undigested organic solids, as well as high concentrations of heavy metals, were present in the effluent from the acidogenic digester. As these were then pumped into the methanogenic digester, the heavy metals began to accumulate in this digester. The reason why these heavy metals were carried over is because the acidogenic digester was not run under optimum conditions. It appears that the HRT of 25 days was too short to degrade the organic solids present in the tannery sludge. Further research is therefore necessary to determine the optimum operating conditions in order to achieve the maximum acidification of the organic solids.

Although a great deal of success was achieved with this investigation, a lot of further research is necessary if anaerobic digestion is to be used in the tanning industry. All the experiments carried out in this investigation were operated as acclimatization studies i.e. the bacteria were allowed to adapt to increasing concentrations of the tannery wastewater over a period of time. This obviously requires a fairly long startup period before the digesters are fully operational and is in contrast to aerobic processes which have a very short startup period. This would be a major disadvantage in industry and therefore research is necessary to determine the operating conditions which will ensure the quickest startup period. The use of bacteria that had been previously adapted to tannery wastes could reduce this problem substantially.

The optimum concentration of trace nutrients required also needs to be determined. In this investigation, a concentration of 100 mg/l of each nutrient (Ni, Co, Mo and W) was found to be inhibitory to the metabolism of the methanogenic bacteria. However, when the concentration of nutrients was reduced to 20 mg/l, this had a stimulatory effect on the process. Because trace nutrients can significantly improve the operating efficiency of anaerobic digesters, determining their optimum concentration is very important. Another area for further research includes determining the optimum phosphorous requirements for the anaerobic treatment of tannery wastewaters.

Over the last 10 to 20 years, rapid advances have been made in the technology and understanding of the anaerobic digestion process. Although most of the research has been orientated towards a better understanding of the microbiology and biochemistry of the process, major developments have been made in the engineering and design of anaerobic digesters. At the commencement of this research project, it was decided to make use of Upflow Anaerobic Sludge Blanket (UASB) digesters, as it was felt that they were the best design available at that time (Section 4.1.4.1). However, over the last few years, two new digesters have been used successfully in industry viz. the MARS and ADUF processes (Section 3.5.5). Both of these digesters make use of membranes to retain the active biomass in the digesters. This has ensured that very high loading rates are possible, and an added advantage is that the effluent from these digesters is free of suspended solids. Any solids that do emerge in the effluent are filtered out and returned to the digester for further degradation. This ability to prevent solids from emerging in the digester effluent suggests that these digesters have tremendous potential for use in the anaerobic treatment of tannery wastewaters, especially the two-stage treatment of these wastewaters. They could be used to prevent the carry over of solids and heavy metals into the methanogenic digester and therefore ensure that complete acidogenesis of the waste occurs prior to being supplied to the methanogenic digester. In this way, the methanogenic digester would not be exposed to undigested solids and heavy metals, and these compounds would not accumulate in the digester. The major problem of heavy metal accumulation would therefore be overcome.

In addition to there being major advances in the field of anaerobic wastewater treatment, the past 10 to 20 years has seen tanneries focus a great deal of attention on methods to reduce the effects of their wastewaters on the

environment. This has led to a number of new developments, many of which are still in the developmental phase viz. enzymatic unhairing, the replacement of salt by biocides and the processing of "green" hides, the conservation of Cr^{3+} in the tannery through the use of high-exhaustive floats and the development of chrome recycling and recovery, the development of Cr^{3+} -free tanning methods, and the use of CO_2 as a deliming agent. In view of the trend towards the use of cleaner technology, it seems likely that in the future, tannery wastewaters will become less complex, with many compounds being recycled and reused, and the use of some compounds being phased out altogether. This will ensure that tannery wastewaters will become even more suitable for anaerobic treatment.

In conclusion, despite the many problems that will have to be solved through further research, the findings of this investigation clearly show that anaerobic digestion will play an important role in the treatment and disposal of tannery wastewaters in the future.

APPENDICES

APPENDIX I

GENERAL AND SPECIAL STANDARDS FOR EFFLUENT
DISCHARGE IN SOUTH AFRICA

TABLE A1

GENERAL AND SPECIAL STANDARDS FOR EFFLUENT DISCHARGE IN TERMS OF THE
SOUTH AFRICAN WATER ACT, NO. 54 OF 1956 AS AMENDED (Hart *et al*, 1987)

Parameter	General Standard Limits	Special Standard Limits
Colour, odour, taste	Nil	Nil
pH	5,5 - 9,5	5,5 - 7,5
Dissolved oxygen (% Saturation)	75	75
Temperature (°C)	35	25
Typical faecal coli (per 100 ml)	Nil	Nil
Chemical oxygen demand (COD)	75	30
Oxygen absorbed (PV)	10	5
Conductivity (mS/m)	250; 75 above intake	250; 15 % above intake
Suspended solids (SS)	25	10
Sodium (Na)	90 above intake	50 above intake
Soap, oil and grease	2,5	Nil
Residual chlorine (as Cl)	0,1	Nil
Free and saline ammonia (as N)	10	1
Nitrate (as N)	N.S.	1,5
Arsenic (as As)	0,5	0,1
Boron (as B)	1,0	0,5
Chromium (total, as Cr)	0,5	0,05
Copper (as Cu)	1,0	0,02
Phenolics (as phenol)	0,1	0,01
Lead (as Pb)	0,1	0,1
Sulphides (as S)	1,0	0,05
Fluoride (as F)	1,0	1,0
Zinc (as Zn)	5,0	0,3
Soluble orthophosphate (as P)	N.S.*	1,0*
Iron (as Fe)	N.S.	0,3
Manganese (as Mn)	0,4	0,1
Cyanides (as CN)	0,5	0,5
Cadmium (as Cd)	0,05	0,05
Mercury (as Hg)	0,02	0,02
Selenium (as Se)	0,05	0,05
Hexavalent chromium (as Cr)	0,05	N.S.

* In terms of Government Notice No. 991 of 18 May 1984, effluents draining to certain sensitive areas must have soluble orthophosphate (as P) concentrations of less than 1,0 mg/l.

Note 1. All units mg/l unless specified otherwise
2. N.S. = Not specified

APPENDIX IITHE EFFECT OF CHLORIDES ON THE CHEMICAL OXYGEN DEMAND TEST

The dichromate reflux method is used to measure the oxygen equivalent or chemical oxygen demand (COD) of samples that are susceptible to oxidation by a strong chemical oxidant. The method is based on the principle that almost all organic matter is oxidized by a boiling mixture of chromic and sulphuric acids. Under these conditions, however, it has been shown that pure solutions of chloride (Cl^-) ions are quantitatively oxidized over the range 250 to 20000 mg/l (Moore et al, 1949), while 92 to 100 % of the Cl^- ions are oxidized in synthetic samples containing 8 to 350 mg/l Cl^- (Moore and Walker, 1956). If Cl^- ions are oxidized in the presence of organic matter, the two interact and alter the physical and chemical properties of the components. This can significantly affect the extent to which a particular compound can be oxidized. For example, it has been shown that benzene is only oxidized 8 % while chlorobenzene is oxidized 40 % (Moore et al, 1951). In order to account for this oxidation of Cl^- ions, a Cl^- correction was introduced. However, it was found that significant errors occurred when high Cl^- : high COD and high Cl^- : low COD samples were tested. A more reliable test method was therefore required.

