Development of single-stage solar-supported hyper-thermophilic anaerobic reactor for biogas production and disinfection of black water. A pilot case study of Terterkessim slum, Elmina – Ghana

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By

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Declaration

I hereby declare that I have never had any permission to the final examination for a doctoral degree cancelled nor I have had a doctoral degree disqualified as a result of an attempt to deceive. I never made any attempt of deceit after entering the International PhD Programme ERM at the BTU Cottbus-Senftenberg.

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Dedication

This thesis is dedicated to my wife, Mrs Rita Mbir Bryant and our three boys, Samuel Fanyinka Bryant, Nhyiraba Joseph Kingsley Bryant and Matthias Hayford Bryant for having the understanding and forbearance that I had to leave them in Ghana to pursue this career dream. Also to my late dad (Mr Joseph Kingsley Bryant) and late mum (Madam Sabina Oduro) for bringing forth a son, who can contribute his iota of knowledge to the global wealth of knowledge. Caroline Aboagye can never be left out, since you were committed in helping with caring for my children in my absence. You deserve this accolade as well.

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My favourite quotes:

Your wastes, my sources of energy and fertiliser – Isaac Mbir Bryant

It is worth to try and fail than to fail to try – William F O'Brien

'Then Samuel took a stone and set it up between Mizpah and Shen and called its name Ebenezer, saying, "Thus far the LORD has helped us -- 1 Samuel 7:12' New King James Version (NKJV) of the HOLY BIBLE.

Summary

Rapid urbanisation in Ghana has resulted in individuals expanding the built-up area in the cities to make them their abodes without any considerations of the negative externalities these may have on the environment. One of the major challenges with rapid urbanisation is the formation of urban slums associated with lack of basic sanitation facilities. This has led to recurrent outbreak of a number of water and sanitation-related diseases such as cholera and typhoid fever. The use of a single-stage solar-supported hyper-thermophilic anaerobic biogas digester for the treatment of black water has not been investigated. Consequently, this study aimed at development and use of such biogas digester in Terterkessim slum in Elmina – Ghana, for simultaneous treatment of black water for biogas production and disinfection of digestate for agricultural use.

The performance of three seeding sludge under three different hyper-thermophilic temperatures (60 ^{o}C, 65 ^{o}C and 70 ^{o}C) were tested in batch tests based on Verein Deutscher Ingenieure (VDI) 4630 (2006) guidelines. The three seeding sludge were sewage sludge, sludge from maize silage and cow manure. The results from the batch tests showed cow manure at 65 ^{o}C as the preferred seeding sludge and optimal hyper-thermophilic temperature when a bigger set-up is to be considered. In locations where cow manure is not available to be used as seeding sludge, sewage sludge operating at 60 ^{o}C can be used. It was ascertained that treating black water at hyper-thermophilic temperature of 70 ^{o}C was not feasible irrespective of the seeding sludge used.

A 50 L single-stage laboratory-scale hyper-thermophilic continuous stirred tank reactor (HT-CSTR) with a revolution of 50 rpm was operated to treat only black water for 10 weeks using cow manure at 65 ^{o}C as the seeding sludge and optimal hyper-thermophilic temperature. Afterwards, co-digestion of blended kitchen food waste and black water was also practised for 12 weeks. With a mean hydraulic retention time (HRT) of 23.3 days, a mean total COD removal of 86.3 % was achieved. The reactor had an average COD volumetric loading rate of 6.22 kgCOD/(m^3.d) and remained uninhibited. It also had organic loading rate of 0.3 kgVS/(m^3.d) and a degradation performance (R) of 5.43 kgCOD/(m^3.d). Treatment of only black water produced biogas with less methane content of 34.9 % even though a stable pH of 6.9 was recorded both in the reactor and in the effluent. Co-digestion of the reactor with mixed substrate of BW and kitchen food waste increased the percentage content of methane in the biogas by 77 % from 34.9 % to 61.8 %. The cumulative methane productivity over the period for the HT-CSTR was 1.25 $Nm^3CH_4/(m^3.d)$, with daily average being 0.06 $Nm^3CH_4/(m^3.d)$ while its cumulative methane yield was 55.43 Nm^3CH_4/kgVS, averaging 2.52 Nm^3CH_4/kgVS per day within the study period. The effectiveness of the HT-CSTR to hygienise the effluent for agricultural purpose was assessed by spiking the reactor with 200 ml each of 2 x 10^9 CFU/ml

Salmonella senftenbergensis and 8 x 10^8 CFU/ml *Escherichia coli*. The laboratory-scale single-stage HT-CSTR was able to hygienise all bacteria of *Salmonella senftenbergensis* and *E. coli*. A simulation test confirmed that between 30 minutes and 1 hour, all the cells of *Salmonella senftenbergensis* and *E. coli* in the treatment system were killed at 65 ^oC. The seeding sludge for the HT-CSTR was also analysed to ascertain which methanogens were present. Eubacteria (EUB338 I), *Methanosarcina spp.* (MS821), *Methanomicrobium spp.* (MG1200) and *Methanococcus spp.* (MC1109) were identified in the seeding sludge at the hyper-thermophilic temperature of 65 ^oC.

The design, construction and performance of a pilot manually-stirred, fixed pyramidal-dome-shaped solar-supported hyper-thermophilic anaerobic biogas digester (SSHTABD) in Terterkessim slum in Elmina, Ghana was based on results from the laboratory-scale HT-CSTR. The SSHTABD had average daily flow rate of 182.1 L/d and mean HRT of 51.3 days. The mean daily COD volumetric loading rate and mean daily organic loading rate were 0.97 kgCOD/(m^3.d) and 0.06 kgVS/(m^3.d), respectively. The pilot single-stage SSHTABD achieved 97 % removal of the influent total COD and could produce about 2.52 Nm^3 CH_4/(kgCOD.d) which could be burned for at least 8 hours. The effluent produced contained less concentrations of metals compared with the effluent discharge standards set by the Environmental Protection Agency of Ghana, however, it cannot be used for cultivation of leafy vegetables such as cabbage and lettuce since it had some concentrations of pathogens like *Salmonella spp.* and *E. coli* but can be used for cotton crop.

Social survey was carried out to assess the perception of residents of Elmina on the concept of biogas technology and their willingness to accept it in their homes. Structured questionnaire were administered to 219 respondents in Elmina. About 43.8 % of the respondents in Elmina town did not have toilets in their homes. About 40.6 % used only charcoal for cooking while 29.7 % used only LPG, with 18.7 % using both charcoal and LPG for cooking. About 5.9 % used firewood while 4.6 % used electricity for cooking. The level of knowledge of the respondents on the concept of biogas technology was very low, particularly on co-digestion. Even though the respondents had little knowledge on biogas technology, about 86.3 % of them expressed their willingness to adopt and invest into having the single-stage SSHTABD technology in their homes. The potential to save money was the major reason that influenced the respondents' willingness to adopt and invest into having this technology in their homes. Easy access to cooking gas and availability of the gas in their homes also motivated the respondents' willingness to adopt the technology. However, 13.7 % were unwilling to have the technology in their homes for fear of explosion. **Keywords:** Black water, single-stage, anaerobic digestion, batch tests, solar-supported, hyper-thermophilic temperature, methane yield, methane productivity, degree of COD degradation.

Zusammenfassung

Die schnelle und unkontrollierte Urbanisierung führt in vielen Städten Ghanas zur Vergrößerung der Bebauungsfläche für individuelle Unterkünfte ohne Berücksichtigung der negativen Effekte für die Umwelt. Eine der größten Herausforderungen der schnellen Verstädterung ist die Ausbildung von Slums, die nicht über eine grundlegende sanitäre Infrastruktur verfügen. Dies führt zu wiederkehrenden Ausbrüchen von Abwasser- und Fäkalienassoziierten Krankheiten wie Cholera und Typhus Fieber. Eine Möglichkeit der Abwasserbehandlung liegt in der Anwendung von einstufigen solar-geheizten hyperthermophielen anaeroben Bioreaktoren, die bisher jedoch nicht untersucht wurde. Daher liegt der Fokus dieser Arbeit in der Entwicklung eines solchen Reaktors in einem Modellstandort in Ghana, dem Terterkessim Slum, um Schwarzwasser und Gärreste zu desinfizieren und für die Landwirtschaft nutzbar zu machen und gleichzeitig Biogas herzustellen. Die Betriebseigenschaften von drei Inokulationsschlämmen wurden unter drei verschiedenen hyperthermophielen Temperaturen (60 °C, 65 °C und 70 °C) nach der Richtline Verein Deutscher Ingenieure (VDI) 4630 (2006) in Batch-Ansätzen untersucht. Dabei handelt es sich um Belebtschlamm einer Kläranlage, Mais-Silage sowie Kuhdung. In den Batch-Ansätzen erwies sich Kuhdung bei 65 °C am besten als Inokulationsschlamm geeignet, wenn optimale hyperthermophiele Temperaturen und ein größerer Reaktor in Betracht kommen. In Gegenden ohne verfügbaren Kuhdung kann Klähranlagenschlamm bei 60 °C alternativ als Inokulum verwendet werden.

Ein einstufiger hyperthermophieler 50 Liter Laborreaktor (HT-CSTR) mit kontinuierlicher Rührung bei 50 Umdrehungen je Minute wurde mit Kuhdung inokuliert und 10 Wochen betrieben, um Schwarzwasser zu behandeln. Anschließend wurden zusätzlich über 12 Wochen zerkleinerte Lebensmittelabfälle zugegeben. Der Reaktor wurde im Mittel mit einer volumenbezogenen Beladungsrate von 6,22 kg CSB/m³·d beladen und blieb durchgängig ungehemmt. Die organische Beladungsrate betrug 0,3 kg suspendierte Trockenmasse je Kubikmeter und Tag. Der Reaktor erreichte eine Abbaurate (R) von 5,43 kg CSB/(m³·d). Bei einer hydraulischen Retentionszeit von 23,3 Tagen wurden durchschnittlich 86,3 % des Chemischen Sauerstoffbedarfs abgebaut. Bei der Behandlung von Schwarzwasser konnte nur ein Biogas mit geringem Methangehalt von 34,9 % erzeugt werden, obwohl stabile pH-Werte von 6,9 im Reaktor und im Reaktorabfluss gemessen wurden. Die Co-Vergärung von Schwarzwasser und Küchenabfällen erhöhte den Methangehalt im Biogas um 77 % von 34,9 auf 61,8 %. Die Methanproduktivität über die gesamte Versuchsdauer des HT-CSTR betrug 1,25 $Nm^3CH_4/(m^3.d)$ mit einer durchschnittlichen täglichen Produktivität von 0,06 $Nm^3CH_4/(m^3.d)$. Insgesamt wurden während der Versuchsdauer 55,43 $Nm^3CH_4/kgVS$ produziert mit einem durchschnittlichen täglichen Ertrag von 2,52 $Nm^3CH_4/kgVS$.

Die Effektivität der Hygienisierung durch den HT-CST, um dessen Abfluss zur landwirtschaftlichen Bewässerung zu nutzen, wurde durch die Zugabe von je 200 ml einer $2 \cdot 10^9$ KBE/ml *Salmonella senftenbergensis* und $8 \cdot 10^8$ CFU/ml *Escherichia coli* Kultur untersucht. Der einstufige HT-CSTR Laborreaktor konnte die Bakterien der beiden Referenzstämme vollständig abtöten. Eine Simulation mit beiden Stämmen bestätigte eine vollständige Desinfektion nach 30 bis 60 Minuten bei 65°C. Der Inokulationsschlamm für den Reaktor wurde mittels FISH auf verschiedene Methanogen Bakterien untersucht. Es konnten *Methanosarcina spp.* (MS821), *Methanomicrobium spp.*(MG1200) und M*ethanococcus spp.* (MC1109) im Inokulationsschlamm bei hypertermophielen Temperaturen von 65 °C identifiziert werden, die Sonde (EUB338 I) für Eubacterien diente als Kontrolle.

Nach den erfolgreichen Laborversuchen wurde im Terterkessim Slum in Elmina, Ghana, ein manuell gerührter, pyramidal-kuppelförmiger, Solar-geheizter, hypothermophieler anaerober Biogasreaktor als Pilotanlage errichtet, dessen Design, Konstruktion und Betriebseigenschaften auf dem Laborreaktor basieren. Der Pilotreaktor hatte ein durchschnittliches tägliches Beladungsvolumen von 182,1 L/d mit einer durchschnittlichen hydraulischen Retentionszeit von 51,3 Tagen. Die mittlere volumenbezogene CSB-Beladungsrate betrug 0,97 kg COD/(m^3.d), die Beladung mit Trockenmasse betrug 0,06 kg VS/(m^3.d). Die Pilotanlage konnte 97 % des eingehenden CSB abbauen und produzierte 2,52 Nm^3 CH_4/(kgCSB.d). Die Metallkonzentrationen im Abwasser des Reaktors unterschritten die Grenzwerte der Umweltbehörden von Ghana. Allerdings waren pathogene Bakterien wie *Salmonella spp.* und *E. Coli* nachweisbar, weshalb der Ablauf nicht für die Bewässerung von Blattgemüse wie Kohl verwendet werden kann, für Baumwolle hingegen schon.

Eine Umfrage mittels strukturierten Befragungen von 219 Einwohnern von Elmina sollte deren Einstellung zum Konzept der Biogastechnologie und deren Bereitschaft zur Nutzung untersuchen. 43,8 % der Befragten verfügen über keine Toilette im eigenen Haushalt. 40,6 % nutzten ausschließlich Holzkohle zum Kochen, 29,7 % nutzten nur LPG und 18,7 % nutzten beide Energieträger. Nur 5,6 % nutzten Feuerholz und 4,6 % Elektrizität zum Kochen. Der Wissensstand zum Thema Biogas, speziell zur Co-Vergärung, war unter den Befragten sehr gering. Trotzdem zeigten sich 86,3 % der Befragten zu Veränderungen bereit und würden in die SSHTABD-Technologie für ihre Häuser investieren. Die Hauptmotivation lag in der Möglichkeit des Geldsparens neben der einfachen Verfügbarkeit von Heizgas. Die anderen 13,7 % lehnten die Technologie für ihre Häuser aus Angst vor Explosionen ab.

Keywords: Schwarzwasser, einstufig, anaerobe Vergärung, Batch-Tests, Solar-beheizt, hyperthermophil, Methanertrag, Reaktorproduktivität, CSB-Abbaurate

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List of equipment

OHAUS, Adventurer Pro top pan balance (MODEL AV313, SERIAL NUMBER 8732199166, Switzerland)

Oven (Heraus Function Line UT12, Fabric No. 40309216, maximum temperature, 250 ^{o}C, Made in Germany)

Desiccator (Vakuumfest DURAN, Made in Germany)

Furnace (Nabertherm-More than Heat 30-3000 ^{o}C, B180, S/N. 223453, L-150KICN, L-15/11/B180, Nabertherm GmbH, Made in Germany)

Gas flow meter (Ritter, TG05/5, made in Germany)

Gas Analyser: Geotechnical Instrument – Biogas Ansyco, UK.

WTW Series InoLab pH/Conductivity 720 equipment (S/N. 09041928, Made in Germany)

Merck COD-Thermoreaktor TR300 MERCK ((MERCK, 64293, Darmstadt, Made in Germany)

COD Spectroquant NOVA 60 by MERCK (MERCK, 64293, Darmstadt, Made in Germany)

CEM MARS Xpress Microwave (S/N. BR601050, made in USA)

Shaker (Heidolph REAX 2000, S/N. 119552376-11/95, maximum speed 7, Made in Germany)

UV-2450 UV-VIS Spectrophotometer-SHIMADZU (model UV-2450 (PC), S/N A10834732924 CS, Manufactured by Shimadzu Suzhou Instruments Mfg. Co. Ltd., Suzhou Jiangsu-China, SHIMADZU Corporation, Kyoto Japan)

Respirometer (BOD measuring system BSB digi, SELUTEC GmbH, 72379, Hechingen, HRB: 381947 AG Stuttgart, USt-ID-Nr.: DE 198 436 332, Germany)

Gerhardt Vapodest equipment (Gerhardt Vapodest, Made in Germany)

Behrotest tube (Düsseldorf aufschlußgefäß SR 3i)

TITRONIC® (Titronic® Universal TZ 3260 Nr. M003189 SI Analytics, S/N 00693908, D-55122, Mainz-Germany)

Centrifuge (Hettich-Universal 320 R, S/N 000-3247-0200, Made in Germany)

Eppendorf Thermostat Plus

Water bath (GD120-Grant S/N-GM0920015, Grant Instruments – Cambridge Ltd, Cambridgeshire SGB 6GB England

Microscope (Nikon H600L, MODEL: Nikon Eclipse LV100)

Photovoltaic panel (model number 3-01-001260, made by offgridtec® AGM GmbH, CMK ENERGY, Germany)

Solar charge controller (Stecca PR1010 756.477 by Solar Electronics, PV offGrid, PV Autarke systeme, made in EU)

AGM gel battery series (by offgridtec AGM GmbH, Germany)

NP series pure sine wave inverter (Model number NP 300, made by Solartronics, Leipzig-Germany)

Atomic Absorption Spectrophotometer (AAS) (Model: BUCK 210 VGP, serial number 922, 58 Fort Point St. East Norwalk, CT 06855, U.S.A.)

List of chemicals

Tartrate solution (p.a. by MERCK, KGaA, 64271, Darmstadt, Germany)

Nesslers reagent (p.a by S.No.: 1.09028.0500, MERCK, KGaA, 64271, Darmstadt, Germany)

Ascorbic Acid (p.a. by VWR-PROLABO-BDH)

Molybdenum (Reagent A: 13 g Ammonium heptamolybdate (≥ 99 %) x $10H_2O$ in 100 ml H_2O solution and 300 ml 50 % H_2SO_4. Reagent B: 0.35 g Potassium antimony(III) oxide tartrate-hydrate 99 % in 100 ml H_2O and mixed thoroughly) (Produced by Sigma-Aldrich).

2N NaOH (purity 99.3 % by VWR-PROLABO-BDH, Made in Czech Republic)

Salicylic powder (purity 99.8% by VWR-PROLABO-BDH, Made in EC-EMB 45053)

H_2SO_4 (purity 95-97 %, CAS No. 7664-93-9, MERCK, KGaA, 64271, Darmstadt, Germany)

1 % sulphanilamide in 1.5 N HCl and 0.02 % NED (SA/NED-reagent)

H_3PO_4 (85 %) (purity, 85 %, CAS No 7664-38-2 by VWR Chemicals-PROLABO-BDH, Made in EC)

NaOH used (p.a., 1.0M diluted in water AVS TITRINORM, Reag. Ph Eur.USP) (CAS No 1310-73-2, S/N: UNI824 by VWR Chemicals-PROLABO-BDH, Made in EC)

Phenolphthalein indicator (1 % in ethanol, pH 8.2-9.8, by MERCK, KGaA, 64271, Darmstadt, Germany)

Nutrient Broth II (SIFIN, D-13088, Berlin – Germany)

Brilliant green powder (Modified) (OXOID Ltd, Wade Road, Basingstoke, Hants, RG24 8PW, UK)

Endo selective media (SIFIN, D-13088, Berlin - Germany)

Phosphate Buffer Solution (PBS) (2.3 g $Na_2HPO_4x7H_2O$, 16 g NaCl and 0.4 g KH_2PO_4 in 200 ml distilled water)

Paraformaldehyde (PFA) – (Carl ROTH GmbH & Co. KG, reinst., Schoemperlenstrasse 3-5, D-76185, Karlsruhe, Germany).

Sodium Dodecyl Sulphate (SDS) (purity 98 %, by Sigma-Aldrich, D-89555, Steinheim – Germany; Fluka BioChemika, product of Japan).

Tris (hydroxymethyl) aminomethane (purity 99.8 % - 100.1 % by MERCK, KGaA, 64271, Darmstadt, Germany)

EDTA (purity 99 % by Riedel-de Haeen, Wunstorfer Str. 40, 30926, Seelze-Germany)

List of abbreviations

AD = Anaerobic Digestion

Avg. = Average

BOD = Biological Oxygen Demand

BTU = Brandenburg University of Technology

BW = Black Water

CM = Cow Manure

COD = Chemical Oxygen Demand

CSTR = Continuous Stirred Tank Reactor

CT = Contact Time

DM = Dry Matter

DOC = Dissolved Organic Carbon

Eff = Effluent

EPA = Environmental Protection Agency

EU = European Union

FW = Food Waste

GIS = Geographic Information System

HRT = Hydraulic Retention Time

Inf = Influent

K.E.E.A. Municipality = Komenda Edina Eguafo Abirem Municipality (Name of the District where Terterkessim is found)

LCFAs = Long-Chain Fatty Acids

LWG = Lausitzer Wasser GmBH & Co. Kg

MY = Methane Yield

OLR = Organic Loading Rate

POC = Purgeable Organic Carbon

SRT = Sludge Retention Time

SSHTABD = Solar-Supported Hyper-Thermophilic Anaerobic Biogas Digester

STAR = Solar Thermophilic Anaerobic Reactor

TC = Total Carbon

TIC = Total Inorganic Carbon

TOC = Total Organic Carbon

TS = Total Solids

TVS = Total Volatile Solids

VDI = Verein Deutscher Ingenieure (Association of German Engineers)

VFAs = Volatile Fatty Acids

VLR = Volumetric Loading Rate

VOC = Volatile Organic Carbon

VS = Volatile Solids

Glossary of terms

'Akasha'**:** *A locally-manufactured bleaching agent used in cleaning bathrooms and sinks in Ghana*

'Akpeteshie': *A locally-manufactured alcoholic gin prepared from sugarcane*

Anaerobic Digestion*: This is a biological process used in the breakdown of organic matter in the total absence of oxygen.*

'Banku'*: A local food in Ghana prepared from ground corn and cassava*

Batch fermentation test: *This is an anaerobic digestion process carried out on a substrate in an air-tight container already seeded with a sludge and ensuring that the entire process is completed before another test could be done.*

Biogas: *This is a form of gas composed of methane, carbon dioxide, water vapour, hydrogen, ammonia and hydrogen sulphide.*

Digestate*: This is a thick paste-like remains enriched with nutrients after anaerobic digestion process.*

'Fufu': *A local food in Ghana prepared from pounded plantain and/or cassava, yam/cocoyam*

Hygienisation: *This is a process of eliminating all the pathogenic agents in a digested material to ensure environmental quality.*

Hyper-thermophilic temperature*: This is a temperature regime that exceeds 60 °C but is less than 90 °C.*

Methane Content*: This is the percentage of methane present in the biogas*

Pathogen: *These are organisms that are infectious in nature and can cause diseases.*

Single-stage anaerobic digestion: *This is a type of anaerobic digestion where all the four steps (hydrolysis, acidogenesis, acetogenesis and methanogenesis) occur in the same reactor.*

Total Solids: *These are all the solids present in the waste materials.*

Volatile Solids: *These are components of waste materials that can be degraded microbiologically.*

CHAPTER ONE

Introduction

1.1 Background

Rural-urban migration is the movement of people from the rural areas to the cities which are considered to be urban centres (Nsiah-Gyabaah, 2003). Rapid urbanisation has resulted in individuals expanding the built-up areas in the cities to make them their abodes without any thorough considerations of the negative externalities these may have on the environment as a whole (Nsiah-Gyabaah, 2003). One of the major challenges with rapid urbanisation is inadequate housing, and when housing is available it is rather sub-standard and overcrowded for the urban dwellers leading to what is known as urban slums. Urban slum is described by the United Nations Agency UN-HABITAT as a run-down area of a city characterised by sub-standard housing and filth and lacking tenure security (UN-HABITAT, 2003). In Ghana, there are a number of urban slums in the major cities like Accra, Kumasi, Takoradi, Cape Coast and Tamale. Formation of urban slums comes with its associated poor sanitation and waste management problems, as well as air and water pollution. In most developing countries such as Ghana, access to sanitation facilities is a problem and this is exacerbated in the urban slums (Songsore, 2009). For example, it is estimated that about 38 % of the Ghanaian population dispose-off their domestic wastewater by throwing them on the un-tarred streets (outside their houses), while 21 % discharge their domestic wastewater directly into open drainage systems without any form of pre-treatment, with 31 % of the domestic wastewater being discharged at other places. Only about 10 % of the wastewater from municipal and domestic sources are channelled through sewers to treatment plants for some form of treatment (Obuobie *et al.,* 2006).

Thus, almost all generated black water (BW) in Ghana are likely to find their way into nearby water bodies, the sea or buried directly in the ground without any pre-treatment. The question is: What are the possible treatment facilities available for pre-treatment of BW in urban slums in Ghana that enable recovery of included resources such as renewable energy, water and fertiliser? The use of biogas plant such as the Solar Thermophilic Anaerobic Reactor (STAR) has been identified as one of the technologies for treating liquid and solid manure for the production of bio-energy (El-Mashad, 2003). This can be adopted in dealing with this menace of poor sanitation associated with urban slums and more especially with the discharge of untreated BW in urban slums in Ghana. BW, which is part of this domestic wastewater has high organic matter in the form of chemical oxygen demand (COD) which can be harnessed for methane production for domestic usage. Thus, cheap and adequate energy sources from

biogas production are imperative for socio-economic development of the world, especially for people in developing countries like Ghana (NAS, 1977). In addition, the digestate obtained when hygienised can be used for (peri)-urban agriculture. The development of a solar-supported high temperature anaerobic digestion system for BW would not only provide biogas to be used for cooking but also a digestate, free of pathogens, for safe use as fertiliser. Previous works have concentrated on treating black water with mesophilic temperatures (van Lier, 2008). However, these have not been effective in disinfection of the digestate. The high concentration and therefore low volume of faeces and urine enable the increase of the temperature using a solar collector with a relatively small surface area and application of high temperature (up to 70°C or more) in anaerobic treatment. Thermophilic anaerobic treatment (50-55°C) of especially animal manure is well established (Lv *et al.*, 2013) but hyper-thermophilic anaerobic treatment (≥ 60°C) of BW is hardly or not researched. Ahring *et al.* (2001), reported on the digestion of cattle manure at temperatures of 55 °C and 65 °C and concluded that it is possible to treat cattle manure at 65°C, nonetheless a lower methane yield is expected as well as an increased amount of volatile fatty acids in the effluent compared with treatment operation at 55°C. Kjerstadius *et a*l. (2013), confirm lower methane production by 10 % when sewage was treated at a thermophilic temperature of 60 ^{o}C compared with treatment temperatures of 55 ^{o}C and 35 ^{o}C at the same HRT and OLR.

This study focuses on BW because its management is a huge challenge in most developing countries including Ghana. Most people in Ghana lack the technical knowledge regarding anaerobic treatment technology. This research will not only provide knowledge on the anaerobic treatment of BW using biogas digester for CH_4 production but also disinfection of the digestate using solar energy for its application on (peri)-urban agricultural lands. This makes this study very important to be carried out in an urban slum of Ghana.

1.2 Statement of the problem

Urban slums in Ghana like most urban slums in the world have poor sanitation as one of their major problems. This poor sanitation has its negative health implications such as outbreak of diseases: cholera, typhoid, dysentery and malaria. According to the World Health Organisation (WHO) at least 1.6 million of children under the age of five years are dying annually, attributable to lack of basic sanitation (World Health Organization, 2006). These poor sanitation-related diseases have killed many people living in these slums and the cities in general without their awareness. There is little or no information related to the design, testing and usage of hyper-thermophilic household biogas digester for anaerobic treatment of BW for

combined biogas production (energy) and disinfection of digestate for agricultural usage. It is therefore important to have a thorough study to address these issues.

There exist single, double and multiple stages of anaerobic treatments of wastewater. In the double and multiple stages of anaerobic treatment using mesophilic, thermophilic or hyper-thermophilic conditions, acidogenesis usually occurs in the first phase at a much higher temperature of 70 ^{o}C while methanogenesis occurs in either a normal mesophilic temperature of 37 ^{o}C or optimal thermophilic temperature of 55 ^{o}C (Lee *et al.,* 2009).

The use of a single-stage hyper-thermophilic anaerobic treatment for BW has not been investigated. However, single-stage systems are considered to be simple, easy to design and less expensive to be constructed and operated making them common in the anaerobic treatment technology applications (Foley *et al.,* 2015; Rapport *et al.,* 2008). Considering small scale anaerobic treatment systems, single-stage reactors have been often used compared to large scale reactors (with a capacity of more than 50 000 tons/year) that use multi-stage systems (Vögeli, Riu, Gallardo, Diener, & Zurbrügg, 2014). Consequently, in this study, a single-stage process will be adopted for both the laboratory and pilot research. In addition, producing a hygienised digestate fit for usage for agricultural purposes is worth mentioning if hyper-thermophilic temperature is used, hence the need to carry out this research. The type of seeding sludge to be used when employing single-stage hyper-thermophilic anaerobic wastewater treatment of BW is very paramount since absence of microbial flora in the sludge at hyper-thermophilic temperatures implies no degradation and removal of organic matter nor production of biogas (methane). It is, therefore, important first to know the type of seeding sludge suitable for use when considering the use of hyper-thermophilic temperature for anaerobic treatment of BW in a single-stage reactor for biogas production and the use of hygienised digestate for agricultural purposes.

1.3 Justification and benefits of the study

This research is worth pursuing because the outcome will seek to:

a. generate primary data on a suitable seeding sludge for laboratory-scale experiment and the performance of a laboratory-scale single-stage HT-CSTR for BW treatment co-digested with kitchen food waste.
b. generate primary data on design, construction and performance of a single-stage SSHTABD for treatment of BW for a household in Terterkessim slum which can be replicated in other parts of Ghana and Africa.

c. generate primary data from residents of Elmina on their knowledge on biogas technology and their willingness to adopt and invest into having the technology in their homes.
d. produce biogas for domestic cooking and reduce death from kitchen smoke inhalation.
e. minimize the outbreak of cholera as well as water and sanitation-related diseases in Ghana.
f. contribute to the production of clean and safe salt devoid of faecal matter contamination in the Elmina Salt Industry.
g. contribute to the production of sustainable clean and safe leafy vegetables in Ghana.
h. reduce deforestation in the quest for fuelwood and charcoal in Ghana.

1.4 Hypothesis of the study

The application of a single-stage solar-supported hyper-thermophilic anaerobic biogas digester for BW treatment in an urban slum of Ghana will result in economically feasible production of CH_4 gas for energy production and hygienised digestate for safe usage in (peri)-urban agriculture.

1.5 Objectives of the study

The purpose of this research is to design and test a simple single-stage BW anaerobic biogas digester operating at a hyper-thermophilic temperature with the use of solar energy for a household in Terterkessim urban slum in Elmina, the administrative capital of Komenda Edina Eguafo Abirem Municipality in the Central Region of Ghana. In order to achieve the above purpose, the following specific objectives were set:

1. To assess optimal hyper-thermophilic temperature for cumulative methane content, cumulative methane yield and degree of COD degradation in batch tests.
2. To use batch tests to determine the type of seeding sludge suitable for laboratory-scale continuous experiment in a single-stage hyper-thermophilic anaerobic digester.
3. To determine optimal parameters necessary for pilot-project in Ghana for methane productivity, yield and pathogen decay based on results from a laboratory-scale single-stage HT-CSTR.
4. To assess the type of methanogens present for methanisation in the sludge of the laboratory-scale single-stage HT-CSTR.

5. To assess the perception of residents of Elmina on their knowledge, willingness to accept and adopt a single-stage solar-supported hyper-thermophilic anaerobic biogas digester in their homes.
6. To design and construct a prototype pilot single-stage solar-supported hyper-thermophilic anaerobic biogas digester (SSHTABD) for one household in Terterkessim urban slum in Elmina, Ghana.
7. To determine the composition and quality of BW for some residents of Terterkessim urban slum, Elmina.
8. To assess the performance of a household single-stage SSHTABD for the treatment of BW co-digested with household food waste with respect to biogas potential and effluent quality.
9. To assess the cost-effectiveness of optimised single-stage SSHTABD for one household and its potential to reduce deforestation for charcoal production.

CHAPTER TWO

Literature review

2.1 Energy crisis in developing countries like Ghana

Globally, about one out of seven persons (1 billion people) lack access to electricity while more than two out of five persons (2.9 billion people) use a traditional source of energy for domestic purposes (Foley *et al.,* 2015). Foley *et al.* (2015), reported that deaths from breathing fumes and smoke from indoor cooking are expected to kill most Sub-Saharan African girls than malaria and malnutrition. Of the number that lacks access to electricity in the world, more than half (55 %) live in Sub-Saharan Africa followed by South Asia (34 %) and 11 % for others. A total of 87 % of the percentage without access to electricity in these regions live in rural areas while the remaining 13 % live in urban areas (Foley *et al.,* 2015). On the contrary, South America records the region with greater proportion of people without access to clean energy for cooking (38 %), followed by Sub-Saharan Africa (26 %), East Asia (21 %) and others (16 %) (Foley *et al.,* 2015). About 83 % of the people without access to clean energy for cooking live in rural areas while 17 % live in urban centers (Foley *et al.,* 2015). Despite Africa having about 15 % of the global population, its contribution to global electricity is only 4 % (147 GW), less than the amount generated by Germany alone, which is 188 GW (Foley *et al.,* 2015; Federal Ministry of Economic Affairs and Energy (bmwi), 2014). The total amount of renewable energy generated by 47 Sub-Saharan African countries (excluding South Africa) is 23 GW (with Ghana contributing only 5 MW; 3 MW solar, 2 MW biomass) (Ahiataku-Togobo, 2014), less than one-third of the total generated by India. However, the average per capita energy consumption of Africa is higher than South Asia which includes countries like Afghanistan, Bangladesh, Bhutan, India, Nepal, Pakistan, and Sri - Lanka (Foley *et al.,* 2015). These figures clearly indicate that renewable energy is highly under-developed in Sub-Saharan Africa and especially, Ghana. Ghana has been distressed with severe electricity challenges since 2006 resulting in the country losing a daily average of $ 2.1 million in the production sector (Kumi, 2017). Apart from the use of obsolete equipment for the distrbribution of electricity and non-payment of electricity bills accounting for inadequate supply of electricity in Ghana, the country also over-relies on thermal (fossil-based) and hydro for electrical power generation (Kumi, 2017). It is, therefore, very crucial for Ghana to expand the production of renewable energy such as biogas from BW for both industrial and household consumption. Consequently, the choice of a good technological process like anaerobic digestion for BW treatment for biogas production complimented with solar energy to hygienise the digestate is very relevant.

2.2 History of anaerobic digestion and biogas digester installations

The process of Anaerobic Digestion (AD) for wastewater treatment dates back to 1859 in Mumbai, India, where AD reactor was built for the treatment of sewage and later, it spread across to some Asian countries like China, Vietnam, Cambodia, Laos and Indonesia (Vögeli, *et. at.,* 2014). In China alone, around 40 million biogas installations have been recorded since 2011. In India and Vietnam, there were roughly 4 million and 10,000 biogas systems installed respectively, as at 2010. In Cambodia, Laos and Indonesia, even though there is the drive to use renewable energy but the number of biogas plants installations is not very high; each country has about 1000 biogas installations as at the year 2010. In 2010 alone, 25,000 biogas digesters were installed in Nepal, increasing the number of installations in that country to 330, 000 as at 2015. The sudden increase of biogas plants installations in Nepal in the past 10 years was as a result of a support program called Biogas Support Program that helped with financial commitments relating to biogas digester installations (Foley *et al.,* 2015). Apparently, the private sector, microfinance organizations, community groups and NGOs play a major role with the expansion, adoption and installations of this technology for renewable energy production (Ahmed *et al.,* 2011).

In European countries like Germany and the Netherlands, Costa *et al.* (2014), report on the proliferation of AD treatment technology for harnessing energy and nutrients from both liquid and solid organic substrates. For example, in Germany, there are more than 6, 000 functional AD plants that are producing biogas for usage since 2010 whereas the Netherlands records about 2, 766 small-scale to large-scale operational anaerobic wastewater treatment facilities for biogas production (van Lier, 2008). There are a number of biogas plants in Sweden to the extent that there are cars that run solely on biogas. This has resulted in the proliferation of many private to public biogas filling stations in especially, the south-west of Sweden (Bagge, 2009).

In Africa, the technology of AD for wastewater treatment for biogas production is emerging, however, since the launch of the African “Biogas for Better Life” in May 2007, the continent is targeted at having about two million biogas plants installed in rural households by 2020 (Vögeli *et al.,* 2014). As a result of this target, in Kenya and Tanzania for example, it is reported that about 2, 000 and 5, 000 biogas plants, respectively, have been constructed while only 250 biogas plants are said to have been constructed in Ghana (Bensah *et al.,* 2011). The number is very low in Ghana because of factors such as poor commitment from government, lack of or no follow-up services on the part of biogas companies, absence of policy on biogas, absence of well-tested standardised designs in the country, and lack of financial support

schemes for persons interested in using biogas digesters in the country (Bensah *et al.,* 2011). Therefore, it is very imperative to undertake this study which will lead to the installation of a single-stage SSHTABD in Ghana, not only for BW treatment but also for biogas production and digestate hygienisation. This will help to enlighten and convince Ghanaians on the possibility of this treatment technology for BW treatment at the household level. When the population accepts the technology and adopts, it will go a long way to first improve the sanitary conditions in Ghana and also help to reduce the energy crisis situation.

2.3 Anaerobic digestion process

Anaerobic digestion (AD) is a microbiological process by which organic matter either in the form of liquid or solid is broken down in the absence of oxygen. Technologically, AD process is employed in many sectors fundamentally as waste treatment approach to produce energy in the form of biogas and nutrient-rich digestate (van Lier, 2008). AD process is preferred to aerobic process (which requires oxygen for organic breakdown) because of high efficiency of COD removal, production of reduced quantities of excess sludge (Dereli *et al.,* 2012), reduction of dependency on fossil fuels, creation of jobs and closing the loop for nutrient cycle (Vögeli *et al.,* 2014). Other advantages include smaller reactor volume, rapid reactor start-up, little or no use of chemicals (van Lier, 2008), reduction of greenhouse gas like methane (CH_4) being released into the atmosphere and generation of renewable energy in the form of biogas which is a mixture of CH_4, CO_2, H_2S, O_2, H_2 and N_2 (Vögeli *et al.,* 2014). The biogas produced serves not only as a renewable energy source but also contributes to the protection of natural resources like forests by reducing deforestation for fuel wood especially in the developing countries (Vögeli *et al.,* 2014). The anaerobic digestion process can be subdivided into the following four phases: hydrolysis, acidogenesis, acetogenesis and methanogenesis with each stage requiring its own characteristic group of bacteria (Bajpai, 2017; van Lier, 2008).

2.3.1 Hydrolysis

Hydrolysis is the first step in the anaerobic process and it is the conversion of non-soluble biopolymers (solid organic matter) to water-soluble organic compounds by exo-enzymes which are secreted by hydrolytic and fermentative bacteria. Obligate hydrolytic fermentative bacteria such as *Bacteriocides, Clostridia* and *Bifidobacteria* and facultative type like *Streptococci and Enterobacteriaceae* secrete hydrolytic enzymes such as cellulases,

cellobiase, xylanase, amylase, lipase and protease to help breakdown of both monomers and polymers present in the substrate for AD (Bagi *et al.,* 2007). In the hydrolytic step, proteins are converted into amino acids, carbohydrates (polysaccharides) into glucose and lipids into long chain fatty acids. Temperature is one factor that affects this step and very important for consideration in the design of anaerobic digesters for wastewater with high suspended solid to COD ratio such as distillery. Higher temperatures generally ensure faster hydrolysis than lower temperatures. Consequently, hydrolysis is rate limiting step (Bagge, 2009; Bajpai, 2017).

2.3.2 Acidogenesis

In the acidogenesis step, amino acids, glucose and fatty acids from the hydrolysis step are converted intracellularly to volatile fatty acids (VFAs) which consist of acetate, propionate and butyrate. Also CO_2, H_2, some lactic acids, ethanol, H_2S and ammonia are produced. Conversion of fatty acids by beta-oxidation is a major source of acetate in the process (Bajpai, 2017; Vögeli *et al.,* 2014). The acidogenesis step is facilitated by hydrogen-producing acidogenic bacteria and is the most rapid step in the entire process. As carbonic acid is the main product in this step, the fermentative bacteria responsible for this step are considered to be acidogenic or acidifying (Bajpai, 2017; Vögeli et al., 2014).

2.3.3 Acetogenesis

In acetogenesis, hydrogen-consuming acetogenic bacteria convert short-chain fatty acids (except acetate) to acetate, hydrogen gas and carbon dioxide. Intermediate products such as propionate and butyrate are the most important substrates in this step as they are converted to acetate. Lactate, ethanol, methanol, H_2 and CO_2 are also converted to acetate. Acetogenic bacteria use beta-oxidation to convert long-chain fatty acids (LCFAs) such as oleate and linoleate to intermediate products such as propionate and butyrate, which are further converted to acetate and hydrogen for the next step of methanogenesis (Bagge, 2009; Bajpai, 2017; Vögeli *et al.,* 2014). Hydrogen is added to the LCFAs to make them saturated before their conversion (Vögeli *et al.,* 2014). The acetogens involved in this step are sensitive to the accumulation of hydrogen and thus any accumulation of hydrogen inhibits this step (Bagge, 2009). This is because, the acids are only converted if the partial pressure in the reactor is reduced by hydrogen-scavenging bacteria. Thus, lower concentration of hydrogen in the

reactor indicates the activeness of the hydrogen scavenging bacteria, for that matter, the reactor is functionally in good condition and vice versa (Vögeli *et al.,* 2014).

2.3.4 Methanogenesis

The final and most important step in the entire anaerobic conversion of organic matter is methanogenesis, as methane (energy) is produced. In the methanogenic step, methanogenic archaea-bacteria (*e.g. Methanosarcina* spp. and *Methanosaeta* spp.) convert both the acetate (main substrate which yields about 70% of the methane produced) and carbon dioxide by reduction reaction to biogas (mostly methane and carbon dioxide). The bacteria accomplish this by using hydrogen as electron donor. It is reported that biogas which has methane content of about 45 % is flammable (Bagge, 2009; Bajpai, 2017; Vögeli *et al.,* 2014). Methanogens have narrow substrate range making them obligate anaerobes with some depending solely on acetate, methylamines, methanol, formate and H_2/CO_2 or CO (Bagge, 2009; Bajpai, 2017; Vögeli *et al.,* 2014). This makes them vulnerable to factors such as low pH, organic loading rate, sudden changes in temperature, C/N ratio (organic carbon:organic nitrogen), as well as inhibitory substances for example, heavy metals and chlorinated organic compounds (Bagge, 2009). Methanogenic archaea are classified into two groups based on which reaction pathway they mediate during the process of methanogenesis. The first group is called acetoclastic or acetate-converting methanogens or acetotrophic methanogens, which convert acetate to methane and carbon dioxide while the second group, hydrogenotrophic methanogens (hydrogen-utilizing) convert carbon dioxide and hydrogen (formate) to methane (Bagge, 2009; Bajpai, 2017; Vögeli *et al.,* 2014). Hydrogenotrophic methanogens have higher growth rate with doubling time of about 4-12 hours compared to acetoclastic methanogens which have doubling time of several days. Thus, high sludge concentrations and long start-up time is needed for unadapted inoculum for anaerobic reactors (Vögeli *et al.,* 2014). Lignin disturbs the AD process by the foam it forms since it is not broken down during the digestion process (Bagge, 2009). Thus, it should be carefully considered that the substrate being used for the AD process does not contain lignin. Examples of those substrates include wood and its products as well as bark of a tree.

2.4 Factors that affect anaerobic treatment of wastewater (BW)

AD process in biogas digesters is noted to be easily disrupted by changes in external conditions as well as toxic levels of inhibitory substances like metal concentrations (Vögeli *et al.,* 2014). Performance of any reactor using the AD process with respect to wastewater treatment depends on several factors such as sludge biomass characteristics and retention, high salt concentration, temperature and pH. Other factors include toxicity, hydraulic retention time (HRT) and organic loading rate. When optimal conditions are attained, the efficiency of the reactor is also enhanced (Ozgun *et al.,* 2013).

2.4.1 Sludge biomass characteristics and retention

The efficiency of AD treatment technology for the treatment of wide range of wastewater in recent past is as a result of high retention of slow-growing methanogenic biomass, for example in the granular form. In a review by van Lier (2008), it is reported that raising the temperatures to extreme values may disturb the performance of the sludge bed systems and thus affecting the stability of methanogenic granular sludge and biofilm. For this reason, the use of membrane to retain the biomass has been reported (Dereli *et al.,* 2014). Consequently, some of the present day high rate anaerobic treatment technology use membrane for complete biomass separation from the effluent thereby producing high quality effluents which are free of pathogens and solids (Dereli *et al.,* 2014). Due to its efficient nature, this anaerobic membrane bioreactor can be used for different wastewater with characteristics such as high concentrations of suspended solids, high salinity and high temperature (Ozgun *et al.,* 2013). Membrane bioreactors are also used when the micro-organisms are slow-growing (Vögeli *et al.,* 2014). It is therefore recommended that biogas digesters are started at least 3 months before they become operational as methanogens have slow growth rate (Deublein and Steinhauser, 2011; Sheth, 2009).

2.4.2 Effect of salt concentration

High salt concentration has been reported to inhibit the activities of non-adapted biomass in the reactor during AD. This is caused by the stress on the microbial enzymatic activities leading to decay of the cell thus decreasing the performance of the anaerobic reactor (Ismail *et al.,* 2010). High salinity of the wastewater prevents granulation of the sludge and the overall performance of the anaerobic reactor. The inhibition caused by high salinity increases when other ions are also present in the reactor and thus in the absence of other ions, the anaerobic treatment of saline wastewater is possible when the biomass have been able to adapt to the

high salinity concentration (Ozgun *et al.,* 2013). Long-term adaptation of biomass to high salinity concentration results in high sodium tolerance and has little negative effects on the specific methanogenic activity of the biomass between 10 g Na+/L and 20 g Na+/L (Ismail *et al.,* 2010). However, in a review by van Lier (2008), it is reported that raising the salinity to extreme values may disrupt the performance of the sludge bed systems, thus affecting the stability of methanogenic granular sludge and biofilm.

2.4.3 Temperature

The influence of temperature on the AD process cannot be over-emphasised since it affects the percentage concentration of methane in the overall biogas produced (Arikan *et al*., 2015). The digestion temperature also influences the biogas yield as well as anaerobic digestion rate. Different temperature regimes affect biogas yield in the anaerobic digestion process. The higher the temperature, the better the methane yield but this also depends on the SRT. However, the yield does not linearly increase with increasing temperature (Chae *et al.*, 2008). With optimal mesophilic temperature occurring mostly between 35 ^{o}C – 37 ^{o}C, Bergland *et al.*, (2015) and Arikan *et al.,* (2015) reported in separate works that mesophilic temperatures within a range of about 22 ^{o}C - 30 ^{o}C could produce methane yield similar to that of 35 ^{o}C – 37 ^{o}C. However, the yields at 30 ^{o}C and 35 ^{o}C are higher than the yield at 25 ^{o}C by more than 13 –17 % (Chae *et al.*, 2008).

In terms of mesophilic anaerobic digestion rate, every 1 ^{o}C decrease in temperature below the optimum range (30 ^{o}C to 40 ^{o}C) reduces the digestion rate by 11 % based on Arrhenius equation, thus hydrolysis of organic matter at lower temperatures is very slow (Deublein and Steinhauser, 2011; Sheth, 2009). In spite of this, anaerobic treatment can still be applied to temperature ranges of 10 ^{o}C and 80 ^{o}C based on research from bench and pilot scale systems (van Lier *et al.,* 1997). Contrary to what was reported in van Lier *et al.* (1997), it is reported in the work of Vögeli *et al.* (2014), that there are two main ideal temperature ranges for efficient performance by anaerobic bacteria. These are mesophilic range of 30 ^{o}C to 40 ^{o}C with optimal being 37 ^{o}C, and thermophilic range of 45 ^{o}C to 60 ^{o}C with optimal being 55 ^{o}C. It is also reported that operations of anaerobic digesters in mesophilic condition are more stable compared to thermophilic as the microbes are more tolerant to changes in the mesophilic temperature range. Consequently, higher biodiversity of microbes have been reported for mesophilic condition as opposed to thermophilic. In addition, AD operations at mesophilic condition consume less energy and are not often inhibited by ammonium, however, the process requires longer residence time for maximum biogas production since the microbes are slow growing compared to those of thermophilic condition (Deublein and Steinhauser,

2011; Sheth, 2009). Slight increase in temperature in the thermophilic regime (52 ^{o}C – 57 ^{o}C) causes a change in the methane yield (Navickas *et al*., (2013). However, this result does not mean that the higher the temperature the more optimal the production of biogas, due to the larger energy requirement at higher digesting temperatures (Deublein and Steinhauser, 2011; Sheth, 2009; Chae *et al.,* 2008). Singh (2008), extensively studied on extreme environments and extremophiles and reported that the majority of thermophilic bacteria have optimal growth temperature ranging from 50 ^{o}C to 70 ^{o}C even though some could grow slowly at a lower temperature of 40 ^{o}C. Others can also grow at very high extreme temperatures of 80 ^{o}C and 110 ^{o}C, with examples being eubacteria and archaeabacteria. Consequently, the term hyper-thermophiles was used to describe micro-organisms that can only grow at temperatures 60 ^{o}C and above while most extreme hyper-thermophiles are micro-organisms that cannot grow below 90 ^{o}C, with an example being *Pyrolobus fumarii* (Singh, 2008).

Even though thermophilic methanogens are considered to be more temperature-sensitive (within a range of +/− 2 °C) than mesophilic ones, they have about 50 % higher rate of organics degradation and thus higher biogas yield. Another advantage with the thermophilic digestion is the hygienisation of the digestate for agricultural use. Deublein and Steinhauser (2011) and Sheth (2009), all reported that pathogenic microbes are totally destroyed at thermophilic temperature greater than 55 °C with a hygienisation retention time of 24 hours. Furthermore, due to less solubility of oxygen in the thermophilic temperature range, optimal anaerobic operational conditions are easily reached. Conversely, at higher temperatures, CO_2 concentration increases by 2 to 4 % because of its lower solubility and this may increase the percentage of CO_2 in the biogas produced (Vögeli *et al.,* 2014). Thermophilic digestion of wastewater like BW can be feasible in a tropical developing country like Ghana if external renewable energy source like the use of solar energy (which can be readily available in Ghana) is used for heating during thermophilic digestion and the digester is well insulated and buffered by soil temperature. Biogas digesters built underground are less susceptible to sudden temperature fluctuations as they use the temperature of the soil as temperature buffer (Vögeli *et al.,* 2014). In this way, thermophilic or hyper-thermophilic digestion will be preferred to mesophilic digestion because of its safe digestate and higher biogas produced.

2.4.4 Effect of pH

The first three steps of anaerobic digestion can occur in a wide range of pH values, while methanogenesis only proceeds when the pH is close to neutral. For pH values outside the range 6.5 - 7.5, the rate of methane production is lower (Kjerstadius *et al.,* 2013) and if the pH

is lower than 5, methane production stops altogether when acetoclastic methanogens are involved. However, hydrogen utilizing methanogens can still be active even at pH lower than 5, making them more tolerant to more acidic conditions than acetoclastic methanogens (Kim, *et al.,* 2004). The lowering of the pH occurs because acid forming bacteria grow much faster than methane forming bacteria. In that case, if acid-producing bacteria grow too fast, they may produce more acid than the methane forming bacteria can consume and thus excess acids (VFAs) build up in the reactor. The pH drops and the system may become unbalanced (Kim, *et al.,* 2004). Acidic pH can be neutralized by the addition of sodium salt, lime, sodium bicarbonate ($NaHCO_3$) or sodium hydroxide (NaOH) (Kjerstadius *et al.,* 2013). Sodium bicarbonate and sodium hydroxide are, however, expensive and not easily available especially, in developing countries like Ghana, thus lime or sodium salts will be much recommended.

2.4.5 Hydraulic Retention Time (HRT)

The Hydraulic Retention Time (HRT) describes how long the liquid component of the influent stays in the reactor. The HRT is the ratio of the active reactor volume to the flow rate of the substrate. Most anaerobic systems are designed to retain the waste for some time. The HRT is important since it establishes the time available for bacterial growth and subsequent conversion of the organic material to biogas. Biomass retention in the reactor requires that the system has different HRT and SRT. Low HRTs with the intention of promoting granulation lead to washout of some microbial flora that may help with the removal of other harmful pollutants in the wastewater (van Lier *et. al.,* 1997). Unlike shorter regeneration times for hydrolytic and acid-forming bacteria, it is reported that thermophilic methanogenic bacteria take a longer time to regenerate and thus HRT of 10 to 15 days are recommended to avoid washout from the reactor especially, for digesters that do not have the facilities to retain and re-circulate the biomass, as opposed to mesophilic HRT of 20 to 30 days (Deublein and Steinhauser, 2011; Sheth, 2009). A direct relationship exists between the HRT and the volatile solids converted to biogas. Thermophilic digestion, therefore, operates on shorter HRT with higher biogas production compared with mesophilic digestion but this is also associated with higher ammonia production which may also inhibit the process (Kjerstadius *et al.,* 2013). Unlike UASB which normally has different HRT and SRT (the HRT in UASB reactor is much shorter than the SRT as solids settle by gravity in the UASB reactor), the HRT and SRT for a CSTR is generally considered to be the same and thus the shorter the HRT, the shorter the SRT will be (Tchobanoglous *et al.,* 2004). A careful consideration is therefore needed with the operation

of a thermophilic CSTR which will have same HRT and SRT at a shorter time vis-a-vis conversion of the COD to methane and hygienisation of the digestate.

2.4.6 C/N ratio and ammonia inhibition

AD at thermophilic temperatures even though is faster than mesophilic, it is more sensitive to toxic compounds, metal ions, substrate pH and C/N ratio, making it difficult for mesophilic bacteria to easily adapt to thermophilic condition (Bagge, 2009). The carbon-nitrogen ratio defines the relationships between the amount of organic carbon and organic nitrogen present in the substrate to be used by the micro-organisms and its implication for the performance or possible inhibition to the microbes in the reactor. According to Bagge (2009), a C/N ratio between 16 and 19 is optimum for the performance of methanogenic micro-organisms in an anaerobic reactor. Similar to what was reported by Bagge (2009), Vögeli *et al.,* (2014) reported 16 and 25 as the optimal C/N ratio in anaerobic reactors. For a single-stage systems of the AD process, a C/N ratio of between 15-25 is required while double stage systems require a C/N ratio of between 10-45 for the first stage and 20-30 for the second stage (Dobre *et al.,* 2014). When the C/N ratio value is high, it implies less degradation of the carbon is occurring unlike nitrogen and may result in low biogas production. On the other hand, when the C/N ratio decreases, it implies more of the carbon is being degraded unlike the nitrogen. The latter could lead to a sudden build-up of ammonia in the reactor and consequently leading to an increase in the pH level to a basic medium, inhibiting methanogenic bacteria (Vögeli *et al.,* 2014).

Work by Kroiss (1985) and reported in a review by Weiland (2010) emphasised ammonia could build-up in the reactor and become toxic with increase in temperature in the thermophilic or hyper-thermophilic regime. The build-up of free or un-ionised ammonia could cause inhibition when it reaches a concentration of 80 mg/L (Lu, 2017; Kroiss, 1985 in Weiland, 2010), which could lead to build-up of VFAs. As a result of the build-up of VFAs, the reactor might be able to auto-recover from ammonia inhibition even though this may not necessarily happen as the substrate has its own alkalinity for buffering the pH especially for animal-based substrate like cow manure, however, it is recommended that diluting the biomass with the effluent from the reactor could restore the reactor from ammonia inhibition (Nielsen and Angelidaki, 2008).

2.4.7 Nutrients demand

Methanogens, particularly archaeabacteria methanogens rely on different substrates for their anaerobic activities leading to methane production. Some of the core compounds methanogens rely on to convert to methane are simple carbon-based structures which serve as energy sources. These include acetate, formate, carbon dioxide, carbon monoxide, methylamines and methanol (Jarrell and Kalmokoff, 1988). Methanogens are considered to be fastidious anaerobes and thus require certain specific nutrients for their proper functioning in terms of methanisation, even though they are known to be resistant to most common antibiotics. The type and origin of the methanogen influence the specific nutrient requirements. Methanogens need specific nutrients for example, yeast extract, trypticase, peptone, cysteine, acetate, 2-methyl butyrate, isoleucine, isovalerate, tryptophan, rumen fluid for organic growth. They also require organic stimulants (examples include: acetate, cysteine, organic nutrients, rumen fluid, 4-6 amino acids, leucine, casamino acid, trypticase, tryptose, sludge supernatant, soya broth, co-enzyme M and Titanium) for their methanogenic activities. Other specific nutrients include vitamins (examples: riboflavin, folic acid, folate, B_{12}, biotin, CoM, Thiamine, B-vitamins, pantoic acid, pantoyllactone stimulate) and trace elements (minerals) (examples are: Nickel (Ni), Tungsten (W), Iron (Fe), Cobalt (Co), Molybdenum (Mo), Magnesium (Mg), Manganese (Mn), Selenium stimulate (Se), Copper (Cu), Calcium Chloride ($CaCl_2$), Chlorine (Cl), Potassium (K) and Zinc (Zn). Varied concentrations of Sodium Chloride (NaCl) ranging from 0.07 M to 2.1 M are also required by methanogens for their methanogenic activities (Jarrell and Kalmokoff, 1988). Depending on the concentrations of these trace elements, the methanogens may either be inhibited or enhanced during the AD process for methane production (Lu, 2017). Each of the trace element has a specific function for the methanogens. For example, Mn is an activator of many enzymes, Mo is used by some formate dehydrogenases, Ni influences coenzyme F_{430} of methanogens and it is also used by most hydrogenases as well as carbon monoxide dehydrogenase. Thungsten (W) influences oxotransferases of hyper-thermophiles as well as formate dehydrogenases (Lu, 2017).

2.4.8 Mixing

The contact between the biomass and COD in the reactor is very important for the hydrolysis process. Conversion of dissolved components of wastewater is low when there is little or no contact between the organic substrate with the biomass (Kujawa-Roeleveld & Zeeman, 2006). However, homogenous distribution of influent over the bottom of the reactor using magnetic

stirrers ensures good contact between sludge and COD in the influent and thus ensuring good conversion of COD into biogas. Mixing also ensures that there is no temperature differences established in the reactor. In addition, it prevents formation of scum layer in the reactor and thus ensuring that the bacteria are constantly flocculating for effective methanisation (Vögeli *et al.,* 2014). Avoiding scum and foam formation is relevant for mechanical functioning of the reactor, since corrosion and blockages of pipes will be minimized (Deublein and Steinhauser, 2011; Sheth, 2009). Recirculation of the digestate into the reactor and bubbling in the reactor can also ensure some amount of mixing, particularly in a situation where mechanical mixers are not available (Vögeli *et al.,* 2014).

2.4.9 Organic loading rate

The organic loading rate (OLR) could be explained as the amount of organic matter that is introduced into the reactor volume per day. In an anaerobic reactor that operates continuously, the measurement of the OLR is critical since it determines whether the anaerobic bacteria have received too much of the substrate and for that matter, 'they' are 'shocked' to have any further conversions or not. Failure of the anaerobes to further convert the already produced acids in the reactor lead to accumulation of VFAs and thus, lowering the pH in the reactor. This is inhibitory to the methanogens and could negatively affect the production of methane (Vögeli *et al.,* 2014). About 50-70 % removal of volatile solids in an anaerobic reactor is considered ideal for normal functioning of the microbes, an indication of good loading rate. Maximum organic loading rate of 4.0 kgVS/(m^3.d) is recommended for all wet fermentation processes (Weiland, 2010).

