



Faculty of Engineering & Technology
Department of Civil and Environmental Engineering

**Evaluation of the Dosage of Nickel and Cobalt Nanoparticles
on Energy Generation and Enhanced Anaerobic Digestion of
Abattoir Effluent**

by

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A Thesis Submitted to the Faculty of Engineering & Technology in Partial Fulfilment of the
Requirements for the Award of the Degree of Master of Engineering in Civil and
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May, 2022

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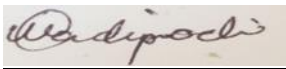
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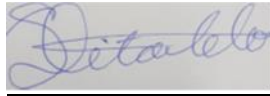
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DEDICATION

This thesis is dedicated to my loving sister Sale ‘Mmabana’ Radipodi. You are more than a sister to me; you are a friend and a blessing I can rely on anytime.

And

A feeling of thankfulness and appreciation is given to

Mom and Dad

‘You can be anything you want; you are only limited by your imagination’

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LIST OF ABBREVIATIONS

AD	Anaerobic Digestion
BOD	Biochemical Oxygen Demand
BOBS	Botswana Bureau of Standards
BMC	Botswana Meat Commission
BMP	Biochemical methane potential
CHP	Combined Heat and Power
CH ₃ COOH	Acetic acid
CH ₃ -S-CoM	Methyl Coenzyme M
Co	Cobalt
COD	Chemical Oxygen Demand
CODH	Carbon Monoxide Dehydrogenase
CO ₂	Carbon dioxide
CH ₄	Methane
C/N	Carbon to Nitrogen ratio
CSTR	Continuous Stirred Tank Reactor
DAF	Dissolved air flotation
DO	Dissolved Oxygen
EDTA	Ethylenediaminetetraacetic acid
F ₄₃₀	Coenzyme F ₄₃₀
FS	Fixed solids
GHG	Greenhouse gases
H ₂	Dihydrogen
H ₂ S	Hydrogen sulphate

HRT	Hydraulic Retention Time
Ni	Nickel
NPs	Nanoparticles
OLR	Organic Loading Rate
OHPA	Obligate hydrogen-producing acetogens
pH	Potential hydrogen
SMA	Specific methanogenic activity
TE	Trace Elements
TEA	Trade Effluent Agreement
TS	Total Solids
UASB	Up-flow Anaerobic Sludge Bed Reactor
VFA	Volatile Fatty Acids
VitB12	Vitamin B12
VS	Volatile Solids
VSS	Volatile Suspended Solids
WUC	Water Utilities Corporation

ABSTRACT

Abattoirs have many activities that produce biodegradable organic waste, which can be utilized in anaerobic digestion as it is energy rich. Anaerobic digestion of abattoir waste (blood, rumen contents, cow dung) has positive environmental effects since it simultaneously achieves waste management, biogas, and fertilizer production. Abattoir effluent has large amounts of organic matter, suspended solids, and it is associated with repugnant odour. It is paramount to find ways of its sustainable management. During anaerobic digestion of abattoir waste, when proteins and lipids are degraded there is formation of potentially inhibitory compounds. These inhibitory compounds can make the process unstable and prone to failure. This research was carried out to investigate the effects of different concentrations of nickel and cobalt nanoparticles on biogas production, as a means to make the process more stable and enhance biogas production. In this study, the effects of individual and combined dosage of nickel and cobalt nanoparticles on AD of abattoir effluent was investigated when operated under mesophilic condition (37°C) in a batch mode.

A concentration of 20 mgL⁻¹ of nickel, cobalt nanoparticles and their combination was observed to be the most effective as compared with 0.5, 2 and 11 mgL⁻¹. Addition of 20 mgL⁻¹ of cobalt, nickel and both nickel and cobalt nanoparticles produced 386, 448 and 438 mL⁻¹ of methane, and 595, 695, and 682 mL⁻¹ of biogas, respectively. The individual dosage of cobalt nanoparticles increased the methane and biogas by 194.65 and 180.65 %, the individual dosage of nickel nanoparticles increased the methane and biogas by 241 and 226.8 %, the digester dosed with both nickel and cobalt nanoparticles increased methane and biogas by 234 and 220 % as compared with the control (131 mL of methane and 213 mL of biogas). Individual dosage of nickel nanoparticles yielded the highest biogas and methane production as compared with individual dosage of cobalt nanoparticles and a combination of both nickel and cobalt nanoparticles. The addition of nanoparticles was observed to increase not only the biogas and methane production but also the degradation rate of volatile solids and the energy potential of the abattoir waste.

Keywords: Abattoir effluent, Anaerobic digestion, Cobalt, Nanoparticles, Nickel

CHAPTER 1. INTRODUCTION

1.1 BACKGROUND INFORMATION

Botswana is known for its export driven cattle industry. Agriculture contributes about 2 % to the country's Gross Domestic Product (GDP) and 80 % of the agricultural GDP is for livestock production mainly cattle (ITA, 2021). The country is a semi desert hence the climate favours rearing of livestock as opposed to growing of crops. Domestic beef market is also growing due to urbanisation, income, and preferences of Botswana (Englen *et al.*, 2013). Botswana has about 1, 400 000 $\pm$ 30 000 cattle per annum (Statistics Botswana, 2019). The growth in the beef industry has led to the development of state-of-the-art infrastructure such as abattoirs some of which are under the BMC (Botswana Meat Commission). There are about 99 abattoirs in Botswana, 12 municipal, 80 rural, and 7 private abattoirs (Engelen *et al.*, 2013). The BMC has three abattoirs with Lobatse abattoir being the largest and slaughters 1, 200 cattle per day (Darkoh and Mbaiwa, 2002). Before, during, and after slaughter inorganic and organic waste are produced. About 35 % of the total weight of the cow is waste (Neboh *et al.*, 2013). Organic waste produced in abattoirs includes blood, cow dung and gut/rumen contents. The cow dung is found in holding pans before the animals are slaughtered. During slaughter, blood is produced as a waste product. During cleaning of rumen and other internal organs undigested feed and semi-digested feed (rumen/gut contents) is collected.

Waste produced from the abattoir pollutes the environment and poses a health hazard to the public (Naegele *et al.*, 2013, Worku *et al.*, 2017). Proper management and treatment of abattoir effluent is required to mitigate the environmental effects they pose. Treatment of abattoir waste for bioenergy production will create new employment opportunities and reduce the health hazard the waste poses to the public. The Botswana Standards number 93 (BOS: 93) regulates the physical, chemical, and microbiological requirements of wastewater effluents of industries before discharge into the sewer line and environment (BOBS, 2018). Industries sign Trade Effluent Agreement (TEA) with Water utilities Corporation (WUC), the agreement allows industries to discharge wastewater that is compliant with BOS: 93 (BOBS, 2018; UNDP, 2021). Industries are encouraged to install onsite treatment strategies to be compliant (UNDP, 2021). Failure to comply industries will face fines and/or not being allowed to discharge into the sewer line.

In Botswana there is very little reusing and recycling of waste for energy production (Charis *et al.*, 2020). The need to develop sustainable energy sources is eminent to reduce dependency on fossil fuels and the threat to climate change. One of the objectives of the Botswana National Energy Policy is to provide a safe, sustainable, and reliable energy supply (Leete *et al.*, 2013). One of the sustainable energy sources is biomethane which can be obtained from anaerobic digestion of abattoir waste. Employing anaerobic digestion as an onsite waste treatment strategy will help reduce energy and waste disposal costs and it will ensure energy independence of the Botswana Beef Industry.

1.2 PROBLEM STATEMENT (MOTIVATION OF STUDY)

There are many microbiological problems involved in the digestion of abattoir effluent. The problems include foaming of digesters, low degradation rates, production of volatile fatty acids (VFAs), ammonia and ammonium nitrogen (Diaz, 2010). The produced ammonia, ammonium nitrate and VFAs may be hazardous to methanogens as they are very sensitive (Chen *et al.*, 2008). These problems make the process unstable and prone to failure therefore new approaches have to be developed, such as catalysation of the process through supplementation of trace metal ions to the reactors. There might be trace elements ions present in the abattoir wastewater, but it is unknown whether micronutrients that are present are in a form the microbes can utilize i.e., bioavailable. Anaerobic treatment of this effluent, therefore, requires nutrient supplementation.

Microorganisms are responsible for the actual decomposition by utilizing the volatile solids as nourishment. For these microorganisms to function well, a variety of macronutrients and micronutrients are required for their metabolic and enzymatic functions (Fermoso, 2009). The processes involved in the production of the waste determine the nutrients present in the waste based on the chemical and biological components of these processes (Diaz, 2010). Essential micronutrients required in the degradation of organic matter may be completely absent in abattoir effluent, hence the need for supplementation. There have been no studies which report the addition of nanoparticles to abattoir waste, also their prospective impact on AD of this high strength organic effluent. Information is limited on the role of metal ions in the conversion of the raw abattoir waste to biogas and on how to improve this conversion.

1.3 RESEARCH AIM

To determine the effects of dosage of Nickel and Cobalt nanoparticles on biogas production potential from a laboratory-scale mesophilic anaerobic abattoir waste digester.

1.3.1 Specific Research Objectives

- i. To find the optimum concentration of the effluent to use.
- ii. To determine the effects of Ni nanoparticles on biogas production from the abattoir waste.
- iii. To determine the effects of Co nanoparticles on biogas production from abattoir waste.
- iv. To determine the effects of combined dosage of Ni and Co nanoparticles on biogas production from the abattoir waste.
- v. To determine the baseline concentration of the Ni and Co in the abattoir effluent.
- vi. To determine the energy potential of abattoir waste digestion and the possibility of the abattoir powering itself through the generated energy from the combustion of biogas in a Combined Heat and Power plant.

1.4 RESEARCH HYPOTHESIS

As observed from the literature review anaerobic digestion the abattoir waste has high content of lipids and proteins. During digestion of the above-mentioned organics anaerobic digestion of abattoir waste is prone to failure. Addition of Ni, Co and Ni & Co nanoparticles is expected to increase the biodegradability of the abattoir effluent, increase biogas production and volatile solids degradation rate, and improve process stability. Due to their (Ni & Co) synergistic effects, their combined dosage is expected to produce more biogas as compared to individual dosage. Individual dosage of Ni will produce more biogas as compared with Co NPs, because Ni is directly involved in the last step of methane production (synthesis of methyl-coenzyme-M to produce methane).

Dosage of the nanoparticles (NPs) is expected to follow the dose response curve depicted in Figure 1.1. The curve is a depiction of the change in the rate of specific methanogenic activity (biological response) as a function of the dosed micronutrients. The biogas production rate can be used as the indicative parameter of this biological reaction. At lower concentrations the NPs are expected to show no observable effect or limiting conditions (1st region). In this first region the specific methanogenic activity triggered by that concentration produces change in biogas production that is not detectable. In the second region as the concentration increases it is expected that it will cause a biological response that will effect a detectable change in the biogas production. As the

concentration exceed the optimum and/or excess concentration (3rd region), it is not expected to immediately translate into a detectable change in biogas production. Toxicity (4th region) depends on the interaction of the microbial cell with the substance/ion. The microbial cell is expected to withstand/tolerate metal ions until the saturation concentration (3rd region). If the concentration surpasses the 3rd region the reactor performance will decrease as the specific methanogenic activity will be reduced due to the toxicity of the nanoparticles.

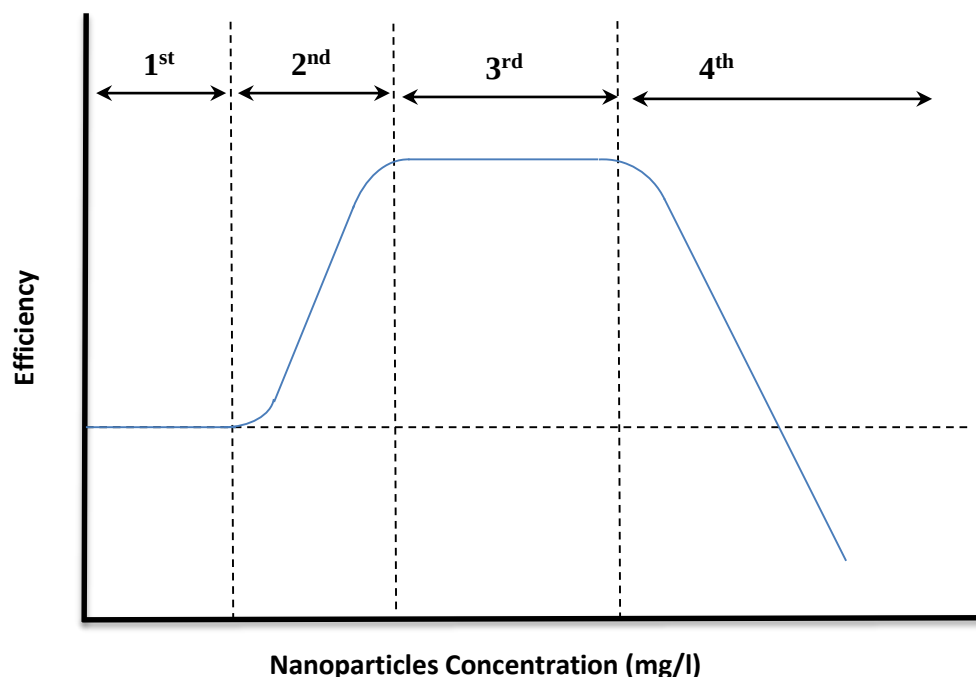


Figure 1.1 Hypothetical dose response curve 1 = no observable effect level, 2 = effective concentration, 3 = saturation/excess concentration, 4 = Toxic concentration.

1.5 THESIS PRESENTATION

This section is a short summary of the major parts of the thesis. Chapter 1 which is the introduction, gives a brief explanation on the motivation of the work (problem statement), the aims, the hypothesis made, the need for this research. Existing literature was reviewed and presented on Chapter two. This Chapter also includes background information on anaerobic digestion, supplementation of Ni and Co to anaerobic reactors. Identified knowledge gaps are also presented in this Chapter. Chapter 3 is the materials and methods that were chosen to carry out this research.

Chapter 4 shows the findings, their interpretation, discussions, and significance. Chapter 5 is the conclusions, as well as the recommendations for further work.

CHAPTER 2. LITERATURE REVIEW

2.1 OVERVIEW OF THE ANAEROBIC DIGESTION PROCESS

This section overviews and discusses the anaerobic digestion process as understood to date. It also elucidates areas in Ad that can be improved to achieve overall performance improvement.

Anaerobic digestion means the degradation of organic materials in an oxygen-free environment. The products of anaerobic digestion can be classified into two categories: biogas and digestate. The biogas produced comprises of methane (CH_4), carbon dioxide (CO_2), water vapour (H_2O) and hydrogen sulphide (H_2S). Methane is the main component of biogas (Laskri *et al.*, 2015). Biogas is a combustible gas which can be used as cooking fuel, natural gas or to produce electricity by combustion. To expand the heating value of biogas and, extend its use, it has to be purified through the removal of carbon dioxide and hydrogen sulphide. There are many methods for purification of biogas some of which are water washing, pressure swing adsorption, polyglycol adsorption, chemical treatment, and chemo-autotrophic purification (Ghorbanian, 2014; SGC, 2012). The digestate can be used for the production of fertilizer for agriculture as it has high nutrient value.

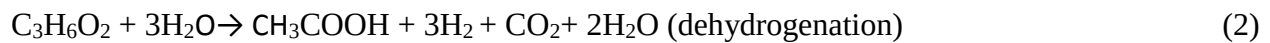
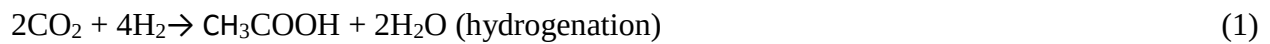
Anaerobic digestion process occurs naturally and can be operated under controlled atmosphere (anaerobic digester). The digester is specially designed and insulated to facilitate the process. The digester systems can either be wet (low-solid) or dry (high-solid) systems (Capareda, 2014; Greene, 2015). Low-solid system consists of solid content below 15% while high-solid has solid content that is above 15% (Ostrem, 2004; Arsova, 2010). Wet digestion ensures better mixing hence improving the degree of digestion as compared with dry digestion which would require additional mixers since the material is dense.

Anaerobic digestion process consists of four stages being hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Gould, 2015). In hydrolysis stage large polymers being proteins, fats and carbohydrates, are broken down into amino acids, fatty acids, and simple sugars (smaller molecules) respectively. This is the reaction with water. Enzymes, acids and bases may be used to speed the reaction (Ersahin *et al.*, 2011).

The products of the first step are then broken down further in the second step (acidogenesis/fermentation). During this process H_2 , CO_2 , H_2S , VFAs, carbonic acids, ammonia, alcohols, and a

small number of other by-products are produced. These are created by the fermentation bacteria which make the environment in the digester acidic (Gould, 2015).

After acidogenesis follows the formation of acetate (acetogenesis), a conjugate base of acetic acid. The products of the fermentation process are catabolized by acetogens into acetate, CO₂, H₂ (Gould 2015). Obligate hydrogen-producing acetogens (OHPA) and homoacetogens are the main types of acetogens involved in this step. The processes of generating acetic acid are hydrogenation and dehydrogenation (Capareda, 2014). The hydrogenation (Wood-Ljungdahl pathway) process is the reaction of carbon dioxide with hydrogen gas to produce acetic acid and water. Hydrogen is the electron source in this reaction. Dehydrogenation involves the reduction of organic acids such as propionic acid to produce acetic acid, hydrogen and carbon dioxide. The dehydrogenation process is done by the hydrogen producing acetogens.



During the dehydrogenation process there is accumulation of hydrogen. The hydrogen pressure can lead to the termination of the activity of acetate forming bacteria (Gerardi, 2003; Capareda, 2014). To reduce the hydrogen pressure the methanogens, use the hydrogen to convert carbon dioxide into methane.

The last stage is the methanogenesis stage (methane production). In this stage, methane and carbon dioxide are produced from the final products of fermentation and also from some of the intermediate products from the first and second steps of the AD process (Ersahin *et al.*, 2011; Laskri *et al.*, 2015). This stage is facilitated by the two main groups of methanogens acetoclastic and hydrogenotrophic. Methanosarcina and Methanosaeta convert acetate to methane. Methanococcus and Methanospirillum convert CO₂ and H₂ to CH₄. The above pathways are the two general ways of producing methane (Laskri *et al.*, 2015). A schematic diagram for describing the interrelationship between these four steps is shown in Figure 2.1.

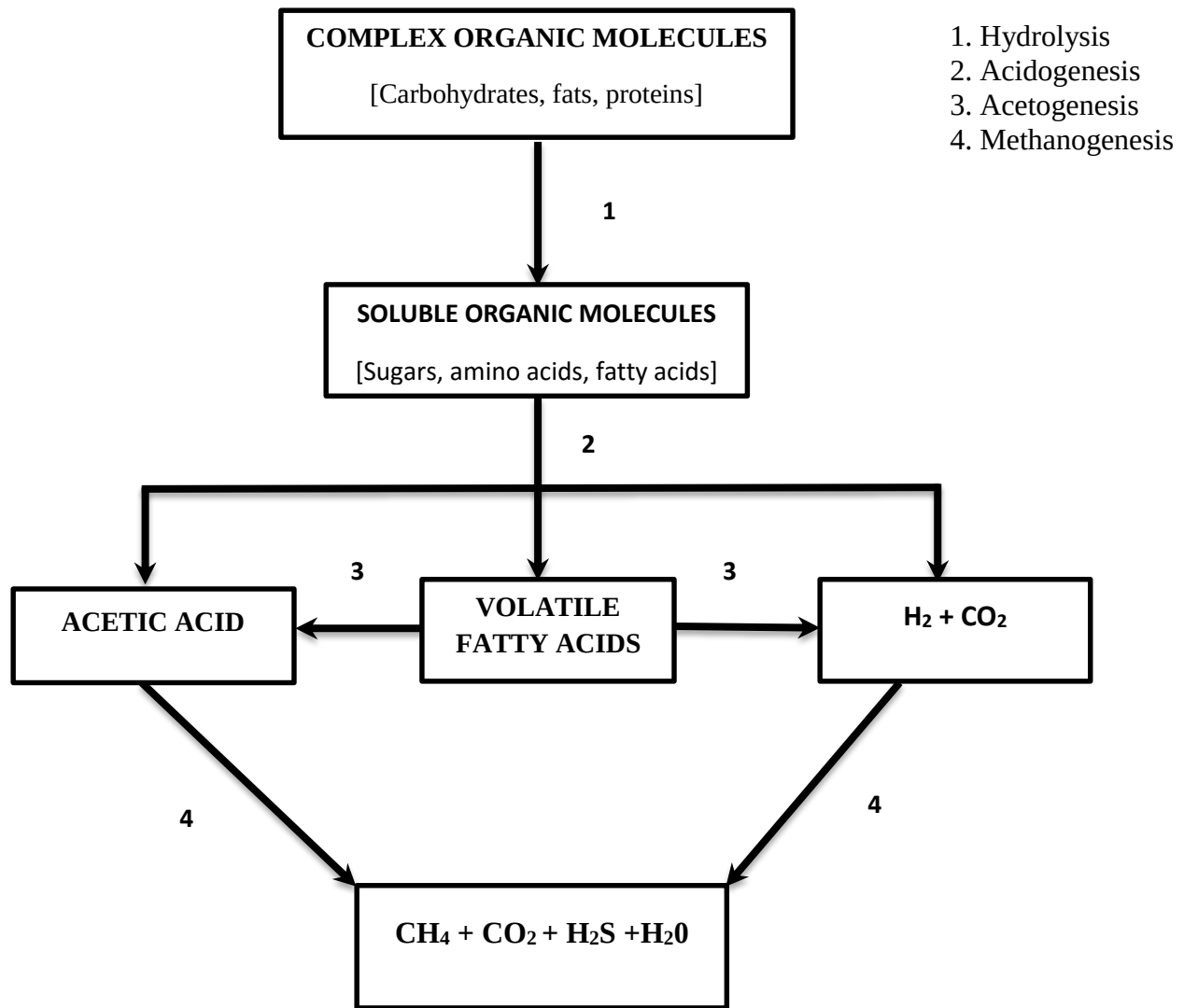


Figure 2.1 Steps that occur during the anaerobic digestion process (EPA, 2015; Greene, 2015)

2.2 PARAMETERS THAT AFFECT ANAEROBIC DIGESTION PROCESS

2.2.1 Biochemical Oxygen Demand

This represents the amount of dissolved oxygen (DO) utilized by microorganisms in the biological oxidation of organic matter measured as a fraction of time (Capareda, 2014; Mitchell *et al.*, 2015). A higher BOD indicates that there is more organic matter in the sample and hence more treatment is required. The standard time for measuring BOD is 5 days (BOD₅) (Delzer *et al.*, 2003; Jouanneau, 2014). The ratio of BOD₅ and chemical oxygen demand shows the amount of organic matter that the microorganisms can decompose.

2.2.2 Chemical Oxygen Demand

This is the quantification of dissolved oxygen required to chemically oxidise organic matter in the effluent and is usually higher than the BOD (Capareda, 2014). If the chemical composition of a compound is known its COD can be calculated. COD values (before and after digestion) shows how efficient the degradation process is. The level of oxidation is affected by the type of substances, pH value, temperature, reaction time and concentration of the oxidising agent. Methane production estimation can be determined from the COD stabilisation (Kumar, 2008).

2.2.3 Total solids (TS), Volatile solids (VS) and fixed solids (FS)

The total solids can be referred to as the entire dry matter of the sample, both inorganic and organic. The inorganic portion is called fixed solids. Volatile solids correspond to the fraction of the total solids that can be digested during anaerobic treatment (Mitchell *et al.*, 2015). The more volatile solids get depleted the more biogas is produced. They are an indication as to whether the treatment process is taking place or not and the rate at which the process is taking place. The TS, VS and FS are needed in anaerobic digestion because they can be used as monitoring tools, and they are required in determining proper loading rates to avoid overfeeding or underfeeding.

2.2.4 Volatile Fatty Acids (VFA)

VFAs play a significant part in anaerobic digestion as they are important during the conversion of organic waste into methane. Microorganisms use them as carbon and energy sources. These microorganisms are responsible for the biological abstraction of nitrogen and phosphorus (nutrients) in wastewater treatment. VFA production rates and VFA utilization rates should be balanced, high VFAs leads to instabilities and toxic conditions in the reactor (Siegert *et al.*, 2005; Mayer *et al.*, 2010; Franke-Whittle *et al.*, 2014). Mostly VFAs are produced during hydrolysis, acidogenesis and acetogenesis in anaerobic digestion, and are used in methanogenesis (Mitchell *et al.*, 2015). The concentrations of VFAs are seen as one of the important variables in the monitoring of anaerobic digestion since they respond to process imbalances, thus showing the ability of the digesters to resist change caused by the disturbances and impending digester failures (Sun *et al.*, 2016).

2.2.5 Macro nutrients

Wastewater treatment processes need micronutrients and macronutrients in correct concentrations for bacterial nourishment, metabolism, activity and growth (Fermoso, Collins, Bartacek *et al.*,

2008a). The required macronutrients are evaluated depending on bacterial composition, growth yield and the composition of the biomass (Vintilou *et al.*, 2012). There are mainly six macronutrients required by biological cells for the synthesis of proteins, lipids, carbohydrates and nucleic acids, being carbon, oxygen, hydrogen, nitrogen, sulphur and phosphorus (Vintilou *et al.*, 2012). Nitrogen and phosphorus are the most important macronutrients that are required during most biological treatment processes (Gerardi, 2003; McCarty, 1964). They are available for methanogens as ammonical-nitrogen (NH_4^+ - N) and orthophosphate-phosphorus (HPO_4^- - P). Methanogens prefers (NH_4^+ - N) as the nitrogen nutrient (Gerardi, 2003).

Methanogens possess several micronutrients needs that are different from those of bacteria present in digesters (Demirel and Scherer, 2008; Zieminski and Frac, 2012). The inclusion of micronutrients in enzyme systems is important in ensuring proper decomposition of the substrate and improving methane production. Proper decomposition of the substrate results in the reduction of volatile fatty acids accumulation hence preventing souring and foaming of digesters, leading to efficient digester operation (Lindorfer *et al.*, 2012; Rousell *et al.*, 2018). Co, iron, Ni, and sulphide are highly required micronutrients (Rousell *et al.*, 2018). Methanogens require the above-mentioned micronutrients during the conversion of acetate to methane. The bacteria produce and excrete an extracellular slime that binds to and transports the required micronutrients into the cell (Gerardi, 2003; Ishaq, 2012; Rousell, 2013). Limitation of these trace elements in anaerobic digestion leads to excessive production of volatile fatty acids such as propionic, acetic acid, butyric and veric acid (Lindorfer *et al.*, 2012; Rousell *et al.*, 2018). The VFAs are the major intermediate product in anaerobic digestion but in high concentrations, they cause inhibition in methanogens metabolism (Sibiya *et al.*, 2015).

The ratio of carbon to nitrogen (C/N ratio) is important to the decomposition of organic material in the anaerobic digestion process (Adekunle, 2015). Every substrate has its optimal ratio. If this C: N ratio is higher than 30 there will be slower multiplication of microorganisms. This is due to the low formation of proteins which results in lower energy and reduced process of acquiring nutrients by microorganisms (Zupancic *et al.*, 2012).

2.2.6 Operation of Anaerobic digestion systems.

2.2.6.1 Organic loading rate

The organic loading rate is the application of soluble and organic matter (volatile solids) into the digester (Babaee *et al.*, 2011). It relies on the nature of waste that is fed to the digester because it influences the level of biochemical activity which occurs inside the digester (Babaee *et al.*, 2011). There's an optimum value for the loading rate and if there are fluctuations in loading rate from the optimum value it will reduce the biogas production. An increase in organic loading rate increases acidogenic bacteria which will create a decrease in pH and rapid production of acids (Ostrem., 2004). The drop in pH will not favour methanogens as they require higher pH (7.8 to 8.2) (Mao *et al.*, 2015), hence leading to low biogas production. The pH drop can kill methanogens leading to system crash. Lower OLR will lead to low biogas production due to low concentration of organic matter.

2.2.6.2 Hydraulic Retention Time (HRT)

This refers to the time that a material is retained in a treatment reactor. HRT used is determined by the biodegradability of the material and conditions in the digester, for example, it reduces when there is the addition of TEs since they will speed up the digestion process hence reducing the time needed to retain the sample (Abdelsalam *et al.*, 2017). It may also depend on the mixing of the sample because a highly biodegradable sample with low solids will have shorter retention times (Mitchell *et al.*, 2015).

2.2.6.2 Temperature, alkalinity & Ph

Anaerobic digestion process generally occurs at the following temp ranges; thermophilic (45°C - 70°C), mesophilic (25°C - 45°C), and psychrophilic (below 25°C) (Mao *et al.*, 2015). Most methane-forming bacteria are mesophiles (Mao *et al.*, 2015). The AD process depends directly on the temperature. There should not be rapid temperature fluctuations during the digestion process, as relevant microorganisms cannot efficiently break down organic materials above or below optimum temperature ranges. Psychrophilic microbes are the less efficient, thermophilic reactors are more efficient than mesophilic reactors. Methanogens are inhibited between 40°C and 50°C (Gerardi 2003). Although thermophilic digesters are effective than mesophilic digesters, they have a lower bacterial diversity due to their high temperature (Mao *et al.*, 2015). Decreased bacterial diversity leads to decreased digester stability. For this research mesophilic temperature (37⁰) was

chosen because of easy maintenance, richness in bacteria and better process stability. For optimal conditions in anaerobic digestion, it's better to use a two phase. Hydrolysis and acidogenesis are optimal at thermophilic temperatures and mesophilic temperature is suitable for methanogenesis (Mao *et al.*, 2015).

Favourable pH range for microbial anaerobic respiration ranges from 6.8 to 7.4 (Mao *et al.*, 2015; Polprasert, 2007), anything lower or higher may inhibit the bacteria. Anaerobes are the ones which depend mostly on the pH. They can be divided into two functional pH groups: acidogens (pH -5.5 to 6.5) and methanogens (pH-7.8 to 8.2) (Mao *et al.*, 2015).

Alkalinity is required for proper pH regulation as it acts as a buffer which impedes rapid changes in pH. The alkalinity is introduced when wastes containing proteins are decomposed, and amino groups ($-NH_2$) and ammonia (NH_3) are produced (Gerardi, 2003). If alkalinity in a digester drops (as it is consumed in the acidogenesis step), VFAs increase and the digester pH drops (Spanjers and Van Lier, 2006). As methanogens convert VFA to CH_4 alkalinity (bicarbonate, carbonate and ammonia) is produced (Spanjers and Van Lier, 2006; Pereira *et al.*, 2013). A drop in pH implies that the alkalinity of a digester is reduced while an increase in pH indicates high alkalinity. A high alkalinity also indicates that the material has the potential to neutralise acids hence reducing pH fluctuations. For high digester stability it is important that alkalinity and production of VFAs are balanced. High alkalinity means digester stability while low concentrations indicate pending failure (Gerardi, 2003).

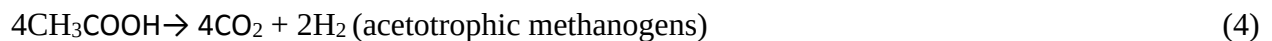
2.2.7 Microorganisms

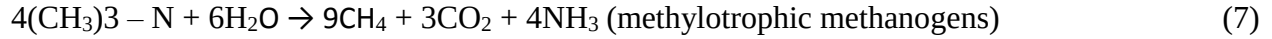
There are acid, acetate, and sulphide forming bacteria present in a reactor, which are favoured by different temperatures in wider pH ranges (Demirel and Scherer, 2008). Since the process of AD is reliant mainly on proper functioning of microorganisms, other operational parameters that affect AD such as pH, temperature and OLR are chosen based on the requirements of microorganisms. Temperature and pH in the digester directly affect the speed of metabolism and growth of microorganisms (Westerholm and Schurer, 2019; De Vrieze and Verstraete, 2016). Changes in temperature and pH will denature microorganisms, their growth will cease, and speed of metabolism will be reduced (Westerholm and Schurer, 2019). Acetogens have a mutually beneficial relationship with methanogens (Demirel and Scherer, 2008; Zieminski and Frac, 2012). During the production of acetate hydrogen is also produced, when this hydrogen accumulates and

a hydrogen pressure occurs, the activity of acetate-forming bacteria is then terminated. This results in less acetate being produced. The hydrogenotrophic methanogens then produce methane using hydrogen and CO₂ and the large hydrogen pressure stops (Gerardi, 2003; Demirel and Scherer, 2008). They are needed for a stable and efficient AD process. The anaerobic digestion process relies on microorganisms, microorganisms need micronutrients for nourishment, biological growth, and optimal functioning. Therefore, the AD process requires the presence of micronutrients for optimal digester performance.

Methanogens are obligate anaerobes, with complicated nutritional requirements and are free-living terrestrial, aquatic organisms and with remarkably high sulphur content (Demirel and Scherer, 2008; Zieminski and Frac, 2012). Its other features include degrading of substrates which results in methane production as specialized coenzymes (Zandvoort, 2005; Zieminski and Frac, 2012). These coenzymes include coenzymes M, F₄₂₀ and F₄₃₀. Coenzyme F₄₃₀ contains nickel in the centre of its structure. The ability of coenzyme F₄₃₀ to convert acetate to methane depends on the bioavailability of nickel (Fermoso, 2009; Ishaq, 2012). Coenzyme F₄₂₀ is involved in the conversion of hydrogen and CO₂ to methane (Demirel and Scherer, 2008). Coenzyme M is involved in the reduction of methyl coenzyme M to methane (Fermoso, 2009).

Methanogen's cell walls are not protected; therefore, they are sensible to the toxicity of various fatty acids (Zieminski and Frac, 2012; De Vrieze and Verstraete, 2016). Substrates must be retained for longer times in AD digesters, to promote the growth of a larger population of the methanogens for the degradation of organic compounds. They have slow growth rate (De Vrieze *et al.*, 2012). For them to survive the temperature has to be held comparatively constant in a small range of pH conditions (Gerardi, 2003). They are mainly responsible for methane production. Reactions involved are shown below together with their respective methanogenic groups;





Sulphate-reducing bacteria use the same substrates utilised by methanogens for reproduction i.e., hydrogen and acetate. They use hydrogen to reduce sulphate into hydrogen sulphide, hence they compete for hydrogen with methane forming bacteria (Gerardi, 2003; Zandvoort, 2005). Figure 2.2 shows this competition for the same electron acceptor substrate by the different bacteria groups;

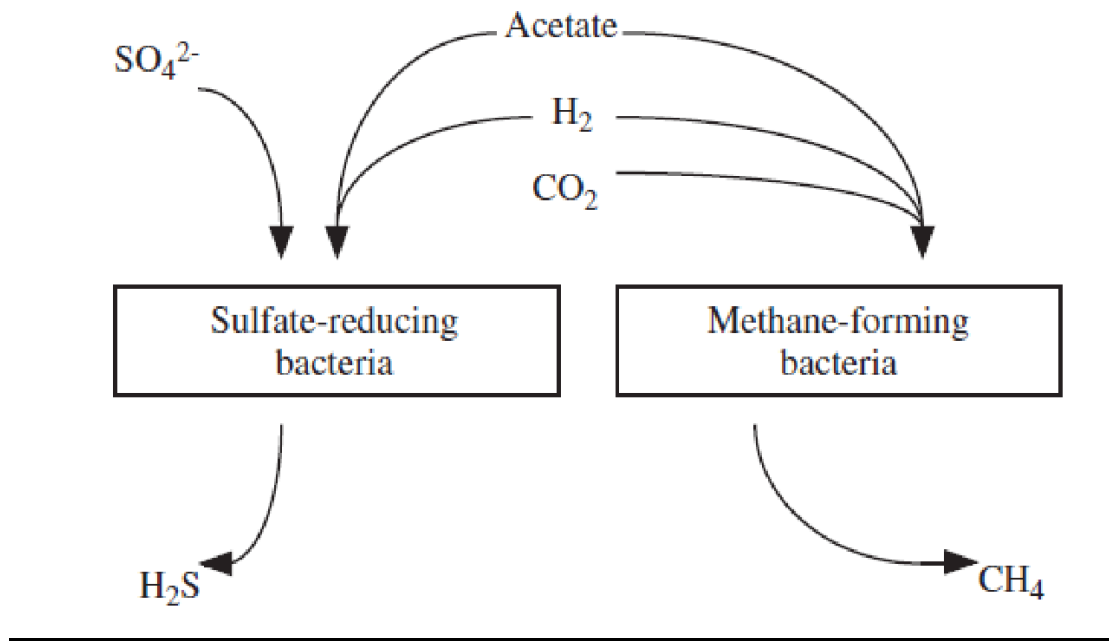


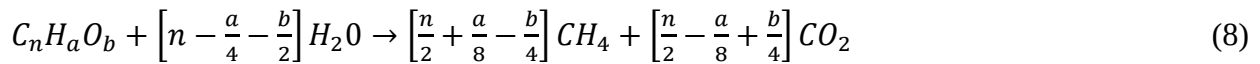
Figure 2.2 Bacterial reactions in a reactor (Adapted from Gerardi, 2003)

The conclusion that can be drawn from this Chapter is that all the parameters that affect anaerobic digestion are interlinked. Microorganisms require very specific temperature and pH ranges; they are also sensitive to high VFA concentration. The relationship between the alkalinity and VFAs determines the pH of the system. Microorganisms also require micro and macro nutrients for metabolism and growth. For efficient digester operation a conducive environment for optimal microorganism operation must be provided. These parameters can be used as a monitoring tool for the efficiency of the anaerobic digestion process. In this research the degradation of volatile solids and concentration of micronutrients (nickel and cobalt) were monitored to determine the efficiency of the process.

2.3 BIOCHEMICAL METHANE POTENTIAL

This is the maximum CH₄ production capacity of a substrate during anaerobic decomposition (Raposo *et al.*, 2018). There are two ways of determining the methane potential being the theoretical methane potential and through experiments. The methane potential through experiments is determined by measuring the methane volume produced during substrate digestion (Da Silva *et al.*, 2017; Raposo *et al.*, 2018; Holliger *et al.*, 2016). The BMP tests are dependent on factors such as the inoculum, substrate and experiment conditions.

