# PRODUCTION OF BIOGAS FROM THE ANAEROBIC CO-DIGESTION OF TANNERY FLESHING AND COW-DUNG ENHANCED BY SODIUM AND CALCIUM ALGINATES.

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NIGERIA.

FEBRUARY, 2015.

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NIGERIA.

FEBRUARY, 2015.

# DECLARATION

I declare that the work in this Thesis entitled 'Production of Biogas from the Anaerobic Co-digestion of Tannery Fleshing and Cow-dung Enhanced by Sodium and Calcium Alginates' was carried out by me in the Department of Chemical Engineering. The information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this thesis work was previously presented for another degree or diploma at this or any other institution.

| _____ | _____ | _____ |
|---|---|---|
| Name of Student | Signature | Date |

# CERTIFICATION

This thesis entitled 'PRODUCTION OF BIOGAS FROM THE ANAEROBIC CO-DIGESTION OF TANNERY FLESHING AND COW-DUNG ENHANCED BY SODIUM AND CALCIUM ALGINATES' by Bukola Titilayo SILAS meets the regulations governing the award of the degree of Master of Science in Chemical Engineering of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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# DEDICATION

This thesis work is dedicated to my husband, Engr. Patrick Imokha Kingsley for believing so much in me and for his push, prayers, love and support that saw me through till the end.

# ACKNOWLEDGMENT

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# ABSTRACT

The subjects of interest in this work were the production of biogas via anaerobic co-digestion of tannery fleshing and cow-dung; and the reduction of sulphide concentration by direct inclusion of sodium and calcium alginate. Comparison of the efficacy of various tannery beam house effluents as substrate diluents indicated that the soaking liquor was most favourable. Use of soaking liquor gave highest methane concentration of 11.8%v/v as against 8.8%v/v, 1.2%v/v, and 4.1%v/v when deliming liquor, liming liquor and a combination of the entire beam house liquors were used respectively. Effect of various fleshing to cow-dung ratio of 1:0, 1:0.5, 1:1 and 1:2 were investigated. The optimum was established to be ratio 1:2 as indicated by the highest methane concentration of 45%v/v as against 1.9%v/v, 5.1%v/v, 7.3%v/v for 1:0, 1:0.5 and 1:1 respectively. The ability of minute concentration of sodium and calcium alginates (0.01% wt/v) introduced from the beginning of the anaerobic digestion cycle to increase methane concentration and reduce hydrogen sulphide concentration was ascertained. The alginates acted as chelating ligands thereby boosting methane production and reducing sulphide concentration. Methane concentration was significantly boosted to 70.1%v/v and 63.8%v/v with the addition of sodium alginate and calcium alginate beads respectively, as against 45%v/v for a similar digestion sample without alginate. Generally, sodium alginate performed better than calcium alginate beads both of 0.01% wt/v. However, an increase in concentration of calcium alginate beads to 0.03% wt/v performed better than sodium alginate of 0.01% wt/v. In addition, inclusion of the alginates to the digestion system shortens the retention time for biogas production and hydrogen sulphide evolution. This work thus recommends soaking liquor as diluent in the anaerobic digestion of tannery fleshing, use of tannery fleshing and

cow-dung in the ratio of 1:2 for anaerobic co-digestion and the use of sodium and calcium alginates (0.01% wt/v) acting as chelating ligands to remove hydrogen sulphide and boost methane production above 20%.

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# ABBREVIATIONS

| | |
|---|---|
| BOD | Biological Oxygen Demand |
| BTU | British Thermal Unit |
| C | Carbon |
| CDTA | 1:2 diaminocyclohexane-N |
| $CH_4$ | Methane |
| $CO_2$ | Carbon(IV)oxide |
| COD | Chemical Oxygen Demand |
| C/N | Carbon to Nitrogen Ratio |
| DP | Degree of polymerization |
| EDTA | Ethylenediamine tetra-acetic acid |
| FEPA | Federal Environmental Protection Agency |
| $g/m^3$ | Gram per cubic metre |
| HRT | Hydraulic Retention Time |
| $H_2S$ | Hydrogen sulphide |
| IR | Infra-red |
| $J/m^3$ | Joules per cubic metre |
| mg/L | Milligram per litre |
| N | Nitrogen |
| $NH_3$ | Ammonia |
| NILEST | Nigerian Institute of Leather and Science Technology |

| | |
|---|---|
| NTA | Nitrilotri-acetic acid |
| $O_2$ | Oxygen |
| P | Phosphorus |
| $R^2$ | Correlation Co-efficient |
| S | Sulfur |
| SSA | 5-sulphosalicyclic acid |
| UK | United Kingdom |
| USA | United States of America |
| VFA | Volatile Fatty Acid |
| W.H.O. | World Health Organization |

# CHAPTER 1

## INTRODUCTION

### 1.1 Preamble

Leather processing is one of the earliest industrial activities taken up by humans (Germann, 1999). Leather processing otherwise called tanning, is a chemical process that converts animal hides and skin into leather and related products. The tanning (leather) industry is of economic importance; should all the hides and skins processed in Nigeria be converted to footwear, the total foreign exchange will amount to 96 billion naira per annum (Paiko, 2002). According to ComTrade for 2010, Nigeria exports around 40 million skins valued in excess of 480 billion naira and representing about 2.9% of global trade in hide and skin. Despite the foregoing economic viability of the industry, it suffers serious environmental shortcomings in terms of the intensity of hazardous pollutants ranging from solids to heavy metals. Presently, most tanneries within the country have poor effluent treatment approach, which do not take cognizance of reuse, reduce or conversion of its waste to wealth. However, uncontrolled waste dumping is no longer an acceptable practice and incineration of organic wastes is now outdated in environmental control parlance. Environmental standards are increasingly getting stricter, while energy recovery and recycling of nutrients and organic matter is being promoted (Teodorita *et al*., 2008).

A detailed overview of tanning industry reveals the beam house to be the major source of solid pollutant and equally produces the largest part of the effluent load. The characteristic foul odour of tanneries is due to high sulphide load and putrefaction of fleshing and trimmings which constitute 58% of its total solid waste (Ozgunay, 2007). Researchers have

exploited pyrolysis, aerobic and anaerobic digestion to reuse solid waste from the tanning industry (Colak *et al*., 2005, Gil *et al*., 2009, Thangamani *et al*., 2009, Srinivasan *et al*., 2011, and Eva *et al*., 2012) .They have also studied several treatment methods for removing sulphides from gases (Robert *et al*., 1977, Mesdaghinia *et al*., 1991, Jensen *et al*., 1995, Janssen *et al*., 1999, Iliev *et al*., 2002, and Linkous *et al*., 2004). However, much hasn't been documented on the production of a useful byproduct (biogas) and a simultaneous treatment of sulphides in the digesting substrates.

In many parts of the developing world, cow-dung is used as fertilizer, fuel, insect repellant, thermal insulator and as sealant for smoke boxes on steam locomotives. In recent times, cow-dung is collected and used in producing biogas to generate electricity and heat. Its heating value is about 600BTUs per cubic foot ($2.2 \times 10^7 J/m^3$) (Ajayi *et al*., 2012).

Sodium alginate, is the sodium salt of alginic acid derived from selected varieties of brown seaweed with empirical formula - $NaC_6H_7O_6$. It is a giant chelating ligand; and in the food industry, it is used for gelling, thickening, stabilizing and film forming properties. It has found uses in textile industry to improve brightness and strengthen yarns, in the printing and dyeing industry as a thickening agent. It also finds uses in paper, dental, cosmetic, water treatment and medical industries (Draget *et al*., 2005, Debbie, 2008, Khanedan *et al*., 2011 and Joseph, 2013). Its use as an efficient sulphide remover from anaerobic digesters is relatively a new comer (Dublein and Steinhauser, 2008). Sodium alginate has a unique ability to form gels when combined with calcium ions without heating to form calcium alginate. This reaction is widely used throughout the food industry to regulate texture and

stabilize many food products. Calcium alginate is derived from the replacement of the sodium ions of sodium alginate with calcium ions and has the chemical formula - $C_{12}H_{14}CaO_{12}$. It is used in different types of medical products, including burn dressings that promote healing and can be removed with less pain than conventional dressing. It finds extensive applications in immobilized catalysis (Kasra *et al*., 2010).

This study couples biogas production through anaerobic co-digestion of fleshing and cow-dung with the reduction of sulphide concentration and boosting of methane production using sodium and calcium alginates. The case study considers the solid tannery effluent from Nigerian Institute of Leather and Science Technology (NILEST), whose current practice is channeling such solid waste into a sewage pit. This work will contribute immensely to the Institute's vision to be a renowned centre of excellence in the field of tannery effluent monitoring and control.

## 1.2 Research Problem

The beam house in tanneries which is characterized by the cleaning and conditioning of hides and skins produces the largest part of the effluent load in a tannery. Putrefaction of fleshing which constitutes 58% of the total solid waste from tanneries causes a foul smell if not properly disposed. Sulphide load of about 500ppm still remains in the fleshing and can be later released to the atmosphere as hydrogen sulphide gas on contact with any acidic medium or during anaerobic digestion.

## 1.3 Justification

This study has a number of objectives it meets that make it a worthwhile area of research. It is therefore justifiable because:

a) Globally, renewable sources of energy are currently being exploited. The beam house solid effluent constitutes potential materials for methane production through anaerobic digestion.

b) The co-digesting substrate (cow-dung) is readily available in abundance in Nigeria especially in the Northern parts of the country where cattle are most commonly reared.

c) Very minute quantities of sodium alginate are required for boosting methane production and reducing the sulphide gas emitted (Dublein and Steinhauser, 2008).

## 1.4 Aim and Objectives

The primary aim of this investigation is to produce biogas by the anaerobic co-digestion of tannery fleshing and cow-dung; and enhance it by introducing sodium and calcium alginates. This can be achieved through the following objectives:

a) To produce biogas from fleshing with cow-dung as carbon booster and carrier of anaerobic microbes.

b) To increase methane production by introducing sodium and calcium alginates into the digesters.

c) To ascertain desulphurizing properties of sodium and calcium alginates within the digesting system.

## 1.5 Scope

The scope of this research work is limited to the following areas of study:

i. Sample and sample collection:

The fleshing and beam house liquors were collected from the tannery in NILEST, from the tanning process of goat skins procured. The skins were procured from the Abattoir in Zango, Zaria. The cow-dung was obtained from Samaru, Zaria environs. The fresh innoculum was obtained from the Abattoir in Zango, Zaria.

ii. Data collection:

Anaerobic conditions were maintained with a total Hydraulic Retention time of 50 days. Constituent gases in the biogas produced were daily analyzed for their concentration using Biogas 5000 gas analyzer and daily gas production was measured using downward water displacement method.

iii. Data Analysis:

The effect of the following on the concentration of methane and hydrogen sulphide gases were considered: different effluent streams as diluents; co-digestion of fleshing with cow dung in different ratios; sodium alginate introduced directly into the digester and calcium alginate introduced as beads directly into the digester.