2.5 Anaerobic microbial flora and shapes in high-temperature anaerobic reactors

Not all microbial populations can be applied or used in any anaerobic treatment process. Depending on some conditions such as optimal temperature, the bacteria cannot be efficient for the intended purpose. A greater proportion of the methanogens are mesophilic while others are thermophilic. Most of the thermophilic anaerobes reported in literature are hydrogenotrophic types. Anaerobic thermophilic strains such as *Syntrophomonas species* have been identified to produce hydrogen for the utilization by other thermophilic strains like *Methanospirillum archeon* for methane production at a temperature of 55 ^oC. *Methanospirillum* combines H_2 gas produced by the *Syntrophomonas* species with CO_2 to form methane (Toumi

et al., 2015). *Firmicutes* were identified to be able to produce cellulases, proteases and other extracellular enzymes and thus aid in the hydrolysis of complex organic substrates like cellulose containing materials. Bovine rumen bacteria have been identified to also break down cellulose and could have synergistically aided the *Firmicutes* to breakdown cellulose present in the substrate (Toumi *et al.,* 2015). Major Archaeon species that thrive during mesophilic anaerobic digestion include *Methanobacteriales*, *Methanomicrobiales* and *Methanosarcinales* with specific example being *Methanosarcina sp.* which are VFA loving. *Thermovirga* is a protein-fermenting microbe that can survive under moderately thermophilic temperatures (Toumi *et al.,* 2015). Strains of thermophilic methanogens *Methanothermobacter thermoautotrophicus* and *Methanothermobacter*-related strain have been found to be efficient in removing dye in azo-contaminated wastewater but only in the presence of riboflavin (dos Santos *et al.,* 2006). *Thermoanaerobacterium thermosaccharolyticum* is a thermophilic hydrogen producing bacteria that can produce high yield of hydrogen at a temperature of 60 ^{o}C and a pH of 6.25 (Chong *et. al.*, 2009). Members of the family *Thermotogales with examples* being *Thermotoga maritima, T. neapolitana, and T. elfii* are considered as hyper-thermophilic eubacteria that are efficient with the production of hydrogen for methanization during anaerobic digestion. *T. neapolitana,* for example, is very tolerant to oxygen availability and it is never inhibited by the presence of oxygen during its hydrogen production. At hyper-thermophilic temperatures of 75 ^{o}C to 80 ^{o}C and pH range of 6.5 to 7, *Thermotoga maritima and Thermotoga neapolitana* are still able to produce hydrogen. *Thermoanaerobacter thermohydrosulfuricus* and *Caldoanaerobacter subterraneus* are another group of thermophilic bacteria that have been isolated from hot spring environment. These two hydrogenotrophic thermophiles are also functional at an average temperature of 78 ^{o}C for the production of hydrogen. The hydrogen produced by these thermophiles is very crucial since it could be combined with CO_2 to produce methane (Chong *et al.,* 2009).

Methanogens appear in varied shapes ranging from longitudinal-shape, rod-shape, bamboo-shape (cylindrical), lobed cocci-shape and oval shape (Panawala, 2017). Methanogens in the form of archaeabacteria are between 0.1 μm to 15 μm in diameter (Orell *et al.,* 2013; Panawala, 2017). Acetoclastic methanogens such as *Methanosarcina spp* are non-motile, irregularly coccoid, sarcina or pseudosarcinal-shaped. Other strains of *Methanosarcina* such as *Methanosarcina semesiae* are obligately methyl-loving and are considered to be methylotrophic methanogenic archaeon. They range from 0.8 μm to 2.1 μm (with average diameter of 1.4 ± 0.2 μm) and are considered to be gram positively-stained (Lyimo *et al.,* 2000; Mizukami *et al.,* 2006). *Methanosaetae spp* (another example of acetoclastic methanogens) and those in the order Methanobacteriales (hydrogenotrophic methanogens) are known to be rod-shaped (Zhu *et al.,* 2012; Orell *et al.,* 2013). Methanogens of the order

Methanobacteriales, Methanosarcinaceae (Methanosarcinales) and Methanomicrobiales (oval-shaped) are mostly found under thermophilic temperature of 55 ^{o}C. Specific examples of these groups include *Methanosarcina spp and Methanothermobacter spp.* The dominant species of methanogenic archaea mostly found at mesophilic temperature of between 35 ^{o}C and 37 ^{o}C are *Methanobacterium spp.* (Orell *et al.,* 2013).

2.6 Microbial composition of BW in Ghana

It is obvious that BW generated in Ghana may have different microbial composition and characteristics from BW generated in temperate regions, like Germany. In addition, it is reported that the mass of BW produced per individuals in low income countries (for example, Ghana) is twice greater than that of their counterparts in high income countries, such as Germany (Rose *et al.,* 2015). The type and quantity of BW produced in domestic wastewater streams depends on the sex and health status of the person (Heaton *et al.,* 1992). Women for example (more especially those of child-bearing age), produce harder faeces compared with men (Degen and Phillips, 1996; Heaton *et al.,* 1992; Vandeputte *et al.,* 2016). The health status of a person has an influence on the BW output within a day (Rose *et al.,* 2015), however, the bacteria population amongst healthy and sick population remain relatively the same. A study conducted in Ghana (Accra, Kumasi and Tamale) indicated that some leafy vegetables (lettuce, cabbage and spring onions) on the Ghanaian market that were irrigated with BW-contaminated stream had total coliforms, faecal coliforms as well as various strains of helminth populations (Obuobie *et al.,* 2006). *Ascaris lumbricoides*, hookworm, *Trichostrongylus*, *Schistosoma heamatobium* and *Trichuris trichiura* were some examples of helminth populations found on the vegetables. Other microbes found include *Strongyloides stercoralis* (high occurrence in the samples) and nuaplius larvae. About 85% of all the contaminated vegetables contained *A. lumbricoides* and thus pose serious health implications to Ghanaians as these worms have high infective dose over their host (Obuobie *et al.,* 2006).

Another study by Feglo and Sakyi (2012), on the bacterial contamination of food on street-vending food in Kumasi, Ghana showed that isolated strains of coagulate negative *staphylococci*, *Bacillus species*, *Klebsiella pneumoniae and Aeromonas pneumophila* were present in the sampled food. *Enterobacter cloacae*, *Enterobacter sakazzkii, Staphylococcus aureus* (which produces heat-resistant toxins in food), *Escherichia coli* (indication of faecal contamination) and *Pseudomonas aeruginosa* were also found in the samples. Some of these microbes in these foods being sold would eventually find themselves in BW (after the people have visited the toilet) which will drain into surrounding water bodies like streams once the BW

has been discharged into the environment without any pre-treatment. When these streams are used for urban or peri-urban irrigation, the microbes get into humans by eating uncooked leafy vegetables.

Mensah *et al.* (2002), reported on contamination of lettuce, cabbage and tomatoes used for salad sold on the Ghanaian market by *Salmonella* species*, Shigella flexneri* and *Escherichia coli.* The presence of these pathogens indicates strongly of faecal contamination and poor sanitary conditions. Analyses of Health Benefits (HB) versus Costs of Interventions (CoI) in the Urban water system in Accra using Quantitative Microbial Risk Assessment were carried out (Labite, 2008). The results showed presence of *Escherichia coli*, Total coliforms, *Salmonella* and Helminths eggs in the storm drain waters of Accra, indicative of BW contamination of the storm drain water. Unfortunately, peri-urban and urban farmers in Accra utilise this water from the storm drains to irrigate their vegetable crops. Open drain water was considered the most hazardous pathway to burden disease on the people of Accra, contributing about 55 % of total burden disease (Labite, 2008). Other micro-organisms that may be present in the Ghanaian BW are viruses which include Hepatisis A virus, *Coronavirus* and *Rotavirus* (Labite, 2008) but they are not of interest in this study.

AD at a thermophilic temperature of 60 ^{o}C with minimum exposure time of 2 hours is reported to eliminate these organisms of pathogenic contamination (Kjerstadius *et al.,* 2013). Kjerstadius *et al.* (2013), further suggest that pre-digestion for the hygienisation of BW from organisms of pathogenic contamination at a temperature of 70 ^{o}C for 1 hour is enough to eliminate all pathogens before the process of methanisation is carried out. Similarly, results from Bagge (2009), also confirmed that pre-pasteurisation of the digestate from cattle manure and waste from a slaughter house at 70 ^{o}C for 1 hour before anaerobic digestion reduces non-spore producing bacteria like *Salmonella spp.* but does not kill all the pathogens including spore-forming *Bacillus spp*. and *Clostridium spp.* Taking this study in perspective, the substrate to be used is BW with much emphasis on BW from Ghana, since the pilot-scale research is focused in Ghana. Thus, the theoretical microbes present in the Ghanaian BW are the target for the hygienised digestate. Table 2.1 shows the summary of pathogens found in literature in Ghanaian BW and possible inhibitory conditions to eliminate them. Once the temperature and other inhibitory substances that can destroy the most tolerant pathogens are known, all other less tolerant pathogens can also be destroyed.

Table 2.1: Microbial composition of BW generated in Ghana and their possible inhibitory conditions

Pathogen			**Temperature, T (°C), contact time (CT) (hr) and HRT (days) based on Swedish EPA and EU regulations.**
Bacteria	Total Coliform	*Escherichia coli* *Enterobacter cloacae*, *Enterobacter sakazzkii* eg. *Citrobacter sp*	*E. coli* : Temp = 55 CT = 2, HRT = 7
	Faecal Coliform	eg. *Escherichia coli*	
	Salmonella species	eg. *Salmonella* species	*Salmonella spp*.: Temp = 60 CT = 2 or CT = 1.
	Shigella	*eg. Shigella flexneri*	
	Cocci	Coagulate negative *staphylococci* *Staphylococcus aureus and*	*Enterococcus* : Temp = 55 or 60 CT = 2 HRT = 7
	Others	*Bacillus, Klebsiella pneumoniae, Aeromonas pneumophila, Pseudomonas aeruginosa*	
Protozoa		*Entamoeba sp* *Giardia sp* *Cryptosoridium sp*	
Helminths		*Ascaris lumbricoides* *Ancylostoma sp* *Necator sp* *Strongyloides stercoralis* *Trichuris trichiura* *Schistosoma heamatobium,* nuaplius larvae *Trichostrongylus* Hookworm	
Virus		Hepatisis A virus *Coronavirus* *Rotavirus*	

C. perfringens (EU requirements for hygienised digestate). C. perfringens: Temperature greater than 60 °C, higher contact time but should not be used as indicator for hygienisation since eggs spores are not destroyed even at 60 and MET of 2 h.

2.7 Black water treatment systems

Wastewater is any water that has been adversely affected in quality by anthropogenic influence. This includes municipal wastewater which comprises wastewater from small enterprises, stormwater runoff and household connections (domestic wastewater). Domestic wastewater consists of grey water (all wastewater other than water from the toilet) and black water (Hernández Leal *et al.,* 2010; Kujawa-Roeleveld and Zeeman, 2006). Black water consists of faeces, urine and flush water. Even though black water represents a very small volume of domestic wastewater, it contains the main components of domestic wastewater such as organics, nutrients (nitrogen, phosphorus, potassium and sulphur), pathogens, pharmaceutical residues and hormones (Hernández Leal *et al.,* 2010; Kujawa-Roeleveld and Zeeman, 2006). In the advanced countries for instance, the use of activated-sludge as part of the conventional system of domestic wastewater treatment had existed. With the conventional system, domestic wastewater is collected and transported to a central place, for example, in the community, where it is treated and discharged to the environment in general but sometimes reused (Kujawa-Roeleveld and Zeeman, 2006). The conventional system however, requires high electrical energy, maintenance and operation cost. Consequently, in the past few decades, most of these developed countries have adopted other technologies which require less energy for its operational processes and which could also recover valuable resource like biogas (van Lier, 2008). These technologies include Upflow Anaerobic Sludge Bed (UASB) reactor, Continuous Stirred Tank Reactor (CSTR), Expanded Granular Sludge Bed (EGSB) reactor, Internal Circulation (IC®) reactor (type of EGSB with biogas-driven hydrodynamics) and Anaerobic Filter (AF) reactor. Others are Anaerobic Lagoon (LAG), Combined Hybrid System with the sludge bed at the bottom and a filter on top (HyBR), Fluidized Bed (FB) reactor, Anaerobic Bioreactors and Sequencing Batch Reactor (SBR). Most of these reactors mentioned above operate using the anaerobic digestion process and thus require no energy for aeration during removal of organics in the BW (van Lier, 2008). Anaerobic digestion is also used for treatment of surplus sludge, waste treatment and for biogas production (van Lier, 2008). The use of septic tanks and UASB-septic tanks have been reported for domestic wastewater (for example black water) treatment in developing countries (Kujawa-Roeleveld and Zeeman, 2006). Consequently, the selection of a BW treatment technology for an urban slum in Ghana requires critical considerations.

2.8 Selection of a BW treatment system for a slum in Ghana

The choice of the type of reactor and the technological process for BW treatment in Ghana is crucial because of the economic implications for the citizens; for example, affordability, its technical complexity for operation and maintenance, its efficiency and also the applicability in a developing country like Ghana. The choice and adoption of different BW treatment technologies mentioned in section 2.7 of this work together with Membrane Bioreactor (MBR), Anaerobic Membrane Bioreactors (AMBRs) is very essential. A critical evaluation of some of these technologies and their easy applicability in Ghana is very crucial.

2.8.1 Membrane bioreactors

Akanyeti *et al.* (2010), considered the use of membrane bioreactor (MBR) for sewage treatment and improved energy recovery by bioflocculation of the sewage. At least 35 % of methane could be recovered from the COD in the sewage at extremely short SRT and HRT. MBR has been considered a suitable technology for concentrating COD in wastewater for methane recovery without extensive mineralisation (Hernández Leal *et al.,* 2010). At a methanisation level of 64 %, anaerobic degradation potential of concentrate (COD concentration of 3,588 mgL^{-1}) from MBR has the potential to produce biogas that has 84 % of methane after 20 days of incubation and a mesophilic temperature of 35 ^{o}C (Akanyeti *et al.,* 2010). In addition, MBR also produces higher quality effluent suitable for water reuse after sufficient post-treatment (Hernández Leal *et al.,* 2010). However, the aerobic mineralisation stage of the MBR technology consumes energy (Hernández Leal *et al.,* 2010) and thus making this technology unsuitable for its implementation in Ghana where energy crisis is prevailing. However, there are anaerobic membrane bioreactors (AMBRs) that have the ability to retain specifically required slow-growing active methanogenic biomass and are able to withstand extreme conditions such as high temperature and salt concentrations but they are not economically feasible due to their low achievable fluxes that depend on cake layer deposition in the reactor (Jeison and van Lier, 2007).

2.8.2 Granular sludge-based reactors

The reactors that operate by the granular sludge-based approach have different SRT and HRT. Sludge is retained in the reactor by sedimentation, granulation or immobilisation on a fixed material. Reactors such as expanded bed reactors with examples being EGSB and IC® are seeded with excess granular sludge on fixed media such as rocks, plastic rings and modular cross-flow media. Alternatively, advanced fluidized bed (FB) reactors use suspended media like sand or porous inorganic particles for biomass attachment and development. FB reactor was reported to have many technological challenges with full scale applications (van Lier, 2008) and as a result not suitable for Ghana. EGSB and IC® have been reported to have higher availability of the methanogenic granular sludge, efficient retention of active biomass and good contact between wastewater and biomass (van Lier, 2008), however, adoption of such a technology in an urban slum of Ghana is not feasible because of the size, high installation cost and maintenance cost.

2.8.3 Septic tanks

The use of septic tanks to pre-treat domestic wastewater with post-treatment by soil absorption is another technology that has been used to treat BW (Kujawa-Roeleveld and Zeeman, 2006). However, this technology has the disadvantage of low conversion of dissolved components in the BW as a result of horizontal flow of influent because it has little or no contact with the sludge (Kujawa-Roeleveld and Zeeman, 2006). In addition, there is accumulation of sludge to form sludge bed and thus reducing the volume and HRT of the septic tank and consequently, requires frequent removal of the sludge, with cost implications (Kujawa-Roeleveld and Zeeman, 2006).

2.8.4 UASB reactor

For some time now, anaerobic treatment of domestic wastewater using UASB reactor has been in the limelight, most especially, in tropical countries (Kujawa-Roeleveld and Zeeman, 2006). The UASB reactor uses a single-stage process for wastewater treatment. In this reactor, influent wastewater enters into the reactor from the bottom and flows upwardly throw a sludge bed of micro-organisms for the removal of volatile organic matter to produce biogas. The average flow velocity operational for the UASB is usually, 0.7 to 1 m/h (Tilley *et al.,* 2014). The reactor can be applied at any scale as it has less functional units [the primary clarifier, the biological reactor (secondary treatment), the secondary clarifier and the sludge digester] in a single-stage (van Lier and Huibers, 2003). The use of UASB for wastewater treatment has

been often applicable to large industrial scale treatment (Tilley *et al.,* 2014) but for domestic wastewater treatment, it has been most applicable in warm climates in developing countries as less energy would be required for heating. In addition, it has low operational cost and also produces less sludge compared to the conventional system of wastewater treatment (Sperling *et al.,* 2010). About 13.5 MJ CH_4 energy/kgCOD (Kujawa-Roeleveld and Zeeman, 2006) is recovered from domestic wastewater with the use of UASB reactor but the effluent requires further post-treatment such as the use of slow sand filters or wetlands which produce effluent with BOD concentration of 20 mg/L from influent BOD of 175 mg/L. The presence of pathogens in the effluent from UASB reactor is another setback from the use of this technology (Sperling *et al.,* 2010; Tilley *et al.,* 2014; Vögeli *et al.,* 2014). The UASB operates in a continuous inflow and outflow like the CSTR but the upflow velocity and the movement of biogas aid in the mixing of the influent with the sludge bed biomass (Tilley *et al.,* 2014).

2.8.5 UASB septic tank

This combines the features of UASB reactor and a septic tank. In UASB reactor, the continuous up-flow mode of wastewater helps with removal of suspended solids and effective biological conversions of dissolved components. There is accumulation of solids in this system (Kujawa-Roeleveld *et al.,* 2006). This technology has the advantage of producing stabilised sludge (as SRT is longer than in the septic tank system), biogas and the slurry is nutrient-rich for agriculture (Kujawa-Roeleveld *et al.,* 2006). However, the slurry and the stabilised sludge are not hygienically safe to be applied on agricultural lands since they may contain pathogenic materials and heavy metals.

2.8.6 Continuous Stirred Tank Reactor (CSTR)

The laboratory-scale research in this study will use CSTR since its operations are similar to the fixed-dome biogas digester to be used in the pilot-scale research in this study. The CSTR usually has a mixing device (mechanical stirrer) that ensures that the influent and the existing biomass in the reactor come into contact (Tchobanoglous *et al.,* 2004). The mixing in the CSTR can also be enhanced by bubbling gas such as the methane produced (Kujawa-Roeleveld *et al.,* 2006). Due to the continuous mixing in the CSTR, there is a possibility of biomass washout in the effluent. It is therefore advisable to have a sedimentation tank to ensure that the biomass settles and used for possible recirculation. Since the fixed-dome

digester to be used in the pilot-scale research operates like the CSTR, it is practical to use CSTR in the laboratory-scale experimental research work.

2.8.7 Fixed-dome digester (Deenbandhu type)

According to Vögeli *et al.* (2014), a fixed-dome (Deenbandhu type) is a closed-dome shaped digester which has an immovable rigid gas-holder. It has an influent inlet and a displacement pit called the compensation tank where the effluent and the digestate exit the reactor. The gas holder is designed to be on top of the digestate in the reactor. With a closed gas valve, higher production of biogas could cause a displacement of the digestate into the compensation tank (Tilley *et al.,* 2014; Vögeli *et al.,* 2014).

The choice of a fixed-dome biogas digester plant for the pilot-scale study in this research for the treatment of household BW in Terterkessim slum in Elmina - Ghana, is based on the following reasons: the user interface is directly connected to the biogas digester (Tilley *et al.,* 2014), the digester can work with or without urine, the reactor can be built underground protecting it from temperature variations (Vögeli *et al.,* 2014) and also implies little space is required (making it feasible in a densely populated area like a slum) (Tilley *et al.,* 2014). Other advantages include: the reactor functions on a wide range of organic input such as animal manure, kitchen waste and BW. Thus, co-digestion would be done to enhance biogas production. It also supports pour flush toilet system (less water used – concentrated BW, higher biogas production), surrounding soil help to counter the in-built pressure in the reactor, moderately not expensive (the use of local materials and labour), has a life span of between 15 to 20 years as there is no corrosion (Vögeli *et al.,* 2014). Figure 2.1 below shows a picture of the fixed-dome biogas digester to be used for the pilot study in this study.

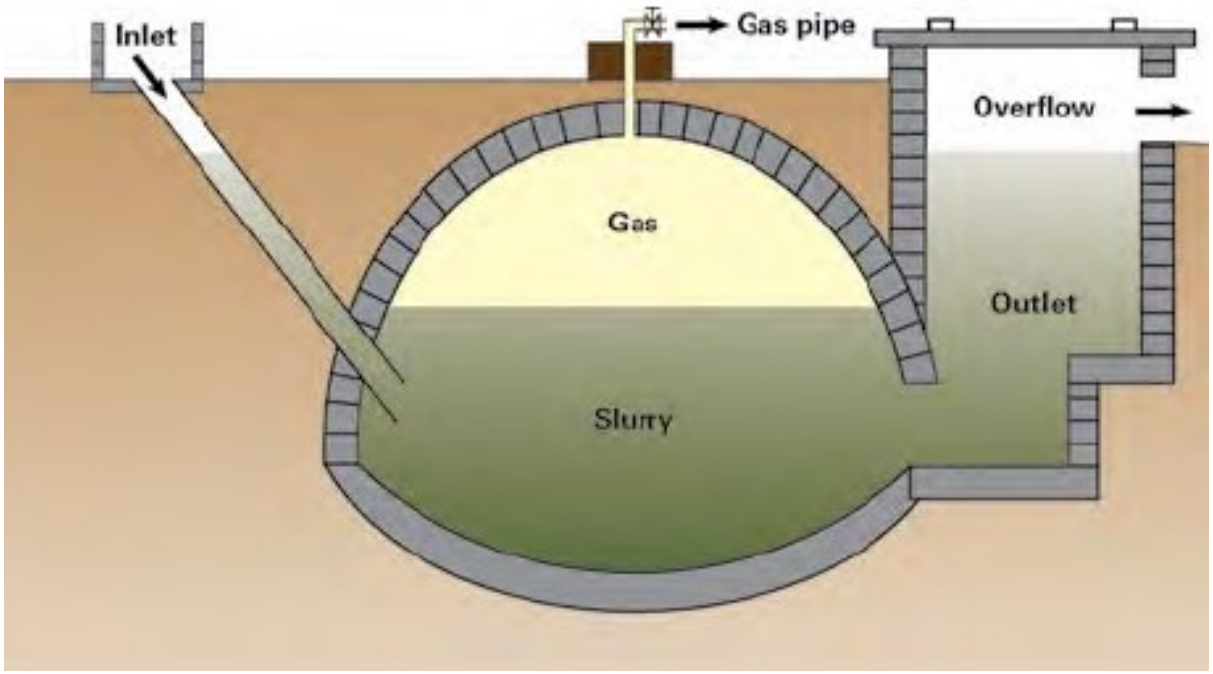

Figure 2.1: Fixed-dome biogas digester for the pilot study in Ghana (Source: Vögeli *et al.,* 2014)

There are other types of anaerobic biogas digesters such as the floating-drum digester, puxin digester and the tubular digester (Kjerstadius *et al.,* 2013).

2.9 Trade-off between high biogas production and disinfection of digestate

According to Bartkowska (2015), the least expensive method of sludge handling after treatment of wastewater is to return it to the environment as soil conditioner. However, the presence of pathogens like *Salmonella spp* and eggs of intestinal parasites like *Ascaris, Trichuris* and *Toxocara* raises safety concerns making it mandatory to hygienise the digestate before it can be reused on agricultural lands. One system that has been identified and applied in Olecko, Poland is the Autothermal Thermophilic Aerobic Digestion (ATAD) process which operated on a temperature of 59 ^{o}C to 65 ^{o}C. This was used after a pre-treatment in a sequential biologic reactor (SBR) operating at a temperature of 40 ^{o}C to 55 ^{o}C for stabilisation of sludge from a wastewater treatment plant (Bartkowska, 2015). The ATAD had a COD removal of 52.4 % however, no information was provided concerning the bacterial growth and survival vis-à-vis with their methanogenic activities (Bartkowska, 2015). Dereli *et al.* (2012), reported that thermophilic temperatures produce similar amounts of methane as mesophilic temperatures at double organic loading rates. However, thermophilic temperatures produce mobile anaerobic biomass since the sludge is dispersed and has poor settling characteristics. This may affect the overall conversion of organics to methane even though the digestate will

be safe for application on agricultural lands. It is very important to consider at which optimal temperature can both higher methane be produced and at the same time producing pathogen-free digestate. Failure to identify this optimal temperature implies trading-off either large volume of methane for pathogen free digestate or vice versa.

2.10 Economical benefits of thermophilic BW treatments using anaerobic reactors

The use of anaerobic reactors for wastewater treatments under thermophilic conditions has enormous benefits. The first most important benefit is ensuring clean and safe water for domestic and agricultural uses. In most developing countries, pollution of drinking water sources by contaminants like BW has led to a number of sanitation-related diseases such as cholera and typhoid fever. Consequently, development of proper BW treatment technologies is one of the many ways of efficiently curbing diseases associated with water and sanitation (Bryant, 2012) in Ghana. This becomes possible when all the generated BW undergo some form of treatments before they are finally discharged into the environment. Clean and safe environment is a requisite to good health. It is also a factor that can ensure productivity for any nation. In addition, less money would be spent on treating sanitation-related diseases which would have occurred as a result of the use of water bodies and food contaminated with BW.

Secondly, the hygienised digestate that would be produced after the thermophilic BW treatment is a good material to fertilize agricultural lands. In most countries, most farmers use inorganic fertilisers as soil amendment material, however, they are expensive to produce and not environmentally friendly. Thus, the production of the digestate from the thermophilic BW treatment will ensure a pathogen-free organic fertiliser to boost agriculture and food production. About 80 - 95 % of nutrients can be recovered from BW when it is collected from separate streams and the BW co-digested with kitchen waste (Kujawa-Roeleveld and Zeeman, 2006). In each day, healthy adult human produces 1.5 L of urine and 0.17 L of faeces. These streams contain about 90 % of nitrogen, 80 % each of phosphate and potassium and 70 % of COD which can be recovered (Bryant, 2012; Kujawa-Roeleveld and Zeeman, 2006). Assuming all the nutrients in the BW are recovered, it can be estimated that, each healthy adult person can produce about 55.85 KgN/p/yr, 49.64 KgP/p/yr and 49.64 KgK/p/yr. The recovery of these nutrients from treated BW can go a long way to improve soil conditions as well as food production when the whole population of a particular country is considered.

In addition, adoption of this treatment technology in Ghana will help solve some of the current energy crisis as the reliance on fossil fuels for energy would minimise. Up-scaling this technology is useful as concentrated BW has higher COD and can produce 16.8 L CH_4 p^{-1} d^{-1} at a COD load of 80 g p^{-1} d^{-1} and a methanisation rate of 60 % (0.35 L CH_4(g COD)$^{-1}$) (Tervahauta *et al.,* 2014). Theoretically, 1 L of BW can produce 2.5 $L^{-1}p^{-1}d$ of methane (Kujawa-Roeleveld, 2005). About 70 % of potential energy in the form of COD can be recovered from BW when it is collected from separate streams and co-digested with kitchen waste (Kujawa-Roeleveld and Zeeman, 2006). According to Elmitwalli and Otterpohl (2007), an important parameter for evaluating anaerobic treatment of wastewater is maximum conversion of total COD present in the influent to methane.

Even though thermophilic and hyper-thermophilic treatments of BW has some enormous economic benefits, a critical economic analyses such as assessing the costs of construction vis-à-vis the anticipated benefits in terms of methane for cooking, fertiliser for agriculture and the payback period need to be done before its adoption. This is because of the extra energy that would be required to heat the reactor to the thermophilic or hyper-thermophilic conditions. However, in a tropical country like Ghana, solar energy is readily available and thus combination of the anaerobic digester with solar energy is feasible, nonetheless, the cost of installing the solar panels as well as technical management of the hyper-thermophilic treatment of BW should also be carefully considered.

CHAPTER THREE

Materials and methods

3.1 Selection of seeding sludge and hunger stage test

Three different seeding sludge were chosen from different sources and tested to assess their methanogenic potentials and thus their possibility to be used as a seeding sludge for the laboratory-scale HT-CSTR experiments and subsequently for the pilot project. The seeding sludge that had better degree of COD degradation, cumulative methane yield and productivity (based on series of batch experiments conducted with the seeding sludge and three different hyper-thermophilic temperatures, described subsequently in section 3.2) was chosen for the laboratory scale experiments and later for the pilot scale project.

3.2 Collection of seeding sludge (inoculum)

About 30 L of wastewater was collected from the effluent buffer tank of the Lausitzer Wasser GmbH & Co. KG (LWG) wastewater treatment plant in Cottbus and was called **LWG**. The average volatile solids present in it was 0.679 % and a pH of 7.27. In addition, 10 L of sieved cow manure (1.0 mm diameter sieve) which had been stored for 6 months in a fridge at 4 ^{o}C in the laboratory of the Chair of Waste Management in Brandenburg University of Technology (BTU), Cottbus-Senftenberg was also collected and named **CM**. The CM had average volatile solids of 1.899 % and a pH of 7.34. Finally, 10 L of effluent from a thermophilic reactor treating maize silage in the laboratory of the Department of Waste Management in Brandenburg University of Technology (BTU), Cottbus-Senftenberg, that operated on a thermophilic temperature of 55 ^{o}C was collected and was called **BTU**. It had volatile solids of 0.612 % and a pH of 7.77. These three seeding sludge were selected based on their easy availability for the purpose of this research. In addition, literature suggest there exist a consortium of methanogens in both sludge from wastewater treatment plant and in cow manure, making them suitable for investigation in this research (Christy *et al.,* 2014). The seeding sludge from the thermophilic reactor in BTU had a stable methane content of at least 65 v-%. Thus comparing these three inocula is not out of place.

3.3 Collection of source-separated concentrated black water (BW)

Source-separated concentrated BW was collected from household black water treatment plants in Cottbus and Komptendorf, Hornower Weg 2, Cottbus, a neighbouring village of Cottbus. One of the sources has 1.5 L per flush while the other source had 4 L per flush. The two streams were put together and homogenised. The homogenised mixture of BW was then stored in a refrigerator in the laboratory of Chair of Waste Management at an average temperature of 4 ^{o}C (to prevent microbial activity) before they were used for the experiments [both the batch fermentation tests based on guidelines proposed by the Association of German Engineers, 2006 - Verein Deutscher Ingenieure (VDI 4630) (2006) and the laboratory-scale continuous experiments using HT-CSTR].

3.4 Hunger stage test

About 10 L each from 3 different seeding sludge collected was put in 3 respective well-insulated containers embossed with the names of the seeding sludge and were heated to achieve optimum thermophilic temperature of 55 ^{o}C (for acclimatisation before they were increased to the hyper-thermophilic temperatures) for 3 weeks. Initial biogas volumes from the date when the hunger stage tests were started were noted for the 3 inocula. Final biogas volumes on the date when the hunger stage tests ended were also recorded for the 3 seeding sludge. The hunger stage test was stopped when the biogas productions from the seeding sludge were at least 1% of the cumulative biogas produced over the three-week period for the tests (Verein Deutscher Ingenieure (VDI 4630), 2006). The hunger stage tests were carried out to ensure that the biogas produced from the substrate was devoid of the contribution from the seeding sludge.

3.5 Fermentation batch tests using the three seeding sludge

The purpose for carrying out the fermentation batch tests was to assess the performance of the 3 seeding sludge with respect to their methane production, methane yield and degree of COD degradation of the substrate, BW. This was done to help ascertain which seeding sludge was best suited for a single-stage hyper-thermophilic treatment of BW in a continuous fermentation experiments both on a laboratory scale and on a pilot level.

3.5.1 Ratio of substrate and seeding sludge for fermentation batch tests

The Association of German Engineers (Verein Deutscher Ingenieure (VDI 4630) (2006), guidelines recommend that the quantity of seeding sludge and substrate to be used for the batch fermentation tests should comply with the equation below:

$$\frac{VSsubstrate}{VSseeding\ sludge} \leq 0.5 \quad (1)$$

Where:

$VS_{substrate}$ is the volatile solids present in the substrate,

$VS_{seeding\ sludge}$ is the volatile solids present in the seeding sludge (inoculum)

0.5 is the desired substrate/seeding sludge ratio for effective performance of the fermentation batch test.

Consequently, based on the values obtained for the volatile solids of the substrate and the different inocula, a $\frac{VSsubstrate}{VSseeding\ sludge} = 0.49$ was calculated and chosen for all the 3 different inocula under the same hyper-thermophilic temperature and pressure conditions.

3.5.2 Determination of COD, Total Solids (TS) and Volatile Solids (VS) for the substrate

COD for the BW was measured using the New Spectroquant® tests kits for COD. The sample was dispensed in the test kit containing potassium dichromate ($K_2Cr_2O_7$) reagent and was mixed thoroughly on the shaking mixing equipment. The cuvette containing the mixed sample was placed in a Thermoreactor (TR 300, MERCK) at 148 ^oC and digested for 2 hours. After 2 hours, it was allowed to cool to room temperature and measured in the COD Spectroquant NOVA 60 (MERCK). Total Solids (TS) representing the dry matter (DM) and volatile solids (VS) were measured following the APHA/AWWA/WEF (2012) standard methods and have been described in details in the sub-section 3.5.3 below.

3.5.3 Determination of TS and VS for inoculum, substrate (influent) and effluent

Dried crucibles were labelled and weighed using OHAUS, Adventurer Pro top pan balance (MODEL AV313, SERIAL NUMBER 8732199166, Switzerland) and their weights noted as **M1.** Each sample (about 50 g) from the inocula was also weighed using the top pan balance and the weight was noted as **M2.** The samples in the crucibles were dried overnight in an oven

(Heraus Function Line UT12, Fabric No. 40309216) at a temperature of 105 °C. The samples together with the crucibles were removed and placed in a desiccator (Vakuumfest DURAN) to cool. Each cooled sample was then weighed and the weight was recorded as **M3**.

The weighed crucibles together with the samples were put in a furnace (Nabertherm-More than Heat 30-3000 °C, B180, S/N. 223453, L-150KICN, L-15/11/B180, Nabertherm GmbH) at a temperature of 550 °C for two hours to burn to ashes. After two hours, they were then cooled in a desiccator and weighed again as **M4**.

The fraction of TS, FS (fixed/inorganic solids or ash) and VS were determined as:

$$TS = \frac{M3 - M1}{M2} * 100\ (\%) \quad (2)$$

$$FS = \frac{M4 - M1}{M2} * 100\ (\%) \quad (3)$$

$$VS = TSS - FSS\ (\%) \quad (4)$$

where:

M1, weight of dried crucible (g)

M2, weight of sample (g)

M3, weight of (crucible + sample) after 105 °C (g)

M4, weight of (crucible + sample) after 550 °C (g)

The same procedure was followed to determine the TS and VS for the BW used as the substrate for the batch fermentation tests and the laboratory-scale HT-CSTR. It was also used to determine the TS and VS of the effluent from the HT-CSTR as well as the mixture of the BW and kitchen food waste (MIX) used for the co-digestion (details of co-digestion for the single-stage HTCSTR are found in section 3.6.5). The procedure is also described in summary by APHA/AWWA/WEF (2012).

3.5.4 Batch fermentation tests for three different inocula at three hyper-thermophilic temperatures

The 3 different seeding sludge were subjected to 3 different hyper-thermophilic temperatures of 60 ^{o}C, 65 ^{o}C and 70 ^{o}C and were compared to assess which of them ensured higher degree of COD degradation of the substrate (BW), produced higher net normalised cumulative methane content and cumulative methane yield. This was done by subjecting the 3 seeding sludge to the same treatments (3 different temperatures of 60 ^{o}C, 65 ^{o}C and 70 ^{o}C and the same ratio of BW to seeding sludge; 0.49). The batch fermentation tests were done based on guidelines from the Association of German Engineers, translated in German as Verein Deutscher Ingenieure (VDI 4630) (2006). The set-up of the batch fermentation tests is seen in figure 3.1. Each set-up consisted of a coated steel water bath (Grant, GD120) of dimensions 738 mm (length) by 289 mm (breadth) by 184 mm (depth). Each water bath was fully covered with a white, double-glazed, heat-resistant polyprolyne (PP) plastic material of thickness 10 mm. Holes of diameter 90 mm were drilled through the double-glazed heat-resistant plastic material to facilitate easy passage of the neck of the batch fermentation bottles and also the eudiometer tubes. Top-up of the water in the water bath (after loss of water through evaporation as a result of high temperatures) was done through one of the holes which was covered with a polyvinyl chloride (PVC) cap. A temperature regulator was affixed on each covered water bath and was set depending on the temperature being investigated. For e.g. the regulator was set at 37 ^{o}C or 65 ^{o}C when that temperature was under investigation. Each batch fermentation bottle was 500 ml while each eudiometer tube connected to the fermentation bottle was 400 ml. Each eudiometer tube affixed on top of a batch fermentation bottle was connected to a 500 ml DURAN bottle containing concentrated H_2SO_4 coloured with methyl orange. Using Gas Analyser (Geotechnical Instrument), the acid was pumped from the DURAN bottle into the eudiometer tube (at a set volume considered to be the initial volume) at the beginning of the experiment. This was used to assess the daily biogas production based on the volume of acid displaced from the eudiometer tube back into the DURAN bottle (by subtracting the initial volume from the final volume). Biogas composition was assessed using Gas Analyser: Geotechnical Instrument.

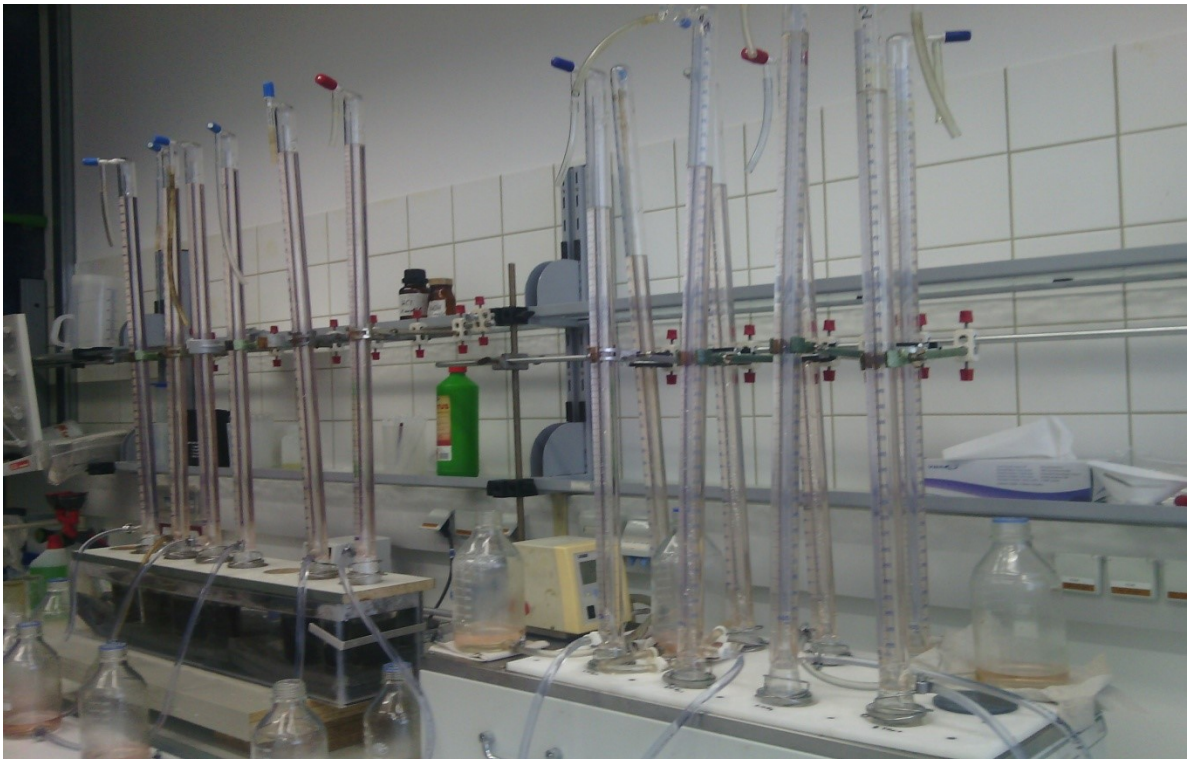

Figure 3.1: Picture of the batch set-up used in this research

Each batch fermentation test for the different temperatures and inocula ran for at least 24 days. According to the Verein Deutscher Ingenieure (VDI 4630) (2006), when the daily biogas production is observed to be at least 1% of the overall cumulative biogas produced, the test can be stopped. The results in terms of degree of COD degradation, net cumulative methane yield and net normalised cumulative methane content were assessed at the end of the tests. Based on the VS present in both the BW and the different seeding sludge, specific mass of the substrate and seeding sludge was weighed in accordance with the Verein Deutscher Ingenieure (VDI 4630) (2006) specification as was proposed in equation 1 above. NB: in this report, i = inoculum, s = substrate. Thus, **Mi** represents mass of the inoculum (g), whereas **Ms** is the mass of the substrate (g). **T (s+i)** is the total mass (g) of the inoculum and substrate in the batch fermentation bottle. **Cmi** represents inoculum for cow manure, **Cmi+s** represents both the cow manure inoculum and substrate. **BTUi** represents inoculum for **BTU**, **BTUi+s** represents both the BTU inoculum and substrate. Finally, **LWGi** represents inoculum for **LWG**, **LWGi+s** represents both the LWG inoculum and substrate. In order to compare the performance of the optimal hyper-thermophilic temperature with optimal thermophilic temperature of 55 ^{o}C and optimal mesophilic temperature of 37 ^{o}C with respect to degree of COD degradation, net cumulative methane yield and net normalised cumulative methane content, batch fermentation tests were also performed for temperatures 55 ^{o}C and 37 ^{o}C using the same substrate and inoculum ratio of 0.49. The tests were repeated three times and their

average values were used for analyses. Tables 3.1 gives details of average mass of substrate and inoculum used in this research for the optimal mesophilic temperature of 37 °C and optimal thermophilic temperature of 55 °C as well as hyper-thermophilic temperatures of 60 °C, 65 °C and 70 °C. Values written in brackets are the standard deviations.

Table 3.1: Average quantities of substrate and inoculum used for batch tests at 37 °C, 55 °C, 60 °C, 65 °C and 70 °C

Name of Sample (ID)	Mass of the inoculum, Mi (g)	Mass of Sample, Ms (g)	Total of inoculum and sample, T (s+i), (g)
Cmi	400.2 ± (0.4)	0.0 ± (0.0)	400.2 ± (0.4)
Cmi+s	243.4 ± (48.6)	156.7 ± (48.6)	400.1 ± (0.1)
BTUi	400.0 ± (0.0)	0.0 ± (0.0)	400.0 ± (0.0)
BTUi+s	307.6 ± (2.5)	98.7 ± (4.7)	406.3 ± (7.3)
LWGi	400.5 ± (0.4)	0.0 ± (0.0)	400.5 ± (0.4)
LWGi+s	306.7 ± (11.2)	93.4 ± (11.2)	400.0 ± (0.1)

The net normalised cumulative volume of methane content in the batch fermentation test is given by:

$$\begin{aligned}&\text{Net normalised cumulative volume of methane content } (\text{mLNCH4} - \%)\\&= \text{Total of normalised cumulative volume of methane content in sample}\\&- \text{Total of normalised cumulative volume of methane content in seeding sludge}\end{aligned} \quad (5)$$

Methane yield, methane productivity as well as degree of COD degradation were calculated using equations 6, 7 and 8 respectively.

Methane Yield (MY) is given by:

$$\text{MY} = \frac{\text{Net cumulative Normalised Volume of Methane after the batch test (mLNCH4)}}{\text{Mass of volatile solids (gVS)}} \quad (6)$$

Methane Productivity (MP) is given by:

$$\text{MP} = \frac{\text{Normalised Volume of Methane (mLNCH4)}}{\text{Active reactor volume (mL)} * \text{Time (d)}} \quad (7)$$

Degree of COD degradation (η) is given by:

$$\eta = \frac{\text{Volume of gas} * \text{Amount of methane} * 100\%}{320 * \text{mass of substrate} * \text{COD in substrate}} \quad (8)$$

320 = 1 g COD will practically produce about 320 mLN of methane gas under complete degradation.

3.6 Hyper-Thermophilic Continuous Stirred Tank Reactor (HT-CSTR) treatment

The use of continuous stirred tank reactors for wastewater treatment has been in existence, however, the use of a single-stage hyper-thermophilic continuous stirred tank reactor (HT-CSTR) is novel. Consequently, in this study, a single-stage HT-CSTR was employed for the treatment of BW on a laboratory-scale.

3.6.1 Description of the laboratory-scale single-stage HT-CSTR

A single-stage hyper-thermophilic continuous stirred tank reactor (HT-CSTR) with a total capacity of 50 L and active reactor volume of 35 L was used for this experiment. The reactor was made of aluminium metal tube with inner diameter of 300 mm and height of 700 mm. The reactor had a double wall, wrapped with a copper pipe for heating. It was well covered with thermo-insulator of thickness 80 mm for insulation and temperature control of 65 ^{o}C. Biogas was collected from the top of the reactor through biogas vents through a gas tube of length 2000 mm connected to a gas flow meter. Biogas produced was displaced via a tube of wall thickness 2 mm and diameter 15 mm to a gas flow meter (Ritter TG05/5), thence into a biogas bag for storage. Liquid effluent from the reactor was collected from an effluent outlet of diameter, 35 mm (made of PVC pipe) affixed at the side of the reactor. The reactor had a thermometer probe for measuring the temperature and an electric stirrer installed on it to ensure continuous mixing of the influent and the active sludge in the reactor. On the reactor was also an inlet pipe made of PVC pipe (35 mm in diameter), inserted 450 mm deep into the reactor for manual feeding of the reactor with the influent. Figures 3.2 and 3.3 show the picture and schematic diagram, respectively, of the single-stage HT-CSTR used for the laboratory-scale research.

Figure 3.2: Picture of the laboratory-scale single-stage HT-CSTR used in this research

The detailed schematic representation of the 50 L single-stage HT-CSTR with a total active reactor volume of 35 L together with all the accessories used in this study for the laboratory-scale experiment for BW treatment is seen in Figure 3.3.

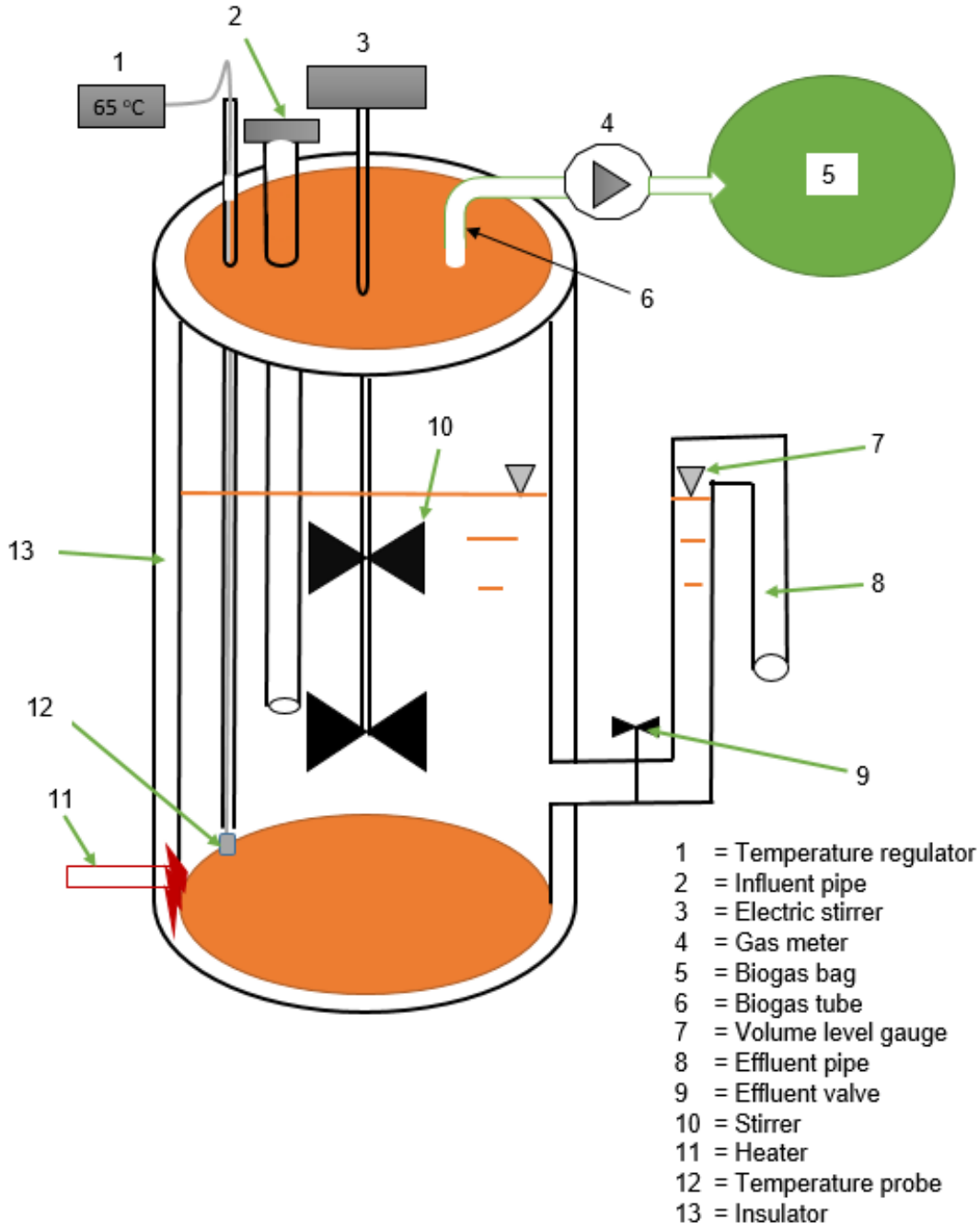

Figure 3.3: Schematic diagram of the laboratory-scale single-stage HT-CSTR (50 L) used in this research

3.6.2 Characteristics of seeding sludge for the laboratory-scale single-stage HT-CSTR

The laboratory-scale single-stage HT-CSTR was seeded with liquid CM based on the performance of CM in the batch fermentation tests, compared with LWG and BTU seeding sludge. The CM used had a percentage VS of 1.5 % of the TS or DM which was 2.1 %; consequently, the ratio of VS to TS was 71.4 %. The seeding sludge had a COD of 26.5

gCOD/L. The volume of CM used was 25 L with a pH of 8.75 and thus 5 ml 2M H_2SO_4 was used to adjust the pH to 7. 99. This was subsequently diluted with deionized water until the pH was 7.56 and the active reactor volume was 37.5 L. The temperature for the reactor was set at 65 ^oC (based on results from the batch fermentation tests) while the electric stirrer was set at 50 revolutions per minute. The seeding sludge was subjected to a hunger stage (as was described in section 3.4) and was allowed to stay in the reactor for at least 24 hours, to ensure that a stable optimised hyper-thermophilic temperature of 65 ^oC was maintained before the BW was introduced.

3.6.3 Single-stage Hyper-thermophilic treatment of source-separated concentrated BW

The source-separated BW (section 3.3) obtained for the batch fermentation tests was also used for the laboratory-scale single-stage HT-CSTR continuous experiments for hyper-thermophilic treatment of BW. The BW (influent) was analysed to assess its physico-chemical composition as well as volatile fatty acids, specifically acetic acid. The effluent from the HT-CSTR was also analysed for physico-chemical composition, acetic acid as well as pathogen concentrations. The volume of influent introduced manually into the reactor per day for treatment was equal to the volume of effluent discharged manually daily so as to keep the active volume of the reactor the same. The single-stage hyper-thermophilic treatment of source-separated concentrated BW was ran for 10 weeks, after which co-digestion with kitchen food waste (FW) was introduced to the reactor for 12 more weeks. The quality and composition of biogas produced was measured daily using Geotechnical Instrument – Biogas Ansyco while the volume of biogas produced was measured using gas meter reader (Ritter, made in Germany).

3.6.4 Analyses of influent and effluent composition of the single-stage HT-CSTR

Physico-chemical parameters for the influent (only BW or later mixture of BW and FW in a ratio of 1:1, v/v) samples (22 samples; one sample per week) like pH, COD, TN, NH_4-N, NO_3-N, NO_2-N, TP, PO_4-P were measured. Concentration of acetic acid present in the influent was also measured (described in sub-section 3.6.4.9). Standard deviations for the sample mean values were calculated (equation 9) and presented in brackets.

Standard deviation is given by:

$$S = \sqrt{\frac{\sum(yi - \bar{y})^2}{n - 1}} \quad (9)$$

Where:

S is the sample standard deviation

yi is the sample value

ȳ is the sample mean

n is the number of samples.

The quality of effluent produced from the laboratory-scale single-stage HT-CSTR was also assessed by measuring the physico-chemical parameters mentioned in section 3.6.4 above. In addition, BOD_5, as well as microbiological parameters such as the concentration of pathogens like *E.coli.* and *Salmonella senftenbergensis* that may be present after the single-stage hyper-thermophilic treatment were also measured.

3.6.4.1 Measurement of pH

The pH for both the influent and effluent were measured weekly (22 samples; one sample per week) following standard methods using a Lab pH/Conductivity 720 (S/N. 09041928, WTW) equipment.

3.6.4.2 Measurement of COD

Merck COD-Thermoreactor TR300 was used for the digestion of the influent and effluent samples prior to COD measurement. The New Spectroquant® tests kits for COD was used and the samples were measured in COD Spectroquant NOVA 60 by MERCK (MERCK, 64293, Darmstadt, Made in Germany). A total of 22 samples (one sample per week) were analysed for each of the influent and effluent samples during the study period.

3.6.4.3 Measurement of total nitrogen (TN) and total phosphorus (TP)

Ten millilitres each of the samples were pipetted into cylindrical vessels of the CEM MARS Xpress Microwave (S/N. BR601050, made in USA). Ten millilitres of distilled water was

pipetted and treated in the same way as the samples. The samples (22 samples; one sample per week) were pre-treated with 100 - 200 mg (1 or 2 measuring spoon affixed in the cap) oxidising decomposition reagent. The cylindrical vessels in the Xpress Microwave were positioned to balance each other during the heating process. They were digested by oxidative decomposition for 3 minutes at a power of 1600 W and a temperature of 170 ^{o}C to heat up the sample in a CEM MARS Xpress Microwave (S/N. BR601050, made in USA) under pressure. The digested samples were allowed to cool for 40 minutes prior to their measurement. The digested samples were prepared according to standard methods and measured as NO_3-N and PO_4-P (detailed descriptions of measurements for NO_3-N and PO_4-P are found in the sub-sections 3.6.4.5 and 3.6.4.7, respectively) using UV-2450 UV-VIS Spectrophotometer (SHIMADZU Corporation, Kyoto Japan). Conversion factors based on the molecular weight of NO_3-N and PO_4-P were used to calculate the nitrogen and phosphorus in the NO_3-N and PO_4-P. The weekly samples and control were measured in duplicates and averages calculated to represent the value for the sample for the week. Mean and standard deviation values were calculated for the values obtained for the 22 weeks of the study.

3.6.4.4 Measurement of NH_4-N

Other parameters like NH_4-N was prepared by following standard methods. Five millilitres of the sample was pipetted and 0.2 mL Tartrate solution was added. The mixture was shaken on a shaker (Heidolph REAX 2000, S/N. 119552376-11/95, maximum speed 7) for chemical analyses. Exactly 0.2 mL Nesslers reagent was added to the mixture and allowed to stand for 5-10 minutes before measuring in UV-2450 UV-VIS Spectrophotometer (SHIMADZU Corporation, Kyoto Japan) at a wavelength of 425 nm. The nitrogen present in NH_4-N was calculated using a conversion factor NH_4= 0.7778 mgN since 1 mgN is equivalent to 1.2857 mg NH_4. The samples and control were measured in duplicates.

3.6.4.5 Measurement of NO_3-N

The NO_3-N was prepared by pipetting 50 µL of the sample and 0.2 mL 5% of Salicylic acid (0.2 g of salicylic powder into 4 mL of H_2SO_4) was added and mixed thoroughly and allowed to stay for 20 minutes. After 20 minutes, 4 mL 2N NaOH was added and mixed thoroughly. It was then measured in the UV-2450 UV-VIS Spectrophotometer (SHIMADZU Corporation, Kyoto Japan) at a wavelength of 410 nm after the samples had attained room temperature.

The samples (22 samples; one sample per week) and control were measured in duplicates and averages for each week calculated.

3.6.4.6 Measurement of NO_2-N

In measuring the NO_2-N, 5 mL of the sample was pipetted and 2 mL diluted SA/NED-reagent (which is a mixture of 1 % sulphanilamide in 1.5 N HCl and 0.02 % NED in a ratio of 1:1, all with v/v ratio 1:1 with distilled water) was added and mixed vigorously on a mixer. The mixture was allowed to stand for 20 minutes and measured in the UV-VIS spectrophotometer (SHIMADZU Corporation, Kyoto Japan) at wavelength of 540 nm. The samples and control were measured in duplicates and averages for each week calculated.

3.6.4.7 Measurement of PO_4-P

The PO_4-P was measured following the standard procedure. Five (5) mL of the sample was pipetted and 100 µL of Ascorbic Acid was added and mixed thoroughly before 200 µL of Molybdenum was also added and mixed thoroughly. The mixture was allowed to stand for 20 minutes and measured in the UV-2450 UV-VIS Spectrophotometer (SHIMADZU Corporation, Kyoto Japan) at wavelength of 880 nm. The samples and control were measured in duplicates and averages for each week calculated.

3.6.4.8 Measurement of BOD_5 using Respirometer by SELUTEC

Exactly 250 g (250 mL) each of the effluent sample was weighed and placed in three BOD_5 amber bottles. The same weight of water was also weighed and used as blank. Two drops of nitrogen were added to each of the bottle containing either the sample or the water. One magnetic stirrer was put in each of the bottle. Three pellets of sodium hydroxide (absorbing agent) were put in each perforated rubber septum affixed on each bottle before the bottles were tightly capped. Each bottle containing the prepared sample was incubated at 20 ^oC for 5 days in a BOD_5 digital equipment (BOD Measuring System BSB digi, SELUTEC - Germany). After waiting for 1 hour all the oxygen present in the bottles were released and the equipment was restarted for BOD_5 measurement. This method uses the power consumption to calculate the concentration of oxygen consumed in the samples and the blank (coulomb-metric oxygen measurements). With the help of the magnetic stirrers in the bottles, each sample in each

bottle is stirred to consume oxygen to reach a saturation limit. Carbon dioxide is consequently produced in the process but it is absorbed by the sodium hydroxide pellets in the septum. These processes of oxygen consumption and carbon dioxide production result in creation of a vacuum leading to electrolytic generation of oxygen using a contact pressure gauge. The concentration of oxygen consumed is directly related to the concentration of organic matter in the sample. For example, 1 mgO_2/L is equal to 1 mgBOD/L. The concentration of oxygen consumed over the five-day period is measured and recorded on an online computer control system connected to the BOD_5 digital equipment. After 5 days of incubation, oxygen concentrations in the samples and the blank that had been consumed by bacteria and measured from the beginning to the end of the test via the online system were used to calculate the BOD_5 values at 20 °C on the online computer control system as:

$$BOD_{5,\,20\,^{o}C} = (Initial\ concentration\ of\ oxygen\ in\ the\ sample - Final\ concentration\ of\ oxygen\ in\ the\ sample) * Dilution\ factor \quad (10)$$

The values of the BOD_5 were recorded based on the positions of the sample bottles in the BOD_5 equipment cell chamber and the average BOD_5 was calculated for the effluent.