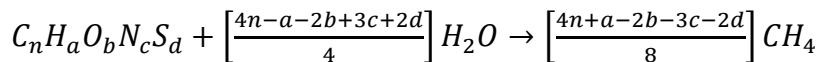
The theoretical BMP is used to predict the methane production of a substrate and is expressed as mL CH₄ at STP per gram of organic material added or removed (VS or COD basis). The chemical components of the substrate have to be known when using this model. The model considers only carbon, hydrogen, oxygen (C_nH_aO_b) as input elements (Raposo *et al.*, 2011). The formula was developed by Buswell and Mueller, (1952). The formula assumes that all of the organic matter in the sample is converted into carbon dioxide and methane, and this leads to an overestimation of gas yields. The formula can be expressed as follows;



The methane yield can then be calculated as:

$$\frac{\left[\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right] * 22400}{12n + a + 16b} \quad (9)$$

Boyle modified the chemical reaction and included sulphur and nitrogen (C_nH_aO_bN_cS_d) to find the fraction of NH₃ and H₂S in the produced biogas (Nielfa *et al.*, 2014; Achinas *et al.*, 2016). This formula does not distinguish between biodegradable and non-biodegradable matter. This formula neglects the presence of other trace elements in the waste and does not consider the temperature and bacterial conditions in the digester. The formula can be expressed as follows;



$$+ \left[\frac{4n-a+2b+3c+2d}{8} \right] CO_2 + cNH_3 + dH_2S \quad (10)$$

The methane yield can then be calculated as:

$$\frac{\left[\frac{n}{2} + \frac{a}{8} + \frac{b}{4} + \frac{3c}{8} + \frac{d}{4} \right] * 22400}{[12n+a+16b+14c+32d]} \quad (11)$$

With the organic fraction of the substrate the methane yield can be estimated as follows (Raposo *et al.*, 2018; Nielfa *et al.*, 2014; Achinas *et al.*, 2016);

$$CH_4 \text{ ml/g of VS} = (415 \cdot \% \text{ Carbohydrates} + 496 \cdot \% \text{ Proteins} + 1014 \cdot \% \text{ Lipid}) / 100 \quad (12)$$

With the experimental and theoretical values of methane the biodegradability ($BD_{CH_4}(\%)$) of the substrate can be calculated as follows;

$$BD_{CH_4}(\%) = (Bo - Exp / Bo - Th) \cdot 100 \quad (13)$$

The above equation assumes that a large amount of organic matter decomposed is converted into methane, and the remaining amount of the organic matter is used for nourishment of the microorganisms and maintenance of cellular metabolism (Raposo *et al.*, 2011).

2.3.1 Specific Methanogenic Activity

Specific methanogenic activity (SMA) tests are often used in determining the methane-producing abilities of a substrate (Hussain *et al.*, 2014). This test can be used to assess the performance of the substrate in the presence of inhibiting compounds (Harada *et al.*, 1994; McHugh *et al.*, 2004; Hussain *et al.*, 2015), to monitor the changes in sludge activities, to designate the reduction rate of various components. Due to the importance and sensitivity of the methanogenic step the SMA test has been found to be very effective as a digester monitoring tool (Ishaq, 2012).

SMA makes it possible to establish the potential loading capacity of the anaerobic treatment systems, hence allowing use of suitable organic loading rates (Jawed *et al.*, 1999; Hussain *et al.*, 2015). Anaerobic biodegradability is an important parameter for determining the SMA. When the

substrate biodegradability increases, it results in the bio-methanation rate also increasing (Hussain *et al.*, 2015). Higher SMA shows that the system has a high conversion rate of active biomass to methane. The simplicity of the experimental setup of the SMA serum bottle test has very few gas leaks and hence the relatively low variation between replicate serum bottles (Ishaq, 2012).

2.4 ANAEROBIC DIGESTION AND ABATTOIR EFFLUENTS

The wastewater generated from the various abattoir/Slaughterhouse activities is a very energy-rich stream (Ek *et al.*, 2011). It comprises of water produced from cleaning, dissolved solids, blood, oil, gut contents, and urine i.e., it is characterised with a high organic level (Budiyono *et al.*, 2011; Kwadzah *et al.*, 2015; Kosamu *et al.*, 2011). Other major characteristics such as abundant organic biological nutrients, adequate alkalinity, oxygen-consuming wastes, relatively higher temperatures and high biodegradability make the waste well suited to anaerobic treatment (Worku *et al.*, 2017). The waste produced contains a high methane potential when treated anaerobically which ranges between 120-160 m³ biogas per tonne of waste (Kunal *et al.*, 2017).

The variation of the strength and characteristics of waste is determined by several features such as the slaughterhouse's production capacity, animal weight and type, transportation methods used, animal receiving and holding, technologies used during processing, carcasses size, washing temperature and cleaning procedures (Budiyono *et al.*, 2011). The wastewater has high values of BOD ranging from 150 to 8500 mg/L, with COD ranging between 500 and 16 000 (Bustillo-Lecompte *et al.*, 2015; Bustillo-Lecompte *et al.*, 2017; Kwadzah *et al.*, 2015).

There are several possible microbiological problems linked with anaerobic digestion of abattoir effluents including protein content, high accumulation rates of volatile fatty acid and high decomposition. During anaerobic degradation, the high protein content can lead to sulfides formation in the reactor. The sulphides will lead to high production of H₂S in the biogas and inhibition of methanogens (Ek *et al.*, 2011, Chen *et al.*, 2008). These problems arising from sulphides can be reduced by precipitation of sulphides with trace metals. Also, during anaerobic degradation ammonia is formed which in turn leads to high pH in the reactor. The pH can be above 8 which can limit VFA consuming methanogens (Ek *et al.*, 2011). High fermentation rate of proteins, fats and high pH (above optimal) can increase the accumulation of volatile fatty acids. The organic load has to be decreased because if not, the overload will occur leading to pH drop, inhibition of methanogenesis and lastly process collapse (Ek *et al.*, 2011).

Supplementation of trace metals (Fe, Ni, Co, Mn, and Mo) during anaerobic digestion of abattoir wastewater ensured an increased degradation efficiency and biogas production, a stable process, high OLR, reduced hydraulic retention times. VFA accumulation at lower organic loading rates and low daily biogas produced (84% less) in the control as compared to the reactors supplemented with trace elements, which shows process stability in the latter (Schmidt *et al.*, 2018). Lower concentrations of H₂S in digesters with TE were observed, because of metal sulphides formation from the supplemented trace metals. The formation of metal sulphides reduces the available sulphur that is used for the formation of H₂S (Nagele *et al.*, 2017; Schmidt *et al.*, 2018).

2.5 TRACE ELEMENT SUPPLEMENTATION TO ANAEROBIC REACTORS

When metals are fed into anaerobic reactors they interact with the liquid phase, solid phase and the microorganisms present in the reactors (Fermoso, Bartacek, Jansen *et al.*, 2008), at first, they have to be internalised by microorganisms. Factors that influence metal uptake are the speciation of the metal and reactor pH (Ishaq, 2012). They have to be available as free forms or bound with biological ligands to be accessible to microorganisms (Rousell, 2013), to obviate any precipitation which may occur or complexation before any uptake. Microorganisms use two forms of metal uptake mechanisms. The first one is unspecific, very quick, constitutively expressed and controlled by the chemiosmotic gradient across the cell membrane, while the latter is energy-dependent, slow and substrate-specific (Nies, 1999 cited by Ishaq, 2012).

Lindorfer *et al.* (2012) showed that dosage of trace elements resulted in increased microbial biomass, low VFA accumulation, and improved performance in digesters. Improved performance in digesters is witnessed during the first few days after supplementing trace elements, and the process maintains stability for at least three months (Rousell *et al.*, 2018).

The major issue in considering trace elements supplementation in anaerobic digesters is that the saturation concentration lies between deficiency and toxicity concentrations; hence it is not easy to determine. Supplementation of high doses TEs may lead to toxic inhibition of microbiological activities, and to avoid this suitable dosing strategy have to be developed (Rousell *et al.*, 2008). They are usually dosed to anaerobic reactors as salts. However, the rate at which they dissolve is still a challenge and consequently, limited bioavailability of metal ions can lead to reduced enzyme activity (Tsapekos, 2018).

2.5.1 Cobalt

Methanogens require Co for activation of enzyme systems as it is a good methyl transfer catalyst. Dosing of Co into enzyme systems increases efficiency in converting acetate to methane. Role of cobalt in multiple enzymes involved in anaerobic reactions include; B12-enzymes, CO-dehydrogenase, methyltransferase (Fermoso *et al.*, 2008). Cobalt is also required as a nutrient for microbes, activity of carboxypeptidase formation of methylcobalamin, synthesis of corrinoid protein compounds (Bhattacharya *et al.*, 1995; Florencio *et al.*, 1995). Methanogens need a high amount of Co during methylotrophic growth, because of the involvement of corrinoids in the methylotrophic pathway. The corrinoid compound vitamin B12 has three components: a central ring, an adenosyl moiety and a nucleotide loop and Co as the central atom (Fermoso, 2009). Cobalamin catalyses the transfer of methyl-groups (Aresta *et al.*, 2003). Blaylock and Stadtman, (1963) discovered that methane can be produced from the methyl moiety of methyl-cobalamine.

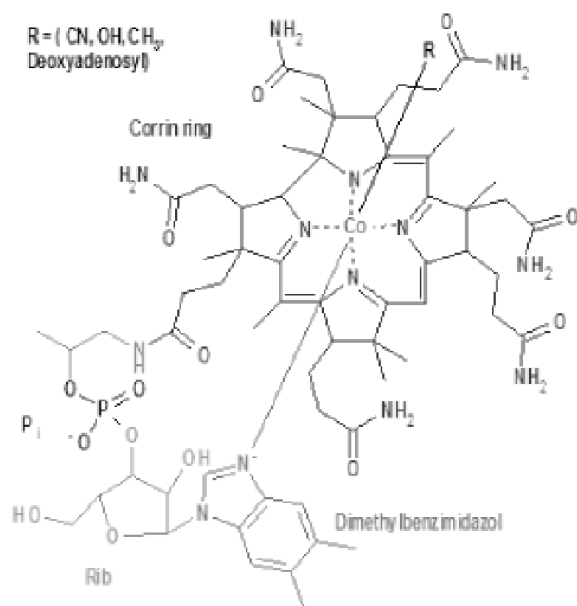


Figure 2.3 Coenzyme B12 structure adapted from Fermoso, (2009)

To maintain a high SMA and prevent reactor acidification, supplementation of Co is needed (Zandvoort *et al.*, 2004). It is needed at lower concentration for optimal acetate utilisation. Also, that cobalt has no effect on generation time since addition or omission of cobalt to acetate digesters showed no effect on the time it took to reach peak biogas production (Ditalelo *et al.*, 2013).

Supplementation of cobalt does not immediately reduce the acetate (Fermoso, Bartacek, Chung *et al.*, 2008).

Florencio *et al.*, 1995 showed Co accelerated both acetogenesis and methanogenesis significantly on the anaerobic degradation of methanol. The reactors with adequate Co produced almost triple the methane as compared to the cobalt deprived reactors. The methanogenic activity was poor in cobalt deprived reactors. Gustavsson *et al.* (2013) found that acetate utilizing methanosarcinales were dominant with Cobalt and Nickel addition. A significant increase in hydrogenotrophic methanomicrobiales, as well as volatile fatty acids concentrations under Cobalt and Nickel deficiencies. Cobalt dosing during methanol conversion in thermophilic reactors helps achieve higher performance and a reduced VFA accumulation (Paulo *et al.*, 2003). Under Cobalt limiting conditions, the methanogenic activity and Methanosarcina population decreases significantly (Fermoso *et al.*, 2010).

2.5.2 Nickel

Nickel is a distinctive micro-nutrient required by methane forming bacterium and a good methyl reducing catalyst. Role of nickel in multiple enzymes systems in anaerobic reactions and transformations include CO-dehydrogenase, acetyl-CoA synthase, methyl-CoM reductase (F₄₃₀), urease, stabilization of DNA, RNA, hydrogenase (Fermoso, Collins, Bartacek *et al.*, 2008b; Rousell, 2013). One mol of methyl-S-CoM reductase contains 2 mol of tightly bound coenzyme F₄₃₀ (Fermoso, 2009). F₄₃₀ is a nickel porphinoid with low molecular mass and detection at its absorption maximum of 430nm (Ishaq, 2012). As shown in Figure 2.4 nickel is in the centre. The supplementation of Ni can enhance acetate digestion rate of methane forming bacteria. Deficiencies of sufficient bioavailable Ni in anaerobic digesters consequently leads to a notable decrease in the rate in which methane is produced, i.e., enzyme's ability to convert acetate to methane is minimized.

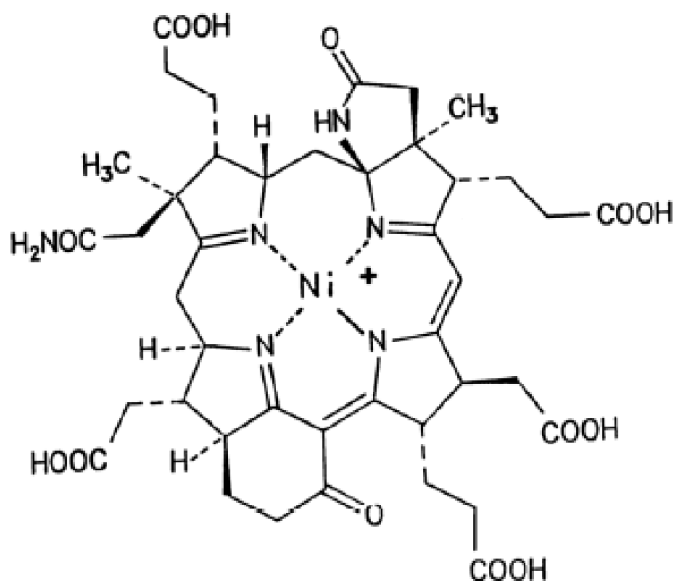


Figure 2.4 Coenzyme F₄₃₀ structure in the oxidation state of Ni²⁺ (Fermoso, 2009)

Nickel is also an essential component of carbon monoxide dehydrogenase (CODH). In methanogenesis from acetate by acetogens (H₂/CO₂ and methanol) CODH is a key enzyme (Ishaq, 2012). Dehydrogenase (CODH) has two nickel-containing metallocentres, available in both acetoclastic methanogens and acetogenic microorganisms (Fermoso, 2009). It uses both Ni and Fe, as it is made up of two enzyme components: a Ni/Fe-sulphur component and a corrinoid/Fe-sulphur component (Ishaq, 2012).

Studies have been performed which investigate the addition of organic agents (ethylenediaminetetraacetic (EDTA), citric and nitrilotriacetic acid) as Ni chelators in the anaerobic digestion process. It was observed that they increase the production of methane from 10 to 49%. Babich and Stotzky, (1983) demonstrated reduced toxicity to microorganisms from high nickel concentrations with the addition of EDTA as cited by Ishaq, (2012).

Jansen *et al.* (2007) showed total cobalt and nickel concentrations increases methanogenic activity and decreases when sulphides concentrations increase in a methanosarcina species enriched culture. The absence of Ni leads to the reduction /limitation of the methanogenic activity (Fermoso, Collins, Bartacerk *et al.*, 2008a). The limitation in the methanogenic activity led to the reduced removal rate of methanol. Addition of 0.5 µM Ni as NiCl₂ increased the removal rate of methanol to 99 % and reduced the accumulation of VFAs. A 99% methanol removal efficiency minimised

accumulation of VFAs and an increase in size of the *Methanosarcina* population was observed from the SMA of the sludge with complete TE solution. From these studies it shows that supplementation of micronutrients is important as it influences the specific methanogenic activity.

Ditalelo, (2017) established that nickel and cobalt were co-required for anaerobic digestion of acetate under mesophilic conditions. The dosage of Ni and Co (combination) produced more biogas than their individual dosage. This supports the concept of them having additive/synergistic effects. The percentage of methane in the biogas was constant at 64% regardless of the dosage. A combined concentration of 31.50-504.00 μM in all CSTR digesters led to inhibition of biogas production.

2.5.3 Application of Nanoparticles in AD

In the batch AD of fresh raw manure (urine and faeces), the most effectual concentrations of NPs were observed as follows 1 mg/L Co NPs, 2 mg/L Ni NPs, 20 mg/L Fe NPs and 20 mg/L Fe_3O_4 NPs. 2 mg/L Ni NPs produced 658 ml biogas which was recorded as the highest production as compared with the other NPs. 1 mg/L Co NPs, 20 mg/L Fe NPs and 20 mg/L Fe_3O_4 NPs yielded 596, 580 and 633.3 ml of biogas respectively. On average the control produced 159.3 ml of biogas. This shows a significant increase in the biogas volume by 1.7(Co), 1.8(Ni), 1.5(Fe) and 1.7(Fe_3O_4) times the biogas production of the control (Abdelsalam *et al.*, 2017).

Abdelsalam *et al.* (2016) observed that addition of 0.5 and 1 mg/L Co NPs showed a notable enhancement on biogas production start-up, with the latter giving the highest value. The supplementation of 1 mg/l of Co NPs increased the biogas 1.64 and methane by 1.86 as compared to the control. It helped achieve a long-term stable production of biogas, which helped reduce the lag-phase and the time to reach the peak of biogas production. The supplementation of 2 mg/L Cobalt NPs affects both the methane and biogas production negatively. It was observed that it increased biogas and methane by 0.95 and 0.87 in the order given. It decreased both the biogas and methane production by 0.69 and 0.99 as compared to dosing of 1 mg/l. This shows that as you move from 1mg/l it's the toxic concentration phase hence 1 mg/l being the saturation concentration. The saturation concentration provided the highest degradation of TS and VS corresponding to 5.38% and 4.09%, respectively.

Supplementation of 0.5, 1 and 2 mg/L of Ni NPs, showed stimulation in biogas volume by 1.46, 1.72, and 1.74 times the control production, respectively. Methane production increased by 1.74, 1.96, and 2.01 times the volume of the control, respectively. Values obtained for 1 and 2 mg/l were the highest with a small difference between them for both methane and biogas. The highest decomposition of the organic matter i.e., TS and VS were observed during treatment with 2 mg/L Ni NPs and were 4.58% and 3.94%, respectively (Abdelsalam *et al.*, 2016). Faisal *et al.* (2019) reported that addition of NPs to anaerobic digestion can stimulate biogas and methane production by up to 200 %. Ni NPs dosed digesters produced more methane as compared with Co NPs, it was found that Ni stimulate key enzymes activities involved in hydrolysis, acidification and methanogenesis (Abdelsalam *et al.*, 2017; Hassanein *et al.*, 2019) From previous research different percentages increases due to addition of NPs were observed. Micro *et al.* (2018) and Liu *et al.* (2021) indicated that the differences in the influence of NPs can depend on several factors such as digester type, inoculum and substrate origin, operating parameters, and bioavailability of the TEs. Different feedstocks require different concentrations for improved methane and biogas production. A summary of the effects of NPs on different feedstocks is summarized on the Table 2.2.

Table 2.2 Effects of NPs on different feedstocks (adapted from Ajay *et al.* 2020 and edited)

NPs	Size (nm)	Temp. °C	Feedstock	Conc.	Effect	Reference
Co	30.0-80.9	35	Poultry litter	5.4 mg/l	Increased CH ₄ production by 29.7 %	Hassanein <i>et al.</i> 2019
				2.7 mg/l	Increased CH ₄ production by 26 %	
				1.4 mg/l	Increased CH ₄ production by 29 %	
	< 100	37	Green micro-algae	1 mg/l	Increased CH ₄ production by 9.6 %	Zaidi <i>et al.</i> 2018
				1.34 mg/g o VS	Increased CH ₄ production by 31.73%	
	28	37	Fresh raw manure	2 mg/l	Reduction in CH ₄ production by 4.9 %	Abdelsalam <i>et al.</i> 2017
					Reduction in CH ₄ production by 12.7 %	
				1 mg/l	Increased biogas production by 64.12 %	
				0.5 mg/l	Increased CH ₄ production by 85.6 % Increased biogas production by 36.5 %	
Ni	17	37	Fresh raw manure	2 mg/l	Increased biogas production by 78.53 %	Abdelsalam <i>et al.</i> 2017
					Increased CH ₄ production by 116.76 %	
				1 mg/l	Increased biogas production by 72.35 %	
	30.0-80.9	35	Poultry litter	0.5 mg/l	Increased biogas production by 46.47 %	Hassanein <i>et al.</i> 2019
				12 mg/l	Increased CH ₄ production by 38.87 %	
					Increased CH ₄ production by 95.1% (first 29 days)	
				6 mg/l	Increased CH ₄ production by 26.8 %	
	>20 nm	37°C	Dairy manure	3 mg/l	Increased CH ₄ production by 27.5 %	Hassanein <i>et al.</i> 2019
				0.5-2 mg/l	Increased CH ₄ production by 55-101 %	
	20 nm	37°C	Industrial wastewater	60 mg/l	Increased biogas production by 23 %	Elreedy <i>et al.</i> 2017

NPs	Size (nm)	Temp. °C	Feedstock	Conc.	Effect	Reference
	<100 nm	37°C	Green micro algae	1 mg/l	Increased CH ₄ production by 26.6 %	Zaidi <i>et al.</i> 2018
	-	40°C	Activated sludge and wastewater	1.25 g/l	Increased biogas production by 133.33 %	Amo-Duodo <i>et al.</i> 2021
				2.5 g/l	Increased biogas production by 100 %	
	<100 nm	37°C	Waste activated sludge	0.16mg/g of TS	Increased biogas production by 100 %	Zaidi <i>et al.</i> 2018

2.6 ENERGY AND COMBINED HEAT AND POWER (CHP)

The energy capacity of the gas relies mainly on its methane percentage; hence high methane content is required. The calorific (average) value of biogas is around 21-23.5 MJ/m³. One cubic metre (m³) of biogas corresponds to around 6 kWh and 1 m³ of methane has an energy content of about 9.97kWh (FNR, 2009; DIMPL, 2010).

Combined Heat and Power (CHP) can be explained as generating two different types of useful energy by utilising a single primary energy source such as natural gas, biomass, and biogas (Turner, 2001). The systems generate mechanical energy by burning fuel which is used to drive an alternator/generator to produce electricity. During the combustion of the fuel, there are hot exhaust gases. A heat recovery unit then captures heat from the combustion system's exhaust stream. This heat is then turned into useful thermal energy, generally in the form of hot water or steam. CHP is one of the economical and environmentally friendly alternatives available, for accomplishing high efficiency by depleting one fuel to generate two forms of energy as compared to fossil fuel power plants (Deublein *et al.*, 2008; U.S. Environmental Protection Agency, 2007).

The two main kinds of CHP cycles are a bottoming cycle and a topping cycle (Hite, 2002). In a bottoming cycle, fuel is first used to produce heat, the system then recovers the portion of the rejected heat and uses it for power production. The primary product is thermal energy. The topping cycle uses a prime mover (turbine/engine) that generates electrical or mechanical power. The hot exhaust gases are then captured and used to supply process heat and/or hot water for the site as shown in Figure 2.5. Here electricity is the primary product, hence the topping cycle is more

relevant in this research as it focuses on the ability of the abattoir powering itself. Topping cycle approaches produce far more energy (Turner, 2001).

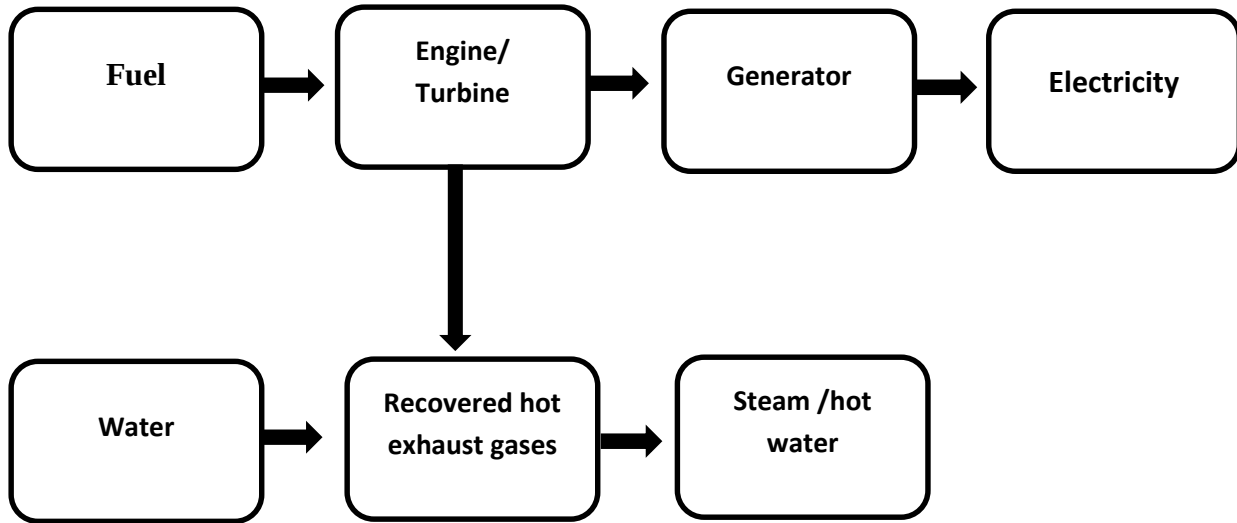


Figure 2.5 Gas turbine/engine CHP system.

CHP is more effective than the production of thermal and electrical energy separately because it utilises less fuel to produce a given energy output, minimises losses (distribution and transmission) and decreases the impact of outages. It also lessens air pollution and greenhouse gas emissions since a small amount of fuel is burnt to produce energy. The high efficiency translates to low operation costs and minimised emissions of pollutants (U.S. Environmental Protection Agency, 2014). Biogas produced from AD is not pure it contains droplets, dust, mud, or trace gases such as H_2S . Hydrogen sulphide in biogas decreases the lifetime of pipework and all installations because it is toxic and highly corrosive to different types of steel. During combustion of the biogas, hydrogen sulphur is turned into sulphur oxides, which corrode metallic components. All the above contaminants must be removed for further utilisation of the biogas in a CHP plant (Deublein *et al.*, 2008).

2.7 KNOWLEDGE GAPS IDENTIFIED

There are various investigations of anaerobic digestion of abattoir effluents (Diaz, 2010; Ek *et al.*, 2011; Worku *et al.*, 2017). These indicated that there are many challenges encountered during anaerobic digestion of abattoir effluent which make the process prone to failure (Diaz, 2010; Ek *et al.*, 2011). Therefore, an opportunity to optimise anaerobic digestion of abattoir effluent as a means of solving the problems exists.

Individual effects of nickel and cobalt nanoparticles on many different feedstocks have been investigated (Abdelsalam *et al.*, 2017; Zaidi *et al.*, 2018; Hassanein *et al.*, 2019; Elreedy *et al.*, 2019), but no research has been done on anaerobic digestion of abattoir effluent. In addition, suitable ranges for dosage or concentrations of individual nickel and cobalt nanoparticles to anaerobic digesters of raw abattoir effluent has not been well documented.

Effects of co-dosage of Fe, Ni, Co, Mn and Mo in anaerobic digestion of abattoir effluent have been investigated (Schmidt *et al.*, 2018). The abattoir effluent used in this research was treated to recover fat, and since the five trace elements were combined in one solution it is difficult to single out the effects of one element. The effect of dosage of cobalt and nickel nanoparticles has not been investigated on abattoir effluent. This research aims to fill the above-mentioned gaps experimentally.

CHAPTER 3. MATERIALS AND METHODS

3.1 INTRODUCTION

This Chapter outlines how the experiment was conducted to achieve the set aims and objectives. The research was divided into three parts. The first part was to determine the optimum concentration of the waste streams to use. The other part was to determine and evaluate the effects of individual and combined dosage of nickel and cobalt nanoparticles on biogas production also on batch experiments. The last part was to determine the energy potential generated and the possibility of the abattoir powering itself through the generated energy from the combustion of methane in a Combined Heat and Power plant.

3.2 SOURCE OF BIOMASS

The biomass was collected from JAGO Beef abattoir effluent. The abattoir produces three waste streams: cow dung from the holding pen, rumen contents and blood and washing water from the slaughterhouse. 3*10L PVC plastic containers were used to collect the different samples from the site to the laboratory.

The Co NPs used were purchased from intelligent materials Pty. Ltd. (prepared by Nanoshell). The NPs are grey-blackish in colour, with a molecular weight of 58.93 g/mol, purity of 99.9 % and 40-80 nm in size. The Ni NPs were also from the same company, they are grey in colour, with a molecular weight of 58.69 g/mol, purity of 99.9 % and 40-70 nm in size.

3.3 DETERMINATION OF THE OPTIMUM CONCENTRATION OF FEED

The experiment was conducted in batch form using Automatic Methane Potential Test 11 (AMPTS 11) with 2.0 L reactors shown in Figure 4.1, with a 1.7 L sample volume and 0.3 L headspace. The objective of this part is to determine a suitable ratio of water and the sample (rumen contents, water and blood and cow dung). Thus, multiple ratios of the above streams were tested, and one was selected for the rest of the research. The selection was based on the biomethane potential of the different ratios, gas production and the ease at which the mixer was able to mix the contents uniformly.

Table 3.1 Composition of the sample to water ratio in the 5 reactors used

Reactor	Mass of sample (g)	Water added (ml)	Waste stream to water ratio (v/v)	Sample concentration (g/ml)
1	300	1400	1: 5	0.214
2	500	1120	1: 2	0.446
3	700	1050	1: 2	0.667
4	900	900	1: 1	0.942
5	1100	660	2: 1	1.667

3.4 DOSAGE OF COBALT AND NICKEL NANOPARTICLES

3.4.1 Experimental Setup and Reactor Operation

Four sets of digesters (and serum bottles) were used for digestion. The first were for testing nickel nanoparticles alone, the second one for cobalt nanoparticles alone. The third combined the two (nickel and cobalt nanoparticles). The last one was the control reactor in which there was no addition of nanoparticles. For each set for trace element supplementation, there were 4 different concentrations tested. The four concentrations used are 0.58, 2, 11 and 20 mg/l. The automated Methane Potential Test 11 Light was used for the first two concentrations (0.58 and 2 mg/l). 250 ml serum bottles were used for the 11 and 20 mg/l concentrations. Abdelsalam et al., 2017 showed that 0.5 mg/l of nickel and cobalt nanoparticles as the stimulatory concentration whereas 2 mg/l as the saturation concentration. Large doses lead to inhibition of biogas production. Based on the above information the first dose chosen was 0.58 mg/l.

3.4.2 The schematic diagram of the AMPTS 11

Automated Methane Potential Test 11 Light digesters were used for large scale digestion. Figure 3.1 shows the schematic diagram of the AMPTS 11. It shows the connection of the components of the system.

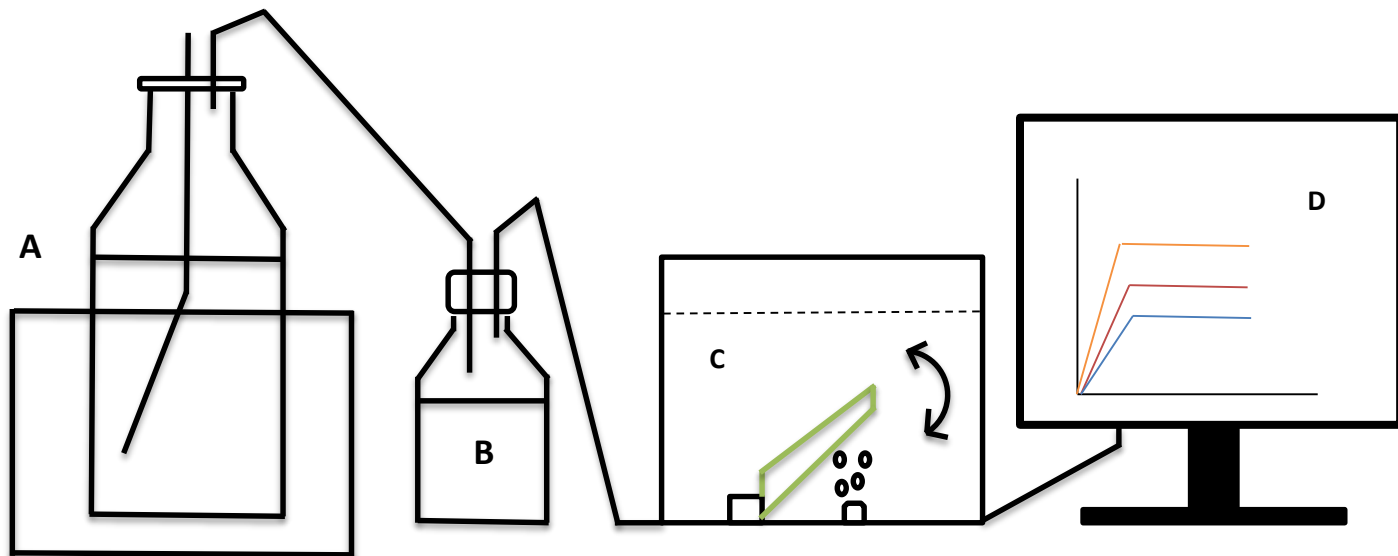


Figure 3.1 The different components of the Automated Methane Potential Test 11 Light (photo and schematic diagram).

Schematic diagram components,

- A-Sample Incubation Unit – Consists of 6*2L digesters immersed in a water bath with regulated temperature to 37⁰C. A mixer is inserted in the digester, which mixes the digester contents at intervals to keep it homogeneous.
- B-CO₂-Absorption Unit - 3 M NaOH solution 0.4 % Thymolphthalein. The sodium hydroxide reacts with CO₂ from the biogas produced. The reaction that occurs is as follows;

$$2\text{NaOH}(\text{aq}) + \text{CO}_2(\text{g}) \rightarrow \text{NaCO}_3(\text{aq}) + \text{H}_2\text{O}(\text{l}) \quad (14)$$
It also reacts with hydrogen sulphide as follows,

$$2\text{NaOH}(\text{aq}) + \text{H}_2\text{S}(\text{g}) \rightarrow \text{Na}_2\text{S}(\text{aq}) + 2\text{H}_2\text{O}(\text{l}). \quad (15)$$
Due to very low solubility of methane, it is not absorbed in the sodium hydroxide solution. It passes to the gas volume measuring device.
- C-Gas Volume Measuring Device- The system uses buoyancy and liquid displacement to measure the mass flow of the methane produced. The CH₄ collects in the bells therefore displacing the water. A certain volume of the gas will make the bell float, as it does so it will flip and let the gas bubble go and it falls back. The system then uses the laminar flow element and the gas temperature measured to convert the gas mass volume to STP

conditions. The system measures flow mass as opposed to volume flow hence the use of the “N” in units (Nml/day). The N indicates that the units are normalised to standard temperature and pressure.

- D-The Computer for biogas volume and flow rate recording- Connected to the gas measuring device with an ethernet cord the computer makes online recording of the gas measurement. The recordings are made every minute and the recorded data will be stored as quarter of an hour, hourly and daily.

3.4.3 Schematic diagram of the 250 ml serum bottles.

Four sets of 250 ml serum bottles were used for digestion. The digesters were sealed with rubber stoppers to cover for leakages. The 250ml bottles were also used in conducting the research, with a 220ml sample volume and 30ml headspace. They were sealed with a butyl rubber stopper, connected with a 5mm ID PVC plastic tube which was closed on the other side with a 1ml syringe. The syringe was used to sample the biogas produced and deposit in into the gas meter to determine the percentage of methane. The serum bottles were incubated in a Thermo Scientific Heratherm IMC-18 Incubator at 37⁰C. A picture and a schematic diagram of the flow measurement mentioned is shown on Figure 3.2;

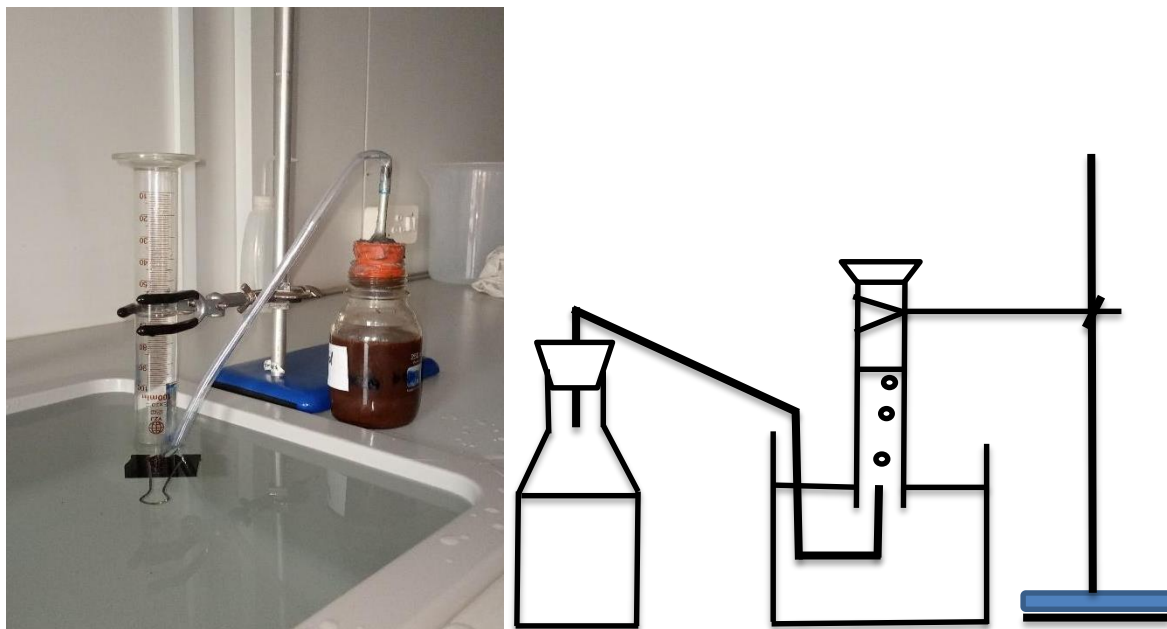


Figure 3.2 Laboratory experimental set-up for anaerobic digestion using 250ml serum bottles.

3.5 GAS MEASUREMENTS

The volume of biogas produced was measured daily using a water displacement method. An inverted measuring cylinder filled with water was placed and held firmly on a basin, also filled with water. The gas produced from the serum bottles was released into the measuring cylinder and as the gas accumulates it replaced the water in the cylinder. The water replaced will indicate the volume of the gas produced. Percentages of CH₄ was determined using a voice type multifunctional gas detector.

3.5.1 Calibration of the gas meter

OC-840 Portable Voice Type Multi Gas detector was used to measure the percentage of methane in the biogas produced. The gas meter measures Lower Explosive Level (LEL) of the combustible gas produced. In this case the combustible gas in the biogas is the methane. To calibrate this gas meter a combustible gas (Liquified Petroleum Gas) was used. 1.00 ml of the gas was drawn into a syringe, then a desired amount of the gas was emptied out of the syringe. Dry laboratory air was then drawn in to fill the syringe to 1.00 ml. The mixture of the combustible gas and dry laboratory air (1.00 ml) was then emptied into the gas meter and a reading of the lower explosive level was read from the meter. The percentage of the lower explosive level read from the gas meter was plotted against the known percentage of the combustible gas injected into the gas meter. A calibration graph of the gas meter was produced as shown in Figure 3.3. The calibration was done from 0% and up to 100%. This calibration was then used in determining the combustible gas (methane) percentage in the biogas produced.