# CHAPTER 2

## LITERATURE REVIEW

### 2.1 Background

In Nigeria, tanning and finishing of leather in the mechanized way started in Kano in 1949, and currently not less than 32 tanneries have been established in the country. The installed capacity of the tanneries for the production of wet-blue leather is above 8.5 million kilograms (17 million pieces) per annum. If processed to finished leather, this will earn the country about 12 billion naira per annum at the selling price of 400 naira per square foot (Paiko, 2002). Nigeria's leather industry export potential is currently valued at 6.9 trillion naira in comparison to the world's current production value of 11.5 trillion naira (Siaka, 2012). The economic benefits derived from the leather industries are enormous; however, the negative environmental impact of its effluent is a major setback (Rao *et al*, 1997). During a typical tanning process at least 300kg chemicals (lime, salt etc.) are added per ton of hides. The beam house is the source of all non-limed and limed solid wastes such as fleshing, trimming and waste split (Buljan, 1994). According to Srinivasan *et al*., (2010), 15% of total thickness of raw skin/ hides is removed as fleshing; this translates to about 37.5kg of fleshing daily from Nigerian Institute of Leather and Science Technology, Zaria, with installed capacity of 250kg.

### 2.2 The Tanning Process (a general overview)

The processing of hides and skins in a tannery can be split into the following four main headings:

1. Preservation of hides and skins.

2. Beam house operations.

3. Tanning operations.

4. Post-tanning and finishing operations.

### 2.2.1 Beam house operations

Cleaning and conditioning of hides and skins takes place at this stage and it produces the largest part of the effluent load. It involves soaking, unhairing and fleshing (liming), deliming and bating, and pickling.

## 2.3 Pollutants in a Tannery

Solid waste is inherent to manufacture of leather from skin and hide. Solid wastes generated at various unit operations of the tanning process considerably vary in quantity and composition. Statistically, the quantity of the world leather produced is about $1.5 \times 10^{10}$ kg per day. Out of 1000kg of raw hide, nearly 850kg is generated as solid wastes in leather processing. Only 150kg of the raw material is converted into leather. Tannery generates huge amount of solid waste with the following approximate composition (Kanagaraj *et al*., 2006):

i. fleshing 50-60%;
ii. chrome shaving, chrome splits and buffing dust, 35 -40%;
iii. skin trimmings, 5-7%; and
iv. hair, 2-5%.

Solid waste generation from tannery process is estimated at $6 \times 10^{9}$kg per year (Rajamani *et al*., 2009). Solid waste includes salt (sodium chloride) from preserved raw skin/hide,

dusting from raw skin/hide trimmings; hair from the liming/unhairing process which may contain lime and sulphide; and fleshing from raw skins/hides.

### 2.3.1 Fleshing

Fleshing is a type of animal tissue waste generated during the preparatory leather processing stage in relatively large quantities as compared to other types of solid waste in the tanning industry. Fleshing mainly contains fat and protein and residual chemicals such as lime and sulphide used in the 'unhairing' process. Fleshing contain a significant quantity of volatile solids amenable to biodegradation. Biomethanation of fleshing and solid sludge from primary and secondary treatments is an economically viable option for secured disposal (Colak *et al*., 2005). Different proportions of waste fleshing and primary sludge can be subjected to anaerobic digestion in a laboratory scale reactor with the aim of developing an appropriate technology for recovery of bio-energy from the waste and subsequently ensure their safe disposal.

### 2.3.2 Sulphide ($S^{2-}$)

The sulphide content in tannery effluent results from the use of sodium sulphide and sodium hydrosulphide in the unhairing process. The sulphides pose many problems. Under alkaline conditions, sulphides remain largely in solution. When the pH of the effluent drops below 9.5, hydrogen sulphide evolves from the effluent: the lower the pH, the higher the rate of evolution. This gas produces a noxious smell and in acid media liberates poisonous hydrogen sulphide gas, in which one gram per cubic metre ($1g/m^3$) in air is lethal even after a short exposure.

$$S^{2-} + 2H^{+} \rightarrow H_2S\ (g) \qquad \text{--- 2.1}$$

The gas characterized by a smell of rotten eggs causes a severe odour problem. Comparable in toxicity to hydrogen cyanide, even a low level of exposure to the gas induces headaches

and nausea, as well as possible damage to the eye. At higher levels, death can rapidly set in and countless deaths attributable to the build-up of sulphide in sewage systems have been recorded (Speece, 1996). Table 2.4 shows the toxicological exposure limits by World Health Organization (W.H.O.).

**Table 2.1: Health Effects Due to Exposure to Different Hydrogen sulphide Concentration Levels (W.H.O., 1981).**

| Parts per million (ppm) | Health Effects |
|---|---|
| 0.01 – 0.3 | • Odor is detectable. |
| 1 – 10 | • Moderate to strong odor.<br>• People may experience nausea, tearing of the eyes, headaches and loss of sleep following prolonged exposure effects appear to be reversible and not serious for the general population, although more susceptible individuals may respond more severely. |
| 10 – 150 | • Increasing degree of irritation to eyes and lungs. |
| 150 – 750 | • Severe health effects, which may lead to death, become more likely as concentration and exposure time increase. |
| > 750 | • Death may occur in minutes or less. |

*2.3.2.1 Hydrogen sulphide standards for tannery effluent*

Government at Federal, State and Local levels has the responsibility to protect its citizens and environment from harmful compounds in a bid to achieving this, the Federal

Environmental Protection Agency (FEPA) in Nigeria stipulates tolerance levels for ambient air pollutants such as hydrogen sulphide as shown in Table 2.2.

**Table 2.2: Tolerance Limits for Ambient Air Pollutants (FEPA, 1991).**

| Pollutants | Long Term $mg/m^3$ | Limits +(hours) | Short-Term $mg/m^3$ | Limits +(min.) |
|---|---|---|---|---|
| Ammonia | 0.20 | 24 | 0.2 | 30 |
| Chromium | 0.001 | 24 | 0.0015 | 30 |
| Hydrochloric acid | 0.006 | 24 | 0.006 | 30 |
| Hydrogen sulphide | 0.008 | 24 | 0.008 | 30 |

## 2.4 Technologies in Solid Waste Treatment

There are various technologies in treatment of tannery's solid waste. Some of them are discussed below:

### 2.4.1 Landfill disposal

Diversion of land for waste disposal would mean use of vacant land available for dumping. The implication therefore, is that if the current methods of solid waste disposal persist, the waste would have to be carried over long distance, which would require the creation of a great deal of transport facilities and infrastructure. This would involve enormous additional finances. Land filling scenario faces the highest cost. Some harmful compounds like lead also seep into ground water after activities like rainfall which contaminates it and is detrimental to the health of consumers.

### 2.4.2 Thermal incineration

Tannery wastes like sludge, shavings and buffing dust can be thermally treated to reduce the volume to be disposed off. The thermal treatment of wastes involves incineration, gasification and pyrolysis as a means of disposal, while also recovering energy from waste. So, thermal incineration is considered as the cheapest alternative and attractive method for its simultaneous energy production and volume reduction of solid waste. The thermal incineration of solid wastes from tanneries needs a special attention on the issues such as release of toxic chromium (VI), halogenated organic compounds and poly aromatic hydrocarbons into the environment (Gil *et al*.,2009) . Solid wastes created by the tannery can be gasified following pre-treatment methods such as maceration, drying and subsequent densification or briquetting (Salman, 2010) or subjected to pyrolysis (Gil *et al*., 2009).

### 2.4.3 Anaerobic digestion

Anaerobic digestion is a microbiological process of decomposition of organic matter, in the absence of oxygen, common to many natural environments and largely applied today to produce biogas in airproof reactor tanks, commonly named digesters. A wide range of micro-organisms are involved in the anaerobic process which has two main end products: biogas and digestate.

Biogas is a combustible gas consisting of methane, carbon dioxide and small amounts of other gases and trace elements. Digestate is the decomposed substrate, rich in macro and micro nutrients and therefore suitable to be used as plant fertilizer.

Anaerobic digestion is considered as one of the best treatment method for the organic fraction of the segregated waste. Anaerobic digestion technologies ensure recovery of energy in the form of biogas, which is a clean fuel as compared to other conventional solid or liquid fuels.

Anaerobic co-digestion is same as anaerobic digestion but for the inclusion of one or more different type of substrate. This method resulted from the need to improve on the product qualities of singular substrates. Various substrates like waste from different animals, food waste and industrial wastes can be combined together in a digester. Cow-dung is an animal waste of high carbon to hydrogen content which results in high methane yields. According to Ramanujam (2011), cow manure is an excellent basic substrate for co-digestion of industrial waste, which could otherwise be difficult to process alone with carbon-nitrogen (C/N) ratio of 25:1 to 32:1 has a positive effect on the methane yield and that of cow-dung is 25:1 (Ramanujam, 2011). Nigeria produces about 227,500 tons of fresh animal (cow) waste daily (Akinbami *et al*., 2001).

Reasons for selecting cow-dung as co-digesting substrate include:

a) It is readily available at every season.
b) It is obtained free.
c) It has high Carbon to Nitrogen ratio of 25:1 and has been found to give methane yields of 70%v/v and above.
*d)* It also has very low sulphide concentration on the average.

*2.4.3.1 Products of anaerobic digestion*

The three principal products of anaerobic digestion are biogas, digestate, and water. Among the many biological treatment methods experimented so far, anaerobic digestion possesses several advantages such as low energy requirement, low sludge production, low nutrient requirements and possibility of operation at high organic loading rate at a relatively low hydraulic retention time (Fergusen and Mah, 2006). Anaerobic digestion is a favorable technological solution which degrades a substantial part of the organic matter contained in

the sludge and tannery solid wastes, generating valuable biogas, contributing to alleviate the environmental problem, giving time to set-up more sustainable treatment and disposal routes. Chrome free digested tannery sludge has a definite value as a fertilizer based on its nutrient content.

Populations of anaerobic microorganisms typically take a significant period of time to establish themselves to be fully effective. Therefore, common practice is to introduce anaerobic microorganisms from materials with existing populations, a process known as "seeding", typically accomplished with the addition of sewage sludge or cattle slurry (Fergusen and Mah, 2006), cow cud can also be used as an innoculum (Thangamani *et al*.,2009).

*2.4.3.2 Stages in anaerobic digestion*

There are four key biological and chemical stages of anaerobic digestion:

i. Hydrolysis.
ii. Acidogenesis.
iii. Acetogenesis.
iv. Methanogenesis.

Figure 2.1, illustrates the four stages of anaerobic digestion and the conversion of carbohydrates, fats and proteins to methane and carbon dioxide gases.

