Enhancement of Anaerobic Digestion of Banana Waste by Reactor Design and Substrate Pre-treatment for Improved Biogas Production

By

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A Dissertation Submitted for the Award of the Degree of Doctor of Agricultural Sciences (Doktor der Agrarwissenschaften (Dr. agr.) of the University of Kassel, Witzenhausen Germany

University of Kassel

Dissertation **Witzenhausen 2021**

Date of defence: 23rd September 2021

Dedication

Dedicated to:
My dear wife; Mrs Rossette Katushabe Gumisiriza,
My Beloved Children: Joseph Godsend, Nobert Nobel, Valeria Josephine,
Livinus Elizabeth, Robert Brian and any others to come
&
To the scientific community all over the globe

Acknowledgement

I am very gratified to the RELOAD project, Makerere University, Uganda, for offering me the financial support and the entire unique opportunity to undertake and accomplish this study, while being part of the research team under the post-harvest loss reduction and value addition (RELOAD) Project in East Africa. This was a fantastic real journey that has not only broadened my horizons in knowledge and research career but a whole life changer!

I am specially thankful to my mentors; Prof. Dr. Oliver Hensel, Assoc. Prof. Dr. Susan Balaba Tumwebaze and MSc. Michael Hermann Hesse for all the subvention from reload project. Your mentorship, parental guidance, prayers, having open heart , being positive and having confidence in my abilities kept me motivated and focused up to this epitome of academic excellence. Your support is exceedingly appreciated and may almighty bless you and reward you abundantly.

My sincere and great appreciations go to my supervisors; Prof. Dr. Oliver Hensel, Assoc. Prof. Dr. Eng. Mackay Okure, Dr. Joseph Fuuna Hawumba and Dr. Peter. C. Vuzi for the inspiring guidance and dynamic supervision. Your thoroughness, thoughtful attitude and substance of a genius have made me grow professionally as well as personally.

My deepest and heartfelt appreciations go to my dear wife; Mrs Rossette Katushabe Gumisiriza and children; Joseph Godsend, Nobert Nobel, Valery Josephine, Livinus Elizabeth and Robert Brian for your sincere prayers, encouragement and patience towards the accomplishment of this study. You had long time of endurance and sacrifice for the success of this academic career and indeed nothing would have been possible without your care and love. Madam Rossette, thank you for keeping the family intact while I was away pursuing this study. May the heavenly Almighty God bless you abundantly and grant you all the desires of your heart (Psalms 20:4-5).

I am grateful to Rev. Dr. Florence Isabirye Muranga (Director), Dr. J. Bahati (Deputy Director) and entire management of PIBID and TBI–Bushenyi banana processing industry for being positive towards my research, especially their support during sampling. Special thanks to Dr. Eng. J. B. Kawongolo for well wishes in my academic career.

I acknowledge with great appreciations the crucial roles of members of Biochemistry Department, Makerere University, and different members of RELOAD family in Uganda, East Africa and Germany. The support of the lab technicians, fellow students and colleagues who helped me to accomplish my tasks, is highly appreciated. The interesting and inspiring discussions and social environments that I enjoyed with all of you comrades were brain refreshments and made a great positive effect on my research.

Finally, I extend my sincere gratitude to my parents, siblings and friends for their prayers, affirmative backyard and moral support. Candidly speaking, it's the power of the almighty God that brought me this far (1 Samuel 2:8) and hence glory back to God who strengthened me throughout this study.

Publications and preliminary remarks

This thesis is mainly based on the results presented in articles published as journals, conference posters, book of abstracts and manuscripts prepared for publication in peer-reviewed journals. These articles are referred to in the text by their chapter Arabic numbers as shown below.

Chapter 2: **Gumisiriza, R**., Hawumba, J.F., Okure, M., and Hensel, O (2017), Biomass waste-to-energy valorization technologies: A review case for banana processing in Uganda. Biotechnology for Biofuels 10:11. DOI 10.1186/s13068-016-0689-5.

Chapter 4*: **Gumisiriza, R**., Hawumba, J.F., Balyeidhusa, A..S.P., Okure, M., and Hensel, O (2019), Processing of East African Highland Green Bananas: Waste Generation and Characterisation as a Potential Feedstock for Biogas Production in Uganda, American Scientific Research Journal for Engineering, Technology, and Science; Global Society of Scientific Research and Researchers; ISSN (Print) 2313-4410, ISSN (Online) 2313-4402. http://asrjetsjournal.org/

Chapter 5: **Gumisiriza, R**., Hawumba, J.F., Okure, M., and Hensel, O. Design, Construction and Operation of a Novel Hybrid Upflow Anaerobic Sludge Blanket (hUASB) Reactor System for Enhanced Anaerobic Digestion of Green Banana Waste with Improved Biogas Recovery (Draft manuscript to be submitted).

Chapter 6: **Gumisiriza, R**., Hawumba, J.F., Kawongolo, J.B., Okure, M., and Hensel, O Optimisation of Operational Parameters of the Novel hUASB Reactor System for Enhanced Biomethanization of Banana Waste (Draft manuscript to be submitted).

Chapter 7: **Gumisiriza, R**., Hawumba, J.F., Vuzi, P.C., Okure, M., and Hensel, O**:** The Effect of Pre-Treatment of Banana Waste by Pre-Fermentation and Co-Digestion on Quality and Quantity of Biogas Production (Draft manuscript to be submitted).

* Part of this chapter was presented at the Tropentag 2017 annual conference as a poster and published in the book of abstracts, page 108.
Online: https://www.tropentag.de/2017/proceedings/proceedings.pdf

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List of acronyms

Acronym	Written in full
AD	Anaerobic Digestion
ASP	Aerated Static Pile
BW	Banana Waste
CH_4	Methane gas
COD	Chemical Oxygen Demand
C:N	Carbon to Nitrogen ratio
CSTR	Continuously Stirred Tank Reactor
D	Day
EC	Enzyme Code
EGSB	Expanded Granular Sludge Bed
HRT	Hydraulic Retention Time
hUASB	hybrid Up-flow Anaerobic Sludge Blanket
Kg	Kilogram
L	Litre
OLR	Organic Loading Rate
P_{H_2}	Hydrogen Partial Pressure
TOPs	Torrefied Pellets
UASB	Up-flow Anaerobic Sludge Blanket
VFA	Volatile Fatty Acids
VS	Volatile Solids
WtE	Waste-to-Energy

Brief abstract

This research study entitled “Enhancement of Anaerobic Digestion of Banana Waste by Reactor Design and Substrate Pre-treatment for Improved Biogas Production” prior noted that Banana industry in Uganda is grappling with a joint problem of lack of energy, especially for efficient drying of pulp, and large emission of banana waste. The study thus focused at investigating an appropriate option for recovery of energy from banana waste. The results showed that anaerobic digestion was the most appropriate technology for recovery of energy from banana waste. The study further developed a novel hybrid Up-flow Anaerobic Sludge Blanket (hUASB) reactor system that could optimally and efficiently recover biofuel in form of biomethane from banana waste. Conversion of such recovered biofuel could potentially produce a net energy of 675.12 kWh or 2,430.432 MJ per tonne of waste. Hence, anaerobic digestion of banana waste using the developed hybrid Up-flow Anaerobic Sludge Blanket (hUASB) reactor system could recover enough biofuel in form of biomethane which is able to produce sufficient and sustainable energy for safe drying of banana chips as well as conversion into electricity for powering the entire banana industry. Since the developed hybrid Up-flow Anaerobic Sludge Blanket reactor system can maximise energy recovery from biowastes, dissemination of this technology to the farmers dealing with agro-produce drying and processing is the future perspective to undertake. This study was funded by the RELOAD project, Makerere University, Uganda; and was part of bigger research team under the post-harvest loss reduction and value addition (RELOAD) Project in East Africa, supported by the Government of Germany.

1 General introduction

1.1 Background

Globally, nations at different stages of development are facing two major challenges; energy crisis and proper waste disposal (Ali *et al.,* 2014). In Uganda, one of the East African nations, banana industry is grappling with inadequate and expensive hydro-energy, and the high cost of imported petroleum products for fuel. The Uganda national research on banana industrialization through the Presidential Initiative on Banana Industrial Development (PIBID) has shown that conversion of banana waste into energy is among the top-most priorities for achieving low-cost technology for drying of banana pulp into chips prior to further processing into various value-added products. Banana waste (BW) is defined as the composite waste from both banana production and processing and comprises peels, rotten fruits, fruit-bunch-stem (stalk or the peduncle), leaves, fibers, pseudo-stem, and rhizome (Abdullah *et al.,* 2014). Banana industrialisation in Uganda is estimated to generate more than three million tonnes of banana waste annually (Spilsbury *et al*., 2002; Tumutegyereize *et al.,* 2011). Unfortunately, this banana waste is improperly managed through uncontrolled dumping, composting and to a small extent used as animal feeds. These activities are suspected to contribute to the spread of banana bacterial wilt disease (Kagezi *et al.,* 2006; Gumisiriza *et al.,* 2019), thereby calling for integration of eco-friendly waste-to-energy technologies into banana industrialisation development initiatives. This would be in line with the Uganda national development plan and vision 2040.

Besides, banana wastes are highly biodegradable and if dumped or left to decompose in uncontrolled manner emit large volumes of Green House Gases (GHGs) especially methane and carbon dioxide that are major drivers of climate change through global warming. Moreover, the leachate from BW dump sites contains high biological oxygen demand (BOD) and nutrients which if channelled into water bodies aggravate climate change through eutrophication (Muyodi *et al*., 2004; Chen *et al.,* 2013; Salemdeeb *et al.,* 2017). In addition to the environmental risks prior mentioned, management of banana waste by cultural methods such as direct use as mulches, compost manure and animal feeds are discouraged due to the association of such methods with the rapid spread of plant diseases like the devastating banana bacterial wilt (Tushemereirwe *et al*., 2001). Therefore there is a dare need to integrate eco-friendly and appropriate waste value-addition technologies with energy generation into banana industrialisation development initiatives. This will be in line with both; the Uganda vision 2040 and the Millennium Development Goal number seven (MDG7) that respectively, emphasises waste value-addition and ensuring environmental sustainability.

It should be noted that technologies for conversion of waste into renewable energy are often prioritized in efforts to mitigate the environmental pollution and greenhouse effect (Chynoweth *et al.,* 2001), owing to there being eco-friendly. In tandem with such technologies, a number of potential eco-friendly waste-to-energy (WtE) technologies that can be used in management of BW with energy generation have been reported by a number of researchers. They include anaerobic digestion (Tock *et al.,* 2010), pyrolysis and gasification (Abdullah *et al.,* 2014), bioethanol fermentation (Velasquez-Arredondo *et al.,* 2010; Graefe *et*

al., 2011 and Hossain *et al.,* 2011), and briquetting (Lee *et al*., 2011 and Sellin *et al.,* 2013). In recent past, research studies by Omulo *et al.,* 2018 on banana waste -value addition reported that using pyrolysis as waste-to-energy technology, banana waste (banana peels) can be a potential bio-resource for generation of biofuel in form of biodiesel as well as other high commercial value biochemicals such as bio-oil and bio-tar.
However, among these waste-to-energy technologies, anaerobic digestion has a superior advantage due to coupled energy (biogas) generation with production of plant organic fertilizer (bioslurry) at minimal net operational energy requirement. Moreover, since banana waste is a wet waste that is purely biodegradable with high concentrations of carbohydrates especially starch and lignocellulose, the net potential for production of energy in the form of biogas is high. The sludge produced in the anaerobic processes can safely be used as bio-fertilizer to boost crop production while the produced biogas can be used to power the industrial production processes thus offsetting the industrial energy needs. Other advantages of anaerobic digestion technology include reduction in wastes' pathogens and disease-spreading potential, smaller land suitability and decrease in waste's pollution potential to levels that are non toxic to the environment (Moody and Raman, 2001). Hence anaerobic digestion is the most promising waste-to-energy eco-friendly technology appropriate for treatment of BW.

Nevertheless, the use of banana waste as a substrate feed for biogas production is majorly limited to reports on co-digestion studies by Bouallagui *et al.,* 2005; Gomez *et al.,* 2006; Kirtane *et al*., 2009 and Tumutegyereize *et al.,* 2011. Moreover, these studies only investigated on the anaerobic digestion of banana peels alone. Anaerobic digestion of banana waste from mixed streams containing whole-damaged fruits, peels, peduncle, stem fibers, pseudo-stems and corms had never been investigated. In addition, the high ligno-cellulose content of waste substrate such as plant biomass has been reported to slow down the bio-gasification process primarily due to limited microbial hydrolysis of complex polysaccharides abundant in such waste (Patrick *et al.,* 2011). A study by Martin-Ryals, 2012 however, reported that an eco-friendly and inexpensive way of effective hydrolysis of ligno-cellulosic biopolymers can be achieved by microbial pre-treatment. Even then, lignocellulolysis has been reported to proceed at low rates with simple microbial pre-treatment methods (Martin-Ryals, 2012). This implies that effective microbial hydrolysis of such complex biopolymers can be achieved through synergistic interactions and co-metabolism involving fungal and bacterial strains (Yan *et al.,* 2012). Furthermore, the efficiency of anaerobic digestion can be enhanced through optimisation of bioreactor environmental conditions such as pH, temperature and concentrations of feed substrates (Bilibio, Hensel and Selbach; 2011), along with optimisation of operational parameters such as loading rate, pH and hydraulic retention time. Moreover, integration of optimised environmental and operational parameters with appropriate bioreactor systems gives the utmost AD process efficiency.
On the other hand, biogas plant defects mainly from technical and inappropriate designs may cause significant gas leaks, mainly methane that compromises the plant efficiency and overall economic value (Hensel, 2014). Typically, methane has a global warming potential of 21-56 times higher than that of carbon dioxide, and is estimated to contribute to 18-21% of the overall global warming (Ayalon *et al.,* 2001). Thus, the rising emissions of climate-impacting greenhouse gases from biogas plants can be mitigated through designing of a feed-tailored

biodigester/anaerobic bioreactor that is biogas leak-proof with measurement systems that enable easy and timely detection and quantification of the escaping gas. The increase in energy efficiency by the resulting technical improvement of the system components and the option of a uniform certification of biogas plants in terms of efficiency and amount of current gas outlet are economic as well as climate policy-relevant innovations (Hensel, 2014).

Most studies on waste biogasification and anaerobic treatment have been conducted with continuous stirred tank reactors, fixed film reactors, up-flow anaerobic sludge blanket (UASB) reactors and anaerobic fluidized bed reactors (Rajeshwari *et al.,* 2000). High rate anaerobic bioreactors such as UASB and continuously stirred tank reactor (CSTR) have been increasingly employed for agro-process, municipal and industrial wastewater treatment (Chan *et al.,* 2009). The up-flow anaerobic sludge blanket reactors like fluidized bed technology provides an opportunity for higher loading rates and resistance to inhibitors (Rajeshwari *et al.,* 2000). Additionally, separating acidogenesis from methanogenesis during anaerobic digestion allows the reactor to operate efficiently and at low costs without the associated environmental control problems (Nachaiyasit and Stuckey,1995; Uyanik *et al.,* 2002a,b). However, the anaerobic digestion of feed substrate from plant origin such as banana waste in conventional reactors including the high-rate bioreactors is generally nuisance and problematic due to physical nature of the substrate, since these fibre-rich plant biomass materials tend to build up a persistent float layer. Physically, the floatation of the feed substrate leads to wash out of active biomass (inocula seeding) that results into digester failure (German agency report, 2005). Generally typical biogas digesters in use today cannot efficiently digest lignocellulosic biomass from plant origin such as energy crops without modifications (Gumisiriza *et al.,* 2017; Leibniz Institute for Agricultural Engineering Potsdam-Bornim (ATB). A well designed biodigester should have the potential advantages of good stability under hydraulic shock loading, low sludge generation, low capital and operation costs coupled with mechanical simplicity (Bilibio *et al.,* 2011). Thus, in this study it was very imperative to design, engineer and optimise a novel hybrid UASB bioreactor system for improved recovery of biogas from banana waste.

1.2 The energy value of biogas

The energy value (or calorific value) of biogas can be defined as the amount of energy or power that can be obtained from one cubic meter (1 m^3) of biogas. The energy value of biogas is contributed by the biomethane since it is the combustible (fuel) component in the biogas. The percentage of methane in biogas on average is in the range of 55 – 70 Volume-% (Deublein and Steinhauser, 2011). Biogas with a methane content higher than 45 % is flammable; the higher the CH4 content the higher the energy value of the gas. The calorific value of biogas is in the range of 6.0 – 6.5 kWh/m3 but usually approximated to a net of 6.0 kWh/m3 which is equivalent to 21.6 MJ of energy (Vogeli *et al*., 2014). The net calorific value depends on the efficiency of the biogas burners or other appliances used to convert the biogas into energy. A gas generator, for example, can convert about 2 kWh out of 6.0 kWh/m3 into useable electricity while the remaining energy is emitted as heat. The calorific values of different common fuel sources have been highlighted in table 1.1 and their energy values compared to 1 m3 of biogas. As rule of thumb; roughly 10 kg (wet weight) of biowastes (e.g. kitchen and market waste) are needed to produce 1 m3 of biogas. This amount

of biogas contains approximately 21.6 MJ of energy, equivalent to 6 kWh of power (Vogeli *et al.*, 2014).

Table 1:1 Calorific value of different fuel sources as compared to 1 m^3 of biogas (Vogeli *et al.*, 2014)

Fuel Source	Approximate Calorific Value	Fuel Equivalent to 1 m^3 of Biogas
Biogas	6 kWh	1.00 m^3
Liquefied Petroleum Gas (LPG)	26.1 kWh/m^3	0.20 m^3
Natural Gas	10.6 kWh/m^3	0.60 m^3
Propane Gas	25.0 kWh/m^3	0.24 m^3
Hard Coal	8.5 kWh/kg	0.70 kg
Diesel, Kerosene	12.0 kWh/kg	0.50 kg
Dry Cow dung	5.0 kWh/kg	1.20 kg
Dry Wood	4.5 kWh/kg	1.30 kg
Dry Plant residues	4.5 kWh/kg	1.30 kg

1.3 Statement of the problem

In Uganda, banana processing into dried chips for banana flour production is grappling due to lack of cheap and sustainable energy for fruit pulp drying (Gumisiriza *et al.,* 2017). Moreover, the banana industry generates large quantities of banana waste that are a potential bio-resource for generation of energy (Tumutegyereize et al., 2011). Decomposition of these banana wastes in uncontrolled manner leads to spread of plant diseases (Gumisiriza *et al.,* 2019) and emission of green house gases such as carbon dioxide and methane (Salemdeeb *et al.,* 2017). Although anaerobic digestion is the most eco-friendly waste to energy option for abatement of such lignocellulose-rich waste with generation of renewable energy in form of biogas, the direct anaerobic digestion process is limited by the recalcitrance of the lignocellulose to microbial hydrolysis leading to low biogas yield (Patrick *et al.,* 2011). Besides, the anaerobic digestion of plant biomass including banana waste in conventional high rate bioreactors such as up-flow anaerobic sludge blanket bioreactors has been reported to often result into failure due to the build up of a persistent fibre-rich float layer that leads to early wash out of active biomass (German agency report, 2005). Generally typical biogas digesters in use today cannot efficiently digest lignocellulosic biomass from plant origin such as energy crops without modifications (Gumisiriza *et al.,* 2017). There had been no yet reported bioreactor system appropriately designed for efficient anaerobic digestion of lignocellulose-rich plant biomass such as banana waste. Therefore, this study was undertaken with the aim of improving biogas production from banana waste through enhanced microbial hydrolysis of lignocellulose and improved bioreactor design.

1.4 Significance of the study

Banana production in Uganda generates large quantities of banana wastes that have the potential for generating energy in form of biogas to run the banana processing industrialisation. Never the less, these biomass wastes were never used as substrates for anaerobic digestion due to low biogas yields resulting from poor digester designs and limited hydrolysis of ligno-cellulose abundant in such waste. Development of a technology for enhancement of biogas production from banana waste therefore motivates banana processing industry and banana farmers to generate an eco-friendly and sustainable energy needed for sufficient drying of banana fruit pulp. Sufficiently dried banana pulp chips have extended shelf life and can be safely stored without deterioration prior to industrial processing into banana flour for bakery, among other applications. Furthermore, the findings from this study also form a technological basis for large scale bioconversion and exploitation of abundant plant biomass by agro-industries and municipalities to generate their own energy and bio-fertilizer to boost agricultural production. Ultimately, this study contributes towards creating an efficient strategy for integration of agro-waste mitigation with production of renewable energy for economic growth.

1.5 Study objectives

1.5.1 Main objective

The main objective of this study was to investigate options for improving biogas production from banana waste through substrate pre-treatment and digester design

1.5.2 Specific objectives

1) To evaluate the appropriate waste-to-energy technology for harnessing energy from the banana waste **(Chapter 2).**

2) To determine the physico-chemical characteristics and biochemical methane potential (BMP) of banana waste from industrial processing of East African highland green bananas **(Chapter 4).**

3) To design a banana waste-tailored bioreactor system for enhanced anaerobic digestion of banana waste and improved production of biogas **(Chapter 5).**

4) To optimise the operational parameters of a bioreactor system treating banana waste for enhanced anaerobic digestion and biogas production **(Chapter 6).**

5) To investigate the effect of waste pre-treatment and co-digestion on anaerobic digestion of banana waste for enhanced biogas production **(Chapter 7).** .

1.6 Thesis structure

The above specific objectives (Section 1.5.2) were accomplished by carrying out research activities as explained in paragraphed road map below.

Chapter 1 explains the general background and magnitude of the problem that motivates the research carried out. It gives the real field situation, problem description as well as highlighting of the research objectives and activities carried out to achieve the research aims.

Chapter 2 is typically the state of the art and it elucidates the most appropriate waste valorization technology for recovery of energy (fuel) from banana waste. It also gives the comprehensive literature review on the potential methods to enhance the anaerobic digestion of biowaste including lignocellulosic plant biomass. This chapter has been published in a peer-reviewed journal as: Gumisiriza, R., Hawumba, J.F., Okure, M., and Hensel, O (2017), Biomass waste-to-energy valorization technologies: A review case for banana processing in Uganda. Biotechnology for Biofuels 10:11. (DOI 10.1186/s13068-016-0689-5).

Chapter 3 highlights the standard materials and methods used in experimentation and analysis of banana waste substrate, digester slurry and biogas composition. Physico-chemical analytical methods used to accomplish different activities are well explained in this chapter.

Chapter 4 entails characterisation of banana waste and determination of the waste's biochemical methane potential (BMP). Within this chapter, the physico-chemical and biochemical methane potential of banana waste is established. This chapter has also been published as: Gumisiriza, R., Hawumba, J.F., Balyeidhusa, A..S.P., Okure, M., and Hensel, O (2019), Processing of East African Highland Green Bananas: Waste Generation and Characterisation as a Potential Feedstock for Biogas Production in Uganda, American Scientific Research Journal for Engineering, Technology, and Science; Global Society of Scientific Research and Researchers; ISSN (Print) 2313-4410, ISSN (Online) 2313-4402. http://asrjetsjournal.org/

Chapter 5 focuses on developing a bioreactor system tailored to enhanced anaerobic digestion of banana waste with improved production of biogas. This chapter builds on the fact that anaerobic digestion of lignocellulosic waste such as banana waste in conventional reactors is problematic due to floatation and recalcitrance of the lignocellulose to microbial hydrolysis. To circumvent such challenges, this chapter focuses at developing an efficient bioreactor system for harnessing biogas from banana waste. The developed novel bioreactor system is termed as hybrid Up-flow Anaerobic Sludge Blanket (hUASB) reactor system. Its principle of operation and superior performance in anaerobic digestion of banana waste is explained in this chapter.

Chapter 6 describes the effect of the operational parameters on the performance of the previously developed novel hybrid Up-flow Anaerobic Sludge Blanket (hUASB) reactor system. Typically, the optimal hydraulic retention time and organic loading rate of the novel reactor system are determined in this chapter.

Chapter 7 aims at investigating the effect of co-digestion and pre-treatment on anaerobic digestion of banana waste for enhanced biogas production. This chapter elucidates the effect of co-metabolism of chicken manure with banana waste on the anaerobic digestion. It further expounds on the effect of bioaugmentation of banana waste with microorganisms native to banana waste dumpsite. Ultimately, this chapter explicates the optimised protocols for banana waste pre-treatment and co-digestion to enhance biogas yield from banana waste.

Chapter 8 highlights the overall findings of the entire research study and interlinks all the chapters into a complete and compact discussion. It also describes the optimal conditions that lead to enhanced biogas production from anaerobic digestion of banana waste. Finally this chapter illustrates the implication of the research findings to the energy needs for the banana industry in Uganda.

Chapter 9 points out key conclusions drew from the entire study and future investment perspectives to undertake as regards to the dissemination and application of the findings of the study. This chapter also highlights the fascinating scientific concepts that emerged out aside the set objectives and are recommended as a subject of future scientific research perspective worth undertaking. Study limitations that were encountered and out manoeuvred are expressed in this chapter.

Chapter 10 gives the summary of the entire study. It gives a brief description of what motivated the research, state of the art, methodology, results, key conclusions and recommendations for dissemination, application and investments as well as future scientific research perspective.

1.7 References

1. Abdullah, N., Sulaiman, F., Miskam, M.A and Taib, R. M (2014), " Characterization of Banana (*Musa spp.*) Pseudo-Stem and Fruit-Bunch-Stem as a Potential Renewable Energy Resource", International Journal of Biological, Veterinary, Agricultural and Food Engineering Vol: 8 No:8, 2014

2. Ali,N., Ubhrani, P., Tagotra, M and Ahire, M (2014), "A Step Towards Environmental Waste Management And Sustainable Biofuel (Ethanol) Production From Waste Banana Peelings" , American Journal of Engineering Research (AJER).Volume-03, Issue-05, pp-110-116

3. Ayalon, O., Avnimelech, Y., Shechter, M (2001), "Solid waste treatment as a high-priority and low-cost alternative for greenhouse gas mitigation", Environmental Management, 27, 697-704

4. Bilibio, C., Hensel, O. and Selbach, J (2011), "Sustainable water management in the tropics and subtropics (and case studies in Brazil)", Vol. 2 Jaguarão/RS, Fundação Universidade Ederal Do Pampa; Unikassel - Pgcult-UFMA 2011

5. Bouallagui, H., Touhami, Y., Cheikh, B. R and Hamdi, M (2005), "Bioreactor performance in anaerobic digestion of fruit and vegetable wastes: Review", Process Biochemistry 40; pp 989–995

6. Chan,Y.J., Chong, M. F., Law, C.L and Hassell, D.G (2009), "A review on anaerobic–aerobic treatment of industrial and municipal wastewater", Chemical Engineering Journal 155 (2009) 1–18

7. Chen, Y., Liu, C., Nie, J., Luo, X. and Wang, D (2013), "Chemical precipitation and biosorption treating landfill leachate to remove ammonium-nitrogen", Clean Techn Environ Policy 15, 395–399 (2013) https://doi.org/10.1007/s10098-012-0511-4

8. Chynoweth, D.P., Owens, J.M. and Legrand, R (2001), "Renewable methane from anaerobic digestion of biomass", Renewable Energy 22, 1–8

9. Deublein D. and Steinhauser A. (2011). Biogas from waste and renewable resources: an introduction, Wiley -VCH Verlag GmbH & Co. KGaA.

10. Dierks, S., Retz, S., Klaus, D., Supply, C., Hensel, O. und A. Kroll: Schwachstellenanalyse und fernmesstechnische Ermittlung von Methanemissionen aus Biogasanlagen (Weak spot analysis and remote methane emission measurement of biogas plants). gwf-Gas+Energie, 9, (690-695), 2016

11. German Agency for Renewable Energy. 2005. http://global.mongabay. com/news/bioenergy/2007/07/new-biogas-reactor-for-energy-crops. html.

12. Gómez X., Cuetos, M. J., Cara, J., Morán, A. and García, A. I (2006), "Anaerobic co-digestion of primary sludge and the fruit and vegetable fraction of the municipal solid wastes: Conditions for mixing and evaluation of the organic loading rate", Renewable Energy, Volume 31, Issue 12, Pp 2017-2024.

13. Graefe. S., Dufour .D., Giraldo .A., Muñoz , L. A., Mora P., Solís H., Garcés H., and Gonzalez A., "Energy and carbon footprints of ethanol production using banana and cooking banana discard: A case study from Costa Rica and Ecuador," Biomass and Bioenergy, vol. 35, no. 7, pp. 2640-2649, 2011

14. Gumisiriza, R., Hawumba, J.F., Balyeidhusa, A.S.P., Okure, M and Hensel, O (2019), "Processing of East African Highland Green Bananas: Waste Generation and Characterisation as a Potential Feedstock for Biogas Production in Uganda", American Scientific Research Journal for Engineering, Technology, and Science; Global Society of Scientific Research and Researchers; ISSN (Print) 2313-4410, ISSN (Online) 2313-4402; http://asrjetsjournal.org/

15. Gumisiriza, R., Hawumba, J.F., Okure, M and Hensel, O (2017) "Biomass Waste-to-Energy Valorisation Technologies: A Review Case for Banana Processing in Uganda" Biotechnology for biofuels 10:11. DOI 10.1186/s13068-016-0689-5

16. Hensel., 2014: Hermann Sewerin GmbH, FG Mess- und Regelungstechnik & FG Agrartechnik "Project report: Measurement of biogas emissions"; sponsored by Deutsche Stiftung Umwelt

17. Hossain A. B. M. S., Ahmed S. A., Alshammari A. M., Adnan F. M. A., Annuar M. S. M., Mustafa H., and Hammad N., (2011), "Bioethanol fuel production from rotten banana as an environmental waste management and sustainable energy," African Journal of Microbiology Research vol. 5, no. 6, pp. 586-598,.

18. Kagezi, G.H., Kangire, A., Tushemereirwe. W., Bagamba, F., Kikulwe, E., Muhangi, J., Gold, C.S., and Ragama, P. C., (2006), Banana bacterial Wilt incindece in Uganda. African crop Science Journal. vol. 14, No.2 pp 83-91

19. Kirtane, R. D., Suryawanshi, P.C., Patil, M.R., Chaudhari, A.B and Kothari, R.M (2009), "Optimization of Organic Loading Rate for different fruit waste during biomethanization", Journal of Scientific & Industrial Research Vol.68, pp 252-255

20. Lee H., and Smith Z., (2011), Feasibility of Biomass Fuel Briquettes from Banana Plant Waste, Cincinnati, USA.: Engineers Without Borders Greater Cincinnati Professional,.

21. Leibniz Institute for Agricultural Engineering Potsdam-Bornim (ATB): "Project report" Neues Hochleistungsverfahren zur Vergärung von Nachwachsenden Rohstoffen - Versuchsdurchführung.

22. Martin-Ryals, (2012), "Evaluating the potential for improving anaerobic digestion of cellulosic waste via routine bioaugmentation and alkaline pretreatment", A thesis Submitted in partial fulfillment of the requirements for the degree of Master of Science in Agricultural and Biological Engineering in the Graduate College of the University of Illinois at Urbana-Champaign, 2012

23. Moody, L.B. and Roman, D.R (2001), "A dual-anaerobic system for complete treatment of food processing waste", Journal of Agricultural Engineering Research 80, 293-299

24. Muyodi FJ, Rubindamayugi MST, Semesi AK (2004). Effects of water Hyacinth on the distribution of sulphate reducing bacteria in sediments of Lake Victoria. Water SA.30: 421-425.

25. Nachaiyasit, S and Stuckey, D. C (1997b), "The effect of shock loads on the performance of an anaerobic baffled reactor (ABR), and transient hydraulic shocks at constant feed strength. Water Research 31(11): 2747-2754.

26. Omulo, G., Banadda, N., Kabenge, I. and Seay, J (2018) "Optimizing slow pyrolysis of banana peels wastes using response surface methodologyGodfrey", Environ. Eng. Res. 2019; 24(2): 354-361. https://doi.org/10.4491/eer.2018.269

27. Patrick, F., Mtui, G., Mshandete, A.M and Amelia Kivaisi, A (2011). Optimization of laccase and manganese peroxidase production in submerged culture of Pleurotus sajor-caju, African Journal of Biotechnology Vol. 10 (50), 10166-10177.

28. Rajeshwari, K. V., Balakrishnan, M., Kansal, A., Lata, K. and Kishore, V.V.N (2000), “State-of-the-art of anaerobic digestion technology for industrial wastewater treatment”, Renew. Sustain. Energ. Rev. 4: 135 – 156.

29. Salemdeeb, R., zu Ermgassen, E. K. H. J., Kim, M. H., Balmford, A. and Abir Al-Tabbaa, A (2017), “Environmental and health impacts of using food waste as animal feed: a comparative analysis of food waste management options”, J. Clean Prod. 2017 Jan 1; 140: 871–880. doi: 10.1016/j.jclepro.2016.05.049

30. Sellin N., De Oliveira B. GMarangoni., C., Souza O., De Oliveira A. P. N, and Novais T. M. De Oliveira,(2013), “Use of banana culture waste to produce briquettes,” Chemical Engineering Transactions, vol. 32, pp. 349-354.

31. Spilsbury, J.S., Jagwe, J.N., Ferris, R.S.B (2002), “Evaluating the Marketing Opportunities for Banana & its Products in the Principle Banana Growing Countries of ASARECA (Uganda)”, International Institute of Tropical Agriculture. Foodnet. P: 60.

32. Tock J. Y., Lai C. L., Lee K. T., Tan K. T., and Bhatia S., (2010), “Banana biomass as potential renewable energy resource: A Malaysian case study,” Renewable & Sustainable Energy Reviews, vol. 14, no. 2, pp.798-805.

33. Tumutegyereize, P., Muranga, F.I., Kawongolo, J. and Nabugoomu, F (2011), “Optimization of biogas production from banana peels”, African Journal of Biotechnology Vol. 10 (79), pp. 18243-18251

34. Tushemereirwe, W. K, Kangire, A. Smith, J., Nakyanzi, M., Karyeija, R. Kataama, D and Musiitwa, C., (2001), An outbreak of Banana bacterial wilt in Mukono and Kayunga district: A new and devastating disease NARO/KARI

35. Uyanik S., Sallis P.J. and Anderson G.K., (2002), “The Effect of Polymer Addition on Granulation in an Anaerobic Baffled Reactor (ABR). I. Process Performance", Water Research, 36, 933-943.

36. Velasquez-Arredondo H. I., Ruiz-Colorado A. A., and De Oliveira Junior S., (2010) “Ethanol production process from banana fruit and its lignocellulosic residues: Energy analysis,” Energy, vol. 35, no. 7, pp. 3081-3087.

37. Vögeli Y., Lohri C. R., Gallardo A., Diener S., Zurbrügg C. (2014). “Anaerobic Digestion of Biowaste in Developing Countries: Practical Information and Case Studies”. Swiss Federal Institute of Aquatic Science and Technology (Eawag), Dübendorf, Switzerland.

38. Yan, L., Gao, Y., Wang, Y., Liu, Q., Sun, Z., Fu, B., Wen, X., Cui, Z and Wang, W., (2012), “Diversity of a mesophilic lignocellulolytic microbial consortium which is useful for enhancement of biogas production” Bioresource Technology 111 pp 49-54.

2 State of the Art

2.1 Introduction

2.1.1 Background

Globally, energy crisis and proper waste disposal are among the major challenges facing most nations (Ali *et al.,* 2014). Uganda is the second largest global producer of bananas after India and the leading in Africa (Tripathi *et al.,* 2008), with annual production estimated at 9.77 million tonnes (FAOSTAT, 2012). The most widely grown cultivars are cooking types belonging to the East African highland banana (EAHB) subgroup. The other banana cultivars grown in Uganda include the dessert bananas locally known as *Sukali Ndizi* and *Bogoya* and some other plantain cultivars for roasting such as *Gonja* and *Kivuuvu* while '*Kayinja*' and *'Kisubi'* are mainly for making local beer. The EAHB cooking banana (AAA-EA group), locally called *matooke,* is the leading staple food (Tumutegyereize *et al.,* 2011) with the annual production of over 6 million tonnes (Spilsbury *et al.,* 2002). Banana growing in Uganda is either cultivation by smallholders in association with other food crops at low densities (as shade trees for perennials such as coffee) or in commercial plantations at high densities in a monoculture system.

Banana processing in Uganda, like other agro-processing, relies mainly on costly imported petroleum products for energy. Cheap and sustainable energy is critically essential in banana processing for efficient drying of banana fruit-pulp into chips prior to processing into value-added products such as starch and flour for export as well as local food security. Scarlat *et al.,* (2015) pointed out that access to cheap, reliable and sustainable energy is an important factor that makes agricultural and industrial processes more efficient. For instance, in the processing of banana, energy would be required for processes such as: drying, milling and also in conversion of the flour into valuable products: starch, bread, and cakes, among others. Besides, energy is needed in households' utilities such as cooking, lighting, and refrigeration. The biggest challenge facing banana industry is the fact that banana-growing areas, that are concentrated in the rural as well as remote parts of the country, are not connected to the national electricity grid. This makes banana processing not only expensive but also rather incomplete as there many wastages. Typically, electricity distribution in Uganda is one of the lowest in Africa; estimated at only 9-12% of the total Ugandan population (Tillmans and Scheweizer, 2011; Lee, 2013) and at only 2-3% in the rural areas (Twaha *et al.,* 2016). This is complicated by the fact that most banana farmers have limited financial capacity to access modern solar energy technologies that would generate sufficient energy for industrial processing. Therefore, such limited and unreliable energy access translates into underutilization of the banana crop, excessive wastage, as well as emission of large volumes of banana waste, leading to the under-development of the banana industry. This, in turn, contributes to limited employment opportunities, and poverty that are the major impediments to economic growth (IRENA, 2012).

As already pointed out from the foregoing, banana production and banana-fruit processing are not only faced with energy scarcity and unreliability, but also they are accompanied by generation of vast quantities of waste. Banana Waste (BW) comprises the following

fractions: rotten/damaged fruits, peels, fruit-bunch-stem (stalks), leaves, fibers, pseudo-stem, and rhizome (Abdullah *et al*., 2014). These fractions of banana wastes are generated from both, banana production and fruit processing. The waste category generated from the former includes all the off-cuts such as pseudo-stem, leaves, fibers and rhizome that remain in the garden after harvesting fruit-bunches, while the latter generates residues such as peels, fruit-bunch-stem (stalks) and rotten/damaged fruits. Uganda's banana-fruit processing alone is estimated to generate more than three million tonnes of banana waste annually (Spilsbury *et al*., 2002; Tumutegyereize *et al.,* 2011), which means that it is possible to think of the waste as a resource for waste-to energy conversion. Nevertheless, banana waste is currently heaped to decompose in uncontrolled manner thereby emitting large volumes of Green House Gases (GHGs) especially methane and carbon dioxide that are major drivers of climate change. In addition, leachate from BW dump sites contains high biological oxygen demand and nutrients which if channelled into water bodies aggravate climate change through eutrophication (IRIN News, 2016). Since the main problem of banana industrialisation in Uganda is dual comprising: lack of cheap sustainable energy coupled with emission of large quantities of organic waste residues, yet the solution to these problems seems to lie in the ability to convert banana waste into valuable energy. The development of either new or the adaptation of existing waste-to-energy technologies would not only solve the energy needs of the banana industry, but would also eliminate the waste burden with its accompanying environmental pollution. This review explores the various waste-to-energy technologies and evaluates their suitability in the generation of energy for use in the banana processing industry.

2.1.2 Management of Banana Waste in Uganda

Banana waste comprises rejected fruits, peels, fruit bunch stems, leaves, pseudo-stems and fibres. The management of banana waste has been largely by cultural means such as: a) direct use pseudo-stems, fibres and leaves to mulch the plantations; b) banana peels, leaves and fruit-bunch stalk are composted for manure; and c) banana peels, rejected fruit fingers are fed to animals. However, cultural methods of managing banana wastes have recently been discouraged due to association with the rapid spread of plant diseases like the devastating banana bacterial wilt. Applying banana waste from infected banana plants into banana fields as mulches or compost manure is one of the leading means of transmitting banana bacterial wilt (Tushemereirwe *et al*., 2001; Kagezi *et al.,* 2006). There have been efforts towards utilizing of banana fibres in the production of such products as paper, rope, table mats and handbags (Preethi and Balakrishna, 2013; Mohiuddin *et al.,* 2014). Even these efforts are not economically viable since such products have very short life-span. Hence, utilisation of banana waste through energy conversions could be the most appropriate venture for Uganda's banana industrialisation.

2.1.3 Energy Requirement for Banana Processing

Banana processing in Uganda starts with cutting of mature banana fruit bunches from the pseudo-stems in the plantation. Subsequently, the fruit is de-bunched to separate fruit-fingers; the fingers are peeled to get the pulp; the pulp is sliced, and finally dried into banana chips. The banana chips serve as the raw material for industrial banana processing into value-added products such as starch and flour, for both export and local food security. The drying of banana fruit pulp into chips is the step that requires reliable energy in order to produce

consistently standard quality products. Moreover, it has been established (Roberts *et al.,* 2008; and Islam *et al.,* 2012) that the drying of banana pulp consumes more energy than that of other related fresh foods such as pineapples and potato. This is so because the activation energy (E_a) for diffusion of water in green banana is 51.21 KJ/mole which is higher than that for potato [32.24 KJ/mole], pineapple [35.17 KJ/mole], and grape seeds [30.45 KJ/mole] (Islam, 1984; Uddin and Islam, 1985; Roberts *et al.,* 2008; and Islam *et al.,* 2012). The differences in activation energy values can be attributed to the differences in chemical composition and cellular structure (Islam, 2012). In Uganda, the drying of banana pulp is done by directly spreading fresh banana fruit pulp on the mat and exposed directly to sunshine. Nevertheless, although Uganda is located on the equator, the number of hours of sunshine per day varies significantly depending on the season. During rainy season, there are few hours of sunshine that make the traditional drying method take many days resulting in the pulp either rotting, or infested with moulds that produce aflatoxins. Aflatoxin contamination is one of the major hindrances to the development of the banana industry as the products thereof would not meet the minimum standards for human consumption. Therefore direct sunshine drying, as done locally, does not meet the energy requirements for efficient and safe drying of the pulp for subsequent processing. Other options would be: a) the use of modern solar dryers. This, however, has not been massively adopted due to the high cost of installation; and b) hot air convection drying. This is one of the oldest methods that have been used to preserve agricultural products like banana (Samadi *et al.,* 2013) and relies on the flow of hot air over the sliced pulp. Its application is, however, hampered by the high energy of operation (Alibas, 2007; Koyuncu *et al.,* 2007; Lewicki, 2006; Motevali *et al*., 2011). Therefore the conversion of waste biomass to energy would offer a cheap and affordable alternative source of energy for drying the pulp by banana growers and processors.

2.1.4 Waste Valorisation: A Concept

Waste valorisation has been defined as the process of converting waste materials into more useful products such as chemicals, materials and fuels (Arancon *et al.,* 2013). Waste valorisation as a concept relies on the assumption that even after the intended use, the residue/waste still contains un-tapped polymeric substance that can be converted to either energy or other chemical forms. Such products make waste a valuable resource that should not be left un-harnessed. This concept is currently being applied on both synthetic as well as bio-waste, with promising success, and it is the basis of the current waste-to-energy (WtE) approaches. Moreover, due to the fast depletion of natural/primary resources, waste valorisation is not a luxury for academic exploration but rather a much needed technology for cost- effective and sustainable waste management options and generation of renewable energy as well as production of high-value chemicals such as ethanol and materials such as nano-bioplastics (Figure 2.1**).** Apart from renewable energy and high-values chemicals, waste valorisation offers additional advantages including: amelioration of waste mal-odours and environmental pollution, and reduction of the volume of waste, resulting in recovery of more space for other uses. In a typical process, high-value chemicals are produced from waste residues through any of the four downstream processing i.e. using inorganic and organic chemicals, a combination of chemicals and enzymes, biotechnological approach using genetically engineered organisms, and green processing technologies whereby only water is used as a reagent in waste volarisation (Arancon *et al.,* 2013).

Waste-to-Energy (WtE), defined as the process of recovering energy in the form of either electricity and/or heat from waste, (Bosmans *et al.,* 2013) applies the waste valorisation concept to generate renewable energy such as heat, and biofuels (biogas, syngas and bioethanol). Waste-to-Energy technologies are categorised into two major groups namely; a) thermo-chemical processes comprising combustion, pyrolysis and gasification; and b) biological processes comprising anaerobic digestion and bio-ethanol fermentation. These WtE technologies provide cheap sources of energy that is crucial for industrial processes such as drying, packaging and preservation of industrial products. As already highlighted, the banana industry releases a large volume of waste that is currently neglected and left to decompose in an uncontrolled manner. Besides, the development of this industry is hampered by both scarcity and costly energy inputs. The application of this volarisation concept, particularly the green processing options, would solve both of these hindrances to the banana industrial development. Scarlat *et al.,* (2015) reported that the energy content of such wastes as banana waste can be recovered by employing appropriate WtE technologies. A number of studies have been conducted to establish the best way to harness energy from banana waste. For instance, banana wastes has been used to make briquettes that store energy for further uses in industrial and domestic heating (Wilaipon *et al.,* 2009; Lee *et al.,* 2011; Sellin *et al.,* 2013). In a separate study, Tock *et al.,* (2010) applied direct combustion of pseudo-stems and leaves to generate heat energy. The green-processing option has been attempted (Clarke *et al.*, (2008) and Tock *et al.*, (2010) whereby microorganisms have been employed to anaerobically convert banana peels into methane, in one study, and banana fruits residues fermented into ethanol (Velasquez-Arredondo *et al.,* (2010); Graefe *et al.,* (2011); and Hossain *et al.,* (2011) in another study. Thus, recovery of energy from waste can play a role in minimising the impact of waste on the environment with the additional benefit of providing a local source of cheap energy (Scarlat *et al.,* 2015).

Figure 2:1 A scheme of green processing technologies for waste valorisation (Arancon *et al.*, 2013)

Development of innovative technologies with high WtE efficiencies is largely dependent on two major but inter-linked factors namely, the type of waste to be harnessed (Van Passel *et al.,* 2013) and the available legislation. The legislation for environmental pollution abatement compels the waste sources (industries) to employ the most eco-friendly technologies for waste management. In addition, the physico-chemical nature of the waste dictates the choice of the technology appropriate for treating such a waste. As already mentioned in the foregoing, the WtE options are most preferred due to recovery of energy that can offset the cost of waste treatment. The energy content of waste is usually recovered by means of either thermo-chemical processes such as combustion, pyrolysis and gasification or biological processes such as anaerobic digestion. A possible algorithm (Figure 2.2) for selecting or developing a suitable WtE technology has been described by Stehlik (2009). In this algorithm, the waste is first assessed for its suitability for thermal processing due to ease of application of thermal conversion technologies. Wastes that cannot be appropriately degraded by thermal means, the emitting industry either employs the existing non-thermal convenient technologies such as anaerobic digestion or supports research for development of new WtE technologies tailored to the type of waste emitted. On the other hand, wastes that are suited for degradation by thermal means are further evaluated for use as alternative fuels. Wastes that are not amenable for use as alternative fuel are degraded via incineration while for those that conform to use as alternative fuel are converted to energy via other WtE technologies such as pyrolysis, gasification as well as thermo-mechanical pulverisation to form refuse derived fuel. Furthermore, the algorithm supports the need for research and development of new technologies in order to either improve on the efficiency of the available technologies and/or innovate new appropriate WtE technologies for waste managent. These new technologies need to prove their economic viability prior to full-scale implementation. Generally, the simpler design has low propensity for technological failure.

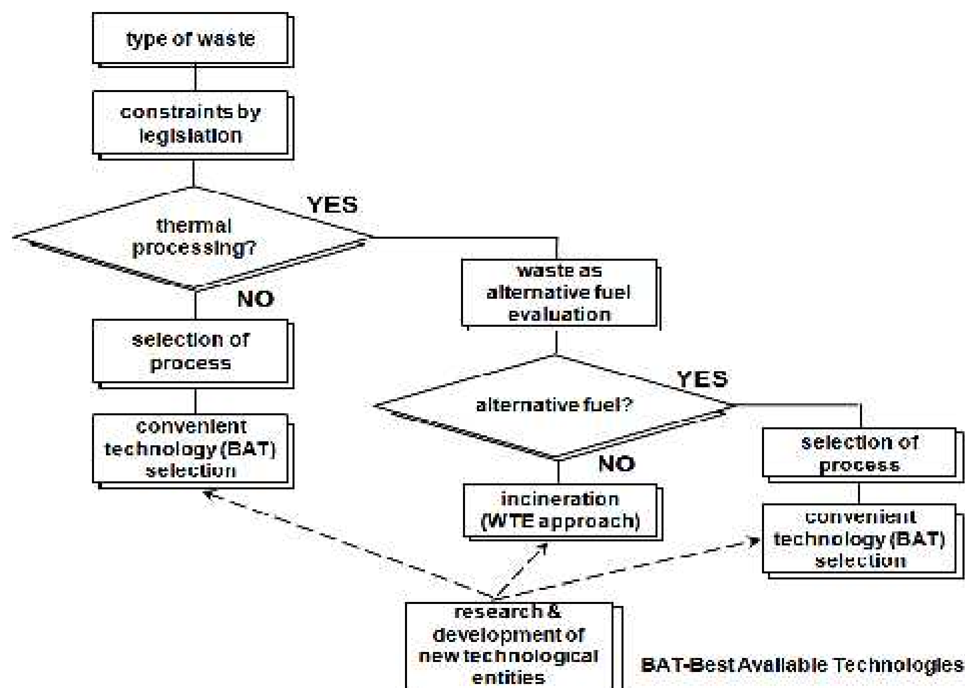


Figure 2:2 Algorithm for convenient WtE technology selection based on Stehlik (2009).

2.2 Potential waste to energy technologies for banana waste valorisation

The potential Waste to Energy (WtE) technologies that can be used in valorisation of BW can be grouped into: Direct Thermal (Direct combustion and Incineration); Thermo-chemical (Torrefaction, Plasma treatment, Gasification and Pyrolysis) and Biochemical (Composting, Ethanol fermentation and Anaerobic Digestion) (Bosman *et al.,* (2013) Figure 2.3. Generally, thermal technologies convert the waste directly into heat energy while thermo-chemical and biochemical ones first convert the waste into secondary energy carriers such as Syngas, torrefied pellets, biogas, bio-ethanol and bio-oil, which can subsequently be burnt (in furnaces, steam turbine, gas turbine or gas engine) to produce energy in form of heat and/or electricity. The conversion of solid wastes into secondary energy carriers allows for a cleaner and more efficient energy harnessing process.

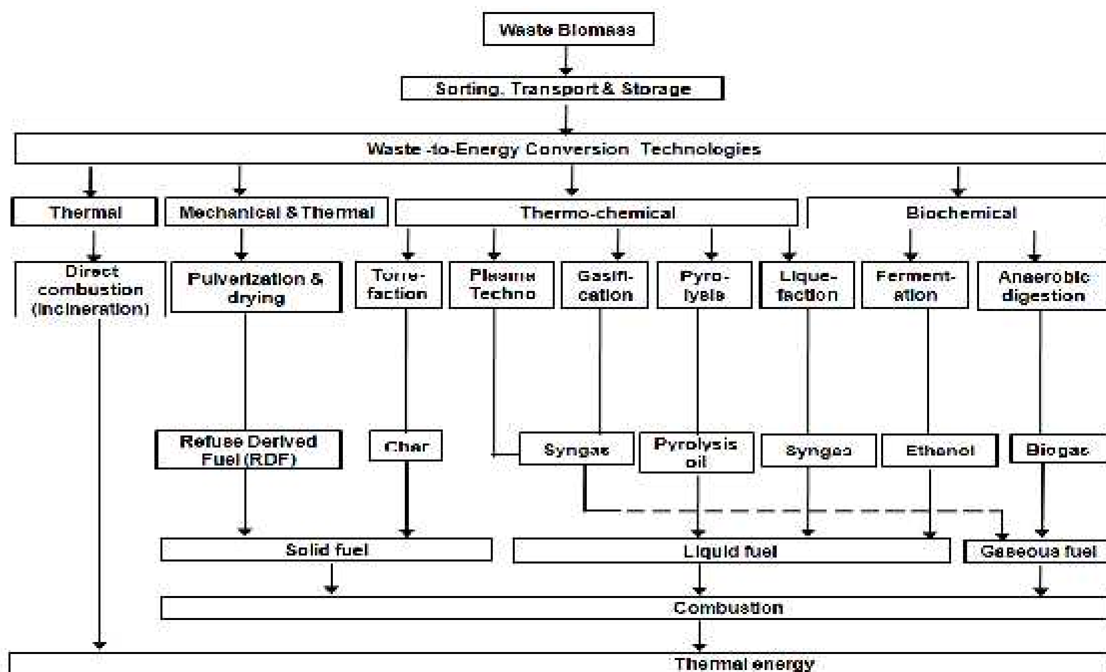


Figure 2:3 Potential WtE technologies for valorisation of banana waste (Bosmans *et al.,* 2013).

2.3 Direct thermal conversion technologies

This is the full oxidative combustion of waste biomass mainly to generate heat energy. This is done by either direct combustion or incineration. Direct combustion is the burning of biomass directly to convert chemical energy stored in plants into heat and electricity (Clini *et al.,* 2008). The direct burning of dry biomass to generate heat energy for mainly cooking and lighting has been practised globally for years. Dry banana waste such as leaves, fibres and fruit-bunch stems can be used as a source of heat energy in domestic cooking and industrial boilers. Industrially, biomass is burnt in the furnace to generate thermal energy that subsequently heats boiler to produce steam. The pressure of the steam can be used to turn a turbine that is attached to an electrical generator which subsequently generates electricity (Chambers, 2003). The potential of banana residue to be directly combusted for energy

generation strictly depends on its energy content or heating value (Tock *et al.,* 2010). However, banana residues have very high moisture content which lead to low net energy efficiency when combusted without prior drying process. Moreover, open burning of waste is particularly discouraged due to emission of harmful compounds such as dioxins, acid gases and furans that cause air pollution (Scarlat *et al.,* 2015). Hence, direct combustion is not a suitable technology for harnessing energy from banana biomass.

Waste incineration, on the other hand, is a full oxidative combustion of the waste in an engineered structure called an incinerator with the purpose of generating thermal energy and simultaneous destruction of pathogenic waste material under emission control. During incineration, the biomass is converted either directly into CO_2 and water vapour or indirectly into CO, H_2 and Char (Figure 2.4). The concentration of oxygen available for the process is the major determining factor. The direct step is favoured at higher oxygen concentrations while the latter occurs when there is limited oxygen supply. Waste incineration is common practice in the developed countries (EU, US, Japan) where waste-related policies limit waste disposal on land (Scarlat *et al.,* 2015). Although waste incineration appears simple and applicable for Uganda's banana processing waste, the technology can be challenged by a number of bottlenecks. The high capital, maintenance, and operation costs of waste incineration plants prevent the large scale application of this technology as an energy recovery option (UN-HABITAT, 2010; UNEP, 2013). As with direct combustion, incineration is also affected by the high moisture content of banana waste, which makes continuous and optimal plant operation difficult to achieve owing to the requirement of additional fuel to support the process. Besides, without proper controls, waste incineration can be highly polluting, generating harmful emissions, such as dioxins and heavy metals.

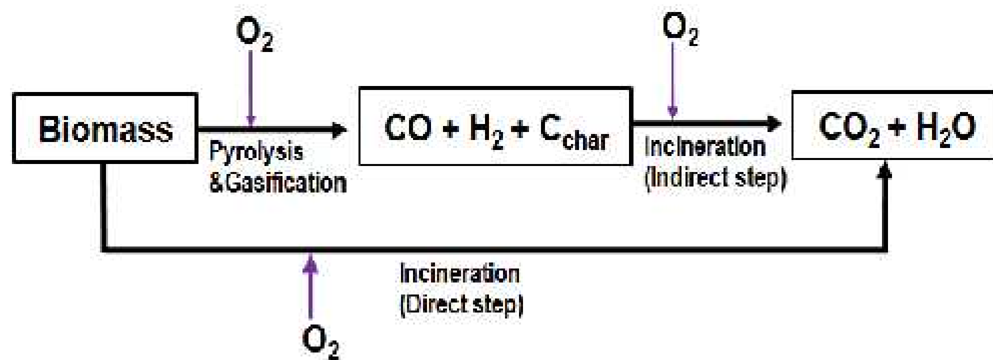


Figure 2:4. Key reaction steps and products from biomass combustion

2.4 Thermo-chemical conversion technologies

Unlike incineration and open combustion, thermo-chemical conversion technologies employ a series of chemical reactions occurring at different temperature and may require partial oxidation as in gasification or proceed in absence of oxygen as in pyrolysis. These conversion technologies are temperature depended and proceed through overlapping spatial and temporal stages of drying and degassing, pyrolysis and gasification and finally full oxidative combustion that turns the organic waste into ash (Figure 2.5). All these technologies require strict control of process conditions in specially designed reactors that are able to separate temperature accordingly. Without temperature separation and proper air rationing, thermo-

chemical reactions do not occur ultimately, turning the process into incineration or combustion.

