

Hygienization of Enteropathogens Contaminated Biowaste for Safer Biological Treatments

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Hygenisierung von mit enteropathogenen Mikroorganismen kontaminierten organischen Abfällen für ungefährliche biologische Verwertungsstrategien

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Declaration

I hereby declare that this Ph.D. thesis has not been previously published or written by another person; neither has it been submitted nor accepted for any other academic award. It is the result of my original work carried out at Brandenburg University of Technology, Cottbus-Senftenberg, Germany, within the framework of the Ph.D. Programme Environmental and Resource Management. All materials from other sources have been duly and adequately acknowledged.

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**** Exodus 14:14 ****

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Summary

Evolution and prevalence of resistant pathogen strains in biowaste pose health risks to humans and environment upon using the biowaste as resource during biotreatment or farm application of the resulting compost and/or digestate. With the promotion of composting and anaerobic digestion of biowaste as environmental friendly tactics toward management of biowaste, application of scientific strategies to curb the anticipated global threats of spreading potentially infectious diseases become of public interest. In this study, the aims were to investigate the die-off levels of pathogens attainable under conventional biotreatments, introduce alternative hygienization options and study their performance at eliminating pathogens from biowaste. In addition, the study investigated feasibility of biotreatment of potato peels waste (PPW) as management option for the peels waste generated in Dar es Salaam city.

Analysis of poorly managed biowaste confirmed high enteropathogens contamination up to 6.3logCFU/g which propagates in soil and leachate to 6.5logCFU/g and 7.8logCFU/g respectively. Pathogens inactivation studies using simulated biowaste (moisture >77%w/w) composed of PPW spiked with *Escherichia coli* and *Salmonella senftenbergensis* at laboratory-scale resulted into low temperature-time patterns of the aerobic In-vessel composting system and turned the process anaerobic thus demonstrated inefficiency at eliminating the spiked bacteria. Likewise, two-phase anaerobic digestion at continuous stirred tank reactor confirmed incapable at effecting complete inactivation of the bacteria unless substrate residence is prolonged above 72hrs. These distasteful confirmations inevitably advocate the importance of applying either pre-and/or post-biotreatment hygienization processes on biowaste for safer biotreatments. Existing hygienization options including acid/lime conditioning and batch pasteurization were applied, their hygienization efficacy investigated and the latter validated. Acetic acid treatment of biowaste at pH value 4 for 5days or lime treatment at pH value 11-12 for 1day brought complete die-off in *E. coli* and *S. senftenbergensis*. Temperature-time combination at 65°C for 30min was sufficient at effecting >1log cycle die-off of the spiked enteropathogens with z-Value of 11°C for *S. senftenbergensis* hence validates the temperature-time regime of 70°C for 30min and 70°C for 60min set by U.S-EPA/625/R-92/013 and Regulations (EC) No. 208/2006 respectively. In addition, alternative hygienization options namely UV radiation and passive solar heating (PSH) were proposed, applied and evaluated. Modes of application and achievable bacteria die-off for

all hygienization options are given in Chapter 2 to 6 of this document. Owing to its small investment capital (mostly do yourself construction), independence from fuel supplies and low running costs, the study highly recommend PSH as environmental friendly and economic viable pre- and post- biotreatment hygienization option especially for tropical climates and developing countries with adequate sunshine.

In vessel co-composting of PPW with yard-waste at 6:1 ratio gave microbiologically safe and good quality compost. Endowed with higher CO₂ removal, mesophilic/thermophilic two-phase anaerobic processing of PPW showed comparable results to biogasification from other biowaste. Biogas productivity from PPW was 2.3l_Nbiogas/l.day with an average CH₄ yield of 0.13-0.35m³/kgoTS input having 58% CH₄. The digestion resulted into H₂S removal from above LoQ to less than 230ppm with 81% VS removal at substrate OLR of 2.4kgoTS/m³.day input without sign of inhibition. It is estimated that 1.0m³biogas with 5hrs burning potential and equivalent to 3.5kg firewood can be produced from 10.2kg of PPW anaerobic digested. The resulting biogas production would complement household energy need thus conserve forest resources which are subjected to high deforestation rate estimated at 4.1kilotons of wood per day as fuelwood. Digestate resulting from the anaerobic reactors would make valuable fertilizer to urban agriculture thus enhance soil fertility and productivity. Based on these advantages, the study recommends anaerobic digestion as sustainable and best management strategy for handling PPW and possibly adaptable to other organic waste substrates as a means of improving living situation of the urban poor in Dar es Salaam.

Keywords: potato peels waste; enteropathogens; hygienization; composting; two-phase biogasification

Zusammenfassung

Die Entsorgung organischer Abfälle mittels nachhaltiger und umweltfreundlicher Verfahren, wie landwirtschaftliche Verwertung, Kompostierung oder Biogasproduktion werden verstärkt gefördert. Jedoch begünstigen diese Entsorgungsstrategien die Entwicklung und Verbreitung resistenter, pathogener Mikroorganismen und bergen somit ein erhöhtes Gesundheitsrisiko für Mensch und Tier. Um der erwarteten globalen Ausbreitung potenziell pathogener Keime wirkungsvoll zu begegnen, sind daher Verfahren und Strategien von gesteigertem Interesse. Ziel dieser Arbeit war es, die Sterilisationsrate pathogener Mikroorganismen sowohl bei konventioneller Behandlung zu untersuchen als auch alternative Hygienisierungsverfahren zu entwickeln und deren Wirksamkeit hinsichtlich der Eliminierung pathogener Organismen in organischem Abfall zu überprüfen. Darüber hinaus wurde die Tauglichkeit biologischer Verwertungsverfahren am Beispiel von Kartoffelschalen (potato peels waste, PPW), als alternativer Entsorgungsweg für Kartoffelabfälle des Einzugsgebiets der Stadt Dar es Salaam analysiert.

Die Untersuchungen von kaum behandeltem organischem Abfall ergab eine hohe Kontaminationsrate durch enteropathogene Mikroorganismen. In den ausgewerteten Proben wurden bis zu $6,3 \log \text{KBE/g}$ nachgewiesen. Im umgebenden Bodenbereich und im Sickerwasser beliefen sich die Zahlen auf $6,5 \log \text{KBE/g}$ bzw. $7,8 \log \text{KBE/g}$. Bei Inaktivierungsversuchen mit simuliertem organischem Abfall (PPW angereichert mit enteropathogenen Mikroorganismen: *Escherichia coli* und *Salmonella senftenbergensis*, Feuchtigkeit von 77% w/w) in einem aeroben geschlossenen Kompostierungsbehälter wurden Zeiträume mit niedrigen Temperaturen und anaeroben Verhältnissen beobachtet. Folglich ist diese Art der Entsorgung nicht geeignet, die angereicherten pathogenen Bakterien wirkungsvoll zu eliminieren. Ein weiter Versuch mittels Zwei-Phasen-anaerob Faulung in einem kontinuierlich gerührten Tank führte zu gleichen Resultaten. Trotz einer Aufenthaltszeit des Abfalls von über 72 Stunden, konnten auch bei dieser Versuchsanordnung die Konzentration der zugesetzten pathogenen Organismen nicht effektiv gesenkt werden. Diese besorgniserregenden Resultate machen deutlich, dass eine Vor- und/oder Nachbehandlung organischer Abfälle für eine sichere Entsorgung unumgänglich ist. Für die Entwicklung alternativer Hygienisierungsverfahren wurden im Vorfeld herkömmliche Methoden, wie Konditionierung mit Säure bzw. Kalk und

Pasteurisierung des Abfalls hinsichtlich ihres Hygienisierungseffektes analysiert und ausgewertet. Sowohl eine Säurebehandlung (pH 4; 5 Tage) des organischen Abfalls als auch eine Kalkbehandlung (pH 11 – 12; 1 Tag) führte zu einem umfassenden Absterben der zugesetzten pathogenen Keime (*E. coli* and *S. senftenbergensis*).

Die Hygenisierung bei einer Betriebstemperatur von 65°C für 30 Minuten erbrachten eine effektive Sterilisationsrate von >1log Zyklus-Sterberate der zugesetzten Organismen mit einem Z-Wert von 11°C für *S. senftenbergensis*. Dieses Ergebnis bestätigt sowohl die Vorgaben der amerikanischen Umweltschutzbehörde (US-EPA/625/R-92/013) als auch der Europäischen Union (Regulations (EC) No. 208/2006), welche ein Temperaturregime von 70°C für 30 Minuten bzw. 70°C für 60 Minuten vorgeben. Darüber hinaus wurde die Wirksamkeit alternativer Hygenisierungsmethoden, wie UV-Bestrahlung und passiver solarer Erhitzung (PSH) untersucht. Versuchsdurchführung und erhaltene Sterilisationsraten werden in den Kapiteln 2 bis 6 vorgestellt. Hinsichtlich der Gegebenheiten in Entwicklungsländern (kaum vorhandene Sachmittel, geforderten Unabhängigkeit der Energiequelle, niedrige Betriebskosten sowie vorherrschende tropische klimatische Verhältnisse mit hinreichender Sonnenscheindauer) wird PSH als das umweltfreundlichste und nachhaltigste prä-/ und post-Hygenisierungsverfahren empfohlen.

Biologische Verwertungsmöglichkeiten von Kartoffelschalen (PPW) wurden mit verschiedenen Kompostierungsverfahren untersucht. Die Kompostierung im geschlossenen Kompostierungsbehälter von PPW mit Gartenabfällen im Verhältnis von 6:1 ergab aus mikrobiologischer Sicht einen sicheren und qualitativ hochwertigen Kompost. Ausgestattet mit erhöhtem CO₂-Rücklauf zeigte die mesophile/thermophile Zwei-Phasen Vergärung von PPW vergleichbare Resultate wie eine Faulung mit konventionellen organischem Abfall. Die Biogasproduktion basierend auf PPW betrug 2,3L_Nbiogas/L*Tag mit einem durchschnittlichen Methanertrag von 58% bei 0,13-0,35m³/kgoTSEingang. Die Faulung hatte zur Folge, dass die H₂S Konzentration sank, von jenseits der Bestimmungsgrenze zu weniger als 230ppm mit 81% organischem Feststoffrückhalt bei einer Beschickungsrate von 2,4kg Raumbelastung*m³oTS ohne Anzeichen von Hemmung. 1,0 m³ Biogas entspricht einer potenziellen Brenndauer von 5 Stunden. Dies ist gleichzusetzen mit 3,5 kg Feuerholz und äquivalent zu 10,2 kg PPW aus anaerober Faulung. Das gewonnene Biogas würde zur Deckung des häuslichen Energiebedarfs beitragen und auf diese Weise erheblich die Nutzung der schwindenden

Waldreserven eindämmen (4,1kilotonnen Feuerholz/Tag). Zusätzlich stellt der verbleibende Gärrest einen wertvollen Dünger dar und trüge dazu bei, die Landwirtschaft in ländlichen Gebieten entscheidend zu verbessern. Als Ergebnis der vorliegenden Analysen wird die anaerobe Faulung als aussichtsreichste Strategie für eine nachhaltige Verwertung der anfallenden Kartoffelschalen in Dar es Salaam empfohlen. Möglichweise kann das Verfahren auch auf andere organische Abfälle übertragen werden. Das entwickelte Verfahren trägt dazu bei, die Lebenssituation der armen städtischen Bevölkerung deutlich zu verbessern.

Schlagwörter: Kartoffelschalenabfall; enteropathogene Mikroorganismen; Hygienisierung; Kompostierung; Zwei-Phasen-Biogasproduktion

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List of abbreviations/ acronyms

AD	Anaerobic digestion
ARU	Ardhi University
BSI	British Standards Institution
CFU/g	colony forming unit per gram
CSTR	continuous stirred-tank reactor
CV	Coefficient of variation
DF	Dilution factor
FM	Fresh material
HRT	Hydraulic retention time
LoQ	Limit of quantification
LPG	Liquefied petroleum gas
MMAD	mesophilic/mesophilic anaerobic digestion
MTAD	mesophilic/thermophilic anaerobic digestion
n	Number of samples
n/a	Not analyzed
OLR	Organic loading rate
oTS	Organic Total Solids
PPW	Potatoes peels waste
rpm	rotational per minutes
TOM	Tradition open market
Tsh.	Tanzanian Shillings (currency)
UNEP	United Nations Environment Programme
WEEE	Waste electric electronic equipment
WTP	Willingness to pay

Glossary of terms

biowaste	<ul style="list-style-type: none">• biodegradable organic waste that is capable of undergoing anaerobic or aerobic decomposition.
biotreatment	<ul style="list-style-type: none">• aerobic or anaerobic process that use microorganisms to biodegrade biowaste.
compost	<ul style="list-style-type: none">• humus-like, biologically stable, hygienized, free from odours having soil-like characteristics and rich in organic matter resulting from aerobic decomposition of biowaste used as organic fertilizer.
digestate	<ul style="list-style-type: none">• nutrient-rich hygienized final product (effluent) resulting from anaerobic digestion of biowaste used as soil conditioner.
biogas	<ul style="list-style-type: none">• a product from anaerobic digestion of biowaste mainly composed of gases mixture in which methane and carbon dioxide are predominant.
hygienization	<ul style="list-style-type: none">• process /treatment(s) or combination thereof applied to biowaste aiming at killing pathogens of public health significance to a level that the risk of carrying disease to human and environment in connection with further treatment, trade and use is minimized.
pathogen	<ul style="list-style-type: none">• potentially infectious microorganism that causes illness and/or infection in human, plants and/or animals directly or through contaminated environmental components.
producer	<ul style="list-style-type: none">• person in a biotreatment facility responsible for carrying out biological treatments of biowaste.
fluence	<ul style="list-style-type: none">• total radiant power of all wave lengths passing from all directions through an infinitesimal sphere of cross-sectional area as function of intensity (W/m^2) and exposure time (s).

1 Introduction

1.1 General overview of the research

This PhD Thesis is a compilation of both empirical studies and field survey carried out between October 2013 and July 2016 at Brandenburg University of Technology, Cottbus-Senftenberg, Germany and Dar es Salaam city in Tanzania. The bold idea behind this study is to advocate proper management of municipal solid waste (MSW) addressing challenges associated with its poor management in general. More emphasis was on possible strategies for achieving biosecurity in biodegradable organic fraction otherwise known as biowaste with potential for recycling and recovery into compost, digestate and biogas through biological treatments. Improper management of biowaste attract pathogens and therefore the use of such contaminated biowaste as feedstock or substrate during composting and anaerobic digestion process pose threats of spreading infectious diseases of public health significance and also contamination to the environment and its components (soil, water and air). This opens therefore possibility of incorporating potentially pathogenic microorganisms into food chain through irrigation and/or application of unhygienized compost and digestate into croplands and animal grazing fields.

In this study, laboratory-scale based biowaste hygienization experiments were carried out using potato (*Solanum tuberosum* L.) peels simulated to contaminated biowaste through artificial spiking with *Escherichia coli* (*E. coli*) and *Salmonella enteric* serover Senftenberg (scientifically known as *Salmonella senftenbergensis* (*S. senftenbergensis*)). In this thesis, feasibility of managing environment by making use of potato peels as waste resource for composting and anaerobic digestion has been studied and the results documented as part of best management strategies, restoration of soil essential nutrients, recovery of energy in the form of biogas, ensure city sanitation, minimize the volume of waste to be disposed of and more important curb the outbreak of refuse based diseases thus protection to the public health. The field survey successfully quantified and qualified the peels generated in Dar es Salaam and investigated feasibility for their biotreatment for biogas and compost, analyzed and confirmed the prevalence and levels of enteropathogens contamination in

biowaste haphazardly dumped in open dumpsites and waste transfer stations as well as subsequent contamination in leachate and soil upon which the biowaste are dumped.

Similarly, the performance and enteropathogens die-off extent achievable in conventional biological treatment i.e. biowaste composting based on In-vessel composting technology and anaerobic digestion based on two-phase anaerobic digestion systems (mesophilic/mesophilic reactor and mesophilic/thermophilic reactor) at inactivating *E. coli* and *S. senftenbergensis* have been investigated. Of paramount to this research was to achieve total biosecurity during biological treatments through hygienization thus prevent the risks associated with producing unhygienized compost/digestate for our farms. The diverse in temperature-time regimes as specified in various specifications developed by both national and regional regulatory bodies advocating hygienization of biowaste through pasteurization were examined and the process efficacy at inducing pathogens die-off validated. A number of hygienization options: acid/alkali conditioning, passive solar heating and ultraviolet radiation have been studied as alternative to pasteurization process. The application procedures of each option at inactivating pathogens in contaminated biowaste are now documented and their performance verified. To this end, some of the early findings from this research have been published in international peer-reviewed journals, some are still under review and other manuscripts are on preparation for public dissemination through scientific conferences and symposia.

1.2 Background and Study Motivation

While the world accept composting and anaerobic digestion as strategies to sustainably managing and handling of the biodegradable waste fraction, it is unfortunate that malfunction of systems such as combined sewer, poor management and/or inefficient treatments applied to other waste streams such as wastewaters [1,2], biomedical waste [3,4] and industrial generated wastes [5] can contaminate the biowaste. This cross contamination not only pose an invariable biosecurity risks to MSW handlers during collection, transportation and treatment of MSW but also limit the possibility of resource recovery as well as use of the resulting by-products namely compost and digestate [6–8]. This is due to the involving threats of contamination and spreading of the contaminants including toxic chemicals, organic contaminants [9], inorganic contaminants (heavy

metals and metalloids) [10] and potentially pathogenic bacteria [11–13]. For many years now, outbreaks of food-borne illness and fatality cases in many parts of the world are being reported in connection with potentially pathogens such as *Salmonella spp.*, *Listeria monocytogens*, *Shigella spp.*, some enterotoxigenic *E. coli* and many other enteropathogens of public health concern [14–18]. Consumption of fresh produce, partially cooked or improperly blanched agricultural produce grown on farmlands that has been preconditioned with unhygienized compost/digestate produced from home or less monitored biotreatment facilities as illustrated in Figure 1.1 after [19–22] being the major named contributing factor.

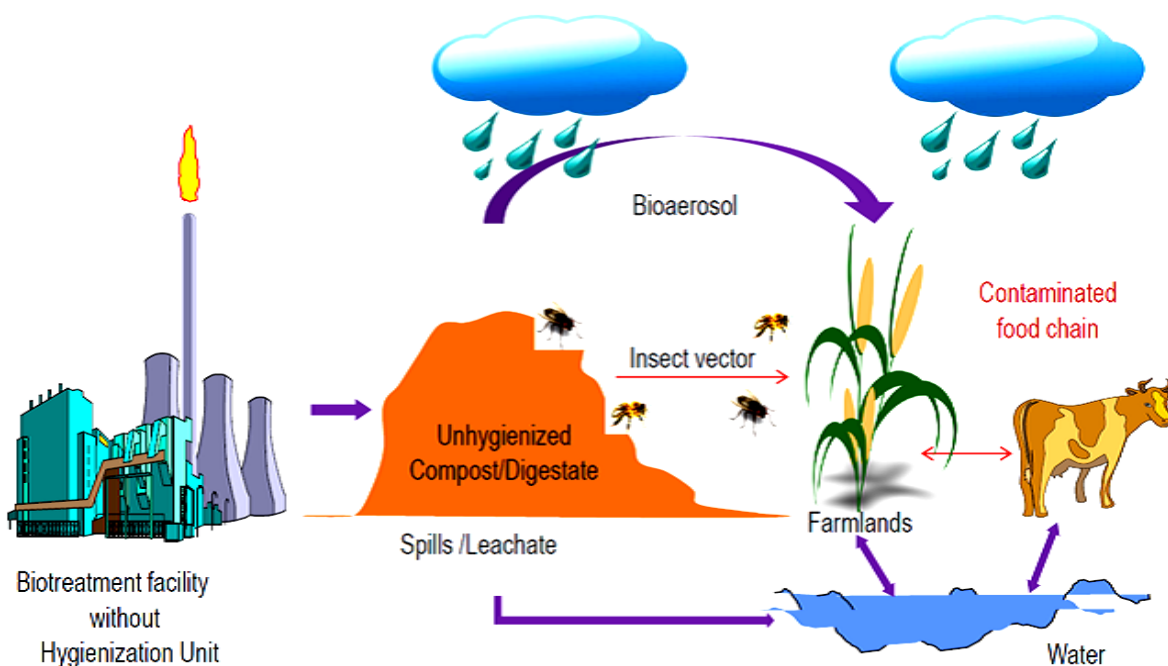


Figure 1.1: Illustration of pathogens contamination to the environment and food chain

Biotreatment facilities operating without hygienization unit generally expose the workers into risk of microbial contamination while the unhygienized compost and/or digestate produced create a contamination cycle to the environment. Upon transportation through air (bioaerosol), insects and rainfall, the unhygienized byproducts become sources of infection for instance to crop fields, live stock grazing sites, air and water bodies.

In some areas, the practice of applying rotten garbage collected from dumpsite for mulching and soil conditioner with the intension of optimizing soil properties result into spreading of infectious agents as well. These constitute health risks to humans, plants and animals which stir-up high morbidity and mortality rates particularly in third-countries thereby exacerbate the poverty rate among the community.

According to DG ENV.A.2/LM [23], hygienization entails treatment applied to pathogens contaminated biowaste during composting or anaerobic digestion with the aims of complete inactivation of the pathogens to a level that risk of carrying disease in connection with further treatment, trade and use is minimized. Therefore, in line with the third Post-millennium 2015 to 2030 Sustainable Development Goals (SDG 3) which promote good health and well being, and also the SDG 7 which advocates ensuring access to affordable, reliable sustainable and modern energy for all, sustainably management of biowaste in order to maximize agro-produce and scale-up energy production by incorporating bioenergy in the energy-mix thus combat desertification, halt both biodiversity and reverse land degradation is considered to be the right choice only if all safety conditions during and after biotreatment processes are exercised and met.

The motivation behind carrying this research was therefore to study the hygienization efficacy of the conventional biological treatments of biowaste and contribute alternative hygienization options that could be used to curb the threats associated with spreading of pathogenic microorganisms for achieving total biosafety during biotreatment processes, the environment and general public in line with the EU commission proposal through SANCO 2632/2634:2005 which recommends the use of any hygienization alternative before or after biotreatment process provided there is a demonstrated pathogens reduction of up to 3log-cycle. In this scientific work, potatoes (*Solanum tuberosum* L.) peel waste was used as feedstock and substrate for biotreatment representing the treatment challenging pathogens contaminated organic waste fractions with high moisture content.

1.3 Research Problem

Presently, various composting and anaerobic digestion systems ranging from decentralized and technically-simple to complicated commercial facilities treating biodegradable fractions of MSW are in operation. However, given their mode of operation to global evolution and amplification of resistant pathogen strains in biowaste, these conventional biotreatment systems prove inefficiency to fully inactivate some of the pathogenic microorganisms. In most cases, compost and digestate produced as final products of biotreatment still contain potentially pathogenic microorganisms viable and above the infectious doze passed the biotreatment process unaffected. This obliged some regulatory bodies to mandate installation of pasteurization unit as part of the biotreatment facilities, and formulated some temperature-time specifications as guide to be met by the biotreatment facilities. However, not all biological treatment facilities comply due to the fact that application of high heat in anaerobic digestion process can facilitate methane oxidation and the fact that thermal treatment necessitates energy use that can be expensive and most of the time unavailable in places with energy constraints such as low-income countries in particular. In addition, the existence of numerous proposed temperature-time regimes with no scientific acknowledged proof on producing microbiological safe compost and digestate has raised many biosecurity concerns which this research ought to answer:

- i- Are the existing temperature-time regimes specified in some national guidelines promising enough at completely inactivating pathogenic microorganisms in the biowaste and the resulting biotreatment by-products?
- ii- Is there possibility of validating and harmonizing the existing temperature-time specifications to universal microbicidal regime for safety and quality assurance in compost and digestate?
- iii- Apart from pasteurization, is there possibility of introducing other affordable, easy-to-use with low empirical know-how hygienization alternative(s) for inactivation of pathogenic microorganisms in biowaste yet with uncompromised biosecurity to the end users?

1.4 Aims and objectives of the research

The main aims of this research were to advocate biosecurity during biotreatment of biowaste through hygienization, introduce alternative hygienization options and in addition, investigate the feasibility for biotreatment of potato peels waste as management option for the potato peels produced in Dar es Salaam, Tanzania. The objectives of the research were to:

- i. Investigate occurrence and levels of enteropathogen contamination in local MSW and use it as benchmark for laboratory simulation of contaminated biowaste.
- ii. Determine performance of conventional biotreatment methods at inactivating enteropathogens from biowaste.
- iii. Determine hygienization efficacy of existing options, validate temperature-time regimes based on batch pasteurization, introduce alternative hygienization means and study their hygienization capacity on biowaste and byproducts.
- iv. Determine the potential biotreatment of potato peels waste as means to properly manage this increasingly organic waste fraction in Dar es Salaam.
- v. Quantify potato peels waste generation in Dar es Salaam and qualify the fraction readily available for biotreatments.

1.5 Preview of Chapters in this Thesis

This thesis is accomplished in nine chapters. Due to the specificity of each experiment carried out which in most cases required unrelated set of equipment, chapters in this thesis are organized based on the objectives of the research. Chapter 1, 2 and 3 address challenges associated with management of MSW and strategies toward safe recovery of resources. Specifically, Chapter 1 presents an overview of the study giving general introduction and rationale of the research. Included to this chapter are description of the problem and the objectives of the research. Chapter 2 presents theoretical background of the existing global challenges associated with MSW generation and management.

Similarly, the role by which biowaste could complement in solving the challenges related to food and energy insecurity whereby Chapter 3 presents the extent of pathogens contamination on environment by portraying population of enterobacteria in biowaste, soil and leachate the levels that were used as benchmark for laboratory simulation of contaminated biowaste.

Simulation of pathogens contaminated biowaste for subsequent laboratory and pilot-scale experiment is covered in chapter 4 while chapter 5 is dedicated on investigation of the extent of pathogens die-off that would be achieved using the selected conventional methods of composting and anaerobic digestion. Chapter 6 presents performance of hygienization option at eliminating enteropathogens from contaminated biowaste. In this chapter, efficacy of existing options in biowaste hygienization process namely acid/lime conditioning and pasteurization at inactivating enteropathogens have been studied and the latter validated. Similarly, alternative options including UVC radiation and passive solar heating were identified and their efficacy at effecting elimination of *E. coli* and *S. senftenbergensis* have been studied and fully documented. Chapter 7 is dedicated to the practical aspects on co-composting of fresh potato peels waste with yard-waste to final compost using In-vessel composting system and biogasification of the fresh peels using two-phase mesophilic/thermophilic anaerobic digestion. Equally, the description of the process control, microbiological safety of the resulting compost and/digestate, proportion of biogas and methane at the quality of households cooking energy requirement are also covered here.

Chapter 8 explores feasibility of co-composting and anaerobic digestion of potato peels waste as a means of proper managing of this waste resource and improve living situation of urban poor in Dar es Salaam city. In this chapter, results from field survey in Dar es Salaam based on quantification of potato peels readily available for biotreatment process and assurance of sustainable availability of substrate is presented. Lastly in this report are the general conclusions and recommendations given in Chapter 9. Following to this chapter are the cited references and appendices.

2 Theoretical Background

2.1 Global production and challenges in managing municipal solid waste

All over the world, food and energy security are the most pressing demands for humanity in as much the world population keeps on increasing [24,25]. With the challenge of feeding the ever growing population from the fixed agricultural land, factors such as decrease in agriculture production due to soil infertility and bad agricultural practices are turning most of cropland areas into barren lands and respectively reduce the size of farmlands [26–28]. Lands setting for dumpsites and landfills to accommodate the ever increasingly MSW equally reduce land that would be used for other purposes including agriculture. This simply means low food production per unit of cultivated land thus necessitates usage of thousands of tones of inorganic fertilizers for busting soil fertility in every farming season. On the other hand, the world demand for energy has resulted into an over exploitation of forest resources and fossils which for millennia have served as the sources of energy for humankind [29]. Eventually the outputs have been destruction of biodiversity, increase in GHGs emission and the onset of climate change [30–32]. Proper management of MSW in particular the biodegradable organic fraction could be the input and output of the whole process without underestimating the role of protecting public health from refuse based diseases which are weakening and/or reducing the human workforce day after the other especially in developing countries [33].

Municipal solid waste comprises byproduct of society daily material consumption or system redundancy [34] which are part and parcel of our everyday consumerism such as food scraps, product packaging, yard-waste, wood, textile, plastics, leather, commercial and institutional waste (papers, food processing byproducts, glass, metals, hazardous waste, plastics), waste originating from construction and demolition (bricks, steel, wood, concrete), waste from municipal services (market waste, tree trimming, street cleaning, landscaping), waste from treatment plants (residual sludge, treatment plant wastes) and the like [26,35,36]. In terms of composition (see Figure 2.1), MSW varies greatly and depends on geographical zones, economic status, cultural habits, waste management approaches and consumer preferences [37]. Proportionally between 30% and 70% of the world generated MSW is the biodegradable organic fraction [26,38] which has the

potential for biotreatments into renewable green energy (biogas) and organic fertilizer/soil conditioner (compost and digestate). The World Bank and UNEP report of year 2012 on global status of MSW (see Table 2-1) project a double increase in generation of MSW from the current 1.3 billion tons/year by 2025 [39]. Unless otherwise, this quantity of MSW implies a continual expenditure of tax payer’s money in the course of daily waste management involving routine collection, handling and transportation to final disposal.

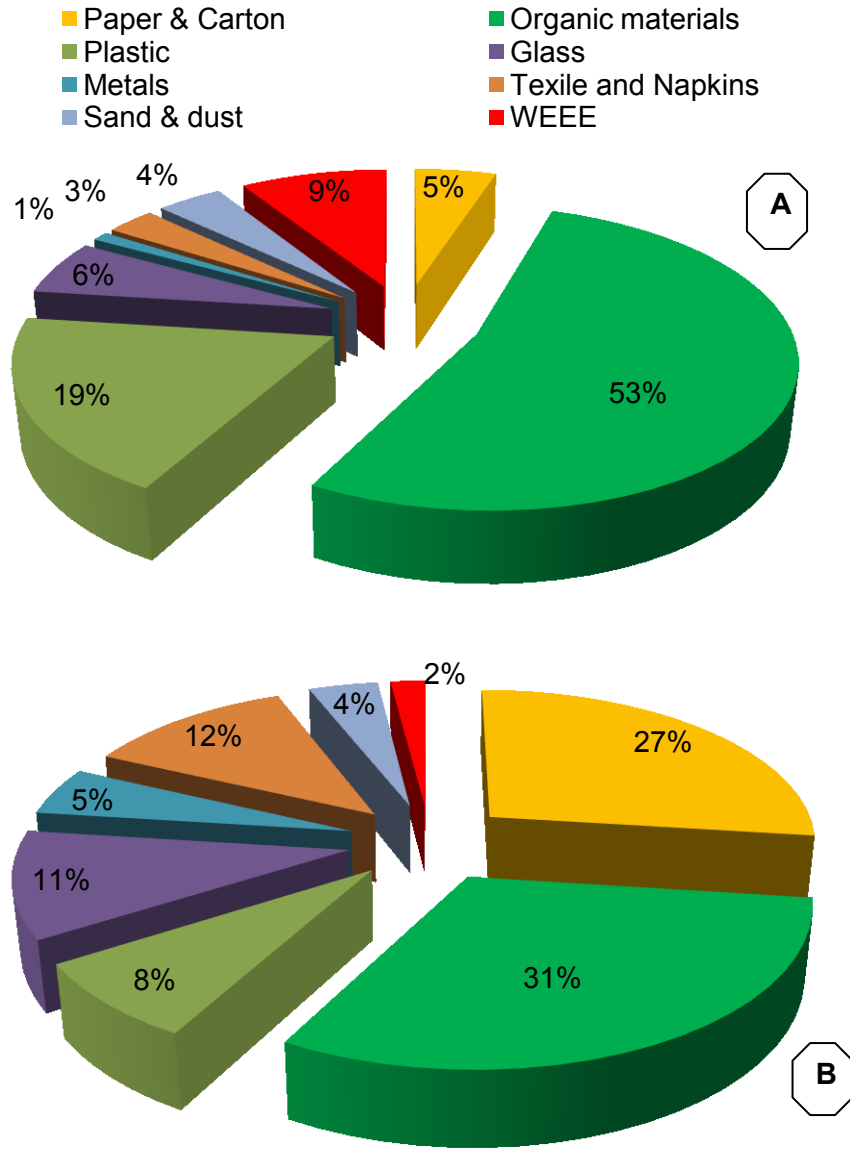


Figure 2.1: MSW composition for low-income countries (A) and industrialized countries (B)

Source: Karak et al.,[26], Troschinetz and Mihelcic [36], Okot-Okumu [37]

2.2 Interlink of poor waste management to poverty

Solid waste management stands to be a challenge and a daily demanding task to waste management authorities in both developed and developing countries (see Picture 2-1). In many cities of developing countries, increasing waste generation and poor community attitude towards management of waste seem to have outweighed the budget and waste management strategies of most MSW management authorities [40] exclusively focused on waste collection, transportation to disposal sites without any form of treatment [7]. In this regard, waste management is named among the sectors that consume large portion of national budget directly in the course of waste handling or indirectly in the course of remediating environmental components and preventing recurring diseases outbreaks.