It has been theorized that if the Cl^- ions were complexed and therefore removed from the reaction, Cl^- interference could be prevented and a simpler and more accurate assessment of organic COD would be possible. Mercuric sulphate has been shown to complex Cl^- ions, and its use in the COD test eliminates chloride ion-organic matter interactions (Dobbs and Williams, 1963). This modification has resulted in a more realistic evaluation of the organic content of wastewater samples, and has been incorporated into the standard analytical method for COD determinations (APHA, 1980).

In the synthetic study (Chapter 5), the effect of heavy metals on anaerobic digestion was investigated. Heavy metal chlorides were used throughout, as under anaerobic conditions, sulphates are reduced to sulphides, and these sulphides, which are toxic, could have adversely effected the toxicity results obtained. The use of these heavy metal chlorides, however, resulted in extremely high concentrations of Cl^- ions in the samples to be analyzed, and therefore it was important to determine whether the Cl^- ions had any

statistically significant effect on the COD test. Each different heavy metal chloride was tested for its effect on the COD test. Glucose was used as a control at the same concentration as that present in the synthetic waste i.e. 2,5 g/l. The small-sample t test ($\alpha = 0,10$) was determined for each sample relative to the control solution (Freund, 1979).

(a) The Effect of $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$

TABLE A2

THE EFFECT OF $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ ON THE COD TEST

Sample	Cl^- Concentration (mg/l)	COD (mg/l)	Mean COD (mg/l)	Standard Deviation	t _{0,050}	Statistically Significant (Yes/No)
Glucose	0	2640 2680 2704 2657	2670	24,1	-	-
Glucose + 1000 mg/l Cr^{3+}	2046	2640 2657 2641 2680	2655	16,2	0,89	No
Glucose + 2000 mg/l Cr^{3+}	4092	2649 2640 2720 2673	2671	31,0	-0,04	No
Glucose + 3000 mg/l Cr^{3+}	6138	2680 2650 2642 2678	2663	16,8	0,41	No
Glucose + 4000 mg/l Cr^{3+}	8184	2720 2657 2753 2693	2706	35,3	-1,46	No
Glucose + 5000 mg/l Cr^{3+}	10230	2726 2720 2688 2673	2702	22,0	-1,70	No
Glucose + 6000 mg/l Cr^{3+}	12276	2656 2720 2627 2678	2670	33,9	0,00	No

Note: 2 ml and 5 ml samples of the above solutions were analyzed.

(b) The Effect of NaCl

TABLE A3

THE EFFECT OF NaCl ON THE COD TEST

Sample	Cl ⁻ Concentration (mg/l)	COD (mg/l)	Mean COD (mg/l)	Standard Deviation	t _{0,050}	Statistically Significant (Yes/No)
Glucose	0	2680 2678 2680 2640	2670	17,1	-	-
Glucose + 1000 mg/l Na ⁺	1564	2720 2753 2649 2664	2697	42,0	-1,03	No
Glucose + 2000 mg/l Na ⁺	3128	2600 2704 2716 2641	2665	47,2	0,17	No
Glucose + 3000 mg/l Na ⁺	4692	2716 2600 2752 2716	2696	57,3	-0,75	No
Glucose + 4000 mg/l Na ⁺	6256	2600 2720 2723 2738	2695	55,4	-0,75	No
Glucose + 5000 mg/l Na ⁺	7820	2752 2649 2790 2664	2714	59,0	-1,24	No
Glucose + 6000 mg/l Na ⁺	9384	2752 2753 2640 2664	2702	51,0	-1,03	No

Note: 2 ml and 5 ml samples of the above solutions were analyzed.

(c) The Effect of AlCl_3

TABLE A4
THE EFFECT OF AlCl_3 ON THE COD TEST

Sample	Cl^- Concentration (mg/l)	COD (mg/l)	Mean COD (mg/l)	Standard Deviation	t 0,050	Statistically Significant (Yes/No)
Glucose	0	2642 2627 2565 2534	2592	44,2	-	-
Glucose + 1000 mg/l Al^{3+}	3950	2550 2534 2611 2627	2581	39,3	0,32	No
Glucose + 2000 mg/l Al^{3+}	7900	2611 2611 2642 2627	2623	12,9	-1,17	No
Glucose + 3000 mg/l Al^{3+}	11850	2580 2642 2627 2627	2619	23,3	-0,94	No
Glucose + 4000 mg/l Al^{3+}	15800	2673 2650 2650 2580	2638	34,9	-1,41	No
Glucose + 5000 mg/l Al^{3+}	19750	2604 2611 2573 2627	2604	19,6	-0,43	No
Glucose + 6000 mg/l Al^{3+}	23700	2604 2599 2580 2611	2599	11,5	-0,27	No

Note: 2 ml and 5 ml samples of the above solutions were analyzed.

(d) The Effect of CaCl_2

TABLE A5

THE EFFECT OF CaCl_2 ON THE COD TEST

Sample	Cl^- Concentration (mg/l)	COD (mg/l)	Mean COD (mg/l)	Standard Deviation	t 0,050	Statistically Significant (Yes/No)
Glucose	0	2688 2560 2672 2706	2657	57,0	-	-
Glucose + 1000 mg/l Ca^{2+}	1778	2704 2688 2563 2577	2633	63,4	0,49	No
Glucose + 2000 mg/l Ca^{2+}	3556	2600 2736 2600 2704	2660	61,1	-0,06	No
Glucose + 3000 mg/l Ca^{2+}	5334	2760 2752 2560 2704	2694	80,3	-0,65	No
Glucose + 4000 mg/l Ca^{2+}	7112	2720 2752 2688 2620	2695	48,9	-0,88	No
Glucose + 5000 mg/l Ca^{2+}	8890	2736 2640 2672 2563	2653	62,3	0,08	No
Glucose + 6000 mg/l Ca^{2+}	10668	2760 2640 2720 2506	2657	97,0	0,00	No

Note: 2 ml and 5 ml samples of the above solutions were analyzed.

(e) The Effect of NH_4Cl

TABLE A6
THE EFFECT OF NH_4Cl ON THE COD TEST

Sample	Cl^- Concentration (mg/l)	COD (mg/l)	Mean COD (mg/l)	Standard Deviation	$t_{0,050}$	Statistically Significant (Yes/No)
Glucose	0	2640 2688 2642 2680	2663	21,7	-	-
Glucose + 1000 mg/l NH_4^+	1941	2720 2720 2627 2611	2670	50,8	-0,21	No
Glucose + 2000 mg/l NH_4^+	3882	2640 2627 2688 2657	2653	22,8	0,55	No
Glucose + 3000 mg/l NH_4^+	5823	2688 2642 2650 2657	2659	17,4	0,25	No
Glucose + 4000 mg/l NH_4^+	7764	2611 2642 2726 2673	2663	42,5	0,00	No
Glucose + 5000 mg/l NH_4^+	9705	2752 2688 2684 2560	2671	69,5	-0,19	No
Glucose + 6000 mg/l NH_4^+	11646	2600 2703 2703 2734	2685	50,7	-0,69	No

Note: 2 ml and 5 ml samples of the above solutions were analyzed.