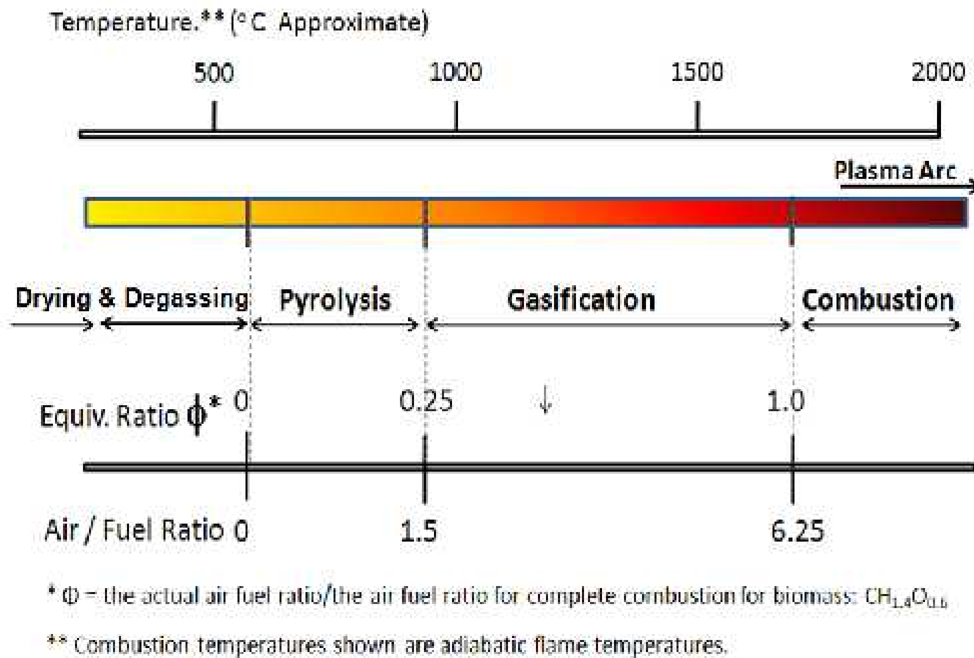


Figure 2:5. The temperature dependence and overlapping of thermo-chemical conversion technologies

Pyrolysis and gasification differ from incineration in that the former may be used for recovering the chemical value of the waste, while the latter is used to recover its energy value. The chemical products generated from pyrolysis and gasification may be either used as fuel to generate heat energy or as secondary feedstocks (char) for subsequent fuel generation (Figure 2.6). The products from incineration are generally non-fuel and include ash and flue gas that mainly consists of carbon dioxide and water vapour.

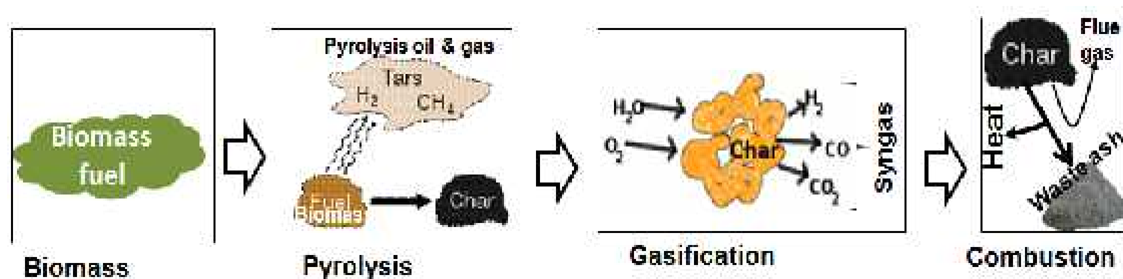


Figure 2:6. Sequential product generation during pyrolysis and gasification

Like incineration, pyrolysis and gasification also release carbon dioxide. A comparison of pyrolysis, gasification and combustion based on generated products is as shown in Table 2.1. The principles underlying the application of each of the thermo-chemical conversion technologies in harnessing energy from biomass are here-below described in detail:

2.4.1 Pyrolysis

Pyrolysis is the thermal degradation of organic material in the absence of oxygen. It occurs at relatively low temperatures (400-900 ^{0}C) (Bosmans *et al.,* 2013). In pyrolysis, biomass is subjected to an optimal temperature of 700 ^{0}C in the absence of oxygen resulting into production of pyrolysis oil (bio-oil), char and synthesis gas (Syngas). Syngas is a mixture of majorly CO, CO_2, H_2, H_2O, CH_4, trace amounts of higher hydrocarbons such as ethane and propane; as well as various contaminants such as small char particles. These can be used as secondary fuel to generate electricity. In a typical process the biomass is transformed into high quality fuel without creating ash or emitting large volumes of flue gas as in combustion. The process proceeds through the following basic process stages: 1) grinding to increase the surface area for improved heat transfer and reaction; 2) drying to increase the efficiency of gas-solid reactions within the reactor; 3) anoxic thermal degradation of organics to generate pyrolysis products (pyrolysis gas, bio-oil and char); and 4) ultimate secondary treatment of pyrolysis gas and pyrolysis char. The last step involves the condensation of the gases for the extraction of energetically usable oil mixtures and/or combustion of gas and char as secondary energy products. The major gases generated from pyrolysis are methane; carbon monoxide and hydrogen are shown by reaction equations 1 and 2 (Figure 2.7). Pyrolysis offers a flexible and attractive way of converting solid biomass into an easily stored and transportable fuel, which can be successfully used for the production of heat, power and chemicals. Pyrolysis gas, for example, may be used to power gas engines and gas turbines to generate electricity more efficiently than conventional steam boilers. Moreover, pyrolysis of biomass may lead to recovery of organic liquid fraction as fuel in form of methanol that can be distilled for use in various industries. Notably too, combustion of pyrolysis products emits smaller volumes of flue gas compare to direct combustion and incineration of biomass and hence pyrolysis reduces the flue gas treatment capital costs. Despite the advantages of pyrolysis, biomass with high ash content such as straw and banana waste are not good feed stocks for pyrolysis process due to reactor blockage by ash accumulation. Besides, pyrolysis is an expensive technology that requires high investment costs before it can be carried out commercially for energy harnessing.

2.4.2 Gasification

Gasification is a partial oxidation of organic substances at elevated temperature (500 ^{0}C - 1800 ^{0}C) to produce syngas. Biomass gasification occurs as the char reacts with carbon dioxide and water vapour (steam) to produce carbon monoxide and hydrogen via the reaction equations 3 – 6 (Figure 2.7). In addition, the concentrations of carbon monoxide, steam, carbon dioxide and hydrogen are balanced very fast at the temperatures in a gasifier via the equilibrium reaction equation 7 (Figure 2.7). Syngas can be used as a fuel for efficient production of electricity and/or heat (UBA, 2001). A gasifier can use oxygen, steam, carbon dioxide or a mixture of these as gasification agents.

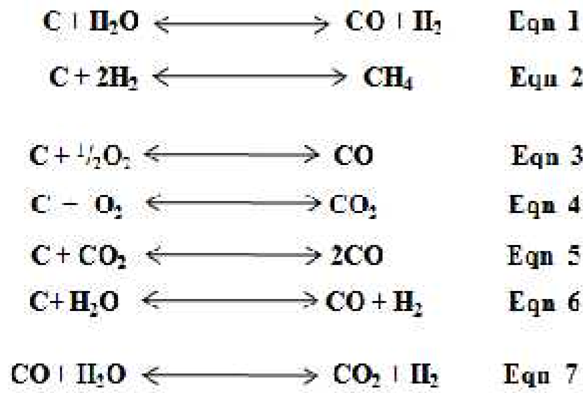
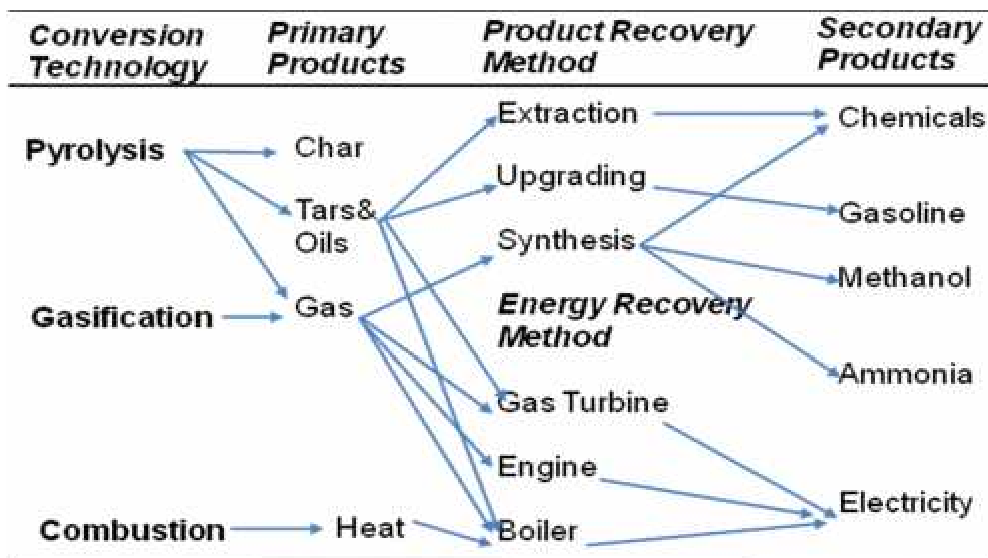


Figure 2:7. Major Reactions of pyrolysis and Gasification Conversion Technologies

On the other hand, banana waste being a wet biomass is not regarded as a promising feedstock for direct utilization or application of the conventional thermo-chemical gasification processes due to its high moisture content (Tock *et al.,* 2010). This problem can be circumvented by employing a recently developed technology referred to as supercritical water gasification (SCWG) whereby water is used as a reaction medium. In this technology, gasification of wet biomass may be accomplished without having to dry the material and thereby avoid the high processing costs associated with the drying process. Supercritical water gasification of wet biomass, as an advanced technology, has drawn the attention of a few research groups in the USA, Germany, Japan and the Netherlands (Tock *et al.,* 2010). The main advantage of using SCWG is that the technology does not require drying of wet biomass prior to gasification (Gasafi *et al.,* 2008). As a matter of fact, water in wet biomass is essential for the chemical reactions. Moreover, the SCWG of wet biomass results into high yields of hydrogen (H_2) and very low yield of carbon monoxide (CO) when compared to the ‘‘dry processes’’ in which syngas is produced with CO as the main product. Besides, in SCWG less tar and coke are formed and inorganic ingredients such as salts remain in aqueous solution, thus corrosion problem during gas treatment can be avoided. Nevertheless, SCWG is an expensive technology which requires high capital investment before put into operation.

Table 2:1. Thermo conversion processes and products (Adapted from Bridgwater, 1994)



2.4.3 Plasma Technology

Plasma technology relies on the physical principle that matter changes its state when energy is supplied to it: solids become liquid, and liquids become gaseous. When more energy is supplied to a gas, it is ionized and goes into the energy-rich plasma state, the fourth state of matter (Nandkumar, 2014). The initial energy required to create plasma can either be thermal or electric current or electromagnetic radiations. The presence of charged gaseous species makes the plasma highly reactive and causes it to behave significantly different from other gases, solids and liquids. The peculiar advantage of this technology is that the energy contained in the plasma allows the use of low-energy biomass that would otherwise not be suitable as feedstock for energy generation using gasification technology. The high temperature conditions that are reached in plasma results in the decomposition of organic compounds into their elemental constituents and ultimately forming a high-energy synthesis gas, constituted of mainly of hydrogen and carbon monoxide. Nevertheless, the application of plasma-based systems for waste management is challenging. For instance, the use of electricity as an initial energy vector is expensive, turning economic considerations into the strongest barrier for using plasmas for waste treatment. Moreover, the inorganic fraction (glass, metals and silicates) that is melted and converted into a dense, inert, non-leaching vitrified slug can be hazardous when released to the environment.

2.4.4 Torrefaction

Torrefaction is defined as the thermal upgrading of biomass into a more homogeneous product that is densified through pelletisation to generate a more energy-dense product called torrefied pellets (TOPs) or briquettes, with similar properties to coal (Batidzirai *et al.,* 2013). The energy derived from biomass through thermal upgrading (heating) is concentrated into an energy-dense and homogeneous product (TOPs) useful for further thermo chemical conversions (Yan *et al.,* 2010). Torrefaction technology is also referred to as mild-pyrolysis and is a thermochemical process conducted in the temperature range between 200 ^{0}C and 300 ^{0}C under an inert atmosphere and low heating rate (Medic *et al.,* 2012). The process involves biomass chipping to allow efficient drying; screening for impurities before sizing (Schorr *et al.,* 2012) and drying to 20% moisture content (Figure 2.8). A small fraction of the feedstock biomass is used as fuel for the drying and torrefaction process. Torrefied biomass (briquettes) which retains up to 96% of its chemical energy is hydrophobic and resistant to biodegradation. Therefore it can be used as substitute for coal/charcoal for domestic heating, co-firing power generation and gasification (Agar and Wihersaari, 2012; Boyd *et al.,* 2011; Phanphanich and Mani, 2011 and Prins *et al.,* 2006). A study by Sellin *et al.,* (2013), in the Northern region of Santa Catarina in Brazil, revealed that banana wastes including leaves and pseudo stems can be used to produce briquettes as fuel for energy generation. Briquettes produced from this waste at low cost are an excellent source of cheap renewable energy which is regarded as environmentally clean. Despite the potential of torrefaction technology, there are still several technical and economic challenges that need to be overcome before the technology is fully commercialised in banana industry (Nordin, 2012). Firstly, banana waste like other plant biomass is highly heterogeneous in quality and nature, and is mostly available in low energy density form (Delivand *et al., 2011*; Münster *et al.,* 2011 and Wannapeera *et al.,* 2012). Secondly, it has relatively high moisture content and consequently lower heating value compared to fossil fuels (Pimchuai *et al.,* 2010; Ben and Ragauskas, 2012; Chen and Kuo, 2011). It, therefore, needs to be pre-treated to improve handling (Rentizelas *et al.,* 2009;

Luo, 2011; Park and Jang, 2012). Pre-treatment such as pre-drying to 20% moisture content is energy consuming and significantly reduce the energy efficiency of the technology.

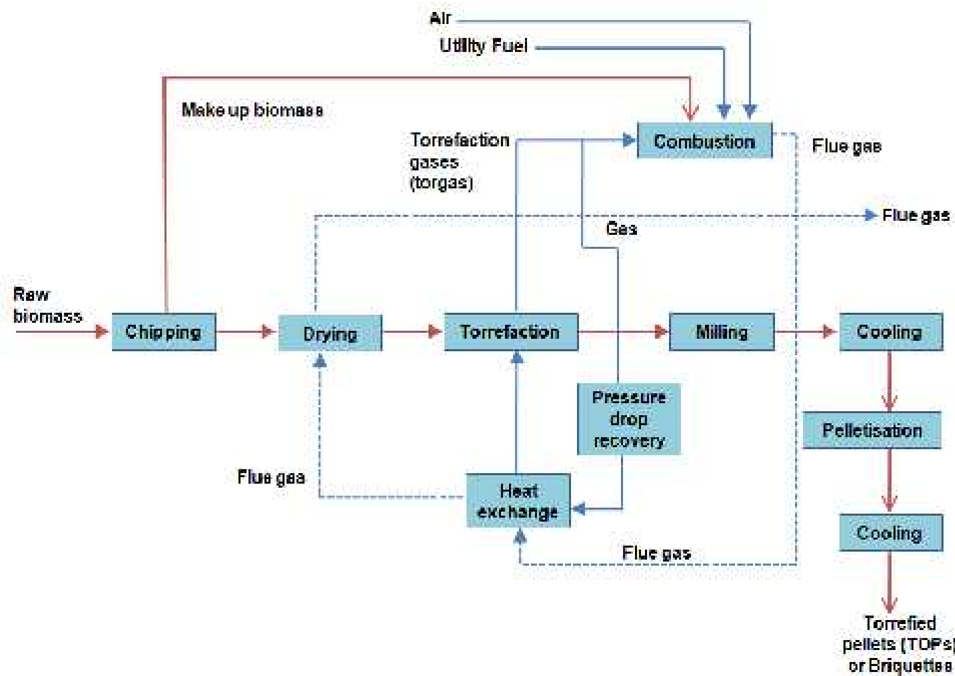


Figure 2:8. A flow scheme of an integrated torrefaction process based on Batidzirai *et al.,* 2013)

2. 5 Biochemical conversion technologies

Biochemical conversion technologies of waste-to-energy venture are much more eco-friendly as compared to the thermal and thermo-chemical techniques discussed in the foregoing. The advantages and disadvantages of different waste-to-energy technologies are highlighted in table 5.2. Biochemical conversion primarily involves the action of enzymes derived from microorganisms to harness the energy stored in biomass. The techniques falling under this category are: composting to generate heat energy, bioethanol fermentation and anaerobic digestion for biogas production.

2.5.1 Composting

Composting, defined as the biological decomposition of biodegradable solid waste under predominantly aerobic conditions, transforms the biomass into: carbon dioxide, water, heat and a more stable solid product called compost. The compost is nuisance-free, easy to handle, and can be safely used in agriculture to ameliorate the soil (Irvine *et al.,* (2010; Annepu, 2012; Kalyani and Pandey, 2014). Recently, there has been increased attention given to heat recovery from aerobic composting systems as a way to improve their economic viability (Smith and Aber, 2014). Generally, the composting process is optimized by having the starting carbon to nitrogen ratio in the range of 30:1 and the moisture and oxygen levels and temperatures that are closely managed and monitored (Fagundes, 2012). Three categories of microorganisms, namely, bacteria, actinomycetes and fungi are involved in the composting process. In the initial phase of composting, mesophilic microorganisms such as bacteria,

Bacillus, Clostridium, Alcaligenes, Serratia and *Pseodomonas,* degrade biomass. This is accompanied by generation of heat owing to their metabolic activities, causing the ensuing rise in temperature (≥ 45°C) in the composting heap. This gives way to the second phase, whereby thermophiles take over the composting process. Thermophilic fungi such as *Aspergillus fumigates*, *Humicola sp, sporotrichum thermophile* and *Myriococcum thermophilum,* and *Streptomycetes thermofuscus, S. Rectus, Nocardia sp,* and *Thermoactinomycetes sp* continue with the process until the temperature of ≥ 50°C is reached above which most of them are either inhibited or remain dormant as spores. Above 50°C themophilic bacteria belonging to such genera as *Bacillus* (*Bacillus stearothermophilus*), *Thermus*, *Clostridium* continue with the process to temperatures ranging from 60 to as high as 65 ^{0}C (Figure 2.9) and then starts to fall within a couple of months (Singh, 2011). This sets in the third and final phase of the composting process. During this final stage, the actinomycetes, initially, followed later by fungi proceed with the composting process until the temperature falls to mesophilic range, after which both mesophilc fungi and bacteria re-colonise the compost heap to complete the process.

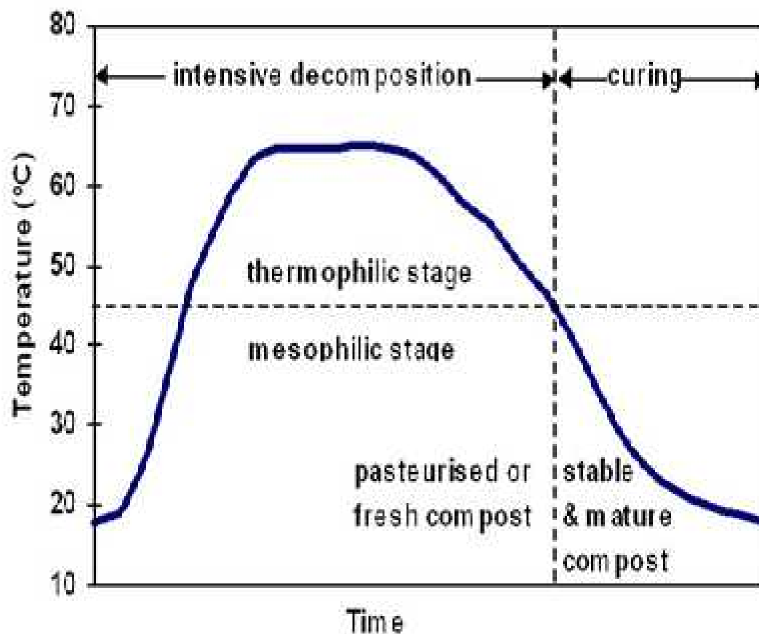


Figure 2:9. Heat generation during composting

The mechanism of heat transfer has been described by Shaw and Stentiford, (1996); Themeli, (2005) and Tucker, (2006) and involves convection and conduction, with radiation effects being assumed negligible. There are three components of energy balance namely; energy transfers into, within and out of a composting system which together equate to the change in energy stored within the system that ultimately dictates the temperature within the composting substrate. A study by Smith and Aber, (2014) reported an operational system capturing thermal energy in the hot air generated by the composting process, installed at research farm of University of New Hampshire (UNH) in the United States.

The system consists of an aerated static pile (ASP) of biomass or compost housed in a concrete insulated compost bay (Figure 2.10). The hot vapour from the ASP is collected through PVC pipes that passes through manifold and connects to the heat exchange system. The condensate from the manifold and heat exchange system is collected through condensate sump and ultimately pumped back to the ASP in the compost bay. The heat exchange system operates by blowing hot compost vapour (110-170ºF), against an array of two-phase super-thermal conductor heat pipes termed as Isobars. These Isobars are 30ft long containing within 24-inch diameter vapour duct and housed inside a 295-gallon water tank. Isobars provide thermal uniformity across the entire length of the pipe, thus heat energy is evenly distributed across the entire length of the pipe (Acrolab, 2013). When compost heated vapor is applied to the evaporator side of the pipe (portion contained within the 24-inch diameter pipe), the refrigerant inside the Isobar heats up and vaporizes. The vapor stream within the Isobar travels up the pipe, condensing on the cooler side, releasing its energy inthe bulk storage water tank through the latent heat of condensation. After condensing, the refrigerant is returned to the warm end of the pipe through gravity, repeating the process without any moving parts.

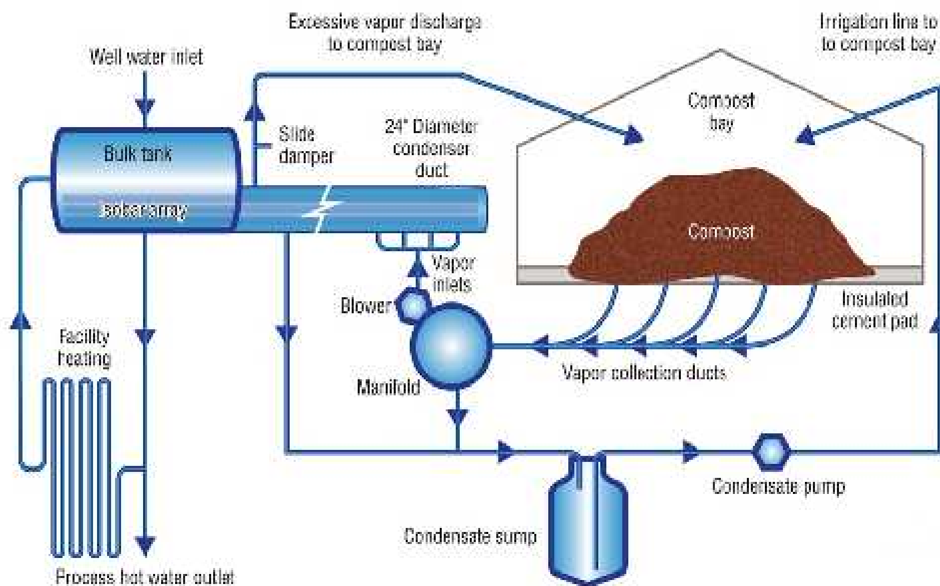


Figure 2:10. Flow diagram of UNH heat recovery system (Smith and Aber, 2014)

The system captures the metabolic heat produced by microorganisms during aerobic composting, through a negatively aerated fan system, and blows the hot compost vapour (110-170ºF) against the heat exchange system to heat water for radiant floor heating, feed preparation and sanitation of equipment. However, the success in application of composting technology to generate thermal energy has been scantily reported elsewhere in the world. Moreover, composting of mixed wastes generates low quality compost which can introduce heavy metals into human food chain.

2.5.2 Bioethanol fermentation

Ethanol produced from different renewable feedstock constitutes an alternative fuel for spark ignition engines (Velasquez-Arredondo *et al.,* 2010). This ethanol is considered as biofuel due to the vegetative origin of its carbon and, therefore, when it is released during the combustion process, it will not contribute to the increase in CO_2 emissions (Kadam, 2002; Hsieh *et al.,* 2002). The most suitable feedstock for ethanol production are high sugar-content crops such as sugarcane, sugar beets and fruits, since they majorly contain simple sugars such as glucose and fructose, that can be readily converted into ethanol by alcohol fermenting microorganisms (Ensinas *et al.,* 2009). Two groups of microbes: saccharolytic and ethanologenic, are important in ethanol production. These groups operate on the principle of co-metabolism, whereby, when saccharolytic microbes break down complex polymeric carbohydrates (starch, cellulose, hemicelluloses, etc) to simpler utilisable forms the ethanologenic converts them to ethanol. Many promising saccharolytic and ethanologenic microbes fall within, respectively, to the phyla Neocallimastigomycota and Ascomycota, for fungi, Proteobacteria and Fibrobacteres, for bacteria. Notably, *Saccharomyces cerevisiae*. (Ascomycota) and *Zymomonas mobilis* (Proteobacteria) are the only microbes naturally capable of producing ethanol close to theoretical maximum, with *Saccharomyces cerevisiae* predominant for current ethanol production based on starch and sugar feedstocks.

To enable cellulosic ethanol technologies, microbial capability and efficiency must be enhanced by appropriately designed mixed-culture systems and/or genetically modified microbes. Since banana associated residual biomass are generally starchy (amylaceous) and lignocellulosic materials; they can give high yields of glucose after successful hydrolysis which may further be fermented to produce ethanol. The conversion of starch-based crops such as corn, grains, and potatoes, among others, involves the enzymatic breakdown of strong 1,6 glycosidic bonds in starch into simple sugars (glucose) prior fermentation into ethanol (Shapauri *et al.,* 2002). On the other hand, lignocellulosic feedstock such as banana fruit-bunch stem contains cellulose, hemicellulose, and lignin which are more difficult to breakdown than starch and may require concerted efforts involving consortia of microorganism. While one consortium may breakdown the lignin wall, another may be required to hydrolyze the polymer into simpler units for the next consortium. Details of the interplay of these microbial consortia are covered below under the pre-treatment options. Nevertheless, the application of bioethanol fermentation as a waste to energy approach has limitations. For instance, conversion of biomass into bioethanol generates other forms of highly polluting wastes such as distillery slope that cannot be directly applied to the fields as biofertilizer or bioslurry. Moreover, the use of bioethanol as engine fuel for generating electricity negatively affects the electric fuel pumps by increasing internal wear and undesirable spark generation. In addition, ethanol is hygroscopic a property that makes it absorb water from air leading to high corrosion progression of energy generating engines and power machines (Masjuki and Kalam, 2013).

Table 2:2. Advantages and disadvantages of different WtE technologies (Kalyani and Pandey, 2014)

Technology	Advantages	Disadvantages
Anaerobic digestion	•Energy recovery with production of high grade soil conditioner •No power requirement for sieving and turning of waste pile •Enclosed system enables trapping the gas produced for use •Controls GHG emissions •Free from bad odor, rodent and fly menace, visible pollution •Compact design needs less land area •Net positive environmental gains •Can be done in small scale	•Unsuitable for wastes containing less organic matter •Requires waste segregation for improving digestion efficiency
Landfill with gas recovery	•Least cost option •Gas produced can be utilized for power generation or direct thermal application •Skilled personnel not required •Natural resources are returned to the soil and recycled •Can convert marshy lands to useful areas	•Surface runoff can cause pollution •Soil and groundwater may get polluted by the leachate •Yields only 30%–40% of the total gas generated •Large land area required •Significant transportation costs •Cost of pre-treatment to upgrade the gas to pipeline quality and leachate treatment may be significant •Spontaneous explosion due to methane gas build up
Incineration	•Most hygienic & suitable for high calorific value waste •Units with high throughput and continuous feed can be set up •Thermal energy for power generation or direct heating •Relatively noiseless and odorless •Low lands are required •Can be located within city limits, reducing transportation costs	•Least suited for aqueous, high moisture content, low calorific value and chlorinated waste •Toxic metal concentration in ash, particulate emissions, SOx, NOx, chlorinated compounds, ranging from HCL to dioxins •High capital and O&M costs •Skilled personnel required
Pyrolysis/ Gasification	•Production of fuel gas/oil, which can be used for various purpose •Superior Control of pollution as compared to incineration	•Net energy recovery may suffer in waste with excessive moisture •High viscosity of pyrolysis oil may be problematic for during transportation

2.5.3 Anaerobic Digestion (AD)

2.5.3.1 Biochemical and Microbial Fundamentals of Anaerobic Digestion

Anaerobic digestion (AD) is the anoxic biological decomposition of organic matter by a complex microbial ecosystem through parallel sequences of metabolic pathways involving different kinds of synergistic microbial trophic groups leading to the formation of methane and carbon dioxide (Gumisiriza, 2009). The mixture of methane and carbon dioxide is referred to as biogas (Cirne, 2006). Anaerobic digestion offers the opportunity to produce renewable energy and a higher quality of treatment for agro-waste. The technology has recently become an attractive method in Europe for the biodegradation of organic fractions derived from municipal solid waste (Scarlat *et al.,* 2015). The AD process is driven by concerted action of highly varied microbial population, consisting of several groups of both strict and facultative bacterial strains. The process is carried out in well designed vessel referred to as anaerobic digester/anaerobic bioreactor. The entire system consisting of the feedstock, digester, biogas holder and digestate reservoir is called a biogas plant. The complete AD process of a ligno-cellulose rich substrate such as banana waste can be divided into four main stages (Figure 2.11) namely: hydrolysis, acidogenesis (or fermentation), acetogenesis and methanogenesis.

Stage one: Hydrolysis

During hydrolysis, the insoluble complex biopolymers such as polysaccharides, proteins and lipids are broken down into simple soluble monomeric biomolecules such as sugars, amino acids, fatty acids and glycerol. It should be noted that organic wastes are a complex mixture of mainly carbohydrates (starch cellulose, hemicellulose), proteins and lipids; with their relative concentrations being dependent on the nature and origin of the waste. Owing to their structural complexity, the bio-polymers are not only too large for microbial uptake through the cell membrane for the subsequent intracellular biotransformation steps, they are also either sparingly soluble or completely insoluble in aqueous medium. Therefore, in order to utilise these biopolymeric organics, uptake must hydrolysed them to smaller units and solubilised, to enable membrane uptake and their availability to further metabolic degradation.

Biopolymer hydrolysis is accomplished by means of extracellular hydrolytic enzymes such as laccase, cellulases, amylases, proteases, and lipases, which may be either secreted into the environment or secreted but remain bound to cell membrane as protuberances (Morgenroth *et al.,* 2002; Marta-Alvarez, 2003; Parawira *et al.,* 2005a). In the digester system, both mesophilic and thermophilic microbes work synergistically to hydrolyze the biopolymers into simple units (oligomers and monomers). For instance, after the pre-treatment step, the lignin layer would have been removed thereby exposing cellulose, which is a substrate to a number of bacterial genera in the digester. *Clostridium Acetivibrio*, *Bacteroides*, *Selenomonas,* and *Ruminococcus* are some of the most common hydrolytic bacteria in the anaerobes bioreactors (Balagurusamy and Ramasamy, 1999; Balagurusamy, 2007).

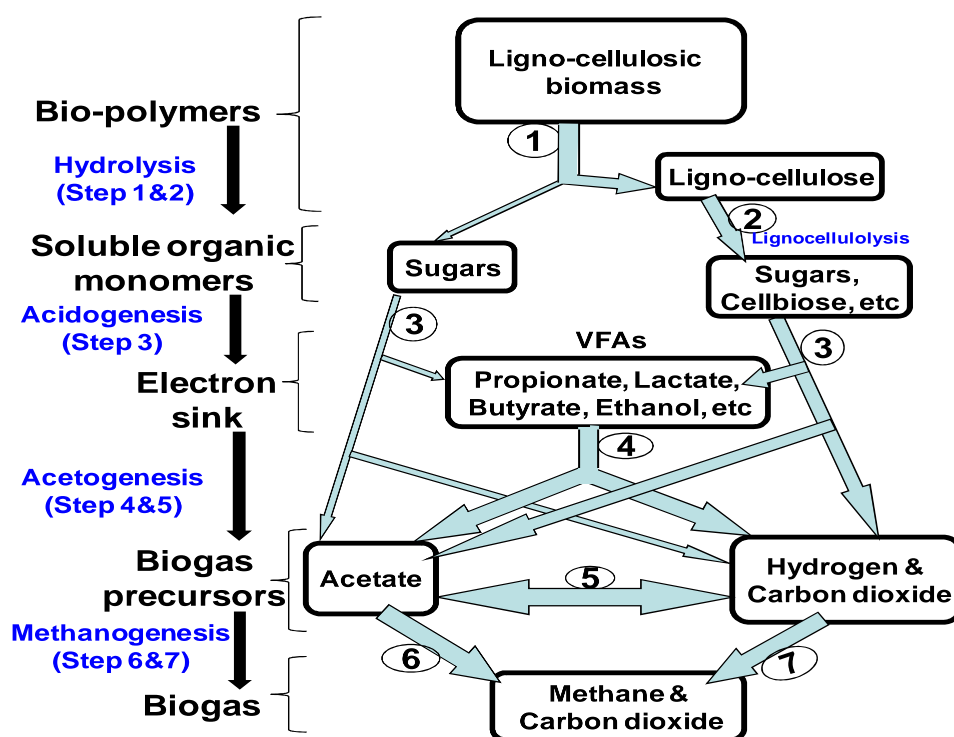


Figure 2:11. Scheme of anaerobic biodegradation process of lingo-cellulosic substrate 1. Hydrolysis; 2. ligno-cellulolysis; 3. Acidogenesis (fermentation); 4. Acetogenesis; 5. Homo-acetogenesis; 6. Aceticlastic methanogenesis; 7. Reductive methanogenesis (adapted from Matta-Alvarez, 2003; Parawira, 2004 and Cirne, 2006).

In the rumen, the most similar natural environment to biodigesters, *Ruminococcus albus* and *R. flavefaciens* are the predominant gram-positive, fiber-degrading bacteria, while *Fibrobacter succinogenes* is the most abundant Gram-negative (Wanapat and Cherdthong, 2009). Typically, hydrolytic bacteria adhere to the substrate particles, which subsequently induce the production and secretion of the specific hydrolytic enzymes. Starch is broken down by a mixture of amylolytic enzymes that hydrolyse the α-1,4 and α-1,6 glucosidic bonds of amylose and amylopectin. This enzyme mixture include α-and β-amylase, which exhibit specificity to α-1,4 glucosidic bonds, and glucoamylase (amyloglucosidase), which exhibit specificity to both the α-1,4 and α-1,6 glucosidic bonds (Lehninger *et al.,*1993; Bobleter, 1998). Starch hydrolysis releases a mixture of sugars; notably maltose and glucose. On the other hand, cellulases; which are sub-divided into three main groups namely: endocellulase or endo-β-1,4-D-glucanase, (EC 3.2.1.4), exocellulase or exo-β-glucanase, also called cellobiohydrolase (EC 3.2.1.91) and β-glucosidases (EC3.2.7.21), are also secreted by microorganisms in the digester. The degradation of cellulose is effected by the cooperative action of both endocellulase and exocellulases, whereby, the endocellulases randomly hydrolyze internal glycosidic linakages, which is accompanied by a rapid decrease in polymer length and gradual increase in the reducing sugar concentration, while the exocellulases hydrolyze the oligosaccharides released by the endocellulases to produce cellobiose from a non-reducing end. Completed hydrolysis is achieved when β-glucosidase hydrolyzes cellobiose to glucose monomers (Hreggvidsson *et al.,* 1996; Li et al., 2003). The cellulase enzyme system is enclosed in a cellulose-binding multicellulase-containing protein complex called a cellulosome. The cellulosome is responsible for the adherence of the bacterial cell to cellulose and to hydrolyze the cellulose thereafter. It should also be noted that

the cellulosome complex retains the ability to bind to and hydrolyze cellulose when present in the extracellular medium as it does when it is cell-bound (Bayer *et al.,* 1985; Bayer *et al.,* 1985). Similar surface structures exist among different cellulolytic bacteria. Typical examples include: a) glycocalyses, which have been observed in rumen bacteria, b) fibrous and membranous structures of *Bacteroides succinegenes* and c) spherical bodies, vesicular structures, lobes, and tubelike appendages, which have been observed in *Ruminococcus albus*. The presence of these structures strongly supports the widely held view that a single enzyme is incapable of extensive solubilisation of complex substrates, but rather, multiple enzyme system that act synergistically are required (113). Micro-organisms produce both intracellular and extracellular proteases contemporaneously (Harwood, 1992). As with other classes of enzymes, proteases likewise, play major roles in microbial physiology and as such, their production is highly regulated to suit particular needs. The synthesis of extracellular proteases, for example, is also tightly regulated. Their production has been linked to their participation in physiological activities such as sporulation (Priest, 1977), cell wall turnover and autolysis (Stephenson *et al.,* 1999), nutrition and overall protein turn-over (Mala *et al.,* 1998). Lipases (triacylglycerol acylhydrolase; EC.3.1.1.3) hydrolyze lipids or triacylglycerols to diacylglycerides, monoacylglycerols, fatty acids and glycerol. In comparison, hydrolysis of proteins and lipids is faster (Ortega-Charleston, 2008) Proteins are generally hydrolyzed to amino acids by proteases. Microorganisms that are responsible of this reaction include species of the genera *Bacteroides, Butyrivibrio, Clostridium, Fusobacterium, Selenomonas*, and *Streptococcus* (Amani *et al.,* 2010).

Stage Two: Acidogenesis

In acidogenesis, soluble monomers: simple sugars, amino acids, glycerol and fatty acids released from the hydrolysis stage, are biodegraded by fermentative organisms and anaerobic oxidizers (β-oxidisers) to produce different organic acids. Representatives of domain Bacteria, especially microbial genera inhabiting the rumen: *Clostridium, Eubacterium*, and *Bacteroides,* are largely responsible for acid generation. Fermentative species typical of the rumen include species of *Clostridium* and *R. Albus* (Sivakumaran *et al.,* 1991; Delbes *et al.,* 2000), while *Streptococcus* sp., *Lactobacillus* sp. and *Propionibacterium* are also fermentative microorganisms associated with the biodigesters, probably originating from the environment. Their degradative products of metabolism include acetate, lactate, ethanol, CO_2 and H_2 (Insam *et al.,* 2010).
On the other hand, the de-amination process in the degradation of amino acids also produces ammonia. Microbial fermentation of glucose and 5-carbon atoms sugars such as xylose and ribose mainly proceed through Embden Meyer-hof Pathways (EMP), generating pyruvate as an intermediate pathway product. However, the formation of pyruvate depends on the conditions prevailing in the bioreactors and the microbial species present. Pyruvate is a central molecule in terms biochemical interconversions and can be converted into different compounds such acetate, propionate, butyrate, formate, lactate, alcohols, ketones and aldehydes (Pavlostathis and Giraldo-Gomez, 1991). The amino acids originating from protein hydrolysis can be degraded either through fermentation following either stickland reactions or via anaerobic oxidation linked to hydrogen production. The protein biodegradation products are volatile fatty acids (VFAs), ammonia, sulphide, carbon dioxide and hydrogen depending on the amino acid present, microbial diversity and the path way. Butyrate and

valerate are typical products of valine and leucine amino acid biodegradation (Bryant, 1979; Mclnery, 1988; Nagase and Matsuo, 1982). The acidogenic microbial population can constitute up to 90% of the total microbial populations present in the anaerobic digesters (Pereira, 2003). These microbes have a short doubling time that makes acidogenesis not regarded as a limiting step in the process of anaerobic digestion.

Stage three: Acetogenesis

Acetogenesis is the degradation of reduced fermentation intermediates (electron 'sink') from the previous stage, i.e. volatile fatty acids (VFAs) such as propionate and butyrate to acetate, carbon dioxide and hydrogen by obligate hydrogen producing acetogens (OHPA). This intermediate bioconversion is a crucial process for the successful production of biogas; since these compounds cannot be utilized directly by methanogens. However, the acetogenic reactions (Table 2.3) are not energetically feasible under standard conditions because the reactions are energy consuming (endothermic; +ve values of ΔG). Therefore, a syntrophic microbial interdependency is required for the reactions to proceed.

Table 2:3. Free energy values of some key acetogenic and methanogenic reactions of anaerobic digestion (Adapted from Matta-Alvarez, 2003; Cirne, 2006)

AD step	Reaction	G^0 (kJ mol^{-1}) *
Acetogenesis		
Propionate → Acetate	$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + H^+ + HCO_3^- + 3H_2$	+76.1
Butyrate → Acetate	$CH_3CH_2CH_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + H^+ + 2H_2$	+48.1
Ethanol → Acetate	$CH_3CH_2OH + H_2O \rightarrow CH_3COO^- + H^+ + 2H_2$	+9.6
Lactate → Acetate	$CH_3CHOHCOO^- + 2H_2O \rightarrow CH_3COO^- + H^+ + HCO_3^- + 2H_2$	-4.2
Formate → Acetate	$2HCO_3^- + 4H_2O + H^+ \rightarrow CH_3COO^- + 4H_2O$	-104.6
Methanogenesis		
Acetate → Methane	$CH_3COO^- + H_2O \rightarrow HCO_3^- + CH_4$	-31.0
H_2/CO_2 → Methane	$4H2 + CO_2 \rightarrow CH_4 + 2H_2O$	-131.0
Formate → Methane	$HCO_3^- + 4H_2 + H^+ \rightarrow CH_4 + 3H_2O$	-135.6

* **Temperature 298K, pH 7, 1M for solutes and 1 atm for gases**

According to Björnsson (2000) and Cirne (2006), the reactions become feasible when the hydrogen partial pressure (P_{H_2}) is low (10^{-4}-10^{-5} atm). Acetogens are slow growing microorganisms and depend on a low hydrogen partial pressure in order for acetogenic biodegradation to yield energy required to move the reaction forward (Björnsson, 2000). This low (P_{H_2}) is achieved by the syntrophic association of obligate hydrogen-producing

acetogens (OHPAs) with hydrogen-consuming bacteria (hydrogen scavengers) such as the hydrogenotrophic methanogens (Schink, 1997). However, the thermodynamic feasibility of acetogenic reactions is inversely proportional to that of methanogenic reactions. This means that hydrogen producing acetogenic reactions become more favourable at low P_{H_2} (Figure 2.12) whereas hydrogen-consuming methanogenic reactions become less favourable at the same PH_2. Thus, syntrophic reactions occur within a narrow range of very low P_{H_2} (between 10^{-4} and 10^{-5} atm).

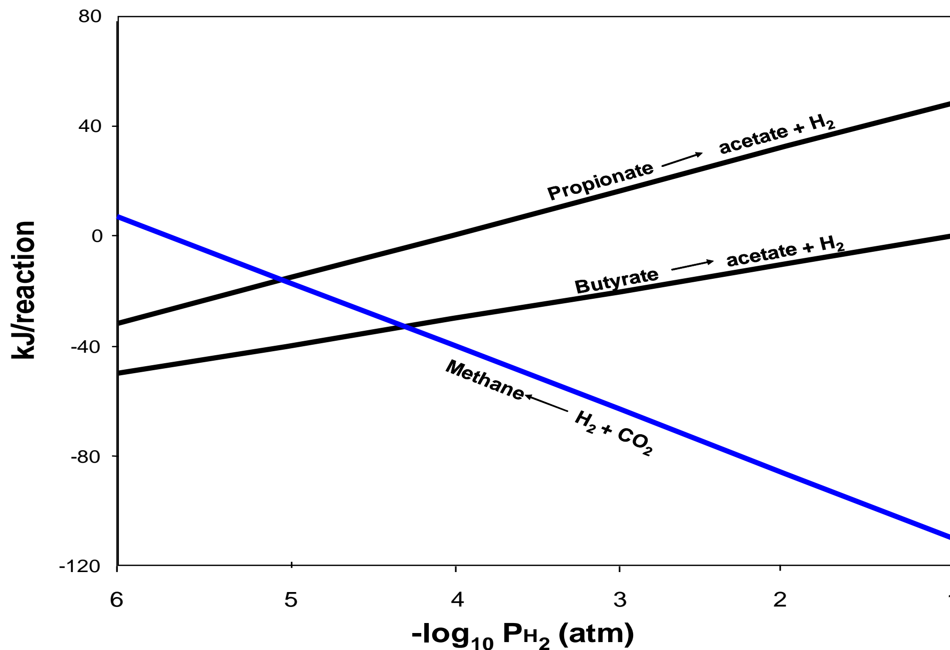


Figure 2:12. The energetics and effect of hydrogen partial pressure on syntrophic degradation in anaerobic digestion (adapted from Björnsson, 2000).

Syntrophic acetogenic bacteria include; a) the butyrate-degrading acetogenic bacteria such as *Syntrophomonas wolfei, Syntrophomonas sapovorans* and *Syntrophomona bryantii*; b) the propionate-degrading acetogenic bacteria such as *Syntrophobacter wolinii*, *Syntrophobacter phenigii* (Cirne, 2006) c) the primary alcohol-degrading bacteria encompassing such species as: *Syntrophobacter fumaroxidans, Desulfovibrio vulgaris*, *Thermoanobacterium brockii* and *Plobacter venetianus*; and d) homoacetogenic bacteria (hydrogen utilizing acetogens such as strain AOR) which are responsible for converting acetic acid into hydrogen and carbon dioxide. Acetogenesis is a low energy yielding anaerobic biodegradation step. This makes acetogenic microbes very slow growing and sensitive to changes in organic loads, flow rate and environmental conditions (Xing *et al*., 1997). Acetogenic bacteria, therefore, require long periods to adapt to new environmental conditions in order to optimize acetogenesis in the bioreactor.

Stage Four: Methanogenesis

Methanogenesis is the biomethanisation step in which organic substrates: acetate, H2/CO2, methanol and formate, the end products of the acetogenesis, are converted into methane (Gujer and Zehnder, 1983). Unlike in the previous stages, the microorganisms responsible for

the methanogenic stage belong to the domain archaea and they produce methane via two major pathways: acetotrophic (or acetoclastic) and hydronenotrophic methanogenic pathways (Figure 2.12). It has been estimated from stoichiometric reactions that about 70% of the methane is produced via the acetotrophic pathway (Lalmand and Bagley, 2001). Nevertheless, very few known species can perform acetotrophic methanogenesis, whereas nearly all known methanogenic species are hydrogenotrophic methanogens (Björnsson, 2000). Bioenergetically, hydrogenotrophic methanogenic reactions are more favourable (ΔG^0 = -131.01 KJ/mol for H_2/CO_2 and ΔG^0 = $^-$135.6 KJ/mol for H_2/HCO_3), while acetoclastic (acetotrophic) methanogenic reactions are least favourable (ΔG^0 = -31.0 KJ/ mol for CH_3COOH) as shown in Table 1.5. The hydrogenotrophic methanogenic pathway is more energy yielding than acetotrophic methanogenic pathway and is normally not rate limiting but rather fundamentally important in keeping the PH_2 low in bioreactor system, allowing syntrophic acetogenesis to proceed. Hydrogen is recognized as the controlling parameter in the overall scheme of waste biodegradation; but rarely detected in well-functioning methanogenic biodigesters (Archer *et al.*, 1986; Björnsson, 2000). Unlike the acetoclastic methanogens, the hydrogenotrophic methanogens are among the fastest-growing organisms in the anaerobic biodegradation process and the accumulation of hydrogen may only occur during process overloads or toxic microbial inhibition. The minimum doubling time for the hydrogenotrophic methanogens has been estimated to be 6 hours compared to 62.4 hours (2.6 days) for the slow-growing acetoclastic methanogens (Björnsson, 2000). Furthermore, hydrogenotrophic methanogens are more resistant to environmental changes while acetoclastic methanogens are more sensitive which makes their reactions more rate limiting in several cases of anaerobic digestion of organic wastes (Björnsson, 2000). The genera *Methanosaeta* and *Methanosarcina* are the only two groups known to carry out the acetotrophic methanogenesis (Garcia *et al*., 2000). The microorganisms of the genus *Methanosaeta* have a lower maximum growth rate than those belonging to the genus; *Methanosarcina* hence the former dominates the bioreactor at high acetate concentrations and the latter at low acetate concentrations. Other methanogenic groups include methylotrophic methanogens, which utilize methane-containing compounds such as methanol, methylamine and dimethylsulphides (Deppenmeir *et al*., 1996).

2.5.3.2 Energy and Other Key Products Derived from Anaerobic Digestion Technology

In AD, organic waste is fed to the process as feedstock and acted upon by microorganisms in absence of oxygen (Igoni *et al.,* 2008; Iglesias *et al.,* 2000; Amblker and Shakdar, 2004; Elango *et al.,* 2007) to produce biogas and bio slurry. The digestate (bioslurry) can be dewatered and converted through thermal conversion technologies into other forms of fuel including refuse derived fuel (Figure 2.13). The remaining inorganic and the inert waste are either incinerated or gasified to generate more energy. Apart from energy generation, the bioslurry can safely be used as bio-fertilizer in agricultural production as well as animal feed especially for piggery, fisheries and aquaculture. This makes anaerobic digestion one of the best waste-to-energy technologies with superior advantage of coupling energy generation with generation of valuable bi-products such as plant organic fertilizer (bioslurry) at minimal net operational energy requirement. Furthermore, a study by Tock *et al*., (2010) reported that AD is usually a preferred WtE technology for biomass with high water content (including banana waste). It is a low-temperature process that can process wet or dry feeds (with added

water) economically at a variety of scales. Results from previous studies on AD of banana peels (Clarke *et al.*, 2008) suggest the high potential and suitability of banana waste as a feedstock for economically viable waste treatment technology like anaerobic digestion for the purpose of energy generation in the form of methane (Tock *et al.,* 2010). The composition of the gas produced is primarily carbon dioxide and methane with small traces of hydrogen sulphide

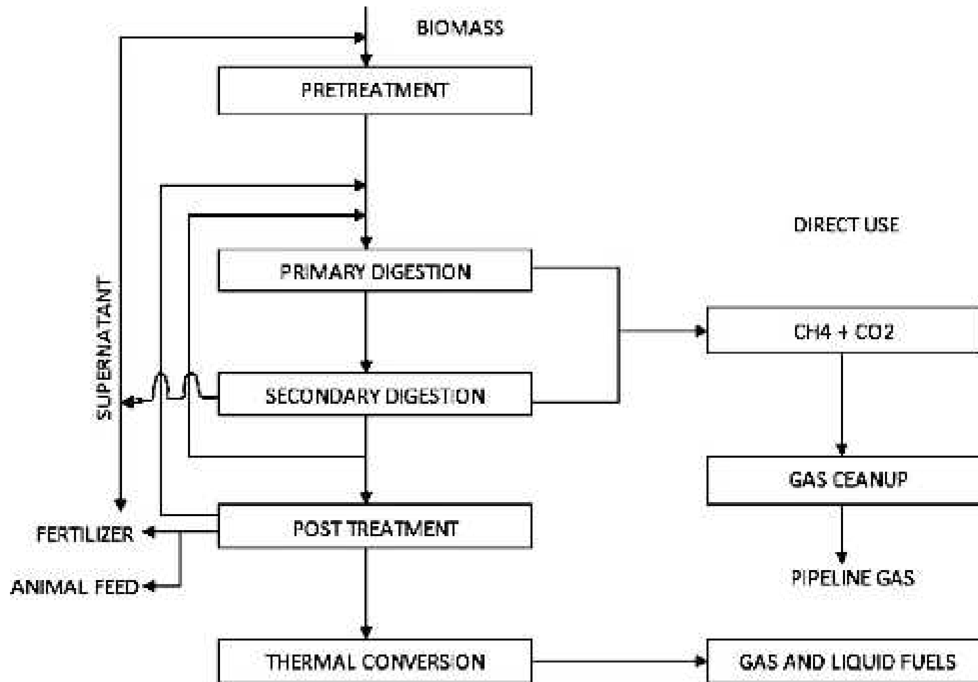


Figure 2:13. Generalized scheme of major products from anaerobic digestion (Tock *et al.*, 2010)

Besides, the AD of banana waste also reduces global warming and air pollution since the methane produced is considered a clean gas with a zero carbon cycle. Notably, the banana biogas has been proven as a perfectly feasible option to run tractors, farm machinery and vehicles (Biopact News, 2008), thus offsetting the industrial energy needs. Other advantages of AD process are: reduction in wastes' pathogens, smaller land suitability and decrease in waste's pollution potential to levels that are non toxic to the environment (Moody and Raman, 2001).

2.5.3.3 Challenges of using lignocellulosic biomass as feed stocks for anaerobic digestion

Anaerobic digestion of plant biomass as digester feedstocks can be limited by three typical challenges, namely: limited microbial hydrolysis of lignocellulosic biomass; floatation of feed slurry; as well as unbalanced C:N ratio. Limited microbial hydrolysis is one of the major hindrances to AD of lignocellulosic plant biomass such as banana waste, whereby, as much as 50% of the feed substrate could be left undigested.

Ligno-cellulosic substrates are complex polymeric substances that are insoluble and too large to be taken up by microbial cells for the subsequent intracellular anaerobic degradation steps. Moreover, lignin degradation is primarily an aerobic process, and in an anaerobic environment lignin can persist for very long periods (Van Soest, 1994). Therefore to use these lignocellulosic biopolymers as substrates for anaerobic digestion, they must undergo prior solubilisation under aerobic environment. Since biogas digesters are anaerobic, lignocellulosic feedstocks have to first be degraded through pretreatment stages such as biological hydrolysis under aerobic conditions prior to anaerobic digestion. A research by Mshandete *et al.,* 2005 reported that ligno-cellulosic rich wastes such as solid sisal residues have high suitability as feedstock for biogas production, after effective hydrolysis. The microbial hydrolysis of lignocellulosic biomass involves several steps, including enzyme production, diffusion, adsorption, reaction and enzyme deactivation step (Batstone *et al*., 2002). Hydrolytic enzymes include lacase, cellulase, xylanase and amylase for degrading lignin, cellulose, xylan and starch into oligosaccharides and simple sugars; protease for degrading protein into amino acids, and lipase for degrading lipid into glycerol and long-chain fatty acids (Parawira *et al*., 2005). The overall hydrolysis rate depends on organic material size, shape, surface area, enzyme production and adsorption (Batstone *et al*., 2000). Moreover, competitive adsorption of enzyme on the inert substrate like lignin can also decrease hydrolysis efficiency (Converse and Optekar, 1993). Hydrolysis has been shown to be a rate-limiting step for digestion of high particulate substrate like agro-industrial residues, municipal solid wastes, swine waste, cattle manure and sewage sludge while methanogenesis is the rate-limiting step for readily degradable substrate, due to inherent slow growth nature of methanogens (Björnsson *et al*., 2001).

Floatation of feed slurry in bioreactors digesting plant biomass is another challenge limiting use lignocellulosic material as feedstocks for biogas production. The anaerobic digestion of biomass from plant origin in conventional reactors including the high-rate reactors is generally nuisance and problematic due to the physical nature of the biomass, since these fibre-rich plant biomass materials tend to build up a persistent float layer. The floatation of the feed substrate leads to wash out of active biomass (inocula seeding) that results in digester failure. When feed substrates are discharged early from the reactor, the active flora adsorbed on to the biocarrier gets lost as well, further reducing the efficiency (German Agency for Renewable Energy, 2005*)*. This has limited the application of high-rate digesters such as upflow anaerobic sludge blanket (UASB) and expanded granular sludge bed (EGSB) reactors, in the treatment of buoyant waste biomass from plant origin and lipid-rich wastes such as fish processing and slaughter house effluents (Hwu, 1997; Cammarota *et al.,* 2001; Pereira, 2003). In order to prevent flotation, intensified agitation and stirring has been recommended and this can demand up to 10% of the electric energy produced after conversion of the produced biogas into electricity. Intensive mixing can also negatively affect the substrate decomposition process by inhibiting microbial flocculation and adsoption apart from taking up a considerable amount of energy that makes the system economically unattractive. Generally typical biogas digesters in use today cannot efficiently digest lignocellulosic biomass from plant origin such as energy crops without modifications (Leibniz Institute for Agricultural Engineering Potsdam-Bornim (ATB). Other research studies reported that AD can proceed at high rate when carried out in appropriately designed

bioreactor system with fully optimised environmental and operational parameters (Mshandete, 2005; Bilibio *et al.,* 2011).

In addition, unbalanced C:N ratio is the other typical challenge faced during anaerobic digestion of lignocellulosic feedstocks from plant biomass. Hydrolysis of lignocellulosic plant biomass mainly releases a lot of sugars comprising simple sugars and oligomers such as multitrioses, with limited nitrogen-rich biomolecules such as amino acids. This implies that there is a high C:N ratio in lignocellulosic plant biomass which can lead to acidic and inhibitory growth conditions for methanogenic bacteria in anaerobic digesters. Successful hydrolysis of lignocellulosic feed stocks such as banana waste can yield a lot of sugars which if converted into organic acids by the acidogenic bacteria, results into bioreactor acidification and inhibition of methanogenesis step. Therefore, before one uses lignocellulosic biomass such as banana waste as a feedstock for biogas production, such apparent challenges ought to be overcome.

2.5.3.4 Options for enhancement of AD of lignocellulosic feedstock

The AD process is influenced by a number of factors leading to varying rates of methane production from a feedstock. The total methane yield and the rate of production, which are a measure of the degree of feed stock microbial digestion, is affected by factors namely: physical-chemical composition of feedstock (feedstock particulate nature), C:N ratio, operating temperature, retention time, inhibitors, agitation (rate of stirring), loading rate, and bioreactor configuration. Hence, the AD of plant biomass feedstock such as banana waste can be enhanced through optimisation of: a) feedstock pre-treatment, b) C:N ratio by co-digestion; c) bioreactor design; and d) environmental and operational parameters.

a) Feedstock pre-treatment

Pre-treatment is generally feedstock deformation to increase its ability for hydrolysis and absorption by living cells. For lignocellulosic feedstock, an ideal pre-treatment method would increase surface area and reduce lignin content and crystallinity of cellulose (Fan *et al.*, 1981). Lignocellulosic biopolymer pre-treatment can be divided into three categories (Table 2.4) namely: a) physical methods such as mechanical (milling and grinding), irradiation, steam explosion and hydrothermolysis; b) thermo-chemical methods (treatment with alkali, dilute acid, oxidizing agents, organic solvents, and wet oxidation); and c) biological methods such as whole microbial pre-treatment, enzymatic hydrolysis and bio-augmentation (Mshandete *et al.,* 2005, 2006; Björnsson *et al.,* 2005). Physical/mechanical and chemical pre-treatment methods have been quite intensively studied with the aim of improving the hydrolysis of lignocellulosic substrates. However, these methods have the disadvantages of being either energy intensive or costly and resulting into residual disposal problems (Takashima *et al.,* 1996). Nevertheless, many researchers have reported that feedstock particle size directly affects the performance of anaerobic bioreactor operating on solid wastes, especially those with a high fibre content (Palmowski and Muller, 2000; Sanders *et al.,* 2003; Yadvika *et al.,* 2004; Tumutegyereize *et al.,* 2011). The mechanical size reduction of the particles and the resulting increase in the available surface area represents an option for increasing biodegradation yields and accelerating the AD of substrates that have high fibre

content such as banana waste, sisal fibres and straw (Hartmann *et al.,* 2000; Angelidaki and Ahring, 2000; Mshandete *et al.,* 2005). A research study by Mshandete *et al.,* (2005) demonstrated that feedstocks with high content of fibres such as hay, seeds and leaves give improved digester gas production after mechanical pre-treatment. This leads to a decrease in the amount of residues to be disposed of, and to an increase in quantity of useful digester gas. Therefore it is imperative to pulverise fibrous feedstocks prior to other pre-treatment methods and subsequently anaerobic digestion.