(i) Dresden, Germany



(ii) Dar es Salaam, Tanzania

Picture 2-1: MSW collection as part of daily waste management process

Existing of legal and illegal established open dumping sites and even poorly managed landfills play as potential sources of cross contamination of zoonotic diseases from both domestic and wild animals scavenging on piles of poorly managed MSW composed of commingled and contaminated market waste, waste from hospitals and food leftovers (see Picture 2-2). Groups at very high risks of contracting infections are the MSW handlers, waste pickers and biowaste treatment facility workers normally operating without or with minimum personal protection equipment as well as families living closer to dumpsite owing to their long time exposure to the contaminants.



Picture 2-2: Scavengers activities at an open dumpsite in Tanzania: waste picking and pigs feeding on dumped biowaste

Photo courtesy: Mwaikono, 2014

There exists therefore a strong connection between poor waste management to poverty (see Figure 2.2). In many developing countries, changing consumers eating habits and high population connected to an increase in rural-urban migrants with expectations of employment and better life in the cities are named among the key factors resulted into increase in MSW generation, poor waste management and degradation of natural resources bases. Poor attitude and practices towards waste management among city inhabitants are the reasons behind haphazardly dumping of waste any-where and any-how. Lack of funds to enable MSW authorities adequately maintain the waste disposal sites and service vehicles used in waste collecting leads to piles of MSW at transfer station thus worsening the situation and cities outlook day by day [41,42]. Uncollected waste undergo natural aerobic decomposition giving out gasses mainly CO_2 and CH_4 which are GHGs which are the input and output to nowadays experienced weather variability and climate change.

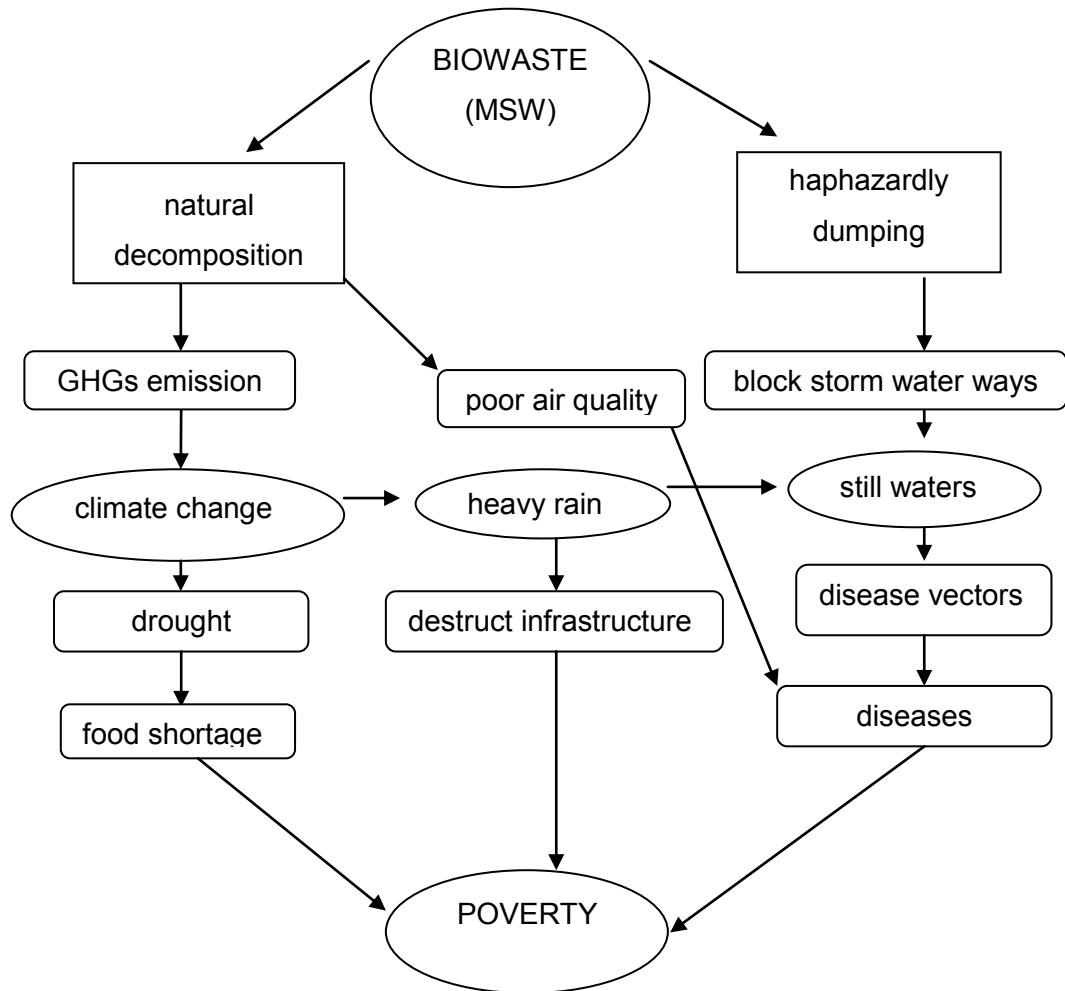


Figure 2.2: Interplay model of evolving threats following poor management of biowaste

Mismanagement of waste result into outbreak of refuse based diseases as well as increases in vectors and small animals whose survival and life cycles depend on waste. In addition, production of nuisance odour and leachate from decomposing organic waste cause pollution to air and contamination of water bodies respectively. Commingled dumping makes biowaste a good source of infectious microorganism and hazardous due to toxic chemicals and heavy metals. Groups of pathogenic microorganisms characterized in biowaste includes viruses, yeast, protozoa, bacteria, filamentous fungi and helminthes [19,43] play a major role in the transmission of infections for instance enteric bacterial including Salmonellosis (*Salmonella spp*), dysentery (*Shigella spp*), diarrhea (*E. coli*), cholera (*V. cholera*) and so on. Species of fungus, protozoa (*Enteromeba histolytica*,

Giardia intestinalis) and many enteric viruses have been reported in soil upon which the contaminated biowaste or its final product after biotreatment have been disposed of [44]. The risk of contamination is even higher in places where farmers collect rotten garbage from the dumpsite and/or less managed landfills and use for farm application as compost manure. Intestinal worms, schistosomiasis and skin diseases caused by fungal infection are common diseases reported to contribute more than 70% of all daily medical cases to most developing countries. Flies and other flying insects spread microbial pathogens from various dirty places including illegal dumps to food sold at the tradition open markets (TOM) leading into outbreak of diseases like cholera, diarrhea and dysentery. These challenges and the like are the precursor to the reduced life expectancy, low productivity intertwined to increased man-days spent by people attending hospitals altogether contributing to the low-pace of development within the respective communities [45].

2.3 Drivers to biotreatment and use of biowaste

In solid waste management hierarchy, composting and anaerobic digestion technologies are intertwined to waste recycling and energy recovery respectively and have been accepted as environmental friendly resorts for managing the organic fraction of municipal solid wastes and eliminate negative environmental impacts [26,46–49] capable at transforming the biowaste from its useless state into valuable commodities including compost, biogas and digestate. The observed pace and promotion towards using biowaste as resource has been connected to a number of driving forces which include:

- a) introduction of strict environmental regulations by various national and regional regulatory bodies on proper management of MSW,
- b) decrease in land space for landfills and increase in community awareness on the environmental menace of dumping raw waste
- c) promotion of waste-to-energy [25,50] and waste-to-wealth initiatives [51],
- d) increasing cost and challenges in managing solid waste that has resulted into environmental pollution and health risks to human, animals and plant,
- e) the need for alternative sources of energy caused by escalating cost of energy and fuel shortage that developed during and after the second world war,

- f) the need to conserve forest resources and biodiversity following global threats on the climate, environment and its components [52].
- g) The need to scale up sustainable bioenergy share in the energy-mix and fertilizer demand on agricultural lands.

2.4 The role of biowaste in solving food and energy insecurity

Three options for biological treatment of biowaste namely composting, anaerobic digestion [53] and mechanical-biological treatment (MBT) exist [52,54]. The first two present the common and well long known and applied cost effective options in both developing and developed countries. The efficiency in volume reduction of biodegradable waste, reduction in pathogenic microorganisms, stabilization of the final waste products, recycling of biowaste into organic fertilizer and recovery of renewable energy source in the form of biogas [26] makes the options attractive. Although MBT equally present several advantages including reduction of the volume of waste and stabilize emission in the final byproduct, operation specificity of the technique makes it presently less popular with exception to European countries like Germany, Austria, Italy and the like [55]. Composting of biowaste results into compost which according to European Commission [23], it is a stable, sanitized and humus-like material rich in organic matter, free from pathogens and offensive odors which is applied on farmlands and grazing areas as organic fertilizer. Anaerobic digestion of biowaste results into biogas and the effluent after substrate digestion called digestate. Biogas which mainly composed of methane and carbon dioxide is a promising renewable source of energy with potential at replacing natural gas and petroleum based fuel, minimize the use of fossil and wood-based fuels and therefore accommodate the global shortage of energy [56]. Digestate is the nutrient-rich final products of anaerobic digestion highly in mineral nitrogen (N) in the plant usable form of ammonia and soil organic matter used as soil conditioner thus improves soil properties by replenishing nutrients into infertile agricultural land, close the yield gap and therefore increase soil productivity [57]. Weiland [58] pointed out that biogas in developed countries like Germany, Sweden, Denmark, Belgium, Spain and possibly elsewhere, is used interchangeably with natural gas in many applications including running of the natural gas vehicles, heating and electricity generation. The ongoing global increase in natural gas vehicles reflect the wealth upon which biowaste have a potential role to play [59].

2.5 Abatement of contaminants in biowaste

2.5.1 Source segregation of waste

The first economical and environmental viable option towards achieving contamination free biowaste therefore is to practice strictly separation of waste at source. High percent of the disposed of MSW could be recycled and thus reused provided they are not exposed to contaminants, though this is not always the case. Source segregation of waste entails a holistic separation of waste into different components related to their specific treatment to ensure correct treatment and disposal route thus reduce cross contamination [60]. Chemical contamination including heavy metals and metalloids for instance can be avoided in biowaste through strictly segregation of waste at the point of generation. In addition, preventing dippers from disposed of in the same container with other household wastes definitely prevents introduction of possible disease agents' in particular enteric pathogens in the biowaste. Industrialized countries such as Germany, Japan, USA and the like have achieved great results in harnessing biogas and organic fertilizer through composting and anaerobic digestion of source segregated MSW. As an example, Germany uses plastic containers of various colors and size with labels and clear instructions (see Picture 2-3) to facilitate source segregation of respective waste components namely paper waste, glasses, packaging materials and residual waste.



Picture 2-3: Waste segregation containers at BTU Cottbus, Germany

Source segregation of waste plays an important role in increasing resource recovery rate and ensuring high quality biowaste for subsequent biological treatments. It also minimizes contaminations on biowaste and thus protects human, animals, vegetation and environmental components (water, air and soil) from contamination. Inorganic contaminants for instance and other undesirable pollutants are much reduced in compost and digestate produced from source segregated biowaste compared to those prepared from generally mechanical sorting of biowaste from commingled disposed-off MSW. EU member states through Article 22 of the Waste Framework Directives 2008/98/EC [61] for instance have been directed to strictly undertake separation of waste with a view of composting and anaerobic digestion, and treat the biowastes in such a way that high level of environmental protection is fulfilled and carry out land application or any other use of the byproducts from biotreatment facilities only after confirming their microbiological safety. It was from this understanding that in their biotreatment facilities Sweden only accept biowaste separated at source as substrates for biogas production [62] as a means to curb spreading of pathogens and other chemical contaminants in the course of land application of the resulting final digestate.

2.5.2 Microbial biodegradation of contaminants in biowaste

With exception to microbial pathogens, the accumulation and toxicity of chemical contaminants both organic and inorganic depend on the dosage [63] and some of the organic contaminants in biowaste can be neutralized through microbial activities thus converted into harmless products. For instance, Bagge [13] expounded that useful microbes during anaerobic decomposition of biowaste are capable at converting polychlorinated biphenyls (PCB) into harmless byproducts. Studies explain and confirm that even though the levels of inorganic contaminants such as trace metals and metalloids could be equally high in the biowaste, the environmental impact of such contaminants depend on the fraction that is reversibly incorporated in the solid matrix and available for remobilization down the soil profile through soil solution, or be up-taken via plant roots and become incorporated into food chain [64]. The use of well established mechanisms such as phytoremediation, soil stabilization options through sorption and desorption reduces trace metals and metalloids from leaching as well as their bioavailability and therefore their adverse impacts [65].

2.5.3 Pasteurization of biowaste

Pasteurization which is a mild heat-treatment below 100°C at a specified time uses both external and internal generated heat to kill pathogens in contaminated biowaste and can be effectively used either before or after biological treatment of biowaste. Similarly, depending on the pathogens type and contamination levels, conventional biotreatments are promising methods for abatement of microbial contaminants in the biowaste. In addition, pasteurization not only elimination of pathogenic microorganisms but also expose fermentable sugars of the biowaste and render the biowaste more decomposable by reducing the lignin content of lignocellulosic on biowaste [66] thus increase hydrolysis during digestion therefore enhance recovery of bioenergy.

2.5.4 Incineration of biowaste

Total combustion of the collected biowaste separately or in commingled form with other waste components for energy entails the production of microbial free ashes though with no guarantee to attenuation of toxic substances as well as inorganic substances (heavy metals). Incineration with or without energy recovery presents one disadvantage which is the possibility of emissions to the environment hazardous substances in case of burning the waste in uncontrolled conditions.

2.6 Distribution of pathogens in the environment

The presence of potentially infectious pathogenic microorganisms (bacteria, virus, parasites, fungi) and many vectors in biowaste have been extensively researched, isolated, confirmed and documented [67–71]. This is simply because biowaste are characterized by having high contents of biodegradable organic matter, acceptable range of carbon-nitrogen ratio, enough moisture content, compromised pH with good proportion of macro and micro-nutrients thus provides enough nutrients, conducive environment and attraction to potentially pathogenic microorganisms for their reproduction/growth and proliferation [26,72]. Application of unhygienized biowaste on arable land, forestry, play grounds and gardens as direct mulching, soil conditioner or organic fertilizer through its byproduct (compost and digestate) even after biological treatments is known to be the major route of disseminating the pathogens into ecosystem [73]. Gerba et al., [11] ranks

pets' excreta as the largest contributor of *Salmonella spp*, human enteroviruses and protozoa in MSW collected in cities and towns. Traditional open markets that deal with livestock business greatly contribute pathogens in market wastes. Other deadly contributors of enteric pathogens in MSW include: septage and human open defecation, poorly managed wastewater, pit latrine emptying behavior, septic tanks flooding out [74] and comingled dumping of diapers together with household waste [33].

2.7 The concept of biowaste hygienization

For many past years, more attention concerning the risks of pathogens contamination was on composts prepared from sewage sludge. However, findings reveal the presence of high levels of pathogenic microorganisms in the unhygienized composts and digestate produced from biowaste both locally and commercially presenting possibility of contamination and cause global health threats to both human and the environment [22]. Studies reported among others Muhondwa et al., [75], Déportes et al., [19], Astals et al., [76], Jones and Martin [77], Aitken et al., [78] and possibly more, confirm that even after aerobic composting or anaerobic digestion of pathogens contaminated biowaste, the biosafety of compost and digestate remain in limbo and cannot be guaranteed unless confirmed through laboratory tests owing to the possibility of the pathogens to survive the treatments. Islam et al., [79], Cordano and Jacquet [80], Lemunier et al., [14], Solomon et al., [81] and Beuchat [82] report the entry of pathogens from contaminated organic soil amended with compost and digestate into food chain through consumption of fresh agriculture produce (vegetable and fruits) including lettuce, carrots, tomatoes, salad, radishes, etc.

Therefore owing to their high pathogenic contaminations level, biowaste for biological treatment and the byproducts (compost and digestate) unless they are treated and well hygienized are not recommended for use in any way for instance enhancing soil fertility, support organic farming¹ nor for any land application so as to curb the risks of spreading potentially pathogenic microorganisms. Over the past years, it has been wrongly taken that composting and anaerobic digestion process inevitably eliminates all pathogenic

¹ <http://www.farmsreach.com/welcome/wp-content/uploads/2014/11/CompostingOrganicFarms.pdf>

(retrieved on May 12, 2015)

microorganisms during treatment of the biowaste. Lately research revealed that there evolving unique and more resistant pathogens species with public health significance that passes anaerobic and aerobic decomposition process unaffected. Continual application of such un-hygienized compost and/or digestate in arable lands and garden result into spread of pathogens and therefore greatly pose health risks to consumers of fresh produce such as leafy vegetables, fruits, tubers and root crops [44]. Hygienization as earlier on stated is intentionally applied to ensure that the potentially pathogens are completely inactivated or reduced to a level that is no longer potent thereof, thus making a hygienization unit an integral part of every biological treatment facility not to be bypassed.

2.8 Hygienization versus pre-biotreatment processes

In bioresource technology, a number of biological treatments based studies particular on aerobic composting and anaerobic digestion of biowaste, see Kim et al., [83], Luste et al., [84], Mosier et al., [85] and Zaher et al., [86] refer mechanical processes such as particle size reduction, pulping with gravity separation, waste sorting, drum screening and addition of water in sample substrates before the process as pre-treatment methods. To avoid such confusion in terminologies since these processes do not necessarily induce any form of pathogens inactivation in biowaste, the word pre-biotreatment hygienization has been therefore used in this research to mean those processes/treatment(s) or combination thereof applied to biowaste aiming at inactivation (complete die-off) of pathogenic microorganisms of public health significance to a level that the risk of carrying disease to human and environment in connection with further treatment, trade and use is minimized.

3 Prevalence and levels of enteropathogens in poorly managed biowaste

3.1 Enteropathogens benchmarking for simulation of contaminated biowaste

The investigation of enteropathogens prevalence and levels in contaminated biowaste was conducted in Dar es Salaam, Tanzania between June and August 2014. The essence of undertaking this investigation was to confirm the existence of pathogens and their levels in biowaste collected from commingled dumped MSW and therefore use this pre-established microbial population from the field as point of reference during laboratory simulation of contaminated biowaste that was used for investigating hygienization efficacy of various options described in subsequent chapters of this thesis. The family *Enterobacteriaceae* consisting gram-negative rods, mostly excreted micro-flora from the intestinal tract was preferred as it consists most of potentially infectious strains also referred to as marker bacteria (see Figure 3.1) commonly isolated from environmental samples and mainly highly abundance in poorly managed biowaste.

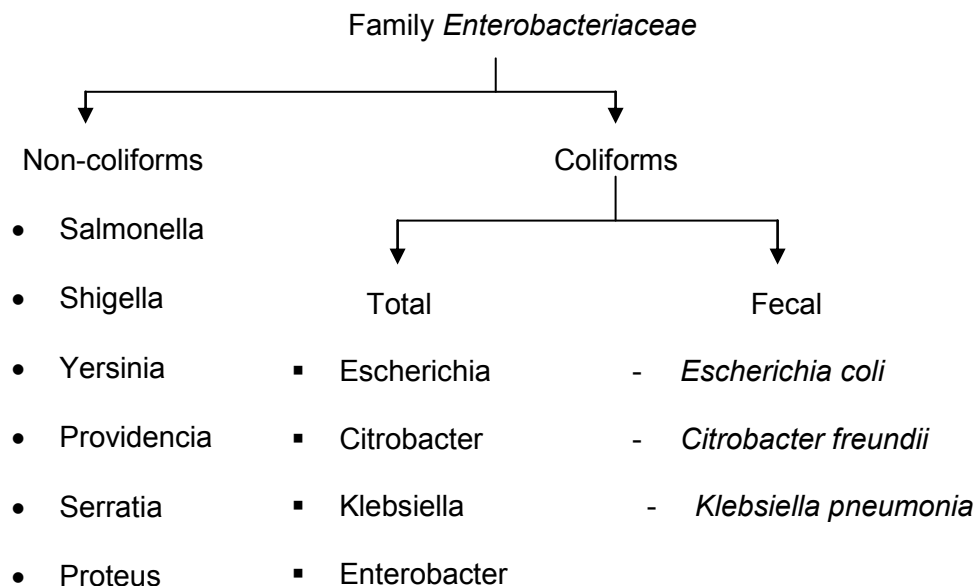


Figure 3.1: Predominant members of the family *Enterobacteriaceae*

Source: Adapted from Hach [87].

These enteropathogens exhibit facultative anaerobes as their mode of respiration, with most of the members having the ability to reduce nitrates to nitrites and ferment glucose. However, the scope of this bacteriological experiment did not include specie identification, of interest to this research were *Salmonella spp* and *E. coli* given their low infectious doze, high mortality rate up to 15% of the infected people [59] and that they are globally accepted as surrogate and marker microorganisms.

3.2 Local sampling site investigation: Description of a case study area

Dar es Salaam is an administrative capital of Tanzania and one of the fast growing cities within Sub-Saharan Africa. Apart from being the home town of the author, Dar es Salaam city was selected for exploratory case study because of its economic potential and the fact that it is used as reliable market for agricultural products and goods from other regions within and outside of Tanzania. Administratively the city is divided into three municipalities: Kinondoni, Ilala and Temeke (Figure 3.2). The responsibility of the MSW authorities among others include the day-to-day collection, handling, transportation and disposal of the MSW generated in 73 Wards composed of more than 276 Streets [88].



Figure 3.2: Map of Dar es Salaam city showing Municipality boundaries

3.3 Materials and Methods

3.3.1 Sample collection points

Sample of MSW for benchmarking of enteropathogens contamination was collected from three locations of Dar es Salaam city that include: Pugu municipal dumpsite (Picture 3-1) and transfer station of two open markets namely Manzese and Buguruni (Picture 3-2).



Leachate drains at Pugu dumpsite



Dippers disposed at the dumpsite

Picture 3-1: Existing MSW management situation at Pugu municipal dumpsite



Transfer station, Buguruni market



Leachate draining from Manzese market

Picture 3-2: Existing situation at open markets in Dar es Salaam city

Pugu municipal dump is an open dump situated in Ilala municipality and collects all MSW generated in the city [40]. Manzese and Buguruni market are within Kinondoni and Ilala municipality respectively. The two markets represent activities almost similar to all other open markets in Dar es Salaam city and have many things in common. Firstly, the two markets have waste transfer station situated within market premises where commingled market waste are dumped and stays for some days awaiting collection by the MSW collectors for disposal. Secondly, both markets are famous for selling common fresh agricultural food items (fruits and vegetables), live animals: rabbits and fowls (ducks, pigeons and chicken both indigenous and exotic breeds). Therefore it is obvious that the droppings and cleansing water from the fowls and chicken are disposed together with other market waste at the waste transfer points where the pathogens multiply given the nutrient composition in the disposed biowaste and the conducive temperature ranging 28-32°C in Dar es Salaam which accelerate both growth and multiplication of the pathogens.

3.3.2 Sampling process

Three samples composed of decomposing organic waste (rotten fruits, peels and vegetables), leachate draining from decomposing organic food materials and drainage from chicken processing area as well as soil sample surrounding the waste transfer station at the two markets were collected. Similarly, leachate, soil on the bank of channels collecting the leachate and visible decomposing organic fractions at the Pugu dumpsite were sampled. A total of 9 pre-sterilized glass bottles of capacity 100mL were used in carrying and handling the collected sample portions.

About 75g of each sample was aseptically collected in individual bottle using pre-sterilized laboratory spoon in which three bottles (Picture 3-3) were used for each sampling location. The bottles containing samples were capped and preserved in ice cooler before and during transportation to the microbiological laboratory of Sokoine University of Agriculture (SUA). The samples were refrigerated at 4°C until bacteriological analysis on the following day.



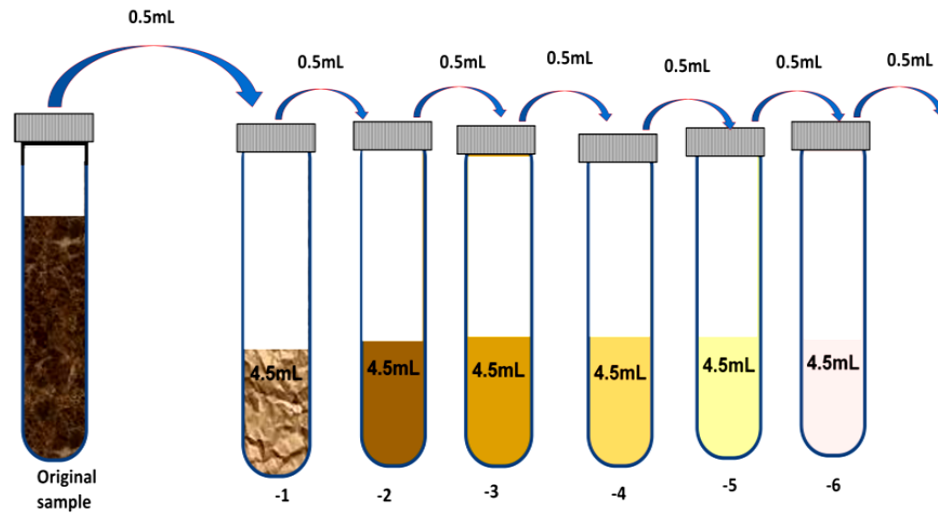
Picture 3-3: Sample collected for enteropathogens quantification

3.3.3 Sample analysis and enumeration of enteric pathogens

In this experiment, disposable petri plates (90mm diameter) were purchased from P&K Laboratory equipment store in Morogoro town. Other consumables such as glassware sterile pipette, test tubes, golf stick and reagents including sterile physiological saline (0.86-0.90% NaCl) were prepared in laboratory. Nutrient Broth (Oxoid) for cultivation and pre-enrichment of microorganism strains having the following composition: Enzymatic Digest of Gelatin 5g, Beef extract 3g and final pH 6.8 ± 0.2 at 25°C was used. MacConkey Agar (Oxoid, CM 0115, UK) with the following typical composition: Peptone 20g/L, Lactose 10g/L, Bile salt mixture 5g/L, sodium chloride 5g/L, neutral red 0.03g/L, Crystal violet 0.001g/L, Agar 15g/L and final pH 7.1 ± 0.2 at 25°C was used for isolation, growing and differentiation of Gram-negative enterobacteria based on their ability to ferment lactose (e.g. *E. coli*) or inability to ferment lactose (e.g. *Salmonella spp*).

3.3.4 Serial dilution and spread plating of sample

Approximately 1g of each well homogenized sample was weighed on analytical balance (Mettler Toledo-OHaus AB204 with a precision of $\pm 0.1\text{mg}$). The sample were then dissolved in Phosphate Buffered Saline solution (PBS, Mumbai, India) in order to isolate bacteria cells from sample matrices and at the same time provide isotonic medium between the bacteria cells and the surrounding for cell integrity and viability. The PBS solution had the following typical composition: Dipotassium Hydrogen Phosphate 1.1g/L, Potassium Dihydrogen Phosphate 0.32g/L, Sodium Chloride 8.5g/L making a final pH 7.2 ± 0.1 at 25°C . The samples were mechanically agitated for 3min and thereafter 0.5mL of the prepared sample was transferred into pipette containing 4.5mL sterile physiological saline solution and serially decimal diluted (10-folds) from 0 to 10^{-6} (see Picture 3-4) as described in Ewell [89].



Picture 3-4: Serial 10-folds decimal dilution of samples prior to plating

3.3.5 Identification and enumeration of viable colony units

In this investigation, 100 μ L of the appropriate serially decimal diluted samples were spread in duplicates on petri-plates with solidified MacConkey agar that was prepared on the previous day. The petri-plates were incubated (Hareaus/SUA/B066/FVM) at 37°C for 18-24hrs. Counts of viable bacteria colony (see Picture 3-5) based on spread plate technique as described in Ewell [89], Spencer et al., [90] and Benson [91] was employed.



Picture 3-5: Author enumerating enteropathogens cultured on MacConkey Agar, SUA.

The use of MacConkey Agar was helpful in isolation and discrimination of gram-negative enteric bacteria from the observable colour of the resulting colony units and swarming characteristic exhibited by some members of the family *Enterobacteriaceae*. Depending on their ability to ferment lactose (the fermentable carbohydrate ingredient in the Agar), the colony units resulted were colorless, pink and some showing swarming character as stated in Acumedia [92]. From the observed colony units it was concluded that the colorless colony units were *Salmonella spp* due to their inability to ferment lactose, the pink colony units were confirmed to be *E. coli*; their ability to ferment lactose caused acidic media that masked the pinkish colour, while the pink swarming colony units were identified to be *Klebsiella pneumonia*. After enumeration, the petri-plates with viable bacteria colony units were re-incubated for longer time up to 48hrs to confirm the maximum possible bacteria population existing in the sample. To all petri-plates, longer incubation time did not alter the counts; therefore the first bacteria colony counts were taken for calculation of the colony forming units (CFU) per gram of sample applying equation (3.1) below as specified in ISO 16649-2:2001. In this investigation, the results in CFU/g values were reported using scientific notation of writing large numbers and conveniently reported in \log_{10} units as $\log\text{CFU/g}$.

$$CFU / g = \frac{\sum a}{V(mL) * (n_1 + 0.1 n_2)} * DF \quad (3.1)$$

Whereby:

$\sum a$ =sum of the CFU counted in the MacConkey Agar petri-plate,

n_1 =number of petri-plates retained at the first dilution,

n_2 =number of petri-plate retained in case of duplicates,

V =volume of inoculum (100 μ L) used on each petri-plate after dilution²

DF= dilution factor corresponding to the retained test-tube.

² 1mL of water at STP is assumed equal to 1g

3.4 Results and Discussion

3.4.1 Enteric bacterial contamination levels in poorly managed MSW

Figure 3.3 presents summary of results (Confidence interval (CFU/g) \pm Uncertainty for 95% confidence level, n=3) for levels of total *Enterobacteriaceae* contamination in biowaste, leachate and soil sample collected from MSW transfer station at Buguruni and Manzese traditional open markets and Pugu dumpsite in Dar es Salaam city.