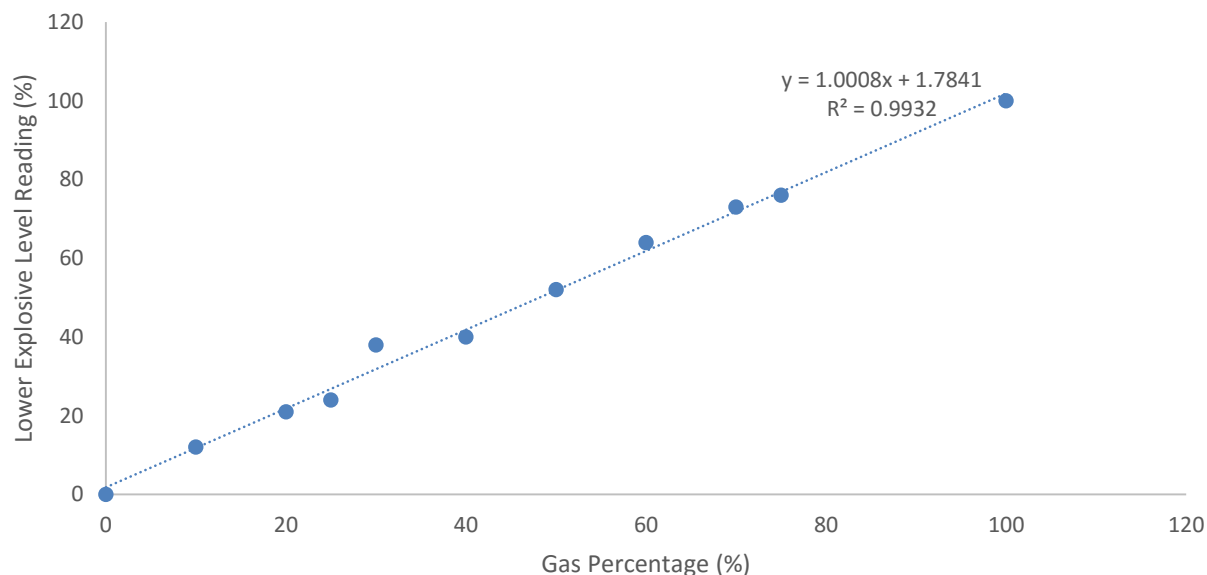


Figure 3.3 Calibration of the gas meter with laboratory air.

3.6 STATISTICAL ANALYSIS

The gas produced was measured daily and the results were statistically analysed using excel to determine the significance of the trace element dosage. The experiment was conducted to determine various regions of the hypothetical dose-response curve. A paired two sample t-test for means with a significant level of 5% ($\alpha = 0.05$) was performed on the BMP means and total biogas and methane to assess the significance of the effect caused by the added TEs. Simple linear regression was used to test the individual effects of Co and Ni nanoparticles in a co-dosed digester.

3.7 MEASUREMENT OF PHYSICAL PARAMETERS

Total and volatile solids were measured according to Standard Methods (Eaton *et al.*, 2005), while pH was measured using a multi-parameter PCS tester 25. Sludge metal content was determined using inductive coupled plasma-optical emission spectroscopy (ICP-OES). These physical conditions of a digester affect its performance as well as biogas production.

3.7.1 Total solids and volatile solids

These were determined according to standard methods (Eaton *et al.*, 2005; Ditalalelo, 2017). 40 ml of digestate was collected from each digester for analysis. Clean and dry glass Petri dishes were

weighed to obtain their empty mass (D_{mass}). A measured volume (V) of the sample was poured into the dish and weighed and then heated in the oven for at least 6 hours at 105°C . At this temperature and time negligible amounts of volatile solids will volatilize. After cooling for about 2 hours, the dishes now containing total solids were weighed again for the mass of the dish plus total solids (Dish + TS). The dishes with the total solids were then placed in a furnace at 505°C for at least 6 hours. This was done to burn off the volatile solids in the total solids and leave the fixed solids. The dishes were then weighed to obtain their mass plus that of fixed solids (Dish + fixed solids) after being cooled for about 2-3 hours. These analyses were done at the start and end of the experiment to relate the degradation of the solids to biogas/methane production.

The total and volatile solids content of the reactor was then calculated as follows;

Volatile solids

$$vs = \frac{[dish + ts] - [dish + fixed\ solids]}{sample\ volume} \quad (16)$$

Total solids

$$TS = \frac{[dish + ts] - [D_{mass}]}{sample\ volume} \quad (17)$$

3.7.2 Measurement of the metal content in the samples

3.7.2.1 Preparation of the sample with Acid Digestion

This was done using the standard method 3030F as described in Standard Methods for Water and Wastewater Treatment (APHA-AWWA-WEF, 1998) and modified by Smith, (2006). The modifications made include the use of a fixed ratio of $1(\text{HNO}_3):3(\text{HCL})$. The acids and distilled water are added at the same time rather than separately. The solution is heated two times only after the addition of acids and distilled water. Also, a fixed sample size was introduced. 0.5-0.8g equivalence of total solids was weighed and added to a 250ml beaker. 30 ml distilled water, and 6ml HCL and 2ml HNO_3 were also added to the same beaker. The solution in the beaker was then heated using a hotplate and allowed to evaporate until 10 ml remains. It was then cooled. After

cooling 30 ml distilled water, and 6 ml HCL and 2 ml HNO₃ were added again to the remaining 10 ml solution. It was then heated and evaporated again until 10 ml of the solution remained. The remaining solution was cooled and then filtered through Whatman filter (110mm) into a 50 ml volumetric flask. Distilled water is then added (approximately 40 ml) to bring the total volume to 50ml. The sample was then re-filtered using 0.45µmm polytetrafluoroethylene (PTFE) disc before being taken for analysis using ICP-OES/MS.

3.7.2.2 Measurement of the metal content using ICP-OES

Measurements of the trace metal ions in the wastewater was done using the inductive coupled plasma-optical emission spectroscopy (ICP-OES) to determine the baseline of the trace elements (nickel and cobalt) in the abattoir effluent. This is important because it will give insight of whether an opportunity to supplement exists or the already available TEs are of required/toxic concentrations. At the end of the experiments the concentrations of nickel and cobalt were determined. The comparison made between the initial and final concentrations will give an amount of used concentrations of nickel and cobalt during biogas production. This method depends on the emitted photons from ions which have been excited in the radio frequency discharge. To identify the elements present in the sample, the spectroscopy uses the wavelengths of these photons. There is direct proportionality between the total number of the photons and concentration of the originating element in the samples (Hou and Jones, 2000).

3.7.2.3 Calibration of the ICP-OES

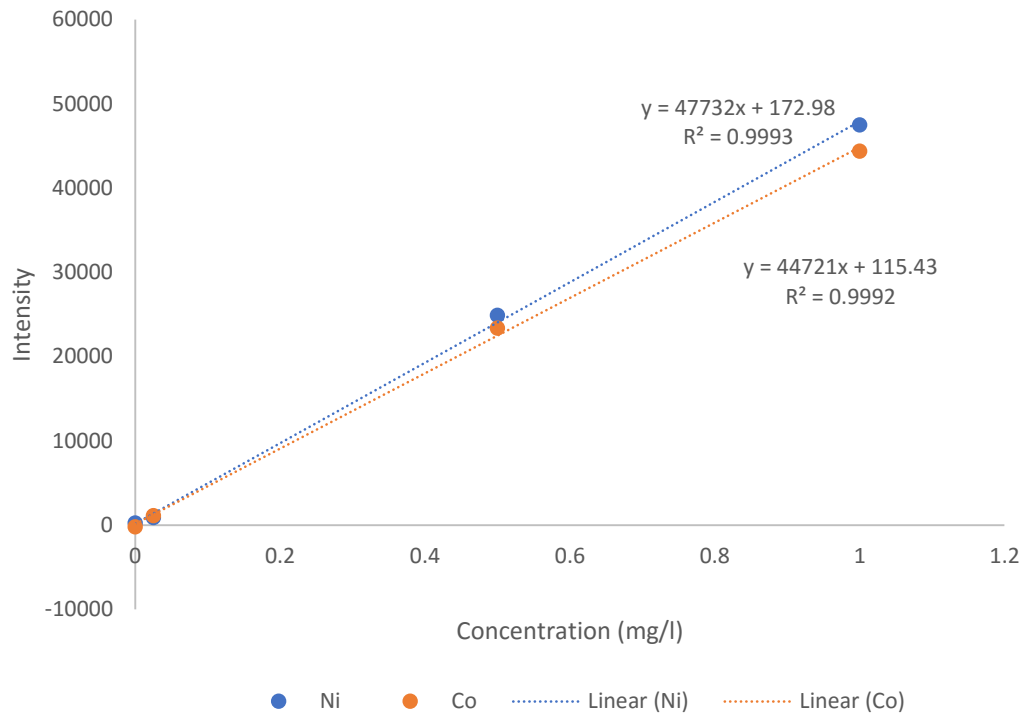


Figure 3.4 The calibration of the ICP-OES (Co and Ni)

Three standards and a blank (0.00 mg/l) were used for the calibration of the ICP-OES. Standard 1 and 2 had a concentration of 0.025, 0.50 and 1 mg/l, respectively for both Ni and Co. As shown in Figure 3.4, above the Ni curve has an R-squared value of 0.9993. The Co R-squared value is 0.9992. These values are all close to 1, this shows that the regression predictions satisfactorily fit the data.

3.8 THE ENERGY POTENTIAL GENERATED AND THE POSSIBILITY OF THE ABATTOIR POWERING ITSELF

The daily electricity consumption of the abattoir was obtained from the abattoir to use it for sizing. From the literature review, the amount of energy produced per kg of biogas/ methane was determined. The conversion rate of this energy to electricity was also identified from literature. From the batch experiments, the biogas volume was recorded. The energy potential of the biogas produced was determined and converted to electricity. The following steps were followed to size the biodigester that will be required to power the abattoir as adopted from Wresta *et al.* (2015).

Based on the amount of electricity in kWh a 100g (0.1 kg) of the feed will produce, the mass to be fed to the digester was estimated using the following formula;

$$ms = \frac{E}{0.0039722} * 0.1 \quad (18)$$

Where ms = mass of the abattoir effluent (kg/day), E is the electricity required by the abattoir (kWh/Day), 0.0039722 kWh is the amount of electricity that can be produced from 100g of the abattoir effluent, determined from the biogas potential from the batch experiments. The biogas potential in ml of biogas/g of sample was converted to energy potential (MJ/g), which was then converted to kWh/g. The optimum ratio of water to feed was also determined in the batch experiment and found to be (5:1). The amount of water to be added to the digester was computed using the following Equation;

$$ma = \frac{water(5)}{sample(1)} * ms \quad (19)$$

Where ma is the mass of water to be added to the digester (kg/day), ms is as determined by Equation 3.3.1. The total feeding (mt) in kg/day was determined by;

$$mt = ma + ms \quad (20)$$

The total volumetric feeding rate was computed by;

$$F = \frac{ms+ma}{\rho} \quad (21)$$

Where F is the volumetric feeding rate (m³/day), ρ is the density of the slurry (kg/m³). The volume of the digester was calculated using Equation 3.3.5.

$$V = (1 + \alpha) \left\{ \tau \left(\frac{ms+ma}{\rho} \right) \right\} \quad (22)$$

Where V is the volume of the digester (m³), α is the safety factor and τ is the hydraulic retention time (days). To ensure the safety of the digester the volume should be multiplied with a safety factor.

CHAPTER 4. RESULTS AND DISCUSSION

This research was conducted to determine and evaluate the effects of individual and combined dosage of nickel and cobalt nanoparticles on biogas production as well as evaluation of the energy potential of a laboratory-scale mesophilic anaerobic abattoir effluent digester. It was carried out in batch experiments using Automatic Methane Potential Test 11 (AMPTS 11) and 250 ml self-assembled biogas potential system as explained in Chapter 3.4.2. The first part shows the findings of the selected ratio (feed to water). The second part was done to determine and evaluate the effects of individual and combined dosages of nickel and cobalt nanoparticles on biogas and methane production. Here the digesters were dosed in incremental amounts of either: Ni, Ni & Co, Co nanoparticles and no Co or Ni nanoparticles (Control). The last part was to determine the energy potential generated.

4.1 DETERMINATION OF THE OPTIMUM CONCENTRATION OF FEED

This experiment was done to determine the optimum feed to water ratio (g/ml). Five reactors with different masses were used, with 200 grams increments. The feed to water ratios used in the reactors are 0.241, 0.446, 0.667, 0.942 and 1.667 g/ml with 82, 66, 62, 57, 39 % water content, respectively. The 0.241 g/ml reactor had a pH value of 7.14, 0.446 g/ml had 7.12, 0.667 g/ml had 7.23, 0.945 g/ml had 7.15 and lastly 1.667 g/ml had 7.18. As indicated the pH range for anaerobic bacteria to function efficiently in a digester is from 6.8 to 7.4 (Mao *et al.*, 2015). All the pH values of the 5 reactors used are within the specified range hence they provided a conducive environment for the AD process. The average concentrations of the TS, VS and FS of the sample used in the studies are 13.25 ± 0.11 , 11.06 ± 0.21 and 2.19 ± 0.16 % respectively. The effluent has high organic content as indicated by the high volatile solids in the sample of about 83.5% of total solids. Fixed solids only accounts for the remaining 16.5 %.

4.1.1 Cumulative Biogas and Methane Production

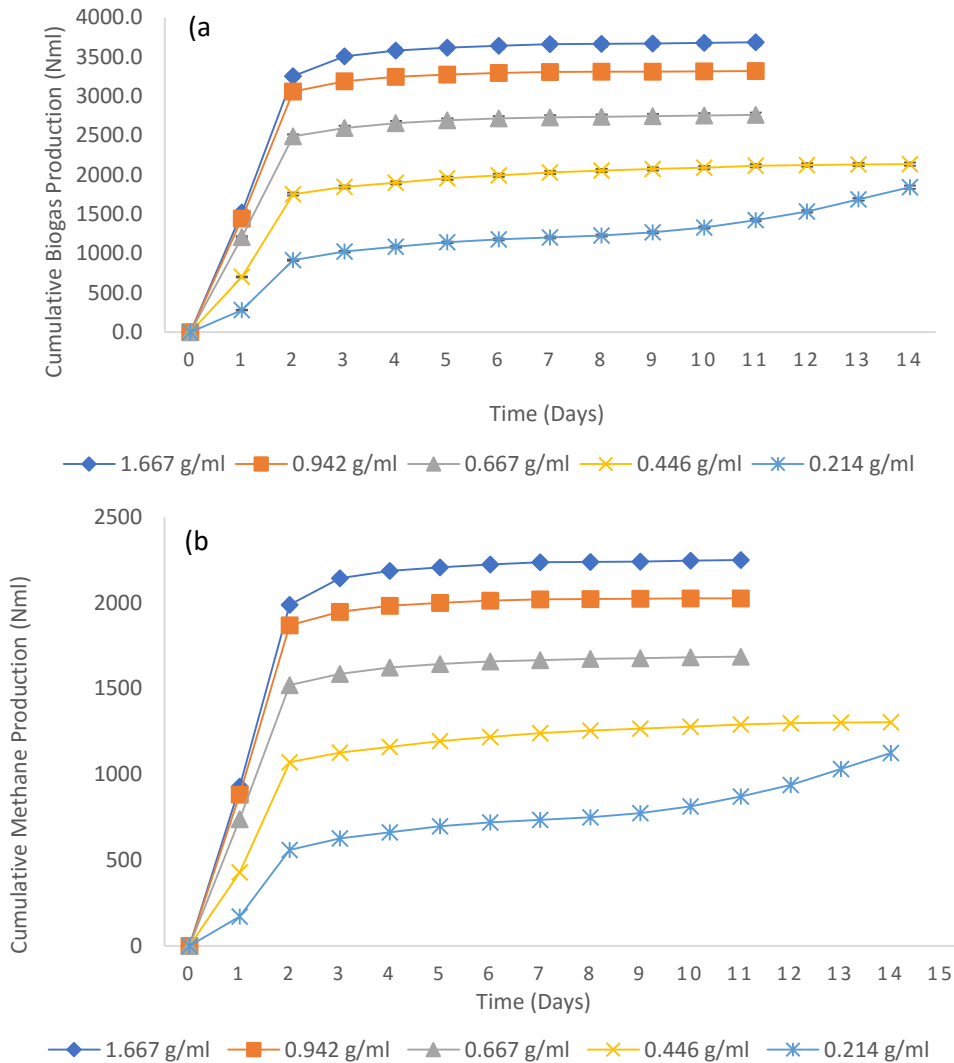


Figure 4.1 Cumulative biogas and CH₄ production (a=cumulative biogas volume, b= cumulative methane volume).

This was done to relate the feed to water ratio to cumulative biogas and methane production. The cumulative biogas and methane production recorded are shown in Figure 4.1. A rapid increase was observed in the first two days in both methane and biogas production. From the 3rd day both the cumulative methane and biogas increased steadily throughout the experiment. For the 0.667, 0.942 and 1.67 g/ml digesters the production stopped on the 11th day. For the 0.446 g/ml digester production continued steadily until the last day of the experiment. Production on the 0.214g/ml

digester continued steadily from the 3rd day until the 7th day. On day 8 it started to increase until the last day of the experiment. As VS increased the methane and biogas production increased. The 1.667 g/ml reactor produced the highest biogas and methane followed by the 0.942 g/ml, then the 0.667 g/ml, then the 0.446 g/ml and lastly the 0.214 g/ml digester.

4.1.2 Daily Biogas and Methane Production

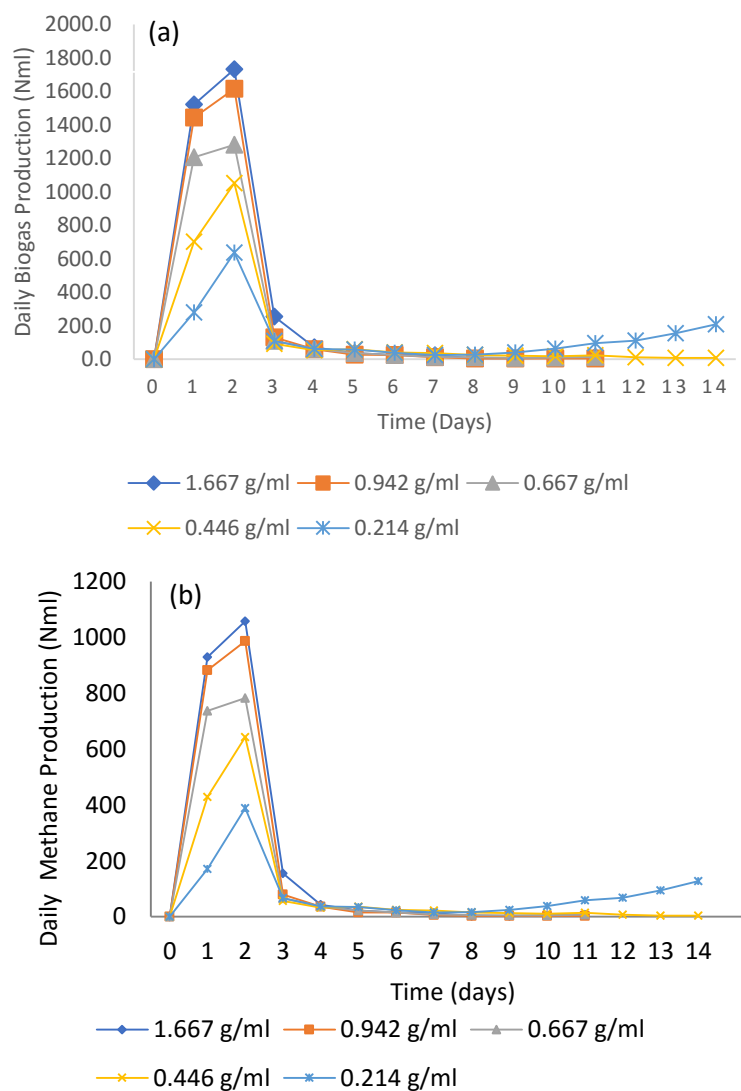


Figure 4.2 Daily methane and biogas production (a= biogas, b= methane).

The methane accounted for 61.1 ± 0.1 % of the total biogas produced, its curve is represented by Figure 4.2b. As on the biogas production the methane production reached peak on the 2nd day of the experiment. From the 3rd day methane production reduced until the reactors reached their

lowest production on their respective days. The production of methane stopped on the 11th day of the experiment for the 0.667, 0.942 and 1.667 g/ml digesters. The production for the 0.442 and 0.214 g/ml continued until the last day of the experiment, with the 0.446 g/ml reaching its lowest production on the 13th and 14th day. On the 7th day the methane production of the 0.214 g/ml digester increased from 15 Nml to 127.7 Nml on the 14th day. The days (day 3 to 6) before this increase, it is anticipated that the microorganisms were still adapting to the changes of the conditions in the digester. After adaptation there is an exponential growth of microorganisms leading to the recovery of methanogenic activity (Mao *et al.*, 2015) hence an increase in biogas production.

The stopping of biogas and CH₄ production on the 11th day, the last 3 reactors (0.667, 0.942 and 1.667 g/ml) is due to several problems encountered during anaerobic digestion. An above optimal pH can limit the production of CH₄ since it is not conducive for VFA consuming methanogens (Ek *et al.*, 2011), the pH may be increased by the formation of ammonia (organic overload). There is a high formation rate of VFAs during the AD of abattoir effluent hence if they are not consumed, they accumulate in the reactors. The fatty acids attach themselves to the cell walls of the methane-forming bacteria, they then dissolve in the wall, damage the cell, and limit the nutrients and inhibit the activity of the microorganisms (Gerardi, 2003; Pereira, 2005). Accumulation of VFAs will later lead to instability in the reactors. There is also the formation of sulfides from the degradation of protein which inhibit methanogens (Chen *et al.*, 2008). Since a higher mass of the feed was used in these reactors as compared with the first two it is anticipated that the intoxicants be greater in them. As indicated in the above reasons the last step of methane/biogas production is the one that is mostly affected hence the need to improve it. Addition of Ni & Co nanoparticles is expected to catalyse it. Addition of NPs is expected to recover the system and efficiently degrade the VFAs.

4.1.3 Total Biogas, Methane Produced and Biomethane Potential

The total methane and biogas produced is highest in the digester with the higher sample mass and lowest in the digester with the least sample mass. This is shown by Figure 4.3(a). This is because the amount of the organic material (VS) increases as the sample size increases. The 0.214 g/ml digester produced a total of 1849.1 ± 18.9 Nml and 1124.6 Nml of biogas and methane respectively. The 0.442 g/ml produced 2135.6 ± 21.9 Nml's of biogas and 1303.9 Nml's of CH₄, with a difference of 286.5 (15.9 %) and 179.3 (15.9 %) Nml in biogas and methane respectively.

The 0.667 g/ml digester produced 2762.1 ± 28.3 Nml's of biogas and 1686.4 Nml's of methane thus increasing the production as compared to the 0.446 g/ml digester by 626.5 (29%) and 382.5 (29%) Nml's of biogas and methane respectively. The 0.942 g/ml digester had a difference of 555.8 (20 %) Nml in biogas and 339.4 (20%) Nml in methane as compared to the 0.667 g/ml digester. The 1.667 g/ml digester produced 3684.5 ± 37.7 Nml's of biogas and 2249.6 Nml's of methane thus increasing the production as compared to the 0.942 g/ml digester by 366.6 (11%) and 223.8 (11%) Nml's of biogas and methane respectively. On average the 200g increments increased the biogas by $458.85 (18.75\%) \pm 158.9 (8.6\%)$ Nml and methane by $281 (18.75\%) \pm 95.4 (8.4\%)$ Nml.

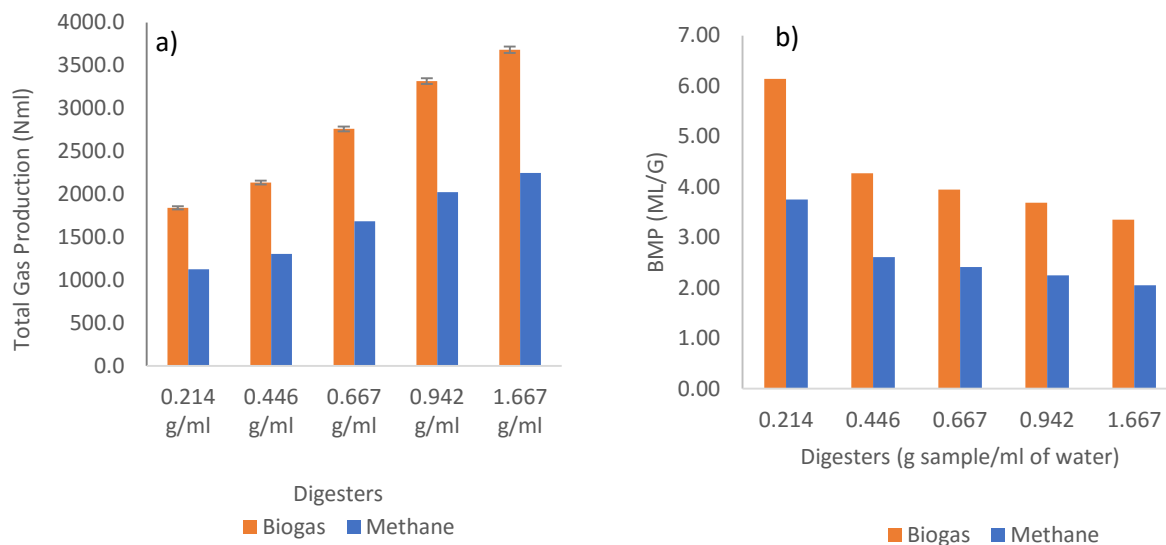


Figure 4.3 Total Biogas, Methane Produced and Biomethane Potential (a = total biogas and methane, b = biomethane potential)

BMP gives the capacity of a substrate to be converted into methane and the possible methane yield (Angelidaki *et al.*, 2009; Diaz, 2010). It is used to assess the biodegradability of the abattoir effluent. It is affected by: mixing, availability of nutrients, digestion time and particle size (Diaz, 2010). To determine how the feed to water ratio affects methane yield all the other factors that affect anaerobic digestion were held constant except for mixing. As opposed to the total methane and biogas produced, the biomethane potential of the sample (expressed in Nml produced per gram of sample) decreases, as the reactor sample mass increases. The 0.214 g/ml reactor produced 3.75

and 6.14 Nml/g of methane and biogas, respectively. This suggests that reactor conditions were more favourable in this reactor as compared to the others, it was the highest BMP recorded in the experiment. The 0.446 mg/l reactor was the second highest with a difference of 30.4% for methane and 30 % for biogas as compared with the 0.214 g/ml digester. In the 0.667 g/ml reactor, it was 2.41 and 3.95 Nm/g, with a difference of 7.7 % for CH₄ and 7.5 % for biogas, respectively as compared with the 500g reactor. The 0.942 g/ml reactor recorded 2.25 (methane) and 3.69 (biogas) Nml/g, which is 6.6 (methane) and 6.6 (biogas) % less than the 0.667 g/ml reactor. The 1.667 mg/l had the lowest BMP of 2.05 Nml/g for methane and 3.35 Nml/g for biogas, with a difference of 8.9 % for methane and 9.2 % for biogas from the 0.942 g/ml reactor. This indicates that as the mass of the sample increased the conditions in the digesters became less and less conducive for methane and biogas production. Abattoir effluent is rich in fats which have high methane potential but when in excess they inhibit methane production due to the increase in the formation of long-chain fatty acids (Chen *et al.*, 2008; Diaz 2010; Ek *et al.*, 2011). As the size of the sample increases, also the fat content in the sample increases hence the reduction in the methane production. The first (0.214) mg/l and (last) 1.667 mg/l reactors had a difference of 1.7 Nm/g (45%) for methane and 2.79 Nm/g (45%) for biogas. A large difference was noticed between the 0.214 g/ml and the 0.446 g/ml of 30 %, while from the 0.446 g/ml until the 1.667 g/ml it was below 10%. On average the 200g increments reduced the BMP by 0.43 (11.4%) $\pm$ 0.48 (12.8%) Nml/g. The 0.241 g/ml ratio was better to use since it had the highest BMP.

4.1.4 Total, Volatile solids and Biodegradability

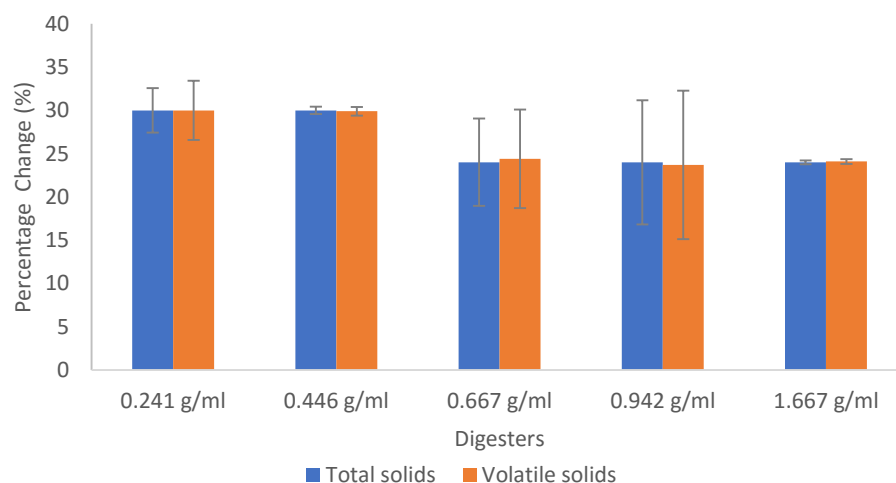


Figure 4.4 Changes in total and volatile solids concentration

Total and volatile solids were determined before and after the experiment to account for the digested VS throughout the experiment. Initially the abattoir effluent contained 13.25 % of TS, 11.06 % of VS and 2.2 % of FS. The VS occupied 83.47 % of the total solids. Figure 4.4 shows how the FS, VS and TS were in each digester, as they all had different masses. At the end of the experiment the 1.667 g/ml digester had 76 % of the initial TS. The 0.214, 0.446, 0.667 and 0.942 g/ml remained with 70, 70.1, 75.6 and 76.3 % of the initial TS, respectively. The 0.214 g/ml digester reduced the VS by 30%. The 0.446, 0.667, 0.942, and 1.667 g/ml digesters reduced the VS by 29.9, 24.4, 23.7, and 24.1 %, respectively.

Table 4.1 Biodegradability of the abattoir effluent

Reactors (g/ml)	Experimental Methane yield (ml of CH ₄ /g of VS)	Deviation of experimental methane yield values from the theoretical Value (%)	Biodegradability (%). Organic Fraction
0.214	113.1	82.5	17.5
0.446	78.9	87.8	12.2
0.667	89.3	86.2	13.8
0.942	89.3	86.2	13.8
1.667	76.7	88.1	11.9

Xu *et al.* (2018) reported that abattoir waste contains 0-27 % carbohydrates, 2-38.9 % proteins and 1-40 % lipids. Based on this and Equation 12 theoretical BMP was computed and found to be 645.561 ml of CH₄ /g of VS. The 0.241 g/ml digester produced 1125.6 ml of methane, based on the theoretical BMP this then suggests that only 1.74 g of the sample is needed to produce that amount of gas and 94.75 % of the VS is then left. The 0.446 g/ml digester produced 1303.9 ml of methane, with the theoretical BMP being 645.561 ml of CH₄ /g of VS the VS destructed will then be 2.02 g and 96.35 % left after the experiment. The 0.667 g/ml digester produced 1686.4 ml of methane, this then suggests that only 2.61 g of the sample is needed to produce that amount of gas and 96.63 % of the VS is left. The 0.942 g/ml digester produced 2025.8 ml of methane, with the theoretical BMP being 645.561 ml of CH₄ /g of VS the VS destructed will then be 3.14 g and 96.85 % left afterwards. The 1.667 g/ml digester produced 2249 ml of methane, this then suggests that

only 3.49 g of the sample is needed to produce that amount of gas and 97.14 % of the VS is left. All the values of the remaining VS (theoretical) are higher than those obtained experimentally. This may be because the theoretical methane content assumes full digestion (ideal bacterial conditions), constant temperature and optimum mixing hence a higher conversion rate (Achinas *et al.*, 2016). The biodegradability ($BD_{CH_4}(\%)$) of the substrate was then calculated using the experimental and theoretical values of methane. The BD_{CH_4} according to the organic fraction of the sample for the 0.241, 0.446, 0.667, 0.942 and 1.667 g/ml digesters were 17.5, 12.2, 13.8, 13.8 and 11.9 %, respectively. This shows that a digester with low sample mass (0.241g/ml) had a high biodegradability than the one with higher sample mass. A higher solid content digester has a small amount of water added to it, a lower BD_{CH_4} shows the ratio was not favourable for methane production.

4.2 DOSAGE OF COBALT AND NICKEL NANOPARTICLES

Based on the BMP results of the experiment from part 1, 500 g was selected as sample size, to be added to 1400ml distilled water to achieve a working volume of 1700 ml and headspace of 300 ml from the 2 L reactors. Here different dosages (0.58, 2, 11, and 20 mg/l) of the Nanoparticles were tested. The concentrations were selected based on Abdelsalam *et al.* (2016), which shows that a concentration of 0.5 mg/l of Ni and Co nanoparticles was stimulatory to biogas production of fresh raw manure.

4.2.1 Dosage of 0.58 mg/L of Ni, Ni & Co, Co nanoparticles to the digesters

4.2.1.1 Cumulative methane and biogas production

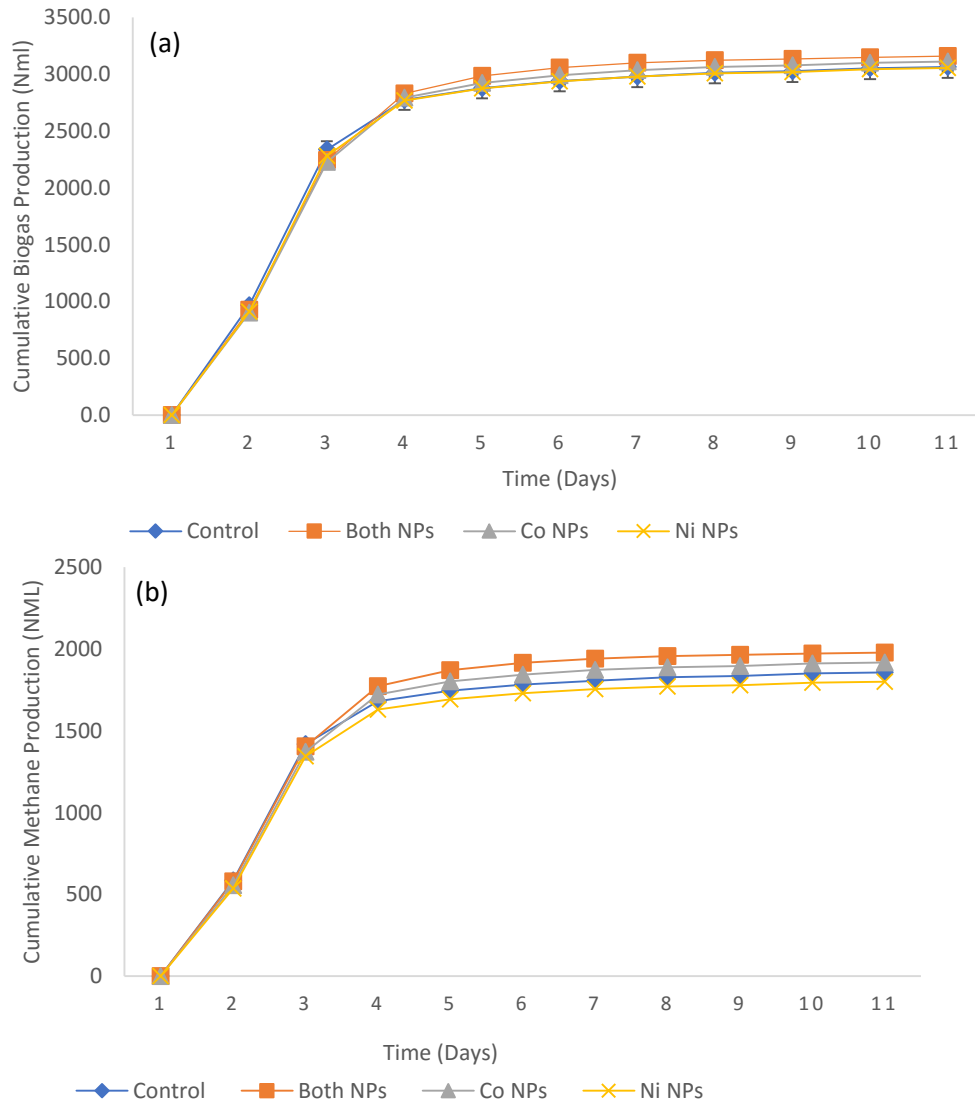


Figure 4.5 Performance of digesters fed with different Nanoparticles (a = cumulative biogas volume, b = cumulative methane volume).

To test the effects of individual and combined dosage of Co and Ni nanoparticles, at first the lowest measurable concentration was used. Figure 4.5 shows the biogas (a) and cumulative methane (b) of the digesters fed with nanoparticles as compared with the control. 1 ml of biogas was sampled from the tube connecting the digesters and NaOH bottles and bubbled through the handheld gas

meter to determine the methane percentage in the biogas produced. Gas sampling was done in duplicates. For all the digesters the gas production increased from the first day until the 3rd day. From the 4th day to the last day of the experiment a steady production was observed. The digester dosed with both NPs (Ni & Co) produced more gas (3157.9 Nml) than the other three digesters, followed by the Co nanoparticles digester then the control and lastly the Ni NPs fed digester. The trends in Figure 4.5 are similar to those of Figure 4.1. Addition of 0.58 mg/l of Ni, Co (individual and their combination) had an increase of less than 10 % in biogas production when compared with the control. The effected change is small, that is why the trends are similar to Figure 4.1 where all digesters were not dosed. The sample ratio used in Figure 4.5 was selected from Figure 4.1.