(f) The Effect of FeCl_3

TABLE A7
THE EFFECT OF FeCl_3 ON THE COD TEST

Sample	Cl^- Concentration (mg/l)	COD (mg/l)	Mean COD (mg/l)	Standard Deviation	t _{0,050}	Statistically Significant (Yes/No)
Glucose	0	2640 2642 2680 2688	2663	21,7	-	-
Glucose + 1000 mg/l Fe^{3+}	1941	2650 2688 2611 2642	2684	53,6	0,74	No
Glucose + 2000 mg/l Fe^{3+}	3882	2688 2734 2719 2596	2684	53,6	-0,63	No
Glucose + 3000 mg/l Fe^{3+}	5823	2765 2673 2642 2519	2650	88,0	0,25	No
Glucose + 4000 mg/l Fe^{3+}	7764	2688 2688 2673 2650	2675	15,5	-0,78	No
Glucose + 5000 mg/l Fe^{3+}	9705	2688 2673 2688 2688	2684	6,5	-1,61	No
Glucose + 6000 mg/l Fe^{3+}	11646	2749 2719 2611 2596	2669	66,3	-0,15	No

Note: 2 ml and 5 ml samples of the above solutions were analyzed.

From the above data it is evident that under the conditions used throughout this investigation i.e. the use of 2 ml and 5 ml samples, high chloride ion concentrations do not statistically interfere with the chemical oxygen demand test. The use of 2 ml and 5 ml samples ensured that the mercuric sulphate : chloride ratio was greater than 10 : 1, which is essential in order to complex the chloride ions (APHA, 1980). Alternatively, larger aliquots of diluted samples could have been used to maintain the 10 : 1 ratio.

APPENDIX III

VOLATILE FATTY ACID GAS CHROMATOGRAM

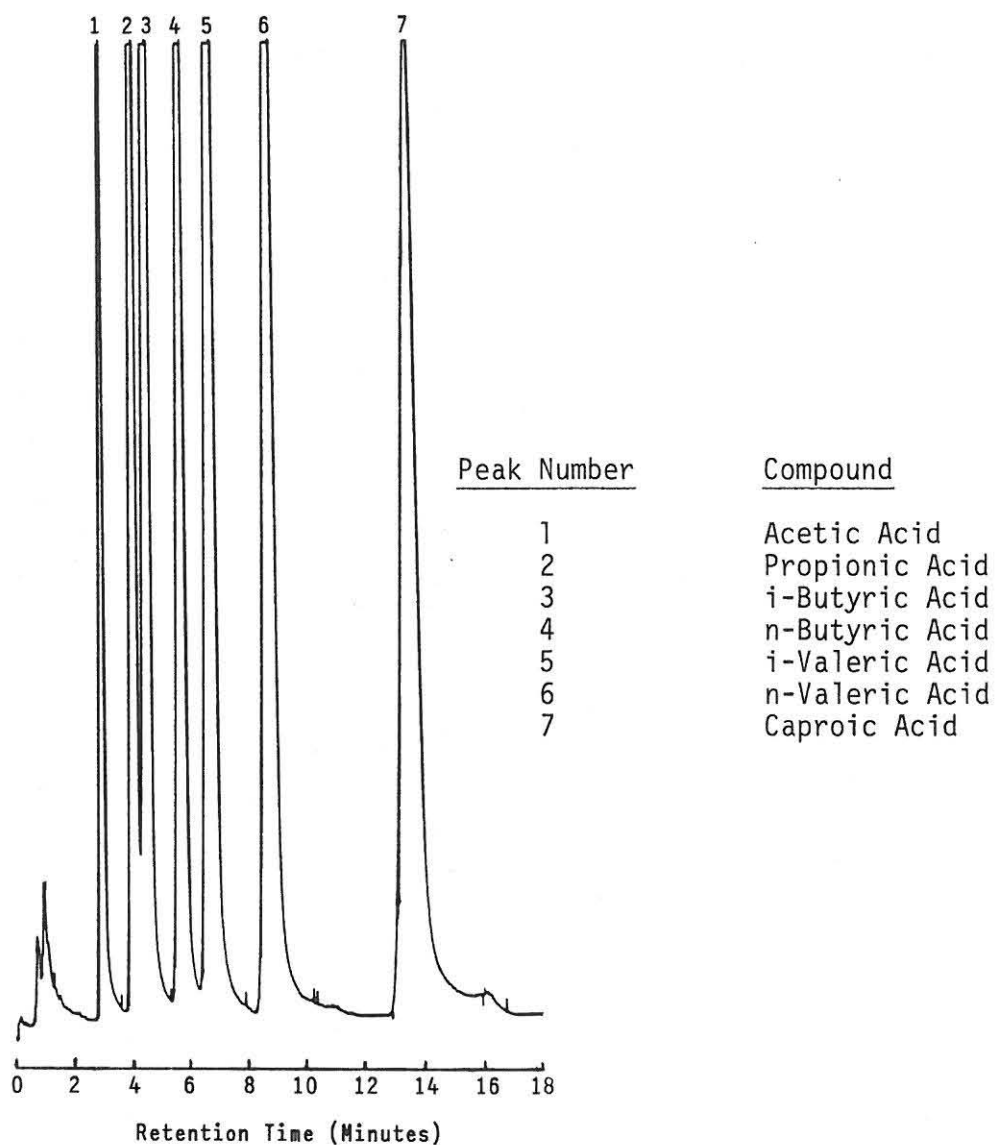


FIGURE A1 - GC Chromatogram of a Volatile Fatty Acid Standard

Operating Parameters

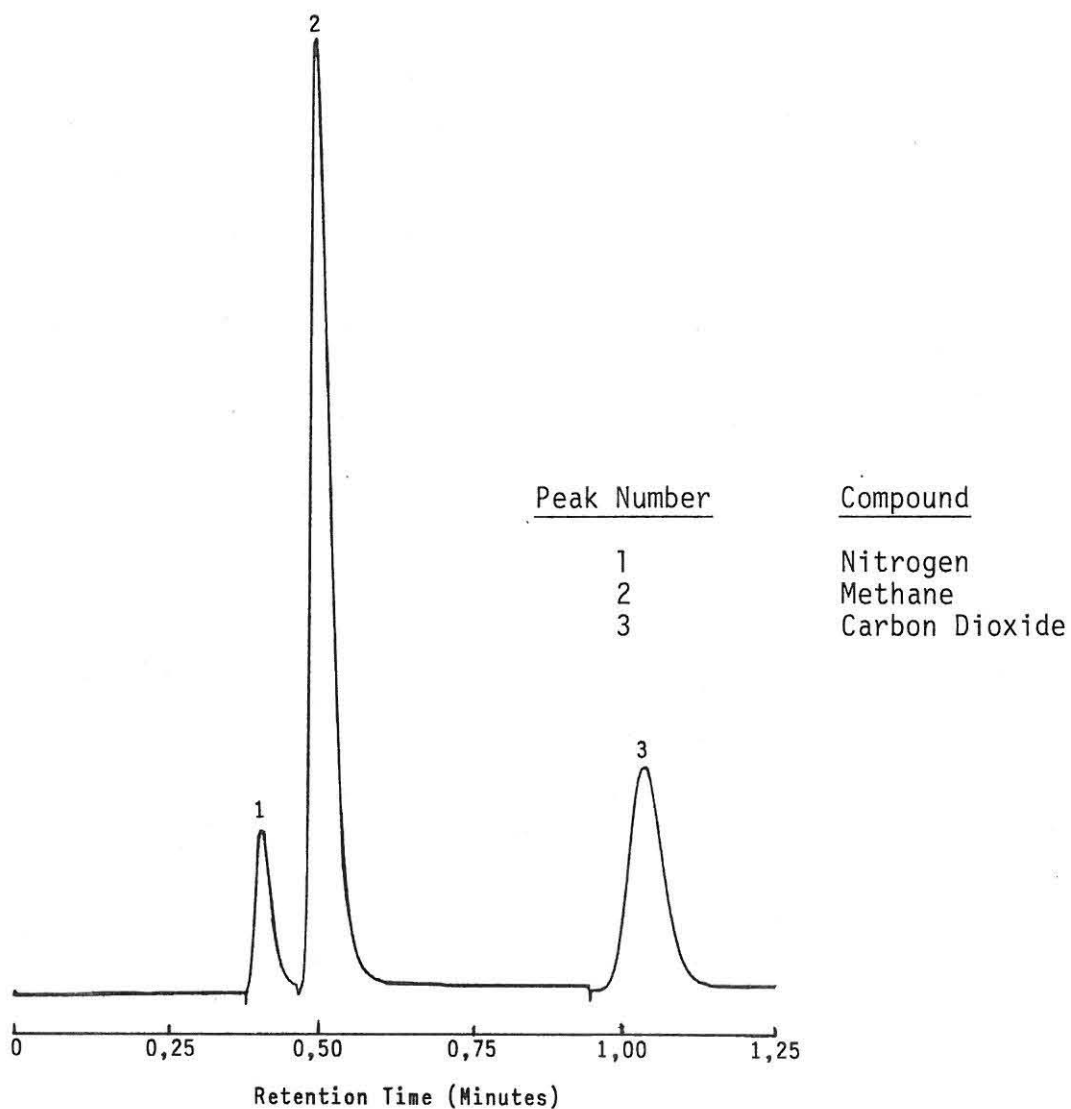
Column: 10 % SP 1000 - 1 % H_3PO_4 on Chromosorb W-AW 100/120
 Column Length: 2 m
 Carrier Gas: Nitrogen
 Carrier Gas Flow Rate: 40 ml/minute
 Sample Volume: 0,5 μ l
 Detector: FID
 Injection Temperature: 170°C
 Column Temperature: 150°C
 FID Temperature: 180°C