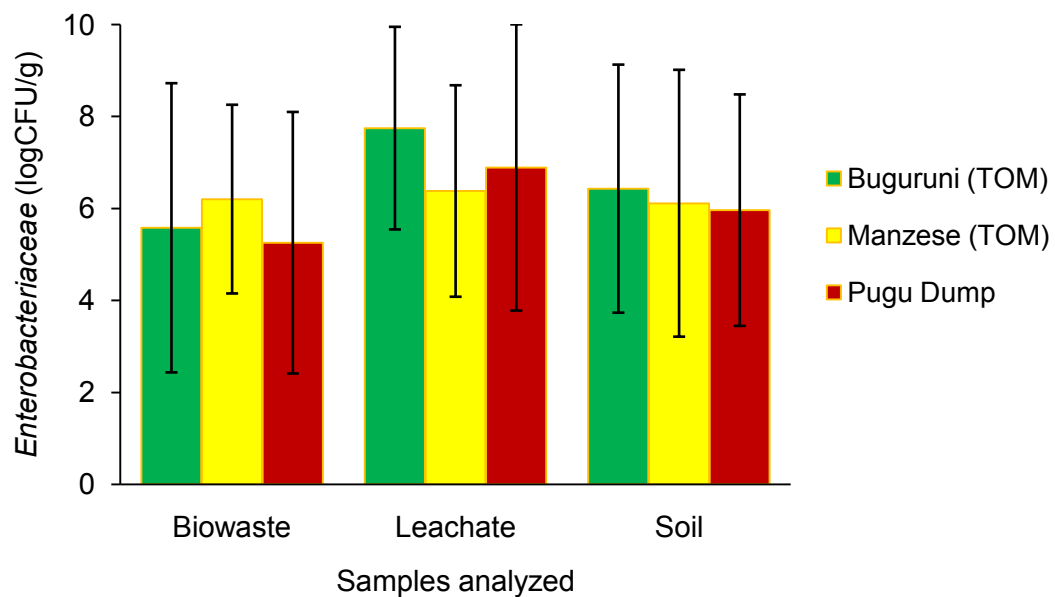


Figure 3.3: *Enterobacteriaceae* contamination levels used for benchmarking laboratory simulation of contaminated biowaste

As expected, there were high contamination levels of *Enterobacteriaceae* observed in all three sampling points i.e. Pugu dumpsite as well as the transfer stations at Manzese and Buguruni markets. Similar to the earlier reported findings in Bagge [13], Aderemi et al., [67] and Vital et al.,[93]. Once shed out from their hosts, enterobacteria have the ability to colonize and live in soil, organic matter fractions and in water for about 56 days according to Johansson et al.,[94]. On average, enterobacteria contamination levels (CFU/g) from Buguruni, Manzese and Pugu dump were determined as follows: in biowaste, total viable *Enterobacteriaceae* ranged between 1.8×10^5 CFU/g to 1.6×10^6 CFU/g in which Pugu dump presented the lowest contamination whereby

Manzese TOM exhibited the highest level of *Enterobacteriaceae* contamination. Enterobacteria contamination level in soil from the three sampling points ranged between 9.2×10^5 CFU/g to 2.7×10^6 CFU/g in which Pugu dump presented lower contamination whereby Buguruni TOM exhibited the highest level of *Enterobacteriaceae* while the contamination level in leachate from all three sampling points ranged between 2.4×10^5 CFU/g to 5.2×10^7 CFU/g in which Manzese TOM presented the lowest contamination and Buguruni TOM exhibited the highest level. These *Enterobacteriaceae* contamination levels were used as benchmark during laboratory preparation of study sample in which potato peels waste was artificially spiked with *E. coli* and *S. senftenbergensis* for the simulation of the contaminated biowaste.

The results in this study agree with those in Aderemi et al., [67] who observed *Enterobacteriaceae* contamination up to 1.26×10^5 CFU/mL equivalent to 5.2logCFU/g in leachate collected from unlined MSW landfill in Lagos, Nigeria. Similarly, Lepeuple et al., [95] report between 6.0-7.3logCFU/g as the expectable range of bacterial contamination including (Total coliforms, Faecal coliforms and *Enterococci*) in biowaste. Flores-Tena et al., [96] report gram-negative bacteria contamination of 3.0×10^8 CFU/g equivalent to 8.5logCFU/g and 1.5×10^6 CFU/g equivalent to 6.2logCFU/g in soil and leachate samples respectively. In this investigation, leachate from all three sampling points showed high total *Enterobacteriaceae* contamination followed by soil sample. The possible explanation for comparatively lower contamination levels in soil and biowaste than in leachate could be caused by percolation of bacteria cells down the soil profile since the sampling was done just after the end of rain season. The rainfall equally neutralized the leachate thus optimized conditions for the proliferation of bacteria. Lower *Enterobacteriaceae* counts in the OFMSW collected from Pugu dumpsite could be influenced by the factor of time. The duration MSW stay at transfer station until collected transported until the time dumped greatly affect the life cycle of bacteria. In the environment outside their host for instance, *E. coli* which is the marker bacteria for others can possibly survive for not more than 60days [94] provided no new contamination takes place.

Thomas and Young [33] expounded that water and sanitation diseases such as cholera, Typhoid fever, skin infections, eye infections, malaria and filariasis are endemic in several cities of Tanzania including Dar es Salaam. The severity of the problem is even worse during rainy season due to poor sanitation. It is estimated that about 70-80% of the city

population lives in squatter settlements, use un-improved pit latrines and about 43,700 occupants equivalent to 1% lives without the necessary solid waste management infrastructures and sanitation options thus increasing threats to environmental and public health caused by the complex cross contamination. In most places of the city there exists illegal dumping sites which operate haphazardly thus increase contamination threat to soil, water sources both surface and ground waters.

Personal observation during sample collection process revealed that with exception to used plastic bottles, aluminum bottles and metals (see Picture 3-6) which are nowadays hard to be seen in streets of the city as they are raw materials demanded by the few existing recycling facilities, other MSW resources largely produced in the city including waste papers comprising 9% of MSW, biodegradable domestic and market garbage comprising 70-80% of biodegradable MSW [26,36], non-degradable thin carrier plastic bags and others comprising 9% of the MSW [37] are haphazardly disposed of untapped simply because to the general public, waste is considered as dirty and not as resource. This results into crude dumping of waste and mostly at illegal places.



Plastic bottles



Aluminium bottles/cans

Picture 3-6: Waste recycling in Dar es Salaam: collected bottles and cans for sell

This attitude of waste disposal in which waste is always disposed of in commingled with non-biodegradable materials (nylons, thin-plastic carrier bags, diapers) and plastic based medical wastes such as drip sets, gloves, bandages, urine bags, injections syringes, blades and blood bags negatively affect the possibility of using biowaste as resource.

3.5 Conclusive summary

High population of enteropathogens was confirmed in all samples drawn from three different sampling points that was for the pathogens benchmarking experiment thus confirmed the existence of a challenge as far as biological treatment of biowaste is concern. High population of enteropathogens observed simply indicates the possibility of finding other potentially infectious microorganisms and therefore an indicator of the magnitude of the challenges facing the biological treatment facilities. Unless treated, there exist high chances of incorporating these pathogens into food chain right from the garden following practicing urban agriculture in the city where some farmers make use of unhygienized rotten garbage from open dumps as bio-fertilizer into vegetable gardens or through domestic animals grazing around contaminated places such as dumpsites. Other routes of pathogens contamination could be right at the tradition open markets following the common practice of selling food items openly displayed at the interface of live animals some with compromised health status.

In support to the world's struggle to ensure food and energy security within dilapidated and infertile agricultural lands, as well as finding a solution to cut down the existing deforestation rate fueled in quest for energy, the biodegradable fraction of MSW becomes an input and output of the whole process. The greatest challenge of our time that prevents full utilization of the biowaste resource is the high levels of contamination and particularly from pathogenic microorganisms. In this study, generation of MSW and the magnitude of the microbiologic contamination risks to both public and the environment due to poor management of MSW were found threatening and directly linked to economic status of both individual and the community at large. The study anticipate that, if the waste management becomes an integral part of the day to day community life and the safety aspect of byproducts of biotreatment are strictly observed, then biowaste could be the suitable and sustainable resource in tackling food and energy insecurity which are among the major world threatening challenges through enhanced crop production by making use of compost manure and digestate, and the later tackled through biogas.

4 Simulation of Pathogen Contaminated Biowaste for Hygienization Studies

4.1 Enteropathogens contamination of potato peels waste

Potato peels waste a by-product from potatoes (*Solanum tuberosum* (L.) processing after peeling off the skin from the tubers were used as biomass for simulation of contaminated biowaste that was used as study sample in all subsequent investigation. The simulation of study sample used enteropathogens (*E. coli* and *S. senftenbergensis*) as contaminants on the biowaste for which biotreatment and hygienization experiments were to be applied, their performance at effecting pathogens die-off determined and evaluated.

The reasons behind choosing potato peels was that, firstly, in Dar es Salaam city, potato peels as part of the biodegradable fraction of MSW is produced at high quantity by chips processing vendors and partly as peels waste from kitchen at homes, restaurant, hotels, bars or elsewhere. Each year million metric tons of potato peels waste are generated [97] but ends up being disposed of with no promising strategy on how best and economically worthwhile could be fully utilized as input resource in the biological treatment. Secondly, potato peels contain high moisture which if not properly optimized would affect the biotreatment process and result into unhygienized and poor quality final products. Furthermore, this study promotes sustainable pro-poor growth through recovery of resources (biogas, compost and digestate) through biotreatments of potato peels waste with conformity to hygiene requirements for the feedstock/substrate, biotreatment process and the final byproducts being an input of the whole process.

4.2 Materials and Methods

4.2.1 Preparation of potato peels waste for laboratory simulation of bacteria contaminated biowaste

Fresh potato peels waste for the study were mainly obtained from Restaurant (*Schlodder and Schlodder GbR Kartoffelkiste*, Spremberger Str. 37, 03046 Cottbus) dealing with preparation of potatoes based recipes. In case of shortfall, addition peels were prepared through manual peeling of potatoes bought from tradition open market at Stadthalle,

03046 Cottbus. The formulation of study samples and simulation of contaminated biowaste was done as described in the following Figure 4.1.

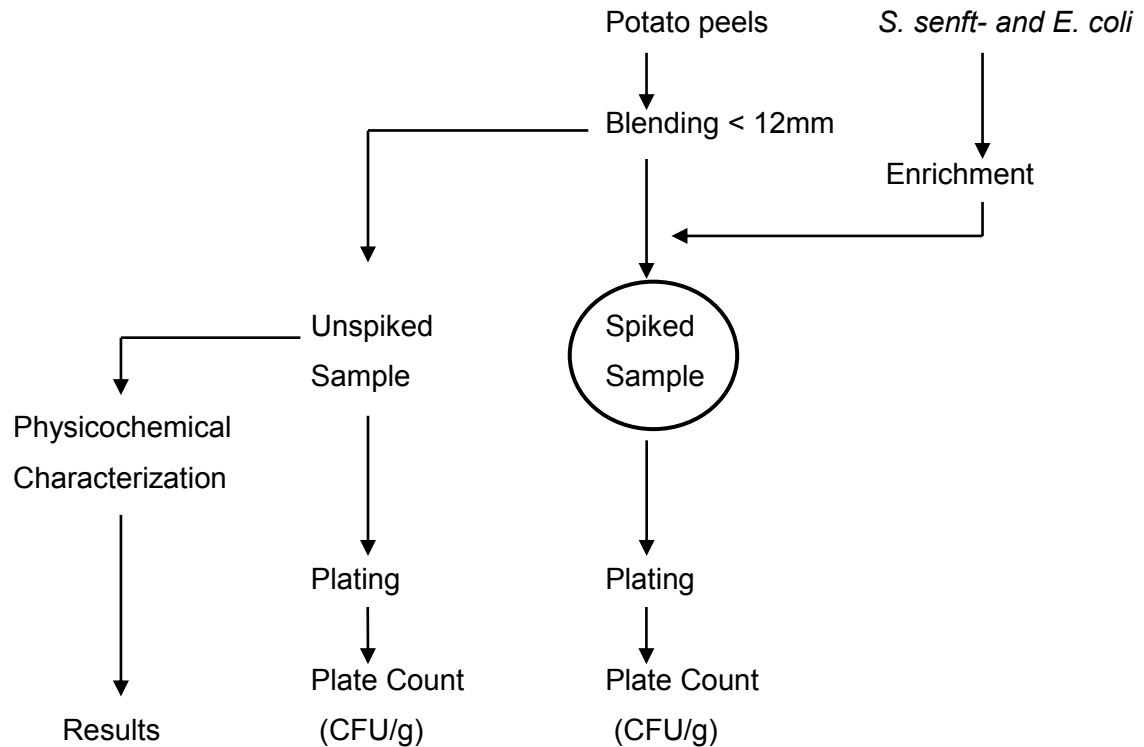


Figure 4.1: Layout for formulation of simulated study sample

To minimize sample heterogeneity, after received from the supplier before any treatment, fresh potato peels were firstly blended (Multipro compact blender) of 1.5L capacity to reduce the size to about 10-12mm as recommended in Regulation (EC) No. 208/2006 and SANCO 7066/2010. The sample of blended and homogenized potato peels was divided into two portions. One portion was analyzed for bacteriological contamination and physicochemical characteristics of the peels that reflect the potential of the potato peels for biotreatments prior to the spiking process. The other remained sample portion was used for simulation process in which the enriched *E. coli* and *S. senftenbergensis* were artificially spiked resulting into the so called contaminated biowaste.

4.2.2 Physicochemical analysis of potato peels prior to bacteria spiking

Suitability of materials as feedstock and substrate for biotreatment process depends much on its physical and chemical attributes. These attributes among others dictate process capability and efficiency at performing the required process. In all experiments that required physicochemical attributes of the feed materials, individual analysis were performed and the confidence intervals were derived based on the mean of results, standard deviation (sd), degree of freedom (k) number of repetitions (n) and students'-t for bilateral distribution at 95% level of confidence.

Determination of physicochemical attributes of potato peels waste was carried out to assess and fit the waste to disposal category and also affirm the waste attributes during investigation on feasibility of using peels as feedstock for aerobic composting and substrate for anaerobic digestion. The Germany legislation for waste dumping (Ordinance on environmentally Compatible Storage of Waste from Human Settlements and on Biological Waste Treatment Facilities (*AbfAbIV - Abfallablagerungsverordnung*³) was used for that purpose. In this experiment a number of parameters including: moisture content (Mc), total solids (TS), sample pH, ash content, organic matter content, total organic carbon (TOC), Nitrogen as TKN, carbon-nitrogen ratio, total chemical oxygen demand (COD) and Macronutrient content of the potato peels waste including: Calcium (Ca), Sodium (Na), Potassium (K), Phosphorus (P) and Magnesium (Mg) were determined. Samples for physicochemical characterization were analyzed according to Christensen et al.,[98] and *Bundesgütegemeinschaft Kompost eV*, (1994) as translated and described in Burkhardt [100].

4.2.2.1 Moisture and Total Solids determination

Crucibles for moisture determination were conditioned by drying them in an oven (Heraeus 230V 50/60 Hz class II, Germany) at 105°C for two hours followed by cooling in decicator. An approximate 50g mass of the pre-blended and homogenized PPW and compost was weighed in pre conditioned crucibles. Sample weighing was by difference without tare the analytical balance Model Adventurer Pro AV 313 (Max wt. 310±0.001g) in

³ <http://www.gesetze-im-internet.de/bundesrecht/abfablv/gesamt.pdf> (Accessed on April 21, 2014).

order to minimize systematic error and increase precision on results. The crucibles with sample were then dried at 105°C for 24hrs. Thereafter the sample moisture content (Mc) was determined using the following equation:

$$Mc(\%) = \left(\frac{Mass_{Sample} - Mass_{drysample}}{Mass_{Sample}} \right) * 100 \quad (4.1)$$

The individual result from the moisture content determination was used to calculate total solids (TS) in samples applying the following equation:

$$TS(\%) = \frac{Mass_{dry}}{Mass_{Sample}} * 100 \quad (4.2)$$

4.2.2.2 Determination of pH values

The pH of feedstock for anaerobic digestion, aerobic composting and co-composting was determined in water extract using 1:10 ratio. In this experiment, an approximately 20g of sample was mixed with 200mL water or 0.01M CaCl₂ solution. The solution of 0.01M CaCl₂ was used for PPW prior to aerobic composting and to the final compost while water was used for PPW to be used for all other hygienization options. In both cases before pH reading the samples were continually stirred using magnetic stirrers or a glass-rod for about 30min. The pH meter (WTW inoLab) was initially calibrated using buffer solutions pH 4.01 and pH 7.0 and thereafter the pH readings were taken at an interval of 5-10min.

4.2.2.3 Determination of ash contents

The percentage of the residual remains after completely ignition of sample in the muffle furnace at 550°C for 6 hours is described as ash content. Ash content in samples was calculated using the following expression:

$$Ash(\%) = \frac{Mass_{ash}}{Mass_{Sample}} * 100 \quad (4.3)$$

4.2.2.4 Determination of organic matter content

Sample organic matter also referred to as the sample volatile solids was determined as a difference between total solid and ash content of sample in the respective run. It was calculated using the following equation:

$$OM(\%) = \left(\frac{Mass_{dry\ sample}}{Mass_{Sample}} - \frac{Mass_{Ash}}{Mass_{Sample}} \right) * 100 \quad (4.4)$$

And therefore, the percentage of organic total solid from the Total solid (TS) was calculated applying the following equation:

$$oTS(\%TS) = \frac{oTS}{TS} * 100 \quad (4.5)$$

4.2.2.5 Determination of Total Organic Carbon

In this experiment, the infrared (IR) spectroscopy which works based on absorption of electromagnetic radiation within the infrared region of the spectrum was used. Thermal digestion of potato peels waste was carried out in ELTRA-high temperature oven (Robax, Germany) operated at 1100°C using oxygen gas flowing through Micro N/C measuring apparatus (Picture 4-1) as a carrier gas.



(i) Micro N/C Spectrometer



(ii) ELTRA-high temperature oven

Picture 4-1: Instrumentation for TOC determination

The resulting Carbon dioxide gas was purified by filters, dried, and flushed into the non-dispersive infrared (NDIR) detector. The detector is usually of Inorganic carbon determined by NDIR detection following the carbon dioxide formed by sample acidification and subsequent purging. In each respective experiment conducted, a sample of 0-20mg CaCO₃ was weighed for low measurement and another sample of 20-150mg CaCO₃ was weighed for high measurement range (Ballance Pioneer™ Ohaus 110±0.0001g, NJ, USA) in order to calibration a day factor applying the following equation:

$$F_{new} = (C_{st,real} / C_{st,m}) * F_{old} \quad (4.6)$$

Whereby:

F_{new} stands for new day factor,

$C_{st,real}$ stands for the real concentration of carbon in standard CaCO₃

$C_{st,m}$ stands for the average concentration of carbon measured in standard CaCO₃ and

F_{old} stands for the old day factor (during this determination was =1)

Total Organic Carbon (TOC) which is the difference between total carbon to total inorganic carbon was determined applying the following equation:

$$TOC_{g/kg} = TC_{g/kg} - TIC_{g/kg} \quad (4.7)$$

4.2.2.6 Determination of Total Kjeldahl Nitrogen

Kjeldahl apparatus for total nitrogen determination was used in this determination. The method is based on three steps including digestion, distillation and titration. Approximately 250mg of the sample was weighed then transferred into 250mL of digestion tube followed by addition of 15mL of concentrated H₂SO_{4(aq)}. One tube treated in the same way but without sample was used to give blank reading. Digestion was done by warming in the heating block until clear solution appeared. Then the test tubes were cooled down followed by 20 mL addition of distilled water. During distillation, 20mL of boric acid H₃BO₃ and approximate 0.5mL of indicator were added into 130mL Erlenmeyer flasks. Then the flasks were put on the distillation chamber and NaOH was added in surplus to elute ammonia. After distillation the flasks with distillate were taken for titration of ammonia (Picture 4-2).



Picture 4-2: Automatic titration of distillates for TKN determination

Values for total nitrogen content in potato peels waste and in finished products (compost and digestate) were determined using the following equation:

$$\text{TKN}(\%) = \left(\frac{\text{Vol}_{\text{mL}(\text{sample})} - \text{Vol}_{\text{mL}(\text{blank})}}{\text{Mass}_{\text{sample}}} \right) * K_N * 100 \quad (4.8)$$

Whereby:

$K_N = 1.4$ factor for nitrogen equivalent in mg for 1mL of acid used

$\text{Vol}_{\text{mL}, \text{S}}$ = represents amount of titer used for titrating sample

$\text{Vol}_{\text{mL}, \text{b}}$ = represents amount of acid used for titrating blank

4.2.2.7 Potato peels C/N ratio

The determination of Carbon to Nitrogen ratio of the potato peels waste was vital in order to know the proportion of nutrients to be supplied to the microorganism during composting process. Since total Kjeldahl nitrogen (TKN) is expressed in percentage (%), it was of the essence to convert total organic carbon (TOC) which is expressed in mg/kg by a division factor of 10. Then the ratio of carbon to nitrogen (C/N ratio) was calculated as per the following equation:

$$C / N_{\text{Ratio}} = \frac{\text{TOC}(\%)}{\text{TKN}(\%)} \quad (4.9)$$

4.2.2.8 Chemical Oxygen Demand

The chemical oxygen demand in the PP was determined in accordance of DIN 38414 Teil 9 methods. In this method, the peel were firstly dried at 60°C for 6 hrs followed by 10-15 minutes grinding at 10000 rpm grinder (Retsch ZM 1000, Germany) then sieved through 0.5mm to obtain sample powder. About 2g of the powder was mixed with 10mL water, 0.33M K₂Cr₂O₇ solution followed by 95-97% H₂SO₄ (behr DT 20). The mixture was cooked at 155°C for 2hrs in behr TRS 300 then titrated with 0.1M Ammonium (III) sulphite. The total chemical oxygen demand (COD) was determined as average of two determinations using the following expression:

$$COD_{(T)mg/g} = \frac{c * f}{Mass_{sample(g)}} * (V_T - V_B) \quad (4.10)$$

Whereby:

c = 0.33mole/L concentration of potassium dichromate solution

f = equivalent factor = 48g/M

V_T = titre volume of potassium dichromate solution used for sample

V_B = titre volume of potassium dichromate solution used for blank

4.2.2.9 Macronutrients contents in potato peels

The determination of specific trace elements was done using inductively coupled plasma atomic emission spectrometry (ICP-AES Model iCAP 6000, Thermo Scientific). In principle, the inductively coupled plasma excites the atoms and characteristic emission spectra that are produced are the ones related to specific elements [101]. In this study, the macronutrients essential for microorganism activity including calcium, magnesium, phosphorous, potassium and sodium were analyzed.

4.2.3 Acquisition, storage and bacteria enrichment for spiking potato peels

Strain of *E. coli* originally from Leibniz Institute, Inhoffen Straße Braunschweig, Germany that has been enriched and stock maintained in the laboratory of the Chair of Biotechnology for Water treatment of the BTU Cottbus-Senftenberg, and a pure culture of *S. senftenbergensis* supplied by the Institute of Bioanalysis, Environmental toxicology and Biotechnology (*Institut für Bioanalytik, Umwelt-Toxikologie und Biotechnologie*) IFB

Schiepziger Straße 35, Halle, Germany were used in this study. The choice for using *E. coli* and *S. senftenbergensis* was based on the fact that the two enterobacteria have specific and understandable detection methods. In addition, *S. senftenbergensis* has been named in EU 2000 working document on sludge as an indicator and surrogate microorganism that reflects conditions to be met for inactivation of other heat resistant species. High resistance to heat makes *S. senftenbergensis* the most difficult strain to eliminate. This supposes that whenever a certain hygienization option manages to completely inactivate this bacteria strain, the process will equally be effective to many other potentially infectious microorganisms including viruses, bacteria, parasites and worms that are commonly present in poorly managed biowaste. Other facts include:

- i) *Salmonella* and *E. coli* exist in different strains. Even though few enterotoxigenic *E. coli* exists, both *Salmonella* and *E. coli* are well known for ability of causing outbreaks of diseases such as cholera, bloody diarrhea, urinary tract infections (UTI) and typhoid fever [102,103] therefore potentially enteric pathogens to both human and animals.
- ii) Enteric pathogens generally are resistance to natural die-off even in environment with depletion of substrate [20]. The pathogens can long survive outside their host and live in waste and in environmental components including soil and water for not less than 50 days.
- iii) Germany Biowaste Ordinance, and SANCO7066/2010 both mention *S. senftenbergensis* as a suitable test/indicator organisms in hygienization based investigations while DG ENV.C2/BZ/tb and Regulations (EC) No. 208/2006 amending Regulations (EC) No. 1774/2002 set specifications for monitoring *Salmonella spp* and *E. coli* in compost/digestate to be applied on arable lands.
- iv) U.S-EPA Scientific Advisory Panel declares *Salmonella* and *E. coli* as enteropathogens of public health concern that should be monitored and that the use of compost or digestate on arable land should comply with the requirements for *E. coli* and *Salmonella spp* contaminations.

- v) Detection of both *Salmonella* and *E. coli* on samples confirm fecal contamination and therefore used as marker that signifies a greater chance of detecting other groups of pathogenic microorganisms [68].

The bacteria strains were preserved in refrigerator at 4°C for the whole research period except during subculture and enrichment experiments where strains were aseptically handled at room temperature 23.2±1.8°C. Microbial strains were individually enriched in Nutrient Broth (supplied by Carl Roth GmbH +Co. KG Schoemperlen, Karlsruhe) a non-selective enrichment medium having the following composition: Peptone from casein 3.5g/L, peptone from meat 2.5g/L, peptone from gelatin 2.5g/L, yeast extract 1.5g/L, sodium chloride 5.0g/L and pH 7.2±0.2. A smear loop (3mm diameter, 10µL) was sterilized on flame and allowed to stand for few seconds to cool down to room temperature. Enteropathogens enrichment was carried out as described in Benson [91] and Spencer et al., [90] in which the loop aseptically scooped a single isolated colony of bacteria culture from streaked petri-plate. Adapting quantitative surface plating technique [104], a uniform bacteria smear was inoculated in two test tubes containing Nutrient Broth (Carl Roth). The test tubes were then incubated (Memmert Incubator Model 600) overnight at 36°C.

4.2.4 Agar selection test for excellent bacteria growth response

A total of four culture media were used in this experiment, these include: (i) Endo Agar (SIFIN), a semi-selective agar specifically for the isolation and detection of *E. coli* having the following composition: Pancreatic Peptones (Meat, Gelatine) 7.7g/L, Yeast extract 2.3g/L, Lactose 10.0g/L, Dipotassium hydrogen phosphate 3.0g/L, Potassium dihydrogen phosphate 0.5g/L, New fuchsin 0.15g/L, Sodium sulfite 1.0g/L and Agar 10.0g/L with pH value 7.5±0.2 at 25°C. (ii) Salmonella Brilliance Agar (Oxoid), a highly selective agar for growing and identification of *Salmonella spp* having pH 7.3±0.1 at 25°C with the following composition: Inhibigen™mix 14g/L, Chromogenic Mix 25g/L and Agar 15g/L. (iii) Gassner Agar (Oxoid) also known as Water-Blue Metachrome Yellow Lactose Agar, medium for growing and isolation of pathogenic *Enterobacteriaceae* having pH 7.2±0.2 at 25°C with the following composition: Meat Peptone 7.0g/L, Sodium Chloride 5.0g/L, Lactose 50.0g/L, Metachrome Yellow 1.25g/L, Water Blue 0.625g/l and Agar 13.0g/L and (iv) Xylose lysine deoxycholate (XLD) agar (Carl Roth GmbH Karlsruhe), a medium for

detection and isolation of *Salmonella* and *Shigella* having the following typical composition: pH 7.4 ± 0.2 at 25°C and composed of Yeast extract powder 3.0g, NaCl 5.0g, Xylose 3.75g, Lactose 7.5g, Sucrose 7.5g, L-Lysine hydrochloride 5.0g, Sodium thiosulfate 6.8g, Iron (III) ammonium citrate 0.8g, Phenol red 0.008g, Sodium deoxycholate 1.0g and Agar 13.5g.

In this investigation, quadrant streak plate technique described in Katz [105] was used. The streaked petri-plates were incubated (Mettler Incubator Model 600) at 36°C for 24hrs. On the following day, the resulted bacterial colony units were observed thus confirming viability of enteropathogens strains. The petri-plates that showed well isolated bacterial strains were stored and used as seed for more enrichment of bacteria cells to be used for the laboratory simulation of contaminated biowaste.

4.2.5 Determination of bacteriological quality of potato peels before spiking

Analysis of bacterial contamination in potato peels to be used in subsequent experiments as simulated biowaste was done to assess if the peels are in any way contaminated with *E. coli* and *Salmonella spp* so that the initial bacterial population in the study sample can be quantified for easy monitoring of their die-off during hygienization process. This experiment was to avoid the presence of such pathogens in the original sample from interfering with the laboratory-established enteropathogens level following artificial spiking. In this experiment, a qualitative detection of the presence or absence of *E. coli* and *Salmonella spp* in the blended potato peels was carried out based on quadrant streaking plate technique as described in Katz [105]. In this method, disposable sterile petri-plates (90mm diameter) with solidified agar media, Endo agar and modified BGA prepared on the previous day were used. A wire loop was firstly sterilized on flame and then cooled to room temperature followed by aseptically scooping of a liquid part out of the blended potato peels. A smooth smear was done on the edge of the plate and then streaked across the solidified agar on the petri-plate at a time. The streaked petri-plates were incubated (Mettler Incubator Model 600) at 36°C overnight, the qualitative visual observation of bacteria viability was done after 24hrs.

4.2.6 Bacterial spiking of the blended potato peels: simulation process

Simulation of contaminated biowaste was done by artificially spiking of the peels with pre-enriched *E. coli* and *S. senftenbergensis* to every sample portion to be used in lab-scale or pilot scale experiments. The contamination levels was referenced to the benchmarked enterobacteria levels observed in the real organic fraction samples of MSW analyzed from Dar es Salaam City. The required weight of freshly blended potato peels for spiking process was in the ratio of 100:1w/v (peels to bacteria suspension). After spiking, the mixture were evenly homogenized using scoops or spoons in order to ensure an evenly distribution of the bacteria. The resulting samples after spiking were referred to as potato peels waste. Due to the fact that several laboratory and pilot-scale investigations were to be performed; there were several batches of potato peels waste without knowledge of botanical classification of the potato tubers. On that regards, some physicochemical characteristics of the studied samples within the research period might have slightly varied but with no impact on the simulation process.

4.2.7 Establishing enteropathogens level in the simulated biowaste

The confirmation of enteropathogens population in potato peels following spiking was a primary and an important experiment prior to any subsequent investigation. As a general procedure to this and all other experiments requiring estimation of pathogens population in this research, total bacteria count technique based on bacteria cultivation on petri-plate containing Endo agar and modified BGA was used. In all tests, serially (10-folds) dilution as earlier on described was adhered in which 0.5mL of the sample was drawn and transferred to 4.5mL of sterilized water (*leitungs wasse*) in the test tubes to make $\times 10^{-1}$ to 10^{-7} dilutions. From these dilutions, 100 μ L was drawn and surface spread on pre-prepared agar media followed by incubation of the plates at 36°C for 24 hrs. The contamination level of the simulated biowaste (potato peels waste) was confirmed through total bacteria enumeration on the following day.

4.2.8 Quantitatively determination of viable bacteria population

4.2.8.1 Microscopy enumeration method

Viable bacterial population density in cells per mL⁴ was determined using Fluorescent Microscope (NIKON Eclipse LV 100, Tokyo, Japan) for which three test samples i.e. dilution 10⁻¹, 10⁻² and 10⁻³ were made from each strain of bacteria suspension to avoid inducing factors such as microbial antagonism and formation of toxic compound during microbial propagation process that would hamper cell growth and multiplication.

From each sample, a volume of 0.1mL was mixed with 10µL of the live/dead dye solution (supplied from Probes Invitrogen, USA). In order to allow absorption and equilibration of the dye into bacterial cells for microscopy quantitative assay, the prepared solution was left to stand for 10min then exactly 110µL of the formed solution was filtered through Isopore membrane filters of 2.1mm diameter. Viable bacteria population was studied under epi-fluorescent light beam with 2 phases contrast at 20-fold magnifications of the Microscope. Depending on their spatial distribution of live bacteria cells on Syto9 and propidium iodide, the viable cells were counted using NIS Element BR 3.1 software. The counting was either done on a full screen picture or on segments of 50x50mm² or 100x100mm². The final total bacteria cells viable for spiking process were calculated according to the mathematic expression (4.11) below:

$$\text{Cells/ mL} = \frac{\sum \text{Cells}}{\text{Segment/ Area}(\text{mm}^2)} * \text{Filter}(\text{mm}^2) \frac{1}{\text{Sample}(\text{mL})} * \frac{1}{DF} \quad (4.11)$$

4.2.8.2 Plate enumeration of viable bacteria colony units

Agar Preparation

In this research, two culture media, Endo Agar recommended in ISO 16649-2: 2001(E) and Brilliant Green Agar (modified BGA) recommended in ISO 6579: 2002(E) were preferred in growing and differentiating *E. coli* and *S. senftenbergensis* for all hygienization based experiments. Modified BGA a highly selective medium for plating of

⁴ 1mL of H₂O at 40°C is equivalent to 1g

Salmonella spp (other than *Salmonella typhi*) had the following typical composition: 'Lab-Lemco' powder 5.0g/L, Peptone 10.0g/L, Yeast extract 3.0g/L, Disodium hydrogen phosphate 1.0g/L, sodium dihydrogen phosphate 0.6g/L, Lactose 10.0g/L, sucrose 10.0g/L, Phenol red 0.09g/l, Brilliant green 0.0047g/L, Agar 12.0g/L with a final pH of 6.9±0.2). The two media had excellent results observed following preliminary experiments in terms of viability, colony units recovery, selectivity, colony colors development thus easily for discrimination from other bacteria strains following strong inhibition. In addition, reliability in terms of suppliers' and affordable price were the reasons behind selecting these two media for all investigations done in this research. The agar were prepared and used in accordance with manufacturer instructions and observing all aseptic techniques. The agar enough to be used for any particular experiment was prepared and poured unto disposable petri plates to solidify overnight before use in the following day.

