Cape Peninsula
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BIOLOGICAL NUTRIENT REMOVAL FROM MUNICIPAL SEWAGE USING ACTI-ZYME: RECOVERING BIOGAS AND BIO-SOLIDS FROM SEWAGE SLUDGE

By

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DECLARATION

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M. M. Manyuchi, R. L. Marisa, D. I. O. Ikhu-Omoregbe and O. O. Oyekola, *Design and development of an anaerobic bio-digester for application in sewage sludge digestion for biogas and bio-solids generation using Acti-zyme as bio-catalyst*, 2nd International Renewable Energy Conference and Exhibition, Meikles Hotel, Harare, Zimbabwe, 29 July-1 August, 2015.

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ABSTRACT

Water scarcity is a global problem hence the need for sustainable wastewater management. Sewage, a form of wastewater is being disposed-of to river bodies untreated. Additionally, disposal of sewage sludge, a by-product from the sewage treatment process, is resulting in landfilling problems. This study focused on the sustainable anaerobic treatment of sewage, co-harnessing biogas and bio-solids as value added products utilizing Acti-zyme, an enzyme bio-catalyst through bio-augmentation. Emphasis was given to the optimum sewage treatment conditions for removal of bio-nutrients, biogas and bio-solids generation, kinetic and statistical modelling of the bio-nutrient removal in sewage as well as the biogas and bio-solids production from sewage sludge. A techno-economic analysis was then done to check the viability of applying this technology on a large scale.

The biochemical properties for Acti-zyme were characterized for potential use in anaerobic sewage treatment with the aim of producing biogas. Acti-zyme was then used for sewage treatment at a temperature of 37 °C, agitation rate of 60 rpm, Acti-zyme loadings of 0-0.070 g/L and retention times of 0-60 days. The total Kjeldahl nitrogen (TKN), biochemical oxygen demand (BOD_5), total suspended solids (TSS), total dissolved solids (TDS), electrical conductivity (EC), pH, chloride ions concentration (CI^-), total phosphorous (TP), sulphate ions concentration (SO_4^{2-}), dissolved oxygen (DO) and the chemical oxygen demand (COD) of sewage were measured using standard methods. The bio-nutrient removal ratios from the sewage were determined and statistical modelling was carried out for the bio-nutrient removal ratios: The COD/BOD_5, BOD_5/TKN, COD/TKN and the COD/TP. The sewage sludge was anaerobically digested using Acti-zyme in order to obtain biogas and bio-solids. Sewage sludge loading of 5-10 g/L.d and mesophilic and thermophilic temperatures of 37 °C and 55 °C were applied. The biogas quantity produced was measured using the water displacement method. Samples of the biogas were analysed for bio-methane (CH_4), carbon dioxide (CO_2) and traces gases composition using gas chromatography. The bio-solids obtained were tested for nitrogen, phosphorous and potassium (NPK) content using $uv - vis$ spectrophotometry. Kinetic modelling was carried out in MATLAB R2013A to simulate bio-methane production from sewage sludge. Statistical models for anaerobic sewage sludge digestion for generation of biogas and bio-solids utilizing Acti-zyme, were then simulated from the experimental data. SPSS Statistics 19.0 was used as the statistical modelling package at a p-value of 0.05. Capital budgeting techniques were then used for techno-economic assessment of sewage treatment recovering biogas and bio-solids.

Acti-zyme was found to be immotile and contained catalase, proteolytic enzymes and amylase. Acti-zyme did not promote H_2S production, making it useful in sewage treatment producing biogas. Sewage treatment using Acti-zyme resulted in >60% decrease of the sewage contaminants through bio-augmentation. Optimum sewage treatment conditions were obtained at 0.050 g/L Acti-zyme loading and retention time of 40 days. The COD/BOD_5, BOD_5/TKN, COD/TKN and the COD/TP ratios obtained were > 1.2, 4.0, 8.0 and 15.0 respectively. The COD/BOD_5, BOD_5/TKN, COD/TKN and the COD/TP bio-nutrient removal models were developed. Optimum biogas production was obtained at a sewage sludge loading of 7.5 g/L.d and Acti-zyme loading of 0.050 g/L with a 78% bio-methane composition was achieved at mesophilic temperatures of 37 °C. Bio-solids with 8.17, 5.84 and 1.34 % of NPK respectively were produced. The bio-methane production was simulated to the linear, exponential, logistics kinetic, exponential rise to a maximum, first order exponential model and the modified Gompertz kinetic models. The logistics kinetic model accurately simulated the bio-methane production with a k-value of 0.073 day^{-1}. Furthermore, linear, quadratic, compound and exponential statistical models were tested and validated for the biogas and the bio-solids generation. The quadratic statistical models were significant for simulating biogas and bio-solids production respectively.

A sewage plant with a capacity of 19.6 600 ML/day was considered for techno-economic assessment, with an operating efficiency of 60% and a life span of 20 years. $5.125/day of Acti-zyme were required for production of 12 769.69 kg/day for biogas costing $1.50/kg and 672.08 kg/day of bio-solids costing $16.00/50kg. A net benefit of $5 656 363.92 per annum for using the Acti-zyme technology in sewage digestion was forecasted, whilst a net energy of 1 387.33 KWh was set to be produced. An investment of $22 199 501.40 was required for kick-starting the project. A positive net present value of $1 186 239.23 was realized with an internal rate of return of 17.6% and a payback period of 5.9 years. For breakeven to be realised, only 183 059.16 KWh must be produced. The techno-economic assessment indicated it is viable to treat sewage using Acti-zyme as bio-augmentation catalyst; co-harnessing biogas and bio-solids as valued added products to an extent of contributing 0.04% to the Zimbabwe gross domestic product.

Keywords: Acti-zyme, bio-augmentation, biogas, bio-nutrients, bio-solids, sewage treatment, statistical modelling, techno-economic analysis

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DEDICATION

To: Celine, Danielle, Ethan and Sean

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ABBREVIATIONS

A_o	Sewage Treatment without Acti-zyme added
A_1	Sewage Treatment with Acti-zyme added
ANOVA	Analysis of Variance
BOD_5	Biological Oxygen Demand
COD	Chemical Oxygen Demand
df	Degrees of Freedom
DO	Dissolved Oxygen
EC	Electrical Conductivity
E. coli	*Escherichia coli*
EMA	Environmental Management Agency
EPA	Environmental Protection Agency
GDP	Gross Domestic Product
g/L.d	Grams per litre per day
IRR	Internal Rate of Return
KWh	Kilowatt Hour
MPN	Most Probable Number
MSDS	Material Safety Data Sheet
NPV	Net Present Value
NTU	Nephelometric Turbidity Units
p-value	Probability Value
R^2	Regression Coefficient Squared
SI	Statutory Instrument
SIM	Sulphide Indole Motility Agar
SSE	Sum of Squares Error

TDS	Total Dissolved Solids
TKN	Total Kjeldahl Nitrogen
TP	Total Phosphates
TSS	Total Soluble Solids
TS	Total Solids
TVS	Total Volatile Solids
µS/cm	Micro Siemens per Centimetre
UV	Ultra Violet Light
WHO	World Health Organisation

GLOSSARY OF TERMS

Acti-zyme	A solid bio-catalyst that is a combination of several enzymes and bacteria used in wastewater treatment
Anaerobic digestion	This is the process by which organic wastes are biologically transformed in the absence of oxygen
Batch system	This is a system whereby microbial culture is produced by inoculating a closed culture vessel containing a single batch of medium. The conditions within batch culture change dynamically with time
Bio-catalyst	A biological micro-organism used to speed up a reaction
Biogas	Gas produced from digestion of any organic material
Bio-nutrients removal	A process for removal of nitrogen and phosphorous in wastewater
Bio-solids	Solids obtained from the digestion of organic material
Mesophilic conditions	These are conditions whereby an organism that can grow in the temperature range 20-45 °C
Methanogenesis	The process by which sewage is degraded to form bio-methane. This is the last stage of the anaerobic digestion involving bio-methane-producing microorganisms called methanogens
Model	A statistical or kinetic description of a system using statistical concepts quantitatively
Wastewater	This is any water whose set quality parameters have been altered
Sewage	Wastewater from domestic and municipal uses
Sewage Effluent	Treated sewage at a sewage treatment plant
Sewage Influent	Untreated sewage at a sewage treatment plant

Thermophilic conditions	Describes conditions whereby an organism that can grow at high temperatures ≥45 °C

CHAPTER 1. INTRODUCTION

1.1 BACKGROUND

Water scarcity and pollution have become global issues and there is a need to address in wastewater treatment issues using economical treatment technologies. A good example of a municipality facing wastewater treatment challenges is Chitungwiza; a satellite town in Mashonaland East in Zimbabwe where sewage is being disposed of without treatment (Nhapi, 2009). This has posed environmental challenges to the receiving water body, Lake Chivero which is one of the major lakes providing drinking water to people in Chitungwiza and Harare, Zimbabwe (Nhapi, 2009). There is therefore a need to find an economic and alternative method for treating this sewage, using biological means such as Acti-zyme that are environmentally friendly and sustainable.

Acti-zyme is an organic bio-catalyst bacterial that has been used successfully for treating wastewater in numerous parts of the world including Australia, Canada and Chegutu in Zimbabwe (Tshuma, 2010). Acti-zyme has been shown to decrease the river water pollutants based on the biological oxygen demand (BOD_5), chemical oxygen demand (COD), total dissolved solids (TDS), total Kjeldahl nitrogen (TKN) and total phosphate (TP) (Tshuma, 2010). However, no work has been done in terms of bio-nutrient removal in sewage while harnessing biogas and bio-solids as value added products.

Although Acti-zyme has been proven to be a successful bio-catalyst for wastewater treatment, the optimal operating conditions of the Acti-zyme on the sewage treatment are yet to be established as well as the optimal biogas and bio-solids production conditions. Furthermore, the kinetic and statistical modelling during the bio-nutrients removal and biogas production still need to be established as well as the techno-economic analysis to check the viability of applying this technology in sewage treatment recovering value added products. This therefore formed the basis of this study.

1.2 RESEARCH MOTIVATION

Water is a scarce commodity globally; hence sustainable use of any water resources is essential. A good example is the Chitungwiza Municipality in Zimbabwe where poorly treated water comes from Harare City Council which is located 30 km away, with the satellite town consumption at 30 to 45 mega litres per day (ML/day) (Nhapi, 2009). Water is available to residents only once a week and is priced at $\$0.38/m^3$ (Nhapi, 2009). Due to the water challenges faced by the Chitungwiza Municipality in Mashonaland East, Zimbabwe and all other developing country municipalities, there is a need to utilize sustainable sewage treatment methods to ease water problems and recover value added products from wastewater.

The untreated sewage effluent from the Chitungwiza sewage plant is disposed into Lake Chivero whose water is used for human consumption. This poses problems with the Environmental Management Agency (EMA) which controls water pollution in accordance with the statutory instrument (SI) 274 of 2004 on Effluent and Waste Disposal Regulations (Nhapi and Gijzen, 2002). Bio-nutrient removal in sewage using viable bio-catalysts at the same time harnessing value added bio-gas and bio-solids therefore makes sewage treatment economically friendly and sustainable. Statistical and kinetic modelling of the biogas and bio-solids during the process is also critical for simulating the real life situations from experimental data during sewage treatment.

Using Acti-zyme for possible treatment of sewage will ease the sewage treatment problems as well as energy problems that are affecting Zimbabwe. Furthermore, recovering of biogas may reduce the sewage treatment costs as the bio-methane obtained can be used for energy generation at the sewage plant. In addition, the bio-solids obtained during the sewage treatment can be used as a bio-fertiliser. Most sewage treatment approaches have been for environmental purposes in order to prevent pollution, preserve the ecosystem and protect public health. However, an integrated approach to the management of this waste which focuses on energy recovery will have a significant positive economic and environmental impact of the country.

1.3 RESEARCH QUESTIONS

The following research questions were formulated as a basis of this study:

i. Do the properties of Acti-zyme allow it to be used in sewage treatment and biogas generation?
ii. Can sewage be effectively treated using Acti-zyme?
iii. Can the bio-nutrient removal in sewage using Acti-zyme be optimal?
iv. Can the biogas production during sewage treatment with Acti-zyme be optimised?
v. Can the bio-solids obtained during sewage treatment with Acti-zyme be used as bio-fertilisers?
vi. Can the bio-nutrient removal during sewage treatment using Acti-zyme be kinetically and statistically modelled?
vii. Can the biogas and bio-solids generation from sewage sludge using Acti-zyme be modelled?
viii. Is the sewage treatment at the same time co-harnessing biogas and bio-solids using Acti-zyme an economically feasible technology?

1.4 RESEARCH OBJECTIVES

The following were the research objectives for this study:

i. Determine the Acti-zyme biochemical characteristics
ii. Optimize sewage treatment using Acti-zyme under different operating conditions
iii. Benchmark the treated effluent quality to the EMA guidelines which is the regulatory body
iv. Generate biogas and bio-solids from the anaerobic treatment of sewage sludge using Acti-zyme and determine its composition
v. Develop statistical models for bio-nutrient removal from sewage treatment with Acti-zyme as well as biogas and bio-solids generations
vi. Develop kinetic and statistical models for biogas, bio-methane and bio-solids generation from sewage sludge digestion using Acti-zyme

vii. Determine the techno-economic feasibility of sewage treatment co-harnessing biogas and bio-solids using Acti-zyme as bio-catalyst

1.5 RESEARCH HYPOTHESES

The following hypotheses for this study were therefore formulated.

i. Acti-zyme has properties that allows it to be used for anaerobic sewage treatment and bio-gas generation
ii. Biogas and bio-solids can be generated during treatment of sewage using Acti-zyme.
iii. Statistical models can be used for simulation of bio-nutrient removal in sewage using Acti-zyme as bio-catalyst
iv. Kinetic and statistical models can be used for simulation of biogas and bio-solids production from sewage sludge digestion using Acti-zyme
v. Sewage treatment using Acti-zyme co-harnessing biogas and bio-solids is an economically feasible technology

CHAPTER 2. LITERATURE REVIEW

2.1 SEWAGE WASTEWATER TREATMENT

Wastewater is any water whose quality parameters have been altered and contains a mixture of organic and inorganic pollutants. Wastewater sources include domestic, sewage, municipal, chemical and mining industries as well as surface runoff and groundwater. Sewage wastewater contains pollutants that are physical, chemical or biological in nature. Wastewater must be treated for environmental purposes such as the prevention of groundwater contamination and natural water bodies pollution, preserving the soil, aquatic life as well as protection of public health (Manyuchi and Phiri, 2013; Muserere *et al*., 2014). Furthermore, wastewater treatment is essential for re-use in agriculture as well as in industry.

2.2 SEWAGE WASTEWATER CHARACTERISTICS

Sewage is wastewater containing human waste in suspension that is removed from kitchens, lavatories, communities, rainwater flowing into drains and industries, it is mainly composed of 99.9% water and 0.1% faecal matter and urine (Mahmoud *et al*., 2003; Manyuchi and Phiri, 2013; Muserere *et al*., 2014). The objective of sewage treatment like any treatment of wastewater is to produce a disposable effluent without causing harm to the surrounding environment by modifying the water quality parameters. The water quality parameters considered in sewage treatment include total faecal coliforms, temperature, dissolved oxygen (DO), biological oxygen demand (BOD_5), chemical oxygen demand (COD), total phosphates (TP), total Kjeldahl nitrogen (TKN), total suspended solids (TSS), total dissolved solids (TDS) and pH.

An overall summary of raw sewage characteristics is shown in **Table 2.1** in comparison to the set standards acceptable for effluent disposal by EMA. In order, to make sewage disposal safe and environmentally friendly, economic sewage treatment is necessary. The set standards for effluent disposal for EMA are also indicated. Currently the sewage

that is being disposed of in Zimbabwe as indicated in **Table 2.1**, does not meet the required EMA guidelines.

Table 2-1: Raw sewage physicochemical characteristics

Raw sewage parameter	Composition range (Muserere *et al*., 2014)	EMA effluent disposal guidelines (Nhapi and Gijzen, 2002)
TSS	250-600 mg/L	25-50 mg/L
Faecal content	200 colonies/100 Ml	-
Appearance	Dark cloudy	-
Temperature	15-35 ˚C	25-35 ˚C
pH	5.5-8.0	6.0-9.0
Total Kjeldahl Nitrogen	20-50 mg/L	10-20 mg/L
Phosphorous	5-10 mg/L	0.5 – 1.5 mg/L
Sulphate	50-200 mg/L	-
Chloride	100-200 mg/L	-
BOD_5	100-400 mg/L	30-50 mg/L
COD	200-700 mg/L	60-90 mg/L

2.2.1 Faecal coliform content

Faecal coliform content indicates bacteria found in faeces of human beings which contain pathogenic micro-organisms. The higher the faecal coliform, the more contaminated the water and therefore hence not useful for human consumption. Recommended faecal coliforms must be less than 200 colonies / 100 mL of water sample (Tshuma, 2010).

2.2.2 *E. coli* content

Escherichia Coli (E. coli) is a gram negative *Bacillus* in the family *Enterobacteriaceae*, which can be classified as diarrheogenic or non-diarrheogenic according to the influences on the human host. The normal flora of the human intestine harbours non-diarrheogenic *E. coli* that is considered relatively harmless to the host. These generally harmless bacteria are frequently used as indicators of faecal contamination. Most strains will grow under conditions for faecal coliform analysis, but are differentiated by the following characteristics: Methyl Red Positive, Voges-Proskauer negative, does not use citrate as the sole carbon source and are Indole positive are *E. coli* type 1 and associated with mammalian intestines (Doyle and Padhye, 1989). An ideal sample size for *E. coli* content in water treatment will result in 20 to 80 coliform colonies per dish (Doyle and Padhye, 1989).

2.2.3 Temperature

Extremely low temperatures in sewage effluent affect the efficiency of biological treatment systems and the efficiency of sedimentation. The same also applies if the temperatures are on the high side. Acceptable temperature for sewage influent and effluent ranges between 15-35 °C (Mahmoud, 2002). Increase in the sewage temperature from 15-35 °C increases the TSS and COD removal (Ahsan *et al*., 2005).

2.2.4 Organic material in raw sewage

Sewage is mainly composed of organic material. BOD_5 and COD tests are used to measure the oxidation properties of the organic material in sewage. Sewage generally has a COD/BOD_5 ratio of around 1.7 (Attiogbe *et al*., 2005). Babaee and Shayegan (2011) used 1.4-2.27 kg/VS (m^3.d) and the amount of biogas produced decreased with increase in organic loading rate.

2.2.4.1 Sewage BOD_5

Sewage BOD_5 refers to the amount of oxygen required for the biological decomposition of organic matter under aerobic conditions. The oxygen consumed is related to the

amount of decomposable organic matter and higher BOD_5 are an indication of high bio-contaminants in the wastewater and this has a deadly influence on aquatic life (Muserere *et al*., 2014). BOD_5 ranges in sewage are between 100-400 mg/L (Manyuchi *et al*., 2013; Muserere *et al*., 2014).

2.2.4.2 Sewage COD

The COD refers to oxygen required for chemical oxidation in sewage. COD does not differentiate between biological oxidizable and non-oxidizable material. The COD values of raw sewage ranges between 200-700 mg/L and the higher the COD values, the more contaminated the wastewater is (Mahmoud, 2002).

2.2.5 Sewage total Kjeldahl nitrogen content

The sources of nitrogen compounds in raw sewage are proteins, amines, amino acids and urea. Ammonia nitrogen results from bacterial decomposition of these organic constituents. Total Kjeldahl nitrogen therefore consists of nitrates, nitrites; ammonia and ammonium salts and these have potential to cause eutrophication if disposed of in water bodies at high concentration (Muserere *et al*., 2014). Nitrogen is essential for biological protoplasm hence it is very important for proper functioning of biological systems. Raw sewage contains approximately 20-50 mg/L of nitrogen and is measured as TKN (Mahmoud, 2002).

2.2.6 Sewage total phosphates content

The sources of total phosphates (TP) in raw sewage are mainly food residues containing phosphorous and their breakdowns, which if disposed of in water bodies can result in eutrophication (Muserere *et al*., 2014). Increased use of synthetic detergents also results in increased phosphorous quantities in sewage. The phosphorous concentration in sewage ranges from 5-10 mg/L and this concentration is adequate to support biological wastewater treatment (Mahmoud, 2002).

2.2.7 Sewage pH

The general range for raw sewage is 5.5-8.0 (Muserere *et al*., 2014). Decomposition of organic matter lowers the sewage pH whilst the presence of industrial wastewater may also produce extreme fluctuations. However, pH values of greater than 7.2 are ideal for biogas generation in anaerobic conditions (Mahmoud, 2002).

2.2.8 Sewage colour and odour

Raw sewage has a soapy and cloudy appearance depending on its concentration. As time progresses sewage become stale and darkens in colour due to microbial activities (Manyuchi *et al*., 2013; Muserere *et al*., 2014).

2.2.9 Sulphate concentration

Sulphate reducing bacteria thrive in a pH range of 5-9. Sulphate concentration is generally low in sewage i.e. 50-200 mg/L and does not affect methanogenic bacteria (Mahmoud, 2011). Groundwater infiltration increases the sulphates content leading to excessive generation of hydrogen sulphide (H_2S). Furthermore, excessive sulphates hinder methanogenic activity (Apte *et al*., 2011).

2.2.10 Sewage solids

Raw sewage contains about 0.1% solids and these comprise of TDS and TSS, although these are present in small quantities they need proper disposal. Sewage solids comprise of dissolved solids, suspended solids and volatile suspended solids. Knowledge on the organic fraction of the solids is necessary as this determines the extent of biological treatment required (Mahmoud, 2011).

2.2.11 Chlorides

Chloride concentrations in sewage are greater than the normal chloride content supply. The daily contribution to the chloride concentrations averages 8 gm per person. Any abnormal increase in chloride concentration indicates chloride bearing wastes or saline ground water infiltration (Apte *et al*., 2011).

2.2.12 Toxic metals and compounds

Toxic metals and compounds such as chromium, copper and cyanide may find their way into municipal sewage through industrial discharges. The concentration of metals must be monitored to make the biological treatment process efficient as these have a negative impact on the human health (McBride, 2003).

2.3 NUTRIENT REMOVAL IN SEWAGE USING BIO-CATALYTIC METHODS

Sewage treatment involves the removal of contaminants from the wastewater and includes physical, chemical and biological processes. A safe effluent is normally produced as well as bio-solids (Wei *et al*., 2003; Mahmoud *et al*., 2003; Tas *et al*., 2009; Lai *et al*., 2011; Palanisamy and Shamsuddin, 2013; Lee and Nikraz, 2014). The treated effluent can be used for agricultural purposes such as irrigation water (Manyuchi and Phiri, 2013). Typical sewage treatment process is shown in **Figure 2.1** and the various stages incorporated are dependent on the treatment method and the biogas obtained can be further converted to electricity.

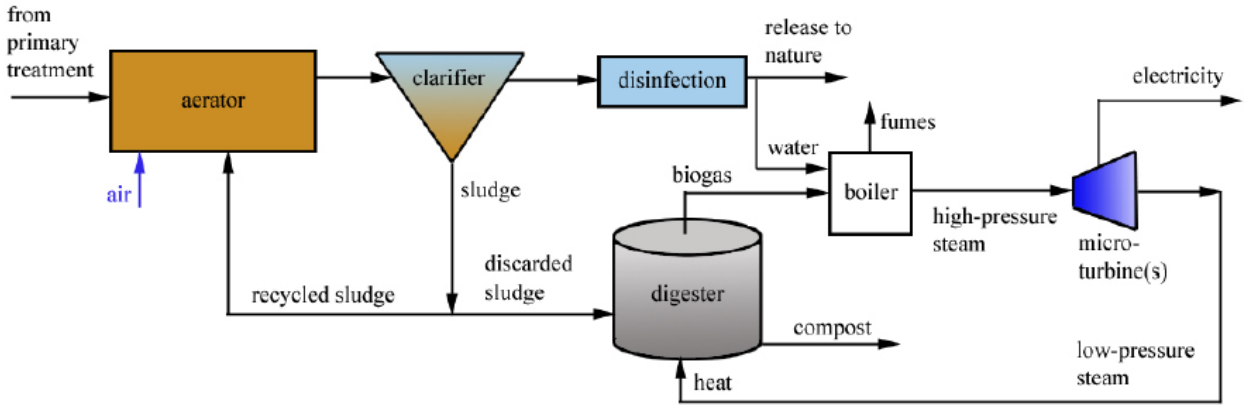


Figure 2-1: Conventional sewage treatment process

Sewage is normally treated in septic tanks, bio-filters or aerobic treatment systems. Biological treatment of sewage removes nutrients such as total nitrogen content, phosphorous content, BOD_5, COD, TSS, TDS, volatile content, *E. coli*, total coliforms

and turbidity (Barker and Dold, 1996; Mace and Mata-Alvarez, 2002; Ahn *et al*., 2003; Kraume *et al*., 2005; Mohan *et al*., 2005; Kampas *et al*., 2007).

Sewage treatment is normally done in 3 steps which are the primary, secondary and tertiary stages like any other wastewater treatment process.

2.3.1 Primary sewage treatment

The primary treatment stage consists of temporary holding tanks where heavy solids settle and floating matter is removed from the top. Large objects are also screened from the influent, and a total of about 40-60% of the suspended solids are removed during this stage. The heavy solids have been recommended for use in biodiesel production as they are rich in lipids (Kargbo, 2010). Primary treatment of sewage removes about 25-35% of sewage suspended solids from the system and about 25-30% of the sewage BOD_5 (Gupta *et al*., 2012). The removed solids during primary sewage treatment can also be sent for digestion such that value added products are obtained.

2.3.2 Secondary sewage treatment

The secondary treatment stage involves the removal of dissolved and suspended solids as well as biological matter by biological techniques. This is done by either aerobic or non-aerobic bacteria and is normally carried out in activated bio-solids systems, trickling filters and rotating biological contactor systems. This is usually done by water borne micro-organism in a controlled habitat. This stage may also require the separation of micro-organisms before tertiary treatment. Secondary sewage treatment removes about 75-95% of the sewage BOD_5 and it is at this stage that there biogas production for anaerobic treatment (Manyuchi and Phiri, 2013). Acti-zyme can be used at this stage as a biological method for removal of biological contaminants in the wastewater (Dzvene, 2013).

2.3.3 Tertiary sewage treatment

The tertiary treatment stage is the final stage of sewage treatment. This is done to ensure safe disposal of the sewage effluent into streams, wetlands or for irrigation

purposes. The treated effluent can be disinfected chemically or physically by the use of lagoons or processes such as micro-filtration. This stage is essential if the end result of the sewage effluent is to use it for domestic purposes.

2.4 SEWAGE TREATMENT CONDITIONS

Sewage treatment can take place either aerobically or anaerobically. Anoxic and anammox conditions can also be considered.

2.4.1 Aerobic conditions

In aerobic conditions, dissolved oxygen (DO) is available and aerobic bacteria survive by metabolising with the oxygen while at the same time producing carbon dioxide and water as products. The reaction that occurs during aerobic conditions is indicated in **Reaction 2.1**. The bacteria that facilitate the reaction are called aerobes (Mittal, 2011). Besides the presence of oxygen, the rate of the aerobic reaction is also dependent on the retention time, temperature and the biological activities of the bacteria (Gupta *et al*., 2012). Aerobic conditions favour the removal of BOD_5 and COD, dissolved and suspended organics, nitrates as well as phosphates. However, aerobic treatment of wastewater results in large quantities of bio-solids which may pose problems in terms of disposal. Aerobic treatment of wastewater is normally done in trickling filters, activated bio-solids ponds and oxidation ponds (Gupta *et al*., 2012).

$$Organic\ Matter + O_2 + Bacteria \rightarrow New\ cells + CO_2 + H_2O + By - products \ldots\ldots\ldots\ldots\ldots\ldots (2.1)$$

2.4.2 Anaerobic conditions

During anaerobic conditions free DO is not available but oxygen is present in the form of sulphate. Anaerobic bacteria can utilize the oxygen bound in the sulphate but sulphidogenesis must be avoided since this can inhibit methanogenesis process especially at temperatures of 30 °C and pH of 7-8 (Molwantwa, 2002). H_2S, carbon dioxide and bio-methane are the major products as indicated in **Reaction 2.2** (Daelman

et al., 2012). These gases are collectively termed biogas and the bacteria which facilitate the anaerobic reactions are called anaerobes (Coppen, 2004; Mittal, 2011).

$$Organic\ Matter + Bacteria \rightarrow Organic\ acids + CH_4 + H_2S + H_20 + CO_2 + By - products \ldots\ldots\ldots (2.2)$$

2.4.3 Anoxic conditions

In anoxic conditions, dissolved oxygen is not available but oxygen is present in the form of nitrate (NO_3). Facultative bacteria utilize the oxygen bound up in the nitrate for breathing which result in the release of nitrogen gas (N_2) as a product (Gupta *et al*., 2012).

2.4.4 Anammox conditions

Anaerobic ammonium oxidation reaction takes place in the absence of oxygen. During this process, nitrite and ammonium are converted directly to dinitrogen gas and has been reported for about 4-37% loss of nitrogen (Hu *et al*., 2011). In addition, anammox reactions best occur at 20-85 ◦C. Anammox reactions are facilitated by a group of bacteria called planctomycetus and these include: *Brocadia, Kuenemia, Anammoxglobus* and *Jettenia*. Anammox reactions promote anaerobic COD removal and biogas production at the same time hindering nitrification and denitrification (Rich *et al*., 2008

2.5 ANAEROBIC TREATMENT OF WASTEWATER USING ACTI-ZYME

Several new methods are being applied in wastewater treatment. These include adsorption, disinfection, flocculation, nutrient removal techniques, membrane systems, oxidation, lagoon systems, reed bed technology, vermifiltration technology and water treatment using Acti-zyme among others (Zhou and Smith, 2002; Coppen, 2004; Huang *et al*., 2010; Tshuma, 2010; Manyuchi and Phiri, 2013; Manyuchi *et al*., 2013). Anaerobic treatment of wastewater is becoming increasingly popular since it provides process stability and control for wastewater treatment plants (Lettinga, 1995; Mahmoud *et al*., 2003; Wei *et al*., 2003; Hospido *et al*., 2005; Coelho *et al*, 2006). In anaerobic treatment of wastewater there is no additional nutrient requirement and valuable by-products such

as biogas are produced (Lettinga, 1995; Mahmoud *et al*., 2003; Wei *et al*., 2003; Hospido *et al*., 2005; Coelho *et al*, 2006). Therefore, anaerobic treatment of wastewater using biocatalysts such as Acti-zyme is an attractive treatment option.

2.5.1 Acti-zyme biochemical properties

Acti-zyme is a bacterial enzyme that degrades organic waste anaerobically through bio-augmentation (Duncan, 1970; Powell and Lundy, 2007; Tshuma, 2010; Dzvene, 2013; Wa *et al*., 2013; Hesnawi *et al*., 2014; Yang *et al*., 2014). Acti-zyme also has the potential to biodegrade wastewater aerobically. During the digestion process, Acti-zyme multiplies and reproduces due to the availability of nutrients in the wastewater. Acti-zyme can reproduce as much as 2 billion bacteria colonies per gram added to water in 48 hours (Tshuma, 2010). Acti-zyme exists in solid form and has a neutral Ph when in solution (**Table 2.2**). The physicochemical characteristics of Acti-zyme are indicated in **Table 2.2**. The microbiological characteristics of Acti-zyme forms part of this study as there is no sufficient literature available in this area. Biochemical tests such as Catalase, Motility, Gelatine Liquefaction, Methly Red and Voges, Starch, Indole, Urea and H_2S production need to be performed in order to determine the suitability of Acti-zyme for application in anaerobic sewage treatment and biogas production (Schreckenberger and Blazevic, 1974, Woodland, 2004; Segal and Potter, 2008; Vashist *et al*., 2013).

Table 2-2: Acti-zyme physicochemical characteristics (Ecolab, 2006)

Parameter	Description
Physical state	Solid
Colour	Brown (Light)
Odour	Faint odour
Boiling point	>100 ˚C
Melting point	Not available
Vapour pressure	Not available
Flash point	Product does not support combustion
Density	0.99 g/Ml
Vapour density	Not available
pH	7 when in solution
Solubility	Slightly soluble in cold water, hot water

2.5.2 Use of Acti-zyme in wastewater treatment

Anaerobic Acti-zyme action on wastewater results in enhanced water treatment physicochemical properties due to reduced BOD_5, nitrates, phosphates and TSS by more than 40% according to studies that were conducted on Mid-Mupfuure Dam water, on piggery wastewater in Zimbabwe and on wool scouring wastewater (**Table 2.3**) (Cail *et al*., 1986; Tshuma, 2010; Dzvene, 2013). Acti-zyme also neutralises the wastewater pH and increase the wastewater effluent DO by more than 100% (Tshuma, 2010). (**Table 2.3**)However, from the studies on wastewater using Acti-zyme, no optimal treatment conditions have been recommended as well as the interactions between Acti-zyme and the other process parameters still need to be understood.

Table 2-3: Influence of Acti-zyme addition on wastewater contaminants

Parameter	Cail *et al*.(1986)	Tshuma (2010)	Dzvene (2013)
Type of wastewater	Wool scouring	Dam water	Piggery
BOD_5	62.4	96	58.1
COD	58.4	-	-
Total nitrogen	-	46	35.6
Total phosphates	-	67	-
Ammonia	-	53	48
Nitrates	-	80	-
E. coli	-	Positive	-
TSS	-	97.6	88.5
TDS	-	-	76.6
pH	-	6.8-7.4	7.82-7.84
Temperature	-	18-24 °C	-
DO		100 increase	-
Acti-zyme loading	1 % (v/v)	4 kg/day for 1 month in a 5 000m^3 dam (0.024 kg/m^3) (0.02 g/L)	300m^3 for 150 days (2 m^3/day) (0.044 g/L)
Retention time	207-211 days	60 days	150 days

At the end of the treatment process, when all the nutrients in the wastewater are spent, the Acti-zyme dies and becomes part of the bio-solids thus there is no need for an extra separation process (Tshuma, 2010; Dzvene, 2013). Acti-zyme can also be used to treat

blocked sewer pipes (Tshuma, 2010). **Figure 2.2** indicates the cleaning capacity of Acti-zyme on sewage pipes in Chitungwiza by introducing the Acti-zyme along the pipes in different doses under aerobic conditions (Powell and Lundy, 2007). Acti-zyme also helps in eliminating odour, which is a challenge in sewage treatment and can also be used for treating municipal, agricultural, and commercial and food industries wastewater (Powell and Lundy, 2007). Treatment of wastewater with Acti-zyme results in nitrogen, clean water, micronutrients and biogas (Powell and Lundy, 2007). Acti-zyme loading in wastewater ranges between 0.03-0.050 g/L at stirring rates of 40-60 rpm (Powell and Lundy, 2007).

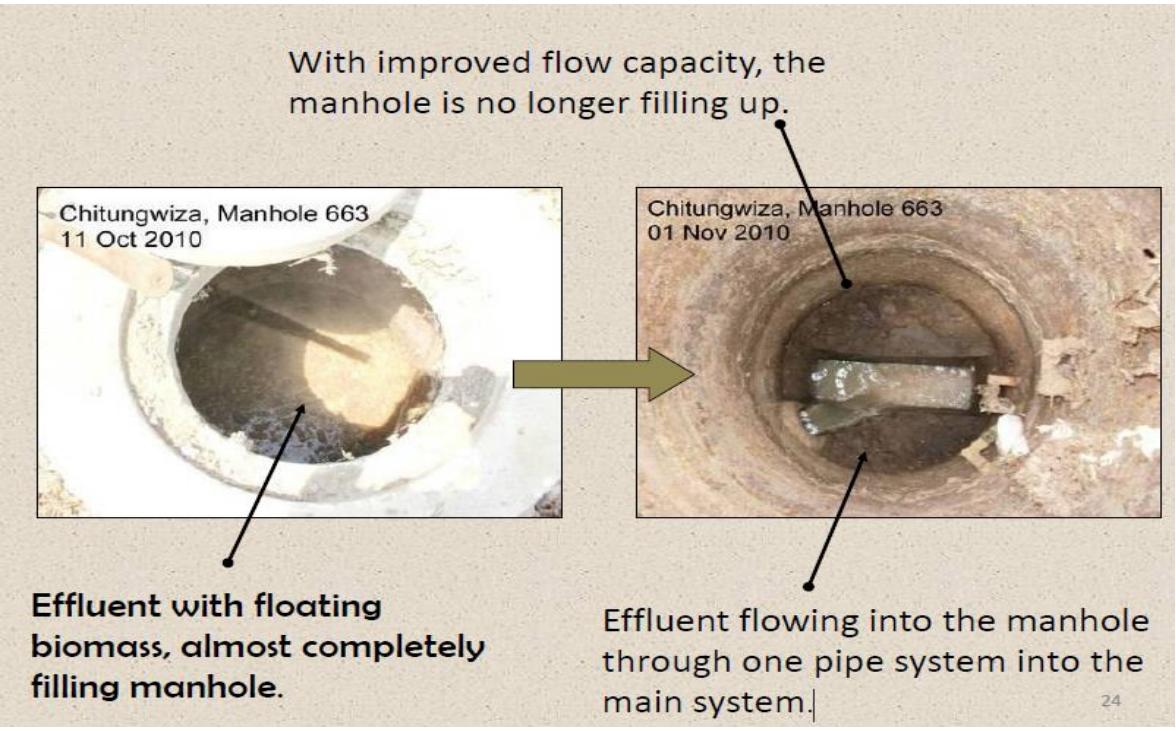


Figure 2-2: Acti-zyme influence on blocked pipe systems (Powell and Lundy, 2007)

Water management including wastewater treatment is rapidly becoming a critical issue world-wide therefore using Acti-zyme in sewage treatment will provide a simple and cost effective way to treat wastewater (Powell and Lundy, 2007).

2.5.3 Acti-zyme kinetics during wastewater treatment

Acti-zyme has been employed for wastewater treatment in batch systems (Cail *et al*., 1986; Tshuma, 2010; Dzvene; 2013). Acti-zyme like any other cell that is introduced in a batch culture with limited nutrients it goes through various stages of growth in the batch culture, in this case the nutrients being the bio-contaminants in the wastewater. When Acti-zyme cells are inoculated into a batch reactor containing fresh culture medium and their increase in concentration is monitored, several distinct phases of growth can be observed. The various phases are shown in **Figure 2.3.** There is an initial lag phase, which is of variable duration. This is then followed by the exponential growth phase, where cell number (and dry weight) increases exponentially. This is also referred to as the logarithmic phase, the name arising from the common method of plotting the logarithm of cell number against time. Following this is a short phase of declining growth, and then the stationary phase. Here the cell numbers are highest. Finally the cell numbers decline during the death phase (**Figure 2.3**).

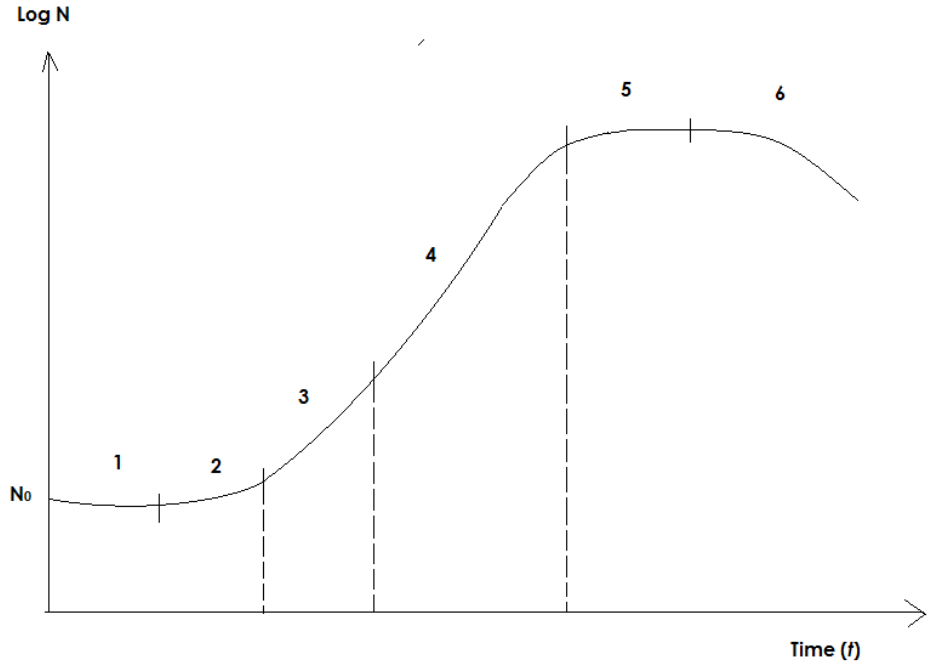


Figure 2-3: Acti-zyme batch cell kinetics during wastewater treatment

Where: N = no of cells, 1 = lag phase, 2 = acceleration phase, 3 = balanced growth, 4 = deceleration phase, 5 = stationary phase and 6= death phase

The *lag phase* results from several factors. When cells are placed in fresh medium, intracellular levels of amino acids and ions (e.g. Mg^{2+}, Ca^{2+} etc.) may be transported across the cell membrane and thus their concentration may decrease appreciably. If intermediates in metabolic pathways are required for enzyme activity, this dilution may reduce the rate at which various pathways operate. Cells must then metabolize the available carbon sources to replenish the intracellular pools prior to initiating cell division. Similarly, if the inoculum is grown in a medium containing a different carbon source from that of the new medium, new enzymes may need to be induced to catabolize the new substrate and this will also contribute to a lag. This exponentially-derived inoculum will have adequate concentrations of intermediates and will not suffer from the dilution influence. If an inoculum is placed in a *rich* medium, one containing amino acids and other complex carbon and nitrogen sources, a shorter lag phase results as the intermediates of metabolism are already provided. Cell division occurs in the *exponential phase*. The rate of increase of cell number (N) is proportional to the number of cells and is represented by **Equation 2.1**.

$$\frac{dX}{dt} = \mu X \quad \ldots\ldots (2.1)$$

Where: μ is the *specific growth rate* of the cells and X is the number of cells per given time.

Following the exponential growth phase, the rate of exponential growth decreases (*declining growth phase*) and is followed by the stationary phase. The duration of the stationary phase may vary with cell type, previous growth conditions etc. Some cells may lyse, releasing nutrients that can be consumed by other cells, and thus maintain the cell population. Following this is the *death phase*; during the death phase, it is thought that cell lysis occurs and the population decreases. Intracellular metabolites are scavenged by different enzyme systems within the cell and toxic metabolites may

accumulate. The rate of decline is also exponential, and is represented during the death phase by **Equation 2.2**:

$$\frac{dX}{dt} = -K_d X \quad (2.2)$$

Where K_d is the death constant for the cells

2.5.4 Acti-zyme technology as applied bio-augmentation

The process of Acti-zyme action in enhancing wastewater treatment can be related to the bio-augmentation process. Bio augmentation is the practice of adding actively growing, specific microbial strains into a microbial community in an effort to enrich the ability of the microbial community to respond to process fluctuations or to degrade certain compounds, resulting in enhanced treatment (Ma *et al*., 2013; Hesnawi, 2014; Yang *et al*., 2014). By changing the microbial community to include specific microbes, the characteristics of the microbial community can be improved thereby improving the wastewater treatment efficiency (Ma *et al*., 2013; Hesnawi, 2014; Yang *et al*., 2014). Various bacteria, besides Acti-zyme have been employed in wastewater treatment and have resulted in improved wastewater treatment as indicated in **Table 2.4.** A minimum of 40% removal of the contaminants was achieved depending on the type of bacterial used and the wastewater through bio-augmentation.

Table 2-4: Impact of bio-augmentation on wastewater treatment

Reference	Wastewater	Parameter observed	% Decrease	Bacteria used
Ma *et al*., 2013	Sewage	Total phosphate	96.4	-
Yang *et al*., 2014	Sewage	Chemical Oxygen demand	83.7	*Pseudomonas and Bacillus*
		Total phosphates	67.8	
		Total nitrogen	58.7	
Hesnawi *et al*., 2014	Municipal wastewater	Total organic carbon	70	*SludgeHammer,*
			54	*B. subtilis*
			52	*B. laterosponus*
			42	*P. aeruginosa*

2.6 ANAEROBIC BIOLOGICAL SEWAGE TREATMENT VALUE ADDED PRODUCTS

Treating sewage anaerobically results in a clean disposable effluent, biogas and low quantities of bio-solids (Lettinga, 1995; Mahmoud *et al*., 2003; Wei *et al*., 2003; Hospido *et al*., 2005; Coelho *et al*, 2006). These are the products that are also promoted by treating wastewater with Acti-zyme (Powell and Lundy, 2007). The bio-solids produced are in minute quantities as most of them are degraded during the decomposition of the sewage into biogas. Anaerobic digestion of sewage has many advantages which include environmental and economic benefits from the biogas production (Lettinga, 1995;; Hospido *et al*., 2005; Coelho *et al*, 2006).

The sewage effluent can be used for irrigation purposes or can be disposed of in water bodies depending on the extent of treatment (Manyuchi and Phiri, 2013; Muserere *et al*., 2014). Sewage effluent characteristics for use in irrigation and decorative purposes are shown in **Table 2.5** post treatment of the wastewater.