4.2.1.2 Daily methane and biogas production

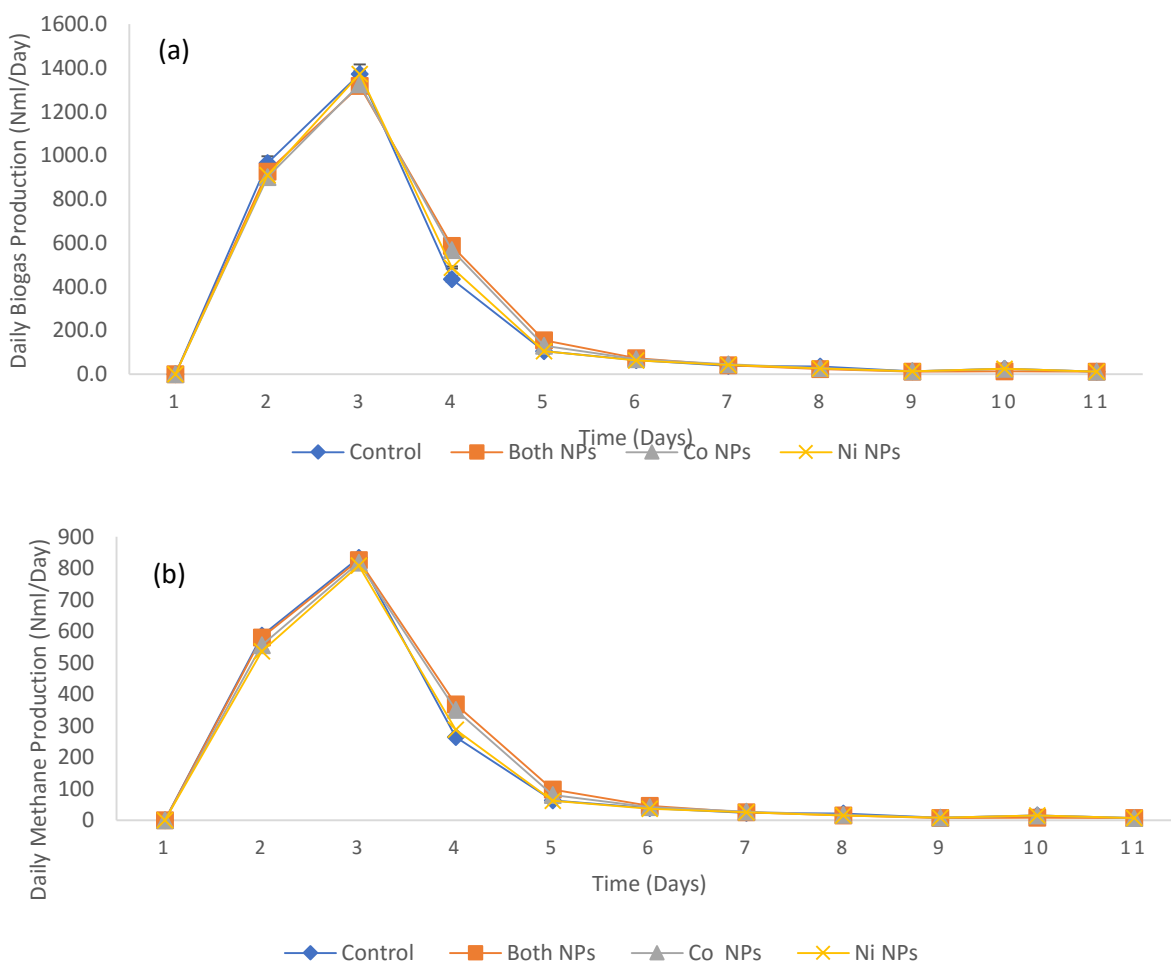


Figure 4.6 Daily methane and biogas production (a= biogas, b= methane).

There was a gradual increase in daily methane and biogas production from the first day until the third day of the experiment. From there a decrease in production was observed which continued until the last day of the experiment. Microorganisms are very sensitive to temperature and pH. The pH of the system is dependent on the balance between the alkalinity and VFAs. Methanogens require pH that is between 7.8 to 8.2 (Mao *et al.*, 2015). If the pH is not within this range it will hinder methanogenesis step hence low biogas production. The control digester produced 832.6 Nml of methane and 1372.4 ± 43.2 Nml of biogas on the peak day. The control digester's methane value was recorded as the highest peak across all digesters. The digester dosed with both NPs produced the second amount after the control digester, with a difference of 6.8 Nml. On the peak day, a digester dosed with both NPs (Ni and Co) produced 1317.8 ± 7.9 Nml. This was 55 (4%) Nml less than that of the control. The biogas from the control digester contained 61 % of methane whilst in the digester with both NPs methane occupied 63 % of the biogas. Therefore, there was more methane in the digester fed with both NPs (based on the percentage of methane) as compared with the control.

The Co nanoparticles dosed digester produced the third largest volume of 817.1 Nml on the 3rd day, with a difference of 155 Nml as compared with the control. On this day, the biogas it produced was 1325 ± 29.9 Nml, 47.4 Nml less than that of the control. The methane produced occupied 62 % of the total biogas produced which is higher than that of the control with 1 %. The Ni nanoparticles dosed digester produced 807.9 and 1371.2 ± 14.9 Nml of methane and biogas, respectively. The methane produced occupied 59 % of the total biogas produced. Due to the low percentage of CH₄, on the peak day the Ni NPs dosed digester produced the lowest methane as compared with other digesters. The biogas it produced on this day was 1.2 Nml less than the control, making it the second highest biogas production on peak day. Addition of micronutrients reduces the time taken to reach peak daily production and reduces hydraulic retention times (Zandvoort, 2005; Nordell *et al.*, 2016; Facchin *et al.*, 2011; Zaidi *et al.*, 2018). This was not the case as it is depicted by Figure 4.6 (a and b) the control and the dosed digesters reached peak on the same day. The micronutrients might be added to the reactors, but their performance will be determined by their bioavailability (Nordell *et al.*, 2016).

4.2.1.3 Effects of Ni and Co nanoparticles in total methane and biogas production

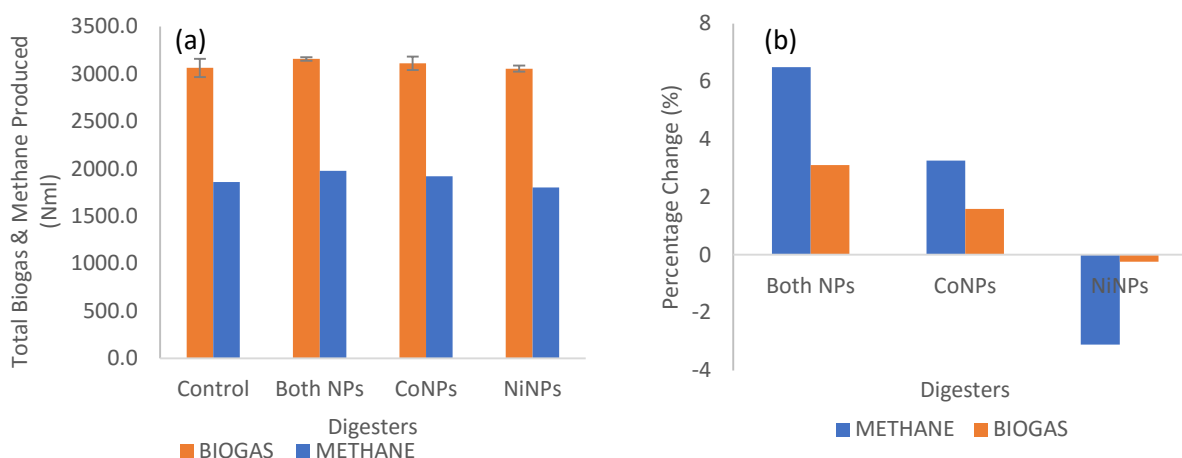


Figure 4.7 Total methane and biogas production and percentage change in Nanoparticles dosed digesters as compared with the control (a= total methane and biogas volume, b= percentage change).

Figure 4.7 shows the total methane and biogas produced by the four digesters. The percentage change in methane and biogas was calculated with respect to the control. It was done to show the change the nanoparticles had on the total gas production. The control digester produced total methane of 1858.2 Nml and biogas of 3062.9 ± 96.4 Nml. The digester dosed with both NPs produced the highest total methane and biogas of 1978.9 and 3157.9 ± 18.8 Nml, respectively. This digester produced 95 (3%) and 120.7 (6%) Nml of biogas and methane respectively, more than the control. The Co nanoparticles dosed digester produced 1918.7 and 3111.4 ± 70.2 Nml of methane and biogas, respectively. The Co NPs effected a change of 3 and 2 % above the control for methane and biogas, respectively. These results agree with those obtained by Facchin *et al.* (2011), who reported an increase of 11 % in methane production from the addition of 0.1 mg of Co/g of TS during mesophilic anaerobic digestion of food waste. These results also agree with Abdelsalam *et al.* (2017) who reported an increase of 36.5 % and 41.8 % in biogas and methane production, respectively. The increase was due to the addition of 0.5 mg/l of less than 28nm Co nanoparticles during the digestion of raw manure. Also found that 0.014 mg of Co/g of TS increased methane production by 85.6 %. This concentration was the saturated/excess concentration from this experiment. Zaidi *et al.* (2018) showed that the addition of 1 mg/l of less

<100 nm Co NPs during digestion of green microalgae increases CH₄ by 9.6 % when compared with the control. Nordell *et al.* (2016) reported that the addition of Co 0.0002 – 0.0005 mg/g to anaerobic digestion of food waste increases biogas production by 10-24 % as compared with the control. Liu, Wei and Leng, (2021) indicated that the stimulatory concentration of Co ranges from 0.06 to 6 mg/l, this agrees with the results of the experiment. The differences in the responses of the feed to the same or similar dosage of NPs may be due to the distinctive characteristics possessed by the substrates. Micro algae have a highly resistant cell wall (cell wall consists of two layers of which NPs must penetrate), which will be difficult as compared with other substrates (Zaidi *et al.*, 2018). Each feed has its own specific trace element requirements (Abdelsalam *et al.*, 2017), depending how the feed was produced.

The digesters dosed with Co NPs and both NPs were the only ones that produced more methane and biogas when compared with the control. Both NPs digester produced more biogas and methane when compared the Co NPs dosed digester. The Ni nanoparticles dosed digester was the only digester to produce both methane (1800.4 Nml) and biogas (3055.7 ± 33.2 Nml) less than the control hence the negative percentage change in Figure 4.7 (b). These results do not agree with Schattauer *et al.* (2011) who reported that a concentration of 0.005 to 0.5 mg/l (Ni) is stimulatory and leads to an increase in biogas and methane production. Abdelsalam *et al.* (2017) reported that addition of 0.5 mg/l of Ni NPs (<17nm) leads to an increase in biogas production of about 46.47 % as compared with the control. He also effected that a concentration of 1 mg/l improves biogas production by 72.35 %. This does not agree with the results attained in this experiment as 0.58 mg/l of N NPs (< 100nm) was not stimulatory to both CH₄ and biogas production. The concentration of toxic materials in abattoir waste is not equal to that of animal manure. High amounts of fats and proteins in abattoir effluent (Diaz, 2010), can be the cause of the difference in the results. The performance of NPs in an anaerobic system depends also on the temperature and size of the NPs used (Abdelsalam *et al.*, 2016; Dehghani *et al.*, 2019), Addition of 1 mg/l of Ni NPs to anaerobic digestion of green microalgae under mesophilic conditions (37⁰C) led to the production of 618 ml of CH₄ as compared to the control which produced 488 ml, an increase of 26.6 % (Zaidi *et al.*, 2018). From previous research an increase of 30% and 100% in CH₄ production was observed during anaerobic digestion of maize silage due to the addition of 10µm and >20nm of Ni (Abdelsalam *et al.*, 2017; Pobeheim *et al.*, 2010).

4.2.1.4 Volatile Solids and their relationship with nanoparticles concentration and Biomethane Potential

The biomethane potential represents the capacity of the substrate to be converted to methane and/or biogas. It is expressed in ml of gas / g of VS. In this experiment, the digesters dosed with NPs were compared with the control to assess the influence of NPs on BMP. The control had a BMP of 26 ml/g of VS, the same as the digester dosed with Ni NPs. The digester dosed with both NPs had the highest BMP of 28 ml of CH₄/g of VS followed by Co nanoparticles digester which recorded 27 ml of CH₄/g of VS.

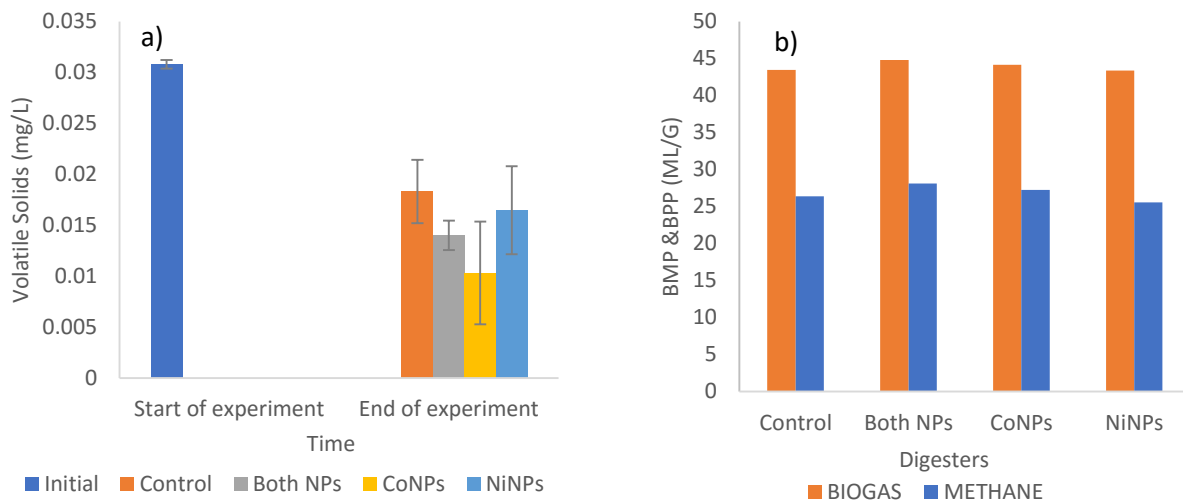


Figure 4.8 Volatile Solids from the start and at the end of the experiment and the biomethane potential (a= Volatile Solids and b= Biomethane Potential).

The volatile solids represent the organic (digestible) material in the sample. As they are depleted biogas is produced. They are used as a monitoring tool in anaerobic digestion as they indicate if the digestion process is taking place (Mitchell *et al.*, 2015). The addition of NPs was expected to improve the start-up of gas production and degradation rate of VS when compared with the control. The control degraded 0.012 mg/l of VS from the initial 0.0308 mg/l (40%) over a period of 11 days. The digester fed with both NPs degraded 54 % of the VS, as this digester produced the highest biogas and methane it was expected that VS degraded will also be higher. The Ni nanoparticles dosed digester degraded 46 % of the VS. The Ni NPs dosed digester had the lowest percentage of degraded VS hence the lowest produced biogas as compared with the control, Co

NPs, and both NPs digesters. The Co NPs dosed digester degraded 0.02 mg/l of VS and was left with 0.01 ± 0.005 mg/l at the end of the experiment.

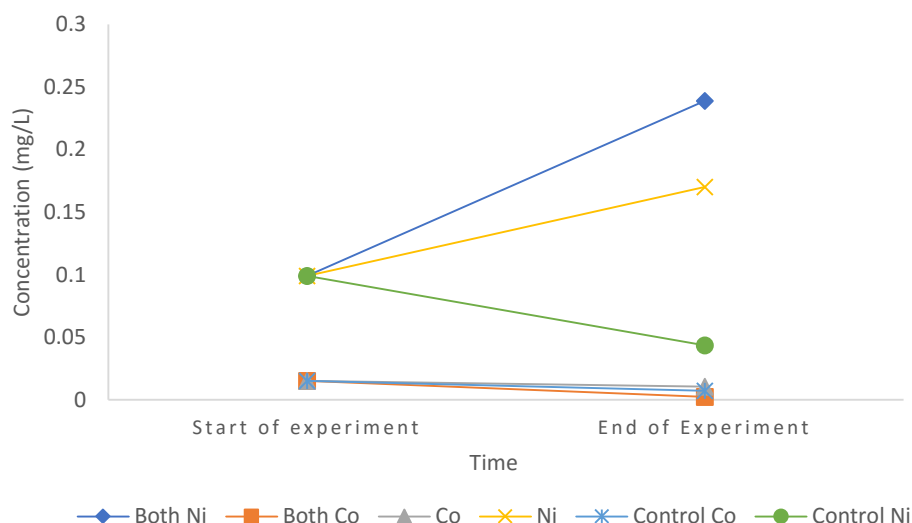


Figure 4.9 Initial and Final concentration of TEs in the dosed digesters.

The initial concentration of trace elements of the abattoir effluent was 0.015 mg/l of Co and 0.099 mg/l of Ni. One digester was fed with 0.58 mg/l of Ni NPs, another with 0.58 mg/l of Co NPs and the other with 0.58 mg/l of both NPs (nickel and cobalt). The relationship between VS (VS reflects the biodegradable component of the sample) and TEs is very important in anaerobic digestion as it depicts the interaction of TEs with microorganisms (Metcalf and Eddy, 2003; Ditalelo, 2017). A stimulatory concentration reflects a high association of TEs and microorganisms and leads to increased biogas and a stable AD process (Ditalelo, 2017). The initial concentration (of NPs/Vs) in relation to volatile solids were 0.014 mg of Co/g of VS and 0.014 mg of Ni/g of VS. Figure 4.9 shows how the concentration of the Ni and Cobalt ions changed from the start of the experiment and the end of the experiment time (11 days). The Ni (ions) in the Ni NPs dosed digester after retention time of 11 days was 0.17 mg/l and 0.24 mg/l in the Both NPs digester. The final concentration of Ni ions in both NPs digester increased by 140 % more than the initial concentration. Both NPs digester had the highest biogas production. The final concentration Ni NPs dosed digester increased by 71 % more than the initial concentration. This digester produced the least biogas as compared with the control, Co NPs, and co-dosed digesters. The Co (ions) in the Co NPs dosed digester after day 11 was 0.02 mg/l and 0.002 mg/l in the Both NPs digester.

4.2.1.5 Comparison of the effect of the sum of the individual and combined supplementation of Co and Ni NPs

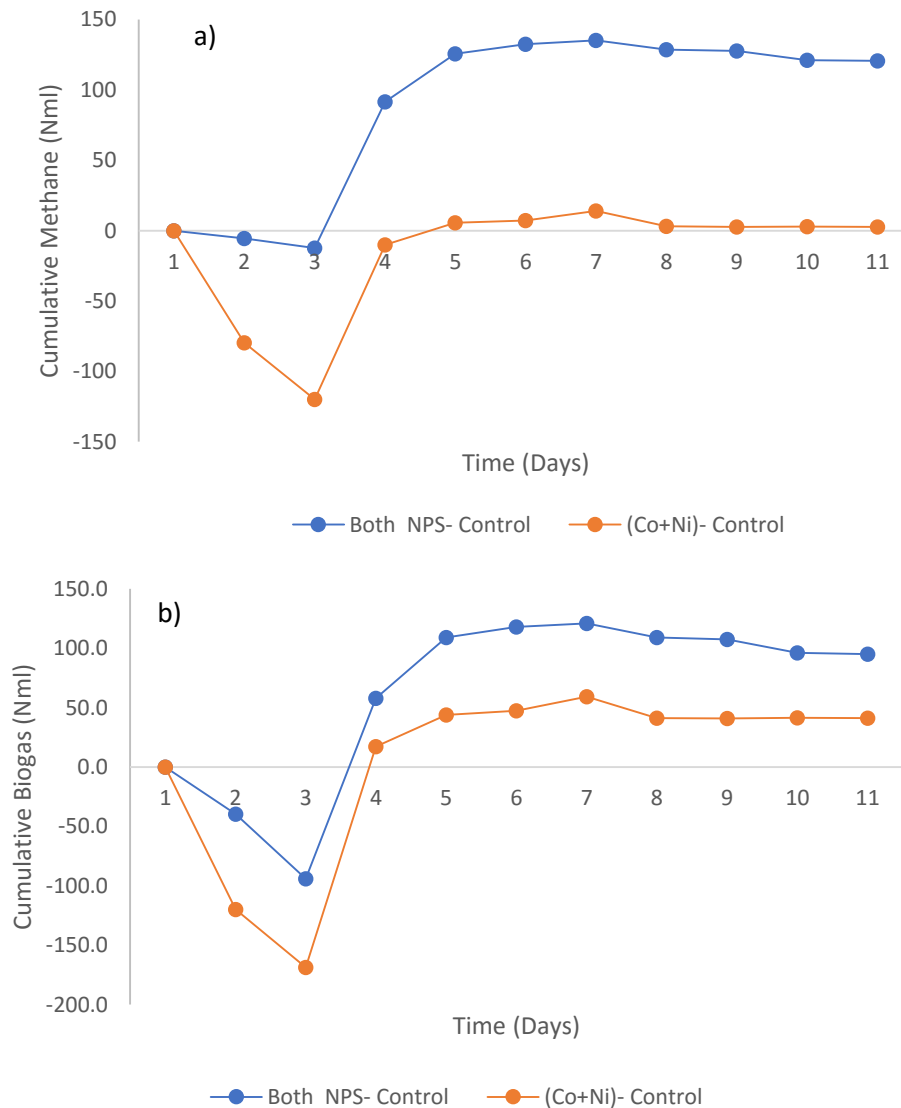


Figure 4.10 Comparison of the sum of the individual effect of Ni and Co NPs dosage to that of their combined dosage (a = methane, b = biogas)

This was done to test the equality of the sum of biogas and methane volume by which individual Co and Ni NPs dosed digesters exceeded the control to that by which both NPs digester exceeded the control. This is to determine if the additive nature of Co and Ni was maintained throughout the experiment (Ditalelo, 2017). During anaerobic digestion of acetate under mesophilic conditions cobalt and nickel are found to be co-required (Ditalelo, 2017). Cumulative methane and biogas

were used for this analysis as shown in Figure 4.10 (a and b). In the first three days of the experiment the control produced more methane and biogas than the individual Ni and Co NPs dosed digesters and both NPs dosed digesters. Both NPs dosed digester produced more methane and biogas than the individual combination, this was true throughout the experiment as shown on Figure 4.10 (a and b). This is because the control produced more methane than the Ni nanoparticles dosed digester and equal amount of biogas. Addition of the effect of individual Co and Ni NPs would then be reduced because of the negative change mentioned above.

4.2.1.6 Effects of nanoparticles (Ni and Co) in a co-dosed digester

This part of the Chapter tests the additive nature of cobalt and nickel nanoparticles. Cumulative biogas production was used to assess the additive nature of cobalt and nickel nanoparticles. This analysis is based on the impact each of the Nanoparticles had when co-dosed and dosed individually.

4.2.1.6.1 Effect of cobalt nanoparticles in a co-dosed digester

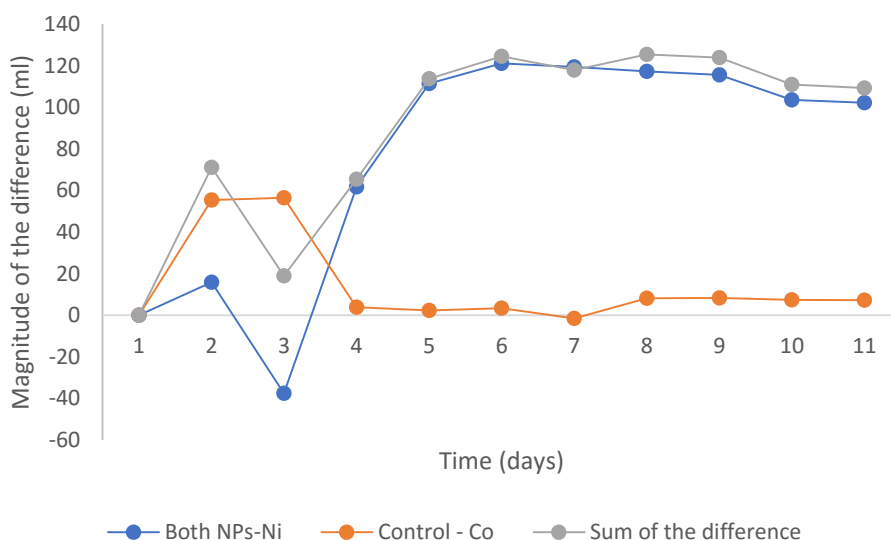


Figure 4.11 Magnitude of the difference of the control and Co NPs dosed digester, both NPs and Ni NPs digester and the sum of the difference.

This was obtained by comparing the difference in time and amount of biogas of both NPs dosed digester and Ni NPs and that of control and Co NPs dosed digester. The first difference gives the biogas volume in the presence of Co NPs while the latter gives the biogas volume in the absence

of Co NPs. The sum of the differences is expected to be close to zero. The magnitude of the difference of the two pairs was not similar throughout the experiment and the sum of the magnitude of the differences is not close to zero. From day 4 until the end of the experiment all the differences (both NPs-Ni NPs and Control-Co NPs) were found to be positive leading to a high sum of the differences as it can be observed on Figure 4.11.

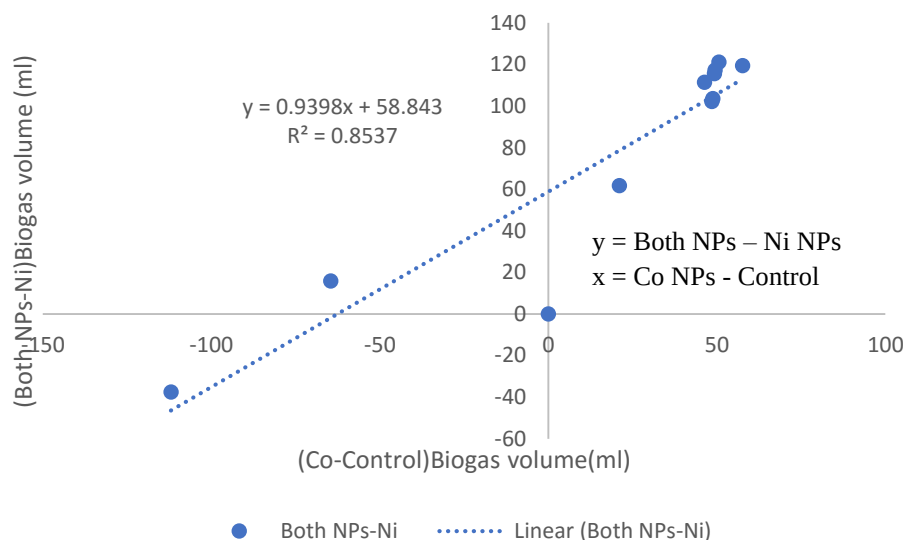


Figure 4.12 A line fit plot of the linear regression that correlates the effect of Co in both NPs dosed digester and Co dosed digester.

The effects of cobalt nanoparticles in a co-dosed digester were tested by comparing the difference in biogas production between Co NPs digester and the control digester (control- Co NPs), to that of the both NPs digester and the Ni NPs dosed digester (both NPs-Ni NPs). The linear regression made gave an R^2 value of 0.8537. An R^2 value closer to 1 will verify the additive effects of cobalt nanoparticles in a co-dosed digester. The Equation; $y = 1.9398x + 58.843$ can be used to predict the difference in biogas production between both NPs digester and Ni NPs based on the difference between the control and the Co NPs.

4.2.1.6.2 Effect of nickel nanoparticles in a co-dosed digester

This was obtained by comparing the difference in the biogas profile (time of experiment and amount of biogas) between Both NPs digester and Co NPs, i.e. (Both NPs-Co NPs) and that of control and Ni dosed digester (Control-Ni NPs). The first gives the biogas volume in the presence of Ni NPs while the latter gives the biogas volume in the absence of Ni NPs. The sum of the

differences is expected to be around zero. The sum of the differences was found to be high throughout the time of the experiment because after day 4 all the differences were positive. The Ni NPs (0.58 mg/l) only digester did not stimulate biogas production when compared with the control.

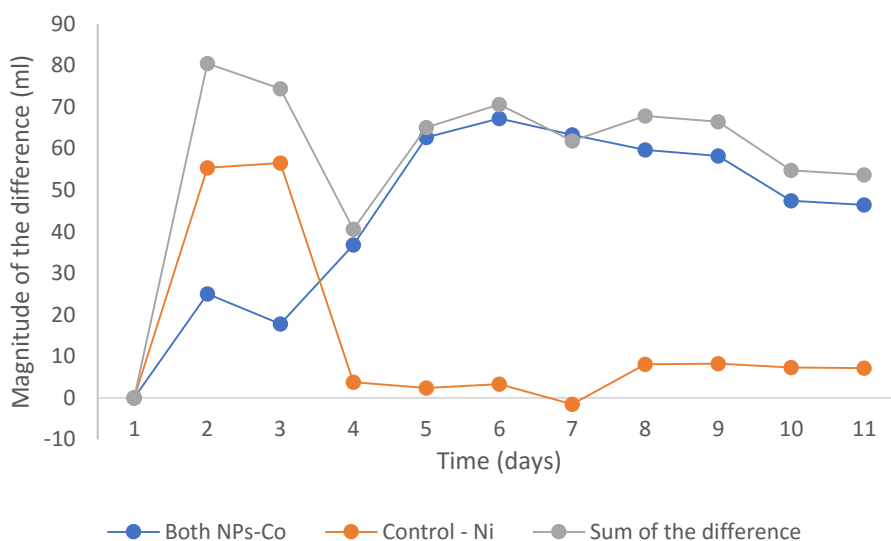


Figure 4.13 Magnitude of the difference of the control and Ni NPs dosed digester, both NPs and Co NPs digester and the sum of the difference.

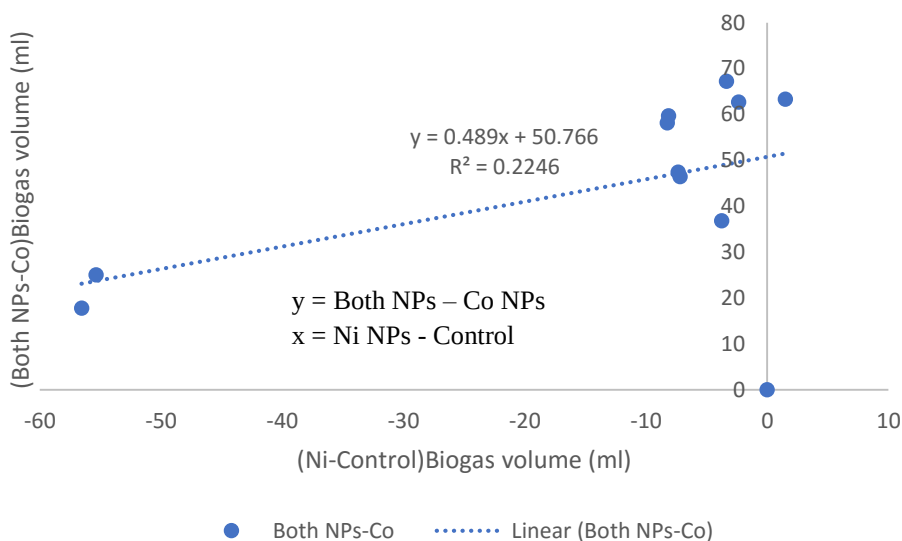


Figure 4.14 A line fit plot of the linear regression that correlates the effect of Ni in both NPs dosed digester and Ni dosed digester.

Figure 4.14 presents the linear regression computed to verify the effects of Ni NPs in a co-dosed digester. An R^2 value of 0.2236 was computed, lower than that of the effect of Co NPs (0.8537) in a co-dosed digester. This shows that the effects of cobalt nanoparticles in a co-dosed digester are higher than that of nickel nanoparticles at the concentration used (0.58 mg/l). The Equation; $y = 0.489x + 50.766$ can be used to predict the difference in biogas production between both NPs digester and Co NPs based on the difference between the control and the Ni NPs digester. The Ni NPs only digester produced less cumulative biogas than the control digester hence the negative x-axis (Ni-Control) values obtained as observed in Figure 4.14.

4.2.2 Dosage of 2 mg/L of Ni, Ni & Co, Co nanoparticles to the digesters

4.2.2.1 Cumulative methane and biogas production

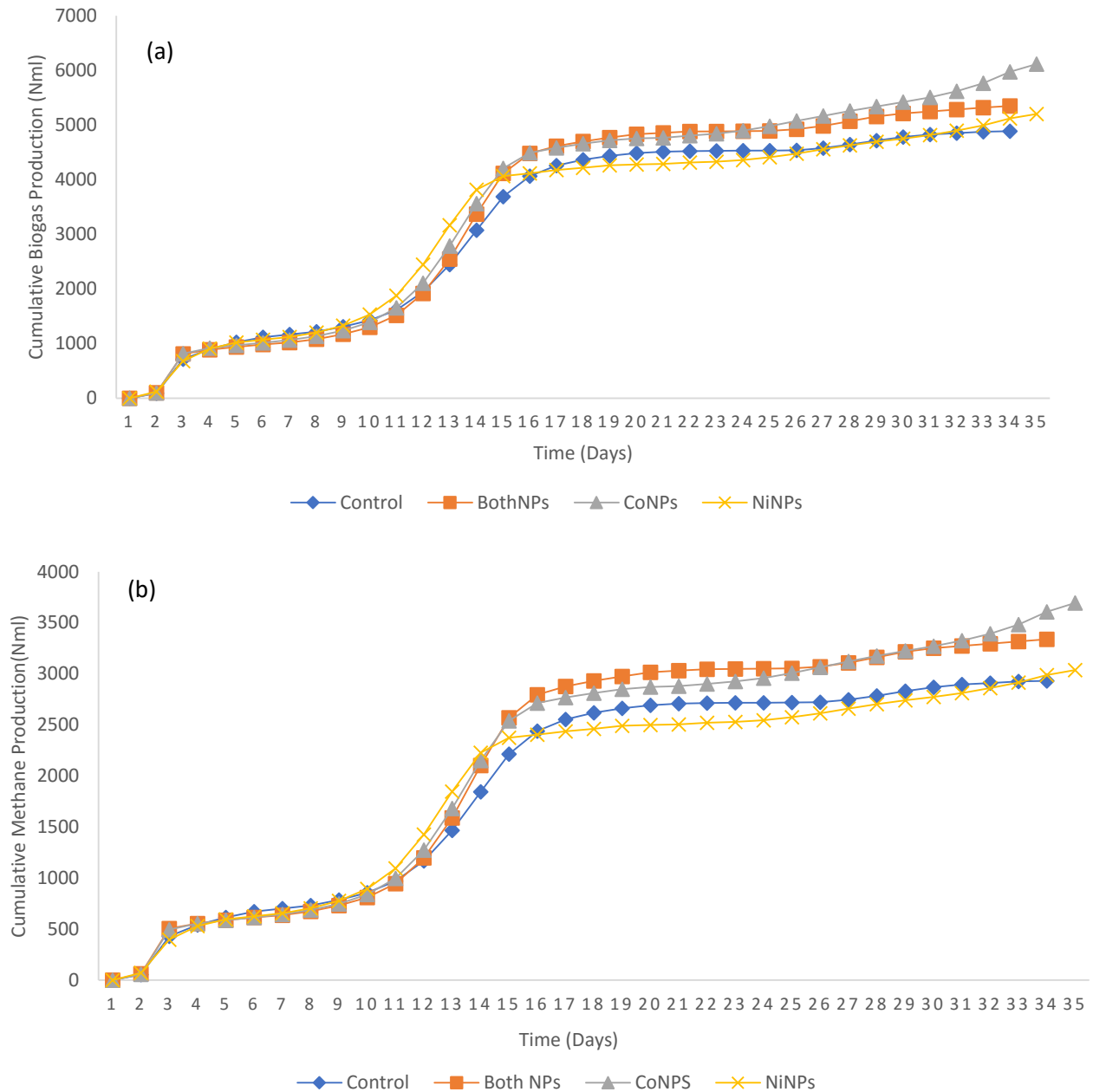


Figure 4.15 Performance of digesters fed with different Nanoparticles (a = cumulative biogas volume, b = cumulative methane volume)

Cumulative methane and biogas production is shown on Figure 4.15. One digester was dosed with Ni nanoparticles only, the other with both Ni & Co nanoparticles, another with Co nanoparticles and the last one was the Control (no NPs added). To determine the methane percentage in the biogas produced, ml of biogas was sampled from the tube connecting the digesters and NaOH bottles and bubbled through the handheld gas meter. This was done in duplicates. The curves had a gradual increase for the first three days of the experiment, followed by a steady increase until day 10. Another gradual increase from day 11 was witnessed which continued for 5 days. On day 17 until the last day of the experiment all the digesters showed a steady increase. From day 1 until day 10 there was not much of a difference in the methane and biogas production between the control and the Nanoparticles dosed digesters. From day 11 until day 14 the Ni nanoparticles dosed digester produced the highest methane and biogas as compared to the other three digesters. On day 15 the Co and Ni & Co NPs digesters produced the highest methane and biogas as compared to control and Ni NPs dosed digesters. They continued producing high volume until the end of the experiment. On day 25 cobalt NPs dosed digester exceeded the digester dosed with both Co & Ni NPs. On day 24 of the experiment the production stopped for both the control and the digester dosed with both Co & Ni nanoparticles. There are several possible reasons this could have happened such as accumulation of VFAs. The activity of volatile fatty acids consuming methanogens will be reduced (Gerardi, 2003), due to the pH drop (Ek *et al.*, 2011). The system will then crash due to the toxicity to methanogens caused by accumulation of VFAs (Pereira, 2005; Ek *et al.*, 2011).