On the other hand, biological pre-treatment methods have been reported to be cost effective and the methods employed are usually simple and involve mild conditions (Mendes, *et al.,* 2005). Biological pre-treatment includes pre-composting and feedstock pre-hydrolysis by either hydrolytic enzymes or pre-culture with hydrolytic enzyme-producing microorganisms (van Lier *et al*., 2001). These strategies involve the utilization of specific microorganisms and/or microbial derived materials (enzymes) as a means of improving a specific step in the AD process that limits the process. Based on operational approach, the biological strategies include; addition of micro-organisms or enzymes prior to AD process (Chipasa and Medrzycka, 2006; Jeganathan, *et al.,* 2007; Valladao *et al.,* 2007). Others include addition of enzymes directly into the reactor in either a free or an immobilized form (Cirne *et al.,* 2006; Jeganaesan *et al.,* 2007) and bioaugmentation where specific microorganisms are introduced directly into the digester (Cirne *et al.,* 2007). Microorganisms, which are naturally growing in ligno-cellulose rich waste and other phytomass rich dumping site, get adapted to degrade ligno-cellulose waste. A number of microorganisms with potential for lignocellulose hydrolysis have been previously isolated from such environment and characterized. They include the white rot fungi of the genera *Phanerochaete, Lentinus and Trametes* (Wu *et al.,* 2005) and *pleurotus* (Patrick *et al.,* 2011), and bacterial cellulase producers from the *Bacillus subtilis* (Krishna 1999). Nevertheless, the only organisms known to extensively degrade lignin are fungi (Kirk and Farrell, 1987). Notably, white rot fungi are the only known living microorganism capable of complete lignin degradation, and their application has been suggested for delignification of lignocellulosic substrates such as wheat straw (Muller and Trosch 1986) prior to AD. The initial reactions are mediated by extracellular lignin and manganese peroxidases, primarily produced by white-rot fungi (Kirk and Farrell, 1987). Actinomycetes can also decompose lignin, but typically degrade less than 20 percent of the total lignin present (Crawford, 1986; Basaglia *et al.,* 1992). Because lignin is an insoluble polymer, the initial steps in its biodegradation must be extracellular. Many enzymes are involved in the oxidative degradation of lignin, including lignin peroxidases (LiP), manganese peroxidase (MnP), and laccase (Sugiura *et al*., 2003).

b) Substrate Co-digestion

Co-digestion is the anaerobic treatment of a mixture of at least two different nutrient-complementary substrates or waste types. Co-digestion can overcome carbon or nitrogen deficiencies (Wei and Brune, 2007). The mixing of several waste types has a positive synergy on both the AD process itself and on economy of the treatment (Hwu *et al*, 1997). Abundance of nitrogen in the substrate can lead to excessive ammonia formation leading to ammonia toxicity and AD process inhibition. Conversely, too little nitrogen creates a risk of nutrient limitation and low buffering capacity incapable to neutralise the volatile fatty acids produced

by fermentative bacteria, ultimately resulting into a more pH sensitive and inhibited AD process (Mshandete, 2005).

During AD, the microbial community utilizes carbon 25-30 times faster than nitrogen (Yadvika *et al.,* 2004). Since not all the carbon and nitrogen in the substrate are available for digestion, the actual C:N ratio is a function of the substrate characteristics and digestion operational parameters.

Table 2:4. Common pretreatment methods for lignocellulosic biomass Adapted from: Zheng, *et al.,* 2009; Alvira, *et al.*, 2010; Khalid, *et al.,* 2011; Takara, *et al.,* 2012; Martin-Ryals, 2012

Pretreatment Method	Advantages	Disadvantages
Physical:		
Mechanical: Physical reduction in substrate particle size by grinding, milling, etc	- Reduced cellulose crystallinity and degree of polymerization - Increased surface area	- Usually negative energy balance
Irradiation: Biomass undergoes high energy radiation (i.e. γ-ray, ultrasound, electron beam, pulsed electrical field, UV, microwave heating)	Results in one or more changes to biomass: - Increased surface area - Reduced cellulose crystallinity and polymerization - Partial depolymerization of lignin	- Slow - Energy intensive - Prohibitively expensive
Steam explosion: Substrate particles rapidly heated by high-pressure saturated steam. Explosive decompression caused by quick release of pressure Acids released aid in hemicellulose hydrolysis	- Causes hemicellulose solubilization and lignin transformation - Cost effective	- Destruction of a portion of the xylan fraction - Generation of toxic compounds
Hydrothermal: Substrate is subject to high-temperature/high pressure water	- Hemicellulose solubilization - Partial delignification	- High water and energy demand
Chemical:		
Alkaline: Addition of base causes swelling, increasing internal surface of cellulose which provokes lignin structure disruption (NaOH, KOH, Lime, $Mg(OH)_2$, NH_4OH)	- Lignin solubilization - Reduced cellulose crystallinity and degree of polymerization - Increased surface area - Can be done at ambient temperature - Relatively inexpensive	- Relatively long residence times required - Irrecoverable salts formed&incorporated into biomass

Pretreatment Method	Advantages	Disadvantages
Acid : Addition of dilute or concentrated acid solutions results in hemicellulose hydrolysis (H_2SO_4, HCl, HNO_3, H_3PO_4)	- Hemicellulose hydrolysis and conversion to fermentable sugars - Alters lignin structure - With high acid concentrations can be done at room temp.	- Relatively expensive - Corrosive - High operational and maintenance costs - Some inhibitory compounds formed
Catalyzed steam explosion: Similar to steam explosion with addition of acid catalyst (SO_2, H_2SO_4, CO_2, oxalic acid)	- Hemicellulose solubilization	- Some inhibitory compounds formed - Portion of xylan fraction lost - Incomplete disruption of lignin-carbohydrate matrix
Ammonia fiber explosion (AFEX): Substrate is exposed to hot liquid ammonia under high pressure. Pressure is released suddenly breaking open biomass structure	- Delignification - Increases surface area - Reduced cellulose crystallinity - Low formation of inhibitors	- Hemicellulose not significantly removed - Very high pressure requirements - Expensive
Wet Oxidation: Dissolved oxygen oxidizes substrate	- Efficient removal of lignin - Low formation of inhibitors - Exothermic	- High cost of oxygen and alkaline catalyst - High temps & pressures
Organo- solvent extraction: Organic solvents are applied, with or without addition of an acid or alkali catalyst to degrade internal lignin&hemicelluloses bonds	- Delignification - Some hemicellulose solubilization - Recovery of relatively pure lignin as by-product	- Solvent removal is necessary - Relatively expensive
Biological:		
Fungi and Actimycetes: Microorganisms degrade/alter biomass structure (white-, brown-, soft-rot fungi, & bacteria)	- Degrades lignin and hemicellulose - Low energy consumption	- Low rate of hydrolysis

Substrates high in nitrogen can be combined with substrates high in carbon in order to attain the desired C:N ratio for optimal AD process. In general, a C/N ratio of 20-32 has been reported to be the optimal for anaerobic digestion (Bouallagui *et al.,* 2003; Zaher *et al.,* 2007; Tumutegyereize *et al.,* 2011; *Chandra et al.,* 2012). Furthermore, co-digestion enables treatment of organic waste with high methane yield due to positive synergies established in the bioreactor (Hartmann *et al.,* 2003; Murto *et al.,* 2004). Therefore a suitable ratio of biodegradable carbon to nitrogen can be maintained by co-digestion for efficient AD process. Highly lignocellulosic feedstocks such as wood dust, cotton residues, among others which are rich in carbon but poor in nitrogen should be co-digested with those rich in nitrogen but poor

in carbon such as chicken droppings, pig slurry among others. Despite the benefits of co-digestion, co-digestion of mixtures of different wastes including banana waste is seldom reported (De Baere, 2000).

c) Appropriate bioreactor design

An anaerobic bioreactor or biogas digester is an enclosed chamber that uses microorganisms to degrade organic matter with production of biogas. Most farm-based biogas digesters are generally designed for the fermentation of liquid manure and include the traditional floating dome Indian digesters, fixed dome Chinese digester and tubular type. Although these digester types are commonly used in domestic biogas generation, they are associated with significant gas leaks, mainly methane and such defects mainly arise from technical and inappropriate designs which ultimately compromise the efficiency and overall economic value of the digester (Hensel, 2014). This indicates that they are not appropriate for industrial application in the current form and may either be modified or new designs made for large scale industrial applications. Similarly, the high-rate and hybrid digesters that have been modified from conventional digesters to improve anaerobic digestion by sustaining inoculum-substrate exposure and sludge retention are inappropriate for AD of plant biomass and only best suitable for liquid wastes such as waste water effluents. These bioreactors include; upflow anaerobic sludge blanket (UASB) and expanded granular sludge bed (EGSB) reactors. When anaerobic digestion of plant biomass is carried out in these conventional bioreactors, the feed substrate slurry tends to build up a persistent float layer that results into discharge of effluent slurry containing partially digested feed substrate and wash out of active biomass (inocula seeding) and ultimately causing AD process failure. Therefore, the efficient anaerobic digestion of lignocellulosic biomass with enhanced biogas production rates requires an appropriate digester design the can circumvent the above highlighted challenge.

Biogas digester design must address three major considerations, namely; physical nature and solid content of feedstock, operating configuration mode and bioreactor accessory devices. These factors need to be considered interdependently when designing a bioreactor. The physical nature of feedstocks for anaerobic digestion can be categorised as either solid feedstocks such as fibrous (lignocellulosic) plant biomass, animal tissues (from rendering plants) or liquid feedstock such as high strength wastewaters and sludge. These physical characteristics dictate the design of bioreactor to be used for anaerobic digestion with less complications and optimal biogas production. Generally, feedstocks with less than 15 % solid content are termed as wet-pumpable substrates and are appropriately digested by wet bioreactors. On the other hand, feedstock with solid content of over 25% is termed as dry – stackable substrate and is appropriately digested by dry bioreactors. Bioreactors can be designed, engineered and configured to operate in either batch or continuous process mode. In a batch system, biomass is added to the bioreactor at the start of the process and then sealed for the duration of the process. All the four anaerobic digestion stages occur in one chamber. Batch bioreactors are feasible for highly malodorous and infectious feedstocks such as hospital wastewaters. Constant production of biogas is achieved by using more than one batch reactor in series and consequently requires a lot of space. In continuous digestion process mode, organic matter is simultaneously added as the digested material is being

removed usually by an automated system. Examples of this form of anaerobic digestion include continuous stirred-tank reactors, up flow anaerobic sludge blankets, expanded granular sludge beds and internal circulation reactors. Such bioreactors are appropriate for liquid slurry such as wastewaters and have constant biogas production. Thick slurry with high solid content (between 15-25%) can be digested by wet bioreactors with more energy input to pump the substrate during feeding and slurry removal. The thickness of the material may also lead to bioreactor abrasion and clogging of pipes. On the other hand, dry bioreactors are designed to digest solid substrates of solid content between 25-40% without the addition of water, in a process termed as solid-state anaerobic digestion. The primary styles of dry bioreactors are continuous vertical plug flow and batch tunnel horizontal dry bioreactors. Continuous vertical plug flow dry bioreactors are upright, cylindrical tanks where feedstock is continuously fed into the top of the digester, and flows downward by gravity during digestion. In batch tunnel dry bioreactor, the feedstock is deposited in tunnel-like chambers with a gas-tight door. Another design consideration is the necessary accessory device to be fitted with the bioreactor for optimal operation. This consideration is majorly linked with the physical nature of the feedstock to be digested. These devices include feed macerator to reduce particle-size and increase surface area for microbial attachment degradation; mixer to re-circulate the feed with micro-organism as well as foam reduction; foam controller to disintegrate foam header on the surface of bioreactor liquor; and grit remover to trap sand and other indigestible material from entering the bioreactor.

Besides, the anaerobic digestion (AD) of feedstock in single-phase bioreactors, where all the four stages of AD process occur in one un-partitioned chamber, is always prone to up-sets due to contrasting optimal conditions required for both acid and methane formation. The hydrolytic and acid forming bacteria differ from the methane-forming bacteria in terms of their nutritional needs, growth kinetics and sensitivity to environmental (bioreactor liquor) conditions such as pH. In conventional single-phase bioreactor, the system operates in a narrow delicate balance between acid phase and methane phase (Figure 2.14) that must be maintained within the reactor in order to in avoid system failure due to acidification. After successful pre-treatment, the hydrolysis stage of lignocellulosic feed stocks such as banana waste can yield a lot of sugars that when converted into organic acids by the acidogenic bacteria can result into bioreactor acidification and failure. These problems can be circumvented by carrying out a two-phase anaerobic digestion. In the two-phase anaerobic digestion, the process is physically separated into two reactors which offer a method for optimizing the operating conditions for the various groups of microorganisms involved in the digestion process. In the two-phase system the first reactor, referred to as the acid-phase reactor is operated under optimal conditions for hydrolysis and acidogenesis while the second reactor is operated under optimal conditions for methanogenesis and is referred to as the methane-phase reactor. In this case, pH and temperature conditions can be maintained at appropriate levels in either reactor. Two-phase digestion can also increase process stability by optimizing the hydraulic retention time (HRT) for either phase of the process. Typically, HRT is shorter in the acid-phase and longer in the methane-phase to accommodate for the variation in growth rate between the rapidly regenerating acidogens and slow growing methanogens. This can help prevent organic overloading or toxic acid build-up in the methane-phase (Demirer, 2005).

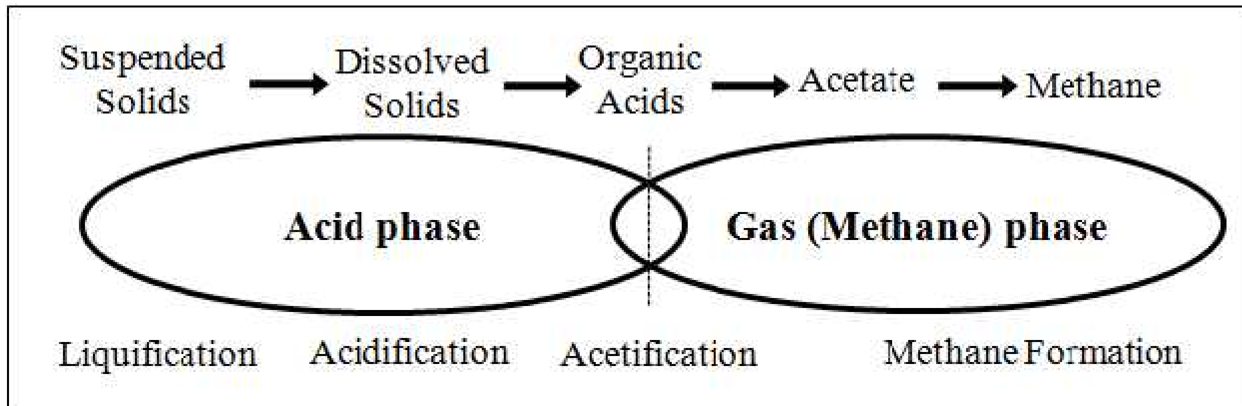


Figure 2:14. Phase separation of anaerobic digestion system. Adapted from Aslanzadeh, 2014

Ultimately, two-phase operation allows for the selection and enrichment of different bacteria in each phase. Previous research has shown that two-phase anaerobic digestion can be successful in treating lignocellulosic substrates such as forest residues (Hooper and Li, 1996) and wood hydrolysate (Chakrabarti, 1999). A report by Zhang, (1991) also revealed that the acetate-utilizing methanogens was 2-10 times higher in the two-phase system than in the single-phase system. Therefore a well designed two-phase bioreactor system can circumvent the problems associated with bioreactor acidification and enhance the AD process leading to high methane yields.

d) Optimisation of operational parameters

Operational parameters are conditions that can be routinely modulated (optimized) either manually or automatically to create suitable environmental conditions for reactor microorganisms and consequently enhancing the anaerobic digestion process (Frick and Uppsten, 1999; Cirne, 2006). These environmental conditions include: concentration of volatile fatty acids (VFAs), pH, temperature, alkalinity and microbial granulation (Table 2.5); and are closely affected by the operational parameters. These operational parameters include Organic loading rates (OLR), agitation/stirring, hydraulic retention time (HRT), biomass retention, and effluent recirculation, among others. Disturbances in reactor equilibrium can result in process inhibition and possible reactor failure.

i) Retention Time (RT)

In anaerobic digestion, retention time is defined as the average time spent by the substrate inside the digester before it comes out after the action of microorganisms in the bioreactor. Retention time is one the key factors that controls the extent to which volatile solids in the substrate are converted to biogas. In typical continuous stirred tank anaerobic digestion systems the solids retention time (SRT) is equal to the hydraulic retention time (HRT). HRT is directly related to reactor volume, by the equation:

$HRT = (V)/(Q)$
Where V is reactor volume and Q is influent flow rate

Short HRT results into faster wash out of active biomass than they can reproduce, consequently causing prolonged lag phase of some steps such as fermentative step (Frick and Uppsten, 1999). However, shorter retention times are preferred for waste treatment in order to reduce system costs and increase process efficiency. Shorter HRT is achieved at higher anaerobic digestion rate that is mainly influenced by substrate characteristics. Substrates containing high amounts of lignocellulose require relatively long HRTs in the range of 60-90 days in order to achieve nearly complete digestion of lignocellulosic substrates (Rivard, Bordeaux et al. 1988). AD carried out in conventional bioreactor requires sufficient volume to give long retention time enough for efficient and effective biodegradation of organics. However, too long HRT requires large volume of the digesters that are limited by cost, treatment capacity, net energy yield and operational skills. Conventional anaerobic digestion processes operate at an HRT in the optimal range of 15-30 days (USDA, 2009). For continuous waste generating industrial processing, an HRT of 15 days would be optimally ideal although it may be practically impossible for AD of lignocellulosic waste without pre-treatment.

In addition to substrate characteristics, short HRT is also limited by microbial regeneration rates. Methanogens are relatively slow growers and require at least 10-15 days of retention in order to regenerate. Due to this slow regeneration time of methanogens, reactor start-up require longer HRTs in order to allow enough time for inoculum sludge to reach a steady-state population (Chandra *et al.* 2012). Limitation of slow microbial regeneration rates can also be overcome by appropriate reactor design containing microbial attachment biocarriers and membrane filters that retain microbial biomass during effluent slurry discharge. However, this might result into sludge build-up leading to bioreactor clogging. Thus typical retention time for biogas units is in the range of 20-60 days (Gunnarsson and Mattsson, 1997). Moreover, optimal HRT may vary from 30-50 days in tropical countries and goes up to 100 days in colder climates (Yadvika *et al.,* 2004).

Table 2:5. Optimal environmental parameters for a stable anaerobic digestion

Environmental Parameter	Stage of anaerobic digestion process	Optimal range	Reference
pH	Hydrolysis & acidogenesis (Two-phase anaerobic digestion)	5.5 - 6.5	Khalid *et al.,* 2011
	Methanogenesis (Two-phase anaerobic digestion)	6.5 - 8.5	Aslanzadeh, 2014 Khalid *et al.,* 2011
	Mixed reactor liquor (One-phase anaerobic digestion)	6.7 - 7.8	Bjornsson, 2000 Cirne, 2006
Hydrogen Partial pressure (P_{H_2})	Mixed reactor liquor (One-phase anaerobic digestion)	10^{-4} - 10^{-5} atm	Bjornsson, 2000 Cirne, 2006
Alkalinity	Mixed reactor liquor (One-phase anaerobic digestion)	1,200 - 2,300 mg $CaCO_3$ per litre	Mshandete, 2004

Environmental Parameter	Stage of anaerobic digestion process	Optimal range	Reference
C:N ratio	Mixed reactor liquor (One-phase anaerobic digestion)	20 - 30	Aslanzadeh, 2014 Chandra et al., 2012
NH_3-Nitrogen	Mixed reactor liquor (One-phase anaerobic digestion)	50 – 200 mg per litre	Mshandete, 2004
Free NH_3	Mixed reactor liquor (One-phase anaerobic digestion)	< 150 mg per litre	Mshandete, 2014
H_2S	Mixed reactor liquor (One-phase anaerobic digestion)	< 200 mg per litre	Eldem *et al.,* 2004
Heavy metals	Mixed reactor liquor (One-phase anaerobic digestion)	$< 10^{-4}$ M	Bjornsson, 2000

ii) Organic Loading Rate

Organic loading rate (OLR) is defined as the amount of volatile solids or chemical oxygen demand fed to the system per unit volume per day (Martin-Ryal, 2012). There is a balance between OLR and HRT that must be determined in order to optimize digestion efficiency and reactor volume. As a consequence, conventional high-rate reactors digesting energy crops can only handle around 3 to 4 Kg of organic dry matter per cubic meter of working volume and per day (German Agency for Renewable Energy, 2005*)*. Higher OLR can lead to an inhibition of the AD process due to the build-up of volatile fatty acids. At higher OLRs, retention times must be long enough such that the microorganisms have enough time to sufficiently degrade the material. A study by Kirtane *et al*., (2009) established that bioreactors fed with lignocellulosic biomass such as, fruit residues, banana waste among others at higher OLR of over 3.5 results into decrease in methane yield due to microbial inhibition by tannins, alkaloids, flavonoids and terpenoids originating from degradation of plant cell wall. Nevertheless, higher OLRs can allow for smaller reactor volumes thereby reducing the associated capital cost for waste treatment through anaerobic digestion.

iii) Feedstock C:N ratio

Ccarbon to nitrogen ratio (C/N) is defined as the relative amounts of elemental carbon and nitrogen present in the substrate (Martin-Ryal, 2012). In general, a C/N ratio of 20-30 is considered optimal for anaerobic digestion (Chandra *et al.* 2012, Zaher *et al.* 2007). Substrates with high C/N ratios, such as paper and most crop residues are usually deficient in nitrogen, which is an essential nutrient for microbial cell growth. Thus, anaerobic digestion of very high C/N ratios such sisal waste, wood dust and banana fruit-stalks may be limited by nitrogen availability. In the case of substrates with low C/N ratios, such as some animal manure, toxic ammonia build-up may become a problem. To overcome deficiencies in either

carbon or nitrogen, co-digestion of low C/N ratio substrates with high C/N ratio substrates has been proven as an effective solution (Hartmann, Ahring 2005).

iv) Bioreactor Liquor mixing

Mixing of bioreactor contents is an important factor in achieving optimal biodegradation of substrate and enhanced methane yield (Frick and Uppsten, 1999). The mixing assures that all biodegradable matter (metabolites) comes into contact with the biocatalysts (bacteria or enzymes) and removes products (such as biogas) from the system. Mixing also serves to prevent pronounced temperature gradients within the digester and provides a uniform bacterial population density as well as preventing scum formation and decantation of organic matter. Gentle or slow mixing is necessary to maintain process stability within the reactor (Zaher et al. 2007) and hence improving anaerobic digester performance (Vavilin 2004, Chen et al. 1990). However, excessive mixing especially stirring at high rate using mechanical devices can disrupt the anaerobic microorganisms, and therefore consideration must be taken in terms of intensity and duration of mixing. Effective mixing of digester contents can be carried out in a number of ways such as stirring using mechanical devices and flushing nozzles, recirculation of biogas and effluent slurry as well as using a wave of feed influx (Van and Faber, 1996; Yadvika *et al.,* 2004). Mshandete *et al.,* (2004) reported that regular shaking (either manually or automatically by shakers) of batch bioreactors especially at laboratory scale can enhance anaerobic digestion. Other related studies have revealed that optimal mixing can be achieved by bioreactor stirring at 60 rpm for 15 min/hr (Willkie *et al.,* 2004). In addition to convention bioreactor liquor mixing, liquid recirculation is often adopted for upflow anaerobic sludge blanket (UASB) reactors treating acidic waste such as high carbohydrate wastes to achieve the re-use of the internally generated alkalinity to maintain the pH around neutral in the sludge bed (Mshandete, 2005). This leads to reduction in the operational costs of treatment due to savings in alkalinity addition. Furthermore, recirculation of effluent liquor or leachate back to the top of the same bioreactor promotes the dispersion of inoculants, nutrients and acids. The performance of dry batch anaerobic digestion has been reported to be enhanced by leachate recirculation (ten Brummeler, 2000). The same study also reported that the leach-bed bioreactor design uses recirculation of leachate between new and mature bioreactors to inoculate, moisturize and provide nutrients for rapid start-up of new bioreactors (fresh waste bed) during anaerobic digestion of solid organic waste. Ultimately, recirculation of leachate removes any build-up of solubilised products, which might otherwise inhibit degradation. The organic acids produced during start-up are conveyed to the mature bed where they converted to methane (Lai *et al.,* 2001).

v) pH

The pH influences the activity of microorganisms and enzymatic activity as they are both active within certain narrow pH ranges (El-mashd, 2004; Cirne, 2006). However, due to the formation of different intermediates, pH varies within each phase of anaerobic digestion. At the same time, the different microbial groups involved in each phase require different pH conditions for optimum growth. This stratification of pH along phases of anaerobic digestion affects the growth of certain microorganisms differently. In general, hydrolytic and acidogenic bacteria prefer slightly acidic conditions near pH 6. Optimal pH for acidogens has

been reported in the ranges of pH 5.5 to 6.5 (Khalid *et al.,* 2011) and 5.8 to 6.2 (Zoetemeyer *et al.,* 1982) . In contrast, acidic conditions are toxic to methanogenic bacteria, which prefer neutral conditions in the range of pH 6.5 to 8.2 (Khalid et al. 2011). The growth rate of methanogens falls sharply below pH 6.5 (Mosey and Fernandes, 1989). The pH-related inhibition of microorganisms in anaerobic digestion process is caused by reactor imbalances between compounds such as ammonia and volatile fatty acids. As a result, acid accumulation is one of the biggest potentials for anaerobic digester failure. Thus to ensure stable operation in batch bioreactors (one-stage anaerobic digestion process), pH should be maintained between 6.7 and 7.4 (Björnsson, 2000; Cirne, 2006). In a properly balanced reactor, pH is buffered through the generation of bicarbonate by methanogens (Zaher *et al.,* 2007). Providing excess alkalinity through blending of high carbohydrate waste feedstock with alkaline compounds or appropriate substrate co-digestion can buffer the AD process against inhibition due to excess acid accumulation.

vi) Temperature

Microorganisms are divided into three groups depending on their optimal growth temperature: psychrophilic (10-15 ^{0}C), mesophilic (30-40 ^{0}C) and thermophilic (45-65 ^{0}C). Similarly, anaerobic digestion occurs over a large range of temperature (Figure 2.15); from psychlophilic temperature at around 10 ^{0}C to some extreme thermophilic temperatures over 70 ^{0}C (Ahring, 1994; Scherer *et al.,* 2000). However, anaerobic digesters are usually operated in the mesophilic range with the optimal at 35 ^{0}C, or in the moderate thermophilic range with the optimal at 55 ^{0}C (van Lie *et al.*, 2001, Mata-Alvarez, 2003). Temperature significantly influences anaerobic reactions both from the kinetic and thermodynamic point of view. Hydrolytic and methanogenic biodegradation rates increase with temperature up to certain temperature optima.

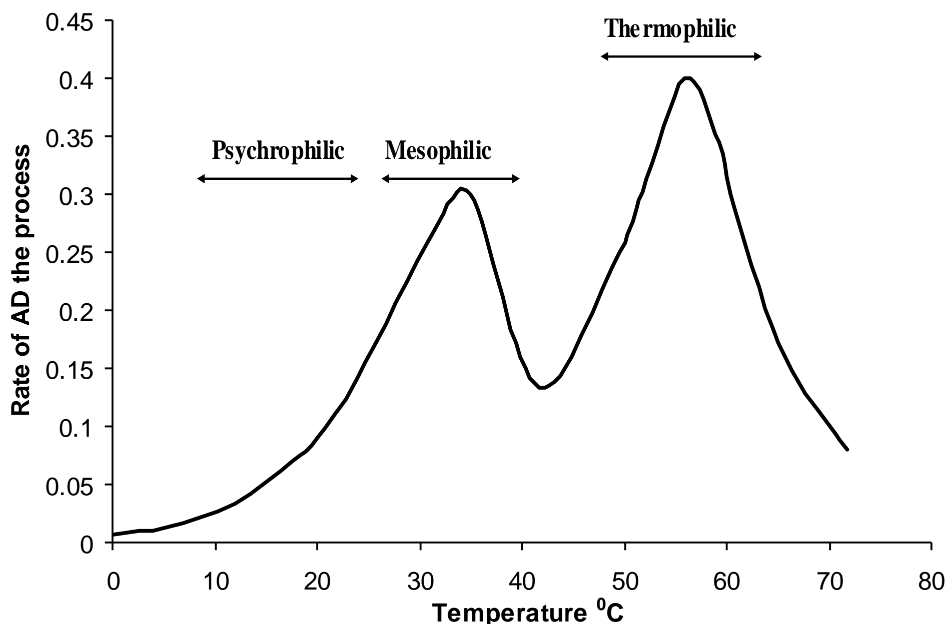


Figure. 2:15. Temperature ranges for anaerobic digestion; optima are 35 ^{o}C for mesophilic range and 55 ^{o}C for thermophilic range. (adapted from Mata-Alvarez, 2003).

In general, higher organic loading rates can be applied in the thermophilic range because of higher microbial growth rate and activity (El-Mashad *et al*., 2004). However, the activity of other groups of bacteria such as propionate and acetate degradation has been shown to decrease when temperature increase above 60°C (van Lier *et al.,* 2001). In addition, the process reactions occurring in the thermophilic range are also more sensitive to toxicity (Angelidaki and Ahring, 1994; El-Mashad, *et al.,* 2004). At higher temperatures, some imbalances can occur such as those resulting from higher acidogenesis (over VFA production) than methanogenesis (low conversion of VFA at higher temperature). Most conventional anaerobic digestion processes occur under mesophilic temperatures due to stability mesophilic conditions that requires less energy input compared to operation under thermophilic conditions, and results in a higher degree of digestion compared to operation under psychrophilic conditions (Khalid *et al.,* 2011; Chandra *et al.,* 2012). Within each temperature range, fluctuations in temperature by even a few degrees can affect microbial activity. A study by Chae *et al.,* (2008) reported that a fluctuation from 35 to 30˚C caused a significant reduction in biogas production rates. It is therefore important to maintain temperature constant and uniform throughout the digestion process.

2.6. Future trend

This review has indicated that anaerobic digestion is the most appropriate eco-friendly WtE option for valorisation of banana waste. However, application of this technology to realise high energy yields in form of methane requires a lot of modification with the feedstock, bioreactor design and optimisation of operational parameters. Although a number of lignocellulosic pre-treatment methods have been greatly studied, there are still challenges that need further investigation and improvement. Chemical pre-treatment generally leads to residual chemical disposal problems and extra cost for neutralisation of chemical –treated feedstock prior to anaerobic digestion. Hence, further research is needed to focus on microbial pre-treatment especially focusing on development of a viable microbial consortium with efficient ligno-cellulolytic activity, since lignocellulosic degradation require sequential interplay of different individual microbial strains. Furthermore, the problems associated with plant biomass clogging of conventional high rate bioreactors and process failure due to feedstock floatation need for more research into development of solid state anaerobic digesters that are more tailored for biomethanisation of high solid feedstocks such as plant biomass including energy crops and banana waste. Since banana waste has high moisture content, it could be digested without additional water requirement. The design and engineering of a future solid state digester tailored for anaerobic digestion of plant biomass should ensure that it:

- Operates in a semi-continuous mode to allow sustainable gas production all throughout without interruption like that caused by batch reactors
- Has mixing devices to mingle in-coming (fresh) solid feedstock with the leachate inoculums
- Re-circulates effluent slurry or leachate back to the digester to re-inoculate the in-coming solid feedstock and minimise water usage

Lastly, further research into standardisation of optimal operational parameters for anaerobic digestion of lignocellulosic feedstocks will be imperative for full scale application of the technology for industrial and large scale energy generation.

2.7. Conclusion

In this review, the waste-to-energy technologies that are potentially applicable to Uganda's banana industrialisation were highlighted. Generally, both thermal and thermo-chemical conversion technologies can positively generate net energy if the processes do not require additional fuel input. Direct thermal and thermo-chemical conversion technologies would be inappropriate Waste-to-Energy options for wastes with high moisture content such as banana waste due to low net energy yield despite their superior potential for complete pathogen destruction. The net energy yield of biomass through thermal conversions is directly related to the moisture content of substrate. Banana waste can be on positive net energy balance through direct thermo-chemical conversions when the substrate had prior drying before thermal degradation. Therefore, thermo-conversion options seem less favoured due to the high moisture content of banana waste. On the other hand, biochemical conversion technologies are more favoured by such moisture content in addition to being more eco-friendly. Among these technologies, anaerobic digestion stands out as the most feasible waste to energy technology for Uganda' banana industrialisation mainly due to limited technical knowledge and economic capability to employ more sophisticated energy conversions such as supercritical water gasification, pyrolysis and bioethanol production. Moreover, anaerobic digestion is a more appropriate waste to energy technology for banana waste since the latter is high organic and purely biodegradable with release of carbohydrates especially starch and lignocelluloses that have high net potential for production of energy in the form of biogas. Besides, the effluent digestate waste from anaerobic digestion is a cheap source of nutrient-rich plant bio-fertilizer which can be re-applied to plantation to boost crop production.

2. 8 References

1. Abdullah, N., Sulaiman, F., Miskam, M.A and Taib, R. M (2014)," Characterization of Banana (Musa spp.) Pseudo-Stem and Fruit-Bunch-Stem as a Potential Renewable Energy Resource", International Journal of Biological, Veterinary, Agricultural and Food Engineering Vol: 8 No: 8, 2014

2. Agar, D and Wihersaari, M (2012), "Bio-coal, torrefied lignocellulosic resources. Key properties for its use in co-firing with fossil coal-their status", Biomass Bio-energy; 44:107-11

3. Ahring, B.K (1994), "Status on science and application of thermophilic anaerobic digestion", Water Science and Technology 30, 241-249

4. Ali, N., Ubhrani, P., Tagotra, M and Ahire, M (2014), "A Step Towards Environmental Waste Management And Sustainable Biofuel (Ethanol) Production From Waste Banana Peelings" , American Journal of Engineering Research (AJER).Volume-03, Issue-05, pp-110-116

5. Alibas, 1., (2007), "Energy consumption and colour characteristics of nettle leaves during microwave, vacuum and convective drying" Biosystems Engineering, Vol. 96 (4), 495–502

6. Alvarado, A., Hernández, L.E.M., Molina, S.L.P., Navarro, R.O., Escareño, M.P.L., and Balagurusamy, N (2014). Microbial trophic interactions and mcrA gene expression in monitoring of anaerobic digesters, Frontiers in Microbiology 5: 597

7. Alvira, P., E. Tomas Pejo, M. Ballesteros and M. J. Negro. 2010. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review. Bioresource technology 101(13):4851-4861.

8. Amani T., Nosrati M., Sreekrishnan T. R. (2010). Anaerobic digestion from the viewpoint of microbiological, chemical, and operational aspects – a review. Environmental reviews 18: 255–278

9. Ambulkar, A and Shekdar, A (2004), "Prospects of biomethanation technology in the Indian context: a pragmatic approach", Resource Conservation and Recycle;40:111–128.

10. Angelidaki, I. and Ahring, B.K (1994), "Anaerobic thermophilic digestion of manure at different ammonia loads: Effect of temperature", Water Research 28, 727-731

11. Angelidaki, I. and Ahring, B.K (2000), "Methods for increasing the biogas potential from the recalcitrant organic matter contained in manure", Water Science and Technology 41, 189-194

12. Annepu, R.K (2012), Sustainable Solid Waste Management in India. New York: Columbia University in the city of New York, Department of Earth and Environmental Engineering; 2012.

13. Arancon, R.A.D., Lin, C.S.K., Chan, K.M., Kwan, T.H and Luque, R (2013), "Advances on waste valorization: new horizons for a more sustainable society", Energy Science & Engineering 1(2):53-71

14. Archer, D.B (1983), "The microbial basis of process control in methanogenic fermentation of soluble waste", Enzyme and Microbial Technology 5, 162-169

15. Aslanzadeh, S (2014). Pretreatment of cellulosic waste and high-rate biogas production, Ph.D Thesis submitted to School of Engineering, University of Borås. SE-501 90 Borås (Sweden). Printed in Sweden by Ineko AB Borås 2014; ISBN 978-91-87525-11-7 (pdf)

16. Balagurusamy N., Ramasamy K. (1999). Biogas technology: an Indian perspective. Current Science 77, 44–55.

17. Balagurusamy, N (2007). A preliminary study on molecular characterization of the eubacteria in a thermophilic, poultry waste fed anaerobic digester. Revista mexicana de ingeniería química 6, 237–242.

18. Basaglia, M. , G. Concheri, S. Cardinali, M.B. Pasti-Grigsby, and M.P. Nuti. 1992. Enhanced degradation of ammonium-pretreated wheat straw by lignocellulolytic Streptomyces spp. Canadian Journal of Micorbiology 38(10):1022-1025.

19. Batidzirai, B., Mignot, A.P.R., Schakel, W.B., Junginger, H.M., and Faaij, A.P.C (2013), “Biomass torrefaction technology: Techno-economic status and future prospects”, Energy 62: 196-214.

20. Batstone, D. J., J. Keller, et al. (2002), "The IWA Anaerobic Digestion Model No 1 (ADM1)”, Water Science and Technology 45 (10): 65-73.

21. Batstone, D. J., Keller, J., Newell, R. B. and Newland, M (2000), “Modelling anaerobic degradation of complex wastewater I: Model development” Bioresource Technology, 75, 67- 74

22. Bayer, A. E. and Lamed, R. (1986) Ultrastructure of the cell surface cellulosome of Clostridium thermocellum and its interaction with cellulose. J. Bacteriol. 167 (3), 828-836.

23. Bayer, A. E., Setter, E. and Lamed, R. (1985) Organization and distribution of the cellulosome in Clostridium thermocellum. J. Bacteriol. 163 (2), 552-559.

24. Ben, H and Ragauskas, A.J (2012), “Torrefaction of Loblolly pine”, Green Chemistry; 14:72-76

25. Bilibio, C., Hensel, O. and Selbach, J (2011), “Sustainable water management in the tropics and subtropics (and case studies in Brazil)”, Vol. 2 Jaguarão/RS, Fundação Universidade Ederal Do Pampa; UNIKASSEL - Pgcult-UFMA 2011

26. Biopact news. Banana biogas to power, heavy-duty farming equipment and vehicle; 2006. Available at: http://news.mongabay.com/bioenergy/2006/10/ banana-biogas-to-power-heavy-duty.html.

27. Biopact news. Growcom trials commercial biogas production from banana waste—high potential yields; 2008. Available from World Wide Web: http://news. mongabay.com/ bioenergy/ 2008 /01/ growcom-trials-commerical-biogas.html.

28. Björnsson, L (2000), Intensification of the biogas process by imposed process monitoring and biomass retention, Doctoral dissertation, Department of Biotechnology, Lund University, Sweden.

29. Björnsson, L., Murto, M., Jantsch, T. G. and Mattiasson, B (2001), “Evaluation of new methods for the monitoring of alkalinity, dissolved hydrogen and the microbial community in anaerobic digestion”, Water Research 35 (12), 2833-2840.

30. Bobleter, O (1998), “Hydrothermal degradation and fractionation of saccharides and polysaccharides”, In: Polysaccharides, Structural diversity and versatility. Dumitriu, S. (ed). Dekker Inc., New York, Basel, Hong Kong.

31. Boominathan, K., and C. A. Reddy (1992). Fungal degradation of lignin: biotechnological applications, p. 763–822. In D. K. Arora, R. P. Elander, and K. G. Mukerji (ed.), Handbook of applied mycology, vol. 4. Fungal biotechnology. Marcel Dekker, Inc., New York.

32. Bosmans, A., Vanderreydt, I., Geysen, D and Helsen, L (2013), " The crucial role of Waste-to-Energy technologies in enhanced landfill mining: a technology review", Journal of cleaner production; 55 (10-23).

33. Bouallagui H, Cheikh RB, Marouani L, Hamdi M (2003). Mesophilic biogas production from fruit and vegetable waste in a tubular digester. Bioresource Technology. 86: 85-85.

34. Boyd, T., de Vries, D., Kempthorne, H., Wearing, J and Wolff, I (2011), "Mass & energy balance for torrefied pellet production; 2011.

35. Bryant, M.P (1979), "Microbial methane production-theoretical aspects", Journal of Animal Science 48, 193-201

36. Cammarota, M.C., Teixeira, G.A. and Freire, D.M.G (2001), "Enzymatic pre-hydrolysis and anaerobic degradation of wastewaters with high fat contents", Biotechnology Letters 23, 1591-1595

37. Capela, I.F., Azeiteiro, C., Arroja, L. and Duarte, A.C. (1999) Effects of pre-treatment (composting) on the anaerobic digestion of primary sludges from a bleached kraft pulp mill. In II International Symposium. Anaerobic Dig. Solid Waste, held in Barcelona

38. Chae, K. J., A. Jang, S. K. Yim and I. Kim. 2008. The effects of digestion temperature and temperature shock on the biogas yields from the mesophilic anaerobic digestion of swine manure. Bioresource Technology 99(1): 1-6.

39. Chakrabarti, S. K. 1999. Biphasic biomethanation of wood-hydrolysate effluent. Artificial Cells, Blood Substitutes, and Immobilization Biotechnology 27(5-6): 461-467.

40. Chambers, A (2003), "Renewable energy in non-technical language", PennWell Books; 2003

41. Chandra R., Takeuchi H., Hasegawa T. (2012): Methane production from lignocellulosic agricultural crop wastes: A review in context to second generation of biofuel production. Renewable and Sustainable Energy Reviews 16(3): 1462-1476.

42. Chen Y., Cheng J. J., Creamer K. S. (2008). Inhibition of anaerobic digestion process: a review. Bioresource Technology. 99 4044–4064.

43. Chen, T., Chynoweth D. P and Biljetina, R (1990). Anaerobic Digestion of Municipal Solid Waste in a Nonmixed Solids Concentrating Digester. Applied Biochemistry and Biotechnology 24(25): 533.

44. Chen, W.H and Kuo, P.C (2011), "Torrefaction and co-torrefaction characterization of hemicellulose, cellulose and lignin as well as torrefaction of some basic constituents in biomass", Energy 2011a; 36:803-811.

45. Chipasa, K.B and Medrzycka, K (2006), “Behaviour of Lipids in Biological Wastewater Treatment Processes”, Journal of Industrial Microbiol Biotechnology 33, 635-645

46. Cirne, D.G (2006), Evaluation of biological strategies to enhance hydrolysis during anaerobic digestion of complex waste, Doctoral dissertation, Department of Biotechnology, Lund University, Sweden.

47. Cirne, D.G., Lehtomäki, A., Björnsson, L. and Blackall, L.L (2007), “Hydrolysis and microbial community analyses in two-stage anaerobic digestion of energy crops”, Journal of Applied Microbiology 103, 516-527

48. Clarke, W.P., Radnidge, P., Lai, T.E., Jensen, P.D and Hardin, M.T (2008), “Digestion of waste bananas to generate energy in Australia”, Waste Management, 28: 527-533

49. Clini, C., Musu, I and Gullino, M.L (2008), “Sustainable development and environmental management: experiences and case studies”, Springer; 2008. Pp. 228–9.

50. Converse A.O., Optekar J.D (1993). A synergistic kinetics model for enzymatic cellulose hydrolysis compared to degree-of-synergism experimental results. Biotechnology and Bioengineering 42:145–148.

51. Crawford, D.L. 1986. The role of actinomycetes in the decomposition of lignocellulose. FEMS Symp. 34:715-728.

52. De Baere, L (2000), “Anaerobic digestion of wastes: State of the art”, Waste Science Technology 41, 283-290

53. Delbes C., Moletta R., Godon J.-J. (2000). Monitoring of activity dynamics of an anaerobic digester bacterial community using 16S rRNA polymerase chain reaction–single-strand conformation polymorphism analysis. Environmental Microbiology 2; 506–515

54. Delivand, M.K., Barz, M and Gheewala, S.H (2011), “Logistics cost analysis of rice straw for biomass power generation in Thailand”, Energy 36(3):1435-1441.

55. Demirer GN, Chen S (2005). Two-phase anaerobic digestion of unscreened dairy manure. Process Biochemistry 40:3542-3549.

56. Deppenmeir, U., Müller, V. and Gottschalk, G (1996), “Pathways of energy conservation in methanogenic archea”, Archaea Microbiology 165,149-163

57. Drake H. L., Küsel K., Matthies C. (2013). “Acetogenic prokaryotes,” in The Prokaryotes eds Rosenberg E., DeLong E. F., Lory S., Stackebrandt E., Thompson F., editors.(Berlin:Springer) 3-60

58. Elango, D., Pulikesi, M., Bakaralingam, P., Ramamurthi, V and Sivanesan, S (2007),"Production of biogas from municipal waste with domestic sewage", Journal of Hazardous Material;141:301–304.

59. El-Mashad, H.M., Zeeman, G., Van Loon, W.K.P., Bot, G.P.A. and Lettinga, G (2004), "Effect of temperature and temperature fluctuation on thermophilic anaerobic digestion of cattle manure", Bioresource Technology 95,191-201

60. Ensinas, A.V., Modesto, M., Nebra, S and Serra, L (2009), "Reduction of irreversibility generation in sugar and ethanol production from sugar cane. Energy; 34:680-688.

61. Fagundes, M (2012), Factors affecting the reduction of carbon to nitrogen ratio in composting separated solids. Senior Project Presented to the Faculty of the Dairy Science Department California Polytechnic State University, San Luis Obispo.

62. FAOSTAT (2011): Global banana production by year, Food and Agriculture Organization of the United Nations. Retrieved on 10th July 2016 from http://faostat.fao. org

63. Food and Agricultural Organisation Statistics (FAOSTAT) 2012 data on bananas and plantains production.

64. Frick, M. and Uppsten, M (1999), Alkaline pretreatment of water Hyacinth for improved anaerobic digestion, unpublished Master of Science thesis, Department of Biotechnology, Lund University, Sweden

65. Gallert C., Winter J. (2005). "Bacterial metabolism in wastewater treatment systems," in Environmental Biotechnology: Concepts and Applications eds Jördening H.-J., Winter J., editors. (Weinheim: Wiley-VCH) 1–48.

66. Garcia, J.L., Patel, B.K.C. and Ollivier, B (2000), "Taxonomin phylogenetic and ecological diversity of methanogenetic Archaea", Anaerobe 6, 205-226

67. Gasafi, E., Reinecke, M.Y., Kruse, A and Schebek, L (2008), "Economic analysis of sewage sludge gasification in supercritical water for hydrogen production", Biomass and Bio-energy; 32:1085–96.

68. Graefe, S., Dufour, D., Giraldo, A., Muñoz, L. A., Mora, P., Solís, H., Garcés, H and Gonzalez, A (2011), "Energy and carbon footprints of ethanol production using banana and cooking banana discard: A case study from Costa Rica and Ecuador," Biomass and Bioenergy, vol. 35, no. 7, pp. 2640-2649.

69. Gujer, W. and Zehnder, A.J.B (1983), "Conversion processes in anaerobic digestion", Water Science and Technology 15, 127-167

70. Gumisiriza, R., Mshandete, A.M., Rubindamayugi, M.S.T., Kansiime, F. and Kivaisi, A. K (2009), "Enhancement of Anaerobic digestion of Nileperch fish processing waste water", African Journal of Biotechnology 8: (2) 328-333

71. Gunnarsson, G. and Mattsson, C (1997), Water hyacinth-trying to turn an environmental problem into an agriculture resource, M.Sc. thesis, Swedish University of Agriculture Science, Uppsala, Sweden

72. Hafez, N., Sheikhdavoodi, M.K., Sajadiye, S.M and Ferdavani, M.E.K (2014), "Evaluation of energy consumption of potato slices Drying using vacuum-Infrared method" International journal of advanced biological and biomedical research, Vol.2 (10), 2651-2658.

73. Hartman, H., Angelidaki, I. and Ahring, B.K (2000), "Increase of anaerobic degradation of particulate organic matter in full-scale biogas plants by mechanical maceration" Water Science and Technology 41, 145-153

74. Hartmann, H. and Ahring, B (2005). Anaerobic digestion of the organic fraction of municipal solid waste: Influence of co-digestion with manure. Water research 39(8): 1543-1552.

75. Hartmann, H., Angelidaki, I., Arhing, B.K., 2003. Co-digestion of the organic fraction of municipal waste with other waste types. In: Mata-Alvarez, J. (Ed.), Biomethanization of the Organic Fraction of Municipal Solid Wastes. IWA Publishing, UK.

76. Harwood, R.C. (1992) Bacillus subtilis and its relatives: Molecular biology and industrial workhorses. Trends in Biotechnology. 10, 247-256.

77. Hensel., 2014: Hermann Sewerin GmbH, FG Mess- und Regelungstechnik & FG Agrartechnik "Project report: Measurement of biogas emissions"; sponsored by Deutsche Stiftung Umwelt

78. Hooper, R. and J. Li. 1996. Summary of the factors critical to the commercial application of bioenergy technologies. Biomass Bioenergy 11(6): 469-474.

79. Hossain, A.B.M.S., Ahmed, S.A., Alshammari, A.M., Adnan, F.M.A., Annuar, M. S. M., Mustafa, H and Hammad, N (2011), "Bioethanol fuel production from rotten banana as an environmental waste management and sustainable energy," African Journal of Microbiology Research 5 (6):586-598.

80. Hreggvidsson, O.G., Kaiste, E., Holst, O., Eggertsson, G., Palsdottir, A. and Kristjansson, K. J. (1996) An extremely thermostable cellulose from the thermophilic eubacteria Rhodothermus marinus. Appl. Env. Microbiol. 62 (8), 3047-3049

81. Hsieh, W., Chen, R., Wu, T and Lin, T (2002), "Engine performance and pollutant emission of an SI engine using ethanol-gasoline blended fuels", Atmosphere and Environment; 36:403-410

82. Hwu, C.S., Molenaar, G., Garthoff, J., Lier, J.B., Van and Lettinga, G (1997), "Thermophilic high-rate anaerobic treatment of wastewaters containing long chain fatty acids; Impact of reactor hydrodynamics", Biotechnology letters 19, 447-451

83. Iglesias, J., Castrilloa, L., Pelaez, N., Marana, E., Maison, O and Andres, H (2000), "Biomethanization of municipal solid waste in a pilot plant", Water Research 2000;34:447–454.

84. Igoni, A., Ayotamuno, M., Eze, C., Ogaji, S and Probert, S (2008), "Designs of anaerobic digesters for producing biogas from municipal solid-waste", Applied Energy ;85:430-438.

85. Insam H., Franke-Whittle I., Goberna M. (2010). "Microbes in aerobic and anaerobic waste treatment," in Microbes at Work eds Insam H., Franke-Whittle I., Goberna M., editors. (Berlin: Springer) 1–34.

86. IRENA. Prospects for the African power sector scenarios and strategies for Africa project. International Renewable Energy Agency; 2012.

87. IRIN News, 2008. http://www.irinnews.org/feature/2008/01/23/study-says-quality-nile-water-has-improved. (Retrieved on Friday, August 2016).

88. Irvine, G., Lamont, E.R and Antizar-Ladislao, B (2010), "Energy from waste: Reuse of compost heat as a source of renewable energy", International Journal of Chemical Engineering; 2010; 1-10.

89. Islam, M. N. (1980), "Use of solar energy for development of shelf-stable potato products" PhD thesis, The Royal Veterinary and Agricultural University, Copenhagen, Denmark.

90. Islam, M. N. (1984), "Kinetic analysis of air drying of potato: traditional and improved methods" Journal of Agricultural Engineering, Institute of Engineers Bangladesh, 12:3-11.

91. Islam, M. S., Haque, M. A and Islam, M. N (2012), "Effects of Drying Parameters on Dehydration of Green Banana (Musa sepientum) and its Use in Potato (Solanum tuberosum) Chips Formulation," The Agriculturists, Vol. 10 (1): 87-97.

92. Jeganaesan, J., George, N and Amarjeet, B (2007), "Oil wastewater treatment using a novel hybrid PBR-UASB system", Chemosphere 67, 1492-1501

93. Jenkins, J (2011). "Thermophillic Bacteria, Composting Stages, and the Sanitization of Compost" A growing culture: http://www.agrowingculture.org/2011/08/humanure-part-iii

94. Juan Wu, J., Xiao, Y.Z and Yu, H.Q (2005), Degradation of lignin in pulp mill wastewaters by white-rot fungi on biofilm, Bioresource Technology 96 1357–1363

95. Kadam, K.L (2002), "Environmental benefits on a life cycle basis of using bagasse-derived ethanol as a gasoline oxygenate in India", Energy Policy ;30:371-384.

96. Kagezi, G.H., Kangire, A., Tushemereirwe. W., Bagamba, F., Kikulwe, E., Muhangi, J., Gold, C.S., and Ragama, P. C., (2006), "Banana bacterial Wilt incindece in Uganda", African crop Science Journal vol. 14, No.2 pp 83-91

97. Kalyani, K.A and Pandey, K.K (2014), "Waste to energy status in India: A short review", Renewable and Sustainable Energy Reviews; 31: 113-120.

98. Khalid, A., M. Arshad, M. Anjum, T. Mahmood and L. Dawson. 2011. The anaerobic digestion of solid organic waste. Waste Management 31 (8): 1737-1744.

99. Kirk, T.K and Farrell., R.L (1987). Enzymatic combustion: the microbial degradation of lignin. Annual Reviews in Microbiology, 41: 465-505.

100. Kirtane, R. D., Suryawanshi, P.C., Patil, M.R., Chaudhari, A.B and Kothari, R.M (2009), "Optimization of Organic Loading Rate for different fruit waste during biomethanization", Journal of Scientific & Industrial Research Vol.68, pp252-255

101. Koyuncu, T., Tosun, I. and Pınar, Y (2007), "Drying characteristics and heat energy requirement of cornelian cherry fruits (Cornus mas L.)" Journal of Food Engineering 78 (2), 735–739.

102. Krishna, C (1999), Production of bacterial cellulases by solid state bioprocessing of banana wastes, Bioresource Technology, 69; 231-239

103. Lai T. E., Nopharatana A., Pullammanappallil P. C. and Clarke W. P. (2001). Cellulolytic activity in leachate during leach-bed anaerobic digestion of municipal solid waste. Bioresource Technology, 80(3), 205-210.

104. Lalman, J.A. and Bagley, D.M (2001), "Anaerobic degradation and methanogenic inhibitory effects of oleic and stearic acids", Water Research 35, 2975-2983

105. Lee, H and Smith, Z (2011), "Feasibility of Biomass Fuel Briquettes from Banana Plant Waste", Cincinnati, USA.: Engineers Without Borders; Greater Cincinnati Professional, 2011.

106. Lee, L.Y (2013), Household energy mix in Uganda. Energy Economics ; 39:252–261.

107. Lehninger, A.L., Nelson, D.L. and Cox, M.M (1993), Principles of biochemistry, 2nd edition, Worth Publishers, New York, pp 479-502

108. Lewicki, P.P (2006), "Design of hot air drying for better foods" Trends in Food Science & Technology 17, 153–163.

109. Li, D-C., Lu, M., Li, Y-L. and Lu, J. (2003) Purification and characterization of an endocellulase from the thermophilic fungus Chaetomium thermophilum CT2. Enzy. Microbiol. Technol. 33, 932-937.

110. Liebrand, C.B and Ling, K.C (2009), Cooperative Approaches for Implementation of Dairy Manure Digesters. USDA Rural Development Research Report 217. STOP 3252, 1400 Independence Ave., S.W Washington, DC 20250-3252

111. Luis, L.M., Romeo, M and Raquel R (2009), "Gareta, Fouling control in biomass boilers", Biomass and Bioenergy 33: 854–861.

112. Luo, X (2011) Torrefaction of biomass; a comparative and kinetic study of thermal decomposition for Norway spruce stump, poplar and fuel tree chips. MSc thesis. Uppsala: Swedish University of Agricultural Sciences.

113. Lynd L. R., Weimer P. J., van Zyl W. H., Pretorius I. S. (2002). Microbial cellulose utilization: fundamentals and biotechnology, Microbiology and Molecular Biology Reviews 66: 506–577

114. Mala, B.R., Aparn, M.T., Mohini, S.G. and Vasanti, V.D. (1998) Molecular and biotechnological aspects of microbial poteases. Microbiology and Molecular Biology Review. 62, 597-635

115. Martin-Ryals, (2012), "Evaluating the potential for improving anaerobic digestion of cellulosic waste via routine bioaugmentation and alkaline pretreatment", A thesis Submitted in partial fulfillment of the requirements for the degree of Master of Science in Agricultural and Biological Engineering in the Graduate College of the University of Illinois at Urbana-Champaign, 2012

116. Masjuki, H and Kalam, A (2013), An overview of biofuel as a renewable energy source: development and challenges, Procedia Engineering 56: 39 – 53

117. Matta-Alvarez, J (2003), "Anaerobic digestion of organic fraction of municipal solid waste: a perspective", in: Biomethanization of the organic fraction of municipal solid waste. Matta-Alvarez, J. (Ed). IWA Publishing, London. UK, pp 91-105.