Table 2-5: Sewage effluent physicochemical characteristics used for irrigation and decorative water (Alwadhi, 2013)

Parameter	Irrigation Water	Decorative Waters	EMA disposal guidelines (Nhapi and Gijzen, 2002)
E.coli (per 100 mL)	2.2	< 100	-
BOD_5	< 5 mg/L	< 25 mg/L	30-50 mg/L
Total nitrogen	-	< 10 mg/L	10-20 mg/L
TSS	< 5 mg/L	-	25-50 mg/L
TP	-	< 1 mg/L	0.5-1.5 mg/L
pH	6-9	6-9	6-9
EC	< 700 µS/cm	-	1000-2000 µS/cm
Turbidity	< 0.5 NTU	-	5 NTU
Ammonia-NH_4	< 2 mg/L	-	0.5 mg/L
Chloride	< 100 mg/L	-	-
Sodium	< 70 mg/L	-	-

2.7 BIO-NUTRIENT REMOVAL IN SEWAGE

2.7.1 Bio-nutrient removal ratios considered

Sewage bio-nutrients removal can be measured by its biodegradability and de-nitrification which focuses on organic matter removed per sample of sewage (Tas *et al*., 2009; Lai *et al*., 2011; Lee and Nikraz, 2014). The COD/BOD_5, BOD_5/TKN, COD/TKN and COD/TP ratios are used as good indicators in bio-nutrient removal through biodegradability and de-nitrification during biological sewage treatment (Tas *et al*., 2009). Muserere *et al*. (2014) reported COD/BOD_5 ratios of 1.5-3-5 and COD/TP ratios

of 20-60 for City of Harare in Zimbabwe waster when they were determining their treatability, and the values obtained indicated the wastewater was easily biodegradable.

2.7.2 Bio-nutrient removal ratios statistical modelling

Statistical modelling is a very important statistical tool which is used in almost all wastewater treatment processes for correlating different parameters using either linear or non-linear regression methods (Khambete and Christian, 2014). Multiple linear regression with the help of correlation analysis assists in finding interrelationship between different parameters and also the main parameters affecting the bio-nutrient removal. With the help of multiple linear regression analysis in bio-nutrient removal, one can easily eliminate tedious laboratory work of determining all parameters and can carry out its work only with the parameters that are significantly correlated in the regression equation (Khambete and Christian, 2014). During the multiple linear regression analysis, in regression analysis, there are two types of variables; the variable whose value is influenced is called dependent variable and the variables which influence the dependent variable (Khambete and Christian, 2014). The bio-nutrient removal model equation can therefore be presented in form of **Equation 2.3**.

$$Y = B_0 + \mathrm{B}_1 X_1 + \mathrm{B}_2 X_2 + \mathrm{B}_3 X_3 + \mathrm{B}_4 X_5 + \varepsilon \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots . (2.3)$$

Where: Y is the bio-nutrient parameter, β_0 is a constant, X_1, X_2….are the dependent variables, β_1, β_2……are the regression coefficients from the multiple linear regression analysis andε is the residual error

2.8 BIOGAS FROM SEWAGE SLUDGE

2.8.1 Conditions for biogas production

Anaerobic treatment of sewage results in biogas production. Biogas production is produced at optimum temperature ranges of 35-55 ˚C (Wei *et al*., 2003; Coelho *et al*, 2006; Kaosol and Sohgrathok, 2012). Biogas mainly comprises of bio-methane; CH_4 (≥60 %); carbon dioxide; CO_2 (30-35 %), as well as traces of H_2S and nitrates (Reali *et*

al., 2001; Arthur and Brew-Hammond, 2010; Nazaroff and Alvarez-Cohen, 2013; Neczaj *et al*., 2013). The average biogas composition is indicated in **Table 2.6**.

Table 2-6: Typical biogas composition from sewage (Arthur and Brew-Hammond, 2010)

Gas	% Composition
CH_4	40-75
CO_2	25-40
N_2	0.5-2.5
O_2	0.1-1
H_2S	0.1-0.5
CO	0.1-0.5
H	1-3

The biogas from sewage is generated in a 4 step process, which is described below and indicated in **Figure 2.4**: The four steps involved are hydrolysis, acidogenesis, acetogenesis and methanogenesis.

i. Hydrolysis of carbohydrates into simple sugars, proteins into amino acids and fats into fatty acids
ii. Acidogenesis which involves the decomposition of the lipids, cellulose and proteins into fatty acids using facultative bacteria e.g. *Staphylococcus* into low alcohols and organic acids
iii. Acetogenesis which involves the conversion of the low alcohols and organic acids into acetic acid, CO_2 and H_2 which are required for the methanogenic process.
iv. Methanogenesis which involves the digestion of the fatty acids by methanogenic bacteria e.g. *Methanobacteri* for bio-methane production. During this stage bio-methane is produced from the acetogenesis products as well as by-products from

the hydrolysis and acidogenesis stages. The equations that occur during this stage are described in **Reactions 2.3-2.5** (Ozmen and Aslaunzadeh, 2009).

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad \ldots\ldots (2.1)$$

$$2CH_3CH_2OH + CO_2 \rightarrow CH_4 + 2CH_3COOH \quad \ldots\ldots (2.2)$$

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_20 \quad \ldots\ldots (2.3)$$

Bio-methane generated from sewage plants can actually meet about 60% of the sewer plants energy requirements (Daelman *et al*., 2012).

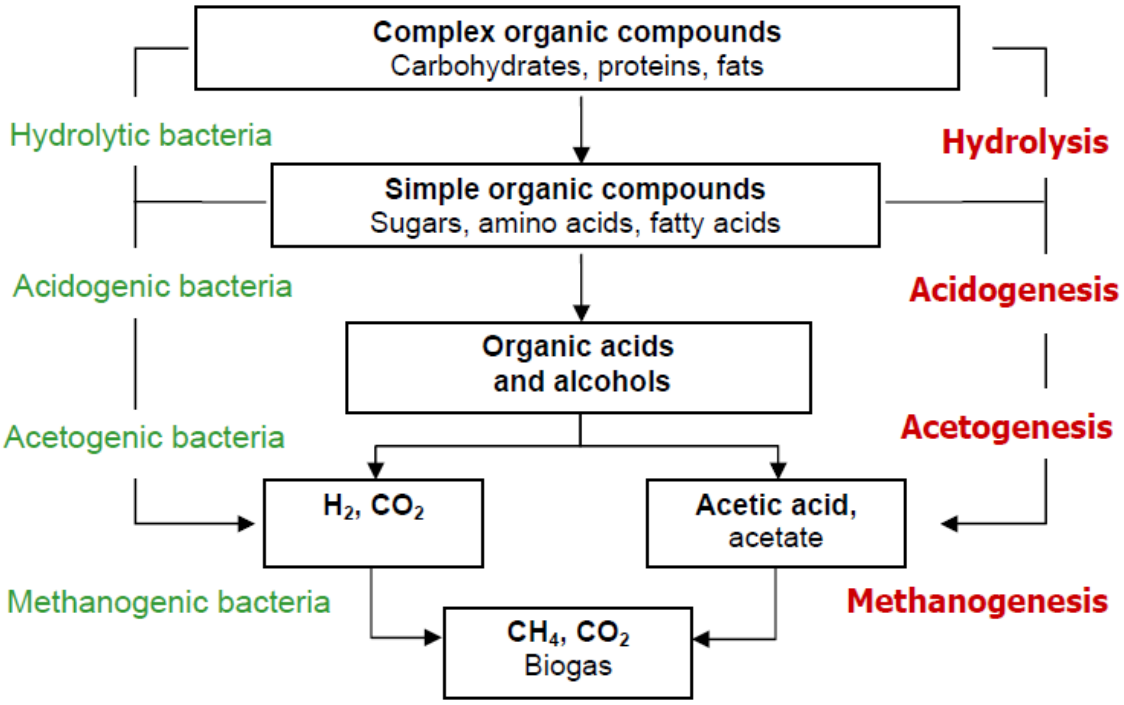


Figure 2-4: Biogas production stages (Schaumann BioEnergy, 2013)

Biogas can be used as an energy source for heating purposes as well as electricity generation to meet the sewage plants electricity demands (Coelho *et al*, 2006B; Malik and Bharti, 2009; Nazaroff and Alvarez-Cohen, 2013). Bio-methane produced from sewage plants have high yields of about 310-740 litres/kg of total volatile solids (TVS) compared to other sources of organic wastes such as pig manure, vegetable residue

and cow manure (**Table 2.7**) (Arthur and Brew-Hammond, 2010). Table 2.7 indicates that the potential for harnessing biogas from sewage treatment with Acti-zyme is attractive (Powell and Lundy, 2007).

Table 2-7: Biogas yield from various types of substrates (Arthur and Brew-Hammond, 2010)

Substrate	Biogas yield (litres/ kg of TVS)
Pig manure	340-350
Vegetable residue	330-360
Sewage bio-solids	310-740
Cow manure	90-310

Bio-methane combustion can result in electricity of 36.5-37.78 MJ/m^3 (Arthur and Brew-Hammond, 2010; Nazaroff and Alvarez-Cohen, 2013). Biogas was produced from sewage in Ghana with retention times of 10, 20 and 30 days and resulted in 170.72 m^3, 341.86 m^3 and 419.46 m^3 of bio-methane for plant capacities of 540 m^3, 1100 m^3 and 1600 m^3 at 30 $^{\circ}$C, respectively (Arthur and Brew-Hammond, 2010). The electricity potential estimation for biogas produced from sewage which is mainly constituted of bio-methane is indicated in **Table 2.8.**

Table 2-8: Potential electricity generation from bio-methane (Arthur and Brew-Hammond, 2010)

Parameter	Values
Bio-methane heating value	37.78 MJ/m^3
Bio-methane content	65 %
Biogas engine efficiency	29 %
Conversion factor	1KWh = 3.6 MJ

A typical bio-methane annual production rate and generator capacity for various biogas digesters from sewage is indicated in **Table 2.9**. High retention times favour increased bio-methane production as well as efficient treatment of the bio-solids in terms of pathogenic organisms' reduction (Driessen *et al*., 2000; Arthur and Brew-Hammond, 2010).

Table 2-9: Annual bio-methane production rates and bio-digesters sizes for varying retention times (Arthur and Brew-Hammond, 2010)

Retention time (days)	Annual bio-methane estimation (m^3)	Annual energy production (MWh)	Generator capacity (kW)	Biogas digester size (m^3)
10	170.719	6 446 779	50	540
20	341.858	12 915 405	100	1100
30	394.710	14 912 143	120	1600

2.8.2 Biogas generation from wastewater using Acti-zyme as biocatalyst

Biogas was generated previously from wastewater using Acti-zyme as bio-catalyst by Cail *et al*. (1986) from wool scouring wastewater and Duncan (1970) in hog wastes (**see Table 2.10**). Although biogas was generated before using Acti-zyme, no attempts to determine the optimum biogas production rate were done. Furthermore, there is still need to understand the statistical and kinetic modelling for the biogas production using Acti-zyme from wastewater so that real life situations at the wastewater treatment plants can be simulated.

Table 2-10: Parameters affecting biogas generation using Acti-zyme

Type of wastewater	Acti-zyme loading	Retention time (days)	Organic loading rate	T (°C)	pH	Biogas production rate	Bio-methane content	Reference
Wool scouring wastewater	1% (w/v)	207-211	0.75-0.99 kg/kg VSS	35 °C	7.1-7.4	2.9-3.3 m^3/ (m^3.day). 30% higher compared to an A_o system	68%	Cail *et al.*, 1986
Hog waste	0.00625%	50	0.5-1.5 L/day	35 °C	7.1-7.2		60% CH_4 , 38% CO_2, 1% N_2, 1% water and H_2S traces	Duncan, 1970

2.8.3 Sewage biogas kinetic modelling

Several kinetic models for sewage sludge digestion have been used to simulate the biogas generation from sewage sludge. The kinetic models can be expressed as linear, exponential, logistics kinetic, exponential rise to a maximum, first order exponential model and the Modified Gompertz equation (Latinwo and Agarry, 2015).

2.8.3.1 Linear kinetic model

The linear kinetic model for biogas production can be expressed in the form indicated in **Equation 2.4** (Latinwo and Agarry, 2015) for both the ascending and descending stages.

$$y = a + bt \quad \dots \dots \quad (2.4)$$

Where: y = biogas production rate in mL/day, t = time in days and a and b = constants obtained from the intercept and slope of y versus t.

2.8.3.2 Exponential kinetic model

In the exponential kinetic model it is assumed that the biogas production rate will increase with increase in time and after a certain period, after reaching the highest point biogas production will decrease to a zero exponentially with increase in time (Latinwo and Agarry, 2015). The exponential model is represented in **Equation 2.5**.

$$y = a + b\exp(ct) \quad \dots \dots \quad (2.5)$$

Where: y= Biogas production rate in mL/day, t = time in days for sewage sludge digestion, a and b = constants in m/day and c = constant in (per day)

2.8.3.3 Logistics kinetic model

The logistics kinetic model is represented by **Equation 2.6**.

$$C = \frac{a}{1 + bexp(-kt)} \quad \dots \dots \quad (2.6)$$

Where: C = cumulative biogas production (mL/day), k = rate constant (per day), t = hydraulic retention time (days) and a and b are constants

2.8.3.4 Exponential rise to a maxima kinetic model

Specific bio-methane production is usually modelled using first order kinetic model with an exponential rise to a maxima model for anaerobic systems whereby hydrolysis is the rate determining step and there is no accumulation of intermediate gases (Rodriguez-Chiang and Dahl, 2015). This kinetic model also further indicates biogas production rises to an exponential maximum and is indicated in **Equation 2.7** (Latinwo and Agarry, 2015).

$$C = A\left(1 - exp(-kt)\right) \dots \dots \dots \dots \dots \dots \dots \dots \dots \dots \dots \dots \dots \dots \dots \dots \dots \dots \dots \dots \dots \dots \dots \dots \dots \dots (2.7)$$

Where: C = cumulative bio-methane yield at time, t (mL/day), A = Constant (mL/day), k = first order rate constant (d^{-1}) and t = time in days for sewage sludge digestion

2.8.3.5 Modified Gompertz kinetic model

The kinetics of biogas production during sewage digestion can also be presented by the modified Gompertz equation (Yusuf *et al*., 2011; Abu-Reesh, 2014). This model assumes biogas production is a function of time and is represented by **Equation 2.8** (Latinwo and Agarry, 2015).

$$B_t = Bexp\left[-\exp\left[\frac{Rb\ x\ e}{B}(\wedge - t) + 1\right]\right] \dots \dots \dots \dots \dots \dots \dots \dots \dots \dots \dots \dots \dots \dots \dots \dots \dots \dots \dots .. (2.8)$$

Where: B_t = Cumulative of biogas (mL/day) produced at any time (t), B = Biogas production potential, R_b = Maximum biogas production rate (mL/day) and ^ = lag phase (days) which is the minimum time required to produce biogas after the Acti-zyme has acclimatized

2.8.4 Sewage biogas statistical modelling

Several methods can be used in bioengineering and these include linear, exponential compound and quadratic models (Ghatak and Mahanta, 2014; Pei *et al*., 2014; Lo *et al*.,

2010). In linear modelling, a linear function is a polynomial function with one variable in which the optimum-degree term is one and takes the form represented by **Equation 2.9**.

$$f(x) = ax + b, \;\; a \neq 0 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots. (2.9)$$

The statistical model is as represented by **Equation 2.10** (Snee, 1977; Fachri *et al*., 2015). Given a random sample $(Y_i, X_{i1}, \ldots, X_{ip}), i = 1, \ldots, n$ the relation between the observations Y_i and the independent variables X_{ij} is formulated as in **Equation 2.10**:

$$Y_i = \beta_0 + \beta_1\phi_1(X_{i1}) + \beta_2\phi_2(X_{i2}) + \cdots \beta_3\phi_p(X_{ip}) + \varepsilon \quad i = 1, \ldots, n \ldots\ldots\ldots\ldots (2.10)$$

Where $\phi_1, \ldots, \phi_p$ may be nonlinear functions in the equation. In **Equation 2.11**, the quantities ε_i are random variables representing errors in the relationship. The linear part of the designation relates to the appearance of the regression coefficients, β_j in a linear way in the above relationship. Alternatively, it can be said that the predicted values corresponding to **Equation 2.10**, are given by **Equation 2.11** are linear functions of the β_j:

$$\hat{Y}_i = \beta_0 + \beta_1\phi_1(X_{i1}) + \beta_2\phi_2(X_{i2}) + \cdots + \beta_3\phi_p(X_{ip}) \quad i = 1, \ldots, n \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.. (2.11)$$

Given that estimation is undertaken on the basis of a least squares analysis (S), estimates of the unknown parameters β_j are determined by minimizing a sum of squares function as indicated in **Equation 2.12**.

$$S = \sum_{i=1}^{n} \left(Y_i - \beta_0 - \beta_1\phi_1(X_{i1}) - \beta_2\phi_2(X_{i2}) - \cdots - \beta_3\phi_p(X_{ip})\right) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2.12)$$

In exponential modelling, the term exponential function is used to mean the natural exponential function e^x as described in **Equation 2.13**.

$$f\,(x) = e^x \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2.13)$$

Where *e* is the Euler's number, such that the function e^x is its own derivative. The exponential function is used to model a relationship in which a constant change in the independent variable gives the same proportional change (i.e. percentage increase or

decrease) in the dependent variable. Whereas in the compound function, is the modified exponential model as defined by **Equation 2.14** with *b* being another constant (Luna-delRisco *et al*., 2011).

$$f\ (x)\ = ab^{x} \ldots \ldots (2.14)$$

Lastly, a quadratic function, a quadratic polynomial, a polynomial of degree 2, or simply a quadratic, is a polynomial function in one or more variables in which the optimum-degree term is of the second degree. A univariate quadratic function has the form represented by **Equation 2.15**.

$$f(x) = ax^2 + bx + c,\ \ a \neq 0 \ldots \ldots (2.15)$$

The quadratic model takes in the form of the **Equation 2.16** (Ghanadzadeh and Ghorbanpour, 2012; Passos *et al*., 2013):

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i\, x_i + \sum_{i=1}^{k} \beta_{ii}\, x_i^2 + \cdots + \sum_{1 \le i \le j}^{k} \beta_{ii} x_i x_j \ldots \ldots (2.16)$$

Where $Y, x_i, \beta_0, \beta_i, \beta_{ii}, \beta_{ij}$ and ε are the biogas quantity, process factors, offset coefficients interaction coefficients, and residuals associated with the experiments respectively.

2.9 BIO-SOLIDS FROM SEWAGE SLUDGE

Bio-solids are produced during aerobic and anaerobic digestion of sewage, though in smaller quantities by anaerobic processes compared to the aerobic processes (Wei *et al*., 2003; Mahmoud *et al*., 2003; Nazaroff and Alvarez-Cohen, 2013). Bio-solids are rich in fertilizer macro and micro nutrients such that they can be utilized as bio-fertilizers (compost) (Gorveno *et al*., 2000; Sohaili *et al*., 2012; Usman *et al*., 2012; Pabsch and Wendland, 2013; Nazaroff and Alvarez-Cohen, 2013). The bio-solids are stabilised to remove pathogens and offensive odours before use as bio-fertilisers (Nazaroff and Alvarez-Cohen, 2013). In previous studies, bio-solids from wastewater treatment were found to contain nitrogen content of about 2.6-3.8 % and phosphorous content of about

2.0-2.2 % and potassium content of 0.2 % (Johannesson, 1999; Pabsch and Wendland, 2013). The typical bio-solids compositions from sewage are shown in **Table 2.11**. From the compositions shown in **Table 2.11**, there is a clear indication that bio-solids from sewage contain fertilizer nutrients that can be exploited for use as agricultural fertilizers with nitrogen and phosphorous having the highest composition (Gorveno *et al*., 2011; Usman *et al*., 2012; Sohaili *et al*., 2012).

Table 2-11: Typical sewage bio-solids composition

Bio-solids parameter	**Composition (Gorveno *et al*., 2000)**	**Composition (Usman *et al*., 2012)**	**Composition (Sohaili *et al*., 2012)**
Total dry solids	2.0-7.0%	-	
Volatile solids	60-80%	-	
Grease and fats	6.0-30.0%	-	
Nitrogen	1.5-4.0%	1.54-1.92%	0.18-0.42%
Phosphorous	0.8-2.8%	0.44-0.70%	0.045%
Potash	0.0-1.0%	0.028-0.41%	0.035%
Iron	2.0-4.0%	2.50%	

Bio-solids from sewage treatment plants also contain traces of bio-methane and pathogens which are further treated (Daelman *et al*., 2012). Bio-solids processing includes stabilisation, dewatering and drying in order to produce bio-solids with low moisture content (Pabsch and Wendland, 2013). The bio-solids dewatering and end use flow is shown in **Figure 2.5**.

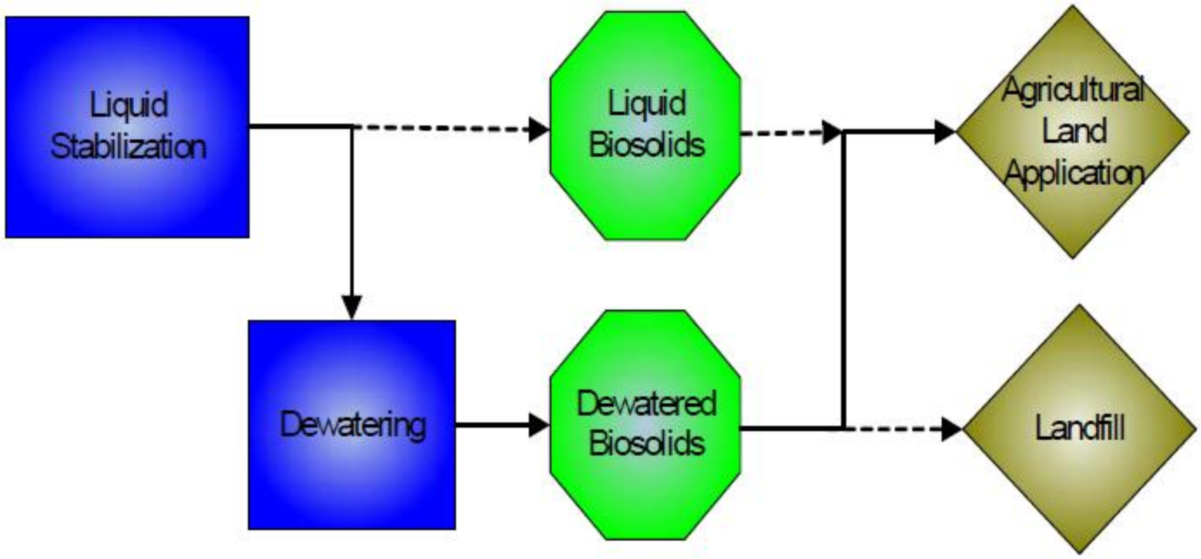


Figure 2-5: Dewatering of bio-solids for agricultural land use (CH2MHILL, 2011)

2.10 ECONOMIC ANALYSES FOR SEWAGE TREATMENT CO-HARNESSING BIOGAS AND BIO-SOLIDS

One of the key concerns of biogas plants is the economic benefits of the technology. The economic performance of anaerobic digestion of a given biogas plant is based on net positive present value (NPV) and the internal rate of return (IRR), breakeven point concepts and the payback period (Gebrezgabher *et al*., 2010).

2.10.1 Net present value

The net present value is normally calculated assuming even flow of income in accordance to **Equation 2.17**.

$$NPV = R\ X\ \frac{1-(1+i)^{-n}}{1} - Initial\ investment \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.. (2.17)$$

Where: R is the net cash inflow expected to be received in each period and i is the required rate of return per period., n are the number of periods during which the project is expected to operate and generate cash inflows usually a maximum of 25 years for biogas plants.

2.10.2 Internal rate of return

IRR is the interest rate at which the net present value of all the cash flows (both positive and negative) from an investment equal zero. IRR is used to evaluate the attractiveness of a project or investment. If the IRR of a new project exceeds a company's required rate of return, that project is desirable. If IRR falls below the required rate of return, the project should be rejected (Gebrezgabher *et al*., 2010). The IRR formula is represented in **Equation 2.18.** The formula for *IRR* is:

$$0 = P_O + \frac{P_1}{(1+IRR)} + \frac{P_2}{(1+IRR)_2} + \frac{P_3}{(1+IRR)_3} + \cdots + \frac{P_n}{(1+IRR)_n} \ldots\ldots\ldots\ldots\ldots\ldots (2.18)$$

Where P_0, P_1, . . . P_n equals the cash flows in periods 1, 2, . . . n, respectively; and IRR equals the project's internal rate of return.

2.10.3 Payback period

The payback period formula is used to determine the length of time it will take to recoup the initial amount invested on a project or investment. The payback period determination is represented by **Equation 2.19.**

$$Payback\ period = \frac{Initial\ investment}{Periodic\ cash\ flow} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2.19)$$

Payback periods of less than five years have been reported for biogas producing plants for various sources sludge as indicated in **Table 2.12**.

Table 2-12: Pay back periods for biogas production reported in literature

Biogas source	Digestion period	Bio-methane quality	Electricity produced	Payback period	Reference
Cattle dung	7 weeks	60%	-	11.5 months	Desai *et al*., 2013
Human excreta Cow dung Chicken manure	50 days	-	2710 KWh/day	2 years	Mukumba *et al*., 2013

2.10.4 Breakeven point

Breakeven refers to the point of balance between making either a profit or a loss. Breakeven point is calculated by dividing the total fixed expenses by the contribution margin. Contribution margin is sales minus all variable expenses, divided by sales. The formula is represented by **Equation 2.20**:

$$Breakeven\ point = \frac{Total\ fixed\ expenses}{Contribution\ margin\ (\%)} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2.20)$$

2.10.5 Sensitivity analysis

Sensitivity analysis is very useful when attempting to determine the impact the actual outcome of a particular variable will have if it differs from what was previously assumed during (Gebrezgabher *et al*., 2010). By creating a given set of scenarios, it can be determined how changes in one variable(s) will impact the target variable e.g. how changes in the payback period, fixed costs, manufacturing costs and loan amount can impact the IRR.

2.11 SUMMARY

Anaerobic treatment of sewage wastewater using Acti-zyme as a biocatalyst co-harnessing biogas and bio-solids is a potential environmentally friendly, sustainable and economic technology that can be explored. However, there is still need to quantify the optimal conditions the sewage treatment using Acti-zyme, at the same time harnessing the biogas and bio-solids produced as value added products. Furthermore, investigation of the statistical and kinetic modelling that can be applied for simulation of the experimental data to real life sewage treatment when utilising this technology is critical. Lastly, the economic assessment is essential for ascertaining the feasibility of the benefits of using this technology in sewage treatment.

CHAPTER 3. MATERIALS AND METHODOLOGY

The materials and methods focused on the methodologies used to analyse biochemical properties of Acti-zyme, physicochemical properties of sewage, quantity and quality of biogas and bio-solids produced in sewage treatment with Acti-zyme. All experiments were repeated thrice to check for repeatability and average values were used. In addition statistical experimental design for optimum sewage treatment, biogas and bio-solids production is discussed for application in the kinetic and statistical modelling of the experimental data.

3.1 DETERMINATION OF ACTI-ZYME BIOCHEMICAL CHARACTERISTICS

3.1.1 Materials

Acti-zyme was obtained from AusTech, an Acti-zyme company based in Australia in September 2013. Acti-zyme was in pellets form and was stored at room temperature and only became active when it was introduced to a media (AusTech, 2010). Peptone water was used as the sterile media for the following agar: Blood, Motility, Gelatine, Indole, Starch and Urease. An Inco Therm Labotec incubator was used for incubation. Pure agars were obtained from Sunfirm Distributors, a chemicals supply company.

3.1.2 Methods

Eight biochemical tests were performed on the Acti-zyme. Acti-zyme was inoculated in a vial containing sterile media containing peptone water at 37.5 $^{\circ}$C for a period of 24 hours. The media was characterized by a pH of 7±0.2. After the 24 hour period, the sample was plated on the following agar: Blood, Motility, Gelatine, Indole, Starch, and Urease. Growth on the various agars was noted in terms of size of colonies, colour and odour. Afterwards various biochemical tests such as Catalase, Motility, Gelatine Liquefaction, Methly Red and Voges, Starch, Indole, Urea and H_2S production were done in order to determine the properties of Acti-zyme especially for application in sewage treatment and biogas production (Schreckenberger and Blazevic, 1974, Woodland, 2004; Segal and Potter, 2008; Vashist *et al*., 2013).

The determined Acti-zyme biochemical tests are shown in **Table 3.1**.

Table 3-1: Determination of various Acti-zyme biochemical properties

Test	Agar	Importance	Reference
Catalase test	Blood agar	Effervescence will indicate positive result showing that the bacteria produces the enzyme catalase	Vashist *et al*., 2013
Motility test	Motility	Used to determine if a bacteria is motile	Vashist *et al*., 2013
Gelatine Liquefaction test	Gelatine	Used to determine if the bacteria produces the enzyme gelatinase which breaks down gelatine	Schreckenberger and Blazevic, 1974
Methyl Red and Voges Proskaeur test	Indole	Used to determine if an organism produces mixed acids from glucose	Vashist *et al*., 2013
Starch hydrolysis	Starch	Shows the ability of a bacterium to breakdown starch using amylase	Schreckenberger and Blazevic, 1974
Urea test	Urease	Shows the ability of the bacterium to break down Urea using the enzyme urease to ammonia	Schreckenberger and Blazevic, 1974
Indole test		Shows the ability of the bacterium to produce indole	Schreckenberger and Blazevic, 1974
Hydrogen sulphide production		Shows the ability of the bacterium to produce Hydrogen sulphide gas	Vashist *et al*., 2013

3.2 DETERMINATION OF INFLUENCE OF ACTI-ZYME PHYSICOCHEMICAL CHARACTERISTICS OF SEWAGE

Raw sewage was obtained from a local sewage plant with a bio-nutrient removal pond treating 19.6 ML/day. The sewage samples were transported from the sewage plant in 5L and placed in a cooler box to deactivate any further microbial activities.

The sewage samples were then stored at 0 °C to deactivate any microbial activity in the sewage before running the experiments. The solids particles in the raw sewage were first reduced by a filtration process to remove the sludge.

The raw physicochemical sewage characteristics were determined in 500 mL volumetric flasks of the sewage loaded with Acti-zyme. A sewage volume of 450 mL was added in the flasks. The flasks were plugged with cotton to stop the addition of oxygen as well as covering with aluminium foil paper. The sewage physicochemical characteristics determined included TP, TKN, COD, BOD_5, TSS, TDS, *E. coli*, total coliforms, EC, pH, SO_4^{2-} ions, Cl^- ions and DO concentrations in the raw sewage were also determined. Physicochemical properties of sewage were measured for Acti-zyme free (A_o) sewage effluent and for effluent at varying Acti-zyme loading and retention time (A_1) for thorough determination of the impact of Acti-zyme. The treated sewage effluent was compared to the EMA Effluent Disposal Guidelines (see **Appendix A for guidelines**).

Acti-zyme loading of 0-0.070 g/L was used for the treatment of the sewage (Duncan, 1970; Powell and Lundy, 2007). Residence times of 0-60 days were employed as this has been reported to be ideal, with Acti-zyme being applied on a weekly basis (Powell and Lundy, 2007; Arthur and Brew-Hammond, 2010; Nazaroff and Alvarez-Cohen, 2013; Wang *et al*., 2013). Furthermore, agitation rates were fixed at 60 rpm (Mahmoud *et al*., 2003). Temperature was fixed at 37 °C as this has been reported to be the ideal temperature for Acti-zyme activity (Tshuma, 2010). An Inco Therm Labotec Incubator was used to maintain temperature at 37 °C for the 250 mL flasks loaded with sewage.

3.2.1 Total nitrogen content by titration

The following materials were used for the determination of the total nitrogen. Digestion block, distillation unit, burette, 10 mL volumetric pipette, 250 mL Erlenmeyer flasks, magnetic stirrers and 200 mL Kjeldahl digestion tubes.

The total nitrogen content was determined using the Total Kjeldahl Method. 1 to 2 g of sewage sample was added to the 200 mL Kjeldahl flask. 15g of anhydrous potassium sulphate were added, together with 0.5g copper sulphate and a generous quantity of anti-bumping granules. 25 mL concentrated sulphuric acid (36N) were then added and swirled gently. The flask was heated gently at first then vigorously until the contents became clear. The flask was placed in ice cold water and 100 mL of water added and 80 mL of 12M sodium hydroxide was slowly added. The blue solution precipitated as it become alkaline. Afterwards, 50 mL of boric acid was added to the collecting beaker and five drops of indicator. The colour of the indicator was noted. The flask was then heated in a distillation apparatus collecting the distillate into the boric acid beaker. The distillation was finished when about 5 mL of steam had distilled and the indicator solution had changed colour. The boric acid solution was titrated with 0.10 M hydrochloric acid back to the original colour. The TKN content was calculated by measuring the amount of acid neutralised during the titration (Ravek and Avnimelech, 1979; APHA, 2005).

3.2.2 Total phosphorous content by titration

Total phosphorous content was determined in accordance to the ascorbic acid method (Watanabe and Olsen, 1965; APHA, 2005). 50 mL of digested sample was pipetted into an acid cleaned and dried 125 mL Erlenmeyer flask. 1 drop of phenolphthalein indicator was then added. When a red color developed, 5N sulfuric acid was added until the color disappeared. 8.0 mL of combined reagent was added and mixed thoroughly and allowed at least 10 minutes (but not more than 30 minutes) for color development. Absorbance was measured at 880 nm using a reagent blank to zero the spectrophotometer. The reagent blank was made using 50 mL of distilled water carried through the digestion step and ascorbic acid procedure. The absorbance of this blank was subtracted from the

absorbance of the sample then checked against the sample's absorbance against the calibration curve. This resulted in the TP concentration determination.

3.2.3 Biological oxygen demand and dissolved oxygen by titration

The Wrinkler Procedure was used for BOD_5 and DO determination (Lotfy and Rashed, 2002; APHA, 2005). 203 mL BOD_5 bottles, pipette, 25 mL burette, 250 mL flasks, measuring cylinders and a 25 $^{\circ}$C incubator were required for BOD_5 determination. Reagents required included phosphate buffer, magnesium sulphate, calcium chloride, alkaline-iodide-azide solution, concentrated sulphuric acid, starch indicator, 0.025N sodium thiosulphate, ferric chloride and manganous sulphate solution. Dilution water was prepared by aerating distilled water for several hours and then transferred to a 2L aspirator bottle and added 2 mL each of magnesium sulphate, phosphate buffer, calcium chloride and ferric chloride. Two bottles designated as control were filled with the dilution water (B_1 and B_2). Carefully add 25 mL of sewage to two bottles and fill them with the dilution water (D_1 and D_2). 2 mL of manganous sulphate solution was added to each bottle B_1 and D_1 immersing tip of the pipette below the surface of the water, furthermore, 2 mL alkali-iodide-azide solution was added to the bottles immersing the tip of the pipette. The bottle were stoppered tight, inverted and mixed thoroughly. When the precipitate settled halfway, 2 mL of conc. Sulphuric acid was added to each bottle and shaken vigorously. The colour of the solution turned orange/yellow. 203 mL of sample was then placed in a flask following a burette being filled with 0.025 N sodium thiosulphate solution and the sample was titrated till a yellow tinge remained. 1 mL of starch was then added and titrated until the solution become colourless. The burette readings were recorded as mg/L of DO for B_1 and D_1. The bottles B_2 and D_2 were incubated for 5 days, after 5 days, DO was determined by the same procedure. The BOD_5 was calculated according to **Equation 3.1** (Lotfy and Rashed, 2002).

$$BOD\left(\frac{mg}{L}\right) = \frac{(D_1 - D_2) - (B_1 - B_2)}{P} \quad \ldots\ldots (3.1)$$

Where: D_1: Initial DO of sample before incubation, D_2: Final DO of sample after incubation period of 5 days, B_1: Initial DO of control before incubation, B_2: Final DO of control after incubation and P = Dilution factor

3.2.4 Chemical oxygen demand by titration

The following materials were required for determination of COD by titration: a reflux apparatus, 0.25N $K_2Cr_2O_7$, sulphuric acid-silver sulphate reagent, 0.1 Fe $(NH_4)_2(SO_4)_2$, ferroin indicator and $HgSO_4$.

0.4g of $HgSO_4$ was first placed in a reflux flask and 20 mL of sewage sample was added to 20 mL with distilled water. After through mixing, 10 mL of 0.25 N $K_2Cr_2O_7$ solution was added to the sample. Some pumice stones were dropped in the sample then 30 mL of H_2SO_4-Ag_2SO_4 reagent were added while swirling the flask. The flask was connected to the condenser and slowly heated. Reflux was carried out for at least 2 hours, followed by cooling and washing down the condenser with distilled water such that the washings fell into the flask. The sample was diluted to 150 mL, cooled and titrated the unreacted $K_2Cr_2O_7$ with 0.1N ferrous ammonium sulphate using ferroin as indicator. The colour change at the end point was from blue green to wine red. The blank sample was done by titration using distilled water. The COD (mg/L) was then calculated according to **Equation 3.2**.

$$COD\left(\frac{mg}{L}\right) = \frac{(V_1 - V_2)\ X\ N\ X\ 800}{x} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots..(3.2)$$

Where: V_1 = Volume of blank experiment run down the burette (mL), V_2 = Volume of sample run down the burette (mL), N = Normality (Concentration) of ferrous ammonium sulphate solution and x = Volume of sample taken (mL)

3.2.5 Total suspended solids by filtration

The ESS Method 340.2 for determination of TSS within 2-20 000 mg/L was used. A well-mixed 100 mL sample of sewage was filtered through a standard glass fibre filter, and the residue retained on the filter was dried in a Germany Mermet Oven to maintain the

weight at 103-105 ˚C for one hour. The increase in weight on the filter represented the TSS. The TSS was calculated according to **Equation 3.3** (Jacobs Engineering Group, 2010).

$$TSS\ \left(\frac{mg}{L}\right) = \frac{(Residue + filter)(mg) - Filter\ (mg)}{Sample\ filtered\ (mL)}\ X\ 1000\ \left(\frac{mL}{L}\right) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.3)$$

3.2.6 Total dissolved solids by filtration

A 100 mL of sewage sample was taken and filtered to remove large particles using TDS-EZ 20 µm filters with a 12 cm diameter. The sewage sample was filtered again to remove smaller suspended particles. The amount of the filtered water was boiled to dryness at 180± 1°C for one hour (APHA, 2005). The amount of residue left after all the water has evaporated is measured as the TDS.

3.2.7 *E. coli* content by total plate count

E. coli content was determined by the total plate count method (George *et al*., 2002). All equipment was sterilised using 70% ethanol. 0.1 mL of sewage was carefully collected as a representative sewage sample. Firstly, the membrane filter was placed in the bottom piece of the filtration unit with the grid side of the filter facing upwards. Sterile forceps were used whenever the membrane filter was handled to prevent contamination and damage to the filter. Afterwards, the membrane filter was placed on the medium in a petri dish using a rolling motion to avoid entrapment of air. A small amount of sample sewage was poured into the petri dish on top of the membrane filter. The sample sewage prevented the bacteria on the filter from going into shock. The petri dishes were closed and sealed by placing two pieces of tape around the dish and then incubated. Incubation was done in an Inco Therm Labotec Incubator at 35±0.5 ˚C for 24 hours. This allowed the bacteria captured by the filter to grow and form a visible colony. After the incubation period was completed the petri dishes were taken out of the incubator and lids removed. The surface of the medium showed both growths of coliforms and other bacteria present. In addition, colonies of coliform bacteria turned a pink with a metallic surface sheen and only these were counted. The dissecting Optika SN 312 477

microscope was set to a 10 to 15× magnification to help count the number of colonies found in each petri dish. The coliform density of each filter was calculated according to **Equation 3.4**.

$$\frac{Coliforms}{100} mL = \frac{(Number\ of\ colonies\ counted)\ X\ 100}{(Sample\ size, mL)} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots . (3.4)$$

3.2.8 Total faecal coliforms by spread count

Two racks with A-1 Medium with Durham tubes were provided. A three-tube most probable number (MPN) rack with a row for each of four dilutions for the total coliform MPN was set up. The first row of three tubes contained 10 mL of 2X or 1X A-1 media for sewage samples. The remaining three rows of three tube contained 10 mL of 1X A-1 media. The tubes were well shaken for proper mixing. Each of the three tubes in the first row containing 2X media was inoculated with 10 mL of undiluted sewage samples. This was followed by inoculation of each of the three tubes in the next rows (containing 1X A-1 media) with 10 mL diluted sewage samples. All tubes were incubated at 35 ˚C (±0.5 ˚C) for 48 hours for total faecal coliforms determination. The number of positive tubes for all dilutions at 24 and 48 hours were noted. MPN values were determined from established tables. Look up the corresponding three-digit number on the MPN table. This number should be reported as an MPN of faecal coliforms per g or mL of sample (Baudisova, 1997).

3.2.9 Electrical conductivity by an electrode

A Hanna HI 9810 EC analyser was used for electrical conductivity (EC) measurement. 100 mL of the sewage sample was used for EC analysis using a calibrated conductivity meter electrode dipped in the sample and the reading.

3.2.10 pH by an electrode

A Hanna HI 9810 pH analyser was used for pH measurement. 100 mL of the sample was used. Using a well calibrated pH meter electrode that was inserted in the sewage sample, readings were taken and noted.

3.2.11 Sulphates by gravimetric method

Sulphate in sewage was determined by a gravimetric method in which sulphate was precipitated as barium sulphate. The method is suitable for sulphate concentrations above 10 mg/L as in the case of sewage (APHA, 2005).

3.2.12 Chlorides by titration

50 mL of the sewage sample was transferred into a 250 mL conical flask. 1 mL of potassium chromate indicator was added and titrated against 1/20N of silver nitrate. The change in mL of the silver nitrate in the burette indicated the chloride content in the sewage sample (Mesquita *et al*., 2002).

3.3 BIOGAS PRODUCTION FROM SEWAGE TREATMENT USING ACTI-ZYME

3.3.1 Experimental conditions

The sewage sludge was filtered and dried to 80% moisture content in a 2000W Mermet oven at 105 °C for 5 minutes. 500 mL flasks representing the digesters were put in a water bath set at 37 °C and 55 °C to create mesophilic and thermophilic conditions respectively at atmospheric pressure. The digesters were closed and sealed with aluminium foil paper to ensure anaerobic conditions. Outlets were created for movement of the biogas and sampling. Acti-zyme loading of 0-0.070 g/L over retention period of up to 60 days was used in the digesters for biogas and bio-solids generation. Agitation in the digesters was fixed at 60 rpm and sewage sludge organic loading ranging from 5-10 g/L.d were employed to determine the optimum biogas production conditions (Hesnawi and Mohamed, 2013).

3.3.2 Biogas quantity and quality measurement

The biogas quantity from the sewage sludge was measured in millilitres per day (mL/day), through the displacement of water, as shown in in **Figure 3.1**. The biogas generated was taken from the sampling points for composition analysis. A GC 5400 gas

chromatography analysis was used for analysing the biogas content and the composition was expressed as a percentage.

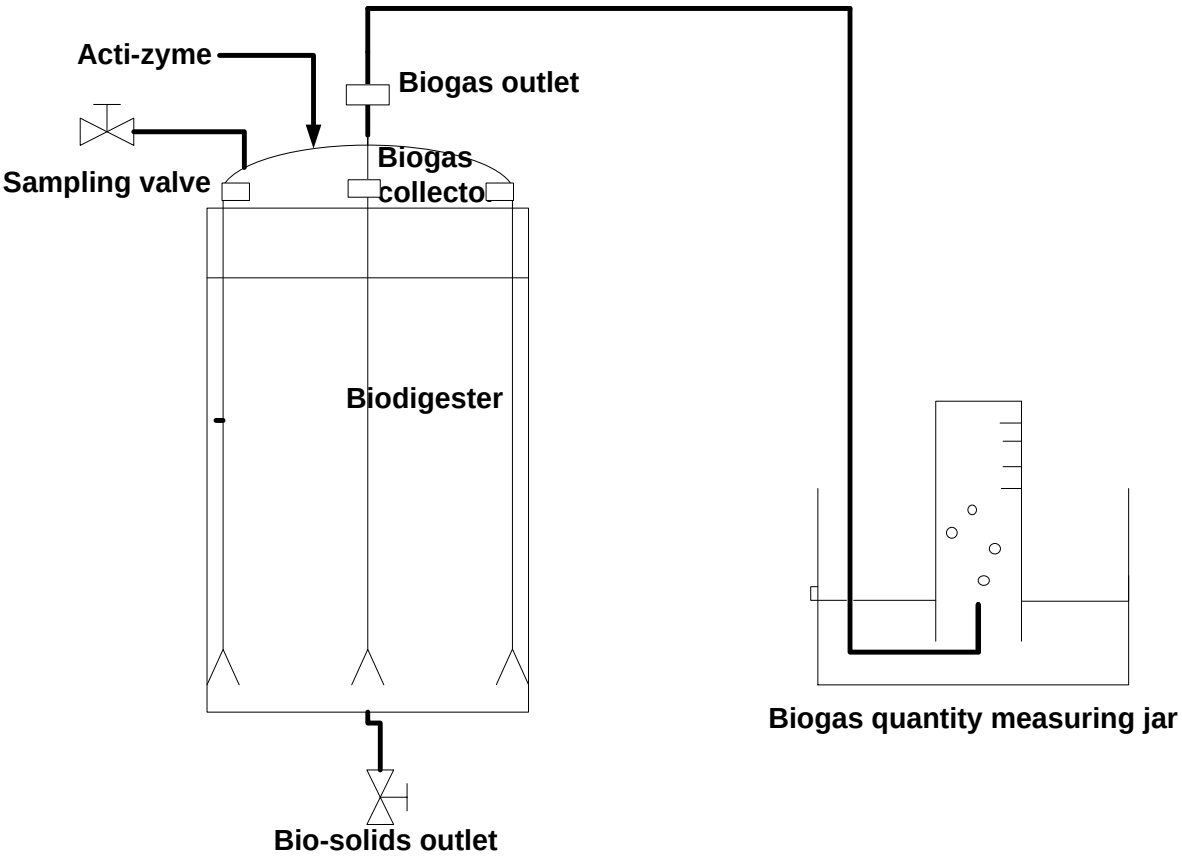


Figure 3-1: Schematic diagram for biogas generation from sewage sludge using Acti-zyme

In large scale application, the geometry of the bio-digester to be considered are as follows: height (H) to diameter (D) ratio of 4 (H/D = 4), baffle width (J) to bio-digester diameter of 1/12 (J/D = 1/12), agitator diameter (D_i) to bio-digester diameter was 1/3 (D_i/D = 1/3), distance between the bottom of the bio-digester and the turbine impeller diameter (E) to the diameter of the bio-digester was 1/3 (E/D = 1/3), the Rushton turbine width (W) to the Rushton turbines diameter was 1/5 (W/D_i = 1/5) and the Rushton

turbine length (L) to the Rushton turbines diameter was 1/4 ($L/D_i = 1/4$). The geometric representation is shown in **Figure 3.2**.