4.2.2.2 Daily methane and biogas production

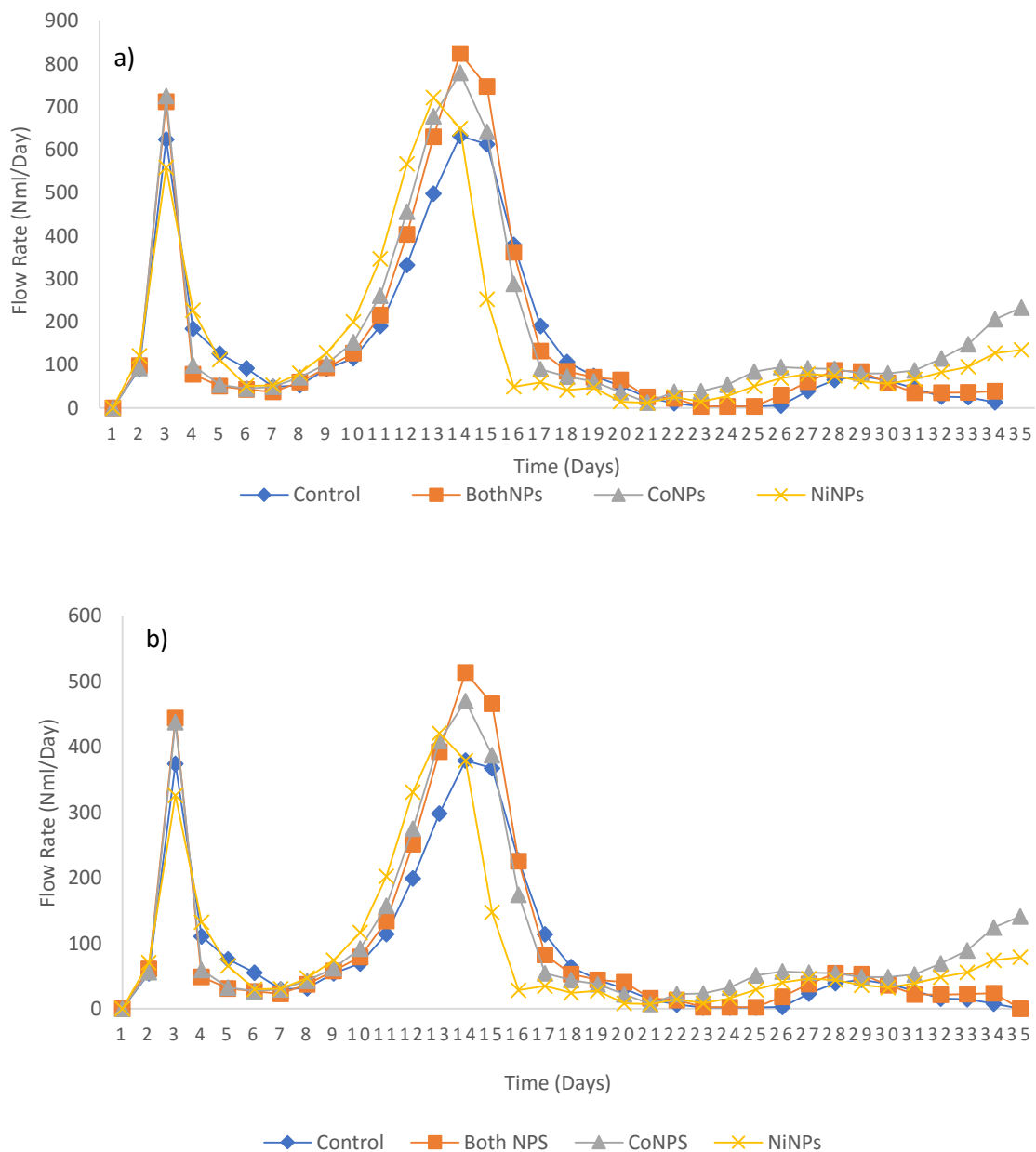


Figure 4.16 Daily methane and biogas production (a= biogas, b= methane).

Figure 4.16 depicts the daily methane and biogas production of the four digesters. Production started on the first day of the experiment for all the four digesters, with the control producing 54.5 and 90.9 ± 2.5 Nml of CH_4 and biogas, respectively. The control production increased on the next day to reach 374.1 (methane) and 623.8 ± 17.2 Nml (biogas); it then continued dropping until the

8th day of the experiment. The production then increased gradually on the 9th day until it reached its highest peak on day 14 of 379.4 and 632.7 ± 17.5 Nml of methane and biogas production, respectively. The control reached its lowest daily production on days 24, 25 and 26 of 1.9 and 3.2 Nml of methane and biogas, respectively. On the first day the Ni NPs dosed digester produced 70.5 (methane) and 120.8 ± 1.2 (biogas) Nml, this shows an increased production as compared with control. The Ni nanoparticles dosed digester reached its peak on day 13, a day before the control reached its peak. On its peak day, the production was 421.3 Nml for methane and 721.8 ± 7.1 Nml for biogas. This shows an increase of 41.9 (methane) and 89 (biogas) Nml on the peak of the control digester. On day 21 the Ni nanoparticles dosed digester produced 12.0 ± 0.1 Nml of biogas and 7 Nml of methane, these were recorded as the lowest productions of this digester. From day 22 onwards the production increased until the last day of the experiment. The 2 mg/l Ni Nanoparticles reduced the time to reach the peak of the control digester and the start-up production of both methane and biogas production, this is in accordance with Abdelsalam *et al.* (2016). On their research the Ni Nanoparticles reduced the time to reach the peak by nine days when dosed during anaerobic digestion fresh raw manure under mesophilic conditions (Abdelsalam *et al.*, 2016).

On the first day Co nanoparticles dosed digester produced 55.9 and 92.6 ± 1.2 Nml of methane and biogas, respectively. This indicates an increase of only 1.4 Nml in methane production as compared with the control. This reactor reached its peak on day 14 of 470.3 and 779.1 ± 21.5 Nml for methane and biogas, respectively. Although it reached its peak on the same day with the control digester there was an increase in both methane and biogas peaks by 90.9 and 146.4 Nml, respectively. The production of the Co NPs dosed digester reduced gradually after its peak for about 8 days until it reached its lowest recorded daily production of 7.6 and 12.6 ± Nml of methane and biogas, respectively. On day 22 the production increased steadily until the last day. From this day onwards the Co nanoparticles dosed digester recorded the highest daily methane and biogas production as compared with the control, Ni NPs and the Ni & Co NPs digesters. It also recorded the highest daily peak as compared with Ni nanoparticles dosed digester with a difference of 49 and 57.3 Nml of methane and biogas, respectively. On the last day of the experiment there was biogas production only in the Co and Ni nanoparticles dosed digesters.

The Ni & Co NPs dosed digester production increased from the first day to the second day where it reached a production of 444.1 and 712.1 ± 2.7 Nml of methane and biogas, respectively. On day 3 the production of both methane and biogas decreased until day 7. On day 8 the production increased until day 14 when the digester reached its highest peak of 513.7(CH₄) and 823.7 ± 3.2 (biogas) Nml. This peak production increased the peak of the control for both methane and biogas by 134.3 and 191 Nml, respectively. On day 15 the production decreased until it reached its daily lowest production on day 24, this production remained constant for the next 2 days (25th and 26th day). The production then increased for the next 4 days, from which it then decreased until it stopped on day 34. The decrease in daily biogas production across all digesters in day 4 to day 7 shows accumulation of VFAs which leads to instabilities and toxic conditions in the reactor hence low production of biogas (Mayer *et al.*, 2010; Siegert *et al.*, 2005). Uptake of the volatile fatty acids by methanogens will then increase the biogas production. As methanogens convert volatile fatty acids to methane alkalinity is produced (Spanjers and Van Lier, 2006; Pereira *et al.*, 2013) hence digester stability.

4.2.2.3 Effects of Ni and Co nanoparticles in total methane and biogas production

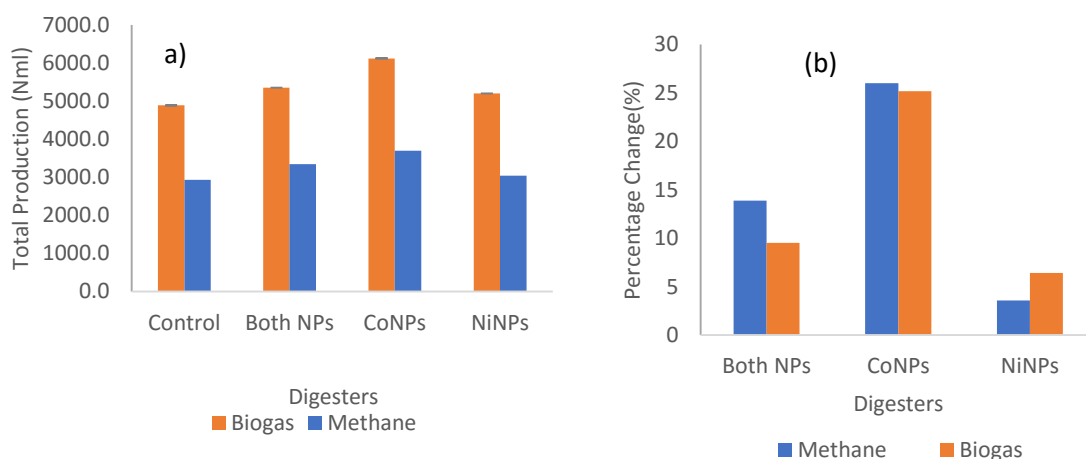


Figure 4.17 Total methane and biogas production and percentage change in Nanoparticles dosed digesters as compared with the control (a= total methane and biogas volume, b= percentage change).

The control digester produced the least total methane and biogas of 2931.2 and 4887.9 ± 4.6 Nml, respectively. The Co NPs dosed digester produced the highest methane and biogas as compared

with the control, Ni and Co nanoparticles dosed digester and Ni NPs only digesters. The Co nanoparticles dosed digester produced 3693.8 and 6118.8 ± 4.6 Nml of methane and biogas, respectively. An increase of the production of the control digester by 26 and 25 % for methane and biogas, respectively. Addition of NPs to anaerobic digesters increases degradation rates of the VS and increases the production of biogas and methane (Hassanein *et al.*, 2019). These results do not agree with the research of Abdelsalam *et al.* (2017). From this research it shows that dosage of 2 mg/l of Cobalt Nanoparticles ($< 28\text{nm}$) when digesting raw manure under mesophilic conditions (37°C) led to the inhibition of biogas and CH_4 production. The CH_4 and biogas produced were 5% and 12 % less than what the control produced. Ni *et al.* (2013) reported that although the Co NPs may be toxic at the initial stages of anaerobic digestion, they become non-toxic during long term contact with the substrate. Hassanein *et al.* (2019) reported that addition of 1.4 mg/l Co NPs (30-80.9 nm) during digestion of poultry litter under mesophilic conditions leads to an increase in CH_4 production by 29 % as compared with the control. Feng *et al.* (2010) indicated that dosing of 0.06 – 6 mg/l of Co increased the CH_4 yield by 7- 15 %, this agrees with my experimental results as 2 mg/l was found stimulatory to CH_4 production. Lo *et al.* (2012) also reported that concentrations of Co between 0.148-5.362 mg/l was stimulatory during AD of organic fraction of municipal solid waste.

The digester dosed with Ni & Co nanoparticles produced 5353.1 ± 0.4 and 3338.5 Nml of total biogas and methane respectively. This was the second highest production after the Co NPs dosed digester. The digester containing both NPs increased the total methane and biogas by 14 and 10 % above the control digester, respectively. The Ni NPs dosed digester increased the total methane and biogas by 4 and 6 % above the control digester, respectively. Of all the three digesters compared with control the Ni nanoparticles dosed digester became the one with the least increments when compared with the control. These results are in line with the observation made by Wu, Yang and Tian, (2011), that a concentration of Ni NPs between 0.5- 16 mg/l is stimulatory to biogas production. Abdelsalam *et al.* (2017) shows that an addition of 2 mg/l Ni NPs (17nm) improved the CH_4 and biogas by 116.76 and 78.53 % when compared with the control. These results also agree with Demirel and Scherer, (2011) who reported that stimulatory concentrations of Ni ranges between 0.013 and 5 mg/l for batch cultures of methanogens. These results do not agree with Zhang, (2003) who reported that the inhibition limit of the Ni NPs is 1.2 mg/l. Bozym *et al.* (2015) reported that addition of Ni NPs improved gas production and the inhibition limit for

Ni as 10 mg/l, this is in line with the results obtained in this study. In this study only a slight difference was observed on the daily production. The reason behind this may be the difference in the composition of substrates digested. The amount of toxic material in a substrate (Bozym *et al.*, 2015), size of the nanoparticles used (Abdelsalam *et al.*, 2016) have an influence in the performance of NPs. Addition of 0.5 – 2 mg/l of Ni NPs (>20 nm) to anaerobic digesters of dairy manure under mesophilic conditions promotes CH₄ yield by 55- 101% (Abdelsalam *et al.*, 2017; Hassanein *et al.*, 2019). Lo *et al.* (2012) also indicated that concentrations between 0.801 to 5.362 mg/l are stimulatory during AD of organic fraction of municipal solid waste. Reactor configuration affects the effectiveness of the NPs. Hydraulic retention time also may be a factor, as it has already been established from literature that NPs are not toxic in longer retention times, hence favouring experiments with longer HRTs (Ni *et al.*, 2013).

4.2.2.4 Volatile Solids and their relationship with nanoparticles concentration and Biomethane Potential

Biomethane potential is a test that assesses the potential for methane and biogas volume from a sample. It evaluates the ability of a substrate to produce biogas and methane (Hollinger *et al.*, 2016). Here the effects of NP addition on BMP were assessed. Different BMPs of the four digesters control, Co NPs, Ni NPs, and both NPs are presented in Figure 4.18(b). The control had the lowest BMP of 40.78 ml/g of VS. The Co NPs dosed digester had the highest BMP of 51.4 ml/g of VS, an increase of 26.0 % as compared to the control digester. These results are in line with those of Zhang *et al.* (2015) who reported an increase of 12.4 % in the BMP due to dosing of 1 m/l of Co to anaerobic digestion of food waste. Zaidi *et al.*, (2018) also reported that addition of 1 mg/l of Co NPs during anaerobic digestion of algal biomass increased the BMP by 28 %. The Ni nanoparticles dosed digesters recorded 46.45 ml/g of VS (13.9 % increase from the control) while the digester with both NPs recorded 42.23 ml/g of VS (3 % increase from the control).

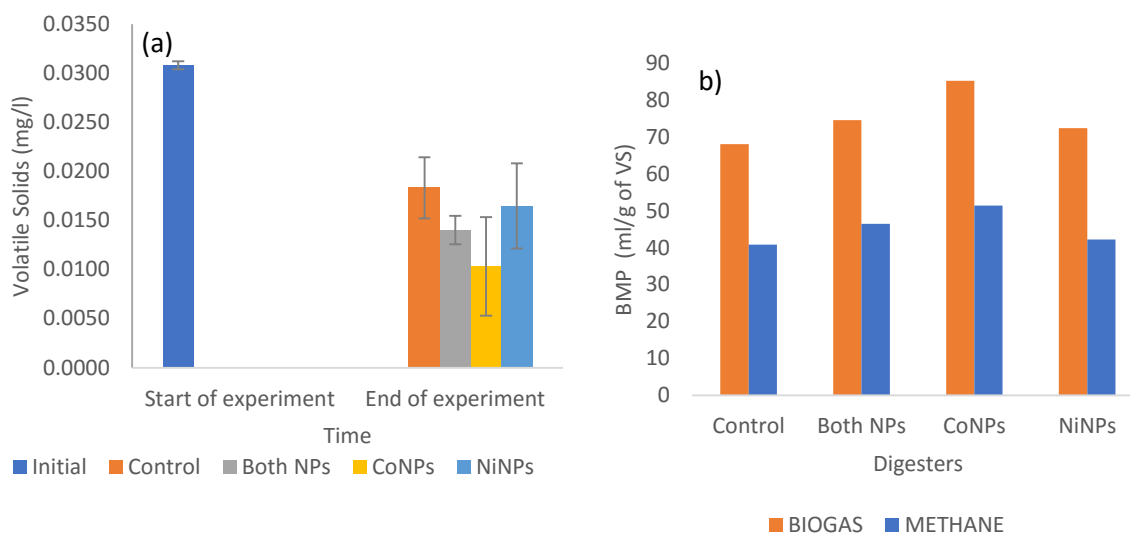


Figure 4.18 Volatile Solids from the start and at the end of the experiment and the biomethane potential (a= Volatile Solids and b= Biomethane Potential).

The volatile solids are needed in anaerobic digestion because they can be used as a monitoring tool. They represent the digestible part of the sample, as they get depleted biogas is produced (Mitchell *et al.*, 2015). Since this was a batch experiment the VS were determined at the start and at the end of the experiment as depicted in Figure 4.18. Before the experiment, the VS contained in the sample were 0.0308 ± 0.0004 mg/l. The control degraded 40.5 % of the volatile solids and remained with 0.0183 ± 0.0031 mg/l. The control digester produced the least biogas and methane that is why it had the lowest degradation of the initial VS. The Co NPs dosed digester recorded 0.0103 ± 0.0005 mg/l, it degraded 60.0 % of the initial VS and 20% more than the control. The Co NPs dosed digester degraded the highest percentage of VS hence the highest production of biogas. The Ni nanoparticles dosed digesters degraded 46.5 % and both NPs degraded 54.5 % i.e., 6 and 13 % more than the control, respectively. Both NPs digester had the second highest degradation after the Co NPs digester hence the second highest biogas production.

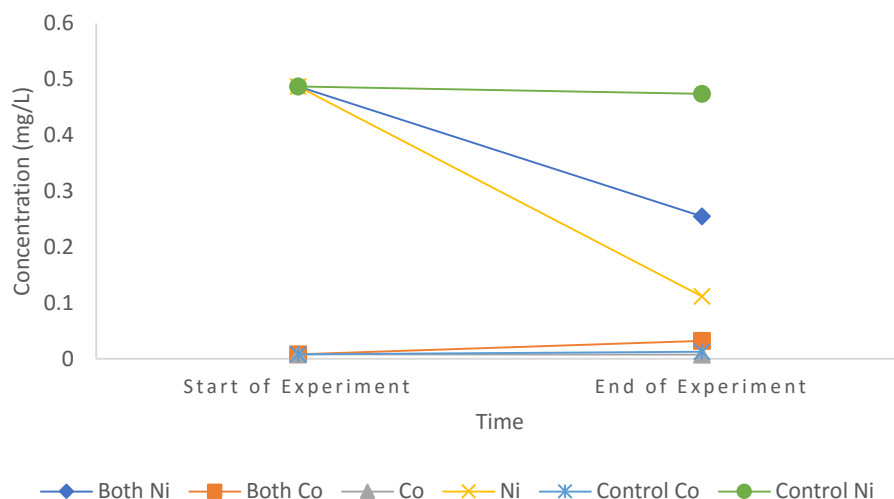


Figure 4.19 Initial and Final concentration of TEs in the dosed digesters.

The relationship between VS and TEs is very important in anaerobic digestion because it reflects the interaction of TEs and microorganisms (Ditalelo, 2017). A stimulatory concentration can be attributed to a high association between microorganisms and trace elements. Initially the abattoir effluent had a concentration (of trace elements) of 0.00786 mg/l of Co and 0.4868 mg/l of Ni. One digester was dosed with 2 mg/l of Ni NPs, another with 2 mg/l of Co NPs and another with 2 mg/l both Co and Ni NPs. It was expected that when they react with material they will disintegrate and release metal ions (Co^{2+} and Ni^{2+}) and the final concentration of Ni and Co will increase (Hassanein *et al.*, 2019). The final concentration of Ni and Co will not increase if the nanoparticles do not release ions. The Co dosed digester produced the highest biogas and methane as compared with the digester dosed with Ni NPs. This could show that Ni is needed in high concentrations as compared with Co, also that Co is more stimulatory than Ni at lower concentrations (Abdelsalam *et al.*, 2017). Figure 4.19 shows the initial and last concentration of TEs in the dosed digesters. At the end of the experiment the Co NPs dosed digester had 0.008 mg/l, the Ni had 0.11 mg/l and both NPs had 0.25 mg/l of Ni and 0.032 mg/l of Co. Only the Co concentration in the digester dosed with both NPs increased by 305% as compared with the initial concentration. At the end of the anaerobic digestion process not all NPs will be converted, some will still be intact (Dehaghi *et al.*, 2019). Because of other possible mechanisms involved in anaerobic digestion process, the NPs supplemented might not release ions but still affect the biogas produced (Wang *et al.*, 2019).

4.2.2.5 Comparison of the effect of the sum of the individual and combined supplementation of Co and Ni NPs

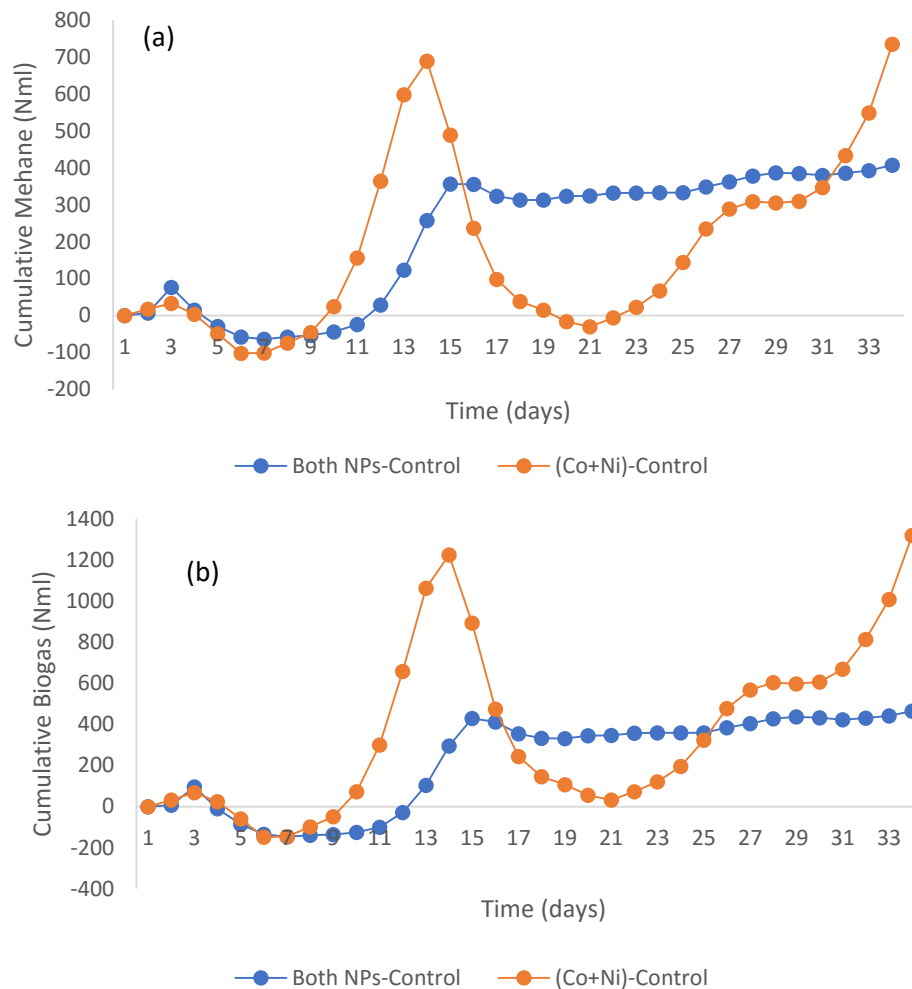


Figure 4.20 Comparison of the sum of the individual effect of Ni and Co NPs dosage to that of their combined dosage (a= methane, b= biogas).

To test the additive effects of Ni and Co nanoparticles the sum of the effects of individual dosage ((Ni + Co)-Control) were compared with the effects of their combined dosage (both NPs – Control). This was done to test the equality of the sum of the biogas and methane volume by which individual Ni & Co NPs dosed digesters exceeds the control to that by which both NPs digester exceeds the control. Cumulative CH₄ and biogas were used in this part of analysis. From day 2 to day 8 the control produced more biogas than the Ni nanoparticles dosed digester hence the negative effect observed on Figure 4.20 (a and b). This was also observed from day 15 until 32. This is what

caused the fluctuations observed in the (Ni + Co) – Control graph. Linear regression analysis gave a coefficient of determination of 0.3155 and 0.252 for biogas and methane, respectively. Throughout the experiment the two did not produce equal amounts of biogas and methane. From previous research it was shown that the two produce almost the same amount of biogas even though by then they were dosed as dissolved salts as compared to Nanoparticles. Ditalelo, (2017) reported that the additive nature of the effect of Ni & Co was held true throughout the experiment during digestion of acetate. He found a coefficient of determination (R^2) of 0.998 during daily dosage of 2.5 μ M. This does not agree with the experimental results obtained reasons may be due to the differences in the form in which the micronutrients were dosed in. The type of experiment may also be a factor Ditalelo, (2017) ran a continuous system while here experiments were conducted in batch mode.

4.2.2.6 Effects of nanoparticles (Ni and Co) in a co-dosed digester

The effects of co-dosage and individual dosage of nickel and cobalt nanoparticles are compared here. The analysis for the comparison is like the one used in Chapter 4.2.1.6.2. Linear regressions were also computed to verify the additive effects of the nanoparticles.

4.2.2.6.1 Effect of cobalt nanoparticles in a co-dosed digester

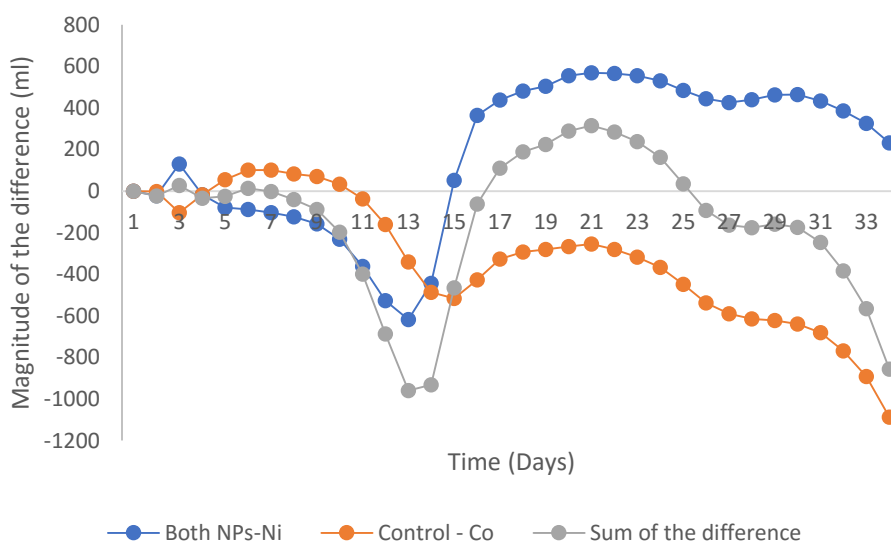


Figure 4.21 Magnitude of the difference of the control and Co NPs dosed digester, both NPs and Ni NPs digester and the sum of the difference.

The magnitude of the difference of the two pairs was not similar throughout the experiment hence the sum of the differences is not close to zero. From Figure 4.15 it can be observed that there is no clear difference in the cumulative methane and biogas production of the four digesters (control, Ni NPs, Co NPs and both NPs). From day 4 to day 14 the Ni NPs only digester had more cumulative biogas than both NPs digester. From day 4 to day 10 the cumulative biogas of the control was higher than that of Co NPs digester. Due to these reasons the magnitude of the difference of the two pairs was not similar for the 34 days of HRT.

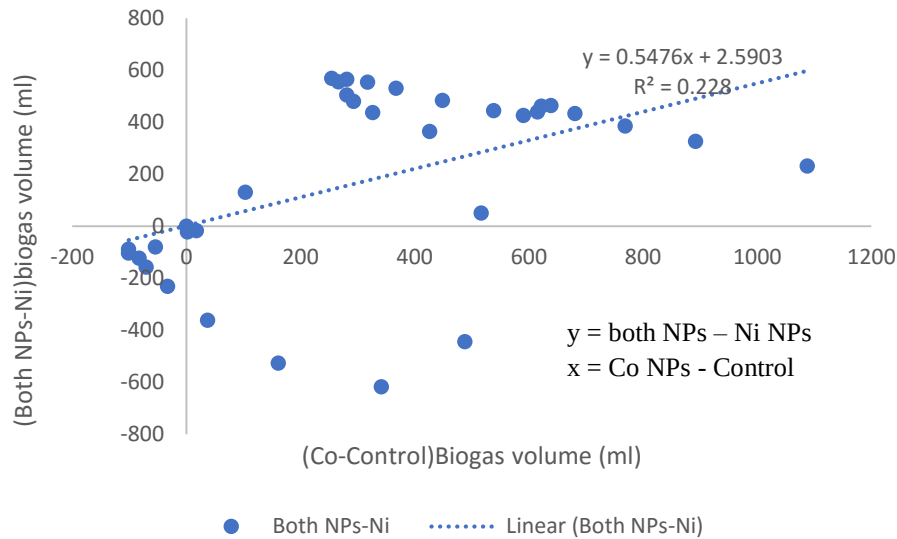


Figure 4.22 A line fit plot of the linear regression that correlates the effect of Co in both NPs dosed digester and Co dosed digester.

Figure 4.22 shows linear regression that correlates the effect of Co nanoparticles in both NPs dosed digester and Co NPs only dosed digester. This linear regression was computed to verify the additive effects of Co NPs in co-dosed digester. The linear regression gave an R^2 value of 0.228 which is lower than 1. This shows that the effects of Co NPs when dosed alone and co-dosed with Ni nanoparticles are not the same. This is because on some days Co NPs were not stimulatory to biogas production as compared with the control. From day 4 to day 10 the cumulative biogas of the control was higher than that of Co NPs digester. To predict the difference in biogas production between both NPs digester and Ni NPs based on the difference between the control and the Co NPs the Equation; $y = 0.5476x + 2.5903$ can be used.

4.2.2.6.2 Effect of nickel nanoparticles in a co-dosed digester

This was also computed as it was at 0.58 mg/l. The sum of the differences was also not close to zero or zero throughout the time of the experiment. The two differences were found to be not similar throughout the experiment. The magnitude of the differences is almost equal at each point and the sum of the differences being almost zero or zero at each point during anaerobic digestion of acetate supplemented with 2.5 μ M of Co and Ni (Ditalelo, 2017). He also found a R^2 value of 0.998 which is almost 1. This proved that the effect of Ni in a Ni only digester and digester dosed with Ni and Co was almost the same and that the effects of Ni can be isolated from those of Co when they are co-dosed.

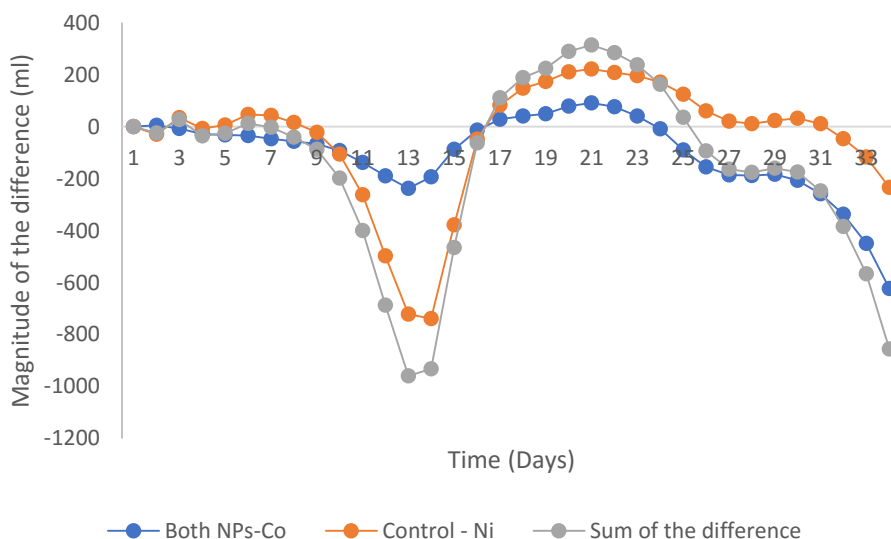


Figure 4.23 Magnitude of the difference of the control and Ni NPs dosed digester, both NPs and Co nanoparticles digester and the sum of the difference.

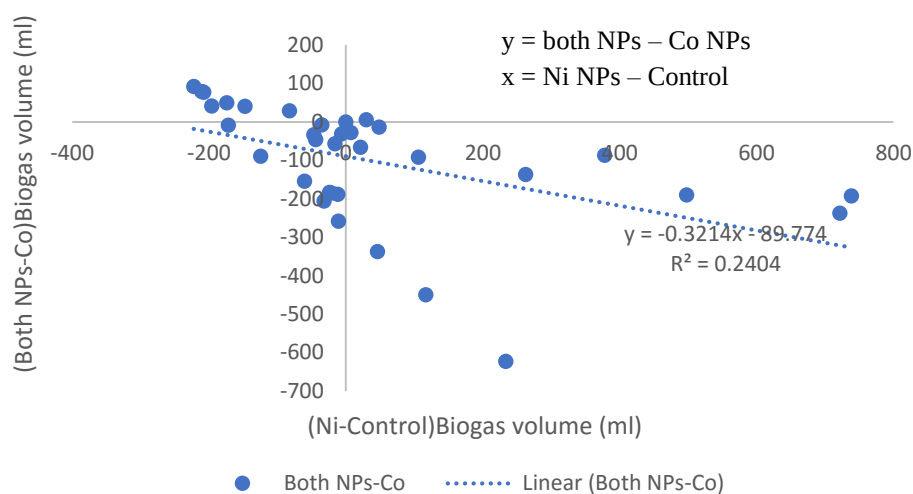


Figure 4.24 A line fit plot of the linear regression that correlates the effect of Ni in both NPs dosed digester and Ni dosed digester.

Figure 4.24 shows a linear regression that correlates the effect of Ni NPs in both NPs dosed digester and Ni NPs dosed digester. An R^2 value of 0.2404 was found which is less than 1. An R^2 value close to one will verify that effects of Ni NPs in a Ni NPs only and both NPs digesters is almost the same. The Equation; $y = 0.31214x - 89.774$ can be used to predict the difference in biogas production between both NPs digester and Co NPs based on the difference between the non-dosed digester (control) and the Ni NPs. The Co NPs only digester produced more biogas (cumulative) than both NPs digester and that is why the biogas volume (y-axis) in Figure 4.24 is mostly recorded negative. Co NPs are more stimulatory at lower concentrations than Ni NPs (Abdelsalam *et al.*, 2017).

4.2.3 Dosage of 11mg/L of Ni, Ni &Co, Co nanoparticles to the 250 ml serum bottles

4.2.3.1 Cumulative methane and biogas production

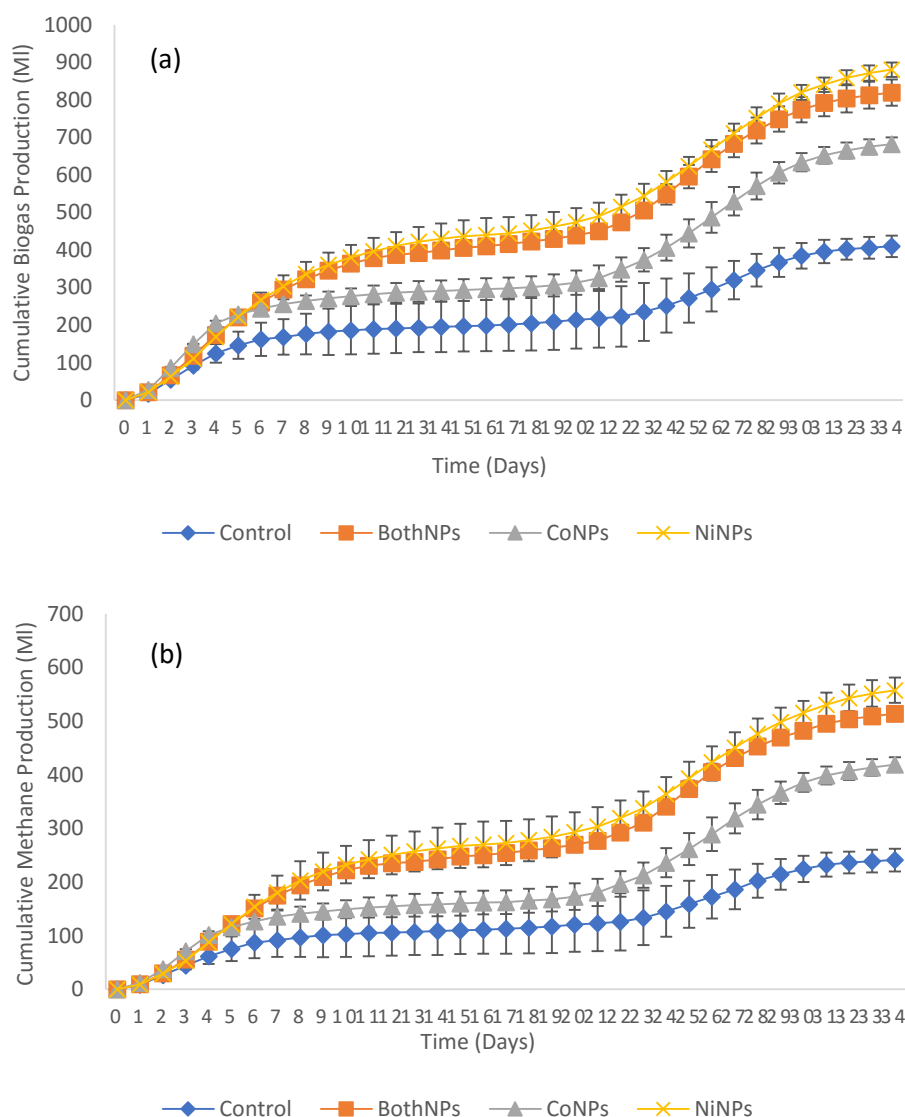


Figure 4.25 Performance of digesters fed with different Nanoparticles (a = cumulative biogas volume, b = cumulative methane volume).

The Figure 4.25 (a and b) shows average results of the 8 serum bottles used in the experiment. Their individual results are presented in appendix A. For measurement of methane in the biogas 1 ml of the biogas was sampled and bubbled through a gas meter. This was done before the gas was released into the inverted measuring cylinder. There was a gradual increase in both the CH₄ and biogas production from day 1 until day 6 for all of the serum bottles. From day 5 the daily

production decreases, which is the part where it shows steady increase in the cumulative production. During this decrease it shows that VFAs have accumulated in the reactor. It was expected that the different NPs will recover the digesters and increase biogas production. The production increased on day 18 until day 25 of the experiment. From the high cumulative methane and biogas production of all digesters fed with NPs, it shows they were able to help with the digestion of VFAs hence preventing acidification of the reactors. The production from the bottles dosed with the Ni NPs was the highest from day 6 until the end of the experiment as seen on Figure 4.25 (a and b). The serum bottles dosed with both NPs had the second highest cumulative methane. This does not agree with the information found from literature which indicates that due to their synergistic effects when co-dosed, they produce more biogas than their individual dosage (Fermoso, 2009; Rousell, 2013; Ditalelo, 2017). From the start of the experiment to the end the control had the lowest production of both daily methane and biogas.