118. Mclnerney, M.J (1988), "Anaerobic hydrolysis and fermentation of fats and proteins", In: Biology of Anaerobic Microorganisms. Zehnder, A.J.B. (ed). John Wiley and Sons, pp. 373-415

119. Mclnerney, M.J. and Bryant, M.P (1981), "Anaerobic degradation of lactate by syntrophic associations of Methanosarcina barkeri and Desulfovibrio species and effect of H_2 on acetate degradation", Applied Environmental Microbiology 41, 346-354

120. Medic, D., Darr, M., Shah, A., Potter, B and Zimmerman, J (2012), "Effects of torrefaction process parameters on biomass feedstock upgrading", Fuel 91: 147-154

121. Mendes, A.A., Castro, H.F., Pereira, E.B., Furigo Jr., A (2005), "Application of lipases for wastewater treatment containing high levels of lipids", Química Nova 28, 296–305

122. Mohiuddin, A.K.M., Saha, M.K., Hossain, S and Ferdoushi, A (2014), "Usefulness of Banana (Musa paradisiaca) Wastes in Manufacturing of Bio-products: A Review", The Agriculturists 12(1):148-158.

123. Moody, L.B. and Roman, D.R (2001), "A dual-anaerobic system for complete treatment of food processing waste", Journal of Agricultural Engineering Research 80, 293-299

124. Morgenroth, E., Kommedal, R. and Harremoës, P (2002), "Processes and modeling of hydrolysis of particulate organic matter in anaerobic wastewater treatment- a review", Water Science and Technology 15, 25-40

125. Morgenroth, E., Kommedal, R., Harremoe, P (2002). Processes and modeling of hydrolysis of particulate organic matter in aerobic wastewater treatment—a review. Water Science Technology. 45 (6), 25 – 40.

126. Mosey, F.E. and Fernandes, X.A (1989), "Patterns of hydrogen in biogas from anaerobic digestion of milk-sugars", Water Science and Technology 21, 187-196

127. Motevali, A., Minaei, S., Khoshtagaza, M.H (2011), "Evaluation of energy consumption in different drying methods" Energy Conversion and Management 52 (2), 1192–1199.

128. Mshandete A,Kivaisi A, Rubindamayugi M, Mattiasson B (2004). Anaerobic batch co-digestion of sisal pulp and fish wastes. Bioresource Technology, 95:19-24.

129. Mshandete, A., Björnsson, L., Kivaisi, A.K, Rubindamayugi, S.T. and Mattiasson, B (2005), "Enhancement of anaerobic batch digestion of sisal pulp waste by mesophilic aerobic pre-treatment", Water Research 39, 1569-1575

130. Mshandete, A., Björnsson, L., Kivaisi, A.K, Rubindamayugi, S.T. and Mattiasson, B (2005), "Enhancement of anaerobic batch digestion of sisal pulp waste by mesophilic aerobic pre-treatment", Water Research 39, 1569-1575

131. Mshandete, A., Björnsson, L., Kivaisi, A.K., Rubindamayugi, M.S.T., Mattiasson, B (2006), "Effect of particles size on biogas yield from sisal fibres waste", Renewable Energy 31, 2385-2392

132. Mshandete, A.M (2005), "Improved anaerobic digestion of agro-industrial waste by enhanced hydrolysis and operational design", Doctoral thesis, University of Dar es salaam, Tanzania

133. Müller, H.W., Trösch, W (1986). Screening of white-rot fungi for biological pretreatment of wheat straw for biogas production. Applied Microbiology and Biotechnology 24, 180–185.

134. Münster, M and Meibom P (2011), "Optimization of use of waste in the future energy system", Energy; 36(3):1612-1622.

135. Murto, M; Bjornsson, L; Mattiasson, B (2004). Impact of food industrial waste on anaerobic co- digestion of sewage sludge and pig manure, Journal of Environmental Management. 70:101–107.

136. Nagase, M. and Matsuo, T (1982), "Interactions between amino acid-degrading bacteria and methaanogenic bacteria in anaerobic digestion", Biotechnology and Bioengineering 24, 2227-2239

137. Nandkumar, N (2014) Plasma – The Fourth State of Matter, International Journal of Scientific & Technology Research, vol. 3, (9): 49-52

138. Nordin A (2012). The dawn of torrefaction BE-sustainableethe magazine of bio- energy and the bioeconomy; 2012. p. 21-23.

139. Odier, E. and Artaud, I. (1992), Degradation of Lignin. In Microbial Degradation of Natural Products, ed G. Winkelmann, VCH Publishers, Inc., New York, pp.161-191.

140. Ohman, M., Pommer, L and Nordin, A (2005), "Bed agglomeration characteristics and mechanisms during gasification and combustion of biomass fuels", Energy & Fuels; 19: 1742–1748

141. Ortega-Charleston L. (2008). Hyperthermophilic Anaerobic Digestion of Food Waste. Ph.D. thesis, McGill University; Montreal.

142. Palmowski, L., Müller, J (200). Influence of size reduction of organic waste on their anaerobic digestion . Water Science and Technology, 41: 155-162

143. Parawira, W (2004), Anaerobic treatment of agricultural residues and wastewater, published Ph.D dissertation, Lundi University, Sweden.

144. Parawira, W., Murto, M., Read J.S. and Mattiasson, B (2005a), "Profile of hydrolases and biogas production during two-stage mesophilic anaerobic digestion of solid potato waste", Process Biochemistry 40, 2945-2952

145. Park, S and Jang, C (2012), "Effects of pyrolysis temperature on changes in fuel characteristics of biomass char", Energy; 39:187-1895

146. Patrick, F., Mtui, G., Mshandete, A.M and Amelia Kivaisi, A (2011). Optimization of laccase and manganese peroxidase production in submerged culture of Pleurotus sajor- caju, African Journal of Biotechnology Vol. 10 (50), 10166-10177

147. Pavlostathis, S.G. and Giraldo-Gomez, E (1991), "Kinetics of anaerobic treatment", Water Science and Technology 24, 35-59

148. Pereira, M.A (2003), Anaerobic Biodegradation of Long Chain Fatty Acids: Biomethanisation of biomass-associated LCFA as a challenge for the anaerobic treatment of effluents with high lipid/LCFA content, Ph.D thesis Minho University, Portugal.

149. Pereira, M.A (2003), Anaerobic Biodegradation of Long Chain Fatty Acids: Biomethanisation of biomass-associated LCFA as a challenge for the anaerobic treatment of effluents with high lipid/LCFA content, Ph.D thesis Minho University, Portugal.

150. Phanphanich, M and Mani, S (2011), “Impact of torrefaction on the grindability and fuel characteristics of forest biomass”, Bioresource Technology; 102(2):1246-1253.

151. Pimchuai, A., Dutta, A and Basu, P (2010), “Torrefaction of agriculture residue to enhance combustible properties”, Energy Fuels; 24(9):4638-4645.

152. Priest FG (1977) Extracellular enzyme synthesis in the genus Bacillus. Bacteriol Rev 41,711-753

153. Prins, M.J., Ptasinski, K.J and Janssen, F.J.J.G (2006), “More efficient biomass gasification via torrefaction”, Energy 2006a; 31(15):3458-3470.

154. Pronobis, M (2006), “The influence of biomass co-combustion on boiler fouling and efficiency”, Fuel 85: 474–480.

155. Rani DS, Nandi, K (2004). Ensilage of pineapple processing waste for methane generation. Waste Management; 24(5):523-8.

156. Rentizelas, A., Tolis, A and Tatsiopoulos, I.P (2009), “Logistics issues of biomass: the storage problem and the multi-biomass supply chain”, Renewable and Sustainable Energy Review;13(4):887-894.

157. Rivard, C., F. Bordeaux, J. Henson and P. Smith. 1988. Effects of Addition of Soluble Oxidants on the Thermophilic Anaerobic-Digestion of Biomass to Methane. Applied Biochemistry and Biotechnology 17(1-3): 245-262.

158. Roberts, J. S., Kidd, D. R and Padilla-Zakour, O (2008), “Drying kinetics of grape seeds” Journal of Food Engineering, 89:460–465

159. Ryan, P., C. Forbes, S. McHugh and C. O'Reilly. 2010. Enrichment of acetogenic bacteria in high rate anaerobic reactors under mesophilic and thermophilic conditions. Water research 44 (14): 4261-4269.

160. Salvachúa, D., Prieto, A., Abelairas, M.L., Chau, T.L., Martínez, A.T and Martínez, M.J (2011). Fungal pretreatment: An alternative in second-generation ethanol from wheat straw Bioresource Technology 102: 7500-7506

161. Samadi, S.H., Ghobadian, B., Najafi, G and Motevali, A. (2014), “Potential saving in energy using combined heat and power technology for drying agricultural products (banana slices),” Journal of the Saudi Society of Agricultural Sciences, Vol. 13 174-182.

162. Sanders, W.T.M (2001), Anaerobic digestion of complex substrates, Ph.D thesis, Wageningen Agricultural University, The Netherlands.

163. Sanders, W.T.M., Veeken, A.H.M., Zeeman, G and van Lier, J.B. (2003). Analysis and optimisation of the anaerobic digestion of the organic fraction of municipal solid waste, In biomethanization of the organic fraction of municipal solid waste. Mata-Alvarez, J. (ed). IWA Publishing .London, UK., PP 63-88.

164. Scarlat, N., Motola, V., Dallemand, J.F., Monforti-Ferrario, F. and Linus Mofor (2015), “Evaluation of energy potential of Municipal Solid Waste from African Urban areas”, Renewable and Sustainable Energy Reviews 50: 1269-1286

165. Scherer, P.A., Vollmer, G.R., Fakhouri, T. and Martensen, S (2000), “Development of a methanogenic process to degrade exhaustively the organic fractions of municipal “grey waste” under thermophilic and hyperthermophilic conditions”, Water Science and Technology 41, 17-39

166. Schorr, C., Muinonen, M and Nurminen, F (2012), “Torrefaction of biomass”, Mikkeli, Finland: Miktech Ltd/Centre of Expertise-programme; 2012. p. 55.

167. Schwartz, W.H. (2001). The cellulosome and cellulose degradation by anaerobic bacteria. Applied Microbilogy and Biotechnology 56, 634-649.

168. Sellin, N., De Oliveira, B. G., Maranon, C., Souza, O., De Oliveira, A.P.N and Novais De Oliveira T. M (2013), “Use of banana culture waste to produce briquettes,” Chemical Engineering Transactions, vol. 32, pp. 49-354.

169. Shapouri, H., Duffield, J.A and Wang, M (2002), The energy balance of corn ethanol: an update. United States Department of Agriculture, USDA; 2002.

170. Singh, R.P., Tyagi, V.V., Allen, T., Ibrahim, M.H and Kothari, R (2011), “An overview for exploring the possibilities of energy generation from municipal solid waste (MSW) in Indian scenario”, Renewable and Sustainable Energy Reviews ;15(9):4797–808.

171. Sivakumaran S., Balagurusamy N., Ramasamy K. (1991). “Cellulase complex in biogas slurry,” in Biological Nitrogen Fixation and Biogas Technology eds Kannaiyan S., Ramasamy K., Ilamuguru K., Kumar K., editors. (Coimbatore: Tamil Nadu Agricultural University) 101–110.

172. Smith, M and Aber, J (2014), “Heat Recovery from Compost: A Step-by-Step Guide on Building an Aerated Static Pile Heat Recovery Compost Facility” Biocycle; 55 (2), Pp. 27

173. Spilsbury, J.S., Jagwe, J.N., Ferris, R.S.B (2002), “Evaluating the Marketing Opportunities for Banana & its Products in the Principle Banana Growing Countries of ASARECA (Uganda)”, International Institute of Tropical Agriculture. Foodnet. P: 60.

174. Stehlík, P (2009), "Contribution to advances in waste-to-energy technologies", Journal of cleaner production: 17,919-931.

175. Stephenson, K., Bron, S. and Harwood, R. C. (1999) Cellular lysis in Bacillus subtilis; the effect of multiple extracellular protease deficiencies. Letters in Applied Microbiology. 29, 141-145.

176. Sugiura, M., Hirai, H. & Nishida, T (2003), "Purification and characterization of a novel lignin peroxidase from white-rot fungus Phanerochaete sordida YK-624", FEMS Microbiology Letters, 224:285–290

177. Takara, D. and S. Khanal. 2012. Biomass Pretreatment for Biofuel Production. In Sustainable Bioenegry and Bioproducts: Value Added Engineering Applications, 59. ed. Anonymous , Springer London.

178. Takashima, M., Kudoh, M.J and Bildan, M.C (1996). Effect of thermochemical pre-treatment on the anaerobic digestion of waste activated sludge, Waste Science and Technology, 35: 209-215

179. ten Brummeler E. (2000). Full scale experience with the BIOCEL process. Water Science and Technology 41:299–304.

180. Ten Brummeler, E., Koster, F.W (1990). Enhancement of dry anaerobic digestion of the organic fraction of municipal solid waste by anaerobic pre-treatment step, Biological Waste 31.199-210

181. Themelis, N.J (2005). Control of Heat Generation during Composting. BioCycle, 46(1), 28-30.

182. Tillmans A, Schweizer-ries P. Energy for sustainable development knowledge communication regarding solar home systems in Uganda: the consumers' perspective. Energy for Sustainable Development, 2011;15(3):337–46.

183. Tock, J.Y., Lai, C.L., Lee, K.T., Tan, K.T and Bhatia, S (2010), "Banana biomass as potential renewable energy resource: A Malaysian case study," Renewable & Sustainable Energy Reviews, vol. 14, no. 2, pp 798-805

184. Tripathi, L., Tripathi1, J. N. and Tushemereirwe, W. K (2008), "Rapid and efficient production of transgenic East African Highland Banana (Musa spp.) using intercalary meristematic tissues", African Journal of Biotechnology Vol. 7 (10), pp. 1438-1445

185. Tucker, M.F (2006). Extracting Thermal Energy from Composting. BioCycle, 47(8), 38.

186. Tumutegyereize, P., Muranga, F.I., Kawongolo, J. and Nabugoomu, F (2011), "Optimization of biogas production from banana peels", African Journal of Biotechnology Vol. 10 (79), 18243-18251

187. Tushemereirwe, W. K, Kangire, A. Smith, J., Nakyanzi, M., Karyeija, R. Kataama, D and Musiitwa, C., (2001), "An outbreak of Banana bacterial wilt in Mukono and Kayunga district", A new and devastating disease NARO/KARI

188. Twaha,S., Ramli, M.A.M., Murphy, P.M., Mukhtiar, M.U and Nsamba, H.K (2016), "Renewable based distributed generation in Uganda: Resource potential and status of exploitation", Renewable and Sustainable Energy Reviews; 57: 786-798.

189. Uddin, M.B and Islam, M. N (1985), "Development of shelf stable pineapple products by different methods of drying" Journal of Agricultural Engineering, Institute of Engineers Bangladesh, 13(1):5-13.

190. UNEP (2013). Municipal solid waste: is it garbage or gold? Available at:. UNEP Global Environmental Alert Series; 2013. ⟨http://www.unep.org/pdf/UNEP_GEAS oct_2013.pdf⟩

191. UN-HABITAT (2010). Collection of municipal solid waste in developing countries. Nairobi, Kenya: United Nations Human Settlements Programme (UN-HABI- TAT); 2010.

192. Valladao, A.B.G., Freire, D.M.G. and Cammarota, M.C (2007), "Enzymatic pre-hydrolysisapplied to the anaerobic treatment of effluents from poultry slaughterhouses", International Biodeterioration & Biodegradation 60: 219–225

193. Van Passel, S., Dubois, M., Eyckmans, J., de Gheldere, S., Ang, F., Jones, P.T., Van Acker, K., (2013), "The economics of enhanced landfill mining: private and societal performance drivers", Journal of cleaner production 55, 92-102.

194. van Lier, J.B., Tilche, A., Ahring, B.K., Macario, H., Moletta, R., Dohanyos, M., Hulshoff Pol, L.W., Lens, P. and Verstraete, W (2001), "New perspectives in anaerobic digestion", Water Science and Technology 43, 1-18

195. Van Soest, P. J. (1994) Nutritional Ecology of the Ruminant, 2nd ed. Cornell University Press, Ithaca, New York

196. Van, D.M. and Faber, J (1996), "Anaerobic fermentation of solid organic waste in a reactor", WO Patent 9607726

197. Vavilin, V. A (2004). Anaerobic degradation of solid material: Importance of initiation centers for methanogenesis, mixing intensity, and 2D distributed model. Biotechnology and bioengineering 89(1): 113-122.

198. Veeken, A., and Hamelers, B. (1999). ''Effect of temperature on hydro-lysis rate of selected biowise components.'' Bioresource Technology, 69, 249–

199. Velasquez-Arredondo, H. I., Ruiz-Colorado, A. A and De Oliveira Junior, S (2010), “Ethanol production process from banana fruit and its lignocellulosic residues: Energy analysis,” Energy, vol. 35, no. 7, pp. 3081-3087, Jul, 2010.

200. Wanapat M., Cherdthong A. (2009). Use of real-time PCR technique in studying rumen cellulolytic bacteria population as affected by level of roughage in buffalo, Current Microbiology 58: 294–299

201. Wang Y., Zhang Y., Wang J., Meng L. (2009). Effects of volatile fatty acid concentrations on methane yield and methanogenic bacteria. Biomass Bioenergy 33 848–853

202. Wannapeera, J and Worasuwannarak, N (2012), “Upgrading of woody biomass by torrefaction under pressure”, Journal of Analytical and Applied Pyrolysis; 96:173-1780

203. Wannapeera, J., Fungtammasan, B and Worasuwannarak, N (2011), “Effects of temperature and holding time during torrefaction on the pyrolysis behaviors of woody biomass” Journal of Analytical and Applied Pyrolysis, Vol. 92, 99-105.

204. Wilaipon, P (2009), “The Effects of Briquetting Pressure on Banana-Peel Briquette and the Banana Waste in Northern Thailand,” American Journal of Applied Sciences, vol. 6, (1) 167-171.

205. Willkie, A.C., Smith, P.H and Bordeaux .F.M (2004), “An economical bioreactor for evaluating biogas potential of particulate biomass”, Bioresource technology 92 pp 103-109

206. Xing, J., Criddle, C. and Hickey, R (1997), “Effects of long-term periodic perturbation on anaerobic community”, Water Research 31, 2195-2204

207. Yadvika, Santosh, Sreekrishnan, T.R., Kohli, S. and Rana, V (2004),Enhancement of biogas production from solid substrates using different techniques-a review”, Bioresource Technology 95,1-10

208. Yamaji, F. M., Chrisostomo, W., Vendrasco, L and Flores, W. P (2010), “The use of forest residues for pellets and briquettes production in Brazil”, Proceedings Venice 2010, Third International Symposium on Energy from Biomass and Waste.

209. Yan, W., Hastings, J.T., Acharjee, T.C., Coronella, C.J and Vásquez, V.R (2010), “Mass and energy balances of wet torrefaction of lignocellulosic biomass”, Energy Fuels ; 24(9):4738-4742.

210. Yen, H.W and Brune D . E (2007). Anaerobic co-digestion of algal sludge and waste paper to produce methane, Bioresource technology 98: 130-134.

211. Zaher, U., Grau, P., Benedetti, L., Ayesa, E., Vanrolleghem, P.A (2007). Transformers for interfacing anaerobic digestion models to pre- and post-treatment

processes in a plant-wide modelling context. Environmental Modelling and Software, 22, 40-58.

212. Zaher, U., Li, R., Jeppsson, U., Steyer, J.P. and Chen, S., 2009. GISCOD: General integrated solid waste co-digestion model. Water research. Vol. 43: 2717-2727.

213. Zhang, T. C. 1991. Comparison of one-phase and two-phase anaerobic digestion processes in characteristics of substrate degradation and bacterial population levels. Water Science and Technology 23(7-9): 1157-1166.

214. Zhang, T.C and Noike T (1991), Comparison of one-phas e and two-phase anaerobic digestion processes in characteristics of substrate degradation and bacterial population levels. Water Science and Technology, 23, 1157-1166.

215. Zheng, M., Li, X., Li, L., Yang, X., He, Y., 2009. Enhancing anaerobic biogasification of corn stover through wet state NaOH pretreatent. Bioresource Technology. 100, 5140–5145.

216. Zoetemeyer, RV Andenheuvel, J. J. C and Cohen A (1982), pH Influence on Acidogenic Dissimilation of Glucose in an Anaerobic Digester. Water research 16(3): 303-311.

3 Materials and methods

3.1 Introduction

This chapter majorly describes the raw material (banana waste) and key analytical methods used in characterization of banana waste as well as highlighting the important formulae for monitoring and determination of operational parameters typical to UASB reactors. Analytical characteristics of the waste help to evaluate the suitability of the substrate for anaerobic digestion while the bioreactor operational parameters affect the progress of anaerobic digestion and rate of biogas production. The banana waste samples analysed in this study were residues from industrial processing of *Musa acuminata* (AAA-EA)-the East African High land cooking bananas and the waste comprised of peduncle, whole fruit rejects, peels and waste pulp (Karamura *et al*., 2012 and ProMusa banana cultivar check list, 2021).

3.2 Banana waste as a raw material for anaerobic digestion

Bananas are long curved fruits of the tropical and subtropical palm-like plant of the genus Musa. These fruits have soft pulpy flesh and yellow skin when ripe, and grow in clusters on a single peduncle to form a bunch of banana fruits per plant. Uganda is the second largest global producer of bananas after India and the leading in Africa (Tripathi *et al.,* 2008), with annual production estimated at 9.77 million tonnes (FAOSTAT, 2012). The most widely grown cultivars are cooking types belonging to the East African highland banana subgroup. Among these East African highland bananas, the green cooking banana (AAA-EA group), locally called *matooke,* is the leading staple food (Tumutegyereize *et al.,* 2011) in Uganda with the annual production of over 6 million tonnes (Spilsbury *et al.,* 2002).
Processing of the green cooking bananas (*matooke*) involves peeling of green fruits to finally expose the brown fleshy pulp that is the edible component when cooked. The non-edible components constitute the banana waste investigated in this study as raw materials for biogas production. During processing of green cooking bananas, about 60% of a bunch of banana fruits is disposed off as banana wastes, majorly comprised of peels, peduncle and damaged fruit fingers. The banana waste investigated in this industry was collected from a banana processing industry located at TBI-Nyaruzinga, Bushenyi, Western Uganda. The waste mainly comprised of peels, peduncle and fruit rejects. Banana peels constituted the major percentage followed by the peduncle and lastly the fruit rejects. The different fractions of the banana waste are illustrated in chapter 4, section 4.3.

3.3 Determination of physico-chemical parameters

3.3.1 Determination of pH

The pH of samples was determined after suspension of the homogenised sample into distilled water and left to stabilise for 1 hour at room temperature. The pH readings were recorded directly from a digital pH meter (*HANNA,* UK) before anaerobic digestion of the biomass. The alkalinity was evaluated as partial alkalinity (PA) by titration to pH 5.75 and total alkalinity (TA) by titration to pH 4.3 according to Mshandete, *et al*., (2004).

3.3.2 Determination of Total Solids (TS)

Total solid (TS) and volatile solids (VS) of the substrate and inoculum were determined gravimetrically by the oven-drying and ignition method respectively, according to standard methods, APHA (1998). The porcelain crucibles to be used were pre-heated at 550 ^{0}C for 1 hour and cooled to room temperature in desiccators.

To determine % TS, the previously prepared empty crucibles were weighed, and then a known weight of fresh sample added and oven dried for 24 hours at 105 ^{0}C in a Gallen-kamp Hotbox Oven (Gallenkamp & Co. Ltd, and London, UK). The %VS were determined by ignition of the previously oven-dried samples for 2 hours at 550 ^{0}C in a Carbolite-1100 ^{0}C furnace (Chelmsford, England). The samples were cooled in the desiccators to room temperature for 1hour and then re-weighed. The entire experiment was done in triplicate and the average weight recorded.

$$\text{Total solid (\%)} = \frac{(B-A)}{\text{Weight of fresh Sample}} \times 100$$

Where: A = average weight of empty Crucible

B = average weight of residue dried at 105 ^{0}C + Crucible

3.3.3 Determination of Moisture Content (MC)

The dry matter that remains after moisture removal is commonly referred to as total solids. This implies that a fresh biological sample is primarily comprised of moisture and total solids that together make up 100%.

$$\text{Moisture Content (\%)} = 100 - \%TS$$

3.3.4 Determination of Volatile Solids (VS)

Volatile solids (VS) are the organic biodegradable fraction of the total solids (TS) or total dry matter content of a substrate that contributes to biogas production. The volatile solid is the parameter commonly used to characterise the organic waste for anaerobic digestion. Suitable substrates for anaerobic digestion have the volatile solids content ranging from 70 % to more than 95 % of the TS (Vögeli *et al.,* 2014).

$$\text{Volatile Solids (\% of TS)} = \frac{\text{Weight difference between TS\&Fixed Solids at 550 } ^{0}\text{C}}{\text{Weight of dried Sample (TS)}} \times 100$$

$$\text{Volatile Solids (\% of TS)} = \frac{(B-A) - (C-A)}{(B-A)} \times 100$$

Where: B= average weight of residue dried at 105 ^{0}C (before ignition) + Crucible

C= average weight of residues/ ash after ignition at 550 ^{0}C + Crucible

3.3.5 Determination of Fixed Solids

$$\text{Fixed Solids (\%)} = \frac{(C-A)}{\text{Weight of dried Sample (TS)}} \times 100$$

Where: A = average weight of empty Crucible
C= average weight of residues/ ash after ignition at 550 ^{0}C + Crucible

3.3.6 Determination of Total Organic Carbon (TOC)

Total organic carbon was determined by dry combustion method described by Allen (1989). The dried samples were apportioned from the total solid (TS) determination (Previously described). One gram of the dry, pounded sample was placed into pre-weighed porcelain crucibles (heated to 600 ^{0}C for 1 hour and cooled to room temperature in a desiccators) and transferred to muffle furnace. The crucibles were heated at 600 ^{0}C for 5 hours and thereafter cooled to room temperature for 1 hour in desiccators and the weight of the ash recorded.

$$\text{Total Organic Carbon (\%)} = \frac{(100 - \%\ \text{ash})}{1.8.}$$

The denominator 1.8 is used to correct for organic carbon during combustion.

$$\%\ \text{ash} = \frac{(C-A)}{\text{Weight of dry Sample (TS)}} \times 100$$

Where: A= average weight of empty Crucible.
C= average weight of residues/ ash after ignition at 600 ^{0}C + Crucible

3.3.7 Determination of Total Organic Matter Content (OM)

The organic matter content of banana waste samples was determined by the dry combustion method (Lyimo *et al.* 2002). One gram of sun-dried sample was pulverized and placed in a weighed porcelain crucible and further dried at 80 ^{0}C for 24hours to a constant weight. The samples were further heated at 550 ^{0}C for 4 hours. The total organic matter content was then calculated as the difference in weight between dry weight at 80 ^{0}C and ash weight (550 ^{0}C).

$$\text{Total Organic Matter (\%)} = \frac{(B-A) - (C-A)}{\text{Weight of dry Sample at 80 } ^{0}\text{C}} \times 100$$

Where: A= average weight of empty Crucible.
B= average weight of residue dried at 80 ^{0}C (before ignition) + Crucible
C= average weight of residues/ ash after ignition at 550 ^{0}C + Crucible

3.3.8 Determination of Total Kjeldah Nitrogen (TKN)

Total Kjeldah Nitrogen (TKN) was determined by the Kjeldahl method according to standard methods (APHA 1998).

Sample digestion*:* The sample (0.2g) plus 9.6 g anhydrous sodium Sulphate (Na_2S0_4), 0.5g anhydrous copper Sulphate ($CuSO_4$) and 0.2 g of selenium powder was placed in a 250-mL Kjeldahl flask. Concentrated sulphuric acid (H_2S0_4) (20 mL) was added. The flask was heated at 300 ^oC until no more frothing and fumes came off. Heating was continued to obtain a yellowish green liquid that was later cooled to room temperature to solidify into crystals that formed a mass of solid cake.

Distillation: To the flask containing the sold cake, 300 mL of distilled water was added in small quantities while cooling the flask under the tap. The solution was transferred to a 500-mL distillation flask along with the boiling chips and neutralised by adding 100mL sodium hydroxide (NaOH) solution into the flask using the funnel and thereafter the funnel was sealed. Ammonia (NH_3) was trapped in the 500-mL receiver flask with 100mL saturated boric acid prepared by adding 4 g boric acid to 100 mL of distilled water, and three drops of an indicator prepared by dissolving one part of methyl red mixed with three parts of bromocresol green in 95 % ethyl alcohol. Distillation was carried out until when 200mL distillate was collected, and stopped when the distillate turn universal indicator paper neutral.

Titration: The 300 mL distillate containing the borate ions formed by the reaction of the liberated ammonia with boric acid was titrated against 0.1 M HCl. Titration was repeated three times, with the end point of the titration being indicated by a greyish colour at pH 4.6. Urea $CO(NH_2)_2$, which was used as the standard, was treated separately in exactly the same way as the sample. The percentage total nitrogen was calculated using the equation:

$$\text{Total nitrogen \%} = \frac{T \times M_a \times 1.4007}{W}$$

Where:

T = Sample titre (mL)

M_a = Molarity of HCL solution used in the titrations

W = Weight of sample (g)

1.4007 = Milliequivalent weight of N × 100

3.3.9 Determination of Ammonium Nitrogen (NH4-N)

Ammonia nitrogen in the samples was determined by titrimetric method, (APHA, 1998). **Sample preparation*:*** The samples were first dechlorinated by adding 1ml dechlorinating reagent (comprised of 3.5g $Na_2S_2O_3.5H_2O$ per litre) to 500 ml of the sample. To the dechlorinated sample, 25 ml of borate buffer solution (comprised of 88 ml of 0.1M NaOH solution added to 500 ml of 0.025M $Na_2B_4O_7$ and solution diluted to 1 litre) was added and the pH adjusted to 9.5 with 6M NaOH using a pH meter.

Distillation: The prepared sample was transferred to the distillation flask and distilled at the rate of 6 to 10 ml/minute with the tip of the delivery tube below the surface of acid receiving solution. A volume of 200 ml distillate was collected in a 500 ml Erlenmeyer flask containing

50ml indicating boric acid solution. The indicator boric acid was comprised of 10g of H_3BO_3 and 10 ml of mixed indicator solution, mixture diluted to 1,000 ml. The mixed indicator was consisted of 200 mg of methyl red indicator in 100 ml of 95 % ethyl alcohol, the mixture combined with 50 ml of methylene blue (100 mg) in 95 % ethyl alcohol.

Titration: The 200 ml of the distillate was diluted to 500 ml with distilled water and titrated against standard 0.02N H_2SO_4 (0.01M H_2SO_4) titrant until indicator turns pale lavender.

$$NH_4\text{-N (mg /Litre)} = \frac{(A-B)}{\text{ml sample}} \times 280$$

Where: A = ml volume of H_2SO_4 titrated for sample

B = ml volume of H_2SO_4 titrated for blank

280 = Constant; for 0.02N H_2SO_4 acid titrated, 1.00 ml =280µgN.

3.3.10 Determination of Organic Nitrogen

The organic nitrogen of the sample was determined by Kjeldahl method as the difference between total Kjeldahl nitrogen (TKN) and ammonium-nitrogen (NH_4-N).

Organic nitrogen (%) = (TKN) – (NH_4-N)

3.3.11 Estimation of Total Proteins

The protein in the sample was estimated based on the organic nitrogen content, in accordance with AOAC (2002). The following conversions were applied:

Total protein (mg/L) = (Organic Nitrogen) $\times$ 6.25

Total protein (mg/L) = (TKN-[NH3-N]) $\times$ 6.25

3.3.12 Determination of Total Carbohydrates as Total Sugars

The total concentration of sugars in the samples was determined by the phenol-sulphuric acid method with glucose as the standard (Dubois *et al.* (1956). Five milliliters (5 ml) of the sample extract was hydrolysed with 2.5 ml of 0.1M sulphuric acid into a homogeneous solution and thereafter phenol-sulphuric acid reagent was added. After colour development, the absorbance and concentration of total sugars in the sample was measured at 490 nm using a DR 2010 spectrophotometer (Hach Co. Loveland, CO, USA).

3.3.13 Determination of Starch Content

Starch content in the banana waste samples was analysed using two methods namely: determination of starch content as total sugars and determination of starch content as Amylose and Amylopectin, and the mean value from the two methods calculated to ensure validity of the results.

Method I: Determination of starch content as total sugars

In this method, starch content was determined according to Smith and Zeeman (2006) and involved conversion of starch in the sample into free glucose that was subsequently analyzed colorimetrically by the phenol-sulphuric acid method with glucose as the standard (Dubois *et al.,* (1956). Starch in the fresh homogenised sample was extracted by boiling the sample in 80% ethanol to remove free glucose, pigments and membranes. The starch extract was then solubilised to total glucose by heating and digestion of the ethanol-extract sample with 0.1M sulphuric acid. The solution was thereafter mixed with phenol-sulphuric acid reagent for colour development. Starch content was then estimated colorimetrically as total sugars measured at 490 nm using a DR 2010 spectrophotometer (Hach Co. Loveland, CO, USA).

Method II: Determination of starch content as Amylose and Amylopectin

In this method, starch content was estimated by iodine-starch colorimetric assay according to Hovenkamp-Hermelink *et al.,* 1988). Starch in the fresh homogenised sample was extracted by boiling in 80% ethanol to remove free glucose, pigments and dissolution of cell membranes (Smith and Zeeman, 2006). The extracted starch was then solubilised to amylose and amylopectin by boiling the solution with 90% dimethyl sulfoxide (Carpita & Kanabus, 1987). The soluble extract was then mixed with iodine solution for colour development and starch content measured colorimetrically at 620 nm, with standard starch solutions (Hovenkamp-Hermelink *et al.,* 1988 and Fajardo *et al.,* 2013).

3.3.14 Determination of Crude Fat

A sun-dried sample was pounded and fat extracted with diethyl ether, which dissolves fats, oils, pigments and other fat soluble substances, (Undersander *et al.*, 1993). The ether was then evaporated from the fat solution. The resulting residue were weighed and referred to as ether extract or crude fat.

Sample drying: The beaker to be used for fat extraction was oven-dried for 1hour at 100 ^{0}C and thereafter cooled to room temperature in desiccator before its weight ($\mathbf{W_1}$) recorded. A mass of sun-dried pounded sample was put into the beaker and the new weight ($\mathbf{W_2}$) recorded. The non-lipid soluble material in the sample was washed off with de-ionized water. A second sample for dry matter (DM) determination was also put into a beaker and its weight $\mathbf{W1_{dm}}$ recorded. The samples were oven-dried for 5hours at 100 ^{0}C and thereafter cooled to room temperature for 1 hour in a desiccator. The cooled beakers were weighed and the weight recorded as $\mathbf{W_3}$ and $\mathbf{W2_{dm}}$, respectively for the first and second samples.

Lipid Extraction: The dry sample (W_3-W_1) was transferred to a separating funnel. The beaker previously containing sample was carefully rinsed with 30 ml of diethyl ether (extracting solvent) and the solvent washings added to the separating funnel. The separating funnel was shaken vigorously for 2 minutes and allowed to settle in order for layers to separate. The solvent layer was drained through a funnel containing solvent-rinsed filter paper and 10g Na_2SO_4, into a clean, dry, pre-weighed distillation flask. The aqueous layer was recombined together with any remaining emulsion or solids in separating funnel and the extraction repeated twice more with 30 ml solvent portion. The total extracts were put into

the distillation flask including the final rinsing of filter and Na_2SO_4 with an additional 20 ml solvent. The solvent was recovered from the distillation flask by distilling the mixture in a water bath at 85 ^{0}C. The distillation flask was fitted with a distillation adapter equipped with a drip tip that directed the solvent into an ice-bath cooled receiver to maximise solvent recovery. When visible solvent condensation stopped, the distillation flask was removed from the water bath and dried on top of water bath cover at 85 ^{0}C for 15 minutes. The air was drawn off the flask with an applied vacuum for 1 minute and the flask cooled in the desiccator for 1hour before re-weighing. The distillation flask containing solvent blank was similarly treated and the solvent recovered through an ice-bath as for sample extraction.

$$\text{Total crude Fat (\%)} = \frac{(A - B)}{W_2 - W_1} \times 100$$

Where A = Average weight gain of distillation flask due to sample
B = Average weight gain of distillation flask due to solvent blank
W1 = Weight of beaker in grams
W2 = Weight of Sun-dried sample + beaker in grams

$$\text{Crude Fat (\%DM basis)} = \frac{(A - B)}{(W3 - W1) \times \text{Lab DM}/100} \times 100$$

Where W3 = Weight of Oven-dried sample + weight of beaker in grams
DM = Dry Matter

$$\text{Dry Matter (\%)} = \frac{(W2_{dm} - W1)}{(W1_{dm} - W1)} \times 100$$

3.3.15 Determination of Fibre and Lignin (lignocellulose) Content

The fibre and lignin contents of banana waste were determined by two methods namely; acid detergent solubilisation method and Gravimetric method, and the average of the values from the two methods calculated.

Method I: Determination of lignocellulose content by Acid-Detergent Solubilisation Method

The fibre and lignin contents of banana waste were determined in triplicates according to Goering and Van Soest (1970). The procedure was based on the ability of detergent to solubilise non-fibrous components and separate the fibre by filtration, as particulate material. The determination involved analysis of Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF).

a) Determination of acid detergent fibre (ADF)

Dried pounded banana waste sample of 1 g (W_1) was put in a 250-mL reflux flask fitted with a condenser at the top. To the sample, a volume of 50 mL of acid detergent solution (composed of 49.04 g (26.65 mL) H_2S0_4 (95-97%) and 20 g cetyltrimethly ammonium bromide (CTAB) per 1 litre of distilled water); 2 mL decahydronaphthalene and one drop of antifoam were added. The reflux fitted with a condenser was placed on a heating mantle in the fume cupboard. The mixture was brought to boiling within 5-10 minutes. Boiling was then maintained for another 60 minutes. The contents of the flask were poured into glass crucibles of porosity 2 (40-100µm pore diameter, which had been dried overnight at 100 ^{0}C and weighed while hot), and filtered using a suction pump without letting the sample dry. The sample was washed with hot distilled water (90-100 ^{0}C), stirred and left to soak for 5 minutes. The water-washed sample was then dried by vacuum, and the above step repeated. The sample was then washed with acetone and vacuum dried for 10 minutes. The crucibles with the sample were oven dried overnight at 100 ^{0}C, transferred to a desiccator, cooled to room temperature and weighed (W_2). The percentage ADF was calculated using the formula:

$$\% \text{ ADF} = \frac{(W_2)}{W_1} \times 100$$

Where: W_1 = Initial sample weight (g)
W_2 = Acid-digested Oven dried sample weight (g)

b) Determination of neutral detergent fibres (NDF)

Neutral detergent fibre (NDF) was determined the same way as acid detergent fibre (ADF) but an NDF solution instead of ADF solution, was used. The NDF solution was composed of 30g SDS (sodium dodecyl sulphate), 18.61g ethylene diamine tetra acetic acid disodium salt ($Na_2EDTA.2H_2O$) and 6.31g Na_2HPO_4 (pH 6.9-7.1).

c) Determination of Hemicellulose

Hemicellulose was determined as the difference between the percentage NDF and the percentage ADF.

$$\% \text{ Hemicellulose} = \% \text{ NDF} - \% \text{ ADF}$$

d) Determination of Lignin

Lignin was measured as the weight lost by dissolving the deposited manganese and iron oxides, resulting from oxidation of lignin by an excess of acetic acid buffered $KMnO_4$. Three solutions namely; lignin buffer, oxidising solution and demineralisation solution, were used to dissolve lignin prior its determination. were prepared as follows: Solution 1 (Lignin buffer) was prepared by dissolving 6 g $Fe(NO_3)_3.9H_2O)$ in 100 mL of distilled water, and then

added 0.15 g $AgNO_3$ and 500mL glacial acetic acid.,5 g potassium acetate and 400 mL tertiary butyl alcohol was then be added. Solution 2 was prepared by mixing 50 g KMO_4 , 0.05 g Ag_2SO_4 and double distilled water to a final volume of 1 litre. Solution (2) and the lignin buffer (1) were mixed at a ratio of 2:1 just before use. Demineralisation solution composed of 50 g of Oxalic acid ($C_2H_2O_4$.$2H_2O$) in 700 mL 95% ethanol and 50 mL 12 N HCL and 250 mL distilled water.

The crucibles containing the ADF fraction were placed in a pan containing cold water. About 25 mL of the mixture of solutions 1 and 2 were added to the ADF fraction to make the water level of the pan 2-3 cm higher. A glass bar was placed in each crucible to mix the solution. The crucibles were then sucked dry and placed in a clean pan and half-filled with demineralisation solution. The crucibles contents were filtered dry after 5 minutes and the procedure were repeated until the residue is white. The crucibles contents was then be filled with 80% ethanol and the content washed thrice and then twice with acetone after which, the glass crucible containing the sample were dried in an oven at 105 ^{0}C overnight. The oven-dried weight constitutes lignin.

e) Determination of cellulose

The crucibles containing the residues obtained after lignin extraction were weighed and thereafter heated in a muffle furnace at 500 ^{0}C for 2 hours, and weight of the residual ash recorded. The weight loss on sample conversion to ash was the cellulose.

Method II: Determination of lignocellulose content by Gravimetric method

The gravimetric method for lignocellulosic compositional analysis (cellulose, hemicelluloses and lignin content) was done as described by Ayen *et al.,* (2015). Sun-dried pounded sample was weighed and loaded into a cellulose thimble and extractives (sucrose, nitrate/nitrite, protein, chlorophyll and waxes) removed by Soxhlet extractor using boiling acetone (70 ^{0}C) for 4 hours. The extractive-free biomass was oven dried at 105 ^{0}C for 24 hours prior to re-weighing using a precision balance. The difference in weight between the raw extractive-laden biomass and extractive-free biomass was expressed as the % content of extractives.

a) Gravimetric Determination of % Hemicellulose

One gram of extractive-free sample was digested by boiling with 0.5M NaOH for 3.5 hours (Aveni *et al.,* 2013); cooled down and washed with distilled water to neutral pH prior to vacuum filtration. The residue was dried to a constant weight at 105 ^{0}C in a convection oven and reweighed using a precision balance. The difference in sample weight before and after alkali treatment, expressed as a percentage was the hemicellulose content in the sample.

b) Gravimetric Determination of % Lignin

The dried extractive-free sample was weighed into glass test tube and digested with 72% H_2SO_4 in an autoclave for 1 h at 121 ^{0}C. The slurry was cooled at room temperature, residues filtered through vacuum using a filtering crucible. The lignin content was

determined by oven drying the residues at 105 ^{0}C for 24 hours prior to re-weighing. The ash content was determined by ignition of the dried acid hydrolyzate residues at 575 ^{0}C in a muffle furnace for 2 hours (Sluiter *et al.,* 2008).

c) Gravimetric Determination of %Cellulose

The cellulose content of the sample was estimated as a percentage difference from total summation of % extractives, % hemicellulose and % lignin.

3.4 Estimation of methane percentage content in the biogas

The composition of biogas produced during anaerobic digestion was estimated by the concentrated alkaline absorption method using serum bottles as described by Ergüder *et al.* (2001). In this method only methane is determined and other biogas components such as CO_2 and H_2S are dissolved in the concentrated alkaline solution. A volume of 5 ml biogas sample was injected into a closed 11-ml serum bottle containing 8 ml of KOH solution (20 g/L) at atmospheric pressure. The bottles were vortexed for 4 minutes and allowed to settle for one more minute. A 5 ml syringe was inserted through the gas-tight rubber stopper and the pressure of the undissolved gas (methane) in the headspace pushed the piston of the syringe upwards until no more sliding was observed (Gumisiriza *et al.,* 2009).

$$\%\ \text{Methane} = \frac{V_2}{V_1} \times 100$$

Where: V_1= initial volume of biogas sample (5ml)
V_2= final volume of the gas after shaking.

3.5 Determination of operational parameters and evaluation of progress of anaerobic digestion

The main formulae for determination of operational parameters and evaluation of progress of anaerobic digestion are shown in table 2.1 and have been highlighted by previous researchers.

Table 3:1 Main formulae for evaluation of performance of anaerobic digestion systems (Mata-Alvarez, 2003; Vogeli *et al.,* 2014)

Operational Parameter	Formula	Description	Units
Hydraulic Retention Time (HRT)	HRT = V/Q	HRT = Time the Slurry spends in the Reactor	Days
		V = Reactor Volume	M^3
		Q = Substrate Flow rate	M^3 /Day
Organic Loading Rate (OLR)	OLR = [Q x S]/V	OLR = Quantity of Substrate introduced into a volume of Reactor per given Time	KgVolatile Solids (VS) /M^3/Day
		Q = Substrate Flow rate	M^3 /Day
		S = Inflow Substrate Concentration	KgVS/M^3
		V = Reactor Volume	M^3
Gas Production Rate (GPR)	GPR = Q_{biogas}/V	GPR = Volume of biogas produced per Volume of reactor per given Time	M^3 biogas/M^3 Reactor/Day
		Q_{biogas} = Biogas Flow Rate	M^3 /Day
		V= Reactor Volume	M^3
Specific Gas Production (SGP)	SGP = GPR/OLR	SGP = Volume of biogas produced per Kg of volatile solids fed into the Reactor	M^3 biogas/KgVS inflow substrate
		Q_{biogas} = Biogas Flow Rate	M^3 /Day
	Or SGP = Q_{biogas}/QxS		
		Q = Substrate Flow Rate	M^3/Day
		S = Inflow Substrate Concentration	KgVS/M^3

3.6 References

1. Allen, S.E. (1989) “Chemical analysis of ecological material. (2nd ed). Blackwell Scientific Publications; Oxford, UK.

2. AOAC (2002) Association of Official Analytical Chemist. Official Methods of Analysis (17th ed.). Washington, DC.

3. APHA (1998). Standard methods for examination of water and wastewater. 20 Edn. American Public Health Association, Washington, D.C

4. Ayeni, A.O., Adeeyo, O.A., Oresegun, O.M and Oladimeji, T.E (2015) “Compositional analysis of lignocellulosic materials: Evaluation of an economically viable method suitable for woody and non-woody biomass” American Journal of Engineering Research Vol.4, (4) pp-14-19

5. Carpita, N. & Kanabus, J (1987) Extraction of starch by dimethylsulfoxide and quantitation by enzymatic assay. Analytical Biochemistry. 161, 132–139.

6. Dubois, M., Gilles,K.A., Hamilton, J.K., Rebers, P.A and Smith, F (1956) “Colorimetric methods for determination of sugars and related substances. Analytical Chemistry 28: 350-356.

7. Erguder, J.F., Tezel, U., Guven, E., Demirer, G.N (2001), “Anaerobic biotransformation and methane generation potential of cheese whey in batch and UASB reactors”, Waste management. 21: 643-650.

8. FAOSTAT 2012: Food and Agricultural Organisation Statistics; data on bananas and plantains production.

9. Fajardo, D., Jayanty, S.S and Jansky, S.H (2013). Rapid High Throughput Amylose Determination in Freeze Dried Potato Tuber Samples, Journal of Visual Experiments, (80): 50407

10. Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analysis. USDA Agric. Handbook No. 379. USDA-ARS, Washington, DC.

11. Gumisiriza, R (2009), Auditing of fish wastes generated along lake Victoria and enhancement of anaerobic digestion of fish processing wastewater,” MSc. Thesis submitted to the University of Dar es Salaam.

12. Hovenkamp-Hermelink J, Devries J, Adamse P, Jacobsen E, Witholt B, Feenstra W. (1988) Rapid estimation of the amylose amylopectin ratio in small amounts of tuber and leaf tissue of the potato. Potato Res.;31:241–246.

13. Karamura, D. A., Karamura, E and Tinzaara, W (2012) “Banana Culltivar Names, Synonymes and their Usage in East Africa” Bioversity International, Uganda.

14. Lyimo, T.J, Pol, A and Opden Camp, H.J.M (2002a) “Methane emission, sulphide concentration and redox potential profiles in Mtoni mangrove sediment, Tanzania”. Western Indian Ocean Journal of Marine Science 1: 71–80.

15. Mata-Alvarez J. (2003). Biomethanization of the organic fraction of municipal solid wastes.IWA publishing. London.

16. Mshandete, A., Kivaisi, A., Rubindamayugi, M. and Mattiasson, B (2004), “Anaerobic batch co-digestion of sisal pulp and fish wastes”, Bioresource Technology 95, 19-24

17. ProMusa: *"Banana cultivar checklist on ProMusa"*. *Retrieved 26th October 2021.* https://www.promusa.org/tiki-index.php?page=Banana+cultivar+checklist&f_87=EAHB

18. Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J and Templeton, D (2008) Determination of structural carbohydrates and lignin in biomass: laboratory analytical procedure (LAP). Golden, CO: National Renewable Energy Laboratory; NREL Report No.: TP-510-42618. Contract No.: DE-AC36-99-G010337, Sponsored by the U.S. Department of Energy.

19. Smith, A. M and Zeeman, C.S (2006), Quantification of starch in plant tissues. Protocol; 2006 Nature Publishing Group; http://www.nature.com/natureprotocols.

20. Spilsbury, J.S., Jagwe, J.N., Ferris, R.S.B (2002), “Evaluating the Marketing Opportunities for Banana & its Products in the Principle Banana Growing Countries of ASARECA (Uganda)”, International Institute of Tropical Agriculture. Foodnet. P: 60.

21. Tripathi, L., Tripathi1, J. N. and Tushemereirwe, W. K (2008), “Rapid and efficient production of transgenic East African Highland Banana (Musa spp.) using intercalary meristematic tissues”, African Journal of Biotechnology Vol. 7 (10), pp. 1438-1445

22. Tumutegyereize, P., Muranga, F.I., Kawongolo, J. and Nabugoomu, F (2011), “Optimization of biogas production from banana peels”, African Journal of Biotechnology Vol. 10 (79), 18243-18251

23. Undersander, D., Mertens, D. R and Thiex, N (1993) “Forage Analysis procedures; National Forage Testing Association, Omaha, USA

24. Vögeli Y., Lohri C. R., Gallardo A., Diener S., Zurbrügg C. (2014). “Anaerobic Digestion of Biowaste in Developing Countries: Practical Information and Case Studies”. Swiss Federal Institute of Aquatic Science and Technology (Eawag), Dübendorf, Switzerland.

4 Characterisation of banana waste as a potential feedstock for biogas production

4.1 Introduction

Banana production systems and banana fruit processing accumulate large quantities of waste residues due to high quality demands of the markets (Graefe *et al.,* 2011). The East African highland cooking banana subgroup (AAA-EA group) locally called *matooke*, is the major grown variety and a leading staple food (Tumutegyereize *et al.,* 2011). Studies on banana production have shown that over 70% of the farmers in major producing districts within the Lake Victoria basin grow bananas as a primary crop and over 50% depend on banana for food and income security (Bagambe *et al.,* 2006). Uganda is the second largest global producer of bananas after India and the leading in Africa, with annual production estimated at 9.77 million tonnes (Tripathi *et al.,* 2008; FAOSTAT, 2012). Generally, crop production and processing produce huge amount of waste termed as agricultural waste (Padam *et al.,* 2014). Banana production, post-harvest handling (market value chain) and the ultimate processing to generate edible fruit pulp are all accompanied by release of large volumes of inedible residues that constitute the banana waste. Banana waste (BW) (Abdullah *et al.,* (2014) comprises: rotten/damaged fruits, peels, fruit-bunch-stem (stalks), leaves, fibers, pseudo-stem, and rhizome. As a matter of fact, it is estimated that more than three million tonnes of banana waste are generated annually in the country (Spilsbury *et al.,* 2002; Tumutegyereize *et al.,* 2011). Studies on banana post harvest losses (PHL) by Asha *et al.,* (2015) revealed that poor banana handling methods along the market value chain can lead to a loss of 9.6% of mature banana fruits mainly as a result of short shelf life and rapid ripening. Such PHL that mainly occur during high production with limited market, can be circumvented by industrial banana processing into dried banana chips that can serve as the raw material for value-added products such as starch and flour, for both export and local food security. Thus, the production and processing that release major waste streams remain the major challenge. However, Uganda's banana industrialization relies mainly on costly imported petroleum products for fuel energy and is grappling with inadequate and expensive energy (Gumisiriza *et al.,* 2017). Hence, utilization of banana waste as feedstock for energy production to relieve the banana industry from both energy scarcity and reliability can be the best option and first priority for managing banana waste in Uganda. Among the applicable waste-to-energy technologies, anaerobic digestion to generate biogas has been recommended as the most appropriate for banana waste due to it being rich in organic matter with high moisture content (Tock *et al.,* 2010; Gumisiriza *et al.,* 2017).

In biogas milieu, the term feedstock is defined as any substrate that can be converted to methane by anaerobic bacteria (Steffen *et al.,* 1998). Generally, biogas feedstock comprises of all compounds with a substantial amount of organic matter that is finally converted to mainly methane and carbon dioxide through anaerobic digestion. Biogas feedstocks range from readily degradable animal manure, wastewater sludge, and agricultural wastes to complex lignocellulosic biomass that contains high-solid content. Besides, toxic compounds that contain organic matter may also be biomethanised depending on the technology applied (Steffen *et al.,* 1998). Nevertheless, traditional feedstock for anaerobic

digestion has mainly been associated with animal manure (pig, cattle, and poultry) and sewage sludge from wastewater treatment plants. The use of these feedstocks in anaerobic digestion has been mainly to promote good sanitation and local utilization of biogas. However, the increased craving for renewable energy forms for industrial purposes accompanied by the demand for new eco-friendly waste management strategies has broadened the search for alternative biogas feedstocks. This has introduced new field of feedstock sources such as energy crops and the industrial wastes such as residues from agro-processing, slaughterhouses and diaries as well as organic fraction of municipal solid wastes (OFMSW) as shown in Figure 4.1. Clearly, agriculture accounts for the largest potential sources of feedstocks for biogas production and includes the harvest remains, animal manure, weeds and energy crops.

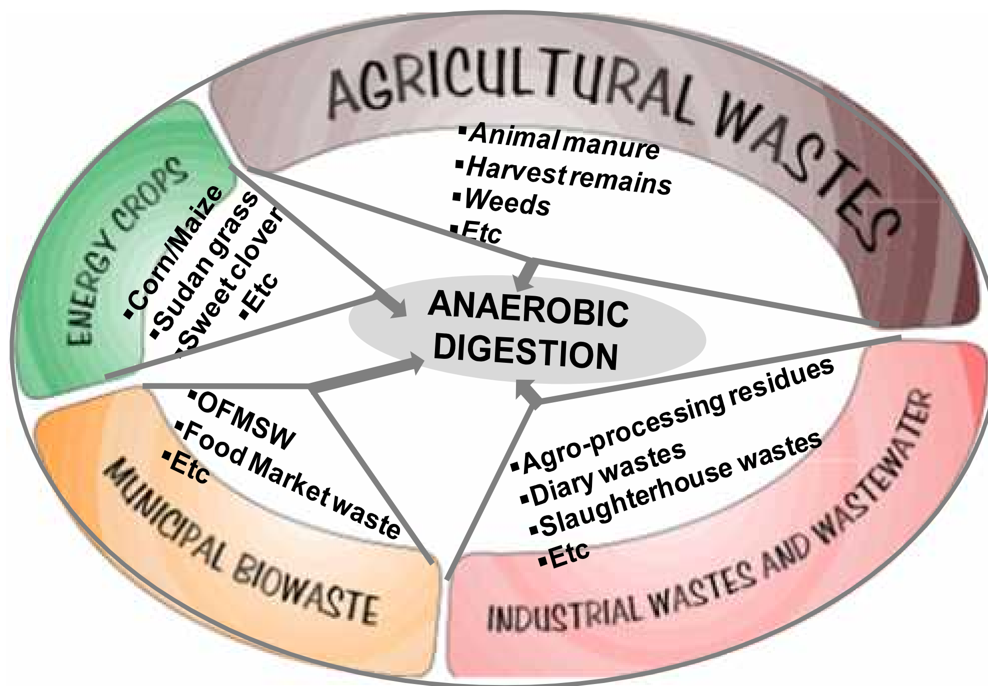


Figure 4:1. Major sources of feedstocks for anaerobic digestion (adapted from Steffen et al., 1998)

Animal manure, as feedstock for biogas production, is popular mainly due to the biotechnological ease of handling during anaerobic digestion. For instance, cow slurry has inherent microbial flora necessary for anaerobic digestion of the feedstock to generate biogas. Typically, cow's rumen is one of the excellent rich sources of methanogenic bacteria required for bioreactor start-up and hence using such animal manure offsets the requirement for feedstock inoculation. However, using animal manure as biogas feedstocks generates less biogas when compared with fresh plant biomass. This low biogas yield may be attributed to the fact that animal manure, probably is not well balanced in other nutrients required for balanced microbial growth, but rather containing complex polysaccharides such as lignocelluloses. These are not only hard to digest, but they also require consortia of microorganisms for complete breakdown. The high ligno-cellulose content of waste substrate such as plant biomass has been reported to slow down the bio-gasification process primarily due to limited microbial hydrolysis of complex polysaccharides abundant in such waste (Patrick *et al.,* 2011). A research study by Martin-Ryals, 2012 however, reported that an eco-

friendly and inexpensive way of effective hydrolysis of ligno-cellulosic biopolymers can be achieved by microbial pre-treatment. Effective hydrolysis is only by synergistic interactions and co-metabolism of different microbial strains mainly of fungal origin and a few rare bacterial strains (Yan *et al.,* 2012).