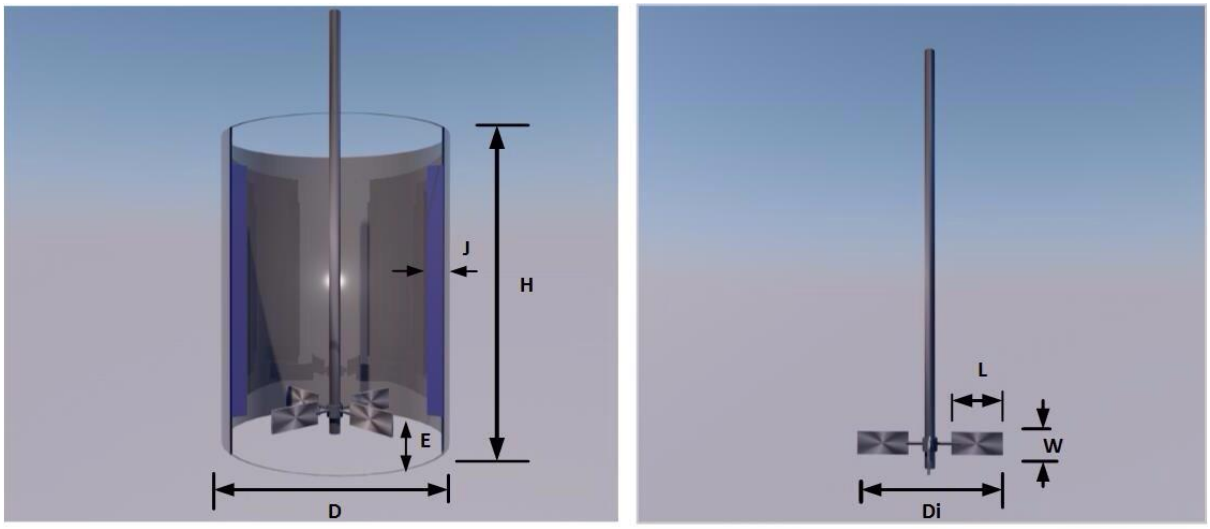


Figure 3-2: Bio-digester for sewage sludge digestion using Acti-zyme design considerations

3.4 BIO-SOLIDS QUALITY MEASUREMENT

The sewage sludge and bio-solids pH was measured using an HI 9810 Hanna pH electrode. The moisture content in the sewage sludge was determined by heating 5g of sewage sludge sample at 105 °C for 10 minutes and then recording the difference in weight in an AND moisture analyser. After the digestion process, the bio-solids were dried as a measure to reduce moisture content from 80% to 20%. Nitrogen, phosphorous and potassium (NPK) content in the bio-solids was measured using a Labtronics double beam *uv-vis* spectrophotometer content. *E. coli* content was measured through the total plate count procedure (Lang *et al*., 2007; Lang and Smith, 2007).

3.5 EXPERIMENTAL DESIGN AND ANALYSES

Full factorial experimental designs were used for the determination of sewage optimum treatment conditions and, biogas and bio-solids production from the sewage treatment using Acti-zyme. All experiments were repeated thrice to check repeatability and the

average value used for analyses. The standard deviations between the data was also calculated and noted.

3.5.1 Experimental design for physicochemical parameters treatment and optimum bio-nutrient removal in sewage

Raw sewage was biologically treated using Acti-zyme of different loadings and residence times. Acti-zyme loading of 0-0.070 g/L was used for the anaerobic treatment of sewage (Powell and Lundy, 2007). Residence time of 0-60 days was used as this has been reported to be ideal in biogas production (Arthur and Brew-Hammond, 2010; Nazaroff and Alvarez-Cohen, 2013; Wang *et al*., 2013). Stirring rate was fixed at 60 rpm and operating temperature at 37 °C The optimum treated sewage bio-nutrient characteristics was determined by monitoring the TKN, TP, COD, BOD_5, TSS, TDS, *E. coli*, total coliforms, EC, pH, sulphates, chlorides and DO content. These physicochemical properties are the ones that are mainly affected during biological treatment of sewage especially TP and TKN (Barker and Dold, 1996; Mace and Mata-Alvarez, 2002; Ahn *et al*., 2003; Kraume *et al*., 2005; Mohan *et al*., 2005; Kampas *et al*., 2007).

2^2 full factorial experimental designs were first used to determine the optimal conditions for sewage treatment using Acti-zyme. Experiments were repeated thrice and the average used also taking note of the standard deviation. For purposes, Acti-zyme loading (g/L) was denoted as A and residence time (days) was denoted as (B) (**Table 3.2**). The lowest level investigated for the dependent parameters was denoted as (-1) whilst the highest level investigated was denoted as (1) (**Table 3.3**). The response outputs were TKN, TP, COD, BOD_5, TSS, TDS, pH, EC, *E. coli* and total coliforms. Further tests were conducted at 0.070 g/L to check the impact of Acti-zyme at higher loadings and observe the trend

.

Table 3-2: Parameters varied during optimum bio-nutrient removal using Acti-zyme

Factor	Low level (-1)	High level (1)
A. Acti-zyme loading rate (g/L)	0.035	0.050
B. Residence time (days)	7	40

Table 3-3: Factorial designs for determination of optimum bio-nutrient removal from sewage using Acti-zyme

Factor		Treatment Combination
A	B	
-1	-1	A 0.035 g/L; B 7 days
1	-1	A 0.050 g/L; B 7 days
-1	1	A 0.035 g/L; B 40 days
1	1	A 0050 g/L; B 40 days

3.5.2 Experimental design for biogas and bio-solids quantity and quality production from sewage sludge

Acti-zyme loadings of 0-0.070 g/L and retention times of 0-60 days were used. Temperature was varied at 37 ^{o}C and 55 ^{o}C to determine the influence of mesophilic and thermophilic conditions on biogas and bio-solids production during sewage treatment with Acti-zyme as bio-catalyst (**see Table 3.4**). Optimum Acti-zyme loading of 0.070 g/L and retention period of 60 days were further used in the digesters for biogas and bio-solids generation to determine optimal production conditions. Furthermore, digesters which were free of Acti-zyme were also set so as to determine the actual impact of activating sewage sludge with Acti-zyme for biogas production. Agitation in the

digesters was fixed at 60 rpm and sewage sludge organic loading ranging from 5-10 g/L.d were employed to determine the optimum biogas production conditions (Hesnawi and Mohamed, 2013).

Table 3-4: Parameters varied during biogas and bio-solids production from sewage sludge digestion

Factor	Low level (-1)	High level (1)
A. Acti-zyme loading rate (g/L)	0.035	0.050
B. Residence time (days)	7	40
C. Temperature	37 ^{o}C	55 ^{o}C
D. Sewage loading	5 g/L.d	10 g/L.d

3.6 STATISTICAL MODELLING

A statistical package, Statistical Package, SPSS Statistics 19.0 was used as the tool for development of the models mainly focusing on the ANOVA, F values and the *t-tests* for the analyses (Fachri *et al*., 2015). This was done at 95% confidence interval. SPSS Statistics 19.0 was ideal to use for the statistical analysis due to the small sample size in this study. The empirical data derived from the models was compared to the experimental data to check for validity of the models.

3.6.1 Statistical modelling for bio-nutrient removal

Four assumptions were considered during the development of the bio-nutrient removal model and these included linearity and additivity, statistical independence of variables, homoscedasticity i.e. constant variance of the errors and normality of the error distributions. The covariances and correlation between the COD/BOD_5, BOD_5/TKN, COD/TKN and COD/TP ratios were determined and the significance of each model was validated using the *t-test*. The bio-nutrient ratios based on the bio-nutrients removal can therefore be represent in the form of **Equation 2.1**.

3.6.2 Statistical modelling for biogas and bio-solids production

Linear, quadratic, exponential and compound models described in **Section 2.8.4** were evaluated for suitability in simulation biogas and bio-solids generation from sewage sludge digestion using Acti-zyme using SPSS Statistics 19.0.

3.7 KINETIC MODELLING FOR BIO-METHANE PRODUCTION

Five models for possible application in biogas generation were applied i.e. the linear, exponential, the logistics kinetic equation, the exponential rise to a maximum equation and the modified Gompertz equation were considered (**See Section 2.7.3**). MATLAB R2013A was used in development of the kinetic models with the relevant algorithm being applied. The data derived from the kinetic models was compared to the experimental data to check validity of the models. The non-least squares method in MATLAB was used to fit the models to the bio-methane generated.

CHAPTER 4. BIOCHEMICAL PROPERTIES OF ACTI-ZYME: POTENTIAL FOR USE IN SEWAGE TREATMENT CO-GENERATING BIOGAS

4.1 INTRODUCTION

Acti-zyme, a biocatalyst has been used for vast applications such as wastewater treatment, drains cleaning and odor elimination since 50 years ago (Tshuma, 2010). Acti-zyme has been reported to treat wastewater either aerobically or anaerobically, reducing wastewater contaminants properties such as TKN, TP, nitrates, ammonia, BOD_5 and TSS by >40%. Acti-zyme also increases DO by > 100% in treated dam water promoting aquatic life (Tshuma, 2010). The use of Acti-zyme like any biological catalyst under anaerobic conditions has the potential to favor biogas production (Tshuma, 2010; Reali *et al*., 2001). On the other hand sewage is generated every day. There is a need for sustainable and economical ways of treating this sewage; harnessing biogas being one of them (Malik and Bhart, 2009; Neczaj *et al*., 2013). Acti-zyme therefore poses an economic and environmentally friendly way of treating sewage utilizing its biochemical properties. Although Acti-zyme has been used for over 5 decades in one way or the other, its biochemical characteristics were not investigated especially its potential and suitability to be used in sewage treatment for biogas production. This chapter focuses on biochemical analysis of Acti-zyme so that its use in sewage treatment as a biological technique can be validated. The biochemical analyses provided an understanding of the sewage pollutants Acti-zyme targets during its bio activity in biological treatment.

4.2 EXPERIMENTAL APPROACH

Eight biochemical tests were performed on the Acti-zyme. Acti-zyme was inoculated in a vial containing sterile media containing peptone water. The vial was incubated at 37.5°C for a period of 24 hours. The media was characterized by a pH of 7±0.2. After the 24 hour period, the sample was plated on the following agar: Blood, Motility, Gelatine, and Indole. Growth on the various agar was noted in terms of size of colonies, colour and

odour. Afterwards various biochemical tests such as the Catalase, Motility, Gelatine, Methly Red and Voges, Starch, Urea, Indole and H_2S were performed in order to determine the properties of Acti-zyme for application in sewage treatment and biogas production (Schreckenberger and Blazzevic, 1974; Vashist *et al*., 2013).

4.3 RESULTS AND DISCUSSION

4.3.1 Catalase test

This test is used to determine if a bacterium contains catalase (Schreckenberger and Blazzevic, 1974; Vashist *et al*., 2013). Effervescence will indicate a positive result showing that the Acti-zyme contains the enzyme catalase. Rapid effervescence was observed when Acti-zyme was added to the catalase media. This indicated the presence of the biocatalyst component catalase which breaks down hazardous compounds and facilitates detoxification according to **Reaction 4.1**. Catalase optimally works at pH of 7 (Yumoto *et al*., 2000), which was an indication that sewage treatment and detoxification of contaminants will optimally take place at this pH when Acti-zyme was employed.

$$2H_2O_2 \xrightarrow{Catalase} 2H_2O + O_2 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (4.1)$$

4.3.2 Motility test

The motility test is used to determine if a bacterium is motile through the identification of *Flagella* (Schreckenberger and Blazzevic, 1974; Vashist *et al*., 2013). The motility test showed that no *Flagellum* was present in Acti-zyme. This implied that Acti-zyme was immotile hence it was not capable of moving freely which makes agitation necessary during sewage treatment and biogas production. Immotility promotes Acti-zyme's stability in sewage treatment at increased catalyst loading as indicated by Nisha *et al*. (2012) on the advantages of using immotile bacterial in bioprocesses.

4.3.3 Gelatine liquefaction test

The gelatine liquefaction test is used to determine if a bacterium contains the enzyme gelatinase which breaks down gelatine, a form of protein through gelatinase

(Schreckenberger and Blazzevic, 1974; Vashist *et al*., 2013). Gelatinase breaks down proteins to polypeptides then to amino acids. Acti-zyme tested positive for the Gelatine liquefaction test through hydrolysis using a nutrient gelatine medium on which a positive test results in a liquid medium on Acti-zyme addition. This indicated that Acti-zyme contained the proteolytic enzyme which can break down proteins in accordance to **Reaction 4.2**. Proteolytic enzymes allow the hydrolysis of amino acid sequence due to their bio catalytic activity in hydrolysis amino acid esters, amides and peptides bonds optimally at pH ranges of 6-11 (Cheu *et al*., 2004). Acti-zyme will promote biogas production due to its hydrolytic behaviour thus promoting the generation of amino acids during the hydrolysis stage (see **Figure 2.4**). In addition, proteolytic enzymes favour high quality bio-methane production due to improved digestion (Prabhudessai *et al*., 2014).

$$Protein + H_2O \xrightarrow{Gelatinase} Polypetides + H_2O \xrightarrow{Gelatinase} Amino\ acids \ldots\ldots\ldots\ldots\ldots\ldots (4.2)$$

4.3.4 Methyl Red and Voges Proskaeur test

The Methyl Red and Voges Proskaeur test was used to determine if a bacterium produces mixed acids from glucose (Schreckenberger and Blazzevic., 1974; Vashist *et al*., 2013). The Methyl Red and Voges Proskaeur test for Acti-zyme tested negative for mixed acids production. However, non-production of mixed acids does not have an effect on biogas production rate and quality (Frauke-Whittle *et al*., 2014).

4.3.5 Starch hydrolysis test

The starch hydrolysis test shows the ability of a bacterium to breakdown starch using amylase (Schreckenberger and Blazzevic, 1974; Vashist *et al*., 2013). Acti-zyme broke down starch into constituent sugars such as maltose and glucose which can easily be metabolized due to the presence of amylase (**Reaction 4.3**). Amylase hydrolyses polysaccharides into smaller units during sewage treatment making its presence in Acti-zyme critical (Faccin *et al*., 2014). Amylase optimally functions at a pH of 6-7 (Oboh, 2005).

$$Starch \xrightarrow{Amylase} Maltose + Glucose + Dextrin \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4.3)$$

This result indicated that Acti-zyme can treat sewage that is highly organic substances contaminated due to its resistance to harsh conditions.

4.3.6 Indole test

The Indole test which is also known as the Tryptophan Hydrolysis shows the ability of a bacterium to break down Tryptophan using the enzyme tryptophanase (Schreckenberger and Blazzevic, 1974; Vashist *et al*., 2013). The Indole test can be used to indicate which Enterobacteriaceae is present, i.e. Enterobactor or *E. coli*. A negative test indicates that is present and a positive test indicate the presence of *E. coli*. In this study, the test was found to be negative in Acti-zyme, indicating that the Enterobacteriaceae species present was Enterobacter.

4.3.7 Urea test

The urea test shows the ability of the bacterium to break down urea to ammonia and carbon dioxide, using the enzyme urease (Schreckenberger and Blazzevic, 1974; Vashist *et al*., 2013). Acti-zyme did not test positive for urease. The absence of urease is good for biogas production in sewage treatment. The presence of urease would have resulted in the release of ammonia gas which in-turn increases the pH of the sewage away from the optimum, to acidic conditions, hence reducing the amount of biogas produced.

4.3.8 Hydrogen sulphide production test

The hydrogen sulphide production test shows the ability of the bacterium to produce hydrogen sulphide gas by checking for the enzyme thiosulfate reductase (Schreckenberger and Blazzevic, 1974; Vashist *et al*., 2013). Acti-zyme did not promote the production of H_2S gas on testing; this revealed that the enzyme thiosulfate reductase was absent. The presence of thiosulfate reductase is known to impair bio-methane production during anaerobic digestion (Madden *et al*., 2014). Therefore its absence in Acti-zyme is good for quality biogas production. Also, since H_2S is a known greenhouse

gas, is malodorous, is inhibitory to methanogenesis and can corrode sewage plants equipment. Acti-zyme's capability of not promoting H_2S production makes the use of Acti-zyme environmentally attractive in sewage treatment.

4.4 SUMMARY

Acti-zyme was found to be an immotile biocatalyst. In addition, it contained several enzymes that can be used in sewage treatment such as catalase which has a detoxifying influence, proteolytic which breaks down proteins into amino acids in sewage and amylase which breaks down the complex sugars to simple sugars in sewage. However, Acti-zyme tested negative for urease, which promotes ammonia production which has a potential to cause eutrophication. From the biochemical tests, Acti-zyme does not promote hydrogen sulfide production meaning that the biogas produced will be of good quality and will not need to go through further treatment processes.

CHAPTER 5. INFLUENCE OF ACTI-ZYME LOADING AND RETENTION TIME ON PHYSICOCHEMICAL PROPERTIES OF SEWAGE DURING ANAEROBIC TREATMENT

5.1 INTRODUCTION

Poorly treated sewage is being disposed of in water bodies in developing countries due to poor treatment techniques (Manyuchi and Phiri, 2013; Muserere *et al.,* 2014). This poses an environmental threat to the water bodies hence need to utilize environmentally friendly treatment techniques. Acti-zyme, an enzyme biocatalyst which possesses biochemical properties that allows it to be used for sewage treatment can be considered as an alternative sewage treatment method (Powell and Lundy, 2007). Acti-zyme contains enzymes such as catalase, proteolytic substances and amylase which promote detoxification of biological contaminants in sewage and has a potential to treat wastewater aerobically or anaerobically (**Chapter 4**). However, there is need to determine the optimum conditions that can be applied for sewage treatment using Acti-zyme as bio-catalyst especially the Acti-zyme loadings required and retention time. In this chapter, Acti-zyme was applied for sewage treatment and the impact on the physicochemical parameters of the effluent sewage determined so as to ascertain the optimum conditions in sewage treatment as a biological method.

5.2 EXPERIMENTAL APPROACH

The raw sewage was tested for pH, TP, TKN, BOD_5, COD, TSS, TDS, EC, CI^-, SO_4^{2-} and the DO (Singh and Singh, 2010; Popa *et al*., 2012). The sewage was then treated with Acti-zyme loadings of 0-0.060 g/L between 0-70 days (Powell and Lundy, 2007). All parameters were in mg/L except for pH and the EC which was measured in µS/cm. Temperature was fixed at 37 °C and agitation rate in the 500 mL flasks was maintained at 60 rpm. The TKN, TP, BOD_5, DO, COD, SO_4^{2-} and CI^-ions concentration were measured using titration methods, TSS and TDS were measured by filtration using the

APHA Standards Methods of determination in wastewater using a 20 µm filter (APHA, 2005). The EC and pH were measured by the Hanna HI electrode probe. Lastly, the *E. coli* content was measured through total plate count and the total coliforms by the spread count method. Sewage physicochemical properties were measured for Acti-zyme free (A_o) sewage effluent and for effluent at varying Acti-zyme loadings and retention time (A_1) for thorough determination of the impact of Acti-zyme on sewage treatment. All experiments were replicated thrice and the average values were used. The raw data for the raw sewage and treated sewage physicochemical properties is given in **Appendixes B and C**.

5.3 RESULTS AND DISCUSSION

5.3.1 Raw sewage and treated effluent characteristics

The raw sewage physicochemical characteristics obtained are indicated in **Table 5.1**. Some the physicochemical characteristics were out of range in terms of the EMA guidelines for effluent disposal (**Appendix A**). This was in exception for pH which was at 9.0 which the upper limit of the set guideline and also the TDS which were at 535 mg/L (**Table 5.1**). This was an indication that the treatment of sewage through bio-augmentation in this study using Acti-zyme was essential with the physicochemical parameters showing a decrease ranging from 20-97%. Although a decrease in the physicochemical properties was achieved for Acti-zyme free effluent (A_o) (**Table 5.1**), there was a clear indication that the effluent treated with Acti-zyme resulted in all the physicochemical properties being in the range acceptable by EMA (**Table 5.1**). The decrease in the sewage physicochemical parameters in an Acti-zyme free system was due to the presence of native microbes and enzymes in sewage. Bio-augmentation aids the treatment of wastewater by adding additional micro-organisms such that the treatment process is enhanced (Ma *et al*., 2013; Hesnawi *et al*., 2014; Yang *et al*., 2014).

Table 5-1: Sewage characteristics for systems with Acti-zyme and without Acti-zyme in reference to disposal guidelines after 40 days

Parameter	Raw sewage	Sewage effluent (A_o)	Sewage effluent (A_1)	EMA Guidelines	% Decrease in sewage effluent after adding Acti-zyme
TKN (mg/L)	245±5.5	39.9±0.20	9.4±0.36	10-20	77
BOD_5 @20°C (mg/L)	557±15.3	312.6±0.20	41.8±1.08	30-50	87
TSS (mg/L)	608±16.1	397.9±0.35	37.3±1.02	25-50	91
TDS (mg/L)	535±13	253.7±0.25	59.4±0.53	500-1500	77
E. coli	TMC*	TMC*	TMTC	-	-
EC @25°C (µS/cm)	3887±32.1	2186.2±0.21	1070.4±0.36	1000-2000	51
Cl^- (mg/L)	833±11.2	673.6±0.50	263.3±4.02	-	61
pH @25°C	9±0.3	7.9±0.20	6.3±0.1	6.0-9.0	24
Coliforms (cfu/ mL)	$1x10^{11}$	$1x10^{10}$	$1x10^{8}$	≤1000	20
TP (mg/L)	52±3.0	29.1±0.20	1.4±0.24	0.5-1.5	64
SO_4^{2-} (mg/L)	1192±70.8	776.9±0.35	53.6±2.71	-	93
DO (% Saturation)	7±0.2	20.9±0.26	87.0±0.20	≥ 60	316 increase
Temperature (°C)	22±1.5	37±0.5	37±0.5	< 35	-
COD (mg/L)	738±12.6	409.5±0.38	77.9±2.24	60-90	81

A_o: Acti-zyme free sewage effluent
A_1: Sewage effluent with Acti-zyme treatment
*Too many to count

5.3.2 Influence of Acti-zyme loadings, retention time and their interaction on sewage effluent physicochemical characteristics

Further work was done in terms of quantifying the influence of Acti-zyme loading and retention time on physicochemical properties of sewage for an acceptable effluent to be achieved. The trends on the sewage effluent physicochemical was then determined upon increasing Acti-zyme loading and retention time in the digesters. The variations in the experimental results of the physicochemical properties were within 7% in accordance to the standard deviation indicating the accuracy of the results.

5.3.2.1 Influence of Acti-zyme loading and retention time on pH

On treatment of sewage with Acti-zyme, the pH changed from being alkaline to almost neutral linearly. The change in pH varied from 8.3 to 6.3 in the sewage effluent with increased Acti-zyme loading and retention time for Acti-zyme loadings of 0 g/L to 0.050 g/L and retention times of 0 to 70 days (**Figure 5.1**). Increase in Acti-zyme loading and increase in the retention time in the digester had a positive influence on pH neutralization and this can be due to the removal of contaminants in the effluent due to Acti-zyme activity. Increased Acti-zyme loading increases the rate of biodegradation of bio-contaminants as the Acti-zyme will be feeding on the bio-contaminants in the sewage eventually decreasing the pH especially at longer retention times. The pH decreases from 7.4 to 6.8 were observed by Tshuma (2010) for Acti-zyme loadings of 0.02 g/L for a period of 60 days in dam water treatment indicating an agreement on the impact of Acti-zyme on pH. Dzvene (2013) also reported pH decreases from 7.84 to 7.82 upon adding 0.04 g/L of Acti-zyme for 150 days to piggery wastewater which had a similar trend like observations in this work. The reduction was due to the removal of TSS and TDS which are contaminants in the sewage and effectively result in increased pH (Mahmoud, 2002; Mandal, 2014; Muserere *et al*., 2014). Mandal (2014) reported a pH change from alkaline to neutral and attributed this to removal of TSS and TDS.

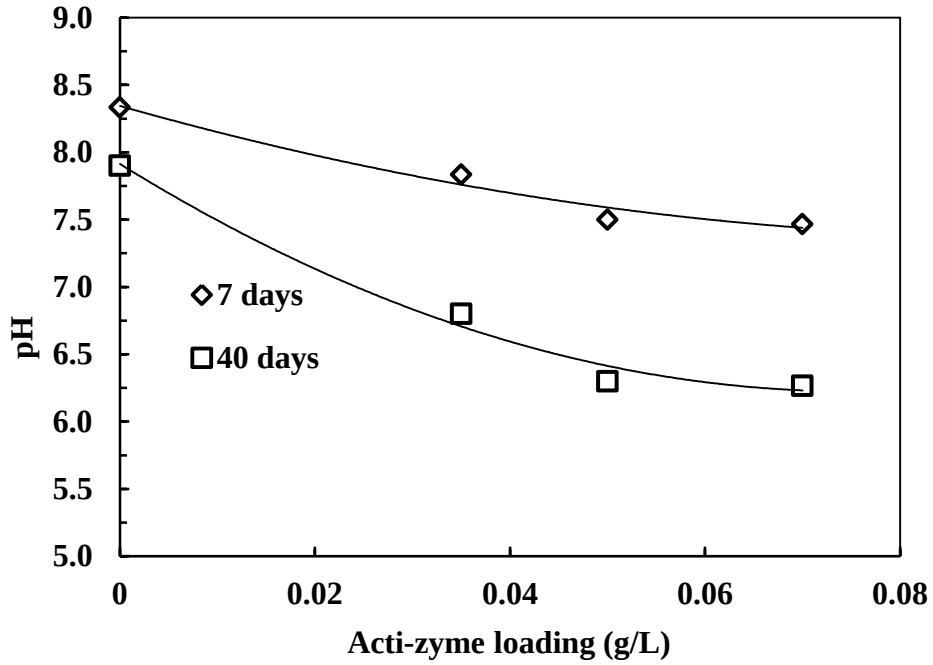


Figure 5-1: Influence of Acti-zyme loading and retention time on pH

5.3.2.2 Influence of Acti-zyme loading and retention time on TP

Total phosphates (TP) in sewage exist as phosphorous. The presence of phosphorus in raw sewage is found both as phosphates and as organically bound phosphorus and they have a potential to cause eutrophication if uncontrolled. The TP in the sewage effluent decreased by an overall 97% with increase in Acti-zyme loading and the retention time to around 1.4 mg/L (**Figure 5.2**). Increase in Acti-zyme loading and increase in the retention time for Acti-zyme loadings of 0 g/L to 0.050 g/L and retention times of 0 to 70 days had a positive influence on the TP reduction. The TP reduction is due to the uptake of nutrients in the sewage by Acti-zyme especially at increased loadings and retention times. A 67% reduction in TP was also reported by Tshuma (2010) after addition of 0.02 g/L of Acti-zyme per day over a 60 day period; however, the reduction in this study is 30% higher because of the higher Acti-zyme loadings reemployed. TP reduction after bio-augmentation with bacterial were also reported by Wa *et al*. (2013) and Yang *et al*. (2014) after inoculating *Pseudomonas* and *Bacillus*

then a 96.4% and 67.8% TP decreases were observed and this was due to the bacterial utilising the wastewater nutrients during its metabolism. The same was concluded to be the Acti-zyme effect on sewage TP during biological treatment.

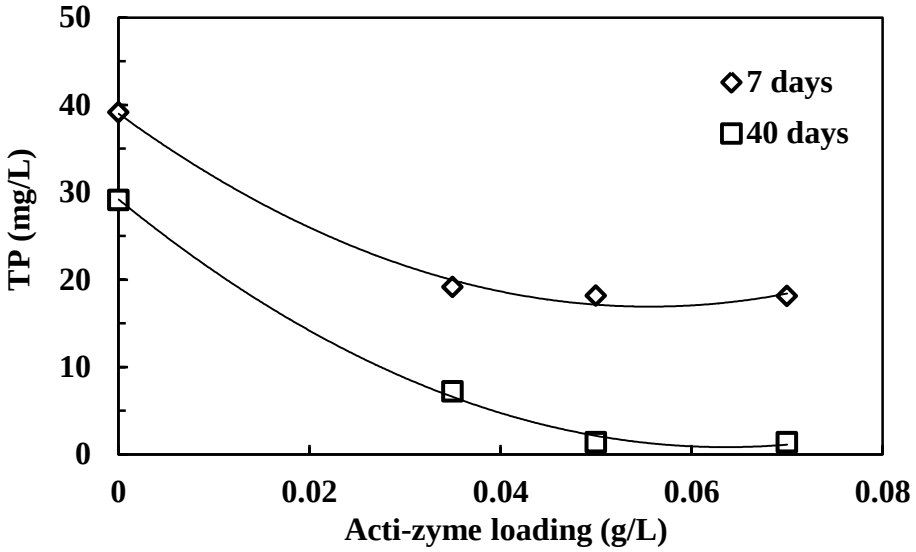


Figure 5-2: Influence of Acti-zyme loading and retention time on sewage TP

5.3.2.3 Influence of Acti-zyme loading and retention time on TKN

TKN is the measure of the total Kjeldahl nitrogen which comprises of ammonia nitrogen and organically bound nitrogen. TKN if left uncontrolled have a potential to cause eutrophication and excessive nitrogen compounds release to the environment. Furthermore, the part of free ammonia of the total ammonia content is related to increase in both water pH and temperature for the prevailing conditions in Zimbabwe with water temperatures > 20 °C. The amount of TKN in the sewage in overall decreased to 9.4 mg/L with increase in Acti-zyme loading and the retention time for Acti-zyme loadings of 0 g/L to 0.050 g/L and retention times of 0 to 70 days (**Figure 5.3**). An overall 96% TKN decrease was achieved for Acti-zyme loading of 0.035 g/L to 0.050 g/L at retention times of 7 and 40 days. Increase in Acti-zyme loading and increase in the retention time had a positive influence on the TKN reduction. Tshuma (2010) and Dzvene (2013) also reported a 47% and 48% decrease in TKN for dam water and

piggery wastewater respectively and for a treatment period of 60 and 150 days respectively as well as Acti-zyme loadings of 0.02 g/L and 0.044 g/L respectively. The TKN reduction in this study is 38% higher as compared to the studies by Tshuma (2010) and Dzvene (2013) due to higher Acti-zyme loadings used resulting in increased bio-activity due to bio-augmentation. This clearly indicates the importance of using Acti-zyme in bio-nutrient removal since Acti-zyme utilizes the TKN during its metabolism especially at higher Acti-zyme loadings and retention times of 40 days. The same argument was brought forward by Yang *et al*. (2014) when they used *Pseudomonas* and *Bacillus* as bio-augmentation organisms for sewage treatment and a 58.7% reduction in the total nitrogen was observed.

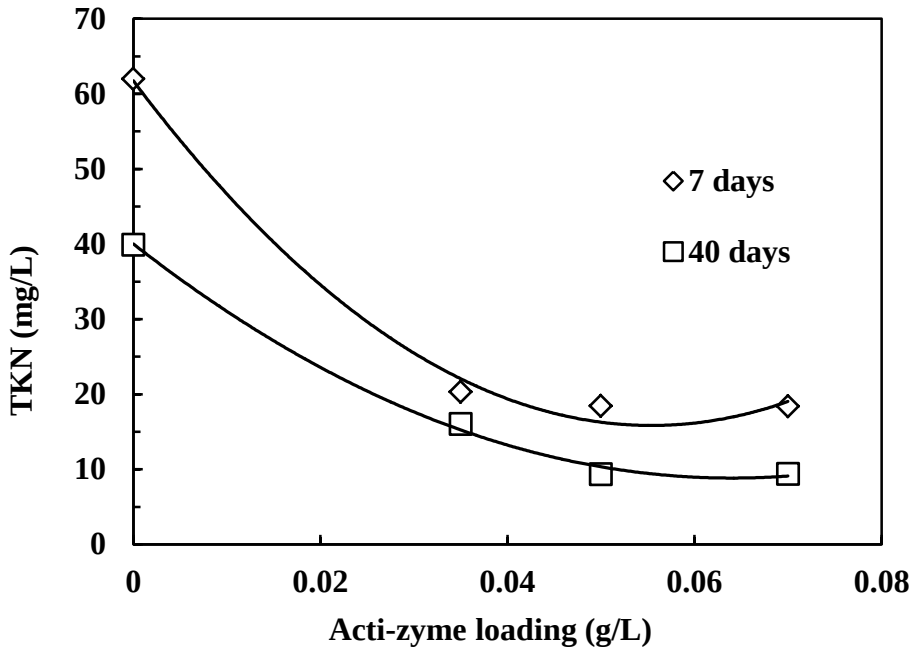


Figure 5-3: Influence of Acti-zyme loading and retention time on sewage TKN

5.3.2.4 Influence of Acti-zyme loading and retention time on BOD_5

BOD_5 refers to the amount of dissolved oxygen required by aerobic bacteria to break down organic matter. High BOD_5 and COD values negatively impact aquatic life in wastewater by lowering the oxygen levels in the wastewater killing aquatic life. High

TKN and TP values can also increase the BOD_5. The BOD_5 in the sewage effectively decreased by 92% to 41.8 mg/L with increase in Acti-zyme loading and the retention time with in the sewage effluent for Acti-zyme loadings of 0 g/L to 0.050 g/L and retention times of 0 to 70 days (**Figure 5.4**). Cail *et al*. (1986); Tshuma (2010) and Dzvene (2013) also observed a 68%, 96% and 58% decrease in BOD_5 upon addition of Acti-zyme to wool scouring wastewater, dam water and piggery wastewater for retention periods of 207 days, 60 days and 150 days respectively. An almost equation BOD_5 reduction (>90%) in this work was reported by Tshuma (2010) as the retention periods were almost equal. The decrease in BOD_5 was due to the increased bio-degradation activity of Acti-zyme. However, one observation to note is at longer retention periods above 60 days, the Acti-zyme activity decreased possibly due to degeneration to depletion of nutrients other contamination from the local microbes in the wastewater.

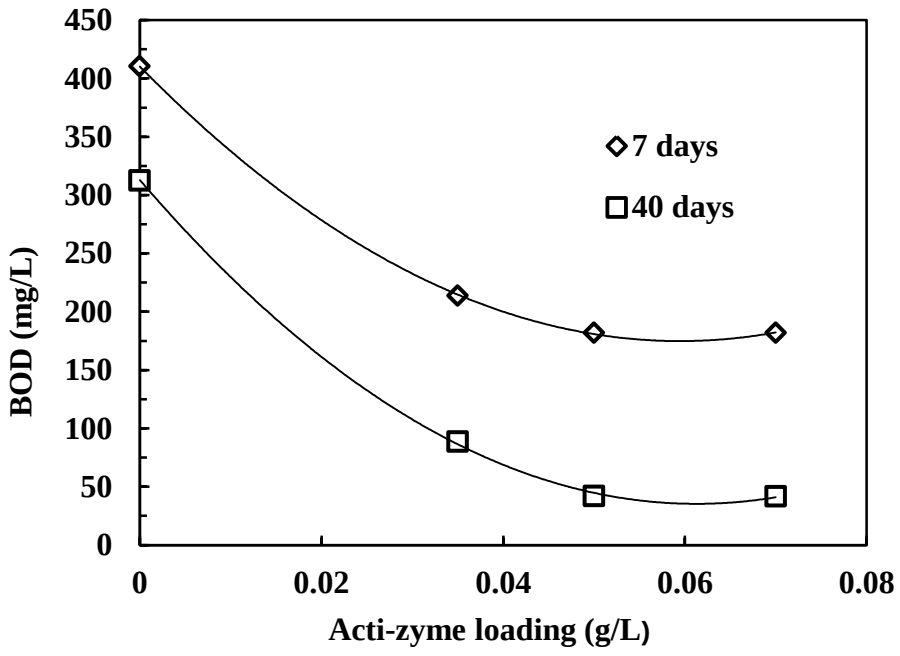


Figure 5-4: Influence of Acti-zyme loading and retention time on sewage BOD_5

5.3.2.5 Influence of Acti-zyme loading and retention time on COD

COD measures the amount of organic pollutants in sewage. The COD in the sewage decreased linearly by 89.4% to 77.9 mg/L with increase in Acti-zyme loading and the retention time in the sewage for Acti-zyme loadings of 0 g/L to 0.050 g/L and retention times of 0 to 70 days (**Figure 5.5**). Only increase in Acti-zyme loading and increase in the retention time had a positive influence on the COD reduction. The decrease is associated with Acti-zyme activity which promotes bio-degradation in sewage removing bio-contaminants which also contribute to the COD concentration especially at higher loadings and retention times. Cail *et al*. (1986) also observed a 58% decrease in COD after treating wool scouring wastewater using Acti-zyme loadings of 1% (v/w) over a period of 207 days. However, this work reported a 30% higher in COD reduction as compared to Cail *et al*. (1986) since higher Acti-zyme loadings were used and optimal removal achieved at 0.050 g/L and retention time of 40 days whereby, at this stage the Acti-zyme will not have reached the death phase. An 83.7% reduction in COD was also reported by Yang *et al*. (2014) for sewage treated through bio-augmentation with *Pseudomonas* and *Bacillus* and the same argument was brought forward showing the importance of added micro-organisms is sewage treatment.

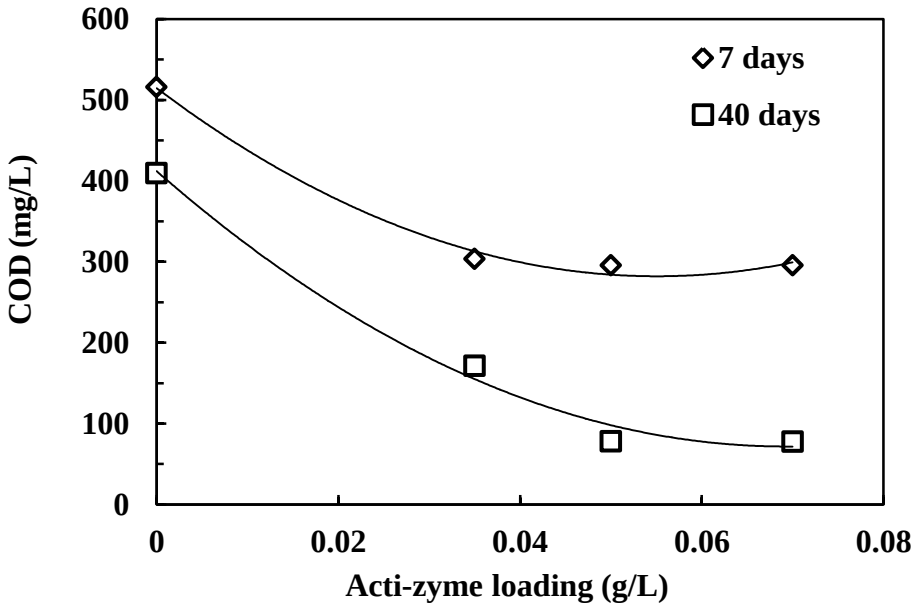


Figure 5-5: Influence of Acti-zyme loading and retention time on sewage COD

5.3.2.6 Influence of Acti-zyme loading and retention time on TSS

TSS measures the turbidity of water. High TSS and TDS values if uncontrolled can promote the limitation of dissolved oxygen available to aquatic life. The TSS in the sewage decreased in total by 94% to 37.3 mg/L with increase in Acti-zyme loading and the retention time for Acti-zyme loadings of 0 g/L to 0.050 g/L and retention times of 0 to 70 days (**Figure 5.6**). Increase in Acti-zyme loading from 0.035 g/L to 0.050 g/L and increase in the retention time from 7 days to 40 days had a positive influence on TSS reduction. The results for TSS reduction in this work are similar to the work of Tshuma (2010) and Dzvene (2013). Tshuma (2010) and Dzvene (2013) reported a decrease of at least 88% in dam waster and piggery wastewater upon Acti-zyme loading of 0.02 g/L and 0.044 g/L respectively for 60 and 150 days respectively. This indicates the ability of Acti-zyme to biodegrade the solids during wastewater treatment.

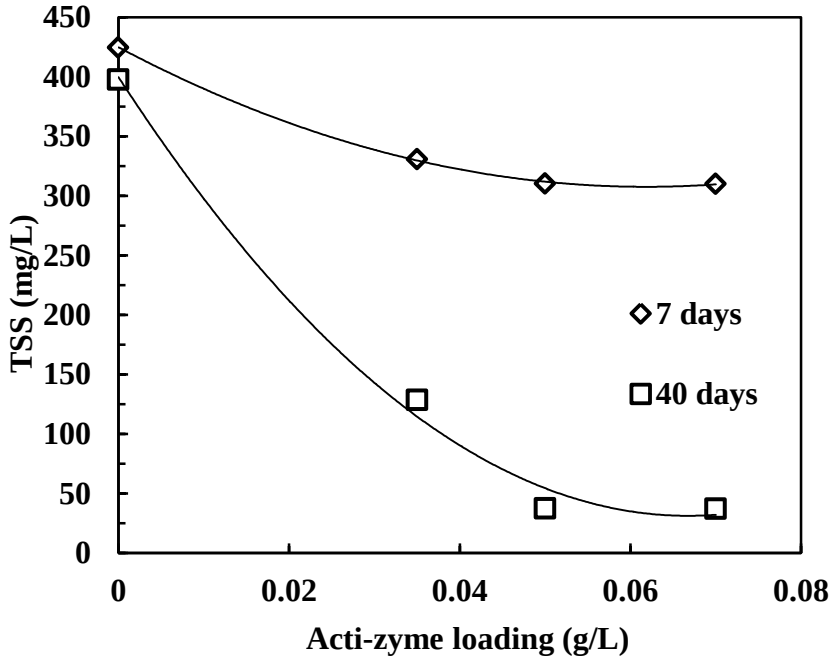


Figure 5-6: Influence of Acti-zyme loading and retention time on sewage TSS

5.3.2.7 Influence of Acti-zyme loading and retention time on TDS

TDS refer to the amount of mobile charged ions including minerals, salts and metals. The TDS in the sewage decreased linearly by 88.9% to 59.4 mg/L with increase in Acti-zyme loading and the retention time for Acti-zyme loadings of 0 g/L to 0.050 g/L and retention times of 0 to 70 days (**Figure 5.7**). The 88.9% TDS decrease was due increase in Acti-zyme loading and increase in the retention time. The TDS reduction in this study is 12% higher in comparison to the work of Dzvene (2010). Dzvene (2010) reported a 76.6% decrease in TDS upon Acti-zyme loading of 0.044 g/L in a 4.500 L pond to piggery wastewater for 150 days. This indicates the possibility of Acti-zyme's reducing influence on TDS due to its metabolism, however, at very long time retention times, the activity decreases possibly due to the fact that all the bio-contaminants will be used up.

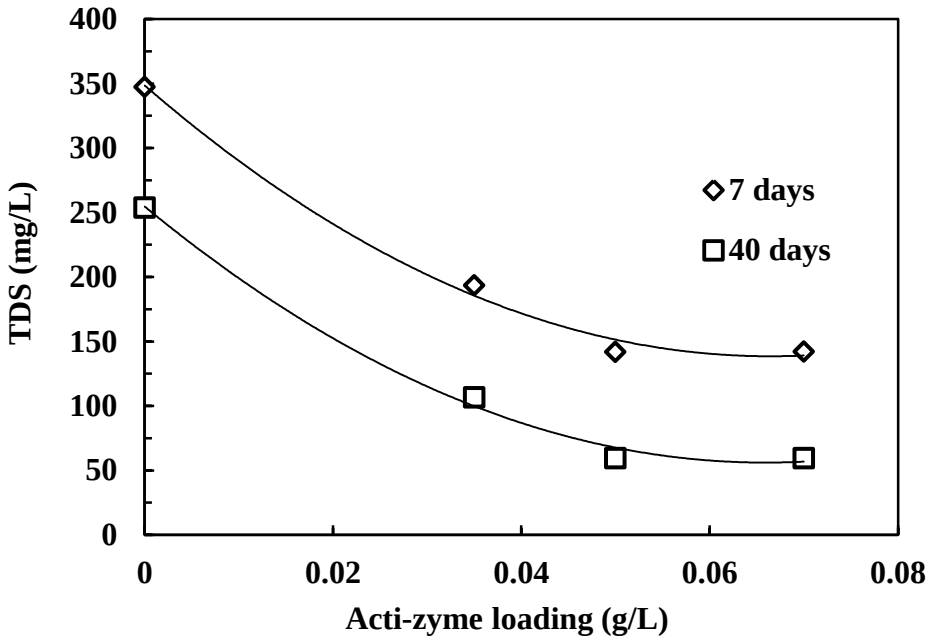


Figure 5-7: Influence of Acti-zyme loading and retention time on sewage TDS

5.3.2.8 Influence of Acti-zyme loading and retention time on EC

Electrical conductivity (EC) refers to the amount of dissolved ions in water that have the potential to conduct electricity. The EC is also directly affected by ions in wastewater such as chlorides, ammonia and sulphates. The EC in the sewage decreased by 72% to 1070.4 µS/cm with increase in Acti-zyme loading and the retention time (**Figure 5.8**). Increase in Acti-zyme loading from 0.035 g/L to 0.050 g/L and increase in the retention time in the digester from 7 days to 40 days had a positive influence on the EC reduction. EC reduction has a direct relationship to TSS and TDS reduction, which are reduced to Acti-zyme action on the sewage contaminants.