4.2.3.2 Daily methane and biogas production

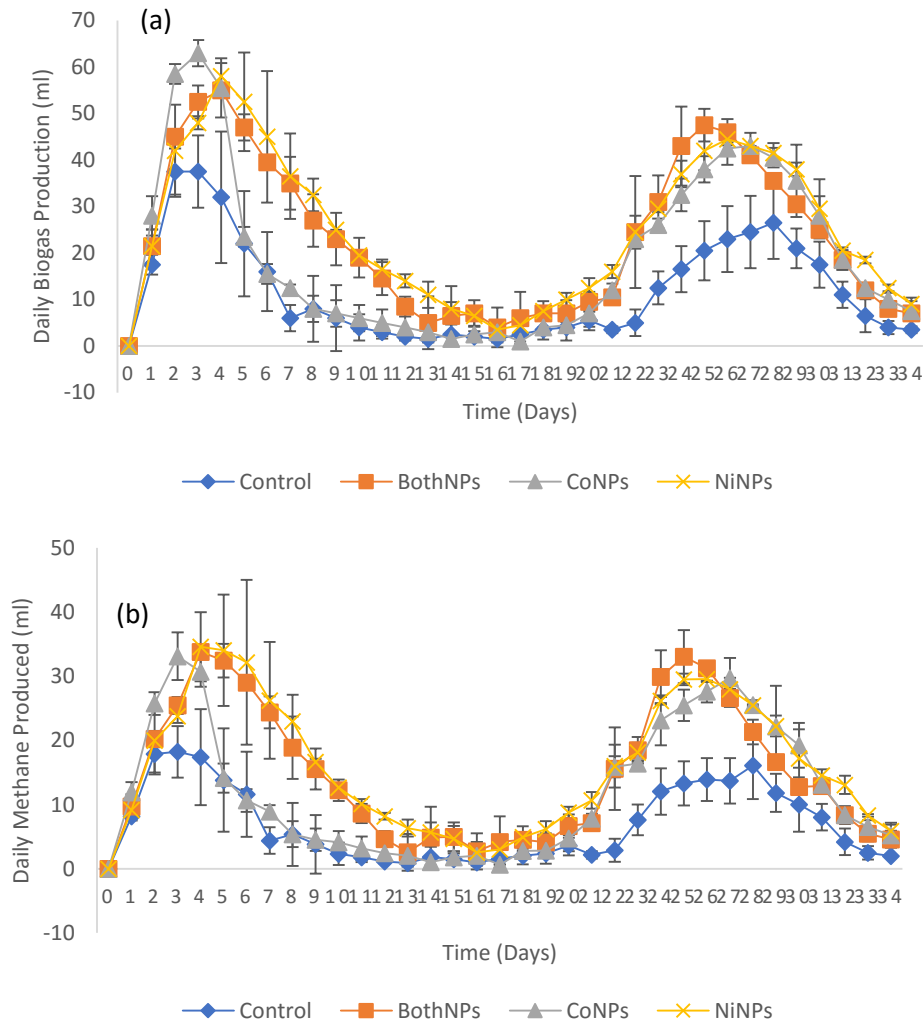


Figure 4.26 Daily methane and biogas production (a= biogas, b= methane).

Figure 4.26 (a and b) depicts the daily production of biogas and CH_4 , respectively. On the first day the control produced an average of 17.5 ml with a standard deviation of ± 2.12 ml. CH_4 occupied 46.66 % of the biogas produced which is 8.165 ± 0.007 ml. For the first four days the methane percentage of the control digesters was less than 50 %. On the 5th day it passed 50 %. The highest recorded percentage of methane in the control was 73% recorded on day 15 and 24. Two peaks were observed on day 2 and 28. On day 2 the biogas produced was 37.5 ± 4.95 ml, the CH_4 produced on this day was 17.865 ± 3.15 ml (73 % of the biogas produced). The same amount of biogas was produced on the 3rd day, the daily production reduced from this day until it reached its

all-time low of 0.87 ± 0.52 ml on day 13. Day 7 to day 22 the production was less than 10 ml; this is the time where the VFAs have accumulated causing instability in the reactor hence low biogas production (Diaz, 2010). Accumulation of VFAs can be a result of deficiency of micronutrients in the digester (Rousell *et al.*, 2015; Nordell *et al.*, 2016). Addition of micronutrients will reduce VFAs accumulation. Nordell *et al.* (2016) reported a reduced concentration of VFAs during anaerobic digestion of dairy and swine manure with 0.06 and 0.51 mg/kg of Co and Ni, respectively. On the day, the control serum bottles reached their second peak the dosed serum bottles had already reached their second peak. Both the peaks the control reached were the lowest recorded as compared with the dosed serum bottles. On the first day the Co NPs dosed serum bottles produced 11.885 ± 1.6 and 28 ± 4.24 ml of CH₄ and biogas, respectively. It reached its peak on the 3rd day, and it was the highest peak recorded. It produced 10.5 and 3.72 ml of biogas and methane, respectively more than what the control produced on this day. The CH₄ produced occupied 44 % of the biogas produced on peak day, for the first three days of the experiment the percentage of CH₄ in the biogas produced was less than 45 %. After the peak day, the daily production reduced until it reached its lowest production on day 16 of 1 ml for biogas and 0.65 ± 0.014 ml for CH₄. After day 17 the daily production gradually increased until it reached its second peak on day 26 (Figure 4.26 a). This peak was reached 2 days before the control reached its peak. For this second peak the CH₄ and biogas produced were 27.6 ± 1.7 and 42.5 ± 3.53 ml, respectively. Despite the biogas peak being reached on day 26 the CH₄ peak was reached on day 27 (Figure 4.26 b). This was because on day 26 (65%) the percentage of CH₄ produced was lower than that of day 27 (69%). This second peak was not higher than its first peak as the gas produced on the first peak was 33.125 ± 3.71 ml (CH₄) and 63 ± 2.83 ml (biogas). As depicted by Figure 4.26 (a) the biogas of the Co nanoparticles dosed bottles was the highest of all the digesters but due to a low methane percentage in the gas produced, the methane peak is not the highest.

The Ni nanoparticles dosed serum bottles produced two peaks as shown in Figure 4.26 (a and b). The digesters reached their first peak on day 4. The methane produced on this day was 59.7 % of the biogas produced. For the first three days the methane percentage was less than 50 %. The production on this day was 58 ± 2.828 and 34.6 ± 5.37 ml of biogas and CH₄, respectively. On this day, the control produced 17.39 ± 7.48 and 32 ± 14.14 ml of CH₄ and biogas, respectively. This shows an increase of 98.96 % (CH₄) and 81.25 % (biogas) above the control by the Ni NPs dosed serum bottles. The daily production then dropped from the 5th day until the 16th day where it

reached its lowest recorded gas production. The lowest recorded production was 3.5 ± 2.12 ml of biogas and 2.52 ± 1.67 of CH_4 . These digesters reached their second peak in biogas and methane production on day 26 of 44.5 ± 0.707 and 29.585 ± 0.474 ml, respectively. This peak was reached two days before the control digesters reached their peak (reduced lag-phase). Juntupally *et al.* (2017) reported a reduction in time to reach peak day by addition of Ni and Co. Ni reduced the lag phase by 3 days and Co by 2 days. This was during anaerobic digestion of cattle manure at 37°C . The micronutrients increase the rate of biogas production (Abdelsalam *et al.*, 2016; Juntupally *et al.*, 2017), that is the reason behind the control serum bottles having the least daily production of biogas as compared with the dosed serum bottles (throughout the 34 days). The serum bottles dosed with both NPs have two peaks, the first one on day 4 and the other on day 25. On the 4th day the production was 55 ± 1.41 ml for biogas and 33.8 ± 1.07 ml for CH_4 . On day 25 the production was 47.5 ± 2.828 and 33.075 ± 4.14 ml of biogas and methane, respectively. These peaks were the highest recorded as compared with other digesters. The second peak of these digesters reduced the lag-phase of the control by 3 days. The shape of the daily methane and biogas graph represents the changes in the parameters (pH, temperature, VFAs) in the digester that affect anaerobic digestion. The peaks represent the time when conditions are optimal and microorganisms are performing well hence high biogas production (Palatsi *et al.*, 2010; Mao *et al.*, 2015). The peaks also represent the exponential growth of microorganisms and increased methanogenic activity (Mao *et al.*, 2015). The low production of biogas between the two peaks shows the inhibition stage (Chen *et al.*, 2008, Palatsi *et al.*, 2010).

4.2.3.3 Effects of Ni and Co nanoparticles in total methane and biogas production

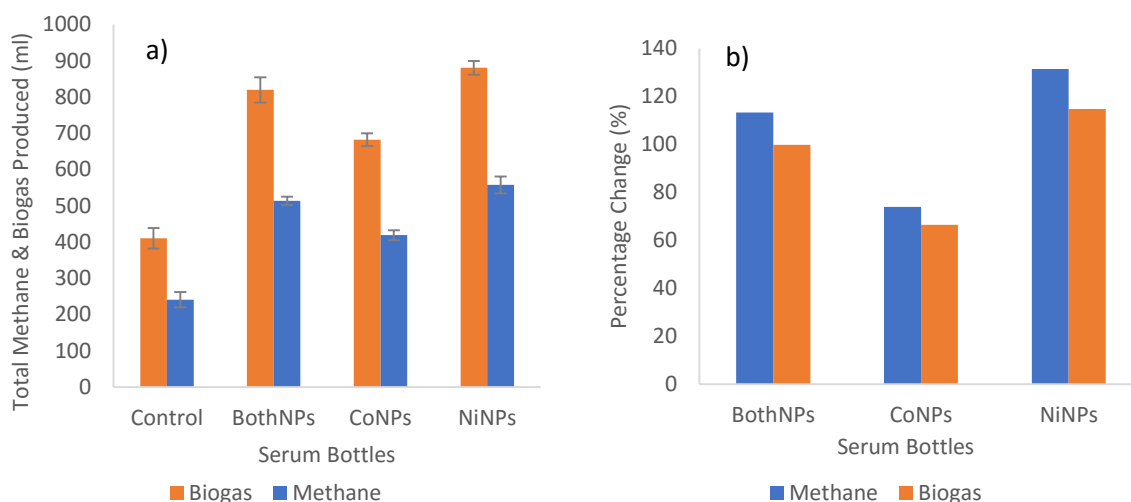


Figure 4.27 Total methane and biogas production and percentage change in Nanoparticles dosed digesters as compared with the control (a= total methane and biogas volume, b= percentage change).

The total biogas and CH₄ produced by the control during the 34 days of the experiment were 410 ± 28 and 241 ± 21 ml, respectively. The CH₄ produced was 58.78 % of the total biogas produced. The Co NPs dosed bottles produced 683 ± 18 and 419 ± 14 ml of biogas and CH₄, respectively. From the total biogas produced here 61.35 % is CH₄. The Co NPs dosed serum bottles (0.34 mg of Co NPs/g of VS) increased the methane and biogas by 73.9 and 66.46 % more than the control serum bottles, respectively. Co is used in the generation of methyl coenzyme M, which is needed to produce biogas (Fermoso, 2009; Ditalelo, 2017). This shows that Co is not directly involved in the last step of biogas production. The results do not agree with those of Garuti et al 2018 who reported that only a concentration between 0.024-10 mg/l of Co is stimulatory to CH₄ production. Also, Lo *et al.* (2012) reported that a concentration of 7.239 mg/l is inhibitory. The differences seen between results can be caused by several factors such as digester geometry, substrate origin and composition, operating conditions, and bioavailability of the supplemented NPs (Garuti *et al.*, 2018; Liu Wei and Leng, 2021). Hassanein *et al.* (2019) reported that a concentration of 0.30 mg of Co NPs/ g of VS increased the CH₄ production by 26 % more than the control. The serum bottles fed with both NPs produced total methane of 514 ± 12 ml, 113.28 % more than what the control produced. It also produced 820 ± 35 ml of biogas. The biogas produced is 99.88 % more than that

of the control and methane occupied 62.68 % of the biogas produced. These results do not agree with Lo *et al.* (2012) who effected that inhibitory concentration of Co ($\text{CoSO}_4 \cdot 6\text{H}_2\text{O}$) is 8.625 mg/l.

The Ni NPs dosed serum bottles produced the highest total CH_4 and methane more than other digesters. It produced 881 ± 19 and 557 ± 23 ml of biogas and CH_4 , respectively. Ni is involved in the reduction of methyl coenzyme M to produce biogas which is the last step in AD (Ditalelo, 2017). The CH_4 and biogas produced were 131.5 % and 114.4 % more of what the control produced. The CH_4 produced by the Ni NPs dosed bottles occupied 63.22 % of the total biogas. Figure 4.27(a) shows percentage change effected by the NPs added on both the total methane and biogas produced. As shown on Figure 4.27(b) the Ni NPs dosed bottles produced the highest total methane and biogas produced, followed by the bottles dosed with both nanoparticles. These results agree with those of Abdelsalam *et al.* (2017) and Hassanein *et al.* (2019) from which Ni dosed digesters had the highest biogas and CH_4 production, more than the Co and Fe dosed digesters. Ni NPs stimulate microbial bacteria by increasing key enzyme activities in the different anaerobic digestion steps such as acidification and methanogenesis (Abdelsalam *et al.*, 2017; Hassanein *et al.*, 2019). Altas, (2009) reported that stimulatory concentration of Ni to anaerobic digesters ranges from 0.5-16 mg/l. Hassanein *et al.* (2019) found that a concentration of 0.34 mg of Ni/g of VS was stimulatory during anaerobic digestion of poultry litter, as it produced 27.5 % CH_4 more than the control. This agrees with the results obtained in this experiment, the 11 mg/l dosed to the digesters is equivalent to 0.34 mg of Ni/g of VS. Zhang *et al.* (2015) and Xu *et al.* (2018) indicated that a stimulatory concentration for Ni ranges from 0.4-7 mg/l. The Co NPs dosed bottles were the least of the dosed digesters.

A paired two sample test for means at a 5% significance level ($\alpha = 0.05$) was performed to assess if the addition of Co and Ni nanoparticles had a significant impact in the total biogas and CH_4 production. A significant difference was observed between the mean CH_4 produced by the control (240.76) and the serum bottles fed with Ni NPs (513.51) with the t- statistics =11.84, $P(T \leq t) = 2.7 \times 10^{-2}$ and t-critical = 6.31. Also, a significant difference was observed between the biogas produced by the control (mean = 410) and Ni NPs (mean = 819.5) with the t- statistics =9.20, $P(T \leq t) = 3.45 \times 10^{-2}$ and t-critical = 6.31. A significant difference was also observed between the mean biogas produced by the digesters dosed with Co NPs (418.68) and the control (410), with the t- statistics =36.33, $P(T \leq t) = 8.76 \times 10^{-3}$ and t-critical = 6.31. Also, a significant difference in

the CH₄ production was observed between the digesters dosed with Co NPs (682.5) and the control (240.76), with the t- statistics =34.92, $P(T \leq t) = 9.11 \times 10^{-3}$ and t-critical = 6.31. A significant difference was observed between the mean CH₄ produced by the control (240.76) and the serum bottles fed with both NPs (557.37) with the t- statistics =181.44, $P(T \leq t) = 1.75 \times 10^{-3}$ and t-critical = 6.31. Also, a significant difference was observed between the biogas produced by the control (mean = 410) and both NPs (mean = 880.5) with the t- statistics =72.38, $P(T \leq t) = 4.4 \times 10^{-3}$ and t-critical = 6.31. The p-value of all the comparisons between the total methane and biogas of the control serum bottles and the NPs dosed serum bottles are less or equal to α (0.05). From this it can be concluded that addition of nanoparticles (Ni, Co and both Co and Ni NPs) had a significant impact on the biogas and methane production.

4.2.3.4 Volatile Solids and their relationship with nanoparticles concentration and Biomethane Potential

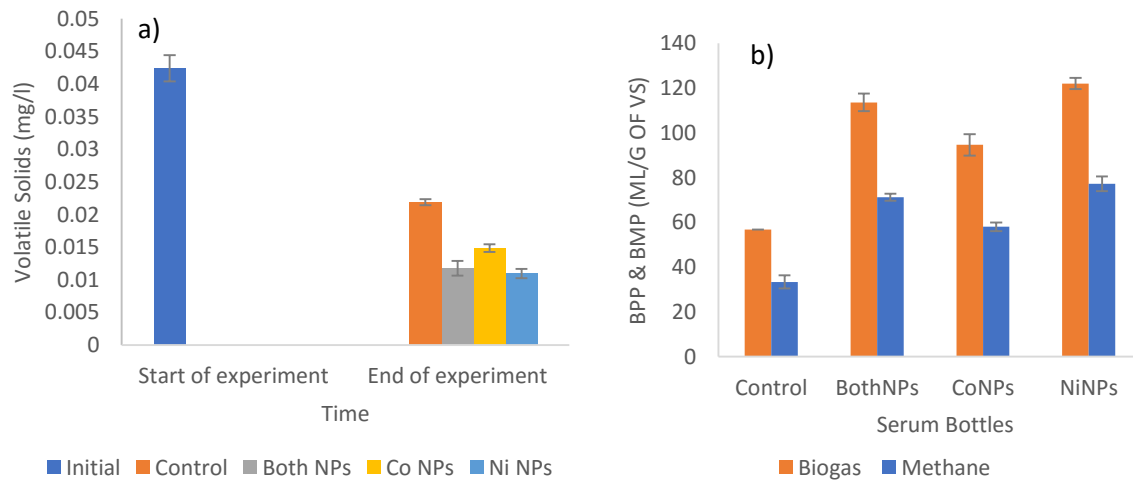


Figure 4.28 Volatile Solids from the start and at the end of the experiment and the biomethane potential (a= Volatile Solids and b= Biomethane Potential).

This is the maximum methane production capacity of a substrate during anaerobic decomposition (Raposo *et al.*, 2018). It was done through batch experiments, and it is expressed in ml/g of VS as shown in Figure 4.28 (b). The Ni nanoparticles dosed serum bottles recorded a BMP of 77 ± 3 ml/g of VS (133.3 % increase as compared with the control), this was the highest recorded in the experiment. The bottles fed with both NPs recorded a BMP of 71 ± 2 ml/g of VS, this effected a change of 115.2 % as compared with the control. The Co nanoparticles dosed bottles increased the

BMP of the control by 75.8 % after it recorded 58 ± 2 ml/g of VS. The control produced the least methane hence the lowest recorded BMP of 33 ± 3 ml/g of VS. Zaidi *et al.* (2018) reported an increase of 12 and 5 % in the biomethane potential due to the addition of 10 mg/l of Ni and Co during digestion of algal biomass, respectively. This agrees with my experimental results.

A paired two sample test for means at 5 % confidence level ($\alpha = 0.05$) was performed to assess the influence of addition of Co and Ni NPs on the biomethane potential (ml produced per g of VS) of the abattoir effluent. To verify if the NPs influenced the BMP. The p-value obtained must be less than α (0.05) to show that the different NPs had a significant effect on the biomethane potential as compared with that (BMP) of the control serum bottles. A significant difference was identified between the control serum bottles and those fed with both NPs. The mean for control bottles is 33 ± 4 and both NPs is 71 ± 2 ml/g of VS. From the statistical test t-statistics = 11.84, $P(T \leq t) = 2.68 \times 10^{-2}$ and t-critical = 6.31. There is also a significant difference of the methane output in ml per gram of VS between the control (33 ± 4) and Co NPs dosed serum bottles (58 ± 2) with t-statistics = 34.91, $P(T \leq t) = 9.11 \times 10^{-3}$ and t-critical = 6.31. A significant difference of the biomethane potential (ml produced per g of VS) means was also observed between control (33 ± 4) and the Ni NPS dosed bottles (77 ± 3) with t-statistics = 181.44, $P(T \leq t) = 1.75 \times 10^{-3}$ and t-critical = 6.31.

Volatile solids correspond to the fraction of the total solids that can be digested during anaerobic treatment of a substrate. They are directly proportional to biogas and methane production. The more volatile solids depleted the more biogas produced leading to a decrease in their initial values together with those of the total solids. They are an indication that the anaerobic digestion process is taking place and the rate at which the process is taking place (Mitchell *et al.*, 2015). The reduction of VS throughout the experiment is shown in Figure 4.28 (a), at the start of the experiment the VS in every serum bottle was 0.0424 ± 0.002 mg/l. The Ni NPs dosed serum bottles reduced the VS by 74.14 %, the highest reduction in this experiment. The Ni NPs had the highest biogas production hence highest Vs reduction. This shows an increase of 25.71 % from the control. At the end of the experiment the Co NPs dosed serum bottles had 0.015 ± 0.0006 mg/l, which indicates a reduction of 64.93 %. The serum bottles fed with both NPs reduced the VS by 72.25 % after recording a final concentration of 0.012 ± 0.0011 mg/l. Both NPs serum bottles increased the

destruction rate of the control by 23.8 %. The control produced the lowest methane and biogas hence the low usage of VS. 0.022 ± 0.0005 mg/l (48.43 % reduction of initial VS).

Volatile solids have a direct proportional relationship with the TEs, microorganisms consume TEs during the degradation of the VS. The VS depicts the concentration of bacteria in the sample. The relationship between VS and TEs is important because it shows the actual interaction of TEs with the microorganisms. An increase in the concentration of TEs increases their bioavailability to microorganisms (Ditalelo, 2017). The abattoir effluent in this experiment contained 0.044 Ni and 0.007 Co mg/l prior to supplementation of TEs. Zandvoort *et al.* (2006) indicated that addition of TEs increases specific methanogenic activity. Zandvoort *et al.* (2006) and Ditalelo, (2017) reported that an effluent with initial concentration will have no decrease in Ni concentration, this symbolising that although it is present its not available to methanogens hence the need to dose and increase its bioavailability.

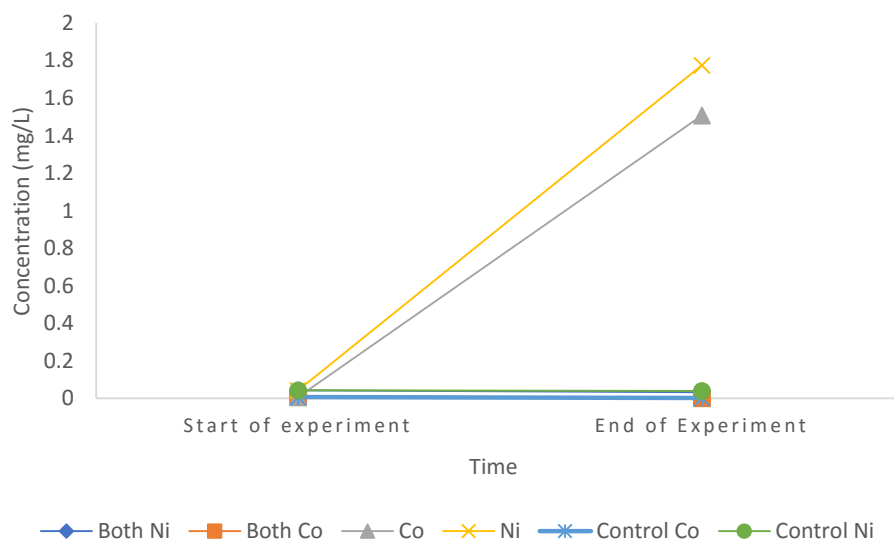


Figure 4.29 Initial and Final concentration of TEs in the dosed digesters.

Initially the abattoir effluent used contained 0.04 mg/l of Ni and 0.00735 mg/l of Co (ions) prior to addition of the different NPs. Supplementation of 11 mg/l of Ni NPs to the Ni and both NPs serum bottles lead to a concentration of 0.34 mg of Ni NPs/g of VS. Supplementation of 11 mg/l of Co NPs to the Co and both NPs serum bottles lead to a concentration of 0.34 mg of Co NPs/g of VS. It is anticipated that the supplemented NPs will disintegrate and release metal ions (Dehhagi *et al.*, 2019; Hassanein *et al.*, 2019). Released metal ions will then increase the initial concentration

of TEs. At the end of the experiment the digesters fed with both NPs had a concentration of 0.033 mg/l Ni and 0.0027 mg/l Co. The Co NPs dosed serum bottles had 1.5 mg/l of Co i.e., 20417 % more than the initial concentration. The Ni NPs dosed serum bottles had 1.77 mg/l at the end of the experiment as depicted in Figure 4.29. The Ni NPs dosed serum bottles had 3964 % increase in Ni concentration more than the initial concentration. After the AD process there are converted NPs and NPs that are still intact (Dehhagi *et al.*, 2019). NPs might not release ions but still influence biogas production because there are other mechanisms involved in the AD process (Wang *et al.*, 2016).

4.2.3.5 Comparison of the effect of the sum of the individual and combined supplementation of Co and Ni NPs

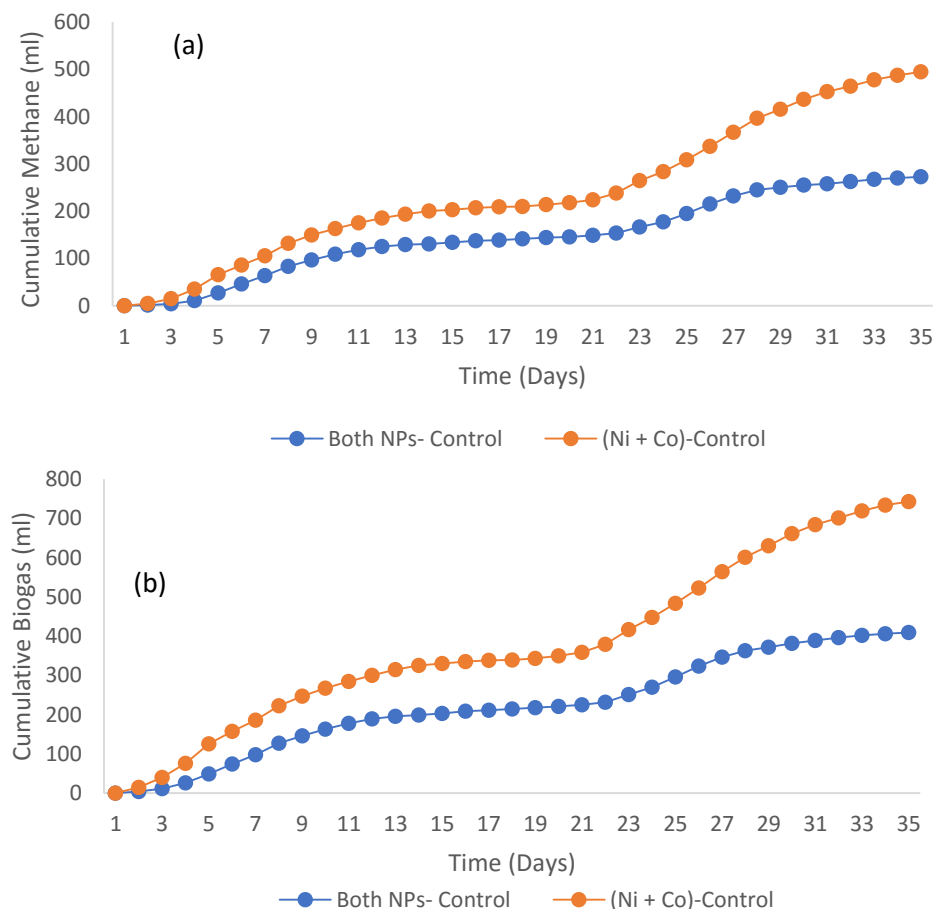


Figure 4.30 Comparison of the sum of the individual effect of Ni NPs and Co NPs dosage to that of their combined dosage (a =CH₄, b = biogas)

Cumulative CH₄ and biogas was used to test the equality of the sum of biogas and methane volume by which individual Co and Ni NPs exceeded the control to that by which both NPs serum bottles exceeded the control. This is to determine if the additive nature of Co and Ni NPs was maintained throughout the experiment. Figure 4.30 (a and b) shows this comparison. Throughout the incubation days effects of individual Ni and Co nanoparticles were high in both methane and biogas production. The serum bottles dosed with both NPs produced less biogas and CH₄ than the serum bottles dosed with individual Ni NPs. This is the reason behind both NPs exceeding the control less than how Ni and Co NPs exceeds the control. The additive nature of Ni and Co NPs was not held true throughout the experiment. Linear regression analysis gave a coefficient of determination (R^2) of 0.9879 and 0.9821 for biogas and methane, respectively.

4.2.3.6 Effects of nanoparticles (Ni and Co) in co-dosed serum bottles

This part of the Chapter tests the additive nature of cobalt and nickel nanoparticles based on the impact each of the Nanoparticles had when co-dosed and dosed individually. The linear regression computed verifies the individual effect and the co-dosage effect of each of NPs added.

4.2.3.6.1 Effect of nickel nanoparticles in co-dosed serum bottles

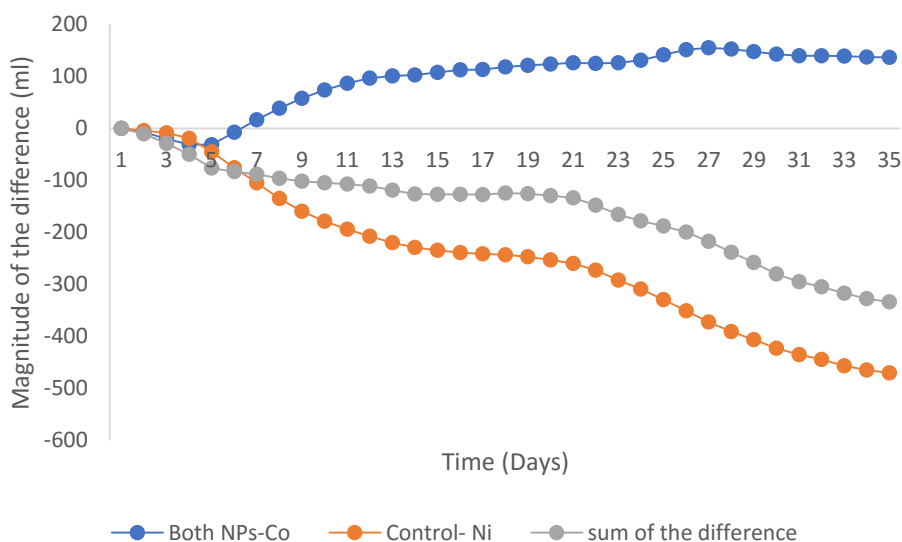


Figure 4.31 Magnitude of the difference of the control and Ni NPs dosed digester, both NPs and Co NPs digester and the sum of the difference.

This was obtained by comparing the time of experiment and amount of biogas difference between Both NPs serum bottles and Co NPs, i.e. (Both NPs-Co NPs) and that of control and Co NPs dosed serum bottles (Control-Ni NPs). Both NPs minus the Co NPs gives the biogas volume in the presence of Ni NPs while the latter gives the biogas volume in the absence of Ni NPs. The sum of the differences is expected to be close to zero or zero. The sum of the differences was also not close to zero or zero throughout the time of the experiment. The sum of the difference is closer to the Control minus the Ni NPs, which indicates Ni NPs had a higher impact when dosed alone.

Figure 4.32 shows a linear regression that correlates the effect of Ni in both NPs dosed digester and Ni NPs dosed digester. An R^2 value of 0.8363 was determined. The coefficient of correlation (R) is 0.91 (91%). To predict the difference in biogas production between both NPs dosed digester and Co NPs based on the difference between the control and the Ni NPs the Equation; $y = 0.385x - 1.9713$ can be used.

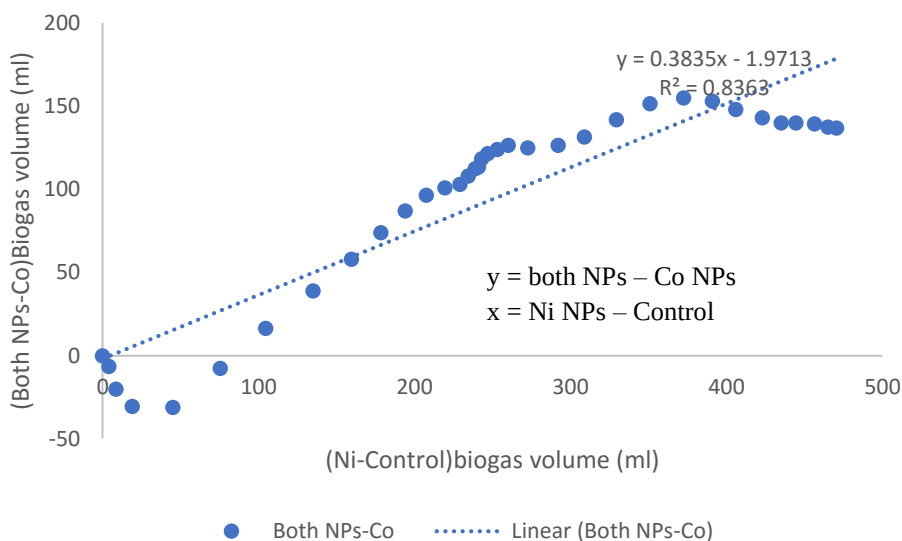


Figure 4.32 A line fit plot of the linear regression that correlates the effect of Ni in both NPs dosed digester and Ni dosed digester.

4.2.3.6.2 Effect of cobalt nanoparticles in co-dosed serum bottles

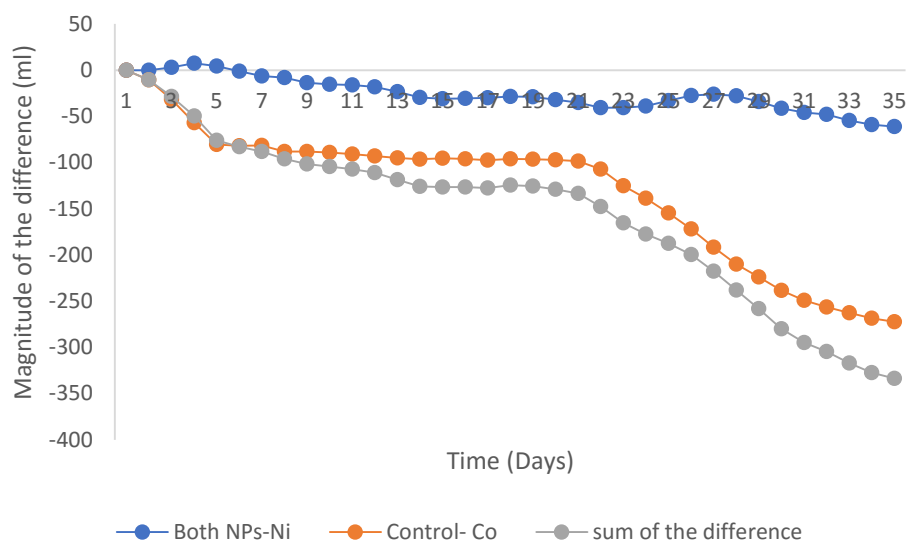


Figure 4.33 Magnitude of the difference of the control and Co NPs dosed digester, both NPs and Ni NPs digester and the sum of the difference.

This was obtained by comparing the biogas profile (time and amount) of Both NPs serum bottles and Co NPs, i.e. (Both NPs-Ni NPs) and that of control and Co NPs dosed serum bottles (Control-Co NPs). Both NPs minus the Ni NPs gives the biogas volume in the presence of Co NPs while the latter gives the biogas volume in the absence of Co NPs. The sum of the differences is expected to be close to zero or zero. The magnitude of the difference of the two pairs was not similar throughout the experiment hence and their sum of the differences is not close to zero. The magnitude of the differences was all found to be negative.

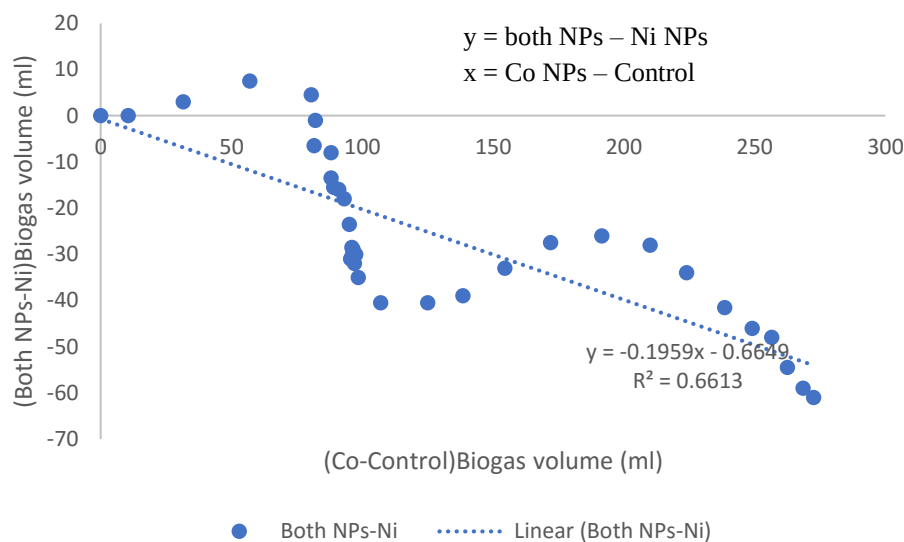


Figure 4.34 A line fit plot of the linear regression that correlates the effect of Co in both NPs dosed digester and Co dosed digester.

An R^2 value of 0.6613 lower than that of the effect of Ni nanoparticles in a co-dosed digester was found when computing linear regression analysis. This shows that the effects of Co nanoparticles when individually dosed and co-dosed with Ni NPs are not the same. The Ni NPs dosed serum bottles produced more daily biogas than the serum bottles dosed with both NPs, hence the negative values in the y-axis. Ni nanoparticles were more stimulatory to biogas production when dosed alone than in combination with Co NPs. To predict the difference in biogas production between both NPs serum bottles and Ni NPs only serum bottles based on the difference between the control serum bottles and the Co NPs only serum bottles the Equation; $y = 0.1959x - 0.669$ can be used.

4.2.4 Dosage of 20 mg/L of Ni, Ni &Co, Co nanoparticles to the 250 ml serum bottles

8*250ml serum bottles were used as digesters with a working volume of 220ml and 30ml headspace. They were dosed with Ni, Ni &Co, Co nanoparticles and no Co or Ni nanoparticles (Control). Each test was performed in duplicate for statistical purposes. The averages of the duplicate results are presented in this Chapter. The results of individual reactors are presented in appendix B.

4.2.4.1 Cumulative methane and biogas production

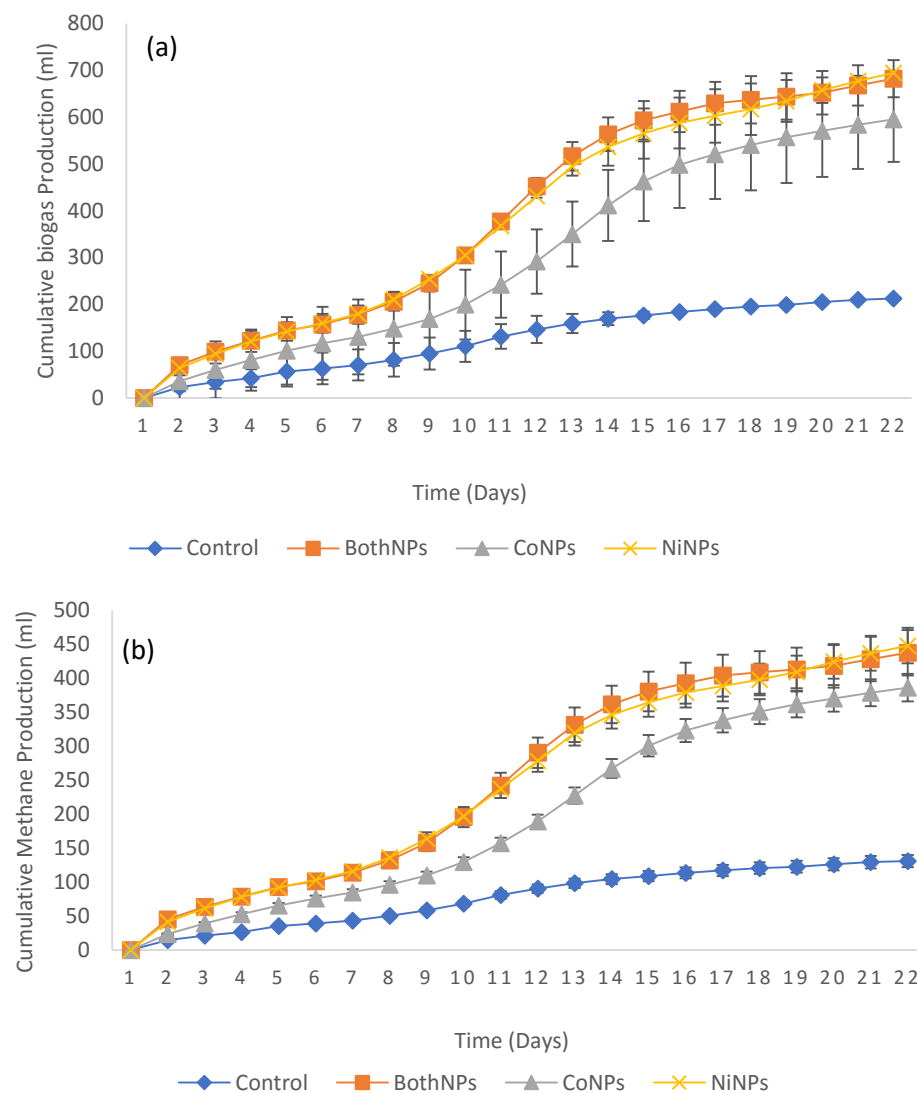


Figure 4.35 Performance of digesters fed with different Nanoparticles (a=cumulative biogas volume, b= cumulative methane volume).