Moreover, anaerobic digestion has a superior advantage of coupling energy (biogas) generation along with plant organic fertilizer (bioslurry) generation at minimal net operational energy requirement. Other advantages of anaerobic digestion (AD) process are: reduction in wastes' pathogens, smaller land suitability and decrease in waste's pollution potential to levels that are non toxic to the environment (Moody and Raman, 2001). However, physical-chemical nature of the feedstock influences the bioreactor configuration (bioreactor design and operational parameters) and has a comprehensive effect on liquor microbial biochemistry that ultimately alters the overall AD process.

Thus banana waste must be characterized prior to use as feedstock for biogas production. Banana waste characterization and use as substrate feed for biogas production is limited to biovalorization studies by Salyeem *et al.,* (2014) and co-digestion experiments by Kirtane *et al*., 2009 and Tumutegyereize *et al.,* 2011). However, thorough characterization of banana waste from mixed streams containing fruit bunch stalks, pseudo-stems and stem fibers was never investigated. Besides, the composition of banana waste varies considerably depending on the variety/cultivar grown, soil, agronomic practices, type of processing, season, geographical origins and also the varying degree of ripeness and post-harvest handling (Salyeem *et al.,* 2014). As such, each waste fraction from banana processing needs to be characterized separately, to provide baseline data for future value addition. Hence, a comprehensive assessment of the quantity and composition (quality) of the feedstock is required prior anaerobic digestion. The objective of this research study was to assess the key steps in processing of green bananas into pulp, and auditing and characterization of the major resulting residual wastes namely peels, peduncle (fruit-bunch stalk) and fruit discard, in order to evaluate their potential as feedstocks for biogas production. Therefore, the physicochemical analysis of composite banana waste and the biochemical quality and feasibility for use of banana waste as a feed stock for biogas production are reported.

4.2 Methodology

4.2.1 Assessment of banana processing and banana waste audit

A banana waste audit was done through a reconnaissance visit to western Uganda, one of the most banana producing regions in the country (Asha *et al.,* 2015). Information regarding the nature and type of processing, quantity and quality of waste generated, and current waste management methods was collected through guided survey along the processing plant, open-ended interviews, photography and sampling for laboratory analysis (Newenhouse and Schmit., 2000). Waste quantification and characterization was done by integration of qualitative and quantitative methods, and ultimately laboratory analysis for evaluation of biochemical composition. Banana waste generated from processing of banana fruit bunch into pulp was quantitatively estimated over a period of six months distributed over one year, based on five commonly cultivated clones of *Musa acuminata* (AAA-EA)-the East African

High land cooking bananas. These clones investigated were namely; Mporogoma, Kishansha, Kibuzi, Mbwazirime and Enyeru as specified by Karamura *et al*., 2012 and promusa; https://www.promusa.org/tiki-index.php?page=Banana+cultivar+checklist&f_87=EAHB
The fruit bunches were weighed prior to processing and subsequently de-bunched and fruit-fingers peeled to obtain the fresh pulp as the product. The generated waste residue fractions were weighed using a precision balance and their percentage composition determined. Banana waste samples for laboratory analysis were collected from different processing streams and transported to the laboratory for analysis and biogas production experimentation at the Department of Biochemistry, Makerere University, Kampala-Uganda. Three samples were collected at each stream and sampling was done weekly (four times a month) at an interval of one month for one year; between January and December 2015, following standard methods described by Undersander *et al*., (1993) and APHA (1998). In total, seventy two samples were analysed for each waste stream.

4.2.2 Physico-chemical Characterization

4.2.2.1 Sample preparation

At the laboratory, raw banana waste samples were shredded into a homogeneous paste (Figure 4.2) using an organic shredder (TR 200: Organic Shredder, BrazAfric Enterprises LTD). The samples were frozen if not used immediately and were thawed for 24 hours at room temperature (26±2 ^{o}C) before analysis and use in the subsequent studies.

Figure 4:2. Sample preparation for physico-chemical analysis and feedstock for anaerobic digestion

4.2.2.2 Laboratory analysis

Laboratory analysis of the samples was done in triplicates for physico-chemical parameters namely: moisture content (MC), total solids (TS), volatile solids (VS), ash content (AC), organic carbon (OC), organic matter (OM), total Kjeldahl nitrogen (TKN) and percentage

composition of proteins, starch, sugars, crude fat, cellulose, hemicelluloses and lignin content.

MC, TS, VS and AC were determined gravimetrically by the hot air oven-drying and ignition method according to standard methods described in APHA (1998). Analysis for MC and TS was done by drying pre-weighed fresh samples in a hot air oven (model: Gallenkamp & Co. Ltd, and London, UK) for 24 hours at 105°C to get consistent constant weights (Emaga *et al.,* 2007 and Kiyasudeen *et al.,* 2015). VS and AC were determined by ignition of the previously oven-dried samples for 2 hours at 550 ^{o}C in a muffle furnace (Model: Carbolite 1100 ^{o}C furnace, Chelmsford, England). The ash containing crucibles were cooled in the desiccator to room temperature (25 ^{0}C) before re-weighing (Gumisiriza *et al.,* 2009) using a precision balance.

OC was determined by dry combustion method (Allen, 1989), in which one gram of the oven-dried ground sample was heated at 600 ^{o}C for 5 hours in a muffle furnace and thereafter cooled in the desiccator to room temperature (25 ^{0}C) and the weight of the ash recorded. The OC was calculated as a quotient of percentage weight deficit divided by a factor of 1.8 to correct for organic matter lost to organic carbon during combustion.

OM content was also determined gravimetrically by the dry combustion method previously described by Lyimo *et al*. (2002), in which one gram of ground sample previously dried at 80 ^{o}C for 24hours in hot air oven (model: Gallenkamp & Co. Ltd, and London, UK) was heated at 550 ^{o}C for 4 hours in the muffle furnace. The total organic matter content was calculated as the difference in weight between dry weight at 80 ^{o}C and ash weight at 550 ^{o}C.

TKN was determined by the Kjeldahl acid digestion block method as described by Undersander *et al*., (1993) and Kiyasudeen *et al.,* (2015). One gram dry ground sample was subjected to Kjeldahl acid digestion (combination of 25 mL H_2SO_4 and Kjeldahl catalysts) using Gerhardt Kjeldatherm digester and allowed to cool for 1 hour and subsequently subjected to distillation (32% NaOH and 2% H_3BO_3 combination) and finally titration using 0.1 N HCl.

Crude protein was obtained by multiplying TKN by a factor of 6.25 (AOAC, 2002; Emaga *et al.,* 2007 and Salayeem *et al.,* 2014).

Crude fats were determined by ether extraction method as described by Undersander *et al*., (1993). Fats in dry samples were extracted using diethyl ether and dried at 105 ^{0}C in an oven for 1 h and finally quantified gravimetrically (Emaga *et al.,* 2007).

Sugars were determined according to Dubois *et al*. (1956) by the phenol-sulphuric acid (Anthrone reagent) method with glucose standard. Diluted solution from homogenized sample was mixed with phenol-sulphuric acid reagent and after colour development; the concentration of sugars was measured colorimetrically at 490 nm (Colin *et al.,* 2007) using a spectrophotometer.

Starch content was estimated by iodine-starch colorimetric assay according to Hovenkamp-Hermelink *et al.,* 1988). Fresh homogenised samples were extracted to remove free glucose, pigments and dissolution of cell membranes by boiling in 80% ethanol (Smith and Zeeman, 2006). Ethanol-treated samples were solubilized by boiling with 90% dimethyl sulfoxide (Carpita and Kanabus, 1987). The soluble extracts were mixed with iodine solution for colour

development and starch content measured colorimetrically at 620 nm, with standard starch solutions (Hovenkamp-Hermelink *et al.,* 1988; Fajardo *et al.,* 2013).

Lignocellulosic compositional analysis for cellulose, hemicelluloses and lignin was done using gravimetric method according to Ayen *et al.,* (2015). Dried ground sample was weighed and loaded into a cellulose thimble and extractives (sucrose, nitrate/nitrite, protein, chlorophyll and waxes) removed by Soxhlet extractor using boiling acetone (70 ^{0}C) for 4 hours. The extractive-free biomass was oven dried at 105 ^{0}C for 24 hours prior to re-weighing using a precision balance. The difference in weight between the raw extractive-laden biomass and extractive-free biomass was expressed as the percentage content of extractives.

To determine the percentage of Hemicellulose, one gram of extractive-free sample was digested by boiling with 0.5M NaOH for 3.5 hours (Ayeni *et al.,* 2013); cooled down and washed with distilled water to neutral pH prior to vacuum filtration. The residue was dried to a constant weight at 105 ^{0}C in a convection oven and reweighed using a precision balance. The difference in sample weight before and after alkali treatment, expressed as a percentage was the hemicellulose content in the sample.

To determine the percentage of Lignin, the dried extractive-free sample was weighed into glass test tube and digested with 72% H_2SO_4 in an autoclave for 1 h at 121 ^{0}C; 15 psi. The slurry was cooled at room temperature, residues filtered through vacuum using a filtering crucible. The lignin content was determined by oven drying the residues at 105 ^{0}C for 24 hours prior to re-weighing. The ash content was determined by ignition of the dried acid hydrolyzate residues at 575 ^{0}C in a muffle furnace for 2 hours (Sluiter *et al.,* 2008).

The percentage of Cellulose in the sample was estimated as a percentage difference from total summation of % extractives, % hemicellulose and % lignin.

All the samples of were analyzed in three replicates and the recorded results were the average of the three recordings.

4.2.3. Determination of Biochemical Methane Potential (BMP) of banana waste

Bioreactor Configuration

Anaerobic digestibility of mixed banana waste was tested using a biochemical methane potential (BMP) assay carried out in batch bioreactors as described by Mshandete, *et al.* (2005), Gumisiriza, *et al.,* (2009). The reactors were made from 150ml wide mouth Erlenmeyer conical flasks with a working volume of 100ml at a substrate concentration of 5.0 gVS/L (Prabhudessai *et al.,* 2013). A solution of 5ml $NaHCO_3$ was added to the each reactor to buffer the pH changes during anaerobic digestion, since banana waste had a high C:N ratio. The outside of the flasks was covered with black polythene bags to cut off light and thus prevent the growth of anaerobic phototrophs that could release oxygen, which is toxic to methanogens (Waiswa *et al.,* 1998; Gumisiriza*, et al.,* 2009).

The inoculum

The inoculum was collected from a highly active fixed-dome anaerobic digester receiving a mixture of cow dung and pulverized hey residues as feedstocks, at a dairy cattle farmer in the vicinity of Makerere University. The inoculum was pre-incubated in anaerobic jars for two weeks to deplete the residual biodegradable organic matter prior to use in this experiment. The total solids of the inoculum at the time of loading were 22g/L.

The inoculum-to-Substrate loading (ISL) ratio

The substrate was seeded at an ISL ratio of 1:1, gVS basis according to moody, (2006) and Gumisiriza, *et al.,* (2009) following the calculations below:

If; Total Solids (TS) of Substrate (g/L) = A
Total Solids (TS) of Inoculum (g/L) = B
Volatile Solids (VS) of Substrate (% of A) = C
Volatile Solids (VS) of Inoculum (% of B) = D

Then; gVS/L of inoculum = $D \times B$
gVS/L of Substrate = $C \times A$

And if the volume (in Litres) of Inoculum used = V_i.
Thus; gVS in V_i of inoculum = $[D \times B] \times V_i$.

Hence, for bioreactor ISLR of 1:1 (gVS basis);
The gVS of the substrate = gVS of the Inoculum.
Implying that; gVS of the substrate = $[D \times B] \times V_i$

Therefore; Volume of substrate (in Litres) loaded $= \dfrac{[D \times B] \times V_i}{C \times A}$

All the experiments were carried out in triplicates including a control without substrate to account for any endogenous biogas residual produced from the inoculum. The calculated biogas production was corrected for blank biogas production before data recording. Each bioreactor was manually shaken once a day and further swirled for 1 minute prior to biogas volume measurement.

Measuring biogas production and methane content

The biogas production was measured by water displacement method (Singh *et al.,* 2001; Kirtane *et al.,* 2009). A tube connected to the reactor delivered the produced biogas to an inverted 250 mL graduated measuring cylinder immersed in a 1000 mL beaker filled with water. Biogas produced was collected in the graduated cylinder connected with a water reservoir which allowed volumetric biogas measurements at atmospheric pressure (Prabhudessai *et al.,* 2013). The methane content was estimated according to Erguder *et al.* (2001) and Mshandete *et al*. (2005), by the concentrated alkaline absorption method. Each bioreactor was manually shaken by swirling for 1 minute prior to biogas volume measurement.

Comparison BMP of banana waste with other potential substrates

In addition, the digestibility of banana waste was compared with grass and fish waste (animal waste) by carrying out a BMP of hey grass and fish waste following similar method as for banana waste. The fish waste comprised of trimmings, skin and viscera was collected from the fish market waste bins. Hey grass mainly comprised of *Chloris gayana* residues was obtained from the dairy cattle barn yard at the time of inoculum collection. Samples were pulverized prior to loading into the bioreactor.

4.3 Results

4. 3.1 Banana processing and waste generation

A survey of the major banana producing regions revealed that processing of banana fruit bunches is carried out manually by peeling of fruits to generate fresh pulp for domestic consumption, and is usually done by women (Figure 4.3). The banana waste streams generated at production level mainly include pseudo-stem, leaves, fibers and corm (rhizome) that remain in the garden after cutting off fruit-bunches. The survey also revealed that processing of fruit bunches into fruit-pulp generates residue fractions mainly comprising peels, fruit-bunch-stem (peduncle or stalk) and rotten/damaged fruits.

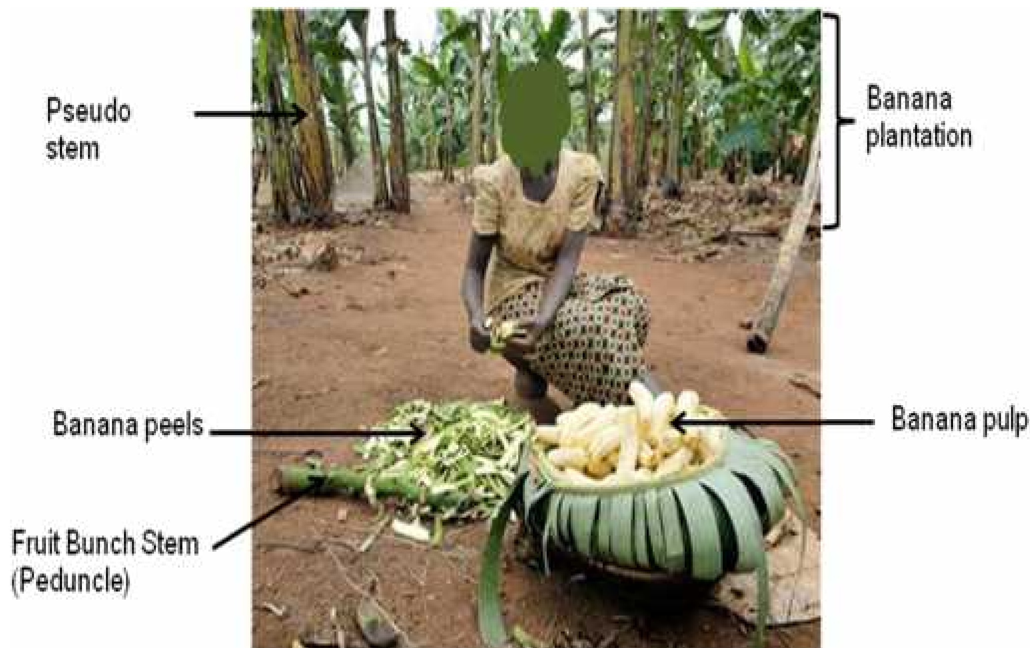


Figure 4:3. Banana peeling: A traditional method for banana processing in Uganda

It was further noted that the Government of Uganda had initiated industrial banana processing, through a organization called Presidential Initiative on Banana Industrial Development (PIBID), into banana chips that could serve as the raw material for value-added products such as starch and flour, for both export and local food security. At this industry, banana processing start with receiving of mature banana fruit bunches that were subsequently de-bunched to separate fruit-fingers from the peduncle (Figure 4.4). Fingers were peeled to get the pulp that was sliced, and finally dried into banana chips. The major waste fractions generated at the banana processing industry mainly comprised peels, peduncle and fruit rejects (Figure 4.5). Banana peels constituted the major percentage of the industrial waste stream followed by the peduncle and lastly, the fruit rejects.

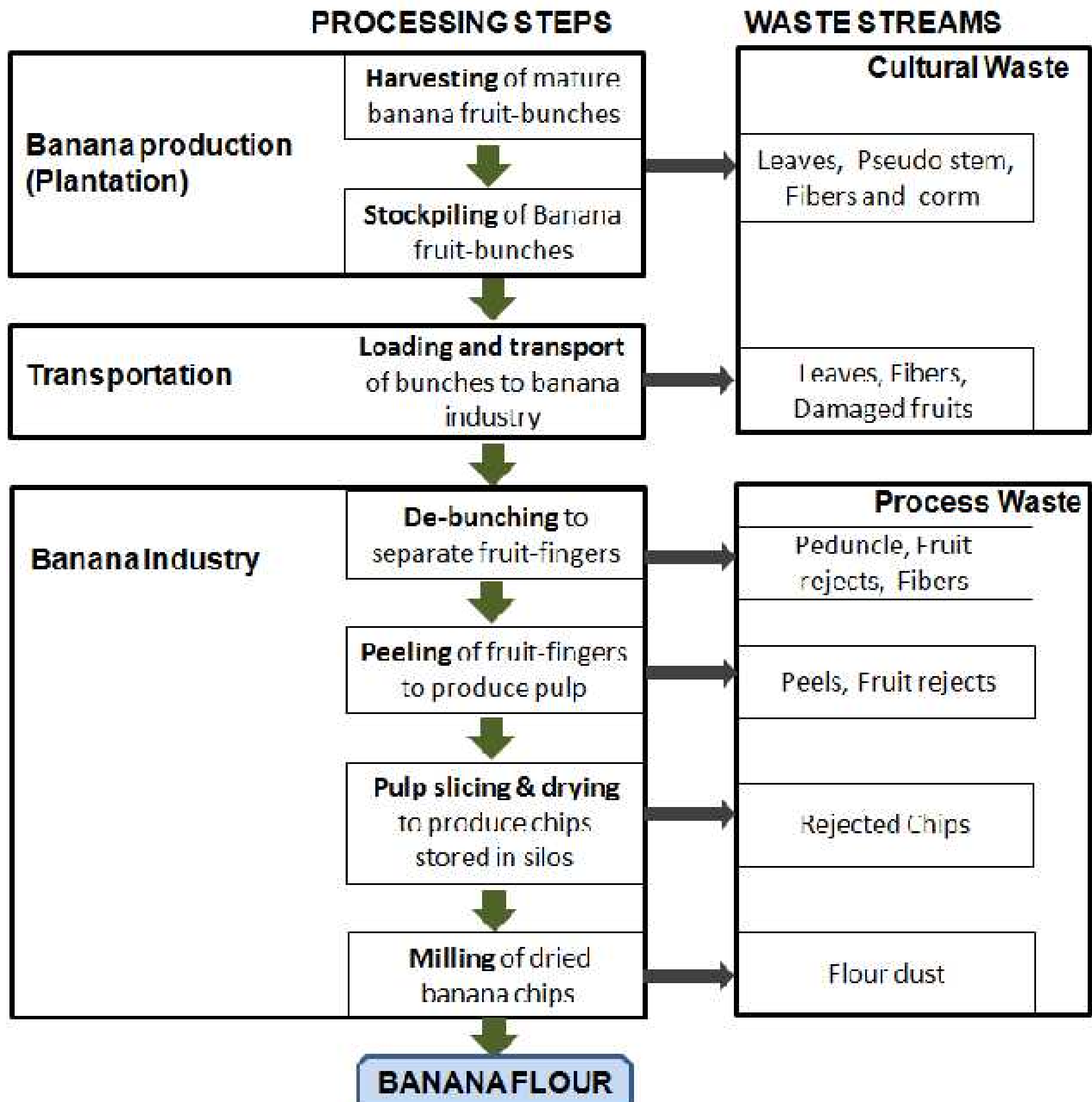


Figure 4:4. Steps for banana industrial processing of East African Highland Green Bananas and the major waste streams

Figure 4:5. Major waste fractions generated from industrial banana processing

4.3.2 Current in-situ methods for management of banana waste

The field survey also noted that banana waste was not utilized properly, both ecologically and economically. The major methods employed in utilization of banana waste (Table 4.1) were, direct application as mulches, dumping on the ground and feeding to animals especially dairy cows.

Table 4:1. Current methods for management of banana waste and associated major challenges

Waste stream	Current Management	Associated Major Challenges
Process wastes		
Peels	▪ Animal feed supplement ▪ Dumping	▪ Only small fraction used ▪ Spread of plant disease such as Banana Bacterial Wilt ▪ Emission of GHGs ▪ Water-body eutrophication by leachate ▪ Spread of plant Disease such as Banana Bacterial Wilt
Peduncle	▪ Dumping ▪ Mulching ▪ Direct use of dried materials for Fuel	▪ Water-body eutrophication by leachate ▪ Emission of GHGs ▪ Spread of plant Disease such as Banana Bacterial Wilt ▪ Air-pollution by smoke emissions
Fruit rejects	▪ Animal feed supplement	▪ Spread of plant Disease such as Banana Bacterial Wilt
Cultural (Production)Wastes		
Leaves, Pseudo-stem, Fibre and Corm	▪ Mulching ▪ Dumping ▪ Direct use of dried materials for Fuel	▪ Spread of plant Disease such as Banana Bacterial Wilt ▪ Water-body eutrophication by leachate ▪ Emission of GHGs ▪ Spread of plant Disease such as Banana Bacterial Wilt ▪ Air-pollution by smoke emissions

4.3.3 Estimation of banana waste generation per unit fruit-bunch

All the banana waste fractions generated from processing of banana fruit bunches into pulp were quantitatively estimated by weighing all the residue fractions and pulp, repeated over a period of six months. The results (Table 4.2) were expressed as a percentage per unit bunch

and indicated that processing of a bunch of green bananas generates 40% as pulp and 60% as total waste residues with peel / pulp ratio of 1.3.

Table 4:2. Percentage residual fractions generated from industrial processing of green bananas

Residues per unit fruit bunch	% Wet Weight
Pulp	40.1 ± 3.5
Peels	50.2 ± 3.4
Peduncle	7.1 ± 1.7
Fruits Rejects	2.6 ± 1.4
Total waste (Peels +Peduncle +Fruit rejects)	59.9 ± 1.5
Total Waste: Pulp Ratio	1.5: 1
Peel: Pulp Ratio	1.3: 1
Peduncle: Pulp ratio	0.2 : 1

Results of percentage residual fractions generated from common banana cultivar clones of *Musa acuminata* (AAA-EA)-the East African High land cooking bananas locally grown in the region (Mporogoma, Kishansha, Kibuzi, Mbwazirime and Enyeru) are shown in table 4.3. The results indicated that Mporogoma had most of the fruit rejects at 8 %, followed by Kishansha at 4.4 % while Kibuzi, Enyeru and Mbwazirime had the least at 0.9 %, 0.7 % and 0.5 %, respectively.

Table 4:3. Common banana varieties and percentage waste fraction per unit fruit bunch (Total waste equals the sum total of Peels, Peduncle and Fruit Rejects)

Banana Variety	Residues per unit fruit bunch (%)						
	Pulp	Peels	Peduncle	Fruit Reject	Total Waste	Peel/pulp ratio	Total Waste/ Pulp ratio
Mporogoma	36.8 ± 3.1	48.0 ± 1.5	7.2 ± 1.1	8.0 ± 1.8	63.2 ± 2.8	1.3 : 1	1.7 : 1
Kishansha	40.0 ± 0.9	50.0 ± 1.6	5.6 ± 0.9	4.4 ± 2.8	60.0 ± 2.2	1.3: 1	1.5 : 1
Kibuzi	36.5 ± 3.7	56.5 ± 0.9	6.1 ± 0.9	0.9 ± 0.2	63.5 ± 0.8	1.5 : 1	1.7 : 1
Mbwazirime	38.9 ± 2.9	50.6 ± 1.5	10.0 ± 1.0	0.5 ± 0.2	61.1 ± 1.0	1.3: 1	1.6 : 1
Enyeru	38.5 ± 1.8	54.1 ± 1.1	6.7 ± 0.6	0.7 ± 0.2	61.5 ± 1.0	1.4: 1	1.6 : 1

4.3.4 Physico-chemical Analysis

Pulverized samples comprising peels, peduncle, fruit rejects, a mixture and pulp were analyzed at the Department of Biochemistry, Makerere University for physico-chemical content analysis. The results (Table 4.4) revealed that banana waste has high moisture content of over 80 % making it unsuitable for direct thermochemical conversion without considerable drying, but rather a high potential substrate for biochemical conversions such as anaerobic digestion for biogas production.

Table 4:4. Physico-chemical composition of residues from industrial processing of green bananas

Parameters	Process streams				
	Peels	**Peduncle**	**Fruit reject**	**Mixed waste**	**Pulp**
MC ^{wb}	83.30 ± 3.04	90.50 ± 2.70	78.61 ± 2.21	85.47 ± 0.35	70.31 ± 4.62
TS ^{wb}	16.71 ± 2.33	9.51 ± 3.10	21.40 ± 2.02	14.55 ± 0.35	29.68 ± 3.11
VS ^{db}	86.78 ± 2.33	80.91 ± 3.02	88.71 ± 2.11	91.79 ± 0.16	96.11 ± 1.12
Ash ^{db}	13.22 ± 2.00	19.11 ± 3.53	11.32 ± 1.91	8.21 ± 0.16	3.90 ± 0.40
OC ^{db}	41.03 ± 4.31	40.02 ± 0.81	53.09 ± 4.71	51.99 ± 0.26	56.13 ± 2.10
OM ^{db}	89.04 ± 1.44	81.12 ± 1.01	87.11 ± 4.32	87.00 ± 0.50	89.83 ±3.33
TKN ^{db}	1.20 ± 0.09	1.93 ± 0.21	0.89 ± 0.32	1.26 ± 0.50	0.74 ± 0.11
C:N ratio	34.19 : 1	20.74 : 1	59.65 :1	41.26 : 1	75.68 : 1
Protein ^{db}	7.53 ± 1.21	12.06 ± 2.00	5.56 ± 1.81	7.88 ± 0.01	4.63 ± 0.62
Starch ^{db}	40.11 ± 2.22	1.73 ± 0.97	51.21 ± 2.13	50.30 ± 2.01	80.70 ± 2.30
Sugars ^{db}	1.42 ± 0.11	0.01 ± 0.01	3.61 ± 0.51	0.29 ± 0.03	4.11 ± 2.11
Cellulose ^{db}	13.09 ± 0.09	31.21 ± 1.50	4. 11 ± 0.13	21.16 ± 2.00	Nil
Hemicellulose ^{db}	14.66 ± 0.31	8.83 ± 0.13	4.88 ± 0.46	10.46 ± 0.51	1.21 ± 0.01
Lignin ^{db}	13.97 ± 0.02	18.77 ± 1.9	4.20 ± 0.20	11.31 ± 1.33	Nil
Crude Fat ^{db}	1.52 ± 0.22	0.33 ± 0.10	1.16 ± 0.19	1.43 ± 0.11	0.71 ± 0.16

MC = Moisture Content; TS = Total Solids; VS= Volatile solids; OC= Organic Carbon; OM= Organic Matter; TKN= Total Kjeldahl Nitrogen; *wb* = wet basis (% wet weight); *db* = dry basis (% TS)

4.3.5 The Biochemical Methane Potential (BMP) of banana waste

The digestibility of banana waste was compared with animal waste and grass using batch-wise anaerobic digestion for 40 days. Based on the total gas yield and quality in terms of methane content, banana waste yielded more methane gas than hey grass and fish waste (Table 4.5). Generally daily methane yield showed variable peaks as a function of retention time (Figure 4.6). Fish waste had one optimal peak at day 10 corresponding to 106 ml CH_4/gVS/day and then the gas production dropped drastically to 23 ml CH_4/gVS/day at day 35.

Table 4:5. The Biochemical Methane Potential (BMP) of banana waste, Hey grass and Fish waste

Retention time (Days)	Cum. Methane Yield (ml CH4/gVS)		
	Banana waste	**Hey grass**	**Fish waste**
0	19.40	17.20	7.80
3	44.06	40.15	21.55
8	95.21	77.52	60.36
10	123.77	101.44	167.24
15	185.87	157.60	269.64
20	264.62	227.28	338.94
24	344.54	273.92	376.88
30	394.38	310.88	404.08
35	436.61	340.00	427.27

The Biochemical Methane Potential of banana waste and grass showed double peaks with related trend. The first peak of daily methane production appeared at day 8 corresponding to 51.2 and 37.4 ml CH_4/gVS/day, respectively for banana waste and grass. In the second peak, both banana waste and grass produced higher methane than first peak. Banana waste produced highest volume of methane at day 24 (79.9 ml CH_4/gVS/day) while hey grass produced 69.7 ml CH_4/gVS/day at day 20 (figure 4.6). This was in agreement with other reported related research on anaerobic digestion of banana waste and grass (Bardiya *et al.,* 1996; Prabhudessai *et al.,* 2013). Moreover, banana waste showed highest cumulative methane yields at 436.61 ml CH_4/gVS, followed by fish waste at 427 ml CH_4/gVS and least by grass at 340 ml CH_4/gVS as shown in figure 4.7.

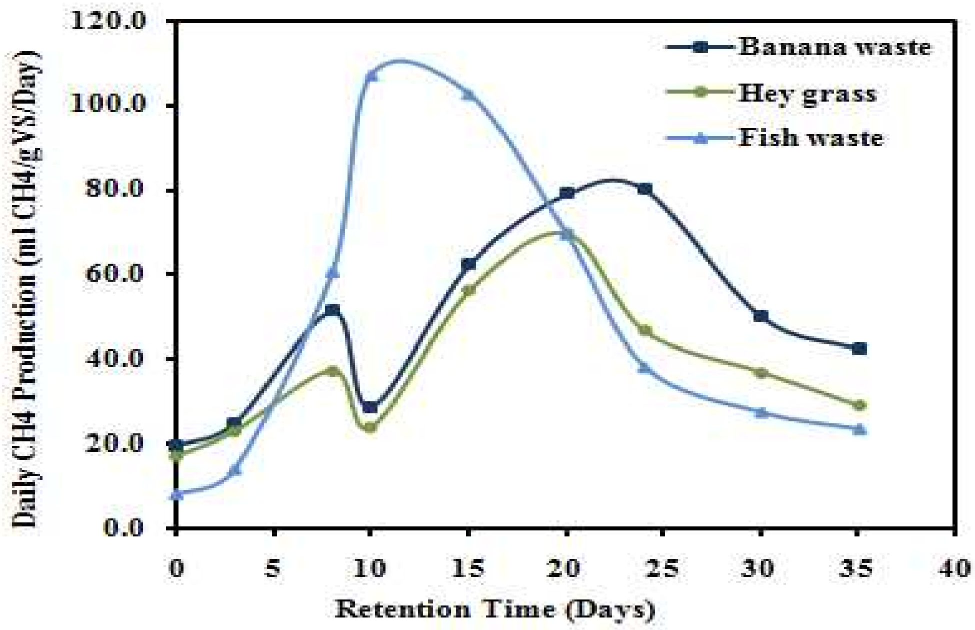


Figure 4:6. Effect of retention time on methane production from banana waste, grass and fish waste

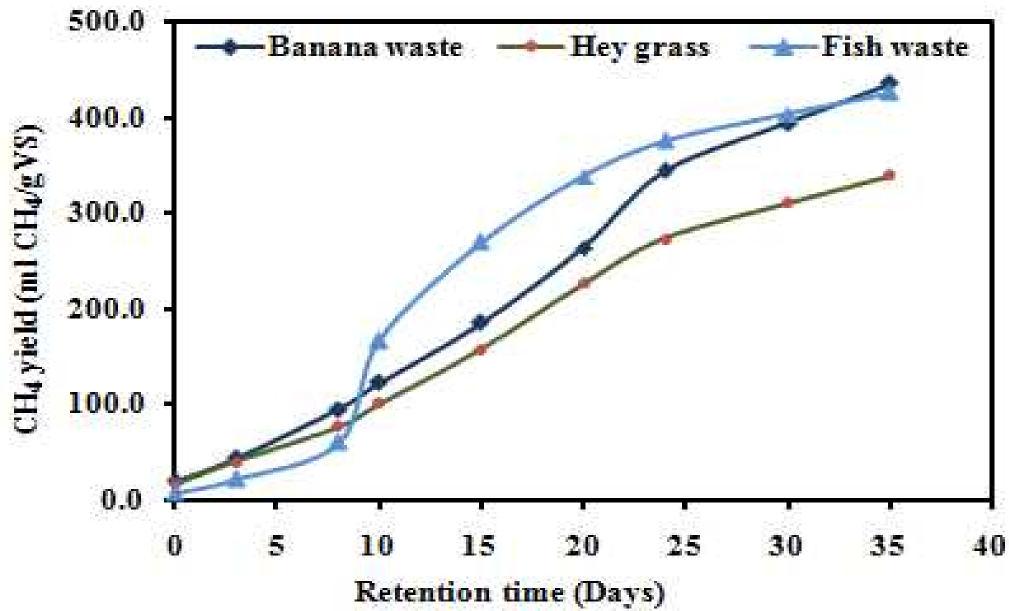


Figure 4:7. Comparison of methane yield from banana waste with grass and fish waste

4.4 Discussion

4.4.1 Waste survey

Results from the survey indicated that banana processing in Uganda is done manually and there is less value addition to the fruits to enhance their shelf life by farmers. A recently installed banana processing factory under Presidential Initiative on Banana Industrial Development (PIBID) is the only industrial enterprise adding value-addition to green bananas through pulp drying and conversion into banana flour. However, a challenge of lack of a 24hour supply of cheap and reliable sufficient energy for complete drying of banana pulp into dried products with consistent standard quality was prominently noted for both industry and local farmers. Local farmers need such energy for drying of banana pulp to sell to the banana industry as a raw material in form of dried chips. Indeed, this survey found out that most of the rural areas with high banana production were not connected to the electricity grid power. For the ones connected, the cost of the grid energy was considered costly and cannot be afforded for use in produce drying. Alternatively, the use of wood and petroleum fuels was undesirable due to high costs and adverse environmental impact. As a result, solar drying of banana pulp by directly spreading the fresh pulp on a mat and exposing it to sunshine was practiced by some rural farmers. This method cannot be easily controlled and its output is not reliable. The practice is considered unhygienic leading to inconsistent and substandard product quality, characterized by rotting and infestation with moulds that produce aflatoxins (Gumisiriza *et al.,* 2017). On the other hand, large quantities of banana waste were generated both at farm production level and during the processing of fruit-bunch into pulp. This was in agreement with findings from previous researchers (Graefe *et al.,* 2011) and is attributed to the high quality standards desired for the market demands of green bananas. Moreover, the short shelf life of mature bananas leads to quick quality deterioration resulting into huge piles of damaged/spoilt fruit waste fraction. The methods for management of banana waste residues were mainly by dumping, reuse as mulching materials and animal feeds, as well as use of dried fibrous fraction for fuel. While these methods are cheap and convenient, they are being discouraged owing to their association with the spread of plant diseases, especially the Banana Bacterial Wilt, as well as their lack of economic value to farmers. The use of dried banana waste as fuel by direct burning was an indication that there was scarcity of energy for both domestic usage and drying of pulp. However, since banana waste has high moisture content it cannot be appropriately utilized via such a waste-to-energy process especially during the rainy season (Gumisiriza *et al.,* 2017).

4.4.2 Physico-chemical analysis

Quantitative analysis based on percent weight by residual fraction revealed that processing of a unit bunch of green bananas generates 40% as pulp and 60% as total waste residues with total waste to pulp ratio of 1.5:1 and peel to pulp ratio of 1.3:1. The high ratio of waste to pulp is attributed to high moisture content of peduncle (MC of 90 %) and peels (MC of 83 %) in freshly harvested fruit bunches (Clarke *et al.,* 2008; Tumutegyereize *et al.,* 2009; Salayeem *et al.,* 2014). The high waste to pulp ratio implied that the waste contained more water than the pulp. Indeed, when bunches were left at room temperature for a day before

processing, the fruits lost more moisture from peels than pulp consequently lowering the ratio of peel to pulp from 1.3 to 1. The high moisture content banana waste suggests that the waste is more amenable to biochemical conversion than thermal technologies and would require minimal additional water thus reducing biogas production costs. On the other hand, the high moisture content of pulp suggests that it requires a lot of energy to achieve complete dryness.

Qualitatively, wastes generated at production level (on farm) are more fibrous and hence highly lignocellulosic. This must be pre-treated for effective energy harnessing through anaerobic digestion. Physical-chemical analysis (Table 4.4) of banana waste fractions from the industrial processing indicated that the residues had organic matter of over 80 %, suggesting that they were highly organic and thus amenable to value addition through bioconversion technologies such as anaerobic digestion. The high moisture content is favorable for biochemical conversion technologies that proceed without any additional water requirement thus reducing on water use and costs. Furthermore, analysis results showed that more than 80 % of the total solids in banana wastes were volatile. This confirms reports by previous researchers (Bardiya *et al.,* 1996; Tumutegyereize *et al.,* 2009; Kirtane *et al.,* 2009; Valasquez-Arredondo *et al.,* 2010). Such waste characteristic indicates that these solids were of organic origin and have high potential for bioenergy production, if efficiently biodegraded through anaerobic digestion. However, the mixed waste had high organic carbon with low nitrogen content resulting into a C:N ratio of 41:1. This ratio is above the range of 20-32 recommended for optimal anaerobic digestion (Zaher *et al.,* 2007; Bouallagui *et al.,* 2003; Chandra *et al.,* 2012; Gumisiriza *et al.,* 2017). The high C:N ratio implies that optimal anaerobic digestion of banana waste requires co-digestion with nitrogen-rich feed substrate such as fish waste, slaughterhouse waste and chicken manure Gumisiriza *et al.,* 2017. The high ratio was attributed to high starch content from fruit reject and the high lignocellulosic content of peduncle. The high carbon content and low TKN was translated into higher carbohydrate content than protein of 50.3 % and 7.8 %, respectively for mixed waste. The sugar content varied depending mainly on the maturity of the fruit bunches and time lag from harvesting to processing. The lipid content was higher in peels than other fractions but generally lower than sugars and protein contents. These findings compare well to the ones reported by Essien *et al*., (2005); Salayeem *et al.,* (2014). Besides, the process of anaerobic digestion of substrates with high C:N ratio is susceptible to failure mainly due to acidification (Mshandete, 2005; Bilibio *et al.,* 2011; Gumisiriza *et al.,* 2017). The lignocelluloses content of mixed waste was high equivalent to 42.93 % total sum of fibres in form of lignocellulose and comprised 21.16 %, 10.46 % and 11.31 % for cellulose, hemicelluloses and lignin, respectively. The results of lignocellulose content of banana waste agree with similar analysis reported by previous researchers (Robio *et al.,* 1998; Monsalve *et al.,* 2006; Jeon *et al.,* 2010; Valasquez-Arredondo *et al.,* 2010; Adebayo and Martinez-Carrera, 2015), and imply that banana waste can generate more biogas through anaerobic digestion, if appropriately pre-treated to optimally solubilize the lignocellulose content.

4.4.3 The Biochemical Methane Potential (BMP) of Banana Waste

Results from BMP assays showed that banana waste has high anaerobic digestibility. The peaks in daily methane production represent retention times that gave optimal gas production of digested substrates. The first peak in both banana waste and grass was likely

due to quick microbial assimilation of soluble sugars released from the substrates during pulverization process while the second peak was related to the lag microbial solubilization of starch and other complex biomolecules in waste substrate (Tumutegyereize *et al.,* 2011). The banana waste gave a methane yield of 0.436 m^3 CH4/KgVS which was higher than 0.340 m^3 CH4/KgVS for grass. Moreover, the daily methane production curve appears superimposed over the one for grass. This was due to the different nature of VS in the two phytomass substrates. That is, the VS in banana waste contained higher starch and sugar content than in grass, in addition to more lignocellulosic content in the latter (Prabhudessai *et al.,* 2013). The values of methane yield from all the wastes assayed were below the theoretical maximum methane production of 0.490 m^3 CH4/KgVS but slightly higher than 0.332 m^3 CH4/KgVS previously reported for grass (Prabhudessai *et al.,* 2013). This could have been due to the inoculum that was already pre-adapted to digest hey at the fixed dome digester. Besides, the single peak for daily methane production exhibited by the BMP assay of fish waste indicated that the nature of VS in such waste has a nearly similar complexity. Implying that, it could be digested continuously once the reactor microorganisms have acclimatized to the substrate. The peak for digestion of fish waste coincided with the retention time at lower methane production from banana waste. This suggested that the fish waste could be a good substrate for co-digestion with banana waste to yield more methane at lower retention time of less than 15 days.

4.5 Conclusion

This study aimed at assessing banana processing, auditing of banana waste generated from banana processing activities in Uganda and evaluation of the waste management options as well as potential for value-addition through biogas production. Findings revealed that the banana industry in Uganda is faced with a challenge of lack of cheap, reliable and sufficient energy for complete drying of banana pulp into chips with consistent standard quality. The huge banana wastes generated and currently underutilized were rich in organic matter with high moisture content and thus a good substrate for biogas production through anaerobic digestion. The high moisture content makes banana waste a better feedstock for biogas production since it would require minimal additional water thus reducing the cost of bioenergy production. The biochemical methane potential assay showed that banana waste has a higher methane yield than grass and fish waste due to high starch and sugar content. The high lignocellulosic content in banana waste however suggested that application of appropriate pre-treatment is necessary to increase nutrient bioavailability that enhance anaerobic digestion and ultimately improves biogas yield from the substrate.

4.6 References

1. Abdullah, N., Sulaiman, F., Miskam, M.A and Taib, R. M (2014)," Characterization of Banana (Musa spp.) Pseudo-Stem and Fruit-Bunch-Stem as a Potential Renewable Energy Resource", International Journal of Biological, Veterinary, Agricultural and Food Engineering Vol: 8 No: 8, 2014.

2. Adebayo, E.A and Martínez-Carrera, D (2015) "Oyster mushrooms (Pleurotus) are useful for utilizing lignocellulosic biomass" African Journal of Biotechnology Vol. 14 (1): 52-67.

3. Allen, S.E.(1989) "Chemical analysis of ecological material. (2nd ed). Blackwell Scientific Publications; Oxford, UK.

4. AOAC (2002) Association of Official Analytical Chemist. Official Methods of Analysis (17th ed.). Washington, DC.

5. APHA (1998). Standard methods for examination of water and wastewater. 20 Edn. American Public Health Association, Washington, D.C.

6. Asha, N., Enoch,K., Kephas, N., Susan, A. and Diego, N. (2015) "Technical report: Structure of the Cooking Banana Value Chain in Uganda and Opportunities for Value Addition and Postharvest Losses Reduction" Expanding Utilization of Roots, Tubers and Bananas and Reducing Their Postharvest Losses (RTB-ENDURE) is a 3 year project (2014-2016) implemented by the CGIAR Research Program on Roots, Tubers and Bananas (RTB) with funding by the European Union and technical support of IFAD. http://www.rtb.cgiar.org/endure

7. Ayeni, A. O., Hymore, F. K, Mudliar, S. N., Deskmukh, S. C., Satpute, D. B., Omoleye J. A, and Pandey, R. A (2013) Hydrogen peroxide and lime based oxidative pretreatment of wood waste to enhance enzymatic hydrolysis for a biorefinery: process parameters optimization using response surface methodology, Fuel, 106, 187-194.

8. Ayeni, A.O., Adeeyo, O.A., Oresegun, O.M and Oladimeji, T.E (2015) "Compositional analysis of lignocellulosic materials: Evaluation of an economically viable method suitable for woody and non-woody biomass" American Journal of Engineering Research Vol.4, (4) pp-14-19

9. Bagambe F, Kikulwe E, Tushemereirwe W K, Ngambeki D, Muhangi J, Kagezi GH, Rugama PE, Eden-Green S (2006). Awareness of Banana Bacterial W ilt Control in Uganda: 1. Farmers' Perspective. Afr. Crop Sci. J. 14(2): 157-164

10. Bardiya, N., Somayaji, D and Khanna, S (1996) "Biomethanation of banana peel and pineapple waste" Bioresource Technology 58 :73-76

11. Bilibio, C., Hensel, O. and Selbach, J (2011), “Sustainable water management in the tropics and subtropics (and case studies in Brazil)”, Vol. 2 Jaguarão/RS, Fundação Universidade Ederal Do Pampa; UNIKASSEL - Pgcult-UFMA 2011

12. Bouallagui H, Cheikh RB, Marouani L, Hamdi M (2003). Mesophilic biogas production from fruit and vegetable waste in a tubular digester. Bioresource Technology. 86: 85-85.

13. Carpita, N. & Kanabus, J (1987) Extraction of starch by dimethylsulfoxide and quantization by enzymatic assay, Analytical Biochemistry 161, 132–139

14. Chandra R., Takeuchi H., Hasegawa T. (2012): Methane production from lignocellulosic agricultural crop wastes: A review in context to second generation of biofuel production. Renewable and Sustainable Energy Reviews 16(3): 1462-1476.

15. Clarke, W.P., Radnidge, P., Lai, E.T., Jensen, P and Hardin, M.T (2008) “Waste Management 28 (3), 527-533.

16. Colin X, Farinet JL, Rojas O, Alazard D (2007). Anaerobic treatment of cassava starch extraction waste water using a horizontal flow filter with bamboo as support. Bioresource Technology. 98: 1602-1607.

17. Dubois, M., Gilles,K.A., Hamilton, J.K., Rebers, P.A and Smith, F (1956) “Colorimetric methods for determination of sugars and related substances. Analytical Chemistry 28: 350-356.

18. Emaga. T.H., Andrianaivo, R.H, Wathelet, B., Tchango, J.T and Paquot, M (2007) “Effects of the stage of maturation and varieties on the chemical composition of banana and plantain peels” Food Chemistry .103 590-600.

19. Erguder, J.F., Tezel, U., Guven, E., Demirer, G.N (2001), “Anaerobic biotransformation and methane generation potential of cheese whey in batch and UASB reactors”, Waste management 21, 643-650
20. Essien, J.P., Akpan, E.J and Essien, E.P (2005) “Studies on mould growth and biomass production using waste banana peel” Bioresource Technology 96: 1451–1456

21. Fajardo, D., Jayanty, S.S and Jansky, S.H (2013). Rapid High Throughput Amylose Determination in Freeze Dried Potato Tuber Samples, Journal of Visual Experiments, (80): 50407

22. FAOSTAT (2012): Global banana production by year, Food and Agriculture Organization of the United Nations. Retrieved on 10th May 2017 from http://faostat.fao. Org

23. Graefe, S., Dufour, D., Giraldo, A., Muñoz, L. A., Mora, P., Solís, H., Garcés, H and Gonzalez, A (2011), “Energy and carbon footprints of ethanol production using banana and cooking banana discard: A case study from Costa Rica and Ecuador,” Biomass and Bioenergy, vol. 35, no. 7, pp. 2640-2649.

24. Gumisiriza, R., Hawumba, J.F., Okure, M and Hensel, O (2017) “Biomass Waste-to-

Energy Valorisation Technologies: A Review Case for Banana Processing in Uganda" Biotechnology for biofuels 10:11. DOI 10.1186/s13068-016-0689-5

25. Gumisiriza, R., Mshandete, A.M., Rubindamayugi, M.S.T., Kansiime, F. and Kivaisi, A. K (2009), "Enhancement of Anaerobic digestion of Nileperch fish processing waste water", African Journal of Biotechnology 8: (2) 328-333

26. Hovenkamp-Hermelink J, Devries J, Adamse P, Jacobsen E, Witholt B, Feenstra W. (1988) Rapid estimation of the amylose amylopectin ratio in small amounts of tuber and leaf tissue of the potato. Potato Research; 31:241–246.

27. Jeon, Y.J., Xun, Z. and Rogers, P.L (2010). "Comparative evaluations of cellulosic raw materials for second generation bioethanol production" Letters of Applied Microbiology. 51: 518-24.

28. Karamura, D. A., Karamura, E and Tinzaara, W (2012) "Banana Culltivar Names, Synonymes and their Usage in East Africa" Bioversity International, Uganda.

29. Kirtane, R. D., Suryawanshi, P.C., Patil, M.R., Chaudhari, A.B and Kothari, R.M (2009), "Optimization of Organic Loading Rate for different fruit waste during biomethanization", Journal of Scientific & Industrial Research Vol.68, pp252-255

30. Kiyasudeen,S.K., Hakimi bin Ibrahim, M and Ismail, S. A (2015) "Characterization of Fresh Cattle Wastes Using Proximate, Microbial and Spectroscopic Principles" American-Eurasian Journal of Agricultural & Environmental Science, 15 (8): 1700-1709.

31. Lyimo, T.J, Pol, A and Op den Camp, H.J.M (2002a) "Methane emission, sulphide concentration and redox potential profiles in Mtoni mangrove sediment, Tanzania". Western Indian Ocean Journal of Marine Science 1: 71–80.

32. Martin-Ryals, (2012), "Evaluating the potential for improving anaerobic digestion of cellulosic waste via routine bioaugmentation and alkaline pretreatment", A thesis Submitted in partial fulfillment of the requirements for the degree of Master of Science in Agricultural and Biological Engineering in the Graduate College of the University of Illinois at Urbana-Champaign, 2012

33. Mohammad J. Taherzadeh, M.J and Karimi, K (2008) "Pretreatment of Lignocellulosic Wastes to Improve Ethanol and Biogas Production: A Review" International Journal of Molecular Sciences 9, 1621-1651; DOI: 10.3390/ijms9091621

34. Monsalve, J.F.G., De perez, V.I.M. and Colorado, A.A.R (2006) "Ethanol production of banana shell and cassava starch" Dyna, Año 73, Nro. 150, pp. 21-27

35. Moody, L., Burns, R., Wu-haan, and Spajic, R (2009), "Use of Biochemical Methane Potential (BMP) Assays for Predicting and Enhancing Anaerobic Digester Performance", Agricultural and Biosystemes Engineering, Lowa State University, Digital Repository: Conference Proceedings and Presentations.

36. Mshandete, A., Björnsson, L., Kivaisi, A.K, Rubindamayugi, S.T. and Mattiasson, B (2005), "Enhancement of anaerobic batch digestion of sisal pulp waste by mesophilic aerobic pre-treatment", Water Research 39, 1569-1575.

37. Mshandete, A., Björnsson, L., Kivaisi, A.K, Rubindamayugi, S.T. and Mattiasson, B (2005), "Enhancement of anaerobic batch digestion of sisal pulp waste by mesophilic aerobic pre-treatment", Water Research 39, 1569-1575

38. Newenhouse SC, Schmit JT (2000). Qualitative methods add value to waste characterization studies. Waste Management Research.18: 105-114.

39. Padam, B.S.,Tin,H.S., Chye,F.Y and Abdullah,M.I (2014), " Banana by-products: an under-utilized renewable food biomass with great potential", Journal of Food Science and Technology 51 (12), 3527-3545.

40. Patrick, F., Mtui, G., Mshandete, A.M and Amelia Kivaisi, A (2011). Optimization of laccase and manganese peroxidase production in submerged culture of Pleurotus sajor- caju, African Journal of Biotechnology Vol. 10 (50), 10166-10177

41. Prabhudessai, V.,Ganguly, A, and Mutnuri, S (2013), "Biochemical Methane Potential of Agro Wastes", Journal of Energy. Vol: 7

42. ProMusa: *"Banana cultivar checklist on ProMusa". Retrieved 26th October 2021.* https://www.promusa.org/tiki-index.php?page=Banana+cultivar+checklist&f_87=EAHB

43. Salyeem, E .J., Mshandete, A.M and Kivaisi, A.K (2014) "Biovalorization of Banana waste: Auditing and improvement of bio-methane production by fungal pre-treatment" Journal of Chemical, Biological and Physical Sciences, Vol. 4, No. 1; 818-833.

44. Singh, S., Kumar, S., Jain, M.C and Kumar, D, (2001) "Increased biogas production using microbial stimulants" Bioresouce Technology Vol. 78 (3): 313-316.

45. Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J and Templeton, D (2008) Determination of structural carbohydrates and lignin in biomass: laboratory analytical procedure (LAP). Golden, CO: National Renewable Energy Laboratory; NREL Report No.: TP-510-42618. Contract No.: DE-AC36-99-G010337, Sponsored by the U.S. Department of Energy.

46. Smith, A. M and Zeeman, C.S (2006), Quantification of starch in plant tissues. Protocol; 2006 Nature Publishing Group; http://www.nature.com/natureprotocols

47. Spilsbury, J.S., Jagwe, J.N., Ferris, R.S.B (2002), "Evaluating the Marketing Opportunities for Banana & its Products in the Principle Banana Growing Countries of ASARECA (Uganda)", International Institute of Tropical Agriculture. Foodnet. P: 60.

48. Steffen, R., Szolar, O. and Braun, R (1998) "Feedstocks for Anaerobic Digestion" University of Agricultural Sciences Institute for Agrobiotechnology. Tulln, Vienna. Q:\RODL\PROJEKTE\AD-NETT\FEEDNEW.DOC

49. Tock, J.Y., Lai, C.L., Lee, K.T., Tan, K.T and Bhatia, S (2010), "Banana biomass as potential renewable energy resource: A Malaysian case study," Renewable & Sustainable Energy Reviews, vol. 14, no. 2, pp 798-805Moody, L.B. and Roman, D.R (2001), "A dual-anaerobic system for complete treatment of food processing waste", Journal of Agricultural Engineering Research 80, 293-299

50. Tripathi, L., Tripathi1, J. N. and Tushemereirwe, W. K (2008), "Rapid and efficient production of transgenic East African Highland Banana (Musa spp.) using intercalary meristematic tissues", African Journal of Biotechnology Vol. 7 (10), pp. 1438-1445

51. Tumutegyereize, P., Muranga, F.I., Kawongolo, J. and Nabugoomu, F (2011), "Optimization of biogas production from banana peels", African Journal of Biotechnology Vol. 10 (79), 18243-18251

52. Undersander, D., Mertens, D. R and Thiex, N (1993) "Forage Analysis procedures; National Forage Testing Association, Omaha, USA

53. Velásquez-Arredondo, H.I., Ruiz-Colorado, A.A and De Oliveira junior, S (2010) "Ethanol production process from banana fruit and its lignocellulosic residues" Energy analysis 35: 3081-3087

54. Waiswa, A.N.A (1998), Anaerobic treatment of distillery slope and landfill leachate using UASB and hybrid reactors, Master of Science thesis, University of Dar es salaam.

55. Yan Q, Hua C, Yang S, Li Y, Jiang Z. (2012) "High level expression of extracellular secretion of a β-glucosidase gene (PtBglu3) from Paecilomyces thermophila in Pichia pastoris". Protein Expression and Purification. 2012;84:64–72. doi: 10.1016/j.pep.2012.04.016.

56. Zaher, U., Grau, P., Benedetti, L., Ayesa, E., Vanrolleghem, P.A (2007). Transformers for interfacing anaerobic digestion models to pre- and post-treatment processes in a plant-wide modelling context. Environmental Modelling and Software, 22, 40-58.

5 Design, Construction and Operation of a novel hybrid Upflow Anaerobic Sludge Blanket (hUASB) Bioreactor System

5.1 Introduction

Lignocellulosic waste and currently the energy crops are an important global raw material for production of biofuels. In Uganda, the banana industry generates voluminous lignocellulosic agro-wastes that can be bioconverted into biofuel in form of biogas through anaerobic digestion. The banana waste is a concentrated source of putrescible organic waste and ideal for anaerobic digestion to produce bio-energy in form of biogas as well as nutrient- rich compost manure (Clarke *et al., 2007*). Biogas generated from such agro-wastes can significantly reduce the industry's import costs on petroleum fuel.

However the optimal biogas production and recovery from a feedstock requires that the anaerobic digestion process must be carried out in a well designed bioreactor system, tailored to the type of the substrate (Mshandete *et al.,* 2005; Gumisiriza *et al.,* 2017). Other factors that enhance biogas production include optimisation of bioreactor operational and environmental parameters, substrate pre-treatment, co-digestion, among others (Gumisiriza *et al.,* 2017). Although different bioreactor types have been used in anaerobic digestion of different wastes, the high rate anaerobic bioreactors such as up-flow anaerobic sludge blanket reactors (UASBR) have been proven for efficient anaerobic treatment of most bio-wastes (Amin and Vriens, 2014). The successful operation of an UASB reactor depends on the retention of highly active, flocculated or compact sludge aggregates called granules (Najafpour *et al.,* 2006; Narihiro *et al.*, 2009). Compared with other reactors, USAB reactors have the advantage of their ability to retain high bio-mass with high void volume, because no support material is externally supplied (Mrunalini *et al.,* 2013), their independence from mechanical mixing of reactor contents (Ghangrekar and Kahalekar, 2003), and their ability to cope with perturbances from temperature fluctuations and high organic loading rates (Nidal 2008; Kovacik *et al.,* 2010).

Nevertheless, the anaerobic digestion of feed substrate from plant origin using almost all bioreactor types reported to date, including the most efficient high-rate reactors such as UASBR, is generally nuisance and problematic due to the physical nature of the substrate: that is, plant biomass materials are more fibre-rich and tend to build up a persistent float layer. Physically, the floatation of the feed substrate leads to wash out of active biomass (inocula seeding) that results in digester failure. When fermentation residues are discharged early from the reactor, the active flora adsorbed on to the biocarrier gets lost as well and ultimately leading to failure of the anaerobic digestion process (German Agency for Renewable Energy, 2005).

In order to prevent flotation, agitation and stirring has to be intensified which may demand more energy (electric energy) input than what is produced from the system. Moreover, intensive mixing inhibits microbial substrate adsorption and granulation, and ultimately affecting the substrate decomposition process negatively. Generally, too much agitation

results into taking up a considerable amount of energy that makes the system unattractive both energetically and economically.