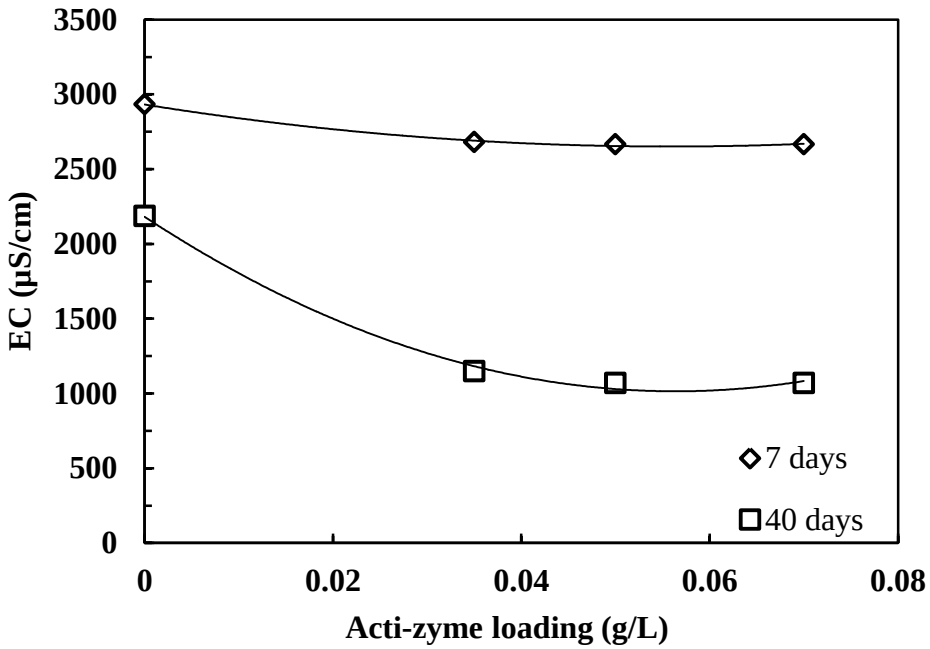


Figure 5-8: Influence of Acti-zyme loading and retention time on sewage EC

5.3.2.9 Influence of Acti-zyme loading and retention time on Cl^- ions concentration

The Cl^- ions concentration in the sewage decreased by 68% to 263.3 mg/L in the sewage effluent with increase in Acti-zyme loading and the retention time with (**Figure 5.9**). Increase in Acti-zyme loading, increase in the retention time and their interaction had a positive influence on the Cl^- ions reduction.

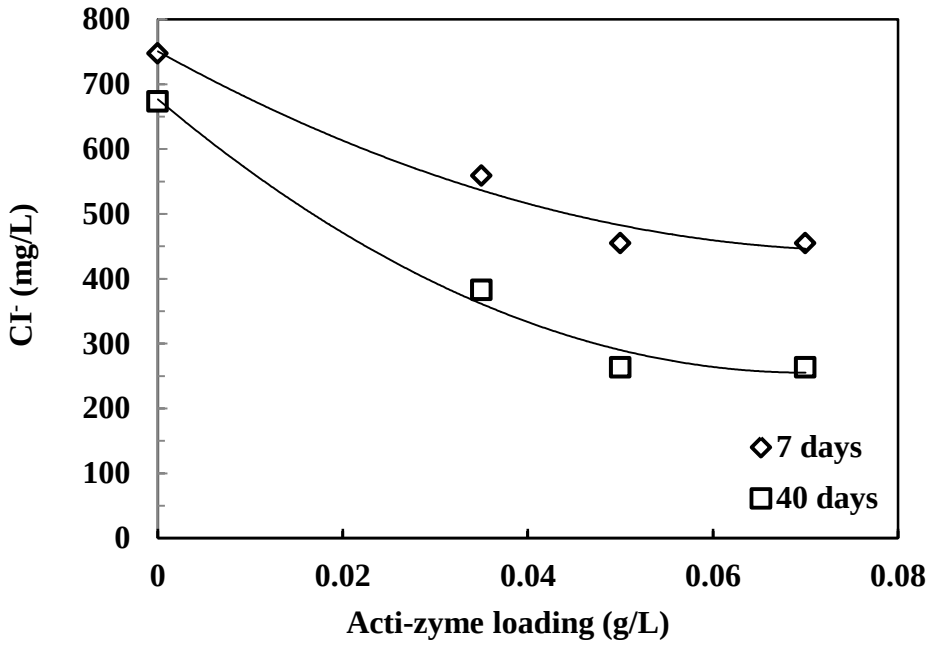


Figure 5-9: Influence of Acti-zyme loading and retention time on sewage Cl^- ions

5.3.2.10 Influence of Acti-zyme loading and retention time on SO_4^{2-} ions concentration

The SO_4^{2-} ions concentration in the sewage decreased by 92% to 53.6 mg/L in the sewage effluent with increase in Acti-zyme loading and the retention time for Acti-zyme loadings of 0 g/L to 0.050 g/L and retention times of 0 to 70 days (**Figure 5.10**) unlike in a system without Acti-zyme (**Table 5.1**). This decrease can be due to the H_2S production hindering activities of Acti-zyme (Cail *et al*., 1986). This is very significant increase in Acti-zyme loading and increase in the retention time.

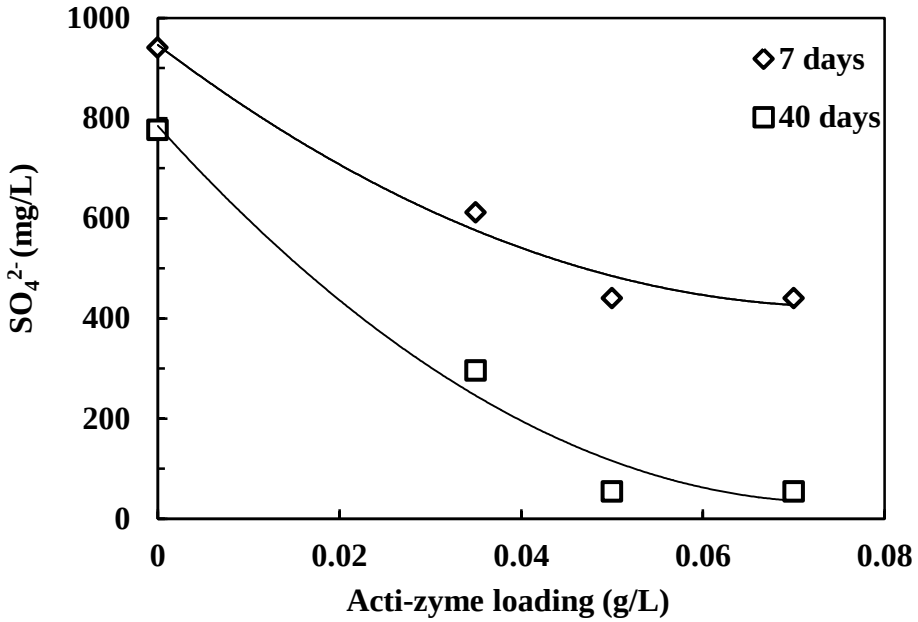


Figure 5-10: Influence of Acti-zyme loading and retention time on sewage SO_4^{2-} ions

5.3.2.11 Influence of Acti-zyme loading and retention time on DO

The DO concentration in the sewage effluent increased linearly by 222% to 87 mg/L with increase in Acti-zyme loading and the retention time for Acti-zyme loadings of 0 g/L to 0.050 g/L and retention times of 0 to 70 days (**Figure 5.11**) unlike in a system without Acti-zyme (**Table 5.1**). Increase in Acti-zyme loading, and increase in the retention time had a positive influence on the DO increase. This was due to the removal of all contaminants due to the Acti-zyme action.

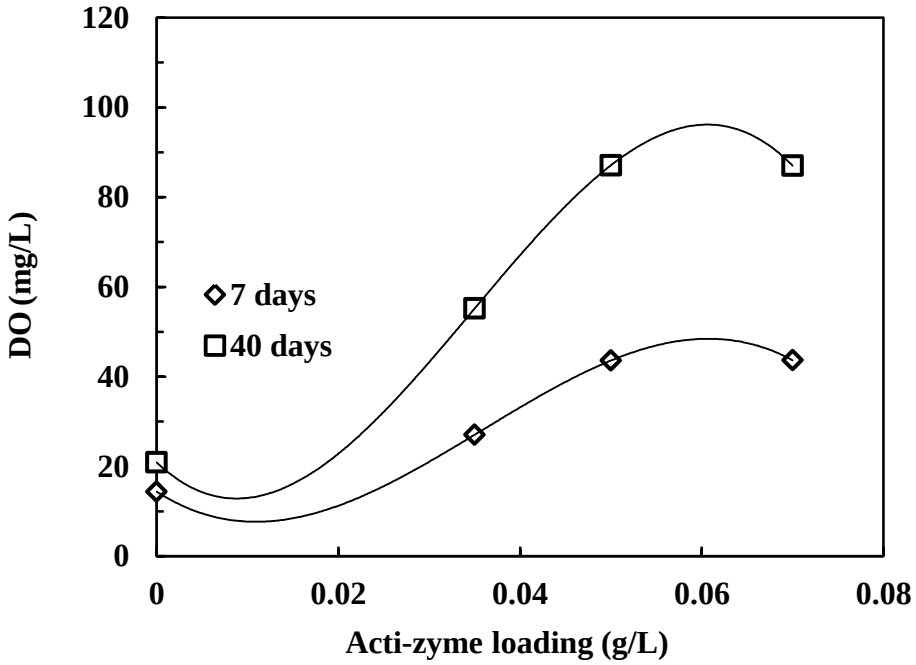


Figure 5-11: Influence of Acti-zyme loading and retention time on sewage DO

5.3.2.12 Influence of Acti-zyme Total E. coli and coliforms content

The *E. coli* in the sewage after treatment with Acti-zyme was too many to count. In addition, the total coliforms value was too high, that was around 10^8 coliforms. Despite, the *E. coli* and total coliforms, the sewage effluent met the prescribed guidelines for sewage disposal either in accordance to EMA (**Table 5.1**). Tshuma (2010) also reported a slight decrease in *E. coli* composition after addition of 0.02 g/L Acti-zyme to dam water, however, after 60 days, the dam water still tested positive for *E. coli.* A summary of the influence of optimal Acti-zyme loading (0.050 g/L) and retention time (40 days) on sewage physicochemical parameters in relation to other wastewater treated with Acti-zyme is given in **Table 5.2.** This indicates the benefit of Acti-zyme bio-augmentation in wastewater as there is a significant reduction in wastewater contaminants.

Table 5-2: Overall summary on influence of Acti-zyme on wastewater treatment

Parameter	**Cail *et al*. (1986)**	**Tshuma (2010)**	**Dzvene (2013)**	**This study**
Type of wastewater	Wool scouring	Dam water	Piggery	Sewage
BOD_5	62.4	96	58.1	92
COD	58.4	-	-	89.4
Total nitrogen	-	46	35.6	96
Total phosphates	-	67	-	97
Ammonia	-	53	48	-
Nitrates	-	80	-	-
E. coli	-	Positive	-	Positive
TSS	-	97.6	88.5	94
TDS	-	-	76.6	88.9
pH	-	6.8-7.4	7.82-7.84	6.8-7.4
Temperature	-	18-24 °C	-	35 °C
DO	-	100 increase	-	222 increase
Acti-zyme loading	1 % (v/v)	0.02 g/L	0.044 g/L	0.550 g/L
Retention time	207-211 days	60 days	150 days	40

5.4 SUMMARY

Acti-zyme effectively treats sewage removing all the wastewater contaminants to meet the required guidelines for effluent disposal compared to treatment without Acti-zyme. Sewage treated with Acti-zyme showed an additional > 50% reduction in the sewage contaminants at optimum loadings of 0.050 g/L and retention time of 40 days except for total coliforms. Acti-zyme utilizes the nutrients during its metabolism making it suitable for biological sewage treatment.

CHAPTER 6. STATISTICAL MODELLING FOR BIO-NUTRIENT REMOVAL RATIOS DURING SEWAGE TREATMENT USING ACTI-ZYME AS BIO-CATALYST

6.1 INTRODUCTION

Excess TKN and TP exist as bio-nutrients in sewage and can result in eutrophication if disposed of untreated to water bodies, therefore leading to a significant destruction of water bodies (Muserere *et al*., 2014). Thus there is need to monitor these bio-nutrients in sewage using statistical models that can be applied in real life situations whereby sewage is being treated using Acti-zyme. Acti-zyme is an enzyme biocatalyst has been found to be useful in wastewater treatment technology as a biological organism (Tshuma, 2010; Dzvene, 2013). Sewage a form of wastewater being generated daily from domestic usage contains biological components as contaminants which result in deteriorated water quality if left untreated (Lai *et al*., 2011; Manyuchi and Phiri, 2014). The biological contaminants removal in sewage can be measured by its biodegradability and de-nitrification which focuses on organic matter removed per sample of sewage (Tas *et al*., 2009; Lai *et al*., 2011; Lee and Nikraz, 2014). The COD/BOD_5, BOD_5/TKN, COD/TKN and COD/TP ratios are used as good indicators in bio-nutrient removal through biodegradability and de-nitrification (Tas *et al*., 2009; Muserere *et al*., 2014). Recently, Acti-zyme has become an interesting bio-catalyst for application in sewage treatment but there is need to understand the statistical modelling for bio-nutrient removal (**Chapters 4 and 5**). Statistical modelling is a very important statistical tool which is used in almost all for correlating different parameters (Khambete and Christian, 2014; Fachri *et al*., 2015). Multiple linear regression with the help of correlation analysis assists in finding interrelationship between different parameters and also the main parameters affecting the bio-nutrient removal. During regression analysis, there are two types of variables, the variable which value is influenced is called dependent variable and the variables which influence the dependent variable (Khambete and Christian, 2014; Fachri *et al*., 2015) such that the bio-nutrient removal model equation can be presented in form of **Equation 6.1**.

$$Bio-nutrient\ removal\ ratio = B_0 + B_1X_1 + B_2X_2 + B_3X_3 + B_4X_5 + \varepsilon \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (6.1)$$

Where: β_0 is a constant, X_1, X_2….are the independent variables, β_1, β_2……are the regression coefficients from the multiple linear regression analysis and ε is the residual error

In this chapter, Acti-zyme was used for sewage treatment and the rate of biodegradability and de-nitrification of the organic pollutants were measured to determine the bio-nutrient removal. Statistical models for the different bio-nutrient removal ratios in sewage were then formulated for simulation of bio-nutrient removal in real life sewage systems and compared to the experimental values obtained.

6.2 BIO-NUTRIENT REMOVAL RATIOS STATISTICAL MODELLING APPROACH

SPSS Statistics 19.0 was used for generation of the statistical models for the COD/BOD_5, BOD_5/TKN, COD/TKN and COD/TP experimental data at a p-value of 0.05 and their validity assessed. The models generated data and were compared to the measured ratios to check their applicability in sewage treatment using Acti-zyme using a *t-test*. The raw data of the bio-nutrient ratios are shown in **Appendixes AD1-AD4**. The models were then validated as a way of measuring their accuracy for possible industrial application (Demey *et al*., 2001; Belia *et al*., 2009; Abyaneh, 2014).

6.3 RESULTS AND DISCUSSION

The raw sewage had a BOD_5, TKN, COD and TP of 557 mg/L, 245.2 mg/L, 52.5 mg/L and 739.1 mg/L respectively. The other raw sewage parameters are given in detail in **Chapter 5, Table 5.1**.

6.3.1 Bio-nutrient removal during treatment with Acti-zyme

Sewage treatment with Acti-zyme (A_1 and A_2) resulted in significant bio-nutrients removal as compared to Acti-zyme free systems (A_o) as shown in **Figure 6.1** and **Figure 6.2**. Significant reduction in TKN, BOD_5, TP and COD was achieved at Acti-zyme loadings of 0.050 g/L especially at 40 days retention time (**Figure 6.1** and **Figure 6.2**). This is due to the bio-catalytic capability of Acti-zyme in reducing organic pollutants which is becomes more effective with increase in retention time during treatment. Acti-zyme successfully reduced the sewage bio-contaminates through bio-augmentation,

whereby inoculated micro-organisms enhance the sewage treatment process. Ma *et al*. (2013) and Yang *et al*. (2014) reported a significant decrease in sewage phosphates composition by at least 67.8%, an 83.7% reduction in COD and a 58.7% of total nitrogen upon adding *Pseudomonas* and *Bacillus* as bio-augmentation bacteria.

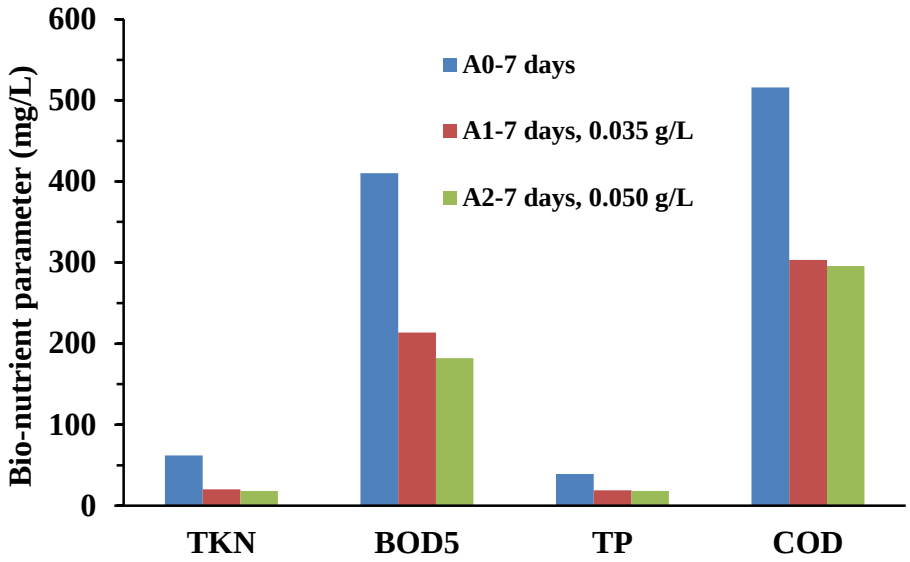


Figure 6-1: Bio-nutrient removal in sewage at 7 days retention time and Acti-zyme loadings of 0.035 g/L and 0.050 g/L

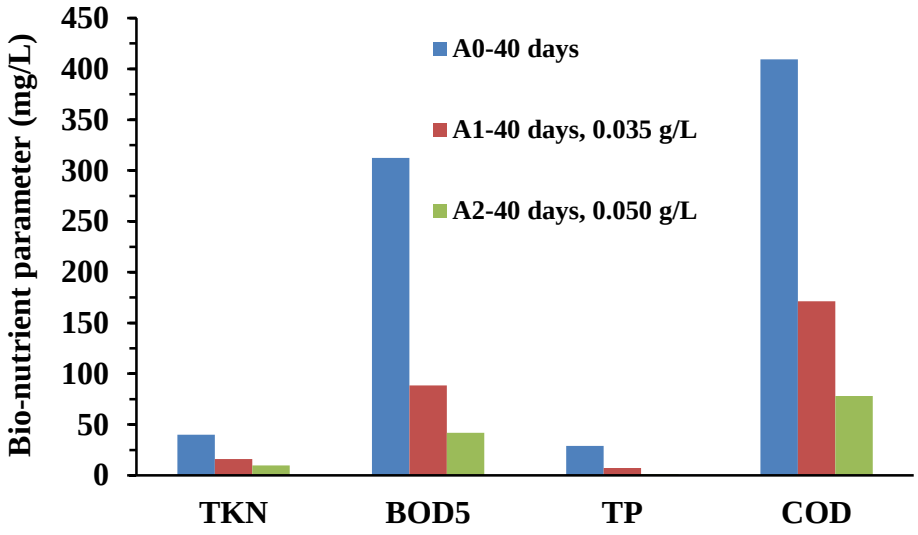


Figure 6-2: Bio-nutrient removal in sewage at 40 days retention time and Acti-zyme loadings of 0.035 g/L and 0.050 g/L

6.3.2 Statistical modelling for the bio-nutrient removal coefficients

Four statistical models for the COD/BOD_5, BOD_5/TKN, COD/TKN and COD/TP bio-nutrient ratios were generated and were in the form represented in **Equation 6.2**:

$$Bio-nutrient\ removal\ ratio = K + X_1TKN + X_2BOD + X_3TP + X_4COD \ldots\ldots\ldots\ldots (6.2)$$

Where: K = Constant, X_1 = TKN constant, X_2 = BOD_5 constant, X_3 = TP constant and X_4 = COD constant

6.3.2.1 Statistical model development assumptions

Four assumptions were considered during the development of the statistical models and these included linearity and additivity, statistical independence of variables, homoscedasticity i.e. constant variance of the errors and normality of the error distributions. The model analyses for the COD/BOD_5, BOD_5/TKN, COD/TKN and COD/TP models coefficients used in development of the models are shown in **Appendixes E1-E4** respectively.

6.3.2.2 COD/BOD_5 ratio model

The COD/BOD_5 ratios were all greater than 1.26 which indicated a high rate of biodegradability in sewage, therefore high bio-nutrient removal especially at higher retention times and increased Acti-zyme loading (**Figure 6.3**). The COD/BOD_5 ratios greater than 0.5 are an indication of high degradability of the wastewater (Gomes *et al*., 2013; Abdalla and Hammam, 2014; Muserere *et al*., 2014). The COD/BOD_5 ratios were greatest at increased Acti-zyme loadings and retention time possibly because Acti-zyme had enough time to acclimatize in the sewage and perform its bio-catalytic role. The COD/BOD_5 ratio model is represented by **Equation 6.3**.

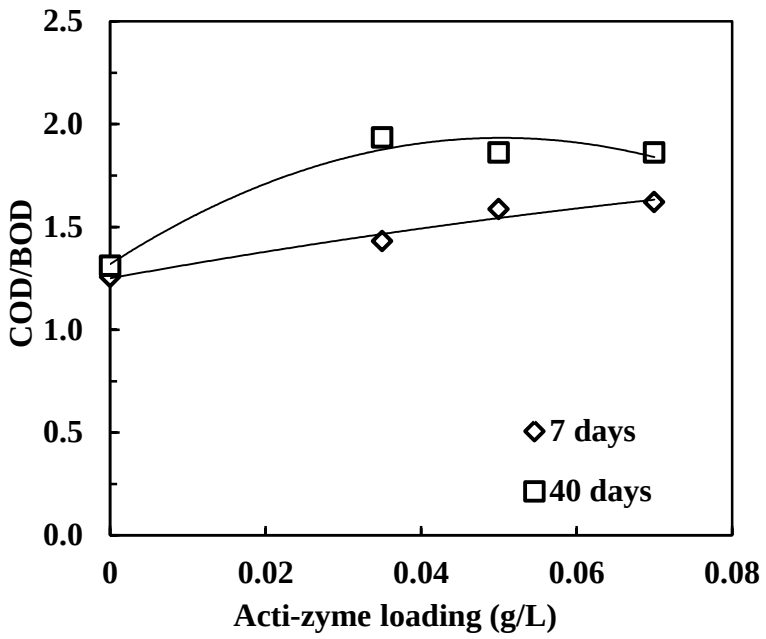


Figure 6-3: Influence of Acti-zyme loading and retention time on COD/BOD_5 ratio

$$\frac{COD}{BOD_5} = 1.669 + 0.008TKN - 0.008BOD_5 - 0.036TP + 0.006COD \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (6.3)$$

6.3.2.3 BOD_5/TKN ratio model

The BOD_5/TKN ratios were >4.0 for all the treatment combinations with Acti-zyme at varying retention times (**Figure 6.4**). All the BOD_5/TKN values were greater than 3.0 which was an indication of low biological de-nitrification in the sewage at the same time achieving clear effluent at the end (Shin *et al*., 2005). However, favorable BOD_5/TKN ratios were achieved at 40 days retention time (**Figure 6.4**). This is an indication that Acti-zyme promotes de-nitrification in sewage and a predictive model for the BOD_5/TKN ratio is indicated in **Equation 6.4**.

$$\frac{BOD_5}{TKN} = 4.967 - 0.245TKN + 0.029BOD_5 + 0.148TP + 0.005COD \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (6.4)$$

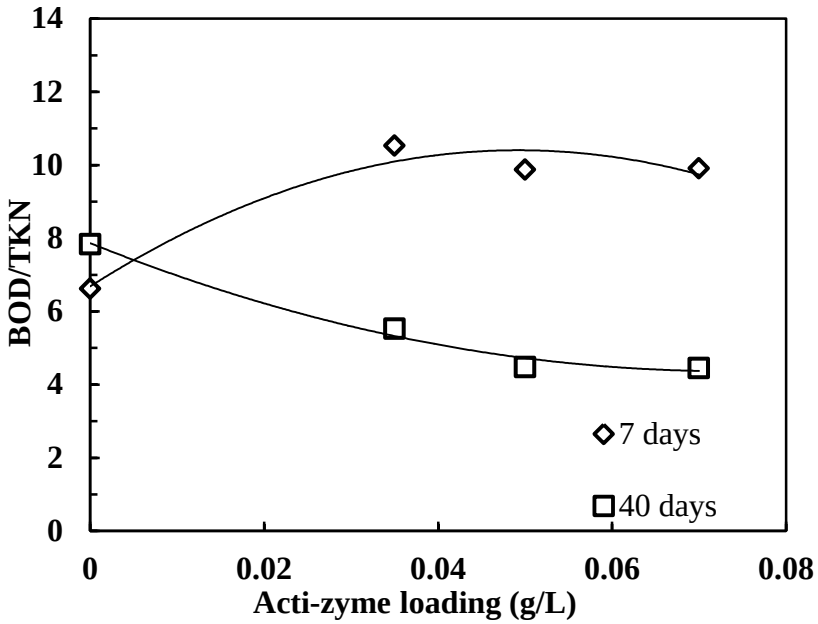


Figure 6-4: Influence of Acti-zyme loading and retention time on BOD_5/TKN ratio

6.3.2.4 COD/TKN ratio model

The COD/TKN ratios were >8.0 for the various Acti-zyme loading and retention time during sewage treatment (**Figure 6.5**). The higher values of the COD/TKN ratio indicated a moderate rate of de-nitrification in sewage (Shin *et al*., 2005). However, high de-nitrification was achieved at increased Acti-zyme loading at the 40 day period. This indicated Acti-zyme has a potential for de-nitrification of nitrogen contaminants in sewage promoting biodegradability. Higher retention times are encouraged as this result in decreased COD/TKN ratios hence improved de-nitrification (**Figure 6.5**). A COD/TKN ratio model represented by **Equation 6.5** was then developed.

$$\frac{COD}{TKN} = 8.677 - 0.408TKN - 0.032BOD_5 + 0.192TP + 0.058COD \ldots\ldots\ldots\ldots\ldots\ldots\ldots (6.5)$$

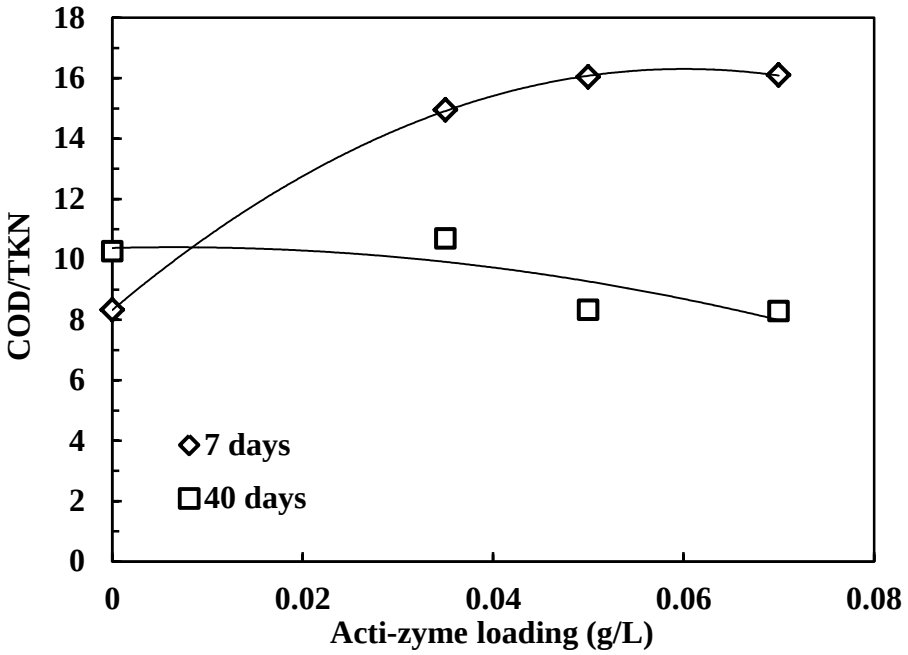


Figure 6-5: Influence of Acti-zyme loading and retention time on COD/TKN ratio

6.3.2.5 COD/TP ratio model

Lastly, the COD/TP ratios were >15.0 for the various Acti-zyme loading and retention times (**Figure 6.6**). COD/TP values of 20-60 were also reported for Harare City waste water in Zimbabwe by Muserere *et al*. (2014) and they indicated that values within this range show that the wastewater has increased biodegradability efficiency. Total phosphate and nitrogen removal in sewage water is essential in avoiding eutrophication in water bodies such as rivers and lakes where it is disposed in. The COD/TP model was developed and is represented by **Equation 6.6**.

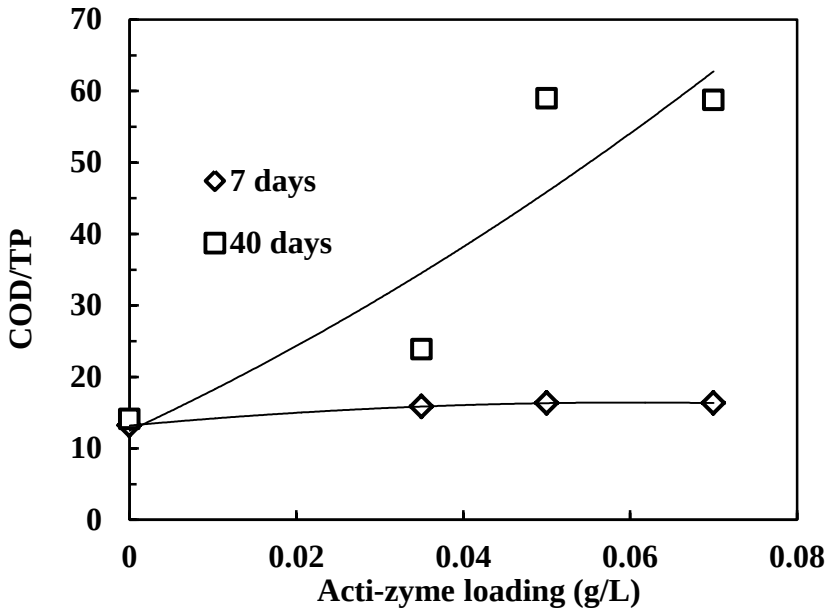


Figure 6-6: Influence of Acti-zyme loading and retention time on COD/TP ratio

$$\frac{COD}{TP} = 48.650 - 10.778TKN + 1.105BOD_5 - 21.8TP + 1.214COD \ldots\ldots\ldots\ldots\ldots (6.6)$$

6.3.2.6 Interactions and covariances between the model ratios

Interactions between the COD/TKN, TKN/BOD_5, BOD_5/TP and the COD/TP had a negative influence on the *COD/BOD_5* ratio whilst the interaction between BOD_5/TKN and TKN/TP had a positive influence on the *COD/BOD_5* ratio. At the same time, the covariances between the data were very small indicating lesser dependency between the variables during model development.

6.3.2.7 Comparison of the bio-nutrient removal statistical models data to experimental data

The COD/TKN, TKN/BOD_5, BOD_5/TP and the COD/TP models were validated using p-plots at 95% confidence interval. All the four models indicated a linear correlation between the measured values and the predicted values.

A significant *t-test* at 95% confidence interval indicated the applicability of the bio-nutrient removal models to any sewage treatment plant. The models are suitable for use at 40 days retention time whereby the required bio-nutrient removal ratios are achieved with Acti-zyme loading of 0.050 g/L (See **Appendix E5** for *t-test* analyses). This is also shown by **Table 6.1** hereby the percentage difference between the experimental data

and those simulated from the model are within ±6%. The detailed standard error estimation is given in **Appendix E65**.

Table 6-1: Comparison of the statistical models data to the experimental data

Bio-nutrient ratio	Experimental	Model	% Difference (±)	R^2	Adjusted R^2	Standard erro estimates
COD/BOD_5	1.86	1.86	0.60	0.983	0.978	0.0281
BOD_5/TKN	4.48	4.22	6.16	1.000	1.000	0.0590
COD/TKN	8.33	8.28	0.60	1	0.990	0.0840
COD/TP	58.98	57.81	2.02	0.9440	0.9270	

6.4 SUMMARY

The addition of Acti-zyme for bio-augmentation purposes during anaerobic treatment of sewage promoted bio-nutrient removal through biodegradability and de-nitrification of the contaminants. This was measured through the COD/BOD_5, BOD_5/TKN, COD/TKN and COD/TP ratios which have interactions between them at 95% confidence interval with estimated errors within 6%. Optimum bio-nutrient removal was achieved at 0.050 g/L and 40 days retention times hence these conditions were considered for the statistical modelling. Statistical models simulated indicated the measured values relate to the predicted values hence, the models can be applied in determining bio-nutrient removal ratios during sewage treatment using Acti-zyme.

CHAPTER 7. BIOGAS AND BIO-SOLIDS GENERATION FROM MUNICIPAL SEWAGE SLUDGE UTILIZING ACTI-ZYME AS BIO-CATALYST

7.1 INTRODUCTION

Municipal sewage sludge management is increasingly becoming a problem in developing countries due to poor wastewater treatment methodologies. About 60% of the sewage sludge ends up in landfills. Sewage sludge, like any other wastewater sludge, can be used for biogas production using Acti-zyme as bio-catalyst anaerobically (Duncan, 1970; Cail *et al*., 1986). Although biogas has been produced from Acti-zyme catalyzed wool scouring wastewater sludge by Cail *et al*. (1986) and from hog wastes by Duncan (1970), the influence of temperature on biogas production under mesophilic and thermophilic conditions still needs to be understood. Furthermore, the quantification, of the bio-solids that are generated from the digestion process still needs to be understood. Mesophilic conditions are normally employed for wet substrates with TSS of less than or equal to 15% of the total composition, residence time of 60-95 days and complete mixing is required whereas thermophilic conditions are required for wet substrates with TSS greater than or equal to 20% and residence times of 9-45 days (Vindis *et al*., 2009; Kardas *et al*., 2011). This chapter therefore focused on sewage sludge digestion utilizing Acti-zyme focusing on the optimum biogas and bio-solids production temperatures.

7.2 EXPERIMENTAL APPROACH

The sewage sludge was filtered and dried to 60-80% moisture content. Moisture content and volatile matter analyses were done using an AND moisture analyser. The %Moisture content (M) was determined by heating 5g of sample at 105 °C for 30 minutes in a Mermet oven with a power rating of 2000 watts and then recording the difference in weight. The %Volatile matter (VS) was determined by heating 5g of sample at 105 °C for 3 minutes and then recording the difference in weight. Sewage sludge digestion was carried out at 37 °C or 55°C to create mesophilic conditions at

atmospheric pressure. The schematic biogas collection method is shown in **Figure 3.1**. The biogas composition was measured in volume per percentage (vol.%). The raw results for the biogas and bio-solids quantity and composition are given in **Appendixes F-J** in triplicate and the average values were used for generating the graphs discussed in **Section 7.3**.

Paired sample *t-test* was used to test the influence of temperature on biogas production. Paired sample *t-test* is a statistical technique that is used to compare two population means in the case of two samples that are correlated. Paired sample *t-test* is used when the samples are the matched pairs, or when it is a case-control study as in this case where temperature is the controlled parameter. To calculate the paired sample *t-test* **Equation 7.1** was used:

$$t = \frac{\bar{d}}{\sqrt{s^2/n}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (7.1)$$

Where $\bar{d}$ is the mean difference between two samples, s^2 is the sample variance, *n* is the sample size and *t* is a paired sample *t-test* with n-1 degrees of freedom. Degrees of freedom are the number of values that are allowed to vary, in this case sample size minus one.

An alternate formula for paired sample t-test is represented in **Equation 7.2**:

$$t = \frac{\sum d}{\sqrt{\frac{n(\sum d^2) - (\sum d)^2}{n-1}}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (7.2)$$

A hypothesis was then developed and quantified with the t-test for the different amount of biogas, bio-methane, carbon dioxide, trace gases and bio-solids produced at the different temperatures at 95% confidence interval.

H_o = Digestion temperature does not have an influence on catalytic municipal sewage sludge digestion using Acti-zyme to biogas and bio-solids

H_1 = Digestion temperature have an influence on catalytic municipal sewage sludge digestion using Acti-zyme to biogas and bio-solids

The *t-test* was carried out at 95% level of significance. If the significant value (sig value) (p-value) is found to be less than 0.05 H_o is rejected otherwise the null hypothesis that the digestion temperature has no influence on municipal sewage sludge digestion utilizing Acti-zyme as bio-catalyst is accepted. The t-test analyses for the biogas and bio-solids quantity and composition at the different temperatures are given in **Appendixes M**.

7.3 RESULTS AND DISCUSSION

The influence of mesophilic and thermophilic conditions on the various biogas constituents is discussed. Furthermore the impact of the mesophilic and thermophilic conditions on bio-solids generation is also discussed.

7.3.1 Characterization of the raw sewage sludge

The sewage had total solids of 1143 mg/L (**Table 5.1**) and the pH changed from being acidic to alkaline during the digestion process with Acti-zyme under mesophilic conditions. The other raw sewage sludge physicochemical characteristics are indicated in **Table 7.1**.

Table 7-1: Raw sewage sludge characteristics

Parameter	Value
pH	6.3-8.3
COD	750±12.5 mg/L
TS	1143±14.35 mg/L
VS	2.5±0.05%
Moisture content	60±20%
TKN	245±5.1 mg/L
TP	52.5±2.7 mg/L
BOD_5	557±2.5 mg/L

7.3.2 Biogas production

Biogas production in a digester with substrate activated with Acti-zyme started immediately resulting in a low lag phase (Duncan, 1970). The biogas obtained had a composition of CH_4 (72-78%), CO_2 (16-20%) and trace gases (8-12%).

Biogas production increased with increase in Acti-zyme loading from 0- 0.050 g/L for both the mesophilic (37 °C) and thermophilic conditions (55 °C) and all sewage sludge loadings. Acti-zyme loadings of 0.050 g/L and sewage sludge loading of 7.5 g/L.d were found to be optimal in terms of bio-degradability of sewage using Acti-zyme reaches its optimum at 7.5 g/L.d as it has the chance to act on the substrate which is sewage sludge in this case unlike at higher loadings of 10 g/L.d where it does not digest all the sewage sludge to biogas. At higher sewage sludge loadings, Acti-zyme does not have the capacity to act on all the sewage sludge thereby decreasing the bio digestion rate.

Sewage sludge loading of 6.9-9.2 g/L.d have been recommended for optimal biogas production (Hesnawi and Mohamed, 2013). However, maximum biogas was achieved at mesophilic conditions with about 50% higher as compared to thermophilic conditions (**Figure 7.1**). This can be due to the Acti-zyme activity being optimum at temperatures ~37 ^{o}C. The biogas produced from Acti-zyme catalyzed digestion trend was unlike in other bio-catalyst free municipal sewage digestion reactors whereby the cumulative biogas amount actually increased by more than four times at thermophilic conditions (Vindis *et al*., 2009; Kardas *et al*., 2011). Paired sample t-test results showed $t(20) = 10.27$, $p<0.0005$ and therefore it was concluded that there is a significant difference in the production of biogas at temperatures of 37 ^{o}C and 55 ^{o}C, since p is less than 0.05.

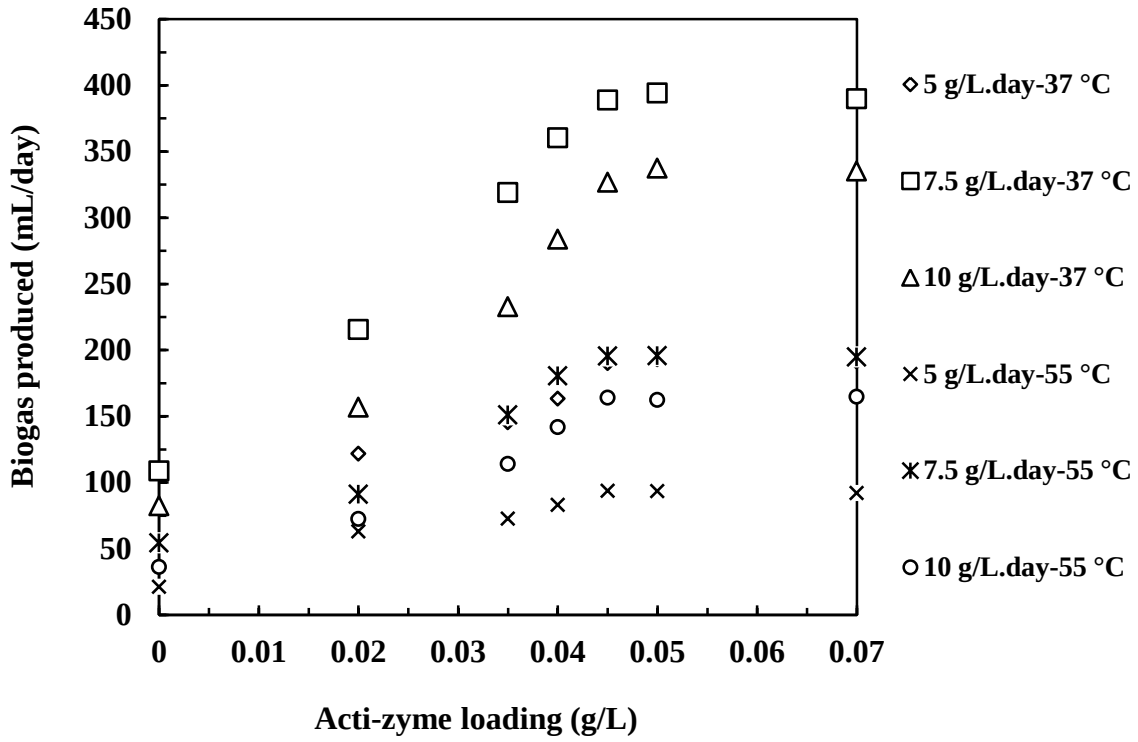


Figure 7-1: Influence of mesophilic and thermophilic temperature on biogas production at varying Acti-zyme and sewage sludge loadings at retention time of 40 days

A bio-methane rich biogas was produced with CH_4 composition ranging from 72-78 % with a peak being obtained for sewage loadings of 7.5 g/L.d at an Acti-zyme loading of 0.050 g/L as compared to Acti-zyme free digesters which had a bio-methane composition of 53-65% (**Table 7.2**). The biogas also contained 16-20 % CO_2 and 8-12% traces amounts of H_2S, N_2 and H_2. The trace gases were in lower quantities in digesters with Acti-zyme due to the ability of Acti-zyme to hinder their production effectively improving the quality of the biogas (**Table 7.2**). The bio-methane was found in high quantities due to the enhanced bio-degradability of the sewage sludge by Acti-zyme.

The amount of bio-methane generated in this study was also 23% higher as compared to previous studies with Acti-zyme by Duncan (1970) whereby they obtained a biogas with a composition of 60% CH_4 for digesting hog waste using Acti-zyme with 0.00625% of Acti-zyme for 50 days at 35 °C for the hog waste loading of 0.75-0.99 kg/kg VSS. Earlier studies by Cail *et al*. (1986) also reported a biogas composition with 68% CH_4 which was 12.8% lower as compared to that obtained in this study after digesting wool

scouring wastewater at 35 °C using 1% (v/w) of Acti-zyme for 207 days for the wastewater loading of 0.5-1.5 L/day. The high bio-methane quality in this study is due to higher Acti-zyme loading which assisted in catalyzed digestion of the sewage sludge.

Table 7-2: Biogas composition from anaerobic digestion of sewage sludge for systems with and without Acti-zyme

Gas	% (Acti-zyme)	% (without Acti-zyme)
CH_4	72-78	53-65
CO_2	16-20	22-27
Traces (H_2S, N_2, H_2)	5-9	8-12

7.3.3 Bio-methane generation

CH_4 production was at its maximum at mesophilic conditions by more than 100% compared to thermophilic conditions since this is where Acti-zyme activity is at its peak (**Figure 7.2**). CH_4 yield was ~78% for the mesophilic conditions whilst it was around 40% for thermophilic conditions at sewage sludge loadings of 7.5 g/L.d. This was a clear indication that mesophilic conditions are favorable for Acti-zyme bio-augmented biogas production from municipal sewage sludge. The t-test analyses showed that there is a significant difference in the bio-methane at varying temperatures since a p-value is less than 0.005 that is $t(20) = 53.27$, $p\text{-value}<0.0005$.