APPENDIX IVVOLATILE FATTY ACID SPECIFIC DENSITIES

TABLE A8

VOLATILE FATTY ACID SPECIFIC DENSITIES

Compound	d ²⁰ ₄
Acetic acid	1,048 - 1,051
Propionic acid	0,993
i-Butyric acid	0,950
n-Butyric acid	0,964 - 0,970
i-Valeric acid	0,925 - 0,928
n-Valeric acid	0,937 - 0,940
Caproic acid	0,927

APPENDIX VBIOGAS GAS CHROMATOGRAMFIGURE A2 - GC Chromatogram of a Biogas SampleOperating Parameters

Column: Poropak Type N
Column Length: 2 m
Carrier Gas: Helium
Carrier Gas Flow Rate: 40 ml/minute
Sample Volume: 0,2 ml
Detector: TCD
Injection Temperature: 80°C
Column Temperature: 60°C
TCD Temperature: 80°C

APPENDIX VITHERMAL CONDUCTIVITY RELATIONSHIP BETWEEN METHANE AND CARBON DIOXIDE

Biogas samples were analyzed using a Hewlett-Packard 5830A gas chromatograph equipped with a thermal conductivity detector (TCD). Thermal conductivity is defined as the time rate of transfer of heat by conduction, through unit thickness, across unit area for unit difference of temperature (Weast, 1983). Different compounds have different thermal conductivities depending on the nature of the compound.

If TCD is being used as the method of detection, the peak area is directly proportional to that particular compound's thermal conductivity. As biogas from anaerobic digesters consists almost entirely of CH_4 and CO_2 , the concentration of these two gases was normalized to 100 %. Methane and carbon dioxide have different thermal conductivities and consequently equimolar concentrations of these two compounds will result in two different peak areas. It is therefore important to develop a relationship between the thermal conductivities in order to quantify each gas in a biogas sample.

Various volumes of a gas standard (Scott Speciality Gases) containing 1 % CH_4 and 1 % CO_2 in nitrogen were analyzed in order to develop a thermal conductivity relationship between the two gases. The results are shown in Table A9.

TABLE A9

THERMAL CONDUCTIVITY RELATIONSHIP BETWEEN METHANE
AND CARBON DIOXIDE

Area CH ₄ Peak	Area CO ₂ Peak	$\frac{\text{Area CH}_4 \text{ Peak}}{\text{Area CO}_2 \text{ Peak}}$
3809	6649	0,57
5024	9108	0,57
2657	4852	0,55
6444	11370	0,57
1973	3391	0,58
3111	5584	0,56
4093	7332	0,56
4100	7192	0,57
3922	6934	0,57
1479	2537	0,58
3097	5451	0,57
3681	6489	0,57
2464	4320	0,57
1990	3571	0,56
2216	3979	0,56
5817	10189	0,57
4404	7569	0,58
3708	6528	0,57
1211	2151	0,56
1610	2806	0,57
2606	4468	0,58
3008	5389	0,56
4111	7361	0,56
3515	6197	0,57
4868	8536	0,57
Mean		0,57

From Table A9, it is evident that CO₂ yields a larger peak area than the equivalent concentration of CH₄. If the determination of concentrations was based on peak area, the concentration of CO₂ would be greater than the concentration of CH₄. However, the standard contained equal concentrations of CH₄ and CO₂ viz. 1 % of each, and therefore by using the data in Table A9, it was possible to develop the following relationship based on the fact that CO₂ yields a greater peak area.

$$\% \text{ CH}_4 = \frac{\text{Area CH}_4 \text{ Peak}}{\text{Area CH}_4 \text{ Peak} + (0,57 \times \text{Area CO}_2 \text{ Peak})} \times 100$$

$$\% \text{CO}_2 = 100 - \% \text{CH}_4$$

The above relationship was used throughout this investigation to calculate the percentage CH_4 and CO_2 present in the biogas samples.

APPENDIX VIITHE EFFECT OF THE CHROMIUM OXIDATION STATE ON THE
VANADOMOLYBDOPHOSPHORIC ACID TEST

Total phosphorous includes all orthophosphates and condensed phosphates, which may be dissolved or particulate, organic or inorganic. Because phosphorous may be bound to organic matter, acid digestion is required to release the phosphorous as orthophosphate. The released phosphorous can then be measured by:-

- (a) the vanadomolybdophosphoric acid colourimetric method
- (b) the stannous chloride method or
- (c) the ascorbic acid method.

In this investigation the vanadomolybdophosphoric acid colourimetric method was used.

This method is based on the principle that under acid conditions, ammonium molybdate reacts with orthophosphate to form molybdophosphoric acid. In the presence of vanadium, yellow vanadomolybdophosphoric acid is formed. The intensity of the yellow colour is proportional to the phosphorous concentration. For samples containing low phosphorous concentrations i.e. less than 5 mg/l, a wavelength of 400 nm should be used (APHA, 1980).

The tannery wastewaters used in this investigation contained significant quantities of particulate matter, and consequently acid digestion was essential in order to release the phosphorous bound to the organic matter. A major problem with these wastewaters, however, is that they contain high concentrations of trivalent chromium (Cr^{3+}). If these wastewaters undergo rigorous acid digestion, the Cr^{3+} is oxidized to hexavalent chromate (Cr^{6+}). This results in a colour change from the green Cr^{3+} to the yellow Cr^{6+} , and the possibility therefore exists that this yellow Cr^{6+} could cause a positive interference with the vanadomolybdophosphoric acid test. In view of this, the effect of different acid digestion techniques required investigation in order to eliminate interference in the test method.