Besides, maximum recovery of biogas from anaerobic digestion requires gas-tight reactors or digesters equipped with accurate gas measuring system. Most traditional digesters such as fixed dome reactors are associated with significant biogas (biomethane) leaks and such defects mainly arise from technical and inappropriate designs which ultimately compromise the plant efficiency and overall economic value of the digester (Hensel, 2014). Since biogas production is proportional to the substrate utilization and microbial growth (Wilkie *et al.,* 2004), accurate gas measurement is required for monitoring the biochemical transformations in the bioreactor. The lack of an inexpensive gas-tight bioreactor system that allows liquor mixing, substrate addition and culture removal has been a barrier to the accurate measurement of gas production in anaerobic digestion (Wilkie *et al.,* 2004).

It was therefore imperative to investigate a bioreactor system that would reduce the early wash-out of active flora and in turn, increases the efficiency of biogas production and recovery from banana waste. Thus, the objective of this study was to design and operationalize a bioreactor system engineered for enhanced anaerobic digestion of banana waste with improved biogas production and recovery.

5.2. Methodology

5.2.1 Principle of operation and Components of the Novel hybrid Up-flow Anaerobic Sludge Blanket Reactor System

A novel two-stage bioreactor system was constructed and set up at the Department of Biochemistry, Makerere University. The construction and operation of the novel reactor was based on the principle that synchronized stirring with up flow movement of feed substrate in a sludge bed column circumvents the floatation and early wash-out of fibrous feed material from the reactor. Moreover, creation of an inoculum reservoir at the base of the hUASB reactor keeps replenishing the lost bio-flora and solves the problem of loss of active microbial biocatalysts , a problem synonymous with the current conventional UASB reactors.
The constructed novel reactor was therefore a hydrid Upflow Anaerobic Sludge Blanket (hUASB) reactor that incorporated an upflow sludge bed column, a Bordeaux stirrer which is distinctive to the Continuously Stirred Tank Reactor (CSTR) and a unique reservoir of the active seed inoculum that would automatically re-seed the in-coming substrate.

The novel reactor system comprised of four sub units, namely: **A**. Hydrolysis tank; **B**. Hybrid Up-flow Anaerobic Sludge Blanket (hUASB) Reactor; **C**. Biogas measuring system; and, **D**. Effluent slurry collection tank (figure 5.1).

5.2.2 Construction of the hydrolysis and effluent slurry tanks

Each of the hydrolysis and effluent tanks were made up of 20 litres, 1meter-tall plastic aspirator bottle. The hydrolysis tank was connected to the hUASB reactor inlet port by an S-shaped transparent plastic tubing with an internal diameter (ID) of 18.75mm. At the base of the hydrolysis tank, a plastic nipple of internal diameter of 12.5mm was fitted on to the wall

of the tank and connected to a tap valve with internal diameter of 12.5mm followed by a hydraulic flow rate monitoring window, a flow-rate regulatory tap valve (with ID of 12.5mm) and an outlet valve (with ID of 12.5mm) that allowed substrate slurry flow into the Hybrid Up-flow Anaerobic Sludge Blanket (hUASB) reactor inlet port.

The effluent slurry tank received the digested slurry from the Hybrid Up-flow Anaerobic Sludge Blanket (hUASB) reactor via the effluent port. The digested slurry flew through a transparent plastic tube that was connected to the base of the bank with a plastic nipple with an internal diameter of 12.5mm. The effluent slurry tank was pre-filled with 5 litres of slurry that enters the connecting plastic tubing to create a liquid seal. The base of hydrolysis tank was positioned at a height of 2meters above the base of effluent slurry tank and bioreactor.

5.2.3 Construction of the Hybrid Up-flow Anaerobic Sludge Blanket (hUASB) Reactor

The bioreactor was a modified UASB reactor incorporated with a Bordeaux stirrer and thus a Hybrid UASB reactor. The reactor vessel was made of transparent polyvinyl chloride (PVC) plastic column, of total height of 115 cm, internal diameter (ID) of 11.30 cm and total volume of 11.5 litres. The total volume of the reactor vessel was calculated using the formula for calculating the volume of a cylinder. All the specifications of the reactor are shown in table 5.1. The column consisted of four parts. From top to bottom, the topmost space of height of 5.0 cm and volume of 0.5 litres was occupied by rubber stopper.

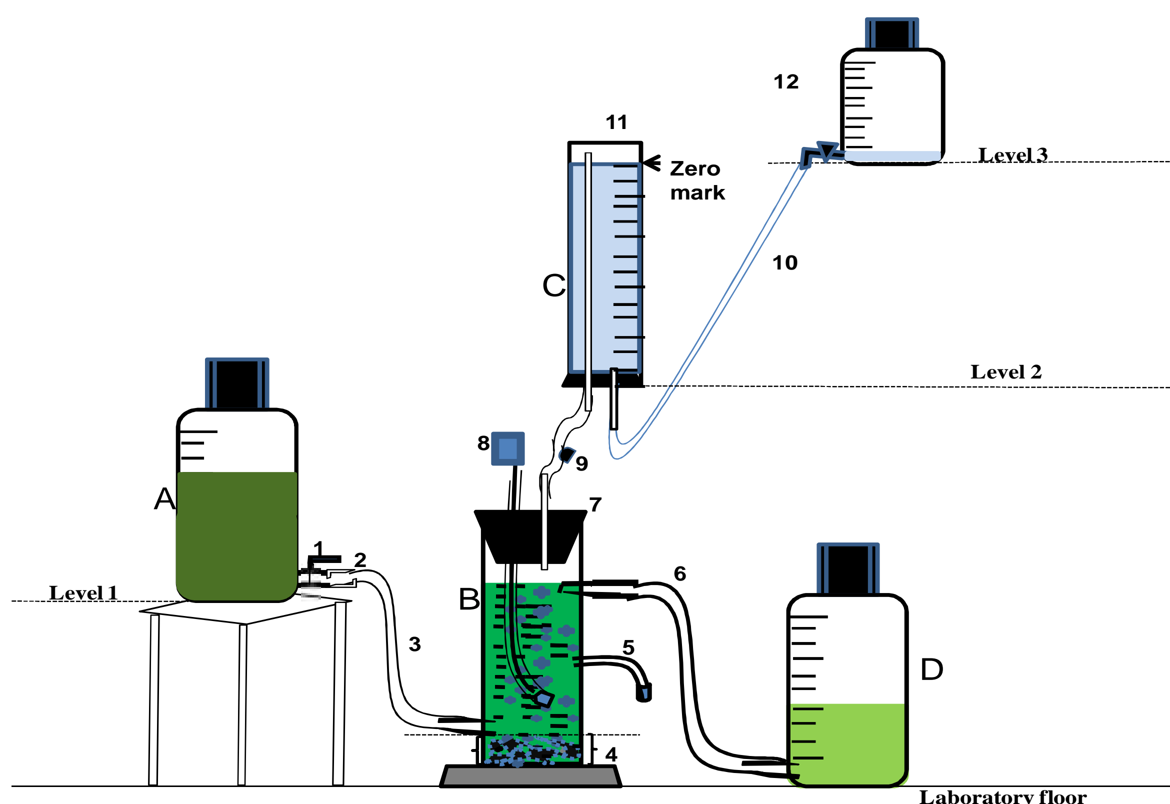


Figure 5:1. Schematic illustration of the designed bioreactor system

The labelled parts are: 1.Tap-valve; 2.Hydraulic flow monitoring window; 3. Flexible plastic tubing connecting inlet port; 4.Sludge bed reservoir; 5.Sampling port; 6.Effluent port; 7.Rubber stopper; 8. Bordeaux stirrer system; 9.Biogas sampling septum; 10.Coloured-liquid tubing; 11.Inverted measuring cylinder; 12.Coloured liquid reservoir

The next space was the gas-phase head space or the gas –liquid-solid separator (GLSS) that occupied a height of 5.0 cm and volume of 0.5 litres. Below the GLSS to the inlet port was the actual bioreactor with the anaerobic sludge blanket of column height of 100 cm and working volume of 10 litres. From the inlet port to the base of the reactor was the bottom portion and contained a slurry reservoir of volume of 0.5 litres and column height of 5.0 cm. Five ports were fitted on to the reactor and located at 2.0 cm, 5.0 cm, 35.0 cm, 65.0 cm and 105.0 cm from the reactor bottom. The ports at the column height of 5.0 cm and 105.0 cm were the substrate inlet and bioslurry outlet, respectively. All the ports were fitted through plastic nipples (with ID of 12.5 mm) that protruded out at 90^0 from the vertical axis. The port nipples were connected to the transparent plastic tubes of internal diameter (ID) of 18.75mm. The bioreactor stopper was a rubber bung of height 6cm, top diameter 11cm and bottom diameter 9.5cm; through which a glass tube (with ID of 11 mm) passed to transfer biogas to the measuring system. The stopper was tightly fitted on to the bioreactor by a thin coat of silicone sealant. The Bordeaux stirrer was fixed in such a way that the stirrer rod reached mid the reactor so that it only effected intermittent mixing at a rate of 60 rpm and an interval of 15min/h (Wilkie *et al.,* 2004).

Table 5:1. Specifications of the constructed novel Hybrid Up-flow Anaerobic Sludge Blanket (hUASB) reactor system

Specification	Magnitude
Total Reactor Vessel Height (h)	115.00 cm
Reactor Vessel Internal Diameter (ID)	11.30 cm
Reactor Vessel Internal Radius (IR)	5.65 cm
Height of stopper	5.00cm
Volume of stopper	0.50 L
Height of Gas-phase head space or GLSS	5.00 cm
Volume of GLSS	0.50 L
Actual Height of Reactor (sludge bed)	100.00 cm
Actual Volume of Reactor (Sludge bed)	10.00 L
Height of Bottom reservoir sludge bed	5.00 cm
Volume of Bottom reservoir sludge bed	0.50 L
Height of Sampling ports from the base:	
Port 1	2.00 cm
Port 2 (Inlet Port)	5.00 cm
Port 3	35.00 cm
Port 4	65.00 cm
Port 5 (Outlet Port)	105.00cm

5.2.4 Construction of the biogas collection and measuring system

The biogas collection and measuring system was modified from water displacement method (Wilkie *et al.,* 2004). It consisted of a 1.0 L inverted plastic measuring cylinder, gas sampling septum, flexible plastic tubing and reservoir tank containing coloured liquid. As shown in figure 5.1, the generated biogas from the bioreactor diffused through the glass tubing (height 15cm; ID 11mm) traversing the rubber stopper and connected via septum to another tall glass

tubing (height 60cm; ID 11mm) traversing the rubber stopper to an inverted measuring cylinder above the bioreactor at level 2. The entry of biogas into the head space in the measuring cylinder displaces the coloured liquid that passes through another flexible plastic tubing (ID 12.5 mm) connected to the reservoir tank above the measuring cylinder at level 3. Biogas volume generated at a certain time interval was measured by the change in the level of meniscus of the coloured liquid in the measuring cylinder. The biogas samples for analysis of percentage gas composition were sucked out through a rubber button on the septum using a syringe and needle.

5.2.5 Feed substrate and Innoculum

Banana waste used in this study was obtained from a banana processing industry at the field station of Presidential Initiative on Banana Industrial Development (PIBID) in Nyaruzinga, Bushenyi-Ishaka Municipality, in the western region of Uganda. Three randomly selected bunches of banana were processed into pulp and all the waste collected. The waste comprised of peels, peduncle and fruit rejects with banana peels constituting the major percentage followed by the peduncle and lastly, the fruit rejects. The waste was transported in cool boxes to the laboratory for analysis and biogas production experimentation at the Department of Biochemistry, Makerere University. The waste was shredded into a homogeneous paste (Gumisiriza *et al.,* 2019), using an organic shredder (TR 200: Organic Shredder, BrazAfric Enterprises LTD). The shredded homogeneous paste constituted the feed substrate. The feed substrate not used immediately was kept in the freezer until it was needed. When required to feed the bioreactor, the substrate would be removed from the freezer and thawed for 24 hours at room temperature before use.

The inoculum was collected from an active fixed-dome anaerobic digester that was fed on a mixture of cow dung and pulverized hey residues, at a dairy cattle farmer in the vicinity of Makerere University. The inoculum was adapted by incubation in the previously constructed anaerobic bioreactors for two weeks to deplete the residual biodegradable organic matter prior to use in this study (Gumisiriza *et al.,* 2019).

Samples from both, the feed substrate and inoculum were analysed in triplicates for physico-chemical parameters according to Gumisiriza *et al.,* 2019 and following standard methods as described by American Public Health Association (APHA), 1998. The parameters analyzed include: Moisture Content (MC), Total Solids (TS), Volatile Solids (VS), Ash Content (AC), Organic Carbon (OC), Organic Matter (OM), Total Kjeldahl Nitrogen (TKN) and percentage composition of proteins, starch, sugars, crude fat, cellulose, hemicelluloses and lignin content.

5.2.6 Initial feeding, start-up and operation of the hUASB reactor

The previously described subunits were connected by transparent plastic tubing and assembled into a unit bioreactor system. The inoculum initially contained in the hydrolysis tank was allowed to flow into the Hybrid Up-flow Anaerobic Sludge Blanket (hUASB) reactor by opening the valves. The Hybrid Up-flow Anaerobic Sludge Blanket reactor was slowly and gradually filled up with an inoculum volume of 10.5 litres creating a slurry column of a height of 10.5cm from the base of the reactor. All the valves were closed and air entrapped at the reactor head-space was removed by suction using a syringe and needle pierced through the sampling septum. The bioreactor was operated in fed-batch upflow mode

with the inoculum retained for two weeks until the residual biodegradable organic matter was depleted to reach a steady state of constant biogas production (Bardiya *et al.,* 1996; Amin and Vriens, 2014; and Gumisiriza *et al.,* 2019). The two-week anaerobically incubated slurry was termed as pre-adapted inoculum. In subsequent experimentation, the entire system was operated in a continuous flow mode with the feed flowing from the hydrolysis tank to the Hybrid Up-flow Anaerobic Sludge Blanket *(*hUASB) reactor by force of gravity.

5.2.7 Optimisation of Organic Loading Rate

Eight triplicate Hybrid Up-flow Anaerobic Sludge Blanket *(*hUASB) reactor sets that had been previously pre-adapted with inoculum were set up and labelled to receive substrate with different concentrations. The eight reactors received substrates at organic loading rate (OLR) concentrations of 1.0, 2.0, 3.0, 3.5, 4.0, 4.5, 5.0 and 6.0gVS/L/Day, respectively. Additionally, two more similar triplicate reactor sets were set up as controls. The positive control contained only the pre-adapted inoculum while the negative one contained only the substrate diluted with distilled water to a final concentration of 3.0gVS/L/Day. The measured weight of fresh feed substrate was diluted with distilled water to appropriate organic loads of concentrations of volatile solids (VS) in g/L and filled into respective corresponding hydrolysis tanks (feed reservoirs). The inflow rate into the bioreactor was adjusted by slowly opening the tap valve to allow the in-flow rate of 0.4 L/day of feed substrate. In accordance with Bardiya *et al.,* (1996), the pre-adapted inoculum was gradually intermingled with the banana waste feed. After a Hydraulic Retention Time (HRT) of 25 days constituting one cycle, the reactor liquor inoculum was fully mixed with the feed substrate. The flow rate was continuously inspected for any particulate blockage by checking the flow through the monitoring window located after the valve. All the bioreactors were operated in a continuous up flow mode with respective concentration of feed substrate up-flowing through the reactor sludge bed column. The experiment was run for 27 weeks.

5.2.8 Monitoring of anaerobic digestion

The progress of anaerobic digestion and biochemical reactions in the reactor liquor were monitored by measuring biogas production according to Wilkie *et al*., 2004 and; Amin and Vriens, 2014. Both, the rate of biogas production and biomethane yield was monitored by measuring daily gas yield that was later analysed for the entire experimental time . Biogas was collected and the volume recorded directly from the measuring system. Biogas samples for analysis of methane content were collected daily by sucking the biogas using the syringe and needle pierced through the rubber septum. The methane yield from the positive control was substracted off from the experimental gas yield before recording the results. Methane content was estimated using KOH solution that absorbs CO_2 following the Erguder *et al.,* 2001 and Gumisiriza *et al.,* 2019 method. Samples for biochemical analysis were collected weekly from bioreactor sampling ports as well as effluent slurry tank and analyzed for pH, Volatile Fatty Acids (VFAs), Volatile Solids (VS) and COD following standard methods (APHA) (1998).The detailed methods are described in chapter three; materials and methods.

5.3. Results

5.3.1 The construction and operation of the hUASB Bioreactor system

The bioreactor system was constructed using materials purchased from local suppliers of water and gas engineering materials in quantities listed in table 5.2. The use of transparent materials enabled quick monitoring for any blockage or loose fixing.

Table 5:2. List of materials for construction of one-unit bioreactor system

Components	Specification	Quantity
1. Hydrolysis tank		
a) Plastic Aspirator	20L capacity	01
b) Plastic Nipple	ID 12.5 mm	06
c) Tap-valve	ID 12.5 mm	03
d) Flexible transparent plastic tube	ID 18.75 mm	2 meters
2. Hybrid UASB bioreactor vessel		
a) Transparent Plastic Vessel	ID 11.30 cm; H 115.00 cm	01
b) Plastic nipple	ID 12.5 mm	03
c) Rubber bang	ID 120 mm	01
d) Flexible transparent plastic tube	ID 18.75 mm	5 meters
3. Biogas measuring system		
a) Sampling septum	Rubber cup	01
b) Measuring cylinder	1L capacity	01
c) Glass tubing	OD 11 mm,600mm tall	01
d) Flexible transparent plastic tube	ID 12.5 mm	2 meters
e) Plastic Aspirator bottle	10L Capacity	01
4. Effluent bioslurry tank		
a) Plastic Aspirator bottle	20L capacity	01
b) Plastic Nipple	ID 12.5 mm	02
c) Tap-valve	ID 12.5 mm	01
d) Flexible transparent plastic tube	ID 18.75 mm	2 meters

ID = Internal Diameter, OD= Outer Diameter, H= Height

The hydrolysis tank, high rate bioreactor and effluent tank were set up at different height levels from the laboratory floor level. A daily feeding cycle started with sealing of bioreactor top with a rubber stopper through which a glass tube directed the produced biogas to the collection system. Distilled water was initially poured into the effluent slurry tank until it entered half of the effluent plastic tube to create a liquid-gas seal. The entire system was subsequently operated in a continuous flow mode with the feed flowing from the hydrolysis tank by force of gravity (figure 5.2) at a flow rate of 0.4 L/day which constituted to a Hydraulic Retention Time (HRT) of 25 days.

Figure 5:2. Experimental set-up of hybrid UASB bioreactor system
Plate (a) Shows the subunit components of the system before feeding: A-Effluent bioslurry tank, B-Hybrid UASB reactor, C-Feed/Hydrolysis tank and D- biogas collection and measuring system; Plate (b) Shows the fed system and measurement of the volume of the produced biogas.

5.3.2 Physico-chemical Characteristics of the feed substrate and Inoculum

Samples from pulverised fresh mixed banana waste, raw inoculum and pre-adapted inoculum were analysed for physico-chemical characteristics. Results revealed that over 90% of solids in the feed substrate were volatile solids (VS). Furthermore, the waste had a high C:N ratio of 41:1 (***Table 5.3***) indicating that the substrate was more rich in carbohydrates than proteins. On the other hand, the inoculum had less volatile solids and organic matter content but with more Total Kjeldahl Nitrogen (TKN) than the substrate. The pre-adapted inoculum had lower solid content than raw inoculum of 5.68 and 17.64 %, respectively. Moreover, inoculum adaptation process almost doubled the Total Kjeldahl Nitrogen content which suggested an increase in microbial growth.

Table 5:3. The Physico-chemical characteristics of the feed substrate and Seed Inoculum

Parameter	Feed Substrate		
	Banana waste	**Raw inoculum**	**Pre-adapted Inoculum**
MC (%Fresh Weight)	85.45 ± 0.35	82.36 ± 0.06	94.32 ±0.05
TS (%Fresh Weight)	14.55 ± 0.35	17.64 ± 0.06	5.68 ± 0.05
VS (%TS)	91.79 ± 0.16	78.81 ± 0.23	72.70 ±0.44
FS (%TS)	8.21 ± 0.16	21.19 ± 0.23	27.30 ±0.44
OC (%TS)	51.99 ± 0.26	43.31 ± 0.23	42.06 ±0.80
OM (%TS)	87.00 ± 0.50	69.90 ± 0.01	57.80 ± 0.42
TKN (%TS)	1.26 ± 0.50	2.03 ± 0.15	3.82 ± 0.13
C:N ratio	41.15: 1	21.33 : 1	19.06 : 1

MC= Moisture Content; TS= Total Solids; VS= Volatile Solids; FS= Fixed Solids (ash content); OC= Organic Carbon; OM= Organic Matter; TKN= Total Kjeldahl Nitrogen; C:N= Carbon to Nitrogen ratio

5.3.3 Determination of Organic Loading Rate

The bioreactors were loaded with the previously characterised fresh banana waste substrates at increasing Organic Loading Rate (OLR) from 1.0 to 6.0 $KgVS/M^3/Day$ (Table 5.4). The Hydraulic Retention Time (HRT) was constantly set at 25 days with a daily feed flow rate of 0.4 L/day. The OLR and flow rate were determined following the standard formulae described by Vogeli *et al*., (2014); that:

OLR = Q * S / V and S = [OLR X V]/Q

Whereby Q is the substrate flow rate (M^3/day),
S is the substrate concentration in the inflow ($KgVS/M^3$) and
V is the reactor volume.

Flow rate, Q = Reactor Volume / HRT

Whereby HRT is the Hydraulic Retention Time equivalent to Solids Retention Time (SRT)

From the table 5.2, physic-chemical analysis, the BW substrate contained a TS of 14.55 % of fresh weight and VS of 91.79 % of TS. This implied that 1.0g fresh weight of BW contained 0.1455 g TS (14.55%) of which 0.1336g (91.79%) is VS. Hence, 1.0 gVS is obtained from 7.50g fresh weight of banana waste substrate.
Similarly, the adapted inoculum used this study contained TS of 5.68% and VS of 72.70 % TS, which implied that 1g fresh weight of inoculum contained 0.0568g TS (5.68%) of with 0.0412936 gVS. Thus, 1.0 gVS is obtained from 24.27g fresh weight of adapted inoculum.

Table 5:4. Concentration of fresh Substrate at different Organic Loading Rates

Bioreactor Set-up	Feed substrate OLR (gVS/L/Day)	Feed substrate concentration (gVS/L)	Fresh substrate concentration (gFwt/L)
1	1.0	25.0	187.50
2	2.0	50.0	375.00
3	3.0	75.0	562.50
4	3.5	87.5	656.25
5	4.0	100.0	750.00
6	4.5	112.5	843.75
7	5.0	125.0	937.50
8	6.0	150.0	1125.00
Negative control (Dil. Substrate only)	3.0	75.0	562.50
Positive control (Inoculum only)	1.65	41.29	1000.00

After anaerobic digestion for 26 weeks, the results showed that biogas production increased consistently with increase in OLR upto 4.0 KgVS/M^3/Day (Table.5.5) and thereafter both the methane content and pH dropped drastically.

Table 5:5. Effect of Organic Loading Rate (OLR) on methane yield and gas quality

OLR (KgVS/M^3/Day)	Variables				
	HRT (25Days)	pH	Av. Biogas Yield (L/KgVS/Day)	Av. Biogas Quality (% CH_4)	Av. CH_4 Yield (L/KgVS/Day)
0.0	25.0	7.60	0.00	74.00	0.00
1.0	25.0	7.60	275.33	73.00	200.99
2.0	25.0	7.50	522.00	72.00	375.84
3.0	25.0	7.40	670.00	72.00	482.40
3.5	25.0	7.40	740.67	72.00	533.28
4.0	25.0	7.40	768.27	72.00	553.15
4.5	25.0	5.40	674.66	56.00	460.00
5.0	25.0	4.50	752.00	46.00	345.92
5.5	25.0	4.00	924.70	32.00	295.89

Although the methane content continued to fall after the OLR of 4.0 $KgVS/M^3/Day$ the total biogas rejuvenated and increased exponentially perhaps due to high rate of acidification characterised by high production of carbon dioxide, hydrogen gases as well as volatile fatty acids (Figure.5.3).

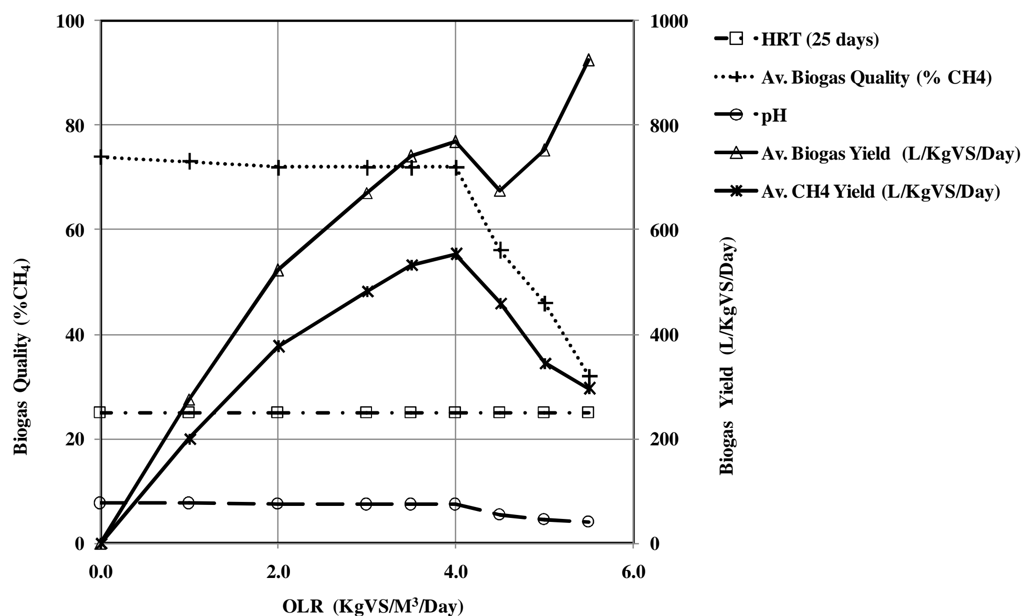


Figure 5:3. Effect of Organic Loading Rate (OLR) on biomethanisation of banana waste at a Hydraulic Retention Time (HRT) of 25 days

5.4. Discussion

5. 4.1 Design and Operation of HUASB reactor system

Successful anaerobic digestion is dependent on the development and use of well designed high rate anaerobic bioreactors (Callaghan *et al.*, 1999; Alvarez *et al.,* 2002; Bouallagui *et al., 2004* and Kirtane *et al.,* 2009). A study by Massart *et al.,* 2006, revealed that reactor design considerations are essential to efficient operation of anaerobic digesters. In this study, a two-stage digester system consisting of a ten-litre laboratory scale Hybrid Upflow Anaerobic Sludge Blanket (hUASB) bioreactor was successfully constructed and setup at the Department of Biochemistry, Makerere University. The separation of hydrolysis stage from methanogenic stage allowed options for application of pre-treatment methods in the hydrolysis tank without causing oxygen toxicity to methanogens. The constructed reactor was a hydrid Upflow Anaerobic Sludge Blanket (hUASB) reactor due to the incorporation of an upflow sludge bed column and Bordeaux stirrer of which the former is typical to Upflow Anaerobic Sludge Blanket (UASB) reactors while the latter is distinctive to the Continuously Stirred Tank Reactor (CSTR). The Upflow Anaerobic Sludge Blanket (UASB) reactor is one of the high rate bioreactors with the major advantage of retaining active biomass in the form of sludge granules thereby achieving highly cost-effective system (Saleh and mahmood, 2004). The incorporation of the Bordeaux stirrer enabled the user of the constructed reactor to set and achieve a controlled appropriate mixing. The feed to the digester should be well mixed, especially if it consists of a heterogeneous particles (Massart, 2006), typical of

pulverised plant biomass like banana waste. To ensure that all solids that enter the digester flow out consistently, adequate mixing is necessary. Moreover, adequate mixing produces a uniform solids concentration throughout the digester liquor, which is important for the optimal bioconversion of volatile solids into biogas (Wilkie *et al.,* 2004; Gumisiriza *et al.,* 2017).

Apart from mixing, other factors considered in the design and construction of the hUASB reactor system in this study include; reduction in energy in-put for consistent continuous flow of feed through the system, effluent overflow, control of organic loading rates as well as foam reduction. The hydrolysis tank was connected to the hUASB reactor inlet port by an S-shaped transparent plastic tubing (ID 18.75mm) which created a gentle flow of substrate into the bioreactor without destabilization of the active biomass in the reactor column. The hydrolysis tank, high rate bioreactor and effluent tank were set up at different height levels from the laboratory floor level which enabled a consistent steady uni-directional flow of substrate by force of gravity without any requirement for a pump. The feed substrate and inoculum successfully flowed from the hydrolysis tank into the bioreactor by of force of gravity after opening the valve. The influx of the inoculum into the bioreactor flushed out air that was sucked out through biogas sampling septum by a syringe needle. The height difference also enabled the biogas measuring system to level to zero mark on the measuring cylinder whenever the gas was withdrawn during experimentation. At day zero of the experiment, the opening of the tap valve of the coloured-liquid reservoir tank held at the topmost height (level 3) enabled the coloured liquid level in the biogas measuring cylinder to raise up and the meniscus coincided with the zero mark of the measuring cylinder. The positioning of the effluent port at the base of the gas-liquid-solid separator (GLSS) enabled the discharge of the effluent slurry from the digester by hydraulic overflow rather than by opening and closing a valve. The difference in hydraulics caused by opening a valve to withdraw sludge could have resulted into variations in the level and volume of the digester contents. By using hydraulic overflow design, the level of sludge in the digester and its volume will always be a constant (Massart *et al.,* 2006).

The major uniqueness of the constructed Hybrid Upflow Anaerobic Sludge Blanket (hUASB) reactor system include; firstly, the use of relatively tall reactor vessel (tall column of granular bioactive sludge blanket) enabled a sustained long time of interface of microbial biocatalyst with feed substrate; secondly, the retention of a seed inoculum reservoir at the base of the reactor created a sufficient amount of active granular seed sludge for faster start-up; and thirdly, the incorporation of Bordeaux stirrer not only allowed good mixing that enhanced microbial biocatalysis, but also reduced the foaming, improved gas escape through the sludge blanket as well as enabling reactor operations at higher OLR. The setting of stirrer motor at 60 rpm created sufficient agitation torque for mixing particulate matter in the feed substrate without destabilization of the inoculum reservoir at the base of the reactor. This was in agreement with previous studies that reported that the Bordeaux stirrer has the ability to offer intermittent agitation of slurries with solid concentrations greater than 10%. Moreover, the stirrer has a distinctive advantage of achieving optimal intermittent mixing of particulate matter at moderate stirrer tip velocities of 40 to 120 cm per second (Fannin, 1987; Smith *et al.,* 1988; Liu and Tay, 2001 and Wilkie *et al.,* 2004). Intermittent mixing is preferred to continuous mixing because the former has been reported to enhance digestion rates while

the latter cause vigorous mixing that disrupts spatial arrangement between bioreactor biocatalysts (bacteria) and feed particulates as well as interruption of microbial syntrophism and grannulation (Fannin, 1987; Wilkie *et al.,* 2004).

Furthermore, the designed Hybrid Upflow Anaerobic Sludge Blanket (hUASB) circumvented the problems of long start-up period and early wash out of active flora common in conventional Upflow Anaerobic Sludge Blanket (UASB) reactors. This was made possible by creation of the reservoir of the active seed inoculum at the base of the novel Hybrid Upflow Anaerobic Sludge Blanket (hUASB) reactor that would automatically re-seed the in-coming substrate. The positioning of the inlet port at a height of 5cm from the bottom led to the retention of the sludge bed of active bioflora for jump starting AD process. As a result the start-up period was shortened from the reported 4-16 days for conventional Upflow Anaerobic Sludge Blanket (Saleh and mahmood, 2004) to 2-3 days obtained this study using the novel Hybrid Upflow Anaerobic Sludge Blanket (hUASB). The reactor also showed quickened recovery from overload and active sludge wash-out due to biomass floatation and especially when operated under low HRTs. The system was operated in a continuous flow mode with the feed flowing from the hydrolysis tank by force of gravity at a flow rate of 0.4 L/day which constituted to a Hydraulic Retention Time (HRT) of 25 days. This helped to maintain a continuous flow of feed substrate at no energy input.

5.4.2 Characteristics of the substrate and Inoculum

The physico-chemical analysis revealed that the banana waste used as a feed substrate constituted over 90% of the solids as volatile solids. This suggested that the substrate was largely organic and a highly valuable substrate for anaerobic digestion (Vögeli *et al.,* 2014). Nevertheless, the high C:N ratio of 41:1 indicated that the substrate was more rich in carbohydrates than proteins. This high C:N ratio could easily cause reactor acidification and process failure especially at high organic loading rate. Pre-adaptation of the raw inoculum caused a threefold reduction in the solid content of raw inoculum thereby depleting the residual organic matter and thus stabilising the inoculum. However, the adapted inoculum had double the Kjeldahl Nitrogen, suggesting that the adaptation process enhanced the growth of microbial biocatalysts leading to the increase in population of microorganisms that carry out the anaerobic digestion.

5.4.3 Optimisation of Organic Loading Rate of HUASB Reactor

Solutions of feed substrate at varying volatile solid concentrations were loaded to separate bioreactors and the progress of the anaerobic digestion monitored. Results indicated that the biogas production and methane content increased with increasing organic loading rate (OLR). The biogas yield increased exponentially with almost doubling the rate of biogas yield as OLR increased from 1.0 to 2.0 KgVS/M^3/Day. The biogas yield increment reached a climax of 0.55315 M^3CH_4/KgVS/Day at OLR of 4.0 KgVS/M^3/Day. This OLR was in the ideal range for continuously stirred reactors. Vandevivere *et al.*, 2003 and Vogeli *et al.,* 2014 reported that the OLR in the range of 4 – 8 kg VS/m^3 reactor per day is ideal for anaerobic treatment of biowastes, and results in the removal of VS in the range of 50 – 70%. However, for non-stirred bioreactor systems, an OLR below 2 kg VS/m^3 reactor per day is recommended and considered suitable. This implied that the designed

hUASB reactor system that reached an OLR of 4.0 $KgVS/M^3/Day$ significantly enhanced the AD of banana waste. The methane yield obtained in this study was higher than the 0.43661 m^3 $CH_4/KgVS/Day$ previously reported from batch-wise anaerobic digestion of banana waste (Gumisiriza *et al.,* 2019) and the 0.36-0.53 m^3 $CH_4/KgVS/Day$ reported for Municipal Solid Waste (Khalid *et al.,* 2011). Since for non-stirred bioreactor systems an OLR below 2 kg VS/m^3 reactor per day is recommended, the designed hUASB reactor system that reached an OLR of 4.0 $KgVS/M^3/Day$ significantly enhanced the anaerobic digestion of banana waste. This high rate of biogas production in this study was attributed among others, to a better reactor design, the use of well pre-adapted inoculum, and to the optimised conditions of the reactor liquor. The initial doubling rate in biogas yield indicated that the used inoculum contained a well activated population of microbial flora that was ready to kick-start the anaerobic digestion process. Moreover due to better bioreactor design, the retained sludge bed of seed inoculum at the bottom served as the back up of active flora for jump-starting anaerobic digestion process thereby shortening bioreactor start-up period. This suggested that the hybrid bioreactor system used in this study was effective in digesting banana waste even at higher organic loading rate (OLR) of over 3.5 $KgVS/M^3/Day$ reported by Kirtane *et al.,* (2009). Further increase in OLR beyond 4.0 $KgVS/M^3/Day$ showed a drastic fall in methane yield perhaps due to the death of methanogens caused by reactor acidification. The concomitant decrease in percent methane content with decrease in biogas production and fall in pH signified acidification of the reactor due to overloading with volatile solids. This was in agreement with studies by Krishnamurthi, 1989 and Kirtane *et al.,* 2009, which noted that the overloading of feed substrate from plant biomass could cause decrease in percentage methane due to accumulation of tannin, alkaloids, flavonoids and terpenoids, which are inhibitory to microbial growth and anaerobic digestion. Further loading during acidification stage resulted in another exponential production of putrid gas of very low percentage methane content but perhaps rich in carbon dioxide, hydrogen and volatile fatty acids that gave the characteristic malodours. The negative control reactor did not yield any methane gas due to lack of seed inoculum that would have supplied the methanogenic bacteria.

5.5. Conclusion

The study findings revealed that a tailored hUASB reactor system was constructed and operated at room temperature of 25^0C. The bioreactor system could be operated in a continuous flow mode using hydraulic flow created by force of gravity. The reactor system could successfully treat banana waste without wash-out of active sludge. The reactor also showed a short start-up period of 3 days and quick recovery from feed overloads. The reactor could optimally digest banana waste at high OLR of $4.0KgVS/M^3/day$ without failure due to acidification. Further studies on the hUASB would be needed to focus on optimization of hydraulic retention time as well as investigating the effect of feed pre-treatment on the performance of the reactor.

5.6 References

1. Alvarez, R.J., Meraz, M., Monroy, O and Velasco, A (2002), "Feedback control design for an anaerobic digestion process", J Chem Technol Biotechnol, 77 Pp 725-734.

2. Amin, G.A and Vriens, S (2014), "Optimization of upflow anaerobic sludge blanket reactor for treatment of composite fermentation and distillation wastewater", African Journal of biotechnology, Vol.13 (10), pp. 1136-1142.

3. APHA. (1998), "Standard methods for the examination of water and wastewater", 20th edition, American Public Health Association, Washington, DC.

4. Bardiya, N., Somayaji, D and Khanna, S (1996) "Biomethanation of banana peel and pineapple waste" Bioresource Technology 58 :73-76

5. Bouallagui, H., Torrijos, M., Godon, J. J., Moletta, R., Cheikh, R.B., Touhami, Y., Delgenes, P.P and Hamdi, M (2004), "Two-phase anaerobic digestion of fruit and vegetable wastes: Bioreactor 's performance", Biochem Eng J, Vol. 21 Pp 193-197.

6. Callaghan, F.J., Wase, D. A. J., Thayanithy, K and Forster, C .F(1999), "Co- digestion of waste organic solids: Batch studies", Biores Technol, Vol. 67: 117-122.

7. Clarke, W.P., Radnidge, P., Lai, T.E., Jensen, P.D and Hardin, M.T (2008), "Digestion of waste bananas to generate energy in Australia", Waste Management, 28: 527-533

8. Erguder, J.F., Tezel, U., Guven, E., Demirer, G.N (2001), "Anaerobic biotransformation and methane generation potential of cheese whey in batch and UASB reactors", Waste management. Vol. 21 Pp 643-650.

9. Fannin, K.F (1987) "Start-up, Operation, Stability, and Control" In: Chynoweth, D.P., Isaacson, R. (Eds.), Anaerobic Digestion of Biomass. Elsevier Applied Sci. Publishers, London, pp.171–196.

10. German Agency for Renewable Energy. 2005. http://global.mongabay. com/news/bioenergy/2007/07/new-biogas-reactor-for-energy-crops. html.

11. Ghangrekar, M.M and Kahalekar, U.J (2003), "Performance and Cost Efficacy of Two-stage Anaerobic Sewage Treatment IE (I)", Journal. EN Vol.84:16-22.

12. Gumisiriza, R., Hawumba, J.F., Balyeidhusa, A.S.P., Okure, M and Hensel, O (2019), "Processing of East African Highland Green Bananas: Waste Generation and Characterisation as a Potential Feedstock for Biogas Production in Uganda", American Scientific Research Journal for Engineering, Technology, and Science; Global Society of Scientific Research and Researchers; ISSN (Print) 2313-4410, ISSN (Online) 2313-4402; http://asrjetsjournal.org/

13. Gumisiriza, R., Hawumba, J.F., Okure, M and Hensel, O (2017) "Biomass Waste-to-Energy Valorisation Technologies: A Review Case for Banana Processing in Uganda"

Biotechnology for biofuels 10:11. DOI 10.1186/s13068-016-0689-5

14. Gumisiriza, R., Mshandete, A.M., Rubindamayugi, M.S.T., Kansiime, F. and Kivaisi, A. K (2009), "Enhancement of Anaerobic digestion of Nileperch fish processing waste water", African Journal of Biotechnology 8: (2) 328-333

15. Hensel., 2014: Hermann Sewerin GmbH, FG Mess- und Regelungstechnik & FG Agrartechnik "Project report: Measurement of biogas emissions" ; sponsored by Deutsche Stiftung Umwelt

16. Kirtane, R.D., Suryawanshi, P.C., Patil, M.R., Chaudhari, A.B and Kothari, R.M (2009), "Optimization of Organic Loading Rate for different fruit waste during biomethanization", Journal of Scientific & Industrial Research Vol.68, pp252-255

17. Kovacik, W.P., Scholten, J.C.M., Culley, D., Hickey, R., Zhang, W and Brockman, F.J (2010) "Microbial dynamics in upflow anaerobic sludge blanket (UASB) bioreactor granules in response to short-term changes in substrate feed", Microbiology Vol. 156: 2418–2427.

18. Krishnamurthi, A (1989), "The Wealth of India", [NISCAIR (CSIR) Vol. VIII Publ, New Delhi, India].

19. Liu, Y and Tay, J.H (2001), " Metabolic response of biofilm to shear stress in fixed-film culture", J. Appl. Microbiol. 90 (3), 337–342.

20. Massart, N., Black and Veatch (2006), "Design and Operational Considerations to Avoid Excessive Anaerobic Digester Foaming", Water Environment Foundation, WEFTEC(R), 06: Pp 2553-2575

21. Matta-Alvarez, J (2003), "Anaerobic digestion of organic fraction of municipal solid waste: a perspective", in: Biomethanization of the organic fraction of municipal solid waste. Matta-Alvarez, J. (Ed). IWA Publishing, London. UK, pp 91-105

22. Mrunalini, M.P., Vijay, S.K., Sunanda, V.K and Girish, S.K (2013), "Review on application of UASB technology for wastewater treatment", Inter. J. Adv. Sci. Eng. Technol. Vol. 2(2): 125-133.

23. Najafpour, G.D., Zinatizadeh, A.A., Mohamed, A.R., Isa, M.H and Nasrollahzadeh, H (2006), "High-rate anaerobic digestion of palm oil mill effluent in an upflow sludge-fixed film bioreactor", Process Biochem. Vol. 41:370-379.

24. Narihiro, T., Terada, T., Kikuchi, K., Iguchi, A., Ikeda, M., Yamauchi, T., Shiraishi, K., Kamagata, Y., Nakamura, K and Sekiguchi, Y (2009), "Comparative analysis of bacterial and archaeal communities in Methanogenic sludge granules from upflow anaerobic sludge blanket reactors treating various food-processing, high-strength organic wastewaters", Microbes Environ. Vol. 24:88-96.

25. Nidal, M (2008), "High strength sewage treatment in a UASB reactor and an integrated UASB-digester system", Bioresour. Technol. Vo1.99:7531–7538.

26. Saleh, M.M.A and Mahmood, U.F (2004) "Anaerobic Digestion Technology for Industrial Wastewater Treatment", Eighth International Water Technology Conference, IWTC8 2004, Alexandria, Egypt Pp 817-833.

27. Smith, P.H., Bordeaux, F.M., Goto, M., Shiralipour, A., Wilkie, A., Andrews, J.F., Ide, S., Barnett, M.W., 1988a. Biological production of methane from biomass. In: Smith, W.H., Frank, J.R. (Eds.), Methane From Biomass: A Systems Approach. Elsevier Applied Science Publishers, London, pp. 291–334.

28. Smith, P.H., Bordeaux, F.M., Wilkie, A., Yang, J., Boone, D., Mah, R.A., Chynoweth, D., Jerger, D., 1988b. Microbial aspects of biogas production. In: Smith, W.H., Frank, J.R. (Eds.), Methane From Biomass: A Systems Approach. Elsevier Applied Science Publishers, London, pp. 335–353.

29. Vögeli Y., Lohri C. R., Gallardo A., Diener S., Zurbrügg C. (2014). "Anaerobic Digestion of Biowaste in Developing Countries: Practical Information and Case Studies". Swiss Federal Institute of Aquatic Science and Technology (Eawag), Dübendorf, Switzerland.

30. Willkie, A.C., Smith, P.H and Bordeaux .F.M (2004), "An economical bioreactor for evaluating biogas potential of particulate biomass", Bioresource Technology, Vol. 92 pp 103-109

6 Optimisation of operational parameters of the novel hUASB reactor system

6.1 Introduction

The Up-flow anaerobic sludge blanket (UASB) reactor belongs to the high-rate bioreactor systems, able to perform anaerobic reaction at reduced hydraulic retention times, when compared to traditional digesters (Mainardis *et al.,* 2020). The main parameters that influence the performance of Up-flow anaerobic sludge blanket reactors are: the operating temperature (psychrophilic, mesophilic or thermophilic regime), pH, Hydraulic Retention Time (HRT), Organic loading Rate (OLR) and up-flow velocity. A stable pH close to neutrality is required to obtain a good-quality granular sludge, with sufficient alkalinity in the feeding substrate (Abbasi *et al*., 2012). Therefore, the Up-flow velocity helps to guarantee and maintain the desired HRT as well as mixing between sludge bed and incoming slurry substrate. The recommended up-flow velocity range for typical Up-flow anaerobic sludge blanket reactor is 0.5–1.5 m/h (Latif *et al*., 2011). It should , however, be noted that Up-flow velocity values above 1 m/h in conventional Up-flow anaerobic sludge blanket reactor systems can lead to granule disintegration and biomass washout (Abbasi *et al*., 2012). Nonetheless, a higher up-flow velocity is generally applied in the reactor start-up phase to select the biomass by removing smaller granules and maintaining the larger ones. Hydraulic retention time (HRT) is the key factor that controls the extent to which volatile solids in the substrate are converted into biogas (Gumisiriza *et al.,* 2017). Besides, a Shorter Hydraulic Retention Time results into faster wash out of active biomass than they can reproduce, consequently causing prolonged lag phase of some steps such as fermentative step (Frick and Uppsten, 1999). Thus, the problem of early discharge of reactor liquor is aggravated by reactors operated at shorter Hydraulic Retention Time. On the other hand, too long HRT requires large volume of the digesters that are limited by cost, treatment capacity, net energy yield and operational skills. Conventional anaerobic digestion processes operate at a Hydraulic Retention Time in the optimal range of 15-30 days (Liebrand and Ling, 2009).

Previous studies have suggested that the problem of feed floatation and early discharge commonly occurring in conventional up-flow anaerobic sludge blanket reactors can be circumvented by carrying out anaerobic digestion in appropriately designed bioreactor system with fully optimised environmental and operational parameters such as Hydraulic Retention Time (Mshandete, 2005; Bilibio *et al.,* 2011). Appropriate bioreactor design, construction and operation have been discussed in the previous chapter. Operational parameters can be defined as reactor engineered controls that can be regulated to stabilise the liquor conditions and biochemical processes (environmental parameters) that in turn lead to enhanced AD and biomethanization process of a given feed substrate. Optimisation of operational parameters such as Hydraulic Retention Time, Organic Loading Rate, liquor agitation, among others contributes to optimal and stability of bioreactor liquor conditions and biochemical processes. The effect of optimisation of organic loading rate, agitation and options for retention of active biomass during anaerobic digestion of banana waste has been investigated and discussed in

the previous chapter of bioreactor design. Therefore, the objective of this section was to determine the optimal hydraulic retention time for enhanced biomethanization of banana waste using the novel hybrid Up-flow anaerobic sludge blanket UASB bioreactor

6.2 Methodology

Seven *Hybrid* Up-flow Anaerobic Sludge Blanket reactors arranged in triplicates with each set containing a pre-adapted inoculum, were set up at room temperature. The reactors were operated in a continuous flow mode with the feed flowing from the hydrolysis tank to the reactors by force of gravity. The reactors were lebelled 1 to 7 and accordingly set to operate at increasing hydraulic retention time of 10, 15, 20, 23, 25, 30 and 40, respectively. The flow of the substrate into the reactors were set at respective inflow rates of 1.00, 0.67, 0.50, 0.43, 0.40, 0.33 and 0.25 litres substrate per day. All of the seven reactors received feed substrate with the same concentration of 4.0gVS/L from the feed reservoir tanks. The experiment was run for 27 weeks and anaerobic digestion process was evaluated by monitoring the rate of biogas production, gas yield and biogas quality as a percentage of CH_4 in biogas generated.

6.3 Results and discussion

6.3.1 Effect of hydraulic retention time on biogas quality

In order to operate a bioreactor in a continuous flow mode, organic loading rate (OLR) and the hydraulic retention time (HRT) are principal parameters to optimise. Organic loading rate is the quantity of organic material added per unit volume of the reactor in a day. The effect of OLR on anaerobic digestion of banana waste has been discussed in the previous chapter. Hydraulic retention time is an average time to which the feedstock remained inside the anaerobic digester (Rittmann and McCarty, 2001). Decrease in the HRT, upsurges the hazard of washout of the active bacterial population. On the contrary, increase in the hydraulic retention time increases the capital cost of the reactor. Hence, there should be an optimum hydraulic retention time to keep the efficient operation of the anaerobic digestion plant (Sahito *et al.,* 2016)

Generally, this study found that anaerobic digestion of solid wastes from industrial processing of green East African highland bananas yielded biogas of low methane content (below 60% methane) at short hydraulic retention time (HRT) of 10 to 20 days (figure 6.1).
The production of biogas with low methane content was more evident in reactors operated at hydraulic retention time between 10 and 15 days in which anaerobic digestion nearly failed especially in the first 8 weeks due to acidication. These observations were similar to what was in agreement with a report by Bardiya *et al.,* (1996), which revealed that biomethanization of banana waste at hydraulic retention time of 10 and 20 days caused digester to turn sour with constant decrease in methane content. This negative effect casued by short hydraulic retention time could be attributed to lack of sufficient retention time required for growth of methanogens leading to process acidification and malodor emmissions.
Moreover, short hydraulic retention time for Up flow anaerobic sludge blanket (UASB) reactors operated in continuous flow mode cause the washout of the reactor liquor leading the escape of all the active microorganisms (Zhang *et al.,* 2006 ; Zaher *et al.,* 2009).

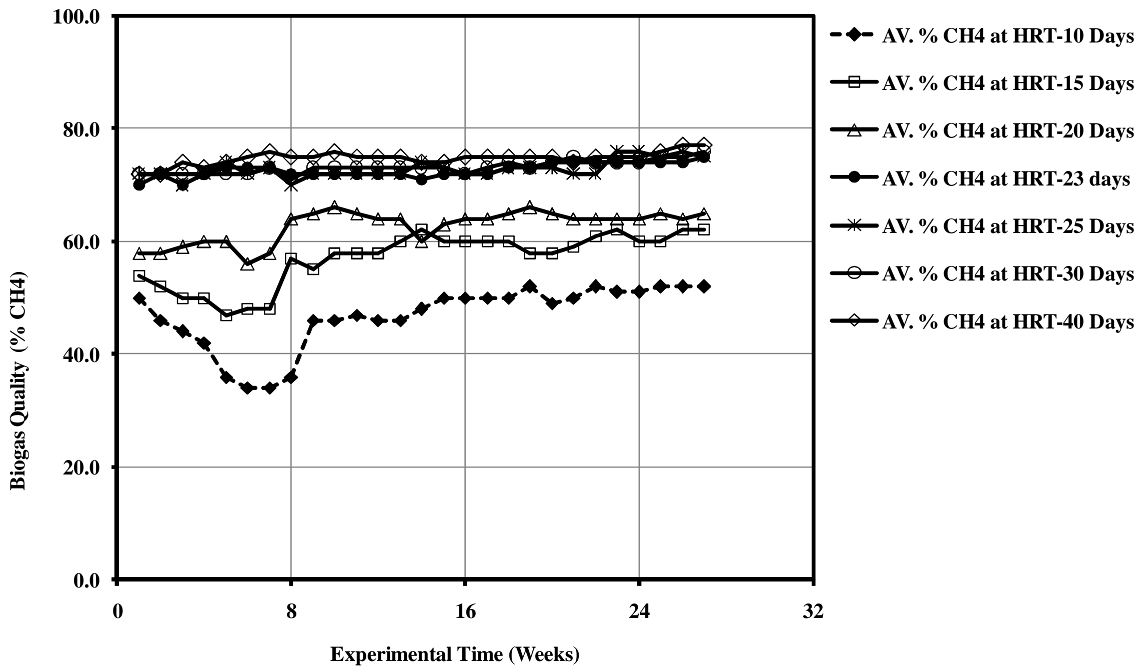


Figure 6:1. Biogas Quality at varying Hydraulic Retention Time (HRT)

In addition, the washout of methanogens favors quick regeneration and survival of acidogenic microorganisms (fermenters) which produce alot of volatile fatty acids (VFAs) that lower the liquor pH to acidic level (6.0–6.5) thereby causing reactor acidification. At short hydraulic retention time, the growth rate of fermenting microorganisms that produce VFAs and hydrogen (fermenters) such as β-oxidixers is much higher than that of hydrogen-consuming bacteria such as hydrogenotrophic methanogens (Zaher *et al.,* 2009). On the contrary, the hydrogen production pattern may shift to methanogenic one when hydraulic retention time is increased (Gumisiriza *et al.,* 2017; Zhang *et al*., 2006).

This study further found out that longer hydraulic retention time resulted into biogas with more methane content (over 70% methane). This was in agreement with findings of other researchers that longer hydraulic retention time enhances growth and dominancy of methanogens, which in turn lead to increased biogas production with high methane content (Huang *et al.,* 2011; Jiang *et al.,* 2020). Despite the high methane content, longer hydraulic retention time was associated with low biogas yield due to sustained substrate depletion from reactor microorganisms at the end of the retention cycle. This study ultimately revealed that a hydraulic retention time of 23 days was the optimal that achieved the highest biogas yield with high methane content. The optimal hydraulic retention time of 23 days was the retention time that favoured the balanced growth of hydrogen- and methane-producing bacteria leading to enhanced biogas yield with high methane content (Zaher *et al.,* 2009).

6.3.2 Effect of hydraulic retention time on rate of biogas production

Generally the trend of biogas production showed characteristic short and tall peaks as well as shallow and deep grooves (Figure 6.2). The variations in peaks and grooves was attributed to the substate composition and feeding cycles as a function of hydraulic retention time. Syntrophically, after reactor feeding with banana waste comprised of some free glucose and large amount complex carbohydrate like starch and lignocellulose, the microbial biocatalysts

in the inoculum quickly and freely utilise the freely available glucose to cause a peak in gas production (short peak). Immediately after the short peak, there was a recession in biogas production indicated by a shallow groove due to deminishing initial free sugar. Moreover the reactor microbial biocatalysts were multiplying exponentially and getting adapted to degrade and utilize starch and lignocellulose leading to the release of more sugars and intermediate metabolites. Consequently, there was a sharp increase in gas production as shown by a taller peak than the previous ones. This trend was in agreement with the findings reported by Bardiya *et al.,* (1996) and Karimi *et al*., (2016); which revealed that the higher initial glucose concentration is beneficial to obtain a higher microbial saccharification of lignocellulosic biomass.

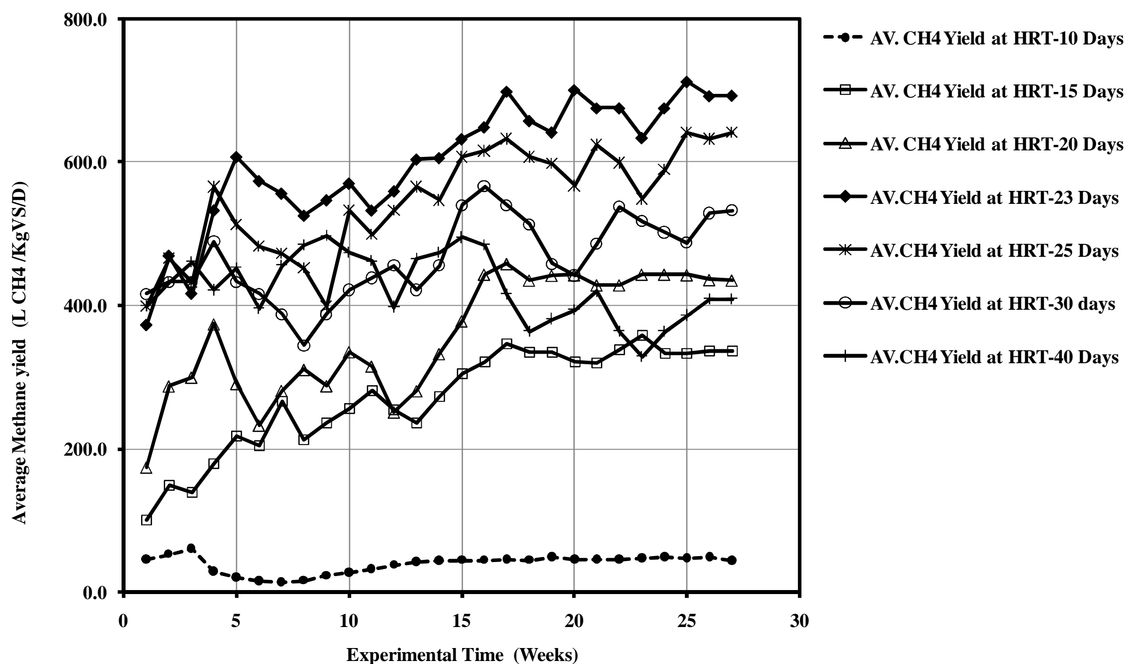


Figure 6:2. Biogas production at varrying Hydraulic Retention Time (HRT)

After the tall peak of gas production, the curves culminated into another sharp grooves which are more prominent with long hydraulic retention time of 30 and 40 days. The sharp drop in gas yield (deep grooves on the curve) likely signified the end of a retention cycle. At long hydraulic retention time cycle, there was low rate of substrate in-flow into the reactor leading to a temporary starvation of microorganisns at the end of every rention cycle, and thus a drop in gas production. On the other hand, the short hydraulic retention time caused the washout of the reactor liquor leading the escape of all the active microorganisms (Zhang *et al.,* 2006; Zaher *et al.,* 2009). The wash out of reactor microorganisms results into reduced efficiency of substrate degradation (Zaher *et al.,* 2009). A short hydraulic retention time cause over feeding of the reactor resulting into over saturation of the few retained microorganisms with feed substrates that distabilises the microbial syntrophic metabolic balance leading to reactor acidification with low methane yields, but with less fluaction in gas production (Bardiya *et al.,* 1996 and Gumisiriza *et al.,* 2019). In this study, the results revealed that the retention time of 23 days was the optimal hydraulic retention time and the biogas production curve showed relatively consistent peaks with less fluctuations (deep grooves) and the highest cumulative methane yield (Figure 6.3). At the optimal hydraulic retention time, there was less

wash out of microorganisms from the reactor thus favouring the establishment of the syntrophic balance between hydrogen producing acetogens and hydrogen consuming methanogens. This balance is the one responsible for enhanced rate of biogas production at optimal hydraulic retention time.