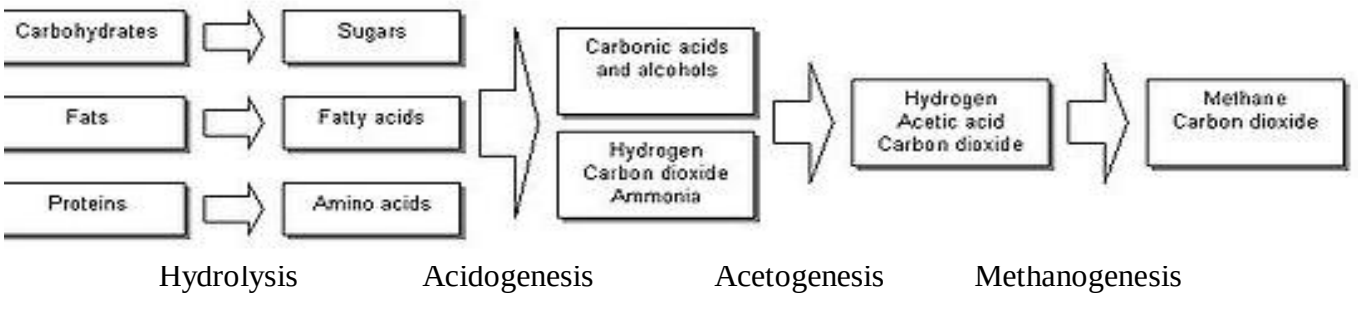


**Figure 2.1: Stages in Anaerobic Digestion (Fergusen and Mah, 2006).**

An anaerobic biodegradation of organic substrate (fleshing) can be estimated using the following stoichiometric relationship (Tchobanoglous *et al*., 1993):

$$C_aH_bO_cN_d + \frac{4a - b - 2c + 3d}{4}H_2O \rightarrow \frac{4a - b - 2c + 3d}{8}CH_4 + \frac{4a - b - 2c + 3d}{8}CO_2 + dNH_3 \quad \text{----2.2}$$

where a, b, c and d are constants.

A simplified generic chemical equation for the anaerobic digestion of carbohydrates is as follows: $C_6H_{12}O_6 \rightarrow 3CO_2 + 3CH_4$ -----2.3

## 2.5 Chemical Characteristics of Biogas Products

Biogas comprises of various gases in different ratios. Table 2.3 shows the various gases obtained in a typical biogas.

**Table 2.3: Typical Composition of Biogas (Igoni *et al*.,2008)**

| Matter | % |
|---|---|
| Methane, $CH_4$ | 50–75 |
| Carbon dioxide, $CO_2$ | 25–50 |
| Nitrogen, $N_2$ | 0–10 |
| Hydrogen, $H_2$ | 0–1 |
| Hydrogen sulphide, $H_2S$ | 0–3 |
| Oxygen, $O_2$ | 0–2 |

### 2.5.1 Methane

Methane ($CH_4$) gas consists of one carbon and four hydrogen atoms and is the main component of natural gas. It is both odorless and colorless. $CH_4$ provides approximately

1000 BTUs of heat energy per cubic foot when burned. One BTU (one Joule) is the energy required to raise the temperature of one pound (one kilogramme) of water to one degree Fahrenheit (one Kelvin). Methane is traditionally produced as a non-renewable fossil fuel that is generated over a period of thousands or millions of years (Zupancic *et al*., 2002).

### 2.5.2 Carbon (IV) oxide

Carbon (IV) oxide is an atmospheric gas consisting of one carbon and two oxygen atoms. Like methane, it is both odorless and colorless. $CO_2$ is produced either by the combustion of organic matter in the presence of oxygen, by microbial fermentation or plant respiration. In biogas, $CO_2$ is produced when methanogenic bacteria break down simple organic compounds, through the process of fermentation. The two main components of biogas are $CH_4$ and $CO_2$, products of the conversion of simple organic compounds by methanogenic bacteria.

### 2.5.3 Trace components

The trace components make up less than 2% of diary manure-digested biogas. The common trace components of anaerobically digested diary manure include ammonia, hydrogen sulphide ($H_2S$), and water vapour. Depending on the use of the biogas, most trace components must be removed from the biogas. Water vapour can be particularly hazardous because it is highly corrosive when combined with acidic components such as hydrogen sulphide ($H_2S$) and to a lesser extent, carbon (IV) oxide ($CO_2$). The major contaminant in biogas is $H_2S$. This component is both poisonous and corrosive, and causes significant damage to piping, equipment and instrumentation. In combustion, $H_2S$ present in the gas is also released as sulfur dioxide, contributing to atmospheric pollution.

## 2.6 Anaerobic Digestion Parameters

The efficiency of an anaerobic digestion is influenced by some critical parameters, thus it is crucial that appropriate conditions for anaerobic microorganisms are provided.

### 2.5.1 Temperature

The Anaerobic Digestion process can take place at different temperatures, divided into three temperature ranges: 25$^{o}$C and below (psychrophilic micro-organisms), 25$^{o}$C – 45$^{o}$C (mesophilic micro-organisms), and 45$^{o}$C – 70$^{o}$C (thermophilic micro-organisms). The temperature stability is decisive for anaerobic digestion (Teodorita *et al*., 2008). Ammonia toxicity increases with increasing temperature and can be relieved by decreasing the process temperature (Angelidaki, 2002). Thermophilic operation temperature results in faster chemical reaction rates, thus better efficiency of methane production, higher solubility and lower viscosity. Thermophilic bacteria are more sensitive to temperature fluctuation (+/-1°C) and require longer time to adapt to a new temperature, in order to reach the maximum methane production. Mesophilic bacteria are less sensitive. Temperature fluctuations of +/-3°C are tolerated, without significant reductions in methane production.

### 2.6.2 pH values and optimum intervals

The pH value is the measure of acidity/alkalinity of a solution. The pH value of the anaerobic digestion substrate influences the growth of methanogenic microorganisms and affects the dissociation of some compounds of importance for the anaerobic digestion process (ammonia, sulphide, organic acids). Experience shows that methane formation takes place within a relatively narrow pH interval, optimally between 7.0 and 8.0 for most methanogens. The optimum pH interval for mesophilic digestion is between 6.5 and 8.0 and the process is severely inhibited if the pH-value decreases below 6.0 or rises above 8.3. The value of pH can be increased by ammonia, produced during degradation of proteins or by

the presence of ammonia in the substrate stream, while the accumulation of VFA decreases the pH-value. Therefore, the VFA readings should be taken alongside pH values from a digesting system. In order to obtain the best-optimized condition for biogas production, where the methane producing bacteria exist, the pH value of input mixture in the digester should be between 6 and 7.

### 2.6.3 Volatile fatty acids (VFA)

The stability of the anaerobic digestion process is reflected by the concentration of intermediate products like the VFA. The VFA are intermediate compounds (acetate, propionate, butyrate, lactate), produced during acidogenesis, with a carbon chain of up to six atoms. In most cases, anaerobic digestion process instability will lead to accumulation of VFA inside the digester, which can lead furthermore to a drop of pH-value.

### 2.6.4 Ammonia

Ammonia ($NH_3$) is an important compound, with a significant function for the anaerobic digestion process. $NH_3$ is an important nutrient, serving as a precursor to foodstuff and fertilizers, and in anaerobic digestion it is normally encountered as a gas, with the characteristic pungent smell. Proteins are the main source of ammonia for the anaerobic digestion process. Too high ammonia concentration inside the digester, especially free ammonia (the unionized form of ammonia), is considered to be responsible for process inhibition. For its inhibitory effect, ammonia concentration should be kept below 80 mg/l.

### 2.6.5 Macro and micronutrients (trace elements) and toxic compounds

Microelements (trace elements) like iron, nickel, cobalt, selenium, molybdenum or tungsten are equally important for the growth and survival of the anaerobic digestion microorganisms as well as the macronutrients carbon, nitrogen, phosphorus, and sulfur. The optimal ratio of the macronutrients carbon, nitrogen, phosphorus, and sulfur (C: N: P: S) is

considered 600:15:5:1. Insufficient provision of nutrients and trace elements, as well as too high digestibility of the substrate can cause inhibition and disturbances in the anaerobic digestion process.

2.6.6 Loading rate

The amount of raw materials fed per unit volume of digester capacity per day is known as loading rate. It is important to optimize the loading rate to avoid overfeeding, which may lead to inhibited methane production. However, underfeeding the plant would lead to low gas production and economically ineffective process as well.

2.6.7 Retention time

The retention time can be significantly distinct in batch type facilities, whereas, by dividing the digester volume by the daily influent rate, the mean retention time can be determined in continuous systems. Based on the vessel geometry, the mixing rate and the actual substrate component, the effective retention time may differ significantly even in continuous systems. Therefore, the appropriate retention time is a function of two processing factors: temperature and substrate type. The amount of time a material substrate spends in the digester obviously has a large effect on the anaerobic digestion process. The longer the retention time, the more likely the substrate will be broken down and stabilized and have proper interactions with the bacteria within the digester. Hence, a longer retention time leads to increased methane production (Rebekkah, 1982).

## 2.7 Hydrogen Sulphide

Sulphide is one of the major components of the tannery effluent obtained from beam-house operations. The use of sodium sulphide and sodium hydrosulphide in tannery for unhairing and liming processes, results in the sulphide content varying from 10 – 5000 mg/l.

### 2.7.1 Removal of hydrogen sulphide

Hydrogen sulphide in biogas should be removed for all processes except in the most simple burner applications. Hydrogen sulphide in combination with water vapor in raw biogas can form sulfuric acid ($H_2SO_4$), which is very corrosive to engine components. There are a variety of $H_2S$ removal methods which include: Various desulphurizing technologies include the Claus process, the use of carbonaceous based materials as adsorbents of sulphide, Membrane separation, the use of metal oxides, direct air stripping using an alkaline scrubber the use of impregnated sorbents and biological desulphurizing methods (Huixing L., 2008). Dublein and Steinhauser (2008) suggested the direct inclusion of sodium alginate in the ratio 0.01%wt/v into the digester to reduce sulphide concentration to about 20ppm.

## 2.8 Sodium and Calcium Alginate

Sodium alginate is the sodium salt of Alginic acid derived from selected varieties of brown seaweed. Its empirical formula is $NaC_6H_7O_6$, boiling point 495.2°C at 760 mmHg and vapor pressure of 6.95E-12mmHg at 25°C. It is one of the structural polymers that help to build the cell walls of these plants. It has some unusual properties and a wide variety of uses. It is a good chelating ligand and finds uses in pharmaceuticals, food, immobilized biocatalysis and paper industries (Chung *et al*., 1996 and Hou *et al*., 2005). Youngsukkasem *et al.*, (2012) in his production of biogas discovered sodium alginate was able to encapsulate methane producing bacteria for up to 3 months without washing out. Imran *et al*., (2012) used sodium alginate to immobilize chemical coagulants such as sulphate, aluminium sulphate, calcium carbonate and sodium citrate for treating tannery wastewater samples.