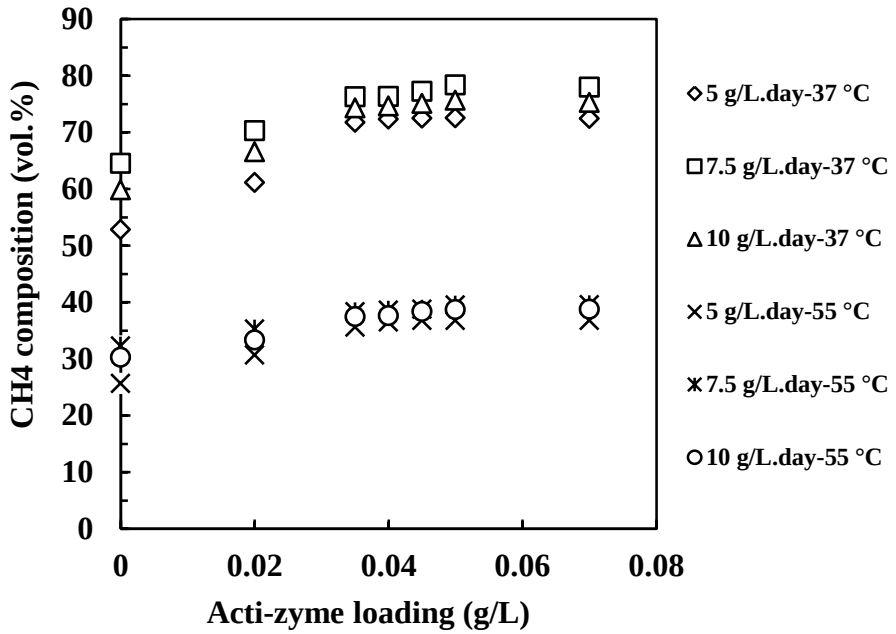


Figure 7-2: Influence of mesophilic and thermophilic temperature on bio-methane production at varying Acti-zyme and sewage loadings at retention time of 40 days

A summary of systems whereby Acti-zyme has been used for biogas generation is shown in **Table 7.3**. For this study only the optimal conditions for maximum biogas yield are presented under mesophilic conditions. In comparison to studies done earlier by Duncan (1970) and Cail *et al*. (1986), sewage sludge digestion under mesophilic conditions results in a biogas which is at least 12.8% richer in terms of bio-methane content (**Table 7.3**).The summary of the biogas generation for various wastewater bio-augmented with Acti-zyme is as shown in **Table 7.3**, where a clear enhancement of the bio-methane from high Acti-zyme loadings in this work is shown.

Table 7-3: Summary of biogas generated in Acti-zyme catalysed systems

Type of wastewater	Acti-zyme loading	Retention time (days)	Organic loading rate	T (°C)	pH	Biogas production rate	Bio-methane content	Reference
Wool scouring wastewater	1% (w/v)	207-211	0.75-0.99 kg/kg VSS	35 °C	7.1-7.4	2.9-3.3 m^3/ (m^3.day). 30% higher compared to an A_o system	68%	Cail *et al*., 1986
Hog waste	0.00625%	50	0.5-1.5 L/day	35 °C	7.1-7.2		60% CH_4 , 38% CO_2, 1% N_2, 1% water and H_2S traces	Duncan, 1970
Sewage sludge	0.050 g/L	40	7.5 g/L.d	37 °C	400 mL/day	400 mL/day	72-78% CH_4 , 16-20% CO_2, and traces (H_2S, N_2, H_2)	Current study

7.3.4 Carbon dioxide generation

The CO_2 produced was 45% higher in mesophilic conditions compared to thermophilic conditions (**Figure 7.3**). This was generally due to the decreased Acti-zyme activity and other naturally existing micro-organisms during the digestion process resulting in the municipal sewage sludge being partially digestion. However, for both mesophilic and thermophilic conditions, the CO_2 produced significantly decreased with increase in Acti-zyme loading from 0.035 g/L to 0.050 g/L due to the Acti-zyme activity which enhances bio-methane production and hinders production of other gases. Paired sample t-test results analyses showed t (20) = 6.22, p<0.0005 and therefore it was concluded that there is a significant difference in the production of CO_2 at temperatures of 37 ^{o}C and 55 ^{o}C, since p is less than 0.05.

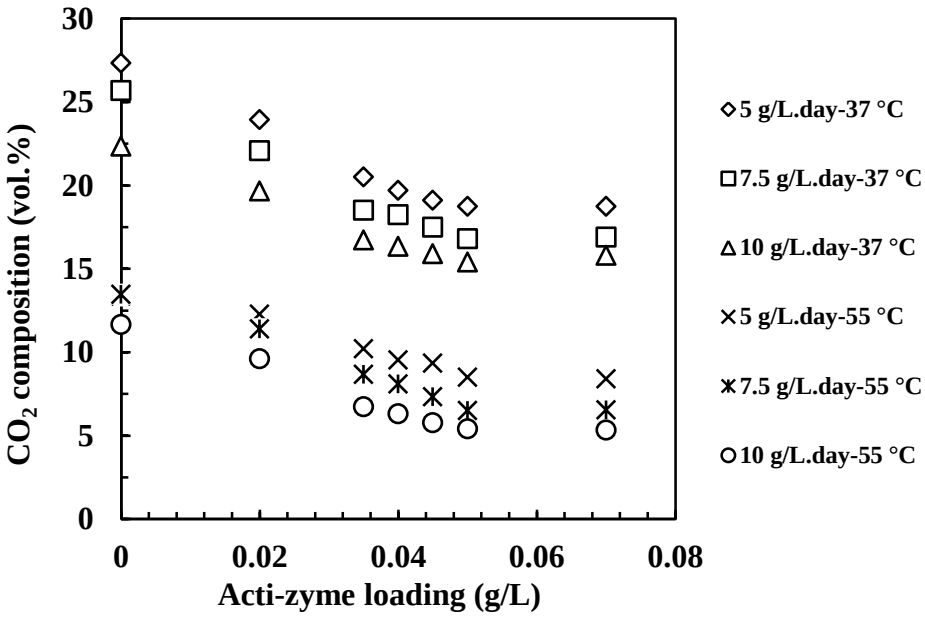


Figure 7-3: Influence of mesophilic and thermophilic temperature on carbon dioxide production at varying Acti-zyme and sewage sludge loading at retention time of 40 days

7.3.5 Trace graces generation

Trace gases included trace amounts of H_2S, ammonia and some water. The amount of trace gases produced was 48% more in in mesophilic conditions as compared to thermophilic conditions (**Figure 7.4**). During thermophilic conditions, the Acti-zyme became inactive hence minimal biogas is produced. Paired sample *t-test* results

analyses showed t (20) = 5.12, p<0.0005 and therefore it was concluded that there is a significant difference in the production of trace gases at temperatures of 37 ^{o}C and 55 ^{o}C, since p is less than 0.05.

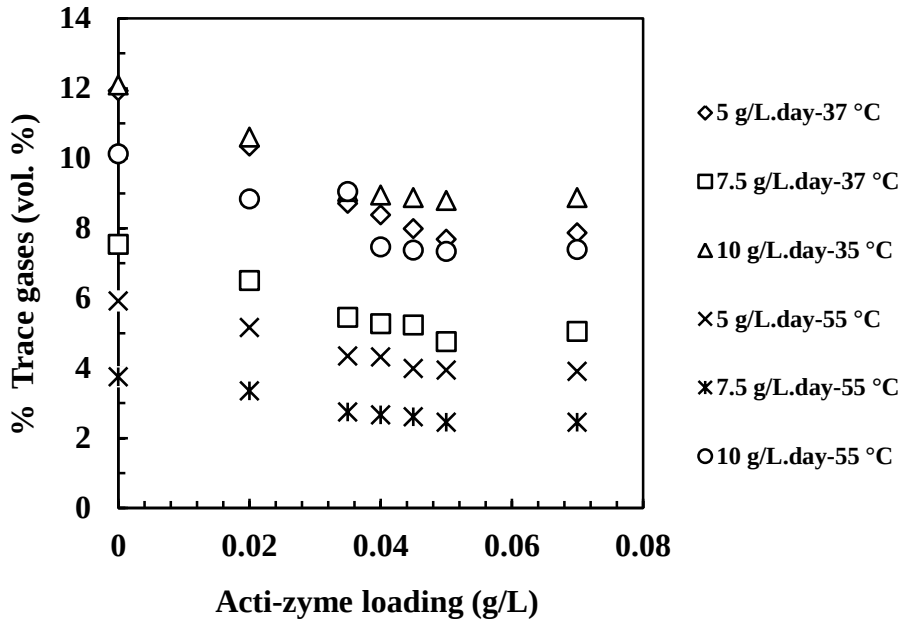


Figure 7-4: Influence of mesophilic and thermophilic conditions on trace gases production at varying Acti-zyme and sewage sludge loadings at a retention time of 40 days

7.3.6 Bio-solids production

Bio-solids are generated as digestate during the Acti-zyme catalysed digestion of sewage sludge (**Figure 7.5**). These bio-fertilizers can be utilized as an alternative source of bio-fertilizers. The bio-solids generated had nitrogen, phosphorous and potassium composition of 8.17±0.15%, 5.84±0.03% and 1.32±0.02% respectively (**Table 7.4**). The bio-solids also contained copper (0.0073±0.0002%), iron (0.0087±0.0003%), calcium (0.0079±0.002%) and magnesium (0.016±0.0021%) which are micronutrients essential for plant growth.

Table 7-4: Sewage bio-solids quality

Parameter	% Composition
Nitrogen	8.17±0.15
Phosphorous	5.84±0.03
Potassium	1.32±0.02
Copper	0.0073±0.0002
Iron	0.0087±0.0003
Calcium	0.0079±0.002
Magnesium	0.016±0.0021

The bio-solids obtained by this treatment method can be classified as high nitrogen content bio-solids and can be used for agricultural purposes (Evanylo, 2009). Bio-solids compositions obtained in this study was more than 50% higher in terms of NPK content in relation to values reported in literature (Johannesson, 1999; Pabsch and Wendland, 2013). This was due to the high nutrient composition in the raw sewage. The reduction of water content in the bio-solids from 80% to 20% as well as the hindering influence of Acti-zyme for *E. coli* activity resulted in a significant decrease in *E. coli* content from 10^{12} to 10^{6} cfu/L. Lower moisture levels and increasing Acti-zyme loading hinder *E. coli* growth making the bio-solids safe for application (Lang *et al*., 2007; Lang and Smith, 2007). The bio-solids had a pH of 7.26±0.54 and if there is a higher pH needed for application, lime stabilization is recommended.

Figure 7-5: Bio-solids generated as digestate during sewage sludge digestion

The amount of bio-solids generated was lowest at 5-10 g/L.d at 37 ^{o}C due to increased digestion due to Acti-zyme activity which was absent at 55 ^{o}C (**Figure 7.6**). As the temperature varied from mesophilic to thermophilic conditions, the amount of bio-solids produced showed an exponential decay trend for all the sewage sludge loadings and Acti-zyme loadings. However, if the target main product is bio-solids, thermophilic conditions can be encouraged since they promote pathogen reduction (Willis and Schafer, 2006). Paired sample *t-test* analysis showed t (20) = -6.59, $p<0.0005$ and therefore it was concluded that there is a significant difference in the production of bio-solids at temperatures of 37 ^{o}C and 55 ^{o}C, since p is less than 0.05.

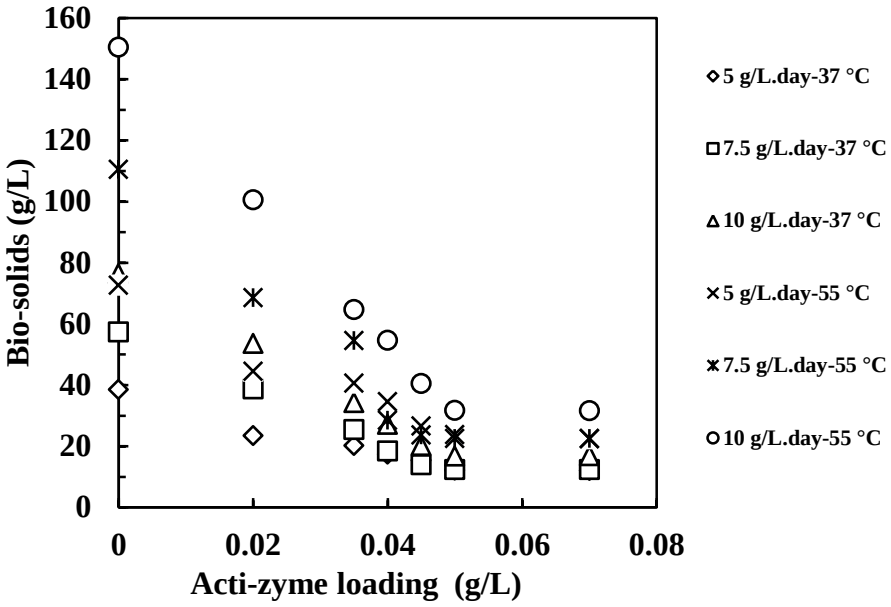


Figure 7-6: Influence of mesophilic and thermophilic conditions on bio-solids production at varying Acti-zyme and sewage sludge loadings and retention time of 40 days

7.4 SUMMARY

Mesophilic anaerobic digestion of sewage sludge utilizing Acti-zyme at 37 °C promotes production of bio-methane rich biogas which is almost free of H_2S and nitrogen and has lower CO_2 composition. Acti-zyme loading of 0.050 g/L and sewage sludge loading of 7.5 g/L.d is essential for optimum biogas production over a 40 day period. Additionally, bio-solids which are rich in fertilizer NPK nutrients are produced and can be utilized as bio-fertilizers. This can result in sustainable management of sewage sludge in developing countries high value added products like biogas being used for micro-energy generation at the same time harnessing bio-solids.

CHAPTER 8. KINETIC MODELLING FOR BIOGAS PRODUCTION FROM SEWAGE SLUDGE DIGESTION UTILISING ACTI-ZYME AS BIO-CATALYST

8.1 INTRODUCTION

Sewage treatment results in unwanted and unutilized municipal sewage sludge which is send off for landfilling resulting in the emissions of greenhouse gases. Of late there has been a push for resources like biogas and bio-solids recovery from municipal sewage sludge as a value addition strategy (**Chapter 7**). Biogas, a form of green gas can be applied for cooking and heating purposes and the bio-solids can be applied as bio-fertilizers. Although, resource recovery from sewage sludge utilizing Acti-zyme digestion is feasible there is still need to understand the kinetic modelling that occur during the digestion process of Acti-zyme bio-augmented sewage. Kinetic models are useful for simulating the trends for bio-methane production from sewage sludge digestion so that they are fully understood. Kinetic models for sewage sludge digestion can be expressed as linear, exponential, logistics kinetic, exponential rise to a maximum, first order exponential model and the modified Gompertz equation (Yusuf *et al*., 2011; Abu-Reesh, 2014; Latinwo and Agarry, 2015; Rodriguez-Chiang and Dahl, 2015). The equations were shown as **Equations 2.7-2.11**.

8.2 KINETIC MODELLING APPROACH

MATLAB R2013A was used as the kinetic modelling tool. Kinetic modelling for bio-methane production was considered at retention time of 40 days, sewage sludge loading of 7.5 g/L.d and Acti-zyme loading of 0.050 g/L since this is where optimum biogas production is achieved (**Chapter 7**). The experimental data was fitted to each of the five models and the coefficient of determination noted. A comparison between the valid kinetic model and the experimental data was also done. The cumulative biogas production was calculated in accordance to **Equation 8.1**.

$$Biogas\ production\ activity = \frac{(Final\ cumulative\ biogas\ amount - biogas\ amount\ produced\ at\ a\ certain\ time)}{Final\ cumulat\square ve\ biogas\ amount} \ldots\ldots\ldots\ldots\ldots\ldots (8.1)$$

8.3 RESULTS AND DISCUSSION

8.3.1 Bio-methane production activity from municipal sewage sludge utilizing Acti-zyme as digestion catalyst

Municipal sewage sludge digestion increased with increase in retention time for all media at the mesophilic and thermophilic conditions irrespective of whether there was Acti-zyme bio-augmentation or not (**Figure 8.1**). However, bio-methane quantity produced was highest in a system containing 0.050 g/L of Acti-zyme and sewage sludge loading of 7.5 g/L.d (**Figure 8.1**) indicating the importance of bio-augmentation in sewage sludge digestion. The bio-methane production from sewage sludge digestion bio-catalyzed with Acti-zyme went through the lag phase, exponential phase, deceleration phase and stationary phase (**Figure 8.1**) (**See Section 2.4.3**). Lag time for biogas production is approximately 10 days for all systems digested at 37 ^{o}C and those digested at 55 ^{o}C, the only major difference is the difference in gas quantity and composition after the 10 days and the rate at which it was produced.

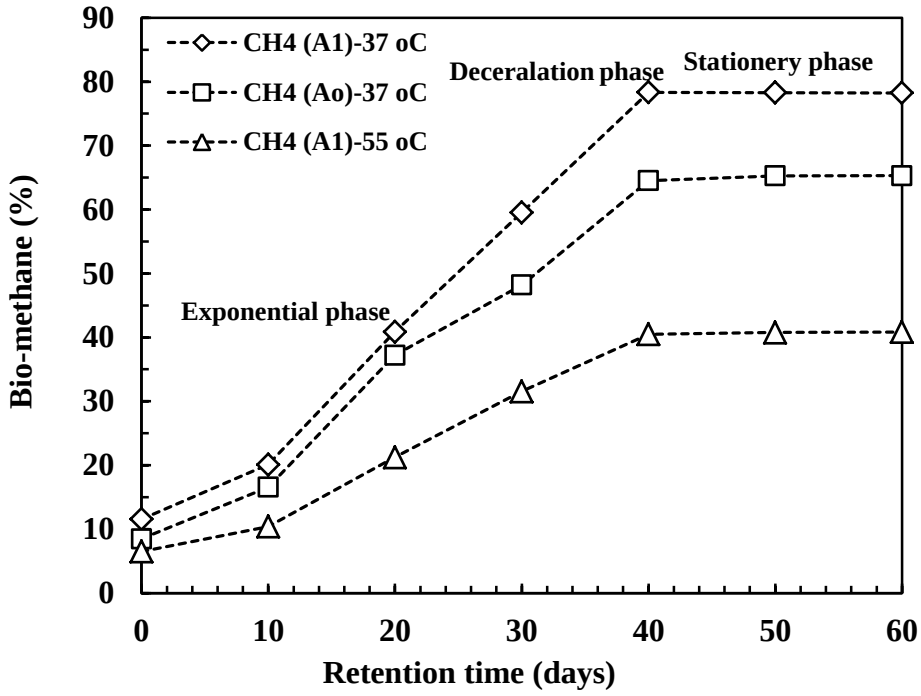


Figure 8-1: Bio-methane production potential from municipal sewage sludge at different temperatures and Acti-zyme loadings

The cumulative bio-methane production activity decreased significantly with increase in Acti-zyme loading and also with decrease in sewage sludge loading at a retention time of 40 days which was noted as the optimal retention time (**Figure 8.2**). This can be due to all the active sites in the sewage sludge being utilized by the Acti-zyme till saturation is achieved.

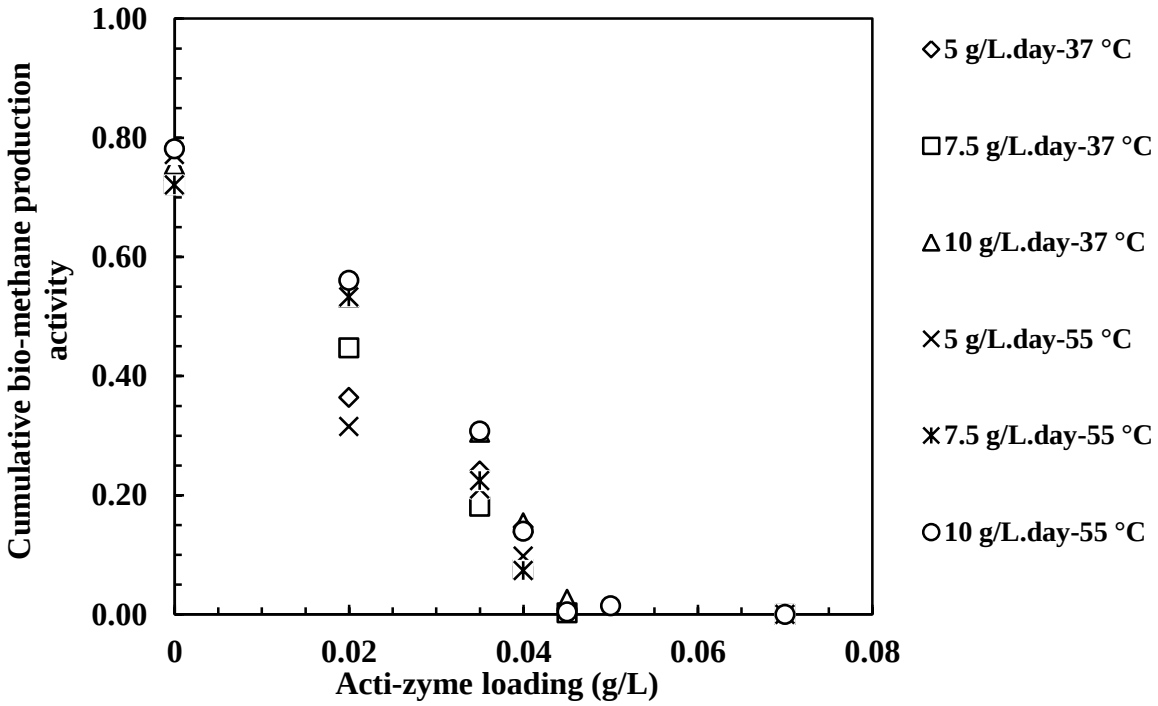


Figure 8-2: Cumulative bio-methane production activity at different temperatures and sewage sludge loading

8.3.2 Kinetic modelling of bio-methane production from sewage sludge

Five kinetic models were applied i.e. the linear, exponential, the logistics kinetic equation, the exponential rise to a maximum equation and the modified Gompertz equation were considered. Experimental data at 37 ◦C and sewage sludge loading of 7.5 g/L.d, Acti-zyme loading of 0.050 g/L for retention times of up to 40 days was considered since the bio-methane production was optimal at these conditions. A lag time of 10 days was considered in relation to **Figure 8.1**.

8.3.2.1 Linear kinetic model

The linear kinetic model for bio-methane production suggest that the cumulative amount of biogas produced increased linearly with increease in digestion time (**Equation 2.7**). The linear kinetic model was fitted on the experimental data and coefficient of determination of R^2 = 0.943 was found, which showed it is a good model for use on explaining the rate of bio-methane generation. The linear model obtained is given by **Equation 8.2** and its fit to the experimental data is shown in **Figure 8.3** with *y* representing the cumulative bio-methane production in mL/day with a = 14.19 and b = 1.229. Estimated coefficient standard errors the linear model were a = 4.87 and b = 0.14.

$$y = 14.19 + 1.229t \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (8.2)$$

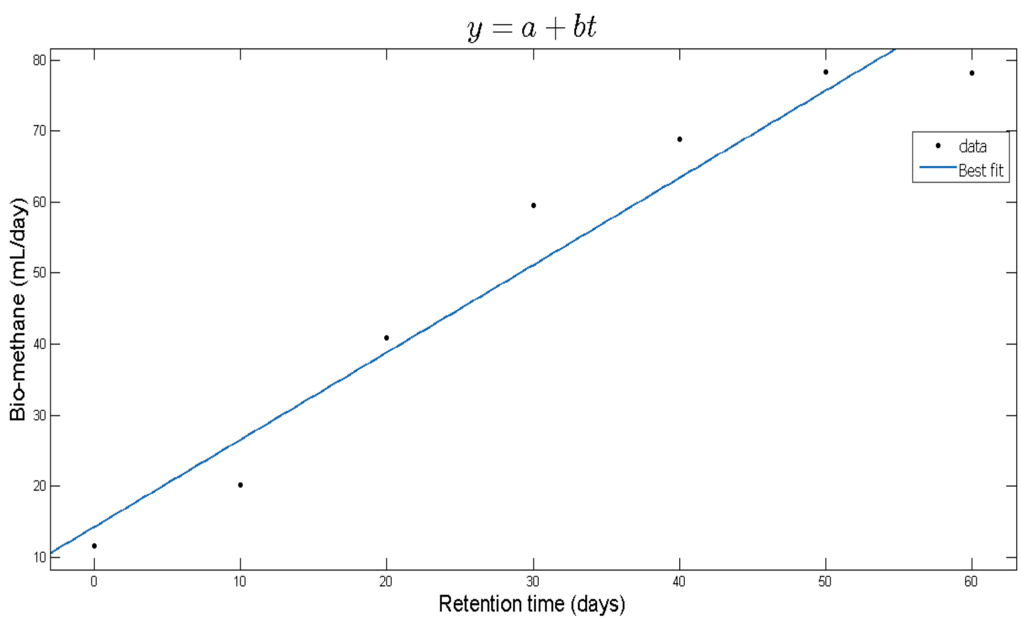


Figure 8-3: Comparison of the linear kinetic model to bio-methane generation experimental data

8.3.2.2 Exponential kinetic model

The exponential kinetic model suggests that cumulative bio-methane production increases exponentially with increase in digestion time (**Equation 2.8**). The non-linear least squares method was used to find the exponential kinetic model that fitted the bio-methane production during sewage sludge digestion. The exponential kinetic model

obtained is represented by **Equation 8.3** and its relation to experimental data is shown in **Figure 8.4** with y representing the cumulative bio-methane production in mL/day with a = -6786, b = 6802 and c = 0.0001738. The coefficient of determination was R^2 = 0.941 which showed that in terms of accuracy in representing the experimental data, the exponential model was a better model than linear model with a sum of squares error (SSE) of 264.8.

$$y = -6786 + 6802e^{0.0001738t} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots . (8.3)$$

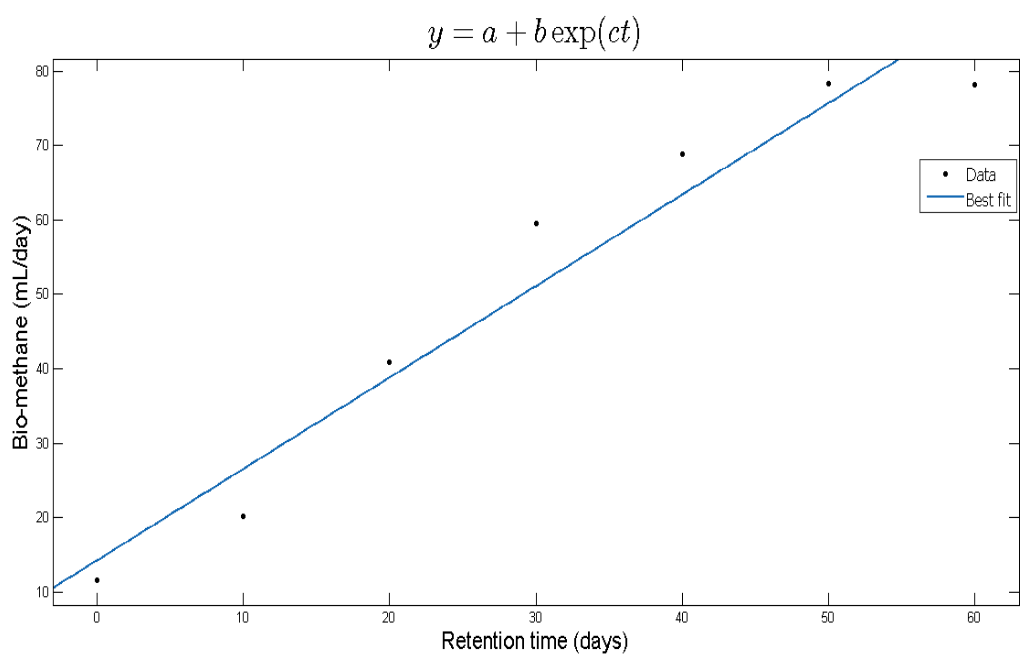


Figure 8-4: Comparison of the exponential kinetic model to bio-methane generation experimental data

8.3.2.3 Exponential rise to a maxima kinetic model

The exponential rise to maxima kinetic model suggest the cumulative bio-methane generation increases with increase in digestion time until a maximum is achieved (**Equation 2.10**). Non-linear least squares method was used to the fit exponential rise to a maximum kinetic model to the bio-methane generation data. The exponential rise to a maximum model was found to have a rate constant, k-value of 0.0003048 day^{-1} with A = 1.788 $x10^4$. The coefficient of determination, R^2 = 0.956, was quite high indicating that it is a good model with an SSE of 5194. The exponential rise to a maximum model is

shown in **Equation 8.4** and its fit to the bio-methane experimental data is shown in **Figure 8.5**, with *C*, being the cumulative amount of biogas produced in mL/day.

$$C = 1.788X10^4[1 - \exp(-0.0003048t)] \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.(8.4)$$

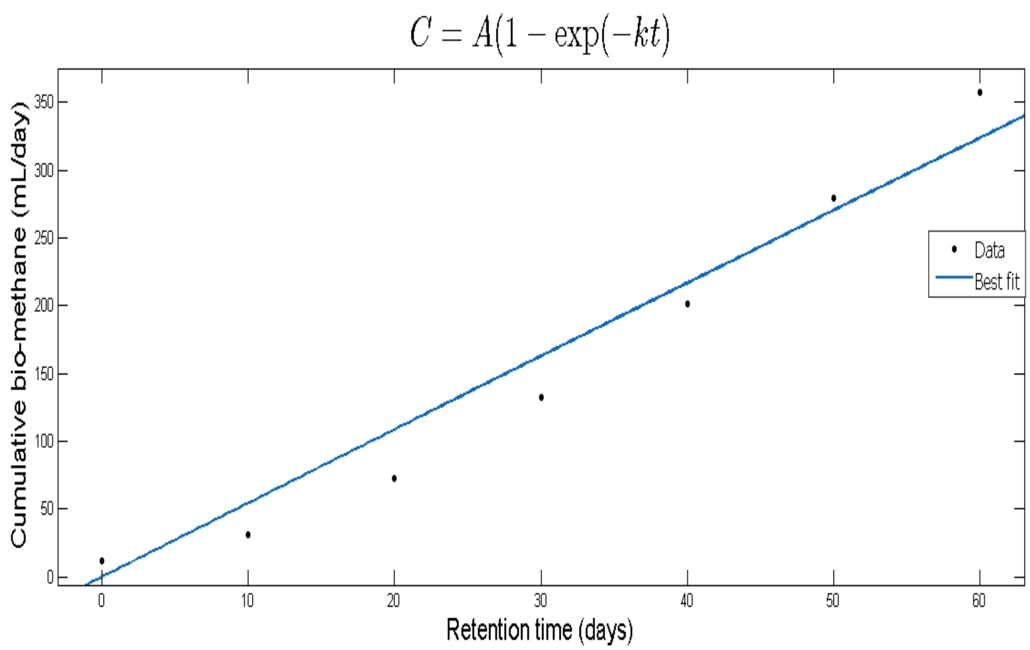


Figure 8-5: Comparison of the exponential rise to a maximum kinetic model and the bio-methane generation experimental data

8.3.2.4 Logistics kinetic model

The logistics kinetic model is used to represent biogas production rate related to microbial activity and it follows the batch kinetics trend of the microbes whereby the trend is defined by the lag phase, exponential phase, deceleration and stationery phases (**Equation 2.9**) (**See Section 2.4.9**). Non-linear least squares method was used to fit logistic kinetic model to the model which represented the bio-methane production utilizing Acti-zyme is represented by **Equation 8.5** with *C*, being the cumulative bio-methane quantity produced per batch in mL/day with a = 458.2, b = 233.96 and k = 0.0735 per day. The fit of the logistics kinetic model in relation to the bio-methane experimental data is shown in **Figure 8.6**. The coefficient of determination is R^2 = 0.9977, this showed that it was a better model than linear, exponential and exponential

rise to a maximum kinetic model. The standard errors a = 31.25, b = 2.77 and k = 0.005 per day.

$$C = \frac{458.2}{1 + 23.96\exp(-0.0735t)} \dots\dots\dots (8.5)$$

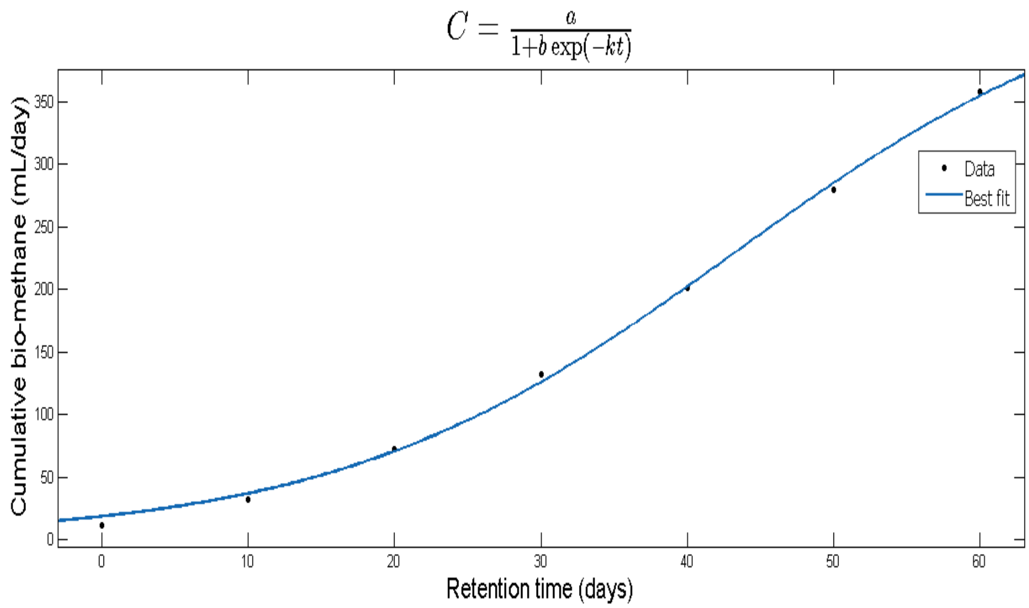


Figure 8-6: Comparison of the logistics kinetic model to the bio-methane generation experimental data

8.3.2.5 Modified Gompertz kinetic model

The modified Gompertz kinetic model was used to represent biogas production rate related to microbial activity (**Equation 2.11**). Non-linear least squares method was used to fit the modified Gompertz equation. The fit of the modified Gompertz equation in relation to the bio-methane experimental data is shown in **Figure 8.7** with *P*, being the cumulative bio-methane quantity produced per batch in mL/day. The coefficient of determination was R^2= 0.548, this showed that the modified Gompertz equation cannot be used for approximation of bio-methane production utilising Acti-zyme as bio-catalyst. An algorithm was run on the experimental data to fit the modified Gompertz equation and the following values were obtained. A = 181.4, r_m= -15.46, e = -1.096 and lambda = 1.361. This result is in contradiction to Ghatak and Mahanta (2014) who indicated that the biogas production process can best be simulated by the modified Gompertz kinetic model under mesophillic conditions.

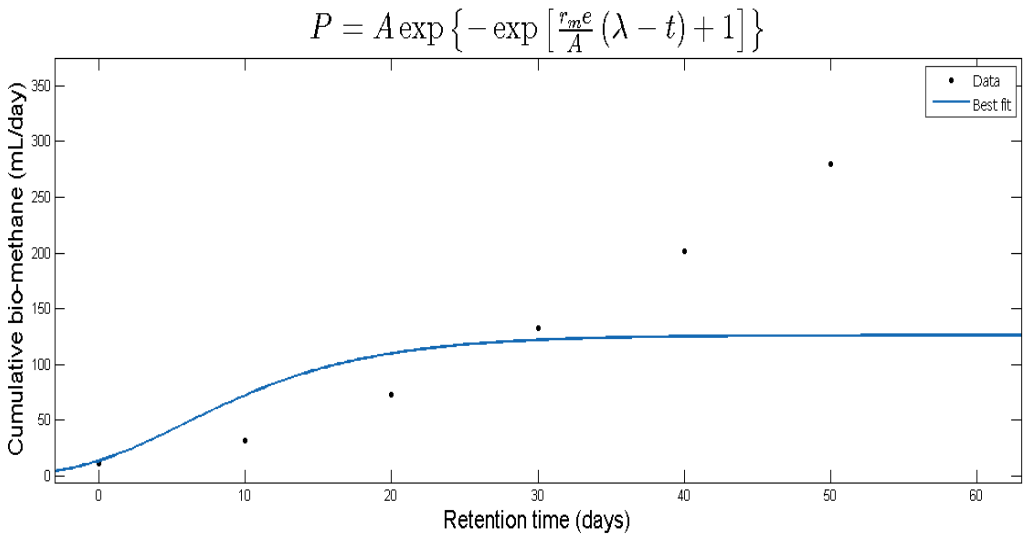


Figure 8-7: Comparison of the modified Gompertz kinetic model to bio-methane experimental data

8.3.2.6 Overview of the bio-methane production kinetic models

All the five models had a low standard error indicating the accuracy of the coefficient. From the coefficients of determination for the five models indicated in **Table 8.1**, the logistic kinetic model had the highest coefficient of determination of 0.999; therefore it is the best model of the five models that can best explain the kinetics of bio-methane generation from sewage sludge utilizing Acti-zyme as bio-catalyst. This is due to the fact that the rate of bio-methane production is determined by the Acti-zyme batch kinetics during the sewage sludge digestion. Furthermore, the logistics kinetic equation had the highest k-value indicating the bio-methane production from sewage sludge follows first order kinetics especially during the exponential phase which is well presented by the logistics kinetic model. However, this is in contradiction with Latinwo and Agarry (2015) who indicated that the cumulative biogas production in municipal waste and sewage biogas digestion is best modelled by the exponential equation.

Table 8-1: Summary of the kinetic models for bio-methane generation

Kinetic model	R^2	Adjusted R^2	Method used in MATLAB	Rate constants (k) (per day)
Linear	0.943	0.931	Linear least squares	-
Exponential	0.941	0.912	Non-linear least squares; Trust region algorithm	-
Exponential rise to a maxima	0.958	0.949	Non-linear least squares; Trust region algorithm	0.00031
Logistic kinetic	0.999	0.998	Non-linear least squares; Levenberg-Marquardt algorithm	0.0735
Modified Gompertz	0.548			-

8.3.2.7: Comparison of the logistics kinetic model to experimental data

The logistics kinetic model which was concluded to be the best fit for modelling the kinetics during bio-methane production only had a 4.9% in the amount of bio-methane in comparison to the experimental data (**Table 8.2**). This validated the applicability of the logistic kinetic model in sewage sludge digestion catalyzed with Acti-zyme loadings of 0.050 g/L, sewage sludge loadings of 7.5 g/L.d and retention times of 40 days.

Table 8-2: Comparison of the logistics kinetic model to bio-methane experimental data at optimum conditions

Method	Bio-methane quantity (ML/day)
Experimental	210.4
Logistics kinetic model	200.0

The link between the experimental cumulative bio-methane and that estimated from the logistics kinetic model is shown in **Figure 8.8**. The relative error between the experimental data and the suggested model was 0.6 with the highest discrepancy being obtained at the initial measurements.

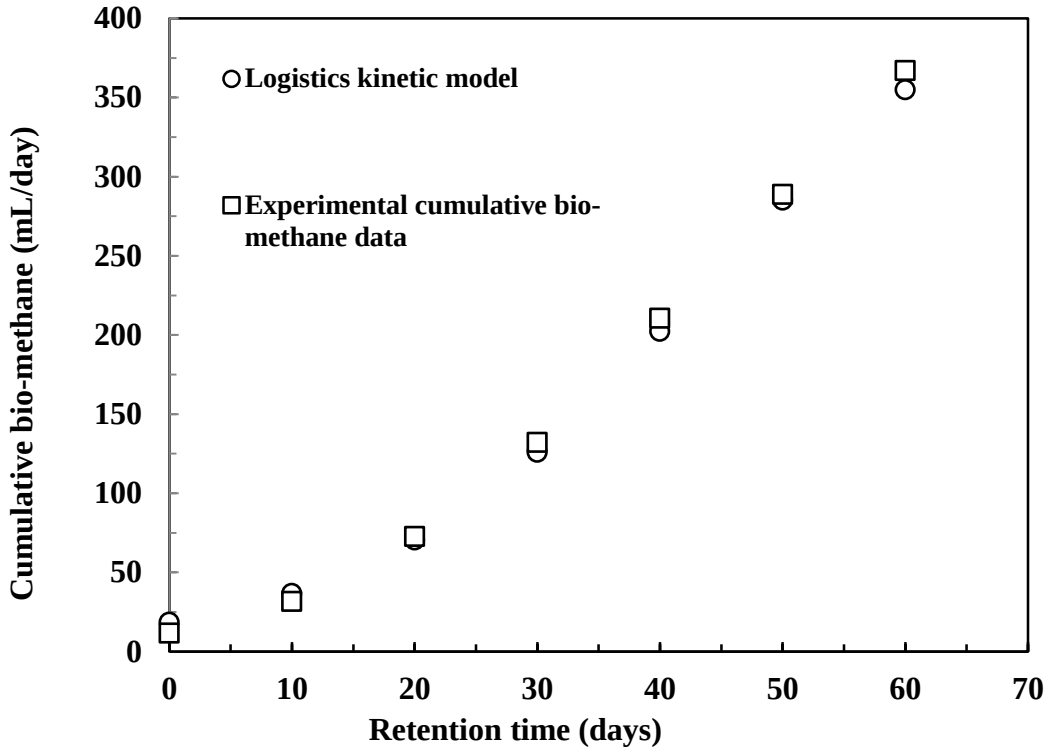


Figure 8-8: Comparison of the empirical logistics kinetic model to the cumulative experimental bio-methane measured

8.4 SUMMARY

The logistics kinetic model can be accurately applied for modelling the experimental data for bio-methane production from sewage sludge utilizing Acti-zyme as bio-catalyst. The logistics kinetic model describes the various batch kinetics stages which Acti-zyme goes through during sewage sludge digestion. The bio-methane production from sewage sludge utilizing Acti-zyme follows the logistics kinetic model with k = 0.073 day^{-1} and has only a 4.9% deviation from the measured data at the optimum conditions which are Acti-zyme loadings of 0.050 g/L, sewage sludge loadings of 7.5 g/L.d and retention times of 40 days.

CHAPTER 9. STATISTICAL MODELLING FOR BIOGAS AND BIO-SOLIDS GENERATION FROM ANAEROBIC SEWAGE SLUDGE DIGESTION USING ACTI-ZYME AS BIO-CATALYST

9.1 INTRODUCTION

Biogas which is rich in bio-methane has been produced from digestion of sewage sludge alongside with some bio-solids. The biogas was found to consist 72-78% CH_4, 16-20% CO_2 and 8-12% traces, whereas the bio-solids had a nitrogen, phosphorous and potassium composition of 8.17%, 5.84% and 1.32% respectively for Acti-zyme loadings of 0.050 g/L, retention time of 40 days and sewage sludge loadings of 7.5 g/L.d (**Chapter 7**). The biogas and bio-solids production from sewage sludge utilizing Acti-zyme under anaerobic conditions can be represented by **Reaction 9.1**.

$$Sewage\ sludge\ + Acti-zyme \rightarrow CH_4\ + CO_2 + H_2 + N_2 + H_2S + bio-solids \ldots\ldots\ldots\ldots\ldots\ldots (9.1)$$

This chapter focused on the statistical modelling for biogas and bio-solids production from sewage sludge utilizing Acti-zyme as biocatalyst so that the experimental data can simulate the real sewage treatment process based on the optimum conditions identified in **Chapter 7**.

9.2 DEVELOPMENT OF STATISTICAL MODELS

SPSS Statistics 19.0 was used as the statistical modelling package for quantifying the biogas and bio-solids production from sewage sludge at a probability value (p-value) of ≤ 0.05 due to the small sample size used (Fachri *et al*., 2015). The Only mesophillic conditions experimental data was considered since Acti-zyme activity is optimal at 37 °C. The four applicable models were used to fit the experimental data obtained (**see Section 2.7.4**).

9.2.1 Statistical modelling assumptions

The most basic assumption made was that there was a relationship between biogas production and Acti-zyme loading and also between the bio-solids production and Acti-zyme loading at all the various sewage sludge loading rates. Another assumption was

that error of estimation (ε) had a normal distribution with mean zero and a constant standard deviation. In addition to that an assumption was made that the error is uncorrelated. For this special case it was assumed that the models were valid in the range of experimentation, meaning that we do not use the model to extrapolate but only to interpolate.

9.2.2 Evaluation of the models

The models considered were linear, exponential compound and quadratic models and their significance (sig) for degrees of freedom (df) between groups and within groups (Snee, 1977). An *F* value was also generated in SPSS 19.0 based on the analyses and the greater the *F* value, the greater the significance. The *F-test* and the *t-test* were used on the basis of a small sample size being analyzed with a p-value of 0.05 (de Winter, 2013). On that basis, the following hypotheses were generated for a fixed retention time in the digesters for the four models tested.