The consistence in gas production at the optimal hydraulic retention time (HRT) was due to synchronization of loading rate with substrate utilization by reactor microorganisms. The timely availability of substrate to reactor microorganisms reduced the recession time especially at the end of every retention cycle leading to a reduction of major grooves exhibited on the biogas yield curves.

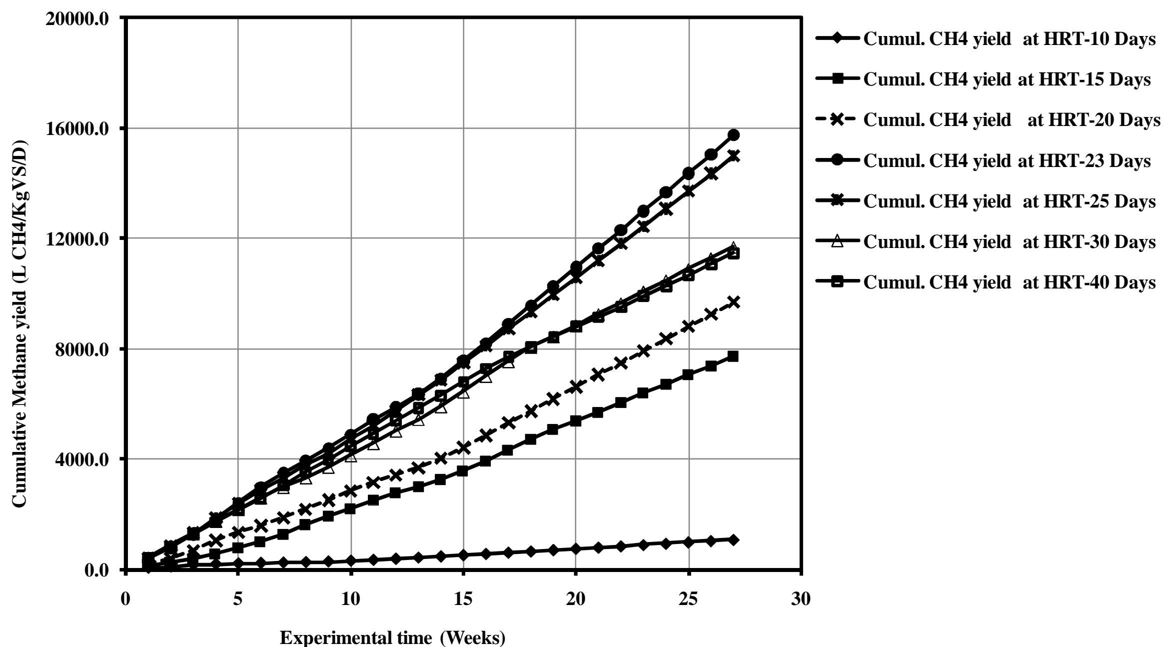


Figure 6:3. Cumulative CH4 yield at varying Hydraulic Retention Time (HRT)

6.3.3 Effect of Hydraulic Retention Time (HRT) on biogas yield

The results showed that the hydraulic retention time (HRT) of 23 days had higher methane yield than HRT of 20 and 25 days as shown in figure 6.4. This was in agreement with the findings reported by other previous studies that lignocellulosic feed stocks like most of the energy crops cannot be digested completely at HRT of less than 20 days (Wolf, 2013). The recommended HRT for wastes treated in a mesophilic digester is in the range of 10 to 40 days (Vögeli *et al.,* 2014). The optimal retention time for complete biological conversion is in the range of 12–24 and 15–30 days respectively, for thermophilic and mesophilic digesters (Mir *et al.,* 2014).

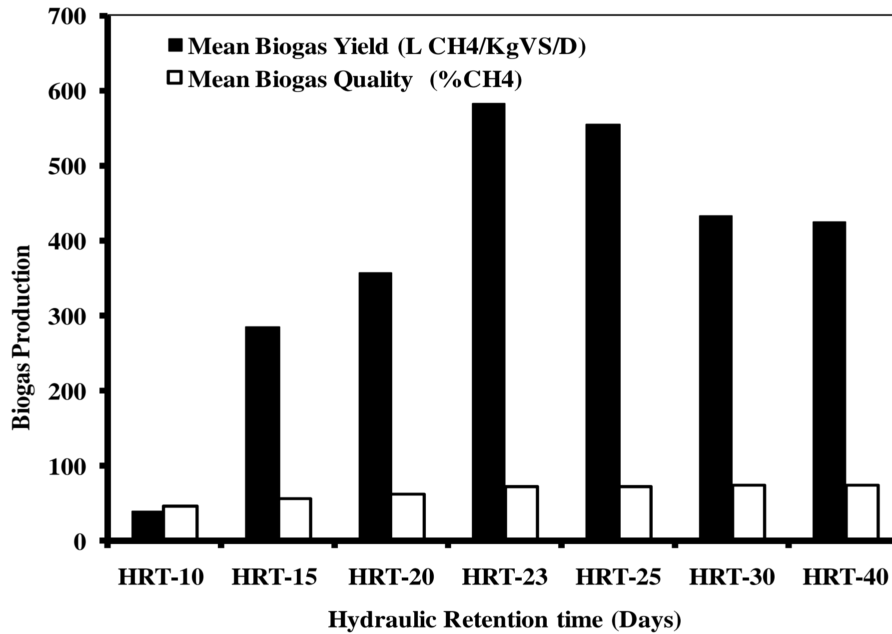


Figure 6:4. Effect of HRT on biogas production from banana waste

The HRT of 23 days yielded 583.08 LCH_4/KgVS/Day (Table 6.1) that is equivalent to 0.535 M^3 CH_4/KgTS/Day. This gas yield was slightly lower than 0.640 M^3 CH_4/KgTS/Day reported for food market waste (Mata-Alvarez *et al.,* 1992). This deviation was perhaps due to higher free sugar content in food market waste than in green banana waste.

Table 6:1. Average methane yields and methane content from banana waste at varying HRT with constant feed concentration

HRT (Days)	Mean methane Yield (L CH4/KgVS/D)	Mean Biogas Quality (% CH4)
10	39.72 ± 12.52	46.74 ± 5.66
15	285.58 ± 78.67	57.00 ± 4.75
20	357.95 ± 87.16	62.74 ± 2.89
23	583.08 ± 10.57	72.63 ± 1.24
25	555.01 ± 80.05	72.85 ± 1.61
30	433.10 ± 51.48	73.19 ± 1.18
40	425.39 ± 44.84	74.78 ± 1.19

However, the methane yield obtained in this study is comparable to what has been reported in the literature. The average methane yield from municipal solid waste was reported to fall between 0.36 and 0.53 m^3/kg VS (Khalid *et al.* 2011). A research by Berlian *et al.*, (2013) on anaerobic digestion of mixed fruit and vegetable waste reported biogas and methane yield ranges of 0.53–0.83 and 0.25–0.55 m^3/KgVS/day, respectively at mesophilic temperature and a hydraulic retention time of 28 days.

However, anaerobic digestion at thermophilic temperatures results into higher biogas yields albeit of low methane content. Typically, a research by Linke (2006), on anaerobic digestion of potato waste in continuously stirred tank reactor (CSTR) at thermophilic temperature of 55°C, yielded biogas in the range between 0.65 and 0.85 m3 kg−1VS, but with 58% methane content. Increasing the organic loading rate up to 3.4 kg VS m−3 day−1, the biogas yield declined due to the accumulation of fatty acids that acted as inhibitors (Linke, 2006).

The results from this study further revealed that increase in hydraulic retention time enhanced both gas yield and methane content. Ultimately, the optimal hydraulic retention time of 23 days was established. Further increase in hydraulic retention time above the optimal resulted into decrease in gas yield with slight increase in percentage methane content as shown in figure 6.5.

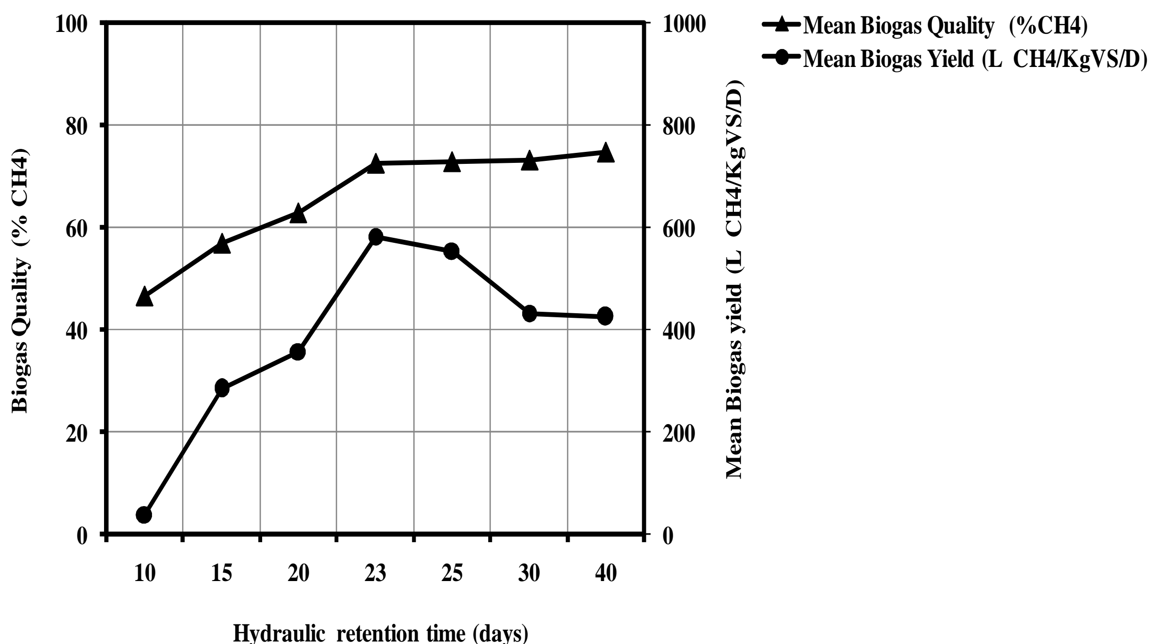


Figure 6:5. Relationship between HRT, gas yield and percentage methane content

The higher percentage methane content at longer hydraulic retention time was likely possible due to growth and stability of methanogenic bacteria at longer retention time (Ariunbaatar *et al.,* 2014 and Guo *et al.,* 2014). The low gas yield at longer hydraulic retention time was attributed to diminishing nutrients caused by slow rate of substrate in-flow at longer hydraulic retention time. On the other hand, the low biogas yield at short hydraulic retention time of less than 23 days was attributed to: the wash out of methanogens that results into reduced efficiency of substrate bioconversion (Zaher *et al.,* 2009); lack of balanced growth of syntrophic hydrogen- and methane-producing bacteria (Zaher *et al.,* 2009); over feeding leading to reactor acidification; as well as lack of sufficient time for effective microbial saccharification of lignocellulose in the substrate. Relatively long hydraulic retention time is needed in anaerobic digestion of lignocellulosic wastes in order to archive effective sacharification of the waste. (Shi *et al.,* 2017).

Hence, the optimal hydraulic retention time of 23 days represented the steady state time that synchronise the optimal reactor microbial activity with timely supply of feed substrate.

Despite the high methane yield at optimal hydraulic retention time of 23 days, the gas production curve still exhibited fluctuations possibly due to microbial relative ease in utilization of free sugar compared to complex lignocellulose. This suggested the need for substrate pre-treatment to enhance sustained availability of sugar to microbial catalysts throughout the anaerobic digestion process.

6.4 Conclusion

The trend of anaerobic digestion of banana waste in continuous flow mode using the novel hybrid up-flow anaerobic sludge blanket (hUASB) reactor showed characteristic short and tall peaks interluded with shallow and deep grooves, respectively. Optimisation of hydraulic retention time (HRT) resulted into enhancement of biogas production and increase in methane content. The optimal HRT of 23 days, gave the highest gas yield of 583.08 LCH_4/KgVS/Day with concomitant gas production at the end of every retention cycle, and thus converting the deep grooves into shallow ones. The tall peaks in gas production indicated a delay in utilization of complex carbohydrates such starch and lignocellulose and thus a need for substrate pre-treatment before feeding into the reactor.

6.5 References

1. Abbasi, T.; Abbasi, S.A. Formation and impact of granules in fostering clean energy production and wastewater treatment in Upflow Anaerobic Sludge Blanket (UASB) reactors. Renew. Sustain. Energy Rev. 2012, 16, 1696–1708.

2. Ariunbaatar, J., Bair, R., Ozcan, O., Esposito, G., Lens, P.N.L and Yeh, D.H (2014), “Pretreatment methods to enhance anaerobic digestion of organic solid waste”, Applied energy 123, 143-156.

3. Bardiya, N., Somayaji, D and Khanna, S (1996) “Biomethanation of banana peel and pineapple waste” Bioresource Technology 58 :73-76

4. Berlian, S., Sukandar, and Seno D. Panjaitan, S.D (2013), ”Biogas recovery from anaerobic digestion process of mixed fruit -vegetable wastes”, Energy Procedia 32 (2013) 176 – 182.

5. Bilibio, C., Hensel, O. and Selbach, J (2011), “Sustainable water management in the tropics and subtropics (and case studies in Brazil)”, Vol. 2 Jaguarão/RS, Fundação Universidade Ederal Do Pampa; UNIKASSEL - Pgcult-UFMA 2011.

6. Frick, M. and Uppsten, M (1999), Alkaline pretreatment of water Hyacinth for improved anaerobic digestion, unpublished Master of Science thesis, Department of Biotechnology, Lund University, Sweden.

7. Gumisiriza, R., Hawumba, J.F., Okure, M and Hensel, O (2017) “Biomass Waste-to-Energy Valorisation Technologies: A Review Case for Banana Processing in Uganda” Biotechnology for biofuels 10:11. DOI 10.1186/s13068-016-0689-5

8. Guo, X., Wang, C., Sun, F., Zhu, W., & Wu, W. (2014), "A comparison of microbial characteristics between the thermophilic and mesophilic anaerobic digesters exposed to elevated food waste loadings", Bioresource technology, 152, 420-428.

9. Huang, Z., Ong, S.L. and Ng, H.Y (2001), "Submerged anaerobic membrane bioreactor for low-strength wastewater treatment: effect of HRT and SRT on treatment performance and membrane fouling" Water Res; 45 (2): 705-713. doi: 10.1016/j.watres.2010.08.035. Epub 2010 Aug 27.

10. Jiang, F., Peng, Z., Li, H., Li, J. and Wang, S (2020), "Effect of Hydraulic Retention Time on Anaerobic Baffled Reactor Operation: Enhanced Biohydrogen Production and Enrichment of Hydrogen-producing Acetogens", Process: 2020, 8, 339; doi:10.3390/pr8030339. www.mdpi.com/journal/processes

11. Karimi, M., Esfaniar, R. and D.Biria, R (2016), "Simultaneous delignification and saccharification of rice straw as a lignocellulosic biomass by immobilized Thrichoderma viride sp. to enhance enzymatic sugar production", Renewable Energy, Vol 104, Pages 88-95

12. Khalid A., Arshad M., Anjum M., Mahmood T., Dawson L. (2011). The anaerobic digestion of solid organic waste - Review. Waste Management Aug; 31(8): 1737–44.

13. Latif, M.A.; Ghufran, R.; Wahid, Z.A.; Ahmad, A. Integrated application of upflow anaerobic sludge blanket reactor for the treatment of wastewaters. Water Res. 2011, 45, 4683–4699.

14. Liebrand CB, Ling KC. Cooperative approaches for implementation of dairy manure digesters. USDA Rural Development Research Report 217. STOP 3252, 1400 Independence Ave., S.W Washington, DC 20250-3252;2009.

15. Linke, B. (2006). Kinetic study of thermophilic anaerobic digestion of solid wastes from potato processing. Bioenergy, 30, 892–896.

16. Mainardis, M., Buttazzoni, M and Goi, D (2020), "Up-Flow Anaerobic Sludge Blanket (UASB) Technology for Energy Recovery: A Review on State-of-the-Art and Recent Technological Advances" Bioengineering 7 (43): 1-49.

17. Mata-Alvarez, J., Llabres, P., Cecchi, F and Pavan, P (1992), "Anaerobic Digestion of the Barcelona food market organic waste: Experimental study. Bioresource Technology 39 :39-48

18. Mir, M.A., Hussain, A and Verma, C (2016), "Design considerations and operational performance of anaerobic digester: A review", Cogent Engineering,3:1, 1181696, DOI: 10.1080/23311916.2016.1181696.

19. Mshandete, A., Björnsson, L., Kivaisi, A.K, Rubindamayugi, S.T. and Mattiasson, B (2005), "Enhancement of anaerobic batch digestion of sisal pulp waste by mesophilic aerobic pre-treatment", Water Research 39, 1569-1575

20. Rittmann, B.E. and P.L. McCarty (2001), "Environmental biotechnology: Principles and applications", (International Ed.), McGraw Hill, Singapore.

21. Sahito, A.R., Mahar, R.B and Ahmed, F (2016), "Optimization Of Organic Loading Rate And Hydraulic Retention Time for Maximum Production of Methane Through Anaerobic Co-Digestion Of Canola Straw and Buffalo Dung" The Journal of Animal & Plant Sciences, 26(2): 2016, Page: 373-381; ISSN: 1018-708.

22. Shi, X.S., Dong, J.J., Yu, J.H., Yin, H., Hu, S.M., Huang, S.X and Yuan, X.Z (2017), "Effect of Hydraulic Retention Time on Anaerobic Digestion of Wheat Straw in the Semicontinuous Continuous Stirred-Tank Reactors", Hindawi, BioMed Research International, Volume 2017, Article ID 2457805, 6 pages. https://doi.org/10.1155/2017/2457805

23. Vögeli Y., Lohri C. R., Gallardo A., Diener S., Zurbrügg C. (2014). "Anaerobic Digestion of Biowaste in Developing Countries: Practical Information and Case Studies". Swiss Federal Institute of Aquatic Science and Technology (Eawag), Dübendorf, Switzerland.

24. Wolf, C. (2013). Simulation, optimization and instrumentation of agricultural biogas plants. Doctoral Dissertation, Department of Electronic Engineering National University of Ireland, Maynooth.

25. Zaher, U., Li, R., Jeppsson, U., Steyer, J.P. and Chen, S., 2009. GISCOD: General integrated solid waste co-digestion model. Water research. Vol. 43: 2717-2727.

26. Zhang, Z., Show, K., Tay, J., Liang, D. T., Lee, D. and Jiang, W (2006), "Effect of hydraulic retention time on biohydrogen production and anaerobic microbial community", Process Biochemistry: 41 (10):2118-2123. DOI: 10.1016/j.procbio.2006.05.021

7 Investigating the effect of pre-treatment of banana waste by pre-fermentation and co-digestion on biogas production

7.1 Introduction

Anaerobic digestion of plant biomass is limited by the recalcitrance of the lignocellulose to bioreactor microbial hydrolysis leading to low biogas yield. To this effect, direct anaerobic digestion of wastes from plant origin , even using conventional high rate bioreactors such as up-flow anaerobic sludge blanket bioreactors, is challenging and ineffective. Moreover, degradation of lignin is primarily an aerobic process and, in an anaerobic environment such as in the bioreactor lignin can persist for very long periods (Van Soest, 1994). Thus, lignocellulosic feed stocks have to first be degraded through pre-treatment stages such as pre-fermentation under aerobic conditions prior to anaerobic digestion. This justifies the preference for a two-stage reactor system to a single-stage or batch reactor systems for optimal biomethanization of agricultural wastes, since the former allows the option for substrate pre-treatment. Microorganisms which are naturally growing in lignocellulose rich waste and other plant biomass dumping site, get adapted to faster degradation and solubilisation of lignin and lignocellulosic biomass (Patrick *et al.,* 2011; Gumisiriza *et al.,* 2017). Thus, pre-treatment of feed substrate from plant biomass using microbial flora from such waste dump site is likely to result into increased lignocellulolysis leading to more availability of sugars and ultimately enhanced anaerobic digestion.

On the other hand, most biogas digester feed substrates from plant origin tend to have higher carbon than nitrogen contents thus an unbalanced C:N ratio for optimal anaerobic digestion process. Research studies have recommended that a C:N ratio in the range of 20 to 30 as being optimal for anaerobic digestion (Bouallagui *et al.,* 2003; Zaher *et al.,* 2007; Tumutegyereize *et al.,* 2011; Chandra *et al.,* 2012). Previous studies on waste characterisation have shown that banana waste has a C:N ratio of 41, implying that such waste was highly unbalanced and skewed to higher carbon than nitrogen contents (Gumisiriza *et al.,* 2019). Nevertheless, other researchers have recommended that co-digestion of carbon-rich substrate with nitrogen-rich substrates can create positive synergies that enhance anaerobic digestion with high methane yield (Hartmann *et al.,* 2003; Murto *et al.,* 2004). Despite the biogas yield enhancement benefits of feeding the digester with mixed nutrient substrates, co-digestion of mixtures of different wastes including banana waste is seldom reported (De Baere, 2000). The objective of this study was therefore to optimise the pre-fermentation time for enhancement of biomethanization of green banana waste and to compare the percentage biogas yield enhancement caused by pre-fermentation and substrate co-digestion with animal manure.

7.2 Methodology

7.2.1 Substrate Pre-treatment by Microbial Pre-fermentation

Freshly generated banana waste was collected from the banana processing industry at PIBID-Bushenyi and pulverised into homogenious paste (Gumisiriza *et al.,* (2019). Waste samples were collected from this industry because it was the only operational banana processing industry in Uganda during entire period of study. The fresh paste was divided into five

portions of three kilograms each and every portion placed into a transparent sterile plastic container. The lid of the container 1, labeled as control,was immediately sealed in a hood and the portion kept in the freezer at 4^0C. The other four containers with the rest of the portions were labeled as ferment-5, ferment-7, ferment-10 and ferment-15 days, respectively. In each container, the fresh paste sample was mixed with wet soil collected from banana waste dumpsite at constant concentration of 1 % w/w. The conatiners with loosely sealed lids were placed in an incubator set at 35 ^{0}C for up to 15 days. The entire set up was done in triplicate to enable reproducibility of data and accuracy of results. Conatiners were removed from the incubator after the 5th, 7th, 10th and 15th day, respectively according to their label and immediately kept in the freezer at 4 ^{0}C until samples required for loading into the hybrid Up-flow anaerobic sludge blanket (hUASB) reactors.

7.2.2 Loading the reactors and monitoring the gas yields

Six hybrid Up-flow anaerobic sludge blanket (hUASB) reactors arranged in triplicates, containing pre-adapted inoculum were set up at room temperature of 25°C and operated in a continuous flow mode. All the reactors were set to operate at constant hydraulic retention time (HRT) of 23 days and received constant substrate feed concentration at OLR of 4.0gVS/L from the respective feed reservoir tanks. Despite the same concentration of feed substrate, each reactor received banana waste substrate pre-treated (fermented) for different periods. Prior to loading, feed substrate samples were removed from the freezer and the container immersed in flowing water at room temperature for 24 hours to enable thawing of the sample. Reactor number 1 was the control and received untreated (unfermented) feed substrate from container 1. The other reactors labelled 2 to 5 received pre-treated (fermented) feed substrates from containers labelled ferment-5, 7, 10 and 15, respectively. The reactor number 6 received a mixture of feed substrate comprising banana waste and chicken droppings mixed in a ratio of 1: 3, respectively (Adeniran *et al.,* (2014). The anaerobic digestion was run for 27 weeks at HRT of 23 days and the biomethanization process was evaluated by monitoring the rate of biogas production, gas yield and biogas quality as a percentage of methane in biogas generated.

7.3 Results and discussion

7.3.1 Effect of substrate pre-fermentation on the rate of biogas production

Lignocellulosic biomass can be a sustainable source of carbon for enhanced industrial processes, including biogas production. Never the less due to its inherent complexity and heterogeneity, efficient biodegradation of such biomass including banana waste requires the actions of different types of hydrolytic enzymes secreted by co-cultures of different microorganisms, both bacteria and fungi (Cortes-Tolapa *et al,* 2017). In nature, efficient biodegradation of lignocellulosic biomass is accomplished by complex microbial communities that work efficiently and often synergistically.

In this study, pre-treatment of banana waste by fermentation using wet soil inoculum from banana waste dump site employed the principle of microbial co-culture synergism for efficient biodegradation of lignocellulosic biomass as recommended by many researchers (Van Dyk and Pletschke, 2012; Mitri and Foster, 2013; Cragg *et al.,* 2015; Deng and Wang,

2016). The wet soil inoculum used in this study was believed to have contained consortia of microorganisms that lived as co-culture at the dump site of mixed substrate and were thus adapted for efficient biodegradation of lignocellulosic biomass along with other, synergistically. The production of biogas as well as the cumulative methane yield are shown figure 7.1. The results clearly demonstrated that substrate pre-treatment enhanced biogas production and methane content when compared to the control reactor. This was most likely attributed to optimal biodegradation of lignocellulose in the waste, rendering it more readily useable by microorganisms in the reactor. Anaerobic digestion of the feed substrate fermented for 7 days showed the highest cumulative gas production followed by co-digestion, yet the substrate fermented for 15 days yielded the least amount of biogas. This was in agreement with other studies which reported that banana waste would be fully colonised by moulds within 6-8 days of fermentation at room temperature (Essien *et al.,* 2005: Shah *et al.,* 2005). Thus, with banana waste pre-fermentated for 7 days, lignocellulolytic microorganisms had optimally degraded substrate to release sugars that enhanced biogas production. Conversely, it was believed that the fermenting microorganisms themselves would start consuming the saccharified sugars from substrate hydrolysis when the substrate was pre-treated (fermented) for longer than the optimal period of 7 days. Thus, longer pre-treatment period reduced the available sugars to reactor microorganisms resulting into low biogas yield, as in the case for substrate fermented for up to 15 days.

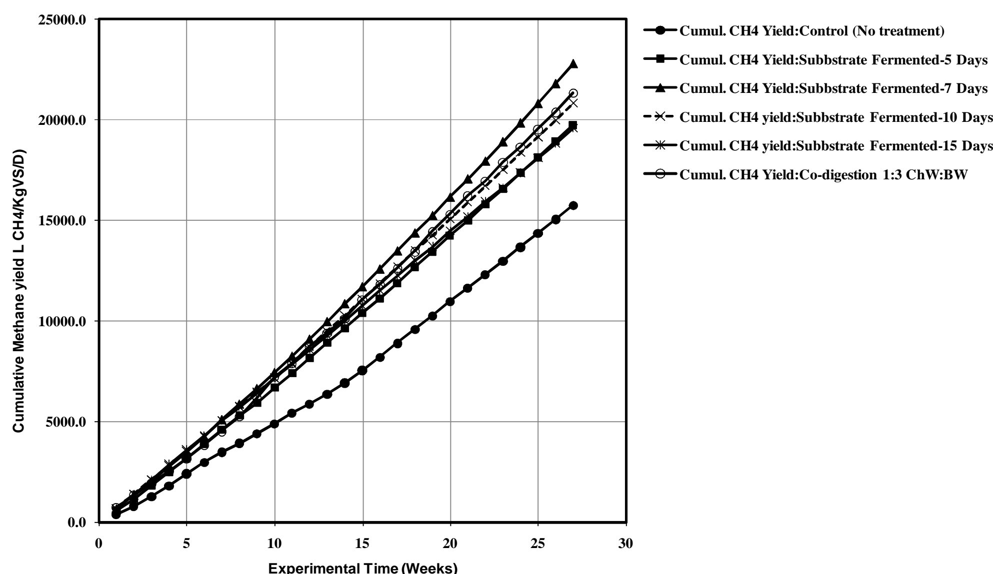


Figure 7:1. Cumulative methane yield from different pre-treatments

In terms of progress of biogas production, the results demonstrated that the substrate pre-treatment (fermented) for 7 days yielded the best overall substrate for high biogas production as well as methane content, from banana waste. The results further showed that the gas production from the reactor receiving substrate fermented for 7 days yielded biogas consistently with fewer fluctuations. This suggested that substrate fermented for 7 days was the most efficiently biodegraded resulting into sustained availability of sugars leading into concomitant gas production with almost similar peaks. This could further suggest that pre-treatment enhanced substrate solubilisation leading to consistent nutrient availability to microorganisms, thus limiting fluctuations in biogas yields. Enhanced solubilisation of

banana waste by microbial co-culture fermentation was similarly reported by Ingale *et al.,* (2014). During their study on saccharification of banana pseudo stems by fermentation with co-culture of *A. ellipticus* and *A. fumigatus*, they observed improved hydrolytic activities under co-culture experiments and reported maximum enzyme production and activity at 8th day of microbial fermentation of banana waste (banana pseudo stem).

Furthermore, all the biogas production curves (figure. 7.2) showed an increasing gradient as the experiment progressed from week 1 to week 27. This suggested an enhancement in anaerobic digestion possibly due to the progressive adaptability of reactor consortia to the efficient substrate biomethanization as a function of experimental time.

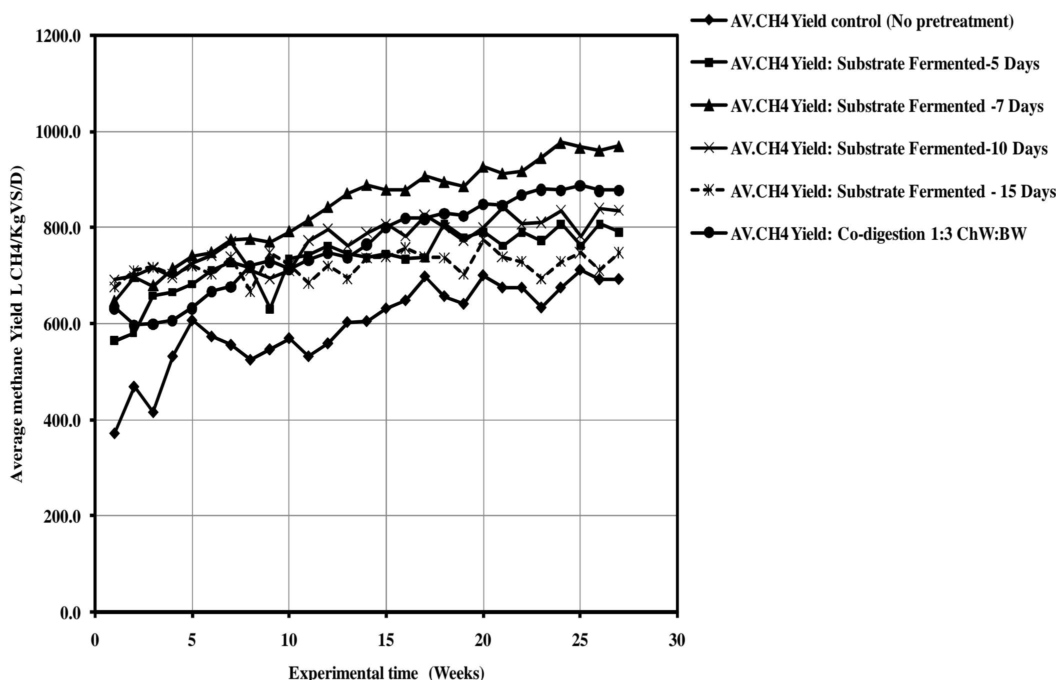


Figure 7:2. Effect of different pre-treatments on the rate (progress) of biogas production

The more the reactor microorganisms were exposed to the substrate, the better adapted to utilization of the substrate, the microorganisms became and hence a progressive increase in biogas production. Moreover, the syntrophic association of acetogenic microorganism (Hydrogen producing bacteria) and methanogens (Hydrogenotrophic methanogens) is stabilised with longer period of anaerobic digestion process (Xing *et al*., 1997 and Chandra *et al*., 2012).

Further analysis using inferential statistics, results confirmed that pre-fermentation retention time significantly (P=0.001, df=26, F=14.9) affect the biogas production and there are significant differences (P=0.001, df=5, F=101.84) in the biogas production for the different fermentation time and treatment applied to the substrate. When the substrate is digested after 7days the production of biogas is highest as shown in table 7.1

Table 7:1. The Effect of Substrate Pre-fermentaion Retention Time on Biogas production

Treatment	Mean Biogas production (L CH4/KgVS/D)	Standard Deviation
Control	600.1[a]	88.22
Fermented5days	731.2[b d]	64.69
Fermented7days	843.9[c]	97.03
Fermented10days	771.6[b]	50.16
Fermented15days	721.6[b d]	25.72
Co-digestion 1:3 ChW:BW	763.1[b]	97.44

Note: Means with the same letters are not significantly different from each other at 5% significance level

7.3.2 Effect of substrate pre-fermentation on methane content

Results of this study further revealed that the percentage methane content in the biogas produced improved and remained stable throughout the experimentation. However, the curve for percentage methane content produced from co-digestion of banana waste with chicken droppings was on topmost of all pre-treatments (figure 7.3). This suggested that where as substrate pre-treatment by fermentation could have increased the availability of sugars for enhanced biogas production, co-digestion stabilised the C:N ratios leading to a more enhanced microbial biochemical reactions. The chicken waste contain high nitrogen content and minerals such as copper, Zinc and manganese that boost microbial activity during anaerobic digestion (Adinirani *et al*., 2014; Diaz-Vazquez *et al.,* 2020; Ojolo *et al*., 2007).

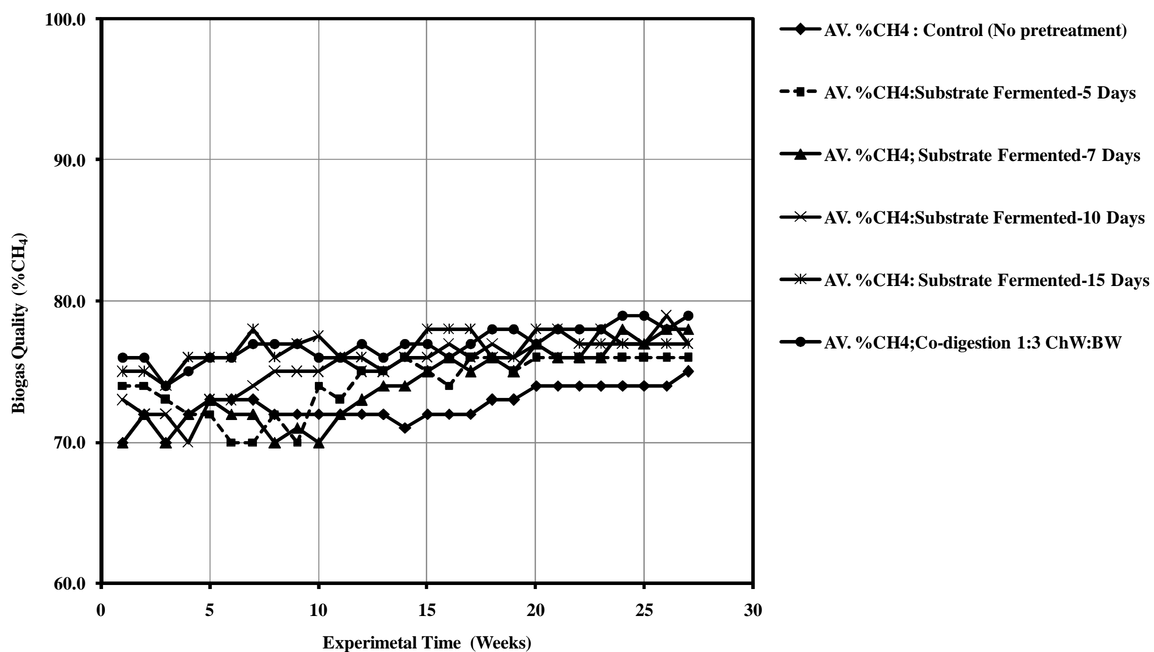


Figure 7:1. Variation of biogas quality at different pre-treatments

Ultimately, the results reveal that the substarte pre-treatment retention time significantly (P=0.001, df=26, F=8.35) affect the biogas quality and there are significant differences (P=0.001, df=5, F=49.78) in the biogas quality for the different fermentation time and

treatment applied to the substrate (table 7.2). Pre-treatment of the substrate by co-digestion with chicken mature in the ratio of 1:3 and longer substarte pre-fermentaion up to 15 days gave the best quality of biogas with methane content of over 76%.

Table 7:2. The Effect of Substrate Pre-treatment Retention Time on Biogas Quality

Treatment	Mean Biogas production (%CH_4 content)	Standard deviation
Control	72.63[a]	1.25
Fermented5days	74.22[b]	2.04
Fermented7days	74.00[b]	2.66
Fermented10days	75.30[c]	2.05
Fermented15days	76.57[d]	1.098
Co-digestion 1:3 ChW:BW	76.96[d]	1.22

Note: Means with the same letters are not significantly different from each other at 5% significance level

7.3.3 Effect of substrate pre-fermentation and Co-digestion on biogas yield

Comparing co-digestion along with pre-treatment by fermentation, substrate fermented for 7 days gave the highest methane yield of 843.89 ± 95.61 L CH_4/KgVS/D with percent methane content of 74 .00 ± 2.66 % while co-digestion gave a yielded of 782.91 ± 90.00 with percent methane content of 76 .89 ± 1.42 %, as shown in table 7.3. This implied that substrate fermentation for 7 days was better option for pre-treatment of banana waste to improve methane yields.

Table 7:3. Biogas yield, % enhancement and % methane content of different pre-treatments

Pre-Treatments	Variables			
	Mean Methane yield (L CH_4/KgVS/D)	**Methane Yield Enhancement (L CH_4/KgVS/D)**	**Methane Yield Enhancement (%)**	**Mean Biogas Quality (%CH4)**
Control (No Pre-treatment)	583.08 ± 10.71	0.00	0.00	72.63 ± 1.24
Subst.Fermented for 5 Days	731.24 ± 64.69	148.17	25.42	74.15 ± 1.99
Subst.Fermented for 7 Days	843.89 ± 95.61	260.82	44.73	74 .00 ± 2.66
Subst.Fermented for 10 Days	770.96 ± 50.16	187.88	32.22	75.30 ± 2.05
Subst.Fermented for 15 Days	724.15 ± 27.20	141.07	24.19	76.59 ± 1.12
Co-digestion- 1:3 ChW:BW ratio	782.91 ± 90.00	199.83	34.27	76.89 ± 1.42

The trend in biogas yields versus methane content against the pre-treatment methods showed that longer fermentation of the substrate yielded less biogas (figure 7.4). This was most likely attributed to the over-utilization of the saccharified sugars and other nutrients in the fermented substrate by the fermentative microorganisms, when the substrate was pre-treated for longer than the optimal period of 7 days (Chukwuma *et al.,* 2020; Tsegaye *et al.,* 2019; Kim *et al.,* 2010; Mussatto and Teixeira, 2010).

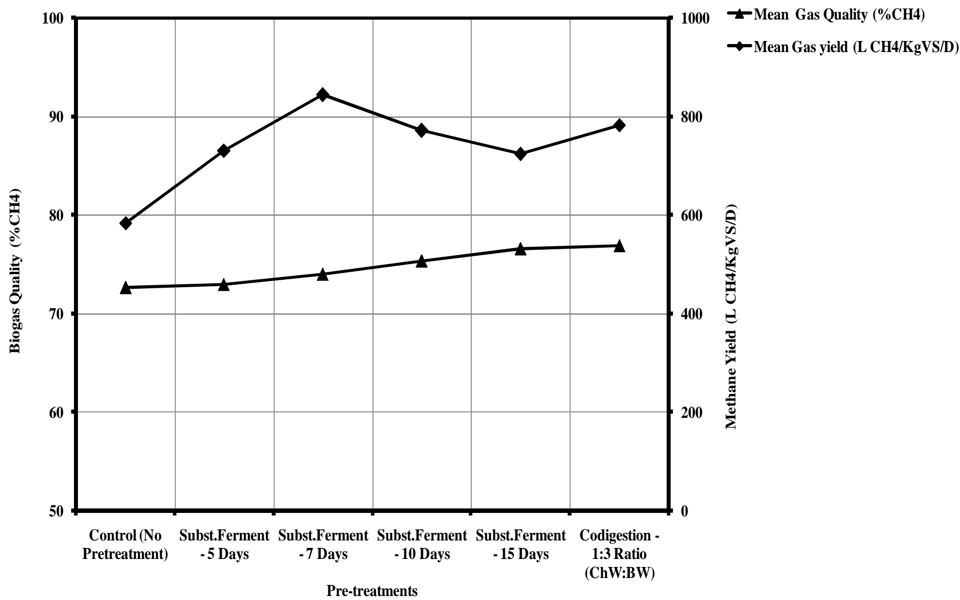


Figure 7:2. Effect of pre-treatments on mean biogas yield and biogas quality

It can therefore be inferred that substrate fermented for 7 days could optimally solubilise the complex substrate to release and preserve the sugars that are subsequently utilized by syntrophic reactor microorganisms to enhance methane yield.

7.3.4 Comparing the Effect of substrate pre-fermentation and Co-digestion on biogas yield enhancement

Substrate fermented for 7 days showed highest gas yield enhancement of 260.82 L CH_4/KgVS/D constituting 44.73 % enhancement as shown in figure 7.5.

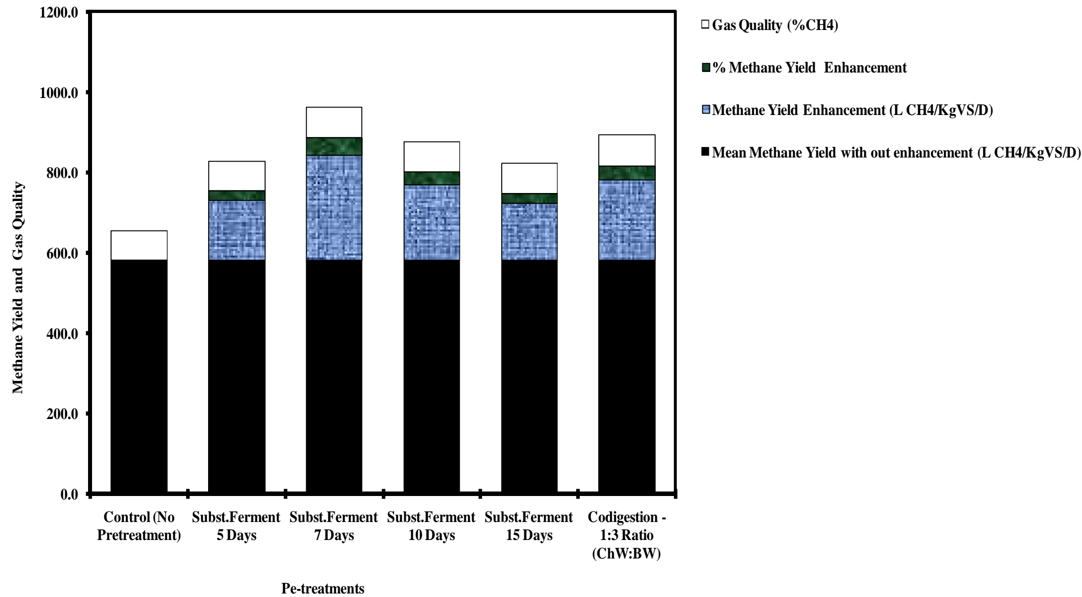


Figure 7:3. Effect of pre-treatment on percentage gas quality and yield enhancement

The enhancement trend showed that increase in substrate fermentation time from day one to day 7 exponentially increased the percentage gas yield enhancement (Figure 7.6). More days from day 7 and above of substrate fermentation, the methane yield dropped probably due to over-consumption of released nutrient by fermentative microorganisms.

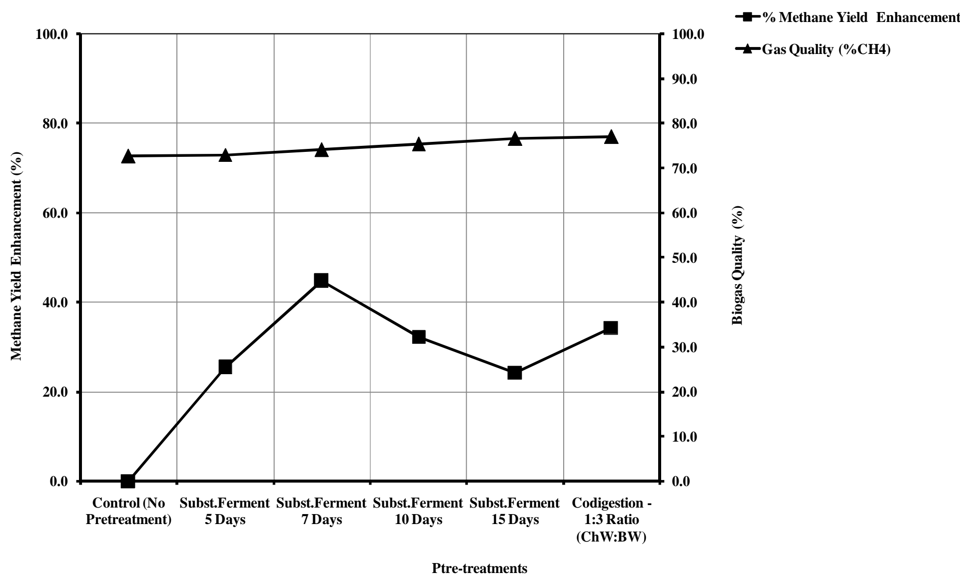


Figure 7:4. Trend in % gas yield enhancement and methane content with different pre-treatments

7.3.5 The Biochemical Effect of Banana Waste Co-digestion with Chicken Manure

Previous studies have reported that good substrates for biogas production should contain adequate amount of carbon, oxygen, hydrogen, nitrogen, sulphur, phosphorous, potassium,

calcium, magnesium and a number of trace elements (Hill and Brath, 1997). Chicken manure has high nitrogen content (Ojolo *et al*., 2007; Thu *et al.,* 2013) that can stabilise the high C:N ratio of 41, previously reported in banana waste by Gumisiriza *et al*., (2019). Carbon and nitrogen often act as limiting factor in anaerobic digestion (Richard, 1998) and for enhanced process, the optimum C:N ratio is between 20 and 30 (Vandevivere *et al.,* 2000). At C:N ratio higher than the optimal, the biogas production is low due to nitrogen deficiencey while a low C:N ratio causes ammonia accumulation leading to a rise in pH to alkaline (pH up to 8.5) condition. This alkaline pH is toxic to reactor microbial consortia especially the methanogens. Hence, an optimum C:N ratio can be achieved by mixing substrate of low and high C:N ratio (Khalid et al., 2011). In most substrates from plant origin, nitrogen is considered as limiting factor and nitrogen sources like urea, bio-solids, and manure could be used as supplements (Richard, 1998) to bioreactors in appropriate proportions to balance the C:N ratio and enhance the anaerobic digestion process. A study by Vincente Jr *et al.,* (2018) reported that chicken manure contains elements and minerals such as copper, Zinc ,manganese, Iron, magnesium, among others, in proportions that are essential for boosting microbial activity during anaerobic digestion (Diaz-Vazquez *et al.,* 2020).

Moreover, micro-nutrient supplementation (in form of Fe, Co, Ni, Se, Mo) was proved to be crucial to enhance methanogenic activity, stimulating methane production (Chan *et al.,* 2019). The addition of metals and natural elements showed to have a positive effect both on chemical oxygen demand (COD) removal and on biogas production in up-flow anaerobic sludge blanket (UASB) reactors as well as co-digestion of high-loaded substrates (Chan *et al.,* 2019; Loizia *et al.,* 2019). Selenium and cobalt are key trace elements found effective in stabilizing digestion mainly during ammonia formation. According to the previous research studies, the recommended concentration of Selenium and Cobalt for kitchen waste is around 0.16 and 0.22 mg /liter, respectively, for moderate organic loading rate. It must be noted that the concentration of Selenium greater than 1.5 mg/liter is found to be toxic to reactor microorganisms (Ray *et al*., 2013). Thus, methane production could be increased by 9-85% by the co-digestion of three or four substrates due to the synergistic effect as a result of increased biodegradability and optimisation of reactor conditions such as balancing C:N ratio (Vincente Jr *et al*., 2018).

7.4 Conclusion

Pre-fermentation enhanced biomethanization process of banana waste with the highest percentage enhancement of 44.73% obtained at optimal fermentation time of 7 days. There was exponential increase in methane yield as substrate fermentation time increased from zero to 7 days. Long period of substrate fermentation from 7 days and above resulted into low methane yields presumably due to over-utilization of released nutrients by fermentative microorganisms. Co-digestion of banana waste substrate with chicken dropping yielded biogas with higher percent methane content perhaps due to stabilisation of reactor pH and liquor biochemical reactions. Future research focusing on isolation and characterisation of microorganisms found in soil from banana waste dump site need to be done.

7.5 References

1. Adeniran, K.A., Yusuf, K.O., Iyanda, M.O and Alo, O.A (2014), "Relative Effectiveness of biogas production using Poultry Droppings and Swine dung", Ethiopian Journal of Environmental Studies and Management; 7 (4): 371-378; ISSN: 1998-0507; doi: http://dx.doi.org/10.4314/ejesm.v7i4.4

2. Bouallagui H, Cheikh RB, Marouani L, Hamdi M (2003). Mesophilic biogas production from fruit and vegetable waste in a tubular digester. Bioresource Technology. 86: 85-85.

3. Chandra R., Takeuchi H., Hasegawa T. (2012): Methane production from lignocellulosic agricultural crop wastes: A review in context to second generation of biofuel production. Renewable and Sustainable Energy Reviews 16(3): 1462-1476.

4. Chandra R., Takeuchi H., Hasegawa T. (2012): Methane production from lignocellulosic agricultural crop wastes: A review in context to second generation of biofuel production. Renewable and Sustainable Energy Reviews 16(3): 1462-1476.

5. Chukwuma, O. B., Rafatullah, M., Tajarudin, H. A and Norli Ismail, N (2020), "Lignocellulolytic Enzymes in Biotechnological and Industrial Processes: A Review", Sustainability 2020, 12, 7282; doi:10.3390/su12187282. www.mdpi.com/journal/sustainability

6. Cortes-Tolapa, L., Salles, J.F and van Elsas, J.K (2017), "Bacterial Synergism in Lignocellulose Biomass Degradation-Complementary Roles of Degraders as Influenced by Complexity of the Carbon Source", Frontiers in Microbiology; 8 (1628):1-14.

7. Cragg, S. M., Beckham, G. T., Bruce, N. C., Distel, D. L., Dupree, P., Etxabe, A. G (2015), "Lignocellulose degradation mechanisms across the tree of life", Curr. Opin. Chem. Biol. 29, 108–119. doi: 10.1016/j.cbpa.2015.10.018.

8. De Baere, L (2000), "Anaerobic digestion of wastes: State of the art", Waste Science Technology 41, 283-290

9. Deng, Y.-J., and Wang, S. Y. (2016). Synergistic growth in bacteria depends on substrate complexity. J. Microbiol. 54, 23–30. doi: 10.1007/s12275-016-5461-9

10. Díaz-Vázquez, D., Alvarado-Cummings, S.C., Meza-Rodríguez, D., Senés-Guerrero, C., Anda, J and Gradilla-Hernández, M.S (2020), "Evaluation of Biogas Potential from Livestock Manures and Multicriteria Site Selection for Centralized Anaerobic Digester Systems: The Case of Jalisco, México", Sustainability 12 (3527): 1-32. Sustainability 2020, 12, 3527; doi:10.3390/su12093527: www.mdpi.com/journal/sustainability

11. Essien,J.P., Akpan, E.J and Essien, E.P (2005), "Studies on mould growth and biomass production using waste banana peel", Bioresource Technology 96 (13): 1451-1456.

12. Gumisiriza, R., Hawumba, J.F., Balyeidhusa, A.S.P., Okure, M and Hensel, O (2019), "Processing of East African Highland Green Bananas: Waste Generation and Characterisation as a Potential Feedstock for Biogas Production in Uganda", American Scientific Research Journal for Engineering, Technology, and Science; Global Society of Scientific Research and Researchers; ISSN (Print) 2313-4410, ISSN (Online) 2313-4402; http://asrjetsjournal.org/

13. Gumisiriza, R., Hawumba, J.F., Okure, M and Hensel, O (2017) "Biomass Waste-to-Energy Valorisation Technologies: A Review Case for Banana Processing in Uganda" Biotechnology for biofuels 10:11. DOI 10.1186/s13068-016-0689-5

14. Hartmann, H. and Ahring, B (2005). Anaerobic digestion of the organic fraction of municipal solid waste: Influence of co-digestion with manure. Water research 39(8): 1543-1552.

15. Hartmann, H., Angelidaki, I., Arhing, B.K., 2003. Co-digestion of the organic fraction of municipal waste with other waste types. In: Mata-Alvarez, J. (Ed.), Biomethanization of the Organic Fraction of Municipal Solid Wastes. IWA Publishing, UK.

16. Hill, J. and Brath, A (1997) 'A Dynamic Model for Simulation of Animal Waste Digestion': Water Pollution Control, Canton, Ohio. www.hach-lange.com.

17. Ingale, S., Joshi, S.J and Gupte, A (2014), " Production of Bioethanol Using Agricultural Waste: Banana Pseudo Stem", Brazilian Journal of Microbiology 45 (3): 885-892.

18. Kim, J., Block, D. E. and Mills, D.A (2010),"Simultaneous consumption of pentose and hexose sugars: An optimal microbial phenotype for efficient fermentation of lignocellulosic biomass", Appl. Microbiol.& Biotechnol (2010) 88:1077–1085.DOI 10.1007/s00253-010-2839-1.

19. Mitri, S., and Foster, K. R. (2013). The genotypic view of social interactions in microbial communities. Annu. Rev. Genet. 47, 247–273. doi: 10.1146/annurev- genet-111212- 133307

20. Murto, M; Bjornsson, L; Mattiasson, B (2004). Impact of food industrial waste on anaerobic co- digestion of sewage sludge and pig manure, Journal of Environmental Management. 70:101–107.

21. Mussatto, S. I. and Teixeira, J. A (2010), "Lignocellulose as raw material in fermentation processes", In : Current Research, Technology and Education; Topics in Applied Microbiology and Microbial Biotechnology, FORMATEX Microbiology, Series Nº 2, Vol. 2, pages 897-907. http://www.formatex.org

22. Ojolo, S.J., Oke, S.A., Animasahun, K. and Adesuyi, B.K. (2007). Utilization of Poultry, Cow and Kitchen Wastes For Biogas Production: A Comparative Analysis. Iran. J. Environ. Health. Sci. Eng., 4(4): 223-228.

23. Patrick, F., Mtui, G., Mshandete, A.M and Amelia Kivaisi, A (2011). Optimization of laccase and manganese peroxidase production in submerged culture of Pleurotus sajor-caju, African Journal of Biotechnology Vol. 10 (50), 10166-10177.

24. Sha, M., Reddy, G.V., Banerjee, R., Babu, P.R and Kothari, I. L (2005), "Microbial Degradation of banana waste under solid state bioprocessing using two lignocellulolytic fungi (Phylosticta spp. MPS-001 and Aspergillus spp. MPS-002)", Process Biochemistry 40 (1): 445-451.

25. Thu, C.T.T., Sommer, S.G and Trach, N.X (2013), "Biogas from chicken, pig and cow manure: Influence of biomass composition on the methane yield", International Conference on Frontiers of Environment, Energy and Bioscience (ICFEEB 2013) ISBN: 978-1-60595-133-1

26. Tsegaye, B., Balomajumder, C. and Roy, P (2019), "Microbial delignification and hydrolysis of lignocellulosic biomass to enhance biofuel production: an overview and future prospect", Bulletin of the National Research Centre, (2019) 43:51, Pg 1-16. https://doi.org/10.1186/s42269-019-0094-x

27. Tumutegyereize, P., Muranga, F.I., Kawongolo, J. and Nabugoomu, F (2011), "Optimization of biogas production from banana peels", African Journal of Biotechnology Vol. 10 (79), 18243-18251.

28. Van Dyk, J. S., and Pletschke, B. I. (2012), " A review of lignocellulose bioconversion using enzymatic hydrolysis and synergistic cooperation between enzymes — Factors affecting enzymes, conversion and synergy", Biotechnol. Adv. 30, 1458–1480. doi: 10.1016/j.biotechadv.2012.03.002.

29. Van Soest, P. J. (1994) Nutritional Ecology of the Ruminant, 2nd ed. Cornell University Press, Ithaca, New York.

30. Vicente Jr, D.V., Costa, M.S.S., Costa, L.A.M., [1], Dercio C. Pereira, D.C and Santos, F.T (2018), "Anaerobic Digestion and Co-Digestion of Poultry Litter Submitted to Different Reuses", Engenharia Agricola, Jaboticabal 38 (6); 961-967. Doi: http://dx.doi.org/10.1590/1809-4430-Eng.Agric.v38n6p961-967/2018

31. Xing, J., Criddle, C. and Hickey, R (1997), "Effects of long-term periodic perturbation on anaerobic community", Water Research 31, 2195-2204

32. Zaher, U., Grau, P., Benedetti, L., Ayesa, E., Vanrolleghem, P.A (2007). Transformers for interfacing anaerobic digestion models to pre- and post-treatment processes in a plant-wide modelling context. Environmental Modelling and Software, 22, 40-58.