3.6.4.9 Measurements of volatile organic acids in influents and effluents for HT-CSTR

Gerhardt Vapodest equipment (Gerhardt Vapodest) was heated until it was ready to be used with programme button signalling P, an indication that it was ready. Exactly 100 mL of distilled water was measured into the behrotest tube (Düsseldorf aufschlußgefäß SR 3i) and placed in the tube section of the equipment. A 500 mL Erlenmeyer flask was placed by the behrotest tube to collect the distillate of distilled water, until the distilled water began to bubble. The behrotest tube and the Erlenmeyer flask were emptied. Exactly 100 mL of the sample (BW, FW, MIX or effluent) was measured into the behrotest tube and 5 mL of 85 % H_3PO_4 was pipetted into the sample. Exactly 50 mL of distilled water was measured into an empty Erlenmeyer flask and placed by the behrotest tube in the Gerhardt Vapodest equipment. The sample was digested for 15 minutes in the Gerhardt Vapodest equipment (Gerhardt Vapodest, Made in Germany) and the distillate was collected in the Erlenmeyer flask containing the 50 mL distilled water. After the distillation process, the distillate tube was rinsed into the Erlenmeyer flask containing the distillate and was further boiled for 9 – 10 minutes (ensuring that the distillate really boils) on a hot plate. After boiling the distillate for 10 minutes, it was allowed to cool to room temperature and 5 drops of phenolphthalein indicator was added and

stirred vigorously using a magnetic stirrer. Drop-wise 1.0 M NaOH solution was titrated into the already cooled distillate containing the phenolphthalein indicator using TITRONIC® (Titronic® Universal TZ 3260 Nr. M003189 SI Analytics, S/N 00693908, D-55122, Mainz-Germany) until a light-pink colour change was observed.

The concentration of the water steam volatile organic acids was calculated as acetic acid (ethanoic acid) or mval/l as:

$$mAC = \frac{V * c * MAC}{Vsample} \quad (11)$$

Where:

mAC = Concentration of acetic acid to be calculated

V = the volume of NaOH that is used in the titration to reach end point.

V_{sample} = the volume of wastewater sample used (100ml)

c = the concentration of NaOH used (1.0M)

MAC = molar mass of acetic acid (ethanoic acid)

3.6.4.10 Spiking of the single-stage HT-CSTR with Salmonella senftenbergensis and Escherichia coli to assess pathogen decay efficiency

A single colony each of *Salmonella senftenbergensis* (origin: Herr Dr. Metzger, UFZ- Halle, Germany; Risk group 2) was picked from a plate of a pure culture using inoculating metal rod and dispersed in a 2 mL of Nutrient Broth II (NB2) medium in a test tube and incubated at 36 ^{o}C for 24 hours. After 24 hours, the bacteria in the 2 mL suspension in the test tube was further inoculated in a 200 mL Nutrient Broth II (NB2) medium and incubated for 36 ^{o}C for another 24 hours. The new suspension of *Salmonella senftenbergensis* (2 x 10^9 CFU/mL) in the 200 mL Nutrient Broth II (NB2) medium was dispensed into the HT-CSTR. This procedure was repeated for the *Escherichia coli* (DSMZ 498, Risk group 1) which had a concentration of 8 x 10^8 CFU/mL before it was introduced into the reactor. The decay efficiency of the model pathogens for the HT-CSTR was monitored after 1, 2, 3, 4, 5 hours right after the spiking was done and 20, 21, 22, 23, and 24 hours.

About 50 mL each of effluent samples from the HT-CSTR was taken in synchronise with the monitoring time mentioned in the afore-mentioned paragraph. The samples were cultured on

Brilliant Green Agar (BGA) and Endo Agar (EA) at a temperature of 36 ^{o}C for 24 hours. The BGA selective medium was used for *Salmonella senftenbergensis* while the EA selective medium was used for *Escherichia coli*. Each culture was monitored with both positive and negative (sterile agar) controls for the two strains (Figure 3.4). Preparations of the EA and BGA are presented in sub-sections 3.6.4.11 and 3.6.4.12, respectively.

Figure 3.4: Researcher preparing different selective media for pathogen decay test in the laboratory of the Chair of Biotechnology of Water Treatment in BTU (Laboratory photograph, 2016)

3.6.4.11 Preparation of Endo selective/differential media (agar)

Endo selective media (SIFIN, made in Germany) was prepared by following Manufacturer's instruction. Exactly 1.73 g of Endo powder was weighed into 50 mL of distilled water (34.6 g in 1 L of distilled water), swirled gently and ensured the pH was 7.5 ± 0.2 (at 25 ^{o}C). It was heated on a water bath at a temperature of 100 ^{o}C for 40 minutes, ensuring all the powder had dissolved and without autoclave. After 40 minutes, it was allowed to cool to 50 ^{o}C. The media was dispensed into different sterilised petri dishes under a fume chamber operating under sterile conditions (the fume chamber was air- cleaned for 10 minutes to avoid contamination) and allowed to cool for about 20 minutes to solidify. The solidified agar was kept at a safe place ensuring that it had no interaction with light. NB: For a guide, *E. coli* on EA appears golden green while *Salmonella Senftenberg* appears light pink or pinkish-white on EA. All precautionary measures to ensure utmost sterility practices were complied with; for

example, the gas burner was on and around the area where water was poured and the media were mixed.

3.6.4.12 Preparation of Brilliant Green agar (modified) selective/differential media

Brilliant green powder (Modified) (OXOID, made in Germany) was prepared by following Manufacturer's instruction. Exactly 2.6 g of the powder of Brilliant green (modified) was weighed into 50 mL of distilled water (500 g in 9.6 L of distilled water), swirled gently and ensured the pH was 6.9 ± 0.2 (at 25 ^{o}C). It was heated on a water bath at a temperature of 100 ^{o}C for 40 minutes, ensuring that all the powder had dissolved and without autoclave. After 40 minutes, it was allowed to cool to a temperature of 50 ^{o}C. The media was dispensed into different sterilised petri dishes under a fume chamber operating under sterile conditions (the fume chamber was air- cleaned for 10 minutes to avoid contamination) and allowed to cool for about 20 minutes to solidify. The solidified agar was kept at a safe place ensuring that it had no interaction with light. *Salmonella senftenberg* on BGA appears pink while *E. coli* is inhibited.

3.6.5 Treatment of source-separated concentrated BW co-digested with kitchen food waste in the laboratory-scale single-stage HT-CSTR

After 10 weeks of treating only BW in the HT-CSTR, co-digestion of kitchen waste was started. A 1:1 (v/v) ratio of assorted kitchen food waste (leftover green waste without cellulose, leftover rice, leftover potato, leftover corn dough, leftover cassava starch and rejected meat pie) to water was thoroughly mixed using a hand mixer. The BW and food waste (FW) were analysed for COD, total carbon (TC), total organic carbon (TOC), total inorganic carbon (TIC), total nitrogen (TN), total phosphorus (TP), ammonium (NH_4-N), nitrate (NO_3-N), nitrite (NO_2-N) and phosphate (PO_4-P). A 1:1 (v/v) ratio of the mixed FW and BW was called **MIX substrate** and the composition making up the physico-chemical parameters stated above was analysed before it was daily fed into the reactor. The performance of the single-stage HT-CSTR operating on the **MIX substrate** was ran for 12 weeks. The volume and quality of biogas produced was measured daily as was described in sub-section 3.6.3.

3.6.6 Assessment of the performance of the laboratory-scale single-stage HT-CSTR

The performance of the laboratory-scale single-stage HT-CSTR that operated on only BW and also MIX substrate was assessed based on methane yield (equation 6), methane productivity (equation 7) and percentage content of methane in the biogas over the period of study. In

addition, COD removal efficiency (Degradation efficiency) (ɳ), HRT and COD or organic loading rate for MIX substrate were calculated based on equations 12, 13 and 14 respectively:

$$COD\ removal\ efficiency\ or\ Degradation\ efficiency\ (\eta) = 100\% - \frac{Eff.conc}{Inf.conc} * 100\% \quad (12)$$

$$\text{HRT (d)} = \frac{Volume\ (L)}{Flow\ rate\ \left(\frac{L}{d}\right)} \quad (13)$$

$$\text{COD Loading Rate}\ \left(\frac{\text{mgCOD}}{\text{d}}\right) = COD\ concentration\ \left(\frac{mgCOD}{L}\right) * \text{Flow rate}\ \left(\frac{\text{L}}{\text{d}}\right) \quad (14)$$

Volumetric loading rate, degradation performance of the reactor and food to mass ratio of the reactor were also assessed for MIX substrate based on the formulae in equations 15, 16 and 17:

$$\text{Volumetric Loading Rate}\ \left(\frac{\text{mg}}{\text{L} * \text{d}}\right) = \frac{COD\ or\ Organic\ concentration\ \left(\frac{mg}{L}\right) * \text{Flow rate}\ \left(\frac{\text{L}}{\text{d}}\right)}{Active\ volume\ of\ the\ reactor\ (L)} \quad (15)$$

$$Degradation\ Performance\ (R)\left(\frac{kg}{m3}.d\right) = \frac{Load\ (influent) - Load\ (effluent)\left(\frac{kg}{d}\right)}{Volume\ of\ the\ reactor\ (m3)} \quad (16)$$

Food to mass (F/M) ratio of the reactor was also assessed using the formula:

$$\frac{\text{F}}{\text{M}}\text{ratio} = \frac{Influent\ concentration. * Flow\ rate}{Volume\ of\ reactor * Concentration\ of\ biomass} \quad (17)$$

3.6.7 Measurement of composition of biogas

The composition of daily biogas production was measured using a portable Gas Analyser: Geotechnical Instrument (Biogas Ansyco, UK). The Gas Analyser measures the percentage of methane, carbon dioxide and oxygen present in the biogas. The inflow pump of the instrument was connected to the outflow tube of the biogas digester or the HT-CSTR ensuring that the biogas measured was for the particular day in which the measurement was done. Biogas that had accumulated in the gas space of the reactor in the day was sucked into the Geotechnical Instrument by pressure to evaluate the composition of the biogas. The analysed biogas was released into the atmosphere through the outflow pump of the instrument. Analyses of biogas composition was done every 24 hours in order to ascertain the percentage composition for a particular day.

3.7 Assessment of biomass present in the hyper-thermophilic seeding sludge

The presence of a good microbial community, particularly, of the methanogenic community has a direct relationship with the performance of the reactor in terms of methane production. Not all methanogens can survive extreme conditions such as hyper-thermophilic treatment regime. Thus, assessing and knowing the type of methanogens that are able to operate under hyper-thermophilic temperature condition is worthwhile. Due to easy availability of chemicals and convenience of the laboratory personnel, Fluorescence In-Situ Hybridisation (FISH) technique was employed to assess the biomass in the seeding sludge of the single-stage laboratory-scale HT-CSTR.

3.7.1 Sample preparation for Fluorescence In-Situ Hybridisation (FISH) technique

The FISH technique was performed based on 16S rRNA gene sequence analyses for archaeabacteria, eubacteria, *Methanosaeta spp., Methanosarcina spp., Methanomicrobium spp., Methanococcus spp., Methanomicrobium spp., Methanogenium spp., Methanoculleus spp., Methanospirillum spp., Methanocorpusculum spp.* and *Methanoplanus*. About 2 mL of the sample was pipetted into a centrifuge tube and was centrifuged (Hettich-Universal 320 R, S/N 000-3247-0200) for 5 minutes at 4 ^{o}C. This was repeated to obtain more pellet or sediment for the hybridisation process. Exactly 1.5 mL of diluted phosphate-based solution (PBS) was added to the pellet (sediments after centrifuging) and mixed thoroughly to wash the sediment. The PBS was composed of 2.3 g sodium phosphate dibasic heptahydrate, 16 g sodium chloride and 0.4 g monopotassium phosphate, all in 200 mL pure water. The prepared PBS was diluted ten times before usage. The sediment in the PBS was centrifuged at 5000 rpm at 4 ^{o}C for 5 minutes to wash it. This process was repeated 3 times. After washing, the sediment was centrifuged again. Paraformaldehyde (PFA) (4 %) of concentration 40 g/L was added to the sediment (to fix the cells) and was allowed to stand for 3 hours at room temperature or at 4 ^{o}C for 12 hours (overnight). The mixture was shaken from time to time to ensure good mixing. After the cells were fixed, the sample was centrifuged for 3 minutes at 10000 rpm at 4 ^{o}C and the supernatant was discarded. The sediment was washed and centrifuged 3 times in PBS at 10000 rpm at 4 ^{o}C (e.g. 1:10 and dissolved on water bath). The pellet was re-suspended gently in 500 μL PBS and 500 μL pure ethanol was added (1:1 ratio of PBS and ethanol or 50 % ethanol). The sample was stored at -21 ^{o}C for the FISH analyses. NB: The prepared sample could be stored in the freezer for at least 2 years. About 5 μL of the prepared sample was pipetted and placed on a slide, dried on Eppendorf Thermostat Plus for about 10 minutes and

the cells were observed under microscope (Nikon H600L, MODEL: Nikon Eclipse LV100) using magnification x20.

3.7.2 FISH performance/techniques

A well suspended sample (washed sediment in PBS) (10 μL) (section 3.7.1) was pipetted onto one side of a glass slide and allowed to air-dry. The sample was dehydrated in 50 % ethanol for 3 minutes and allowed to air-dry on the slide. The process was repeated step-wise for 80 % and 98 % ethanol, respectively, with each step lasting for 3 minutes and allowed to air-dry on the slide before the subsequent step was followed. A hybridisation buffer solution (Formamide) (Table 3.6) was prepared according to the needed stringency or specificity. For example, 20 % for (*Methanomicrobium spp., Methanogenium spp., Methanoculleus spp., Methanospirillum spp., Methanocorpusculum spp., and Methanoplanus spp.*), 35 % for Archaebacteria, 40 % for *Methanosarcina*, 45 % for *Methanococcus spp*., 0-50 % for Eubacteria and 50 % for *Methanosaeta spp.* The prepared hybridisation buffer was put on a water bath (GD120-Grant S/N-GM0920015, Grant Instruments – Cambridge Ltd, Cambridgeshire SGB 6GB England) at 48 ^{o}C for 5 minutes.

Table 3.2: Hybridisation buffer solution based on protocol prepared by Martienssen (2015)

Stringency	**25%**	**30%**	**35%**	**40%**	**45%**	**50%**
NaCl (μL)	360	360	360	360	360	360
Tris/HCl (μL)	40	40	40	40	40	40
Formamide (μL)	500	600	700	800	900	1000
SDS (μL)	2	2	2	2	2	2
Pure water (L)	1098	998	898	798	698	598

NB: The Nucleotide and colour was diluted with pre-warmed (5 minutes) hybridisation buffer to enhance easy visualisation under the microscope. For example, 24 μL hybridisation buffer and 3 μL nucleotide = 27μL, 40 μL hybridization buffer and 5 μL nucleotide = 45 μL.

Exactly 10 μL hybridisation buffer was added on each spot where the samples were assigned. The remaining pre-warmed buffer was put in a tube with a tissue paper to prevent dehydration

and 1 μL (according to 50 ng) oligonucleotide sample was added (work was done in the dark). The slide was placed on a pre-warmed thermomixer at 46 °C, closed carefully and the hybridisation was performed for at least 3 hours (in the dark). After the hybridisation step, the slides were horizontally placed in an Eppendorf tube and kept in an oven at 46 °C. Washing buffer was prepared in a 50 ml Eppendorf tube, heated to 48 °C on a water bath (GD120-Grant, S/N GM0920015) thirty minutes before hybridisation was finished. The slides were taken from the oven at 46 °C and rinsed gently with 1 mL pre-warmed washing buffer (see Table 3.7 for its preparation) (in the dark). After rinsing with 1 mL pre-warmed buffer, the slides were placed in the 50 mL Eppendorf tube containing the washing buffer (in the dark). The Eppendorf tube containing the slides were placed on water bath (in the dark) at 48 °C for 15 minutes. The slides were removed, rinsed with pure water and air-dried in the dark. A maximum of 15 µL 4',6-diamidino-2-phenylindole (DAPI) (1 µg/mL) was added and allowed to stay for about 5-10 minutes before washing with pure water. The slides were then air-dried for about 10 minutes and covered with cover slips. Prior to covering the air-dried slides, anti-fading oil was added to the air-dried spots before covering it with a special cover slip. The cover slip was carefully pressed to remove surplus DAPI and likewise to remove all air. The covered slides were sealed with nail polish and observed under the microscope (Nikon H600L, MODEL: Nikon Eclipse LV100) at x100 magnification to evaluate hybridisation with epifluorescence microscope. The covered slides were later stored at -21 °C.

Table 3.3: Washing solution based on protocol prepared by Martienssen (2015)

Stringency	**20%**	**25%**	**30%**	**35%**	**40%**	**45%**	**50%**
5M NaCl (μL)	2150	1490	1020	700	460	300	180
1M Tris/HCl pH 7.4 (mL)	1.0	1.0	1.0	1.0	1.0	1.0	1.0
MilliQ/Pure water (mL)	46.8	47.46	47.93	48.25	48.49	48.65	48.77
10% SDS (μL)	50.0	50.0	50.0	50.0	50.0	50.0	50.0
Total volume (mL)	50.0	50.0	50.0	50.0	50.0	50.0	50.0

From 20% stringency, a 0.5M EDTA of 500 μL at pH of 7.4 can be used.

EDTA 0.5M 9.306g 50 mL pure water or MilliQ water can be used.

3.8 Pilot project at Terterkessim slum, Elmina - Ghana

3.8.1 Study area

Elmina is a coastal town and the administrative capital of the Komenda Edina Eguafo Abirem (K.E.E.A.) Municipality of the Central Region of Ghana (Ghana Statistical Service, 2014). Elmina is bordered to the South by the Gulf of Guinea, West by Bantoma, East by Abakam and North by Bronyibima townships (Ghana Statistical Service, 2014). Elmina lies within latitudes 5º 05' North and 5º 60' North and longitudes 1º20' West and 1º 22' West (Figures 3.5 and 3.6). The town is one of the biggest fishing hubs of Ghana and thus, the major occupation in the town is fishing. The presence of Brenya lagoon, which stretches and overflows (during high tides) to Terterkessim slum, has also made some of the inhabitants to be involved in salt production at commercial quantities. Temperatures are generally high with average being 27 ºC and annual rainfall ranging between 750 mm to 1000 mm. The vegetation are mostly shrubs and grasses (Ghana Statistical Service, 2014). Elmina has a total population of approximately 34000, of which about 7600 of the Inhabitants live in Terterkessim slum (Personal Communication with Mr. Damptey- K.E.E.A. Municipal Environmental Health Officer, 2016).

Figure 3.5: Map of Ghana showing the District Map of the Study Area, Elmina. Source of the map: Adade, F. (2016). GIS, Department of Fisheries and Aquatic Sciences (DFAS), University of Cape Coast, Cape Coast-Ghana

Figure 3.6: A section of Terterkessim slum in Elmina taken by a drone (DFAS, 2016)

The picture on the ground is significantly different from the picture taken by the drone. Figure 3.7, *a* and *b* show the major drainage canal and type of vegetal cover in Terterkessim slum in Elmina.

a

b

Figure 3.7, *a* and *b*: Terterkessim slum in Elmina showing their major drainage canal to the Brenya lagoon and a grassy vegetation (Field photograph, 2016)

3.8.2 Social survey

3.8.2.1 Assessment of perception of residents on their willingness to adopt and use a single-stage SSHTABD

This part of the report emphasises how the perception of residents of Elmina were sought regarding their willingness to adopt and invest into having the single-stage Solar-Supported Hyper-thermophilic Anaerobic Biogas Digester (SSHTABD).

3.8.2.2 Sources of data

Two data sources were employed in this sub-section of the research to assess the perception of residents of Elmina on their willingness to adopt and invest into having the SSHTABD.

The primary data were obtained by administering structured questionnaire which had both closed and open-ended questions to the respondents in the sampling population. The data obtained from the questionnaire administration include: demographic, educational and occupational data, data on accessibility to toilet facility, energy sources for cooking and sources of fertiliser for agriculture. In addition, knowledge of respondents on SSHTABD for human faeces and food waste treatment was assessed. Furthermore, their willingness to adopt and invest into the SSHTABD technology for human faeces treatment in their homes was also assessed. In addition to the questionnaire, an interview was conducted with the Municipal Environmental Health Officer (Mr Damptey, 2016), who also shared his concerns on the sanitation situation in the Municipality to buttress what has been reported in literature.

The secondary data were obtained by literature review on the demographics of K.E.E.A. Municipality for some vital information such as the population size, type of energy for domestic usage and sanitation-related issues.

3.8.2.3 Sampling population (exclusion criteria for sample size determination)

The sampling population consisted of all residents of Elmina who have reached eligible age for voting in Ghana (18 years and above). Any resident below this age category was considered unfit to participate in the research. In addition, transient population was eliminated in this research. Elmina has a total population of approximately 34000 (Personal

Communication with Mr. Alex Damptey - K.E.E.A. Municipal Environmental Health Officer, 2016), and this represented the sampling population of the social survey.

3.8.2.4 Sample size determination (n).

Based on the information provided by the Municipal Environmental Health Officer (Mr Damptey, 2016) on the population of the Municipality, the sample size (n) was calculated using a mathematical formula proposed by Puopiel and Owusu-Ansah (2014),

$$n = \frac{N}{1 + N(\alpha^2)} \tag{18}$$

Where:

n= sample size,

N= sample population or total population of the area under study (34000)

α = margin of error i.e. 0.07, which is the confidence level of 93 %.

In order to achieve at least 93 % confidence level based on Puopiel and Owusu-Ansah (2014), the sample size for this study was calculated to be 202.86 = 203 individuals who were to be interviewed. Consequently, the sample size for the structured questionnaire survey was supposed to be at least 203 respondents. The researcher therefore decided to administer 219 questionnaire to represent the sampled size. The number of questionnaire was increased to minimise errors and assure accuracy. It noteworthy to mention that because the people in the sample size have different economic status as at the time of the social survey, economic consideration and residential location was factored into the administration of the questionnaire. Consequently, the sampled size within the sampled population was categorised based on their economic status as well as their residential location. This has been described in details in the subsequent sub-section.

3.8.2.5 Sampling approach (administration of questionnaire)

A combination of stratified sampling, simple random sampling, systematic sampling and convenience (accidental) sampling methods were used in this research. The Elmina town was stratified into 3 non-overlapping groups based on the residential and economic strengths

namely: Lower Class Residential Area (LCRA), Middle Class Residential Area (MCRA) and High Class Residential Area (HCRA) adapted from a work by Puopiel and Owusu-Ansah (2014).

The LCRA in this study consisted of individuals whose daily income was below the average minimum daily wage of 1.9 USD (World Bank, 2017). They included suburbs like Terterkessim slum, New Market Area, Cemetery and Zongo. A total of fifty percent (50 %) of all respondents in the sampled size was allocated to members from these communities since their indication of willingness to adopt and invest into having SSHTABD technology would most probably imply the other higher residential classes would also adopt and invest into having the technology. This implied that the sampled size for the LCRA was 109 respondents (Figure 3.8a).

The MCRA in this report consisted of individuals whose average daily income was two to five times higher than the average minimum daily income (3.8 – 9.5 USD daily) proposed by the (World Bank, 2017). These included residents living in suburbs like Estates, Police Station, Brofobobaho, Chapel Square, Fishing Harbour Area and Akotobinsin. They represented twenty-five percent (25%) of the entire sampled size. This gave a proportionate sampled size for the MCRA to be 50 respondents (Figure 3.8b).

Figure 3.8a: Researcher interacting with an inhabitant of the Terterkessim urban slum (LCRA) in Elmina (Field photograph, 2016).

Figure 3.8b: Researcher interacting with some inhabitants of the Chapel Square Area (MCRA) in Elmina (Field photograph, 2016).

The HCRA represented individuals whose average minimum daily wage or income was more than five times (9.5 > USD) the average minimum daily wage of 1.9 USD set by the World Bank (2017). They consisted of residents living in areas like SSNIT Flats, Elmina Beach Resort Area, Ahomka FM, African Pot and Construction Pioneers (CP) residential areas. They also

made up 25 % of the total sampled size interviewed in this study using structured questionnaire and thus the number of individuals who were interviewed in the HCRA was 50.

In Elmina and Ghana in general, most houses are built haphazardly, and thus random sampling technique was used to select the first house within the stratified area. After locating the first house, the systematic approach was then employed where every **n^{th}** (10^{th}) house within the stratified area was selected for the interview using structured questionnaire (to ensure wider coverage of the area). The questionnaire had both closed and open-ended questions. Within the house or household, the convenience sampling method was then used to select any resident in a household who happened to be at home, fell within the inclusion criteria for the interview and was also willing to participate in the research.

3.8.2.6 Processing and analyses of data from the questionnaire

Questionnaire that were administered to the respondents were scrutinised to see whether they were fully filled out and all the questions on the sheet had been answered.

Questionnaire that was not fully filled out was eliminated and new one was administered to a new respondent to replace the spoilt questionnaire. The software, Statistical Package for Social Sciences (SPSS) was used for both quantitative and qualitative analyses of the responses from the questionnaire. Both the quantitative and qualitative data were processed into charts, tables and figures for explanation as part of the results and were later discussed.

3.8.3 Construction of the single-stage SSHTABD

This sub-section describes selection of site for the construction of a household single-stage solar-supported hyper-thermophilic anaerobic biogas digester (SSHTABD), the construction design parameters, the type of inoculum used (based on results from the laboratory-scale HT-CSTR in BTU, Germany), influent flow rate and the parameters needed for optimal performance of the biogas digester.

3.8.3.1 Selection of site for the construction of the single-stage household SSHTABD at Terterkessim slum, Elmina – Ghana

A television station in Ghana, Joynews, on 3rd October, 2016 in one of their repeated news items which had been shown earlier on 10th August, 2016 highlighted the menace BW was causing in Elmina, specifically, in Terterkessim slum. The BW, resulting from open defecation in the Terterkessim slum of Elmina as well as in open gutters of the community was affecting the quality of commercial salt production by Elmina Salt Industry (ESI) in that area. The whole community has only one public toilet (which was in a very bad state) and most individual households do not have toilet facilities, thus giving the residents the impetus to defecate in the open gutters, lagoon and even in and around the salt ponds. Thus the construction of a household toilet facility for both biogas production and disinfection of digestate was imperative for the Terterkessim urban slum in Elmina. The opinion leader of the community who also happens to be the owner of the house where the SSHTABD was constructed had expressed much interest in the technology but did not have the financial capabilities to construct one. Moreover, he showed willingness to take care of the reactor after it had been handed over to his household. In addition, he was willing for the people in his neighbourhood to use the toilet facility that was to be constructed in his home. The nature of soil in the slum is generally sandy, thus the site selected for the construction was also sandy.

3.8.3.2 Description of the household where the SSHTABD was constructed

The household has 10 separate single rooms and had 2 bathrooms which drained into an open gutter located at the back of the house. They had no toilet facility and thus the residents of this household either visited the public toilet which was often in untidy condition and also located 300 m away or they resorted to flying toilets (defecating in polythene bags and flying them over into a gutter or anywhere in the neighbourhood). Some also defecated near or in the lagoon where commercial salt production also occurs. The owner of the house had, however, made efforts to construct a toilet with a three-chambered septic tank system but he could not complete the project due to financial constraint.

The opened septic tanks (three chambers) had received a lot of rain water as well as infiltration water from a gutter which was just adjacent by them. In the opened septic tanks were frogs and also growth of green algae like *Spirogyra sp* (since the colour of the water in the tanks of the chambers was green). The septic tanks had a lot of weeds around it. The biggest chamber of the opened septic tanks was later converted into the main biogas digester for the single-

stage SSHTABD while the other adjoining smaller chambers were converted to the compensation tank and effluent collection tank, respectively, before the effluent was finally used for urban agriculture.

The household selected for the construction of the single-stage SSHTABD was a household with 34 individuals. It comprised 12 male adults, 10 female adults, 9 boys and 3 girls. Figure 3.9 shows the states of the two-seater toilet and the chambers of the opened septic tank that were abandoned by the opinion leader of Terterkessim urban slum (Assembly man and the owner of the house) before their conversions into the single-stage SSHTABD.

Figure 3.9: The original state of the toilets and septic tanks which were converted into the biogas reactor (Field photograph, 2016).

3.8.3.3 Materials for the construction of the SSHTABD reactor

The single-stage SSHTABD was constructed with 6-inch-blocks (moulded sand, cement and water), reinforced with concrete material and plastered with mortar. The reinforcement became necessary due to the dilapidated nature and weakness of the septic tank chambers. The concrete material was made of 10 head pans of quarry sand, 10 head pans of 0.5-inch stones (igneous type), 2 bags of rapid strength Portland cement and 10 L of tap water. Additional mortar and water-proof cements like FEB TANK (UK) were used to stop all water leakages into the reactor chambers (Figure 3.10). The mixture of the mortar was 1 bag of Portland cement (50 kg), 6 head pans of quarry dust, 1 head pan of eroded sand and 2 kg of waterproof FEB TANK cement. About 10 L of water was added and homogenised into a thick paste of mortar for the reinforcement of the weak walls and floor based on the specifications by the manufacturer of the FEB TANK waterproof cement.


Figure 3.10: Reinforcement works on the septic tank chambers which were converted into the biogas reactor (Field photograph, 2016)

Two pour-flush water closet (WC) toilet seats were installed in each of the toilet unit which had been roofed with aluminium roofing sheets. Polyvinyl chloride (PVC) pipes of diameter 4-inches were connected to the toilet seats and into the main chamber of the reactor. Adjoining pipes from the WC into the reactor were connected using 4-inch Tee, 4-inch 45^{o} and 4-inch 90^{o} pipes. The influent pipe was inserted into the reactor to a depth of 450 mm above the floor of the reactor. This was done to ensure that the influent fully covered the pipe to avoid any biogas leakage through the influent pipe (Figure 3.11).

Figure 3.11: Installation of pour-flush water-closet toilet seats and pipe network into the reactor (Field photograph, 2017)

The reactor was a modified form of a circular fixed-dome biogas digester with the circular dome modified into a pyramidal shape roof for biogas storage. The pyramidal shape roof was done instead of the circular dome because the base of the reactor was rectangular, consequently, a pyramidal shape roof on the rectangular base would ensure airtightness. This was because the rectangular base had corners which a circular dome shape could not perfectly fit on without leakages. Ten pieces of 14-ft Wawa wood of dimensions 2-in by 4-in as well as 15 pieces of 14-ft Wawa wood of dimensions 2-in by 2-in were used for the construction of the gable of the pyramidal dome shape of the SSHTABD fastened with 3-in concrete nails. The skeletal structure of the pyramidal-shape roof of the biogas digester was covered with 5 pieces of ¼-plywood. A black thick polythene bag was used to cover the plywood before the concrete layer was formed on the reactor. The 6-in (15.24 cm) concrete layer for the roof of the SSHTABD was made of 15 pieces of 0.6-in (1.5 cm) diameter iron

rods, 1.5-in (3.8 cm) diameter stones (igneous type) and sand (both coarse and fine). A manual stirrer with four (4) galvanised metal blades of dimensions 15 cm by 30 cm each was affixed into SSHTABD. The rotating metal rod of the stirrer was welded into two ball bearings (one affixed to the bottom of the concrete and the other at the top of the metal rod just beneath the pyramidal shape) to enhance easy rotational movement when manually stirred. Detailed structural designs of the pyramidal-shaped roof of the SSHTABD is found in Figure 3.12.

Figure 3.12: Design and construction of an insulated pyramidal-shaped roof and installation of the manual stirring blades in the reactor (Field photograph, 2017)

3.8.3.4 Installation of copper pipes from the SSHTABD to the kitchen

Galvanised copper pipes were used to connect the SSHTABD to the kitchen of the household where potential biogas to be produced was to be used. The copper pipes had diameter of 2

cm. Stop corks or valves were installed at adjoining points to regulate the flow of biogas into a biogas bag to monitor the daily biogas production. The copper pipe was laid into the walls of the restroom to the kitchen at an angle of 45^{o} in order to ensure that all water vapour that could form during the operation of the SSHTABD would trickle down by gravity into a collection tube to be discharged (without losing biogas from the reactor). Figure 3.13 shows the installation of the copper pipes and valves into the kitchen.

Figure 3:13: Installation of the copper pipes and valves from the digester into the kitchen (Field photograph, 2017)

3.8.3.5 Inoculation of the household single-stage SSHTABD

Based on results from the batch fermentation tests and laboratory-scale experiments carried out in Brandenburg University of Technology, Cottbus-Senftenberg, Germany that cow manure was a good seeding sludge for BW treatment at hyper-thermophilic temperature in a single-stage reactor, cow manure was used as a seeding sludge for the pilot-scale research in Terterkessim slum, Elmina-Ghana. A mixture of fresh and dry cow manure (with homogenised TS of 86.8 %, VS of 68.9 % and VS/TS ratio being 79.4 %) from the University of Cape Coast, School of Agriculture Teaching and Research farm was collected, homogenised, mixed with water and sieved (using a 1 mm sieving net). The quality of the filtrate cow manure that was used as the seeding sludge was assessed in terms of its pH, TS, VS, VS/TS ratio, salinity, conductivity, COD and heavy metals. The pH for the diluted filtered cow manure was 8.33 and had TS and VS contents to be 2.7 % and 1.6 %, respectively.

Consequently, the ratio of the VS and TS was calculated to be 59.3 %. The salinity and conductivity were 4.93 ppt and 8795 ds/m, respectively while total dissolved solids was 4376 mg/L. The total COD for the cow manure used as inoculum was 25,600 mg/L. Five hundred litres (500 L) of the mixed cow manure was introduced into the reactor for 1 month (to ensure adaptation of the methanogens in the reactor) before influent (black water and food waste) was introduced into the reactor (Figure 3.14).

Figure 3.14: Researcher sieving diluted cow manure for the inoculation of the pilot-scale single-stage SSHTABD (Field photograph, 2017)

3.8.3.6 Installation of offgrid photovoltaic system on the roof of the toilet

A high quality 50 W offgridtec® autarkic mono photovoltaic panel of dimensions 60.5 cm x 47.5 cm (0.3 m^2) was installed on the roof of the toilet connected to the SSHTABD for heating. The photovoltaic panel was offgrid with model number 3-01-001260 and had a voltage of 22.3 V (made by offgridtec® AGM GmbH, CMK ENERGY, Germany). The photovoltaic panel was connected to a solar charge controller (Stecca PR1010 756.477 by Solar Electronics, PV offGrid, PV Autarke systeme, made in EU) via solar cables. The charge controller was connected to a 12 V /30 A /20 Hours offgridtec AGM gel battery series (by offgridtec AGM

GmbH, Germany). The battery had a constant voltage charge and voltage regulation with cycle use of 14.5 - 14.9 V at 25 ^{o}C and standby use of 13.6-13.8 V at 25 ^{o}C. The battery was connected to an NP series pure sine wave inverter (Model number NP 300, made by Solartronics, Leipzig-Germany) which had a maximum peak power of 600 W and an average current of 300 – 400 W. It also had an input voltage of 12 V and an output voltage of 230 V ~ 50 Hz and efficiency of 84 - 94 %. Figure 3.15 shows the installation of photovoltaic panel and heating system for heating the SSHTABD.

Figure 3.15: Installation of solar photovoltaic heating system on the SSHTABD (Field photograph, 2017)

3.8.3.7 Education of family heads on the usage and operation of the SSHTABD

The family heads of the users of the single-stage SSHTABD were educated on the usage and operations of the entire system. Precautionary measures such as: do not use soapy water in flushing the toilet, do not put used toilet paper in the toilet, do not use detergent and abrasives to clean the toilet bowl were spelt out to them. Other precautionary measures including: do

not put perfumes in the toilet and do not use dirty water from nearby gutters for flushing the toilet were clearly cautioned out to the family heads for onward communication to the members of their families. In addition to the above precautionary measures, the heads were shown the operations of the SSHTABD in terms of how much water they could use for flushing after visiting the toilet and how to turn the manual stirrer for proper mixing of the fresh influent and the seeding sludge after visiting the toilet (Figure 3.16).

Figure 3.16: Orientation and education of family heads on how the entire system operates (Field photograph, 2017)

3.8.3.8 Feeding of the reactor with black water and household food waste

The reactor was fed with human faeces and urine flushed with 4 L water to form BW. Apart from the BW, household food waste (FW) was also introduced into the reactor through a co-digestion inlet (Figure 3.17). The FW was collected from households within the neighbourhood on weekly basis and was mixed with tap water (1:1, w/v) and then fed into the single-stage SSHTABD.

3.8.3.9 Analyses of influent and effluent samples

Influent samples of both the BW and FW were taken (Figure 3.17) and kept in a refrigerator (in the Department of Molecular Biology and Biotechnology, School of Biological Sciences, College of Agriculture and Natural Sciences, University of Cape Coast) at 4 ^{o}C for further chemical and microbial analyses.

Figure 3.17: Collection of influent and effluent samples for chemical and microbiological analyses (Field photograph, 2017)

The influent (BW and FW) samples collected weekly were carried on dry ice and were all stored in a refrigerator at 4 ^{o}C (in the Department of Molecular Biology and Biotechnology, University of Cape Coast). They were all added together at the end of every month and homogenised for the monthly chemical and biological analyses. Each sample was measured in duplicates.

Effluent samples were also taken on weekly basis, carried on dry ice and stored in a refrigerator at 4 ^{o}C (in the Department of Molecular Biology and Biotechnology, University of Cape Coast). At the end of the month, all the collected and stored effluent samples in the refrigerator were also homogenised and analysed in the same way as the influent. Each sample was measured in duplicates.

The stored influent and effluent samples collected from the pilot-scale experimental site were carried on dry ice and transported to the laboratories of Sewerage Systems Ghana Limited (SSGL) in Accra and Ghana Atomic Energy Commission (GAEC), Accra for some chemical and heavy metals analyses, respectively. Some of the samples were also taken to the laboratories of Molecular Biology and Biotechnology and School of Agriculture laboratories in the University Technology Village (University of Cape Coast) for Microbiological and other

physico-chemical analyses, respectively. Total COD, total nitrogen, ammonia nitrogen, nitrate, total phosphorus, phosphate, TS, VS and heavy metals were measured for BW, FW, and a mixture of BW and FW (MIX) (1:1, v/v) as well as the effluent (EFF).

Presence of pathogens like *Salmonella species* and *E. coli* were also cultured and identified in BW, FW and CM to assess their contributions to the concentrations of pathogens in the reactor. Presence of pathogens like *Salmonella species* and *E. coli* were also carried out in the effluent to assess the performance of the single-stage SSHTABD in terms of pathogen elimination as well as the quality of effluent for application as soil nutrients for agricultural purposes (Figure 3.18).

Figure 3.18: Researcher carrying out microbial analyses of samples from the single-stage SSHTABD in Terterkessim slum, Elmina, in the laboratory of Department of Molecular Biology and Biotechnology in the University of Cape Coast (Field photograph, 2017)

3.8.3.10 Analyses of nutrients and heavy metals for SSHTABD

Metals like Copper (Cu), Lead (Pb), Cadmium (Cd), Cobalt (Co), Arsenic (As), Nickel (Ni) and Mercury (Hg) and trace metals (micro-nutrients) like Zinc (Zn), Iron (Fe) and Manganese (Mn) were analysed using standard methods by Flame Atomic Absorption Spectrometry (FAAS).

3.8.3.10.1 Preparation of sample solution for the determination of N,K, Na, Ca, Mg, P, Zn, Cu, Fe

The preparation of sample solutions suitable for elemental analyses involves an oxidation process which is necessary for the destruction of the organic matter, through acid oxidation before a complete elemental analyses could be carried out.

3.8.3.10.2 Sulphuric acid-hydrogen peroxide digestion

The digestion mixture comprised 350 mL of hydrogen peroxide (H_2O_2), 0.42 g of selenium powder, 14 g of lithium sulphate and 420 mL sulphuric acid (H_2SO_4). Between 0.10 to 0.20 g of oven-dried ground sample was weighed into a 100 mL Kjeldahl flask, 4.4 mL of mixed digestion reagent added and the sample was digested at 360 ^oC for 2 hours.

Blank digestions (digestion of the digestion mixture without sample) were carried out in a similar way. After the digestion, the digestates were transferred quantitatively into 100 mL volumetric flasks and made up to volume.

3.8.3.10.3 Determination of organic carbon content from the organic matter content of the sample using Walkley-Black method

A more precise estimate of the organic carbon content from the organic matter content of the sample is given by Walkley-Black method. In this method the carbon content of the soil is determined by wet combustion using chromic acid digestion (chromic acid oxidation). This method does not determine total organic carbon but only the easily oxidisable carbon, which literature indicates is about 77% of the total organic carbon (Pribyl, 2010).

Easily oxidisable carbonaceous material was reacted with chromic acid (oxidising agent). An excess of a standard solution of $K_2Cr_2O_7$ [0.167 M $K_2Cr_2O_7$ (49.04 g $K_2Cr_2O_7$ per L of solution)] in concentrated H_2SO_4 was allowed to react with the sample and the excess dichromate was back-titrated with a standard ferrous iron solution, reducing agent. About [0.5 M ferrous

solution (196.1 g ammonium ferrous sulphate in 800 mL of H_2SO_4 and diluted to 1 L)] was prepared. The addition of concentrated H_2SO_4 to the dichromate solution supplied the heat needed to speed up the reaction. The highest temperature attained was approximately 120 ^oC, which was sufficient to oxidise the active forms of organic carbon but not the more inert form that may be present. The reaction of dichromate ion with organic carbon in the presence of sulphuric acid proceeded as follows:

$$2Cr_2O_7^{2-} + 3C + 16H^+ \longrightarrow 4Cr^{3+}\ 3CO_2 + 8H_2O$$

Excess dichromate ion was titrated with ferrous ion as:

$$6Fe^{2+} + Cr_2O_7^{2-} + 14\ H^+ \longrightarrow 2Cr^{3+}\ 6Fe^{3+} + 7\ H_2O$$

The amount of organic carbon was calculated from the amount of $Cr_2O_7^{2-}$ consumed in the reaction while the amount of organic matter was calculated by multiplying the organic carbon by a conversion factor 1.724 (Pribyl, 2010). Exactly 0.5 g of the sample was weighed and transferred into 500 mL Erlenmeyer flask and the weight was recorded. Ten millilitres (10 mL) of $K_2Cr_2O_7$ solution was pipetted into the Erlenmeyer flask and swirled gently. Twenty millilitres (20 mL) of conc. H_2SO_4 was added to the content of the flask and swirled gently for a minute and allowed to stand for 30 minutes (the reaction was exothermic). After 30 minutes, the content of the flask was diluted with 200 mL of distilled water and swirled again to ensure thorough mixing. Ten millilitres (10 mL) of H_3PO_4, 0.2 g NaF and 1 mL of diphenylamine were added as indicators (the H_3PO_4 and NaF were added to complex Fe^{3+} which otherwise would interfere with the end point). The excess Cr_2O_7 was back titrated with 0.5 M ferrous solution to a green end point. A blank titration was carried out using distilled water in an identical way using the same reagents. Percentage organic carbon was calculated as:

$$\%\ \text{organic carbon} = \frac{(B-S)*\text{Molarity of Fe2+}*0.003*1.299*100}{Weight\ of\ sample} \quad (19)$$

Where:

B = Blank titre value

S = Sample titre value

0.003 = 12/4000= milliequivalent weight of carbon

1.299 = the factor converting the carbon actually oxidized to total carbon

100 = the factor to change from decimal to percent.

$$\%\ \text{Organic matter} = \%\ organic\ carbon * 1.724$$

NB: it is assumed that organic matter for soil, for example, contains 58% carbon hence the use of the factor 100/58 = 1.724 to obtain the % organic matter (Pribyl, 2010).

3.8.3.10.4 Determination of total nitrogen (MICRO-KJEDAHL method) by distillation

A steam distillation apparatus was set up and passed through a steam for about 20 minutes for flushing. After flushing out the apparatus, a 100 mL conical flask containing 5 mL of boric acid indicator solution was placed under a condenser of the distillation apparatus. An aliquot of the sample digestate was transferred into the reaction chamber through a trap funnel and 10 mL of alkali mixture was added and the distillation was commenced immediately. About 50 mL of the distillate was collected and titrated against M/140 HCl from green to the initial red-wine colour of the indicator. The process was repeated for distilled water which served as blank and the titre value from the blank was subtracted from the titre value of the sample and calculation for percentage nitrogen is shown below:

$$\mathrm{N}\ (\%) = \frac{(S - B) * solution\ volume}{102 * aliquot * sample\ weight} \qquad (20)$$

where:

S = Sample titre value

B = Blank titre value

3.8.3.10.5 Colorimetric determination of P using the ascorbic acid method

The procedure required the preparation of colour forming reagents (A and B) and phosphorus standard solutions. Reagent A was made up of 12 g ammonium molybdate in 20 mL distilled water, 0.2908g of potassium antimony tartrate in 100 mL distilled water and 1 L of 2.5 M sulphuric acid (H_2SO_4). The 3 solutions were mixed together in a 2 L volumetric flask and made up to the volume with distilled water.

Reagent B was prepared by dissolving 1.56 g of ascorbic acid in every 200 mL of reagent A. A stock solution of 100 μgP/mL solution was prepared, from which 5 μgP/mL solution was used to set working standards of P with concentrations 0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 μgP/mL in 25 mL volumetric flasks.

A 2 mL aliquot each of the digested samples was pipetted into each 25 mL volumetric flask and 2 mL aliquot of the blank digest was pipetted into each of the working standards to give the samples and the standards the same background solution.

A 10 mL of distilled water was added to the standards and the samples. Afterwards, 4 mL of reagent B was added and their volumes diluted to 25 mL with distilled water and mixed thoroughly. The flasks were allowed to stand for 15 minutes for colour development after which the absorbance of the standards and samples were determined using a spectrophotometer (Model: BUCK 210 VGP, serial number 922, 58 Fort Point St. East Norwalk, CT 06855, U.S.A.) at a wavelength of 882 nm. A calibration curve was plotted using their concentrations and absorbance. The concentrations of the sample solutions were extrapolated from the standard curve based on the calculations below:

$$Concentration\ of\ PO4-P\ \left(\frac{\mu gP}{mL}\right) = \frac{Concentration\ of\ PO4-P\ in\ sample * Dilution\ factor}{Weight\ of\ sample} \quad (21)$$

(International Institute of Tropical Agriculture (IITA), 2000).

3.8.3.10.6 Determination of iron, copper, zinc, lead, chromium, cadmium and cobalt using atomic absorption spectrophotometer

Standard solutions of 1, 2 and 5 µg/mL solutions of Fe, Cu, Zn, Pb, Cr, Cd and Co were prepared based on (APHA/AWWA/WEF, 2012).

The standard solutions were aspirated into the atomic absorption spectrophotometer (AAS) (model: BUCK 210, serial number 922, Made in USA) and the respective calibration curves were plotted on the AAS. As the sample solutions were aspirated their respective concentrations were calculated as shown in equations 22 - 28:

$$Concentration\ of\ Fe\ \left(\frac{\mu g}{mL}\right) = \frac{Concentration\ of\ Fe\ in\ sample * Volume\ of\ solution}{Weight\ of\ sample} \quad (22)$$

$$Concentration\ of\ Cu\ \left(\frac{\mu g}{mL}\right) = \frac{Concentration\ of\ Cu\ in\ sample * Volume\ of\ solution}{Weight\ of\ sample} \quad (23)$$

$$Concentration\ of\ Zn\ \left(\frac{\mu g}{\mathrm{mL}}\right) = \frac{Concentration\ of\ Zn\ in\ sample * Volume\ of\ solution}{Weight\ of\ sample} \quad (24)$$

$$Concentration\ of\ Pb\ \left(\frac{\mu g}{\mathrm{mL}}\right) = \frac{Concentration\ of\ Pb\ in\ sample * Volume\ of\ solution}{Weight\ of\ sample} \quad (25)$$

$$Concentration\ of\ Cr\ \left(\frac{\mu g}{\mathrm{mL}}\right) = \frac{Concentration\ of\ Cr\ in\ sample * Volume\ of\ solution}{Weight\ of\ sample} \quad (26)$$

$$Concentration\ of\ Cd\ \left(\frac{\mu g}{\mathrm{mL}}\right) = \frac{Concentration\ of\ Cd\ in\ sample * Volume\ of\ solution}{Weight\ of\ sample} \quad (27)$$

$$Concentration\ of\ Co\ \left(\frac{\mu g}{\mathrm{mL}}\right) = \frac{Concentration\ of\ Co\ in\ sample * Volume\ of\ solution}{Weight\ of\ sample} \quad (28)$$

3.8.3.10.7 Determination of mercury by cold-vapour atomic absorption spectrometric method

This method used a similar principle like the use of FAAS, however, the sample was made to undergo reduction reaction where the mercury Hg^{2+} was reduced to a ground state mercury (Hg^0) atom. The ground state mercury atom was then transported into an optical cell (230 mm) made of fused silica for detection and measurement at wavelength 253.7 nm. This technique is extremely sensitive for mercury and mercury trace-analyses in ng/L because of its simplicity, robustness, speed, cost-effectiveness and relative freedom from interferences compared with the FAAS (Teledyne, 2017).

3.8.3.11 Economic considerations and possible applicability in Ghana

Economic considerations were assessed based on how much money was involved in the construction of the SSHTADB in the Terterkessim slum of Elmina. Calculations were made

based on how much methane was produced theoretically and how much money they would save if they had used the methane for cooking.

Calculations were also made on how much money the households would save for using the SSHTABD as a toilet facility instead of paying to use public toilet.

Economic calculations were made based on how much money a household would save if they used methane from the household SSHTABD instead of using charcoal and firewood. In addition, the quantity of carbon and carbon dioxide that could be sequestered by a household for adopting and using the SSHTABD was also calculated.

CHAPTER FOUR

Results

4.1 Batch tests for hyper-thermophilic temperatures 60, 65 and 70 °C

Figure 4.1 compares the net normalised cumulative volume of methane content ($C_{CH_4,cum}$) for the three seeding sludge at three different hyper-thermophilic temperatures and BW as the common substrate. For 156.7 g (for CM), 98.7 g (for BTU) and 93.4 g (for LWG) of BW used, CM at 65 °C recorded the highest net normalised cumulative volume of methane content (387 $mLNCH_4$ = 2.47 $mLNCH_4$/gBW), followed by LWG at 60 °C (197 $mLNCH_4$ = 2.11 $mLNCH_4$/gBW) and BTU at 65 °C (164 $mLNCH_4$ = 1.66 $mLNCH_4$/gBW). At 70 °C, BTU was the most inhibited with a net normalised cumulative volume of methane content of 0.0 $mLNCH_4$ followed by LWG (13 $mLNCH_4$) and CM (19 $mLNCH_4$). In this report, the legends for the curves in figure 4.1 **CM60, CM65 and CM70** imply seeding sludge called cow manure at hyper-thermophilic temperatures 60 °C, 65 °C and 70 °C, respectively. Subsequently, **BTU60, BTU65 and BTU70** imply seeding sludge obtained from Brandenburg University of Technology, Cottbus-Senftenberg (BTU) and operated at hyper-thermophilic temperatures 60 °C, 65 °C and 70 °C, respectively. Finally, **LWG60, LWG65 and LWG70** imply sewage sludge obtained from Lausitzer Wasser GmbH & Co. KG (LWG) Wastewater Treatment Plant in Cottbus and operated at hyper-thermophilic temperatures of 60 °C, 65 °C and 70 °C, respectively.

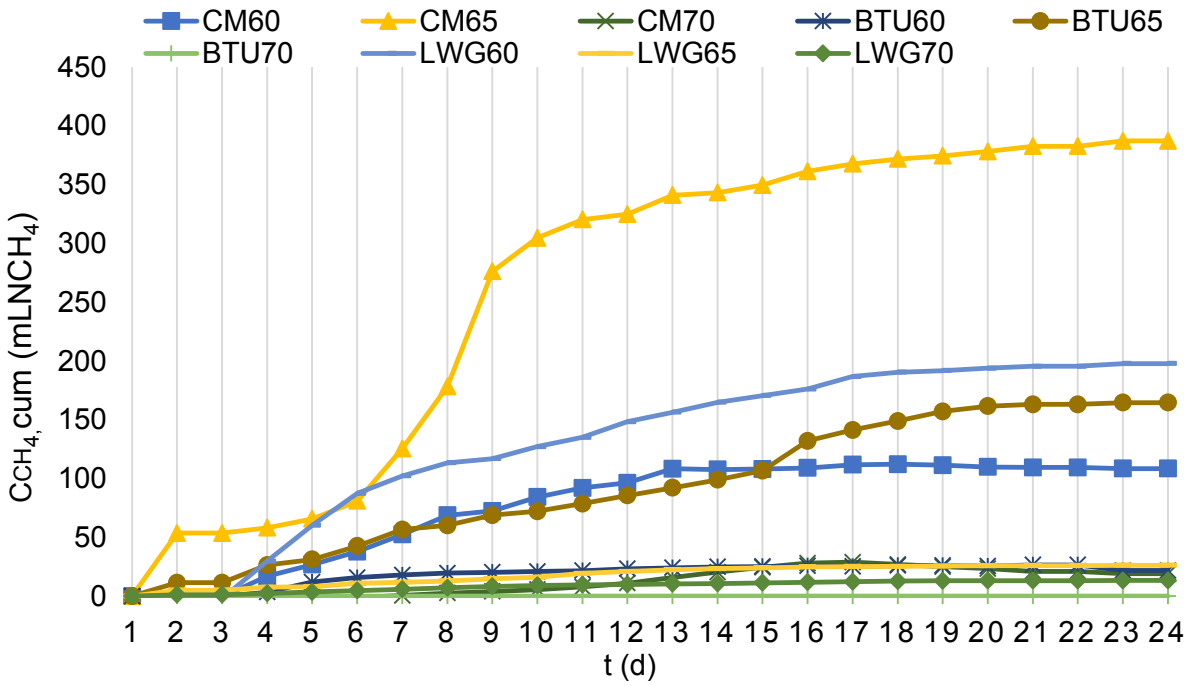

Figure 4.1: Net normalised cumulative volume of CH_4 content for three inocula at three hyper-thermophilic temperatures of 60 °C, 65 °C and 70 °C

Comparing net normalised cumulative volume of methane content of the optimal hyper-thermophilic temperature and inoculum (CM, 65 ^{o}C), to optimal temperatures for mesophilic (37 ^{o}C) and thermophilic conditions (55 ^{o}C) for the 3 seeding sludge, CM at 65 ^{o}C recorded the highest net normalised cumulative volume of methane content of (387 $mLNCH_4$ = 2.47 $mLNCH_4$/gBW). For CM at 65 ^{o}C, net normalised cumulative volume of methane content increased sharply within the first 8 days during the fermentation tests, thereafter, increased gradually till the end of the batch fermentation tests. The net normalised cumulative volume of methane content for LWG at optimal thermophilic temperature of 55 ^{o}C was the second highest (209 $mLNCH_4$ = 2.24 $mLNCH_4$/gBW). LWG at 55 ^{o}C had a curve that increased rapidly within the first 14 days of the batch fermentation tests but slowed down and increased slowly till the end of the batch fermentation tests. BTU at 55 ^{o}C was the third highest with respect to net normalised cumulative volume of methane content (55 $mLNCH_4$ = 0.56 $mLNCH_4$/gBW). It had a curve that increased very slowly even within the first two-weeks and more steady increase till the end of the tests (Figure 4.2). The legends for the curves in Figure 4.2 **CM37, CM55 and CM65** imply seeding sludge called cow manure at optimal mesophilic, thermophilic and hype-thermophilic temperatures 37 ^{o}C, 55 ^{o}C and 65 ^{o}C, respectively. Subsequently, **BTU37 and BTU55** imply seeding sludge obtained from Brandenburg University of Technology, Cottbus-Senftenberg (BTU) and operated at optimal mesophilic and thermophilic temperatures of 37 ^{o}C and 55 ^{o}C, respectively. Finally, **LWG37 and LWG55** imply sewage sludge obtained from Lausitzer Wasser GmbH & Co. KG (LWG) Wastewater Treatment Plant in Cottbus and operated at optimal mesophilic and thermophilic temperatures of 37 ^{o}C and 55 ^{o}C, respectively.

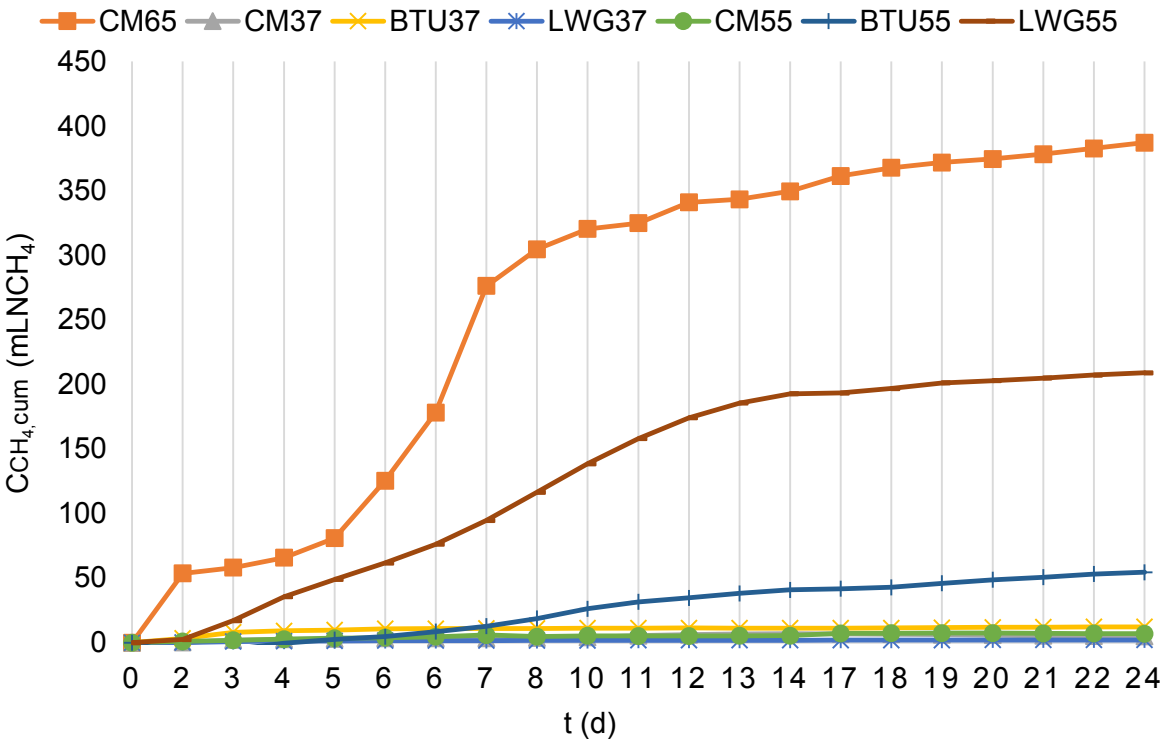

Figure 4.2: Comparison of net normalised cumulative methane volume for three inocula at optimal temperatures of 37 ºC, 55 ºC and 65 ºC

Comparison was made on the net normalised cumulative methane yield ($MYCH_4$,cum) for the three inocula for the five temperature regimes of 37 ºC, 55 ºC, 60 ºC, 65 ºC, 70 ºC. CM at 65 ºC recorded the highest net normalised cumulative methane yield of 232 mLNCH$_4$/gVS, followed by LWG at 60 ºC and 55 ºC which had 217 mLNCH$_4$/gVS and 180 mLNCH$_4$/gVS respectively. The least net normalised cumulative methane yield for the three inocula was recorded by BTU at 70 ºC (0.0 mLNCH$_4$/gVS) followed by CM at 37 ºC which recorded 0.9 mLNCH$_4$/gVS (Figure 4.3).