The polymer can be represented as shown in Figure 2.2.

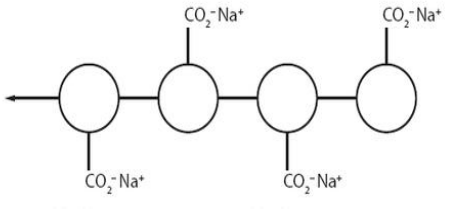


**Figure 2.2: Polymeric Structure of Sodium Alginate.**

The liquid droplet formation method is probably the easiest method for capsule formation amongst other methods to include liquid droplet formation, pregel dissolution, interfacial polymerization and coacervation (Johan *et al*., 2012) and is also called the one-step method. The capsules are instantaneously formed by allowing the cell containing hardening agent (Park and Chang, 2000). The major benefit of cell encapsulation for production purposes is the possibility of re-using the cells in sequential batches or in a continuous process. The reaction system is essentially mass transfer limited.

When sodium alginate is added into a solution of calcium ions, the calcium ions replace the sodium ions in the polymer. Each calcium ion can attach to two of the polymer strands. This is called cross-linking and can be represented as shown in Figure 2.3:

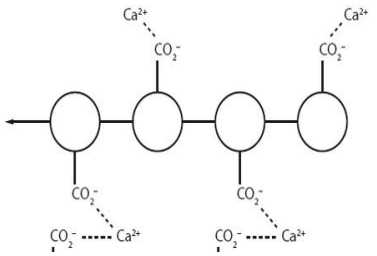


**Figure 2.3: Polymeric Structure of Calcium Alginate.**

Calcium alginate is then formed by the replacement of the sodium ions in sodium alginate. Calcium alginate has been widely used for encapsulation of lactic acid and probiotic bacteria (Kasra *et al*., 2010).

### 2.8.1 Physical properties

Sodium alginate is powder in white or faint yellow and nearly odorless. It is soluble in water but insoluble in such organic solvents such as alcohol, ether and chloroform. It becomes thick liquid when dissolved in water. It is non-toxic and has a strong gel property. The pH value of sodium alginate in 1% distilled water solution is about 7.2. Sodium alginate possesses hygroscopicity and its content of moisture in the balanced state depends on relative humidity. Dry sodium alginate, if stored in a tightly closed container and in temperature 25℃ or below, is very stable. The sodium alginate solution is also stable in pH 5-9. DP (degree of polymerization) and the molecular weight are directly related to the viscosity of its solution. It possesses good thickening property. It is a good chelating ligand like EDTA (ethylenediamine tetra-acetic acid), CDTA 1:2 diaminocyclohexane-N, N-tetra-acetic acid), NTA (nitrilotri-acetic acid), SSA (5-sulphosalicyclic acid).

# CHAPTER 3

## MATERIALS AND METHODS

### 3.1 Introduction

This chapter presents the materials and methods used in the production of biogas from co-digesting tannery fleshing and cow-dung; and reduction of its sulphide concentration. The values of BOD, COD, Chloride, Sulphide, Ammonia-N and Calcium were preliminarily determined for the beam house liquors. These liquors were sourced from the tannery in Nigerian Institute of Leather and Science Technology (NILEST), Zaria, Kaduna state. The liquors characterized were soaking liquor, liming liquor and deliming liquor. The unhairing liquor was not considered due to its inherent high sodium sulphide load and hairs, both of which are inhibitory to methanation. This was followed by a selection of the most favorable liquor for digesting with the substrates. The substrates used were cow-dung (sourced from the Abattoir, Zango Zaria) and fleshing sourced from NILEST, Zaria). The digestion experiments were carried out in the Multiuser Laboratory, Ahmadu Bello University, Zaria in different batches, and every cycle was for a period of 50 days. Biogas concentration readings were taken at intervals of one day using a Biogas Analyzer at the Multiuser Laboratory, Ahmadu Bello University, Zaria. This work adopted the sulphide reduction method using sodium alginate as recommended by Dublein and Steinhauser (2008). The detailed methodology carried out in this order is further explained below.

### 3.2 Materials, Reagents and Apparatus/ Equipment

Below is a list of materials, reagents and equipment used in this study:

3.2.1 Materials

i. Inoculum (cow cud).
ii. Tannery fleshing.
iii. Cow-dung.
iv. Beam house liquors.
v. Sieves.
vi. Bowls.
vii. Digesters (reagent bottles).
viii. Pipes (infusion sets).

3.2.2 Reagents

All reagents used were of analytical grade.

i. Ammonia-free water.
ii. Nessler's reagent.
iii. Rochell's salt solution.
iv. Mercury tetraoxosulphate(VI).
v. Sulphuric acid.
vi. Potassium chromate.
vii. Distilled water.
viii. Ferroin indicator.
ix. Hydrochloric acid.
x. Standard iodine solution.
xi. Potassium Iodide.
xii. Iodine.

xiii. Standard sodium thiosulphate solution.

xiv. Starch solution.

xv. Zinc sulphide.

xvi. Silver nitrate solution.

xvii. Calcium buffer reagent.

viii. Calcium oxalate reagent.

xix. Sodium alginate.

xx. Calcium chloride.

3.2.3 Apparatus/ Equipment

i. Volumetric flasks (2litre).

ii. Refluxing flask.

iii. Conical flasks.

iv. Burette.

v. Pipette.

vi. Syringes (5ml, 2ml).

vii. Measuring cylinders.

viii. Retort stand and clamp.

ix. Beakers.

x. Mortar and Pestle.

xi. Thermometer.

xii. Centrifuge.

xiii. Lovibond comparator.

xiv. Multiparameter bench photometer HI 83200.

xv. pH meter (Jenway 3505, UK) with a pH range of -2 to 16 ± 0.001.

xvi. An Analytical Digital Balance accurate to 0.1mg (Sartorius, USA).

xvii. BIOGAS Analyzer (Biogas 5000, UK).

viii. Milling machine (Retsch RS 200, UK).

### 3.3 Determination of Various Parameters in the Liquor Samples.

Various parameters of the liquor and fleshing samples sourced from the tannery in NILEST, Zaria, were analyzed using the Standard Methods for the Examination of Water and Waste Water, APHA (1985), as compiled in the Laboratory manual at the Department of Water Resources Engineering, Ahmadu Bello University Zaria, Kaduna State in the year 2000. The methodology is as detailed below:

#### 3.3.1 pH determination

The pH was determined analytically, using a pH Meter. The pH meter was standardized using buffer solution. Before measuring the pH for any test sample, the electrode was washed thoroughly first with distilled water and then inserted into the sample solution. The system was allowed to stabilize and then the reading was taken. A duplicate measurement was made and the average recorded.

#### 3.3.2 Ammonia- N determination (Lovibond comparator method)

The Lovibond comparator method using Nessler's reagent without distillation of the sample was used. Reagents used were Ammonia- free water, Nessler's reagent, and Rochell's salt solution. In a typical analysis, 50ml of sample was measured into a beaker, with 5 drops of Rochelle salt solution added and mixed. Then 2ml drops of Nessler's reagent was added and mixed. The mixture was allowed to stand for 10mins for colour development. Two test tubes

one filled with the mixture and the other with a blank sample were inserted inside the comparator to determine colour intensity.

### 3.3.3 Biological oxygen demand (BOD) (BOD incubation method)

BOD is that amount of oxygen required by bacteria, while stabilizing decomposable organic matter under aerobic conditions. It can be expressed as shown in Equation 3.1

$$C_nH_aO_bN_c + \left(n + \frac{a}{4} - \frac{b}{2} - \frac{3c}{4}\right)O_2 \rightarrow nCO_2 + \left(\frac{a}{2} - \frac{3c}{2}\right)H_2O + cNH_3 \quad \ldots(3.1)$$

Where n, a, b, c are constants.

*3.3.3.1 Procedure*

Seeded dilution water was prepared. The dilution required was 1.5 litres. Then the sample was diluted with seeded dilution water in 2 litre volumetric flasks. It was transferred to numbered BOD bottles (4 bottles for each dilution). The bottles were then incubated for 5 days ± 1 hour at 20$^{o}$C. The residual dissolved oxygen was determined after incubation period. This was the result obtained by subtracting the value of the dissolved oxygen after the fifth day from the value just before incubation.

### 3.3.4 Chemical oxygen demand (COD) (Closed reflux method)

This is the oxygen required to oxidize organics to carbon-dioxide and water. In a typical run, 0.4g of $HgSO_4$ was placed in a refluxing flask. 20ml of the sample was also added and mixed. 10ml standard $K_2Cr_2O_7$ solution was added and several pumice granules of glass beads already heated to 600$^{o}$C for 1hr. The flask was attached to the Reflux condenser. 30ml concentrated $H_2SO_4$ was added slowly to the flask containing $HgSO_4$ through the open end of the condenser mixing thoroughly by swirling while adding the acid. The mixture was refluxed for 1hr. The condenser was cooled and then washed with about 25ml of distilled

water. The mixture was diluted to about 100ml with distilled water and then cooled to room temperature. 3 drops (0.15ml) ferroin indicator was added. It was then titrated with $Fe(NH_4)_2(SO_4)_2$, taking as the end point the sharp colour change from blue-green to reddish brown.

3.3.5 Chloride determination ( Mohr's method)

This gives an estimation of chloride ion concentration in a sample. Exactly 50ml of the sample was measured into a conical flask. Then 0.5ml of potassium chromate indicator solution was added. The sample in the flask was titrated with standard silver nitrate solution. The volume of silver nitrate solution consumed at a reddish-brown end point was recorded as $c_1$.

$$Cl^- \text{ (mg/l)} = \frac{C_1}{V_2} \times 10 \quad \ldots (3.2)$$

$v_2$ is the volume of water sample taken for analysis.

3.3.6 Sulphide determination (Iodometric method)

A Multiparameter bench photometer, with a tungsten with narrow band interference filter at 466nm as the light source. The iodometric method of determination was used to determine sulphide concentration. The reagents used were 6*N* Hydrochloric acid, standard iodine solution, 0.025*N* standard sodium thiosulfate solution, and starch solution.

*3.3.6.1 Procedure*

20ml of iodine solution estimated to be in excess over the amount of sulphide present was measured from a burette into a 500ml flask. 2ml of 6*N* HCl was added. Then, 200ml of the sample was poured into the flask, discharging the sample under the solution surface. It was then back titrated with $Na_2S_2O_3$ solution, with a few drops of starch solution as end point was approached and this was continued till the blue color disappeared. The calculation used was

thus:

$$S^{2-}(mg/\text{l}) = \frac{[(\text{A}\times\text{B})-(\text{C}\times\text{D})]\times 16{,}000}{\text{ml sample}} \quad \ldots(3.3)$$

where A = iodine solution in ml

B = normality of iodine solution

C = $Na_2S_2O_3$ solution in ml and

D = normality of $Na_2S_2O_3$ solution.