Figure 4.35 depicts the cumulative methane produced during the 22 days of the experiment. Before the gas was released into the water displacement unit, 1 ml of the gas was sampled and released into the gas meter to determine the methane percentage. As the graph shows, the serum bottles containing both NPs and those containing only Ni NPs were the ones which produced the highest gas, from the day 1 until day 22. Addition of nickel increases biogas production within a matter of hours from the time addition (Nordell *et al.*, 2016). This shows nickel's direct involvement in

biogas production i.e., synthesis of methyl-coenzyme-M to produce methane (Fermoso, 2009; Rousell, 2013). The Co NPs dosed bottles produced both methane and biogas higher than the control. The control serum bottles produced the least gas. A steady increase was observed in the first seven days of hydraulic retention time across all bottles. From day 8 there was a gradual increase in both methane and biogas production until the 16th day of HRT. This increase was observed in the period in which digesters reached their peak production. From day 17 until day 22 daily production reduced which led to the steady increase in cumulative methane production. Low daily production of biogas will not have that much impact on cumulative methane and biogas production, hence a steady increase.

4.2.4.2 Daily methane and biogas production

Mao *et al.* (2015) and Demirel and Scherer, (2008) indicated that the anaerobic digestion process is highly dependent on microorganisms. The daily biogas and methane production is a depiction of the performance of microorganisms throughout the hydraulic retention time. Low gas production indicates unfavourable conditions to microorganisms while high biogas production indicates optimum conditions for microorganisms (Siegert *et al.*, 2005; Mayer *et al.*, 2010; Mao *et al.*, 2015).

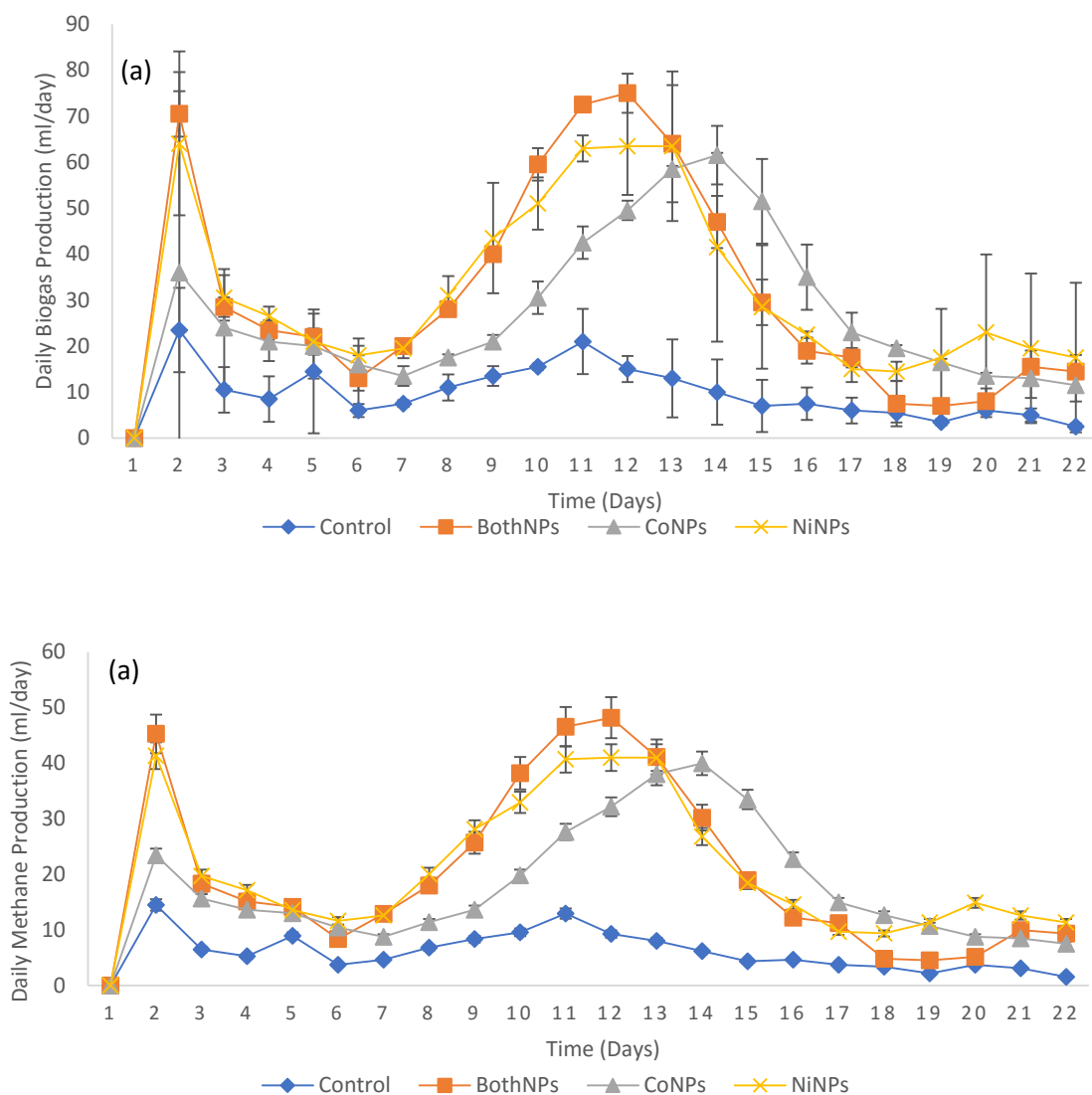


Figure 4.36 Daily methane and biogas production (a= biogas, b= methane).

On the first day the control serum bottles produced 23.5 ± 9.2 and 14.5 ± 1.01 ml of biogas and methane, respectively. These were the lowest productions of the first day as compared with the serum bottles dosed with NPs. From day 2 until day 6 of the experiment there were fluctuations in both the methane and biogas daily production. An increase in daily production was observed from day 7 until day 11. The production on day 11 failed to surpass the production on day 1, hence making the production of day 1 the highest peak of the control. Due to changes in the conditions in the digester, microorganisms might take time to adapt to the new conditions. After adaptation their growth is expected to increase exponentially hence recovery of the system (Mao *et al.*, 2015).

Abattoir effluent has many inhibitory components that are toxic to microorganisms (Diaz, 2010), which may make it difficult for the system to recover. Throughout the experiment the control produced the least daily biogas and CH₄ as compared with the bottles dosed with the NPs. The serum bottles fed with Co NPs produced the second highest daily production on the first day after the control followed by Ni NPs dosed serum bottles. The highest recorded was from the serum bottles dosed with both Ni and Co nanoparticles. Addition of nanoparticles improves the start-up of biogas and methane production (Abdelsalam *et al.*, 2017). They catalyse the process by reducing the accumulation of VFAs and increase key enzyme activities leading to high gas production (Abdelsalam *et al.*, 2017; Rousell *et al.*, 2018). After the first day the Co NPs dosed serum bottles daily production decreased until the 7th day. From the 8th day a gradual increase was observed until the 14th day. On the 14th the Co NPs dosed bottles reached their highest peak of 38±2 and 61.5±6.4 ml for CH₄ and biogas, respectively. This was recorded as the second peak after the control serum bottles. The peak of the Co NPs dosed bottles came three days after the second peak of the control (did not reduce the lag phase). Co is not directly involved in the last step of methane production (it is needed in the production of methyl-coenzyme M) (Fermoso, 2009). The coenzyme M must be converted to methane (Rousell, 2013), depending on the rate of conversion of the methyl-coenzyme M to methane that's when the effects Co had can be noticed. This might make it take time to detect the effects of cobalt from the biogas produced. Although the peak came three days later, on the day that the control reached the second peak i.e., day 11 the production was still lower than that of the Co NPs dosed bottles (it was half of it).

On day 1 the Ni NPs dosed bottles produced the highest methane after the serum bottles dosed with both NPs, which was 41.3 ± 2.41ml of CH₄ and 64 ± 15.6 ml of biogas. From this day there was a gradual decrease in the daily gas production from the Ni nanoparticles dosed bottles until day 6, followed by gradual increase until day 11. On day 12 the serum bottle reached its second peak production of 63.5±10.6 ml for biogas and 40.99 ± 2.39 for CH₄. This peak came the day after the control reached its second peak. It was almost the same as the production on day one with a difference of 0.5 ml (biogas) and 0.31 (CH₄). The peak was almost equal to that of the Co NPs dosed bottles with a difference of 1.05 ml (CH₄) and 2ml (biogas). The second peak of the Ni NPs dosed bottles was 3.02 and 3.17 times higher than the peak of CH₄ and biogas of the control digester, respectively. The lowest daily production of the Ni NPs dosed bottles was observed on day 18 for both methane and biogas, 5 days after the second peak production. The bottles dosed

with both NPs (Ni & Co) produced the highest daily production as compared with the other three digesters, this was a result of the anticipated synergistic effects of the two nanoparticles on anaerobic digestion. This peak was observed on day 12 of the experiment and was 48.15 ± 3.68 and 75 ± 4.2 ml of methane and biogas, respectively.

4.2.4.3 Effects of Ni and Co nanoparticles in total methane and biogas production

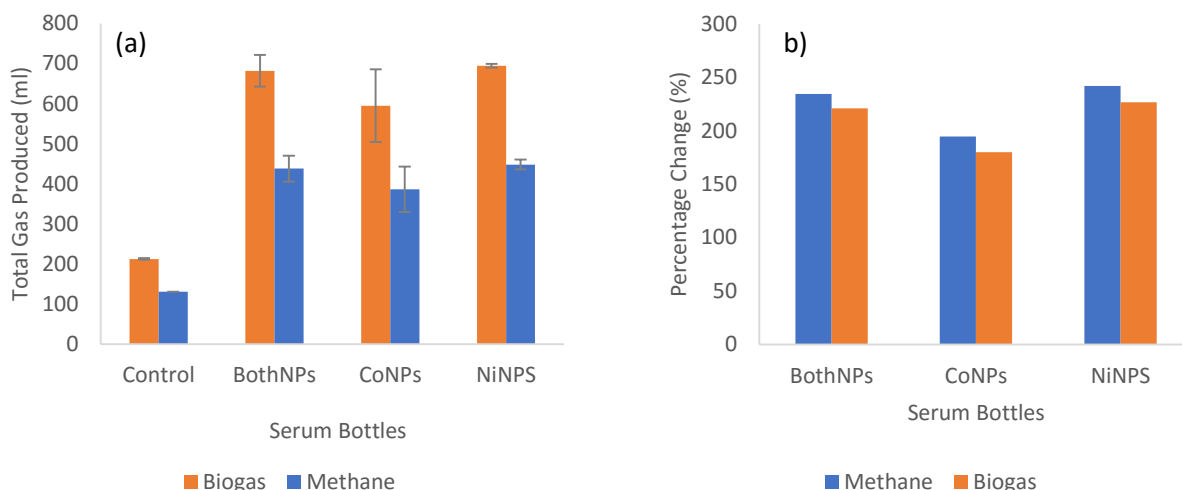


Figure 4.37 Total methane and biogas production and percentage change in Nanoparticles dosed digesters as compared with the control (a= total methane and biogas volume, b= percentage change).

At the end of the experiment (after 22 days) the control bottles produced a total of 131 ± 0.1 ml of CH_4 , and 213 ± 2.1 ml of biogas as shown in Figure 4.37(a). The Co NPs (0.61 mg/g of VS) dosed digesters produced 386 ± 56.7 and 595 ± 90.5 ml of CH_4 and biogas, respectively. They had an increase of CH_4 , and biogas produced by 255 (195%) and 382 ml (180%) above the control, respectively. Hassanein *et al.* (2019) reported that 0.60 mg of Co/g of VS improved methane produced by 29.7 % during digestion of poultry litter and 10 ml of sodium dodecylbenzene sulfonate (for nanoparticles dispersion). Zaidi *et al.* (2019) also reported that addition of 1.34 mg of Co NPs/g of VS during digestion of green microalgae increased methane production by 31.73 % when compared with the control. Garuti *et al.* (2019) and Hassanein *et al.* (2019) indicated that inhibitory concentrations of Co are above 35 mg/l . The previously mentioned studies support our results. The digesters dosed with both NPs produced a total of 438 ± 32.4 and 682 ± 39.6 ml of

CH₄ and biogas, respectively. They increased the methane and biogas by 234.35 % and 220.94 % above the control serum bottles, respectively.

Ni nanoparticles (0.61 mg/g of VS) dosed bottles increased the CH₄ and biogas produced by the control serum bottles by 317 (241.98 %) and 482 ml (226 %), respectively. As shown on Figure 4.37(a) they produced a total of 448 ± 12.4 and 695 ± 4.9 ml of CH₄ and biogas, respectively. These results agree with those of Hassanein *et al.* (2019), who reported an increase in CH₄ by 26.8 % more than the control from a concentration of 0.67 mg of Ni/g of VS. They also reported an increase of 38.87 % from a concentration of 1.34 mg of Ni/g of VS, from this dosage an increase of 95.1 % was observed in the first 29 days of the experiment. Zhang *et al.* (2015) and Xu *et al.* (2018) reported that the toxic threshold of Ni is above 50 mg/l. Although they reported it being above 50 mg/l, Elreedy *et al.* (2017) indicated that addition of 60 mg/l of Ni NPs to anaerobic digestion of industrial wastewater containing mono-ethylene glycol leads to an increase in CH₄ production by 23 % as compared with the control. Addition of 1.25 g/l and 2.5 g/l of Ni NPs during anaerobic digestion of activated sludge and wastewater at 40°C increases biogas production by 133.33 and 100 % above the control, respectively (Amo-Duodu *et al.*, 2021). This shows that it is difficult to compare the performance of micronutrients directly. Depending on the system used, the substrate digested, dosing of micronutrients, biochemical reactions, and substrate characteristics the performance of micronutrients will differ.

Faisal *et al.* (2019) indicated that supplementation of nanoparticles to anaerobic digestion increases degradation rates as the nanoparticles have a high surface to volume ratio which increases chemical reaction sites. This results in increased biogas and methane production of up to 200 %. Amen *et al.* (2018) reported that addition of 1500 mg/l of copper ion based bimetallic (ZVI NPs/Cu) during digestion of municipal sludge enhanced biogas production by 202.62 % above the control. He also reported that zero-valent iron enhanced biogas production by 208.29 % above the control. The results observed in this experiment agree with the above results from previous research that the addition of NPs can increase the biogas and methane production by up to 200 %. Enhancement of biogas production by NPs depends on the bioavailability of the NPs. Bioavailability is dependent on speciation not total concentration. The supplemented concentration of NPs may be the same or similar, but the efficiency will depend on bioavailability of the NPs (Garuti *et al.*, 2018; Liu Wei

and Leng, 2021). Availability of sulphides is also a factor as they precipitate the added metals leading to the reduction of bioavailability of the metals.

To test the effects of the different NPs had on the total biogas and methane production, a paired two sample t-test for means with a significance level of 5 % ($\alpha = 0.05$) was performed. This was performed to verify if they had a significant effect on the total methane and biogas production. To prove a significant difference the p-value obtained should be less or equal to α in the comparison of the total methane and biogas of the dosed serum bottles and the control serum bottles. A significant difference was observed between the total biogas produced by the control (mean = 212.5) and the serum bottles fed with both NPs (mean = 682) with t-statistics = 15.92, $P(T \leq t) = 0.02$ and t-critical = 6.31. Also, a significant difference was observed between the methane produced by the control (mean = 127) and both NPs (mean = 425) with the t- statistics =13.00, $P(T \leq t) = 0.02$ and t-critical = 6.31. A significant difference was also observed between the total methane produced by the control (mean = 127) and the serum bottles fed with Ni NPs (mean = 435.59) with t-statistics = 34.92, $P(T \leq t) = 0.01$ and t-critical = 6.31. Also, a significant difference was observed between the biogas produced by the control (mean = 212.5) and Ni NPs (mean = 694.5) with the t- statistics =96.4, $P(T \leq t) = 0.0033$ and t-critical = 6.31. A significant difference was also observed between the total methane produced by the control (mean = 127) and the serum bottles fed with Co NPs (mean = 425.56) with t-statistics = 6.20, $P(T \leq t) = 0.05$ and t-critical = 6.31. Also, a significant difference was observed between the biogas produced by the control (mean = 212.5) and Co NPs (mean = 595) with the t- statistics = 6.12, $P(T \leq t) = 0.05$ and t-critical = 6.31. All p-values obtained above are less or equal to α (0.05) when comparing the total methane and biogas of the NP dosed serum bottles (Ni, Co and both NPs) to that of the control show a significant difference.

4.2.4.4 Volatile Solids and their relationship with nanoparticles concentration and Biomethane Potential

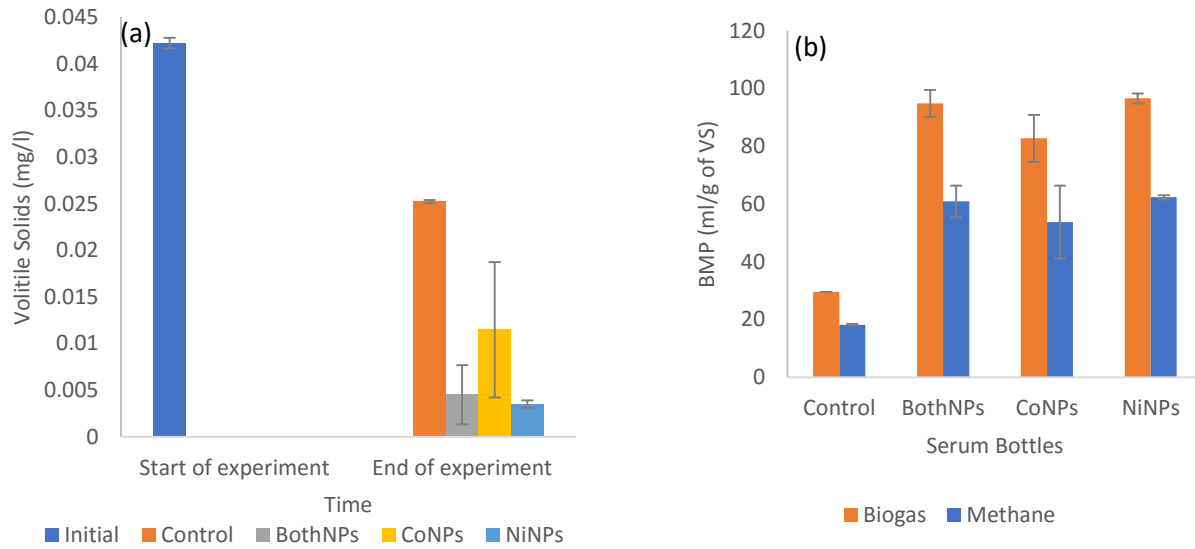


Figure 4.38 Volatile Solids from the start and at the end of the experiment and the biomethane potential (a= Volatile Solids and b= Biomethane Potential).

The BMP is important since it reflects the relationship between VS and methane/biogas produced. A higher BMP indicates a high ability of the digester to convert VS into biogas. Figure 4.38 (b) shows the relationship between volatile solids and biogas production. The control has a BMP of 18.21 ± 0.3 ml/g of Vs, which is the lowest from this study. The Ni nanoparticles dosed bottles recorded the highest BMP of 62.36 ± 0.7 ml/g of VS, an increase of 242.4 % as compared with the control. An increase of 6% in the BMP was reported by Zaidi *et al.* (2018) during anaerobic digestion of algal biomass under mesophilic conditions (37°C). The increase was due to the dosage of 20 mg/l of Ni. The Co NPs dosed bottles recorded a BMP of 53.72 ± 12.59 ml/g of VS (195.0 % increase as compared with the control) while both NPs serum bottles had 60.92 ± 5.5 mg/l of VS (234.5 % increase as compared with the control).

To test the effects of the different NPs had on the biomethane potential, a paired two sample t-test for means with a significance level of 5 % ($\alpha = 0.05$) was performed. A significant difference of the methane output per gram of VS was observed between the control (18.21 ± 0.3) and the serum bottles dosed with both NPs (60.92 ± 5.51) with t-statistics = 13.07, $P(T \leq t) = 2.23 \times 10^{-2}$ and t-critical = 6.31. A significant difference of the methane output per gram of VS was observed

between the control (18.21 ± 0.3) and serum bottles dosed with the Co NPs (53.74 ± 12.59) with t -statistics = 6.20, $P(T \leq t) = 0.05 \times 10^{-2}$ and t -critical = 6.31. Also, a significant difference of the methane output per gram of VS was observed between the control (18.21 ± 0.3) and Ni NPs dosed serum bottles (62.36 ± 0.69) with t -statistics = 35.54, $P(T \leq t) = 8.95 \times 10^{-3}$ and t -critical = 6.31. These were performed to check if the Co and Ni nanoparticles had a significant effect on the biomethane potential as compared with the control. A p -value less or equal to α (0.05) will prove a significant difference between the control serum bottles BMP and that of the dosed serum bottles. All the p -values (are less than 0.05) computed show that addition of Ni, Co and both NPs had a significant increase in the biomethane potential as compared with the control.

The volatile solids available in a digester reflects the concentration of bacteria available (Metcalf and Eddy, 2003; Ditalalo, 2017). Figure 4.38 (a) above shows the volatile solids before and after the experiment, at the beginning the volatile solids contained in all the digesters was 0.0422 ± 0.0006 mg/l. The Ni NPs dosed serum bottles destructed 0.0387 mg/l of Vs which translates to 91.66 % of the initial concentration. The VS destructed reflects the gas produced, the Ni NPs dosed serum bottles produced the highest biogas hence the highest percentage of degraded VS. The serum bottles dosed with both NPs had $4.5 \times 10^{-3} \pm 0.003$ mg/l after the 22 days of HRT. They degraded 89.28 % of their original VS. The Co NPs dosed serum bottles degraded 72.8 % of the initial concentration and remained with 0.011 ± 0.007 mg/l. The control produced the least biogas hence had the low percentage of the destructed VS of destructed 40.28 %.

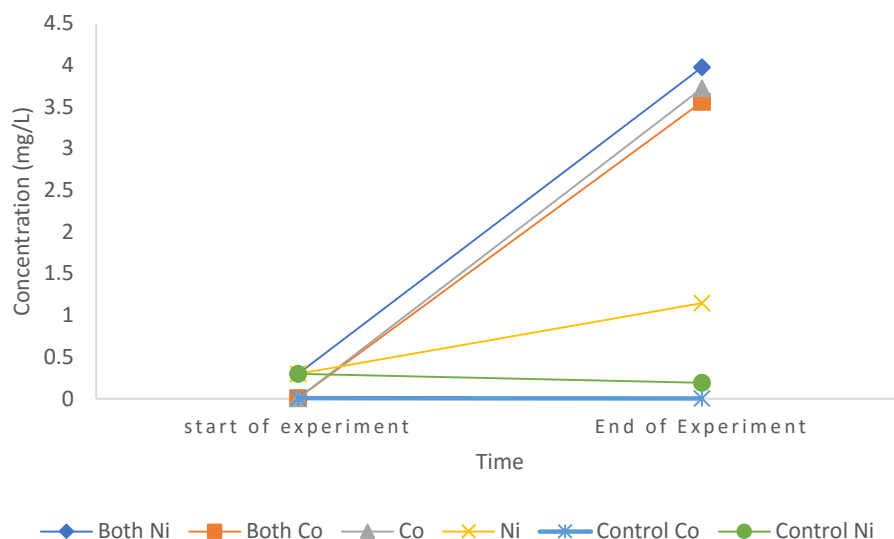


Figure 4.39 Initial and Final concentration of TEs in the dosed digesters.

The abattoir effluent used in this experiment contained 0.3 mg/l of Ni and 0.01 mg/l of Co prior to addition of the different NPs. Supplementation of 20 mg/l to both NPs and Ni serum bottles led to the concentration of 0.61 mg of Ni NPs/ g of VS. Dosing of 20 mg/l of Co NPs to both NPs and Co serum bottles led to the concentration of 0.61 mg of Co NPs/g of VS. Figure 4.39 shows the concentration of the TEs throughout the experiment. When the dosed NPs react with the material they will disintegrate and release metal ions (Co^{2+} and Ni^{2+}) and the final concentration of Ni and Co will increase (Wang *et al.*, 2016; Hassanein *et al.*, 2019). At the end of the experiment the serum bottles dosed with both NPS were left with 3.97 mg/l of Ni and 3.56 mg/l of Co (ions). The Co NPs dosed digester had 3.72 mg/l and the Ni dosed digester had 1.15 mg/l. There will be some disintegrated and intact NPs after digestion (Dehhaghi *et al.*, 2019; Hassanein *et al.*, 2019). Although some NPs are still left intact after digestion this does not mean that they did not enhance methane and biogas production (Wang *et al.*, 2016). There several mechanisms involved in anaerobic digestion that allow NPs to interact with microorganisms whilst still intact (Wang *et al.*, 2016).

4.2.4.5 Comparison of the effect of the sum of the individual and combined supplementation of Co and Ni NPs

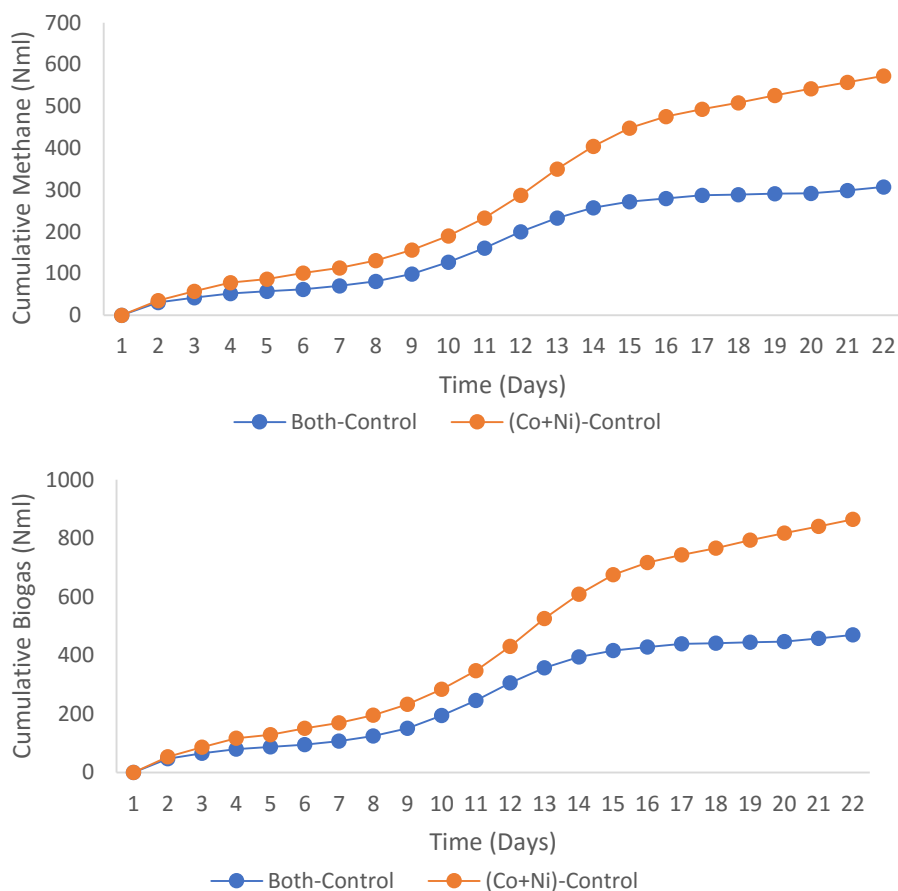


Figure 4.40 Comparison of the sum of the individual effect of Ni and Co NPs dosage to that of their combined dosage (a = methane, b = biogas)

Cumulative CH₄ and biogas were used to test the additive effects of Ni and Co NPs, the effects of individual dosage ((Ni + Co)-Control) were compared with their combined dosage (both NPs – Control). This is done to test the equality of the sum of the biogas and methane volume by which Ni & Co NPs dosed serum bottles exceeds the control serum bottles to that by which both NPs serum bottles exceeds the control serum bottles. As it is shown in Figure 4.40 the effects were not equal throughout the experiment. The serum bottles dosed with both NPs failed to produce more biogas and methane (cumulative) as compared with individual dosage. The serum bottles dosed with Ni NPs only had more cumulative biogas than the serum bottles dosed with both NPs. An R²

value of 0.982 and 0.9827 was obtained from linear regression analysis for biogas and methane, respectively.

4.2.4.6 Effects of nanoparticles (Ni and Co) in co-dosed serum bottles

Based on the impact each of the Nanoparticles had when co-dosed and dosed individually, their additive effects (individually) are computed and presented in this part of the Chapter. To verify the effects of the nanoparticles when co-dosed to their individual effects a linear regression was computed and presented here.

4.2.4.6.1 Effect of cobalt nanoparticles in co-dosed serum bottles

This was done by comparing difference in amount of biogas (throughout the HRT) of Both NPs serum bottles and Ni NPs, i.e. (Both NPs-Ni NPs) and that of control and Co NPs dosed serum bottles (Control-Co NPs). Both NPs minus the Ni NPs gives the biogas volume in the presence of Co NPs while the latter gives the biogas volume in the absence of Co NPs. The sum of the differences is expected to be close to zero or zero. The magnitude of the difference of the two pairs was not similar throughout the experiment hence why the sum of the differences is not close to zero.

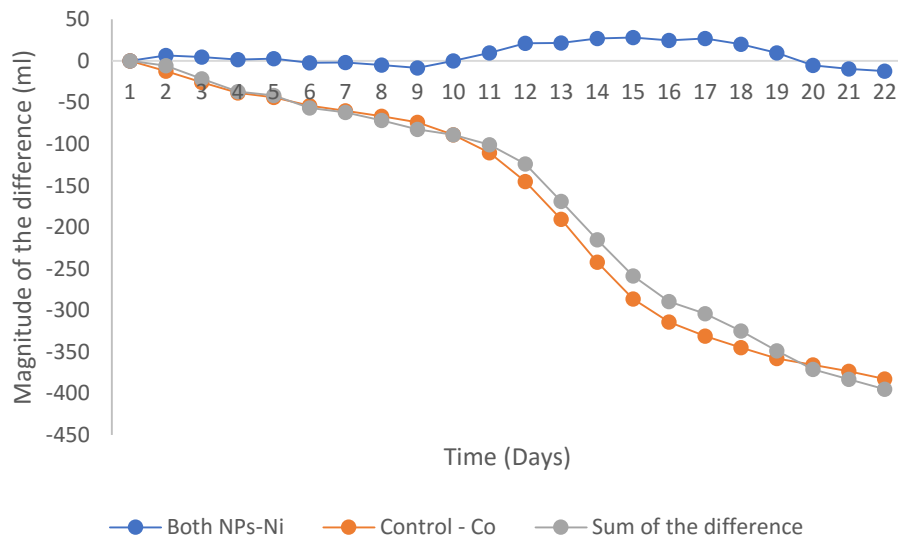


Figure 4.41 Magnitude of the difference of the control and Co NPs dosed digester, both NPs and Ni NPs digester and the sum of the difference.

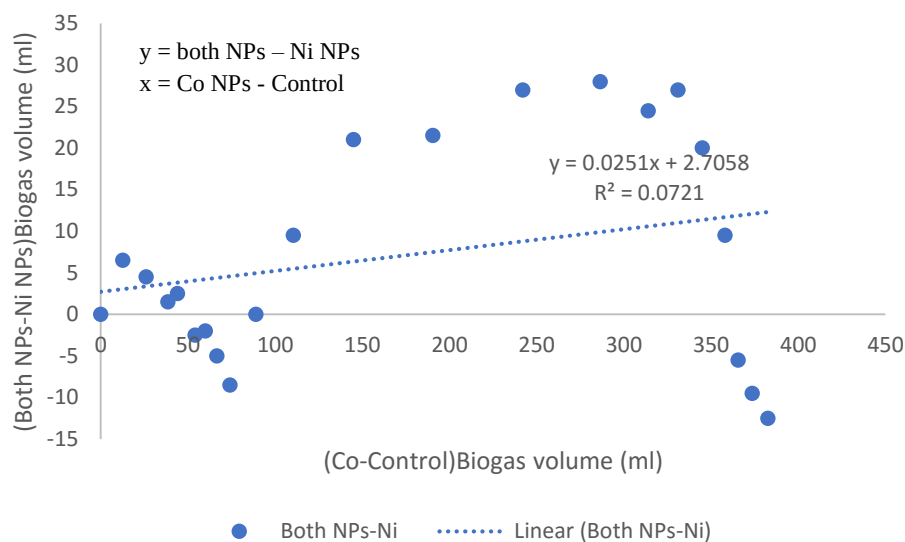


Figure 4.42 A line fit plot of the linear regression that correlates the effect of Ni in both NPs dosed digester and Ni dosed digester.

From the linear regression analysis in Figure 4.42 an R^2 value of 0.0721 was obtained. This shows that the effects of Co NPs when dosed alone and co-dosed with Ni nanoparticles are not the same. From day 5 to day 8 and the last three days of HRT the Ni NPs only dosed serum bottles produced more biogas than the serum bottles dosed with both Co and Ni NPs. The coefficient of determination of the effects of Ni NPs is higher (0.4353) than that of Co NPs (0.0721), which shows that Ni NPs effects in co-dosed bottles was higher. To predict the difference in biogas production between both NPs serum bottles and Ni NPs serum bottles based on the difference between the control serum bottles and the Co NPs serum bottles the Equation; $y = 0.0251x + 2.7058$ can be used.

4.2.4.6.2 Effect of nickel nanoparticles in co-dosed serum bottles

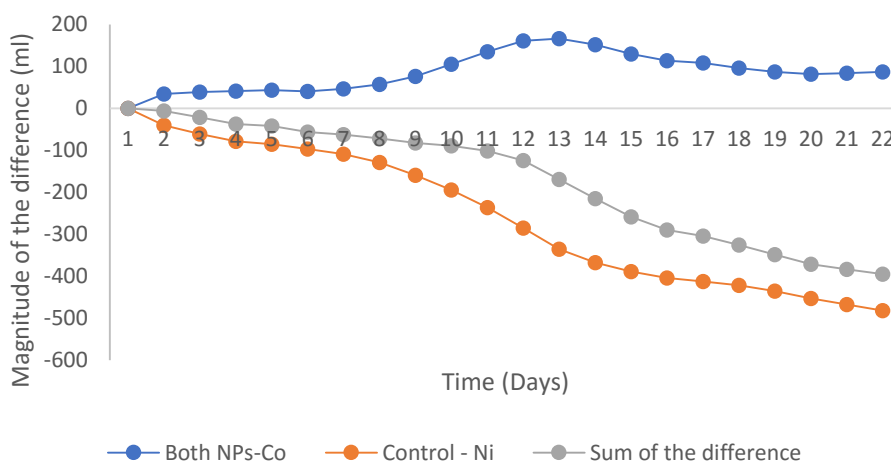


Figure 4.43 Magnitude of the difference of the control and Ni dosed digester, both NPs and Co digester and the sum of the difference.

This was obtained by comparing the biogas profile (time and amount) of Both NPs serum bottles and Co NPs, i.e. (Both NPs-Co NPs) and that of control and Co dosed serum bottles (Control-Ni NPs). Both NPs minus the Ni NPs gives the biogas volume in the presence of Ni NPs while the latter gives the biogas volume in the absence of Ni NPs. The sum of the differences is expected to be close to zero or zero. The magnitude of the difference of the two pairs was not similar throughout the experiment hence why the sum of the differences is not close to zero. The magnitude of the differences was found to be not similar throughout the experiment. The sum of the differences was also not close to zero or zero. The sum of the difference is closer to the Control minus the Ni NPs, which indicates Ni NPs had a higher impact when dosed alone.

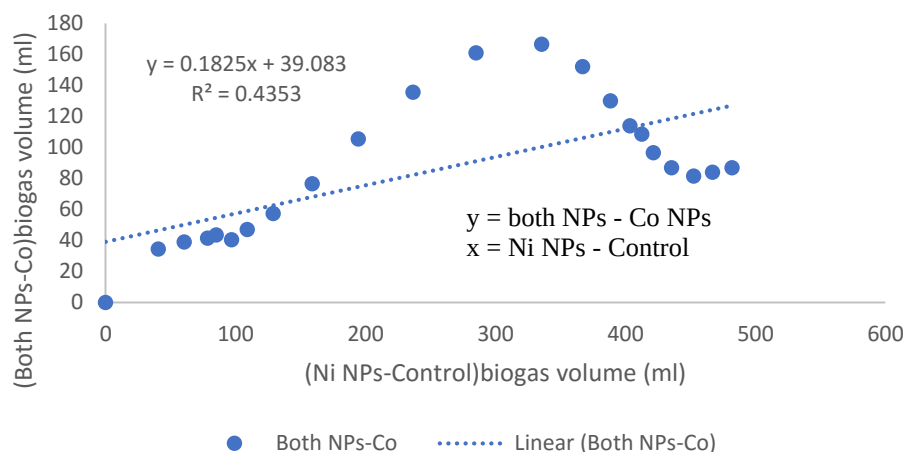


Figure 4.44 A line fit plot of the linear regression that correlates the effect of Ni in both NPs dosed digester and Ni dosed digester.

From Figure 4.44 a coefficient of correlation of 0.66 (66% correlation) was obtained. An R^2 value closer to 1 would effect that the effects of Ni NPs when dosed individually were the same as when co-dosed with Co NPs. This will then support the hypothesis that they (Ni and Co) have additive effects. The serum bottles dosed with NPs alone produced more biogas than the serum bottles dosed with both Ni and Co nanoparticles in this experiment hence not supporting the hypothesis. The Equation; $y = 0.1825x + 39.083$ can be used to predict the difference in biogas production between both NPs serum bottles and Ni NPs dosed serum bottles based on the difference between the control serum bottles and the Ni NPs dosed serum bottles.