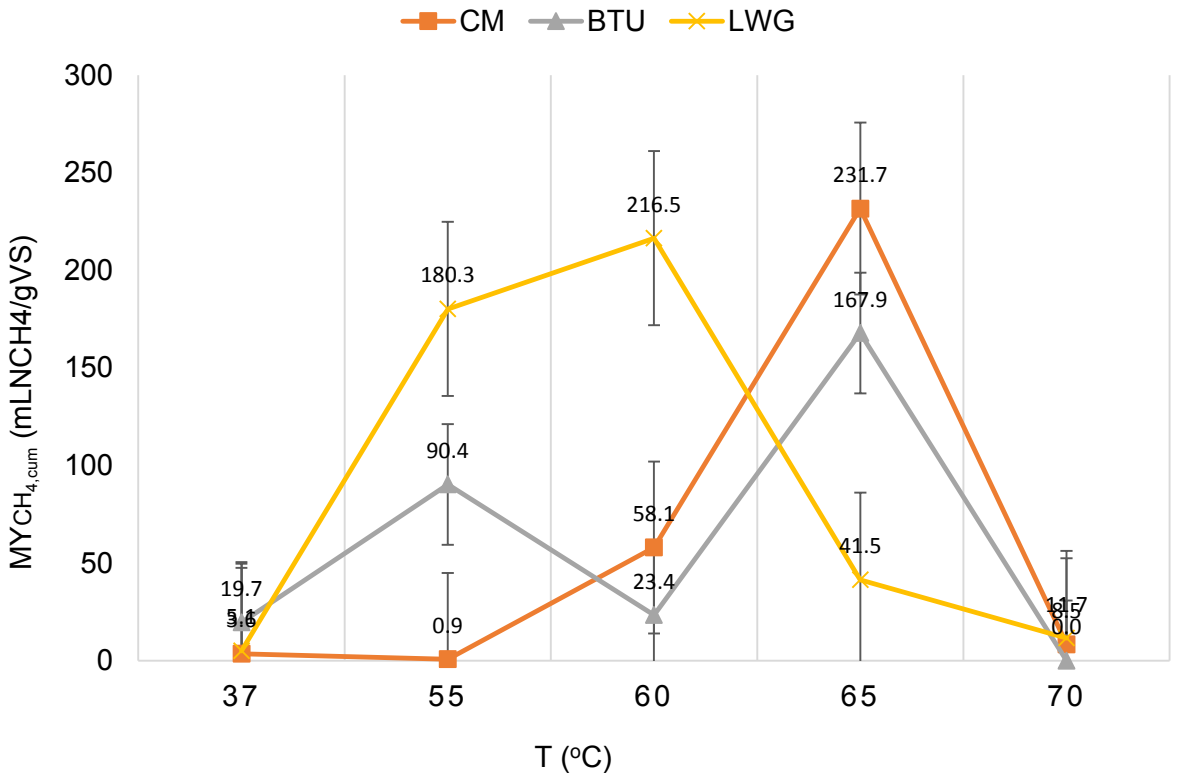

Figure 4.3: Comparison of net normalised cumulative methane yield for three inocula at hyper-thermophilic temperatures and optimal mesophilic temperature of 37 °C and thermophilic temperature of 55 °C

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Comparing net normalised cumulative methane yield of the optimal temperature and inoculum for hyper-thermophilic conditions (CM, 65 °C) to optimal temperatures for mesophilic (37 °C) and thermophilic conditions (55 °C), the optimal temperature for hyper-thermophilic temperature and inoculum, CM at 65 °C recorded the highest cumulative methane yield of 197 $mLNCH_4/gVS$. The methane yield for CM at optimal thermophilic temperature of 65 °C increased sharply within the first week before levelling-off thereafter. LWG at 55 °C recorded the second highest yield of 169 $mLNCH_4/gVS$. The cumulative methane yield increased sharply for LWG at 55 °C increased sharply within the first 2 weeks during the fermentation tests, thereafter, increased slowly till the end of the batch fermentation tests. Inoculum called BTU at 55 °C was the third highest with respect to the cumulative methane yield (79 $mLNCH_4/gVS$). It had a curve that increased very slowly even within the first 2 weeks and more steady increase till the end of the tests. All the remaining inocula at different temperatures were greatly inhibited (Figure 4.4).

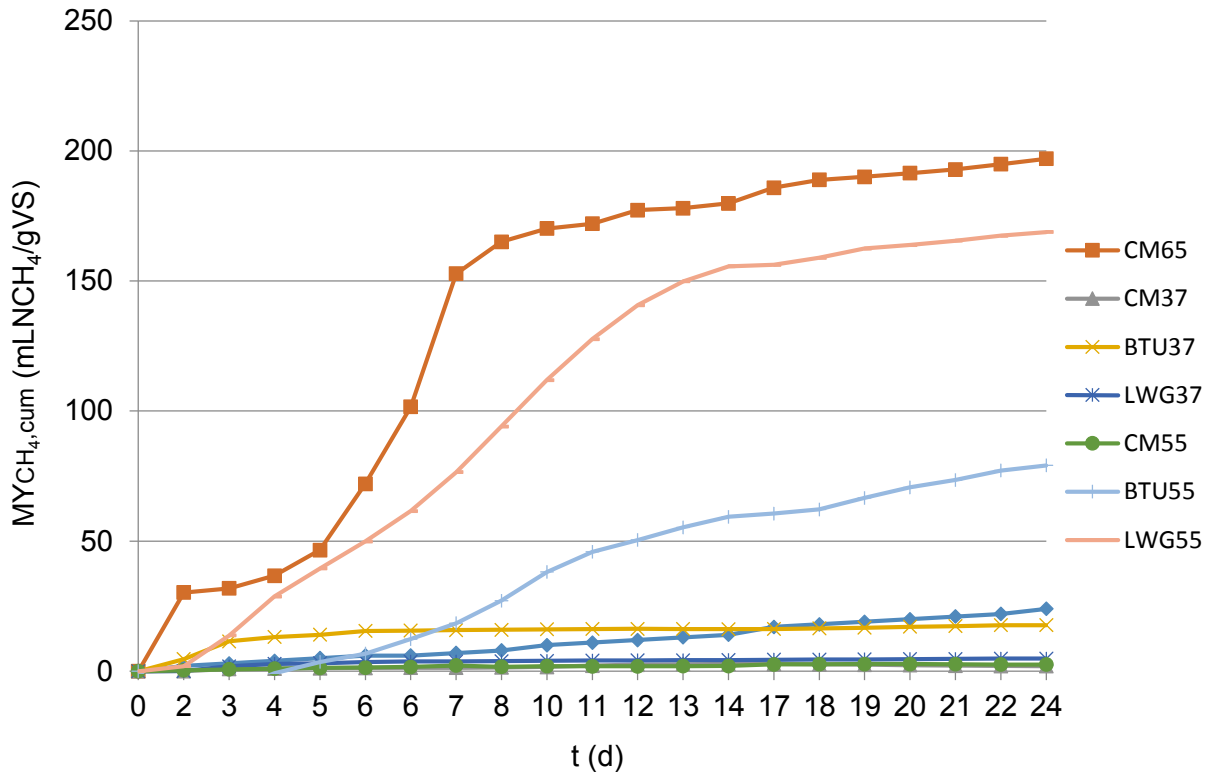

Figure 4.4: Comparison of net normalised cumulative methane yield for three inocula at optimal temperatures 37 ºC, 55 ºC and 65 ºC

Figure 4.5 gives a comparison of degree of COD degradation for the three inocula at different hyper-thermophilic temperatures with optimal mesophilic temperature of 37 ºC and thermophilic temperature of 55 ºC. CM at optimal hyper-thermophilic temperature of 65 ºC had the highest degree of COD degradation of 79.1 %. LWG at hyper-thermophilic temperature of 60 ºC recorded the second highest degree of COD degradation of 73.9 %. This was followed by BTU at 65 ºC which recorded degree of COD degradation of 57.3 % and LWG at 55 ºC (55.2 %). At hyper-thermophilic temperature of 70 ºC, BTU was the most inhibited as it recorded degree of COD degradation of 0.0 %, while CM and LWG recorded degree of COD degradation of 4.3% and 5.9 % respectively.

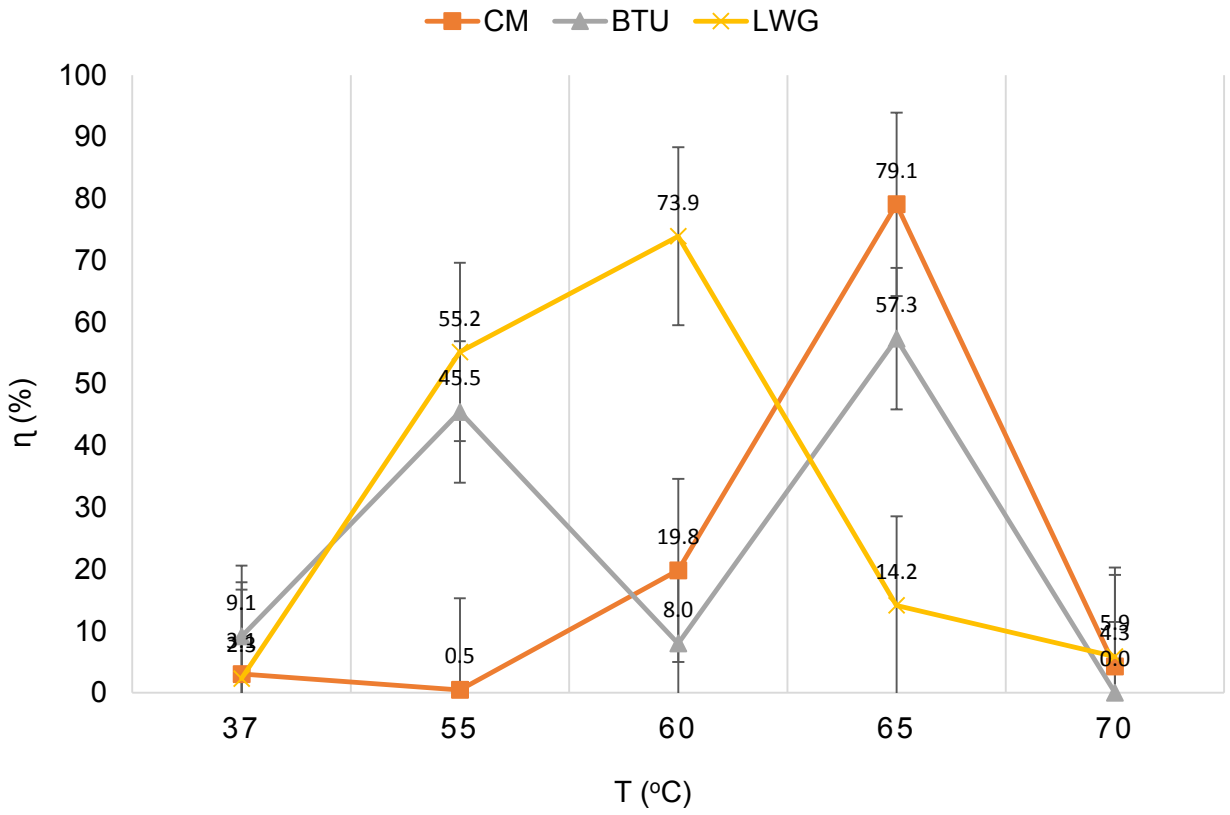

Figure 4.5: Comparison of degree of COD degradation for three inocula at hyper-thermophilic temperatures and optimal mesophilic temperature of 37 °C and thermophilic temperature of 55 °C

4.2 Physico-chemical parameters for the laboratory-scale single-stage HT-CSTR

Different parameters influence the performance of a laboratory-scale single-stage HT-CSTR operating under anaerobic conditions and they influence the usability of the effluent. Some of these parameters include pH, COD, total nitrogen (TN), total phosphorus (TP), ammonium-nitrogen (NH_4-N), nitrate-nitrogen (NO_3-N) and ortho-phosphate (PO_4-P). The average influent pH for BW was 6.9 ± 0.9, FW was 3.7 ± 0.3, MIX was 5.3 ± 0.5 while effluent was 6.9 ± 0.6 (Table 4.1). The average effluent value for the pH fell within the Ghana EPA and WHO effluent discharge standard and Germany wastewater ordinance. The average removal for total COD was lower when only BW was treated in the first 10 weeks of running the single-stage HT-CSTR, as it removed only 24.0 % of the influent total COD. On the contrary, high concentration of total COD (98904.8 ± 15357.6 mg/L) from the influent (MIX) saw average removal of 86.3 % after co-digestion was practised since the average total COD in the effluent was 13571.4 ± 6182.6 mg/L. The average BOD_5 in the effluent was 4584.9 ± 1599.7 mg/L, higher than the BOD_5 required by the Ghana EPA, WHO and Germany wastewater ordinance for effluent

discharge. The TN in the BW was higher than those found in the FW and consequently, the MIX substrate used for co-digestion. However, the TN in the effluent was higher compared to the influent (Table 4.1). The concentration of nitrogen in the form of ammonium (NH_4-N) and nitrite (NO_2-N) in the BW was higher than it was in the FW or the MIX. However, the concentration of nitrogen in the form of nitrate (NO_3-N) in the BW was lower than that of the FW and the MIX. The effluent concentration on the other hand was lower than that of the influent. The concentration of total phosphorus (TP) in the BW was the highest followed by the MIX then the FW. Effluent concentration of TP was lower compared to that of the influent. Phosphorus concentration in the form of phosphate (PO_4-P) and volatile fatty acids (VFA) concentration (in the form of acetate) in the BW was lower compared to that in FW and MIX. However, the effluent concentration of phosphorus in the form of phosphate was lower compared to that in the influent when only BW was treated and during co-digestion. The concentration of volatile fatty acids (in the form of acetate) in the effluent was much higher than it was in the influent (Table 4.1). Average TOC and soluble nitrogen ratio for BW for the laboratory-scale single-stage HT-CSTR was 7.3 ± 1.0 while that of the 1:1 ratio of the MIX was 23 ± 7.8. The TOC and soluble nitrogen ratio for only the FW used for the co-digestion was 43.4 ± 14.2 while that of the effluent was 5.3 ± 1.6. The average TS in the BW was 1.6 ± 0.3 %, of which 1.3 ± 0.3 % was VS, representing 81.3 % of the VS/TS while FW had VS and TS of 9.9 ± 4.0 % and 10.2 ± 4.0 % respectively, representing 97.1% of the organic solids in the dry matter. The MIX had average TS and VS of 4.6 ± 2.0 % and 4.4 ± 2.0 % respectively, representing 95.7 % of the volatile solids in the dry matter (Table 4.1).

Table 4.1: Comparison of selected parameters of black water, food waste, mixture of black water and food waste and effluent from the laboratory-scale single-stage HT-CSTR

Physico-chemical parameter	BW	FW	MIX	EFF	EPA Ghana Effluent Discharge Standard (2010)	Germany Domestic Waste-water Ordinance (2004)
pH	6.9 ± 0.9	3.7 ± 0.3	5.3 ± 0.5	6.9 ± 0.6	6-9	6-9
COD_{total} (mg/L)	26853.7 ± 7609.5	145719.4 ± 39035.1	98904.8 ± 15357.6	13571.4 ± 6182.6	<250	150
TN (mgN/L)	361.0 ± 88.1	276.8 ± 122.2	203.1 ± 58.5	412.2 ± 196.3		
NH_4-N (mgN/L)	243.3 ± 101.3	103.5 ± 32.2	189.9 ± 15.8	344.7 ± 167.3		
NO_3-N (mgN/L)	3.2 ± 1.8	5.94 ± 3.8	4.5 ± 1.9	2.95 ± 1.58		
NO_2-N (mgN/L)	0.12 ± 0.06	0.014 ± 0.009	0.04 ± 0.01	0.064 ± 0.038		
TP (mgP/L)	300.9 ± 102.4	162.0 ± 59.4	238.2 ± 11.0	133.7 ± 46.7		
PO_4-P (mgP/L)	70.4 ± 36.9	117.8 ± 44.9	114.5 ± 29.2	30.5 ± 12.5		
BOD_5 (mg/L)	n.d	n.d	n.d	4584.9 ± 1599.7	50	40
TOC/TNsol ratio	7.3 ± 1.0	43.4 ± 14.2	23 ± 7.8	5.3 ± 1.6		
VFA (Acetate) (g/L)	1.25 ± 0.4	1.36 ± 0.3	1.64 ± 0.3	2.14 ± 0.9		
TS (%)	1.6 ± 0.5	10.2 ± 4.0	4.6 ± 2.0	0.9 ± 0.4		
VS (%)	1.3 ± 0.5	9.9 ± 4.0	4.4 ± 2.0	0.7 ± 0.3		
VS/TS (%)	81.3 ± 4.0	97.1 ± 3.9	95.7 ± 4.6	77.8 ± 3.6		
Cl^-	167.3 ± 33.6	n.d	130.0 ± 89.7	172.7 ± 24.3		
SO_4^{2-}	34.2 ± 25.8	n.d	41.1 ± 0.85	11.5 ± 4.6		
E. coli (CFU/100mL)	9×10^8	n.d	n.d	0.0	1000	1000

*NB: Numbers written after the ± symbol are standard deviation values. n.d = not determined.

4.3 Laboratory-scale hygienisation tests or pathogen decay tests

Figure 4.6 shows growth of *Salmonella senftenbergensis* and *E. coli* on EA and BGA used as a positive control. On EA, *Salmonella senftenbergensis* grew as violet-pink colonies while *E. coli* grew as deep red colony with golden green spots. On the BGA, *Salmonella senftenbergensis* grew as violet-pink colonies while *E. coli* grew as creamy-yellow colonies.

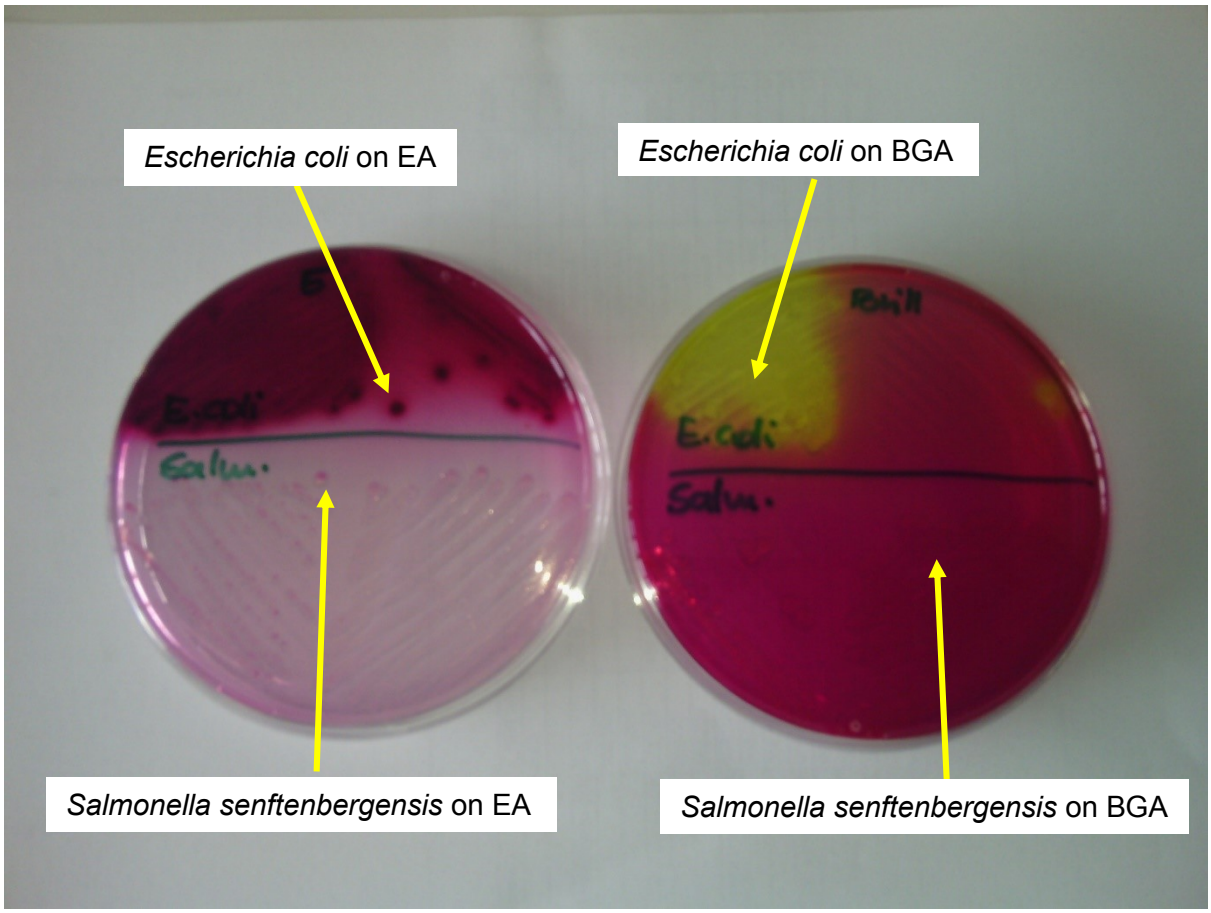

Figure 4.6: Growth of *Salmonella senftenbergensis* and *E. coli* on EA and BGA used as positive control

The initial concentrations of *Salmonella senftenbergensis* and *Escherichia coli* that were spiked into the laboratory-scale single-stage HT-CSTR were $2x10^9$ CFU/ml and $9x10^8$ CFU/ml respectively. Cultures from the effluent from the laboratory-scale single-stage HT-CSTR made on BGA and EA after 30 minutes showed no growth, indicating that all the model pathogens that were spiked into the reactor had been destroyed by the optimal hyper-thermophilic temperature of 65 ^oC.

Figures 4.7 a, b, c show the growth of *Salmonella senftenbergensis* and *Escherichia coli* on EA before and after the hygienisation tests and compared with negative control of the EA and positive control in Figure 4.6 above. It was observed that EA supported more growth of *E.coli* compared to *Salmonella senftenbergensis*, while the reverse was seen on BGA.

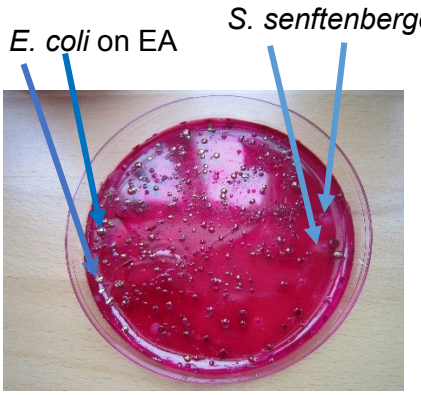

Figure 4.7a: Growth of pathogens on EA before hygienisation test

Figure 4.7b: EA showing no growth of pathogens after hygienisation at 65 °C for 30 minutes

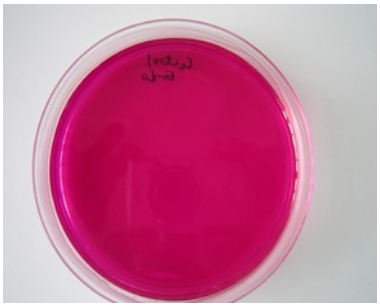

Figure 4.7c: Negative control of EA showing no growth of pathogens

Figures 4.8 a, b, c show the growth of *Salmonella senftenbergensis* and *Escherichia coli* on BGA before and after the hygienisation test and compared with negative control of the BGA and positive control in Figure 4.6 above. All the pathogens that were on the media before hygienisation had died after 30 minutes of heating at 65 °C in the laboratory-scale single-stage HT-CSTR.

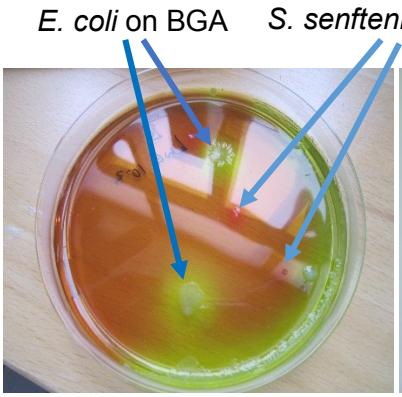

Figure 4.8a: Growth of pathogens on BGA before hygienisation test

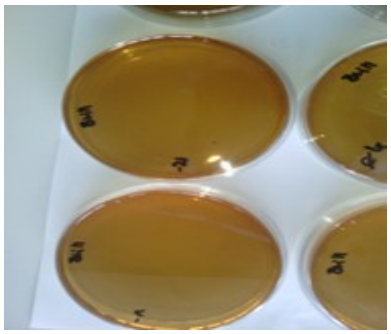

Figure 4.8b: BGA showing no growth of pathogens after hygienisation at 65 °C for 30 minutes

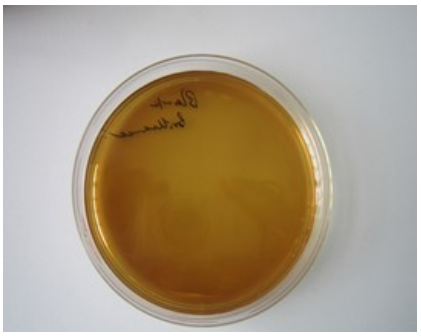

Figure 4.8c: Negative control of BGA showing no growth of pathogens

Biochemical tests with the use of indole-acetate reagent and tryptophan broth showed a cherry-red ring colour on the medium confirming the presence of *E. coli* in the sample before treatment (Figure 4.9a). Golden-yellowish ring on the indole-acetate and tryptophan broth showed the absence of *E.coli* in the sample after hygienisation at 65 ^{o}C for 30 minutes (Figure 4.9b).

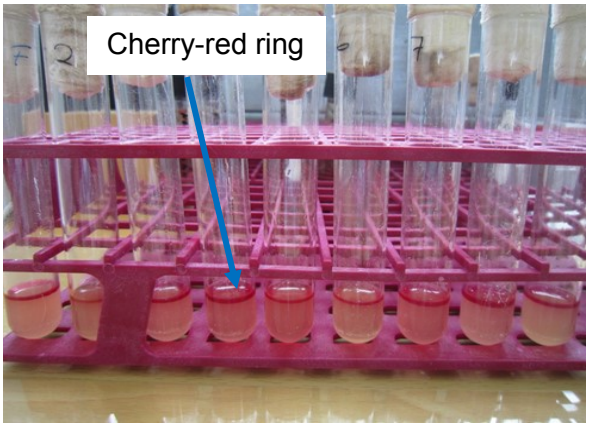

Figure 4.9a: Confirmation of presence of *E.coli* on indole acetate and tryptophan culture broth nutrients media before hygienisation treatment

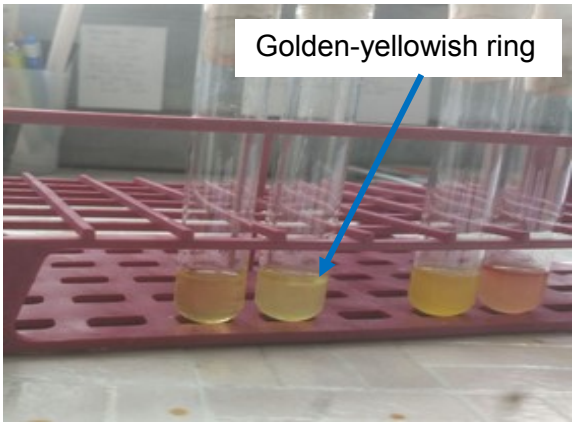

Figure 4.9b: Confirmation of absence of *E.coli* on indole acetate and tryptophan culture broth nutrients media after hygienisation treatment at 65 ^{o}C for 30 minutes

4.4 Biogas production for laboratory-scale single-stage HT-CSTR using only BW and MIX substrates

4.4.1 Relationship between average effluent pH, volumetric loading rate and biogas composition

The average pH value in the laboratory-scale single-stage HT-CSTR which was recorded in the effluent showed a gradual decrease from 7.43 to 6.15 from week 1 to week 7. It then increased gradually from week 7 to week 16 where the average pH recorded in the effluent was 6.79. The average pH in the effluent (Avg. Eff pH) then increased gradually between weeks 16 and 18 from 6.79 to 7.58 and then remained stable till week 22, with an average pH value of 7.65 (Figure 4.10). The decrease in pH in the first 7 weeks corresponded with the build-up of acetate in the effluent as the concentration of acetate in the effluent increased gradually from 2.1 g/L to 3.5 g/L. The average concentration of acetate (Avg. Acetate) in the effluent decreased sharply from week 8 to week 18, with average concentrations being 3.3

g/L to 0.5 g/L, respectively. It then remained relatively stable from week 18 to week 22 where the average concentration was between 0.5 g/L and 0.6 g/L respectively (Figure 4.10).

The percentage of normalised methane content (C_{CH_4}) in the biogas produced from using only BW as a substrate did not necessarily correspond with the decrease of pH and increase in acetate concentrations in the first four weeks, as the percentage of normalised methane in the biogas increased slowly within the first 3 weeks from 0.0 to 0.52. vol-%. The percentages of carbon dioxide in the biogas within the first 3 weeks were between 0.0 to 46.0 vol-% while that of oxygen were between 0.0 to 5.4 vol-%. From the 4th week to the 7th week, the average percentage of normalised methane in biogas increased sharply between the 4th and 5th weeks (5.09 vol-% to 24.9 vol-%). It later decreased slightly in the 6th week (23.6 vol-%) and increased gradually from the 7th week to the 9th week (31.7 vol-% to 34.9 vol-%). The percentages of carbon dioxide in the biogas fluctuated between 41.7 to 59.1 vol-% between week 4 and 9. The percentages of oxygen in the biogas on the other hand, decreased from 5.3 to 0.0 vol-% from the 4th week to the 7th week and remained so till the end of the experiments. Later on, the percentage content of methane decreased slightly in the 10th week with an average normalised methane percentage of 33.6 vol-% recorded. The percentage volumes of carbon dioxide and oxygen recorded in the 10th week of treating only BW were 47.7 vol-% and 0.0 vol-%, respectively.

The influence of co-digestion on the percentage content of methane in the biogas cannot be over-emphasised. From the 11th week when MIX (co-digestion of black water and food waste; 1:1, v/v) was introduced, the average percentage of normalised methane in the biogas increased significantly from 40.9 vol-% to 48.0 vol-% from the 11th to the 14th weeks and decreased slightly (46.1 vol-%) in the 15th week. The percentage volume of carbon dioxide was between 36.8 vol-% and 43.4 vol-%, showing a slight decrease. The percentage of oxygen remained constant at 0.0 vol-%, showing full anaerobic condition had been maintained. The average percentage of normalised methane content in the biogas increased sharply in the 16th and 17th weeks (49.9 vol-% and 58.9 vol-%, respectively) but decreased slightly in the 18th and 19th weeks (56.2 vol-% and 55.3 vol-%, respectively). It fluctuated between 20th and the 22nd weeks from 59.1 vol-% to 61.8 vol-% and 59.1 vol-%, respectively (Figure 4.10). While the percentage of oxygen in the biogas remained constant at 0.0 vol-%, that of carbon dioxide decreased gradually but fluctuated between 41.4 vol-% and 41.7 vol-% in the 16th and 19th weeks. It then decreased further to 37.0 vol-%, 35.2 vol-% and 36.8 vol-% for weeks 20, 21 and 22 respectively.

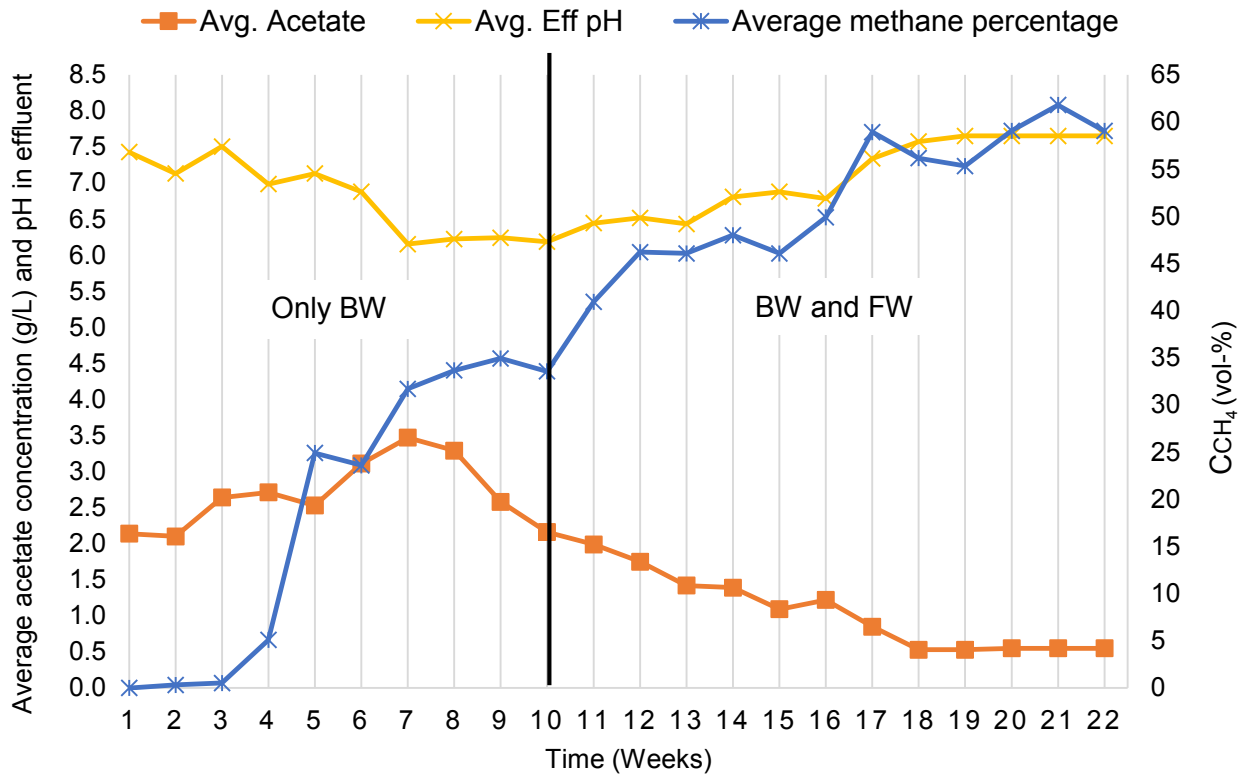

Figure 4.10 Relationship between pH, acetate concentration and percentage of weekly average normalised methane content in biogas

The relationship between the weekly average effluent pH (Avg. Eff pH) in the laboratory-scale single-stage HT-CSTR and the weekly average volumetric loading rate (Avg. VLR) and average acetate (Avg. Acetate) concentration in the effluent of the reactor is established (Figure 4.11). Generally, increase in the volumetric loading rate of COD in the laboratory-scale single-stage HT-CSTR resulted in increase in acetate concentration in the effluent. It also had a corresponding decrease in the pH both in the reactor and in the effluent within the first 7 weeks (Figure 4.11). With weekly average fluctuating volumetric loading rate from the 8th week to the 20th week, weekly average acetate concentration decreased gradually from the 8th week to the 18th week and remained fairly constant from the 18th week to the 22nd week even though the volumetric loading rate only remained constant from the 20th to the 22nd week (Figure 4.11). The weekly average pH in the reactor and the effluent on the contrary, increased slightly from the 8th week to the 18th week before levelling off from the 18th week to the 22nd week (Figure 4.11).

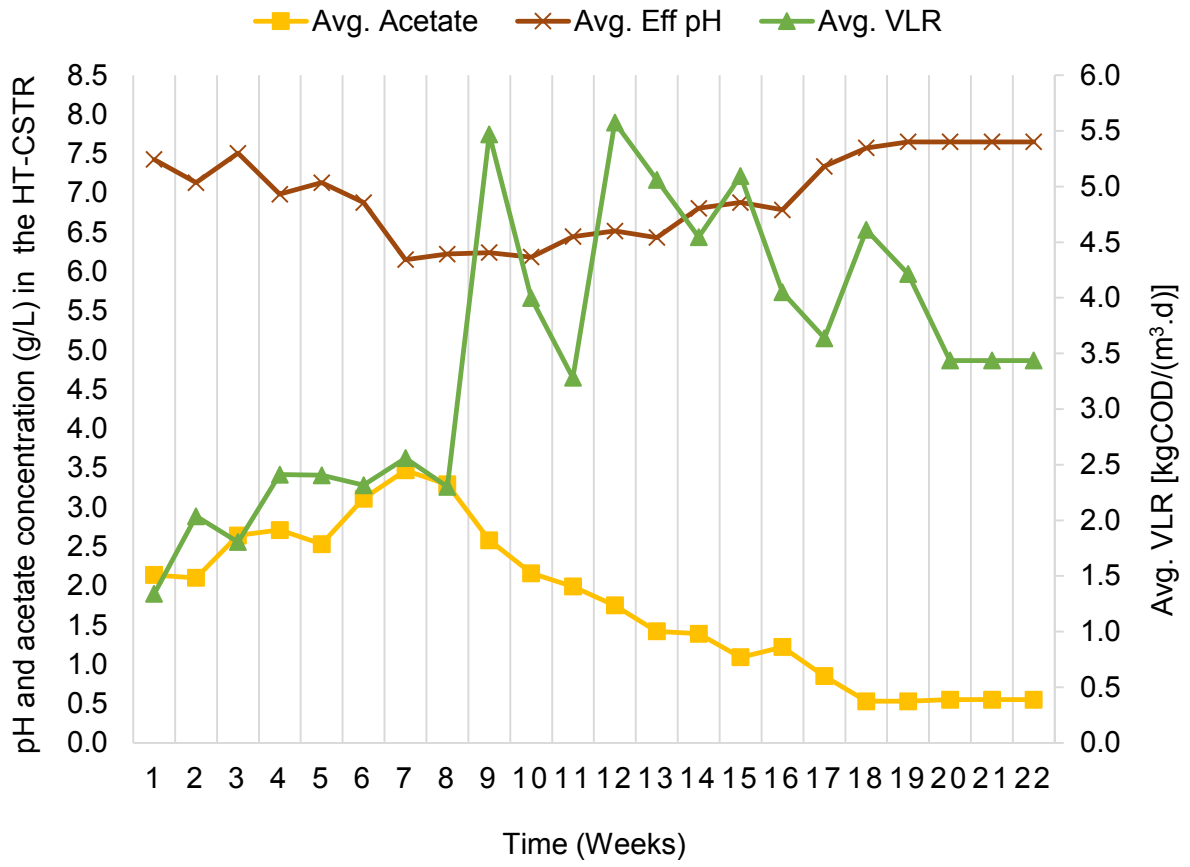

Figure 4.11: Influence of volumetric loading rate on pH and acetate concentration in effluent

When only BW was treated, the weekly average volume of normalised dry methane seemed to have corresponded with the weekly average percentage of methane content measured in the biogas over the study period. The weekly average volume of normalised dry methane increased gradually from the 1st week to the 4th week and sharply from the 4th week to the 6th week before decreasing gradually in the 7th week but increased sharply again in the 8th and 9th weeks (Figure 4.12). The weekly average percentage of methane content in the biogas increased gradually from the 1st week to the 4th week and sharply between the 4th and 5th weeks, where it decreased slightly before increasing gradually again from the 6th to the 9th weeks. Even though the weekly average volume of dry methane decreased sharply in both the 9th and 10th weeks, the weekly average percentage of methane only decreased gradually in only the 9th week then increased gradually in the 10th week (Figure 4.12). After the introduction of the co-substrates, the increase for both the volume of weekly average normalised dry methane and the weekly average percentage of methane content in the biogas fluctuated from the 12th week to the 22nd week. The increase in the volume did not necessarily correspond with the percentage of methane content since in weeks 14 and 17, decrease in

volume saw increases in percentage of methane content while in weeks 18 and 19, increase in volume saw a decrease in percentage of methane content in the biogas (Figure 4.12).

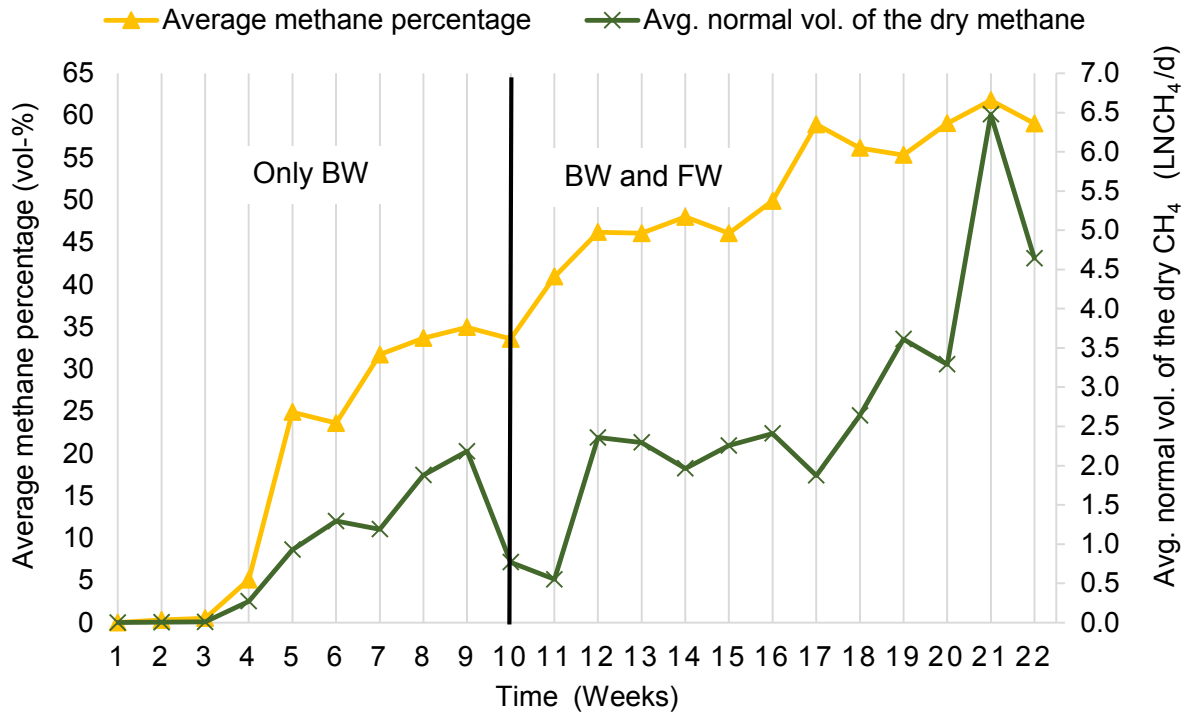

Figure 4.12: Relationship between volume of dry methane and percentage of methane in biogas

Depending on the weekly influent flow rate and the concentration of the substrate at each stage of the experiment, the HRT of the HT-CSTR was influenced and calculated. When only BW was used, the HRT slightly increased from the 1st and 2nd weeks and thereafter decreased sharply from the 2nd week to the 5th week before increasing gradually from the 5th week to the 11th week. Thereafter, it increased sharply from the 11th week to the 13th week when co-digestion of BW and FW was practised. The HRT then decreased sharply in the 15th week and remained constant from the 15th week to the 17th week where it increased gradually from the 17th to 18th week. Finally, it remained steady from the 18th to 20th week but fluctuated from the 20th week to the 22nd week (Figure 4.13a). Treatment of only BW resulted in lower HRT unlike a relatively higher HRT that was observed with the introduction of co-substrate, FW. The percentage content of methane in the biogas on the other hand increased steadily in the first 5 weeks. The increase in the percentage content of methane in the biogas fluctuated from the 5th week to the 22nd week, however, it was observed that treating only BW at optimal hyper-thermophilic temperature resulted in lower percentage content of methane compared with when co-digestion was practised (Figure 4.13a).

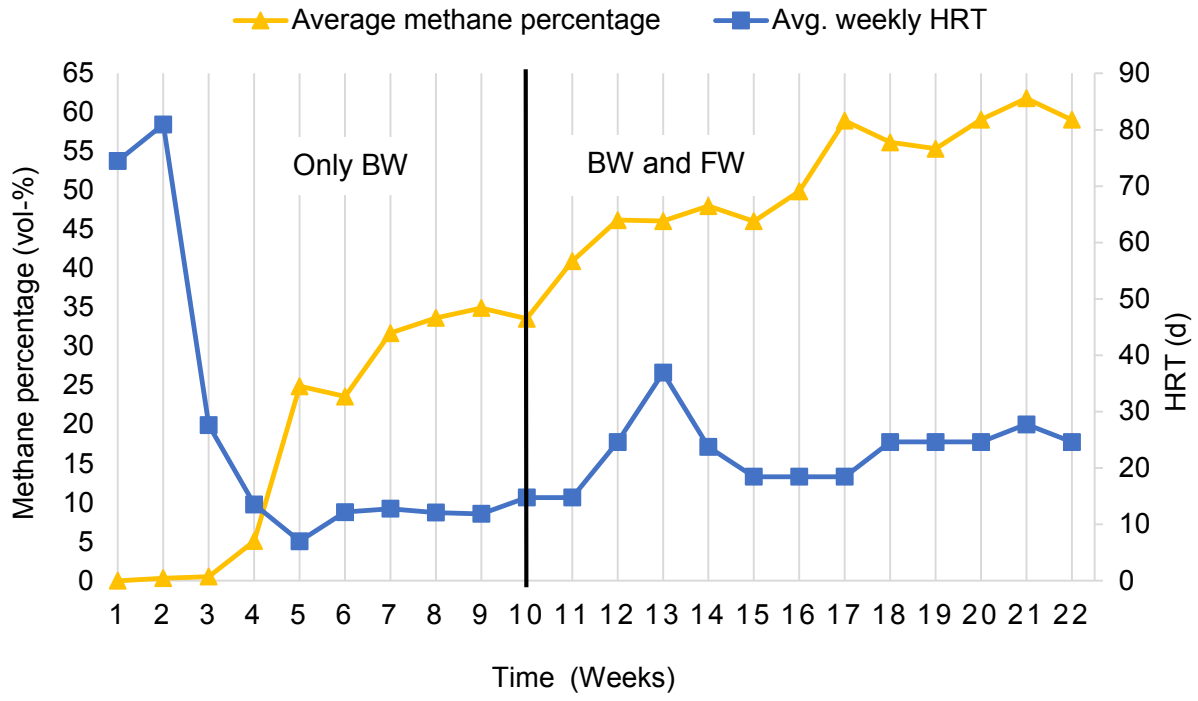

Figure 4.13a: Relationship between HRT and percentage of methane in biogas

The percentage of methane content in the biogas does not necessarily correspond with the increase in the hydraulic retention time (HRT). Small values of methane content such as 0.0 % were recorded for HRTs 74.5 days and 81.0 days. An HRT of 27.6 days was recorded for a percentage methane content of 1.0 % while HRT of 13.5 days recorded percentage methane content 5.0 %. However, HRT of 18.5 days, 24.6 days and 27.7 days recorded high percentage methane content of 59 %, 59 % and 62 %, respectively (Fig. 4.13b).

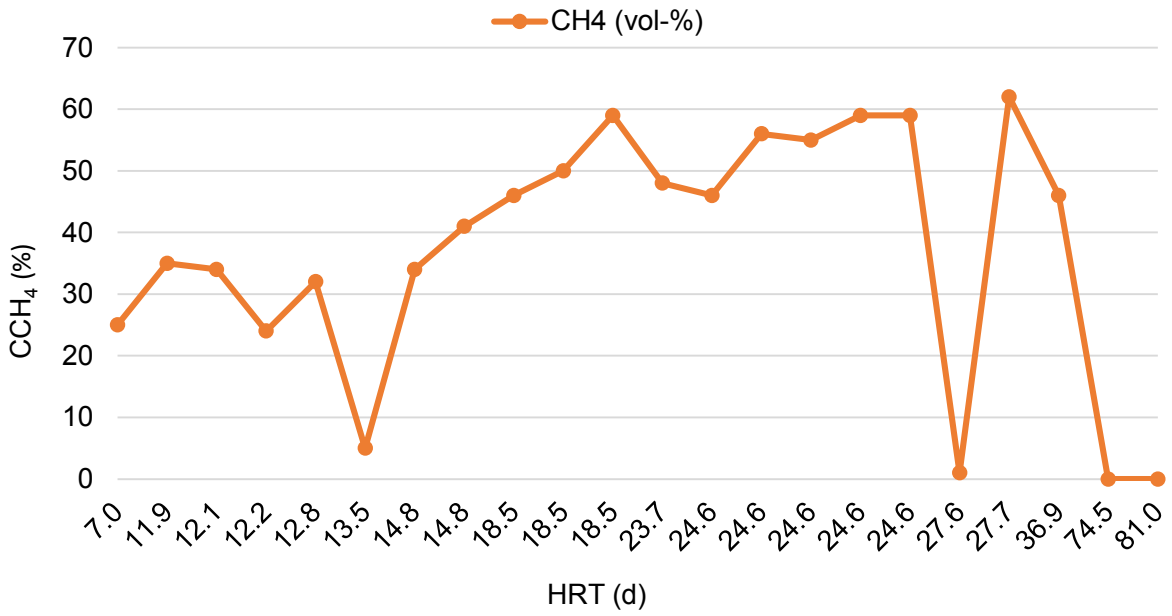

Figure 4.13b: Effect of HRT on percentage of methane content in biogas

The increase in the quantity of weekly average normalised volume of dry methane in the biogas was sharp within the first 6 weeks then decreased gradually in the 7th week before increasing sharply again between the 7th and 9th weeks to about 2.18 $LNCH_4$ when only BW was treated. It had a sudden decrease in the 10th and 11th weeks, respectively (0.77 $LNCH_4$ and 0.55 $LNCH_4$) but thereafter increased sharply again in the 12th week (2.36 $LNCH_4$) after co-substrates were introduced. The increase in the quantity of normalised volume of dry methane in the biogas then fluctuated between the 12th and 17th weeks before experiencing a sharp fluctuated increase from the 18th to the 22nd weeks (Figure 4.14a).

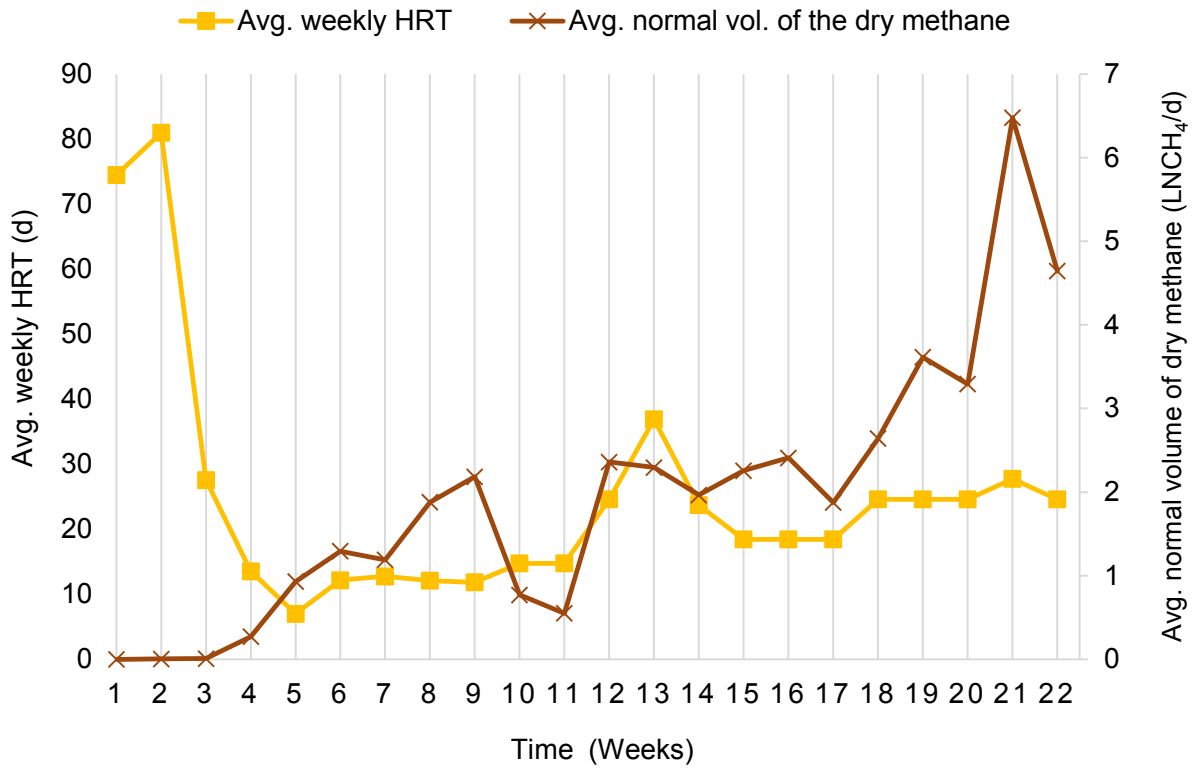

Figure 4.14a: Relationship between HRT and average normal volume of dry methane in biogas

The methane formation rate (MFR) in the biogas did not correspond with the increase or decrease in the hydraulic retention time (HRT). Small values of the MFR such as 0.0 $L_NCH_4/(m^3.d)$, 0.1 $L_NCH_4/(m^3.d)$ and 0.2 $L_NCH_4/(m^3.d)$ were recorded for HRTs 74.5 days, 81.0 days and 27.6 days, respectively. However, HRT of 24.6 days recorded high MFR of 96.3 $L_NCH_4/(m^3.d)$ and 123.8 $L_NCH_4/(m^3.d)$ while HRT 27.7 days recorded the highest MFR of 172.7 $L_NCH_4/(m^3.d)$ (Fig. 4.14b).

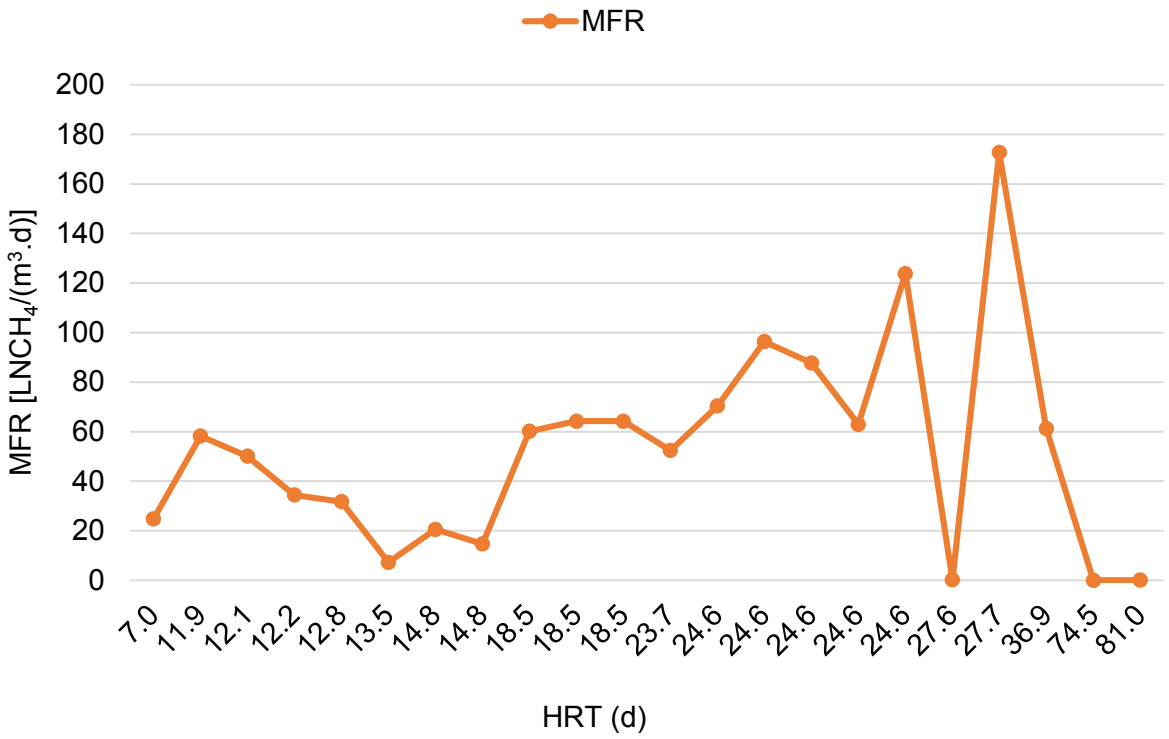

Figure 4.14b: Effect of HRT on methane formation rate (MFR)

The average volumetric loading rate (Avg. VLR) for COD was increased gradually from week 1 to the 9th week with corresponding increase in the normal volume of dry methane. The theoretical dry methane volume did not experience a gradual increase but fluctuated throughout the period. Sudden increase in the VLR from the 8th to the 9th week (2.30 kgCOD/m^3.d to 5.47 kgCOD/m^3.d) did not see sudden increase in the weekly average theoretical dry methane volume as the latter decreased from 9.65 $LNCH_4$/d to 3.24 $LNCH_4$/d (Figure 4.15).

The actual normal volume of dry methane increased from 1.88 $LNCH_4$/week to 2.18 $LNCH_4$/week from the 8th to the 9th week (Figure 4.15). From the 10th week to the 22nd week, increase in the VLR rather saw a decrease in the theoretical dry methane volume and vice versa, with the highest VLR occurring in the 12th week [5.58 kgCOD/(m^3.d)] when co-digestion was started. With regard to the actual normal volume of dry methane, the fluctuations in the increase in the VLR corresponded with the actual normal volume of dry methane as increases and decreases in the VLR saw similar trends for the latter except in weeks 20 and 22 where even though VLR remained constant, the weekly average normal volume of dry methane increased sharply from week 20 to 21 (3.29 $LNCH_4$/d to 6.48 $LNCH_4$/d) and decreased gradually in week 22 (4.64 $LNCH_4$/d) (Figure 4.15).