H_o = Biogas and bio-solids production are dependent on Acti-zyme and sewage sludge loading

H_a = Biogas and bio-solids production are not dependent on Acti-zyme and sewage sludge loading

9.3 GENERATION OF THE BIOGAS FROM SEWAGE SLUDGE

The quantity of biogas generated increased with increase in Acti-zyme loadings (**Figure 9.1**) and retention time with a maxima bio-methane content being obtained at 40 days (**Figure 9.2**) and Acti-zyme loading of 0.050 g/L (**Figure 9.1**). This is due to the fact that Acti-zyme activity increases at the exponential growth phase after it has acclimatized itself to the sewage conditions. In addition, the biogas quantity produced was optimal at sewage sludge loadings of 7.5 g/L.d although a maximum was reached for all the sewage sludge loadings. Low sewage sludge loadings result in low biogas generation, whereas high loadings will also result in lower biogas generation since the Acti-zyme will need longer time to digest the sewage sludge (**Figure 9.1**).

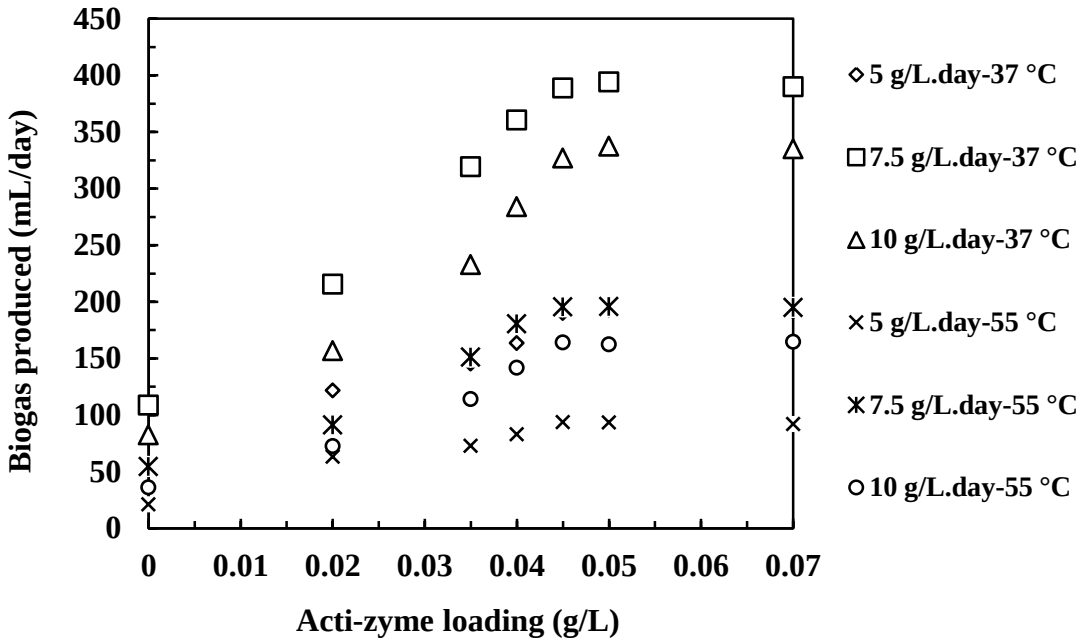


Figure 9-1: Biogas quantity produced at varying Acti-zyme loading at fixed retention time of 40 days

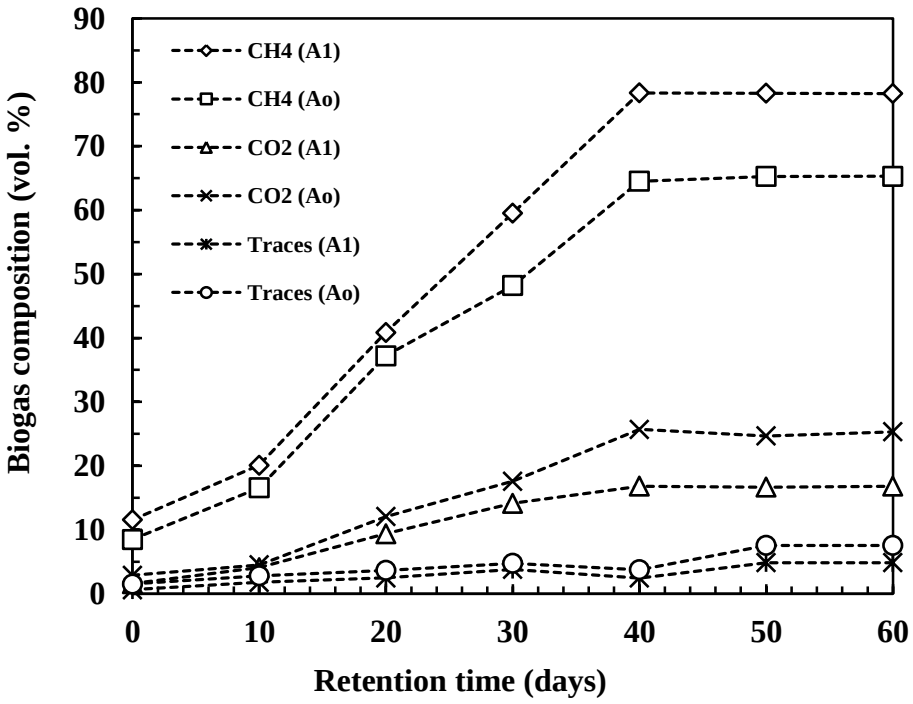


Figure 9-2: Comparison of biogas quality for digestion with and without Acti-zyme loading of 0.050 g/L

9.3.1 Quantifying the impact of Acti-zyme on biogas production

An independent, *t-test* was used to quantify the impact of Acti-zyme on the biogas production for the different Acti-zyme loadings. From **Figure 9.2**, it was observed that at a retention time of 40 days and above there is a significant difference between the production of biogas with Acti-zyme (A_1) and those that are Acti-zyme free (A_o) The *t-test* proved that there is significant difference at 5% level of significant (sig value <0.05) as indicated in **Table 9.1**. Different amounts of Acti-zyme will result in different amounts of biogas, thereby showing the importance of having Acti-zyme catalyzed digestion for achieving high biogas yields.

Table 9-1: Independent *t-test* quantifying impact of Acti-zyme on biogas production

		Levene's Test for Equality of Variances		***t-test* for Equality of Means**						
									95% Confidence Interval of the Difference	
		F	**Sig.**	**T**	**df**	**Sig. (2-tailed)**	**Mean Difference**	**Std. Error Difference**	**Lower**	**Upper**
Biogas	Equal variances assumed	3.014	0.102	51.723	16	0.000	13.256	0.256	12.712	13.799
	Equal variances not assumed			51.723	8.665	0.000	13.256	0.256	12.672	13.838

9.3.2 ANOVA tests for effect of sewage sludge loading on biogas production

An ANOVA test was done on the biogas quantities obtained at the different operating conditions. ANOVA test for biogas produced showed that there is a significant difference (sig.) in amount of biogas produced at sewage sludge loadings of 5 g/L.d, 7.5 g/L.d and 10 g/L.d with increasing retention time (**Table 9.2**); this was indicated by the significant value 0.000. This was a clear indication that different amounts of sewage sludge will give significant different amount of biogas.

Table 9-2: ANOVA test for effect of sewage sludge loading on biogas production at increased retention times

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	273774.508	2	136887.254	18.689	0.000
Within Groups	439477.143	60	7324.619		
Total	713251.651	62			

9.3.3 Statistical modelling for biogas production

Linear, exponential, compound and quadratic models were tested for simulation of the amount of biogas production as indicated in **Table 9.3**. The significant values for all the four models indicated the models were significant with p values of < 0.05 for determination of biogas production rate (**Table 9.3**). The models took the form as indicated by **Equation 9.1** (linear), **Equation 9.2** (quadratic), **Equation 9.3** (compound) and **Equation 9.4** (exponential) with $x_{\text{Acti-zyme}}$ as the loading of Acti-zyme used in the sewage sludge digestion and *y* as the amount of biogas generated in mL/day. However, the quadratic model (**Equation 9.2**) was the most suitable for simulating biogas production with R^2 value of 0.967, the significant value (0.000) and the high *F* value compared to the other models (**Table 9.3**).

$$y_{Biogas} = 142.757 + 4.525x_{Acti-zyme} \quad \ldots\ldots (9.1)$$

$$y_{Biogas} = -0.067x_{Acti-zyme}^2 + 9.101x_{Acti-zyme} + 94.019 \quad \ldots\ldots (9.2)$$

$$y_{Biogas} = 139.855 * 1.02^{x_{Acti-zyme}} \quad \ldots\ldots (9.3)$$

$$y_{Biogas} = 139.855e^{0.019x_{Acti-zyme}} \quad \ldots\ldots (9.4)$$

The quadratic model was also identified as the model that best simulated biogas production by Beevi *et al*. (2014) for digestion of municipal solid waste under mesophilic conditions although their system was cell free at 95% confidence interval.

Table 9-3: Statistical models summary and parameter estimates for biogas production

Model	Biogas model summary					Parameter estimates		
	R^2	F	df_1	df_2	Sig.	Constant	b_1	b_2
Linear	0.854	111.310	1	19	0.000	142.757	4.525	-
Quadratic	0.967	263.272	2	18	0.000	94.019	9.101	-0.067
Compound	0.815	83.904	1	19	0.000	139.855	1.020	-
Exponential	0.815	83.904	1	19	0.000	139.855	0.019	-

9.3.4 Comparison of the quadratic biogas statistical model to experimental data

Evaluation of the relative error for the quadratic model for amount of biogas produced helped to ascertain the usability of this models in real life. The relative error obtained in the biogas quadratic models was very minimal compared to the actual measured values (**Figure 9.3**). The average relative error obtained was 0.0676. The comparison of the biogas quadratic model to the measured experimental data is given in **Appendix N1**.

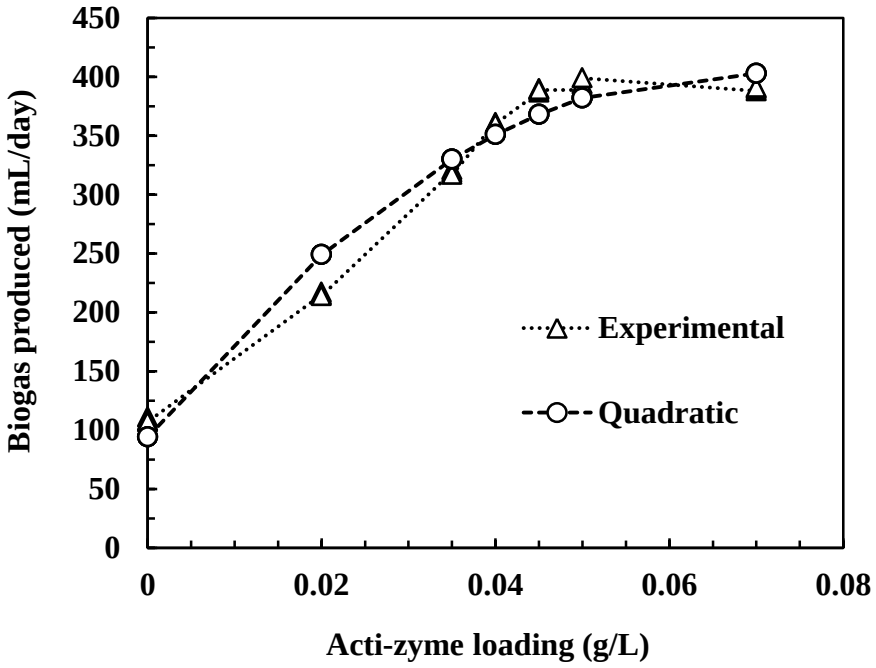


Figure 9-3: Relative error in the quadratic model representing amount of biogas produced from sewage sludge

9.3.5: Comparison of the biogas generation statistical quadratic and the logistics kinetic model

The quadratic statistical model best simulated the amount of biogas produced from sewage sludge digestion for Acti-zyme loadings of 0.050 g/L, sewage sludge loadings of 7.5 g/L.d and retention time of 40 days (**Equation 9.2**) and the logistics kinetic model that best simulated the amount of bio-methane produced at the same experimental conditions (**Equation 8.5)** gave almost the same value of bio-methane produced (**Figure 9.4**). This indicates that we can apply either of the equations in determining the amount of bio-methane produced, which is 78% of the total biogas produced. The two models had a 26% difference in the amount of bio-methane generated with the statistical model giving higher bio-methane values possibly because the model did not account for the 10 days lag phase period where there is no gas being produced. Raw data for the two models comparison is shown in **Appendix AN3**.

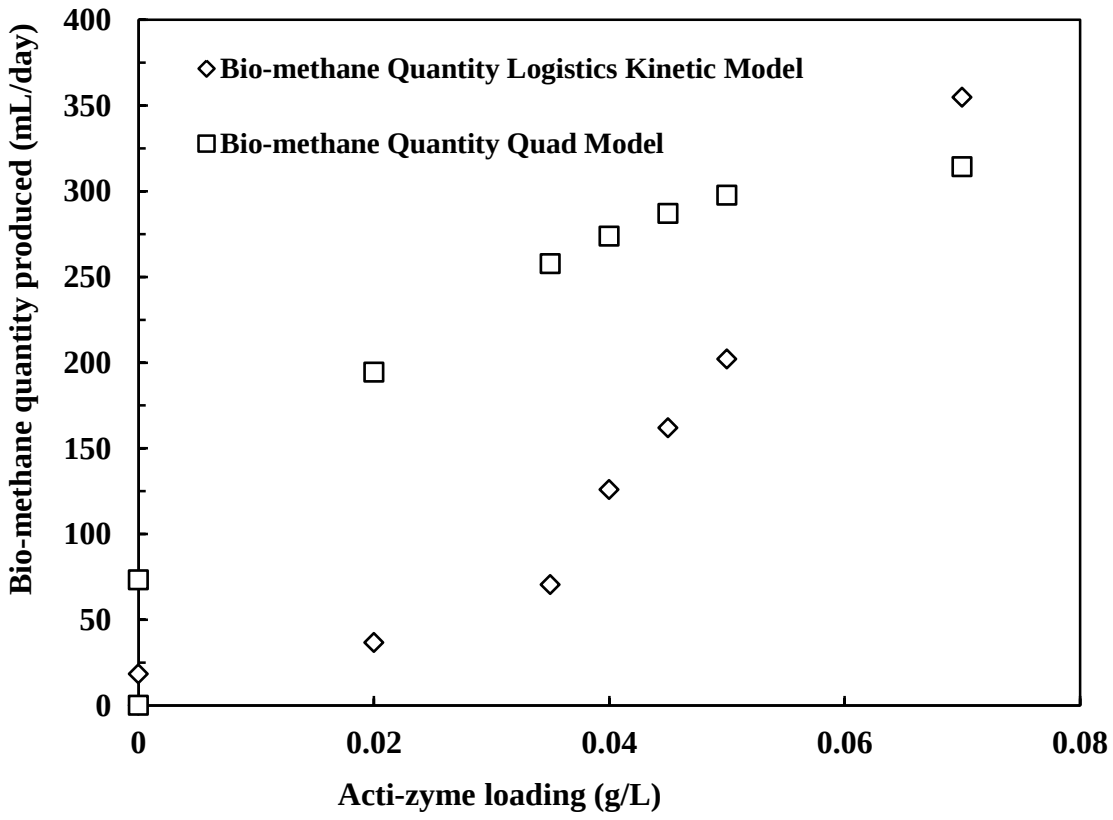


Figure 9-4: Comparison of the kinematic model and the quadratic model used to determine the amount of biogas produced

9.4 GENERATION OF THE BIO-SOLIDS FROM SEWAGE SLUDGE

The bio-solids amount generated decreased with increase in Acti-zyme loading with high sewage loadings of 10 g/L.d generating the highest amount of bio-solids. The bio-solids generated and also became constant for all sewage loadings after 0.050 g/L possibly because Acti-zyme would have digested all the nutrients in the sewage sludge (**Figure 9.5**).

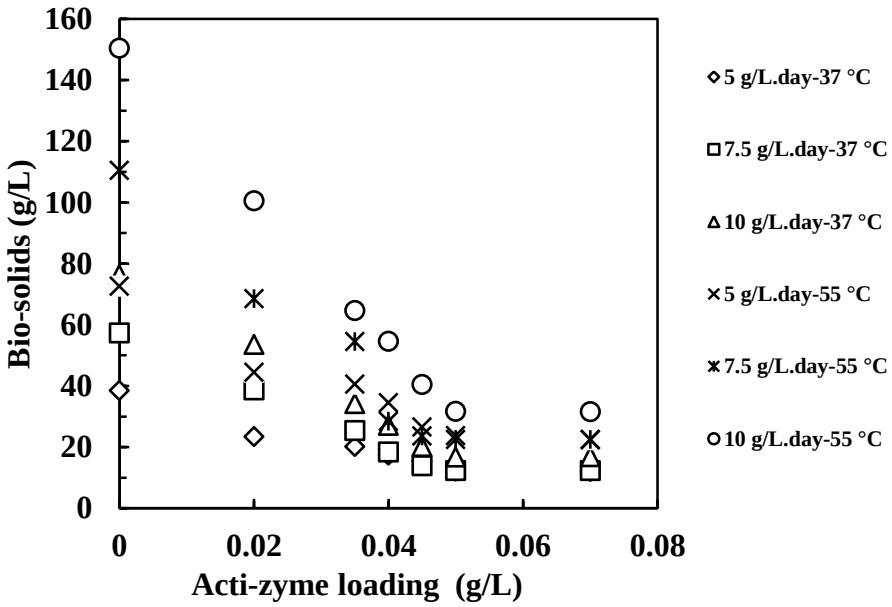


Figure 9-5: Bio-solids produced at increased Acti-zyme loadings and retention time of 40 days

9.4.1 ANOVA tests for effect of increased Acti-zyme and sewage sludge loadings on bio-solids quantity

An ANOVA test conducted for bio-solids showed that there was significant difference in the amount of bio-solids produced at 5 g/L.d, 7.5 g/L.d and 10 g/L.d with increasing Acti-zyme loadings (**Table 9.4**) as indicated by the significant value (sig.) which is 0.013 and a positive *F*-value. This indicated that the amount of Acti-zyme used and the amount of sewage sludge loaded has an impact on the amount of bio-solids produced.

Table 9-4: ANOVA tests for bio-solids produced at increasing Acti-zyme and sewage sludge loading

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2453.990	2	1226.995	4.701	0.013
Within Groups	15661.823	60	261.030		
Total	18115.813	62			

9.4.2 Statistical modelling for bio-solids production

Linear, exponential, compound and quadratic models were tested for simulating the amount of bio-solids production as indicated in **Table 9.6**. The test for significance for all the four models was significant with values < 0.05 (**Table 9.6**). The equations took the form as indicated in **Equation 9.5** (linear), **Equation 9.6** (quadratic), **Equation 9.7** (compound) and **Equation 9.8** (exponential).

$$y_{Bio-solids} = 51.769 - 0.708x_{Acti-zyme} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots (9.5)$$

$$y_{Bio-solids} = -0.010x_{Acti-zyme}^{2} + 1.381x_{Acti-zyme} + 58.936 \dots\dots\dots\dots\dots\dots\dots\dots\dots (9.6)$$

$$y_{Bio-solids} = 55.514 * 0.975^{x_{Acti-zyme}} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots (9.7)$$

$$y_{Bio-solids} = 55.514e^{-0.025x_{Acti-zyme}} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots (9.8)$$

However, the quadratic model was the most suitable for simulating bio-solids production as indicated by the R^2 value of 0.977 and a higher F value in comparison to the other models.

Table 9-5: Models summary and parameter estimates for bio-solids production

Model	Bio-solids model summary					Parameter estimates		
	R^2	F	df_1	df_2	Sig.	Constant	b_1	b_2
Linear	0.875	132.788	1	19	0.000	51.769	-0.708	-
Quadratic	0.977	380.103	2	18	0.000	58.936	-1.381	0.010
Compound	0.894	159.408	1	19	0.000	55.514	0.975	-
Exponential	0.894	159.408	1	19	0.000	55.514	-0.025	-

9.4.3 Comparison of the bio-solids quadratic statistical model to experimental data

Evaluation of the relative error helped to ascertain the usability of these models in real life for bio-solids generation. The relative error obtained in bio-solids quadratic model was very minimal compared to the actual measured values (**Figure 9.6**). The average relative error obtained was 0.115 for the bio-solids indicating the accuracy of the model. The comparison of the bio-solids quadratic to the experimental data is given in **Appendix N2**.

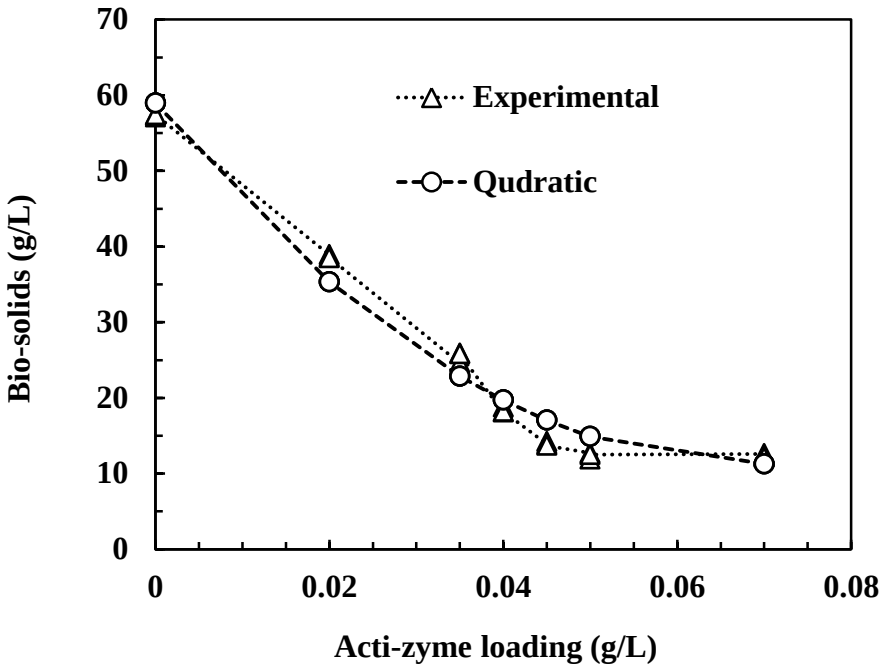


Figure 9-6: Relative error in the quadratic model representing bio-solids production from sewage sludge

9.5 SUMMARY

Biogas and bio-solids production from sewage sludge digestion for increased Acti-zyme loadings up to 0.050 g/L, sewage sludge loadings of 7.5 g/L.d for retention times of 40 days are best simulated by quadratic models. Quadratic models developed indicated the measured values relate well to the predicted values hence, the models can be applied for simulating sewage treatment using Acti-zyme even at industrial scale with relative errors of about 0.1 which is about 10% deviation for both biogas and bio-solids. The quadratic model for amount of biogas produced relates to the logistic kinetic model for bio-methane produced.

CHAPTER 10. TECHNO-ECONOMIC ANALYSIS FOR ANAEROBIC TREATMENT OF SEWAGE USING ACTI-ZYME AS BIO-CATALYST CO-HARNESSING OF BIOGAS AND BIO-SOLIDS

10.1 INTRODUCTION

Wastewater treatment with the recovery of value added products like biogas is increasingly becoming popular. Sewage sludge, though a waste, if properly digested can help to meet the energy demands of a community utilising bio-catalysts like Acti-zyme (Duncan, 1970; Cail *et al*., 1986). Optimum Acti-zyme loading rates of 0.050 g/L, sewage sludge loadings of 7.5 g/L.d and retention times of 40 days have been recommended for biogas production (**Chapters 5-9**). Despite the success stories of Acti-zyme for wastewater and sewage sludge treatment, the techno-economic feasibility has never been investigated. This chapter focused on the techno-economic feasibility of sewage sludge digestion to form biogas and bio-solids utilizing Acti-zyme as the digestion bio-catalyst.

10.2 TECHNO-ECONOMIC ASSESSMENT APPROACH

Capital budgeting techniques were used for the economic assessment for biogas production co-harnessing biogas and bio-solids. A local sewage plant with a bio-nutrient removal plant capacity of 19 600 m^3/day and an operating capacity of 60% was considered for potential sewage treatment using Acti-zyme co-harnessing the biogas and the bio-solids produced. A plant utilisation of 80% was considered resulting in 292 operational days, being operated for 24 hours.

Experimental results for the sewage composition, biogas and biogas solids generated were used as the basis for the techno-economic analysis (See **Chapters 5-9**). A TS content of 1143 mg/L was considered based on the total amount of dissolved and suspended solids in the sewage (**Chapter 5**). In addition, a biogas production rate of 400 mL/day was consideration with a total bio-solids generation amounting to about 10% of the TS in the sewage from this study (**Chapter 7**).

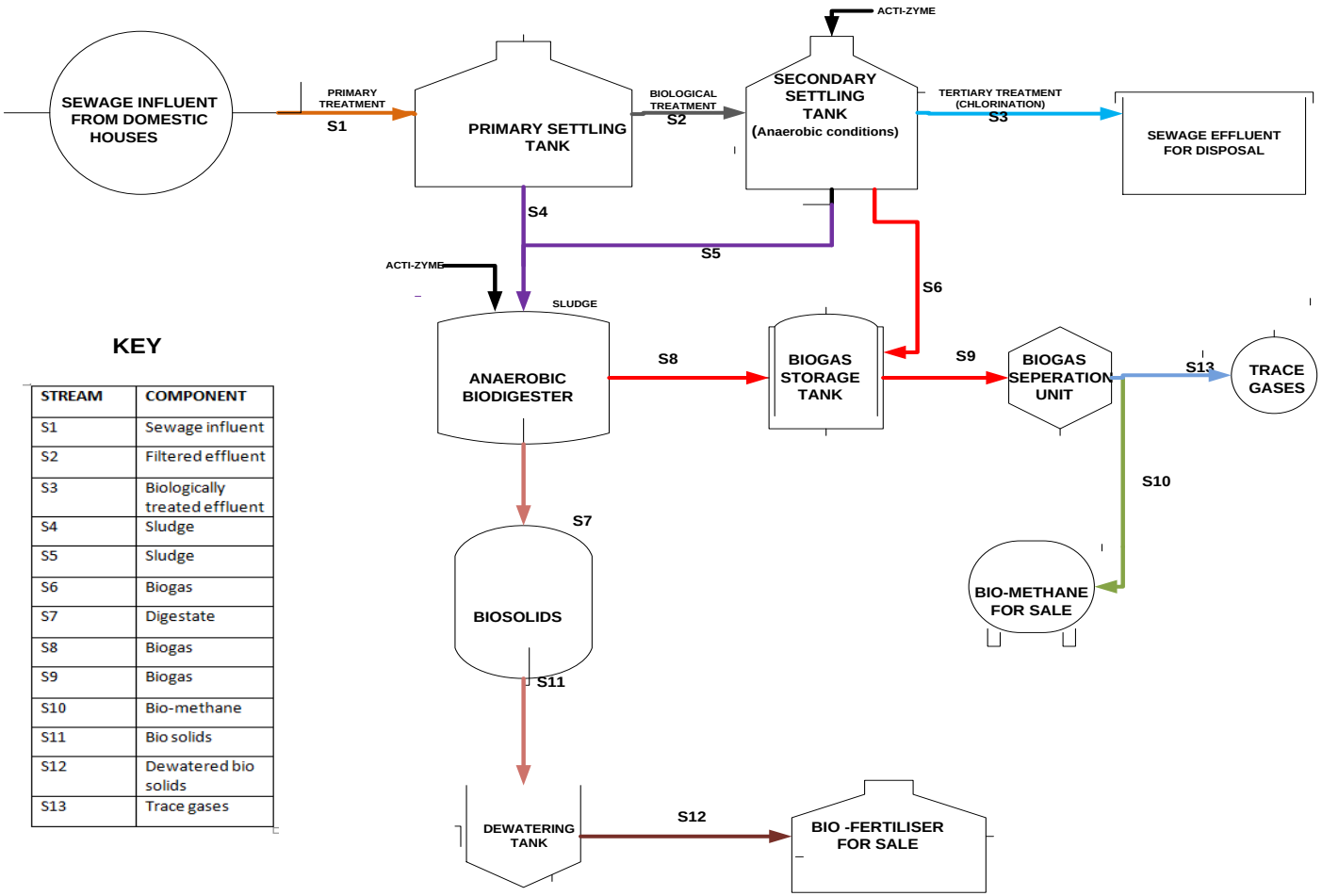
10.3 RESULTS AND DISCUSSION

10.3.1 Process description

Sewage influent is first filtered through a physical separation process of filtration whereby all large objects were removed via a grit filter. The filtrate was fed to the primary settling tank whereby primary sewage sludge is removed and fed to the anaerobic bio-digester. Primary sewage sludge does not comprise of any Acti-zyme. The primary settling tank effluent is passed on to the secondary treatment stage (**Figure 10.1**).

After the primary settling stage, Acti-zyme is added in the secondary clarifying tank at a loading of 0.050 g/L and a maximum retention time of 40 days and agitation is employed so as at to increase Acti-zyme activity in the secondary settling tank. Settle able solids in the secondary clarifying tank are sent to the bio-digester whereby they also acted as feedstock for the biogas and bio-solids production. These were termed as secondary sludge as they comprised of Acti-zyme from the sewage treatment process. Traces of biogas produced from the anaerobic treatment of sewage in the secondary clarifier are also sent to the biogas collection tank for purification. The sewage effluent from the secondary settling tank is sent for chlorine disinfection for reduction of pathogenic bacteria before final disposal to river systems. Alternatively, the sewage effluent can be used for irrigation purposes.

The sewage sludge from the primary and secondary clarifying tanks is fed into a bio-digester where Acti-zyme is also added at 0.050 g/L and a retention time of 40 days. Biogas is produced for further separation into the different constituent gases. The bio-solids formed during biogas production are dewatered and can be utilized as bio fertilizers. On the other hand, the biogas produced is separated into constituent component with the bio-methane being sent for electricity generation. The detailed process design is given in **Figure 10.1**.



STREAM	COMPONENT
S1	Sewage influent
S2	Filtered effluent
S3	Biologically treated effluent
S4	Sludge
S5	Sludge
S6	Biogas
S7	Digestate
S8	Biogas
S9	Biogas
S10	Bio-methane
S11	Bio solids
S12	Dewatered bio solids
S13	Trace gases

Figure 10-1: Process description for sewage treatment using Acti-zyme co-harnessing biogas and bio-solids

10.3.2 Techno-economic analysis for biogas and bio-solids generation

10.3.2.1 Cost analysis of inputs for a conventional system

For a conventional sewage treatment plant, aluminium sulphate is required as the coagulant for removal of the bio-contaminates. The bio-solids generated are sent off for landfilling as they are without further resulting in landfilling problems and also possible health problems. The cost of treating the sewage with the coagulant is pegged at $19.22 per ML of water (Dalton, 2008). For a plant capacity of 19 600 m^3/day with an operating capacity of 60%, the total amount of aluminium sulphate required $226.03 without value addition of sludge.

10.3.2.2 Cost analysis for a sewage plant using Acti-zyme

The amount of Acti-zyme required at optimal process conditions of 0.050 g/L at retention time of 40 days is therefore calculated as follows:

10.3.2.2.1 Acti-zyme required for the plant

Acti-zyme loading required: 0.050 g/L at retention time of 40 days for the 11.7 ML/day sewage is about 588 kg. This converts to $205.00 since Acti-zyme is currently being sold at $0.35/kg (AusTech, 2010).

10.3.2.2.2 Amount of biogas generated

The biogas quantity generated was based on the optimum bio-methane composition which was 78%, which is the main component that is required in the biogas for electricity generation for an optimum sewage loading of 7.5 g/L.d (**Chapter 7**).

Considering a 60% operating capacity and a total solids content of 1143 mg/L, the amount of solids generated was 13 441.68 kg/day. After considering a conversion of 95%, 12 769.60 kg/day of biogas was generated. If a cost price of $1.50/kg of the biogas is assumed which is currently the selling prize of biogas in Zimbabwe, income generated from the biogas per day will amount to $ 19 154.39.

10.3.2.2.3 Amount of bio-solids generated

The bio-solids produced are around 5% of the total amount of solids (Smith, 2009). The bio-solids generated had a nitrogen, phosphorous and potassium composition of

8.17%, 5.84% and 1.32% respectively (**Chapter 7**). The amount of bio-solids produced is around 672.08 kg/day for a capacity of 11.7 ML/day. If a selling price of $16/50kg which is currently the selling price for vermicompost, $215.00 will be realized per day from the bio-fertilizers. The overall cost benefit Analysis for using Acti-zyme in sewage treatment, co-digesting the sewage sludge to biogas and bio-solids is therefore presented in **Table 10.1**.

Table 10-1: Cost benefit analysis for using Acti-zyme in sewage treatment

	Product (per day)	**Cost ($)**
Outputs	Biogas	19 154.39
	Bio-solids	43.01
	Total	19 369.39
Less: Inputs	Acti-zyme	5.125
Net Benefit		19 364.26

If Acti-zyme is used in sewage treatment harnessing biogas and bio-solids, there is a net benefit of $19 364.26 per day which translates to **$5 656 363.92** per annum which will go a long way in improving the economy of Zimbabwe. The income generated from the biogas sales will contribute about 0.04% to the country's gross domestic product which was at 13.66 billion according to Trading Economy (2014).

10.3.3 Financial benefit for electricity generation from bio-methane

The potential for electricity generation from sewage sludge bio-methane is essential. Assumptions from Arthur and Brew-Hammond (2010) (**Table 2.7**) were adopted for determining the amount of electricity generated i.e. bio-methane heating value of 37.78 MJ/m^3, biogas engine efficiency of 29% and conversion factor 1KWh = 3.6 MJ

The bio-methane produced was calculated in accordance to the 78% composition in biogas and also by factoring in the density of the bio-gas which is 1.15 kg/m^3 at standard conditions. The amount of bio-methane generated was equivalent to a quantity

of 455.85 m^3/day. Considering an energy efficiency of 29% of the bio-methane and a conversion of 1KWh being equivalent to 3.6 MJ, the actual amount of bio-energy generated per day was 1 387.33 KWh. The financial benefit for production of electricity from bio-methane considered the investment required against the several costs anticipated.

The amount of money that can therefore be realized from the electricity sales, given that the general price for electricity sales is $9.86/KWh is therefore, **$13 679.06/ day**. The investment that can be made for the sewage plant is $5.48/KWh (Kottner, 2010), which translate to **$7 602.57** per day and **$2 219 950.44** per annum considering a plant operating capacity of 80% and 24 hours of operation.

Several costs must be considered for bio-methane electricity generation from sewage sludge bio-methane utilizing Acti-zyme as digestion catalyst and these are indicated in **Table 10.2**. Maintenance cost is 2% of investment cost (Kottner, 2010), production cost is $0.035/KWh (Gebrezgabher *et al*., 2010), insurance cost is 1-2% of investment cost (Kottner, 2010), labour cost is $ 22/hr (Kottner, 2010) and plant electricity usage is 7% of production cost.

Table 10-2: Expected biogas plant operating costs

Cost	Contribution	Value ($)
Maintenance	2% of investment	44 399.01
Production	$0.035/KWh	14 178.51
Insurance	1-2% of investment cost	44 399.01
Labour	$22/hr	154 176.18
Electricity	7% of production cost	992.50
Total Plant Costs		258 145 .21

Thus the final benefit for electricity generation from bio-methane is indicated in **Table 10.3**. Investment has been taken to be for 10 years Thus it is $2 219 950.44*10 years = $ 22 199 501.40 whilst the other cash flows are still going up to 20 years.

Table 10-3: Cash flows for electricity generation from bio-methane

Item	Cash flow ($)
Initial cash flow (Investment) (2 219 950.44*10)	22 199 501.40
Cash flows per year	
Electricity sold	3 994 285.52
Less: Total expected costs	258 145.21
Net Cash flow	3 736 140.31

10.3.3.1 Net present value

Assuming a discount rate of 15% and a plant operation period of 20 years:

$$NPV = -\text{Initial Cash flow} + \text{Net Cash flow per year}*(1 - 1/(1+0.15)^{20})/0.15$$

$$= -22\,199\,501.40 + 3\,736\,140.31*(1 - 1/(1+0.15)^{20})/0.15$$

$$= \$1\,186\,239.23$$

The project is economically feasible since we have a positive NPV.

10.3.3.2 Internal rate of return

Assuming that the discount rate is increased to 50%:

$$NPV = -22\,199\,501.40 + 3\,736\,140.31*(1 - 1/(1+0.5)^{20})/0.5$$

$$= -14\,729\,467.91$$

$$IRR = I_a + ((NPVa/(NPVa - NPVb)*(I_b - I_a))$$

$$= 15\% + ((1\,186\,239.23/(1\,186\,239.23 + 14\,729\,467.91)*(50\% - 15\%)$$

$$= 17.61\%$$

An IRR of 17.61% shows that the project adds value to stakeholders since it is greater than the cost of capital which was assumed to be 15% hence proving being economical.

10.3.3.3 Payback period

The payback period was calculated considering the initial cash flow against the net cash flow.

Initial cash flow/ yearly net cash flow = 22 199 501.40/3 736 140.31

= 5.94 years

The payback period of 5.94 years showed that it only takes approximately 5.94 years for the project to recoup its initial investment and the rest of the 14 of 20 years are for profit hence the project is beneficial.

10.3.3.4 Break-even point

Break-even refers to the point of balance between making either a profit or a loss and is calculated as follows:

Breakeven point = Fixed cost/contribution per unit

Whereby the fixed costs are a sum of maintenance cost, insurance and electricity for the 20 years this amounts to $ 1 795 810.40.

Contribution/unit = Electricity selling price/KWh – Production cost/KWh – Labour/KWh

= $9.86-$0.035-$22/ (1387.33)

= $9.81/KWh

Breakeven point = $1 795 810.40/$9.81/KWh

= 183 059.16 KWh

Breakeven Sales Value = 183 059.16 KWh X $9.86

= $1 804 963.36

The breakeven point showed that for the project to start making a profit, only 183 059.16 KWh have to be produced with a sales value of $1 804 963.36. The sewage

treatment co-harnessing biogas is capable of producing 1387.33KWh/day and this highly indicated that the technology is profitable. The breakeven chart is indicated in **Figure 10.2.** The following equations were used for determining the breakeven sales value and the values obtained are indicated in **Appendix O1:**

Fixed costs = 1 795 810.40

Variable costs = 0.05*X*

Total costs = 1 795 810.40 + 0.05*X*

Sales = 9.86*X*

9.81 c/KWh is the contribution per unit of electricity that is obtained after subtracting the variable cost (0.05 c/KW) from the selling price whilst *X* is the amount of electricity generated from biogas in KWh.

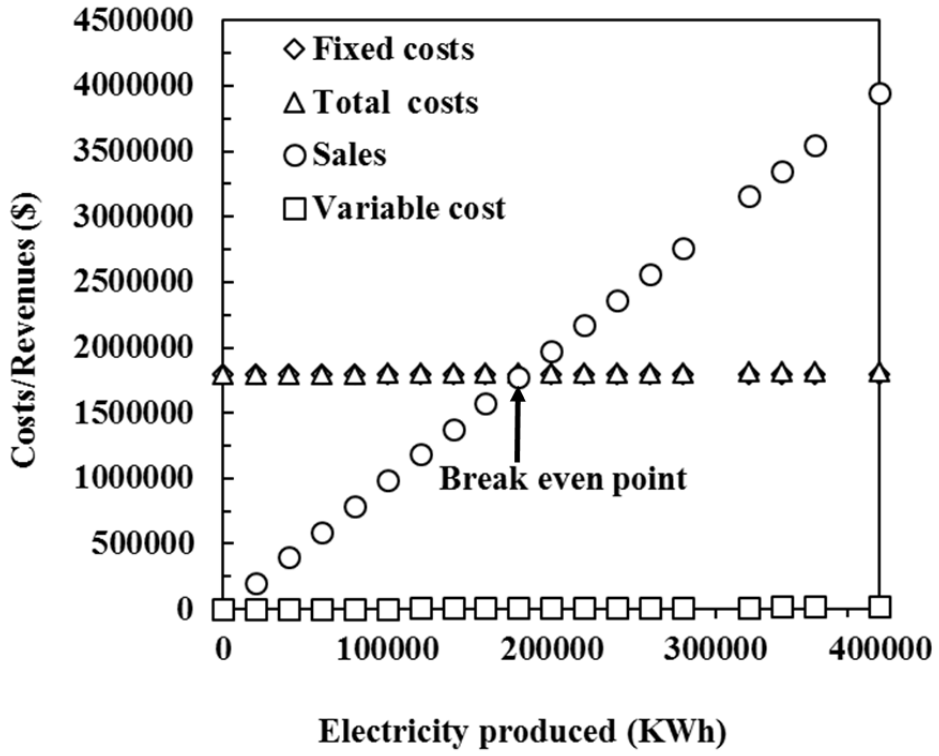


Figure 10-2: Breakeven analysis for sewage treatment co-harnessing biogas and bio-solids

10.3.3.5 Sensitivity analysis

The sensitivity analysis diagram (**Figure 10.3**) shows what happens to the breakeven point when one variable is varied at a time. This is a subset of scenario analysis where

the influence of specific variables on breakeven point is scrutinized. The greater the volatility in breakeven point in relation to a specific variable, the larger the forecasting risk associated with that variable and the more the attention its estimation should be given. Sensitivity analysis was therefore considered for change in unit cost of electricity (**See Appendix O2.1**), change in variable costs (**See Appendix O2.2**) and change in fixed costs (**See Appendix O2.3**) for ±20%.

10.3.3.5.1 Sensitivity analysis for change in electricity costs

Given the deviation of -20% to +20% in unit cost of electricity and constant variable costs and fixed costs, the breakeven point varied from 229 116 KWh (-20%) to 152 420 KWh (+20%) as indicated in **Figure 10.3**.

10.3.3.5.2 Sensitivity analysis for change in variable costs

Given the deviation of -20% to +20% in variable costs and at constant electricity selling price and fixed costs, breakeven varied from 201 203 KWh (-20%) to 183 246 KWh (+20%) as indicated in **Figure 10.3**

10.3.3.5.3 Sensitivity analysis for change in fixed cost

Given the deviation of -20% to +20% in fixed costs at constant electricity selling price and variable costs, breakeven point varied from 146 447 KWh (-20%) to 219 671 KWh (+20%) as indicated in **Figure 10.3**.

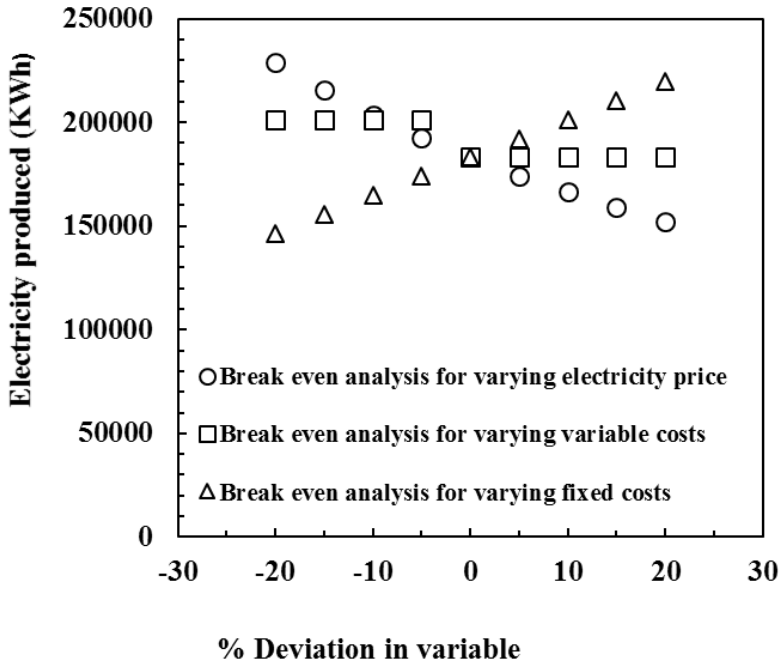


Figure 10-3: Sensitivity analysis for breakeven point

From the sensitivity analysis on the breakeven point, it was noted that increasing the fixed costs, variable costs and decreasing the selling price of the bio-energy will increase the breakeven out (**Table 10.4**).

Table 10-4: Influence of various costs on break even output

Variable	**Direction of change**	**Breakeven output**
Total fixed costs e.g. cost of equipment	Up	Up
	Down	Down
Average variable cost e.g. cost of material	Down	Down
	Up	Up
Product price	Up	Down
	Down	Up

10.4 SUMMARY

Sewage treatment using Acti-zyme as the digestion bio-catalyst is an economically viable technology that can be adopted as a value addition strategy for sewage sludge for sustainable development in developing countries. The project had a positive NPV, IRR of 16.6% and break even sales of 183 059.16 KWh after investing $22 199 501.40 for a project life cycle of 20 years. The project payback period is 5.94 years.

CHAPTER 11. CONCLUSION AND RECOMMENDATIONS

The conclusion and recommendations for bio-nutrient removal in sewage with co-harnessing of biogas and bio-solids are given.

11.1 CONCLUSION

Utilizing Acti-zyme as a bio-augmentation catalyst in sewage treatment co-harnessing biogas and bio-solids is both environmentally and economically feasible. Acti-zyme was found to be an immotile biocatalyst which contained several enzymes that can be used in sewage treatment such as catalase, proteolytic enzymes and amylase. However, Acti-zyme did not produce urease which promotes ammonia production which has a potential to cause eutrophication. From the biochemical tests, Acti-zyme does not promote hydrogen sulfide production meaning that the biogas produced will be of good quality and will not need to go through further treatment processes.