Figure A3 illustrates the absorbance spectra of 100 mg/l solutions of Cr^{3+} and

Cr^{6+} which were prepared by dissolving 513 mg of $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ and 370 mg of K_2CrO_4 per litre respectively. It is evident that the Cr^{6+} has a much greater absorbance than the Cr^{3+} in the wavelength range of the vanadomolybdophosphoric acid test, and therefore the oxidation of the Cr^{3+} to Cr^{6+} should be avoided during acid digestion if this method is to be used.

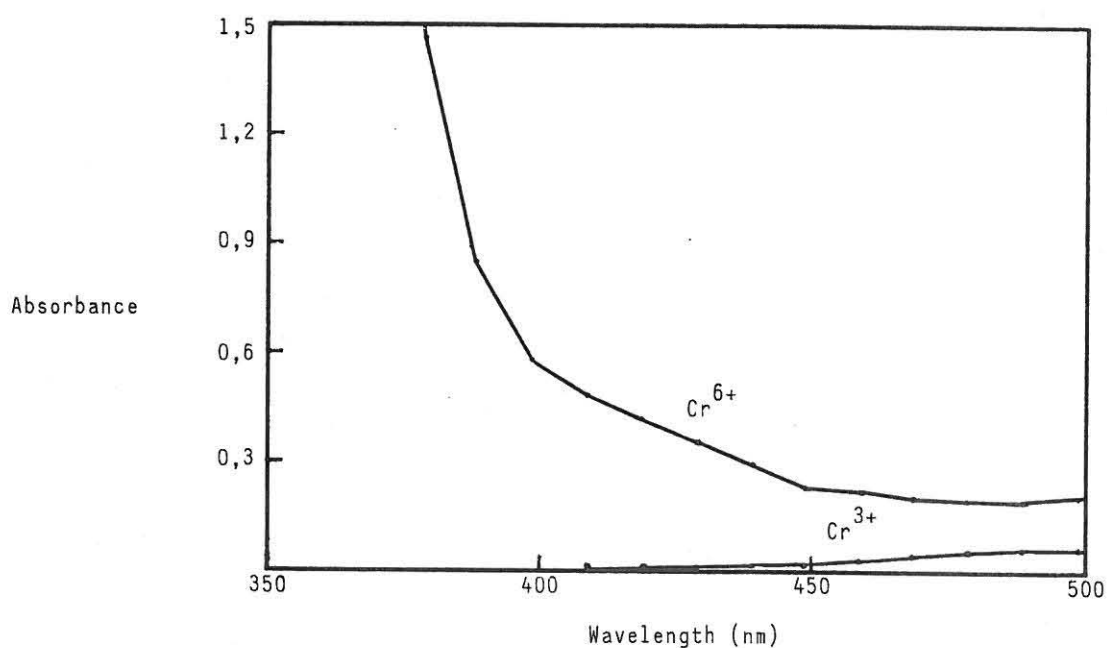


FIGURE A3 - Absorbance spectra of 100 mg/l solutions of Cr^{3+} and Cr^{6+} relative to a water blank

Two different methods of acid digestion were compared in order to determine the one which would least interfere with the vanadomolybdophosphoric acid test. The two different digestion techniques used were:-

- (a) perchloric-sulphuric-nitric acid digestion and
- (b) nitric-hydrochloric acid digestion.

The former method results in complete oxidation of the organic matter while the latter results in only partial oxidation, and is therefore a less rigorous technique.

The data in Table A10 clearly shows the interference caused by the perchloric-sulphuric-nitric acid digestion technique. This interference is directly related to the oxidation of the Cr^{3+} to Cr^{6+} , and leads to a positive interference.

TABLE A10
THE EFFECT OF THE ACID DIGESTION TECHNIQUE ON THE
PHOSPHOROUS CONCENTRATION PRESENT IN TANNERY EFFLUENT

Acid Digestion Technique	Absorbance (400 nm)	Phosphorous Concentration (mg/l)
Perchloric-Sulphuric-Nitric	0,155	3,8
Nitric-Hydrochloric	0,075	1,8

From the above data it can be concluded that the nitric-hydrochloric acid digestion technique should be used for the preliminary digestion of tannery effluents if the vanadomolybdophosphoric acid method of phosphorous determination is to be used. This digestion technique results in only partial oxidation of the Cr^{3+} and therefore results in a very low interference compared with the rigorous perchloric-sulphuric-nitric acid digestion technique.

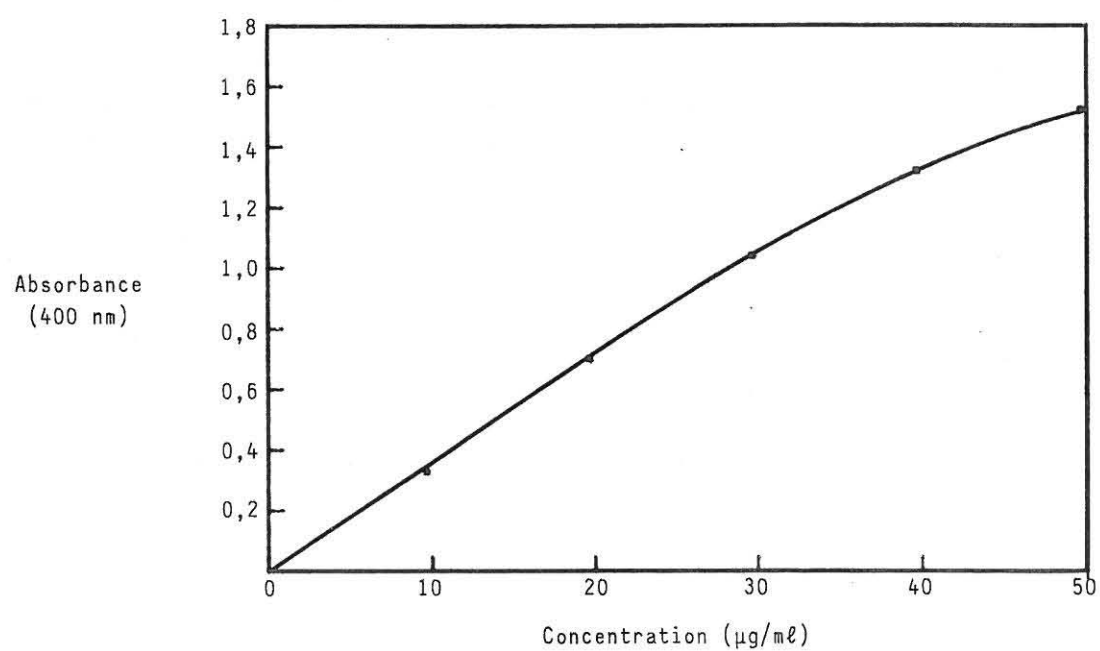
APPENDIX VIIIPHOSPHOROUS STANDARD CURVE

FIGURE A5 - Phosphorous standard curve determined by means of the vanadomolybdophosphoric acid colourimetric method (Means of five readings)