3.3.7 Calcium determination

A Multiparameter bench photometer, with a tungsten with narrow band interference filter at 466nm as the light source. The adaptation of the oxalate method was used to determine calcium ions present in the sample. 4 drops of buffer reagent, 7ml of calcium buffer reagent and 1ml of calcium oxalate reagent were used.

*3.3.7.1 Procedure*

Exactly 3ml of the sample was added to a cuvette using a 5ml syringe. A pipette was used to fill the cuvette up to its 10ml mark with calcium buffer reagent. 4 drops of buffer reagent was added, and the cap was replaced. The cuvette was inverted several times to mix its contents and then placed into the instrument's holder. The machine was zeroed. Using 1ml syringe, 1ml of the calcium oxalate reagent was added and the cuvette was closed and mixed 10 times. The timer was set for 5 minutes and the cuvette was mixed 10 times again. The cuvette was re-inserted into the instrument and the display was read for calcium concentration in mg/l.

## 3.4 The Production of Biogas

The samples- fleshing and cow-dung, to be co-digested were first dried and grinded to reduce their size and aid faster reaction as a form of pre-treatment. The fleshing alone was used as

control. Particle size of 6mm diameter for fleshing using a milling machine and 10mm diameter for cow dung using a mortar and pestle. The digesters used were 2-litre Pyrex bottles covered with rubber bungs so that they were air tight. Little apertures bored through the bungs, allowed for pipes which transferred the gas produced into inverted measuring cylinders filled with water or solutions as required. The second apertures were used to fit in the pipes of the biogas analyzer used for characterizing the gas. A pictorial view of the set-up is shown in Plate 3.1. Different liquors were digested separately with the aim of obtaining the beam house liquor with the highest methane concentration and the lowest sulphide concentration value. After which the co-digestion commenced with different ratios of the two substrates- fleshing and cow-dung. These were weighed with a digital weighing balance. 50g of fleshing and 100g of cow-dung in the ratio 1:2, 50g of fleshing and 50g of cow-dung in the ratio 1:1, 100g of fleshing and 50g of cow-dung in the ratio 1:0.5, were put into different digesters of the same volume (2 litres). 100g of fresh inoculums (cow cud) was also added to the substrates in the digester. The selected liquor of volume 1.5l was added as diluent to the substrates in the subsequent digesters. Gas readings for concentration were taken at one day interval. 0.01% wt/v of sodium alginate in direct form and 0.01% wt/v calcium alginate beads formed from replacing the sodium ions of sodium alginate with calcium ions were added to the fleshing to cow-dung ratio that gave the highest methane concentration and lowest sulphide concentration. Ambient temperature of 25 $\pm 3^{o}$C was observed throughout the digestion cycle which lasted for 50 days depending on when gas evolution ceased and percentage concentration dropped significantly. Volume of the gas was recorded as the volume that displaced water in the inverted cylinder.

**Plate 3.1: Experimental Set-up for the Digestion Process.**

### 3.4.1 Principle of biogas characterization using Biogas 5000

The biogas 5000 analyzer as shown in Plate 3.2 provides an accurate and consistent method of monitoring the composition of the biogas produced from each digester. It operates on the principle of infra-red (IR) absorption for the measurement of $CH_4$, $H_2S$, $O_2$ and $CO_2$. The radiation from a broadband IR source is passed through filters to select only the wavelengths that will be absorbed by $CH_4$, $H_2S$, $O_2$ and $CO_2$. A gas sample is pumped from each digester into the measurement cell of the biogas analyzer where the IR radiation is passed through the gas onto a detector. The detector then identifies and measures each constituent gas present within the sample. The gases of interest in this study were methane measured in %v/v and hydrogen sulphide measured in ppm.


**Plate 3.2: Pictorial view of a Biogas 5000 analyzer.**

3.4.2 Engineering parameters

The following engineering parameters are normally considered in anaerobic digestion: Optimal bacterial growth/process control, dry matter content, organic dry matter content, substrate availability, biogas quantity and quality, hydraulic retention time, and organic loading rate. For this work, substrate quantity, biogas quality and quantity; and retention time were considered. Pretreatment carried out were drying and size reduction.

## 3.5 Determination of Temperature, pH and Volatile Fatty Acid Concentration.

Other parameters that were measured as the digestion progressed were temperature, pH and Volatile Fatty Acid concentration.

3.5.1 Temperature

This was measured using a standard thermometer inserted through one of the apertures on the rubber bung into the substrate mixture.

### 3.5.2 pH

This was measured using a standard pH meter inserted through one of the apertures on the rubber bung into the substrate mixture.

### 3.5.3 Titrimetric method for VFA determination

Volatile Fatty Acids, VFA concentration was measured at intervals within the digestion period according to this procedure.

#### *3.5.3.1 Procedure*

Before analysis, the sample was centrifuged at 300rpm for 15mins and the top decant was obtained. 20ml of the decanted sample was put into a titration vessel, the size of which was determined by the basic requirement to guarantee that the tip of the pH electrode is always immersed below the liquid surface. Initial pH was recorded. The sample was titrated slowly with 0.1N sulfuric acid and stirred until pH 5.0 was reached. The added volume of the titrant was recorded. More acid was slowly added until pH 5.0 was reached. The total volume of the titrant added was recorded. The latter step was repeated until pH 4.0 is reached and the volume of added titrant was recorded once more. A constant mixing of sample and added titrant was ensured right from start to minimize exchange of $CO_2$ with the atmosphere during titration.

The principle behind this method according to Kapp, (1984) is that, the acid required to titrate a sample from pH 5.0 to pH 4.0 can be considered proportional to the content of Sa or VFA present in the sample. This applies because between pH 5.0 and pH 4.0, there is usually no weak acid/base subsystem. Moreover, the dissociation constant of acetic acid, propionic acid, butyric acid and valeric acid are all close to 4.75. Thus they show very similar buffer characteristics and can be lumped together as one parameter.

This method according to Kapp, (1984) was originally developed for the control of mesophilic sludge digesters.

$$Sa = \frac{131{,}340 \times N \times Va_{5-4,measured}}{VS} - 3.08 \times Alk_{measured} - 25 \quad \ldots 3.4$$

where Sa is the Volatile Fatty acid concentration in mg/l.

N is the normality in mmol/l

$Va_{5-4,measured}$ is the measured volume of acid in ml required to titrate a sample from pH 5.0 to pH 4.0 due to Sa buffer.

VS is the volume of the sample which is constant at 20ml.

$$Alk_{measured} = \frac{VA_{4.3\ measured} \times N \times 1000}{VS} \quad \ldots 3.5$$

$Va_{4.3\ measured}$ is the measured volume of acid in ml required to titrate a sample from pH 5.0 to pH 4.3.

## 3.6 Desulphurization Method

Direct use of different weights of sodium alginate and calcium alginate were investigated as a desulfurization technique.

### 3.6.1 Use of sodium alginate

Sodium alginate of 0.01%wt/v was weighed according to as stated in Dublein and Steinhauser (2008). The weight used in proportion to the volume of substrate and diluent, was 0.15grammes for digesters with volumes of 100g fleshing and 200g cow-dung (the ratio 1:2), and diluent of 1.5litres. The biogas composition with the highest methane and lowest sulphide concentrations was analyzed from the digesters directly during the cycle, at intervals of one day.

3.6.2 Use of calcium alginate beads

Calcium alginate was made using sodium alginate and calcium chloride solution. 3% sodium alginate solution and 2% calcium chloride solution were prepared according to the methodology described by Debbie (2008). The alginate suspension was drawn into a 5ml syringe and a beaker of the calcium chloride solution was placed under the syringe. The sodium alginate solution was allowed to drip slowly from the needle into the calcium chloride solution. The calcium chloride solution was swirled gently simultaneously. The alginates will be cross-linked by the calcium ions, trapped the cells in a matrix of calcium alginate. The resulting beads are therefore now calcium alginate beads. The beads were left to harden in the calcium chloride solution for 5 – 10 minutes. Samples of such beads formed are presented in Plate 3.3. The beads from the calcium chloride solution were separated using a sieve and gently washed with cold tap water, weighed and added to the digesters. The biogas produced was analyzed from the apertures of the digesters.

**Plate 3.3: Calcium Alginate Beads in Distilled Water.**

The resulting beads are Calcium alginate beads made from replaced sodium salt, and have the chemical formula $C_{12}H_{14}CaO_{12}$.

# CHAPTER 4

## RESULTS AND DISCUSSION

### 4.1 Introduction

This study focuses on the concentrations of methane and hydrogen sulphide gases obtained from co-substrates in various anaerobic digestion cycles which were analyzed via a gas analyzer, Biogas 5000. Batch systems were applied for each anaerobic digestion process according to Gregor and Viktor (2012) who proffered that a single digester is sufficient for good performance for digestion of protein rich substrates like animal fleshing. Temperature was maintained at room conditions between 29$^{o}$C and 31$^{o}$C throughout the digestion processes, pH between 6.5 and 7.4 were averagely experienced. The volatile fatty acid was also measured 3 times in a cycle following Kapp, (1984) method, and was found to be below inhibitory concentration of 2,000 mg/l. Microsoft Excel graph-plotting, bar chart tools and tables were used to represent the data obtained.

### 4.2 Properties of the Beam House Effluents

The beam house liquors from each waste stream and fleshing were separately analyzed according to basic waste water properties such as BOD, COD, chloride and sulphide concentrations. From the results in Table 4.1, concentrations of the parameters and concentration levels at which they will be inhibitory to methanation were also obtained from previous research work (Ye *et al*., 2005) and shown in the same table. The assay provided a guide to the selection of the beam house liquor that was most suitable as diluent for anaerobic digestion. This differs from works by previous researchers that used the entire primary or secondary sludge as diluents or as substrate for the digestion process (Colak *et al*.,(2005),

Vasudevan and Ravindran (2007), Thangamani *et al*., (2009), Srinivasan *et al*., (2010) and Ramanujam (2011); which will in the long run contribute to an increase in cost of waste water treatment.

**Table 4.1: Properties of Beam house Liquors.**

| Parameter | Inhibitory concentrations (Ye *et al*., 2005) | Soaking liquor | Liming liquor | Deliming liquor |
|---|---|---|---|---|
| BOD (mg/l) | 100 | 90 | 40 | 30 |
| COD (mg/l) | 3,000 – 7,000 | 4,000 | 8,000 | 5,000 |
| Chloride (mg/l) | 5,000 | 4,548.58 | 1,249.61 | 649.79 |
| Sulphide (ppm) | 100 – 150 | 25.2 | 25.4 | 448 |
| Ammonia-N (mg/l) | 1.5 – 3.0 | 1.04 | 0.16 | 0.56 |
| Calcium (mg/l) | 8,000 | 1619.4 | 2,024 | 3,0363 |

Berardino and Martinho (2009) in their work, found out that production of biogas was highly dependent from the fleshing content in the substrate. Addition of 30 % by weight of fleshing to sludge increased the average biogas production by four times.