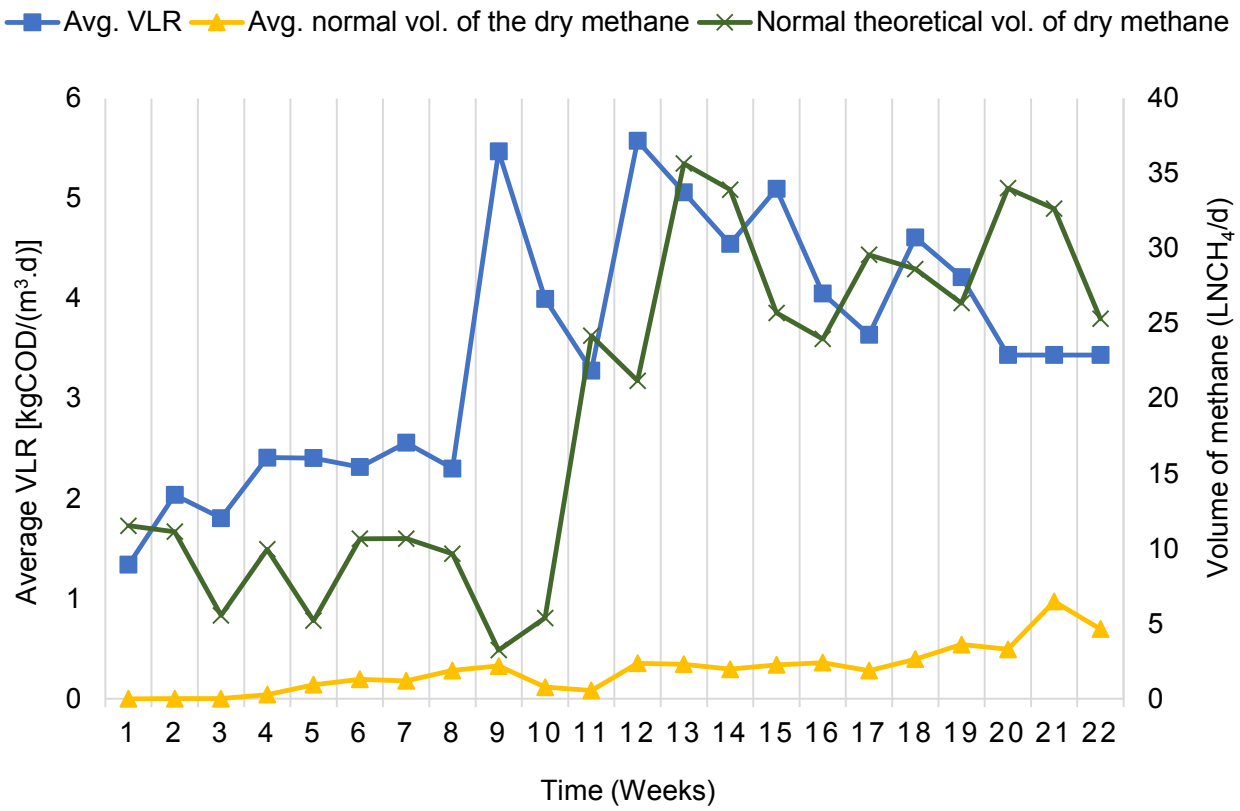

Figure 4.15: Influence of VLR on normal and theoretical volumes of dry methane

The normal dry methane yield and the normal dry methane productivity of the laboratory scale single-stage HT-CSTR correlated with each other except in week 6. From week 1 to week 22, increase in the dry methane yield saw a corresponding increase in the normal dry methane productivity even though the values for the dry methane yield were all higher than the normal dry methane productivity. The highest normal dry methane yield was observed in the 5th week (7.25 $Nm^3CH_4/kgVS$), followed by the 9th week (5.67 $Nm^3CH_4/kgVS$) and 21st week (4.28 $Nm^3CH_4/kgVS$) while the lowest yields were recorded in the first 3 weeks (0.00 $Nm^3CH_4/kgVS$) (Figure 4.16).

When co-digestion was started, the first highest normal dry methane productivity for the first 12-weeks was observed on the 12th [0.065 $Nm^3CH_4/(m^3.d)$] week. Afterwards, the highest normal dry methane productivity were recorded for 15th [0.066 $Nm^3CH_4/(m^3.d)$], 18th [0.085 $Nm^3CH_4/(m^3.d)$], 19th [0.103 $Nm^3CH_4/(m^3.d)$] and 21st weeks [0.185 $Nm^3CH_4/(m^3.d)$]. The lowest were in the first 3 weeks [0.000 $Nm^3CH_4/(m^3.d)$] when only BW was treated (Figure 4.16).

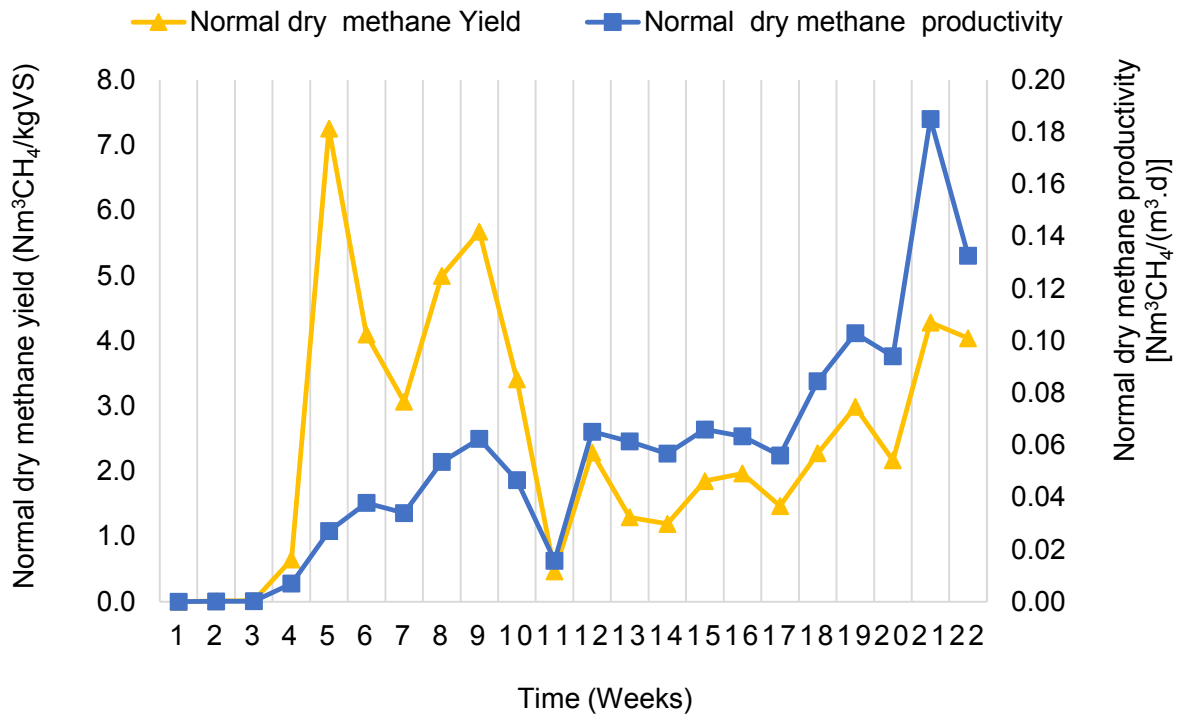

Figure 4.16: Comparison of normal dry methane yield and normal dry methane productivity

The cumulative methane productivity over the period for the laboratory-scale single-stage HT-CSTR was 1.25 [$Nm^3CH_4/(m^3.d)$], with an average of 0.06 [$Nm^3CH_4/(m^3.d)$] while its cumulative methane yield was 55.43 $Nm^3CH_4/kgVS$, averaging 2.52 $Nm^3CH_4/kgVS$ (Figure 4.17).

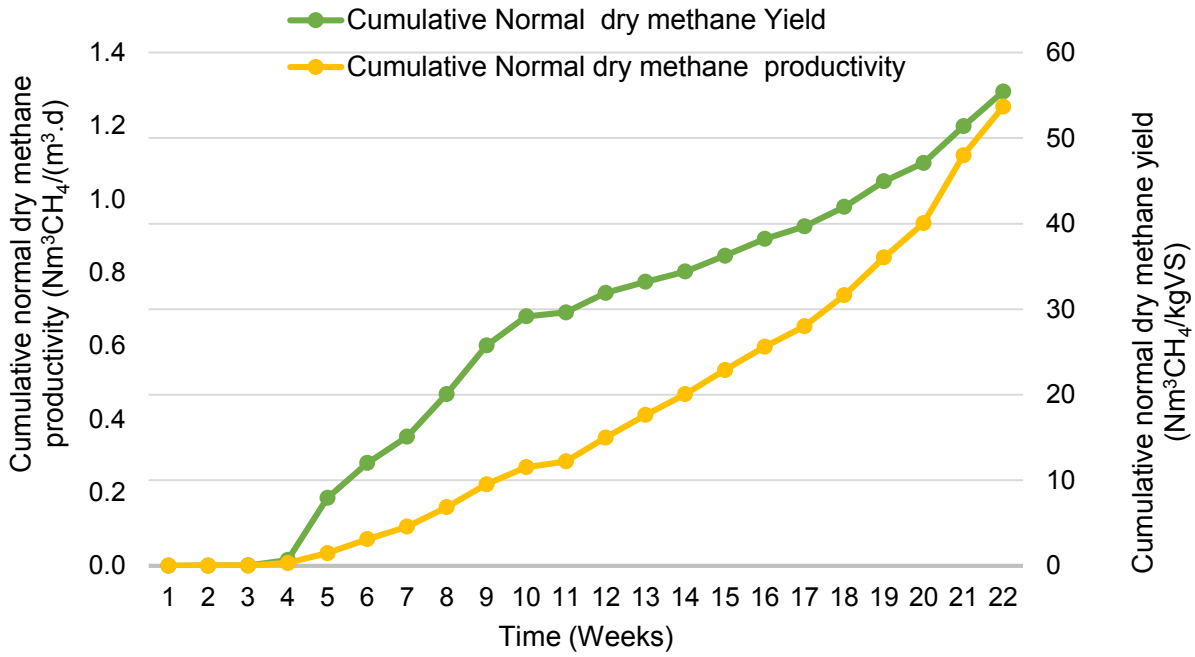

Figure 4.17: Comparison of cumulative normal dry methane yield and cumulative normal dry methane productivity

In the laboratory scale single-stage HT-CSTR, the normal theoretical dry methane yield and the normal theoretical dry methane productivity interrelated with each other except in the 5th, 13th, 18th and 19th weeks where increase in the normal theoretical yield saw decrease in the normal theoretical methane productivity (Figure 4.18). From week 13 to 19, the increase in the normal theoretical methane yield was very gradual before decreasing slightly from the 20th to the 22nd week. The normal theoretical methane productivity, on the other hand, fluctuated within that period with the highest productivity within that period being 1.02 [$Nm^3CH_4/(m^3.d)$] (in the 13th week) and the lowest productivity within that period was 0.68 [$Nm^3CH_4/(m^3.d)$] (in the 16th week) (Figure 4.18).

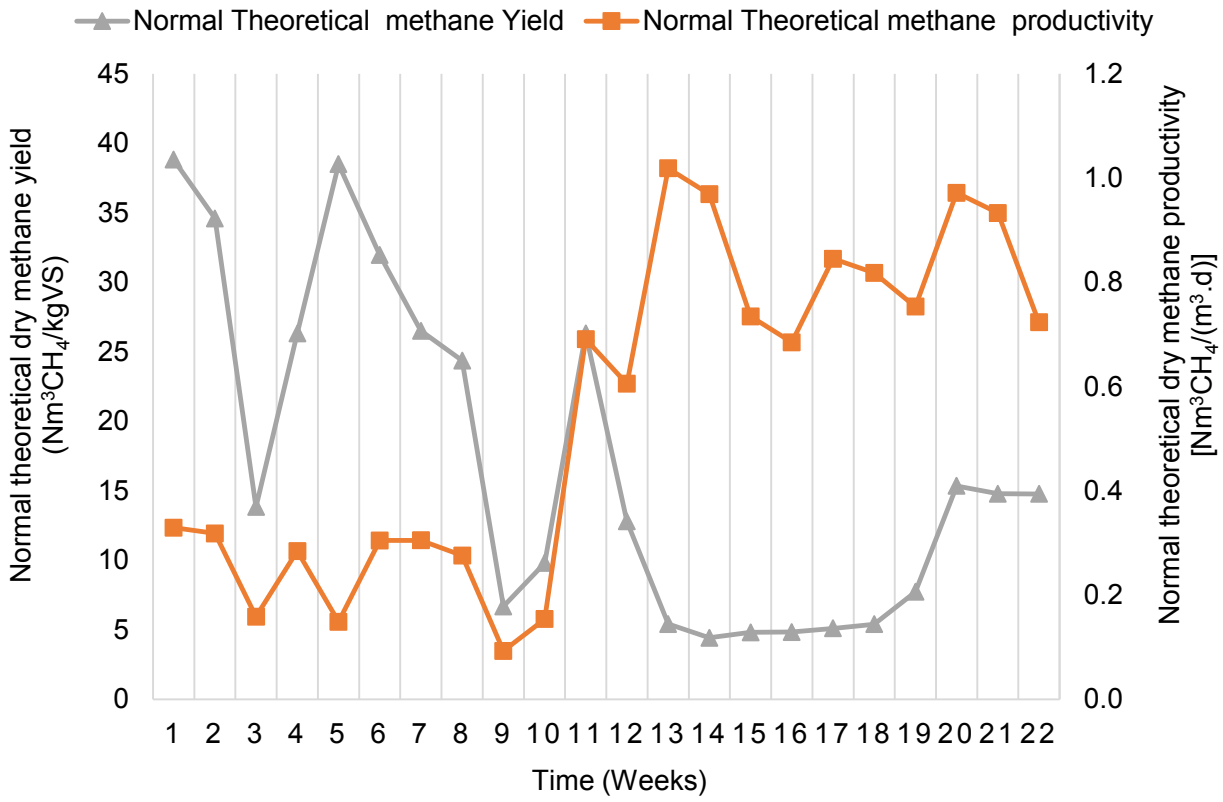

Figure 4.18: Comparison of normal theoretical dry methane yield and normal theoretical dry methane productivity

The calculated COD degradation efficiency (η) in the laboratory-scale single-stage HT-CSTR was 86.3 %. With the average flow rate of 2.2 L/d, average influent COD concentration of 98904.8 mg/L and effluent COD concentration of 13571.43 mg/L, the laboratory-scale single-stage HT-CSTR had an average influent daily load of 0.22 kg/d. The average effluent daily load was 0.03 kg/d, consequently, the reactor had a degradation performance (R) of 5.43

[kgCOD/(m^3.d)]. The food-to-mass (F/M) ratio of the laboratory-scale single-stage HT-CSTR was calculated to be 1428.57 [kg/(kgVS.d)].

4.5 Assessment of microbial biomass present for methanization in the laboratory-scale single-stage HT-CSTR

The type of microbial biomass and methanogens present in the laboratory-scale single-stage HT-CSTR responsible for COD removal and methanisation is very important for effective operation of the reactor. Results from the Fluorescence In-Situ Hybridization (FISH) technique which was targeted at the RNA in the ribosomes in the cytoplasm showed varied microbes that could survive the optimal hyper-thermophilic temperature of 65 ^{o}C for effective COD removal and methanisation. Archaeabacteria (ARCH915) appeared to be longitudinally-cylindrical under the microscope (Nikon H600L, MODEL: Nikon Eclipse LV100). The appearance of archaeabacteria (ARCH915) showed Alexa-594 red colour for FISH (Figure 4.19). The appearance for the other FISH results for the eubacteria (EUB338 I), *Methanosaeta spp. (MX825), Methanosarcina sp.* (MS821) and *Methanococcus spp.* (MC1109) were all orange colour. The appearance for the FISH for *Methanomicrobium spp., Methanogenium spp., Methanoculleus spp., Methanospirillum spp., Methanocorpusculum spp. and Methanoplanus spp. (*which were all classified as MG 1200) was also orange colour. The arrows are pointing to some methanogens exhibiting auto-fluorescence by appearing orange (Figure 4.19).

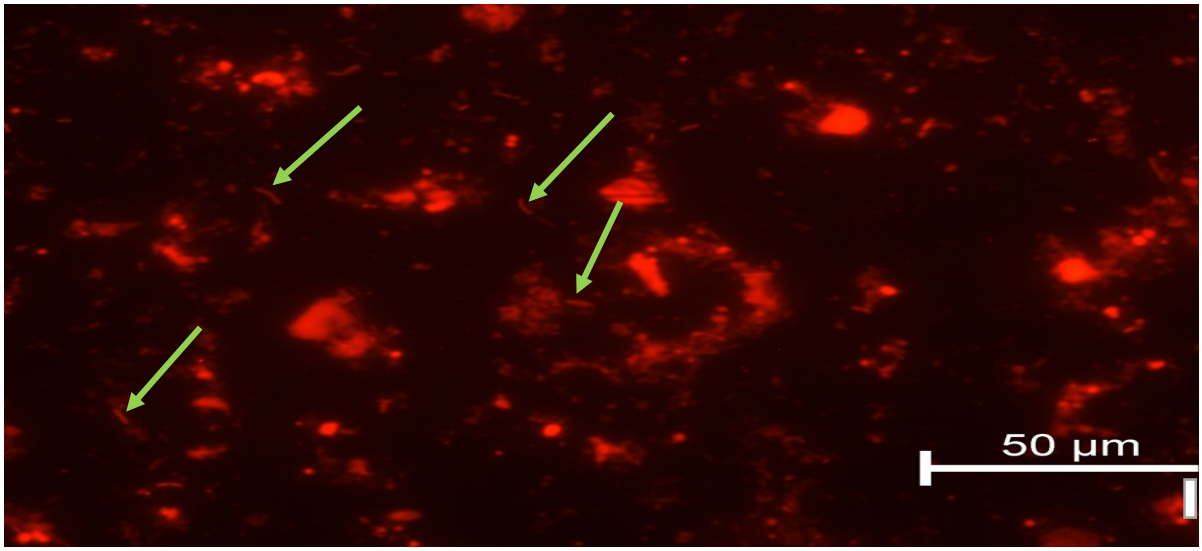

Figure 4.19: FISH staining of archaeabacteria using probe ARCH915. Arrows pointing to the presence of selected individual archaeabacteria

The results from the use of DAPI (4′,6-diamidino-2-phenylindole), which stains DNA in the nucleoid gives blue-fluorescence by binding to the adenine-thymine regions of the double strand of the DNA. The appearance for all the bacteria in the laboratory-scale single-stage HT-CSTR showed blue fluorescence after DAPI staining. The DAPI staining for the archaeabacteria (ARCH915) confirmed the same longitudinal-cylindrical shape as was observed in the FISH image, however, the archaeabacteria picked the colour for the DAPI stain by appearing blue under the microscope (Nikon H600L, MODEL: Nikon Eclipse LV100) (Figure 4.20).

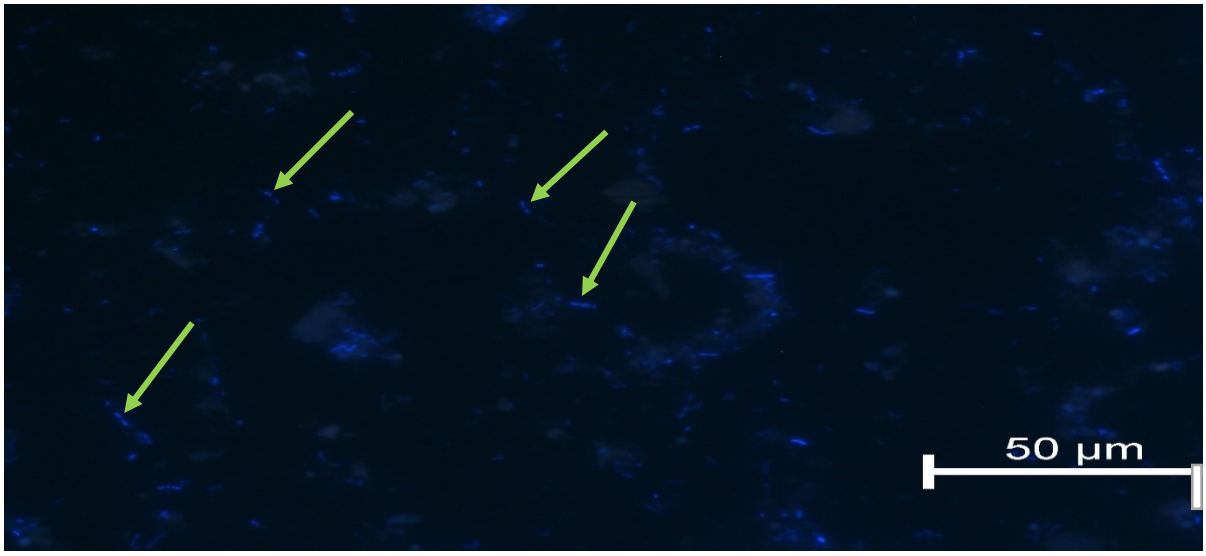

Figure 4.20: DAPI staining of archaeabacteria using probe ARCH915. Arrows pointing to the presence of selected individual archaeabacteria.

An overlay of the DAPI-stained image on the FISH image confirmed the auto-fluorescence of the archaeabacteria present in the sludge of the single-stage HT-CSTR that operated at optimal hyper-thermophilic temperature of 65 ^{o}C. The auto-fluorescence of the archaeabacteria in the overlay image showed a blue-pink longitudinal-cylindrical images. Some of the archaeabacteria also appeared as small blue segmented longitudinal cylinders on a pink longitudinal cylindrical background (Figure 4.21). The appearance of the pink colour is as a result of the mixture of the 598-Alexa red colour and the DAPI-stained blue colour.

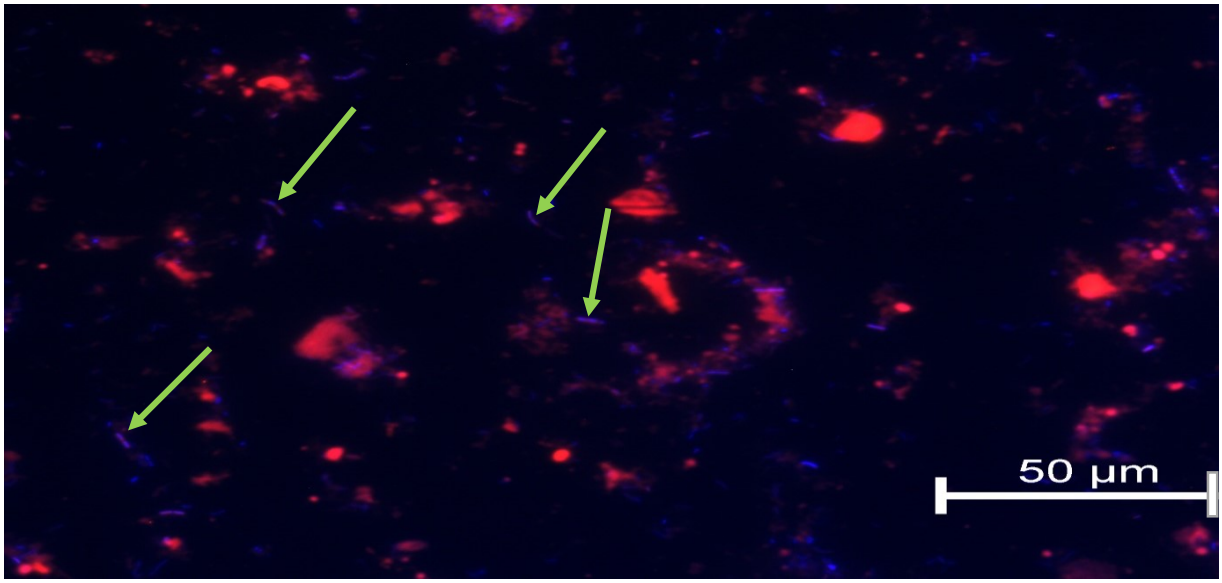

Figure 4.21: FISH-DAPI overlay of archaeabacteria using probe ARCH915. Arrows pointing to the presence of selected individual archaeabacteria.

The appearance for FISH results for the eubacteria (EUB 338I) showed auto-fluorescence of consortium of different bacteria. The normal bacteria in the category of eubacteria exhibited orange-reddish colour with varied shapes when FISH technique was employed. The shapes included rod-like (of varied length), spherical and longitudinally-cylindrical. Some were segmented while others were not (Figure 4.22).

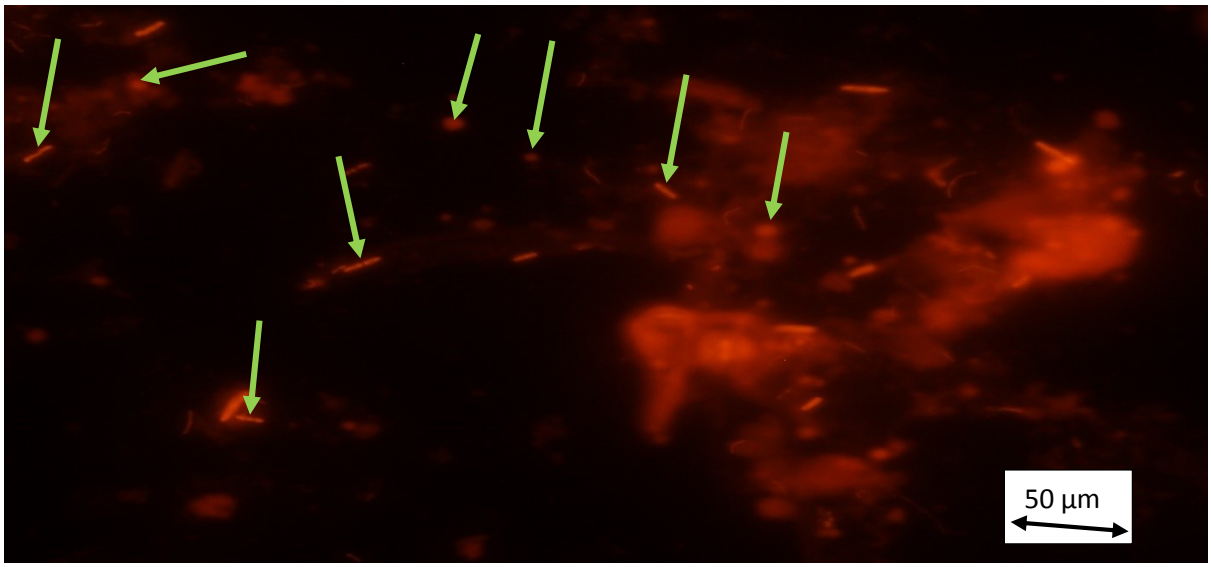

Figure 4.22: FISH staining of eubacteria using probe EUB 338I. Arrows pointing to the presence of selected individual eubacteria.

The appearance of the eubacteria under the DAPI-stained image was similar to the appearance when FISH technique was used except that in the former, the eubacteria appeared bluish while in the latter, they appeared orange-reddish (Figure 4.23).

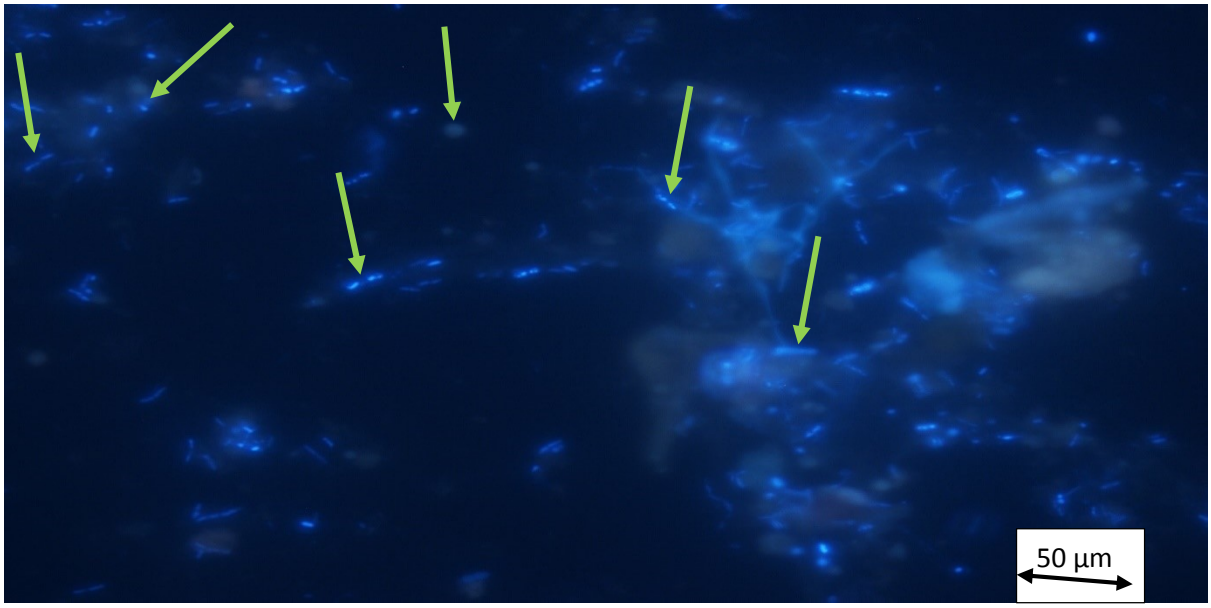

Figure 4.23: DAPI staining of eubacteria using probe EUB 338I. Arrows pointing to the presence of selected individual eubacteria.

An overlay of the DAPI-stained image on the FISH image for eubacteria (EUB 338I) confirms the auto-fluorescence of the eubacteria present in the sludge of the single-stage HT-CSTR at optimal hyper-thermophilic temperature of 65 ^{o}C. The auto-fluorescence of the eubacteria in the overlay image showed a blue-pink longitudinal-cylindrical shapes. Some of the eubacteria also appeared as small blue-pink spherical shapes (Figure 4.24). The appearance of the pink colour is as a result of the mixture of the 598-Alexa red colour and the blue DAPI-stained colour in the overlay.

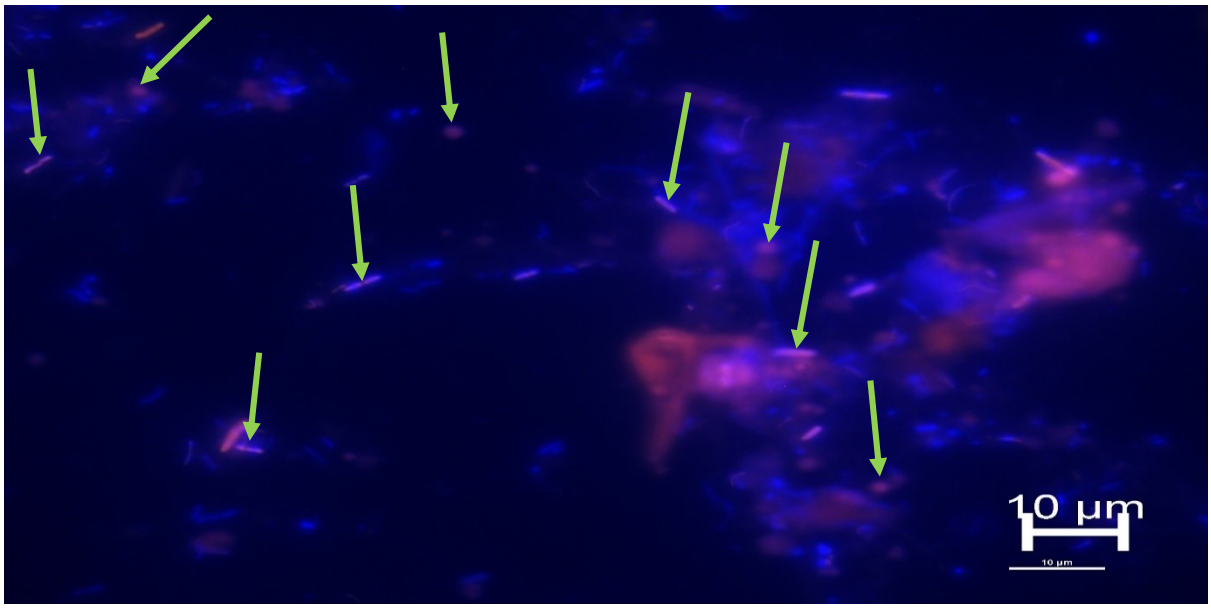

Figure 4.24: FISH-DAPI overlay of eubacteria using probe EUB 338I. Arrows pointing to the presence of selected individual eubacteria.

Unlike archaeabacteria which appeared more elongated as longitudinal cylindrical shapes, the shapes of *Methanosarcina sp.* (MS 821) identified in the seeding sludge of the single-stage HT-CSTR in this research were short and lobed-like. The arrows in figure 4.25 show a number of *Methanosarcina sp.* (MS 821) under FISH observation.

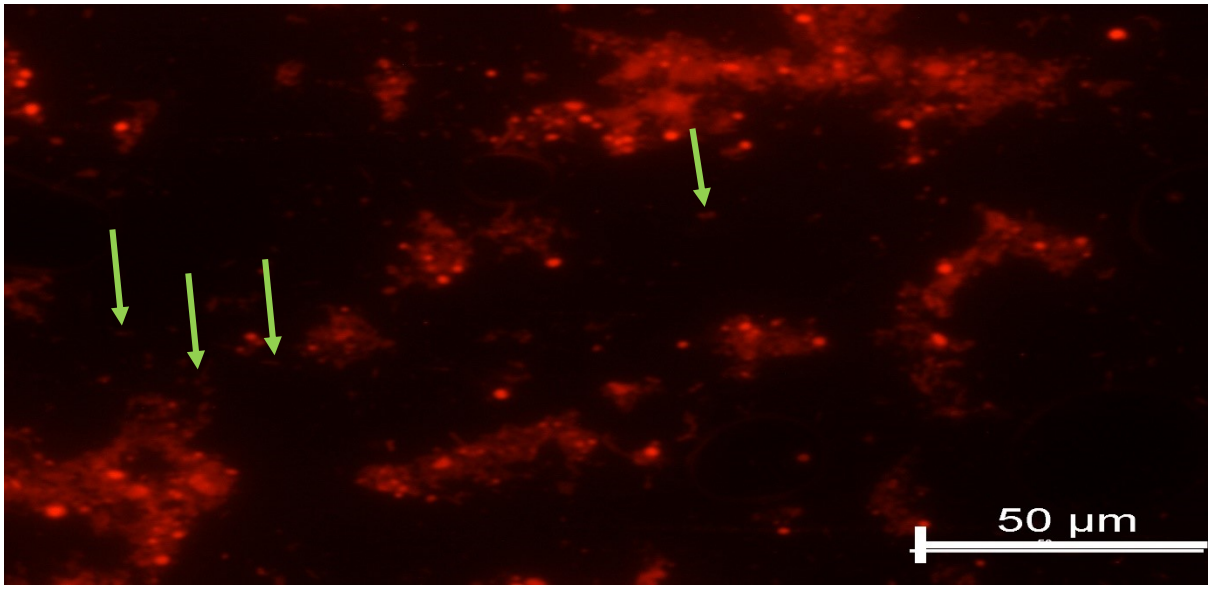

Figure 4.25: FISH staining of *Methanosarcina sp.* using probe MS 821. Arrows pointing to the presence of selected individual *Methanosarcina sp.*

The same short and lobe-like shapes were observed under DAPI-stain. The number of *Methanosarcina sp.* (MS 821) observed were fewer compared to those of archaeabacteria and eubacteria (Figure 4.26).

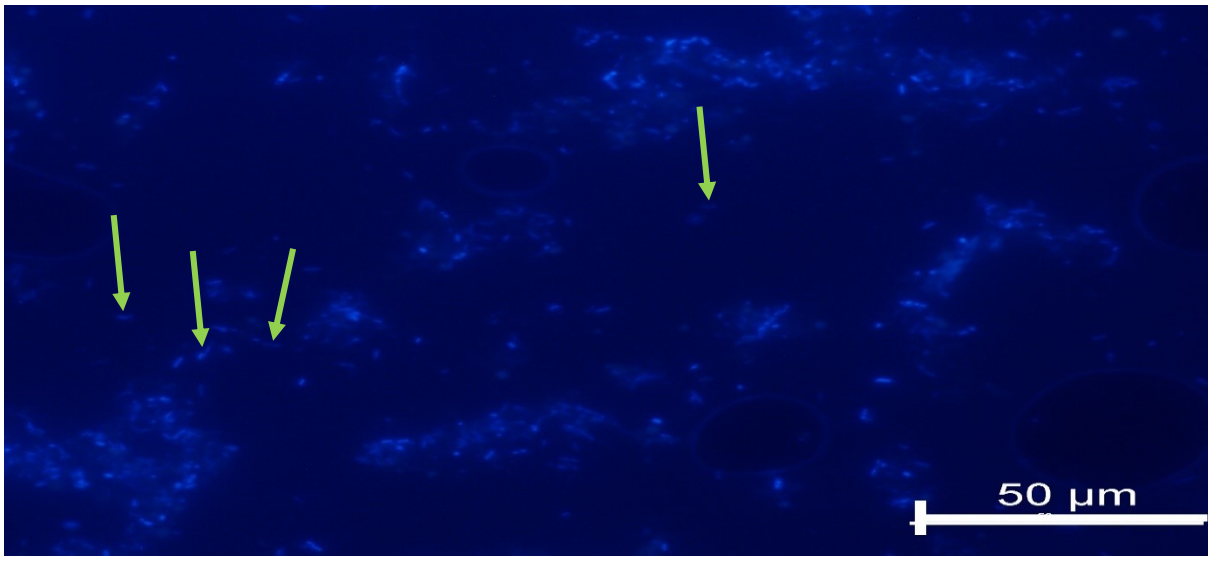

Figure 4.26: DAPI staining of *Methanosarcina sp.* using probe MS 821. Arrows pointing to the presence of selected individual *Methanosarcina sp.*

The FISH-DAPI overlay image of the microscopic picture showed very few sections which had blue-pink colouration. The green arrows on the picture points to the blue-pink two-lobed like shape of *Methanosarcina sp.* (MS 821), even though its number is very few (Figure 4.27).

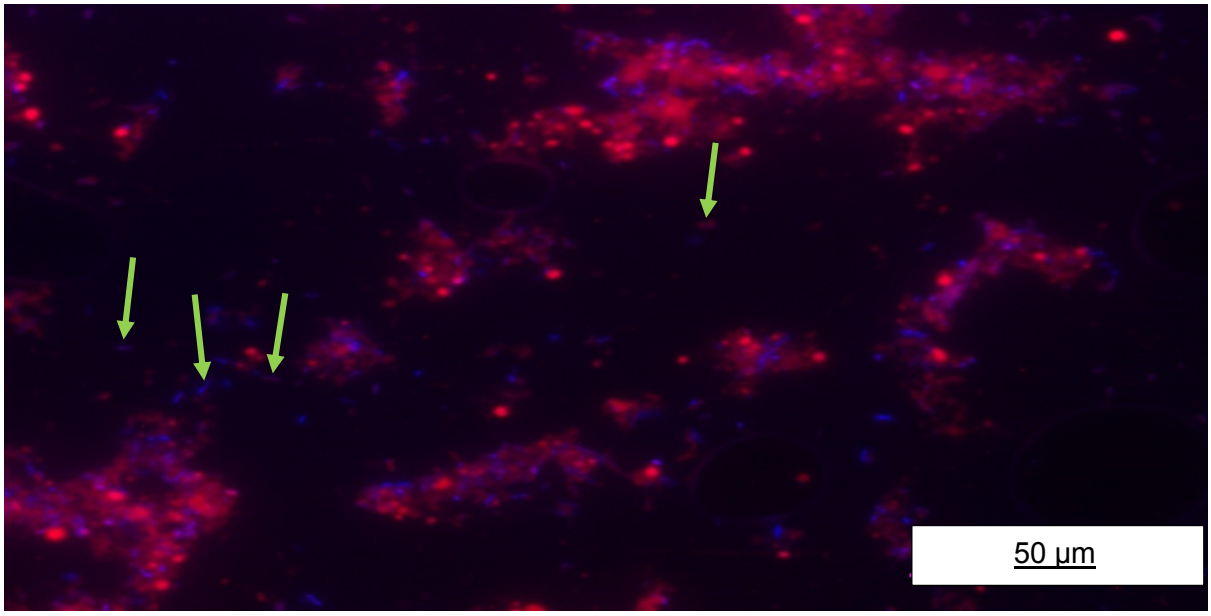

Figure 4.27: FISH-DAPI overlay of *Methanosarcina sp.* using probe MS 821. Arrows pointing to the presence of selected individual *Methanosarcina sp.*

Strains of *Methanococcus spp.* (MC 1109) were observed under FISH image, since only cocci-shaped fluorescence were observed compared to methanogenic archaea which appeared rod-like or longitudinally-cylindrical (Figure 4.28).

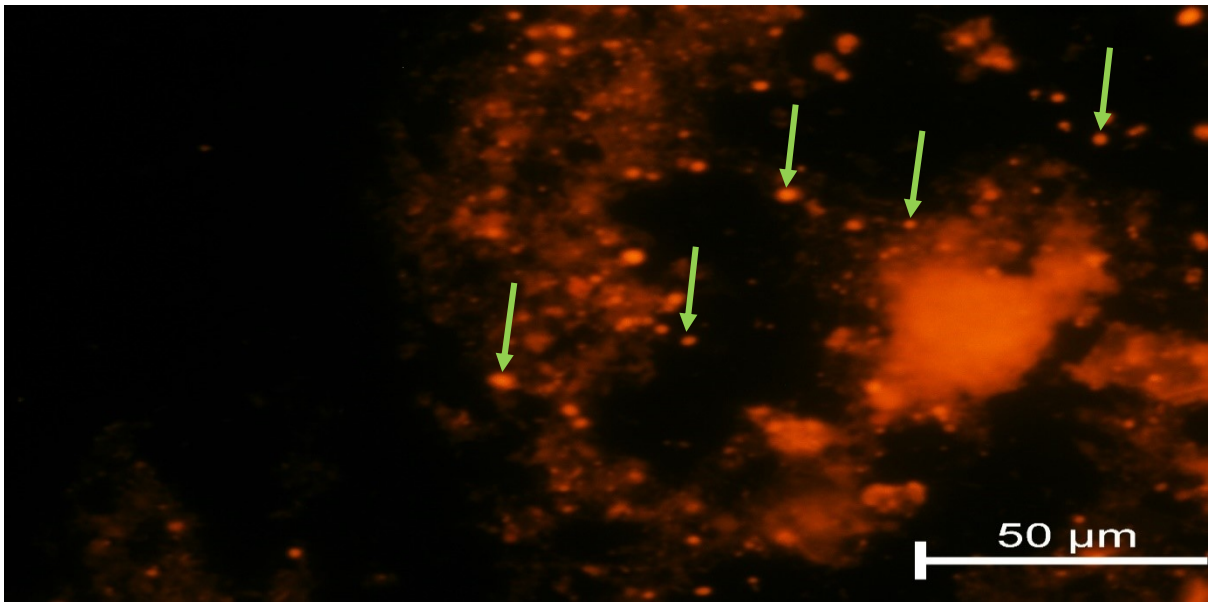

Figure 4.28: FISH staining of *Methanococcus spp* using probe MC 1109. Arrows pointing to the presence of selected individual *Methanococcus spp*.

DAPI-stained image for *Methanococcus spp.* (MC 1109) showed both singly and stringed bluish cocci-shapes and rod-like lobed structures (Figure 4.31), contrary to only singly-cocci shapes observed under the FISH image (Figure 4.29).

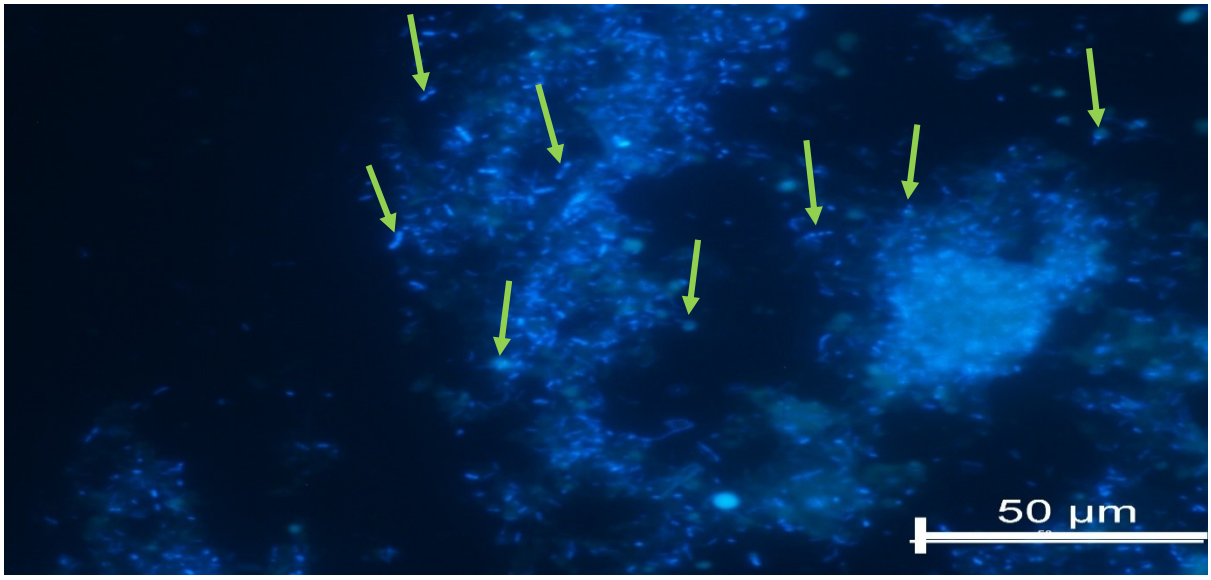

Figure 4.29: DAPI staining of *Methanococcus spp* using probe MC 1109. Arrows pointing to the presence of selected individual *Methanococcus spp.*

The overlay of DAPI-stained image on the FISH image showed bluish-pink singly cocci-shapes. The overlay did not show bluish-pink for the short rod-like shapes in the image confirming that the spherical bluish-pink shapes were *Methanococcus spp* (Figure 4.30).

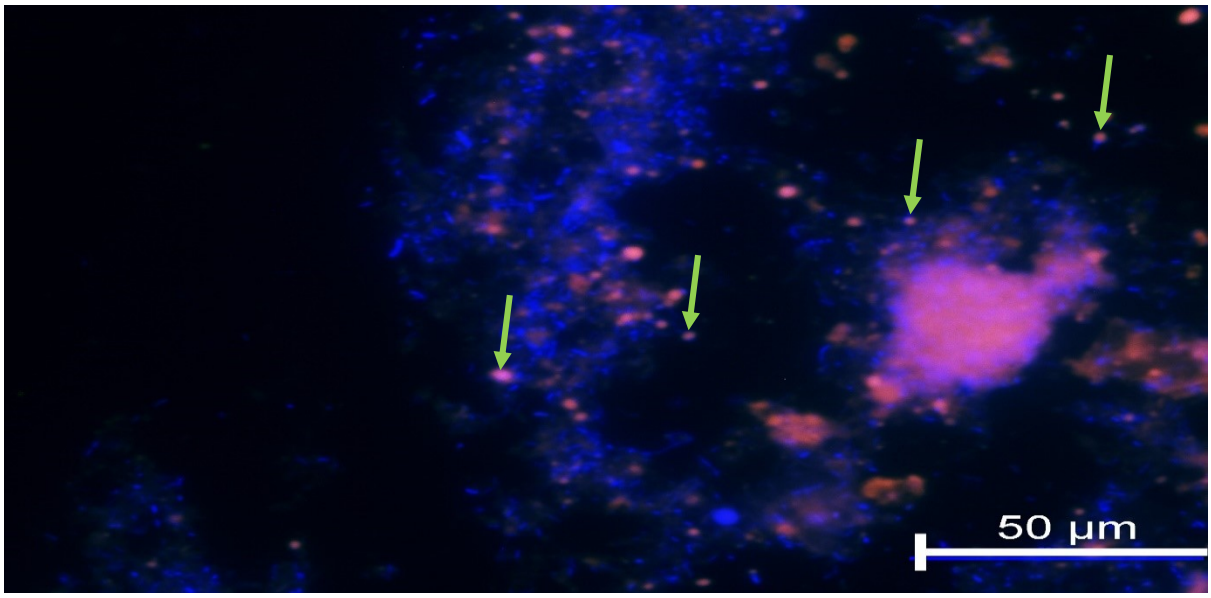

Figure 4.30: FISH-DAPI overlay of *Methanococcus spp* using probe MC 1109. Arrows pointing to the presence of selected individual *Methanococcus spp.*

Results for a group of methanogens such as *Methanomicrobium spp., Methanogenium spp., Methanoculleus spp., Methanospirillum spp., Methanocorpusculum spp. and Methanoplanus spp.* (which were all classified as MG 1200) for FISH image showed mostly spherical to oval shapes which were singly or in clusters, except in one spot where a single rod-like CY3 shape was observed. The oval shapes present confirm the presence of *Methanomicrobium spp.* The green arrows on the image points to the auto-fluorescence by this possible methanogen (Figure 4.31).

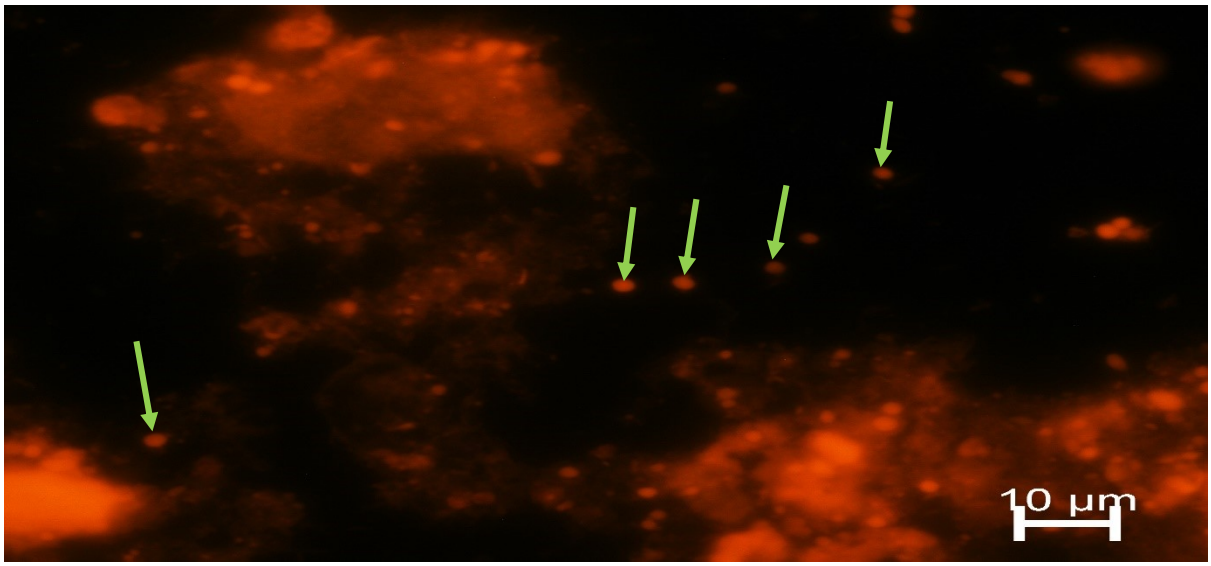

Figure 4.31: FISH staining of *Methanomicrobium spp* using probe MG 1200. Arrows pointing to the presence of selected individual *Methanomicrobium spp.*

DAPI-stained image for MG1200 showed fluorescence of stringed cocci shapes, segmented rod-shapes and spherically-oval shapes which were not conspicuous in the FISH image (Figure 4.32).

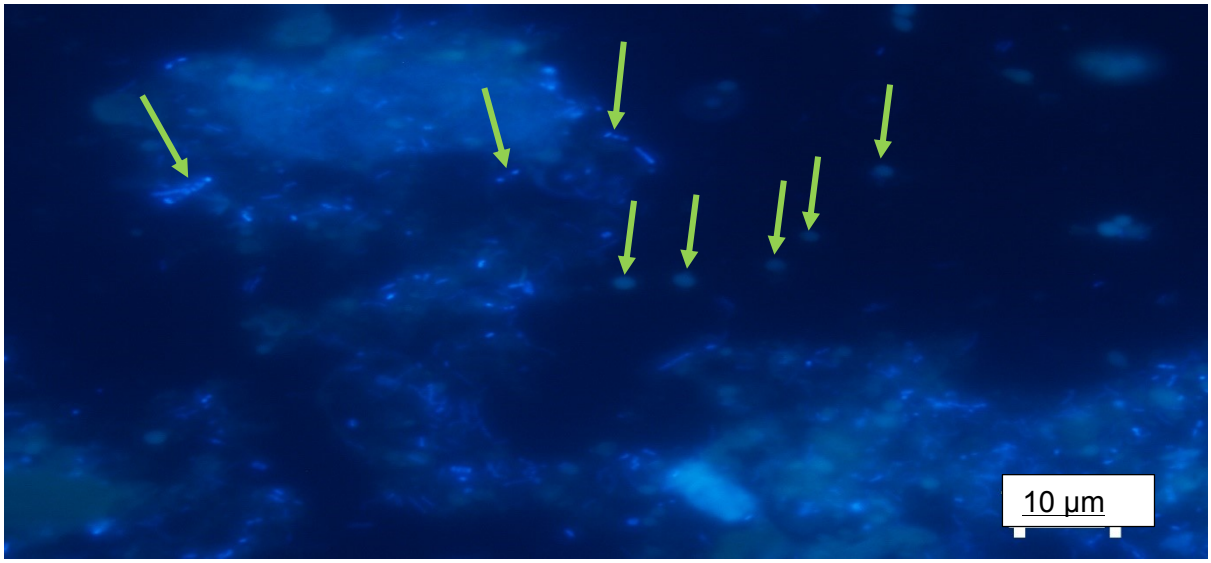

Figure 4.32: DAPI staining of *Methanomicrobium spp* using probe MG 1200. Arrows pointing to the presence of selected individual *Methanomicrobium spp.*

The overlay of DAPI-stained image on the FISH image showed no overlay of blue fluorescence over red fluorescence for rod-like, longitudinally-segmented or longitudinally-cylindrical shapes. Instances where blue fluorescence was superimposed on the red to form the bluish-pink colour were for only spherically-oval shapes confirming the presence of *Methanomicrobium spp* (MC1109) (Figure 4.33).

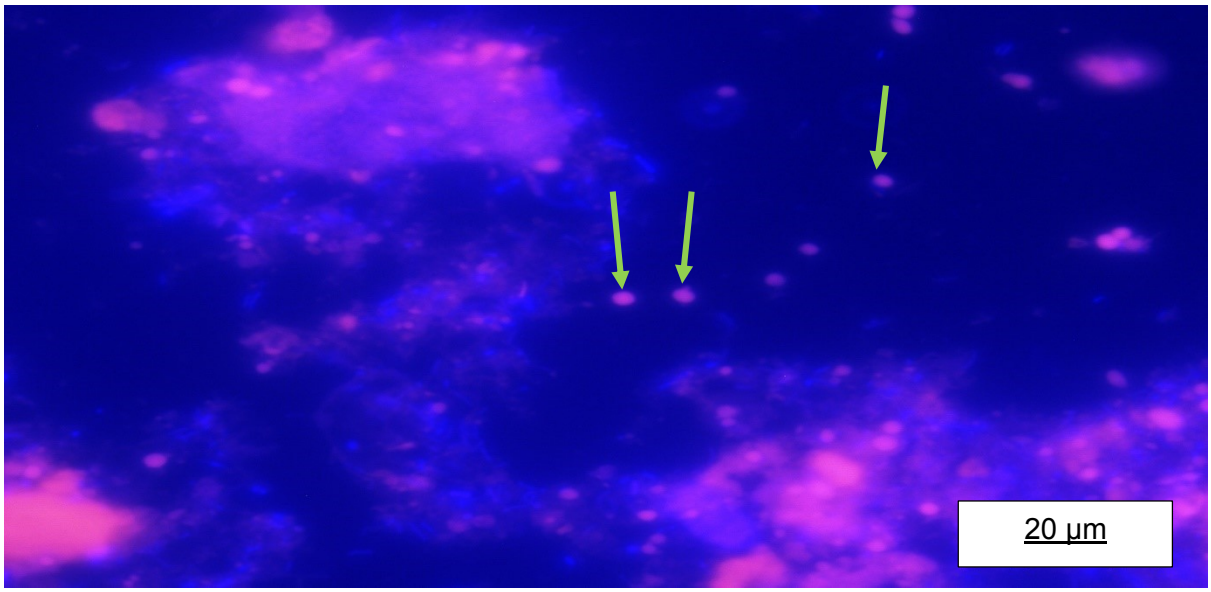

Figure 4.33: FISH-DAPI overlay of *Methanomicrobium spp* using probe MG 1200. Arrows pointing to the presence of selected individual *Methanomicrobium spp.*

4.6 Prototype pilot-scale solar-supported hyper-thermophilic treatment system

The schematic diagram below (Figure 4.34) shows the prototype single-stage hyper-thermophilic biogas reactor for the pilot-scale study research in Terterkessim slum, Elmina – Ghana. The diagram is based on the design of a fixed-dome biogas digester, thus is has all the features of a fixed-dome reactor as described in section 2.8.7 above. In this modified fixed-dome treatment technology, there is incorporation of solar panels for hyper-thermophilic heating. In addition, there is a manual mixer for stirring the influent to the already existing methanogenic biomass in the seeding sludge.

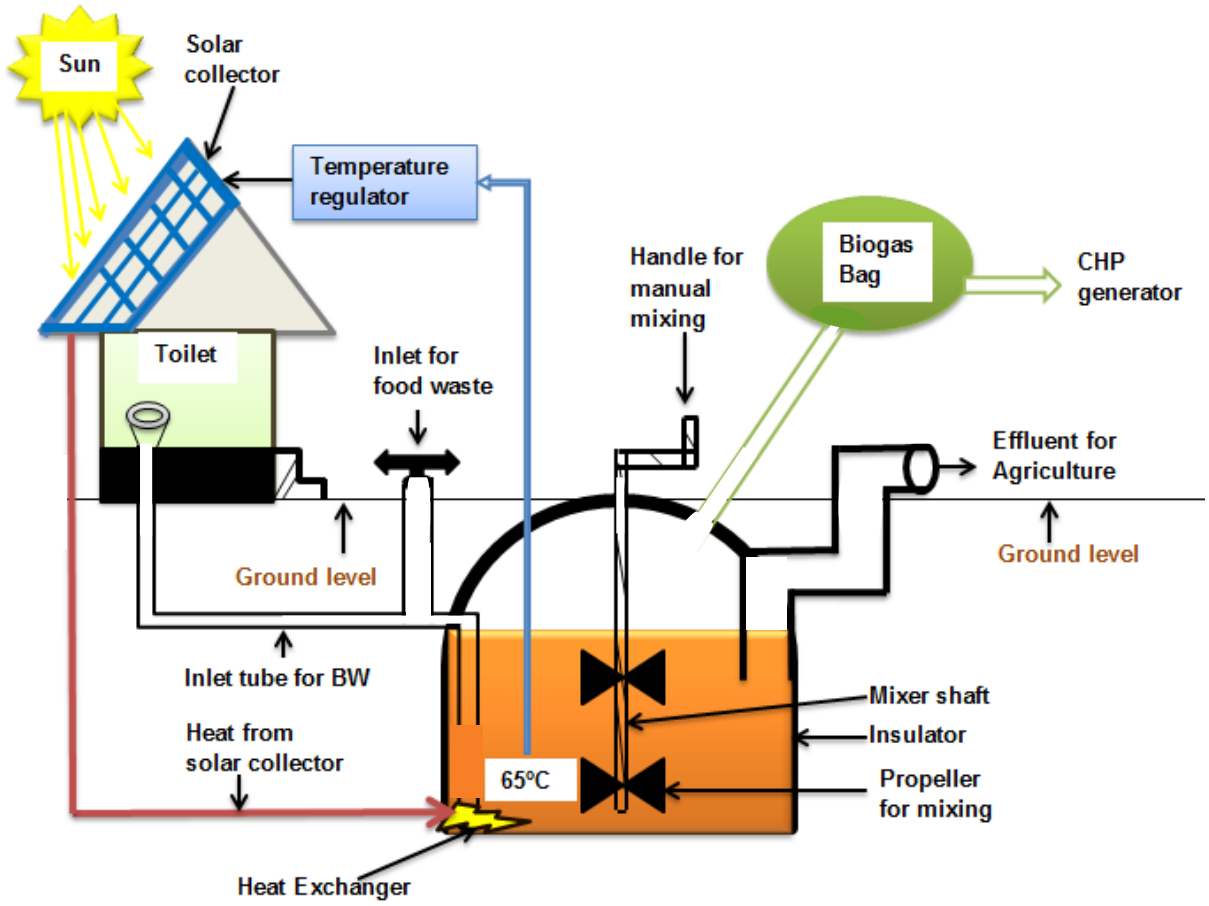

Figure 4.34: Schematic diagram of the prototype pilot scale solar-supported hyper-thermophilic BW treatment system in Terterkessim slum, Elmina-Ghana

4.7 Construction design for the single-stage SSHTABD for the household

The single-stage SSHTABD composed of 3 chambers which were originally designed for a septic tank system. The septic tanks were connected to a two-unit toilet meant for that

household described in section 3.8.3.2 above. The first chamber was the biggest and was converted into the main single-stage SSHTABD in which the AD process occurred. It had a total volume of 8.64 m^3. Adjoining the main reactor was a compensation tank which had a tunnel from the main digestion chamber. The compensation tank was about 3.17 m^3. Within the compensation tank were steps designed to help with settling of particles as well as directing clear effluent to be discharged into the next chamber, the effluent collection and storage tank. The effluent collection and storage tank had a total volume of 4.52 m^3. It had an effluent discharge pipe for overflow into a collection container for agricultural usage. Figure 4.35 gives detailed schematic representation and dimensions of the manually-stirred single-stage SSHTABD that was constructed for the residents of Terterkessim urban slum of Elmina, Ghana.