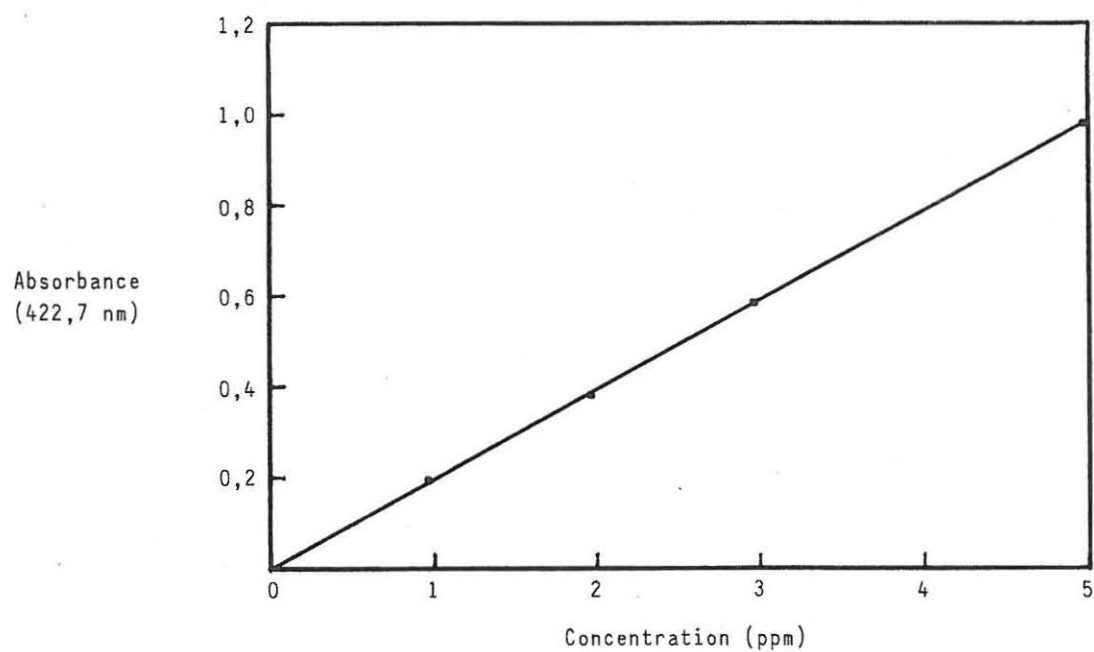
APPENDIX IXMETAL ION ATOMIC ABSORPTION STANDARD CURVES

FIGURE A6 - Calcium atomic absorption standard curve (Means of five readings)

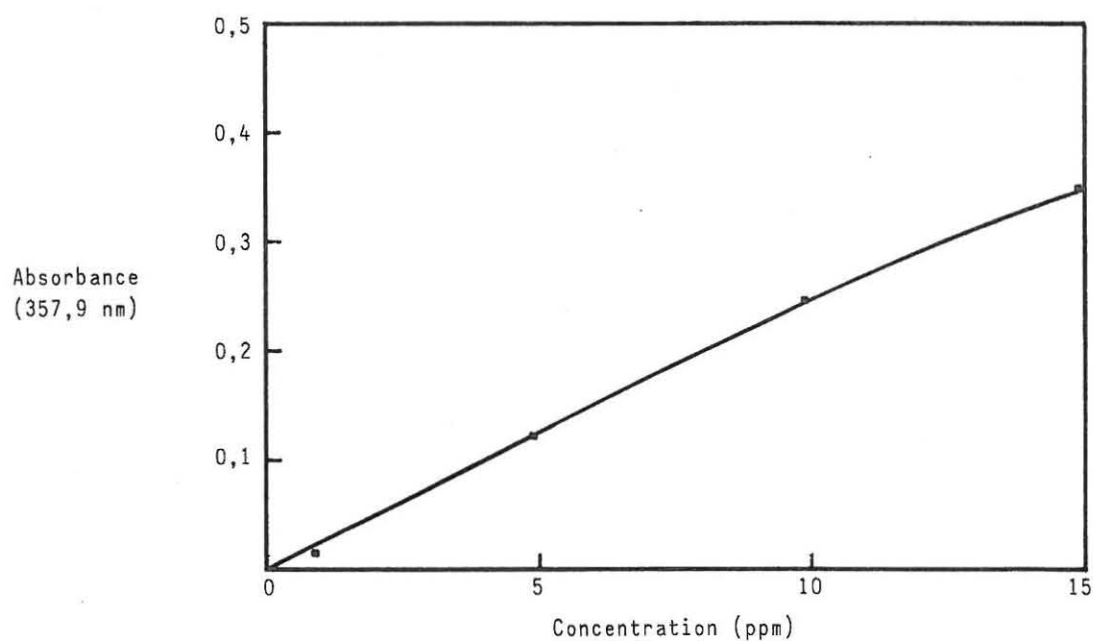


FIGURE A7 - Chrome atomic absorption standard curve (Means of five readings)

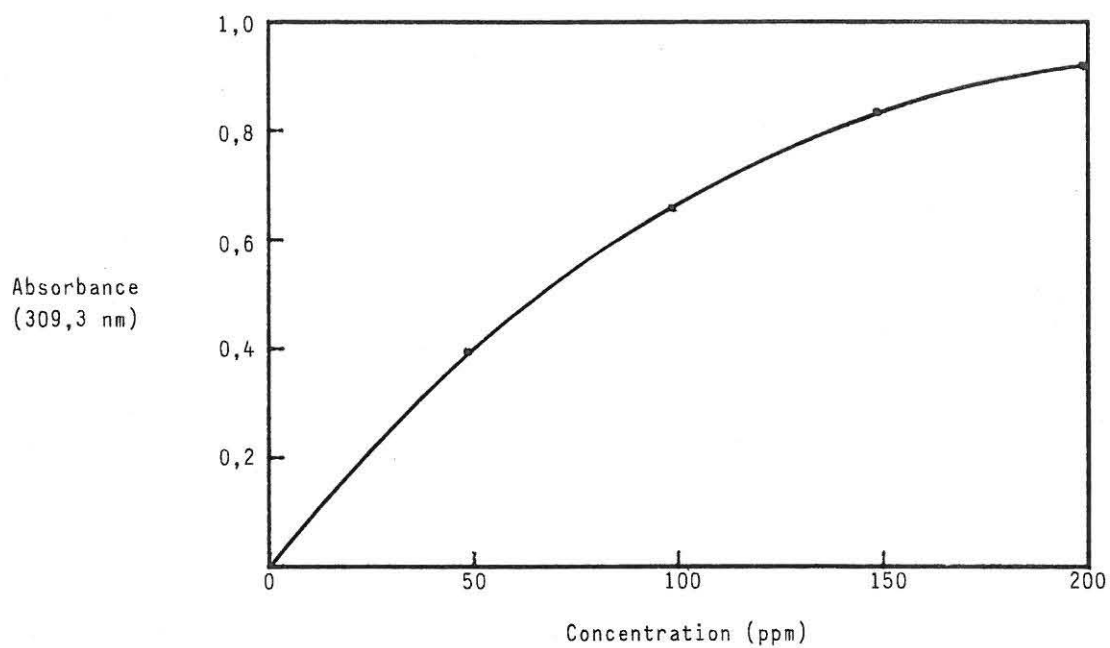


FIGURE A8 - Aluminium atomic absorption standard curve (Means of five readings)