From the assay in Table 4.1, it can be seen that the soaking liquor had its concentrations below or within the range of that given by Ye *et al*., (2005). The concentration of sulphide in the fleshing was also measured prior to the digestion process and was as high as 532ppm. This is because not all of the sodium sulphide used in the tannery unhairing process goes into the waste stream liquor after the unhairing process (Buba, 2004). From literature, sulphide concentrations between 100ppm and 150pm have inhibitory effects to methanation (Ye *et al*., 2005). Therefore, the need to tackle this either prior to digestion or simultaneously is pertinent.

### 4.3 Biogas Composition of Different Diluents

Different diluent stream types were used in the preliminary stages of the study with fleshing as substrate, the methane and hydrogen sulphide concentrations from each digester were measured. The temperature was maintained between 29 $^{o}$C and 31$^{o}$C. The particle size of 6mm diameter for the fleshing, the weight of the fleshing and volume of the diluent were kept constant. The diluent stream types were varied here.

4.3.1 Effect of diluents on methane concentration

The three beam house liquors (deliming, liming and soaking) were individually and jointly used (combined liquor) as diluents for the tannery fleshing. They were digested in equal volumes. Figure 4.1 gives a representation of the highest methane concentrations measured from each digester.

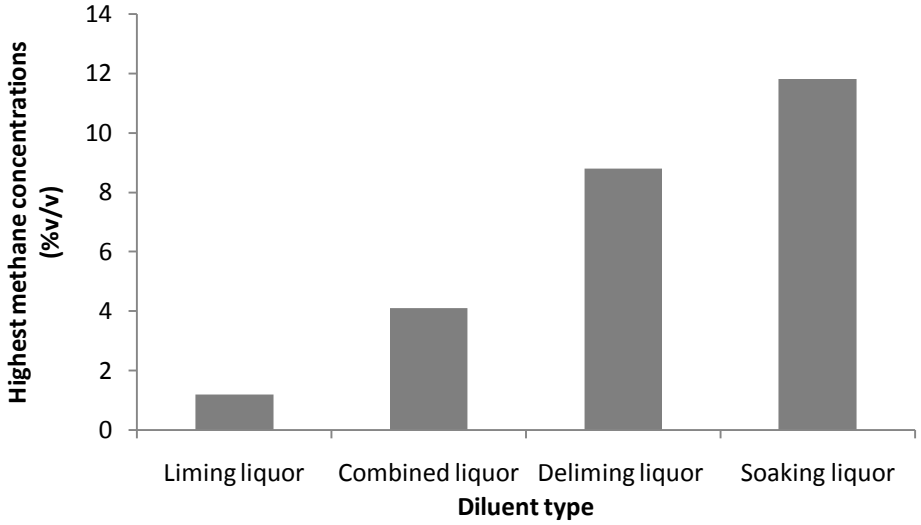


**Figure 4.1: Effect of Beam House Liquors on Highest Methane Concentration.**

From Figure 4.1 it is seen that the methane concentration from soaking liquor as diluents gave the highest concentration of 12% v/v. This could be as a result of substances like residual

blood and dung in it that are amenable to biodegradation. From the assay in Table 4.1, it was also observed that the concentrations of the parameters for the soaking liquor fell within or below inhibitory level. This made it the most favorable to be used as a diluent in subsequent digestion cycles. The highest methane concentration from digesting fleshing with the combined liquors was 4.1% v/v. The deliming liquor gave its highest methane concentration of 8.8% v/v. The liming liquor gave its highest methane concentration was 1.2% v/v which was the lowest methane concentrations amongst the others considered, this is due to its high alkaline nature.

4.3.2 Effect of diluents on hydrogen sulphide concentration

From Figure 4.2, the lowest sulphide concentrations were considered which suggest the most appropriate liquor in terms of toxicity or inhibition to methanation. The soaking liquor gave the lowest concentrations and this is due to the fact it is obtained from processes preceding the use of sodium sulphide in the tannery.

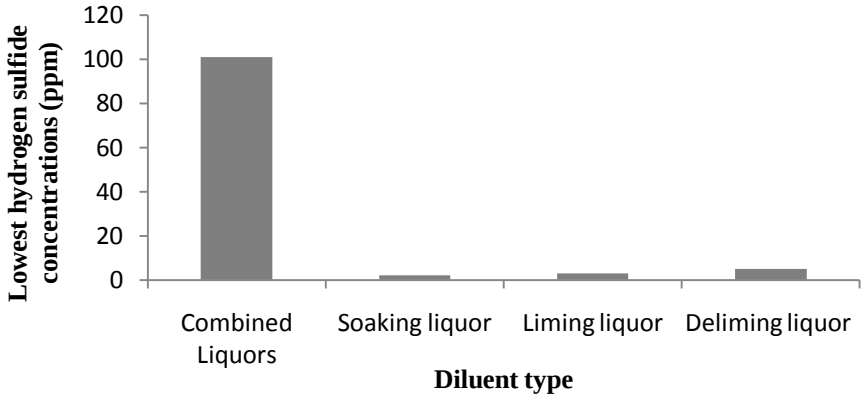


**Figure 4.2: Effect of Beam House Liquors on Lowest Hydrogen sulphide Concentrations.**

A sulphide concentration of 101ppm was obtained from using the combined liquors as diluent, the liming liquor as diluent gave 3ppm, the deliming liquor 5ppm and the least concentration of 2ppm from using the soaking liquor as diluent. This validates the use of the soaking liquor

as the best beam house diluent liquor with fleshing, since its sulphide concentration will not be inhibitory to methanation. The soaking liquor was thus used as diluent in subsequent digestion processes.

### 4.4 Effect of Co-Digestion on Methane Concentration

Considering the highest methane concentrations from Figure 4.1, it is evident that using fleshing alone as substrate in biomethanation will not give methane concentrations that are commercially viable. Methane concentrations between 50 and 75% are ideal for use in burning or power supply (Dublein and Steinhauser, 2008). In order to achieve this, fleshings were co-digested with cow-dung which is a viable source of carbon addition (Thangamani *et al*, 2009). Cow-dung is a carbon rich source. Carbon serves as the basic building block of bacterial cell material and the primary source of energy (Jeanger, 2013). Co-digestion is an appropriate approach for the treatment and disposal of manure along with organic industrial wastes for obtaining higher gas yield (Ramaujam, 2011). There was a steady increase in methane concentration with increased cow-dung ratio as expected. This is because the higher the carbon to nitrogen (C/N) content towards 25:1, the higher the methane concentration. Low C/N ratio which is typical of digesting proteinous matter causes ammonia accumulation and pH values exceeding 8.5 which is toxic to methanogenic bacteria. While, high C/N ratio is an indication of rapid consumption of nitrogen by methanogens which results in lower gas production according to Kangle *et al*., (2012). Carbon to nitrogen ratios between 25:1 and 30:1 have been seen to be optimum for the activity of methanogenic bacteria (Ramaujam, 2011). The ratio of co-digestion blends based on weight revealed that, the ratio 1:2 of fleshing to cow-dung gives the most favorable and highest methane concentration of 45%v/v.

The gas produced may not be fit for electricity supply since it doesn't fall within the range of 50-75% methane concentration but it can be used for heating purposes.

### 4.5 Effect of Adding Alginates to Co-Substrates

Sodium alginate of 0.01%w/v was added to individual digesters with cow-dung and fleshings in different ratios. In each of the blends, the effect of adding sodium alginate on methane and hydrogen sulphide concentrations were compared with when sodium alginate was not added and the results are also shown in Figure 4.3 and Figure 4.4 respectively. The blends are in terms of ratios of fleshing to cow-dung.

#### 4.5.1 Effect of sodium alginate addition on methane concentration of co-substrates

The specific effects shown in Figure 4.3 are as follows: the blend of fleshing to cow-dung ratio 1:1 without sodium alginate has a highest methane concentration of 7.4% v/v, whereas same ratio of co-substrate blend with sodium alginate gave highest methane concentration of 45% v/v which is an approximate increase of six (6) times. There was an approximate increase of one and half (1.5) when sodium alginate was added to the blend of fleshing to cow-dung ratio 1:2 as compared to when it wasn't added and this could be for the same reason mentioned earlier. The blend of fleshing to cow-dung ratio of 1:0.5 without sodium alginate had a highest methane concentration of 5.1% v/v, whereas with sodium alginate gave a highest methane concentration of 42.5% v/v which translates to eight (8) times increase of methane concentration. The control substrate of fleshing only (fleshing to cow-dung ratio 1:0) without sodium alginate had a highest methane concentration of 1.5% v/v without and 10.7% v/v with sodium alginate. The highest methane concentration of 60% v/v obtained from fleshing to cow-dung ratio of 1:2 with the addition of sodium alginate.

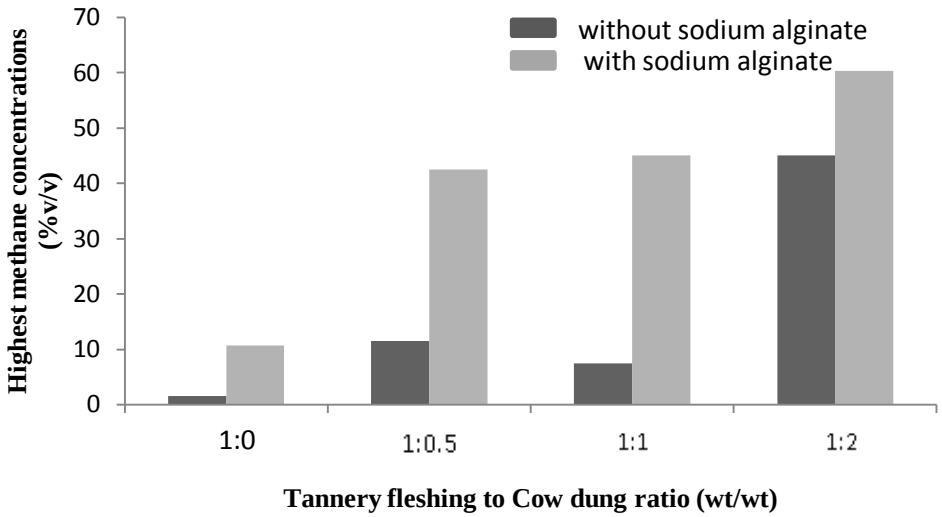


**Figure 4.3: Effect of Sodium Alginate on Highest Methane Concentration from Co-substrate Ratios.**

Considering the minute quantity of the alginate added, it is most probable that the alginate is acting as a co-factor in promoting the activities of the microbes responsible for the methanation of fleshing and not as an additional carbon source. Preferred techniques for increasing the concentration of methanogens in anaerobic digester sludge include the addition of chelating agents to the sludge in order to increase the solubility of inorganic nutrients, and the addition of chelating agents (Kroll, 1952).