Total bacteria counts

In this approach, total bacteria count [89] was used for enumeration of bacteria colony units. The methods used was as described in Benson [91] and Spencer et al., [90]. In this investigation, aerobically enrichment of the indicators bacteria was separately done overnight in Nutrient Broth (Carl Roth GmbH, 36°C) followed by plating 100µL of the resulting culture on either Endo agar or modified BGA and incubated overnight at 36°C. Preparation of Nutrient Broth for enrichment of bacteria strains and agar media for total bacteria counts were done according to manufacturer's instructions. Although Endo agar could support *S. senftenbergensis*, but because of its background colour which mimics much to that of *S. senftenbergensis* colony units and that the reported percent recovery of *Salmonella spp* on Endo Agar is lower. The use of modified BGA was therefore for confirmatory of *S. senftenbergensis* while Endo Agar was specifically for confirmatory of *E. coli*. The resulting culture of bacteria from the enrichment experiment after incubation were mixed followed by 10-folds serially decimal diluted in a range 10^{-1} to 10^{-7} as earlier on described (see clause 3.3.4) and the dilutions from 10^{-3} to 10^{-7} were aseptically spread on petri-plates at a volume of 100µL per plate and 2 plates per dilution (one petri-plate with Endo agar and the other with modified BGA). The cells mass grown as bacteria colony units were enumerated and visual identification on the plate was done after incubation (Incubator Model 600, Memmert) of the petri-plates at 36°C for 24hrs. Total bacteria count technique as earlier on described in clause 3.3.5 above was used. The

resulting viable colony units on the plates where germination was more than 300 colony units were regarded as too numerous to count (TNTC) and therefore not considered in the counting. Only petri-plates with ≤ 300 CFU/g of sample were considered for enumeration. In this case the emerged colony units were counted based on their discriminating colors and the results calculated as colony forming unit per gram (CFU/g).

4.3 Hygienization expression on pathogens inactivation experiments

Performance of any hygienization option in terms of both effectiveness and efficiency at completely elimination or reducing both *E. coli* and *S. senftenbergensis* spiked in potato peels waste that simulated into contaminated biowaste was based on the analysis of the enteropathogens initial population denoted as (N_0) in the sample prior to the application of respective hygienization process and the final population found after completing the process denoted as (N). Normally the CFU/g results represent large range of numbers in thousands enumerated as viable microorganism. In this investigation, the resulting CFU/g values were calculated using scientific notation of writing large numbers and conveniently reported as reduction in \log_{10} CFU/g using equation (4.12) below:

$$Reduction = L = \log_{10} \left(\frac{N_0}{N} \right) \quad (4.12)$$

Whereby:

L = log difference in pathogens level before and after hygienization

N_0 = bacteria population before hygienization process

N = bacteria population survived the treatment

In the course of evaluating the hygienization performances of a specific biological treatment system or particular hygienization option, the results expression in percentage reduction was much preferred to indicate the efficacy and the rate of *E. coli* and *S. senftenbergensis* die-off following the application of the treatment or the hygienization option thereafter. The percent reduction was calculated using equation (4.13) as follows:

$$\% Reduction = 100 * (1 - 10^{-L}) \quad (4.13)$$

L = log difference in pathogens level before and after hygienization

4.4 *E. coli* and *S. senftenbergensis* population spiked in potato peels

In this study, a total of four microscope examination and ten spread-plates were performed to establish bacteria viability and estimation of cells or colony units' population. Table 4-1 summarizes the proportion of *E. coli* and *S. senftenbergensis* population used for the artificial spiking of potato peels waste that was used in various hygienization experiments performed. All hygienization based investigation used the microbial population established from the total bacteria count experiment as the initial contamination levels required to be inactivated. In each experiment, it was worth using a higher population from those observed in real MSW samples so that to create a worse contamination phenomenon in the biowaste and thus assuring reliability, robustness and increased efficacy and verification of the hygienization option under investigation.

Table 4-1: Average enriched enteropathogens population for spiking in potato peels waste

Test	Microscope		Total Bacteria Counts		Application
	Average Cells/mL		Average CFU/g		
	<i>E. coli</i>	<i>S. senft-</i>	<i>E. coli</i>	<i>S. senft-</i>	
i	3.9x10 ⁸	1.6x10 ⁹	1.2 x10 ⁸	2.3 x10 ⁸	Pasteurization
ii	4.7x10 ⁸	2.3x10 ⁹	2.8 x10 ⁸	5.6 x10 ⁸	“
iii	na	na	4.6x10 ⁸	2.2x10 ⁸	Conditioning
iv	na	na	6.3x10 ⁶	2.1x10 ⁶	“
v	4.5x10 ⁹	1.4x10 ⁹	6.3x10 ⁸	1.3x10 ⁹	Composting
vi	5.3x10 ⁸	1.8x10 ⁹	2.7x10 ⁸	5.8x10 ⁸	“
vii	na	na	3.2x10 ⁸	7.9x10 ⁸	Co-composting
viii	na	na	x10 ⁶⁻⁸	x10 ⁷⁻⁸	Anaerobic digestion
ix	na	na	2.3x10 ⁷	1.3x10 ⁸	Solar drying
x	na	na	1.3x10 ⁶	2.4x10 ⁶	“

na = not analyzed

4.5 Results and Discussion

4.5.1 Physicochemical quality of potato peels waste

The summary of results on physicochemical attributes of fresh potato peels as biowaste feedstock for aerobic composting and substrate for anaerobic digestion process are presented in Table 4-2. Similar to the results from other former studies [106,107], moisture content of the potato peels was higher above the recommend ranges 45-60%w/w for aerobic composting process [108,109] while C/N ratio was found lower to the recommended ideal range of 25-35:1[109]. Apart from moisture and C/N content, other parameters among the selected ones were found within acceptable limits confirming therefore the possibility of biological treatment of this organic waste resource.

Table 4-2: Physicochemical characteristics of potato peels waste

Parameter	Composition	Units
Moisture (fresh PPW)	77.3±0.9	%w/w
Total solids (TS)	22.9±0.2	%w/w
Organic matter (OM)	94.7±0.1	%w/w
Total organic carbon	27.7 ±1.8	%
Nitrogen	1.4±0.1	%
C/N ratio	20:1	-
Ash	5.3±0.3	%w/w
pH-H ₂ O	6.5±0.1	-
pH-0.01M CaCl ₂	5.8±0.2	-
COD _(T)	974.2	g/kgTS
Protein ⁵	2.02*	%
Carbohydrate	17.5*	%
Sugar total	0.78*	%
Total fibre	2.2*	%

Results: ± was calculated as confidence Interval with uncertainty for 95%; n=3

⁵ Composition with (*) were adapted from Al-Weshahy & Rao [106] and Schieber & Saldaña [107]

The result of total solids, total COD and organic dry matter as percentage of total solids found in potato peels depict its potential as feedstock/substrate for biological treatments. Vögeli et al., [110] recommend that the organic total solid as percent of total solid (organic matter) in biomass is a best criteria for biodegradability potential of substrate to be used as feedstock for composting or substrate for anaerobic digestion and is therefore supposed to always be above 70%w/w.

The sample pH is an important parameter in biological treatment that determines effectiveness of composting and anaerobic digestion process by providing conducive working environment to microorganisms. For the microorganisms to properly decompose the organic matter, an optimum pH of 6.0-8.0 described as slightly acidic to slightly alkali needs to be maintained. The pH determination using water was found closer to neutral which is good for microbial survival and function. Even when 0.01M CaCl₂ was used as solvent the observed small change in pH signifies good buffering capacity exhibited by the peels waste. The observed decrease in pH was a result of extractable macro and micro nutrients in the peels which is an indication of presence of mineral salt required and necessary for good metabolic function of the microbial cells performing composting and/or anaerobic digestion process. Although potato peels showed low C/N ration in accordance to Cooperband [109] and Darlington [111], Diaz et al., [108] concur the possibility of biotreatment of feedstock with C/N ration up to 20:1 but with loss of more nitrogen through ammonia volatilization. Sánchez-Bascones et al., [112] supported that that higher C/N values decrease decomposition rate making the process to take longer time since it is averaged that microorganisms consume 30 parts of carbon for single part of Nitrogen. Higher nitrogen in the feedstock prevents the formation of enough microbiological biomass, cause release of nitrogen in the form of ammonia that leads to bad odours and results into low quality of the final compost.

4.5.2 Composition of essential soil macronutrients in potato peels

Table 4-3 shows composition of macronutrients in potato peels. The presence of essential nutrients in acceptable range and proportion qualifies biomass for biological treatment without threats of causing inhibition or process failure [113]. During biological treatment processes, the macronutrients are highly required by the microorganisms to enhance their physiological growth and activity but excess supply is reported to cause dehydration in

bacteria due to induced osmotic pressure and also process inhibition due to mineral toxicity. In a nutshell, the physicochemical attributes of potato peels present good proportion of nutrients (N, P and K) which could be recycled back to soil through the use of compost and/or digestate thus replenish soil fertility and productivity on landscape and derelict lands therefore widen arable lands for growing crops and grazing grasses.

Table 4-3: Macronutrients composition of potato peels

Nutrient	Average Composition mg/kg	Limits (mg/L)		
		Composting ⁶	Anaerobic digestion ⁷	
			Moderate inhibitory	Strong inhibitory
Calcium (Ca)	574.9	≤2500	2500-4500	8000
Sodium (Na)	494.9	<3500	3500-5500	16000
Potassium (K)	232.7	<400	2500-4500	12000
Magnesium (Mg)	110.5	<400	1000-1500	3000
Phosphorus (P)	2023	n/a	n/a	n/a
Nitrogen (N)	16500	n/a	n/a	n/a

n/a =regulated values were difficult to find

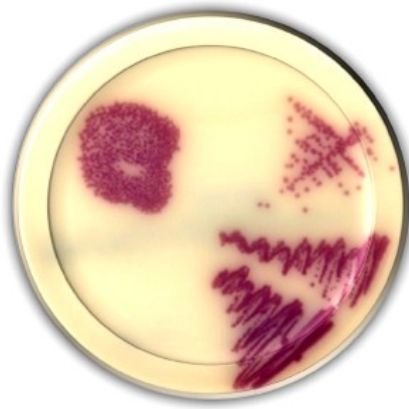
4.5.3 Viability and discrimination of *E. coli* and *S. senftenbergensis* on different culture media

Results for microbial viability following quadrant streaking on petri-plate with various agar media are shown in Picture 4-3. Purely purple colony units typical for *Salmonella spp* was observed on Salmonella Brilliance Agar. This agar being highly selective only allowed *S. senftenbergensis* to grow while *E. coli* was completely inhibited. Gassner lactose agar allowed germination of both bacteria with clear colour differentiation. In this media both *S.*

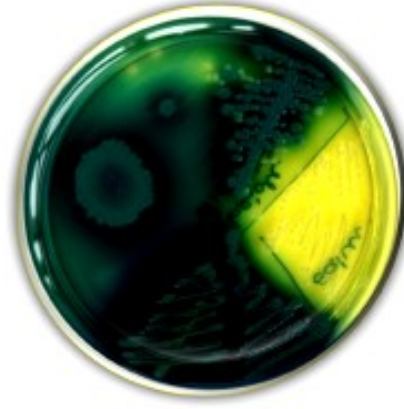
⁶ Source for limits value for macronutrients as given in Chen et al.,[113] and Diaz et al., [108]

⁷ Source for Literature values Zaher et al., [86] and Zupančič & Grilc[159]

senftenbergensis and *E. coli* exhibited a good luxuriant growth with *S. senftenbergensis* giving yellow colour while *E. coli* were identified by dark-green colour. Xylose lysine deoxcholate (XLD) agar and Endo Agar equally allow germination of both *E. coli* and *S. senftenbergensis*. On XLD agar, *S. senftenbergensis* appeared as red/pinkish colony units while on Endo agar the bacteria appeared colourless to pale-pink.



(i) Salmonella Brilliance Agar



(ii) Gassner lactose Agar



(iii) XLD Agar



(iv) Endo Agar

Picture 4-3: Viability and differentiation of *S. senftenbergensis* and *E. coli* on different agar

E. coli on XLD were identified by exhibiting yellow opaque colour while on Endo agar *E. coli* appeared rose-red with permanent green metallic sheen (gold) colour with marked reddening of the background of the media. This is because *E. coli* metabolizes lactose and produce aldehyde and acid. The aldehyde produced then liberates fuchsin from the

fuchsin-sulfite compound. In the case of *E. coli* this reaction is so intense to the extent that the fuchsin crystallizes out giving the *E. coli* colony units permanent green-metallic gold shining sheen. These results confirmed viability of both *E. coli* and *S. senftenbergensis* and thus warranty for their use for biowaste simulation study.

4.5.4 Microbial quality of the potato peels prior to simulation process

The knowledge on microbial quality of the potato peels to be used for biowaste simulation process that prepares feedstock and /or substrate was important in order to establish whether the peels were already pre-contaminated or not. The peels did not indicate *Salmonella spp* or *E. coli* contamination that would results into an interference with the spiked microbes before commencing the hygienization process. After 24hrs of incubation of streaked plates at 36°C, mucoid red-purple colony units (see Picture 4-4) were observed on petri-plate with Endo agar indicating the presence of other coliforms within the family *Enterobacteriaceae* but which are neither *E. coli* nor *Salmonella spp* since there was no greenish metallic sheen (fuchsin luster) on the colony units to confirm the presence of *E. coli* (lactose fermenting gram-negative enteric bacteria).

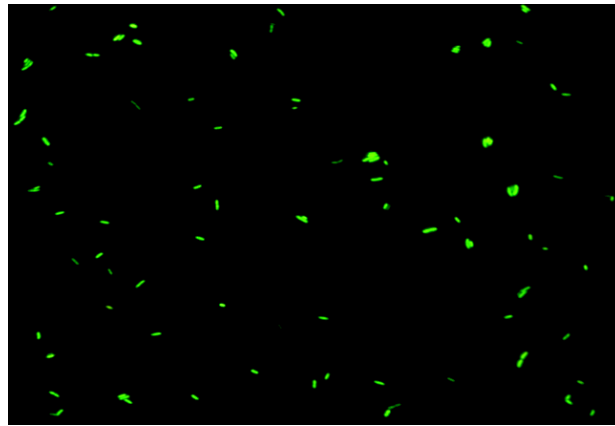


Picture 4-4: Streaking for detection of microbial contamination in potato peels

The absence of *E. coli* and *Salmonella spp* was confirmed on the plate with modified BGA as there was no sign of microbial growth. This was simply attributed by the strong microbial selectivity and inhibition exhibited by the modified BGA that resulted into complete inhibition of all coliforms that were able to germinate on Endo Agar.

4.5.5 Microbes population estimation under microscope and petri-plates

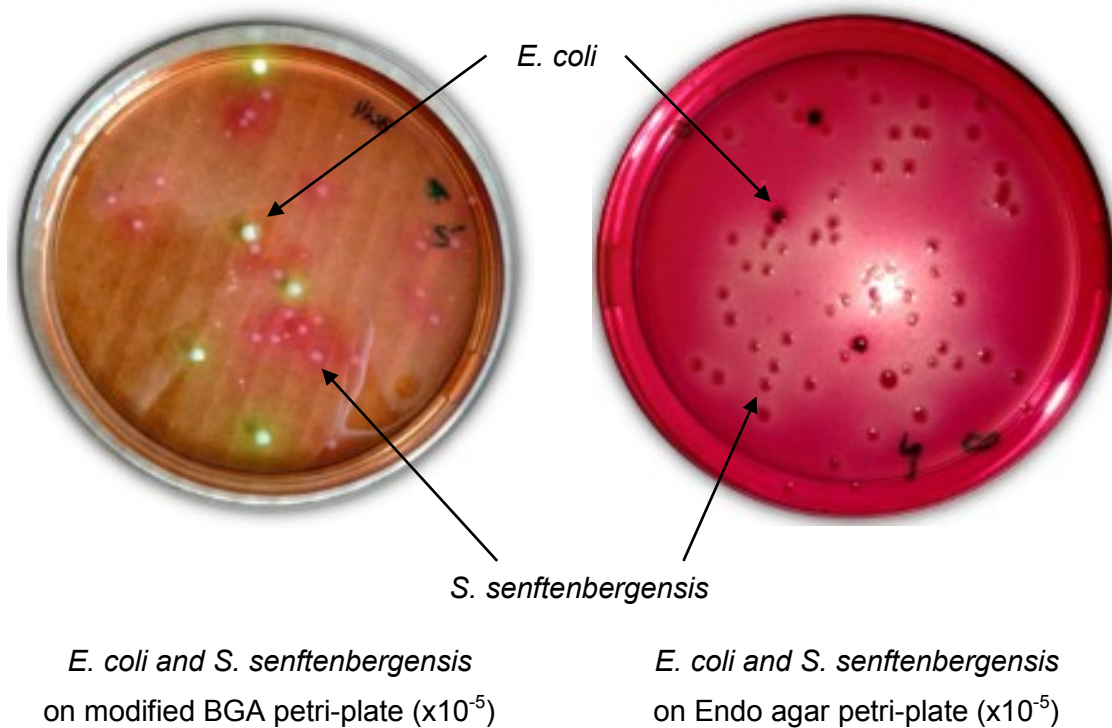
Appearance and population results for live cells of *E. coli* and *S. senftenbergensis* on microscope (NiKON Eclipse LV 100 Microscope (sample biomass $\times 10^{-3}$) are shown in (Picture 4-5). It was impossible to discriminate *E. coli* from *S. senftenbergensis* under microscope snapshot because both microorganisms being viable fluoresce green colour of the live/dead indicator solution. The microscopy results on population estimation based on individual strain showed that under the same media (Nutrient Broth) and similar growing conditions, *S. senftenbergensis* multiply faster than *E. coli* and the results for total viable bacteria population counted on a microscope snapshot were 10- folds higher than those grown on the petri-plates.



Picture 4-5: Segment snapshot of viable *E. coli* and *S. senftenbergensis* cells (green spots) viewed at 20-fold magnification

Possible explanation for this observation could be the fact that *S. senftenbergensis* have great acclimatization and colonization ability than *E. coli* similar to the observation in previous studies including Broennum et al., [114]. Another possibility is that on the microscope, the count was done on individual bacterial cells while on plate-count the bacteria are counted as colony units for which one colony could be formed as a result of combination of several bacterial cells. The other possibility for higher counts in microscope was based on doubling time. The time required for *E. coli* and *S. senftenbergensis* population to double (undergo binary fusion) is very short between 20min and 40min [89,115,116] when growing at $25\pm 5^{\circ}\text{C}$. The process taken in sample

preparation that involves filtration, smearing on the microscope slide until examination might have been prolonged to possibly meet the first doubling time thus leading to the observed higher bacterial cells population. Picture 4-6 shows colony units on spread plate for Modified BGA and Endo agar. Modified BGA a recommended media for the isolation of *Salmonella spp* was much useful in confirmation of bacteria population grown following spread plate method due to its higher percent recovery and clear colour discrimination of the colony units.



Picture 4-6: Discrimination of viable bacterial colony units on modified BGA and Endo Agar

In modified BGA, *S. senftenbergensis* showed a good-luxuriant growth with reddish- pink colour while *E. coli* colony units exhibited yellowish green colour as it can be seen from the two plates above spread with equal sample volume from the same dilution. *E. coli* colony units counted were less compared to those of *S. senftenbergensis* confirming shorter doubling time and quick acclimatization in *S. senftenbergensis* than in *E. coli*. The use of modified BGA was to confirm the presence of *S. senftenbergensis* and validate the population which could have mistakenly overestimated on Endo Agar (slightly selective)

due to its low inhibition nature on coliforms and poor colour discrimination of *S. senftenbergensis* from other Gram-negative rods exhibiting faint pink to colorless colonies.

4.6 Conclusive summary

Potato peels waste presents an ideal organic waste from our kitchen as well as food processing facilities. The use of *E. coli* and *S. senftenbergensis* for simulating contamination in biowaste was fit for purpose as it presents the most commonly and abundant microbial pathogens in poorly managed MSW with public significance and the latter covers the highly thermal tolerant species which elimination requires deliberately application of extra hygienization means in order to achieve total biosecurity. With an exception to coliforms and other useful micro-flora, the absence of *E. coli* and *S. senftenbergensis* and the presence of optimum mineral composition in potato peels made it suitable for the simulation study.

High water composition, easily hydrolysable carbohydrate into simple sugars (glucose) and the observed relatively low C/N ratio in potato peels waste signifies the risks of producing unhygienized compost and digestate as the finished product of biological treatment process due to their direct or indirect effect following increasing microbial tolerance above the die-off causing parameters. This suggests that laboratory based prior knowledge on types of pathogens contamination and their levels on the organic waste are of paramount. Otherwise, achieving complete hygienization in the contaminated biowaste under conventional biotreatments remain questionable and in limbo.

5 Hygienization Performance of Conventional Biotreatment Methods

5.1 Biotreatment of pathogens contaminated biowaste with high moisture content

Potato peels waste presents biowaste containing high moisture above 77%w/w, a typical characteristic of biowastes from food processing plants. If not monitored, composting of these organic waste result into unhygienized final compost due to the fact that the process fail to attain temperature higher enough to eliminate potentially pathogens during aerobic composting process due to the high moisture content. The focus in this investigation therefore was to quantify the rate of pathogens reduction (if any) using aerobic composting technology and anaerobic digestion in conformity to the British Standards Institution's Publicly Available Specification (BSI-PAS 110:2010), Regulation (EC) No. 208/2006 and DG ENV.C2/BZ/tb [117] requirements presented in Table 5-1 below:

Table 5-1: Specified pathogens limits in digestate following anaerobic digestion

Pathogen	DG ENV.	Regulation (EC)	BSI - PAS
	EC 2010	No. 2008/2006	110:2010
	Grab sample 25g wet wt. CFU/g		
<i>Salmonella spp</i>	0	0	0
<i>E. coli</i>	< 5x10 ³	1x10 ³	1x10 ³

So far, diverse composting technologies characterized with different investment and maintenance costs, space requirement, compost time and labor requirements exist and are in use in our daily life. These includes: windrows composting, passively aerated windrows, forced aerated windrows, bin/ vermin composting and in-vessel composting [109]. With exception to in-vessel composting systems, some of the systems are the simplest and probably the cheapest, nevertheless, the composting process in these systems suffer low temperatures on the outer layers of the material where pathogens may survive or even re-grow and re-colonize the final compost thereafter. An isolated reactor system with forced aeration is a more effective way of ensuring evenly distribution of heat in the composting vessel and thus effecting pathogens die-off. In this investigation therefore, aerobic composting technology based on In-vessel composting Technology

(ICT) and anaerobic digestion technology based on two-phase mesophilic/mesophilic and mesophilic/thermophilic system was preferred and therefore investigated for the inactivation potential of both heat sensitive pathogens represented by *E. coli* and heat resistant pathogens represented by *S. senftenbergensis*.

5.2 Enteropathogens die-off in aerobic composting and anaerobic digestion

5.2.1 Aerobic composting based on In-vessel composting system

Within waste management hierarchy, composting present a strategy that stabilize MSW in particular biowaste, minimize the negative impacts such as emission of GHGs and therefore divert the MSW from dumpsite and landfill [97]. The Directorate-General ENV.A.2 [23] define acceptable compost for land application as a stable, sanitized and humus-like material rich in organic matter, resulting from the composting process of separately collected organic residues by naturally found microorganisms and complies with environmental classes of quality and that is free from both pathogenic microorganisms and offensive odours. Aerobic composting process ideally is accomplished through two phases. According to Schaub and Leonard [5], firstly is the pre-composting process in which the physical and chemical parameters of biowaste for composting are adhered such as segregation of waste and removal of unwanted and biologically inert materials, size reduction of the feedstock and laboratory analysis of some physical, chemical and microbiological quality of the feedstock is done to confirm the suitability of the biowaste for composting that would also reflect the expected quality in the final compost. The second phase is the composting process where the feedstock is heated following microorganisms activities and takes place in three phases described as follows:

- Phase 1. - Mesophile microorganisms dominate the process and initiate degradation of easy biodegradable organic fractions in the first 1-2 days. Temperature increases to about 45°C and above to allow growth of thermophile microorganisms.

- Phase 2. - Thermophile microorganisms dominate the process for several weeks and cause the main degradation of organic matter. This cause an

increase in composting temperature between 55°C and 75°C. This temperature inactivates the pathogens, breaks down organic compounds, destroys weed seeds and dries the compost to optimum moisture.

- Phase 3. - Compost undergoes curing and then maturation after passing the active composting stage. During this stage the rate of oxygen consumption decreases, temperature cools down 30-35°C thus attract re-colonization of the final compost by indigenous useful mesophilic microorganisms' that complete decomposition of organic matter into biologically stable humic substances known as the final compost.

5.2.2 Factors inducing hygienization during aerobic composting

During decomposition process other intrinsic factors including microbial antagonistic and antibiotics inhibition take place. However, the heat generated during the active decomposition stage of organic matter in the composting feedstock and the time of exposure are the main contributors in pathogens inactivation [118]. However, it can be observed from Table 5-2 that there exist great variations in temperature-time specifications among countries and regional regulatory bodies concerning appropriate temperature and the required exposure time that can be regarded as standard for achieving a complete inactivation of pathogenic microorganisms in biowaste for quality and hygienized compost. Among the 10 countries cited in which composting process and its quality is regulated, the processing temperature and the residence time differs much. For instance using the same In-vessel composting method, Germany advocate digester temperature above 60°C for not less than 7 days, USA, Canada and The Netherlands recommends temperature of 55°C for 5 days, 3 days and 2 or 3days respectively. The hygienization in aerobic composting process is to a great extent effected by temperature which is internally generated as a result of microbial activity on the substrate depending on the type of the composting technology [19]. Moisture of the feedstock within the reactors is considered critical in both as a factor necessary for microbial survivor and renders lignin, cellulose and hemicelluloses soft thus decomposable provided other factors such as population and types of micro-flora, C/N ratio of the feedstock, air and pH are optimally maintained. It is through decomposition activity by which the composting

microorganisms produce heat energy causing temperature within the composting vessel to rise.

Table 5-2: Selected national compost hygiene Temperature-Time specifications

Country	Composting methods	Temperature-Time
Germany	In-vessel	>60°C for 1 week
	Other methods	65°C for 1 week
Austria	All methods	>60°C for 6 days or >65°C for 3 days
Switzerland	All methods	>55°C for 3 weeks or >60°C for 1 week
Belgium	Piles and In-vessel	60°C for 4days
Italy	Piles and In-vessel	>55°C for 3 days
Netherlands	All methods	55°C for 2 or 3 days
Denmark	All methods	>55°C for 2 weeks
Australia	All methods	>55°C for at least 3 days
USA	In-vessel	55°C for 5 days
Canada	In-vessel	55°C for 3 days

Source: Brinton and Gardner [119], Lepeuple et al., [95], Hogg et al., [120].

A review on composting process by McCartney and Wichuk [20] disclose that some research recommends at least 55°C as sufficient temperature enough to effect pathogens die-off during composting process provided it is maintained for at least 3 consecutive days while on contrary, some other scholars and compost regulatory bodies propose even higher temperature reaching up to 65°C [120]. The existing diverse in specifications not only created ambiguity in application but also doubts based on efficiency and effectiveness of the hygienization recommended following pathogenic resistance under the interface of cross contamination.

The consortium of microorganism taking part in effecting decomposition process include: bacteria, yeast, fungi, actinomyces and protozoa all acting on biodegradable organic materials of the waste at different specified temperature (mesophilic or thermophilic)

range [121,122]. The presence of optimum composting parameters including moisture, pH, C/N ratio, mineral salts both macro and micro nutrients in composting feedstock produces conducive environment for the indigenous microorganisms in facilitating the composting process. After temperature, Other factors causing pathogens die-off include competition among indigenous microorganism with the pathogenic ones Sahlström [69]. Except for enteric microbial pathogens, reviews article by McCartney and Wichuk [20] reveals that antibiotics released by fungi and actinomycetes following stress contribute much on microbial inactivation. Others include natural death as results of accumulation of toxic metabolites such as ammonia, nutrient depletion and antagonistic among microbial population [20]. Vinnerås [123] concurs that these inherent factors though effectively induces die-off in pathogenic microorganisms, their degree of effectiveness and level of pathogens inactivation has not yet been assessed nor documented.

5.3 Composting of fresh and partially open air-dried potato peels

5.3.1 Materials and Methods

5.3.1.1 Choice of composting technology

Among the existing composting technologies expounded earlier on, of interest to this research was the In-vessel composting technology (ICT). ICT was preferred than the other composting systems due to the fact that ICT is more efficiency in killing pathogens as the system can maintain the generated temperature within the vessel for long time independently of the external weather and thus guarantee safety of the final compost [124]. In addition, ICT provides better protection to the personnel working at the composting facility (compost producers) as it is done inside the vessel thus reduces direct exposure to contaminated feedstock biowaste materials for composting. ICT provides a better process control for aeration prevents off-odour, nuisance, dusts and requires less installation space compared to other composting techniques therefore making it possible and ideal to use even in congested cities.

5.3.1.2 Components of In-vessel composting system

In this investigation, a lab-scale forced aerated composting model based on In-vessel composting technology (ICT) described in Figure 5.1 was used. The system is made of

vacuum glass flask cylinders composting reactors of 2.1L capacity inserted in metal cases 28.0cm height with a diameter of 11.0cm and provision for a perforated aeration chamber at the bottom with air pipe that circulate fresh air in the vessel and create aerobic condition.

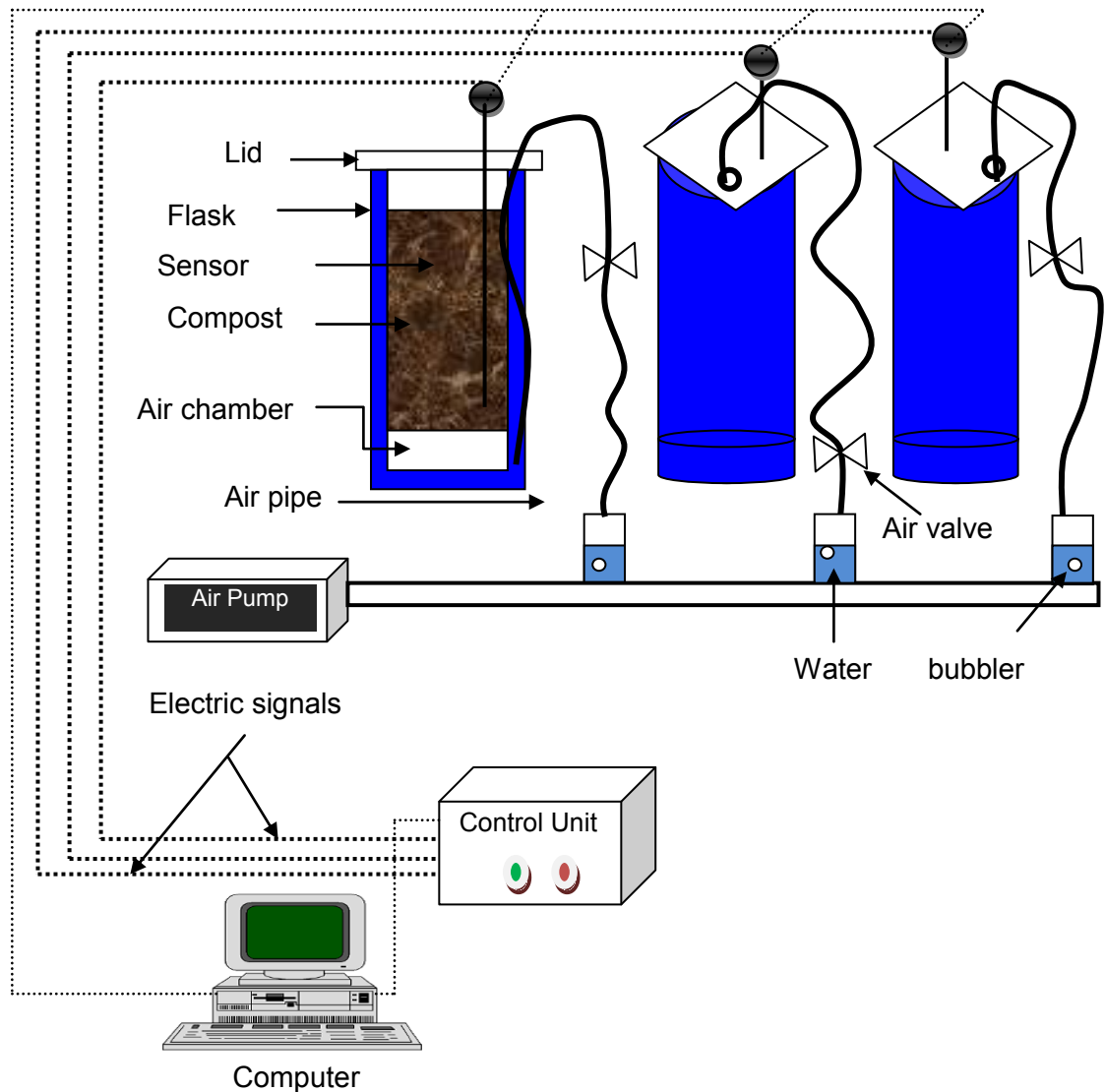


Figure 5.1: Schematic set-up and components for In-vessel composting system

The flasks are covered with lid that control air, moisture and temperature from freely escaping. The system is connected to a control unit which mounts 36 temperature control sensor probes (30-42cm long) which are placed at the middle of the composting flask and

connected to a computer system for automatic monitoring the temperature of composting feedstock inside the composting vessels. Air in the composting flask was supplied using Air pump (Model JUN Aquarium ACO-5502).