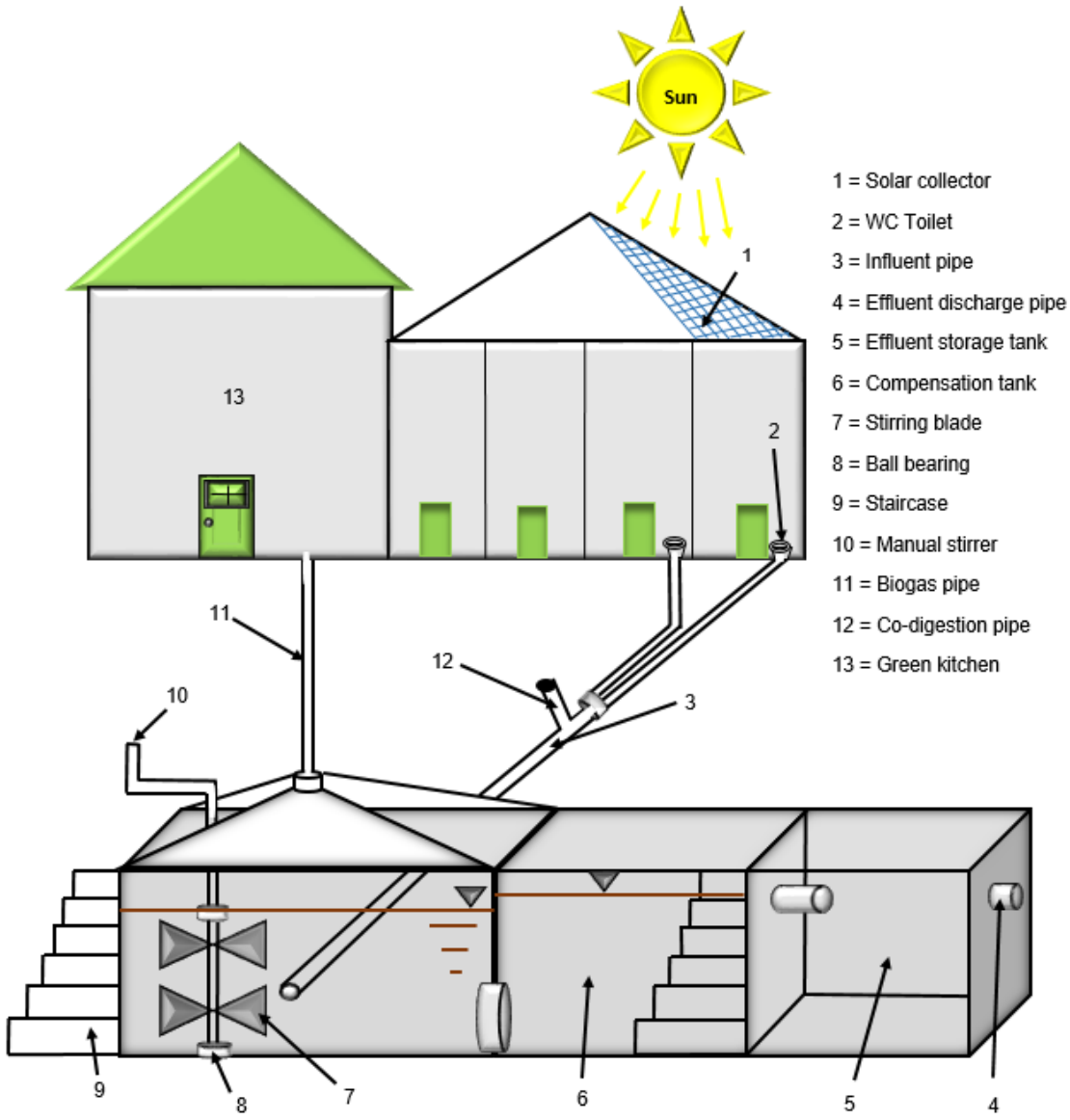

Figure 4.35: Schematic diagram showing the dimensions of the abandoned septic tanks converted into solar-supported single-stage hyper-thermophilic anaerobic biogas digester

4.8 Physico-chemical parameters for the pilot-scale single-stage SSHTABD

Different parameters influence the performance of single-stage SSHTABD operating under anaerobic conditions and these influence the reactor performance and usability of the effluent for purposes such as agriculture. Some of these parameters include pH, COD, total nitrogen (TN), total phosphorus (TP), ammonium-nitrogen (NH_4-N), nitrate-nitrogen (NO_3-N) and ortho-phosphate (PO_4-P). The average influent pH for BW was 6.1 ± 0.4, FW was 4.5 ± 0.3, MIX was 4.8 ± 0.2 while effluent was 6.6 ± 0.2 (Table 4.2). The average effluent value for the pH fell within the Ghana EPA effluent discharge standard as well as Germany Wastewater Ordinance. The high concentration of total COD (48869.2 ± 13297.8 mg/L) from the influent (MIX) saw an average COD removal of 97.6 % in the reactor as the total COD in the effluent was 1190.4 ± 317.4 mg/L (Table 4.2). The average BOD_5 in the effluent was 10.53 ± 3.76 mg/L, lower than the BOD_5 required by the Ghana EPA and Germany Wastewater Ordinance for effluent discharge. The TN in the BW was lower than those found in the FW and consequently, the MIX. The concentration of nitrogen in the form of ammonium (NH_4-N) in the BW was higher than it was in the FW and the MIX used as influent but lower than that of the effluent. However, the concentration of nitrogen in the form of nitrate (NO_3-N) in the BW was lower than that of the FW and the MIX used as influent. The nitrate (NO_3-N) concentration in the effluent was also higher than the concentration present in the influent (Table 4.2). The concentration of total phosphorus (TP) in the BW was the highest followed by the MIX and then the FW. Effluent concentration of TP was, however, higher compared to that of the influent. A similar observation was also made for phosphorus concentrations in the form of phosphate (PO_4-P). The average total solid (TS) in the BW was 4.6 ± 1.4 %, of which 4.1 ± 1.2 % was volatile solids (VS), representing 89.1 ± 3.1 % while FW had VS and TS of 7.7 ± 2.8 % and 7.9 ± 2.8 % respectively, representing 97.5 ± 2.4 % of the volatile solids in the dry matter. The MIX had average TS and VS of 6.0 ± 1.0 % and 5.6 ± 1.1 % respectively, representing 93.3 ± 3.2 % of the volatile solids in the dry matter (Table 4.2).

Table 4.2: Average values of physico-chemical parameters compared with the effluent discharge of EPA Ghana and EU for the pilot project in Terterkessim

Physico-chemical parameter	BW	FW	MIX	EFF	EPA Ghana Effluent Discharge Standard (2010)	Germany Domestic Waste water Ordinance (2004)
pH	6.1 ± 0.4	4.5 ± 0.3	4.8 ± 0.2	6.6 ± 0.2	6-9	6-9
COD (mg/L)	22996.2 ± 13073.3	68349.0 ± 6637.9	48869.2 ± 13297.8	1190.4 ± 317.4	<250	150
TN (mgN/L)	1117.7 ± 637.1	1188.9 ± 305.6	1651.0 ± 755.9	15.8 ± 6.6		-
NH_4-N (mgN/L)	907.4 ± 517.1	28.8 ± 16.8	351.9 ± 286.6	1583.9 ± 1406.4	1.0	-
NO_3-N (mgN/L)	27.4 ± 6.1	27.7 ± 14.6	71.0 ± 34.7	78.4 ± 42.1	<50	
TP (mgP/L)	142.5 ± 8.1	24.6 ± 14.7	63.8 ± 31.6	184.1 ± 15.5	2	-
PO_4-P (mgP/L)	89.3 ± 3.6	16.5 ± 10.5	46.9 ± 24.1	100.9 ± 10.3		
BOD_5 (mg/L)	n.d	n.d	n.d	10.53 ± 3.76	50	40
Electrical Conductivity(µs/cm)	3351.2 ± 505.4	2188.0 ± 183.3	2704.4 ± 356.2	6217.8 ± 1272.9	1500	
Salinity (ppt)	1.8 ± 0.3	1.1 ± 0.1	1.4 ± 0.2	3.4 ± 0.7		
TOC/TNs ratio	11.1 ± 3.7	30.5 ± 6.0	20.0 ± 11.0	18.6 ± 6.3		
Total Dissolved Solids (mg/L)	1579.2 ± 265.8	1038.6 ± 52.2	1180.4 ± 300.1	3091.6 ± 604.6	<1000	1000
TS (%)	4.6 ± 1.4	7.9 ± 2.8	6.0 ± 1.0	0.3 ± 0.1		
VS (%)	4.1 ± 1.2	7.7 ± 2.8	5.6 ± 1.1	0.08 ± 0.03		
VS/TS (%)	89.1 ± 3.1	97.5 ± 2.4	93.3 ± 3.2	26.7 ± 2.4		
E. coli (CFU/100mL)	5 X 10^8	0	n.d.	4 x 10^4	10	

*NB: Numbers written after the ± symbol are standard deviation values. n.d = not determined.

The influence of flow rate and COD removal efficiency in the pilot scale reactor constructed in the Terterkessim urban slum of Elmina shows that as the average monthly flow rate decreased, the removal efficiency of COD increased gradually from 1st to 3rd months where the maximum COD removal was achieved. The COD removal efficiency then decreased from 3rd to 5th months (Figure 4.36).

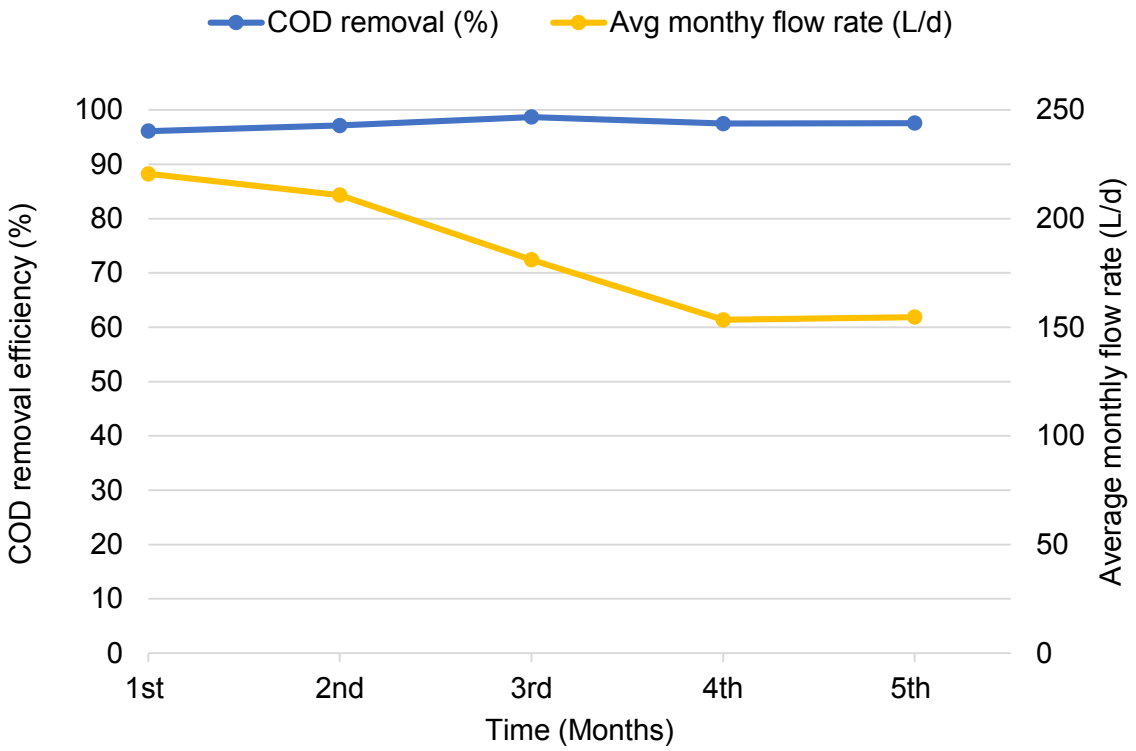

Figure 4.36: Relationship between COD removal efficiency and flow rate of a single-stage SSHTABD

Salinity and electrical conductivity (EC) of the influent and effluent samples were corresponding within the period of sampling. Between 1st and 2nd months, salinity and EC in the influent decreased slightly and then increased gently between 3rd and 4th months before they finally decreased gently in the 5th month. The concentrations of salinity and EC in the effluent followed similar trends as was observed in the influent samples over the period (Figure 4.37).

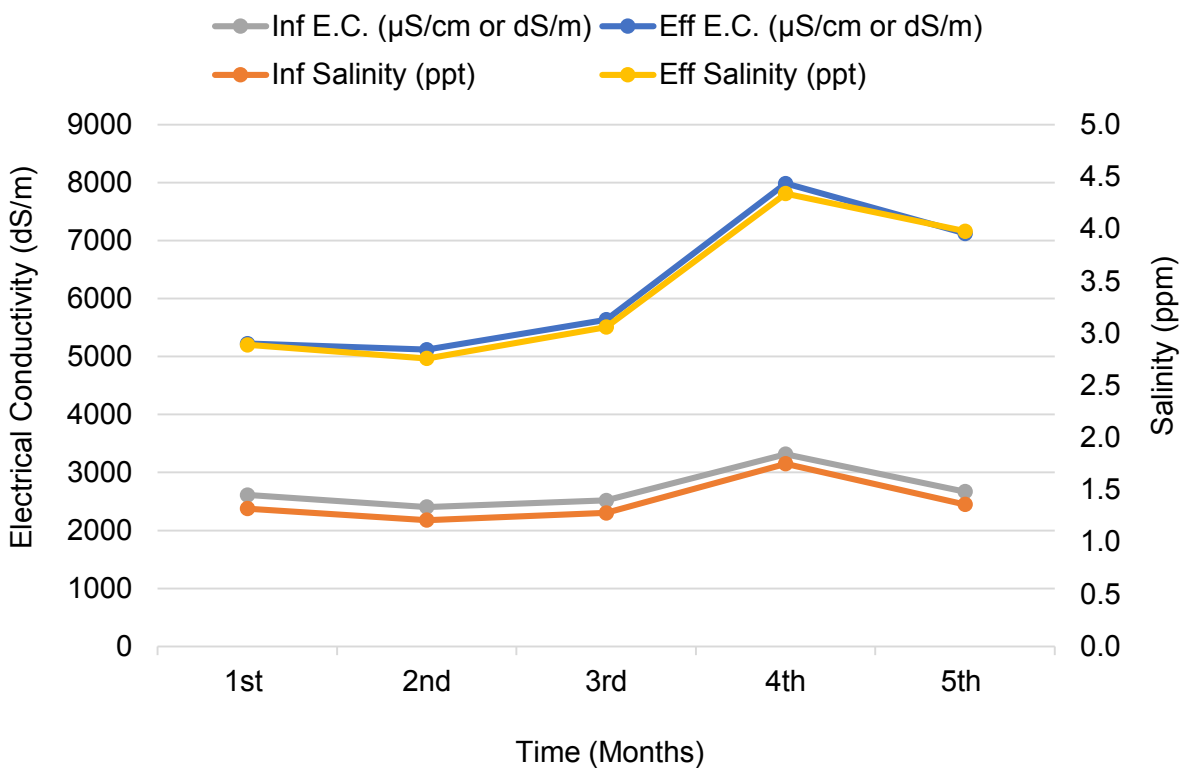

Figure 4.37: Relationship between salinity and electrical conductivity of influent and effluent

The trends for the concentrations of both the total dissolved solids (TDS) and salinity of the influent and effluent for the pilot-scale single-stage SSHTABD were similar from 1st to 5th months. Between the 1st and 2nd months, salinity decreased slightly in the influent before increasing gradually between the 3rd and 4th months and then decreased again in the 5th month. This also affected the concentration of salinity in the effluent.

Unlike salinity in the influent, the TDS in the influent increased gradually from the 1st month through to the 4th month before decreasing a little in the 5th month. The concentration of TDS in the effluent, however, did not follow the same pattern as in the influent since it decreased slightly between the 1st and 2nd months, increased sharply from the 2nd to the 4th months, before decreasing gradually in the 5th month (Figure 4.38).

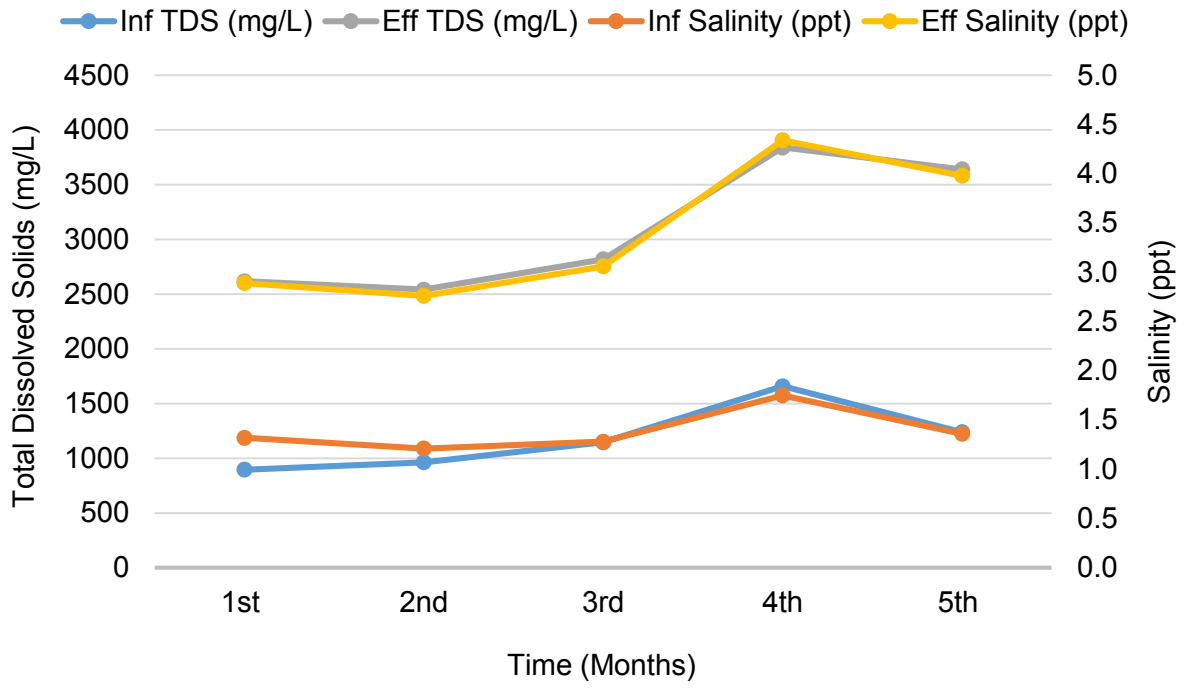

Figure 4.38: Relationship between salinity and Total Dissolved Solids of influent and effluent

4.9 Comparison of design parameters for the lab-scale HT-CSTR and the pilot-scale single-stage SSHTABD

The proper performance and efficiency of any anaerobic digester depends on some important parameters such as the operational temperature of the reactor, the hydraulic retention time (HRT) and the pH of both the influent and effluent. Other important design parameters include the COD volumetric loading rate and the organic loading rate of the reactor.

A comparison was made between the design parameters of the laboratory-scale single-stage HT-CSTR and the pilot-scale single-stage SSHTABD. Table 4.3 shows that the average pH for influent for the lab-scale HT-CSTR was 6.0 ± (1.0) while that of the pilot-scale single-stage SSHTABD was 4.8 ± (0.2). Similarly, the average pH for the effluent for the laboratory-scale HT-CSTR was 6.89 ± (0.56) while that of the pilot-scale single-stage SSHTABD was 6.6 ± (0.2). The organic loading rate for the lab-scale HT-CSTR [0.27 ≈ 0.3 ± 0.1 kgVS/(m^3*d)] was higher than that of the pilot-scale single-stage SSHTABD [0.06 ≈ 0.1 ± 0.0 kgVS/(m^3*d)] (Table 4.3).

Table 4.3: Mean values of design parameters for laboratory-scale HT-CSTR and pilot-scale SSHTABD with their standard deviation values

Parameter	Laboratory-scale HT-CSTR	Pilot-scale SSHTABD
Reactor Temperature (ºC)	65.2 ± (7.0)	37.0 ± (2.7)
HRT (days)	23.3 ± (16.8)	51.3 ± (12.9)
Flow Rate (L/d)	2.2 ± (1.6)	182.1 ± (27.5)
COD Volumetric Loading Rate kgCOD/(m^3*d)	6.22 ± (1.6)	0.98 ± (0.2)
Organic Loading Rate kgVS/(m^3*d)	0.27 ± (0.1)	0.06 ± (0.0)
Influent pH	6.0 ± (1.0)	4.8 ± (0.2)
Effluent pH	6.9 ± (0.6)	6.6 ± (0.2)

*NB: Numbers written in brackets () are standard deviation values. n.d = not determined.

4.10 Theoretical methane productivity and yield for the pilot-scale single-stage SSHTABD

Theoretical Methane productivity was calculated based on the COD removal over the period of study in relation to the active reactor volume (level of liquid in the reactor). The average normalised theoretical methane productivity increased sharply from the 1st month to the 3rd month and then decreased gradually from the 3rd month to the 5th month. This also corresponded with the COD load that was removed by the reactor from the 1st month through to the 5th month (Fig. 4.39).

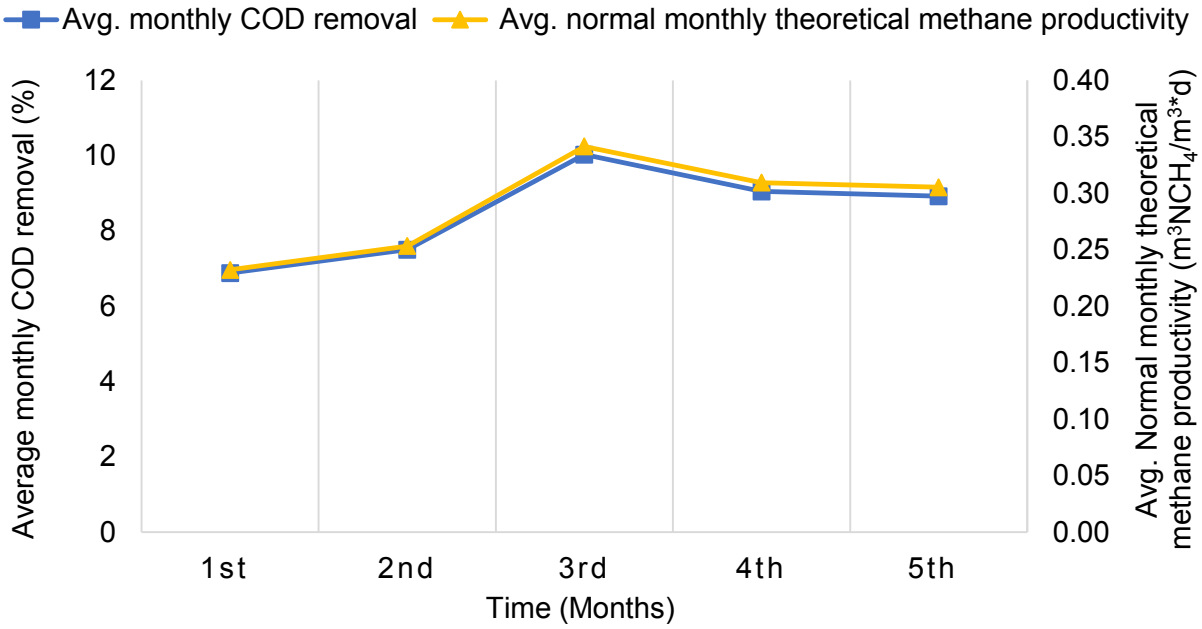

Figure 4.39: Relationship between average monthly COD removal and average monthly normalised theoretical methane productivity

The average monthly normalised theoretical methane yield increased sharply between the 1st and 2nd months and then decreased sharply between the 2nd and the 3rd months. It further decreased gradually from the 3rd to the 5th months. The average monthly COD removal on the other hand, increased gradually between the 1st and 2nd months, sharply between the 2nd and 3rd months and then decreased gradually from the 3rd through to the 5th month (Fig. 4.40).

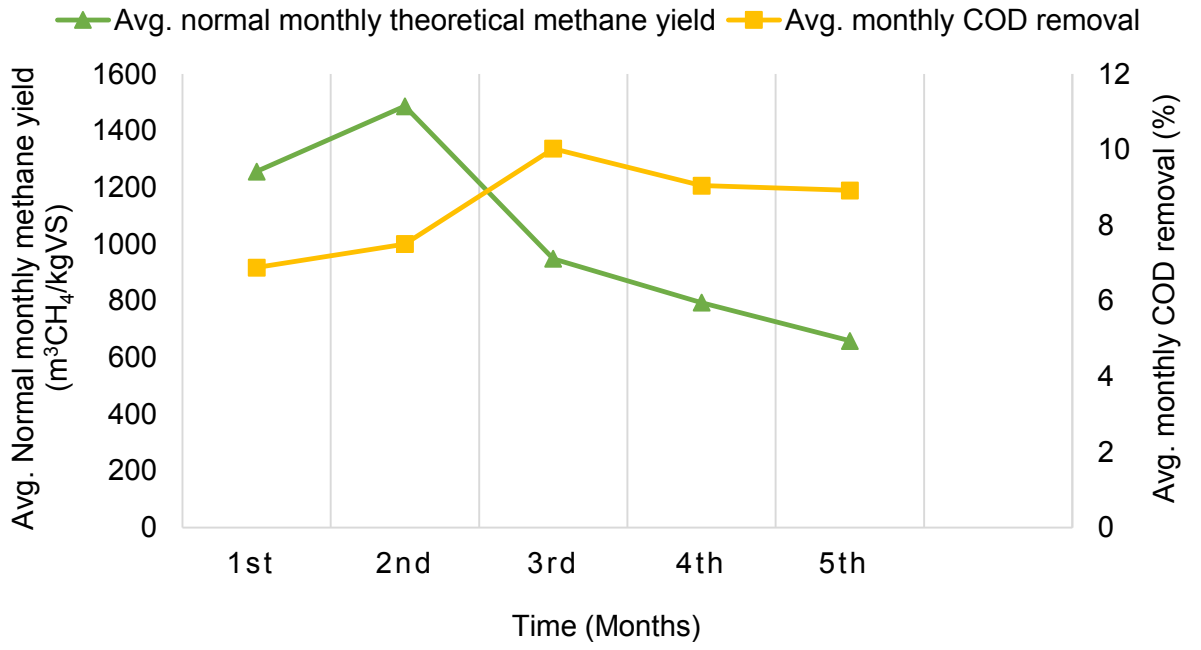

Figure 4.40: Relationship between removed COD and theoretical methane yield

Throughout the study period, the Hydraulic Retention Time (HRT) for the pilot-scale single-stage SSHTABD fluctuated from the first day to the end of the study period. The longest HRT of 96.8 days each was observed for both day 100 and day 106 during the period of study. The shortest HRT of 44.8 days was recorded for day 2. The normalised theoretical methane production, on the other hand, increased gradually from the beginning of the study to the end. Within the first 25 days, the average normalised theoretical methane production was 9.4 LN, which increased gradually to 10.8 LN at the end of the 55th day. This increased sharply to 16.9 LN between day 56 and 85 and then increased gradually again to 18.1 LN from day 86 to the end of the study period (day 149), where an average normalised theoretical methane production was recorded to be 17.7 LN (Figure 4.41).

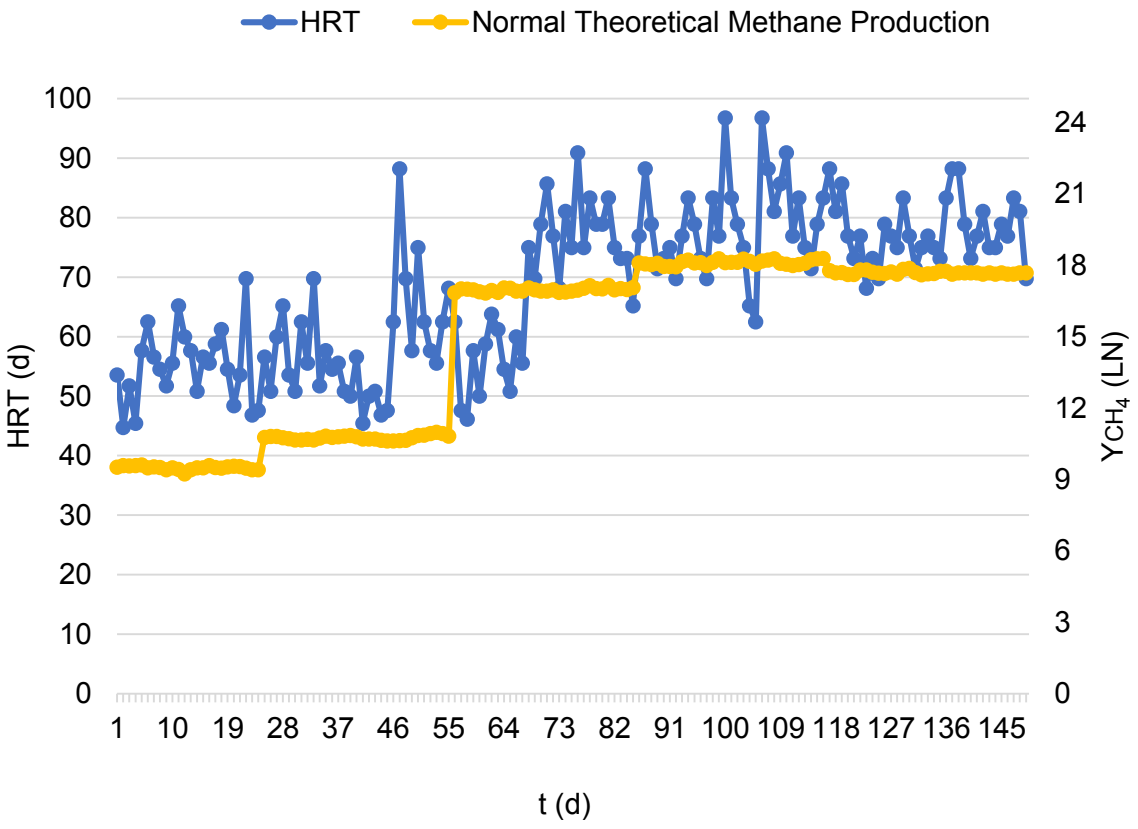

Figure 4.41: Relationship between HRT and Normal Theoretical Methane Production

4.11 Concentrations of heavy metals in the influent and effluent of the pilot-scale single-stage SSHTABD

The influence of black water (BW) on the concentrations of some of the metals in the influent (MIX) and the effluent of the pilot-scale single-stage SSHTABD cannot be overlooked. Average values for some heavy metal concentrations in the BW such as total iron (1.60 ± 0.2

mg/L), total manganese (0.64 ± 0.1 mg/L), total copper (0.23 ± 0.1 mg/L) and total zinc (0.31 ± 0.1 mg/L) were much higher compared to the contributions made by equal volumes of the same metals in the FW. In the FW, total iron was 0.43 ± 0.0 mg/L, total manganese was 0.08 ± 0.0 mg/L, total copper was 0.003 ± 0.0 mg/L while total zinc was 0.07 ± 0.0 mg/L. All the heavy metal concentrations in the effluent were below the Ghana EPA standard as well as the Guideline for wastewater effluent discharge standard in Germany (Table 4.4).

Table 4.4: Average values of heavy metals compared with the effluent discharge of EPA Ghana and Germany Wastewater Ordinance

Heavy Metal	BW	FW	MIX	EFF	EPA Ghana Discharge Standard (2010)	Germany Waste water Ordinance (2004)
Total Iron (Fe)mg/L	1.598 ± 0.15	0.433 ± 0.04	0.483 ± 0.04	0.915 ± 0.05		
Total Manganese (Mn)mg/L	0.636 ± 0.13	0.080 ± 0.01	0.118 ± 0.03	0.176 ± 0.01		
Total Copper (Cu)mg/L	0.233 ± 0.06	<0.003 ± 0.000	<0.003 ± 0.000	<0.003 ± 0.000	1.0	0.5
Total Zinc (Zn)mg/L	0.308 ± 0.07	0.070 ± 0.01	0.084 ± 0.01	0.151 ± 0.05		2.0
Total Lead (Pb)mg/L	<0.001 ± 0.000	<0.001 ± 0.000	<0.001 ± 0.000	<0.001 ± 0.000	<1.0	0.5
Total Chromium (Cr)mg/L	<0.002 ± 0.000	<0.002 ± 0.000	<0.002 ± 0.000	<0.002 ± 0.000		0.5
Total Cadmium (Cd)mg/L	<0.002 ± 0.000	<0.002 ± 0.000	<0.002 ± 0.000	<0.002 ± 0.000	<0.02	0.1
Total Cobalt (Co)mg/L	<0.005 ± 0.000	<0.005 ± 0.000	<0.005 ± 0.000	<0.005 ± 0.000		1.0
Total Arsenic (As) mg/L	<0.004 ± 0.000	<0.012 ± 0.001	<0.010 ± 0.000	<0.004 ± 0.000		0.1
Total Mercury (Hg) mg/L	<0.001 ± 0.000	<0.001 ± 0.000	<0.001 ± 0.000	<0.001 ± 0.000		0.05

*NB: Numbers written after the ± symbol are standard deviation values. n.d = not determined.

4.12 Effluent quality of the pilot-scale single-stage SSHTABD with respect to pathogens concentrations

The applicability of effluent for agricultural purposes depend largely on the quality of effluent, especially when unrestricted irrigation is considered and consequently, the concentrations of influent and effluent were assessed for *Salmonella spp* and *Escherichia coli* for the pilot-scale single-stage SSHTABD. The concentrations of *Salmonella spp* on Brilliant Green Agar (BGA) was $2x10^8$ CFU/mL from the 1st month through to the 4th month and then increased slightly to $5x10^8$ CFU/mL in the 5th month. The concentrations of *Salmonella spp* on Endo Agar (EA) was, however, a little higher ($5x10^8$ CFU/mL) from the 1st month through to the 4th month and then increased considerably to $3x10^9$ CFU/mL in the 5th month. The concentrations of *Escherichia coli* in the influent on BGA were 2×10^8 CFU/mL in the 1st and 2nd months and then increased slightly to $6x10^8$ CFU/mL from the 3rd to the 5th months. The growth of *Escherichia coli* on the EA in the influent sample was a little higher than it was in BGA as its concentrations were $5x10^8$ CFU/mL in the 1st and 2nd months, $6x10^8$ CFU/mL in the 3rd and 4th months and $3x10^9$ CFU/mL in the 5th month (Figure 4.42).

There was a reduction of the concentrations of *Salmonella spp* on BGA in the effluent by 5 logs in the 1st and 2nd months and then 4 logs from the 3rd to the 5th month. On the EA, on the contrary, the reduction of *Salmonella spp was* 4 logs in 1st and 2nd months, then decreased to 3 logs in the 3rd and 4th months and a much higher reduction of 5 logs in the 5th month. The concentration of *Escherichia coli* in the effluent on BGA was reduced by 5 logs in the 1st and 2nd months and then 4 logs from the 3rd to the 5th months while that on EA had reduced by 5 logs from 1st to the 4th months and then 4 logs in the 5th month. The effluent quality with respect to pathogen concentrations could not meet the Ghana EPA effluent discharge standards (Figure 4.42).

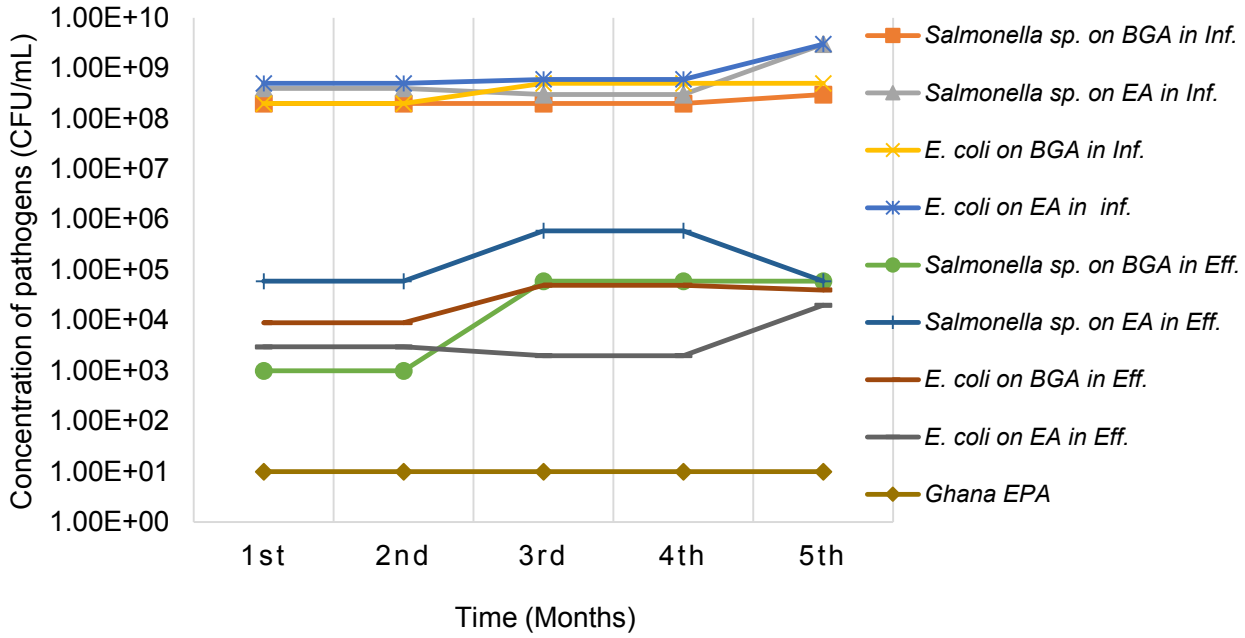

Figure 4.42: Pathogen concentrations in the influent and effluent of the pilot-scale single-stage SSHTABD

4.13 Cost-effectiveness of adopting the pilot-scale single-stage SSHTABD

The cost for constructing, operating and maintaining the single-stage SSHTABD is one important factor that may hinder people from either adopting it or may not motivate people to adopt. Consequently, assessing the financial benefits of adopting the technology vis-à-vis the investment cost is very relevant for any technology implementation and scale-up. The overall cost for only the construction of the single-stage SSHTABD was five thousand two hundred and ninety-one Ghana cedis and five pesewas (GH₵ 5291.5 = € 1058.3). The cost for the solar panel, gel battery, inverter and charge controller was four hundred and twenty-five Ghana cedis only, excluding shipment (€ 85 – Personal Communication- Prof Martienssen). The cost for operating the single-stage SSHTABD depends on which measurement parameters the operator would be carrying out and for how often those parameters would be measured. The maintenance cost would also depend on how the reactor will function or malfunction and also which connecting parts need replacement or otherwise and how often.

The pilot-scale single-stage SSHTABD could produce at least 2.53 $Nm^3CH_4/(kgCOD.d)$. This volume of methane could be burnt for at least 8 hours for cooking or other domestic purposes. On the average, a respondent in Elmina uses fifty-five Ghana cedis (GH₵ 55.00 = € 11.00) of LPG per month. Consequently, if this 55 cedis is saved per monthly, within a year a respondent

using the single-stage SSHTABD would have saved six hundred and sixty cedis only (GH₵ 660.00 = € 132.00). If the biogas digester is to last for at least 25 years, the user of the facility would have saved at least sixteen thousand five hundred Ghana cedis (GH₵ 16500.00 = € 3300.00). This amount does not even factor in inflation of prices which would also affect prices of LPG if the user of the facility had continued using LPG for cooking and other heating chores in the home.

Apart from the benefits the user of the single-stage SSHTABD will have in terms of availability of cooking gas, the user of the facility will also benefit from having access to free toilet facility in the home as well as producing hygienised digestate for urban agriculture. Assuming there is no price increase and a household of four persons pays Fifty Ghana pesewas (GH₵ 0.50) to have access to public toilet facility in Terterkessim slum or Elmina in general, the household would have spent Seven-Hundred and Thirty Ghana cedis (GH₵ 730.00 = € 137.7) per annum and Eighteen Thousand Two Hundred and Fifty cedis (GH₵ 18250 = € 3442.5) within 25 years (assuming a biogas digester lasts for at least 25 years). In addition, the incidence and recurrence of cholera outbreak which hitherto had been the situation in Terterkessim slum in Elmina and most other slums and cities in Ghana would cease, leading to cleaner and safer environment as well as healthy citizens.

4.14 Conclusive summary for all the technical results

The results from the batch tests for the three seeding sludge under three different hyper-thermophilic temperatures showed CM at 65 ^{o}C as the preferred seeding sludge and optimal hyper-thermophilic temperature when a bigger set-up is being considered followed by sewage sludge from wastewater treatment plant, however, its optimal operational temperature was 60 ^{o}C. It was ascertained that treating BW at hyper-thermophilic temperature of 70 ^{o}C was not feasible irrespective of the type of seeding sludge used. The laboratory-scale HT-CSTR had a high mean total COD removal of 86.3 % and an average COD volumetric loading rate of 6.22 kgCOD/(m^3.d). It also had organic loading rate of 0.3 kgVS/(m^3.d) compared with 0.06 kgVS/(m^3.d) of the SSHTABD used for the pilot study. Treatment of only BW at optimal hyper-thermophilic temperature of 65 ^{o}C in the laboratory-scale HT-CSTR produced biogas with less methane content of 34.9 %, however, co-digestion with kitchen food waste increased the percentage content of methane in the biogas by 77 % from 34.9 % to 61.8 %. The normalised cumulative methane productivity over the period for the HT-CSTR was 1.3 $Nm^3CH_4/(m^3.d)$, with an average being 0.06 $Nm^3CH_4/(m^3.d)$ while its normalised cumulative methane yield was 55.4 Nm^3CH_4/kgVS, averaging 2.52 Nm^3CH_4/kgVS. The single-stage HT-CSTR was able to hygienise all bacteria of *Salmonella senftenbergensis* and *E. coli* that were spiked into the

reactor. Microbial flora that were present in the cow manure at the optimal hyper-thermophilic temperature of 65 ^{o}C were Eubacteria (EUB338 I), archaeabacteria, *Methanosarcina spp.* (MS821), *Methanomicrobium spp.* (MG1200) (oval-shaped) and *Methanococcus spp.* (MC1109). Eubacteria were dominant followed by *Methanococcus spp.* (MC1109), *Methanomicrobium spp.* (MG1200), archaeabacteria, and *Methanosarcina spp.* (MS821). The effluent produced from the pilot single-stage SSHTABD contained less concentrations of heavy metals compared with the effluent discharge standards set by the Environmental Protection Agency of Ghana. However, it cannot be used for cultivation of leafy vegetables such as cabbage and lettuce since it had some concentrations of pathogens like *Salmonella spp.* and *E. coli.* On the contrary, the effluent can be used for the cultivation of plantation and cash crops like rubber and cotton or food crop like maize, banana, plantain, avocado, oranges and mango.

4.15 Perceptions of residents of Elmina on their willingness to adopt and use a solar-supported SSHTABD

The willingness and readiness of the citizenry to adopt and use a technology that has been developed is very paramount in every scientific breakthrough. Consequently, the need to assess the perception of some residents of Elmina on their willingness to accept and adopt the single-stage solar-supported hyper-thermophilic manually stirred anaerobic biogas digester.

4.15.1 Demographics of some of the residents of Elmina, Ghana

The ages of respondents in Elmina ranged from 21 to 70 years and above 71 years. Respondents between the ages of 21-30 years formed the highest percentage (47.9 %) followed by 31-40 years (20.1 %) and 41-50 years (17.4 %). The age group with the least number of respondents was above 71 years (1.4 %) (Figure 4.43). Regarding the sex of the respondents, females were dominant (51.1 %) over males (48.9 %).

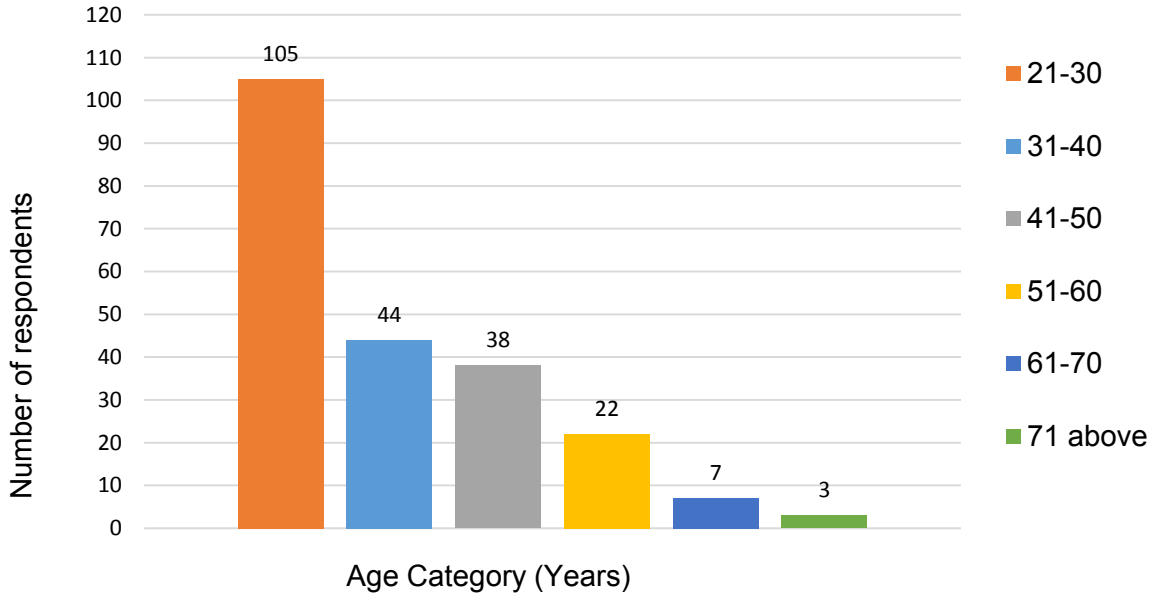

Figure 4.43: Age distribution of respondents in Elmina, Ghana

At least, 90 % of the total respondents had some form of formal education, while 10 % had no formal education of any kind. Concerning the percentage that had formal education, the greater percentage (53 %) had formal education to the secondary level, while 27.9 % and 9.1 % had formal education to the tertiary and primary levels, respectively (Figure 4.44).

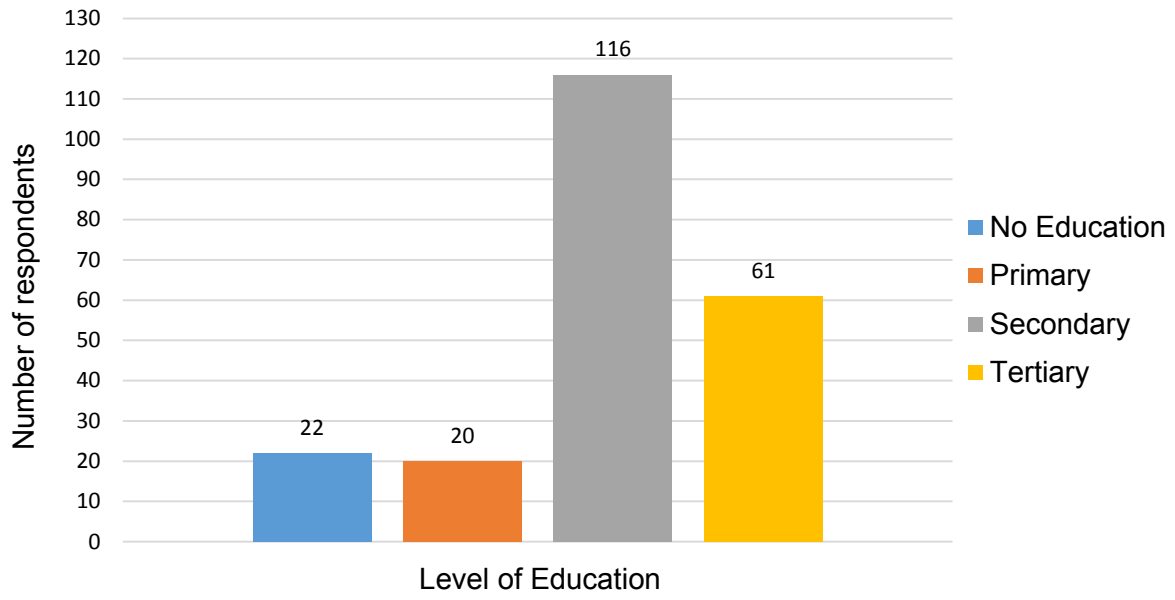

Figure 4.44: Level of education of respondents in Elmina, Ghana

About 79 % of the respondents in Elmina were gainfully employed in the various employment sectors of Ghana while 21 % were unemployed. Regarding those who were gainfully employed, 52.5 % work in the informal private sector, 6.4 % in the formal private sector and 20.1 in the formal government sector (Figure 4.45).

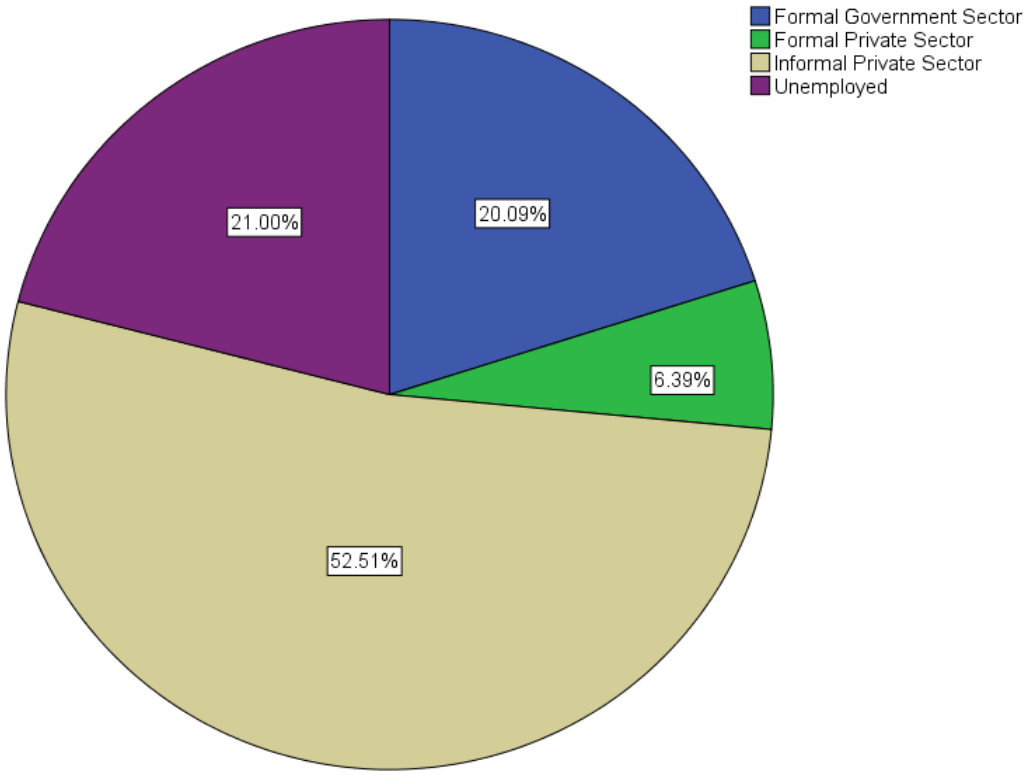

Figure 4.45: Type of employment sector engaged by respondents in Elmina

4.15.2 Responses on accessibility to a household or public toilet facility in Elmina

In Elmina town in general, some residents have household toilet facility while others do not. The percentage of those who have household toilet facility in Elmina town was 56.2 % while those who do not have toilet facility in their homes were 43.8 %. With respect to the residents who have household toilet facilities, about 46.1 % use water closet (WC) connected to a septic tank, 13.2 % use pit latrine while 4.1 % use a modified ventilated improved pits called Kumasi Ventilated Improved Pit (KVIP). About 36.5 % use other types of toilets other than the ones above-mentioned. Examples included deep trenches with concrete slabs, deep trenches with wooden boards or deep trenches with tree branches. In very few occasions, some households mentioned the use of toilets connected to pan latrines, which hitherto, has been banned for usage in Ghana (Figure 4.46).

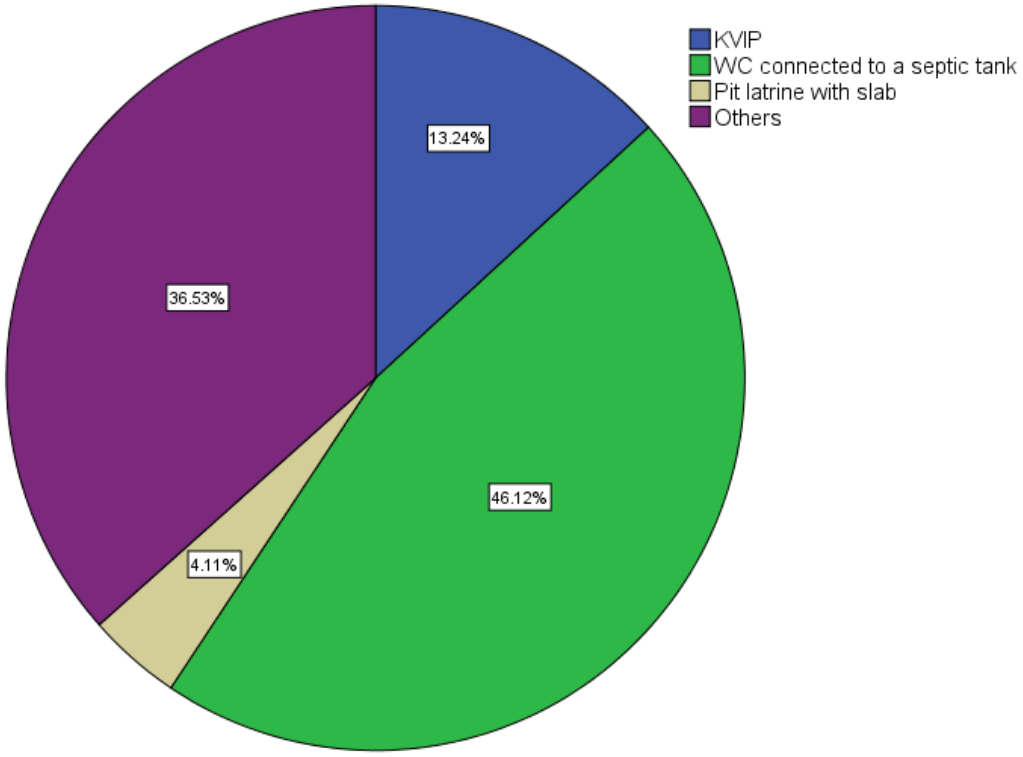

Figure 4.46: Types of household toilet facilities used by residents of Elmina

The households that had toilet facilities in their homes employed different techniques in emptying the toilet when it was full. The use of vacuum trucks or tanker from the K.E.E.A Municipal Assembly to empty the toilets particularly, WCs that were connected to septic tanks dominated with about 30.6 % (Figure 4.47). Depending on the distance of one's house from the Municipal Assembly, varying amounts of One Hundred Ghana Cedis (GH₵ 100.00, equivalent to € 20) to Five Hundred Ghana Cedis (GH₵ 500.00, equivalent to € 100) were paid by households as service fees to the Municipal Assembly. About 10.5 % of the

respondents did not have any idea whatsoever, how their toilets were emptied anytime it was full. In addition, about 8.7 % of the respondents stated that their toilets were not yet full, consequently, they could not mention which ways their toilets would be emptied should it get full (Figure 4.47). More so, about 3.7 % added chemicals to the toilet to dehydrate the faeces even though the names of the chemicals that were mentioned varied from different users. For example, some use *'Akasha'* (a locally manufactured bleaching agent used in cleaning bathrooms and sinks) while others used acids (which they could not mention the specific type) (Figure 4.47). The prices for the *'Akasha'* and the acids ranged from Five Ghana Cedis (GH₵ 5.00, equivalent to € 1) to Twenty Ghana Cedis (GH₵ 20.00, equivalent to € 4). Interestingly, 2.3 % stated that their toilet facilities never gets full, therefore, did not need any emptying. About 1.4 % of the respondents employ the use of human services to carry their excrement to bury in a dugout in a nearby bush anytime their pan latrines were full. These people who carry the pan latrine charge a service fee as low as Twenty Ghana Cedis (GH₵ 20.00, equivalent to € 4) to Fifty Ghana Cedis (GH₵ 50.00, equivalent to € 10) and a bottle of local gin called '*Akpeteshie'*. About 0.9 % had their toilets connected to biogas digesters even though the digesters were not functional. Those who had their toilets connected to biogas digesters had spent at least, Five Thousand Ghana Cedis (GH₵ 5000.00, equivalent to € 1000) in its construction. About 42 % that showed 'not applicable' represented the respondents who did not have toilet facility in their homes and thus did not give any information pertaining to emptying of toilet (Figure 4.47).

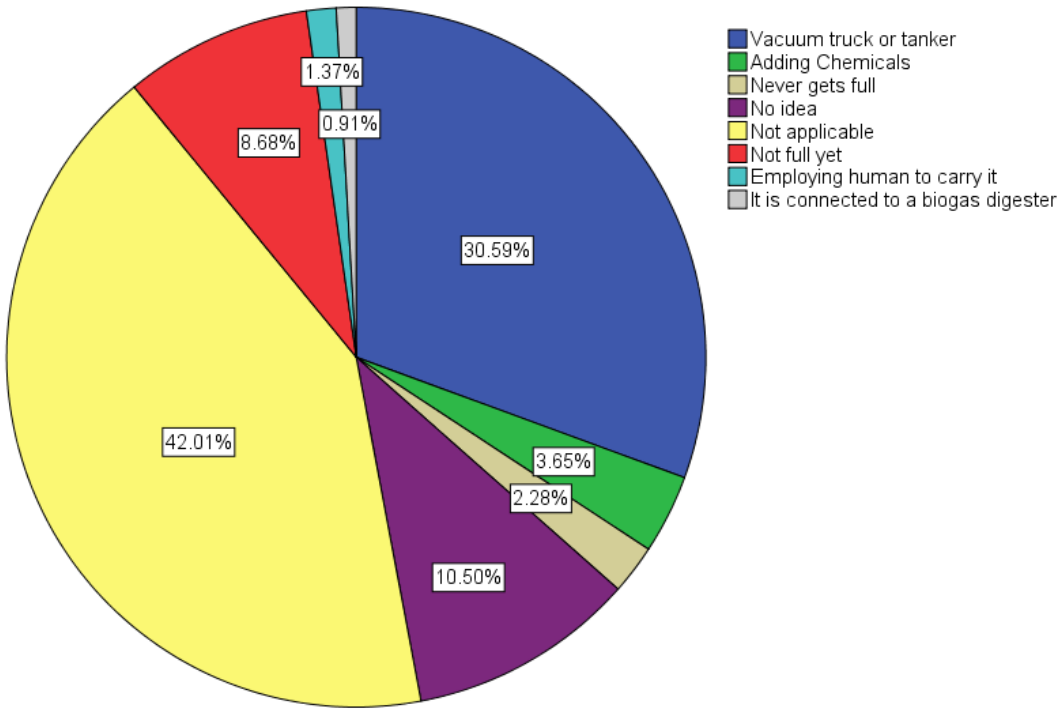

Figure 4.47: Techniques employed by residents of Elmina for emptying toilets when it was full

In instances where there was absence of household toilet facility, residents sought after alternative options for easing themselves in response to nature's call. The greater percentage (33.8 %) resorted to the use of public toilets which were sparsely distributed in the Elmina town. Those who patronise the public toilet facilities paid varying amounts per each visit. Some of the public toilets charged Twenty Ghana pesewas (GH₵ 0.20 equivalent to 4 euro-cent) per a visit, while others charged Thirty Ghana pesewas (GH₵ 0.30 equivalent to 6 euro-cent) per a visit. The most expensive public toilets in Elmina charged Fifty Ghana pesewas (GH₵ 0.50 equivalent to 10 euro-cent) per a visit. Some of the residents practised all forms of open defecation. For example, about 2.7 % practised open defecation in a nearby bushy area while 3.7 % and 1.8 % defecated in the open either by a nearby lagoon used for commercial salt production or at the seashore, respectively. Some of the residents also used private toilet facilities in the homes of other neighbours (0.9 %) as well as private toilet facilities operated by private vendors (1.4 %). About 0.5 % of the residents who do not have toilet facility resorted to defecating in a polythene bag and adding to the household solid waste or defecating in a polythene bag and flying the faeces to any unknown destination (flying toilet) (Figure 4.48). Averagely, the residents of Elmina attend nature's call at least twice daily; one in the morning and one in the evening. In some extreme cases, some residents attend nature's call three or four times in a day.