Acti-zyme successfully treated sewage removing all the contaminants to meet the required guidelines for effluent disposal. Sewage treated with Acti-zyme showed an additional > 50% reduction in the sewage contaminants at optimum loadings of 0.050 g/L and retention time of 40 days.

This makes Acti-zyme suitable for biological sewage treatment especially for removal of bio-nutrients as indicated by the trend of bio-nutrient removal ratios i.e. COD/BOD_5, BOD_5/TKN, COD/TKN and COD/TP ratios. High bio-nutrient removal was achieved at an Acti-zyme loading of 0.050 g/L and 40 days. The sewage effluent obtained can be used for irrigation purposes.

Anaerobic digestion of sewage sludge utilizing Acti-zyme at mesophilic conditions of 37 °C promoted production of bio-methane rich biogas which is almost free of H_2S and nitrogen and has lower CO_2 composition. Acti-zyme loading of 0.050 g/L and sewage sludge loading of 7.5 g/L.d is essential for optimum biogas production over a 40 day retention period.

The bio-methane production kinetics is best modelled by the logistics kinetic model with a rate constant of 0.073 day^{-1}, which also produces results that are comparable to the quadratic statistical model used to simulate biogas production. Additionally, bio-solids

which are rich in fertilizer NPK nutrients are produced and can be utilized as bio-fertilizers. Amount of bio-solids generation can also be simulated by the statistical quadratic models.

Production of biogas from sewage sludge using Acti-zyme as the digestion bio-catalyst is an economically viable process with a positive NPV, IRR of 17.6% and a payback period of 5.94 years for an investment of $ 22 199 501.40 over 20 years. The technology can be adopted as a value addition strategy for sewage sludge for sustainable development in developing countries. This can help to ease the water and energy problems that are being encountered in developing countries.

11.2 RECOMMENDATIONS

Accurate measurement of the biogas using a flowmeter is recommended. The biogas obtained from the anaerobic digestion of sewage sludge can be further purified by separation of carbon dioxide so that its purity is enhanced and various methods of doing this can be investigated in future work. Acti-zyme immobilization and then recovery of the Acti-zyme for re-use in sewage treatment and sewage sludge digestion as a way of minimising costs and making the project more sustainable and economical is also recommended for future work.

REFERENCES

1. Abu-Reesh, I. M. (2014) Kinetics of Anaerobic Digestion of Labaneh Whey in a Batch Reactor. African Journal of Biotechnology, 13 (6), p. 1745-1755.
2. Abyaneh, H. Z. (2014) Evaluation of Multivariate Linear Regression and Artificial Neural Networks in Prediction of Water Quality Parameters. Journal of Environmental Health Science and Engineering, 1, p. 40.
3. Ahn, K., Song, K., Cho, E., Cho, J., Yun, H., Lee, S. and Kim, J. (2003) Enhanced Biological Phosphorous and Nitrogen Removal Using a Sequencing Anoxic/Anaerobic Membrane Bioreactor (SAM) Process, Desalination, 157, p. 345-352.
4. Ahsan, S., Rahman, S., Kaneco, S., Katsumata, S., Suzuki, T. and Onta, K. (2005) Influence of Temperature on Wastewater Treatment with Natural and Waste Materials, Clean Technology Environment Policy, 7, p. 198-202.
5. Al Awadhi, M. A. A. N. (2013) Beneficial Re-use of Treated Sewage Effluent, Director of Sewage Treatment Plant Department, Dubai Municipality.
6. APHA (2005) Standard Methods for the Examination of Water and Wastewater, 21st Edition, America Public Health Association, American Water Works, Association, Water Environment Federation, Washington, DC, USA.
7. Apte, S. S., Apte, S. S., Kore, V. S and Kore, V. S. (2011) Chloride Removal from Wastewater by Bio-sorption With the Plant Biomass, Universal Journal of Environment Research and Technology, 1(4), p. 416-422.
8. Arthur, R. and Brew-Hammond, A. (2010) Potential Biogas Production from Sewage Bio-solids: A Case Study of the Sewage Treatment Plant at Kwame Nkrumah University of Science and Technology, Ghana, International Journal of Energy and Environment, 1 (6), p. 1009-1016.
9. Attiogbe, F. K., Glover-Amengor, M. G. and Nyadziehe, K. T. (2005) Correlating Biochemical and Chemical Oxygen Demand of Effluents- A Case Study of Selected Industries in Kumasi Ghana, West African Journal of Applied Ecology. ISSN: 0855-4307, p. 1-11.

10. Austech Products (2010) Material Safety Data Sheet. Acti-zyme Drain Cleaner and Odor Eliminator.
11. Babaee, A and Shayegan, J. (2011) Influence of Organic Loading Rate (OLR) on Production of Methane From Anaerobic Digestion of Vegetable Waste, World Renewable Energy Congress, Bioenergy Technology, 8-13 May 2011, Linkoping, Sweden.
12. Barker, P. S. and Dold, P. L. (1996) Denitrification Behaviour in Biological Excess Phosphorous Removal Activated Bio-solids Systems, Water Research, 30 (4), p. 769-780.
13. Baudisova, D. (1997) Evaluation of *Escherichia Coli* as the Main Indicator of Faecal Pollution, Water Science and Technology, 35 (11-12), p. 333-336.
14. Belia, E., Amerlinck, Y., Benedetti, L., Johnson, B., Sin, G., Vaurolleghem, P. A., Gernaey, K. V., Gillot, S., Neumann, M., Rieger, L., Shaw, A. and Villez, K (2009) Wastewater Treatment Modelling: Dealing with Uncertainties, Water Science and Technology, 60 (8), p. 1929-1941.
15. Beevi, S., Jose, P. P. and Madhu, G. (2014) Optimization of Process Parameters Affecting Biogas Production from Organic Fraction of Municipal Solid Waste via Anaerobic Digestion. International Journal of Environmental, Chemical, Ecological, Geological and Geophysical Engineering, 8 (1), p. 43-48.
16. Cail, R. G., Barford, J. P., and Linchacz, R. (1986) Anaerobic Digestion of Wool Scouring Wastewater in Digester Operated Semi-Continuously for Biomass Retention, Agricultural Wastes, 18, p. 27-38.
17. CH2MHILL (2011) Niagara Region Bio solids Management Master Plan, Final Report, 2011.
18. Cheu, X. G., Stanbnikova, O., Tay, J. H., Wang, J. Y. and Tay, S. T. (2004) Thermo active Extracellular Proteases of GeoBacillus Caldoproteolyticus. Sp. Nov from Sewage Sludge. Extremophiles, 8 (6), p. 489-498.
19. Coelho, S. T., Velazquez, S. M. S. G., Silva, O. C., Pecora, V and Abreu, F. C. (2006) The Production of Sewage Biogas and its Use for Energy Generation, World Bioenergy Conference and Exhibition, 30 June.

20. Coppen, J. (2004) Advanced Wastewater Treatment System, Courses ENG 411 and 412 Research Project.
21. Daelman, M. R. J., van Voorthuizen, E. M., van Dongen, U. G. J. M., Volcke, E. I. P and van Loosdrecht, M. C. M. (2012) Bio-methane Emission during Municipal Wastewater Treatment, Water Research, 46, p. 3657-3670.
22. Dalton, M. (2008) Potable Water Coagulant Trials Utilising PolyAluminium Chlorhydrate, 33rd Annual Old Water Industry Operations Workshop, Indoor Sports Centre, Carrara-Gold Coast, 3-5 June 2008.
23. De Winter, J. C. F. (2013) Using the Student t-test with Extremely Small Sample Sizes. Practical Assessment, Research and Evaluation, 18 (10), p. 1-12.
24. Demey, D., Vanderhaegen, B., Vanhoore, H., Liessens, J., Van Eyck, L., Hopkins, L. and Vaurolleghem, P. A. (2001) Validation and Implementation of Model Based Control Strategies at an Industrial Wastewater Treatment Plant, Water Science and Technology, 44 (2), p. 144-143.
25. Desai, S. T., Palled, V. and Mathad, R. (2013) Performance Evaluation of Fixed Doom Type Biogas Plant for Solid State Digestion of Cattle Dung, Karnataka Journal of Agricultural Sciences, 26 (1), p. 103-106.
26. Doyle, M. P and Padhye, V. S. (1989) Escherichia Coli. In Food Borne Bacterial Pathogens. Doyle, M .P. Ed. Marcel Dekker, Inc, New York, NY.
27. Driessen, W., Yspeert, P., Yspeert, Y. and Vereijken, T. (2000) Compact Combined Anaerobic and Aerobic Process for the Treatment of Industrial Effluent, Environmental Forum, May 24-26, p. 1-10.
28. Duncan, A. C. (1970) Two Stage Anaerobic Digestion of Hog Wastes, Master of Science Thesis, University of British Columbia, 1970.
29. Dzvene, D. K. (2013) An Investigation on the Effectiveness of Acti-zyme Bacteria to Treat Organic Waste at Triple C Pigs (Colcom), Bachelor of Science Honours Degree in Environmental Sciences and Technology, School of Agricultural Sciences and Technology, November 2013.
30. Ecolab (2006) Material Safety Data Sheet, Acti-zyme Compost Accelerator, 903100, p. 1-3.

31. Evanylo, G. K. (2009) Agricultural Land Application of Bio-solids in Virginia: Production and Characteristics of Bio-solids, Virginia Cooperative Extension, Publication 425-301, p. 1-6.

32. Faccin, S., Alves, P. D. D., Siqueira, F. F., Barraca, M., Victoria, J. M. N. and Kalapothakis, E. (2014) Biodiversity and Secretion of Enzymes with Potential Utility in Wastewater. Biomedical and Life Sciences, Earth and Environmental Sciences, 3 (1), p. 34-37.

33. Fachri, B. A., Abdilla, R. M., Rasrendra, C. B. and Heeres, H. J. (2015) Experimental and Modeling Studies on the uncatalyzed Thermal Conversion of Inulin to 5-hydroxymethylfurfural and Levulinic Acid. Sustainable Chemical Processes, 3 (8), article in press.

34. Frauke-Whittle, I. H., Walter, A., Ebner, C. and Insam, H. (2014) Investigation into the Effect of High Concentrations of Volatile Fatty Acids in Anaerobic Digestion on Methanogenic Communities. Waste Management, 34 (11), p. 2080-2089.

35. Gebrezgabher, S. A., Meuwissen, M. P. M. and Oude Lansink, A. G. J. M. (2010) Cost of producing biogas at Dairy Farms in the Netherlands, International Journal of Food System Dynamics, 1, p. 26-35.

36. George, I., Crop, P. and Servais, P. (2002) Faecal Coliform Removal in Wastewater Treatment Plants Studied by Plate Counts and Enzymatic Methods, Water Research, 36 (10), p. 2607-2617.

37. Ghanadzadeh, H. and Ghorbanpour, M. (2012) Optimization of Ethanol Production from Cheese Whey Fermentation in A Batch-Airlift Bioreactor. Journal of Bioengineering and Biomedical Science, 2 (2), p. 2155-9538.

38. Ghatak, M. D. and Mahanta, P. (2014) Comparison of Kinetic Models for Biogas Production from Saw Dust, International Journal of Research in Engineering and Technology, 3 (7), p. 248-254.

39. Gomes, A. C., Silva, L., Simoes, R., Canto, N. and Alburquerque, A. (2013) Toxicity Reduction and Biodegradability Enhancement of Cork Processing Wastewaters by Ozonation. Water Science and Technology, 68 (10), p. 2214-2219.

40. Gorveno, J., Kiepper, B., Magbanua, B. and Adams. T. (2000) Characterisation and Quantification of Georgia's Municipal Bio-solids Production and Disposal, The University of Georgia, College of Agriculture and Environmental Sciences, Department of Biological and Agricultural Engineering.

41. Gupta, V. K., Ali, I., Saleh, T. A., Nayak, A. and Agarwal, S. (2012) Chemical Treatment Technologies for Waste-water Recycling-An Overview, Royal Society of Chemistry Advances, 2, p. 6380-6388.

42. Hesnawi, R., Dahmani, K., Al-Swayah, A., Mohamed, S. and Mohamed, S. (2014) Biodegradation of Municipal Wastewater with Local and Commercial Bacteria. 12th International Conference on Computing and Control for the Water Industry, CCW12013. Procedia Engineering, 70, p. 810-814.

43. Hesnawi, R. M. and Mohamed, R. A. (2013) Influence of Organic Waste Source on Methane Production during Thermophilic Digestion Process. International Journal of Environmental Science and Development, 4 (4), p. 435-437.

44. Hospido, A., Moreira, M. T., Martin, M., Rigola, M and Feijoo, M. (2005) Environmental Evaluation of Different Treatment Processes for Bio-solids from Urban Wastewater Treatments: Anaerobic Digestion versus Thermal Processes, Urban Wastewater Treatments, Waste in LCA.

45. Hu. B. L., Shen, L. D., Xu, X. Y. and Zheng, P. (2011) Anaerobic Ammonium Oxidation in Different Natural Ecosystems, Biochemical Society Transactions. 39 (6), p. 1811-1816.

46. Huang, X., Xiao, X and Shen, Y. (2010) Recent Advances in Membrane Bioreactor Technology for Wastewater Treatment in China, Frontiers of Environmental Science and Engineering, 4 (3), p. 245-271.

47. Jacobs Engineering Group (2010) Standard Operating Procedure for Total Solids Determination Using Vacuum Filtration and Oven Drying, TVA-KIF-SOP-49, Tennese Valley Authority, Environment and Technology, Environmental Science and Resources, April.

48. Johanneson, G. H. (1999) Sewage Characteristics and Evaluation of P Availability under Greenhouse Conditions, Master of Science Thesis, University of Guelph.

49. Kampas, P., Parsons, S. A., Pearce, P., Ldoux, S., Vale, P., Churchley, J. and. Cartmell, E. (2007) Mechanical Bio-solids Disintegration for the Production of Carbon Source for Biological Nutrient Removal, Water Research, 41 (8), p. 1734-1742.

50. Kardas, L., Jahasz, A., Palko, G.Y., Olah, J., Bakacs, K. and Zaray, G. Y (2011) Comparing of Mesophilic and Thermophilic Anaerobic Fermented Sewage Sludge Based on Chemical and Biochemical Tests. *Applied Ecology and Environmental Research*, 9 (3) 293-302.

51. Kargbo, D. M. (2010) Biodiesel Production from Municipal Sewage Bio-solids, Energy Fuels, 24, p. 2791-2794.

52. Kaosol, T and Sohgrathok. N. (2012) Enhancement of Biogas Production Potential For Anaerobic Co-Digestion of Wastewater Using Decanter Cake, American Journal of Agriculture and Biological Sciences, 7 (4), p. 494-502.

53. Khambete, A. K. and Christian, R. A. (2014) Statistical Analysis to Identify the Main Parameters to influencing WWQI of Sewage Treatment Plant and Predicting BOD, International Journal of Research in Engineering and Technology, 3 (1), p. 186-195.

54. Kottner, M. (2010) BioEnergy Farm, International Training Course, 2010.

55. Kraume, M., Bracklow, U., Vocks, M. and Drews, A. (2005) Nutrients Removal in MBRs for Municipal Wastewater Treatment, Water Science Technology, 51, p. 391-402.

56. Lai, T. M., Shin, J. and Hur, J. (2011) Estimating the Biodegradability of Treated Sewage Samples using Synchronous Fluorescence Spectra. Sensors, 11, p. 7382-7394.

57. Lang, N. L. and Smith, S. R. (2007) Influence of Soil Type, Moisture Content and Bio-solids Application on the Fate of Escherichia Coli in Agricultural Soil under Controlled Laboratory Conditions. Journal of Applied Microbiology, 103, p. 2122-2131.

58. Lang, N. L., Bellet-Travers, M. D. and Smith, S. R. (2007). Field Investigation on the Survival of Escherichia Coli and Presence of Other Enteric Micro-organisms in Bio-solids Amended Agricultural Soil. Journal of Applied Microbiology, 103, p. 1868-1882.

59. Latinwo, G. K. and Agarry, S. E. (2015) Modelling the Kinetics of Biogas Generation from Mesophilic Anaerobic Co-digestion of Sewage Sludge with Municipal Organic Waste. Chemical and Process Engineering Research, 31, p. 43-53.

60. Lee, A. H. and Nikraz, N. (2014) BOD_5: COD Ratio as an Indicator for Pollutants Leaching From Landfill. Journal of Clean Energy Technologies, 2 (3), p. 263-266.

61. Lettinga, G. (1995) Anaerobic Digestion and Wastewater Treatment Systems, Antonie van Leeuwenhoek, 67, p. 3-28.

62. Lo, H. M., Kurniawan, T. A., Sillanpää, M. E. T., Pai, T.Y., Chiang, C. F., Chao, K. P., Liu, M. H.,Chuang, S.H., Banks, C. J., Wang, S.C., Lin, K. C., Lin, C. Y., Liu, W. F., Cheng, P. H., Chen, C. K., Chiu, H. Y and Wu, H. Y. (2010) Modelling Biogas Production From Organic Fraction of MSW Co-digested with MSWI Ashes in Anaerobic Bioreactors. Bioresource Technology, 16, p. 6329-6335.

63. Lotfy, H. R. and Rashed, I. G. (2002) A Method for Treating Wastewater Containing Formaldehyde, Water Research, 36 (3), p. 633-637.

64. Luna-delRisco, M., Normak, A. and Orupõld, K. (2011) Biochemical Methane Potential of Different Organic Wastes and Energy Crops from Estonia. Agronomy Research, 9 (1–2), p. 331–342.

65. Ma, B., Wang, S., Zhang, S., Li, X., Bao, P. and Peng, X. (2013) Achieving Nitrification and Phosphorous Removal in a Continuous Flow Anaerobic/Oxic Reactor through Bio-augmentation. Bioresource Technology, 139, p. 375-378.

66. Mace, S. and Mata-Alvarez, J. (2002) Utilisation of SBR Technology for Wastewater Treatment: An Overview, Industrial and Engineering Chemistry Research, 41, p. 5539-5553.

67. Madden, P., Al-Raei, A. M., Enright, A. M., Chinalia, F. A., De Beer, D., Flaherty, V. O. and Collins, G. (2014) Influence of Sulfate on Low-Temperature Anaerobic Digestion. Frontiers in Microbiology.

68. Mahmoud. N. (2011) Wastewater Characteristics, Ecological Sanitation Training Course, SWITCH PROJECT, 25-27 January.

69. Mahmoud, N. (2002) Anaerobic Pre-treatment of Sewage under Low Temperature (15 ºC) Conditions in an Integrated UASB-Digester System. Ph.D. Thesis, Wageningen University, Wageningen, The Netherlands.

70. Mahmoud, N., Zeeman, G., Gijzen, H and Lettinga, G. (2003) Anaerobic Sewage Treatment in a one stage UASB and a combined UASB-Digester System, Seventh International Water Technology Conference, Egypt, 28-30 March.

71. Malik, D. S. and Bharti, U. (2009) Biogas Production from Sludge of Sewage Treatment Plant at Haridwar (Uttarakhand). Asian Journal of Experimental Sciences, 23 (1), p. 95-98.

72. Mandal, H. K. (2014) Influence of Wastewater pH on Turbidity. International Journal of Environmental Research and Development, 4 (2), p. 105-114.

73. Manyuchi, M. M., Kadzungura, L and Boka, S. (2013) Pilot Studies for Vermifiltration of 1000 m^3/day of Sewage Wastewater. Asian Journal of Engineering and Technology, 1 (1), p. 13-19.

74. Manyuchi, M. M. and Phiri, A. (2013) Application of the Vermifiltration Technology in Sewage Wastewater Treatment. Asian Journal of Engineering and Technology, 1 (4), p. 108-113.

75. McBride, M. B. (2003) Toxic Metals in Sewage Sludge Amended Soils: Has Promotion of Beneficial Use Discounted the Risks. Advances in Environmental Research, 8 (1), p. 5-19.

76. Mesquita, R. B.R., Fernandes, S. M.V. and Rangel, A. O. S. S. (2002) Turbidimetric Determination of Chloride in Different Types Water Using a Single Sequential Injection Analysis System. Journal of Environmental Monitoring, 4, p. 458-461.

77. Mittal, A. (2011) Biological Wastewater Treatment-Water Today, p. 32-44, August.

78. Mohan, S. V., Rao, N. C., Prasad, K. K., Madhavi, B. T. V. and Sharma, P. N. (2005) Treatment of Complex Chemical Wastewater in a Sequencing Batch Reactor (SBR) With an Aerobic Suspended Growth and Configuration, Process Biochemistry, 40, p. 1501-1508.

79. Molwantwa, J. B. (2002) The Hydrolysis of Primary Sewage Sludge under Bio-sulphidogenic Conditions, Master of Science Thesis, Rhodes University.

80. Mukumba, P., ,Makaka, G., Mamphwel, S. and Misi, S. (2013) A Possible Design and Justification for a Biogas Plant at Nyazura Adventist High School, Rusape Zimbabwe, Journal of Energy in Southern Africa, 24 (4), 12-21.

81. Muserere, S. T., Hoko, Z. and. Nhapi, I. (2014) Characterisation of Raw Sewage and Performance Assessment of Primary Settling Tanks at Firle Sewage Treatment Works, Harare, Zimbabwe, Physics and Chemistry of the Earth, 67-69, p. 226-235.

82. Nazaroff, W. W. and L. Alvarez-Cohen (2013) Anaerobic Digestion of Wastewater Bio-solids, Section 6.E.3.

83. Neczaj, E., Grosser, A. and Worwag, M. (2013) Boosting Production of Bio-methane from Sewage Sludge by Addition of Grease Trap Sludge. Environmental Protection Engineering, 39 (2), p. 125-133.

84. Nhapi. I. (2009) The Water Situation in Harare, Zimbabwe: A Policy and Management Problem. Water Policy, 11, p. 221-235.

85. Nhapi, I. and Gijzen, H. (2002) Wastewater Management in Zimbabwe, Sustainable Environmental Sanitation and Water Services, 28th Conference, Calcutta, India, p. 181-184.

86. Nisha, S., Arun Karthick, S. and. Gobi, N. (2012) A Review on Methods, Application and Properties of Immobilized Enzyme. Chemical Science Review and Letters, 1 (3), p 148-155.

87. Oboh, G. (2005) Isolation and Characterization of Amylase from Fermented Cassava (Manihot Esculenta Crants) Wastewater. African Journal of Biotechnology, (4) 10, p. 1117-1123.

88. Ozmen, P. and Aslaunzadeh, S. (2009) Biogas Production From Municipal Waste Mixed With Different Portions of Orange Peel, MSc Thesis, Applied Biotechnology, University of Boras, School of Engineering.

89. Pabsch, H. and Wendland, C. (2013) Sewage Bio-solids Treatment, Lesson B5, Efficient Management of Wastewater.

90. Palanisamy, K. and Shamsuddin, A. H. (2013) Biogas to Energy Potential From Sewage Treatment Plant in Malaysia, 1st Malaysia National Sewage Conference, Sewerage Malaysia-Setting New Frontier Technology, June 2013.

91. Passos, F., Sole, M., Garcia, J and Ferrer, I (2013) Biogas Production from Microalgae Grown in Wastewater: Influence of Microwave Pre-treatment, Applied Energy, 108, p. 168-175.

92. Pei, P., Zhang, C., Li, J., Chang, S., Li, S., Wang, J., Zhao, M., Li, J., Yu, M. and Chen, X. (2014) Optimization of NaOH Pre-treatment for Enhancement of Biogas Production of Banana Pseudo-Stem Fibre using Response Surface Methodology. Bioresources, 9 (3), p. 5073-5087.

93. Popa, P., Timofti, M., Voiculesai, M., Dragan, S., Trif, C. and Georgescu, L. P. (2012) Study of Physicochemical Characteristics of Wastewater in an Urban Agglomeration in Romania, The Scientific World Journal, Article ID 549028, 10 pages. http://dx.doi.org/10.1100/2012/549028.

94. Powell, B. and Lundy, J. (2007). Acti-zyme Agricultural and Municipal Wastewater Treatment. Environment Depot Canada, p. 1-23.

95. Prabhudessai, V., Salgaonkar, B., Braganca, J. and Mutnuri, S. (2014) Pretreatment of Cottage Cheese to Enhance Biogas Production. Biomed Research International, 2014: 374562. doi: 10.1155/2014/374562. Epub 2014 June.

96. Ravek, A. and Avnimelech, Y. (1979) Total Nitrogen Analysis in Water, Soil and Plant Material with Persulphate Oxidation, Water Research, 13 (9), p. 911-912.

97. Reali, M. A., Campos, J. R. and Penetra, R. G. (2001) Sewage Treatment by Anaerobic Biological Process Associated With Dissolved Air Floatation. Water Science and Technology, 43 (8), p. 91-98.

98. Rich, J. J., Dale, O. R., Song, B. and Ward, B. B. (2008) Anaerobic Ammonium Oxidation (Anammox) in Chesa Peak Bay Sediments, Microbiology Ecology, 55, p. 311-320.

99. Rodriguez-Chiang, L. M. and Dahl, O. P (2015) Influence of Inoculum to Substrate Ratio on the Methane Potential of Microcrystalline Cellulose Production Wastewater. Bioresources, 10 (1), p. 898-911.

100. Schaumann BioEnergy. (2013) Biogas Production-A Complex Process.

101. Schreckenberger, P. C. and Blazevic, D. J. (1974) Rapid Methods for Biochemical Testing of Anaerobic Bacteria. Applied Microbiology, 28 (5), p. 759-762.

102. Segal, K. and Potter, B. (2008) Identification of Unknown Bacteria Using a Series of Biochemical Tests, Microbiology 202, p. 1-10.

103. Shin, J., Sang, L., Jung, J., Chung, Y. and Noh, S. (2005) Enhanced COD and Nitrogen Removals for the Treatment of Swine Wastewater by Combining Submerged Membrane Bioreactor (MBR) and Anaerobic Up flow Bed Filter (AUBF) Reactor. Process Biochemistry, 40, p. 3769-3776.

104. Snee, R. D. (1977) Validation of Regression Models: Methods and Examples. Technometrics, 19 (4), 415-428, 1977.

105. Sohaili, J., Zaidi, N. S. and Loon, S. N. (2012) Nutrients Content of Sewage Sludge and Its Utilization Towards Horticulture Plant. Journal of Emerging Trends in Engineering and Applied Sciences, 3 (1), p. 81-85.

106. Singh, S. and Singh, K. N. (2010) Physicochemical Analysis of Sewage Discharged into Varuna River at Varamasi, Current World Environment, 5 (1), p. 201-203.

107. Smith, S. R. (2009) Organic Contaminants in Sewage Sludge (Bio-solids) and their Significance for Agricultural Recycling, Philosophical Transactions A, The Royal Society Publishing, 367 (1904):4005-41. DOI: 10.1098/rsta.2009.0154.

108. Snee, R. D. (1977) Validation of Regression Models: Methods and Examples. Technometrics, 19 (4), p. 415-428.

109. Tas, D. O., Karahan, O., Insel, G., Ovez, S., Orhon, D. and Spanjers, H. (2009) Biodegradability and Denitrification Potential of Settle able Chemical

Oxygen Demand in Domestic Wastewater. Water Environment Research, 81 (7), p. 715-727.

110. Trading Economy (2014) Zimbabwe GDP

111. Tshuma, A. C. (2010) Impact of Acti-zyme Compound on Water Quality along Mid-Mupfuure Catchment-Chegutu, BSc Honours Thesis, Geography and Environmental Studies.

112. Usman, K., Khan, S. G., Ghulan, S., Kha, M. U., Khan, N., Khan, M. A. and Khalil, S. K. (2012) Sewage Sludge: An Important Biological Resource for Sustainable Agriculture and Its Environmental Implications. American Journal of Plant Sciences, 3, p. 1708-1721.

113. Vashist, H., Sharma, D. and Gupta, A. (2013) A Review of Commonly Used Biochemical Tests for Bacteria. Innovare Journal of Life Sciences, 1 (1), p. 1-7.

114. Vindis, P., Marsec, B., Janzekovic, M. and Cus, F (2009) The Impact of Mesophilic and Thermophilic Anaerobic Digestion on Biogas Production. Journal of Achievements in Materials and Manufacturing Engineering, 36 (2), p. 192-198.

115. Wanatabe, F. S. and Olsen, S. R. (1965) Test for an Ascorbic Acid Method for Determining Phosphorous in Water and $NaHCO_3$ Extracts from Soil, Soil Science Society of America Journal, 29 (6), p. 677-678.

116. Wang, C., Yu. X., Lu, X. and Yang, J. (2013) Nitrogen and Phosphorous Removal from Municipal Wastewater by Green Alga Chlorella sp. Journal of Environment Biology, 34, p. 421-425.

117. Wei, Y., VAN Houten, R. T.; Borger, A. R; Eikelboom, D. H and Fan,Y. (2003) Minimisation of Excess Bio-solids Production for Biological Waste Water Treatment. Water Research, 37, p. 4453-4467.

118. Willis, J and Schafer, P. (2006) Advances in Thermophilic Anaerobic Digestion, Water Environment Foundation, WEFTEC 06, 5378-5392.

119. Woodland, J. (2004). Laboratory Procedures Manual, Bacteriology, p. 1-44.

120. Yang, K., Ji, B., Wang, H., Zhang, H. and Zhang, Q. (2014) Bio-augmentation as a tool for improving the modified sequencing batch biofilm reactor. Journal of Bioscience and Bioengineering, 117 (6), p. 763-768.

121. Yumoto, I., Ichihashi, D., Iwata, H., Istokovics, A., Ichise, N., Matsuyama, H., Okuyama, H. and Kawasiki, K. (2000) Purification and Characterization of a Catalase From The Facultative Psychrophilic Bacterium Vibro Rumoiensis S-1^T Exhibiting High Catalase Activity. Journal of Bacteriology, 182 (7), p. 1903-1909.

122. Yusuf, M. O. L., Debora, A and Ogheneruona, D. E. (2011) Ambient Temperature Kinetic Assessment of Biogas Production from Co-digestion of Horse and Cow dung. Research Agricultural Engineering, 57 (3), p. 97-104.

123. Zhou, H. and Smith. D. W. (2002) Advanced Technologies in Water and Wastewater Treatment, Journal of Environmental Engineering and Science, 1, p. 247-264.

APPENDIXES

APPENDIX A: ENVIRONMENTAL MANAGEMENT AGENCY STATUTORY INSTRUMENT (SI) 274 of 2004 for EFFLUENT DISPOSAL

The SI instrument used by EMA for monitoring effluent disposals in Zimbabwe is given. The normal range is the accepted range for wastewater disposal (Nhapi and Gijzen, 2002).

Parameter	**Blue**		**Green**	**Yellow**	**Red**
	Sensitive	**Normal**			
Ammonia (N), mg/L	≤ 0.5	≤ 0.5	≤ 1.0	≤ 1.5	≤ 0.3
Nitrate-nitrogen, mg/L	≤ 3	≤ 3	≤ 5	≤ 8	≤ 10
Nitrogen Total (N), mg/L	≤ 10	≤ 10	≤ 20	≤ 30	≤ 50
Boron (B), mg/L	≤ 0.5	≤ 0.5	≤ 1.0	≤ 1.5	≤ 2.0
BOD, mg/L	≤ 15	≤ 30	≤ 50	≤ 100	≤ 120
COD, mg/L	≤ 30	≤ 60	≤ 90	≤ 150	≤ 200
Conductivity (µS/cm)	≤ 200	≤ 1000	≤ 2000	≤ 3000	≤ 3500
DO% saturation	≥ 75	≥ 60	≥ 50	≥ 30	≥ 15
FC (#/100mL)	≤ 1000	≤ 1000	> 1000	> 1500	≤ 2000
Helmith eggs (#/100 mL)	≤ 1000	≤ 1000	> 1000	> 1000	≤ 2000
Iron (Fe), mg/L	≤ 0.3	≤ 1	≤ 2	≤ 5	≤ 8
Lead (Pb), mg/L	≤ 0.05	≤ 0.05	≤ 0.1	≤ 0.2	≤ 0.5
Oxygen absorbed, mg/L	≤ 5	≤ 10	≤ 15	≤ 25	≤ 40
pH (pH units)	6.0 – 7.5	6 – 9	5 – 6	4 – 5	0 – 4
			9 – 10	10 – 12	12 – 14
Total-PO_4^- (P), mg/L	≤ 0.5	≤ 0.5	≤ 1.5	≤ 3	≤ 5
Potassium (K), mg/L	*	*	*	*	< 500
TDS, mg/L	≤ 100	≤ 500	≤ 1500	≤ 2000	≥ 3000
Temperature, °C	< 25	< 35	< 40	≤ 40	≤ 45
Total heavy metals, mg/L	≤ 1.0	≤ 2.0	≤ 4	≤ 10	≤ 20
TSS, mg/L	≤ 10	≤ 25	≤ 50	≤ 100	≤ 150
Turbidity (NTU)	≤ 5	≤ 5			

APPENDIX B: RAW SEWAGE PHYSICOCHEMICAL PARAMETERS RESULTS

Raw sewage physicochemical characteristics before treatment with Acti-zyme

Parameter	Experiment 1	Experiment 2	Experiment 3	Average	Standard deviation	Standard deviation (%)
TKN (mg/L)	250.1	239.9	245.7	245.2	5.1	2.09
BOD_5 @ 20°C (mg/L)	560.1	540.7	570.2	557.0	15.0	2.69
TSS (mg/L)	620.2	590.8	615.3	608.8	15.8	2.59
TDS (mg/L)	550.3	530.9	525.8	535.7	12.9	2.41
E. coli	TMTC*					
EC @ 25°C (µS/cm)	3910.4	3900.8	3850.9	3887.4	31.9	0.82
Cl^- (mg/L)	830.1	823.9	845.1	833.0	10.9	1.31
pH @ 25°C	9.2	8.9	9.5	9.2	0.3	3.26
Coliforms (cfu/100 mL)	$1x10^{11}$					
TP (mg/L)	49.8	55.2	52.4	52.5	2.7	5.15
SO_4^{2-} (mg/L)	1230.3	1110.9	1235.1	1192.1	70.4	5.90
DO (mg/L)	7.5	7.3	7.6	7.5	0.2	2.05
Temperature (°C)	20.9	22.4	23.8	22.4	1.5	6.48
COD (mg/L)	750.5	740.9	725.8	739.1	12.5	1.68

*Too many to count

APPENDIX C: INFLUENCE OF ACTI-ZYME LOADING AND RETENTION TIME ON SEWAGE EFFLUENT PHYSICOCHEMICAL PROPERTIES

The physicochemical parameters were measured in mg/L except for pH and EC in µS/cm. A temperature of 37 ◦C, agitation of 60 rpm and atmospheric pressure under anaerobic conditions were used. 2^2 full factorial designs were used to determine optimum conditions for Acti-zyme loading of 0-0.070 g/L of sewage and retention time of 7-40 days. The raw data for the sewage effluent are indicated in **Appendixes C1-C11**.

AC1: Influence of Acti-zyme and retention time on pH raw results

	Treatment combinations		**Experimental Conditions**		**Experimental runs**					
Experiment number	***A***	***B***	***A (g/L)***	***B (days)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
0			0.000	7	8.6	8.3	8.1	8.3	0.25	3.02
1	-1	-1	0.035	7	7.8	7.9	7.8	7.8	0.06	0.74
2	1	-1	0.050	7	7.5	7.6	7.4	7.5	0.10	1.33
3			0.070	7	7.6	7.1	7.7	7.5	0.32	4.31
4			0.000	40	7.9	7.7	8.1	7.9	0.20	2.53
5	-1	1	0.035	40	6.7	6.8	6.9	6.8	0.10	1.47
6	1	1	0.050	40	6.4	6.3	6.2	6.3	0.10	1.59
7			0.070	40	6.3	6.2	6.3	6.3	0.06	0.92

AC2: Influence of Acti-zyme and retention time on TP raw results

	Treatment combinations		Experimental Conditions		Experimental runs					
Experiment number	***A***	***B***	***A (g/L)***	***B (days)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
0			0.000	7	39.4	38.9	39.1	39.1	0.25	0.64
1	-1	-1	0.035	7	19.2	19.0	19.2	19.1	0.12	0.60
2	1	-1	0.050	7	18.1	18.4	17.9	18.1	0.25	1.39
3			0.070	7	18.3	17.9	18.1	18.1	0.20	1.10
4			0.000	40	29.1	28.9	29.3	29.1	0.20	0.69
5	-1	1	0.035	40	7.3	6.8	7.5	7.2	0.36	5.01
6	1	1	0.050	40	1.3	1.7	1.1	1.4	0.31	22.35
7			0.070	40	1.6	1.4	1.1	1.4	0.24	17.54

AC3: Influence of Acti-zyme and retention time on TKN raw results

	Treatment combinations		Experimental Conditions		Experimental runs					
Experiment number	***A***	***B***	***A (g/L)***	***B (days)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
0			0.000	7	61.6	63.0	61.4	62.0	0.87	1.41
1	-1	-1	0.035	7	20.5	20.1	20.3	20.3	0.20	0.99
2	1	-1	0.050	7	18.6	18.2	18.5	18.4	0.21	1.13
3			0.070	7	18.2	18.4	18.5	18.4	0.15	0.83
4			0.000	40	39.9	40.1	39.7	39.9	0.20	0.50
5	-1	1	0.035	40	16.2	15.9	16.0	16.0	0.15	0.95
6	1	1	0.050	40	9.6	9.1	9.4	9.4	0.25	2.69
7			0.070	40	9.1	9.3	9.8	9.4	0.36	3.84

AC4: Influence of Acti-zyme and retention time on BOD_5 raw results

	Treatment combinations		Experimental Conditions		Experimental runs					
Experiment number	***A***	***B***	***A (g/L)***	***B (days)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
0			0.000	7	410.4	409.5	411.2	410.4	0.85	0.21
1	-1	-1	0.035	7	214.4	212.9	213.8	213.7	0.75	0.35
2	1	-1	0.050	7	180.3	183.1	182.5	182.0	1.47	0.81
3			0.070	7	181.9	181.6	182.2	181.9	0.30	0.16
4			0.000	40	312.6	312.8	312.4	312.6	0.20	0.06
5	-1	1	0.035	40	90.6	85.8	89.5	88.6	2.51	2.84
6	1	1	0.050	40	42.7	40.7	42.4	41.9	1.08	2.57
7			0.070	40	42.1	41.8	41.6	41.8	0.25	0.60

AC5: Influence of Acti-zyme and retention time on COD raw results

	Treatment combinations		Experimental Conditions		Experimental runs					
Experiment number	***A***	***B***	***A (g/L)***	***B (days)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
0			0.000	7	516.7	514.9	516.3	516.0	0.95	0.18
1	-1	-1	0.035	7	300.5	303.8	305.9	303.4	2.72	0.90
2	1	-1	0.050	7	299.2	298.6	289.4	295.7	5.49	1.86
3			0.070	7	296.2	295.7	295.1	295.7	0.55	0.19
4			0.000	40	409.7	409.1	409.8	409.5	0.38	0.09
5	-1	1	0.035	40	168.4	170.1	175.6	171.4	3.76	2.20
6	1	1	0.050	40	78.1	77.8	78.2	78.0	0.21	0.27
7			0.070	40	78.9	75.3	79.4	77.9	2.24	2.87

AC6: Influence of Acti-zyme and retention time on TSS raw results

	Treatment combinations		Experimental Conditions		Experimental runs					
Experiment number	***A***	***B***	***A (g/L)***	***B (days)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
0			0.000	7	425.6	424.9	423.8	424.8	0.91	0.21
1	-1	-1	0.035	7	334.6	325.1	333.2	331.0	5.13	1.55
2	1	-1	0.050	7	310.1	312.4	309.1	310.5	1.69	0.54
3			0.070	7	310.5	310.2	309.8	310.2	0.35	0.11
4			0.000	40	397.9	398.2	397.5	397.9	0.35	0.09
5	-1	1	0.035	40	129.4	128.1	128.2	128.6	0.72	0.56
6	1	1	0.050	40	37.1	36.8	38.2	37.4	0.74	1.97
7			0.070	40	36.9	38.5	36.6	37.3	1.02	2.74

AC7: Influence of Acti-zyme and retention time on TDS raw results

	Treatment combinations		Experimental Conditions		Experimental runs					
Experiment number	***A***	***B***	***A (g/L)***	***B (days)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
0			0.000	7	349.8	346.2	346.1	347.4	2.11	0.61
1	-1	-1	0.035	7	190.4	196.2	193.5	193.4	1.53	0.79
2	1	-1	0.050	7	141.3	139.1	145.2	141.9	0.53	0.37
3			0.070	7	142.3	141.8	142.1	142.1	0.25	0.18
4			0.000	40	253.7	253.4	253.9	253.7	0.25	0.10
5	-1	1	0.035	40	105.7	108.3	105.6	106.5	0.53	0.50
6	1	1	0.050	40	59.7	58.9	59.9	59.5	0.53	0.89
7			0.070	40	59.4	59.6	59.1	59.4	0.25	0.42

AC8: Influence of Acti-zyme and retention time on EC raw results

	Treatment combinations		Experimental Conditions		Experimental runs					
Experiment number	***A***	***B***	***A (g/L)***	***B (days)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
0			0.000	7	2932.9	2931.8	2934.5	2933.1	1.36	0.05
1	-1	-1	0.035	7	2680.3	2679.5	2685.7	2681.8	10.48	0.39
2	1	-1	0.050	7	2670.6	2660.2	2665.8	2665.5	0.26	0.01
3			0.070	7	2666.1	2666.4	2665.8	2666.1	0.36	0.01
4			0.000	40	2186.5	2185.9	2186.3	2186.2	0.21	0.01
5	-1	1	0.035	40	1140.2	1145.1	1160.3	1148.5	0.36	0.03
6	1	1	0.050	40	1070.4	1070.9	1070.5	1070.6	0.26	0.02
7			0.070	40	1070.3	1070.1	1070.8	1070.4	0.36	0.03

AC9: Influence of Acti-zyme and retention time on Cl^- raw results

	Treatment combinations		Experimental Conditions		Experimental runs					
Experiment number	***A***	***B***	***A (g/L)***	***B (days)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
0			0.000	7	747.9	746.8	747.1	747.3	0.56	0.00
1	-1	-1	0.035	7	560.5	550.8	565.4	558.9	7.43	0.01
2	1	-1	0.050	7	453.2	450.5	460.6	454.8	5.23	0.01
3			0.070	7	455.1	454.8	454.5	454.8	0.30	0.00
4			0.000	40	673.1	673.5	674.1	673.6	0.50	0.00
5	-1	1	0.035	40	380.1	383.3	385.3	382.9	2.62	0.01
6	1	1	0.050	40	260.5	263.9	265.9	263.4	2.73	0.01
7			0.070	40	260.7	267.9	261.2	263.3	4.02	0.02

AC10: Influence of Acti-zyme and retention time on SO_4^{2-} raw results

	Treatment combinations		Experimental Conditions		Experimental runs					
Experiment number	***A***	***B***	***A (g/L)***	***B (days)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
0			0.000	7	941.7	939.6	941.5	940.9	1.16	0.12
1	-1	-1	0.035	7	605.3	613.5	617.1	612.0	6.05	0.99
2	1	-1	0.050	7	440.5	442.6	438.8	440.6	1.90	0.43
3			0.070	7	440.5	439.9	440.7	440.4	0.42	0.09
4			0.000	40	776.5	777.2	776.9	776.9	0.35	0.05
5	-1	1	0.035	40	290.7	301.4	296.1	296.1	5.35	1.81
6	1	1	0.050	40	54.1	53.9	54.2	54.1	0.15	0.28
7			0.070	40	54.4	54.6	54.8	54.6	0.20	0.37

AC11: Influence of Acti-zyme and retention time on DO raw results

	Treatment combinations		Experimental Conditions		Experimental runs					
Experiment number	***A***	***B***	***A (g/L)***	***B (days)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
0			0.000	7	14.5	14.1	14.6	14.4	0.26	1.84
1	-1	-1	0.035	7	27.1	27.2	26.9	27.1	0.15	0.56
2	1	-1	0.050	7	43.2	44.1	43.5	43.6	0.46	1.05
3			0.070	7	43.7	43.5	43.8	43.7	0.15	0.35
4			0.000	40	20.8	21.2	20.7	20.9	0.26	1.27
5	-1	1	0.035	40	55.8	54.4	55.2	55.1	0.70	1.27
6	1	1	0.050	40	86.9	87.3	87.1	87.1	0.20	0.23
7			0.070	40	87.2	87.0	86.8	87.0	0.20	0.23

APPENDIX D: BIODEGRADABILITY AND DE-NITRIFICATION OF SEWAGE USING ACTI-ZYME UNDER ANAEROBIC CONDITIONS OVER TIME

Various ratios were measured to indicate bio-nutrient removal through biodegradability and de-nitrification for Acti-zyme loadings of 0-0.070 g/L and retention time of 0-40 days. These included the COD/BOD_5 ratio, BOD_5/TKN ratio, COD/TKN ratio and COD/TP ratio. The raw results for the ratios are indicated in **Appendixes AD1-AD4**.