5.3.2 Formulation of composting feedstock

In this investigation, seven feedstock recipes were formulated having varying ratio of fresh and partially air-dried for 36hrs drying which was equivalent to 9hrs of open air exposure for 4days at mean temperature of 24.5°C of blended potato peels. The peels were then mixed with inoculating compost (starter) in weight basis as shown in the Table 5-3 below. High water content of the potato peels and low C/N ratio was the reason behind studying the two feedstock formulations. In all two experiments, artificially microbial spiked blended peels <12mm size seeded with compost “*inoculum*” was used as the main feedstock for the composting process. Physicochemical characterization of each formulated compost feedstock was carried out in which moisture content, total solids, organic total solids, pH, organic matter and C/N ratio were determined before commencing the composting process.

Table 5-3: Feedstock formulation for fresh and open air-dried potato peels waste in w/w

Feedstock	Fresh/Air-dried	Inoculum	Ratio
	potato peels (g)	Compost soil (g)	
Recipe -1	1200	0	1:0
Recipe -2	1150	50	23:1
Recipe -3	1100	100	11:1
Recipe -4	1050	150	7:1
Recipe -5	1000	200	5:1
Recipe -6	950	250	4:1
Recipe -7	0	1200	0:1

In both experiments, the ready-made compost soil was then added to provide indigenous micro-flora necessary to effect composting process. Due to the fact that the two investigations were done at different period, the population of enteropathogens that was artificially spiked in the composting feedstock differed. In the first composting experiment

in which fresh potato peels were used, the mixture was spiked with 8.8log *E. coli* (6.3×10^8 CFU/g) and 9.2log *S. senftenbergensis* (1.3×10^9 CFU/g). Whereby in the second experiment air-dried potato peels were used. The mixture was spiked with 8.4log *E. coli* (2.7×10^8 CFU/g) and 8.7log *S. senftenbergensis* (5.8×10^8 CFU/g). The full composting process was carried out as described in Figure 5.2 below.

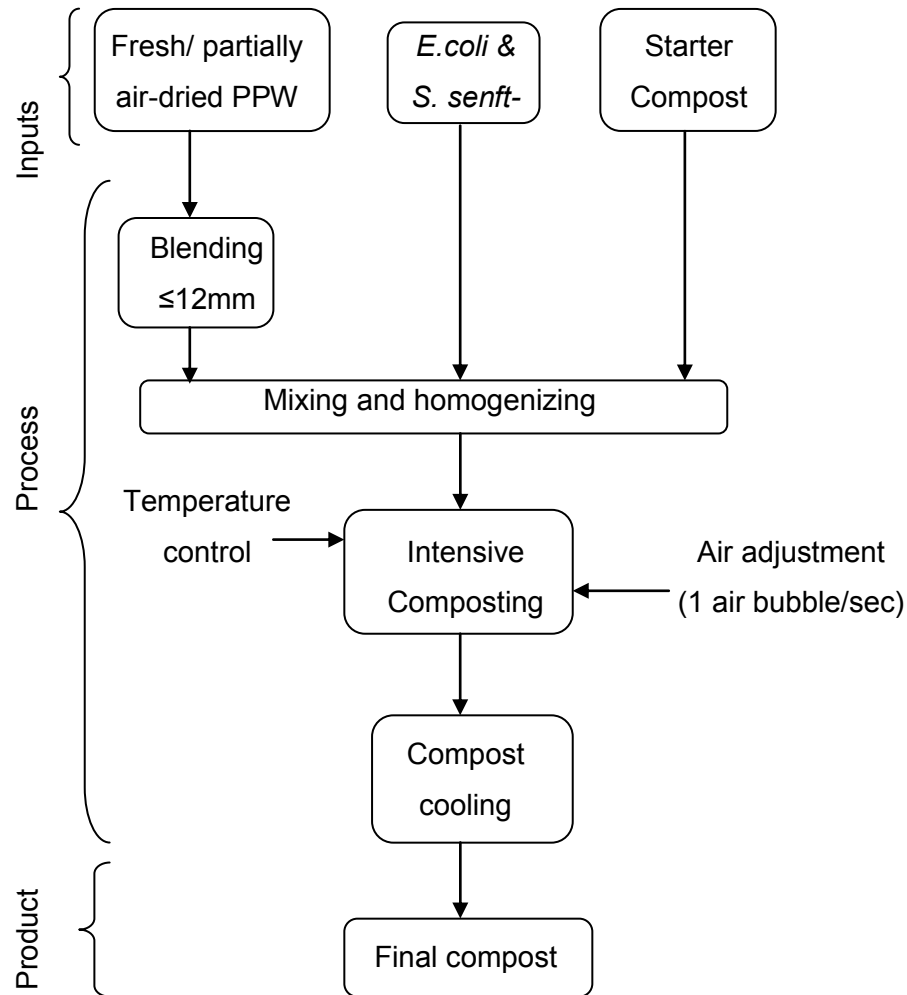


Figure 5.2: Schematic layout of feedstock formulation and composting process

5.3.3 Verification of temperature sensor knobs before composting

Before composting process, the temperature sensing probes were calibrated (LAUDA eco Re.104 at $45.0 \pm 0.2^\circ\text{C}$). Seven out of the calibrated sensors were directly inserted into each composting flask and the one remained was left hanging to record the indoor

temperature within the composting facility. During the whole composting period, temperature was automatically recoded in a computer device at 2hrs interval using data generating software HP VEE-T-Mess Me-2600 V37-2015 programme.

5.3.4 Ensuring aeration in the composting feedstock

The bucket method as described in Chen and Moore [125] was used in determination of percentage porosity of the composting feedstock before starting the composting process. This was an important experiment to ensure that the aerobic condition is created in the composting vessel thus enhancing the composting activity of the aerobic micro-flora within the composting reactor. In this method three composting vessels were used (2.1L capacity, 28.0cm height, diameter of 11.0cm). The flasks were filled with 2L water and the maximum height reached was marked using a marker-pen. Homogenized recipes of formulated composting feedstock sized $\leq 12\text{mm}$ were then filled into the flasks to the marked height in the same way as filling for In-vessel composting process. A known volume of water was then added on top of the filled composting feedstock to saturation that covered the void volume in the flasks. Porosity was then determined in percentage using the following expression (5.1).

$$\text{Porosity} = \frac{\text{Water (mL)}}{\text{Flask Vol (mL)}} * 100 \quad (5.1)$$

Aeration in the composting flask was controlled using a rate of one air bubble per second through adjusting air bubbles evolved through water from several glass troughs into smaller plastic pipes which were inserted deep down in the composting flasks.

5.3.5 Determination of hygienization efficacy and quality of compost

Sample analysis for inactivation status of the spiked enteropathogens was done after the onset of composting process; absence or decrease in bacteria population in the compost from each composted feedstock was analyzed and the results used in evaluating pathogens inactivation efficiency of the In-vessel composting technology. The size for test sample was as recommended in Regulation (EC) No. 208/2006 in this case 25g of the final compost was suspended in 250mL sterilized water (*Leitungswasser*) and agitated (Janke and Kunkel Ika labortechnik Ks 501 digital, Deutschland) at 130rpm for 10min at

room temperature $23\pm 2^{\circ}\text{C}$ followed by decimal (10-folds) serial dilution. From the resulting solution, $100\mu\text{L}$ of the diluents in the range 0 to 10^{-6} were surface spread on Endo agar and modified BGA. The plates were incubated at 36°C and the enumeration of bacteria colony units was done after 24 hrs in the following day. In addition, post composting physicochemical parameters including moisture content, C/N ratio, conductivity, pH, Ash and organic matter content in the final compost were determined to confirm quality attributes of the compost as well as sensory characteristics that include smell and colour of the final composts were equally determined so as to confirm the optimum peels to compost soil ratio that results into microbiologically safe and quality final compost.

5.4 Results and Discussion

5.4.1 In-vessel composting of fresh potato peels

5.4.1.1 Physicochemical quality of feedstock before In-vessel composting

Table 5-4 presents some physicochemical results of the composting feedstock that were formulated using fresh potato peels mixed with compost soil that was used as composting starter by supplying useful composting microorganisms.

Table 5-4: Selected physicochemical attributes of formulated sample before composting

Flask	Feedstock	Mc	TS	oTS	Ash (wt. dry)	C/N	pH-
		Values given as % w/w					CaCl ₂
A	Recipe -1	77.9	22.1	94.8	8.2	18:1	5.7
B	Recipe -2	76.7	23.3	93.6	7.2	18:1	5.7
C	Recipe -3	76.4	23.6	93.0	8.1	17:1	6.4
D	Recipe -4	75.8	24.2	93.5	9.8	17:1	6.3
E	Recipe -5	75.5	24.5	93.7	10.2	16:1	6.2
F	Recipe -6	75.1	24.9	93.5	11.4	19:1	6.1
G	Recipe -7	40.2	59.8	47.7	52.3	15:1	6.8

Key: C/N = Ratio of Carbon to Nitrogen
oTS = Volatile organic matter

Mc = Moisture content
TS= Total solids

Particle size reduction of composting feedstock increase the surface area and expose the peels to indigenous micro-flora and therefore facilitate decomposition process [126]. The use of compost was as both starter and bulking material. The bulking proportionally improved other attributes including total solids, organic matter and ash content of the composting feedstock with only small reduction on moisture content of the feedstock. The results confirm that potato peels contain high moisture above the recommended range of 45-60% which should not be exceeded especially for In-vessel composting process [127]. High moisture in composting feedstock normally results into production of leachate during composting process. Consequently, keeping the leachate within the composting vessels tend to block air passage pores and affect the entire composting process.

The observed moisture reduction was in the range of 0-2.8%w/w indicating that the addition of inoculating soil compost (Recipe 7, moisture = 40.2%w/w) did not affect much the feedstock moisture content. The feedstock total solid was a bit elevated from its original value of 22.1%w/w to 24.9%w/w in Recipe 6 following addition of 250g of inoculating soil compost. The increase in compost soil in the feedstock proportionally caused a decrease in sample organic matter, this is because compost contains low organic matter as decomposition results into mineralization of organic matter and carbon dioxide loss [43,86,128]. The C/N ratio of the feedstock were not much changed and were still in the range of 16-19:1 which is below the recommended composting range of 20-35:1 reported in Baldwin and Greenfield⁸, Jimenez and Garcia [129] and Cooperband [109].

Except for the first two feedstock formulation (Recipe 1 and 2), it was worth noting that other sample formulation in Recipe 3 -6 and the inoculating compost (Recipe 7) exhibited optimum pH value for composting which is 6.0-8.0. The pH determination using 0.01M CaCl₂ was preferred. This is because 0.01mol/L CaCl₂ solution is the perfect neutral salt extractant and has an ionic strength of 0.03M which is closer to the expected value in soil solution upon which the compost is applied [130]. Sample pH determines the effectiveness of composting process by providing conducive environment to the decomposing indigenous micro-flora.

⁸ <http://www.farmsreach.com/welcome/wp-content/uploads/2014/11/CompostingOrganicFarms.pdf>.

Retrieved May 14, 2015

5.4.1.2 Temperature-Time patterns during In-vessel composting of fresh potato peels

The results for temperature patterns for In-vessel composting of feedstock formulations (Recipe 1 to 7) which were coded according to composting flask in the order of Flask A to Flask G in that order using fresh potato peels inoculated with compost soil are shown in Figure 5.3. Flask A contained Recipe 1 which was potato peels alone while Flask G contained Recipe 7 which was the compost soil alone and therefore these two flasks were used as controls.

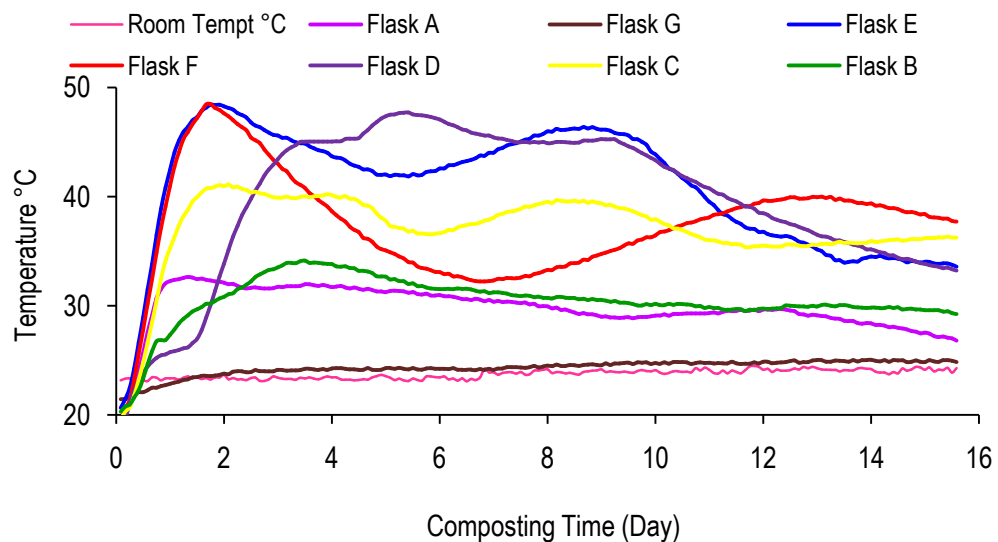


Figure 5.3: Temperature-time patterns for In-vessel composting of potato peels waste with inoculating compost at different feed formulation ratios

The poorer microbial activities in the system the slower was the decomposition rate and therefore a cause for both, lower temperature and irregular temperature-time patterns. The whole composting time in this investigation was 16 days. The observed composting time was definitely short compared to the time reported in other composting trials. For instance, Déportes et al., [19] used 21 days in composting organic fraction of municipal solid waste. Similarly, it took about 26 days for Mbuligwe et al., [122] to obtain compost from domestic waste whereby Tosun et al., [131] spent 32 days to complete composting of olive waste mixed with tomato stalks. From the temperature-time patterns observed in this work, the maximum temperature reached in individual Flasks A to Flask F at the end of

composting process indicate that the composting temperature ranged between 20°C and 50°C. This range of decomposition temperature corresponds to grade III to V of the compost described in *Bundesgutegemeinschaft Kompost e.V.*, 1994. The composting process was inhibited in such a way that the system could not manage to enter thermophilic phase of decomposition regardless of the amount of compost soil added which means that the thermophilic microbes were not able to colonize and take over the composting process. This can be explained by the fact that water in the fresh potato peels drained out during composting process forming leachate that greatly affected other composting parameters in the feedstock and the activity of indigenous composting microflora inside the composting vessels.

The resulting drains blocked air spaces leading to the blockage of air from circulation in the composting vessel therefore created anaerobic condition. Composting temperature being a by-product of microbial activity formed as result of decomposition of biowaste can be optimally reached only if the feedstock moisture content, C/N ratio and aeration in the composting system are optimized and monitored. The maximum attained temperature-time reached in this composting trial was 48°C observed in sample formulation Recipe 5 and Recipe 6 composted in Flask E and F respectively. In these two flasks, the residence time for the maximum temperature was 1 day and 2hrs respectively. This temperature was quite lower than the minimum reported temperature in Bijlsm et al., [132] of 55°C in 4hrs for inactivation of *E. coli* and 60°C in 4hrs for inactivation of *S. senftenbergensis* [71] in the compost. The observed irregular temperature-time pattern was an indication of blocked air-pores within the composting feedstock due to high moisture content that caused oozing of leachate from potato peels. With exception for Recipe 3, which attained a maximum of 41°C, the other sample formulations did not even reach 35°C.

5.4.1.3 Post-composting physicochemical attributes of the resulting compost

Although at the end of day 16, compost were obtained, these compost were unhygienized and therefore not safe for use for instance in land application due to unattained recommended hygienization temperature of at least 55°C for 3 days [20]. Table 5-5 gives the results of some of the analyzed physicochemical quality of the resulted byproducts as final compost in case the potato peels are to be used as exclusive feedstock for compost production.



(i) Peels : Compost ratio (1:0)



(ii) Peels : Compost ratio (23:1)



(iii) Peels : Compost ratio (11:1)



(iv) Peels : Compost ratio (7:1)



(v) Peels : Compost ratio (5:1)



(vi) Peels : Compost ratio (4:1)

Picture 5-1: Attributes of unhygienized compost from fresh composted potato peels

Similarly to Zhu [134] observation, too much moisture higher above 65% in biowaste hamper biowaste decomposition, accelerate onset of anaerobic decomposition in the system due to blockage of pore-spaces, prevent oxygen flow and result into production of foul odour and nutrient leaching producing poor sensory qualities of the resulting compost Bonhotal et al., [135] in their review explained that higher moisture in the composting feed results into slurry compost accompanied by foul odour and leachate a similar phenomenon that was observed to all sample recipes formulated in this study. The

dominant smell was that of rotten eggs which characterizes H₂S formed following electronic acceptor behavior of sulfur and sulfate by sulfur-reducing bacteria (SRB) in an oxygen depleted environment signifying the onset of anaerobic digestion.

5.4.1.4 Pathogens inactivation following In-vessel composting of fresh peels

Reduction of bacterial colony units from the original population was used to judge the performance of each hygienization option. Colony units of the two enteropathogen strains were discriminated based on the colour as they appear on agar plate. *E. coli* and *S. senftenbergensis* reduction extent following aerobic composting of fresh potato peels with the corresponding minimum and maximum temperature generated in the composting vessels are given in Table 5-6. Small *E. coli* reduction of 4.5%, 71.8% and 61.1% was obtained in recipe 4, 5 and 6 only which attained maximum temperatures of 47.5 °C, 48.4°C and 48.5°C respectively. *S. senftenbergensis* increased in almost all Recipes except in 5 and 6 where there was 8.8% and 4.5% reduction in Recipe 5 and 6 in that order. In general, there was percent increase in population of *E. coli* in the order of 14.8% in Recipe1, 2.3% in Recipe 2 and 9.6% in Recipe 3. *S. senftenbergensis* population equally increased in the order of 20.2% in Recipe 1, 9.6% in Recipe 2 and 14.8% in Recipe 3 and 7.2% in Recipe 4 of the formulated composting feedstock.

Table 5-6: Efficacy of In-vessel composting technology on pathogens inactivation in fresh composted potato peels waste

Flask	Feedstock	Min. Tempt °C	Max. Tempt °C	Die-off (logCFU/g) *	
				<i>E. coli</i>	<i>S. senft-</i>
A	Recipe -1	19.5	32.5	-0.06	-0.08
B	Recipe -2	19.5	34.2	-0.01	-0.04
C	Recipe -3	20.4	41.1	-0.04	-0.06
D	Recipe -4	20.3	47.5	0.02	-0.03
E	Recipe -5	22.5	48.4	0.55	0.04
F	Recipe -6	20.4	48.5	0.41	0.02

*Negative sign means bacteria population for which, $N_t > N_0$

The small die-off in *S. senftenbergensis* and *E. coli* observed could be simply connected to low C/N ratio in the peels. Low C/N implies that the peels are nitrogen-rich therefore during composting process, nitrogen are metabolized to release gaseous compounds of ammonia and nitrous oxide which are toxic to the pathogens and even to the indigenous useful micro-flora. In summary, unless otherwise, composting contaminated fresh biowaste at very high moisture content is associated with a danger of increasing pathogens population rather than eliminating them and therefore become potential source of contamination and thus harmful effect to humans and the environment in case of using such by-products as compost in the farmland, a statement equally supported in Albihn and Nyberg [73] and Albihn et al., [129].

5.4.2 In-vessel composting of air-dried potato peels

The physicochemical results for the peels exposed for 36hrs (equivalent to 9hrs of exposure for 4 consecutive days) at an ambient mean temperature of 24.5°C to an initial moisture content of almost 10% less compared to the fresh potato peels leading to a moisture of 67.7%w/w in potato peels are given in Table 5-7 below.

Table 5-7: Physicochemical quality of composting feeds using air-dried potato peels

Flask	Feedstock	Mc	TS	oTS	Ash (dry. wt)	pH- CaCl ₂
		Values given as % w/w				
A	Recipe -1	67.7	32.3	94.7	6.3	5.7
B	Recipe -2	67.2	32.8	93.7	7.1	6.4
C	Recipe -3	66.2	33.8	94.4	5.6	6.3
D	Recipe -4	65.4	34.6	92.5	7.2	6.2
E	Recipe -5	63.3	36.7	93.1	6.9	6.1
F	Recipe -6	61.6	38.4	91.5	9.9	6.4
G	Recipe -7	40.2	59.8	47.7	51.5	6.8

Key: Mc = Moisture content TS= Total solids
 oTS = Volatile organic matter

Addition of inoculum compost soil apart from becoming bulking material [136] it also reduced moisture to a less extent for instance in Recipe 6 where proportionally large fraction of the compost soil was added, the recipe moisture content was found less by 6.1%w/w compared to the moisture in Recipe 1. No great changes in other tested physicochemical parameters were observed.

5.4.2.1 Temperature-Time patterns during In-vessel composting of air-dried potato peels

Moisture reduction following open air-drying of the peels caused improvements in the temperature-time patterns (see Figure 5.4) including increasing composting time from 16 days (the composting time used in aerobic composting of fresh potato peels) to 30 days. At the start of composting there was a sharp increase in composting temperature to all Flasks except in the two flasks set for process control (Flask A which had potato peels waste alone and Flask G which had inoculating compost soil only). The increase in temperature within the composting vessels signifies presence of microbial activities. Similar to the observation in Day et al., [121], the temperature-time patterns for aerobic composting of air-dried peels except for Flask E and Flask F were not that much improved in both temperature and time of heating to effect a complete hygienization to the formulated composting feedstock.

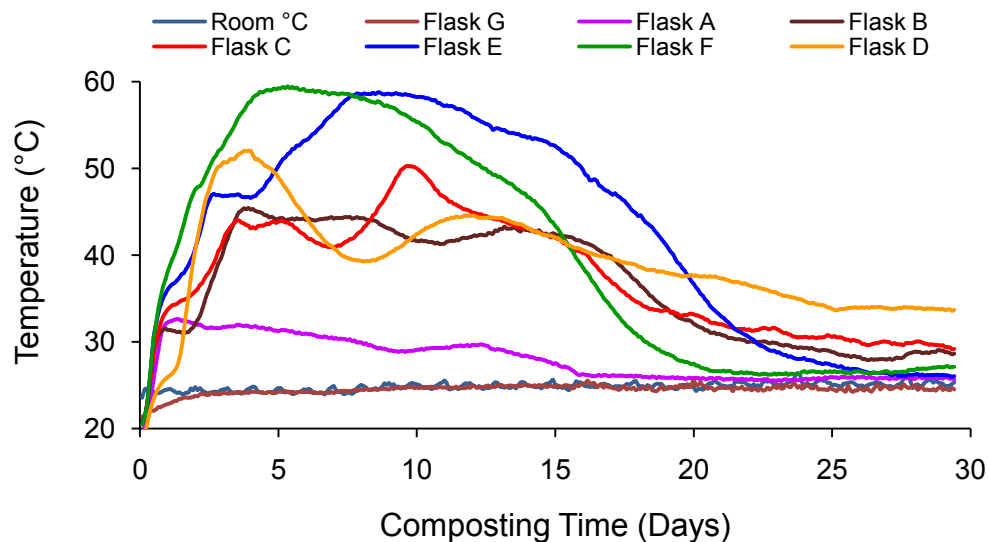


Figure 5.4: Temperature-time patterns for In-vessel reactors composting air-dried potato peels waste mixed with inoculating compost

In the first three feedstock formulation recipes that means in Flask A, Flask B and Flask C corresponding to Recipe 1, 2 and 3 respectively, the maximum composting temperature reached in Recipe 3 for instance was 48.6°C in recipe 2 was 44.2°C and in recipe 1 was 32.6°C. Recipe 5 and 6 that were composted in Flask E and F respectively showed patterns demonstrating composting stages namely latent, mesophilic, thermophilic and second mesophilic which is a characteristic of a good composting process. It is in the latent stage where indigenous composting micro-flora gets acclimatized to the new environment ready to start the composting process. In the first mesophilic stage, normally lasting between 1-4days in which mesophilic bacteria are the mostly decomposers, temperature rose at logarithmic rate ranging 31-45°C.

During the thermophilic stage which is the main degradation stage, the micro-flora involved are mostly thermophilic microorganisms (fungi, bacteria and actinomycetes) from day 5-13 with temperature between 50-65°C and lastly after the intensive composting process. This range of decomposition temperature corresponds to grade II of the compost described in *Bundesgutegemeinschaft Kompost e.V.*, 1994. The second mesophilic process takes place and is mostly dominated by bacteria from day 14 normally characterized and dominated by microorganism including mesophilic bacteria, algae and fungi [122]. In this second mesophilic stage, normally cooling of the compost and degassing of ammonia and H₂S which are plant-harming compounds, and maturation or curing of the compost takes place [71,109]. The temperature-time profiles in four feedstock formulation (Recipe 1, 2, 3 and 4 composted in Flask A, B, C and D) in which moisture was still above 65%w/w even after partially air drying of the feedstock showed irregular patterns confirming that moisture content of feedstock prior to composting process is a critical and a governing factor [75,137].

5.4.2.2 Physicochemical attributes of compost from air-dried potato peels

There was much improvement in moisture content (see Table 5-8) of the compost resulted following aerobic In-vessel composting of pre-air dried potato peels. Improvement in decomposition process equally brought a great change in organic matter due to oxidization process giving out CO₂, H₂O and new microbial biomass [138] and it reflect bacteria activity whose by-product is heat generation which is a desired effect for causing pathogens die-off.



Picture 5-2: Attributes of compost from composting of air-dried potato peels

5.4.2.3 *Enteropathogens inactivation during aerobic composting of air-dried peels*

The distasteful results on composting of fresh potato peels waste was the reason behind adjusting peels moisture by air-drying of the peels waste to enhance the composting process since high moisture content in composting feedstock is associated with leachate formation in the composting vessel leading to the onset of anaerobic digestion as results

of blockage of air pore-spaces. The performance of In-vessel composting technology results for inactivation of *E. coli* and *S. senftenbergensis* in pre-air-dried potato peels waste are given in Table 5-9 below. There was improvement in composting temperature estimated to be improved by 10°C units for every 10% w/w reduction in moisture that was observed in potato peels waste.

Table 5-9: Efficacy of In-vessel composting on pathogens inactivation in air-dried potato peels waste

Flask	Feedstock	Min.	Max.	% Inactivation	
		Temp ^t °C	Temp ^t °C	<i>E. coli</i>	<i>S. senft-</i>
A	Recipe -1	19.5	32.6	85.26%	78.30%
B	Recipe -2	20.9	44.2	93.69%	92.06%
C	Recipe -3	20.5	48.6	96.29%	93.69%
D	Recipe -4	22.6	52.3	99.09%	98.46%
E	Recipe -5	20.4	59.4	99.99%	99.99%
F	Recipe -6	20.5	58.7	99.99%	99.99%

McCartney and Wichuk [20] citing U.S-EPA and the Canadian Council of Ministers of Environment (CCME) concluded that pathogens inactivation is possibly expected at 55°C under a sample residence of 3 days if and only if all compost particles are equally exposed and get heated uniformly. It can be observed from the table that Recipe 1 had low percent inactivation for both *E. coli* and *S. senftenbergensis*. The inactivation kept increasing steadily proportional with the increase in temperature within the vessels up to temperature 52.3°C in formulation Recipe 4 where almost 99% of the die-off was realized in *E. coli* from its original population 2.7×10^8 CFU/g (8.4log) and also about 98.5% die-off in *S. senftenbergensis* from the original population of 5.8×10^8 CFU/g (8.8log). The reason behind the observed low die-off of the enteropathogens in formulation feedstock Recipe 1 could be simply attributed by the low indigenous decomposers micro-flora present in the potato peels waste necessary for the composting process therefore resulting into poor temperature-time pattern. Another possible reason could be the moisture content which was still higher in Recipe 1 above 65%w/w. High moisture as equally affected temperature-time patterns in feedstock formulation for Recipe 2, 3 and 4.

The possible explanation for the general small observed microbial reduction up to 0.8log in *E. coli* and 0.7log in *S. senftenbergensis* compared to the original population spiked at the start of composting process is probably linked to inhibition caused by increased H₂S, NH₃ and possibly other volatile fatty acids following the onset of anaerobic decomposition.

Considering the temperature cut-off point of 55°C, it was observed that only two feedstock which are Recipe 5 and 6 managed to attain this lethal temperature, the rest showed lower temperature readings with some irregularities in their temperature-time patterns. In Recipe 5 and 6, the maximum temperature reached was 59.4°C and 58.7°C with residence of 2 and 3days respectively. Using the 55°C, the holding time for Recipe 5 increased to about 8days while that for Recipe 6 residing for 7days making these composts conform to as many national regulation on compost including USA (55°C for 5 days), Canada (55°C for 3 days), The Netherlands (55°C for 3 days), Italy (55°C for 3 days) and Australia (55°C for 3 days) [20,120] but not acceptable in other nations due to strictly higher temperature –time specification recommended for instance, Germany (60°C for 7 days), Austria (60°C for 6 days), Switzerland (55°C for 21 days), Denmark (55°C for 14 days) and Belgium which advocate 60°C for 4 days [95,120] just to cite a few.

It should be remembered that in these two formulated compostable recipes, together with the air drying of the peels had a higher proportion of compost soil added as inoculum (see Table 5-3). With such combination the sample moisture was adjusted to 63.3%w/w in Recipe 5 and 61.6%w/w in Recipe 6 which is within the recommended range for In-vessel composting process. Great inactivation >6.0log for *E. coli* and about 5.8log in *S. Senftenbergensis* was observed in the feedstock Recipe 5 while in Recipe 6 there was up to 5.2log die-off in *E. coli* and 4.7log die-off in *S. Senftenbergensis*. These two formulations Recipes conform with the National hygienic specifications for UK, USA, Australia, Canada, Netherlands and Italy as stated in Hogg et al., [120], Brinton et al., [119] and Lepeuple [95].

5.5 Conclusive summary

During in-vessel composting process, moisture content in the feedstock was confirmed to be a critical factor towards hygienization and therefore stands a prime parameter for consideration in achieving hygienization in the finished compost. Temperature in the reactor depends on the activity of the microbes that is influenced by conducive environment like optimum moisture, aeration, pH and mineral salts. In case of imbalance to these conditions, there is a great possibility of process inhibition following the disturbance on the composting microbes. High feedstock moisture turns aerobic process into anaerobic, causing formation of offensive off-odours and lower temperature-time profile in the reactor.