8 General discussion

8.1 The energy challenges of the banana industry in uganda

Uganda is the second largest global producer of bananas after India and the leading in Africa (Tripathi *et al.,* 2008), with annual production estimated at 9.77 million tonnes (FAOSTAT, 2012). However, banana industrialisation in Uganda is heavily impeded by the lack of cheap, reliable and sustainable energy mainly needed for drying of flesh banana pulp to convert it into chips before milling into banana flour that has several uses in the bakery industry, among others (Gumisiriza *et al.,* 2017). Sufficiently dried chips have a long stable shelf life and can safely be stored while awaiting subsequent processing into flour used in bakery, glycemic therapeutics, nutritional formulations, among others (Muranga *et al.,* 2007, Saifullah *et al.,* 2009; and Bezeera *et al*., 2013). Drying is one of the oldest technologies employed in the processing of agricultural produce for increasing shelf life and economic value (Kawongolo, 2013). It removes water from produce to a level that greatly minimizes microbial spoilage and deterioration reactions (Doymaz, 2007). Drying has a superior advantage of enhancing produce shelf life with concomitant increase in concentration of nutrients and organoleptic flavours (Kawongolo, 2013). The drying of banana fruit pulp into chips is the step that requires reliable energy in order to produce consistently standard quality products. Moreover, it has been established (Roberts *et al.,* 2008; and Islam *et al.,* 2012) that the drying of banana pulp consumes more energy than that of other related fresh foods such as pineapples and potato. This is so because the activation energy (E_a) for diffusion of water in green banana is 51.21 KJ/mole which is higher than that for potato [32.24 KJ/mole], pineapple [35.17 KJ/mole], and grape seeds [30.45 KJ/mole] (Islam, 1984; Uddin and Islam, 1985; Roberts *et al.,* 2008; and Islam *et al.,* 2012). The differences in activation energy values can be attributed to the differences in chemical composition and cellular structure (Islam, 2012).

However, drying banana pulp into chips using either electricity or solar is challenging in Uganda especially for rural farmers. Uganda has one of the lowest electricity access levels, estimated at only 2-3% in rural areas where most of the banana growing is located (Twaha *et al.,* 2016). Implying that drying banana pulp into chips using electricity by rural banana farmers is almost practically impossible due to lack of grid electricity access. Besides, although Uganda is located on the equator, the number of hours of sunshine per day varies significantly depending on the season. In fact, there are few hours of solar radiations during rainy season that make solar drying take many days leading to inconsistent and substandard product quality, characterized by rotting and infestation with moulds that produce aflatoxins (Cotty and Jaime-Garcia, 2007). Therefore solar drying alone may not meet the entire energy requirements for efficient and safe drying of the flesh banana pulp and other fruits into safe dried chips with long stable shelf life. Moreover, most banana farmers have limited financial capacity to access modern solar driers that would generate sufficient energy for safe pulp drying and general industrial processing.

On the other hand, the hot air convection drying is the most promising and has been one of the oldest methods that have been used to preserve fresh agricultural products (Samadi *et al.,* 2014). It relies on the flow of hot air over the sliced pulp to drive off the water leading to

consistent drying and conversion of the pulp into dried chips. The application of hot air convection drying is however, hampered by the high energy of operation (Alibas, 2007; Koyuncu *et al.,* 2007; Lewicki, 2006; Motevali *et al*., 2011). Since Uganda's banana industrialisation generates huge tones of banana waste, estimated to be over three million tonnes per year (Spilsbury *et al*., 2002; Tumutegyereize *et al.,* 2011), harnessing energy from such wastes would solve the problem of lack of energy especially for pulp drying and ultimately boost the banana industrialisation in Uganda and world over.

Therefore, this study focused at investigating an appropriate Waste-to-Energy option for harnessing energy from banana waste to offer an affordable and reliable sufficient energy required for drying of banana pulp as well as boosting banana industrialisation.

8.2 Major research findings

8.2.1 Evaluation of the potential Waste-to-Energy options for recovery of energy from banana waste

This research study (Chapter 2) evaluated the potential Waste-to-Energy technologies for conversion of banana waste into energy and the results revealed that anaerobic digestion was the most appropriate technology that can generate energy in form of biogas from the voluminous banana waste emitted from the banana industrialisation (Gumisiriza *et al.,* 2017). Since banana waste is a wet biowaste with moisture content of over 80%, its anaerobic digestion would be favourable to generate net positive energy in form of biogas. Anaerobic digestion requires less energy in put than other thermo chemical methods, such as gasification and pyrolysis, due to the low operating temperature (Gunes *et al.,* 2019), and consequently anaerobic digestion application throughout the world has continuously increased in the last decade (Mainardis *et al.,* 2020).
Anaerobic digestion has become one of the most promising technologies used in breaking complex organic substrates into biogas (Rasapoor *et al.,* 2020). Anaerobic digestion, being 100% renewable, is an effective and environmental-friendly waste management technique and can be considered as one of the most important renewable energy sources, due to methane generation during the digestion process (Kumar and Sammader, 2020). Besides highly biodegradable streams, advances in research has allowed application of anaerobic digestion to lignocellulosic substrates, characterized by slow hydrolysis kinetics, such as micro algal biomass (Misson *et al.,* 2020), switch grass (Zhong *et al.,* 2020), and yard waste (Zhang *et al.,* 2018), widening the spectrum of suitable matrices for biogas production. Moreover, production of methane from agricultural waste is getting importance in recent years as it offers considerable environmental advantages and it is an additional source of earning for crop growers. (Chynoweth, 2004; Sahito *et al.,* 2016). Additionally, anaerobic digestion provides pathways to decreasing treatment costs by simultaneously generating energy and high demand products like fertilizers (Gumisiriza *et al.,* 2017; Vincente Jr *et al*., 2018; Diaz-Vazquez *et al.,* 2020).

Generally, anaerobic digestion is considered as the most important and sustainable bioprocess used for treatment of organic wastes such as food wastes, organic fraction of municipal solid waste (OFMSW), farm yard manure, agricultural residues and agro-process wastes such as

banana waste (Ariunbaatar *et al.*, 2014). This is simply so because, anaerobic digestion has the potential for: 1) waste reduction and stabilization; 2) pollution reduction; 3) energy production, which leads to a reduction in consumption of fossil fuel; 4) reduction of greenhouse gas (methane) emissions and release of carbon-neutral carbon dioxide back to the atmosphere through methane flaring; 5) nutrient recovery via utilization of the digestate or the effluent as biofertilizer for agricultural purposes (Ward *et al.,* 2008; Khalid *et al*., 2011; Ariunbaatar *et al*., 2014).

Hence, the conversion of banana waste biomass to biogas would offer a cheap and affordable alternative source of energy which after burning would produce consistent hot air convection for drying of the fruit pulp by rural banana farmers and processors. Besides generating energy, the anaerobic digestion of banana waste would offer an eco-friendly solution to waste burden as well as use of digestate bio slurry effluents from the bioreactor as a cheap source of organic fertilizer for crop production (Gumisiriza *et al.*, 2017).

8.2.2 Banana waste audit and characterisation

A banana-waste audit was conducted through a reconnaissance visit to western Uganda, one of the most banana producing regions in the country (Asha *et al.,* 2015). Additionally, a survey to a banana processing factory located at the Technology Business Incubator (TBI) of Presidential Initiative on Banana Industrial Development (PIBID) in Bushenyi was also conducted to evaluate the general processing of green bananas, pulp drying options and identifying the major waste streams as well as key waste sink (disposal options). As indicated in Chapter four, the results revealed that banana industrialisation generates two broad streams of banana waste; the cultural banana wastes and the process banana wastes. The former was usually left in the plantation and comprised of leaves, dry fibres, pseudo stem and corm. The latter was the one generated during industrial processing of banana fruit into dried chips and majorly comprised of peels, peduncle and rejected fruits. This study focused more on process banana waste since the cultural banana wastes were always left in the plantations with or without management. The study found out that processing of a bunch of green bananas generated wet weight fractions of 40% as pulp and 60% as total waste residues with peel / pulp ratio of 1.3. In terms of wet weight fractions per unit fruit bunch, banana wastes comprised of peels at 50.2 ± 3.4 %, peduncle at 7.1 ± 1.7 % and fruit rejects at 2.6 ± 1.4 % (Gumisiriza *et al.,* 2019). The 60% waste emission indicates that banana processing generates voluminous wastes that needs eco-friendly and appropriate management. Furthermore, the study carried out the physico-chemical characterisation of banana waste to determine the waste's suitability for use as feed substrates for anaerobic digestion and a biochemical Methane Potential (BMP) to determine the biogas yield. BMP is the most common indicator of digester performance and describes the maximum possible volume of methane gas that can be produced per unit mass of solid or volatile solid matter (Buffiere *et al.,* 2006). Waste characterisation is an essential process that guides waste valorisation and reuse (Gumisiriza *et al.,* 2009; Asquer *et al.*, 2019). Proper characterization of wastes is a major factor in designing an efficient, cost-effective, and environmentally compatible waste management system (Rawat *et al*., 2013; Lohri *et al.,* 2014; Shama *et al*., 2019). Characterisation of the banana waste revealed that the waste contained the moisture content in the range of 78.61-90.50 % (mean.85.47%) of wet weight, Total Solids (TS) in the range of 9.5-21.40% (mean 14.55 %) of wet weight and volatile solids (VS) in the range of 80.69-91.79% (mean. 91.79%) of TS. These values were in the same

range as those reported for market waste (TS of 8-20 %; VS of 75-90%) by Deublein and Steinhauser, 2011 and generally showed high suitability for anaerobic digestion. Suitable substrates for anaerobic digestion typically contain VS is the range of 70-95% of TS. Biomass with VS of less than 60 % are rarely considered as valuable substrates for anaerobic digestion (Vogeli *et al*., 2014). The waste also had higher carbon content than total nitrogen that translated into a high C:N ratio of 41:1. The lignocellulose content was generally very high equivalent to 42.93 % total sum of fibres in form of lignocellulose; comprising cellulose 21.16 %, hemicelluloses 10.46 % and lignin 11.31 %. This suggested that appropriate pre-treatment of lignocellulose would be required to enhance feedstock digestibility and improve biogas yield. The Biochemical Methane Potential (BMP) test showed a methane yield of 436.61 L CH4/KgVS which was higher than 0.340 m^3 CH_4/KgVS for grass but in the range of 0.36-0.53 m^3 CH4/KgVS reported for Municipal Solid Waste (Khalid *et al.,* 2011). The BMP describes the maximum possible volume of methane gas that can be produced per unit mass of solid or volatile solid matter (Buffiere et al., 2006). The highest methane production of 79.9 L CH_4/KgVS was recorded at a retention time of 24 days. The BMP value for banana waste obtained in this study was in the range 420 and 465 mlCH_4/gVS_{added}, reported for food waste (Ariunbaatar *et al*., 2014) and it was in a good agreement with other published research (Kapdan and Kargi, 2006; Xu *et al.,* 2011; Kastner *et al.,* 2012; Zhang *et al.,* 2013).

Generally, the physic-chemical characteristics and Biochemical Methane Potential results showed that banana waste was a more favourable feed stock for anaerobic digestion with high potential for recovery of energy in form of biogas. The relatively high content of lignocellulose indicates that with a suitable enhancing method more biomethane can be recovered from BW. This further implied that utilization of banana waste via anaerobic digestion to produce biogas was the most economically viable option towards alleviation of banana industry's energy scarcity. However, due to particulate (lignocellulosic) nature and unbalanced C:N ratio, batch-wise anaerobic digestion of banana waste was challenging, characterised by scum formation, slurry floatation and release of effluent with incomplete digested fibrous biomass. This was in agreement with conclusions from other researchers that it is difficult to recover the entire potential biomethane from a normal unstimulated anaerobic digestion of complex organic substrates (Ariunbaatar *et al*., 2014).This suggested that further studies were needed to investigate the appropriate methods for enhancement of anaerobic digestion of banana waste in order to maximise the recovery of biogas fuel.

8.2.3 Investigating options for enhancement of anaerobic digestion of banana waste

In Chapter five, six and seven, to optimise the performance of anaerobic digestion process and maximise methane recovery in a short retention time, this research further investigated the options for enhancement of anaerobic digestion of banana waste through: 1) Appropriate bioreactor design; 2) Optimisation of operational parameters (Organic Loading Rate, Hydraulic Retention Time and Agitation); 3) Waste pre-treatment by substrate pre-fermentation; and 4) substrate co-digestion.

8.2.4 Enhancement of Anaerobic digestion of banana waste by appropriate bioreactor design

The use of conventional anaerobic digesters for biomethanization of mixed plant biomass including banana waste is generally problematic majorly due to floatation of particulates leading to early wash out of active biomass as well as low digestibility of lignocellulosic content abundant in such feed substrates. To circumvent this problem, a novel high rate hybrid Up-flow Anaerobic Sludge Blanket (hUASB) reactor tailored to biomethanization of lignocellulosic feed stocks such as banana waste was designed and constructed following considerations described by other researchers (Wilkie *et al.,* 2004; Saleh and mahmood, 2004; Mshandete et 2005; Massart *et al.,* 2006; Salehi-Nik *et al.,* 2013). The hybrid Up-flow Anaerobic Sludge Blanket reactor system was comprised of sub units: Hydrolysis tank; high-rate hybrid anaerobic bioreactor; biogas measuring system and an effluent slurry tank. The reactor was a hybrid one due to the incorporation of an upflow sludge bed column and Bordeaux stirrer of which the former is typical to hybrid Up-flow Anaerobic Sludge Blanket reactors while the latter is distinctive to the Continuously Stirred Tank Reactor (CSTR). The hybrid Up-flow Anaerobic Sludge Blanket reactor successfully biomethanised the banana waste substrate up to a maximum organic loading rate of 4.0 KgVS/M^3/Day, giving average methane yield of 553.15 L CH_4/KgVS/Day. This attained organic loading rate was higher than the 3.5 KgVS/M^3/Day reported by Kirtane et al., (2009) but was in the ideal range of 4 – 8 kg VS/m^3 reactor per day for continuously stirred reactors treating biowastes (Vandevivere *et al*., 2003 and Vogeli *et al.,* 2014). The hybrid reactor showed an efficient biogas collection system and start-up period of 3 days that is shorter than the reported 4-16 days typical for Up-flow Anaerobic Sludge Blanket reactors (Saleh and mahmood, 2004).The methane yield obtained in this study was higher than the 0.43661 m^3 CH_4/KgVS/Day previously reported from batch-wise anaerobic digestion of banana waste (Gumisiriza *et al.,* 2019) and the 0.36-0.53 m^3 CH_4/KgVS/Day reported for Municipal Solid Waste (Khalid *et al.,* 2011).

Since for non-stirred anaerobic digester systems, an organic loading rate below 2 kg VS/m^3 reactor and day is recommended and considered suitable, the designed hybrid Up-flow Anaerobic Sludge Blanket (hUASB) reactor system that reached an organic loading rate of 4.0 KgVS/M^3/Day significantly enhanced the anaerobic digestion of banana waste. The high methane yield together with short start-up period obtained using the novel hybrid Up-flow Anaerobic Sludge Blanket (hUASB) reactor system was attributed to two main innovations: 1) better reactor design and; 2) the use of well pre-adapted inoculum. Due to better bioreactor design, the retained sludge bed of seed inoculum at the bottom that served as active flora back up for jump-starting anaerobic digestion process and hence shortening bioreactor start-up period. On the other hand, the initial doubling rate of increase in biogas production indicated an enhanced population of active microbial flora in the seed inoculum that was well adapted but deficient of the feed substrate. This was similarly reported by Vogeli *et al.,(*2014) that during the bioreactor start-up phase, the bacteria population needs to be gradually acclimatised to the feedstock to enhance biomethanization process. Moreover, the entire reactor system was able to be operated in a continuous flow mode using hydraulic flow created by force of gravity. This eliminated the need for energy to drive the pumping system that would be required during substrate loading.

Ultimately, the reactor system could successfully treat banana waste without wash-out of active sludge and thus quick recovery from feed overloads. However, the findings of this chapter showed a need for further shortening of retention time to be in tandem with the rate of waste generation as well as enabling reactor down-sizing.

8.2.5 Enhancement of anaerobic digestion of banana waste by optimisation of operational parameters

Operational parameters can be defined as reactor engineered controls that can be regulated to stabilise the liquor conditions and biochemical processes (environmental parameters) that in turn lead to enhanced anaerobic digestion and biomethanization process of a given feed substrate.

In order to operate the continuous anaerobic digestion plant, organic loading rate (OLR) and the hydraulic retention time (HRT) are principal parameters (Sahito *et al.,* 2016). Organic loading rate is the quantity of organic material added per unit volume of the anaerobic digestion reactor in a day and it is the measure of the biological conversion capacity of the anaerobic digestion system. Organic loading rate is particularly an important control parameter in continuous systems, as overloading leads to a significant rise in volatile fatty acids which can result in acidification and system failure. This study revealed that the optimal organic loading rate of banana waste treated in the hybrid Up-flow Anaerobic Sludge Blanket reactor was 4.0 KgVS/M3/Day, giving average methane yield of 553.15 L CH_4/KgVS/Day. Since for non-stirred anaerobic digestion systems an organic loading rate below 2 kg VS/m^3 reactor and day is recommended and considered suitable (Vandevivere *et al.*, 2003 and Vogeli *et al.,* 2014), the organic loading rate of 4.0 KgVS/M^3/Day obtained by this study was optimal. The optimisation of organic loading rate enhanced the hybrid Up-flow Anaerobic Sludge Blanket (hUASB) of banana waste with increase in methane yield from 436.61 L CH_4/KgVS/Day previously reported from batch-wise anaerobic digestion of banana waste (Gumisiriza *et al.,* 2019) to 553.15 L CH_4/KgVS/Day obtained using novel hybrid Up-flow Anaerobic Sludge Blanket reactor system.

More still, hydraulic retention time (HRT) is another key factor that controls the extent to which volatile solids in the substrate are converted into biogas (Gumisiriza *et al.,* 2017). Although short hydraulic retention time results into faster wash out of active biomass than they can reproduce consequently causing prolonged lag phase of some steps such as fermentative step (Frick and Uppsten, 1999), long hydraulic retention time is not ideal for industrial processing mainly due to high rate of waste generation that must be treated as they efflux out. To this effect, too long hydraulic retention time requires large volume of the digesters or waste storage facilities that are limited by cost, treatment capacity, net energy yield and operational skills. Besides, anaerobic digestion of substrates in high rate reactor operated in continuous flow mode with long hydraulic retention time results into low biogas yields and high fluctuations in the rate of biogas production due to lag phase created between substrate retention cycles. This study therefore investigated the minimum hydraulic retention time for operation of the novel *hybrid* Up-flow Anaerobic Sludge Blanket reactor to give the maximum biomethane yield. The results showed that at organic loading rate of 4.0 KgVS/M^3/Day the biomethanization of banana waste in the novel hybrid Up-flow Anaerobic Sludge Blanket reactor operated in continuous flow mode has an optimal hydraulic retention

time of 23 days.This was in agreement with the findings reported by other previous studies that lignocellulosic feed stocks like most of the energy crops cannot be digested completely at hydraulic retention time less than 20 days (Wolf, 2013). The recommended hydraulic retention time for wastes treated in a mesophilic digester range from 10 to 40 days (Liebrand and Ling, 2009; Mir *et al.,* 2014; Vögeli *et al.,* 2014). The optimisation of hydraulic retention time at constant organic loading rate further increased the methane yield from 553.15 L CH_4/KgVS/Day at hydraulic retention time of 25 days to a maximum of 583.08 LCH_4/KgVS/Day at hydraulic retention time of 23 days and showed minimum fluctuations in biogas production. Operating the reactor in a continuous flow mode below this optimal hydraulic retention time of 23 days, the reactor yielded less gas but less fluctuation in biogas production while operating above the optimal there was high fluctuation in biogas production and similarly low methane yield.
Generally, the anaerobic digestion of banana waste using the designed *hybrid* Up-flow Anaerobic Sludge Blanket reactor was able to maximally recover 583.08 LCH_4/KgVS/Day when operational parameters are optimised to hydraulic retention time of 23 days at organic loading rate of 4.0 KgVS/M^3/Day.

8.2.6 Enhancement of anaerobic digestion of banana waste by Substrate Pre-treatment &Co-digestion

Substrate biological pre-treatment and co-digestion are among the major eco-friendly options for enhancement of anaerobic digestion of plant biomass, especially when the digestate would be used as biofertilizer. Proper pre-treatment application before anaerobic digestion or creating appropriate mixture of complementary substrates for co-digestion can significantly increase process efficiency and ultimately enhancing biogas yield (Gunes *et al.,* 2019).

In this study the ultimate enhancement of anaerobic digestion of banana waste substrate was obtained through substrate pre-fermentation and co-digestion with chicken manure. Comparing substrate pre-treatment and co-digestion, the results showed that pre-fermentation of fresh banana waste substrate optimally for 7 days exhibited the highest methane yield of 843.89 L CH_4/KgVS/Day with the biogas quality of 74% methane while substrate co-digestion with chicken manure in a ratio of 1:3 chicken manure to banana waste, had a gas yield of 782.91 L CH_4/KgVS/Day with the biogas quality of 76% methane. The high methane content recorded from co-digestion of substrate co-digestion with chicken manure was attributed to the supplementation of micro-nutrients present in chicken droppings that boost microbial activity during anaerobic digestion. Studies by Vincente Jr *et al.,* (2018) reported that chicken manure contain elements and minerals such as copper, Zinc,manganese, Iron, magnesium, among others, in proportions that are essential for boosting microbial activity during anaerobic digestion (Diaz-Vazquez *et al.,* 2020). Micro-nutrient supplementation (in form of Fe, Co, Ni, Se, Mo) was proved to be crucial to enhance methanogenic activity, stimulating methane production (Chan *et al.,* 2019). The addition of metals and natural elements showed to have a positive effect both on chemical oxygen demand removal and biogas production from co-digestion of high-strength substrates using up-flow anaerobic sludge blanket reactor (Chan et al., 2019; Loizia et al., 2019). Hence, nowadays trace elements are added as supplement to

food waste for stable and successful digestion at a particular loading rate per day with high biogas yield. Selenium and cobalt are key trace elements found effective in stabilizing digestion mainly during ammonia formation (Ray *et al.*, 2013). Additionally, chicken manure has high nitrogen content (Ojolo *et al.*, 2007) that stabilise the high C:N ratio of 41:1 in banana waste (Gumisiriza *et al.,* 2019). The optimum C:N ratio is between 20:1 and 30:1 (Vandevivere *et al.,* 2002) and at high C:N ratio, the biogas production is low due to rapid consumption of nitrogen while low C:N ratio causes ammonia accumulation leading to alkaline pH of upto 8.5 that is toxic to microbial biocatalysts especially the methanogens. Hence, optimum C:N ratio can be achieved by mixing substrate of low and high C:N ratio (Khalid *et al.,* 2011). Such high nitrogen sources include urea and animal manure such as chicken droppings and could be used as supplements (Richard, 1998) to bioreactors in appropriate proportion s to balance the C:N ratio and enhance the anaerobic digestion process.

On the other hand, the high biogas production from banana waste pre-fermented for 7 days is attributed to optimal biodegradation of lignocellulose in the waste at day 7. This was in agreement with other studies that reported that banana waste would be fully colonised by moulds within 6-8 days of fermentation at room temperature (Essien *et al.,* 2005: Shah *et al.,* 2005). Thus, with banana waste pre-fermentated for 7 days, lignocellulolytic microorganisms had optimally degraded substrate to release sugars that enhanced biogas production. This implied that substrate fermented for 7 days was the most optimally solubilised by the microbial consortia in wet soil inoculum leading to consistent nutrient availability to microorganisms, thus limiting fluctuations in biogas yields. Enhanced solubilisation of banana waste by microbial co-culture fermentation was similarly reported by Ingale *et al.,* (2014) that fermentation of banana pseudo stems with co-culture of *A. ellipticus* and *A. fumigatus*, improved substrate saccharification and hydrolytic activities with maximum enzyme production and activity at 8th day of microbial fermentation with banana waste (banana pseudo stem).

8.2.7 The optimal conditions and highest biomethane yield obtained at varying enhancement option

To arrive at maximum energy recovery from banana waste through anaerobic digestion (AD), an approach of sequential stage enhancement was employed. Table 8.1 shows the optimal conditions and highest methane recovered at each stage. This study ultimately revealed that using the designed novel hybrid Up-flow Anaerobic Sludge Blanket (hUASB) digester operated at optimised operational parameters of organic loading rate (OLR) of 4.0 $KgVS/M^3/D$ and hydraulic retention time (HRT) of 23 days, anaerobic digestion of banana waste pre-treated by fermentation for 7 days yields the highest biomethane at 843.89 L $CH_4/KgVS/D$ that translates into 93.28 % enhancement when compared to batch-wise biomethanization (BMP) of fresh banana waste.

Table 8:1. Optimal conditions and percentage methane yield increment by varying enhancement methods. (The % CH_4 yield enhancement indicated in this table was calculated basing on the methane gas yield from the batch-wise anaerobic digestion of banana waste)

	AD enhancement Method	Optimal Condition	Highest CH_4 Yield obtained	% CH_4 Yield Enhancement	Reference
1	Batch-wise Determination of BMP	Batch-wise AD for 40 days	436.61 L CH4/KgVS/D	No enhancement	Chapter 4, Table 4.5
2	Batch-wise Determination of BMP	Peak biogas production between day 22-25	79.9 L CH_4 /D	No enhancement	Chapter 4, Figure 4.4
3	Enhancement of AD by hUASB Reactor Design	OLR of 4.0 $KgVS/M^3/D$	553.15 L CH_4/KgVS/D	26.69	Chapter 5, Table 5.5
4	Optimisation of hUASB Reactor Design: HRT	HRT of 23 days	583.08 L CH_4/KgVS/D	33.55	Chapter 6, Table 6.1
5	Co-digestion with Chicken manure	Ratio of 1:3 Chicken manure: to Banana waste	782.91 L CH_4/KgVS/D	79.32	Chapter 7, Table 7.1
6	Pre-treatment by substrate fermentation	Substrate Fermented for 7 days	843.89 L CH_4/KgVS/D	93.28	Chapter 7, Table 7.1

8.3 Implication of the research findings to the energy needs of banana industry in Uganda

In this study, biomethanization of banana waste using optimised hybrid Up-flow Anaerobic Sludge Blanket (hUASB) reactor maximally yielded a volume of 0.84389 M^3 CH_4/KgVS/Day which is equivalent to 843.89 ml CH_4/gVS/Day.

From the physico-chemical analysis, banana waste (BW) contains a total solid (TS) of 14.55 % of fresh weight and volatile solids (VS) of 91.79 % of TS. This implied that 1.0g fresh weight of BW contains 0.1455 g TS (14.55%) of which 0.1336g (91.79%) is volatile solids. Hence, 1.0 gVS is obtained from 7.50g fresh weight of banana waste substrate.
From the gas yields, 1 gVS yields 843.89 ml CH_4/Day which is equivalent to 843.89 ml CH_4 per 7.50 g fresh weight of banana waste substrate. This implies that fresh banana waste has

methane yield equal to 112.52 ml CH_4/gFwt or 112.52 L CH_4/KgFwt or 0.11252 M^3 CH_4/KgFwt which translates to 112.52 M^3 CH_4/Tonne of fresh waste.

From energy conversion equivalent described by Vogeli *et al.,* 2014, $1M^3$ of biomethane is equal to 6.0 kWh or 21.6 MJ of energy (1kWh is equivalent to 3.6 MJ) and the 112.52 M^3 CH_4/Tonne of fresh banana waste translates into 675.12 kWh or 2,430.432 MJ of energy.

Therefore, anaerobic digestion of fresh banana waste using the designed novel HUASB bioreactor system can potentially recover a net energy of 675.12 kWh or 2,430.432 MJ per tonne of waste.

Production of East African Highland Bananas (EAHB), cooking banana (AAA-EA group), locally called *matooke,* in Uganda is estimated at over 6 million tonnes per year (Spilsbury *et al.,* 2002; Tumutegyereize *et al.,* 2011). With a waste generation rate of 60 % per fruit bunch during industrial processing, the total banana waste generation can be estimated at 3 million tonnes per year.

The energy recovery from total annual banana waste from industrial processing of banana (*matooke*) would be 3mil tonnes of BW per year multiplied by 675.12kWh per tonne that is equal to 2,025, 360 MWh per year.

Hence, anaerobic digestion of banana waste generated from industrial processing of green bananas can recover enough biofuel in form of biomethane which can produce sufficient and sustainable energy for safe drying of banana chips as well as conversion into electricity for powering the entire banana industrialisation.

8.4 References

1. Ariunbaatar, J., Bair, R., Ozcan, O., Esposito, G., Lens, P.N.L and Yeh, D.H (2014), “Pretreatment methods to enhance anaerobic digestion of organic solid waste”, Applied energy 123, 143-156.

2. Asha, N., Enoch,K., Kephas, N., Susan, A. and Diego, N. (2015) “Technical report: Structure of the Cooking Banana Value Chain in Uganda and Opportunities for Value Addition and Postharvest Losses Reduction”. Expanding Utilization of Roots, Tubers and Bananas and Reducing Their Postharvest Losses(RTB-ENDURE) is a 3 year project (2014-2016) implemented by the CGIAR Research Program on Roots, Tubers and Bananas (RTB) with funding by the European Union and technical support of IFAD. http://www.rtb.cgiar.org/endure

3. Asquer, C., Cappai, G. and Carucci, A (2019), “Biomass ash characterisation for reuse as additive in composting process”, Biom Bioe 123: 186-194 https://doi.org/10.1016/j.biombioe.2019.03.001

4. Bezerra,C.V., Rodrigues, M.A.D., Amante, E.R. and Da Silva, L.H.M (2013), Nutritional Potential of Green Banana Flour Obtained by Drying in Spouted Bed Rev. Bras. Frutic., Jaboticabal - SP, v. 35, n. 4, p. 1140-1146

5. Buffiere P., Loisel D., Bernet N., Delgenes J-P. (2006). Towards new indicators for the prediction of solid waste anaerobic digestion properties. Water Science & Technology, Vol 53, No 8, 233–241.

6. Chan, P.C.; Lu, Q.; de Toledo, R.A.; Gu, J.-D.; Shim, H. Improved anaerobic co-digestion of food waste and domestic wastewater by copper supplementation—Microbial community change and enhanced effluent quality. Sci. Total Environ. 2019, 670, 337–344.

7. Chynoweth, D.P. (2004). Biomethane from energy crops and organic wastes, In: International Water Association (Eds.), Anaerobic Digestion 2004. Proceedings 10th World Congress, Montreal, Canada: 525-530.

8. Cotty, P.J. and Jaime-Garcia, R. (2007), "Influences of climate on aflatoxin producing fungi and aflatoxin contamination" International Journal of Food Microbiology Volume 119, Issues 1–2, 20, Pages 109-115

9. Deublein D. and Steinhauser A. (2011). Biogas from waste and renewable resources:an introduction, Wiley -VCH Verlag GmbH & Co. KGaA.

10. Díaz-Vázquez, D., Alvarado-Cummings, S.C., Meza-Rodríguez, D., Senés-Guerrero, C., Anda, J and Gradilla-Hernández, M.S (2020), "Evaluation of Biogas Potential from Livestock Manures and Multicriteria Site Selection for Centralized Anaerobic Digester Systems: The Case of Jalisco, México", Sustainability 12 (3527): 1-32. Sustainability 2020, 12, 3527; doi:10.3390/su12093527: www.mdpi.com/journal/sustainability

11. Doymaz Ibrahim (2007). The kinetics of forced convection air-drying of pumpkin slices. Journal of Food Engineering, 79, 243-248.

12. Essien,J.P., Akpan, E.J and Essien, E.P (2005), "Studies on mould growth and biomass production using waste banana peel", Bioresource Technology 96 (13): 1451-1456.

13. Food and Agricultural Organisation Statistics (FAOSTAT) 2012 data on bananas and plantains production.

14. Frick, M. and Uppsten, M (1999), Alkaline pretreatment of water Hyacinth for improved anaerobic digestion, unpublished Master of Science thesis, Department of Biotechnology, Lund University, Sweden.

15. Gumisiriza, R., Hawumba, J.F., Balyeidhusa, A.S.P., Okure, M and Hensel, O (2019), "Processing of East African Highland Green Bananas: Waste Generation and Characterisation as a Potential Feedstock for Biogas Production in Uganda", American Scientific Research Journal for Engineering, Technology, and Science; Global Society of Scientific Research and Researchers; ISSN (Print) 2313-4410, ISSN (Online) 2313-4402; http://asrjetsjournal.org/

16. Gumisiriza, R., Hawumba, J.F., Okure, M and Hensel, O (2017) "Biomass Waste-to-Energy Valorisation Technologies: A Review Case for Banana Processing in Uganda" Biotechnology for biofuels 10:11. DOI 10.1186/s13068-016-0689-5

17. Gumisiriza, R., Mshandete, A.M., Rubindamayugi, M.S.T., Kansiime, F. and Kivaisi, A. K (2009), "Nile perch fish processing waste along Lake Victoria in East Africa: Auditing and characterization", African Journal of Environmental Science and Technology Vol. 3 (1), pp. 013-020

18. Gunes, B.; Stokes, J.; Davis, P.; Connolly, C.; Lawler, J. Pre-treatments to enhance biogas yield and quality from anaerobic digestion of whiskey distillery and brewery wastes: A review. Renew. Sustain. Energy Rev. 2019, 113, 109281.

19. Ingale, S., Joshi, S.J and Gupte, A (2014), " Production of Bioethanol Using Agricultural Waste: Banana Pseudo Stem", Brazilian Journal of Microbiology 45 (3): 885-892

20. Islam, M. N. (1984), "Kinetic analysis of air drying of potato: traditional and improved methods" Journal of Agricultural Engineering, Institute of Engineers Bangladesh, 12:3-11.

21. Islam, M. S., Haque, M. A and Islam, M. N (2012), "Effects of Drying Parameters on Dehydration of Green Banana (Musa sepientum) and its Use in Potato (Solanum tuberosum) Chips Formulation," The Agriculturists, Vol. 10 (1): 87-97

22. Kapdan I.K. and Kargi F. Bio-hydrogen production from waste materials, Enzyme and Microbial Technology 2006; 38 (5): 569-582

23. Kastner V., Somitsch W., Schnitzhofer W. The anaerobic fermentation of food waste:a comparison of two bioreactor systems, Journal of Cleaner Production 2012; 34: 82-90

24. Kawongolo, J.B. (2013) Optimization of Processing Technology for Commercial Drying of Bananas (Matooke), a Ph.D dissertation submitted to the Universität Kassel Fachbereich Ökologische Agrarwissenschaften Fachgebiet Agrartechnik

25. Khalid A., Arshad M., Anjum M., Mahmood T., Dawson L. (2011). The anaerobic digestion of solid organic waste - Review. Waste Management Aug; 31(8): 1737– 44.

26. Khalid, A., Arshad, M., Anjum, M., Mahmood, T., & Dawson, L. (2011). The

27. Koyuncu, T., Tosun, I and Pınar, Y (2007), "Drying characteristics and heat energy requirement of cornelian cherry fruits (Cornus mas L.)" Journal of Food Engineering 78 (2), 735–739.

28. Kumar, A.; Samadder, S.R. Performance evaluation of anaerobic digestion technology for energy recovery from organic fraction of municipal solid waste: A review. Energy 2020, 197, 117253

29. Lewicki, P.P (2006), "Design of hot air drying for better foods" Trends in Food Science & Technology 17, 153–163.

30. Liebrand CB, Ling KC. Cooperative approaches for implementation of dairy manure digesters. USDA Rural Development Research Report 217. STOP 3252, 1400

Independence Ave., S.W Washington, DC 20250-3252;2009.

31. Lohri, C. R., E. J. Camenzind, and C. Zurbrugg. 2014. "Financial sustain- ability in municipal solid waste management-costs and revenues in Bahir Dar, Ethiopia." Waste Manage. (Oxford) 34 (2): 542–552. https:// doi.org/10.1016/j.wasman.2013.10.014.

32. Loizia, P.; Neofytou, N.; Zorpas, A.A. The concept of circular economy strategy in food waste management for the optimization of energy production through anaerobic digestion. Environ. Sci. Pollut. Res. 2019, 26,

33. Mainardis, M., Buttazzoni, M and Goi, D (2020), "Up-Flow Anaerobic Sludge Blanket (UASB) Technology for Energy Recovery: A Review on State-of-the-Art and Recent Technological Advances" Bioengineering 7 (43): 1-49.

34. Massart, N., Black and Veatch (2006), "Design and Operational Considerations to Avoid Excessive Anaerobic Digester Foaming", Water Environment Foundation, WEFTEC(R), 06: Pp 2553-2575

methods to enhance anaerobic digestion of organic solid waste, Applied Energy 15:

35. Mir, M.A., Hussain, A and Verma, C (2016), "Design considerations and operational performance of anaerobic digester: A review", Cogent Engineering,3:1, 1181696, DOI: 10.1080/23311916.2016.1181696.

36. Misson, G.; Mainardis, M.; Incerti, G.; Goi, D.; Peressotti, A. Preliminary evaluation of potential methane production from anaerobic digestion of beach-cast seagrass wrack: The case study of high-adriatic coast. J. Clean. Prod. 2020, 254, 120131

37. Motevali, A., Minaei, S., Khoshtagaza, M.H (2011), "Evaluation of energy consumption in different drying methods" Energy Conversion and Management 52 (2), 1192–1199.

38. Mshandete, A., Björnsson, L., Kivaisi, A.K, Rubindamayugi, S.T. and Mattiasson, B (2005), "Enhancement of anaerobic batch digestion of sisal pulp waste by mesophilic aerobic pre-treatment", Water Research 39, 1569-1575

39. Muranga, F.I., Sampath, H., Marlett, J.A., & Ntambi, J.M., (2007). Impact of processing technique on the apparent bioavailability of cooking banana (matooke) starch. African Journal of Biochemistry Research, 1(5), 72-– 77

40. Ojolo, S.J., Oke, S.A., Animasahun, K. and Adesuyi, B.K. (2007). Utilization of Poultry, Cow and Kitchen Wastes For Biogas Production: A Comparative Analysis. Iran. J. Environ. Health. Sci. Eng., 4(4): 223-228.

41. Rasapoor, M.; Young, B.; Brar, R.; Sarmah, A.; Zhuang, W.-Q.; Baroutian, S. Recognizing the challenges of anaerobic digestion: Critical steps toward improving biogas generation. Fuel 2020, 261, 116497

42. Rawat, M., A. L. Ramanathan, and T. Kuriakose. 2013. "Characterization of municipal solid waste compost from selected from selected Indian cities—A case study for its sustainable utilization." Environ. Prot.4 (2): 163–171. https://doi.org/10.4236/jep.2013.42019.

43. Ray, N. H. S., Mohanty, M. K., & Mohanty, R. C. (2013). Anaerobic digestion of kitchen waste: Biogas production and pre treatment waste, a review. International Journal of Scientific and Research Publication, 3, 11

44. Richard, T. L. (1998). Municipal solid waste composting.Physical processing. Retrieved from http://www..dept/compost/msw.factsheets/msw.Fs2.html/

45. Roberts, J. S., Kidd, D. R and Padilla-Zakour, O (2008), "Drying kinetics of grape seeds" Journal of Food Engineering, 89:460–465

46. Sahito, A.R., Mahar, R.B and Ahmed, F (2016), "Optimization Of Organic Loading Rate And Hydraulic Retention Time for Maximum Production of Methane Through Anaerobic Co-Digestion Of Canola Straw and Buffalo Dung" The Journal of Animal & Plant Sciences, 26(2): 2016, Page: 373-381; ISSN: 1018-7081

47. Saifullah, R., Abbas, F. M. A., Yeoh, S.-Y. and Azhar, M. E (2009), "Utilization of green banana flour as a functional ingredient in yellow noodle" International Food Research Journal 16: 373-379

48. Saleh, M.M.A and Mahmood, U.F (2004) "Anaerobic Digestion Technology for Industrial Wastewater Treatment", Eighth International Water Technology Conference, IWTC8 2004, Alexandria, Egypt Pp 817-833.

49. Salehi-Nik, N., Amoabediny, G., Pouran, B., Tabesh, H., Shokrgozar, M. A., Haghighipour, N., Khatibi, N., Fatemeh Anisi, F., Mottaghy, K. and Zandieh-Doulabi, B (2013), "Engineering Parameters in Bioreactor's Design: A Critical Aspect in Tissue Engineering"; Review Article, BioMed Research International, Volume 2013, Article ID 762132, http://dx.doi.org/10.1155/2013/762132

50. Samadi, S.H., Ghobadian, B., Najafi, G and Motevali, A. (2014), "Potential saving in energy using combined heat and power technology for drying agricultural products (banana slices)," Journal of the Saudi Society of Agricultural Sciences, Vol. 13 174-182.

51. Sha, M., Reddy, G.V., Banerjee, R., Babu, P.R and Kothari, I. L (2005), "Microbial Degradation of banana waste under solid state bioprocessing using two lignocellulolytic fungi (Phylosticta spp. MPS-001 and Aspergillus spp. MPS-002)", Process Biochemistry 40 (1): 445-451.

52. Sharma, A., Ganguly,R. and and Gupta, A.K (2019), "Characterization and Energy Generation Potential of Municipal Solid Waste from Nonengineered Landfill Sites in Himachal Pradesh, India" J. Hazard. Toxic Radioact. Waste, 2019, 23(4): 04019008.

53. Spilsbury, J.S., Jagwe, J.N., Ferris, R.S.B (2002), "Evaluating the Marketing Opportunities for Banana & its Products in the Principle Banana Growing Countries of ASARECA (Uganda)", International Institute of Tropical Agriculture. Foodnet. P: 60.

54. Tripathi, L., Tripathi1, J. N. and Tushemereirwe, W. K (2008), "Rapid and efficient production of transgenic East African Highland Banana (Musa spp.) using intercalary meristematic tissues", African Journal of Biotechnology Vol. 7 (10), pp. 1438-1445

55. Tumutegyereize, P., Muranga, F.I., Kawongolo, J. and Nabugoomu, F (2011), "Optimization of biogas production from banana peels", African Journal of Biotechnology Vol. 10 (79), 18243-18251

56. Twaha,S., Ramli, M.A.M., Murphy, P.M., Mukhtiar, M.U and Nsamba, H.K (2016), "Renewable based distributed generation in Uganda: Resource potential and status of exploitation", Renewable and Sustainable Energy Reviews; 57: 786-798.

57. Uddin, M.B and Islam, M. N (1985), "Development of shelf stable pineapple products by different methods of drying" Journal of Agricultural Engineering, Institute of Engineers Bangladesh, 13(1):5-13.

58. Vandevivere P., L. De Baere, W. Verstraete (2003). Types of anaerobic digesters for solid wastes, in Biomethanization of the Organic Fraction of Municipal Solid Wastes, J. Mata-Alvarez, Editor. IWA Publishing: Barcelona. p. 111–140.

59. Vandevivere, P., & De Baere, L. (2002). Types of anaerobic digesters for solid wastes. In J. Mata-Alvarez (Ed.), Biomethanization of the organic fraction of municipal solid wastes (pp. 336–667). London. Retrieved May 20, 2020, from http://espace.library.uq.edu.au/view/UQ:164433

60. Vicente Jr, D.V., Costa, M.S.S., Costa, L.A.M., [1], Dercio C. Pereira, D.C and Santos, F.T (2018), "Anaerobic Digestion and Co-Digestion of Poultry Litter Submitted to Different Reuses", Engenharia Agricola, Jaboticabal 38 (6); 961-967. Doi: http://dx.doi.org/10.1590/1809-4430-Eng.Agric.v38n6p961-967/2018

61. Vögeli Y., Lohri C. R., Gallardo A., Diener S., Zurbrügg C. (2014). "Anaerobic Digestion of Biowaste in Developing Countries: Practical Information and Case Studies". Swiss Federal Institute of Aquatic Science and Technology (Eawag), Dübendorf, Switzerland

62. Ward AJ., Hobbs PJ., Holliman PJ., Jones DL. Optimization of anaerobic digestion of agricultural resources, Bioresource Technology 2008; 99(17): 7928-7940

63. Willkie, A.C., Smith, P.H and Bordeaux .F.M (2004), "An economical bioreactor for evaluating biogas potential of particulate biomass", Bioresource Technology, Vol. 92 pp 103-109

64. Wolf, C. (2013). Simulation, optimization and instrumentation of agricultural biogas

plants. Doctoral Dissertation, Department of Electronic Engineering National University of Ireland, Maynooth.

65. Xu SY., Lam H.P, Karthikeyan P., Wong JWC. Optimization of food waste hydrolysis in leach bed coupled with methanogenic reactor: Effect of pH and bulking agent, Bioresource Technology 2011; 102: 3702-3708

66. Zhang C., Xiao G., Peng L., Su H., Tan T. The anaerobic co-digestion of food waste and cattle manure, Bioresource Technology 2013; 129: 170-176

67. Zhang, L.; Loh, K.-C.; Zhang, J. Food waste enhanced anaerobic digestion of biologically pretreated yard waste: Analysis of cellulose crystallinity and microbial communities. Waste Manag. 2018, 79, 109–119.

68. Zhong, Y.; Chen, R.; Rojas-Sossa, J.-P.; Isaguirre, C.; Mashburn, A.; Marsh, T.; Liu, Y.; Liao, W. Anaerobic co-digestion of energy crop and agricultural wastes to prepare uniform-format cellulosic feedstock for biorefining. Renew. Energy 2020, 147, 1358–1370

9 Conclusions, recommendations and future research perspective

9.1 Key conclusions

This study carried out an extensive research aimed at investigating and evaluation of the most appropriate waste-to-energy technology for recovery of energy from banana waste so as to intervene in the energy crisis affecting the banana industrialisation. Findings of this study led to the inference that:

1) Anaerobic digestion is the most appropriate waste to energy for recovery of energy from banana waste, since the solids in the waste are more than 90% organic, and anaerobic digestion would produce eco-friendly energy together with an organic biofertilizer.

2) Processing of East African Highland green bananas generates about 60% as wastes per fruit bunch which translates into huge volumes of wastes per tonnage; hence banana waste is a sustainable feedstock for anaerobic digestion

3) The novel hybrid Up-flow Anaerobic Sludge Blanket reactor system developed was efficient in anaerobic digestion of banana waste and recovery of biogas generated, since the reactor showed short start-up period and circumvented the challenges of banana waste floatation and early wash-out of the substrate slurry.

4) Optimisation of reactor operational parameters (Hydraulic Retention Time, Organic Loading Rate and Liquor Agitation) enhanced the anaerobic digestion of banana waste and caused a 33.55% increment in methane yield.

5) Co-digestion of banana waste with chicken manure in a ratio of 1:3 chicken manure to banana waste enhances anaerobic digestion and increases the gas yield up to 79.32 % due to balancing of C:N ratio of the substrate as well supplement of essential trace minerals.

6) Substrate pre-treatment by microbial fermentation optimally for 7 days exhibited the highest methane yield increment of 93.28%, and was the most efficient method for enhancement of anaerobic digestion of banana waste with recovery of biogas

7) Anaerobic digestion of pre-fermented banana waste for 7 days using the designed novel hUASB reactor system can potentially recover a net energy of 675.12 kWh or 2,430.432 MJ per tonne of waste; implying that anaerobic digestion of banana waste generated from industrial processing of green bananas can recover enough biofuel in form of biomethane which can produce sufficient and sustainable energy for safe drying of banana chips as well as conversion into electricity for powering the entire banana industrialisation.

9.2 Study limitations

Despite the successful completion, this study encountered the challenge of small size of laboratory reactors. This study was generally done in the laboratory using a 10 litre volume reactor and without any prior computer simulated model or field full scale operational

reactors. This challenge was circumvented by setting reactors in triplicates in order to obtain average values with standard deviations that can be applicable to full scale size reactors. Therefore a large scale model needed to be simulated on a computer to evaluate the likely operation of a full scale size reactor system and ultimately more studies on full scale field size reactors. Other limitations encountered include:

1. Lack of laboratory equipments, especially accessories related to bioreactors and anaerobic digestion. To accomplish this research study, materials had to be bought and reactors and other equipments assembled from scratch.
2. Lack of pure strains of lignocellulolytic isolates. It became inevitable at the last minute that the supplier of the pure isolates turned down the offer. Consequently, a soil sample presumed to contain a consortia of lignocellulolytic microorganisms was used
3. Lack of specialised technical expertise in the laboratory to guide in design, construction and operation of typical bioreactor system. The novel hUASB reactor system developed in this study was built from scratch based on guidance from my supervisors and extensive literature review.

9.3 Recommendations

For research to be useful and have impact to solve problems or provide interventions for which the study was aimed at, study findings should be disseminated to the community or users in the population for application. To this effect the following are the future investment perspectives to undertake as regards to the above findings.

1. To install a pilot-scale of the novel hybrid Up-flow Anaerobic Sludge Blanket reactor system for harnessing biogas from green banana waste

2. To design and construct of a biogas-solar hybrid drier system for efficient drying of green banana pulp.

9.4 Future research perspective

While accomplishment of research activities on this study, certain fascinating scientific concepts emerged and were never elucidated to their logical outcome mainly due to limited time and financial resources. Hence the following are other future scientific research perspectives worth undertaking:

1. To design computer simulation models of the novel hybrid Up-flow Anaerobic Sludge Blanket reactor system for easy sizing and community adoption.

2. To isolate and characterise the microorganisms, from soil at the banana waste dump-site, that natively carry out lignocellulolysis of green banana waste in the natural environment.

3. To characterise the microbial flora existing in the reservoir volume at the base of the novel hybrid Up-flow Anaerobic Sludge Blanket reactor.

10 Summary

Banana industry in Uganda is heavily impeded by the lack of cheap, reliable and sustainable energy mainly needed for drying of flesh banana pulp to convert it into dried chips before milling into banana flour. Sufficiently dried chips have a long stable shelf life and can safely be stored while awaiting subsequent processing into flour used in bakery, glycemic therapeutics, and nutritional formulations, among others. Besides, Uganda has one of the lowest electricity access levels, estimated at only 2-3% in rural areas where most of the banana growing is located ; implying that drying banana pulp into chips using electricity by rural banana farmers is almost practically impossible due to lack of grid electricity access. Moreover, there are few hours of solar radiations during rainy season that make solar drying pulp take many days leading to inconsistent and substandard product quality, characterized by rotting and infestation with moulds that produce aflatoxins. Therefore solar drying alone may not sustainably meet the entire energy requirements for efficient and safe drying of the flesh banana pulp and other fruits into safe dried chips with long stable shelf life. Additionally, most banana farmers have limited financial capacity to access modern solar driers that would generate sufficient energy for safe pulp drying and general industrial processing.

Although hot air convection drying using liquefied petroleum gas (LPG) can lead to consistent drying of banana pulp, the high cost of liquefied petroleum gas makes the process expensive and unsustainable. Hence, the search for sustainable and cheap source of energy for powering banana industry, especially drying of banana pulp, has been very imperative. Incidentally, the industrial processing of East African Highland green bananas generates a lot of banana waste; mainly comprised of banana peels, peduncle, damaged fruits and pulp rejects, which can be converted into energy through application of appropriate waste-to-energy (WtE) valorization technologies. This study therefore aimed at investigating the most appropriate waste-to-energy technology for recovery of energy from such banana waste.

A review of various potential waste- to-energy valorization technologies inferred that anaerobic digestion for biogas production was the most appropriate for conversion of banana waste into energy, since banana waste has high moisture content. Determination of the potentiality of banana waste as a substrate for anaerobic digestion revealed that banana waste has more than 90% organic solids and hence very suitable substrate for anaerobic digestion. Moreover, processing of East African Highland green bananas generates about 60% as wastes per fruit bunch implying that huge volumes of wastes are generated per tonnage of banana fruit bunches; hence banana waste is a sustainable feedstock for anaerobic digestion. Batch-wise anaerobic digestion of the banana waste showed that the waste had a biochemical methane potential of 436.61 L CH_4/KgVS/D suggesting that banana waste is potentially good substrate for anaerobic digestion to recover energy in form of biogas. However, due to unbalanced C:N ratio and lignocellulosic nature, progress of anaerobic digestion of banana waste was challenging, characterised by scum formation, slurry floatation and release of effluent with incomplete digested fibrous biomass.

To optimise the performance of anaerobic digestion process and maximise methane recovery in a short retention time, this research further investigated the options for enhancement of

anaerobic digestion of banana waste through: 1) Appropriate bioreactor design; 2) Optimisation of operational parameters; 3) Waste pre-treatment by substrate pre-fermentation; and 4) substrate co-digestion.

This study designed and constructed a novel bioreactor system, termed as "hybrid Up-flow Anaerobic Sludge Blanket" (hUASB) reactor that was hybrid between conventional Up-flow Anaerobic Sludge Blanket reactor and continuously stirred tank reactor (CSTR). The novel reactor was based on the principle that synchronized stirring with up flow movement of feed substrate in a sludge bed column circumvents the floatation and early wash-out of incomplete digested feed material from the reactor. Moreover, the creation of an inoculum reservoir at the base of the reactor kept replenishing the lost bio-flora thus solving the problem of loss of active microbial biocatalysts to the overflow slurry effluent. The novel (hUASB) reactor system was thus able to: quicken the start-up period to 3 days, efficiently biomethanize high organic load of up to 4.0 KgVS/M^3/D, operate in a continuous flow mode using hydraulic flow created by force of gravity and circumvent the challenges of banana waste floatation and early wash-out of substrate slurry. When operated optimally at hydraulic retention time of 23 days and organic loading rate of 4.0 KgVS/M^3/D, the novel (hUASB) reactor system was able to recover 583.08 L CH_4/KgVS/D of methane, translating into 33.55% gas yield enhancement.

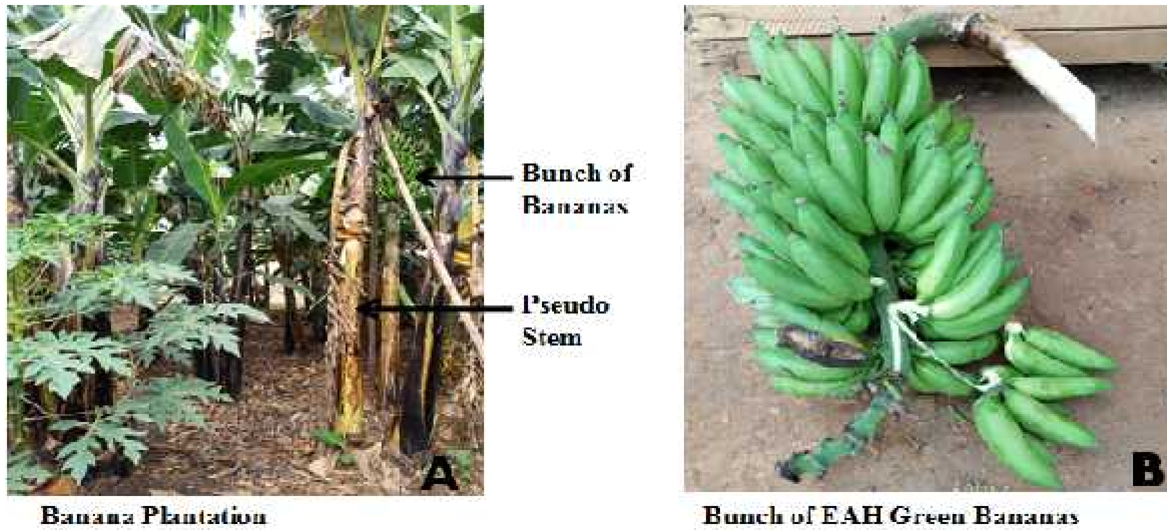
Further enhancement of anaerobic digestion of banana waste using the novel hybrid Up-flow Anaerobic Sludge Blanket reactor showed that substrate co-digestion with chicken manure in a ratio of 1:3 increased methane yields by 79.32 % while substrate pre-treatment by microbial fermentation optimally for 7 days exhibited the highest methane recovery of 843.89 L CH4/KgVS/D constituting to 93.28% methane yield increment.

This study concluded that anaerobic digestion is the most appropriate waste to energy technology for recovery of energy in form of biomethane from banana waste. The novel hybrid Up-flow Anaerobic Sludge Blanket reactor system developed was efficient in anaerobic digestion of banana waste and recovery of biogas generated. Pre-fermentation of banana waste was the most efficient method for enhancement of anaerobic digestion of banana waste. The anaerobic digestion of pre-fermented banana waste using the designed novel hybrid Up-flow Anaerobic Sludge Blanket bioreactor system can potentially recover a net energy of 675.12 kWh or 2,430.432 MJ per tonne of waste; implying that anaerobic digestion of banana waste can recover sufficient and sustainable energy for safe drying of banana chips as well as powering the entire banana industrialisation.

Despite the accomplishment of the set objectives, this study encountered the challenge of small size of laboratory reactors. This study was generally done in the laboratory using a 10 litre volume reactor and without any prior computer simulated model or field full scale operational reactors. It is therefore recommended that a large scale model need to be simulated on a computer to evaluate the likely operation of a full scale size reactor system and ultimately more studies on full scale field size reactors. Further research would focus on installation of a pilot-scale hybrid Up-flow Anaerobic Sludge Blanket reactor system for harnessing energy from banana waste, as well as to isolate and characterise the waste-dump soil microorganisms that natively carry out lignocellulolysis of green banana waste during fermentation.

Appendix

Appendix I: The pictures of the East African High land (EAH) Green Banana used as a raw material for this research



Banana Plantation

Bunch of EAH Green Bananas

Appendix II: The pictures of major fractions of Banana Waste from industrial processing of East African High Land Green Bananas

Peduncle

Fruit Rejects

Peels

Appendix III: Pictures of the developed novel hybrid Up-flow Anaerobic Sludge Blanket (hUASB) reactor system

Set-up of the System

Data collection