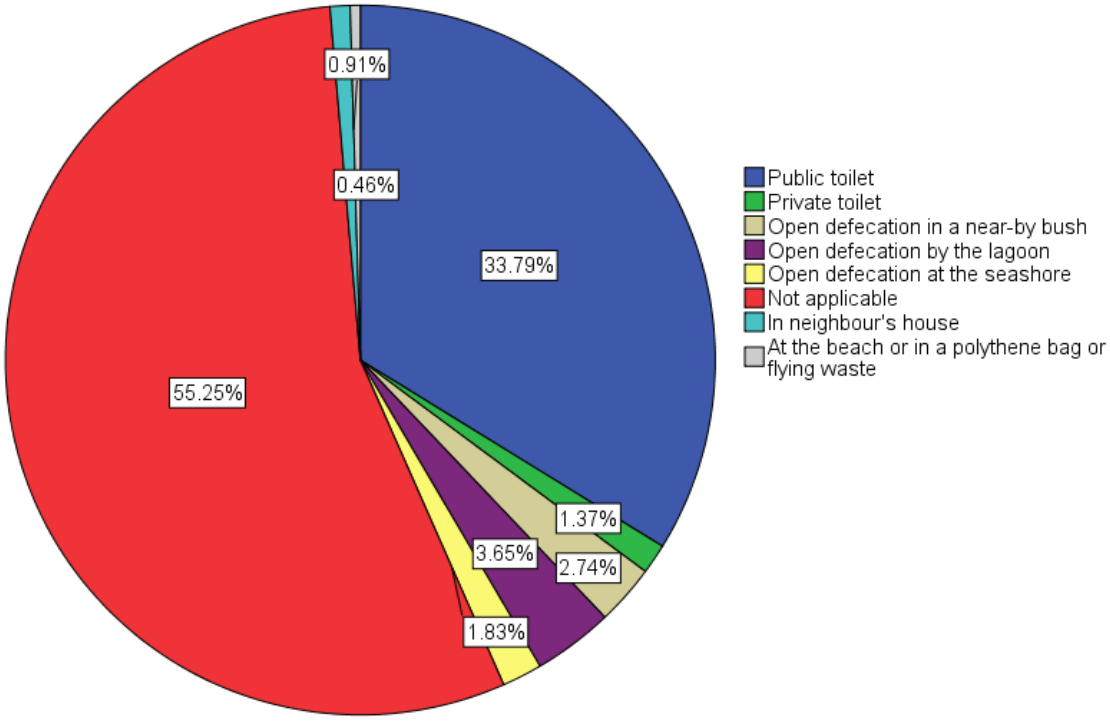

Figure 4.48: Ways by which residents who do not have household toilet facilities attend nature's call in Elmina

4.15.3 Sources of energy for cooking and fertiliser for urban agriculture in Elmina

The residents of Elmina use varying sources of energy for cooking such as the use of liquefied petroleum gas (LPG), firewood, charcoal and electrical energy. The most used source of energy for cooking in Elmina was charcoal (40.6 %), followed by LPG (29.7 %). About 18.7 % of the residents combined the use of charcoal and LPG for cooking; where charcoal was used for cooking food substances like beans and soup which is energy intensive, whereas LPG was used for warming or heating food before eating. About 4.6 % used electrical energy for cooking while 3.2 % combined both charcoal and firewood for cooking. About 2.7 % used only firewood for cooking while the least source of energy was recorded for other sources of energy (0.5 %) like sawdust (Figure 4.49).

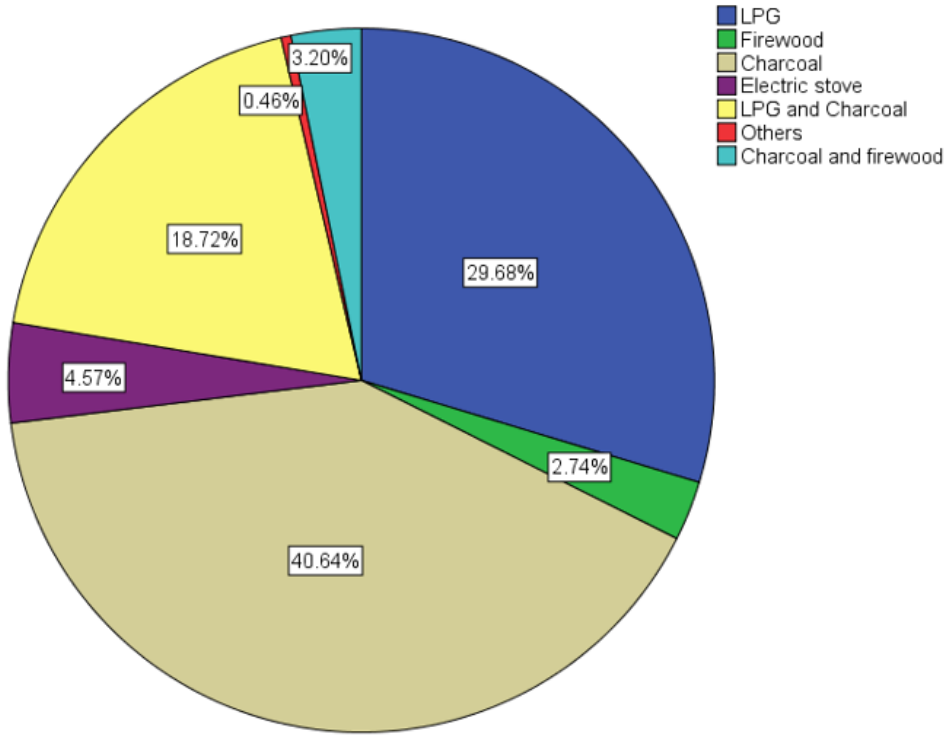

Figure 4.49: Sources of Energy for cooking in Elmina

Most of the respondents had the willingness to use more cooking gas for cooking if they had enough available cooking gas (73.1 %) compared with those who were unwilling to use gas for cooking (26.9 %) (Figure 4.50).

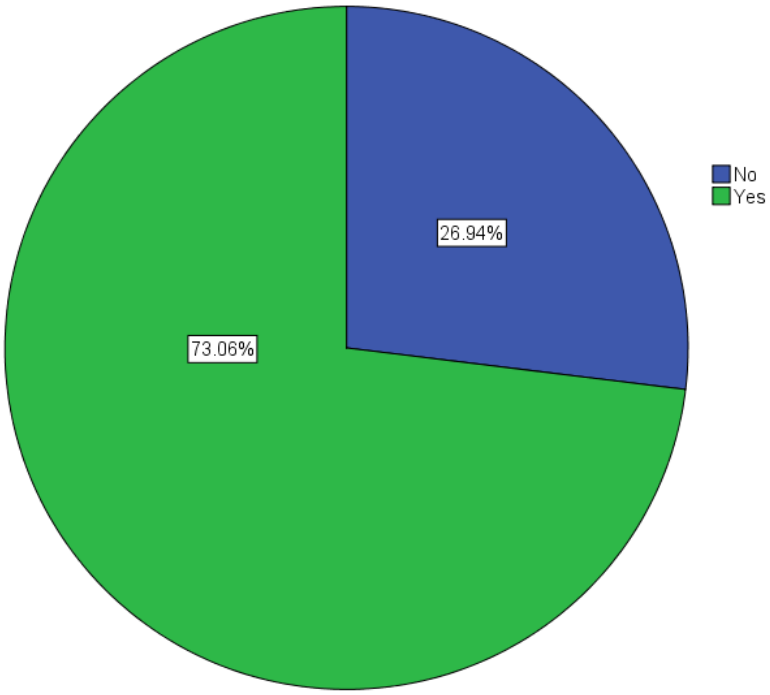

Figure 4.50: Willingness to use more of cooking gas if more is available

About 68.0 % of the respondents confirmed of having heard that human faeces can be used for producing cooking gas like biogas, while 32.0 % did not know about this technology. About 72.2 % strongly agreed to use cooking gas produced from human faeces, 5.5 % agreed, while 21.9 % strongly disagreed to use cooking gas produced from human faeces. About 0.5 % were indifferent about the usage of cooking gas produced from human faeces (Figure 4.51). Varied reasons were given for their willingness to use cooking gas from human faeces such as: there will be continuous availability of cooking gas, it will help save money and the environment, it is very similar to LPG, it will reduce the use of LPG and it will make cooking gas less expensive. Others affirmed that it is an easy source of energy which is not harmful to nature and the fact that it is the methane gas that would be used for cooking and not the human faeces convinced them to be willing to use cooking gas from human faeces. The others who were strongly unwilling to use the cooking gas from human faeces gave various reasons such as: faeces is unhygienic and thus the cooking gas produced from human faeces may also be unhygienic and unpleasant. The fear that the biogas digester may explode, human faeces smells bad and thus the cooking gas may smell bad were some of the other reasons for their rejection. Some of the respondents also said human faeces cannot be converted to cooking gas while others were just not interested in cooking with methane gas. Others preferred cooking with charcoal since it gave the food special aroma and flavour.

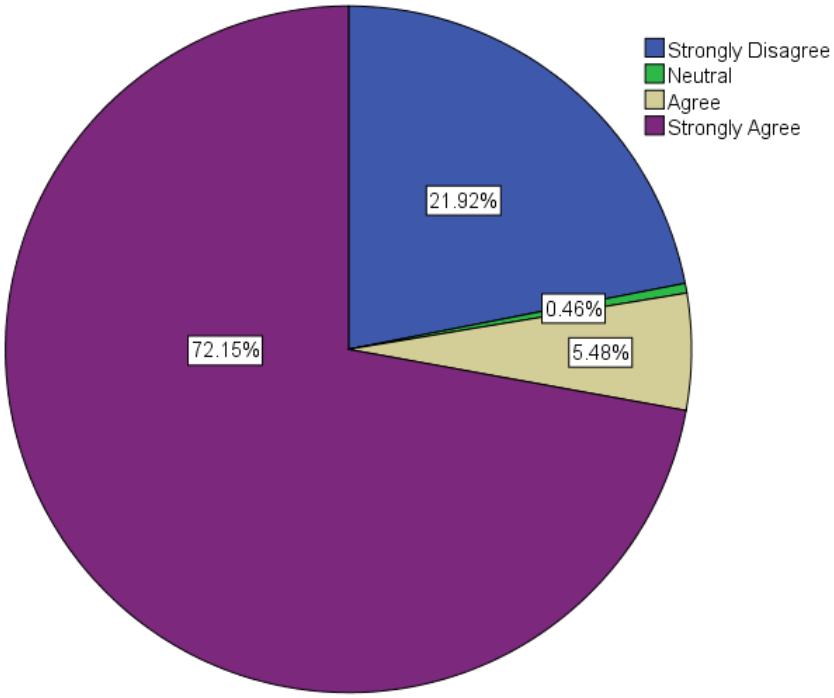

Figure 4.51: Willingness to use cooking gas produced from human faeces

Regarding the percentage of respondents that had knowledge on whether human faeces can be used to produce fertiliser, 60.3 % responded as having knowledge of that while 39.7 % responded as not having any knowledge about that. Even though 60.3 % of the respondents expressed as having knowledge on faeces to fertiliser, 54.3 % of the overall respondents could not explain how human faeces could be turned into organic fertiliser for crop production. Consequently, only 45.7 % of the respondents had some form of knowledge regarding how human faeces can be converted to organic fertiliser. For example, 11.9 % shared that the fresh faeces is ploughed directly into the soil, 7.8 % shared that the faeces is composted and used as organic fertiliser, while 2.7 % shared that the faeces is dried on the field before it is ploughed into the soil as organic fertiliser. The remaining 23.3 % could not explain how human faeces is converted to organic fertiliser even though they had stated earlier as having knowledge on faeces to fertiliser technology. About 71.9 % of the respondent did not need or use fertiliser of any kind, while 28.3 % needed and used fertiliser.

On their response on their willingness to use fertiliser produced from human faeces, 50.2 % strongly agreed to use, 11.4 % agreed, 3.2 % were neutral while 35.2 % strongly disagreed using fertiliser produced from human faeces (Figure 4.52).

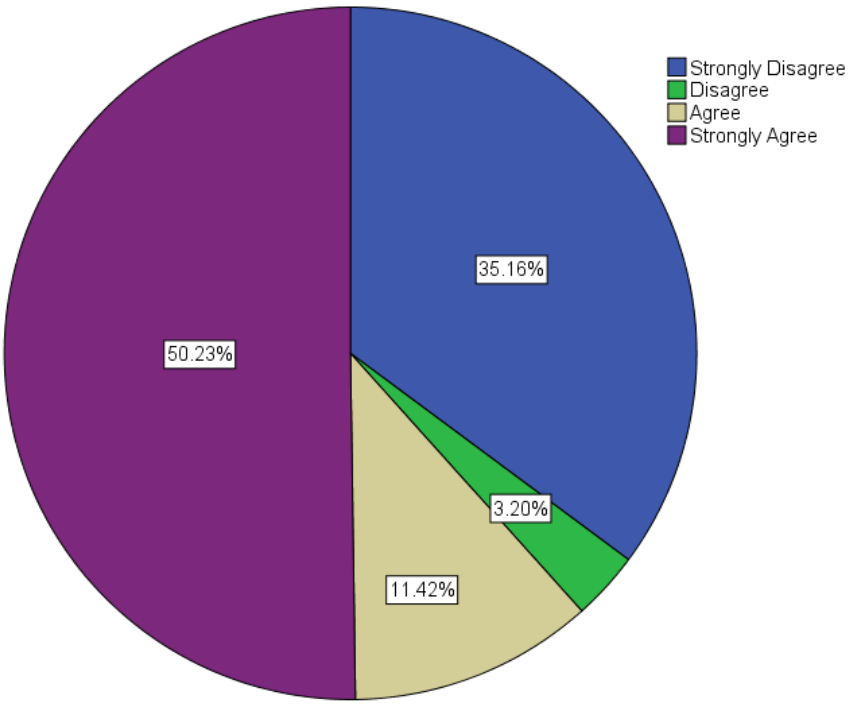

Figure 4.52: Willingness to use fertiliser produced from human faeces

With respect to the respondents' reasons for willing to use or not use human-faeces-based fertilisers, those who were willing to use gave reasons like, it will help crop growth and yield and it will help save money which would have been used for purchasing chemical fertiliser. Other reasons included: it is organic based fertiliser, it is less expensive and that the faeces which is rich in plant nutrients would decompose and become odourless. Concerning those that were against using human faeces as a fertiliser, they gave various reasons such as: faeces is unhygienic and unsightly. Others said for the fact that human faeces was used to produce the fertiliser they cannot use it.

Generally, 78.5 % of the respondents knew the negative health implications of discharging untreated faeces in the environment while 21.5 % did not have any idea. Of those that had knowledge on the health problems with discharging untreated human faeces, 58.5 % stated that it causes cholera, 5.5 % said it causes diseases in general while 4.1 % mentioned dysentery and diarrhoea. Malaria, cancer, heart attack, typhoid fever and offensive odour were some of the other health problems respondents mentioned to be associated with discharging untreated human faeces into the environment (Figure 4.53).

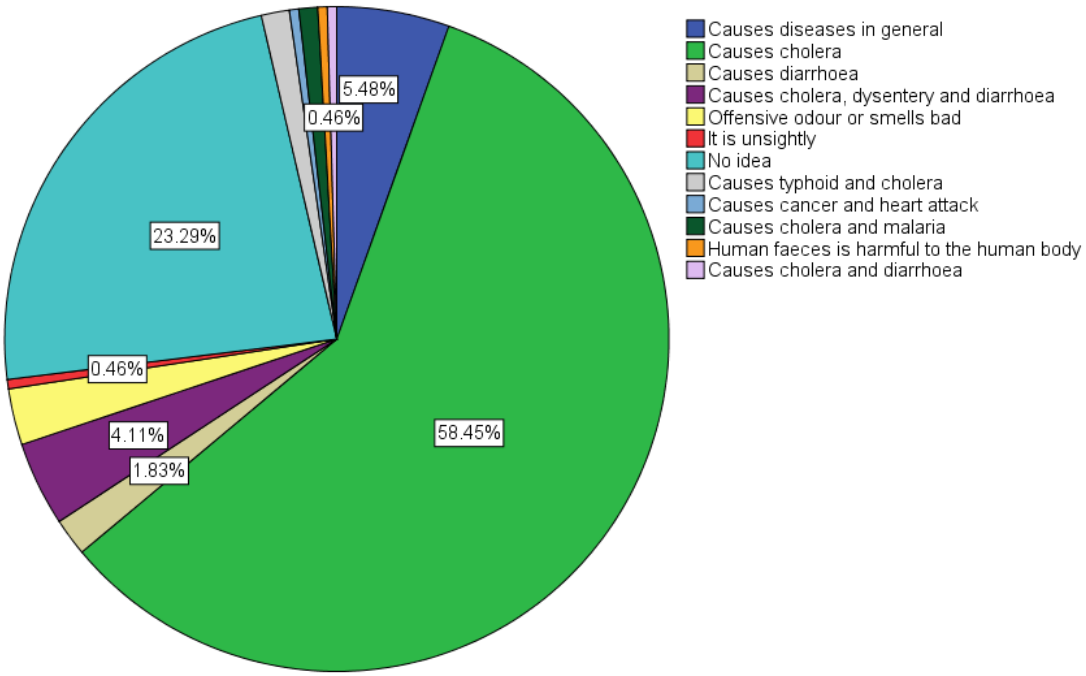

Figure 4.53: Perception on health problems associated with discharge of untreated human faeces

4.15.4 Perception on the use of single-stage SSHTABD for the treatment of black water and food waste

Co-digestion of black water with kitchen food waste to produce biogas is not very popular with most people, especially residents of Elmina. For this reason, knowledge on the perception of residents of Elmina on co-digestion of black water with kitchen food waste for biogas production was assessed. About 55.7 % of the respondents did not have any idea whatsoever about co-digestion of black water with kitchen food waste while 44.3 % had heard of the concept of co-digestion of black water with kitchen food waste (Figure 4.54).

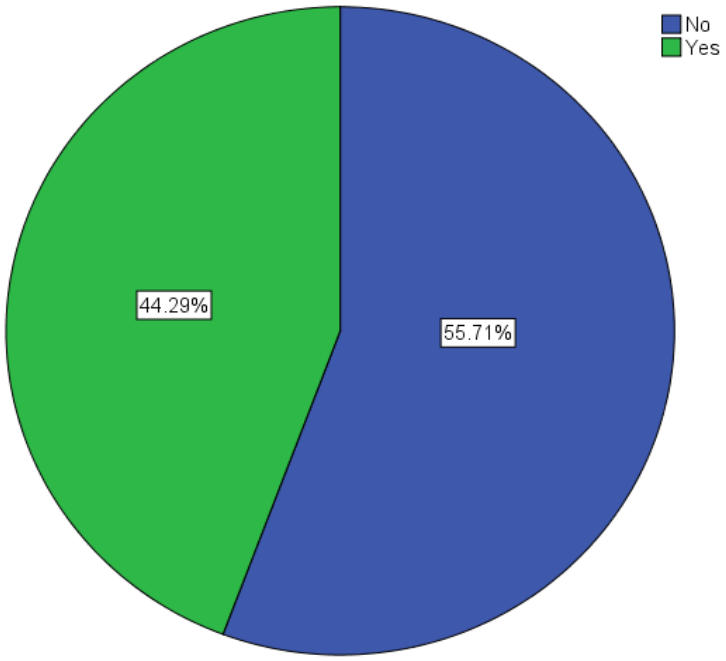

Figure 4.54: Perception on co-digestion of black water and kitchen food waste

Upon probing further for details relating to how co-digestion is done, about 90.4 % explicitly said they had no idea how co-digestion is done while 8.2 % explained it right. About 0.9 % mistook composting for co-digestion while 0.5 % explained co-digestion as addition of chemicals on wastes in a hand dug hole (Fig. 4.55).

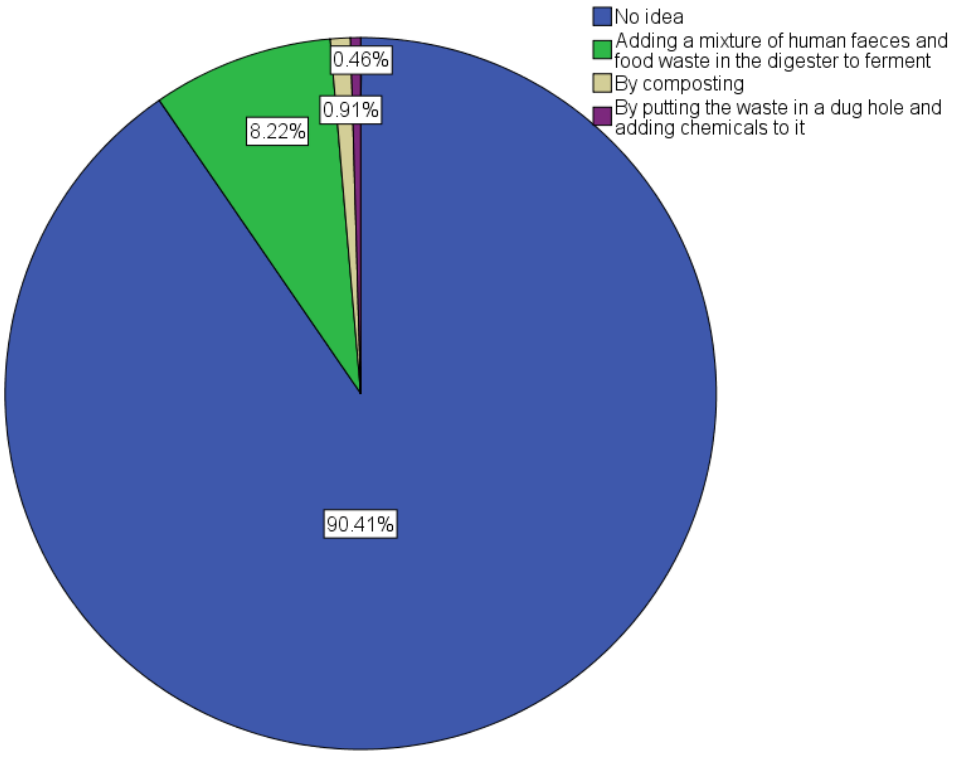

Figure 4.55: Detailed explanation on how co-digestion of black water with food waste is done

The respondents had a split view that the use of treated or digested human faeces co-digested with organic food waste for cultivation of vegetable crops was a good idea. As 47.0 % strongly agreed to the use of treated human faeces co-digested with food waste, 42.5 % strongly disagreed to this application. About 7.3 % agreed to the idea whereas 2.7 % also disagreed, with 0.5 % remaining neutral to the idea (Figure 4.56).

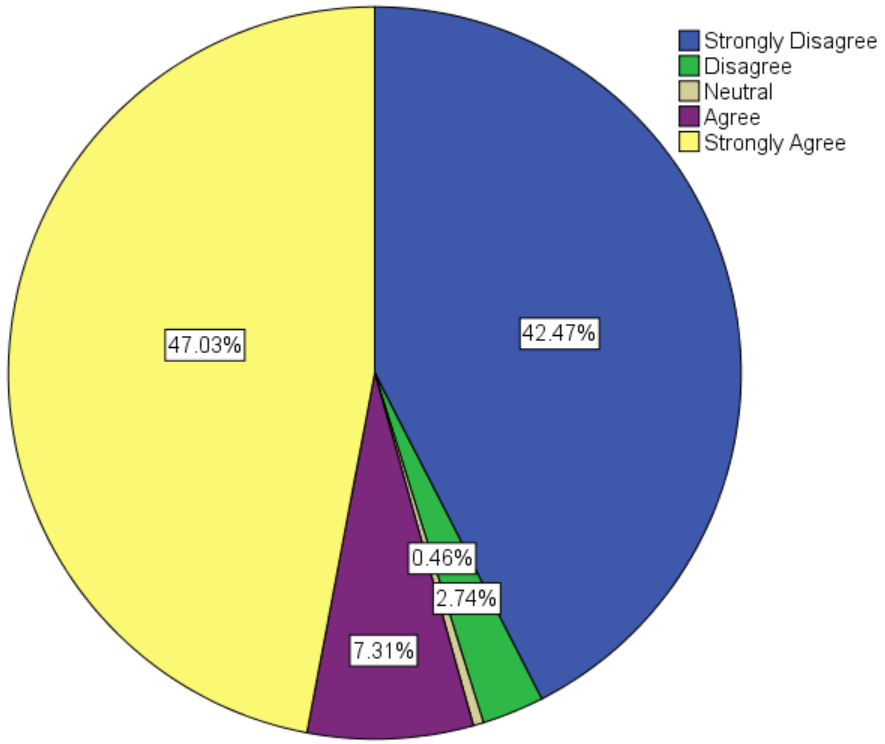

Figure 4.56: Perception on whether it is a good idea to use treated human faeces and food waste for vegetable farming

The majority of respondents (58.0 %) strongly agreed and 10.5 % agreed that once scientists have proven that vegetables cultivated with treated human faeces are safe, they would consume that vegetables. The percentage of respondents who strongly disagreed and disagreed to eating vegetables produced from treated human faeces were 28.3 % and 2.3 %, respectively. About 0.9 % of the respondents were neutral to the idea of eating vegetables produced from treated human faeces (Figure 4.57). The majority of the respondents who agreed to consuming vegetables produced from treated human faeces gave different reasons such as: because scientists have proven, because treated faeces is safe and because of dislike for chemical fertilisers. Others said they would eat the vegetables because of hunger and because the fertiliser that would be produced would be organic, the vegetables would also be organic. Those who disagreed to eat vegetables produced from treated human faeces had reasons such as: faeces is nasty, faeces contains diseases and faeces is waste and should be seen as waste always. Some also said they would not feel comfortable to eat knowing very

well that treated human faeces was used for the cultivation while others said the faeces may not be well treated.

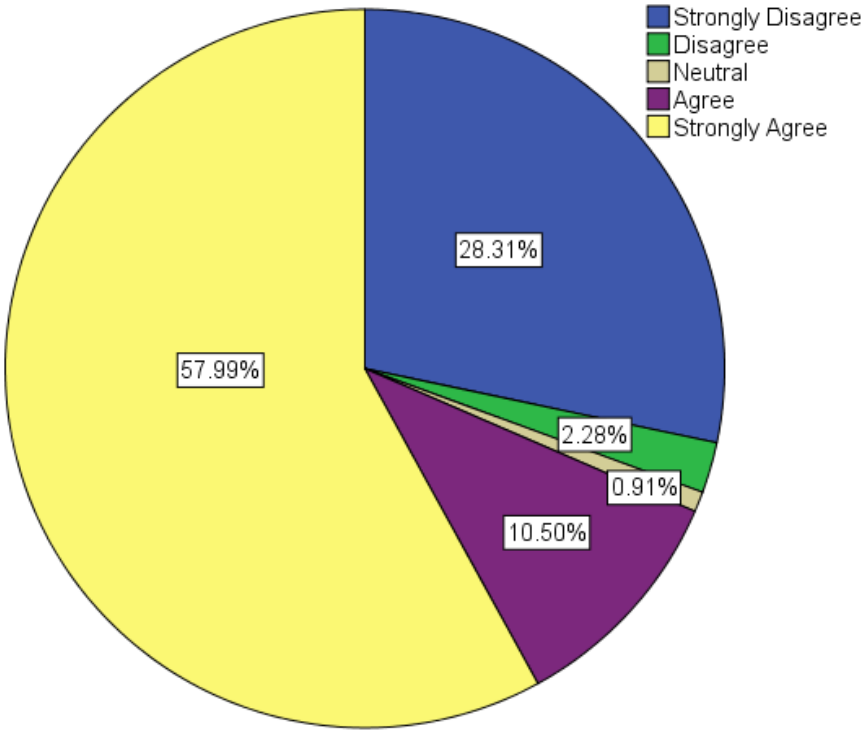

Figure 4.57: Willingness to consume vegetables produced with treated human faeces applied as fertiliser if proven safe by scientists

A greater portion of the respondents in Elmina (86.3 %) expressed their willingness to adopt the single-stage SSHTABD for simultaneous biogas production and disinfection of the digestate for urban agriculture as opposed to those (13.7 %) who were unwilling to adopt the technology in their homes. The respondents that were willing to adopt the single-stage SSHTABD technology in their homes gave varying reasons why they would adopt such a technology. For example, about 39.3 % based their reasons on economic reasons as they would save the money that hitherto would have been used in buying either LPG, charcoal or even firewood. About 25.1 % were willing to adopt the single-stage SSHTABD technology because cooking gas and alternative source of energy would be available always in their homes whereas 7.3 % based their reasons on the availability of a toilet facility in their homes. About 11.4 % had heard of the technology to be safe and proven one, consequently, their willingness to use it while 4.1 % related it to the environment which would be made clean and safe. About 0.5 % were of the view that adopting the technology would reduce the rampant outbreak of faecal-oral diseases such as cholera while another 0.5 % were hopeful of the employment opportunities this technology would create if residents of Elmina were to adopt it (Figure 4.58).

Contrary to those who were willing to adopt the technology were those who were unwilling. Fear or doubt in the single-stage SSHTABD technology (7.8 %) and traditional beliefs about human faeces, biogas and gas for cooking (1.8 %) were some of the reasons some respondents were unwilling to adopt the technology in their homes. About 2.3 % were unwilling to adopt the technology because they were scared of possible disease and negative health impacts from the faces to humans (Figure 4.58).

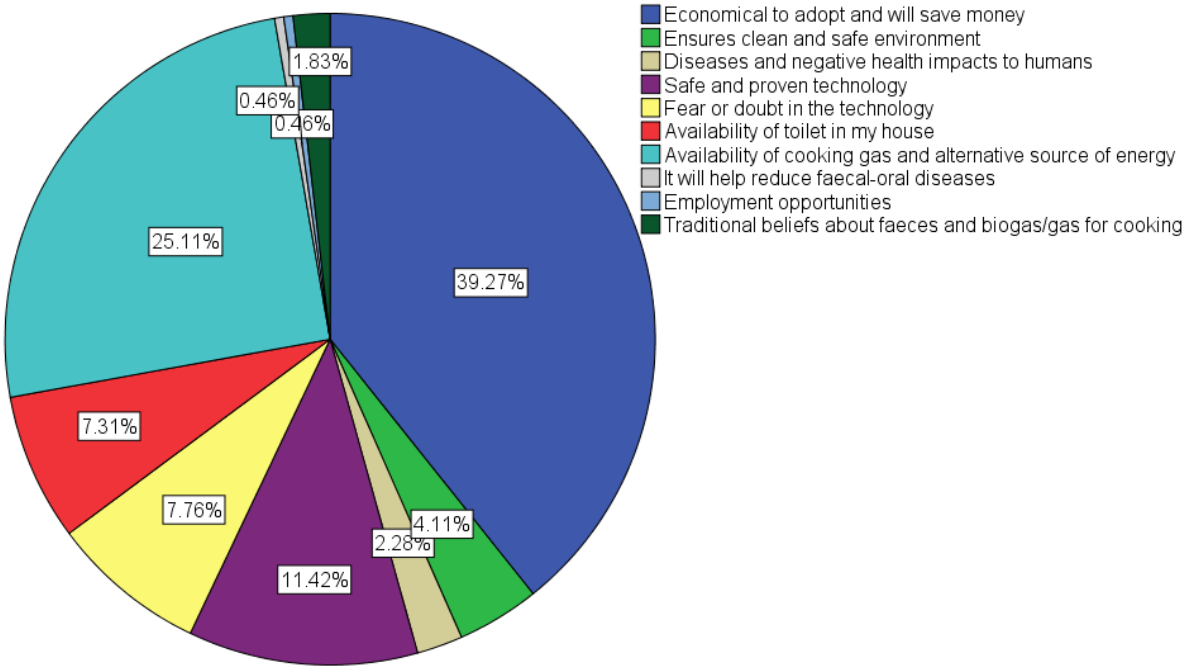

Figure 4.58: Reasons for willingness or unwillingness to adopt the SSHTABD in their homes

The willingness of the residents of Elmina to adopt the single-stage SSHTABD is associated with financial commitment. Thus, the willingness to also invest some money to pre-finance in having the single-stage SSHTABD in their households was also assessed. A greater proportion (24.2 %) were willing to pre-finance with between GH₵ 40.00 -120.00 (€ 8.00 - € 24.00), followed by 19.6 % who were unwilling to pay any money to have this technology. About 16.4 % were willing to invest between GH₵ 1000.00 – GH₵ 2500.00 (€ 200.00 - € 500.00) whereas 14.6 % were willing to pre-finance with GH₵ 130.00 - GH₵ 450.00 (€ 26.00 – € 90.00), followed by 12.8 % who were willing to pay only between GH₵ 2.00 - GH₵ 30.00 (€ 0.40 - € 6.00). In addition, 12.8 % of the respondents were willing to invest GH₵ 460.00 - GH₵ 990.00 (€ 92.00 – € 198.00) to have the technology in their homes while 1.4 % were willing to pre-finance GH₵ 2600.00 – GH₵ 5000.00 (€ 520.00 – € 1000.00) to have the technology in their homes. About 0.5 % of the respondents were willing to pre-finance with more than GH₵ 5000.00 (€ 1000.00) to have the technology in their homes (Figure 4.59).
About 49.1 % of the respondents were willing to make savings from their monthly salaries as well as their incomes from other businesses to use in pre-financing in having the single-stage

SSHTABD technology in their homes. About 16.5 % were willing to do household contributions to raise the money needed for the technology. Furthermore, 9.2 % were willing to go for a loan from the bank or any financial institution to pre-finance in order to have the technology in their homes. About 1.8 % were going to rely on the Government or Non-Governmental Organisations (NGOs) to help them pre-finance for the technology in their homes. However, about 23.4 % could not tell how they would be able to pre-finance it even though they desired to have the technology in their homes.

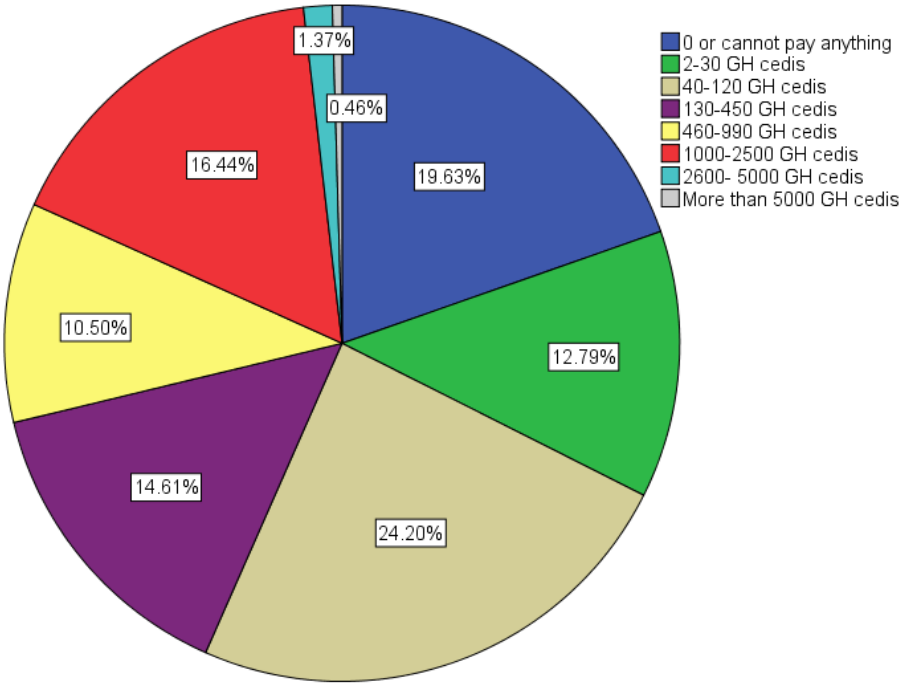

Figure 4.59: Amount of money respondents were willing to pay for SSHTABD in their homes

The income levels of the residents of Elmina has much influence on the readiness and willingness of the respondents in adopting the technology in their homes. Most of the respondents (36.5 %) earned between GH₵ 150.00 - 450.00 (€ 30.00 – € 90.00) monthly, followed by 18.3 % who earned between GH₵ 40.00 – GH₵ 100.00 (€ 8.00 - € 20.00) per month and 16.4 % who earned between GH₵ 500.00 – GH₵ 950.00 (€ 100.00 - € 190.00) monthly. About 13.2 % of the residents of Elmina earned between GH₵ 1000.00 – GH₵ 2400.00 (€ 200.00 – € 480.00) in a month whereas 1.8 % earned between GH₵ 2.00 – GH₵ 30.00 (€ 0.40 – € 6.00) in a month. In furtherance, about 1.4 % received between GH₵ 2500.00 – GH₵ 5000.00 (€ 500.00 – € 1000.00) in a month. About 0.5 % earned more than GH₵ 5000.00 in a month (more than € 1000.00), however, 11.9 % do not earn any money within the month (Figure 4.60).

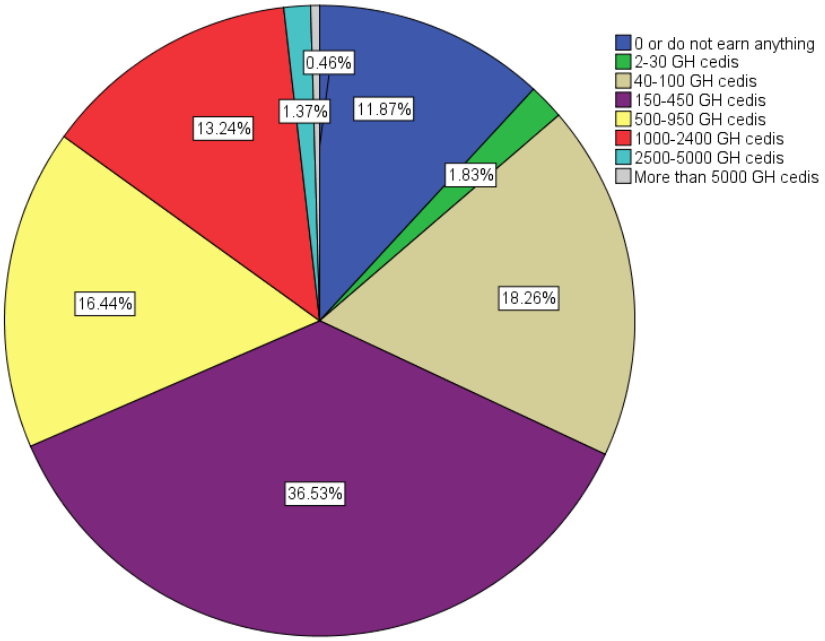

Figure 4.60: Monthly income levels of some residents of Elmina

4.16 Conclusive summary of the social survey

Almost half of the respondents (43.8 %) in Elmina town did not have toilets in their homes. Charcoal is the most used energy source (40.6 %) in Elmina for cooking followed by the use of only LPG (29.7%) and a combination of charcoal and LPG (18.7 %) for cooking. Other residents still used firewood (5.9 %) for cooking while 4.6 % used electricity for cooking even though electricity is expensive. The level of knowledge of the respondents on the concept of biogas technology was low, particularly when the concept of co-digestion was mentioned. About 90.4 % of the respondents indicated that they did not have any idea whatsoever as to how co-digestion is practised. Even though the respondents had little knowledge on biogas technology, about 86.3 % of them expressed their willingness to adopt and invest into having the single-stage SSHTABD technology in their homes. The potential to save money was the major economic reason that influenced the respondents' willingness to adopt and invest into having the technology in their homes. Other reasons such as easy access to cooking gas and availability of the gas in their homes motivated the respondents' willingness to adopt and invest into having the technology in their homes. Respondents' perspectives that the technology could create employment opportunities and could give them easy access to clean household toilet facility further influenced them to accept, invest and adopt the technology in their homes. However, 13.7 % were unwilling to have the technology in their homes for fear of explosion and deviation from their dogmatic societal beliefs such as 'the use of charcoal gives the food special flavour and taste'.

CHAPTER FIVE

Discussion

5.1 Batch tests for mesophilic temperature 37 ºC, thermophilic temperature 55 ºC and hyper-thermophilic temperatures 60 ºC, 65 ºC and 70 ºC

The influence of temperature on the anaerobic digestion process cannot be over-emphasised since it affects the percentage concentration of methane in the overall biogas produced (Arikan *et al.,* 2015). Temperature is also confirmed as the most important factor that influences biogas production and subsequently, methane production and yield (Dobre *et al.,* 2014; Kurbanova *et al.,* 2015). Even though it is reported that optimal mesophilic temperature occurs mostly between 35 ºC – 37 ºC, Bergland *et al.,* (2015) and Arikan *et al.,* (2015) reported in separate works that mesophilic temperatures within a range of about 22 ºC - 30 ºC could produce methane yield similar to that of 35 ºC – 37 ºC. However, the yields at 30 ºC and 35 ºC are higher than the yield at 25 ºC by more than 13 –17 % (Chae *et al.*, 2008). Even though slight changes in the operational mesophilic temperatures affect the methane production and yield, it does not necessarily imply that with an increase in temperature, the methane production and yield also increases, as production at 42 ºC was observed to be lower than 39 ºC (Nielsen *et al.,* 2017). This was also confirmed when Navickas *et al.,* (2013) investigated different thermophilic temperature regimes relating to their biogas yields and observed that slight increases in temperature (between 52 ºC and 57 ºC) resulted in a change in the methane yield.

Methane production is considered to be slower under mesophilic conditions than thermophilic conditions. In addition, build-up and degradation of VFAs such as acetic acids, butyric acids and propionic acids are slower under mesophilic temperatures but faster under thermophilic temperatures (Hegde and Pullammanappallil, 2007). This was confirmed by the batch fermentation tests in this study as the cumulative methane production for all the inocula at mesophilic temperatures were lower compared with some of the inocula at thermophilic temperatures (LWG 55 ºC and BTU 55 ºC) and optimal hyper-thermophilic temperature and inoculum, CM 65 ºC. Increase in temperature is known to increase the hydrolysis rate but not the methane production and yield (Bergland *et al.,* 2015). In terms of mesophilic anaerobic digestion rate, every degree Celsius decrease in temperature below the optimum range (30 ºC to 40 ºC) reduces the digestion rate by 11 % based on Arrhenius equation, thus hydrolysis of organic matter at lower temperatures is very slow (Deublein and Steinhauser, 2011; Sheth, 2009). Consequently, thermophilic condition is considered to produce better biogas yield compared with mesophilic condition (Vindis *et al.,* 2009). Contrary to what was reported by Vindis *et al.* (2009), mesophilic treatment of duckweed was found to have produced higher

biogas with higher methane yield compared with thermophilic temperature treating the same substrate (Ramaraj and Unpaprom, 2016). Thus, the type of inoculum and substrate used for the AD process influence whether the digestion temperature should be mesophilic, thermophilic or hyper-thermophilic.

The results from this research with respect to optimal hyper-thermophilic temperature confirms what was proposed by Singh (2008). In the work by Singh (2008), extensive studies on extreme environments and extremophiles were investigated and reported that the majority of thermophilic bacteria have optimal growth temperature ranging from 50 ^{o}C to 70 ^{o}C even though some thermophilic bacteria could grow slowly at a lower temperature of 40 ^{o}C. In this research, the inoculum, CM, had optimal performance with respect to net normalised cumulative volume of methane content, degree of COD degradation and methane yield at 65 ^{o}C, probably because it had methanogens that had optimum activity levels at 65 ^{o}C. Gupta *et al.,* (2016) reported in their work that cow manure has a consortium of methanogens which could thrive for biogas production at varied conditions and this could account for why CM had the best performance compared to the other seeding sludge. LWG at 60 ^{o}C, on the other hand, was the second highest in terms of net normalised cumulative volume of methane content, degree of COD degradation and methane yield because it also had a consortium of bacteria that could survive under varied conditions for varied purposes. Markiewicz *et al.,* (2014) and Fong and Tan (2000) in separate research isolated microbial consortium in activated sludge that treated sewage and confirmed that about nine different species exist in activated sludge that could degrade organics and even ionic liquids (ILs) (Markiewicz *et al.,* 2014). This could account for why LWG at 60 ^{o}C was the second preferred seeding sludge identified in this study. One other factor that helps with the performance of a seeding sludge is the VS/TS ratio. Once the content of volatile solids in a seeding sludge is above 50 % of the dry matter, bacterial species in the seeding sludge could have enough biodegradable substrates to convert to biogas and methane. The content of the sewage sludge from LWG had more than 50 % of VS/TS ratio and that could also account for its good performance. This was also confirmed in the work by Meisam and Ghanavati (2018).

None of the tested seeding sludge performed very well under a hyper-thermophilic temperature of 70 ^{o}C. BTU at temperature 70 ^{o}C was the most inhibited as it recorded 0.0 mlNCH_4-% net cumulative volume, followed by LWG at 70 ^{o}C (13.3 mlNCH_4-%) and CM at 70 ^{o}C (18.9 mlNCH_4-%). The performance was similar for net normalised cumulative methane yield for the three inocula as well as degree of COD degradation. This could be to the fact that none of the methanogens in the BTU seeding sludge could survive at hyper-thermophilic temperature of 70 ^{o}C. Most research in anaerobic digestion and biogas production have failed to focus on hyper-thermophilic temperature of 70 ^{o}C for methanisation. Forster-Carneiro *et al.,*

(2008) reported on thermophilic digestion of organic waste using activated sludge from wastewater treatment plant in a batch test at 55 ^{o}C and concluded that cumulative volume of 4045.2 mlCH_4 could be produced within 90 days with only 32.4 % of volatile solids removed and methane yield of 0.18 LCH_4/gVS. This is similar to what was reported by Lv *et al.*, (2013) who compared thermophilic and mesophilic digesters operating on dairy manure and concluded that thermophilic temperature of 50 ^{o}C had better removal of volatile solids of 31 % and methane yield of 0.22 LCH_4/gVS.

In the LWG and CM, even though some methanogens could be present and thus produced some volume of methane at that high temperature of 70 ^{o}C, there is an indication that a substantial quantity of the methanogens in the LWG and CM could not survive at that high temperature, consequently, leading to a reduced volume and content of methane. The results from the LWG and CM are in agreement to what was proposed by Singh (2008) that some methanogenic thermophiles can also grow at very high extreme temperatures of 80 ^{o}C and 110 ^{o}C, with examples being eubacteria and archaeabacteria. Consequently, some of the methanogens and the other microbes that existed in the LWG and CM seeding sludge used in this research could either be thermophiles or hyper-thermophiles and could be in the groups of eubacteria and archaeabacteria. The methanogens could not be extreme hyper-thermophiles since most extreme hyper-thermophiles are micro-organisms that cannot grow below 90 ^{o}C, with an example being *Pyrolobus fumarii* (Singh, 2008).

The findings in this research also confirmed what was reviewed by van Lier (2008). In a review by van Lier (2008), it is reported that raising the temperatures to extreme values may disturb the performance of the sludge bed systems and thus affecting the stability of methanogenic granular sludge. This was also confirmed by Ozgun *et al.* (2013), that sludge biomass of anaerobic digestion is greatly affected by thermophilic temperatures. Thus, at hyper-thermophilic temperature 70 ^{o}C, the methanogens in the three seeding sludge were inhibited probably because they were disturbed and thus resulting in no methane production as well as no or low degree of COD degradation as was seen in the seeding sludge, BTU. Dereli *et al.* (2012), further confirmed that thermophilic temperatures even though produce similar amounts of methane as mesophilic temperatures at double organic loading rates, they produce mobile anaerobic biomass since the sludge is dispersed and has poor settling characteristics. Gao *et al.,* (2012) also affirmed that extracellular polymeric substances (EPS) in the bulk sludge, soluble microbial products (SMP) and content of colloidal particles in the seeding sludge and the substrate further increase with increase in temperature of the reactor from mesophilic to thermophilic conditions, thus under hyper-thermophilic conditions more mobile anaerobic biomass is expected with reduced methane volume and content.

This may affect the overall conversion of organics to methane even though the digestate will be safe for application on agricultural lands. Contrary to what El-mashad (2003) reported in his work that increase in temperature from 40 ^{o}C to 60 ^{o}C corresponds with decrease in methane content in biogas, in this research, the content of methane did not depend on the increase in temperature, rather, on the type of seeding sludge. This is because inoculum called CM at a hyper-thermophilic temperature of 65 ^{o}C recorded the highest methane volume of 387.2 mlNCH_4-%, higher than the LWG and BTU at both thermophilic temperature of 55 ^{o}C and hyper-thermophilic temperature of 60 ^{o}C.

The use of thermophilic and hyper-thermophilic temperatures has a positive influence on the quality of sludge that is produced after anaerobic digestion, in terms of pathogen content. According to Bartkowska (2015), the least expensive method of sludge handling after treatment of wastewater is to return it to the environment as soil conditioner. However, the presence of pathogens like *Salmonella spp* and eggs of intestinal parasites like *Ascaris, Trichuris and Toxocara* raises safety concerns making it mandatory to hygienise the digestate before it can be reused on agricultural lands. One system that had been identified and applied in Olecko, Poland is the Autothermal Thermophilic Aerobic Digestion (ATAD) process which operated on a temperature of 59 ^{o}C to 65 ^{o}C. This was used after a pre-treatment of wastewater in a sequential biologic reactor (SBR) operating on a temperature of 40 ^{o}C to 55 ^{o}C for stabilisation of sludge from a wastewater treatment plant (Bartkowska 2015). The ATAD had a COD removal of 52.4%, however, no information was provided concerning the bacterial growth and survival vis-à-vis their methanogenic activities (Bartkowska, 2015).

Deublein and Steinhauser (2011) and Sheth (2009) reported that pathogenic microbes are totally destroyed at thermophilic temperature greater than 55 °C with a hygienisation retention time of 24 hours. This is also confirmed in this study since pathogenic microbes could not survive under any of the hyper-thermophilic temperatures used in this study after 24 hours. It is very important to consider at which optimal temperature both higher methane and pathogen-free digestate can be produced. Failure to identify this optimal temperature implies trading-off either large volume of methane for pathogen-free digestate or vice versa. Apart from ensuring that the sludge is hygienised in terms of pathogens, Tervahauta *et al.,* (2014) proposed that heavy metals in the sludge should also be assessed before its application on agricultural land. Consequently, the use of seeding sludge with less contribution of heavy metals to the effluent and sludge to be applied on agricultural land is worth considering.

The C/N ratio of the substrate also influences methane production. For a single-stage systems of the anaerobic digestion process, a C/N ratio of between 15-25 is required while double stage systems require a C/N ratio of between 10-45 for the first stage and 20-30 for the second

stage (Dobre *et al.,* 2014). Mamimin *et al*., (2015) reported that double-stage processes employed in the treatment of palm oil effluent produce about 34 % better yield compared to single-stage anaerobic digestion processes as a result of the C/N ratio. The BW used as a substrate in this study had an organic C/N ratio of 7.3:1 but the highest methane content in all the inoculum were 43.4 % for BTU, 47.8 % for LWG and 63.5 % for CM. Even though the substrate had organic C/N ratio not within the optimal range proposed by Bagge (2009), Vögeli *et al.,* (2014) and Dobre *et al.,* (2014), the percentage methane content in LWG and CM could be burned since it was more than 45 % (Vögeli *et al.,* 2014). These were possible probably because the inoculum had other organic C/N ratio that could influence the overall organic C/N ratio in the batch systems and consequently, influencing the overall methane percentage content. It is reported that when the C/N ratio (organic carbon:organic nitrogen) decreases, it implies more of the carbon is being degraded unlike the nitrogen. This implies a sudden build-up of ammonia in the reactor and consequently leading to an increase in the pH level to a basic medium, inhibiting methanogenic bacteria (Vögeli *et al.,* 2014). This was most probable in the batch fermentation tests at hyper-thermophilic temperature of 70 ^{o}C, since the measured pH values after the tests period ranged between 8.14 – 8.38 for all the inocula. Ammonia has been reported to inhibit methanogenic bacteria and decrease methane yield by 66 % (Ho and Ho, 2012; Sasaki *et al.,* 2011; Sung and Liu, 2003) while methane production is decreased up to 80 %, especially when co-digestion is practised in a thermophilic anaerobic process (Yenigün and Demirel, 2013). Other factors such as moisture content in the methanogenic vessel and pH have also been identified to influence methane production in a batch test and anaerobic digestion in general. Any pH range below 6.1 or above 8.3 have been considered not to be favourable for methanisation (Lay *et al.,* 1997).

5.2 Co-digestion of laboratory-scale HT-CSTR and the pilot-scale SSHTABD

In any continuous single-stage anaerobic digestion, the influence of the co-digestion substrate, digestion temperature, HRT, pH and VFA affect the overall performance such as the COD removal efficiency, methane content in biogas and methane yield of the reactor. The kind of substrate used for co-digestion influences the methane content and yield. Zamanzadeh *et al.,* (2017) reported that co-digestion of manure with food waste at mesophilic condition could result in 26 % increase in methane content compared with using only food waste or only manure as separate substrates and later summing their percentage methane contents. A study carried out by Dobre *et al.,* (2014); Kurbanova *et al.,* (2015) and Panpong *et al.,* (2017) on different substrates used for co-digestion showed that different co-digestion substrates

have different methane potentials depending on their moisture content, dry matter and volatile solids contents. Moreover, a substrate that is completely degradable anaerobically is preferred for biogas production (Verein Deutscher Ingenieure (VDI 4630), 2006). Various substrates such as residue from fish farming and glycerol (Kuusik *et al.,* 2013), buffalo dung, wastes from banana plant, cotton stalks, canola straw, wheat straw, rice straw and wastes from sugarcane could be used as substrates for co-digestion (Sahito *et al.,* 2013). Other substrates that could be used for co-digestion include organic food waste such as vegetable peelings, leftover rice, bread, meat and pasta (from restaurants), pastry wastes, sweetened creams and bread (from bakery) and fruits and vegetables (from supermarkets) could be co-digested with black water to enhance methane productivity and yield in the biogas during anaerobic digestion (Cabbai *et al.,* 2013). The food waste used for the co-digestion of the blackwater in this study for the laboratory-scale single-stage HT-CSTR was similar to what have been mentioned above as it was a blended product of banana peels, leafy vegetable wastes, pastries like meat pie and expired bread. Other vegetable wastes like carrots (Bunzel *et al.,* 2005; Schäfer *et al.,* 2018), kiwi, radish and asparagus (Bunzel *et al.,* 2005) were excluded as they contain high cellulose and lignin which are not very good for the effective performance of the system. Wood and its products, as well as bark of a tree are other examples of those substrates that contain lignin. Lignin disturbs the AD process by the foam it forms since it is not broken down during the anaerobic digestion process (Bagge, 2009). A little different from what was used for co-digestion in the laboratory-scale single-stage HT-CSTR, the substrates used for the pilot-scale single-stage SSHTABD in Terterkessim slum in Elmina consisted mostly of household food waste and leftovers like cooked rice, cooked corn and cassava dough (*"banku"*), cooked cassava and *"fufu"* (pounded cooked cassava). Unlike the leafy vegetables used as part of the substrates for the laboratory-scale single-stage HT-CSTR, the pilot-scale single-stage SSHTABD received more of carbohydrate-based substrates for co-digestion. As a result, the methanogens in the pilot-scale single-stage SSHTABD may be deprived of other important trace elements that may be present in leafy vegetables to enhance methanisation in the digester. Micro-nutrients such as Nickel (Ni) are needed by methanogens for the production of co-enzyme F_{430} for methanisation while Tungsten (W) is needed by formate dehydrogenases and hyper-thermophiles (Lu, 2017). Consequently, one should carefully consider the kind of substrate being used for a single substrate anaerobic digestion or when co-digestion is being practised in a single-stage system so as not to inhibit the AD process.

Koch *et al.,* (2015) and Nartker *et al.,* (2014) studied the influence of various ratios of inoculum to co-digestion substrate and concluded that the higher the component of food waste or glycerol in the mixture of co-digestion substrate, the higher the methane yield. In this study, however, the ratio of blackwater to kitchen food waste used as a substrate was 1:1, contrary

to what had been proposed by Koch *et al.,* (2015) and Nartker *et al.,* (2014). This negatively affected the methane productivity and yield for both the laboratory-scale single-stage HT-CSTR and the pilot-scale single-stage SSHTABD when only BW was used as the substrate before co-digestion was practised. The BW used as a substrate for the laboratory-scale single-stage HT-CSTR and the pilot-scale single-stage SSHTABD in this study had an average organic C/N ratio of 7.3:1 and 11.1:1, respectively. These recorded the highest percentage methane content in the biogas as 38.1 % for the laboratory-scale single-stage HT-CSTR. The pilot-scale single-stage SSHTABD could not produce any measurable methane when only the BW was used as the substrate. This implies, at hyper-thermophilic treatment of only BW in a single-stage digester, the percentage methane content in the biogas would not be high enough to be burned or methane may not be produced at all (Vögeli *et al.,* 2014) as its C/N ratio falls outside the optimal range of C/N ratio for a single-stage AD process.

The introduction of FW (1:1, v/v) into the laboratory-scale single-stage HT-CSTR and the pilot-scale single-stage SSHTABD resulted in an average C/N ratio of 23:1 and 20.2:1, respectively, available for digestion and was within the proposed optimal range for C/N ratio required for good percentage methane content (Dobre *et al.,* 2014; Kurbanova *et al.,* 2015), resulting in percentage methane content of 61.8 % in the laboratory-scale single-stage HT-CSTR at 65 ^{o}C. This resulted in a percentage increase of methane content by 62.2 % from when only BW was used for the laboratory-scale single-stage HT-CSTR. This was achieved because the mixture in the co-digestion substrate used in this study for the laboratory-scale single-stage HT-CSTR had the right C/N ratio and possibly had other micronutrients necessary for good methane production and yield. Even though the C/N ratio for the pilot-scale single-stage SSHTABD was within the optimal C/N ratio required for good methane production, the reactor could not produce large quantities of biogas with high methane content. This is probably because the co-digestion substrates had more of carbon-based materials with less trace metals and thus the reactor was deprived of important micro-nutrients that may have been needed by the methanogens for methanisation.