4.2.5 Summary

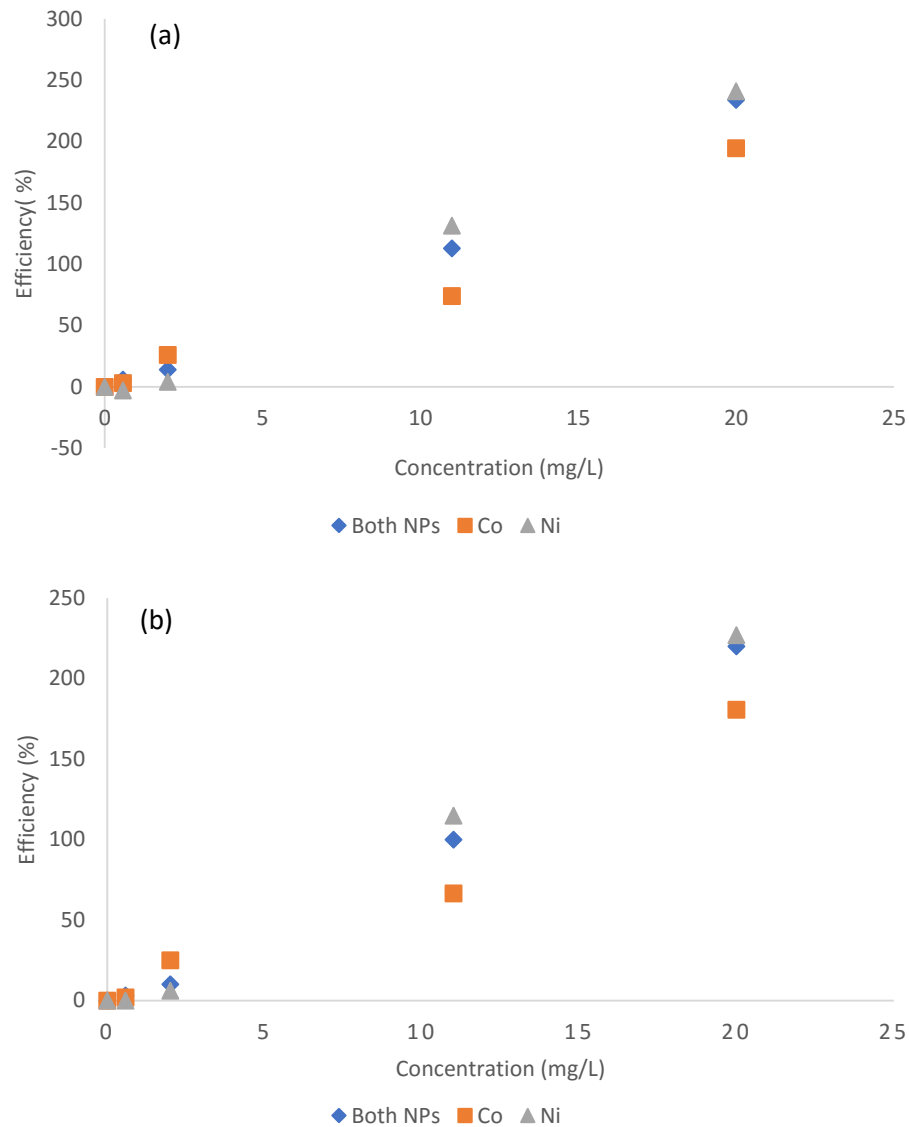


Figure 4.45 Performance of different concentration of NPs (a = methane and b = biogas)

This part of the thesis summarises the effects the different nanoparticle concentrations (0.58, 2, 11 and 20 mg/l) had on methane and biogas production. Figure 4.45 shows the concentration plotted against the efficiency effected by the NPs. The efficiency of NPs was measured as the percentage change effected by the different concentrations as compared with the control. At the lowest dosed NP concentration of 0.58 mg/l the digester dosed with Ni and Co NPs had the highest increase of biogas and methane of 3 and 6 % above the control digester, respectively. The Co NPs dosed

digesters had an increase of 3 and 2 % for methane and biogas over the control, respectively. The Ni NPs dosed digesters did not have any change as compared to the control as shown in Figure 4.25. At a concentration of 2 mg/l the Co dosed digesters had the highest increase of methane and biogas of 26 and 25 % above the control digester, respectively. Both NPs dosed digesters at 2mg/l had an increase of 14% for methane and 10% for biogas. The Ni nanoparticles dosed at 2 mg/l had the least increase of 4 and 6% for CH₄ and biogas, respectively. It was also observed from literature that at lower concentrations, Co is more stimulatory than Ni and for higher concentrations Ni is more stimulatory than Co. The Ni serum bottles dosed with 11 mg/l had an increase of 132 (CH₄) and 115 (biogas) % above the control serum bottles, the highest recorded as compared with the control serum bottles, Co NPs dosed serum bottles, and the both NPs serum bottles. The 11 and 20 mg/l of Co nanoparticles effected the least change as compared with the other dosed digesters. The addition of both NPs in one digester was not found to be as additive as it was observed in literature (Fermoso, 2009; Rousell, 2013; Ditalolo, 2017). In the literature they were reported to have synergistic effects when dosed as metal ions. Their co-dosage was expected to show synergistic effects and produce more biogas than their individual dosage. As it is shown in Figure 4.45 (a and b) only at a concentration of 0.58mg/l Ni and Co nanoparticles (co-dosage) indicated synergistic effects. At 2 mg/l the co-dosed digester effected a change lower than that of Co NPs alone. At 11 and 20 mg/l the co-dosed serum bottles had a change lower than that of individual dosage of Ni NPs.

4.3 THE ENERGY POTENTIAL GENERATED AND THE POSSIBILITY OF THE ABATTOIR POWERING ITSELF

4.3.1 Electricity requirements of the abattoir

For the month of January and February the abattoir used 10 646.77 and 10 979.07 KWh, respectively. On average the abattoir uses 10 812.92 KWh per month which translates to 422.125 KWh per day. On average the abattoir is charged P19 687,625 a month for the electricity. Installation of a biogas plant and a CHP generator could save them a lot of costs on an annual basis. This will also promote energy independence of the abattoir & reduces risk of power disruptions. The abattoir will no longer be affected by uncertainties in electricity prices.

4.3.2 Conversion of the electricity requirements to biogas/methane

The electricity requirements of the abattoir were first converted to energy required and the energy was converted to biogas based on the average caloric value of biogas. A kWh (kilowatt hour) is equivalent to 3.6 MJ (megajoule). The daily consumption of 422.125 kWh of the abattoir is equivalent to 1 519. 6 MJ. For a month, the abattoir requires 38 926. 512 MJ and 467 118. 144 MJ annually. According to IRENA 2016 and FNR 2009 1 m³ of biogas has an average caloric value of about 22 MJ. Based on this the abattoir will need 69.07 m³ (69000 l) of biogas.

4.3.3 Energy potential of the abattoir waste

Based on the control of all the anaerobic digestion batch experiments in this research the energy potential was computed. 1 g of the abattoir effluent produces 6.5×10^{-6} m³ of biogas (0.0065 l). 6.5×10^{-6} m³ of biogas has an average caloric value of 1.43×10^{-4} MJ, which is equivalent to 3.97×10^{-6} kWh. Addition of NPs increased the energy potential of the waste stream. Addition of 20 mg/l of Ni and Co NPs recorded 7.27×10^{-5} kWh (0.000262 MJ) for Co only, 8.49×10^{-5} kWh (0.000306 MJ) for Ni only and 8.34×10^{-5} kWh (0.0003 MJ) for Both NPs. Addition of 11 mg/l of Ni and Co recorded 8.34×10^{-5} kWh (0.0003 MJ) for Co only, 1.08×10^{-4} kWh (0.000387 MJ) for Ni only and 1×10^{-4} kWh (0.000361 MJ) for Both NPs. Addition of 2 mg/l of Ni and Co recorded kWh 9.37×10^{-5} MJ (7.48×10^{-5} kWh) for Co only, 0.000215 MJ (6.72×10^{-5} kWh) for Ni only and 0.00018 MJ (6.54×10^{-5} kWh) for Both NPs.

4.3.4 Sizing of the abattoir digester that will be required if the abattoir is to power itself

This was computed following the steps outlined in Chapter 3.3. Due to the high daily electrical (422.125 kWh) requirements of the abattoir the values obtained are also high. The mass (*ms*) of the abattoir effluent found was 10626.98 kg/day, mass (*ma*) of water to be added to the digester was 20191.27 kg/day, The total feeding (*mt*) in kg/day was 30818.25, The total volumetric feeding rate (*F*) was 58.55 m³/day, and the calculated volume of the digester was 1124.25 m³. Addition of different concentrations of NPs is expected to reduce the size of the digester that will be needed and increase the energy potential of the abattoir effluent. Sizing of the digester using the energy potential of the NPs is summarised in Table 4.2.

Table 4.2 Sizing of the abattoir digester using energy potential of the different concentrations of the NPs

Concentration (mg/l)	NPs	kWh/100g	ms (kg/day)	ma (kg/day)	mt (kg/day)	F (m3/day)	V (m3)
20	Co	0.007272	5804.96	11029.4	16834.4	31.9853	614.118
	Both	0.008336	5064.44	9622.45	14686.9	27.9051	535.778
	Ni	0.008494	4969.71	9442.46	14412.1	27.38315	525.756
11	Co	0.008342	5060.73	9615.40	14676.1	27.88467	535.385
	Both	0.010016	4214.71	8007.94	12222.6	23.22305	445.882
	Ni	0.010762	3922.72	7453.16	11375.8	21.61418	414.992
2	Co	0.007479	5644.82 3 6452.25	10725.1	16369.9	31.10298	597.177
	Both	0.006543	1 6277.97	12259.2	18711.5	35.5519	682.596
	Ni	0.006724	7	11928.1	18206.1	34.59165	664.159

CHAPTER 5. CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

5.1 CONCLUSIONS

With respect to the aims and objectives of this research relevant conclusions are presented in this Chapter. The need to supplement Co and Ni to anaerobic digesters of abattoir effluent in the form of Nanoparticles was established. The combination of both NPs did produce more biogas as compared with individual dosages. This was not expected as literature shows that when Ni & Co are co-dosed, they have synergistic effects. The stimulatory concentrations of Ni and Co observed in this study are specific to the anaerobic digestion system employed. The relevant conclusions are presented as follows:

Objective (i)

As the sample size (also VS) increases the methane/biogas production increases, but the conversion rate (Vs to CH₄) decreases. It is suitable to use smaller sample size for high conversion rate.

Objective (ii)

Supplementation of 11 and 20 mg/l of nickel NPs increased both the biogas production and degradation rate of volatile solids more than the control.

At higher concentrations (11 & 20 mg/l) Ni NPs stimulated both methane and biogas production more than the Co NPs, as the Ni dosed serum bottles produced more biogas and methane as compared with the Co dosed serum bottles.

Objective (iii)

Addition of 11 and 20 mg/l of Co NPs increased the biogas production significantly. An increase in the degradation rate of volatile solids was also observed.

At lower concentrations (0.58 & 2 mg/l) Co NPs stimulated both methane and biogas production more than the Ni NPs, as the Co dosed digester produced more biogas and methane as compared with the Ni dosed digester.

Objective (iv)

Dosage of 11 and 20 mg/l of Co and Ni NPs increased both the biogas production and degradation rate of volatile solids as compared with the control.

Combined dosage of Ni and Co at 2, 11 and 20 mg/l was not found to be additive as it was observed from literature, since it produced low biogas as compared with individual dosage of Co NPs only at 2 mg/l and Ni NPs only at 11 and 20 mg/l.

Objective (v)

On average the abattoir effluent has 0.01 ± 0.004 mg/l of Co ions and 0.23 ± 0.02 mg/l of Ni ions as the baseline concentration of Ni and Co.

Objective (vi)

The abattoir needs a digester of size 1124 m^3 , total feeding in kg/day of 30818 (feed plus water) to power itself. Addition of individual and combined dosage of nanoparticles (2, 11 and 20 mg/l) increased the energy potential of the abattoir effluent hence the reduction in digester size, when compared with those of the control.

5.2 RECOMMENDATIONS FOR FUTHER WORK

The recommendations for future work in relation to dosage of micro-nutrients are presented here. These are made for improving scientific knowledge and applicability to large scale digesters.

- The system used here is a batch system, to improve scientific knowledge and applicability to large scale digesters it is important to use a continuous system also. This will help in monitoring daily biogas production and relate it to daily VS destruction and pH. This will also relate the production with daily dosage of NPs.

- With the available relevant equipment, identification and quantification of methanogens using qPCR is also important. This will relate the different NPs with the methanogens present in the sample. This will also relate the biogas and methane production with the available methanogens.
- Since it is now established that Co and Ni nanoparticles enhance biogas production during anaerobic digestion of abattoir effluent. It is important to find ways to improve the performance of the NPs, such as addition of organic agents.

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APPENDICES

APPENDIX A: DOSAGE OF 11 MG/L OF Ni, Ni & Co, Co TO THE 250 ML SERUM BOTTLES

A total of 8 250 ml digesters were used in the experiment. The serum bottles consist of those fed with Co, Ni, Ni & Co NPs, and the control (non-dosed) each with 2 replicates. Their average is presented in Chapter 4.2.3

Appendix A-1 Cumulative methane and biogas production

The nickel nanoparticles dosed serum bottles had a gradual increase in both methane and biogas production from the first day until the 11th day. The production became steady from the 12th day until the 21st day of the experiment. From the 22nd day a gradual increase in the production was observed. This was the time when the digesters reached their second peak of daily biogas and methane production. From day 1 until day 5 of the experiment the control serum bottles (non-dosed) had a gradual increase in biogas and methane production. The production then became steady until day 24. During this period the control serum bottles recorded the least biogas and methane as compared with the dosed digesters. The serum bottles dosed with both nickel and cobalt nanoparticles had a gradual increase in both methane and biogas production from the first day until the 12th day. The production became steady from the 13th day until the 21st day of the experiment. From the 22nd day a gradual increase in the production was observed due to the second peak the digesters reached at this time. From day 7 until the last day of the experiment the cobalt NPs dosed serum bottles production exceeded the control production only.

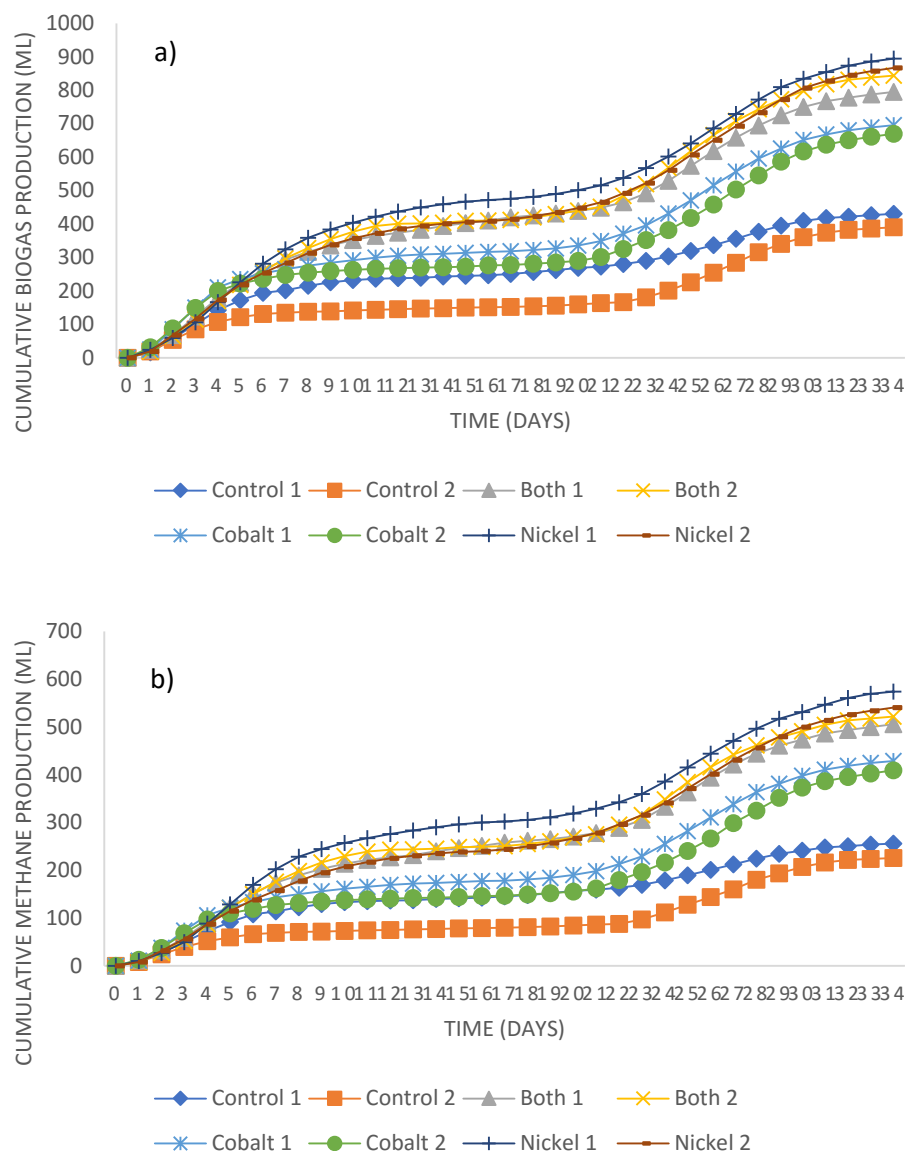


Figure A-1 Cumulative methane and biogas production (b= methane, a = biogas)

Appendix A-2 Daily methane and biogas production

All the digesters reached two peaks as depicted in Figure A-1. With First peak being the highest of the two. The control peaks were the lowest of all the digesters. For the first peak control 2 and 1 recorded 32 and 42 ml of biogas, respectively. For the second peak control 2 and 1 recorded 28 and 21 ml of biogas, respectively. The cobalt nanoparticles dosed serum bottles produced the highest biogas on the first peak cobalt 1 producing 65 while cobalt two produced 61. These were recorded on day 3 of the experiment. All the dosed digesters reduced the time to reach the second peak as compared with the control.

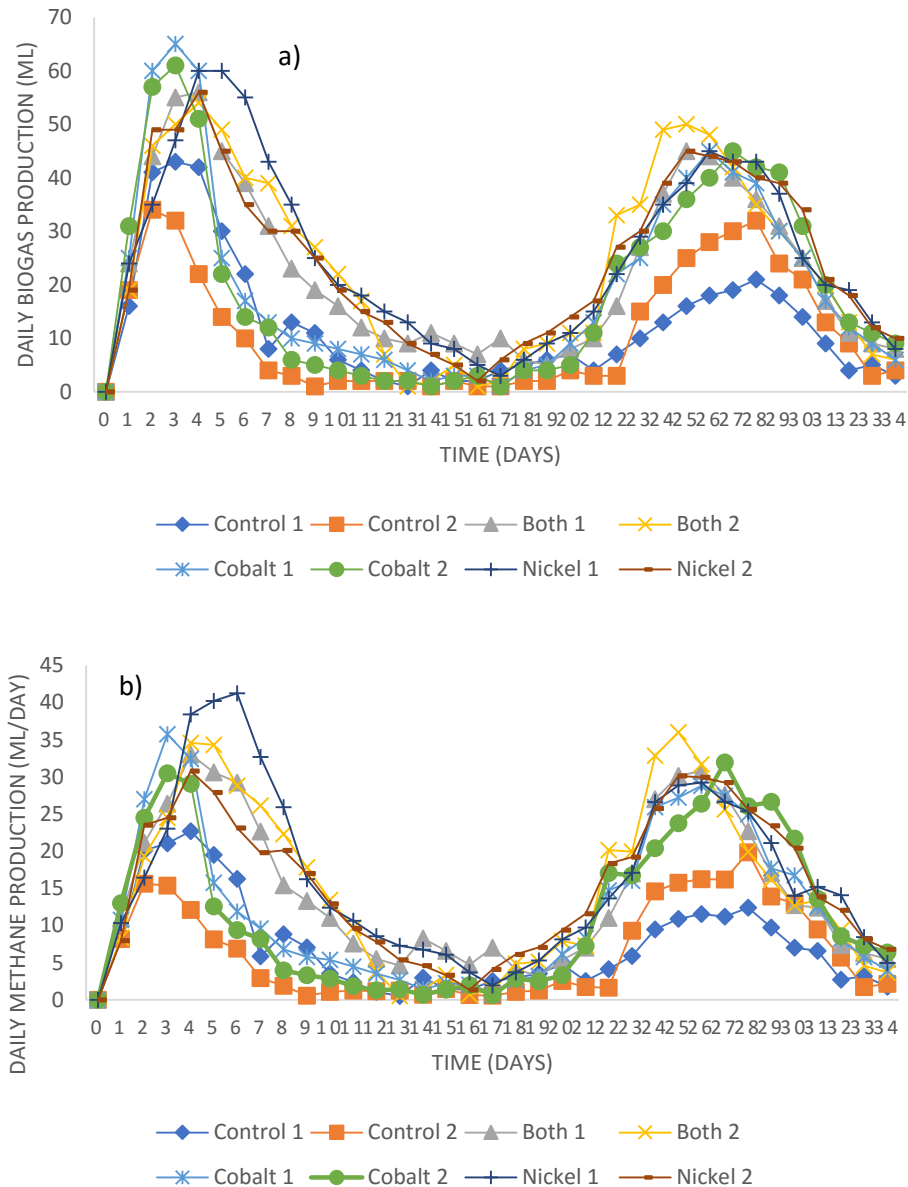


Figure A-2 Daily methane and biogas production (b = methane, a = biogas)

Appendix A-3 Total Biogas, Methane Produced

The control serum bottles (non-dosed) produced the least total biogas and methane as compared with the dosed serum bottles. The serum bottles dosed with cobalt nanoparticles produced the second highest total methane and biogas after the control. The nickel NPs dosed serum bottles produced the highest total methane and biogas. The serum bottles dosed with both nickel and cobalt nanoparticles did not produce more biogas as compared with the individual dosages.

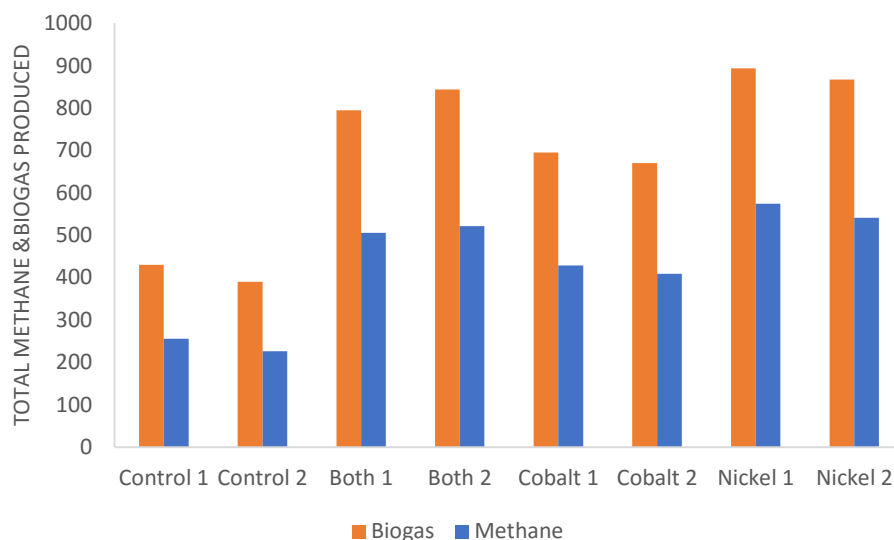


Figure A-3 Total Biogas, Methane Produced

Appendix A-4 Volatile Solids and Biomethane Potential

The control serum bottles were left with 0.021 and 0.0022 mg/l of volatile solids at the end of the experiment. They produced the least biogas hence the least amount of volatile solids depleted. The Ni dosed bottles were left with 0.01 and 0.011 mg/l of volatile solids. They depleted the most volatile solids hence the highest biogas production. 0.013 and 0.011 mg/l of volatile solids were recorded for the serum bottles dosed with both NPs. The cobalt NPs dosed serum bottles were left with 0.014 and 0.015 mg/l of volatile solids. They depleted more volatile solids than the control only.

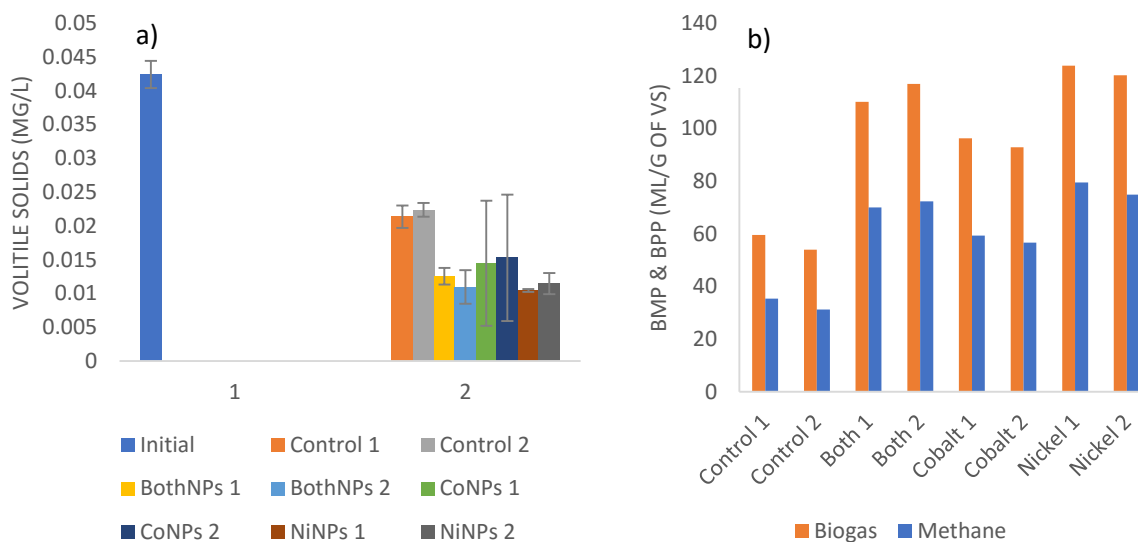


Figure A-4 Volatile Solids and Biomethane Potential (a= volatile solids, b = biomethane potential)

APPENDIX B: DOSAGE OF 20MG/L OF Ni, Ni &Co, Co TO THE 250 ML SERUM BOTTLES

This part shows the data of 8 250 ml digesters used in the experiment. The serum bottles consists of those fed with Co, Ni, Ni & Co and the control each with 2 replicates. Their average is presented in Chapter 4.2.4

Appendix B-1 Cumulative methane and biogas production

The nickel NPs dosed serum bottles had a gradual increase in both methane and biogas production from day 1 to day 13 of the experiment. The production became steady from the 14th day until the 22nd day of the experiment. This was the time after the serum bottles reached their second peak of daily biogas and methane production. From day 1 until day 13 of the experiment the control serum bottles had a gradual increase in biogas and methane production. The production then became steady until day 22. From day 10 to the last day of the experiment the control serum bottles recorded the least biogas and methane as compared with the dosed digesters. The serum bottles dosed with both nickel and cobalt nanoparticles had a gradual increase in both methane and biogas production from the first day until the 15th day. The production became steady from the 16th day until the last day of the experiment.

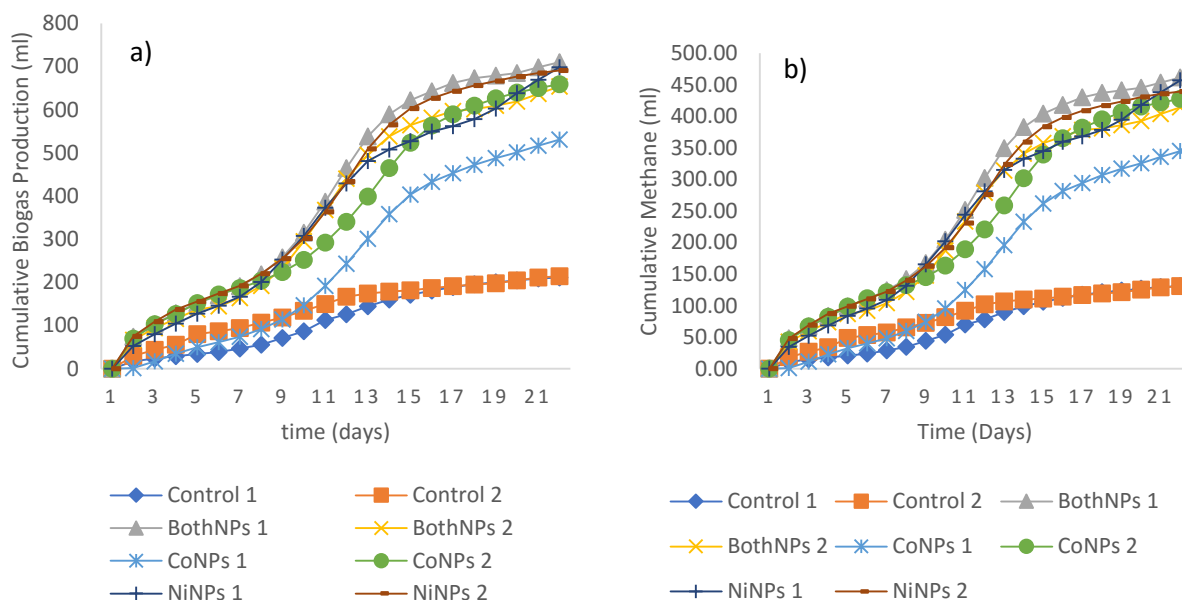


Figure B-1 Cumulative methane and biogas production (a= biogas, b = methane)

Appendix B-2 Daily methane and biogas production

From day 7 of the experiment all the dosed digesters recovered and an increase in daily biogas and methane production was observed. The cobalt dosed digesters reached their peak after the other dosed digesters. Co NPs 1 produced 59 ml of biogas on day 13 (peak day) and Co NPs 2 produced 66 ml of biogas on day 14 (peak day). The serum bottles dosed with both nickel and cobalt NPs reached their peak on day 12 (both NPs 1) and day 11 (both NPs 2). On their respective peak days both NPs 1 and both NPs 2 produced 73 and 78 ml of biogas. Ni NPs 1 produced 65 ml of biogas on day 11 (peak day) and Ni NPs 2 produced 75 ml of biogas on day 13 (peak day). Control 1 reached its peak on day 11 of 26 and 16.16 ml of biogas and methane respectively. Control 2 reached its peak on the second day of the experiment of 30 and 18.36 ml of biogas and methane, respectively. Throughout the rest of the experiment the serum bottle did not recover. From day 11 to day 14 all the other serum bottles reached their second peak except the control 2 serum bottles.

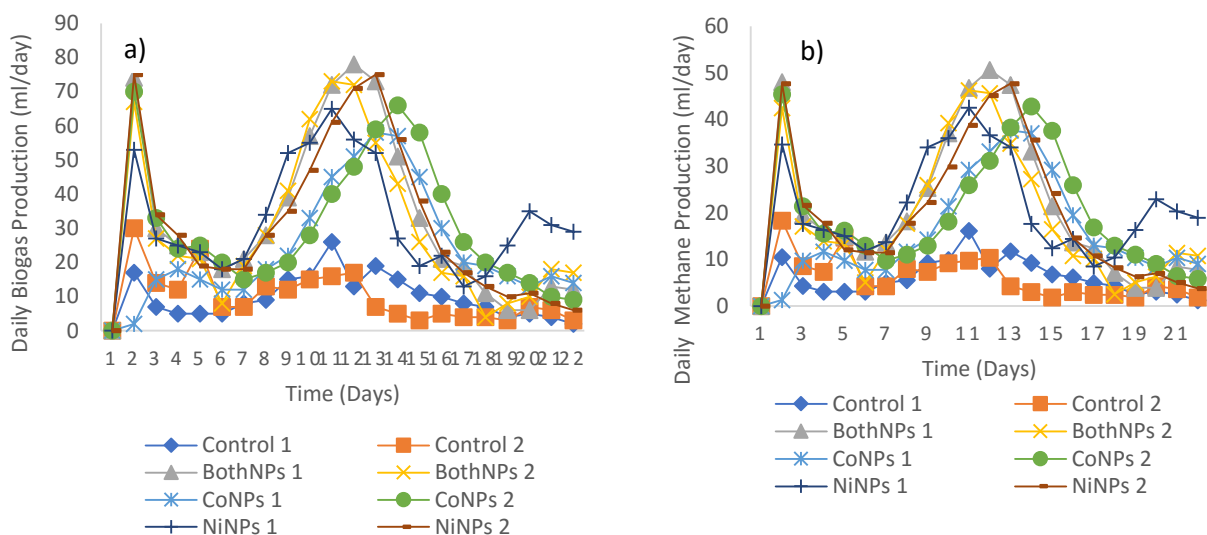


Figure B-2 Daily methane and biogas production (a= biogas, b = methane)

Appendix B-3 Total Biogas, Methane Produced

The non-dosed serum bottles (control) produced the least total biogas (211 and 214 ml) and methane (131 and 131 ml) as compared with the dosed serum bottles. The serum bottles dosed with cobalt nanoparticles produced 345 and 428 ml of methane. They also recorded 531 and 659 ml of biogas. Digesters dosed with nickel and cobalt nanoparticles produced 691 and 698 ml of biogas, respectively. They also recorded 457 and 439 ml of methane. Individual dosage of 20 mg/l of nickel NPs was more stimulatory to both methane and biogas as compared with cobalt NPs dosed serum bottles. 710 and 654 ml of biogas, and 462 and 415 ml of methane were recorded for the serum bottles dosed with both NPs

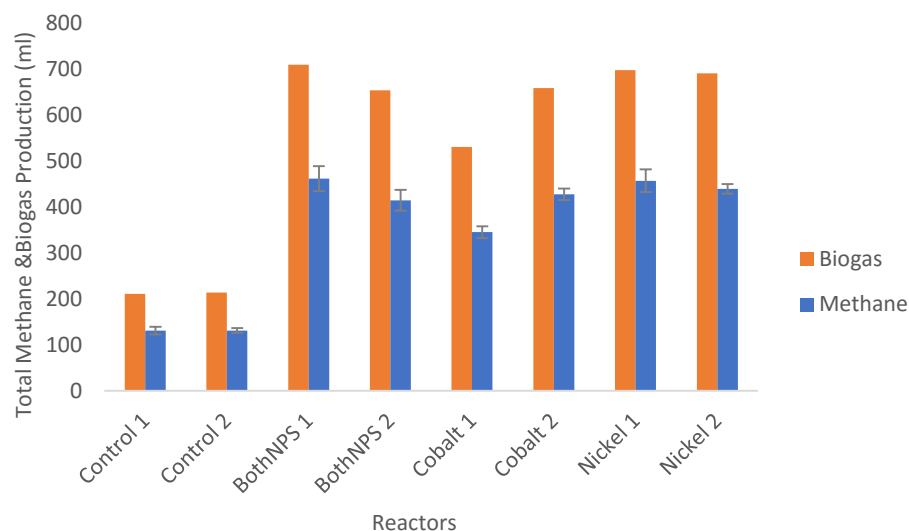


Figure B-3 Total Biogas, Methane Produced

Appendix B-4 Volatile Solids and Biomethane Potential

The control serum bottles were left with 0.0253 and 0.0251 mg/l of volatile solids at the end of the experiment. They produced the least biogas hence the least amount of volatile solids depleted. The Ni dosed bottles were left with 0.0032 and 0.0038 mg/l of volatile solids. They depleted the most volatile solids hence the highest biogas production when compared with the control and other dosed serum bottles. NiNps 1 was the one left with less volatile solids, hence it was the one with higher biogas production between the two. 0.0023 and 0.007 mg/l of volatile solids were recorded for the serum bottles dosed with both NPs. The cobalt NPs dosed serum bottles were left with 0.017 and 0.006 mg/l of volatile solids. CoNps 1 was the one left with more volatile solids, hence it was the one with low biogas production between the two.

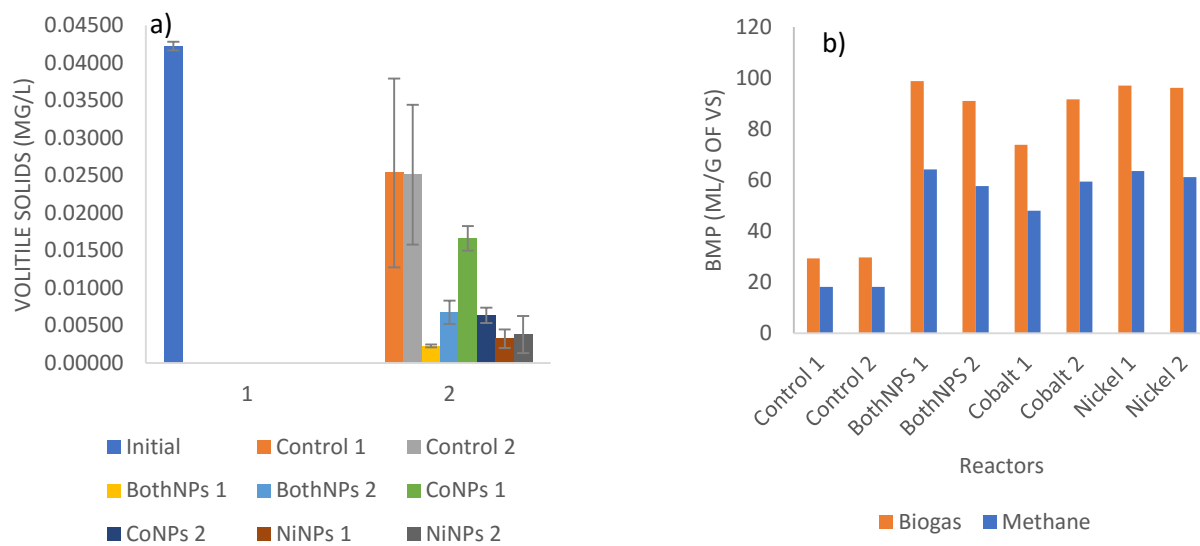


Figure B-4 Volatile Solids and Biomethane Potential (a = VS, b = BMP)

APPENDIX B: SAMPLING AREA



Figure B-4 Aerial view of Jago Beef Abattoir

The letters in the picture represent the different places in the abattoir. A is the holding pen, where animals are kept prior to slaughter. Cow dung was sampled in this area. B and C represent the slaughterhouse. B is where the animals are killed, and the blood is collected. This is where the blood was sampled. C shows where the rumens were emptied, this is where rumen contents were sampled.