AD1: Influence of Acti-zyme and retention time on COD/BOD_5 ratio raw results

	Treatment combinations		**Experimental Conditions**		**Experimental runs**					
Experiment number	***A***	***B***	***A (g/L)***	***B (days)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
0			0.000	7	1.34	1.37	1.27	1.33	0.05	3.75
1	-1	-1	0.035	7	1.26	1.26	1.26	1.26	0.00	0.14
2	1	-1	0.050	7	1.40	1.43	1.43	1.42	0.02	1.12
3			0.070	7	1.66	1.63	1.59	1.63	0.04	2.29
4			0.000	40	1.63	1.63	1.62	1.63	0.01	0.31
5	-1	1	0.035	40	1.31	1.31	1.31	1.31	0.00	0.15
6	1	1	0.050	40	1.86	1.98	1.96	1.93	0.07	3.43
7			0.070	40	1.83	1.91	1.84	1.86	0.04	2.36

AD2: Influence of Acti-zyme and retention time on BOD_5/TKN ratio raw results

	Treatment combinations		Experimental Conditions		Experimental runs					
Experiment number	***A***	***B***	***A (g/L)***	***B (days)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
0			0.000	7	2.24	2.25	2.32	2.28	0.04	1.90
1	-1	-1	0.035	7	6.66	6.50	6.70	6.62	0.11	1.59
2	1	-1	0.050	7	10.46	10.59	10.53	10.53	0.07	0.64
3			0.070	7	9.69	10.06	9.86	9.87	0.18	1.86
4			0.000	40	9.99	9.87	9.85	9.90	0.08	0.80
5	-1	1	0.035	40	7.83	7.80	7.87	7.83	0.03	0.44
6	1	1	0.050	40	5.59	5.40	5.59	5.53	0.11	2.06
7			0.070	40	4.45	4.47	4.51	4.48	0.03	0.71

AD3: Influence of Acti-zyme and retention time on COD/TKN ratio raw results

	Treatment combinations		**Experimental Conditions**		**Experimental runs**					
Experiment number	***A***	***B***	***A (g/L)***	***B (days)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
0			0.000	7	3.00	3.09	2.95	3.02	0.07	2.26
1	-1	-1	0.035	7	8.39	8.17	8.41	8.32	0.13	1.57
2	1	-1	0.050	7	14.66	15.11	15.07	14.95	0.25	1.68
3			0.070	7	16.09	16.41	15.64	16.04	0.38	2.39
4			0.000	40	16.27	16.07	15.95	16.10	0.16	1.02
5	-1	1	0.035	40	10.27	10.20	10.32	10.26	0.06	0.59
6	1	1	0.050	40	10.40	10.70	10.98	10.69	0.29	2.71
7			0.070	40	8.14	8.55	8.32	8.33	0.21	2.49

AD4: Influence of Acti-zyme and retention time on COD/TP ratio raw results

	Treatment combinations		**Experimental Conditions**		**Experimental runs**					
Experiment number	***A***	***B***	***A (g/L)***	***B (days)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
0			0.000	7	15.07	13.42	13.85	14.11	0.86	6.06
1	-1	-1	0.035	7	13.11	13.24	13.20	13.19	0.06	0.48
2	1	-1	0.050	7	15.65	15.99	15.93	15.86	0.18	1.14
3			0.070	7	16.53	16.23	16.17	16.31	0.19	1.19
4			0.000	40	16.19	16.52	16.30	16.34	0.17	1.04
5	-1	1	0.035	40	14.08	14.16	13.99	14.07	0.08	0.60
6	1	1	0.050	40	23.07	25.01	23.41	23.83	1.04	4.36
7			0.070	40	60.08	45.76	71.09	58.98	12.70	21.53

APPENDIX E: STATISTICAL MODELS ANALYSIS DATA FOR BIO-NUTRIENT REMOVAL RATIOS IN SEWAGE TREAMENT USING ACTI-ZYME AS BIO-CATALYST

AE1: COD/BOD_5 ratio model analysis

AE1.1: COD/BOD_5 ratio model summary

Model	R	R^2	Adjusted R^2	Std. Error of the Estimate
COD/ BOD_5	0.991[a]	0.983	0.978	0.0281

AE1.2: ANOVA test for COD/BOD_5 model

Model		Sum of Squares	Df	Mean Square	F	Sig.
COD/ BOD_5	Regression	0.594	4	0.148	188.432	0.000[a]
	Residual	0.010	13	0.001		
	Total	0.604	17			

AE1.3: COD/BOD_5 model coefficients

Model		Unstandardized Coefficients		Standardized Coefficients	T	Sig.
		B	Std. Error	Beta		
COD/ BOD_5	(Constant)	1.669	0.093	-	17.976	0.000
	TKN	0.008	0.012	0.181	0.615	0.549
	BOD_5	-0.008	0.001	-2.909	-5.402	0.000
	TP	-0.036	0.034	-1.537	-1.045	0.315
	COD	0.006	0.002	3.413	2.697	0.018

AE1.4: COD/BOD_5 model coefficients correlations and covariances

COD/BOD_5 Model		**COD**	**TKN**	**BOD_5**	**TP**
Correlations	*COD*	*1.000*	*-0.912*	*0.801*	*-0.975*
	TKN	-0.912	1.000	-0.771	0.872
	BOD_5	*0.801*	*-0.771*	*1.000*	*-0.904*
	TP	-0.975	0.872	-0.904	1.000
Covariances	*COD*	*5.412E-6*	*-2.609E-5*	*2.623E-6*	*-7.814E-5*
	TKN	-2.609E-5	0.000	-1.333E-5	0.000
	BOD_5	*2.623E-6*	*-1.333E-5*	*1.979E-6*	*-4.380E-5*
	TP	-7.814E-5	0.000	-4.380E-5	0.001

AE2: BOD_5/TKN ratio model analysis

AE2.1: BOD_5/TKN model summary

Model	R	R^2	Adjusted R^2	Std. Error of the Estimate
BOD_5/TKN	1.000[a]	1.000	1.000	0.059

AE2.2: ANOVA test for BOD_5/TKN model

Model		Sum of Squares	df	Mean Square	F	Sig.
BOD_5/TKN	Regression	128.747	4	32.187	8974.881	0.000[a]
	Residual	0.047	13	0.004		
	Total	128.793	17			

AE2.3: BOD_5/TKN model coefficients

Model		Unstandardized Coefficients		Standardized Coefficients	T	Sig.
		B	Std. Error	Beta		
BOD_5/TKN	(Constant)	4.967	0.198		25.078	0.000
	TKN	-0.245	0.026	-0.402	-9.354	0.000
	BOD_5	0.029	0.003	0.767	9.747	0.000
	TP	0.148	0.073	0.433	2.015	0.065
	COD	0.005	0.005	0.180	0.974	0.348

AE2.4: BOD_5/TKN model coefficients correlations and covariances

BOD_5/TKN Model			**COD**	**TKN**	**BOD_5**	**TP**
BOD_5/TKN	Correlations	COD	1.000	-0.912	0.801	-0.975
		TKN	-0.912	1.000	-0.771	0.872
		BOD_5	0.801	-0.771	1.000	-0.904
		TP	-0.975	0.872	-0.904	1.000
	Covariances	COD	2.463E-5	0.000	1.194E-5	0.000
		TKN	0.000	0.001	-6.069E-5	0.002
		BOD_5	1.194E-5	-6.069E-5	9.009E-6	0.000
		TP	0.000	0.002	0.000	0.005

AE3: COD/TKN ratio model analysis

COD/TKN model summary

Model	R	R^2	Adjusted R^2	Std. Error of the Estimate
COD/TKN	1.000[a]	1.000	0.999	0.084

AE3.1: ANOVA test for COD/TKN model

Model		Sum of Squares	df	Mean Square	F	Sig.
COD/TKN	Regression	210.273	4	52.568	7463.402	0.000[a]
	Residual	0.092	13	0.007		
	Total	210.364	17			

AE3.2: COD/TKN model coefficients

Model		Unstandardized Coefficients		Standardized Coefficients	T	Sig.
		B	Std. Error	Beta		
COD/TKN	(Constant)	8.677	0.278		31.260	0.000
	TKN	-0.408	0.037	-0.522	-11.089	0.000
	BOD_5	-0.032	0.004	-0.647	-7.496	0.000
	TP	0.192	0.103	0.440	1.865	0.085
	COD	0.058	0.007	1.692	8.343	0.000

AE3.3: COD/TKN model coefficients correlations and covariances

COD/TKN Model			**COD**	**TKN**	**BOD_5**	**TP**
COD/TKN	Correlations	COD	1.000	-0.912	0.801	-0.975
		TKN	-0.912	1.000	-0.771	0.872
		BOD_5	0.801	-0.771	1.000	-0.904
		TP	-0.975	0.872	-0.904	1.000
	Covariances	COD	4.838E-5	0.000	2.345E-5	-0.001
		TKN	0.000	0.001	0.000	0.003
		BOD_5	2.345E-5	0.000	1.769E-5	0.000
		TP	-0.001	0.003	0.000	0.011

AE4: COD/TP ratio model analysis

AE4.1: COD/TP model summary

Model	R	R^2	Adjusted R^2	Std. Error of the Estimate
COD/TP	0.972[a]	0.944	0.927	5.639

AE4.2: ANOVA test for COD/TP model

Model		Sum of Squares	df	Mean Square	F	Sig.
COD/TP	Regression	6971.962	4	1742.991	54.815	0.000[a]
	Residual	413.372	13	31.798		
	Total	7385.335	17			

AE4.3: COD/TP model coefficients

Model		Unstandardized Coefficients		Standardized Coefficients	T	Sig.
		B	Std. Error	Beta		
COD/TP	(Constant)	48.650	18.651		2.608	0.022
	TKN	-10.778	2.470	-2.329	-4.363	0.001
	BOD	1.105	0.283	3.826	3.910	0.002
	TP	-21.800	6.917	-8.425	-3.152	0.008
	COD	1.214	0.467	5.971	2.597	0.022

AE4.4: COD/TP model coefficients correlations and covariances

COD/TP Model		**COD**	**TKN**	**BOD_5**	**TP**
Correlations	COD	1.000	-0.912	0.801	-0.975
	TKN	-0.912	1.000	-0.771	0.872
	BOD_5	0.801	-0.771	1.000	-0.904
	TP	-0.975	0.872	-0.904	1.000
Covariances	COD	0.218	-1.053	0.106	-3.153
	TKN	-1.053	6.103	-0.538	14.903
	BOD_5	0.106	-0.538	0.080	-1.768
	TP	-3.153	14.903	-1.768	47.844

AE5: t-test results for testing applicability of the bio-nutrient models in sewage treatment using Acti-zyme

A significant *t-test* at 95% confidence interval indicated the applicability of the models to any sewage treatment plant was suitable at 40 days retention time whereby the required bio-nutrient removal ratios are achieved with Acti-zyme loading of 0.050 g/L.

t-test for quantifying the influence of retention time on bio-nutrient removal with increasing Acti-zyme loading

Ratio	Time	N	Mean	Std. Deviation	Std. Error Mean
COD/BOD_5	7 Days	9	1.557	0.105	0.035
	40 Days	9	1.886	0.060	0.020
BOD_5/TKN	7 Days	9	10.102	0.337	0.112
	40 Days	9	4.819	0.543	0.181
COD/TKN	7 Days	9	15.697	0.613	0.204
	40 Days	9	9.105	1.213	0.404
COD/TP	7 Days	9	16.168	0.281	0.094
	40 Days	9	47.183	19.544	6.515

APPENDIX F: BIOGAS QUANTITY RESULTS AT VARYING ACTI-ZYME LOADINGS, SEWAGE SLUDGE LOADINGS AND TEMPERATURE

Biogas quantity produced at Acti-zyme loading of 0-0.070 g/L, solids loading of 5-10 g/L.d and retention time of 40 days are indicated in **Appendixes AF1-AF3**.

AF1.1: Biogas quantity produced at 5 g/L.d at 37 ◦C

Conditions		**Experiment number**					
Sewage sludge loading (g/L.d)	***Acti-zyme (g/L)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
	0.000	51	53	52	52	1.00	1.92
	0.020	124	120	121	122	2.08	1.71
	0.035	145	145	146	145	0.58	0.40
	0.040	165	163	162	163	1.53	0.94
	0.045	189	191	190	190	1.00	0.53
	0.050	192	193	194	193	1.00	0.52
5 g/L.d	0.070	191	190	193	191	1.53	0.80

AF1.2: Biogas quantity produced at 5 g/L.d at 55 ◦C

Conditions		**Experiment number**					
Sewage sludge loading (g/L.d)	***Acti-zyme (g/L)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
	0.000	22	21	20	21	1.00	4.76
	0.020	61	60	68	63	4.36	6.92
	0.035	73	71	74	73	1.53	2.10
	0.040	81	85	83	83	2.00	2.41
	0.045	93	91	97	94	3.06	3.26
	0.050	91	94	95	93	2.08	2.23
5 g/L.d	0.070	93	93	91	92	1.15	1.26

AF2.1: Biogas quantity produced at 7.5 g/L.d at 37 ◦C

Conditions		**Experiment number**					
Sewage sludge loading (g/L.d)	***Acti-zyme (g/L)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
	0.000	111	108	107	109	2.08	1.92
	0.020	215	217	214	215	1.53	0.71
	0.035	319	321	317	319	2.00	0.63
	0.040	361	360	360	360	0.58	0.16
	0.045	387	390	389	389	1.53	0.39
	0.050	389	394	399	394	5.00	1.27
7.5 g/L.d	0.070	388	390	391	390	1.53	0.39

AF2.2: Biogas quantity produced at 7.5 g/L.d at 55 ◦C

Conditions		**Experiment number**					
Sewage sludge loading (g/L.d)	***Acti-zyme (g/L)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
	0.000	56	54	53	54	1.53	2.81
	0.020	90	92	91	91	1.00	1.10
	0.035	152	150	151	151	1.00	0.66
	0.040	182	180	179	180	1.53	0.85
	0.045	198	192	196	195	3.06	1.56
	0.050	199	195	193	196	3.06	1.56
7.5 g/L.d	0.070	197	194	193	195	2.08	1.07

AF3.1: Biogas quantity produced at 10 g/L.d at 37 ◦C

Conditions		**Experiment number**					
Sewage sludge loading (g/L.d)	***Acti-zyme (g/L)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
	0.000	80	84	83	82	2.08	2.53
	0.020	156	160	154	157	3.06	1.95
	0.035	232	232	234	233	1.15	0.50
	0.040	284	284	283	284	0.58	0.20
	0.045	328	325	327	327	1.53	0.47
	0.050	338	339	335	337	2.08	0.62
10 g/L.d	0.070	333	337	335	335	2.00	0.60

AF3.2: Biogas quantity produced at 10 g/L.d at 55 ◦C

Conditions		**Experiment number**					
Sewage sludge loading (g/L.d)	***Acti-zyme (g/L)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
	0.000	38	37	33	36	2.65	7.35
	0.020	72	71	74	72	1.53	2.11
	0.035	116	112	114	114	2.00	1.75
	0.040	142	141	142	142	0.58	0.41
	0.045	164	162	166	164	2.00	1.22
	0.050	159	163	165	162	3.06	1.88
10 g/L.d	0.070	169	163	162	165	3.79	2.30

APPENDIX G: BIO-METHANE COMPOSITION RESULTS AT VARYING ACTI-ZYME LOADINGS, SEWAGE SLUDGE LOADINGS AND TEMPERATURE

Bio-methane quantity produced at Acti-zyme loading of 0-0.070 g/L, solids loading of 5-10 g/L.d and retention time of 40 days was determined and the raw bio-methane compositions are given in **Appendixes AG1-AG3**.

AG1.1: Bio-methane quality produced at 5 g/L.d at 37 ◦C

Conditions		**Experiment number**					
Sewage sludge loading (g/L.d)	***Acti-zyme (g/L)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
	0.000	49.9	53.1	55.4	52.8	2.76	5.23
	0.020	61.1	60.9	61.3	61.1	0.20	0.33
	0.035	71.9	71.7	71.6	71.7	0.15	0.21
	0.040	72.3	72.5	72.1	72.3	0.20	0.28
	0.045	72.4	72.6	72.5	72.5	0.10	0.14
	0.050	72.5	72.4	72.7	72.5	0.15	0.21
5 g/L.d	0.070	72.3	72.5	72.4	72.4	0.10	0.14

AG1.2: Bio-methane quality produced at 5 g/L.d at 55 ◦C

Conditions		**Experiment number**					
Sewage sludge loading (g/L.d)	***Acti-zyme (g/L)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
	0.000	25.9	25.3	25.7	25.6	0.31	1.19
	0.020	30.9	30.8	30.3	30.7	0.32	1.05
	0.035	35.7	35.6	35.5	35.6	0.10	0.28
	0.040	36.3	36.5	36.7	36.5	0.20	0.55
	0.045	36.9	36.7	36.7	36.8	0.12	0.31
	0.050	36.8	36.9	36.7	36.8	0.10	0.27
5 g/L.d	0.070	36.9	36.7	36.7	36.8	0.12	0.31

AG2.1: Bio-methane quality produced at 7.5 g/L.d at 37 ◦C

Conditions		**Experiment number**					
Sewage sludge loading (g/L.d)	***Acti-zyme (g/L)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
	0.000	65.1	65.4	63.1	64.5	1.25	1.94
	0.020	70.5	70.3	70.1	70.3	0.20	0.28
	0.035	75.9	76.1	76.8	76.3	0.47	0.62
	0.040	76.3	76.2	76.4	76.3	0.10	0.13
	0.045	77.2	77.4	77.1	77.2	0.15	0.20
	0.050	78.3	78.5	78.2	78.3	0.15	0.20
7.5 g/L.d	0.070	77.9	77.8	78.1	77.9	0.15	0.20

AG2.2: Bio-methane quality produced at 7.5 g/L.d at 55 ◦C

Conditions		**Experiment number**					
Sewage sludge loading (g/L.d)	***Acti-zyme (g/L)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
	0.000	32.2	32.5	32.1	32.3	0.21	0.65
	0.020	35.4	35.1	35.3	35.3	0.15	0.43
	0.035	38.5	38.1	38.3	38.3	0.20	0.52
	0.040	38.3	38.5	38.9	38.6	0.31	0.79
	0.045	38.9	38.8	38.6	38.8	0.15	0.39
	0.050	39.5	39.3	39.6	39.5	0.15	0.39
7.5 g/L.d	0.070	77.9	77.8	78.1	77.9	0.15	0.20

AG3.1: Bio-methane quality produced at 10 g/L.d at 37 ◦C

Conditions		**Experiment number**					
Sewage sludge loading (g/L.d)	***Acti-zyme (g/L)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
	0.000	60.4	59.8	59.3	59.8	0.55	0.92
	0.020	67.5	64.9	67.3	66.6	1.45	2.17
	0.035	74.4	74.2	74.3	74.3	0.10	0.13
	0.040	74.6	74.5	74.8	74.6	0.15	0.20
	0.045	75.1	74.9	75.3	75.1	0.20	0.27
	0.050	75.5	75.6	75.8	75.6	0.15	0.20
10 g/L.d	0.070	75.2	75.5	75.1	75.3	0.21	0.28

AG3.2: Bio-methane quality produced at 10 g/L.d at 55 ◦C

Conditions		**Experiment number**					
Sewage sludge loading (g/L.d)	***Acti-zyme (g/L)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
	0.000	30.3	30.1	30.4	30.3	0.15	0.50
	0.020	33.5	33.3	33.1	33.3	0.20	0.60
	0.035	37.3	37.6	37.5	37.5	0.15	0.41
	0.040	37.4	37.9	37.5	37.6	0.26	0.70
	0.045	38.1	38.4	38.6	38.4	0.25	0.66
	0.050	38.7	38.9	38.5	38.7	0.20	0.52
10 g/L.d	0.070	39	38.5	38.7	38.7	0.25	0.65

APPENDIX H: CARBON DIOXIDE COMPOSITION RESULTS AT VARYING ACTI-ZYME LOADINGS, SEWAGE SLUDGE LOADINDS AND TEMPERATURE

Carbon dioxide quantity produced at Acti-zyme loading of 0-0.070 g/L, solids loading of 5-10 g/L.d and retention time of 40 days was determined and the raw carbon dioxide compositions are given in **AH1-AH3.**

AH1.1: Carbon dioxide quality produced at 5 g/L.d at 37 ◦C

Conditions		**Experiment number**					
Sewage sludge loading (g/L.d)	***Acti-zyme (g/L)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
	0.000	27.5	27.0	27.5	27.3	0.30	1.11
	0.020	23.9	24.1	23.8	23.9	0.15	0.64
	0.035	20.4	20.6	20.5	20.5	0.10	0.49
	0.040	19.5	19.7	19.9	19.7	0.20	1.02
	0.045	19.1	18.9	19.3	19.1	0.20	1.05
	0.050	18.9	18.7	18.6	18.7	0.15	0.82
5 g/L.d	0.070	18.8	18.5	18.9	18.7	0.21	1.11

AH1.2: Carbon dioxide quality produced at 5 g/L.d at 55 ◦C

Conditions		**Experiment number**					
Sewage sludge loading (g/L.d)	***Acti-zyme (g/L)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
	0.000	13.4	13.1	13.6	13.4	0.25	1.88
	0.020	12.2	12.5	12.1	12.3	0.21	1.70
	0.035	10.3	10.1	10.2	10.2	0.10	0.98
	0.040	9.7	9.4	9.5	9.5	0.15	1.60
	0.045	9.6	9.3	9.1	9.3	0.25	2.70
	0.050	8.7	8.5	8.3	8.5	0.20	2.35
5 g/L.d	0.070	8.5	8.6	8.1	8.4	0.26	3.15

AH2.1: Carbon dioxide quality produced at 7.5 g/L.d at 37 ◦C

Conditions		**Experiment number**					
Sewage sludge loading (g/L.d)	***Acti-zyme (g/L)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
	0.000	25.8	25.8	25.5	25.7	0.17	0.68
	0.020	22.1	21.8	22.3	22.1	0.25	1.14
	0.035	18.7	18.5	18.3	18.5	0.20	1.08
	0.040	18.2	18.4	18.1	18.2	0.15	0.84
	0.045	17.5	17.3	17.7	17.5	0.20	1.14
	0.050	16.9	16.7	16.8	16.8	0.10	0.60
7.5 g/L.d	0.070	17.1	16.7	16.9	16.9	0.20	1.18

AH2.2: Carbon dioxide quality produced at 7.5 g/L.d at 55 ◦C

Conditions		**Experiment number**					
Sewage sludge loading (g/L.d)	***Acti-zyme (g/L)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
	0.000	13.8	13.4	13.2	13.5	0.31	2.27
	0.020	11.2	11.6	11.4	11.4	0.20	1.75
	0.035	8.6	8.5	8.9	8.7	0.21	2.40
	0.040	8.1	7.9	8.3	8.1	0.20	2.47
	0.045	7.4	7.1	7.5	7.3	0.21	2.84
	0.050	6.8	6.5	6.2	6.5	0.30	4.62
7.5 g/L.d	0.070	6.9	6.6	6.1	6.5	0.40	6.19

AH3.1: Carbon dioxide quality produced at 10 g/L.d at 37 ◦C

Conditions		**Experiment number**					
Sewage sludge loading (g/L.d)	***Acti-zyme (g/L)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
	0.000	22.6	22.5	22.0	22.3	0.31	1.40
	0.020	19.7	19.5	19.8	19.7	0.15	0.78
	0.035	16.7	16.8	16.7	16.7	0.06	0.35
	0.040	16.2	16.5	16.3	16.3	0.15	0.94
	0.045	15.9	16.1	15.7	15.9	0.20	1.26
	0.050	15.6	15.4	15.2	15.4	0.20	1.30
10 g/L.d	0.070	15.7	15.9	15.8	15.8	0.10	0.63

AH3.2: Carbon dioxide quality produced at 10 g/L.d at 55 ◦C

Conditions		**Experiment number**					
Sewage sludge loading (g/L.d)	***Acti-zyme (g/L)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
	0.000	11.5	11.9	11.6	11.7	0.21	1.78
	0.020	9.6	9.5	9.7	9.6	0.10	1.04
	0.035	6.8	6.5	6.9	6.7	0.21	3.09
	0.040	6.3	6.5	6.1	6.3	0.20	3.17
	0.045	5.8	5.6	5.9	5.8	0.15	2.65
	0.050	5.5	5.4	5.3	5.4	0.10	1.85
10 g/L.d	0.070	5.6	5.1	5.3	5.3	0.25	4.72

APPENDIX I: TRACE GASES COMPOSITION RESULTS AT VARYING ACTI-ZYME LOADINGS, SEWAGE SLUDGE LOADINGS AND TEMPERATURE

Trace gases quantity produced at Acti-zyme loading of 0-0.070 g/L, solids loading of 5-10 g/L.d and retention time of 40 days was determined and the raw trace gases compositions are given in **AH1-AH3**.

AI1.1: Trace gases quality produced at 5 g/L.d at 37 ◦C

Conditions		**Experiment number**					
Sewage sludge loading (g/L.d)	***Acti-zyme (g/L)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
	0.000	11.98	11.7	12.12	11.93	0.21	1.79
	0.020	10.36	10.32	10.35	10.34	0.02	0.20
	0.035	8.73	8.75	8.64	8.71	0.06	0.68
	0.040	8.40	8.38	8.35	8.38	0.03	0.30
	0.045	8.00	8.01	7.95	7.99	0.03	0.40
	0.050	7.77	7.65	7.63	7.68	0.07	0.96
5 g/L.d	0.070	7.89	7.85	7.86	7.87	0.02	0.26

AI1.2: Trace gases quality produced at 5 g/L.d at 55 ◦C

Conditions		**Experiment number**					
Sewage sludge loading (g/L.d)	***Acti-zyme (g/L)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
	0.000	5.94	5.9	5.92	5.92	0.02	0.34
	0.020	5.16	5.14	5.18	5.16	0.02	0.39
	0.035	4.35	4.37	4.32	4.35	0.03	0.58
	0.040	4.24	4.36	4.35	4.32	0.07	1.54
	0.045	4.00	4.01	3.95	3.99	0.03	0.81
	0.050	3.89	3.98	3.96	3.94	0.05	1.20
5 g/L.d	0.070	3.90	3.95	3.86	3.90	0.05	1.16

AI2.1: Trace gases quality produced at 7.5 g/L.d at 37 ◦C

Conditions		**Experiment number**					
Sewage sludge loading (g/L.d)	***Acti-zyme (g/L)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
	0.000	7.55	7.49	7.58	7.54	0.05	0.61
	0.020	6.50	6.48	6.52	6.50	0.02	0.31
	0.035	5.47	5.43	5.45	5.45	0.02	0.34
	0.040	5.27	5.24	5.29	5.27	0.03	0.48
	0.045	5.23	5.26	5.21	5.23	0.03	0.48
	0.050	4.87	4.65	4.75	4.76	0.11	2.28
7.5 g/L.d	0.070	5.05	5.03	5.07	5.05	0.02	0.40

AI2.2: Trace gases quality produced at 7.5 g/L.d at 55 ◦C

Conditions		**Experiment number**					
Sewage sludge loading (g/L.d)	***Acti-zyme (g/L)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
	0.000	3.78	3.72	3.74	3.75	0.03	0.82
	0.020	3.25	3.28	3.52	3.35	0.15	4.42
	0.035	2.74	2.73	2.75	2.74	0.01	0.36
	0.040	2.64	2.66	2.69	2.66	0.03	0.94
	0.045	2.62	2.61	2.59	2.61	0.02	0.59
	0.050	2.44	2.45	2.47	2.45	0.02	0.62
7.5 g/L.d	0.070	2.45	2.43	2.47	2.45	0.02	0.82

AI3.1: Trace gases quality produced at 10 g/L.d at 37 ◦C

Conditions		**Experiment number**					
Sewage sludge loading (g/L.d)	***Acti-zyme (g/L)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
	0.000	12.20	12.18	11.9	12.09	0.17	1.39
	0.020	10.62	10.58	10.61	10.60	0.02	0.20
	0.035	9.03	9.05	9.06	9.05	0.01	0.15
	0.040	8.97	8.92	8.96	8.95	0.03	0.28
	0.045	8.97	8.78	8.88	8.88	0.09	1.05
	0.050	8.76	8.78	8.84	8.79	0.04	0.47
10 g/L.d	0.070	8.89	8.86	8.87	8.87	0.02	0.17

AI3.2: Trace gases quality produced at 10 g/L.d at 55 ◦C

Conditions		**Experiment number**					
Sewage sludge loading (g/L.d)	***Acti-zyme (g/L)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
	0.000	6.30	12.18	11.9	10.13	3.32	32.75
	0.020	5.32	10.58	10.61	8.84	3.05	34.47
	0.035	9.03	9.05	9.06	9.05	0.01	0.15
	0.040	4.52	8.92	8.96	7.47	2.55	34.18
	0.045	4.46	8.78	8.88	7.37	2.52	34.22
	0.050	4.40	8.78	8.84	7.34	2.55	34.69
10 g/L.d	0.070	4.44	8.86	8.87	7.39	2.55	34.57

APPENDIX J: BIOGAS COMPOSITION RESULTS AT VARYING RETENTION TIMES

Bio-methane quantity produced at Acti-zyme loading of 0.050 g/L, solids loading of 7.5 g/L.d and retention time of 0-60 days were determined and are given in **AJ1-AJ2** for systems with and without Acti-zyme respectively.

AJ1: Bio-methane composition with Acti-zyme loadings at 37 ◦C

	Experiment number					
Retention time (days)	**1**	**2**	**3**	**Average**	***Standard deviation***	***% Standard deviation***
0	10.8	12.4	11.5	11.6	0.80	6.93
10	19.9	20.3	20.1	20.1	0.20	1.00
20	40.5	41.2	40.9	40.9	0.35	0.86
30	59.5	59.3	59.8	59.5	0.25	0.42
40	78.3	78.5	78.2	78.3	0.15	0.20
50	78.1	78.3	78.5	78.3	0.20	0.26
60	78.2	78.4	78.1	78.2	0.15	0.20

AJ2: Bio-methane composition without Acti-zyme at 37 ◦C

	Experiment number					
Retention time (days)	**1**	**2**	**3**	**Average**	***Standard deviation***	***% Standard deviation***
0	8.7	8.4	8.3	8.5	0.21	2.46
10	16.8	16.4	16.5	16.6	0.21	1.26
20	37.4	37.3	36.9	37.2	0.26	0.71
30	48.3	47.9	48.4	48.2	0.26	0.55
40	65.1	65.4	63.1	64.5	1.25	1.94
50	65.4	65.5	64.9	65.3	0.32	0.49
60	65.5	65.3	65.1	65.3	0.20	0.31

AJ3: Bio-methane composition with Acti-zyme at 55 ◦C

	Experiment number					
Retention time (days)	**1**	**2**	**3**	**Average**	***Standard deviation***	***% Standard deviation***
0	6.6	6.4	6.5	6.5	0.10	1.54
10	10.8	10.3	10.1	10.4	0.36	3.47
20	21.6	21.2	20.9	21.2	0.35	1.65
30	31.5	31.3	31.9	31.6	0.31	0.97
40	40.3	40.5	40.7	40.5	0.20	0.49
50	40.6	40.8	40.9	40.8	0.15	0.37
60	40.9	40.8	40.8	40.8	0.06	0.14

Carbon dioxide quantity produced at Acti-zyme loading of 0.050 g/L, solids loading of 7.5 g/L.d and retention time of 0-60 days was determined and is given in **AJ3-AJ4** for systems with and without Acti-zyme respectively.

AJ3: Carbon dioxide composition with Acti-zyme at 37 ◦C

	Experiment number					
Retention time (days)	**1**	**2**	**3**	**Average**	***Standard deviation***	***% Standard deviation***
0	1.7	1.4	1.6	1.6	0.2	9.75
10	3.8	4.1	4.3	4.1	0.3	6.19
20	9.1	9.4	9.6	9.4	0.3	2.69
30	14.1	13.9	14.4	14.1	0.3	1.78
40	16.9	16.7	16.8	16.8	0.1	0.60
50	16.7	16.8	16.5	16.7	0.2	0.92
60	16.8	16.9	16.7	16.8	0.1	0.60

AJ4: Carbon dioxide composition without Acti-zyme at 37 ◦C

	Experiment number					
Retention time (days)	**1**	**2**	**3**	**Average**	***Standard deviation***	***% Standard deviation***
0	2.8	2.7	3.1	2.9	0.2	7.26
10	4.3	4.5	4.7	4.5	0.2	4.44
20	11.8	12.1	12.3	12.1	0.3	2.09
30	17.5	17.9	17.4	17.6	0.3	1.50
40	25.8	25.8	25.5	25.7	0.17	0.68
50	24.5	24.8	24.7	24.7	0.2	0.62
60	25.7	25.2	25.1	25.3	0.3	1.27

Trace gases quantity produced at Acti-zyme loading of 0.050 g/L, solids loading of 7.5 g/L.d and retention times of 0-60 days was determined and are given in **AJ1-AJ2** for systems with and without Acti-zyme respectively.

AJ5: Trace gases composition with Acti-zyme at 37 ◦C

	Experiment number					
Retention time (days)	**1**	**2**	**3**	**Average**	***Standard deviation***	***% Standard deviation***
0	0.61	0.64	0.59	0.61	0.03	4.10
10	1.94	1.75	1.63	1.77	0.16	8.81
20	2.53	2.49	2.39	2.47	0.07	2.92
30	3.82	3.91	3.69	3.81	0.11	2.91
40	4.87	4.65	4.75	4.76	0.11	2.28
50	4.83	4.85	4.83	4.84	0.01	0.24
60	4.85	4.88	4.82	4.85	0.03	0.62

AJ6: Trace gases composition without Acti-zyme at 37 ◦C

	Experiment number					
Retention time (days)	**1**	**2**	**3**	**Average**	***Standard deviation***	***% Standard deviation***
0	1.53	1.51	1.49	1.51	0.02	1.32
10	2.78	2.81	2.79	2.79	0.02	0.55
20	3.65	3.61	3.67	3.64	0.03	0.84
30	4.75	4.73	4.79	4.76	0.03	0.64
40	7.55	7.49	7.58	7.54	0.05	0.61
50	7.53	7.58	7.51	7.54	0.04	0.48
60	7.57	7.61	7.48	7.55	0.07	0.88

APPENDIX K: BIO-SOLIDS QUALITY RESULTS

AK1: Bio-solids NPK composition

The raw results for the sewage bio-solids NPK composition are given in **Appendix K1.**

Parameter	*Sample 1*	*Sample 2*	*Sample 3*	*Average*	*Standard deviation*	*% Standard deviation*
N	8.01	8.3	8.2	8.17	0.15	1.80
P	5.81	5.86	5.84	5.84	0.03	0.43
K	1.34	1.36	1.32	1.34	0.02	1.49

AK2: Bio-solids trace elements composition

The raw results for the sewage bio-solids NPK composition are given in **Appendix K2.**

Parameter	*Sample 1*	*Sample 2*	*Sample 3*	*Average*	*Standard deviation*	*% Standard deviation*
Cu	0.0073	0.0071	0.0075	0.0073	0.0002	2.74
Fe	0.0085	0.009	0.0086	0.0087	0.0003	3.04
Ca	0.0079	0.0077	0.008	0.0079	0.0002	1.94
Mg	0.0182	0.0171	0.0149	0.017	0.0017	10.04

APPENDIX L: BIO-SOLIDS QUANTITY PRODUCED AT VARYING ACTI-ZYME LOADINGS, SEWAGE SLUDGE LOADINDS AND TEMPERATURE

Bio-solids quantity in g/L produced at Acti-zyme loading of 0-0.070 g/L, solids loading of 5-10 g/L.d and retention time of 40 days was determined and the bio-solids quantity are given in **AL1-AL3.**

AL1.1: Bio-solids quantity produced at 5 g/L.d at 37 °C

Conditions		**Experiment number**					
Sewage sludge loading (g/L.d)	***Acti-zyme (g/L)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
	0.000	38.5	38.3	38.7	38.5	0.20	0.52
	0.020	23.6	23.4	23.2	23.4	0.20	0.85
	0.035	20.2	19.9	20.3	20.1	0.21	1.03
	0.040	17.7	17.4	17.1	17.4	0.30	1.72
	0.045	15.9	14.8	15.5	15.4	0.56	3.62
	0.050	12.3	12.1	11.9	12.1	0.20	1.65
5 g/L.d	0.070	12.1	11.8	12.2	12.0	0.21	1.73

AL1.2: Bio-solids quantity produced at 5 g/L.d at 55 °C

Conditions		**Experiment number**					
Sewage sludge loading (g/L.d)	***Acti-zyme (g/L)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
	0.000	72.6	72.8	72.4	72.6	0.20	0.28
	0.020	44.5	44.4	44.3	44.4	0.10	0.23
	0.035	40.4	40.9	40.5	40.6	0.26	0.65
	0.040	34.2	34.5	34.9	34.5	0.35	1.02
	0.045	26.3	26.5	26.9	26.6	0.31	1.15
	0.050	23.5	23.7	23.9	23.7	0.20	0.84
5 g/L.d	0.070	22.4	22.7	22.1	22.4	0.30	1.34

AL2.1: Bio-solids quantity produced at 7.5 g/L.d at 37 °C

Conditions		**Experiment number**					
Sewage sludge loading (g/L)	***Acti-zyme (g/L)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
	0.000	57.3	57.1	57.4	57.3	0.15	0.27
	0.020	38.9	38.6	38.5	38.7	0.21	0.54
	0.035	24.6	25.9	25.8	25.4	0.72	2.84
	0.040	18.1	18.8	18.2	18.4	0.38	2.06
	0.045	13.9	14.2	13.7	13.9	0.25	1.81
	0.050	12.7	11.9	12.5	12.4	0.42	3.37
7.5 g/L.d	0.070	12.6	12.3	12.1	12.3	0.25	2.04

AL2.2: Bio-solids quantity produced at 7.5 g/L.d at 55 °C

Conditions		**Experiment number**					
Sewage sludge loading (g/L)	***Acti-zyme (g/L)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
	0.000	110.2	110.7	110.4	110.4	0.25	0.23
	0.020	68.7	68.3	68.5	68.5	0.20	0.29
	0.035	54.9	54.2	54.5	54.5	0.35	0.64
	0.040	28.3	28.8	28.2	28.4	0.32	1.13
	0.045	23.9	23.7	23.3	23.6	0.31	1.29
	0.050	22.5	22.9	22.1	22.5	0.40	1.78
7.5 g/L.d	0.070	22.8	22.3	22.2	22.4	0.32	1.43

AL3.1: Bio-solids quantity produced at 10 g/L.d at 37 °C

Conditions		**Experiment number**					
Sewage sludge loading (g/L)	***Acti-zyme (g/L)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
	0.000	76.5	76.4	76.1	76.3	0.21	0.27
	0.020	53.8	53.6	53.1	53.5	0.36	0.67
	0.035	34.5	34.2	33.9	34.2	0.30	0.88
	0.040	27.1	26.9	27.3	27.1	0.20	0.74
	0.045	20.2	19.9	20.4	20.2	0.25	1.25
	0.050	16.9	16.5	16.7	16.7	0.20	1.20
10 g/L.d	0.070	17.2	16.8	16.5	16.8	0.35	2.09

AL3.2: Bio-solids quantity produced at 10 g/L.d at 55 °C

Conditions		**Experiment number**					
Sewage sludge loading (g/L.d)	***Acti-zyme (g/L)***	***1***	***2***	***3***	***Average***	***Standard deviation***	***% Standard deviation***
	0.000	150.1	150.6	150.7	150.5	0.32	0.21
	0.020	100.9	100.6	100.1	100.5	0.40	0.40
	0.035	64.7	64.2	64.9	64.6	0.36	0.56
	0.040	54.3	54.9	54.7	54.6	0.31	0.56
	0.045	40.4	40.9	40.2	40.5	0.36	0.89
	0.050	31.9	31.5	31.7	31.7	0.20	0.63
10 g/L.d	0.070	31.3	31.6	31.8	31.6	0.25	0.80

APPENDIX M: t-test ANALYSES FOR INFLUENCE OF MESOPHILIC AND THERMOPHILIC TEMPERATURE ON BIOGAS AND BIO-SOLIDS PRODUCTION

AM1: Paired samples test for influence of mesophilic and thermophilic conditions on biogas production

	Paired Differences					T	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1 37^oC-55^oC	121.48	54.23	11.83	96.79	146.16	10.27	20	0.000

AM2: Paired samples test for influence of mesophilic and thermophilic conditions on bio-methane production

	Paired Differences					t	de	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1 37^oC-55^oC	35.29	3.04	0.66	33.91	36.67	53.27	20	0.000

AM3: Paired samples test for influence of mesophilic and thermophilic conditions on carbon dioxide production

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1 37^oC-55^oC	7.10	5.22	1.14	4.72	9.47	6.22	20	0.000

AM4: Paired samples test for influence of mesophilic and thermophilic conditions on trace gases production

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1 37^oC-55^oC	1.53	1.36	0.30	0.90	2.143	5.12	20	0.000

AM5: Paired samples test for influence of mesophilic and thermophilic conditions on bio-solids production

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1 37^oC-55^oC	-24.15	16.80	3.67	-31.80	-16.50	-6.59	20	0.000

APPENDIX N: QUADRATIC MODEL ERROR ANALYSIS DATA

The statistical quadratic models for modelling the biogas and bio-solids experimental data at sewage sludge loadings of 7.5 g/L.d and retention times of 40 days are given.

AN1: Quadratic model for biogas production error analyses

Acti-zyme (g/L)	Biogas quantity actual (mL/day)	Biogas quantity quad model (mL/day)	Biogas relative error (mL/day)
0	108.67	94.02	0.13
0.02	215.33	249.24	0.16
0.035	319.00	330.48	0.04
0.04	360.33	350.86	0.03
0.045	388.67	367.89	0.05
0.05	394.00	381.57	0.03
0.07	389.67	402.79	0.03

AN2: Quadratic model for bio-solids production error analyses

Acti-zyme (g/L)	Bio-solids quantity actual (g/L)	Bio-solids Quantity Quad Model (g/L)	Bio-solids Relative error (g/L)
0.000	57.27	58.94	0.03
0.020	38.67	35.32	0.09
0.035	25.43	22.85	0.10
0.040	18.37	19.70	0.07
0.045	13.93	17.04	0.22
0.050	12.37	14.89	0.20
0.070	12.33	11.27	0.09

AN3: Comparison of bio-methane quadratic model for biogas production and the logistic kinetic model

Acti-zyme (g/L)	Time (days)	Bio-methane Logistics Kinetic Model (mL/day)	Biogas Quantity Quad Model (mL/day)	Bio-methane Quad Mode (mL/day)l
0.000	0	18	94.019	73
0.020	10	37	249.239	194
0.035	20	70	330.479	258
0.040	30	126	350.859	274
0.045	35	162	367.889	287
0.050	40	202	381.569	298
0.070	60	355	402.789	314

APPENDIX O: ECONOMIC ANALYSIS DATA

The economic analysis data for sewage treatment co-harnessing biogas and bio-solids is given in Appendix N.

AO1: Break even chart analysis data

Electricity produced (KWh)	Fixed costs ($)	Variable costs ($)	Total costs ($)	Sales ($)
0	1795810.4	0	1795810.4	0
20000	1795810.4	1000	1796810.4	197200
40000	1795810.4	2000	1797810.4	394400
60000	1795810.4	3000	1798810.4	591600
80000	1795810.4	4000	1799810.4	788800
100000	1795810.4	5000	1800810.4	986000
120000	1795810.4	6000	1801810.4	1183200
140000	1795810.4	7000	1802810.4	1380400
160000	1795810.4	8000	1803810.4	1577600
180000	1795810.4	9000	1804810.4	1774800
200000	1795810.4	10000	1805810.4	1972000
220000	1795810.4	11000	1806810.4	2169200
240000	1795810.4	12000	1807810.4	2366400
260000	1795810.4	13000	1808810.4	2563600
280000	1795810.4	14000	1809810.4	2760800
320000	1795810.4	16000	1811810.4	3155200
340000	1795810.4	17000	1812810.4	3352400
360000	1795810.4	18000	1813810.4	3549600
400000	1795810.4	20000	1815810.4	3944000

AO2: Break even sensitivity analysis data

AO2.1: Break even sensitivity analysis for varying electricity selling price

Deviation	**-20%**	**-15%**	**-10%**	**-5%**	**0%**	**5%**	**10%**	**15%**	**20%**
Unit price ($)	7.89	8.38	8.87	9.37	9.86	10.35	10.85	11.34	11.83
Variable cost ($)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Fixed cost ($)	1,795,810	1,795,810	1,795,810	1,795,810	1,795,810	1,795,810	1,795,810	1,795,810	1,795,810
Breakeven point	229,116	215,558	203,514	192,746	183,059	174,300	166,340	159,076	152,420

AO2.2: Break even sensitivity analysis for varying variable cost

Deviation	-20%	-15%	-10%	-5%	0%	5%	10%	15%	20%
Unit price ($)	9.86	9.86	9.86	9.86	9.86	9.86	9.86	9.86	9.86
Variable cost ($)	0.04	0.04	0.05	0.05	0.05	0.05	0.06	0.06	0.06
Fixed cost ($)	1,795,810	1,795,810	1,795,810	1,795,810	1,795,810	1,795,810	1,795,810	1,795,810	1,795,810
Breakeven point	201,203	201,254	201,305	201,356	183,059	183,106	183,153	183,199	183,246

AO2.3: Break even sensitivity analysis for varying fixed cost

Deviation	-20%	-15%	-10%	-5%	0%	5%	10%	15%	20%
Unit price ($)	9.86	9.86	9.86	9.86	9.86	9.86	9.86	9.86	9.86
Variable cost ($)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Fixed cost ($)	1,436,648	1,526,439	1,616,229	1,706,020	1,795,810	1,885,601	1,975,391	2,065,182	2,154,972
Breakeven point	146,447	155,600	164,753	173,906	183,059	192,212	201,365	210,518	219,671