Therefore the study recommends that prior to composting process the biowaste feedstock should possess the optimum requirements for composting. Also, since high moisture content of the feed hinders pathogens hygienization potential of the In-vessel composting technology, therefore laboratory assessment of biowaste based on physicochemical composition prior to composting should be inevitably carried out. Other practices such as drying of biowaste for moisture adjustment and/ or intentionally mixing of biowaste with other more dry materials for instance agriculture waste and yard-waste before composting process facilitate in optimizing the moisture content and C/N ratio of the formulated feedstock for better composting process.

5.6 Anaerobic digestion based on two-phase continuous stirred tank reactor

5.6.1 Process development and choice of anaerobic digestion system

Anaerobic digestion process is not new [59] and have been in operation for many years in wastewater treatment plants. The main reasons for its use being Stabilization and hygienization of sewage sludge among others [76]. During anaerobic digestion process, a consortium of microorganisms in the absence of oxygen undertake biochemical processes which through four steps namely hydrolysis which involves enzymatic decomposition of polymers, acidogenesis, acetogenesis and methanogenesis [56] in a controlled vessel called reactor or digester degrade natural polymers: polysaccharides, proteins, nucleic

acids and lipids into biogas (mainly methane 60% and carbon dioxide 40% on average) and the effluent (digestate) rich in plants nutrient [57,58,139–142].

Various types of anaerobic digestion systems exist from the simple ones using single stage to complex running as multiple-phase with either mesophilic to mesophilic reactor or mesophilic in tandem to thermophilic temperature co-phased. The mesophilic range ($37\pm 2^{\circ}\text{C}$) and thermophilic range ($55\pm 1^{\circ}\text{C}$) are commonly used depending on the desired process efficiency, quality of the final products and optimization of process running costs in terms of operation energy needed. The choice of the system depends on reliability, technical performance desired as well as level of hygienization required based on pathogens contamination status of the substrate [143]. Song et al., [144] and Astals et al., [76] expound the advantages of using co-phased anaerobic system over the single phased thermophilic digestion as a way to avoid sensitivity associated with operational conditions such as temperature change, organic loading rate and characteristics of substrates unlike the single stage mesophilic anaerobic digestion. In anaerobic digestion several factors that can effect pathogens die-off exist among which pH and temperature are the main.

5.6.2 Contributing factors for pathogens reduction in two-phase anaerobic digestion system

5.6.2.1 Substrate pH in the reactor

pH is an important parameter that influence metabolic rate and ion transport systems in both useful microorganisms and pathogens in the reactor therefore govern their growth and activities [145,146]. Busch et al., [56] pointed out that in two-phase anaerobic digestion process the four complex digestion steps called hydrolysis, acidogenesis, acetogenesis and methanogenesis which involve consortium of specific microorganism for the accomplishment of anaerobic digestion of substrate for biogas equally take place though in separate reactors as shown in Figure 5.5 that has been modified after Nayono [147], Deublein and Steinhausser [59] and Langhans [148].

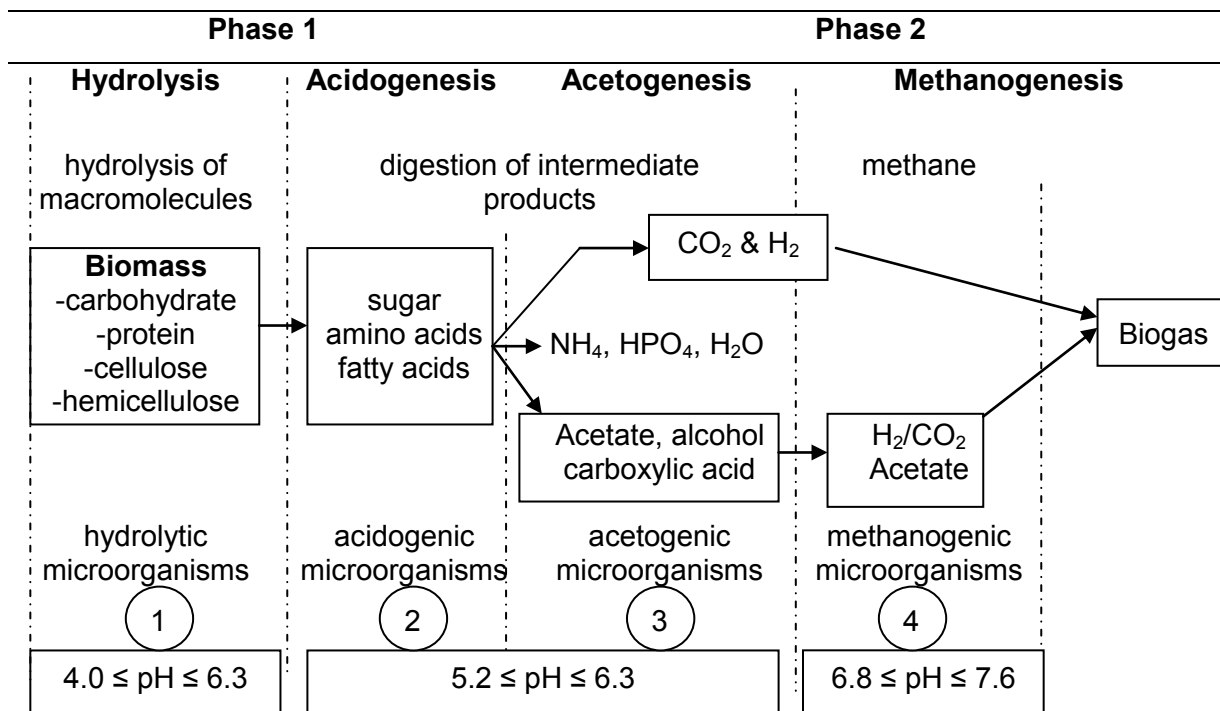


Figure 5.5: Simplified schematic representation of two-phase anaerobic digestion

Deublein and Steinhauser [59] explain that the first three processes involving (i) enzymatic decomposition of polymers (fermentation), (ii) formation of organic (fatty) acids (acidogenic step) and (iii) conversion of higher organic acids and monomers into acetic acid (acetogenic step) together form the hydrogenesis stage and take place in the hydrolysis reactor while (iv) methane generation known otherwise as methanation step takes place in methanogenesis stage within the methanation reactor. Explaining the complexity of the hydrolysis process, Veeken and Hamelers [149] expound that the hydrolysis rate depends on temperature, pH, type of substrate and the concentration of hydrolyzing organic matter in the substrate. The separation of liquefaction and acidification process from methanation process is vital at providing conducive environment to microorganisms which have different nutrient and growth requirements in the two reactors and therefore enhance their microbial activity and bioreactor performance.

More CO₂ up to 50% and other gases including H₂S and NH₃ are released out of acidogenic digestate during hydrolysis stage and therefore allows more methane concentration >72% with more reduction of H₂S than when operating single-phase

anaerobic digestion [56,59]. Unlike the single stage mesophilic anaerobic digestion, Song et al., [144] and Astals et al., [76] explained the advantages of using co-phased anaerobic system as a way to avoid sensitivity associated with operational conditions such as temperature change, organic loading rate and characteristics of substrates which are very challenging when using batch/single phases digestion systems. Separation of the hydrogenesis and methanogenesis process in the two-phase anaerobic digestion process is therefore meant to effect better metabolization of organic compounds by converting them into easily biodegradable materials [58], increase biogas production following creating conducive condition to every indigenous useful micro-flora in accordance to their optimum working environment.

5.6.2.2 Substrate temperature in the reactor

Anaerobic digestion systems are either operated at mesophilic temperature range ($37\pm 2^{\circ}\text{C}$) or at thermophilic temperature range ($55\pm 1^{\circ}\text{C}$). The reactor temperature equally regulates internal temperature of the microbes as well as the pathogens and thus dictates composition of in the biomass, nature and rate of metabolism processes [145] which affects utilization of carbon in the substrate. Apart from enhancing the rate of anaerobic digestion process in both hydrogenesis and methanogenesis stages, temperature especially when operated at thermophilic range plays a major role in elimination most if not all of the epidemics and phytopathogenic microorganisms fed together with the substrate in the reactors [59] and therefore reduce the chance of spreading the potentially infectious pathogens into environment while using digestate for soil amendment or as organic fertilizer.

5.6.3 Substrate for anaerobic digestion

All biomass types composed of biodegradable compounds including carbohydrate/starch, protein and fat fit for anaerobic digestion [150–152] provided proper treatment is applied. Biogas and specifically methane yield potential of any biodegradable biomass is a function of the type of substrate used, the system of digestion used and how long the materials remain in the digester [58]. While the latter two factors are technically based, the first factor dealing with type of substrate depends on what is sustainably available and economically feasible for the digestion process. Reports shows that in the UK, dairy

manure, blood, food wastes and chicken manure are the main substrate used for anaerobic digestion. Denmark use dairy manure, pig manure, poultry abattoir waste and cattle slurry. Spain use pig and dairy slurry, Germany use multiple sources including energy crops, food waste and animal manure, Italy use wastewater sludge and Sweden use mostly food waste and composted wastewater sludge [153].

Successful researches on anaerobic digestion of biowaste have been documented for the past three decades [13]. Briefly, there has been an increase in biogas use recovered as by-product of anaerobic digestion of biowaste in many developed countries including Germany, Sweden, UK, Spain, Poland, The Netherland, etc. Pathogens contamination in the biowaste and the confirmation that pathogens can possibly pass the digestion process unaffected has increased threats of transmitting of these microbiological contaminants and cause global health problems. Two-phase anaerobic digestion system running with one of the reactors at thermophilic temperature is considered effective at pathogens inactivation. The promising hygienization results are rather on the heat sensitive pathogens for example the heat sensitive non-spore forming bacteria and not necessarily on the heat resistant bacteria such as *S. senftenbergensis*, *Clostridium perfringens*, *Bacillus spp* and viruses [154]. This is simply accounted by the small residence time the substrate have in the reactor especially if the substrate feeding and discharge of the digestate takes place in less than 48hrs.

5.6.4 Advantages of using two-phase anaerobic digestion system

A reason behind efficiency in two-phase anaerobic digestion is process separation, improved control and adaptability to various substrates. Normally in this anaerobic digestion system, the biochemical reactions takes place in a separate reactors thus accommodates high organic loading rate (OLR), offer shorter hydraulic retention time (HRT) and reduce chances of process inhibition since each process assures the microorganisms involved with optimum environment as well as nutrient requirements [70]. Besides being in operation for centuries with the endowed advantages such as high biogas production with higher percent of methane proportion, little is known about the extent for which two-phase mesophilic/mesophilic anaerobic digestion (MMAD) and the two-phase mesophilic/thermophilic anaerobic digestion (MTAD) could inactivate or cause complete pathogens elimination in contaminated biowaste.

5.7 Materials and Methods

5.7.1 Pathogens hygienization in two-phase anaerobic digestion system

A two-phase reactor at pilot-scale set-up of anaerobic digestion operated as semi-continuous fed reactor using potato peels (23-25% TS) pre-artificially spiked with *E. coli* and *S. senftenbergensis* at 100:1w/v (blended peels to *E. coli* and *S. senftenbergensis* suspension $\approx 10^6$ - 10^9 CFU/g wet sample) was used in a completely stirred-tank reactor (CSTR) model. In this set-up, hydrogenesis and methanogenesis process takes place in separate reactors. The investigation started on June 01, 2015 and was carried out in the laboratory hall of Chair of Waste Management, BTU Cottbus-Senftenbergensis. Two insulated plastic containers (high density polyethylene) having a dead volume of 70L capacity each were used as bioreactors. One reactor (HY-J) was used for hydrolysis process and the other (ME-P) was used for methanation process. Picture 5-3 illustrates the set-up of reactors, insulation used, substrate inlet and outlet corks, biogas outlet vents, temperature monitoring probes, motor and continuous stirrer rods.



Picture 5-3: Set-up of two-phase CSTR anaerobic digestion system

5.7.2 Design and description of anaerobic digestion process

Mesophilic temperature range of $37\pm 2^{\circ}\text{C}$ for hydrolysis reactor was set throughout the experimental period while the methanation reactor was firstly set at mesophilic temperature that was operated for 10 days (mesophilic/mesophilic anaerobic digestion) then the temperature of methanation reactor was elevated to $55\pm 1^{\circ}\text{C}$ temperature range recommended for mesophilic/thermophilic two-phase anaerobic digestion. Each reactor (see Figure 5.6) was fitted with a gas tight aluminium foil sack and a gas meter (Ritter, Germany) for collecting and quantification of the produced biogas.

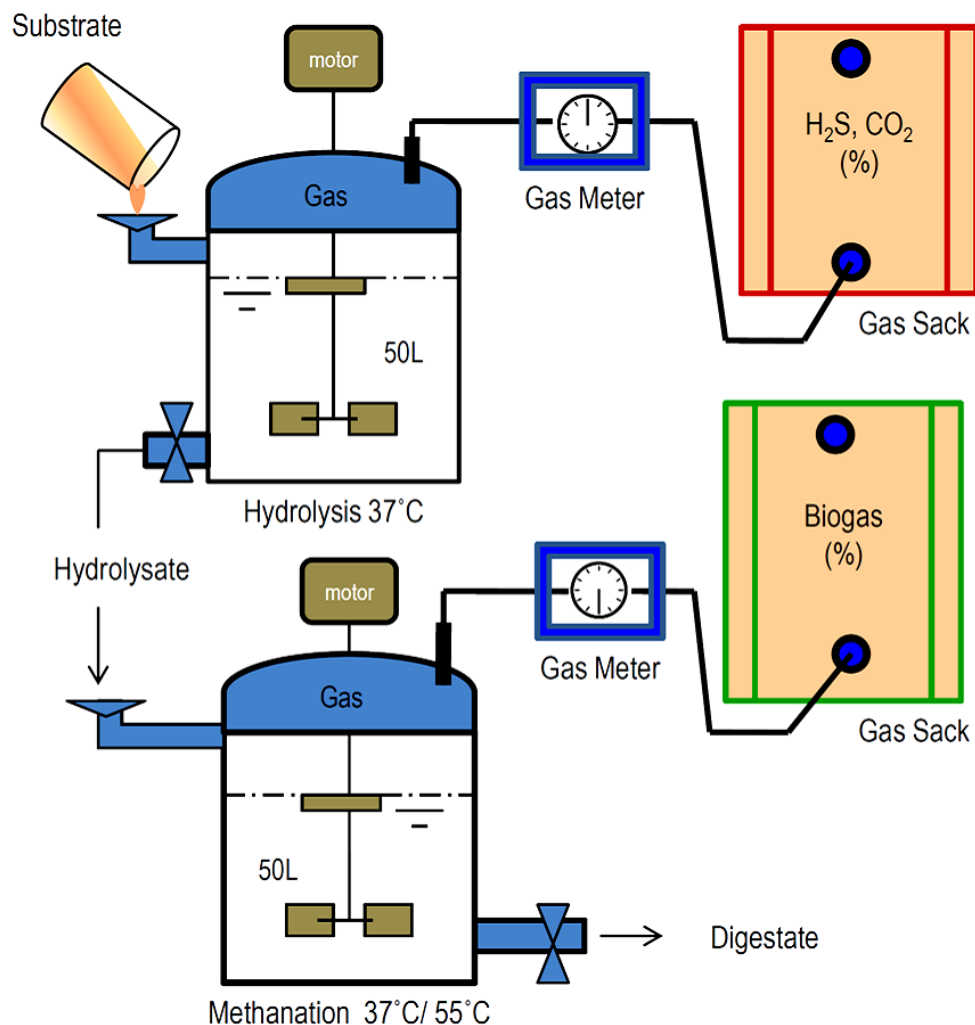


Figure 5.6: Schematic assembly of pilot scale two-phase anaerobic digestion system

The temperature in each reactor was maintained through insulation and constant circulating hot water that was heated and pumped from water-heating baths through the jacket externally connected around each reactor using 13mm diameter vinyl tube. The combination of mesophilic and thermophilic ranges in the second investigation was of interest to maximally collect information on the operating efficacy of the two anaerobic digestion processes at effecting inactivation of pathogenic microorganisms.

5.7.3 Reactor suitability and contamination test

Reactors suitability check for digestion process was carried out for 6 days from June 04-09, 2015. This aimed to observe scenario such as presence of leakage (biogas and substrate), lid tightness, motors performance and stirrer, temperature stability and absence of microbial pathogens contamination in both hydrolysis and methanation reactor before commencing the anaerobic digestion process. The investigation was carried out through thermophilic operation of both reactors using water (pH 7.3, conductivity 5.4 μ S/cm, salinity 0.0‰ and TDS 5.4mg/L) at 50L active volume of both reactors. After day 7 of operation, water sample from both reactors was aseptically sampled and analyzed for *E. coli* and *Salmonella spp* contamination.

5.7.4 Substrate feeding and process monitoring in the reactors

Potato peels artificially contaminated with *E. coli* and *S. senftenbergensis* was the only substrate used in the entire investigation. The hydrolysis reactor was fed once per day with the blended peels whose organic loading rate (OLR) has been pre-determined. The 50L active volume of the methanation reactor was seeded with cow manure slurry (CMS) that was received from one cattle herds within Cottbus. Due to the fact that cattle are among the main reservoir of enteric bacteria in particular *E. coli* [155] whose population can go up to 10⁵-fold CFU per gram and the fact that this investigation involved spiking of *E. coli* in the substrate for investigation of die-off levels, it was of the essence therefore to analyze the CMS for enteric bacterial contamination in this case *E. coli* and/or *Salmonella spp* prior to seeding. The CMS was sieved (5mm diameter mesh) for removal of pebbles and larva (caterpillars). After sieving, the CMS was analyzed for *E. coli* and *S. senftenbergensis* and other physicochemical attributes including moisture, ash, pH, oTS (%TS), conductivity and salinity.

5.7.4.1 Substrate feeding in hydrolysis reactor

In order to easily flush the substrate into the reactor, a wet anaerobic digestion system was preferred in which the substrate total solid of blended peels (77.3% moisture, 22.7% total solids, 94.7% oTS(%TS) suitable for dry digestion was reduced to less than 15% TS through mixing with tap water or process water (hydrolysate) as suggested in Mata-Alvarez [137] and Mata-Alvarez et al.,[156].

5.7.4.2 Substrate feeding in methanation reactor

Prior to substrate feeding, the methanation reactor with cow manure slurry (seeding) was operated by means of hunger phase at mesophilic temperature $37\pm 2^{\circ}\text{C}$. This is a necessary step recommended in VDI 4630[157] to sufficiently allow a complete degradation of organic matter in the cow manure, allow diverse mesophilic micro-flora acclimatize to the new environment and also stabilize the anaerobic digestion process [70,110]. This was also a very important process that avoids over estimation for biogas production from the substrate. During operation the methanation reactor was fed with hydrolysate from hydrolysis reactor based on the fill-and-discharge mode at 24hrs interval following the pre-determined OLR.

5.7.4.3 Substrate flow rate in the reactor

Total solids of the potato peels feeding slurry in the reactor was maintained between 5.2-5.7% with oTS (%of TS) ranging 88.3-88.9%. In this investigation, using an assumption that $1\text{kg} \approx 1\text{L}$ [110], a unit mass of the blended peels (wet wt.) was diluted following Deublein and Steinhauser [59] recommendation in 4units of either tap water or re-mixed with one unit of process water (hydrolysate). The daily mass flow was performed in a fill-and-discharge mode determined in accordance to Vögeli et al., [110] following the following equation:

$$Flow = \left(\frac{Wt.sample + (4L_{water} * Wt.sample)}{1day} \right) = L / d \quad (5.2)$$

5.7.4.4 Organic Loading Rate

The organic loading rate (OLR) of 0.5kgoTS/m³.day proposed in the Association of Germany Engineers, VDI-4630 [157] was used as the benchmark during gasification that was reached after 10days of mesophilic-mesophilic anaerobic digestion process. The OLR for both hydrolysis and methanation reactors were determined applying the following equation:

$$OLR \left(\frac{g \text{ oTS}}{L * day} \right) = \frac{Wt_{feed} * TS(\%) * \text{oTS}(\%TS)}{AV_{reactor}} \quad (5.3)$$

Whereby:

Wt_{feed} =weight of wet peels fed in the reactor per day,

$AV_{reactor}$ =is the active volume of the reactor used, i.e. 50L

oTS = organic total solids of peels

TS = Total solids in the peels

5.7.4.5 Hydraulic Retention Time

At a start-up phase, both reactors were set to operate at mesophilic anaerobic digestion (MAD) temperature range which was later changed to mesophilic and thermophilic combination. The hydraulic retention time (HRT) of 25 days during mesophilic digestion and 12.5 days during thermophilic digestion varying with respect to the substrate flow rate (L/day). The HRT was calculated as a ratio of active reactor volume to the input flow as indicated in the following equation:

$$HRT = \left(\frac{AV_{Reactor} (L)}{Flow(L / day)} \right) = days \quad (5.4)$$

5.7.5 Setting of bacterial contamination benchmark in the reactors

To avoid errors in a course of pathogens inactivation rate of anaerobic digestion process due to bacteria dilution in the 50L of reactor's volume, about 100mL of the enriched and mixed bacteria suspension having 1.4×10^8 CFU/g (8.2log) and 4.8×10^7 CFU/g (7.7log) for *E. coli* and *S. senftenbergensis* respectively was added in the two reactors to benchmark the initial enteropathogens population. Both hydrolysis and methanation reactors at this

time were operated at $37\pm 2^{\circ}\text{C}$ with a HRT of 25 days and a mean cell residence time of 24hrs. Hydrolysis of potato peels was performed for 8days recommended in Busch et al., [56] as the time taken for biowaste to achieve a complete hydrolysis prior to feeding into the methanation reactor.

The efficiency of the hydrogenesis process was monitored through evidence of starch hydrolysis/swelling (Picture 5-4) and by changes in hydrolysate pH (Mettler Toledo, InLab® Expert NTC30), gas production CH_4 , CO_2 and H_2S (Gasiotechnical -Geotech, UK) a confirmatory sign of biodegradation of complex starch compounds in the potato peels to starch-nanocrystals and simple sugars following water uptake during hydrolysis and fermentation process [158].



Picture 5-4: Hydrolysate by solely indigenous micro-flora

On day 20 of operation of hydrolysis reactor, process water (hydrolysate) from another already running hydrolysis digestion reactor adapted to maize substrate was added in order to enhance hydrolysis and bust the indigenous bacteria in the hydrolysis reactor for quicker fermentation of the peels, see Picture 5-5 after observing low hydrolysis/ fermentation following an organic shock load of the indigenous micro-flora in the peels.



Picture 5-5: Hydrolysate after addition of hydrolysate from another anaerobic digester

5.7.6 Mesophilic/Mesophilic anaerobic digestion operation

Mesophilic/Mesophilic anaerobic digestion (MMAD) simply means a two-phase anaerobic digestion systems for which both reactors operates at mesophilic temperature range. Full operation of MMAD including substrate feeding into the methanation reactor started after 8days of operation of the hydrolysis reactor. Therefore, from hydrolysis reactor the initial hydrolysate/process water pH 4.9 at OLR of $0.12\text{kgTS}/\text{m}^3\cdot\text{day}$ was transferred into methanation reactor operating at mesophilic temperature seeded with cow manure slurry (pH 7.4). On the following day, before feeding the methanation reactor, an equivalent

amount of digestate was discharged then the hydrolysate was fed to replace the removed digestate and thus maintained the reactor active volume. Because of the presence of other microorganisms in cow manure slurry that were capable to grow on Endo Agar, all spread plate experiment for digestate from methanation reactor were done on modified BGA which showed great inhibition to other bacteria strain except *Salmonella spp* and *E. coli*. Along with the analysis for microbial reduction, the efficiency of methanation process was monitored through analysis of pH, salinity, conductivity and reduction in volatile solids in digestate as well as the production of CH₄, CO₂ and H₂S. In each reactor, pH was regarded as the first indicator of the process performance. After observing a steady-state in the reactor normally within 5days, the OLR was doubled to 0.24kgoTS/m³.day. Since the MMAD was for evaluating the extent of *E. coli* and *S. senftenbergensis* die-off only, it was operated for only 10 days and thereafter the temperature in the methanation reactor elevated to 55±1°C which is the thermophilic temperature range.

5.7.7 Mesophilic/Thermophilic anaerobic digestion operation

Substrate feeding in the methanation-thermophilic reactor of two-phase anaerobic digestion (MTAD) started 10 days after operation of the mesophilic/mesophilic two-phase anaerobic digestion (MMAD). In this experiment, the heating temperature in methanation reactor was elevated to 55±1°C while that of hydrolysis reactor was maintained at 37±2°C. During this period both reactors were operated at wet digestion process (substrate total solid ranging 5.2-5.7, with 88.9% oTS(%TS) following recommendation in Weiland [58] and corresponded to hydraulic retention time (HRT) of 12.5days. The feed organic loading rate (OLR) of 0.48kgoTS/m³.day that was reached in hydrolysis reactor was assumed for methanation reactor as it was fed with process water (hydrolysate having pH 4.7) from the hydrolysis reactor. Other OLR reached during the experiment were 0.96 kgoTS/m³.day, 1.44 kgoTS/m³.day, 1.92 kgoTS/m³.day and 2.4 kgoTS/m³.day. These OLR are in conformity with the typical loading rate of organic dry matter for wet anaerobic digestion process which should not exceed 4kgoTS/m³.day according to Weiland [58].

5.7.8 Batch operation of two-phase anaerobic digestion

Self-hygienization due to generated low pH medium following fermentation of sugars in the potato peels as substrate was set based on batch operation principle of anaerobic

digestion. On day 49 of operating the two-phase anaerobic digestion processing with daily substrate feeding of potato peels spiked with enteropathogens, about 100mL nutrient broth suspension containing enriched *E. coli* (population level of 4.2×10^8 CFU/g) and *S. senftenbergensis* (population level of 3.7×10^8 CFU/g) was added into each reactor (50L active volume) exhibiting good stability in terms of temperature 36.8°C and 55°C and stable pH values reading at 4.2 and 7.7 for acidogenic digestate and methanogenic digestate respectively.

5.7.9 Process monitoring and Microbiological analysis

In general, reactor temperature, gas temperature, pH, room temperature, pressure, conductivity, salinity and gas generation were analyzed on daily basis as part of the process monitoring activity. Hydrolysate and digestate samples for microbiological analysis were collected in at least 2 working day's interval. Temperatures in both effluents were monitored using automatic sensor inserted inside each reactor. Samples of digestate for analysis of volatile solids removal were daily collected and store in the fridge (approximate 8°C) to be analyzed on weekly basis. All analysis were conducted as stated in Zupan [159], using in-house developed protocols as well as international standard ISO 6579:2002 (E) [160].

5.8 Data analysis and presentation of results

Data from this experiment were analyzed using, MS Excel 2007 spreadsheet and Statistical Package for Social Sciences (SPSS IBM-Statistics 20.0 for Windows). The results have been presented as mean or confidence interval for 95% in case of more than 3 observations as well in figures comprising of bar-charts and graphs and tables.

5.9 Results and Discussion

5.9.1 Characteristics of substrate (wet digestion) and seeding used in anaerobic digestion process

The quality attributes of seeding cow manure slurry and that of hydrated and enteropathogens contaminated potato peels is shown in Table 5-10. The pH in both

substrate and seeding was optimal for bacteria activities which require a range of 6-8 and the seeding sludge was found to comply to the recommended quality by having 75.2% oTS(%TS) over the 50% stated in VDI 4630 [157].

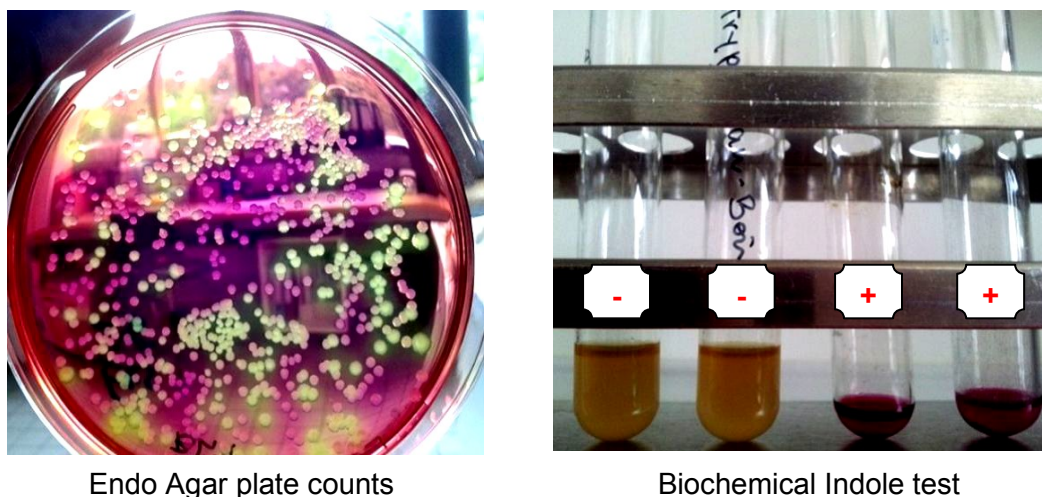
Table 5-10: Selected characteristics of cow manure slurry and substrate

Parameter	Cow manure		Unit
	Potato peels	slurry	
Moisture	96.6	91.9	% w/w
pH	6.3	7.6	-
Conductivity	0.76	13.9	mS/cm
Salinity	0.1	8.1	‰
oTS (%TS)	88.9	75.2	%w/w
<i>E. coli</i>	10 ⁶ -10 ⁸	1.6x10 ³	CFU/g
<i>Salmonella spp</i>	10 ⁶ -10 ⁸	< LoQ	CFU/g

Together with other bacteria which were not identified due to the fact that this research much focused on only two enteropathogens strains i.e. *Salmonella spp* and *E. coli*, the seeding slurry presented a substantial population of *E. coli* which was high above the probable infectious doze specified to be <10 cells [77,161] for pathogenic *E. coli* to humans. The values for conductivity and salinity measured in the cow slurry though presented higher solutes in the solution above the permissible ranges of 0-4 mS/cm specified in Hargreaves et al.,[128] for direct application on agricultural soil, it has been reported from other research including Singleton at al., [162] that microorganism can possibly tolerate up to 25 ‰ salinity without experiencing inhibition.

5.9.2 Microbiological quality of the seeding/inoculum

Results for spread plate of cow manure slurry gave four different colony units which were differentiated based on colours. Exception for the cell mass grown with metallic sheen-gold colour which is a typical identification for *E. coli*, the other resulted bacterial colony units were supposed to fall under other microbial groups and categories within *Enterobacteriaceae* family. The colony units appeared green-gold, pink, red and yellow/colourless (see Picture 5-6).



Picture 5-6: *Enterobacteriaceae* viability and confirmation in cow manure slurry

Since confirmation of the bacteria colony units based on colour discrimination was impossible, the use of a highly selective modified BG agar and Biochemical Indole test was of the essence after an overnight aerobically enrichment (Nutrient Broth, Carl Roth, 36°C) of the colony units and spread of 100µL of the cultures on modified BGA petri-plate. Out of the four colony units isolated, two test tubes displayed indole positive results by the formation of a deep red ring while the other two a yellow-brown ring indicated negative results (see Picture 5-6 above). Therefore, inhibition and selectivity capacity of modified BGA helped to confirm *E. coli* contamination in the cow manure slurry at population density of 1.6×10^3 CFU/g but with the absence of *Salmonella spp.*

5.9.3 Efficacy of two-phase anaerobic digestion at inactivation of enteropathogens

The initial bacteria population analyzed was in average of 2.5×10^4 CFU/g and 1.3×10^4 CFU/g for *E. coli* and *S. senftenbergensis* respectively in the hydrolysis reactor and in the methanation reactor the average population was 6.5×10^6 CFU/g and 4.2×10^6 CFU/g for *E. coli* and *S. senftenbergensis*. The two-phase mesophilic anaerobic digestion reactor seeded with cow manure slurry was operated for 10 days. This anaerobic digestion process present a most widely used process in digestion of biowaste for biogas production all over the world [163], but the hygienization efficiency achieved during mesophilic biogasification is insignificant and in most cases unquantifiable [76].