Marvin, (1989) discovered that addition of certain chelating agents like alginates, to an anaerobic digestion system results in increased production of methane (varying from 5 to 20%), as well as increased destruction of solids. He thus concluded that the addition of chelating agents stimulated methane production, solids destruction and this production tends to increase with increase in concentration at a magnitude of about 20% which shows that the chelating compound is not simply serving as an additional carbon source for methane production. This is due to trace metals like Fe, Ca, Mg, $S^{2-}$ and Co in the substrate solubilized by the addition of chelating agents thereby increasing their availability to methanogenic

bacteria which require them for growth. He found out that iron and sulphide are particularly important for the rapid development of the bacteria. The digesting system in this study was rich in sulphide. He also recorded faster digester start-up, greater digester stability and more rapid digester recovery from upsets from the addition of chelating agents.

### 4.5.2 Effect of calcium alginate addition on methane concentration of co-substrates

Calcium alginate beads of concentration 0.01%w/v were added to a digester with co-substrates in the ratio 1:2 fleshings to cow-dung. The methane concentration obtained was 57.1% v/v which was less than 60%v/v obtained when sodium alginate was used. This was due to mass transfer limitation as calcium alginate beads will have less surface area for any reaction to occur. However, it was still higher in methane concentration than digesting the substrates without alginates.

### 4.5.3 Effect of alginate addition on hydrogen sulphide concentration of co-substrate

The low concentrations of methane from blends without sodium alginate was due to the high sulphide load in the fleshing used (532ppm) which is responsible for inhibiting methanolysis. Attempt was made to reduce the negative effect of hydrogen sulphide by introducing sodium alginate directly to the co-substrate blends. The blends of fleshing to cow-dung with sodium alginate 1:0, 1:0.5, 1:1, and 1:2 all reached hydrogen sulphide concentration of 0ppm in the latter parts of the digestion cycle. Though blends of fleshing to cow-dung – 1:0, 1:1, and 1:2 without sodium or calcium alginates gave hydrogen sulphide concentrations as low as 1ppm, they never arrived 0ppm throughout the digestion cycle. The introduction of either sodium or calcium alginates brought about a complete desulphurization in the biogas produced by a process of absorption. In the absorption reaction, hydrogen sulphide comes in contact with the metal chelate formed as a result of alginate reacting with metals in the substrate, to bring

about the reduction of $M^{n+}$ to $M^{(n-1)+}$ and the oxidation of $H_2S$ to form elemental sulphur with the release of H+ ions; thus the concentration of $H_2S$ gas produced within the digesters is thereby reduced (Gary , 2003). This form of desulphurization is due to the formation of metal chelates. A redox reaction is responsible for the desulphurization observed within the digester systems. Metals (e.g. Fe, Co, $S^{2-}$ and Ni) serving as nutrients already present within the systems, react with the various chelating ligands (alginates) introduced to form metal chelates. These metal chelates then react with the hydrogen sulphide gas produced, absorbing the gas from the system. The reaction sequence for the absorption of hydrogen sulphide by the metal chelates is presented in the equation below:

$$H_2S_{(g)} \leftrightarrow H_2S_{(aq)} \quad \ldots 4.1$$

$$H_2S_{(aq)} + M^{x+}Chelant^{y-} \rightarrow S\downarrow + 2H^+ + 2M^{(x-1)}Chelant^{y-} \quad \ldots 4.2$$

where "x" denotes the charge of the metal cation

"y" denotes the charge of the chelant anion and

"M" represents the metal ion.

This shows that sodium alginate is a viable desulphurizing agent as suggested by Dublein and Steinhauser (2008) and is capable of resulting in sulphide free biogas.

### 4.6 Comparison of the effect of $Na^+$ and $Ca^{2+}$ alginates on biogas volume

Here, the effect of alginates ($Na^+$ and $Ca^{2+}$) of concentration 0.01% wt/v on the biogas volume obtained from fleshing to cow-dung ratio of 1:2 was considered. From data obtained both $Na^+$ and $Ca^{2+}$ alginates gave the same highest volume of 500ml, at the same retention

time of 7 days. Therefore, a cumulative volume was taken to ascertain if there are any differences in total volume of biogas that can be obtained. The cumulative biogas volume from addition of sodium alginate was much higher than that from addition of calcium alginate beads with 2,920ml from addition of sodium alginate and 1,170ml from addition of calcium alginate. This reduction in volume from digesters with calcium alginate beads is due to mass transfer limitations. An increase in the concentration of calcium alginate beads to 0.03% wt/v however increased the biogas volume to 3,975ml. This means higher concentrations will adjust the negative effect caused by surface area available for reaction.

The cumulative volumes were also used to plot empirical equations which reflect experimental data, and $R^2$ values to test if the data are well fitting. This can be seen in Figures 4.4 and 4.5 for sodium and calcium alginates respectively.

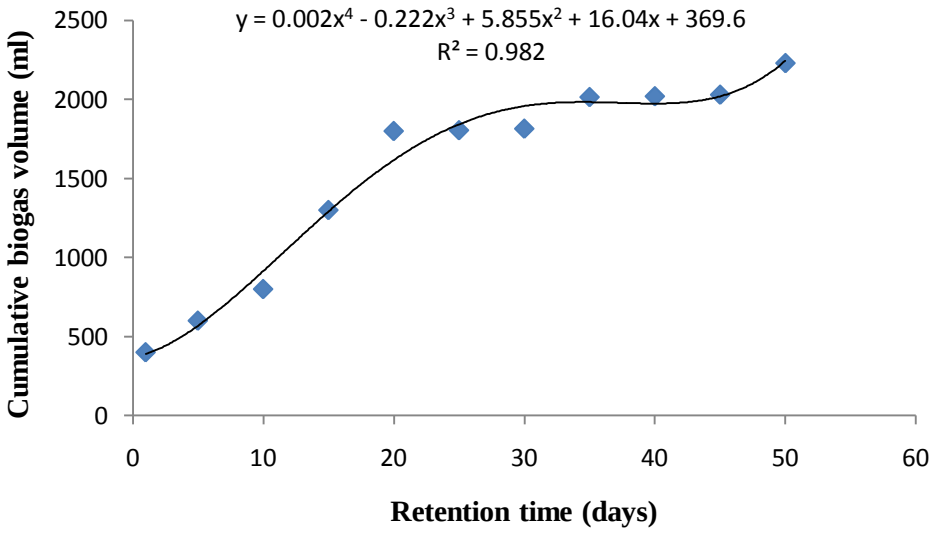


**Figure 4.4: Cumulative Biogas Volume from Co-Substrate with 0.01% wt/v Sodium Alginate**

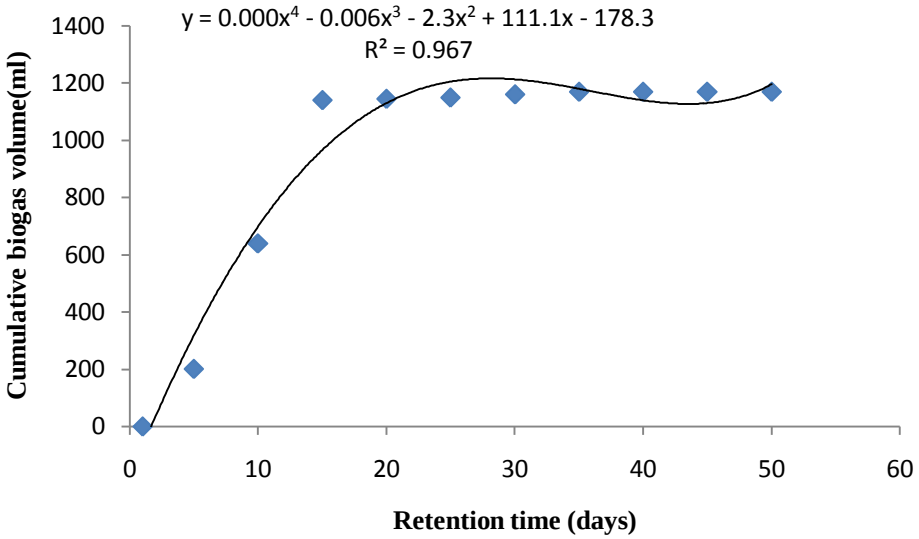


**Figure 4.5: Cumulative Biogas Volume from Co-Substrate with 0.01% wt/v Calcium Alginate**.

From Figures 4.4 and 4.5, $R^2$ values close to 1 show that the data are well fitted. They also give empirical values for the volume of biogas that can be obtained from an experimental set up at any retention time in the digestion cycle.

# CHAPTER FIVE

# CONCLUSION AND RECOMMENDATIONS

This chapter presents a summarized conclusion on this study, recommendations and its contributions to knowledge.

## 5.1 Conclusion

From the work, the following conclusions were made:

i. The soaking liquor obtained from the beam house of Nigerian Institute of Leather and Science Technology, Zaria's tannery, is best suited for use as diluent in the anaerobic digestion. The composition of biogas obtained from using the soaking liquor gives the highest methane and lowest hydrogen sulphide concentrations.

ii. Cow-dung is concluded to be a viable co-substrate in the anaerobic digestion of tannery fleshing. It was able to boost methane concentration about 8 (eight) times. The ratio 1:2 of fleshing to cow-dung can be used to produce biogas of 45% v/v methane concentration.

iii. The use of sodium alginate and calcium alginate beads in the ratio 0.01% wt/v boosted methane production (concentration) above 20% and reduced sulphide concentration reaching 0ppm. Though, addition of sodium alginate gave highest methane concentrations in shorter retention time and higher biogas volume than addition of calcium alginate beads. The calcium alginate beads were generally limited due to mass transfer phenomenon.

## 5.2 Contributions to Knowledge

The following are the contributions this study has provided to knowledge

1. The soaking liquor amongst other beam house liquors is better suited as diluent in the anaerobic co-digestion of tannery fleshing and cow-dung without any form of treatment.
2. Sodium alginate and calcium alginate beads enhanced methane production and inhibited sulphide in biogas produced from the anaerobic co-digestion of tannery fleshing and cow-dung; with sodium alginate performing better.

## 5.3 Recommendations

The following are some recommendations made from this study.

1. Further research should be carried out on the use of sodium alginate in the anaerobic digestion of other substrates, to further ascertain methane boosting and desulphurizing potentials.
2. Equipment for detecting all anaerobic digesting parameters should be readily available and accessible for proper monitoring of the digesting system.
3. A pilot plant that will employ the data obtained from the use of sodium and calcium alginates in the anaerobic co-digestion of tannery fleshing and cow-dung should be designed to fit into tanneries for solid waste treatment.