Micro-nutrients and trace metals are mostly found in cereals like maize, wheat, oat, millet and rice; vegetables like tomatoes, green pepper, bitter gourd; green leafy vegetables such as spinach, cabbage, bitter leaf; legumes and nuts such as lentils, nuts, red kidney beans, soy beans, peanuts, peas; and fruits such as mango, banana and banana peels, water melon, pawpaw, pineapple, orange and tangerine (Basha *et al.,* 2014; Elbagermi *et al.,* 2012; Sobukola *et al.,* 2010). A study carried out by Wang *et al.,* (2014) on co-digestion of dairy manure, chicken manure and rice straw showed that increase in the C/N ratio from 15:1 to 25:1 at mesophilic temperature regime or 20:1 to 30:1 at thermophilic temperature condition resulted in higher methane content. This was also confirmed by Panpong *et al.,* (2017) when

canned sardine wastewater (CSW) and glycerol water (GW) were co-digested at C/N ratio of 43:1 at thermophilic condition resulted in methane content of 68.5 % in the biogas. With higher C/N ratio ammonium-nitrogen and free ammonia become reduced consequently, minimizing ammonia inhibition in the reactor and enhancing methane content. For single-stage systems of the AD process, a C/N ratio of between 15:1 to 25:1 is required while double stage systems require a C/N ratio of between 10:1 to 45:1 for the first stage and 20:1 to 30:1 for the second stage (Dobre *et al.,* 2014).

The type of digestion, whether dry or wet, also influences the methane productivity and yield (Dobre *et al.,* 2014; Kurbanova *et al.,* 2015; Lay *et al.,* 1997). Wet digestion usually has dry matter (DM) content to be 2-15 % while dry digestion has a DM content to be greater than 20 % but usually between 22 – 45 % (Chiumenti *et al.,* 2018; Kothari *et al.,* 2014; Seadi *et al.,* 2008). The volume and percentage of methane content decreases when the total solids or DM content in the substrate is greater than 7.5 %. Total solids content of 7.5 % are considered to be optimal and ensures higher COD removal compared with substrates with total solids greater than 7.5 % (Deepanraj *et al.,* 2014). Sadaka and Engler (2003) evaluated the production of biogas from pig, poultry and cattle manure while Liotta *et al.,* (2014) assessed the role of moisture content on anaerobic digestion of food waste and both concluded that increase in the TS of the substrate above 12 % corresponded with decrease in the quantity of the biogas produced. The TS content for only the BW (first phase) and the MIX (second phase) used as substrates for the laboratory-scale single-stage HT-CSTR were 1.6 ± 0.5 % and 4.6 ± 2.0 %, respectively while that of BW and MIX for the pilot-scale single-stage SSHTABD were 4.6 ± 1.4 % and 6.0 ± 1.0 %, respectively. This implies less revolutions for mixing could be applied since higher TS implies more mixing and subsequently more energy (Liotta *et al.,* 2014). The TS in the substrates in both instances were below the TS of a large-scale reactor that treated food waste and operated in the wet digestion mode with TS ranging from 17 % to 20 % (Angelonidi and Smith, 2015). The water content for the MIX substrate used in this study was 50 % (1:1, w/v) compared with 15 - 40 % of the water content used for the wet digestion in the study by Angelonidi and Smith (2015). The energy demand for heating biogas digesters operating using wet digestion process is smaller compared with digesters using dry digestion processes. This is because in the dry digestion mode, some of the energy is used for both pre and post-heating (Angelonidi and Smith, 2015). Subsequently, operating a hyper-thermophilic anaerobic biogas digester on wet digestion mode may not require much higher energy compared with mesophilic and thermophilic biogas digesters operating on dry digestion mode.

Hydraulic Retention Time (HRT) of a reactor has a direct influence on the COD removal efficiency and consequently, methanisation of the reactor. Work done by Chelliapan *et al.,* (2011) on the effect of Upflow Anaerobic Stage Reactor (UASR) treating brewery wastewater

spiked with recalcitrant Tylosin antibiotic confirmed decrease of HRT from 4 days to 1 day resulted in decrease of COD removal efficiency from 92 % to 77 % but this did not influence the overall methane yield. This was confirmed by Dareioti and Kornaros (2014) when effect of HRT was investigated on anaerobic co-digestion of agro-industrial wastes of a double-stage CSTR that treated olive wastewater, cheese whey and liquid cow manure. They observed that HRT at 25 days had higher methane production compared with HRT of 20 days which resulted in VFA accumulation in the reactor, however, Scoma *et al.,* (2013); Dareioti and Kornaros (2014) concluded that productivity of hydrogen decreased with increase in the HRT. This is contrary to what was observed in this study at hyper-thermophilic temperature of 65 ^{o}C for the laboratory-scale single-stage HT-CSTR where the mean HRT was 23.3 days with mean VFA and NH_4-N concentrations of 2.14 g/L and 344.7 mg/L, respectively. Gaby *et al.,* (2017) investigated COD removal and methane production for thermophilic and hyper-thermophilic temperatures of 55 ^{o}C and 65 ^{o}C at different HRTs and concluded that methane production between the two temperatures were almost the same. They further concluded that methane production increased with decrease in HRT from 17 days to 10 days while ammonium concentration decreased non-linearly with decrease in the HRT from 950 mg/L NH_4^+ to 550 mg/L NH_4^+ for HRTs of 17 days and 10 days, respectively. Due to protein hydrolysis, ammonium in the effluent could increase by 10 % when organic nitrogen is converted into ammonium (Wendland, 2008). This could also account for why methane production in the pilot-scale single-stage SSHTABD had little methane production since it had a mean HRT of 51.3 days and higher ammonium concentration of 1583.9 mg/L NH_4-N, indicating ammonium inhibition. This concentration of NH_4-N in the SSHTABD was higher than 1, 111 mg/L NH_4-N reported by Wendland (2008) when BW was treated in a laboratory-scale CSTR at mesophilic temperature of 37 ^{o}C. In addition, ammonium concentration of 344.7 mg/L NH_4-N was observed when BW was treated at optimal hyper-thermophilic temperature of 65 ^{o}C in the laboratory-scale single-stage HT-CSTR reported in this study.

Concentration of NH_4-N is considered to be beneficial for methanogenesis when it is in the range of 50 mg/L – 200 mg/L. Concentrations of NH_4-N ranging between 200 mg/L – 1500 mg/L are considered not to have any adverse effect on the methanogens. However, when the concentration exceeds beyond 1500 mg/L – 3000 mg/L, it is inhibitory to the methanogens especially, when the pH is higher than 7.4. Concentrations of NH_4-N higher than 3000 mg/L are considered to be toxic to the methanogenic process during AD (Lu, 2017). Unlike ammonium which can be tolerated at a concentration of 1500 mg/L NH_4-N in the AD process, free ammonia at a concentration of 80 mg/L (Lu, 2017) or 300 mg/L (Wendland, 2008) could start inhibiting the AD process and consequently, methanisation.

Organic loading rate (OLR) of between 2.0 and 4.0 kgVS/(m^3.d) is recommended for all wet fermentation processes (Weiland, 2010). A two-stage mesophilic/mesophilic digesters that treated only potato peels had optimal OLR of 0.24 kgVS/(m^3.d) while in the same research a two-stage mesophilic/thermophilic reactor that treated hydrolysate from potato peels had varying organic loading rate with the maximum OLR being 2.4 kgVS/(m^3.d) but had lower reactor pH of 4.2 (Muhondwa, 2016). In this study, increase in volumetric loading rate resulted in lower COD removal within the week of loading as the COD needed some time to be hydrolysed and acidified before eventually the methanogens could convert it to methane. Both the laboratory-scale single-stage HT-CSTR and the pilot-scale single-stage SSHTABD operated on low OLRs of 0.3 kgVS/m^3.d and 0.1 kgVS/m^3.d, respectively. Consequently, both realised little accumulation of VFAs in the reactors. Little accumulation of VFAs in the reactor is associated with less lowering of the pH (Dareioti and Kornaros, 2014). The resultant average pH values in the effluent for the laboratory-scale single-stage HT-CSTR and the pilot-scale single-stage SSHTABD were 6.9 and 6.6, respectively. In addition, because cow manure was used as the inoculum, it could release alkalinity. This could serve as a buffer for the reactor and help regulate the pH and subsequently, methanisation (Weiland, 2010). The OLR recorded in this study was much lower than the 2.0 and 4.0 kgVS/(m^3.d) recommended by Weiland (2010). Cahyari *et al.* (2016) treated water melon wastes in a CSTR using dark fermentation but confirmed optimum OLR to be 1.8 kgVS/(m^3.d) instead of the 2.0 to 4.0 kgVS/(m^3.d) proposed by Weiland (2010). They concluded that increasing the OLR to 2.4 kgVS/(m^3.d) resulted in lowering the pH in the reactor to 4.0 and reduced the volume of gas produced compared with maintaining the OLR at 1.8 kgVS/(m^3.d) which resulted in effluent pH in the range of 5 – 7 and gas volume 20 ml STP higher. Increasing the OLR has nexus with lower VS removal, higher accumulation of VFAs (lower pH value e.g. pH of 4) and lower biogas and methane content (Cahyari *et al.,* 2016). This could account for why in this study higher removals were achieved for both COD (86.3 %) and the VS (83.2 %), respectively.

The values obtained for the removal of the VS were higher than 73.8 % reported by Du *et al.* (2018) and 62 % obtained by Wendland (2008) when BW was treated at mesophilic conditions. The highest normalised dry methane production was 55.4 L_NCH_4/$kgCOD_{removed}$ (62 LCH_4/$kgCOD_{removed}$) smaller than the average theoretical value of 350 LCH_4/kgCOD and 342 LCH4/$kgCOD_{removed}$ reported by Wendland (2008) when concentrated BW was treated at mesophilic condition at HRT of 20 days. The difference in value could be as result of the hyper-thermophilic operational temperature which is known to reduce both the methane volume and content during the AD process. Every 1 ^oC increase in temperature in a thermophilic condition results in 6 % decrease in biogas yield and 2-3 % decrease in methane content in the biogas (Navickas *et al.,* 2013). The operational temperature for the digestion process in a CSTR

influences the methane production and yield. Ho *et al.* (2014), investigated the effect of temperature on acetate production and concluded that increase in temperature from mesophilic to thermophilic conditions increases hydrolysis rate, however, the acetate consumption rate decreased with increase in the digestion temperature.

The pilot-scale single-stage SSHTABD was designed to operate on a similar optimal hyper-thermophilic temperature as the laboratory-scale reactor but it operated within mesophilic and thermophilic temperature regimes. Due to lack of availability of funds, the 0.3 m^2 50 W solar photovoltaic installed on the pilot-scale single-stage SSHTABD could not heat up the digester to the expected optimal hyper-thermophilic temperature of 65 ^oC. As a result, the SSHTABD, on the other hand, operated in a temperature range between 33 ^oC and 45 ^oC. Owing to the fluctuations in temperature in the pilot-scale single-stage SSHTABD, biogas production and methanisation were greatly affected as thermophilic and hyper-thermophilic methanogens are temperature-sensitive (within a range of +/− 2 °C) (Deublein and Steinhauser, 2011; Sheth, 2009). However, thermophilic and hyper-thermophilic methanogens have about 50 % higher rate of organics degradation and thus higher biogas yield (Deublein and Steinhauser, 2011; Sheth, 2009). This does not, however, mean that the higher the temperature the more optimal the production of biogas, due to the larger energy requirement at higher digesting temperatures (Chae *et al.,* 2008). Operations at mesophilic conditions consume less energy and are not often inhibited by ammonium but the process requires longer residence time for maximum biogas production. Mesophilic microbes are slow growing (as was observed in the pilot-scale single-stage SSHTABD) compared with those of thermophilic conditions and even hyper-thermophilic conditions (such as the laboratory-scale single-stage HT-CSTR).

Thermophilic digestion has another advantage of hygienising the digestate for agricultural use (Deublein and Steinhauser, 2011; Sheth, 2009). Pathogenic microbes are totally destroyed at thermophilic temperature greater than 55 °C with a hygienisation retention time of 24 hours (Vögeli *et al.,* 2014). Furthermore, due to less solubility of oxygen in the thermophilic temperature range, optimal anaerobic operational condition is easily reached. On the contrary, at higher temperatures, CO_2 concentration increases by 2 to 4 % because of its lower solubility and this may increase the percentage of CO_2 in the biogas produced (Vögeli *et al.,* 2014). Unfortunately, in the pilot-scale single-stage SSHTABD because the optimal hyper-thermophilic operational temperature was not achieved, the effluent still had concentrations of pathogens such as *Salmonella spp* and *E. coli* compared with the laboratory-scale single-stage HT-CSTR which had effluent devoid of any pathogens. In the situation of the pilot-scale SSHTABD, post-treatment of the effluent using constructed soil filtration or laterite-based constructed soil filter or soil filter bed would remove the remaining pathogens that were in the effluent after the treatment (Jenssen *et al.,* 2010; Kadam *et. al.,* 2009; Kadam *et al.,* 2007).

Hyper-thermophilic digestion of wastewater like BW can be feasible in a tropical developing country like Ghana if external renewable energy source like the use of solar energy (which is readily available in Ghana) is used for heating during the hyper-thermophilic digestion and the digester is well insulated. Biogas digesters built underground are less susceptible to sudden temperature fluctuations as they use the temperature of the soil as temperature buffer (Vögeli *et al.,* 2014). The pilot-scale single-stage SSHTABD, however, was not fully underground because the water table in the Terterkessim slum was very high and thus part of the digester bulged of the ground resulting in temperature fluctuations in the digester.

Methanogens identified in this research were species of archaeabacteria with specific examples being *Methanosarcina, Methanomicrobiales and Methanococcus.* Methanogenic *Archaea* of various species such as *Methanosaeta, Methanolobus, Methanococcoides, Methanohalophilus, Methanosalsus* and *Methanohalobium* have been identified to be responsible for methanogenesis during AD process for methane production (Paritosh *et al.,* 2017). Other examples include *Halomethanococcus, Methanolacinia, Methanogenium and Methanoculleus* (Paritosh *et al.,* 2017). The presence of *Methanosarcina spp* in the HT-CSTR was because they are capable to use both acetoclastic and hydrogenotrophic pathways for methane production, therefore, thrive obligately on methyl and acetate (Lyimo *et al.,* 2000; Ziemiński and Frąc, 2012). *Methanococcus jannaschii* is a known archaeal species and a hyper-thermophile that uses proteasome-activating nucleotidase (PAN) and could operate on a hydrolysis rate at a hyper-thermophilic temperature of 65 ^{o}C (Wilson *et al.,* 2000). A study that assessed the influence of cow manure and sheep manure on mesophilic biogas digester identified more species of *Archaea* such as *Methanomicrobiales* and *Methanobacteriales*. *Methanomicrobiales* were most dominant in the cow manure while fewer of *Methanosaetaceae, Methanococcales* and *Methanosarcinaceae* were identified (Achinas *et al.,* 2018). *Methanosaeta spp* which are filamentous methanogens are considered to be acetate loving and can thrive under many anaerobic conditions (Mizukami *et al.,* 2006) but they were not identified in the sludge of the HT-CSTR. Assorted groups of methanogens in the family of *Methanomicrobiaceae* are reported to be present in cow manure (Kim *et al.,* 2014), confirming the strains of methanogens in the order of *Methanomicrobiales* identified in this study. *Methanothrix sohengenii* are mostly found in biogas digesters that treat substrates like sewage and mango-processing waste and they are usually stimulated to proliferate in growth under iron influence (Preeti Rao and Seenayya, 1994). In this research, they were absent probably because the hyper-thermophilic temperature used was not favourable to them and the sludge may not have sufficient iron required by them. Biogas digesters that run on co-digested substrates had the presence of eubacteria such as *Firmicutes* and *Bacteroidetes. Bacteroidetes* and *Spirochaetes* were found in biogas digesters that run on sewage

(Abendroth *et al.,* 2015; Pampillón-González *et al.,* 2017; Ziganshin *et al.,* 2011). *Methanoculleus spp* were identified in the co-digested digester while *Methanosaeta spp* and *Methanosarcina spp* were identified in the digester that operated on a sewage-sludge (Abendroth *et al.,* 2015; Pampillón-González *et al.,* 2017; Ziganshin *et al.,* 2011). This confirms why eubacteria were identified in the seeding sludge used for the HT-CSTR in this study. Methanogenic bacteria such as *Bacillus spp* and *Proteus spp* have also been identified in cow dung under batch mesophilic conditions (Pratiksha and Gireesh, 2012). Full-scale thermophilic and mesophilic digesters treating food wastewater isolated twenty-seven bacterial genera (Lee *et al.,* 2017). The dominant methanogens were *Methanosaeta harundinacea and M. concilii.* Others included *Methanoculleus bourgensis, M. thermophilus and Methanobacterium beijingense* (Lee *et al.,* 2017).

5.3 Perceptions of residents of Elmina on their willingness to accept and adopt the single-stage SSHTABD in their homes

The effective implementation, acceptance, adoption and usage of any technology that has been developed hangs mostly on the population for which the technology was developed. Consequently, knowing the perception of the target population about the developed technology will facilitate its usage as well as propagating the idea to other unknown users. The age distribution, level of formal education, the type of employment and financial status of the respondents have direct influence on their perceptions of the developed technology. The sex distribution for residents of Elmina showed 48.9 % for males and 51.1 % for females, giving an implied sex ratio of 96 males to every 100 females. The values were very similar to what was reported by the Ghana Statistical Service in a population and housing census carried out in 2010 for Ghana which showed that the percentage of males and females were 48.8 % and 51.2 %, respectively with an implied sex ratio of 95 males to 100 females (Ghana Statistical Service, 2012b). The sex distribution for residents of Elmina were a little different from that of the K.E.E.A. municipality which was 48.1 % for males and 51.9 % for females, implying that the sex ratio for males and females in the entire K.E.E.A municipality was 93 males to 100 females (Ghana Statistical Service, 2014). The higher ratio of males to females in Elmina town obtained in this study compared with the district sex ratio could be as a result of the intense fishing activities and salt production in the town since both fishing and salt production are very backbreaking and require men who are considered to have more energy than women for such a profession in Ghana.

About 95 % of the respondents in Elmina was between 20 years and 60 years which is the age required for working in all sectors in Ghana. This was contrary to the 40.4 % for the entire population of the K.E.E.A. municipality (Ghana Statistical Service, 2014) and 50.5 % for Ghana as a whole (Ghana Statistical Service, 2012b) reported to be between the ages of 20 years and 60 years. The value recorded in this research could be as a result of immigration into the town due to the Christmas and New Year festivities which usually witnessed influx of natives of towns who had travelled away returning to their hometowns. The Ghana Statistical Service further confirms that 28.7 % (42,192) of the population in the municipality in 2010 was as a result of immigration. The number of years the immigrants had spent in the municipality ranged from 1 year to more than 20 years (Ghana Statistical Service, 2014). A period of 6-7 years had elapsed after the 2010 population and housing census in Ghana, consequently, the 23.7 % of the population in the K.E.E.A. municipality who were within the ages of 14 years to 19 years would have matured into the age group between 20 years to 60 years. Some of the population that were nearing their 60 years in 2010 might have also passed the 60 years retirement age (Ghana Statistical Service, 2014). Furthermore, the population growth rate is always higher than population decline rate in the K.E.E.A municipality and Ghana as a whole (Ghana Statistical Service, 2014).

Contrary to the 92.9 % reported by the Ghana Statistical Service for Ghana (Ghana Statistical Service, 2012a), about 90 % of the respondents in Elmina had some form of formal education ranging from primary to post-tertiary level. This was different from the 25.2 % of married males and 41.0 % of married females in the K.E.E.A. municipality who were reported to have had no form of formal education (Ghana Statistical Service, 2014). The massive increase in the percentage of individuals in Elmina that had some form of formal education as at the time of this study could be attributed to Governmental policy interventions in education by successive governments in Ghana. These include 'free, compulsory and available to all' policy in 1992 and Free Compulsory Universal Basic Education (FCUBE) policy in 1996, which was in two phases (UNICEF Ghana Country Study, 2012). The first phase as at 2002 was a mandatory education from 6 years to 11 years through to junior high school while the second phase sought to ensure at least 2 years of pre-primary school. In addition, in the year 2005, basic education at all levels in Ghana was made free, implying that no pupil was made to pay school fees. Furthermore, all operational costs at the basic educational school levels were borne by the Government in the form of a capitation grant (UNICEF Ghana Country Study, 2012). Moreover, in the year 2007/2008, the Government further enforced free compulsory kindergarten education for children between the ages of 4 years and 5 years, hence influencing the percentage of individuals who had received some form of formal education in the K.E.E.A municipality (UNICEF Ghana Country Study, 2012). According to the Ghana

Statistical Service report for K.E.E.A. municipality in 2014, the percentage of males and females who have received tertiary education were 3.2 % and 1.0 %, respectively (4.2 % in total) compared with 27.9 % recorded in this research. The increase in the percentage of residents who have had tertiary education could be as a result of a number of Distance Learning Programmes (DLP) offered by most of the tertiary educational institutions in the various Regional and District capitals of Ghana. This offers residents who work the opportunity to concurrently study at the comfort of their homes. Other factors like higher remuneration for workers who have tertiary education compared with those without tertiary education in both the government and private sectors in Ghana could have influenced residents in Elmina to pursue higher education at the tertiary level. In addition, since Elmina is the administrative capital for the K.E.E.A. municipality, possibility of most of the highly educated and skilled personnel working in the municipality residing in the administrative capital is very high.

Certain employment or jobs are preferably carried out by males or females. According to the Ghana Statistical Service report for the K.E.E.A. municipality in 2010, the percentage of individuals in the municipality who were economically inactive was 32.4 % as opposed to 67.6 % of the residents who were economically active. Of the percentage that was economically active, 93.6 % was reported to have gainful employment compared with 6.4 % who were considered to be unemployed in the municipality (Ghana Statistical Service, 2014). This gives an indication that as at the year 2010, the level of unemployment in the K.E.E.A. municipality was really low compared with what was recorded in this study which showed 21.0 % of the respondents in Elmina were unemployed. The unemployment rate in Elmina had increased because of factors like population growth rate, lack of employable skills by residents, slow growth of businesses in Ghana in general, rural-urban migration and global economic crises (Baah-Boateng, 2013; Twumasi, 2013).

The more educated one becomes, the more enlightened the individual becomes regarding the negative consequences of open defecation in nearby bushes, at the seashore, in polythene bags and flying it over or on open fields. This was opposed to the uneducated individuals who indiscriminately defecated anywhere without any knowledge on the negative consequences to both human health and the environment. Reports by both the Ghana Statistical Service for K.E.E.A. municipality and 2010 Population and Housing Census (PHC) carried out for the entire nation further indicated that about 19.3 % of the inhabitants in the municipality had no access to any form of toilet facility and consequently, resorted to attending to nature's call at the beaches, in nearby bushes and on open fields (Ghana Statistical Service, 2012b, 2014). Factors such as population growth, poor attitudes of people, inadequate toilet facilities, filth on existing public toilets, health-related reasons and economic factors lead to open defecation in Ghana (Ameyaw and Odame, 2017; UNICEF, 2015). Some of the dire consequences of open

defecation include diarrhoea-related diseases like cholera which kills about 1.6 million people annually, of which about 90 % are children below the age of 5 years. Most of these children are from developing countries like Ghana. Globally, there is a risk of about 500 million people being in danger of trachoma disease which has the potential to cause blindness in about 146 million people and threatens to leave about 6 million people visually impaired (Ameyaw and Odame, 2017; UNICEF, 2015). Around 160 million people are infected with schistosomiasis, with several tens of thousands dying from it every year. In every year globally, roughly 133 million people are infected with intestinal helminths such as ascariasis, hook worms and trichuriasis while 1.5 million people are infected with clinical hepatitis A disease (Ameyaw and Odame, 2017; UNICEF, 2015).

The level of education influenced the respondents' perception of their knowledge on whether cooking gas can be produced from human faeces. It also influenced their willingness to use cooking gas produced from human faeces for cooking. This implies that the more educated the respondents were, the more knowledge they had on biogas technology and the more willing they were to use cooking gas produced from human faeces. The percentage of residents in the K.E.E.A. municipality that were reported to use LPG in 2010 was 8.4 % compared with 29.7 % of the respondents reported in this research who used only LPG for cooking and 18.7 % who combined both LPG and charcoal. The significant increase of LPG users over the past few years after 2010 could be attributed to an intervention made by the Government of Ghana in response to the United Nations General Assembly Declaration of 2012 as the International Year of Sustainable Energy for All (SE4ALL). The target for SE4ALL was to ensure there is modern energy services for everyone globally, including Ghana (Energy Commission of Ghana, 2012). Ghana received support from UNDP and other partner agencies to facilitate the country's efforts to achieve universal access to sustainable energy by the year 2030. Consequently, the country implemented a programme called Sustainable Energy for All Acceleration Framework (SEAAF) (Energy Commission of Ghana, 2012). Factors such as increase in the levels of income, availability and easy access to modern fuel (e.g. LPG) and its infrastructure influence the adoption of modern fuels in Ghana (Karimu, 2015; Kwakwa *et al.,* 2013). Other factors include level of education and whether the person lives in an urban or rural areas, family size and employment status also influence the adoption of modern fuels in Ghana (Karimu, 2015; Kwakwa *et al.,* 2013). A review carried out by Muller and Yan (2018), on household fuel use in developing countries showed that as the income levels of individuals increase, they also switch from the use of the 'more dirty' fuel sources like firewood to the 'more clean' fuel sources like LPG in a theory called 'the energy ladder'. Akpalu *et al.* (2011) reported from a survey carried out by the Ghana Living Standard (GLS) that LPG is the most preferred form of energy for household usage in Ghana. On the other hand, the World Bank

reported that in Ghana, charcoal and fuelwood are the most used forms of energy in most households (Heltberg, 2003). This was also confirmed in this study where the use of only charcoal by the residents of Elmina accounted for 40.6 %, with 21.9 % combining the usage of charcoal with either LPG or firewood. Charcoal usage was very high because it is very cheap and easily accessible, however, it is not environmentally friendly as it results in deforestation and greenhouse gas emissions, particularly from carbon dioxide (Akpalu *et al.,* 2011). The reliance on charcoal is, therefore, not sustainable and can adversely affect the health of individuals that use it (Akpalu *et al.,* 2011). Foley *et al.* (2015), projected deaths from breathing fumes from charcoal and smoke firewood from indoor cooking, to kill most Sub-Saharan African girls than malaria and malnutrition.

The level of education also affected the knowledge of the respondents on the issue of conversion of treated human faeces to fertiliser and willingness to use such fertilisers for various crop production. The level of education and the respondents' willingness to consume crops cultivated with treated human faeces has been established. The use of source-separated human faeces and urine for fertiliser production and usage for agriculture in developing countries using composting has been reported (Niwagaba, 2009). The safety and quality of food produced and sold in the market is very important to every consumer as well as experts in the food industries, food technologies and food sciences (Floros *et al.,* 2010). Treated wastewater such as black water used for irrigation purposes comes with a great cause for concern as it may contain pathogenic indicator organisms like *Escherichia coli*, nematode eggs and even hookworms. The presence of these pathogenic indicator organisms gives a clue that further analyses need to be carried out on the treated wastewater stream before it could be applied on crops for human consumption (Miller-Robbie *et al.,* 2017). About 49 % of patients from both poor and middle income countries who are hospitalised based on diarrhoea-related cases are known to be infected with either *E. coli* or *Vibrio cholerae* (O1/O139 strain). The presence of these pathogens in food are associated with contamination from human faeces (Fischer *et al.,* 2010). Apart from causing diarrhoea, pathogenic *E. coli* also cause haemorrhagic colitis, haemolytic uremic syndrome, urinary tract infections and neonatal meningitis in humans. In addition to the diseases caused in humans, pathogenic *E. coli* cause other diseases like post-weaning diarrhoea and edema disease in pigs, peritonitis and airsacculitis in chicken and calf scours and mastitis in cattle (Fratamico *et al.,* 2017). This poses a serious threat to food safety and food security when treated human faeces is being considered as a fertiliser for vegetable crop production as well as cultivation of crops which are eaten uncooked. People who eat human faeces-contaminated food not only suffer from diarrhoea but also typhoid fever and salmonellosis (Farag *et al.,* 2016).

The quality of food crop produced is positively influenced when organic fertiliser like chicken manure and treated human faeces is applied due to nitrate enrichment from a thermophilic treated organic waste (Li *et al.,* 2017). The taste of crops cultivated with treated human faeces is not really influenced by the kind of fertiliser (such as treated human faeces) used, however, the acid content, sugar content or taste of the fruit or vegetable produced may be significantly influenced because of the presence of excess sulphur in the organic fertiliser (Heeb *et al.,* 2006). The case of applying treated human faeces (effluent) on leafy vegetables may affect the smell and the taste of the crop produced as a result of the presence of ammonia and sulphur in the effluent.

Separated domestic wastewater stream comprising concentrated black water and food waste was treated at higher HRT and operational temperature using the Decentralised Sanitation and Reuse (DESAR) concept, however, the effluent was not hygienised enough for safe agricultural use because of the presence of *E. coli* and heavy metals (Kujawa-Roeleveld *et al.,* 2005). It is reported that mesophilic anaerobic digester cannot completely hygienise anaerobic pathogens but can reduce their concentrations by up to 4.58 log CFU/g sample (Smith, 2013). Similar to what was reported in the pilot study, the effluent was contaminated with *Salmonella species* and *E. coli* but heavy metals were below the concentration levels stipulated by the Environmental Protection Agency of Ghana (EPA - Ghana). This was due to inadequate high temperature required for the operation of the digester. The solar photovoltaic (0.3 m^2) installed on the system was inadequate. The total area of solar photovoltaic needed was 3.0 m^2 but because there was no external sources of funding for this research, the researcher could no longer finance this aspect of the study.

Storing the effluent under ambient temperature for at least 40 days could result in 2 logs removal of pathogens like *E. coli* (Wendland, 2008). The use of soil filtration for post-treatment of effluent has been reported to remove between 3 to 5 logs of pathogens (Kadam *et al.,* 2009; Kadam *et al.,* 2007). Consequently, if the effluent from the single-stage SSHTABD is post-treated with slow sand filtration system embedded with activated charcoal, it would be of high quality for application on vegetables but some of the nutrients would be removed (Bryant and Tetteh-Narh, 2015; Reddy *et al.,* 2014), except the organic nutrients like organic phosphorus and organic nitrogen which might have been hydrolysed into phosphate and ammonia, respectively (Khan *et al.,* 2011). The use of Natural Oriented municipal Wastewater Treatment and Reuse concept (NOWTR) where natural UASB pond reactor with nutrient recovery could also be employed for the post-treatment of the effluent. With the NOWTR concept, effluent is hygienised in ponds using sunlight (Abdel-halim *et al.,* 2008).

Some residents of Elmina, Ghana expressed willingness (86.3 %) to accept and adopt the single-stage SSHTABD in their homes because of some socio-economic and environmental reasons. For example, biogas is cheap alternative source of renewable energy and has positive impact on the environment as it reduces the volume of methane gas released into the atmosphere as a greenhouse gas. These were confirmed by Walekhwa *et al.* (2009) that biogas produced at the household level is known to be good alternative source of energy to replace the expensive and unsustainable fossil fuels. However, its adoption and usage is very low especially in Ghana and Africa in general. Ones willingness to accept and adopt household biogas digester has a nexus with the person's need, income level and knowledge about this technology (Jan and Akram, 2018; Walekhwa *et al.,* 2009). Factors such as low technical know-how, lack of capital for installation and socio-cultural set-backs hamper its easy adoption and usage. In addition the higher the income level of a household, the location (remote area or urban area) and bigger the size of a household influence the willingness to adopt the technology (Walekhwa *et al.,* 2009). A study carried out in Pakistan to assess the willingness of households to adopt the biogas technology revealed that the willingness of the people to adopt the technology depended on their educational level, household energy demand in the form of electricity, the effects of energy-deficiency on the education of children and the effects of energy-deficiency on household chores by women (Bonnke, 2014; Jan and Akram, 2018). The issue of normalisation and quality control with respect to the type of biogas digester to be used as well as incorporation of the digestate into farming were reported to also influence the adoption of small-scale biogas digesters by households in Sub-Saharan Africa (Mwirigi *et al.,* 2014). There is the need for governments, for example Government of Ghana to develop a policy on the adoption of biogas digesters at the household levels to help with its proliferation in the country (Amjid *et al.,* 2011). Furthermore, mobilisation of funds from local and international organisations to expand this technology in most willing-households would go a long way to help with its adoption. Moreover, the formation of user groups to help pay the initial installation cost for one household and later for the others would tend to support each household get its digester with less initial financial stress (Mwirigi *et al.,* 2014). Other uses of biogas other than for cooking and lighting for the household should also be encouraged to help with its adoption (Mwirigi *et al.,* 2014).

5.4 Basic calculations for adopting the single-stage SSHTABD vis-à-vis the use of septic tanks for BW treatment

Even though the life span of a household biogas digester is uncertain, it is reported that a well-maintained biogas digester can run effectively for at least 20 years and could even span to 30 years, averaging for 25 years (Raha *et al.,* 2014). The overall cost for the 12 m^3 single-stage

SSHTABD that was constructed at Terterkessim slum in Elmina was five thousand, two hundred and ninety-one Ghana cedis, fifty pesewas (GH₵ 5291.50). With the daily interbank forex rates of the Bank of Ghana quoted on Thursday, 2nd August, 2018 at € 1.0 to GH₵ 5.45, the digester would cost € 970.9 (in euro equivalence). Every household uses some form of energy either in the form of firewood, charcoal, LPG or a combination of the energy streams.

The calculations below were made for a household that uses septic tank system for their on-site BW pre-treatment. Assuming the prices of firewood, charcoal and LPG remain the same for the next 25 years, with the current price of charcoal being GH₵ 40.00/$bag_{charcoal}$ (€ 7. 40/$bag_{charcoal}$) [a bag or sack of charcoal weighs approximately 30 kg (Mwampamba, 2007)] and LPG being GH₵ 70.00/14.5$kg_{cylinderLPG}$ (€ 12.80/14.5$kg_{cylinderLPG}$). If a household uses only charcoal and consumes at least 2 bags of charcoal in a month, the household might have spent at least GH₵ 80.00 (€ 14.80) within a month for energy only for cooking and or heating purposes and thus GH₵ 960.00 (€ 177.60) would have been spent within a year and GH₵ 24,000.00 (€ 4,440.00) within 25 years. In a situation where the household uses only LPG for cooking and heating purposes and consumes at least 14.5$kg_{cylinderLPG}$ per month, the household might have spent GH₵ 70.00 (€ 12.80) and GH₵ 840 (€ 153.60) per annum. Calculating this scenario for the next 25 years, the household might have spent GH₵ 21,000.00 (€ 3,840.00). In addition, assuming that prices of goods and services remain stable for the next 25 years and considering the scenario where people spend at least three hundred Ghana cedis (GH₵ 300.00 = € 55.00) per every two years for sludge removal when their household septic tanks get full, within twenty-five years, the person might have spent at least three thousand seven hundred and fifty Ghana cedis (GH₵ 3750.00 = € 687.5) for only desludging of the septic tanks. However, factoring in the construction of a household septic tank would also involve similar capital investment like what is required for a single-stage SSHTABD. Thus, for 25 years, a household that has a septic tank system and uses either only charcoal or only LPG might have spent GH₵ 27, 750.00 (€ 5, 127.50) or GH₵ 24, 750.00 (€ 4, 527.50), respectively. These figures are all higher than the initial investment cost of GH₵ 5291.5 (€ 970.9) needed for having the single-stage SSHTABD in one's home which would be operational for at least 25 years. Factoring in inflation and price instability of both goods and services even aggravate the expenditure for those who do not adopt the single-stage SSHTABD technology in their homes.

From the foregoing, calculations made could imply that at least between 4.8 and 5.3 years (averagely 5 years) of investing into having the single-stage SSHTABD in a household, the household would have paid back the initial investment cost. Even though initial investment cost for installing a biogas digester in a household is relatively high (as per Ghanaian standard), the payback time is relatively short (approximately 5 years) when the biogas is used

for cooking (Mohammed *et al.,* 2017). The number of years for the payback on investment obtained in this study (averagely 5 years) confirms the short pay-back period (PBP) of 5 years obtained by Mohammed *et al.,* (2017) in a study they carried out in Ghana on PBP for installing a biogas digester. Consequently, it would be economically useful if residents would adopt the single-stage SSHTABD technology rather than relying on the use of septic tanks for some form of household pre-treatment of BW before final discharge into the environment. Even though the level of knowledge and the understanding of the operations of the biogas digester is limited, the Government of Ghana can involve experts in this field to continuously educate, sensitise and implement this technology in any household willing to adopt it as was practised in Assam in India (Raha *et al.,* 2014).

5.5 Potentials of BW and effluent from the single-stage SSHTABD for agricultural purposes

In the pilot-scale single-stage SSHTABD at Terterkessim slum in Elmina, the per capita flow rate for BW was 4 L/p/d. Each litre of BW contained 1117.7 mgN/L with NH_4-N present being 907.4 mg/L NH_4-N and 27.4 mg/L NO_3-N. Consequently, total nitrogen-based nutrients for fertiliser in the form of ammonium and nitrate was 934.8 mgN/L. Thus if each person produces 934.8 mgN/L at a flow rate of 4 L/d, the load of nitrogen-based fertiliser in BW would be 3739.2 mgN/d (3.74 gN/p/d); which was within the 3.0 – 7.0 gN/p/d range proposed by Jonsson *et al.* (2004). This implies annually, 1365.1 gN/p/yr would have been produced in only BW. Since the effluent contained 1583.9 mg/L NH_4-N and 78.4 NO_3-N, the nitrogen-based fertiliser in the effluent from the pilot-scale single-stage SSHTABD would be 1662.3 mgN/L at a flow rate of 4 L/d resulting in a load of 6649.2 mgN/d (6.65 gN/p/d). Thus, the annual quantity of nitrogen-based fertiliser supposed to be discharged in the effluent was 2426.96 gN/p/yr. If an amount of 30 - 70 kgN/yr is needed for 300 – 400 m^2, then about 2.4 kgN from the effluent from the pilot-scale single-stage SSHTABD could support 13.7 m^2 or approximately 14 m^2. A maximum of 170 kg (250 kg on grassland) of animal manure N could be applied per a hectare of land per year (Schröder and Neeteson, 2008). If an average of 183 kgN/ha (range of 174 – 193 kgN per hectare) is required for a hectare of maize farm, then 2.4 kgN could support 131 m^2 of maize farm per person annually. Cotton plantation needs half of the quantity of nitrogen required by maize, therefore, if the effluent is used for cotton plantation, then it could support 262 m^2/p/yr. In terms of phosphorus, BW contains 357.2 mgP/p/d resulting in 130.4 gP/p/yr while the effluent contained 403.6 mgP/p/d or 147 gP/p/yr. These values give the fertiliser potentials of the BW and effluent for urban agriculture.

5.6 Potentials of single-stage SSHTABD for BW and FW treatment towards deforestation prevention and carbon sequestration

Increase in charcoal production and the demand for charcoal for various urban domestic purposes in most developing countries especially, in Sub-Sahara Africa is considered to contribute significantly to deforestation among other factors like increase in agriculture land, urbanisation and increase in grazing lands (Mwampamba, 2007). It is mentioned in the work of Mwampamba (2007) that for every percentage increase in the urban population, the consumption of charcoal also increases by 14 %. Lohri *et al*. (2016) reported in their review that a kilogram of charcoal could be produced from the conversion of between 4 and 6 kilograms of firewood. However, the mass of a single sack of charcoal (M_s) multiplied by the kiln efficiency (E_k) (determined as the kilogram of wood used to produce a kilogram of charcoal) and the inverse of stock density (S) determines the amount of forest needed for a kilogram of charcoal (Mwampamba, 2007). The stock density (S) is determined as the ton of wood per a hectare of forest and a conversion coefficient of 1.075×10^{-3} (which is derived from an assumed 93 % stem harvest and the unit conversion of 1000 kg of wood to 1 ton of wood) (Mwampamba, 2007). Based on the average number of individuals of 4 persons in a household in Elmina, using the monthly charcoal consumption of 2 sacks (60 kg of charcoal) per a household, it implies that in a year, 720 $kg_{charcoal}$ would have been consumed within the household by the 4 persons (180 $kg_{charcoal}$/person/$yr_)$. Urban residents of Tanzania have similar charcoal consumption as their per capita annual charcoal consumption is 180.3 kg/person/yr (Mwampamba, 2007). Both values of annual per capita charcoal consumption reported in this study and that reported by Mwampamba (2007) were much lower than the 1091.4 kg/person/yr reported for residents of Maputo in Mozambique (Brouwer & Falcão, 2004). The over-reliance of urban residents to use charcoal for most domestic purposes such as for cooking and heating is tied strongly to income level, household size, easy access and the area of residence. The use of charcoal is not only associated with the poor but also the rich since they use it for purposes like grilling and barbeque (Brouwer and Falcão, 2004). It is estimated that the area of forest needed for annual charcoal production per a household is 421,000 hectares (Mwampamba, 2007). This implies that a compound house accommodating four different households of about 4 persons each need 1,684,000 hectares of forest annually for charcoal production based on the current charcoal consumption quantity obtained in this study. This implies that if the single-stage SSHTABD is adopted and used by the residents of Terterkessim slum and those of Elmina, a household (of at least 4 persons) could save at least 421,000 hectares of forest annually. The amount of carbon dioxide (CO_2) that a hectare of forest or vegetation can sequester in a year depends on the type of vegetation, soil type, growing conditions and rainfall (Dombro, 2011; Forestry Commission, 2013). For example, the

woodlands of the United Kingdom sequester 5.4 tonnes of CO_2-e grassland per hectare per year equivalent to 1.4 carbon tonnes per hectare per year (1 tonne of carbon is equivalent to 3.7 tonnes of carbon dioxide) (Forestry Commission, 2013). *Pinus massoniana* (Pine) in China is known to sequester 6.3 tonnes of carbon per hectare annually (equivalent to 23 tonnes of CO_2-e per hectare annually) (Justine *et al.,* 2015). In the tropics, between 95 tonnes to 228 tonnes of carbon (representing 351.5 to 843.6 tonnes of CO_2-e) can be sequestered per hectare annually. Consequently, when the residents of Terterkessim slum in Elmina adopt and use the single-stage SSHTABD for BW treatment and biogas production for domestic usage, every household would not only get free available renewable energy for cooking and fertiliser for urban agriculture but also help to sequester between 147,981,500 and 355,155,600 tonnes CO_2-e annually and thus aiding with climate change mitigation.

CHAPTER SIX

Conclusions, recommendations and challenges

6.1 Conclusions

The development of a single-stage solar-supported hyper-thermophilic anaerobic biogas digester treating black water was investigated by first selecting a suitable seeding sludge and appropriate optimal hyper-thermophilic temperature suitable for adoption and application in both a laboratory-scale and pilot-scale experiments. Consequently, the performance of three seeding sludge under three different hyper-thermophilic temperatures were investigated for a common substrate, BW, in batch tests which showed that cow manure (CM) can be considered as the preferred seeding sludge. The optimal hyper-thermophilic temperature of 65 ^{o}C was also selected for setting-up a bigger single-stage hyper-thermophilic digester for both biogas production and disinfection of the digestate for agricultural usage. Consequently, cow manure and hyper-thermophilic temperature, 65 ^{o}C were selected for the laboratory-scale single-stage HT-CSTR experiments. This selection was made because CM produced the highest net normalised cumulative volume of methane content of 387 mLNCH_4-%. In locations where cow manure is not available to be used as a seeding sludge, a probable substitute that can be considered for hyper-thermophilic treatment of BW is sewage sludge from wastewater treatment plant, however, its optimal operational temperature is 60 ^{o}C. It can also be concluded that treating BW at hyper-thermophilic temperature of 70 ^{o}C is not really feasible irrespective of the type of seeding sludge used. This is because at 70 ^{o}C, BTU was the most inhibited in terms of methane content as it recorded 0.0 mLNCH_4-% net normalised cumulative volume of methane content, followed by LWG (13 mLNCH_4-%) and CM (19 mLNCH_4-%). CM at 65 ^{o}C recorded the highest net normalised cumulative methane yield of 232 mLNCH_4/gVS, followed by LWG at 60 ^{o}C and 55 ^{o}C which had 217 mLNCH_4/gVS and 180 mLNCH_4/gVS, respectively. The least net normalised cumulative methane yield for the three inocula was recorded by BTU at 70 ^{o}C (0.0 mLNCH_4/gVS) followed by CM at 37 ^{o}C which recorded 0.9 mLNCH_4/gVS. At least, about 70 % of the COD present in the BW was degraded at both 60 ^{o}C and 65 ^{o}C for LWG and CM, respectively. CM at optimal hyper-thermophilic temperature of 65 ^{o}C had the highest degree of COD degradation of 79.1 % followed by LWG at hyper-thermophilic temperature of 60 ^{o}C which recorded 73.9 %. At hyper-thermophilic temperature of 70 ^{o}C, BTU was the most inhibited as it had degree of COD degradation of 0.0 %, while CM and LWG recorded degree of COD degradation of 4.3 % and 5.9 %, respectively.

The laboratory-scale single-stage HT-CSTR operated for twenty-two weeks with the mean optimal hyper-thermophilic temperature and mean HRT being 65.2 ^{o}C and 23.3 days, respectively. The reactor had an average influent pH of 6.9 ± 0.9 for only BW, 5.3 ± 0.5 for MIX influent (BW:FW, 1:1, v/v) and 6.9 ± 0.6 for effluent. The pH in the reactor was within the optimum pH range for methanogenesis except on days of feeding when it dropped a little but recovered quickly afterwards. The concentration of total COD in the influent (only BW) was 26853.7 ± 7609.5 mg/L and that of the MIX influent was 98904.8 ± 15357.6 mg/L. The mean total COD concentration in the effluent after co-digestion was practised was 13571.4 ± 6182.6 mg/L, showing a total COD removal of 86.3 %. After co-digestion of the BW with kitchen FW, the reactor operated on an average flow rate of 2.2 L/d and an average COD volumetric loading rate of 6.22 kgCOD/(m^3.d) and organic loading rate of 0.27 ≈ 0.3 kgVS/(m^3.d). With the average influent daily load of 0.22 kg/d and average effluent daily load of 0.03 kg/d, the single-stage HT-CSTR had a degradation performance (R) of 5.43 kgCOD/m^3.d. The food-to-mass (F/M) ratio of the laboratory-scale single-stage HT-CSTR was calculated to be 1428.57 kg/(kgVS*d).

The net normalised cumulative methane productivity over the period for the laboratory-scale single-stage HT-CSTR was 1.3 $Nm^3CH_4/(m^3.d)$ with the daily average being 0.06 $Nm^3CH_4/(m^3.d)$. The normalised cumulative methane yield was 55.4 Nm^3CH_4/kgVS and its daily average was 2.5 Nm^3CH_4/kgVS per day. Treatment of only BW at optimal hyper-thermophilic temperature of 65 ^{o}C in a laboratory-scale single-stage HT-CSTR resulted in biogas with less methane content of 34.9% even though the pH in the reactor had stabilised at 6.9. Co-digestion of the reactor with mixed substrate of BW and kitchen FW saw a 77.1 % increase in the percentage content of methane in the biogas from 34.9 % to 61.8 %. This gives an indication that setting-up a single-stage HT-CSTR for a community where the methane would be harnessed for either domestic purposes such as for cooking or heating or for generation of electricity through the use of combined heat and power (CHP) generator, would require the use of co-digested substrates with the BW, instead of using only BW for the AD process.

The laboratory-scale single-stage HT-CSTR was able to hygienise all the strains of *Salmonella senftenbergensis* with initial concentration of 2 x 10^9 CFU/mL and *E. coli* with initial concentration of 9 x 10^8 CFU/mL that were spiked into the reactor. This is because no growth was observed on both the Brilliant Green Agar and the Endo agar after the effluent (treated substrate) was cultured. A simulation test with the optimal hyper-thermophilic temperature of 65 ^{o}C confirmed that, between 30 minutes and 1 hour, all the strains of *Salmonella senftenbergensis* and *E. coli* in the treatment system were killed. This implies that construction of a bigger scale single-stage HT-CSTR treating BW co-digested with household FW can

ensure that renewable energy in the form of biogas as well as hygienised digestate for agricultural use are produced.

The presence of methanogens in the seeding sludge (cow manure) at hyper-thermophilic temperature influences the percentage of methane content in the volume of the biogas. Eubacteria (EUB338 I) were found in the overlay of FISH-DAPI stained image in the sludge of the HT-CSTR. An overlay of the DAPI-stained image on the FISH image showed auto-fluorescence of archaeabacteria present in the sludge of the HT-CSTR that operated at optimal hyper-thermophilic temperature of 65 ^{o}C. *Methanosarcina sp.* (MS821) were identified in the seeding sludge of the HT-CSTR in this research but they appeared irregular coccoid-shape. Other methanogens such as *Methanomicrobium spp.* (MG1200) *and Methanococcus spp.* (MC1109) were also identified in the cow manure used as the inoculum. *Methanogenium spp., Methanoculleus spp., Methanospirillum spp., Methanocorpusculum spp. and Methanoplanus spp.* (which were all classified as MG1200) for FISH image showed a single rod-like CY3 shape. *Methanosaeta spp.* (MX825) and were not identified in the sludge of the HT-CSTR.

The working group of Elmina forms about 95 % of the respondents that were interviewed and had their ages ranging between 20 years and 60 years. About 90 % of the respondents in Elmina that were interviewed had some form of formal education, giving an indication that the respondents were enlightened and could give independent opinion regarding the social survey. About 79 % of the respondents have some form of gainful employment and thus may have the commitment to invest into having the technology in their homes after they get convinced that the technology is good. The majority (40.6 %) of the respondents in Elmina used only charcoal for cooking while 29.7% used only LPG, with 18.7 % using both charcoal and LPG for cooking. About 5.9 % relied on firewood while 4.6 % used electricity for cooking.

The level of knowledge of the respondents on the concept of biogas technology was low, especially when the concept of co-digestion was mentioned, as 90.4 % of the respondents indicated that they did not have any idea whatsoever as to how co-digestion is practised. Even though the respondents had little knowledge on biogas technology, about 86.3 % of them expressed their willingness to adopt and invest into having the single-stage SSHTABD technology in their homes for various economic and environmental reasons while 13.7 % were unwilling to have the technology in their homes for fear of explosion and deviation from their dogmatic societal beliefs such as the use of charcoal gives the food special flavour and taste.

A 2-compartment toilet was constructed on a pilot manually-stirred, fixed pyramidal-dome-shaped single-stage SSHTABD for a compound house of 32 persons in the Terterkessim slum in Elmina but the toilets were used by at least 100 persons within the neighbourhood in a day.

Due to financial constraint, the 0.3 m^2 solar PV installed on the pilot-scale single-stage SSHTABD in Terterkessim slum in Elmina was not able to heat the reactor to the optimal hyper-thermophilic temperature of 65 ^oC used for the laboratory-scale experiment. Consequently, the pilot-scale SSHTABD operated on a mean temperature of 37 ^oC with average daily flow rate of 182.1 L/d and mean HRT of 51.3 days. The mean daily volumetric loading rate and mean daily organic loading rate of 0.97 kgCOD/(m^3.d) and 0.06 kgVS/(m^3.d), respectively, were recorded. These operational values for the reactor give an implication that the reactor had more potential of receiving more organic load for treatment daily. The pilot-scale single-stage SSHTABD removed about 97 % of the influent COD and could produce about 2.52 Nm^3CH_4/(kgCOD.d) which could be burnt for at least 8 hours for purposes such as cooking and heating.

The effluent produced contained less concentrations of heavy metals compared with the effluent discharge standards set by the Environmental Protection Agency (EPA) of Ghana, however, the effluent could not be used for cultivation of leafy vegetables such as cabbage and lettuce since it had some concentrations of pathogens like *Salmonella spp.* and *E. coli,* giving an indication that it was not hygienised enough. On the contrary, the effluent can be used for the cultivation of plantation crops like rubber, cotton or food crops like maize. However, once the required surface area (3.0 m^2) of solar PV that could generate at least 2 kW of power is installed on the single-stage SSHTABD and the optimal hyper-thermophilic AD temperature of 65 ^oC is attained, the effluent would be hygienised enough for application to any kind of crops.

6.2 Recommendations

The recommendations made in this study were classified based on what could be done to improve the single-stage SSHTABD design developed for the household BW treatment in Terterkessim slum in Elmina, Ghana. In addition, research opportunities which could not be investigated in this study were proposed for future study.

6.2.1 Recommendations for future designs and operation

i. The household SSHTABD should be constructed for individuals willing to be educated and those who can adhere strictly to the guiding operations of how the system works and would be committed towards its maintenance.

ii. The services of a caretaker for monitoring how the system is being used and ensuring that all stipulated guidelines and cleanliness are strictly adhered to would go a long way to curtail the numerous challenges encountered in this study regarding the operations of the single-stage SSHTABD in Terterkessim slum.
iii. The household single-stage SSHTABD should be sited at a location where the geology of the area is neither too rocky nor sandy. A slightly hilly clayey soil-dominated area is better compared to a flat, sandy, marshy area like Terterkessim slum.
iv. There could be a buffer to hold the BW while the users continuously use the toilet. In that case, the volume of influents that would be needed per day by the reactor would be discharged into the reactor, consequently avoiding shock loads for the reactor and the methanogens.
v. External source of funding (such as from the local authorities, the government and international organisations) is needed to support the construction, running and maintenance of household single-stage SSHTABD since its target is geared towards improving sanitation for the urban poor and ensuring water quality.
vi. Spherical or circular shape household single-stage SSHTABD is recommended for all future constructions since pressure in a sphere or a circle is the same and would ensure that the gas produced is pushed-up easily by pressure.

6.2.2 Recommendations for further research

i. It is recommended that crops cultivated with the effluent from the digester is evaluated with respect to its quality in terms of pathogens, organic pollutants, heavy metal concentrations and nutritional values.
ii. Even though literature has mentioned the life span for most biogas digesters, this may vary from one climatic conditions to another. It is, therefore, recommended that detailed research is also carried out to assess the robustness and durability of the system with time as against harsh weather conditions in order to evaluate the life span of the digester vis-à-vis the investment cost one has to make.
iii. Further experiments should be carried out on increasing the organic loading rate to assess its effect on effluent quality, methane productivity, methane content and methane yield.
iv. It is recommended that the performance of the system is assessed to evaluate whether COD removal, biogas production and pathogen disinfection improve or decrease with the age of the digester.

v. In order to really convince residents and even farmers in Ghana and most developing countries to use effluent from the digester for agricultural purposes, it is recommended that comparison of the performance of crops cultivated with treated effluent be made with crops cultivated with chemical-fertiliser and normal unfertilised soil.

6.3 Challenges

i. Most or all the residents used more quantity of water for the pour-flush toilet compared to what was proposed by the researcher.
ii. Some of the users used dirty water from a nearby gutter for flushing the toilet and consequently affecting the biogas production and overall performance of the reactor.
iii. Some of the users also used grey water from washed clothes thereby affecting the overall performance of the reactor.
iv. Some of the users also used detergents and perfume for cleaning the toilet. This could have killed the methanogens and consequently, affecting the overall performance of the reactor for methanogenesis.
v. Some of the residents opened the co-digestion pipes, leading to houseflies laying eggs into the system and resulting in maggots in the reactor.
vi. Some residents also opened the effluent pipe regularly, in the name of taking effluent for agricultural purposes but failed to close the biogas valve. This led to escape of already built up biogas in the reactor.

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Appendices

Appendix 1: Structured interview questions (open and closed-ended questions)

Brandenburg University of Technology, Cottbus-Senftenberg
Faculty of Environmental Sciences and Process Engineering
Department of Biotechnology of Water Treatment
P.O. Box 101344 D-03013, Cottbus-Germany

Questionnaire

The purpose of this questionnaire is to assess the perception of residents of Elmina, K.E.E.A. District - Ghana on their willingness to adopt and use a single-stage hyper-thermophilic anaerobic digester and their willingness to invest into having such a technology in their homes. Information provided by respondents will be used solely for academic research work, thus, respondents are assured of anonymity and confidentiality of their information provided.

NB: A **household** in this research is defined as a group of people living together in the same compound sharing the same sanitary facility such as toilet and bathroom.

A) Bio-data of the respondent

1. Age category:
    a. 21-30 b. 31-40 c. 41-50 d. 51-60 e. 61-70 f. 71 above
2. Gender /Sex: Female/Male
3. Level of education:
    a. No education b. Primary c. Secondary e. Tertiary
4. Occupation:
    a. Formal Government Sector b. Formal Private Sector c. Informal Private Sector d. Unemployed
5. How many people are in your household?
    a. Male Adults.....
    b. Male Children.....
    c. Female Adults.....
    d. Female Children.....
    e. Total of all persons in the household.....

B) Accessibility to a household/public toilet facility

6. Do you have a toilet facility in your home? YES/NO
    a. If NO, where do you ´attend to nature´s call´?.....
    b. Do you pay to have access to a toilet facility outside your home? YES/NO
    c. If YES, how much do you pay?
    GH¢.....
7. How many times do you visit the toilet in a day? .....
8. Which type of toilet facility do you use in your home?
    a) KVIP b) WC connected to a septic tank c) Pit latrine with slab d) Others
9. How do you empty the toilet facility in your home when it gets full?
    .....
10. How much do you pay to empty it? GH¢ …….....

C) Energy source for cooking and fertiliser for agriculture

11. Which type of energy source do you use for cooking?
    a) LPG gas b) Firewood c) Charcoal d) Electric stove e) Others……..
12. a) If your answer in 12 is (a), how much LPG gas do you need/use per month? .....
    b) Would you use more of the gas if available?
    …….....
13. Did you know you can produce cooking gas from human faeces?
14. Would you use cooking gas produced from human faeces? YES/NO.
15. Please give reasons for your answer in (14) above……
    ……..
16. a) Do you use or need fertiliser? YES/NO
    b) How helpful is fertiliser?.....
    ……..
17. Did you know you can produce fertiliser from human faeces? YES/NO. If YES, how……
18. Would you use fertiliser produced from human faeces? YES/NO
19. Please give reasons for your answer in (18) above……
    ……..
20. a) Do you know of some health problems associated with human faeces if it is discharged without any treatment? YES/NO.
    b) If YES, please give details……..
    …….

D) Single-Stage Hyper-Thermophilic Anaerobic Treatment Facility (SSHATF) for human faeces and food waste

21. Have you heard of any ways human faeces can be treated to make it safe before discharge?.....
22. a) Have you heard that cooking gas can be obtained from human faeces and food waste? YES/NO
    b) If YES, how.....
23. a) Are you willing to use cooking gas produced from human faeces mixed with food waste if scientists prove it to be safe? YES/NO.
    b) Please, give reasons for the choice of your answer in (23a) above
    .....
24. a) Do you have idea if clean fertiliser can be produced from human faeces and food waste? YES/NO.
    b) If YES, Please explain……..
    .....
25. a) Have you heard of the application of treated human faeces on vegetables/crops in Ghana? YES/NO.
    b) If YES, please provide details.....
    c) Do you think it is a good idea to use treated human faeces in vegetable/crop farming? YES/NO.
    d) Please explain your answer in 25 (c).....
26. a) Will you consume vegetables produced with treated human faeces applied as fertiliser if they are scientifically proven to be safe? YES/NO.
    b) Please give reasons for your choice in 26 (a).....
27. a) Would you eat vegetables if scientists say it does not influence the taste? YES/NO.
    b) Please give reasons in 27 (a)……

E) Willingness to adopt and invest into SSHATF technology for human faeces treatment

28. Are you willing to adopt this technology in your home? YES/NO.
29. Please give reasons for your choice in 28 above.....
    ……..
30. How much are you willing to invest into having this technology in your home……
    GH₵….....

31. How will you pre-finance this investment to acquire this technology in your home?

.....

.....

32. How much do you earn in a month? GH₵ ……

Appendix 2: Schematic Diagram showing the set-up for the Batch Fermentation Tests