5.9.3.1 pH and Temperature behaviors in mesophilic hydrolysis anaerobic digestion

Change in pH as a result of substrate fermentation during hydrogenesis process is considered detrimental to pathogenic microorganisms as the change in pH affect the optimal condition for their survival. The results for pH drop as a function of OLR in the hydrolysis reactor digesting potato peels are presented in Figure 5.7. There was a fast drop in pH at lower loading rate right from day 1 of the process in which the OLR was set at 0.12 4kgoTS/m³.day till the first increase of the OLR to 0.2kgoTS/m³.day. Briefly, during the anaerobic digestion process, degradable organic compounds are broken down, firstly by acetogenic bacteria to methane precursors largely volatile fatty acids (VFAs).

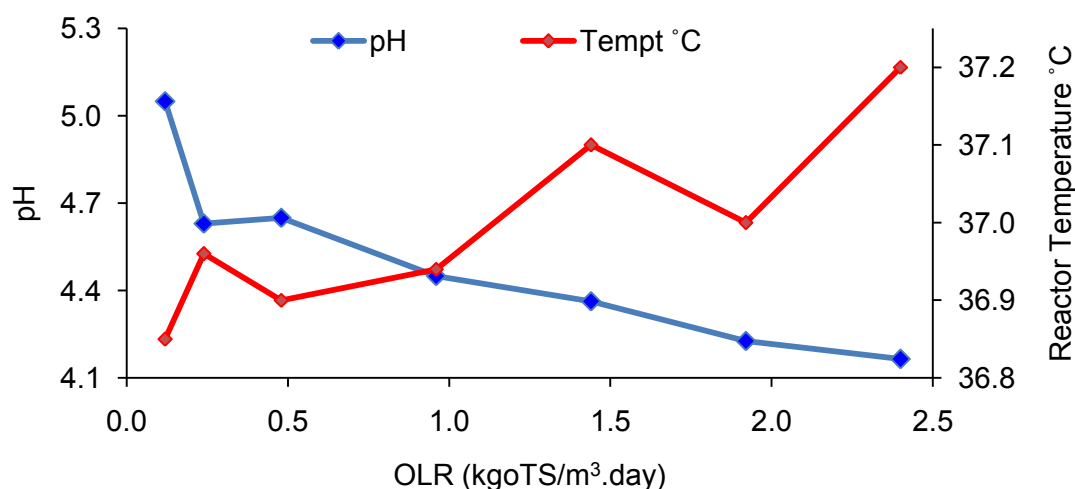


Figure 5.7: Temperature and pH in hydrolysis reactor treating potato peels waste

The acidic condition formed during the hydrogenesis stage causes a drop in pH of the process water. The indigenous microbial community in hydrolysis process (hydrolytic and acidogenic bacteria) breakdown complex compounds (starch, protein and fat) into simple compounds including sugars, fatty acids and glycerol, amino acids and cellobiose. The formation of these simple compounds causes decrease in pH as they ferment. The low pH affects enzymatic activity in microorganisms due to the fact that each microbe has its optimum pH for survive.

Change in OLR from 0.24kgoTS/m³.day to 0.48kgoTS/m³.day was observed to induce organic shock load to the indigenous fermentative micro-flora that lead to a constant pH for 6 days. Upon addition of process water from another digester added more fermentative microbes and resulted into a normal fermentation process leading to pH drop even at the OLR of 2.4kgoTS/m³.day which was the maximum loading reached during the investigation, there was high rate of fermentation with pH 4.2 yet with no sign of inhibition. On the other hand, temperature of the substrate in the reactor was observed to be stable at mesophilic acceptable range between 36.8°C and 37.2°C which is an optimum range for most of the fermentative micro-flora i.e. acidogens, methanogens as well pathogenic microorganism. The by-product of fermentation process which includes volatile fatty acids concentration are said to contribute to the die-off in pathogens. In addition, higher concentration of H₂S produced as by-product of substrate decomposition is considered toxic to pathogenic microorganisms and therefore could have induced inhibition and reduction of *E. coli* and *S. senftenbergensis* even though in this study their fraction contribution could not be quantified.

5.9.3.2 Pathogens reduction in hydrolysis reactor

Since the hydrolysis reactor was operated at mesophilic temperature throughout the investigation, the results for pathogens inactivation presented in Figure 5.8 below covers the hygienization extent of the process in all 49 days of the experiment.

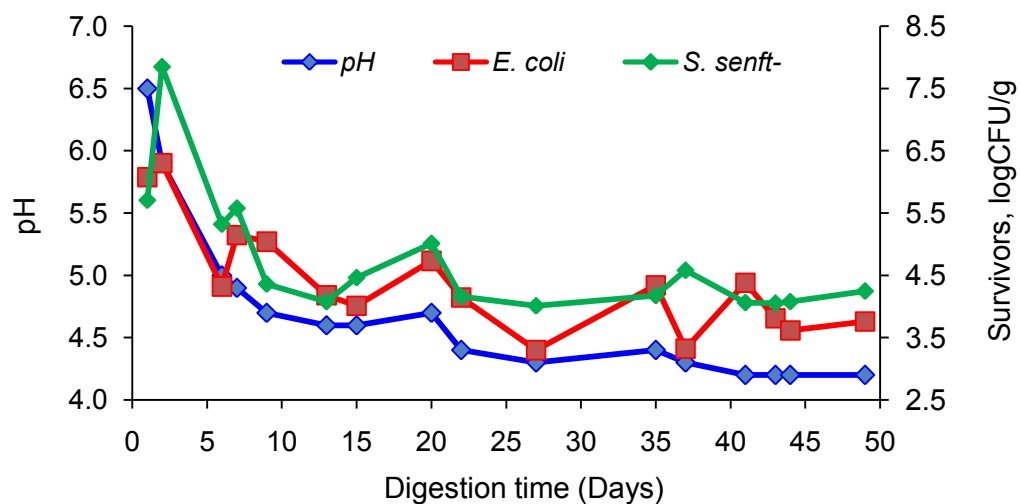


Figure 5.8: Reduction of pathogens load (logCFU/g) in hydrolysis reactor

The population background for *E. coli* in the hydrolysate (pH=6.5) was determined at 1.2×10^6 CFU/g (6.1log) and that for *S. senftenbergensis* was 5.1×10^5 CFU/g (5.7log). Following the presence of conducive growth factors for microorganisms that included optimum temperature of 37°C, pH of 5.9 and the carbon source (substrate), the population of both bacteria initially increased. *E. coli* increased to 6.3logCFU/g while that of *S. senftenbergensis* reaching 7.8logCFU/g on day 2 of the hydrolysis process. In this investigation there was a sharply drop in pH from 6.5 to 4.6 within 2 weeks reflecting a typical characteristics of substrate with higher starch and degradable sugars as a result of the so called “souring” [164]. The pH drops have an impact on the multiplication of the pathogens since lower pH as such does not support the growth in both *E. coli* and *S. senftenbergensis* whose optimum pH is around the neutral zone.

Even though on day 6 there was a pH of 4.9, there was a decrease in population for both *E. coli* and *S. senftenbergensis*. This observed initial decrease in both *E. coli* and *S. senftenbergensis* population could probably be caused by the observed drop in pH as well as high concentration of H₂S which was higher above 4742ppm in the hydrolysis reactor. On day 13 of hydrolysis process (pH 4.6) there was a continual reduction of both bacteria strains until when the increase in substrate loading in the hydrolysis reactor resulted into an organic shock load to the indigenous microorganism performing the fermentation process. This was observed as a delay in fermentation of the added potato peels since up to this time the hydrolysis depended on the normal indigenous micro-flora present in the peels. The delayed fermentation resulted into a slight increase in pH from day 15 to day 19. This resulted into slight increase in *S. senftenbergensis* population for about 0.4logCFU/g although for unknown reasons *E. coli* was slightly reduced by 0.2logCFU/g. Addition of process water from another reactor added more acidogens bacteria which enhanced fermentation process therefore and brought about pH drop to about 4.2 in the hydrolysis reactor which was maintained until the end of the experiment.

Figure 5.9 gives the die-off rate for *S. senftenbergensis* and *E. coli* in terms of D-value that could be achieved during hydrolysis stage of anaerobic digestion reactor operating at mesophilic range of temperature. Generally there was no possibility of achieving a complete elimination of either *E. coli* or *S. senftenbergensis* during this set up of anaerobic digestion system. This is due to the fact that *E. coli* and *S. senftenbergensis*

being facultative anaerobes can generate ATP in the presence of oxygen or switch to anaerobic respiration in the absence of oxygen. Previous studies [165,166] report that as the concentration of acid in the process water increases gradually following the rate of fermentation, so is the acid-adaptability and heat tolerance to pathogenic microorganisms increases.

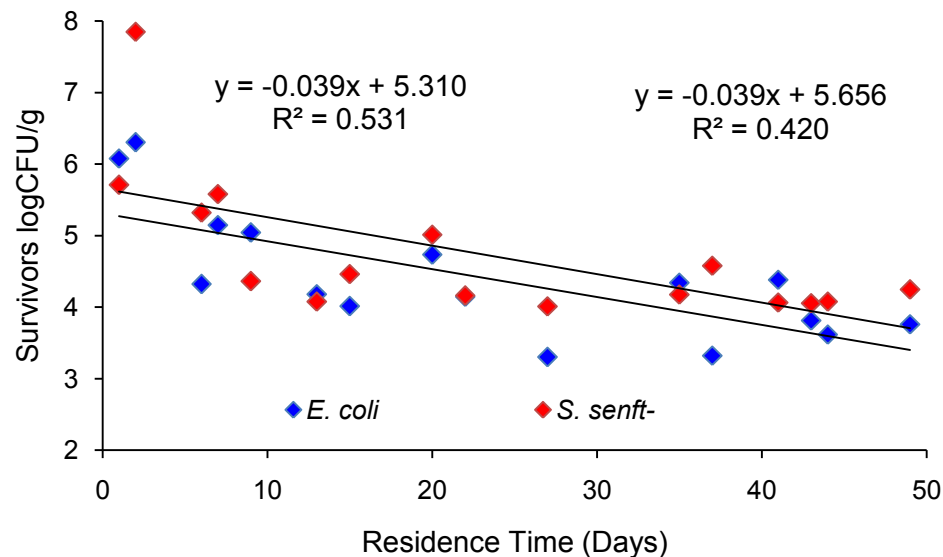


Figure 5.9: Decimal reduction (D-Value) for *E. coli* and *S. senftenbergensis* in hydrolysis stage

Low substrate residence time in the hydrolysis reactor affected much the die-off level of *E. coli* and *S. senftenbergensis*. In the general trend, the extents at which hydrolysis stage of the two-phase anaerobic digestion system reduced enteropathogens in biowaste are shown in Table 5-11. Results in this investigation reveal that *E. coli* and *S. senftenbergensis* tolerated as low as pH value 4.2 thus managed to survive in the hydrogenesis stage. At the end of this investigation, *E. coli* was still surviving and maintaining the population ranging 3.3-4.0logCFU/g while *S. senftenbergensis* population was a bit higher than *E. coli* having population ranging 4.0- 4.2logCFU/g. Therefore, these results make a die-off rate of 1.0 log-cycle equivalent to 90% reduction for 25days to both *E. coli* and *S. senftenbergensis*. The finding on inability of mesophilic anaerobic digestion to eliminate pathogens is not new and has been similarly observed and reported in other studies based on anaerobic digestion field including Bagge [13], Sahlström [69], Kjerstadius et al., [167] and Horan et al., [168].

Table 5-11: Pathogens reduction efficiency of two-phase mesophilic hydrolysis reactor

Residence Time (Day)	log reduction*		% Reduction (logCFU/g)	
	<i>E. coli</i>	<i>S. senft-</i>	<i>E. coli</i>	<i>S. senft-</i>
1	0	0	0	0
2	-0.23	-2.14	-3.7	-37.5
6	1.98	2.52	31.5	32.2
7	-0.82	-0.26	-19.1	-4.8
9	0.10	1.21	2.1	21.8
13	0.86	0.28	17.1	6.5
15	0.16	-0.38	4.0	-9.4
20	-0.07	-0.55	-17.9	-12.4
22	0.59	0.85	12.5	17.0
27	0.84	0.15	20.3	3.6
35	-1.04	-0.16	-31.6	-4.1
37	1.02	-0.40	23.5	-9.7
41	-1.06	0.51	-31.9	11.3
43	0.30	0.01	6.9	0.2
44	0.28	-0.02	6.9	-0.6
49	0.08	-0.10	1.9	-2.5

*Negative sign means bacteria population for which, $N_t > N_0$

The mesophilic temperature $37 \pm 2^\circ\text{C}$ in the reactor presents an optimum surviving range for both *E. coli* and *S. senftenbergensis* and this is the main reason explaining the inefficiency of mesophilic anaerobic digestion to achieve a complete inactivation of pathogenic microorganism. In this case, the main possible responsible factors responsible in reduction of pathogens were pH, antagonism between the pathogens and the indigenous micro-flora as well as inhibition from high concentration of H_2S gas. Although the ammonia concentration was not measured, the determined concentration of H_2S generated during fermentation of potato peels waste which was above 5000ppm (above LoQ of the gas measuring device) almost throughout the hydrogenesis process, and the pH which was fast dropping following an effective fermentation process contributed much to the observed reduction in bacteria population. Even though the retention time for

mesophilic digestion process (37 ± 2) normally ranges between 25-30 days [13], under the normal operation of the two-phase anaerobic digestion system, microbial cells hardly reside in the reactor for 24hrs before a portion of the hydrolysate is transferred to the second phase of the digestion process (methanation stage), this process greatly limits the mesophilic anaerobic digestion (MAD) stage from effecting a complete elimination of pathogenic microorganisms from process water (hydrolysate) besides the exhibited low pH during the operation.

5.9.3.3 Pathogens inactivation in Mesophilic-Methanation Reactor

Relationship between pH and temperature in the methanation reactor is presented in Figure 5.10. The OLR presents a direct proportional relation to pH as there was a rise in pH following increase in substrate feeding which is a sign of absence of inhibition. The mesophilic methanation reactor at this period was operated with two OLR. Throughout 10 days of operation, the confidence interval for temperature in the methanation reactor was 36.9 ± 0.1 for 95% confidence while the confidence interval for digestate pH was 7.3 ± 0.1 for 95% confidence. The population of *E. coli* and *S. senftenbergensis* in the process water (pH 4.8) used to feed the methanation reactor after 7days of fermentation in hydrolysis reactor was analyzed and found to be $5.2\log\text{CFU/g}$ *E. coli* and $5.6\log\text{CFU/g}$ *S. senftenbergensis*.

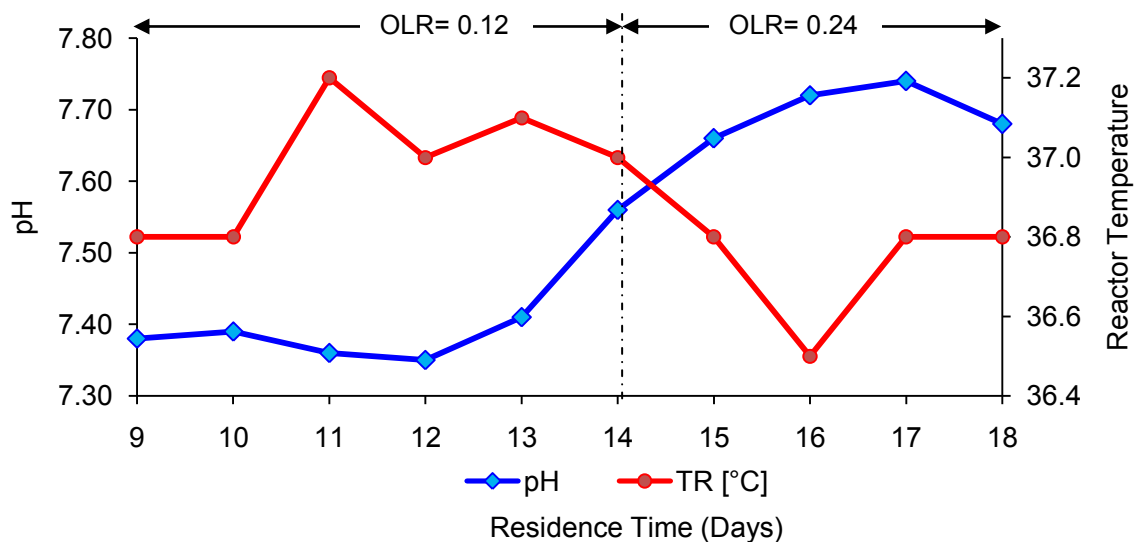


Figure 5.10: pH and temperature trend in methanation reactor of two-phase mesophilic/mesophilic biogasification

Following benchmarking of contamination and the *E. coli* that were confirmed present in the cow manure slurry made the viable enteropathogens population in methanogenic digestate (pH 7.4) to be 5.4logCFU/g and 5.2logCFU/g for *E. coli* and *S. senftenbergensis* respectively. It was observed that the population of *S. senftenbergensis* in the methanation reactor was fairly lower than the population in the hydrolysis reactor while the population of *E. coli* in methanation reactor was higher by 0.2logCFU/g. The reason for higher *E. coli* population in methanation reactor than *S. senftenbergensis* could be attributed by the additional fraction that was earlier present in the cow manure slurry even prior to artificial spiking of the enteropathogens. Results in this experiment for 8 days gave no exactly trend to refer as the reduction of pathogens in MMAD as per the results presented in Table 5-13. As both temperature and pH in this case present the optimum survival ranges for both *E. coli* and *S. senftenbergensis*, this makes difficult to achieve complete inactivation of the pathogens in the mesophilic anaerobic digestion since the optimum growth pH values and temperature for *Salmonella* is reported at 6.0-7.5 and 37°C [169] while pH 6-7 at 37°C present the optimum growth range reported for *E. coli* [170]. Though to some unknown reasons there was some reductions in both bacteria, there was no understandable trends with no significant difference between population means $p > 0.05$ within the four sampling days.

Table 5-12: Reduction of pathogens in methanation phase of two-phase mesophilic anaerobic digestion

Digestion Time (Day)	pH	Pathogens reduction (logCFU/g)	
		<i>E. coli</i>	<i>S. senftenbergensis</i>
1	7.4	5.2	5.4
5	7.4	5.5	4.8
7	7.7	3.9	5.4
8	7.7	4.1	4.6
Population Average		4.7	5.1
Uncertainty (95%)		±1.3	±0.7
p Value		$p > 0.05$	$p > 0.05$

Together with the presence of indigenous *E. coli* in the cow manure slurry that was used for inoculating the methanation reactor, variables and interacting conditions responsible for effecting pathogens die-off in the methanation phase of mesophilic /mesophilic anaerobic digestion (MMAD) are much limited simply because the known principal factors such as temperature and pH both conform with the optimum values in support of growth to both indigenous useful microorganisms and the pathogens [171] thus leaving contributing factors such as retention time, microbial antagonism, competition and presence of inhibitory substances to play the role in hygienization process. The microbial population was observed to be in dynamics, the increase in enteropathogens cells population might be as results of additional bacteria cells from the hydrolysate of the hydrogenesis reactor. In this study, there was no statistical significant difference ($p>0.05$) in the mean reductions for both minimum and maximum *E. coli* and *S. senftenbergensis*.

This could be attributed by either the initial number of pathogens loaded in the reactor, resistance of pathogens on intrinsic inhibitors and/or early adaptability of the pathogens into the new environment and the similar doubling time exhibited by both *E. coli* and *S. senftenbergensis*. On day 5, both numbers of viable cells in both enteropathogens were reduced to around 4.5log. The observed comparative low bacteria population on day 8 of mesophilic methanation could be simply based on the fact that the sample were taken few minutes after temperature transition from mesophilic to thermophilic range. It is theoretically supposed that microbial competition and inhibitions are the intrinsic factors behind the pathogens die-off in MMAD [59]. This is based on the fact that both factors, i.e. pH and temperature are within the optimum range for most of the pathogens that present in the substrate or system thus making it possible to the pathogens pass out the digestion together with the digestate unaffected. The increasing and decreasing trend on population observed in this study brought about an understanding that there also exists pathogens die-off in MMAD but the numbers of few dying cells are replaced by the new cells present in the hydrolysate. The die-off could as well be caused by H₂S inhibition which was observed to be very high in during both hydrolysis phases of potato peels waste and also averaged to 2150ppm in the digestate from methanation reactor. Figure 5.11 below presents the estimated decimal reduction time for both *E. coli* and *S. senftenbergensis* during the methanation stage of the two-phase anaerobic digestion process.

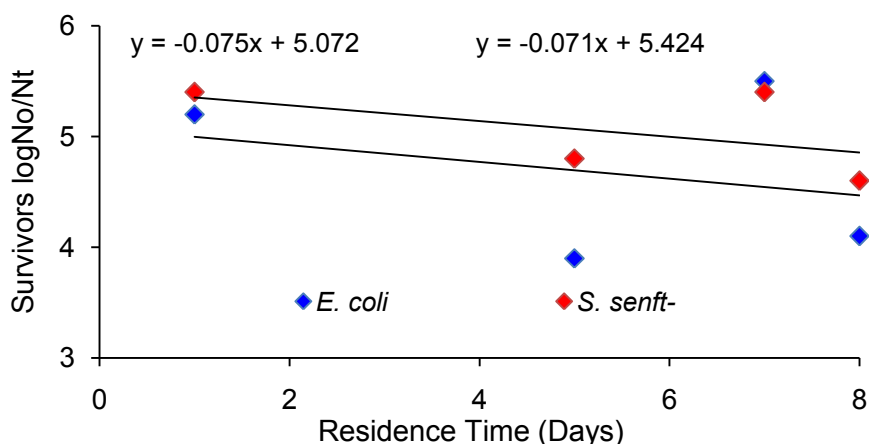


Figure 5.11: Decimal reduction (D-Value) for *E. coli* and *S. senftenbergensis* in methanation stage

The time estimate for reduction of one-log cycle for *E. coli* based on methanation conditions was found to be 14days while that for *S. senftenbergensis* was 15days. Therefore in the same way as explained in the case of retention time, cells residence in the reactor and mode of feeding the digester using process water from the hydrolysis reactor, it become impractical holding the digestate for long time estimated over 2 weeks in the methanation tank as concurred by Fukushi et al.,[165] in their study.

5.9.3.4 Pathogens inactivation in Thermophilic Methanation Reactor

The results for temperature and pH in relation to OLR in the thermophilic operated methanation reactor of the temperature co-phased anaerobic digestion system are presented in Figure 5.12. The pH of the digestate in the reactor was stable and maintained at 7.7 ± 0.2 while the temperature initially at $53.3 \pm 4.2^\circ\text{C}$. The large observed deviation from mean in temperature was results of operation challenges that included unstable weather of the template countries in which there was high drop of the outside temperature thus affected the insulation capacity of the reactor. Secondly was due to technical operation of the water heating jackets in which the temperature was automatically increased from the mesophilic range. This happened at early days of thermophilic operation of the methanation reactor in which the OLR was still $0.48\text{kgoTS/m}^3\text{.day}$.

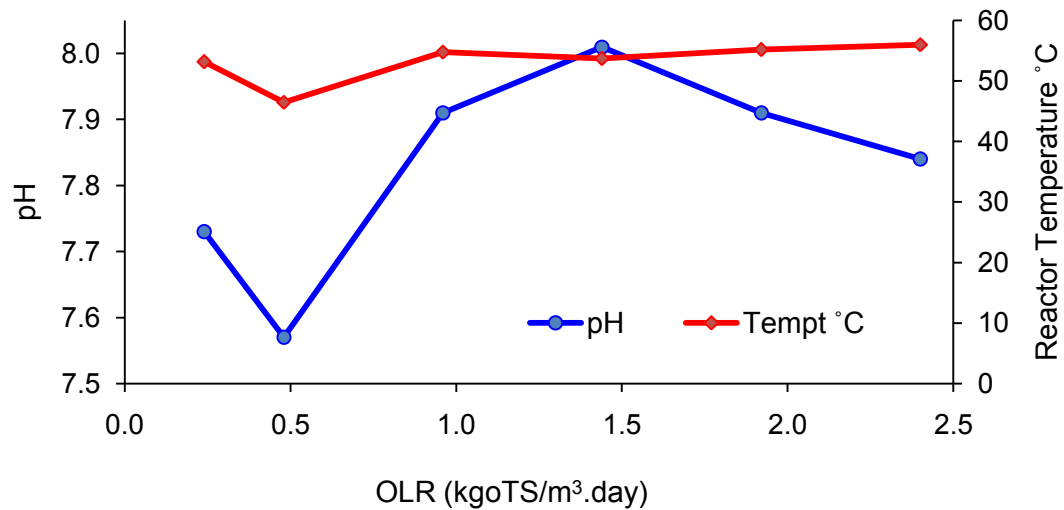


Figure 5.12: Temperature and pH in methanation-thermophilic reactor digesting potato peels waste

After rectification, the including applying more insulation to the reactor the temperature was maintained at $54.7 \pm 1.0^\circ\text{C}$ throughout the experiment independent from the changing OLR. The thermophilic methanation reactor was daily fed with hydrolysate (process water) from hydrolysis reactor which for the entire experiment was set to operate at mesophilic range simulating common systems of two-phase anaerobic digestion gaining interest and mostly operated in UK and EU definitely due to economic advantage as energy supplied is a bit reduced from one reactor but also the pathogenic elimination ability and hygienization effect potential [76]. factor combination including inhibition induced from evolved VFA, ammonia and H_2S concentration [59] and elevated temperature almost above the optimal range to most pathogenic microorganisms are responsible at inactivation of pathogenic microorganisms [76] and ensuring safe digestate.

Figure 5.13 present the results for the *E. coli* and *S. senftenbergensis* die-off levels in the sample of digestate drawn from methanation reactor of the two-phase anaerobic digestion system operating at thermophilic temperature range. Since monitoring of the thermophilic methanation reactor commenced on the day temperature was raised from the mesophilic range (approximate 37°C) to thermophilic range (approximate 55°C), on the first 2days of monitoring bacteria die-off levels, the population in both enteropathogens was still high at about $4.1 \log \text{CFU/g}$ and $3.5 \log \text{CFU/g}$ *E. coli* and *S. senftenbergensis* respectively.

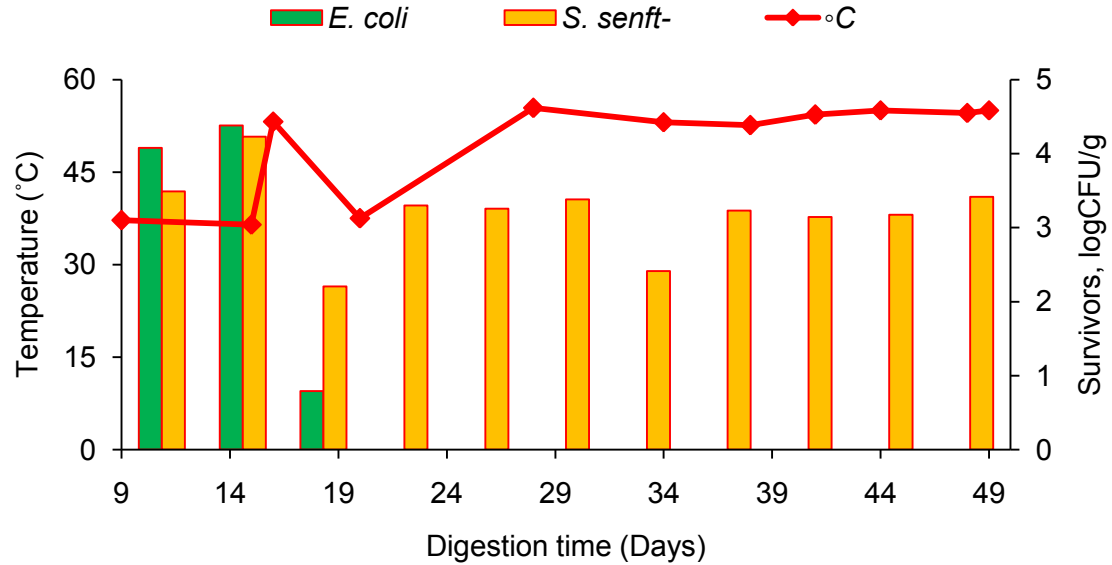


Figure 5.13: Reduction of *E. coli* and *S. senftenbergensis* in methanation-thermophilic reactor of two-phase anaerobic digesting potato peels waste

The temperature result on day 1 was still running as mesophilic range at 37.2°C, this was caused by a strong cooling effect on the digester from the surrounding. On day 2 the reactor temperature even dropped to 36.5°C and the population was maintained to almost the same level with a small increase of about 0.7logCFU/g in *S. senftenbergensis*. After 6days of operation there was still both enteropathogens surviving in the digestate caused by fluctuation of temperature but also probably as a results of acid-adaptation following hydrolysis process in the hydrogenesis reactor which might have induced heat resistant as highlighted by Álvarez-Ordóñez et al., [166]. Poor reactor insulation was a reason behind temperature fluctuation in the methanation reactor; this resulted into high level of bacterial contamination by having up to 4.4logCFU/g of *E. coli* and 4.3logCFU/g *S. senftenberg* in sample of digestate drawn on day 15 and day 20.

Following addition of insulation materials on the methanation reactor, the reactor temperature was raised to thermophilic range and was stable maintained at an average range of 54.3±1.1°C from day 28 till the end of experiment. Temperature increase to about 53.2°C on day 16 was enough to eliminate *E. coli* to a level of about 0.7log CFU of *E. coli* per gram of digestate. Temperature stabilization and maintaining for 24hrs prior to re-

feeding of the methanation reactor with new process water brought about a complete elimination of *E. coli* in all samples of digestate analyzed until the end of the experiment. Unfortunately, this achievement was not similar to *S. senftenbergensis* as there were still few cells surviving the 54.3°C for approximately 24hrs temperature-time regime. On average *S. senftenbergensis* population was maintained at 3.1CFU/g of digestate drained out on each day of the experiment. If post-biotreatment hygienization is not part of the anaerobic digestion process, this population of *S. senftenbergensis* would simply find its way into land where the digestate are to be disposed of or applied thus creating a new source of infection. As per the EU Regulation (EC) No. 208/2006, it is impossible to declare any use for the digestate coming from this process based on the uncertainty involved regarding its biosecurity. The Regulation requires that the digestate leaving the biotreatment facility for land application for instance be confirmed *Salmonella* free by testing 5 samples (n=5) of 25g each and at most 1000CFU/g of *E. coli*.

The observed die-off resistance in bacteria during two-phase anaerobic digestion process could be explained as intertwined to the acid adapted bacteria cells which occur in the hydrolysis reactor and also the presence of simple sugars in the suspension menstruum. In anaerobic digestion process in particular two-phase anaerobic digestion, the acid produced by acidogens in the hydrolysis reactor following fermentation of the substrate pose a great effect in the selected temperature–time combination due to the fact that acidic adapted microbial cells tends to have increased resistant to both temperature and pH [166]. This happens following increased cyclic fatty acids and decrease the ratio of unsaturated fatty acid to saturated fatty acids in the bacteria cells leading to loss of fluidity in cell membrane [172] and therefore induced resistance. Also, high carbohydrate content of about 18% in potato out of which 89% is starch which is normally easily converted into simple sugars during fermentation [173] contributed to the experienced die-off resistance. Jay et al., [174] reported that the presence of simple sugar in the digestate increases heat resistance to microbiological pathogens suspended therein in the order of sucrose > glucose > sorbitol > fructose > glycerol.

This research considers that these two possibilities the major contributing factor to the observed high level of bacteria cells survival and pass to the final digestate unaffected at the end of the anaerobic digestion process although the stage of bacteria especially at

stationary phase is equally associated with resistance to die-off than in the lag and log phases. To effect complete pathogens die-off during biotreatment process therefore, it was of the essence to subject the two enteropathogens under prolonged sample residence assuming batch operation system of the anaerobic digestion process.

5.9.4 Enteropathogens die-off in anaerobic digestion under prolonged substrate residence time simulating batch-reactors

With a daily addition of *E. coli* and *S. senftenbergensis* contaminated feedstock in the reactor and remove of equal volume, the possibility of achieving complete inactivation of pathogens is limited as demonstrated in previous experiments above. Three reasons could be behind the observed failure on achieving total pathogens inactivation in both mesophilic and thermophilic operated anaerobic digestion reactors. Firstly is the fact that an equal amount of the feedstock added in the hydrolysis reactor was to be drawn as feed to the methanation reactor. This feeding regime exposed the substrate to shorter residence time that was insufficient to guarantee pathogens inactivation. Secondly, as the OLR kept increasing, the fresh added contaminated potato peels which had a bit higher pH than the pH already in the acidogenic digestate caused a small increase in pH for which the pathogens could tolerate and regenerate. And lastly, the acid-adapted microorganism have been reported to demonstrate high resistant to die-off when subjected to pasteurization temperature range as well under low pH due to reduced membrane fluid caused by increased cyclic fatty acids.