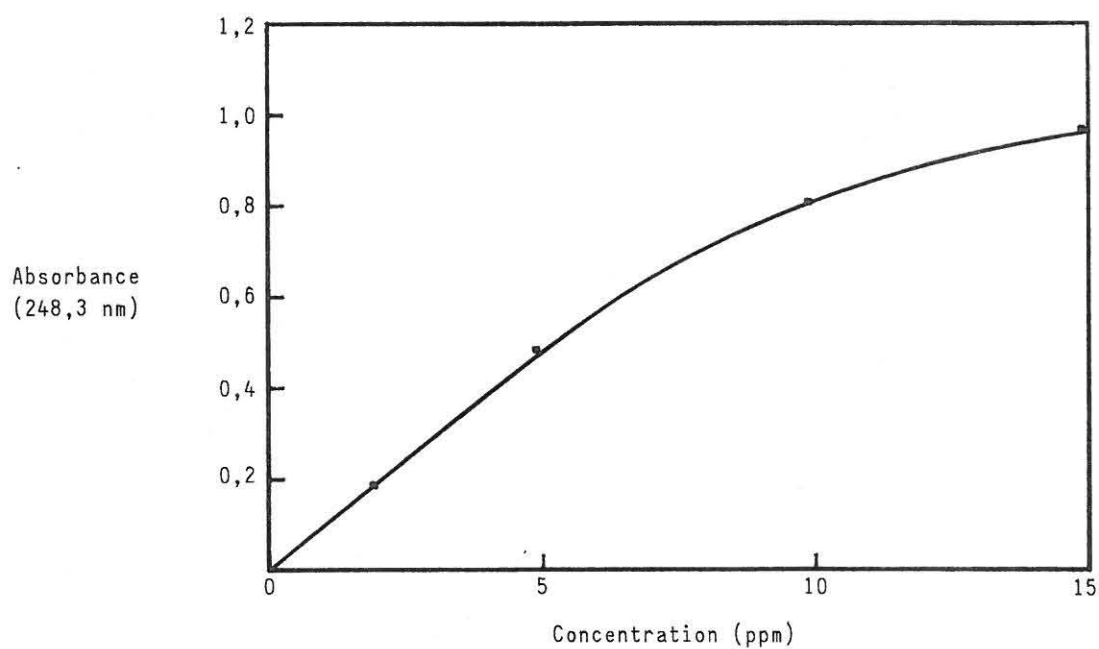


FIGURE A9 - Iron atomic absorption standard curve (Means of five readings)

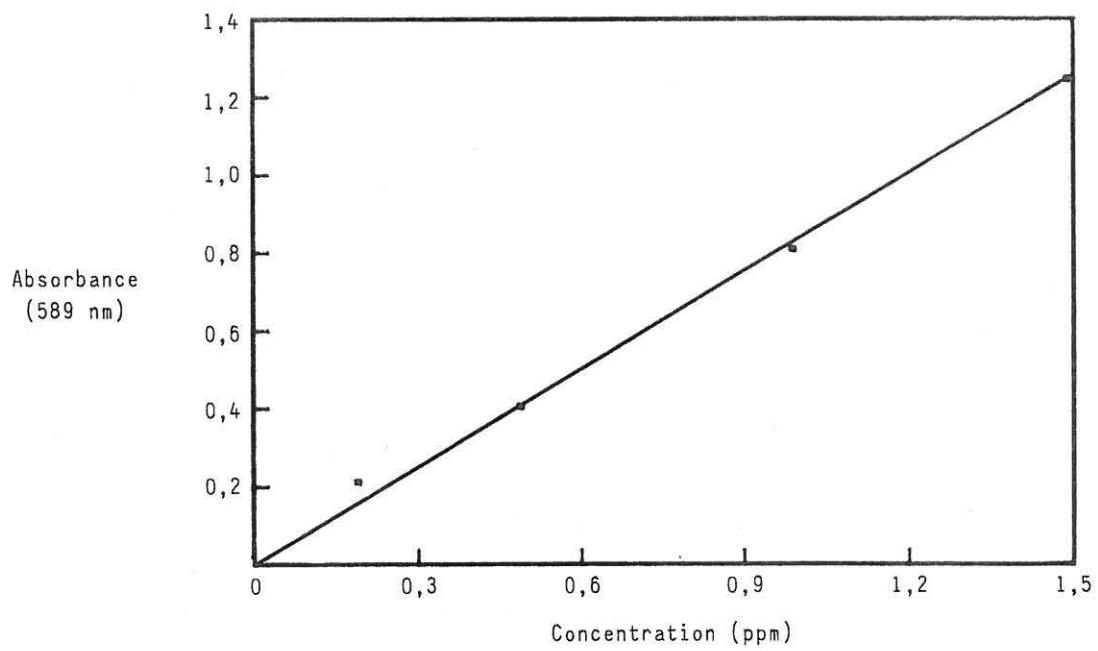


FIGURE A10 - Sodium atomic absorption standard curve (Means of five readings)

APPENDIX XOPERATING CONDITIONS FOR ATOMIC ABSORPTION SPECTROPHOTOMETRY

TABLE A11

OPERATING CONDITIONS FOR ATOMIC ABSORPTION SPECTROPHOTOMETRY

Element	Wavelength (nm)	Lamp Current (mA)	Slit Width (nm)	Gas Mixture	Sensitivity (ppm)
Al	309,3	10,0	0,5	N ₂ O/C ₂ H ₂	0,4 - 200
Ca	422,7	3,5	0,5	N ₂ O/C ₂ H ₂	0,01 - 5
Cr	357,9	7,0	0,2	Air/C ₂ H ₂	0,06 - 20
Fe	248,3	5,0	0,2	Air/C ₂ H ₂	0,05 - 20
Na	589,0	5,0	0,5	Air/C ₂ H ₂	0,003 - 1,5

APPENDIX XIPREPARATION OF THE LABORATORY REAGENTS0,2 N HCl

20 ml 10 N (32 %) HCl

980 ml H₂O6 N HCl

500 ml 12 N (36 %) HCl

500 ml H₂O0,1 N NaOH

4 g NaOH

1000 ml H₂O0,2 N NaOH

8 g NaOH

1000 ml H₂O12 N NaOH

480 g NaOH

1000 ml H₂O0,2 N H₂SO₄6 ml 36 N (98 %) H₂SO₄994 ml H₂O9 N H₂SO₄250 ml 36 N (98 %) H₂SO₄750 ml H₂O

7 N HNO₃500 ml 14 N (65 %) HNO₃500 ml H₂OStandard 1413 µmhos/cm KCl Solution

745,6 mg anhydrous KCl

1000 ml H₂O (atomic absorption grade)Phenol Red Indicator (Vogel, 1961)

1 g phenol red

28,4 ml 0,1 N NaOH

971,6 ml H₂O0,25 N K₂Cr₂O₇12,259 g K₂Cr₂O₇ (Vogel, 1961)1000 ml H₂O0,1 N Fe(NH₄)₂(SO₄)₂39 g Fe(NH₄)₂(SO₄)₂20 ml 36 N (98 %) H₂SO₄980 ml H₂OFerroin Indicator

1,485 g 1,10 - phenanthroline monohydrate

0,695 g FeSO₄·7H₂O100 ml H₂O0,0125 N KMnO₄Stock Solution8 g KMnO₄

1000 ml H_2O

Boil for 2 - 3 hours

Working Solution

50 ml stock solution

950 ml H_2O

0,0125 N $Na_2S_2O_3 \cdot 5H_2O$

3,1 g $Na_2S_2O_3 \cdot 5H_2O$

1000 ml H_2O

3 drops Chloroform

Starch Indicator

Stock Solution

6,7 g soluble starch

40 g NaCl

200 ml H_2O

20 ml 18 N (99 %) glacial acetic acid

Boil together for 5 minutes

Working Solution

10 ml stock solution

190 ml H_2O

0,025 N $K_2Cr_2O_7$

1,226 g $K_2Cr_2O_7$ (Vogel, 1961)