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# APPENDIX

**APPENDIX A: Results from Digestion with Combined Liquors.**

| Retention time(days) | $CH_4$ concentration (%v/v) | $H_2S$ concentration (ppm) |
|---|---|---|
| 1 | 2.4 | 101 |
| 3 | 2.3 | 86 |
| 5 | 2.5 | 188 |
| 7 | 2.7 | 172 |
| 9 | 2.2 | 197 |
| 11 | 2 | 272 |
| 13 | 2.2 | 314 |
| 15 | 2.3 | 400 |
| 17 | 2.3 | 346 |
| 19 | 4.1 | 601 |
| 21 | 2.1 | 644 |
| 23 | 2.5 | 480 |
| 25 | 2.1 | 547 |
| 27 | 2.2 | 690 |
| 29 | 2.1 | 1408 |
| 31 | 2.5 | 1640 |
| 33 | 2.5 | 1740 |
| 35 | 2.1 | 1287 |
| 37 | 2.1 | 1266 |

**APPENDIX B: Results from using Separate Beam House Liquors as Diluent.**

Table B2: Fleshing and Deliming liquor

| Retention time (days) | $CH_4$ concentration (%v/v) | $H_2S$ concentration (ppm) |
|---|---|---|
| 1 | 1.6 | 352 |
| 3 | 2.3 | 44 |
| 5 | 2.5 | 963 |
| 7 | 8.8 | 553 |
| 9 | 1.2 | 92 |
| 11 | 1.2 | 53 |
| 13 | 1.1 | 261 |
| 15 | 0.5 | 184 |

Table B3: Fleshing and Liming liquor

| Retention time (days) | $CH_4$ concentration (%v/v) | $H_2S$ concentration (ppm) |
|---|---|---|
| 1 | 1 | 18 |
| 3 | 1.1 | 6 |
| 5 | 1 | 17 |
| 7 | 1.2 | 26 |
| 9 | 1 | 25 |
| 11 | 1 | 16 |
| 13 | 0.9 | 136 |
| 15 | 1.2 | 496 |

Table B4: Fleshing and Soaking liquor

| Retention time (days) | $CH_4$ concentration (%v/v) | $H_2S$ concentration (ppm) |
|---|---|---|
| 1 | 9.4 | 36 |
| 3 | 9.8 | 72 |
| 5 | 11.8 | 11 |
| 7 | 3.7 | 12 |
| 9 | 2 | 15 |
| 11 | 2.8 | 14 |
| 13 | 3.5 | 16 |
| 15 | 2.6 | 34 |

## APPENDIX C: METHANE CONCENTRATIONS OF FLESHING TO COW-DUNG RATIOS WITH SODIUM ALGINATE.

| Retention time (days) | 1:0 methane concentration (%v/v) | 1:0.5 methane concentration (%v/v) | 1:1 methane concentration (%v/v) | 1:2 methane concentration (%v/v) |
|---|---|---|---|---|
| 1 | 1.7 | 4.4 | 1.3 | 5.9 |
| 3 | 6.3 | 9.9 | 8.8 | 4.7 |
| 5 | 3.5 | 14.1 | 5.9 | 16.9 |
| 7 | 1.8 | 4.2 | 3.1 | 6.2 |
| 9 | 2.4 | 4.7 | 7.4 | 7.5 |
| 11 | 3.5 | 5.2 | 15.2 | 20.3 |
| 13 | 2.1 | 6.9 | 8.8 | 15.4 |
| 15 | 2.4 | 9.5 | 15.4 | 29.1 |
| 17 | 2.3 | 23.2 | 37.3 | 60.3 |
| 19 | 1.9 | 28.8 | 38.8 | 45.5 |
| 21 | 1.9 | 27.5 | 29.5 | 33.1 |
| 23 | 2 | 32 | 23.9 | 48.5 |
| 25 | 6.2 | 23.8 | 16.7 | 34.4 |
| 27 | 10.7 | 42.5 | 45 | 59.4 |
| 29 | 6 | 16.9 | 30.7 | 43 |
| 31 | 2.9 | 38.9 | 39.2 | 8.4 |
| 33 | 3.3 | 37.9 | 42.4 | 39.6 |
| 35 | 8.3 | 26 | 44.9 | 42.4 |
| 37 | 6 | 25.3 | 42.7 | 46.1 |

## APPENDIX D: METHANE CONCENTRATIONS OF FLESHING TO COW-DUNG RATIOS WITHOUT SODIUM ALGINATE.

| Retention time (days) | 1:0 methane concentration (%v/v) | 1:0.5 methane concentration (%v/v) | 1:1 methane concentration (%v/v) | 1:2 methane concentration (%v/v) |
|---|---|---|---|---|
| 1 | 1.1 | 2.1 | 2.2 | 3.9 |
| 3 | 1.5 | 3.7 | 7.3 | 9.7 |
| 5 | 1 | 2.6 | 7.1 | 15.3 |
| 7 | 1 | 1 | 1.7 | 4.9 |
| 9 | 0.9 | 1.4 | 1.3 | 5.4 |
| 11 | 0.9 | 1.5 | 2.1 | 16.5 |
| 13 | 0.8 | 1.6 | 1.2 | 9.5 |
| 15 | 0.8 | 1.7 | 4.2 | 17.3 |
| 17 | 1.9 | 0.8 | 6.9 | 44.6 |
| 19 | 0.8 | 3.8 | 5 | 23.7 |
| 21 | 1 | 4.4 | 7.3 | 31.8 |
| 23 | 1 | 0.9 | 3.3 | 6 |
| 25 | 1 | 6 | 7.4 | 21.7 |
| 27 | 0.7 | 2.8 | 2.8 | 2.2 |
| 29 | 1 | 0.8 | 3.8 | 5.8 |
| 31 | 0.8 | 5.1 | 4.8 | 1.6 |
| 33 | 0.7 | 3.8 | 6.8 | 1.8 |
| 35 | 0.6 | 3.9 | 4.5 | 1.5 |

## APPENDIX E: HYDROGEN SULPHIDE ($H_2S$) CONCENTRATIONS OF FLESHING TO COW-DUNG RATIOS WITH SODIUM ALGINATE.

| Retention time (days) | 1:0 $H_2S$ concentration (ppm) | 1:0.5 $H_2S$ concentration (ppm) | 1:1 $H_2S$ concentration (ppm) | 1:2 $H_2S$ concentration (ppm) |
|---|---|---|---|---|
| 1 | 6 | 31 | 6 | 21 |
| 3 | 25 | 101 | 107 | 26 |
| 5 | 11 | 30 | 15 | 93 |
| 7 | 0 | 2 | 25 | 22 |
| 9 | 3 | 12 | 69 | 15 |
| 11 | 1 | 1 | 1 | 1 |
| 13 | 0 | 2 | 7 | 6 |
| 15 | 1 | 4 | 5 | 7 |
| 17 | 5 | 6 | 13 | 10 |
| 19 | 4 | 10 | 16 | 13 |
| 21 | 0 | 3 | 0 | 2 |
| 23 | 3 | 5 | 7 | 6 |
| 25 | 4 | 5 | 3 | 4 |
| 27 | 0 | 2 | 0 | 1 |
| 29 | 0 | 0 | 0 | 0 |
| 31 | 4 | 5 | 5 | 4 |
| 33 | 0 | 1 | 0 | 0 |
| 35 | 0 | 0 | 0 | 0 |

## APPENDIX F: HYDROGEN SULPHIDE ($H_2S$) CONCENTRATIONS OF FLESHING TO COW-DUNG RATIOS WITHOUT SODIUM ALGINATE.

| Retention time (days) | 1:0 $H_2S$ concentration (ppm) | 1:0.5 $H_2S$ concentration (ppm) | 1:1 $H_2S$ concentration (ppm) | 1:2 $H_2S$ concentration (ppm) |
|---|---|---|---|---|
| 1 | 9 | 10 | 11 | 20 |
| 3 | 53 | 35 | 23 | 63 |
| 5 | 11 | 25 | 451 | 80 |
| 7 | 2 | 4 | 174 | 19 |
| 9 | 4 | 6 | 8 | 29 |
| 11 | 1 | 12 | 2 | 18 |
| 13 | 6 | 3 | 3 | 5 |
| 15 | 3 | 4 | 2 | 10 |
| 17 | 5 | 7 | 10 | 16 |
| 19 | 4 | 9 | 15 | 15 |
| 21 | 3 | 3 | 5 | 6 |
| 23 | 3 | 7 | 10 | 8 |
| 25 | 6 | 6 | 5 | 3 |
| 27 | 5 | 4 | 3 | 6 |
| 29 | 2 | 3 | 1 | 8 |
| 31 | 1 | 6 | 1 | 10 |
| 33 | 5 | 5 | 3 | 13 |
| 35 | 2 | 4 | 55 | 10 |

**APPENDIX G: METHANE CONCENTRATIONS, HYDROGEN SULPHIDE CONCENTRATIONS AND BIOGAS VOLUME FROM CO-SUBSTRATE BLEND WITH SODIUM ALGINATE OF 0.01%wt/v (X) AND CALCIUM ALGINATE OF 0.01%wt/v (Y).**

| Retention time (days) | X $H_2S$ concentration (ppm) | Y $H_2S$ concentration (ppm) | X methane concentration (%v/v) | Y methane concentration (%v/v) | X biogas volume (ml) | Y biogas volume (ml) |
|---|---|---|---|---|---|---|
| 1 | 102 | 202 | 22 | 11.8 | 400 | 0 |
| 3 | 13 | 32 | 30.8 | 22.9 | 200 | 200 |
| 5 | 547 | 268 | 45.1 | 41.4 | 200 | 440 |
| 7 | 190 | 77 | 46.5 | 43.3 | 500 | 500 |
| 9 | 49 | 52 | 56.9 | 55.7 | 500 | 5 |
| 11 | 45 | 20 | 68.4 | 55.7 | 5 | 5 |
| 13 | 36 | 10 | 61.8 | 55.5 | 10 | 10 |
| 15 | 27 | 12 | 59.9 | 57.1 | 200 | 10 |
| 17 | 25 | 9 | 69.1 | 60.1 | 5 | 0 |
| 19 | 25 | 6 | 70.1 | 59.6 | 10 | 0 |
| 21 | 24 | 4 | 69.2 | 62.3 | 40 | 0 |
| 23 | 23 | 3 | 66.3 | 63.3 | 10 | 0 |
| 25 | 19 | 2 | 67.1 | 63.8 | 40 | 0 |
| 27 | 18 | 2 | 54.3 | 61.5 | 10 | 0 |
| 29 | 14 | 1 | 53.2 | 55.7 | 40 | 0 |
| 31 | 12 | 1 | 42 | 45.6 | 400 | 0 |
| 33 | 8 | 1 | 40.1 | 43.7 | 300 | 0 |
| 35 | 5 | 1 | 38.9 | 42.1 | 50 | 0 |
| 37 | 3 | 3 | 38 | 39.6 | 50 | 0 |
| 39 | 0 | 1 | 23.6 | 33.3 | 0 | 0 |
| 41 | 0 | 0 | 22.1 | 31.9 | 0 | 0 |