1000 ml H_2O

10 % $BaCl_2$

100 g $BaCl_2$

1000 ml H_2O

0,025 N Iodine Solution

25 g KI
 3,2 g Iodine
 1000 ml H₂O

0,025 N Na₂S₂O₃·5H₂O

6,205 g Na₂S₂O₃·5H₂O
 0,4 g NaOH
 1000 ml H₂O

Kjeldahl Digestion Mixture

6 g CuSO₄·5H₂O
 60 g K₂SO₄
 250 ml 36 N (98 %) H₂SO₄
 750 ml H₂O

Indicating Boric Acid

40 g H₃BO₃
 6 ml methyl red-methylene blue indicator
 994 ml H₂O

Methyl Red-Methylene Blue Indicator

0,6 g methyl red
 0,4 g methylene blue
 1000 ml 95 % Ethanol

1,41 N Na₂S₂O₃·5H₂O (Dechlorinating Agent)

350 g Na₂S₂O₃·5H₂O
 1000 ml H₂O

0,0141 N AgNO₃

2,395 g AgNO₃
 1000 ml H₂O (Atomic absorption grade)

K₂CrO₄ Indicator

50 g K₂CrO₄
 1000 ml H₂O

0,021 N NH₄VO₃

2,5 g NH₄VO₃
 20 ml 14 N (65 %) HNO₃
 980 ml H₂O

0,04 N (NH₄)₆Mo₇O₂₄·4H₂O

50 g (NH₄)₆Mo₇O₂₄·4H₂O
 1000 ml H₂O

50 µg/ℓ Phosphorous Standard

219,5 mg KH₂PO₄ (anhydrous)
 1000 ml H₂O

1 : 1 HCl-HNO₃ Solution

500 ml 12 N (36 %) HCl
 500 ml 14 N (65 %) HNO₃

3 : 1 60 % HClO₄·2H₂O - H₂SO₄ Solution

750 ml 9 N (60 %) HClO₄·2H₂O
 250 ml 36 N (98 %) H₂SO₄

Synthetic Wastewater Trace Element SolutionStock Solution

20 g		CaCl_2
20 g		NaCl
2 g		FeCl_3
0,2 g		$\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$
0,1 g		$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$
0,1 g		$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$
0,1 g		$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$
0,1 g		$\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$
0,04 g		$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$
0,02 g		$\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$
1000 ml		H_2O (Atomic absorption grade)

Working Solution

5 ml		Stock Solution
995 ml		Synthetic Wastewater

Tannery Effluent Trace Element SolutionStock Solution (1000 ppm of each nutrient)

4,0 g		$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$
1,79 g		$\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$
2,50 g		$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$
4,0 g		$\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$
1000 ml		H_2O (Atomic absorption grade)

Working Solution (10 ppm of each nutrient)

10 ml		Stock Solution
990 ml		Tannery Effluent

Tannery Sludge Trace Element SolutionStock Solution (10000 ppm of each nutrient)

40 g $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$
 25 g $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$
 17.9 g $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$
 40 g $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$
 1000 ml H_2O (Atomic absorption grade)

Working Solution (100 ppm of each nutrient)

10 ml Stock Solution
 990 ml Tannery Sludge

Working Solution (20 ppm of each nutrient)

2 ml Stock Solution
 998 ml Tannery Sludge

Volatile Fatty Acid Standard Solution

10 μl Acetic Acid
 10 μl Propionic Acid
 10 μl i-Butyric Acid
 10 μl n-Butyric Acid
 10 μl i-Valeric Acid
 10 μl n-Valeric Acid
 10 μl Caproic Acid
 10 ml H_2O

Atomic Absorption Spectrophotometry Standard Solutions(a) Aluminium50 ppm Al Standard Solution

5 ml Aluminium nitrate "Spectrosol" standard solution

95 ml H₂O (Atomic absorption grade)

100 ppm Al Standard Solution

10 ml Aluminium nitrate "Spectrosol" standard solution

90 ml H₂O (Atomic absorption grade)

150 ppm Al Standard Solution

15 ml Aluminium nitrate "Spectrosol" standard solution

85 ml H₂O (Atomic absorption grade)

200 ppm Al Standard Solution

20 ml Aluminium nitrate "Spectrosol" standard solution

80 ml H₂O (Atomic absorption grade)

(b) Calcium

20 ppm Ca Stock Solution

5 ml Calcium nitrate "Spectrosol" standard solution

245 ml H₂O (Atomic absorption grade)

1 ppm Ca Standard Solution

5 ml 20 ppm Ca Stock Solution

95 ml H₂O (Atomic absorption grade)

2 ppm Ca Standard Solution

10 ml 20 ppm Ca Stock Solution

90 ml H₂O (Atomic absorption grade)

3 ppm Ca Standard Solution

15 ml 20 ppm Ca Stock Solution

85 ml H₂O (Atomic absorption grade)

5 ppm Ca Standard Solution

25 ml 20 ppm Ca Stock Solution
 75 ml H₂O (Atomic absorption grade)

(c) Chrome20 ppm Cr Stock Solution

5 ml Chromic nitrate "Spectrosol" standard solution
 245 ml H₂O (Atomic absorption grade)

2 ppm Cr Standard Solution

10 ml 20 ppm Cr Stock Solution
 90 ml H₂O (Atomic absorption grade)

4 ppm Cr Standard Solution

20 ml 20 ppm Cr Stock Solution
 80 ml H₂O (Atomic absorption grade)

6 ppm Cr Standard Solution

30 ml 20 ppm Cr Stock Solution
 70 ml H₂O (Atomic absorption grade)

8 ppm Cr Standard Solution

40 ml 20 ppm Cr Stock Solution
 60 ml H₂O (Atomic absorption grade)

(d) Iron20 ppm Fe Stock Solution

5 ml Ferric nitrate "Spectrosol" standard solution
 245 ml H₂O (Atomic absorption grade)

2 ppm Fe Standard Solution

10 ml 20 ppm Fe Stock Solution
 90 ml H₂O (Atomic absorption grade)

5 ppm Fe Standard Solution

25 ml 20 ppm Fe Stock Solution
 75 ml H₂O (Atomic absorption grade)

10 ppm Fe Standard Solution

50 ml 20 ppm Fe Stock Solution
 50 ml H₂O (Atomic absorption grade)

15 ppm Fe Standard Solution

75 ml 20 ppm Fe Stock Solution
 25 ml H₂O (Atomic absorption grade)

(e) Sodium10 ppm Na Stock Solution

5 ml Sodium nitrate "Spectrosol" standard solution
 495 ml H₂O (Atomic absorption grade)

0,2 ppm Na Standard Solution

2 ml 10 ppm Na Stock Solution
 98 ml H₂O (Atomic absorption grade)

0,5 ppm Na Standard Solution

5 ml 10 ppm Na Stock Solution
 95 ml H₂O (Atomic absorption grade)

1,0 ppm Na Standard Solution

10 ml 10 ppm Na Stock Solution
90 ml H₂O (Atomic absorption grade)

1,5 ppm Na Standard Solution

15 ml 10 ppm Na Stock Solution
85 ml H₂O (Atomic absorption grade)

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