Two batch-wise operated anaerobic digestion experiments one for hydrolysis and the other for methanation reactor were set to investigate the effect of long substrate residence time on pathogens inactivation. The tests were carried out at pilot scale with all other factor except reactor feeding regime being constantly maintained and daily monitored. Table 5-13 shows the state of the acidogenic digestate and methanogenic digestate plus the running parameters in the mesophilic hydrolysis reactor and thermophilic methanation reactor before the new spiking.

Table 5-13: Characteristics of hydrolysate and digestate prior to new contamination

Parameter	Acidogenic	Methanogenic	Unit
	digestate	Digestate	
Temperature	36.9	55.2	°C
pH	4.2	7.7	-
Salinity	2.7	5.6	‰
Conductivity	5.5	9.7	mS/cm
<i>E. coli</i>	3.8	Ø	logCFU/g
<i>S. senftenbergensis</i>	4.2	1.9	logCFU/g
VS reduction	n/a	68.3	%

Ø = no growth of *E. coli* was observed

5.9.4.1 Performance of hydrolysis process under delayed feeding regime

The new bacteria spiking caused an increase in the initial population in the hydrolysate to 6.6×10^6 CFU/g for *E. coli* and 2.3×10^6 CFU/g for *S. senftenbergensis*. Table 5-14 presents enteropathogens die-off following long hydrolysate residence time in the hydrolysis reactor, characteristics of the hydrolysate and the hygienization level for which the delayed fed reactors (batch feeding mode) operated at continuous stirring could achieve if operated under optimal and compliant conditions with no interruptions. Although pathogens die-off in the reactors during anaerobic digestion may be contributed by various factors both intrinsic and extrinsic [167,175], as previously stated in Busch et al., [56], Deublein and Steinhauser [59] and Fukushi [165], these researchers concur that pH in the mesophilic hydrolysis stage and temperature in the thermophilic anaerobic process are the most important factors dictating pathogens die-off. Both batch mesophilic and thermophilic anaerobic digestion were operated for 7 days. The pH in the hydrolysate dropped for about 0.1 units on day 3 of the experiment depicting the ongoing fermentation process in the reactor. Having as low pH as 4 is an added advantage in the hydrolysis process making possible for most carbon dioxide generated to exist as free molecule. At the end of the first 24 hrs of hydrolysis batch reactor operation there was about 2.3 log reduction equivalent to 99.5% in *S. senftenbergensis* while in *E. coli* about 3.8 log cfu/g reduction was achieved equivalent to 99.98%.

Table 5-14: Bacteria inactivation under mesophilic hydrolysis reactor (Batch-test)

Days	Hydrolysate characteristics				Enteropathogen levels (logCFU/g)	
	°C	pH	Conductivity (mS/cm)	Salinity (‰)	<i>S. senft-</i>	<i>E. coli</i>
0	37.5	4.2	5.6	3.0	6.4	6.8
1	36.9	4.2	5.8	3.1	4.1	3.0
2	37.1	4.2	5.9	3.2	2.5	1.5
3	37.2	4.1	6.0	3.3	0.9	Ø
7	37.0	4.1	5.4	2.9	Ø	Ø

Ø = no growth of enteropathogen was observed

The second day monitoring of the enteropathogens die-off in the hydrolysis reactor gave 3.8log reduction in *S. senftenbergensis* equivalent to 99.98% whereby in the case of *E. coli* there was more than 5.3log equivalent to 99.999% elimination. There was no viable *E. coli* cell surviving on day 3 (pH value 4.1) of sample residence confirming a complete elimination of *E. coli* in the process. On contrary, few cells 0.9log of *S. senftenbergensis* were able to survive in that low pH but the die-off was higher above 5.5logCFU/g which is almost 99.999% elimination. The die-off monitoring activity was not done on day 4, 5 and 6, as it was during the weekend; the absence of viable cells on day 7 confirmed a complete inactivation of both *E. coli* and *S. senftenbergensis* in the hydrolysis reactor.

In a similar kind of research, Fukushi et al.,[165] in their research simulating acid-phase of anaerobic digester observed a possibility of eliminating a number of microorganisms including *S. senftenbergensis* and *E. coli* in 2 days residence pH 3.5-5.5. Their results differ slightly with the results in this investigation which required one more day especially for *S. senftenbergensis*. The possible explanation for this one day difference could be based on the fact that their study used synthetic organic acid different from this study in which the low pH was as a result of natural fermentation of the potato peels by the fermentative and acidogenic micro-flora. Conner and Kotrola [176] stressed that together with the pH, pathogens die-off differs depending on the type of acidulants. The other

reason for longer time kill-off in *S. senftenbergensis* in this experiment could be connected with induced acid tolerance in the bacteria cell caused by reduced membrane fluid in the cell membrane due to the formation of cyclic saturated fatty acids [166]. Álvarez-Ordóñez, et al.,[166] observed that when microbes are subjected to acidic media for a long time the cells become adapted to low pH (acid-adaptation) leading to loss of membrane fluidity and thus increase cells tolerance to both low pH as well as heat.

5.9.4.2 Performance of methanation process under delayed feeding regime

The initial level for *E. coli* and *S. senftenbergensis* were raised to 2.8×10^5 CFU/g and 1.3×10^5 CFU/g respectively in the methanogenic digestate within methanation reactor following the new bacteria spiking. The pH values in the methanation reactor were stable maintained at the range of 7.5-7.8 which conform within the acceptable pH limits for methanation process according to Deublein and Steinhäuser [59] while electrical conductivity and salinity in both tests did not change much from the values recorded in the former experiments. Table 5-15 below presents the substrate residence time, characteristics of methanogenic digestate and the pathogens levels for which the delayed fed reactors (batch feeding mode) could achieve if operated under optimal and compliant conditions.

Table 5-15: Bacteria inactivation under thermophilic methanation reactor (Batch-test)

Day	Digestate characteristics				Enteropathogen levels (logCFU/g)	
	°C	pH	Conductivity (mS/cm)	Salinity (‰)	<i>S. senft-</i>	<i>E. coli</i>
0	54.8	7.7	8.6	5.0	5.5	5.1
1	55.1	7.7	8.7	5.3	3.1	1.5
2	55.3	7.8	9.6	5.6	0.6	Ø
3	54.6	7.8	9.7	5.6	Ø	Ø
7	54.7	7.5	9.6	5.6	Ø	Ø

Ø = no bacteria growth was observed on petri-plate agar

On average, the temperature in the digestate ranged between 54.8°C and 55.1°C. The hygienization performance of methanation reactor operated at this thermophilic temperature range shows that at the end of day 1, both *E. coli* and *S. senftenbergensis* survived though in small amount. There was 2.4logCFU/g equivalent to 99.6% reduction in *S. senftenbergensis* while a mass reduction of 3.6logCFU/g equivalent to 99.98%) was achieved in *E. coli*. There were no viable *E. coli* cells thereafter except *S. senftenbergensis* which demonstrated a 0.6log of surviving cells per gram of digestate reflecting more than 4.9log reduction (99.999%) in the second day of operation and monitoring of the reactor. On day 3 as well as day 7 there was no observed viable cell survived from the two spiked enteropathogens confirming a complete elimination of both *E. coli* and *S. senftenbergensis*. Bagge [62] concurs that apart from inconsistency in biogas production and variation in biogas composition, batch-wise operated thermophilic anaerobic digestion is capable at achieving high pathogen die-off acceptable to some standards in some regulatory bodies in 24hrs digestate residence time; but a complete elimination of the pathogens is possible if the digestate residence time is prolonged to 48hrs. In addition, batch anaerobic digestion offers a great possibility of monitoring and control of the temperature –time combination among others.

5.10 Conclusive summary

Even though pathogens die-off during anaerobic digestion may be contributed by various intrinsic and extract factors for which pH in the mesophilic hydrolysis stage and temperature in the thermophilic anaerobic process are the principle factors, substrate holding time dictates the extent and hygienization levels the process may be capable at achieving. Two-phase mesophilic anaerobic digestion operated at pH 4.6 and average temperature of between 36.8°C and 37.2°C under continuous stirred tank reactor at HRT of 25days and OLR of 0.24kgoTS/m³.day hardly reduced 35% of both *E. coli* and *S. senftenbergensis* from the original spiked population background determined to be 1.2x10⁶CFU/g (6.1log) and 5.1x10⁵CFU/g (5.7log) for *E. coli* and *S. senftenbergensis* respectively. Upon stabilization, thermophilic temperature 55±2°C was effective at eliminating *E. coli* to a level below LoQ, given the shorter digestion time in the methanation reactor operated at HRT of 12.5 days. However, the same temperature was

insufficient at completely eliminate *S. senftenbergensis* for which on average 2.6logCFU/g was discharged unaffected within the digestate.

Nevertheless, on prolonged substrate residence in both reactors, a complete inactivation of *E. coli* was achieved after 72 hrs while *S. senftenbergensis* was being reduced to 0.9log in hydrolysis phase of mesophilic two-phase anaerobic digestion. A complete *E. coli* elimination was achieved within 24hrs of substrate residence time in methanation mesophilic two-phase anaerobic digestion with *S. senftenbergensis* being reduced to 0.6log confirming that batch operated anaerobic digestion system could be more reliable at effecting total hygienization of enteropathogens provided the substrates residence time is maintained for not less than 72hrs. In a nutshell, from the results of this experiment, the study recommends regular monitoring of potentially pathogens of great abundance on finished digestate to be part of the crucial parameters carried out to confirm microbiological safety of digestate and by so doing avoid chances of land application or deposition of pathogens contaminated materials.

6 Efficacy of Hygienization Options at Eliminating Enteropathogens from Contaminated Biowaste

6.1 Hygienization options, application and effective parameters

Three existing biowaste hygienization options namely heat treatment, acid and alkali as well as two alternative hygienization options namely UVC radiation and passive solar heating (PSH) see Table 6-1 were selected for this experiment. UVC radiation and PSH were selected as alternative options over the existing commonly used pasteurization and chemicals (organic acid and lime) as means hygienization of pathogens contaminated biowaste due to their long known application and operation efficiency in inactivation of pathogenic microorganisms in various matrices such as water [177], wastewater [178], liquid food [179,180] and many other applications. Both existing and alternative hygienization options are meant to be used along with the conventional biological treatment methods to ensure a completely elimination of pathogens from biowaste through pre-or post hygienization of the pathogenic contaminated biowaste.

Table 6-1: Hygienization options, application and effective parameters

	Options	Mode of Application		Hygienization Effective parameters
		Pre-Hygienization	Post-Hygienization	
Commonly used	Pasteurization	Yes	Yes	Temp, Time
	Acid conditioning	Yes	No	pH, Time
	Lime conditioning	Yes	Yes	pH, Time
Alternative	Longer holding time	- within reactors -		pH/Temp
	UVC radiation	No	Yes	Dose, Time
	Solar Heating	Yes	Yes	Time, Temp, Moisture

Briefly, over the past years, pasteurization which entails heating of material to temperature below boiling point and hold for some specified time has remained the most commonly used method for hygienization of contaminated biowaste in many biological treatment facilities. In this investigation, depending on the effect produced by individual

hygienization option that cause inactivation of the pathogens the hygienization process was either applied before biological treatment (Pre-biotreatment) or after biological treatment (post-biotreatment). Pre-biotreatment hygienization of biowaste ensures safe feedstock/ substrate before entering the biological treatments process, in this case, the pre-hygenized feedstock/substrate exempt biotreatment facility workers from exposure to potentially infectious microorganisms and eventually produces safe final by-products if no recontamination takes place in between. On the other side, the post-biotreatment hygienization process is done intentionally to make sure that all pathogens that survived the biological treatment process and find their way into the final by-products of biological treatment process (compost and digestate) are completely eliminated before the products are taken out of the biotreatment facility for being disposed of or used such as on farmlands application.

Acid/lime conditioning and stabilization process in biowaste and solar drying were applied as pre-biotreatment hygienization on potato peels waste and as post-biotreatment hygienization options on compost and digestate. Due to its application mechanism, UVC radiation was applied as post-biotreatment hygienization option on the unhygienized process water (hydrolysate) from hydrolysis reactor of two-phase mesophilic anaerobic digestion before feeding the methanation reactor.

6.2 Batch pasteurization at effecting enteropathogens die-off

For many years, pasteurization which is a thermal treatment of contaminated biowaste based on temperature-time combination continues to be a most recommended, applied and accepted method of hygienization by most regional and national regulatory bodies including Regulation (EC) 2001 [23], Regulation (EC) 2010 [117], BSI: PAS110 [158], Australian Standard 4454-2003, Regulation (EC) No. 208/2006 [181], U.S Environmental Protection Agency, Canadian Council of Ministers of the Environment (CCME) and so many others. On that reason, pasteurization has been the sole hygienization method throughout the history used by most MSW biotreatment facilities as a means of eliminating pathogenic microorganisms. Among the existing national and regional regulations on inactivation of pathogens during anaerobic digestion process, thermophilic stands to be the most promoted option as far as hygienization is concerned. Pasteurization process

has been for long used before biotreatment (Pre-treatment hygienization), during biotreatments or after biotreatment process (post-treatment hygienization) in order to further reduce pathogens below their analytical detection limits and thus ensure safety of the final products and therefore avoid humans contact with pathogens as well as land application of microbiological contaminated final byproducts of biotreatments [183].

6.3 Pasteurization in hygienization of pathogens contaminated biowaste

The concern about diverse in temperature-time combination coupled with absence of scientifically evidence on which temperature specification results into bio-safer results was raised in SANCO/7066/2010 of the EU compost network (ECN). The major challenge to most anaerobic digestion treatment facilities is on meeting the tight microbiological safety criteria of the digestate prior to discharge. The objectives of conducting this experiment therefore were first to investigate the extent of which the various temperature-time specifications can eliminate enteropathogens from artificially contaminated biowaste and second to validate the temperature-time specification so as to harmonize the existing numerous temperature-time specifications which in most cases are questionable in their ability to effect complete or environmentally acceptable results within the increased resistance in pathogenic microorganisms.

The United States Environmental Protection Agency (EPA/832/R-93/003 Guide to EPA 40 Code of Federal Regulation (CFR) part 503 Subpart D of Biosolid Rule provide an ambiguity specification of 55°C for 63hrs as Time-Temperature regime required for achieving Class A pathogens status on biosolid with total solid (TS) \geq 7%. Some countries in which statutory regulations concerning biological treatment in particular anaerobic digestion of biowaste exist e.g. Switzerland [184] and The Canadian Council of Ministries of the Environment (CCME), consider pasteurization at 70°C for 30min as set by the U.S-EPA/625/R-92/013 [185] adequate. In mathematic derivation, USA and Austria advocate 55°C for 63hrs [183] and 24hrs respectively . European Commission (EC) Regulations No. 208/2006 amending Annexes VI and VIII of Regulation (EC) No.1774/2002 promotes Temperature-Time Regime of 70°C for 1hr [181], while the Publicly Available Specification BIS-PAS 110: 2010 accepts Temperature-Time regime of 57°C for 5hrs also 70°C for 1hr. Denmark considers and recommends 55°C for 6hrs. With this diverse of Temperature-

Time regimes, harmonization of these specifications seem to be of paramount importance in order to assure global recycling of biowaste without posing environmental menace and risking the public health.

6.4 Materials and Methods

6.4.1 Pre-biotreatment pasteurization

Set-up of the experiment (see Picture 6-1) involved peels blending, peels weighing, pulping, bacteria spiking, pasteurization at specified temperature and time and finally plating portion of post-pasteurized matrix for verification of bacteria die-off. Approximately 25g of potato peels samples were weighed (Sartorius balance LP 4200S 4200 ± 0.1 g) and suspended into 500mL capacity flasks containing 247.5mL of sterilized normal water (*Leitungswasser*) that was prepared in-house in accordance with Bast [186] having average pH value 7.3 ± 1.1 and conductivity of $514 \mu\text{S}/\text{cm}$. Water was added into potato peels waste to make suspension menstruum of 100-fold dilution for ease mixing and heat transfer during pasteurization process.



(i) Weighing of blended peels



(ii) Batch pasteurization process

Picture 6-1: Experimental set-up for batch pasteurization of potato peels waste

In this experiment, the simulation of contaminated biowaste was prepared through artificial spiking of the peels with 2.5mL of pre-enriched bacterial suspension resulted into 8.7log *E. coli* (5.3×10^8 CFU/g) and 8.8log *S. senftenbergensis* (6.1×10^8 CFU/g) contamination.

A water-bath connected to a digitally controlled stirred water-bath and circulator (Grant Optima™ GD120) with both temperature and time displaying devices was used during pasteurization process for heating and circulation of the heated water in the water-trough. The temperature of the thermostat was set at 2°C higher than the desired pasteurization temperature so as to accommodate heat loss due to water circulation in the pipes. The pasteurization temperature in the sample as well as temperature of water in the water-trough was monitored by external thermometers. In each sample run, countdown of pasteurization time commenced after the sample in individual flask attains the targeted temperature.

6.4.2 Pasteurization Temperature-Time monitoring

Two thermometers were used for recording temperature. One of the thermometers was placed in the water-trough in order to control water temperature while the other was rotating in the pasteurization flasks to control temperatures attained by the study samples. The minimum pasteurization temperature of 50°C was selected as initial heat described in SANCO/7066/2010 based on empirical researches on both anaerobic digestion and aerobic composting reported in The Netherland, Belgium and Germany. This temperature makes 4°C higher than the maximum survivor temperature of *E. coli* which in this investigation represented the most heat sensitive pathogen while *S. senftenbergensis* representing the most heat-resistant among non-spore forming pathogens. The time countdown of pasteurization process started immediately after the temperature inside the pasteurization flask containing the sample reached the desired temperatures which were: 50°C, 55°C, 60°C, 65°C and 70°C to capture the temperature range recommended in Commission (EC) No. 208/2006 [187] and in accordance with the maximum survival temperature for *E. coli* reported at 46°C [188,189] and that of *S. senftenbergensis* estimated as above 65°C following the findings in Ng et al., (1969). The sample holding time during pasteurization was set at 30min, 60min and 24hrs.

6.5 Validation of Temperature-Time Specification following batch pasteurization

Validation of temperature-time regime was performed in accordance to Konieczka and Namiesnik [190]. In order to maintain accuracy and precision in results during validation, five sample units (n=5) as recommended in Regulation (EC) No. 208/2006 were

pasteurized under batch system. Each pasteurization flask contained blended potato peels waste artificially pre-spiked with enteropathogens suspension at population levels of $8.8 \log E. coli$ and $8.7 \log S. senftenbergensis$. Due to its high resistance to thermal death, the pasteurization results for *S. senftenbergensis* were used during validation process supposing that the temperature that will effect a complete die-off in *S. senftenbergensis* will be equally sufficient to completely eliminate *E. coli*.

The validation hypotheses were stated as follows:

Null Hypothesis: H_0 = There is no significant difference in die-off of bacteria cells treated under the same temperature-time regime.

Alternative Hypothesis: H_1 = There is significant difference in the die-off of bacteria cells treated under the same temperature-time regime.

6.5.1 Models for decimal reduction time and bacterial survivor

Four temperatures i.e. 36°C, 50°C, 55°C and 60°C in which *S. senftenbergensis* survived were used in establishing the decimal reduction time (D-value) which refers to the time required to traverse one log cycle or 90% inactivation of initial pathogens population (N_0) from the pasteurized sample at a given temperature [191]. The 5 batch pasteurized samples (n=5 replicates) used in the investigation of pre-biotreatment pasteurization trial was equally used in calculation of the decimal reduction time at constant temperature applying a first –order kinetic equation [192]:

$$\frac{dN_t}{dt} = -kN_0 \quad (6.1)$$

From the inactivation kinetic curve, N_t represents the viable *S. senftenbergensis* colony units at specific given time, k represents the reaction rate constant in (1/min) in which N_0 equal to original population at the beginning of the experiment. The decimal reduction time (D –value) was therefore calculated as a negative inverse of the slope of the best-fit-line for logarithmic reduction in bacteria colony units against sample residence times (min). The counts of viable *S. senftenbergensis* colony units following batch pasteurization were

plotted against residence time starting at 0min, 30min and 60min following the first-order linear regression equation below:

$$\log_{10} N_t = \log_{10} N_o + s \times (t - t_L) \quad (6.2)$$

Whereby:

N_o = initial population of bacterial colony units in the sample

N_t = Survived *S. senftenbergensis* colony units after pasteurization

s = slope of the regression linear line of $\log N_t$ versus time of exposure

$t - t_L$ = retention time during batch pasteurization process (min) ($t > t_L$)

6.5.2 Models for Thermal Inactivation Kinetics and bacterial survivor

In this research, thermal resistance constant, Z-value is the increase in temperature that is required to bring about a one-log cycle or 90% inactivation of initial *S. senftenbergensis* population (N_o) from the pasteurized sample at a given decimal reduction time (D-value). This was calculated as described in Manas et al., [104] from the following equation:

$$\log_{10} D(T_t, T_g) = \log_{10} D(T_{50^\circ C}, T_{36^\circ C}) - T_t / Z \quad (6.3)$$

Whereby:

$\log D$ ($T_t = 36^\circ C, 50^\circ C, 55^\circ C$ and $60^\circ C$,) is the theoretical decimal time reduction time of *S. senftenbergensis* population grown at various specified temperature,

$T_g = 36^\circ C$ is the temperature used for enrichment of *S. senftenbergensis* culture,

z is the negative inverse of the slope,

T_t is the treatment temperature i.e. $50^\circ C$.

The z-Value describes the influence of the temperature on the decimal reduction time for *S. senftenbergensis* population. In this trial, the log of D-values versus temperatures $36^\circ C$, $50^\circ C$ and $55^\circ C$ were used to calculate the z-values as a negative inverse of the slope of the best-fit-line for log D-values obtained from the four selected temperatures against the varied temperature from incubation temperature i.e. $36^\circ C$, $50^\circ C$, $55^\circ C$ and $60^\circ C$.

6.6 Post-biotreatment pasteurization

In another experiment, pasteurization was used to effect post-biotreatment hygienization of hydrolysate from hydrolysis reactor as well samples of digestate from methanation reactor of mesophilic anaerobic digestion. The focus here was to achieve inactivation of *E. coli* and *S. senftenbergensis* that passes the reactors unaffected. This investigation was carried out on hydrolysate and digestate of 24 June 2014 (15days and 7days of operation for the hydrolysis and methanation reactors respectively). The physicochemical and microbiological characteristics of the hydrolysate otherwise known as acidogenic digestate and the effluent from methanation reactor (methanogenic digestate) are given in Picture 6-2 and Picture 6-3 respectively.



Characteristics

Temperature = 37°C

pH = 4.7

Salinity = 1.1 ‰

Conductivity = 2.3mS/cm

5.1log *E. coli* (1.1×10^5 CFU/g)

4.9log *S. senftenbergensis* (7.9×10^4 CFU/g)

Picture 6-2: Digestate from hydrolysis reactor digesting contaminated potato peels



Characteristics

Temperature = 36.8°C

pH = 7.7

Salinity = 7.3‰

Conductivity = 12.5mS/cm

6.4log *E. coli* (2.5×10^6 CFU/g)

6.2log *S. senftenbergensis* (1.3×10^6 CFU/g)

Picture 6-3: Digestate from Methanation reactor digesting contaminated potato peels

The samples were taken for batch-pasteurization process at 50°C, 55°C, 60°C and 65°C using similar time of 30min, 60min and 24hrs. The hygienization equipment such as glassware and the experiment set up was similar to the one during pre-hygenization of contaminated biowaste previously described in Picture 6-1 with exceptional that in this study there was no fresh *E. coli* or *S. senftenbergensis* spiked. The population of survived *E. coli* and *S. senftenbergensis* in the hydrolysate in hydrolysis reactor and digestate from the methanation reactor was used as the initial pathogens load to be inactivated. After pasteurization, the samples were serially decimal diluted in 10-folds followed by triplicate plating of 100µL of the pasteurized solution on both Endo agar and modified BGA. The petri-plates were incubated at 36°C for 24 hrs followed by enumeration of bacteria colonies on the following day.

6.7 Statistical analysis and presentation of results

In order to find whether there exist or not a significant difference in pathogens die-off at the selected temperatures, the validation parameters were calculated based on analysis of variance (ANOVA) for 5 replicate treatments with 4 degrees of freedom using F_{\max} -Hartley's test applying a significance level $\alpha = 0.05$. Validation data were analyzed using MS 2007 Excel spreadsheet.

6.8 Results and Discussion

6.8.1 Performance of pre-biotreatment batch pasteurization option

Results given in Table 6-2 show the average counts for *E. coli* and *S. senftenbergensis* following batch pasteurization of the potato peels suspended menstruum. The results present minimum and maximum values in log CFU/g at four different temperatures selected to be 50°C, 55°C, 60°C and 65°C and sample holding time specified at 30min, 60min and 24hrs. As pointed out in Vinnerås et al., [123] and Wichuk and McCartney [20], there exist threshold temperature-time combination to which every pathogen can be inactivated. Heat above the threshold temperature denatures microbial enzymes therefore prolonged exposure to the temperature higher than the threshold brings about a complete destruction in the enzymes and therefore total inactivation of the microbial cell. At the end

of the batch pasteurization process, the results from laboratory-scale set-up experiment showed difference in inactivation rate between *S. senftenbergensis* and *E. coli*.

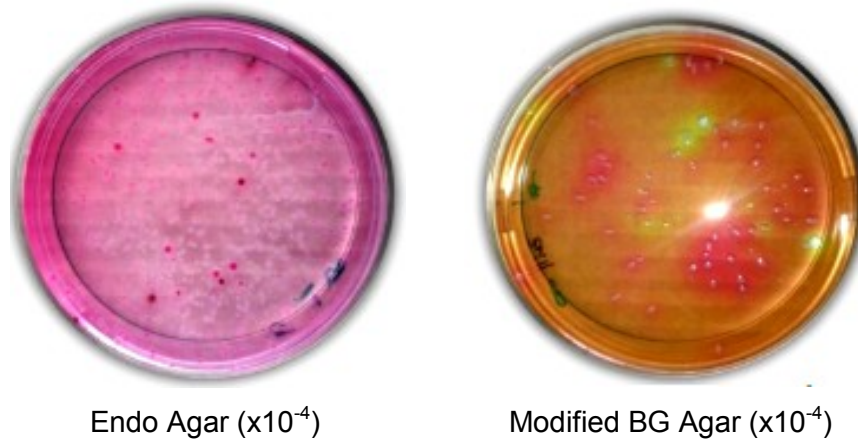
Table 6-2: Enterobacteria die-off (logCFU/g) in potato peels suspended menstruum after batch pasteurization at various temperatures

Temp-Time regime	Bacteria colony counts (logCFU/g)			
	<i>S. senftenbergensis</i>		<i>E. coli</i>	
	Min	Max	Min	Max
50°C for 30 min	8.2	8.5	5.7	6.0
50°C for 60 min	6.9	7.6	3.3	5.2
50°C for 24 hrs	5.4	7.4	Ø	Ø
55°C for 30 min	4.2	5.2	0	2.4
55°C for 60 min	2.7	4.9	Ø	Ø
55°C for 24 hrs	0	0.9	Ø	Ø
60°C for 30 min	Ø	2.6	Ø	Ø
60°C for 60 min	Ø	0.3	Ø	Ø
60°C for 24 hrs	Ø	Ø	Ø	Ø
65°C for 30 min	Ø	Ø	Ø	Ø
65°C for 60 min	Ø	Ø	Ø	Ø
65°C for 24 hrs	Ø	Ø	Ø	Ø

Ø = no growth of bacteria colony was observed, (n=5)

The Initial bacterial population following an overnight aerobically enrichment (Nutrient Broth, Carl Roth GmbH) and incubation at 36°C for 24hrs indicated that the maximum inoculation level of 8.7log was obtained for *S. senftenbergensis* and 8.8log for *E. coli*. Both *E. coli* and *S. senftenbergensis* survived the first two temperature-time specification sets for up to 60min. From a practical viewpoint, it was worth noting that there exists significant difference in the heat resistance between *S. senftenbergensis* and *E.coli* treated at the same temperature and time combination as similarly reported in Ng et al., [193] and Mitscherlich and Marth [194]. At the onset of the process, the population of *S. senftenbergensis* (mean of 8.7log in the original substrate) was not significantly affected (T-test; $p < 0.05$) by pasteurization heat treatment (mean 8.4log and 7.3log after both

30min and 60min at 50°C respectively) although *E. coli* population was significantly affected (T-test; $p > 0.05$) by pasteurization at the same pasteurization holding time. As it has been confirmed from other previous studies [195] that microbial inactivation is not a function of the initial cells population, completely elimination of *E. coli* from the suspension at 50°C for 24hrs confirmed its low resistance to heat. As it can be seen in Picture 6-4, representing *E. coli* and *S. senftenbergensis* colony counts after plating of a 10^{-4} suspension dilution resulting after pasteurization of the potato peels suspending menstruum at 50°C for 60min.



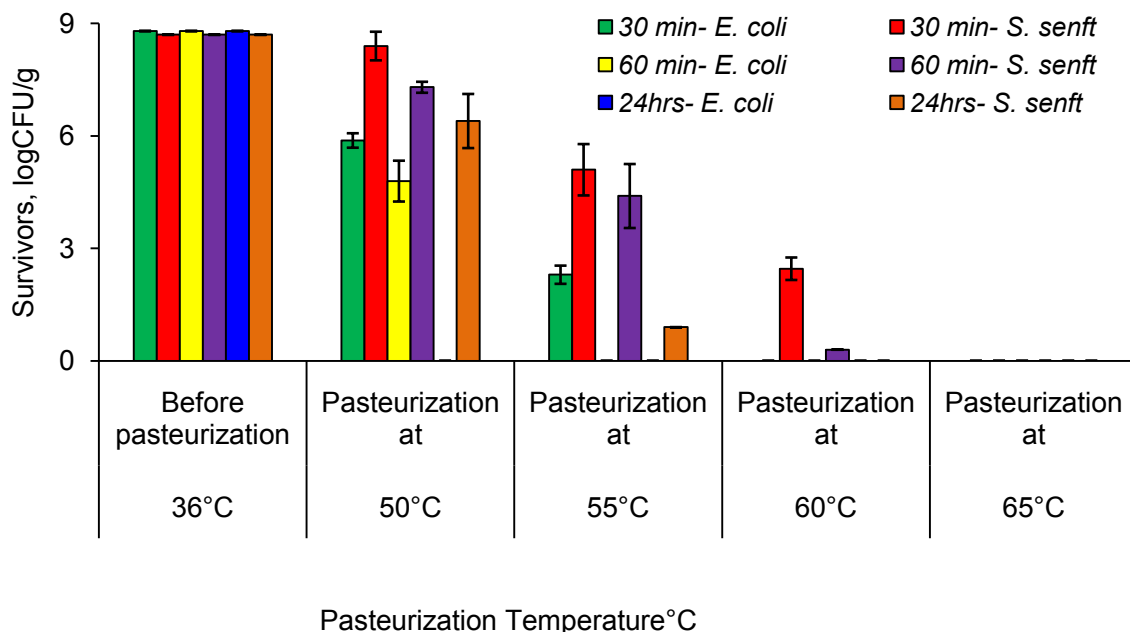
Picture 6-4: Discrimination of *E. coli* and *S. senftenbergensis* on agar following pasteurization

On these two agar plates, *E. coli* population (red with metallic sheen colony units on Endo agar, and yellow colony units on modified BG Agar) was much reduced compared to *S. senftenbergensis* (pinkish-colourless colony units on Endo agar, and red-pinkish colony units on modified BGA) viewed after 24hrs of incubation at 36°C.

6.8.2 Pathogens die-off in batch pasteurization at varied Temperature-Time regime

Confidence interval values and their degree of uncertainties for survive of *E. coli* and *S. senftenbergensis* following inactivation by pasteurization process at various temperature and time regime are as shown in Figure 6.1. The average results of five independent batch pasteurization ($n=5$) shows reduction of the pathogens at various rate even at 50°C. At this initial set minimum temperature, the 30min residence time was enough to cause about 2.8-3.1log reduction in *E. coli* while for *S. senftenbergensis* only 0.2-0.5log

reduction was observed. *E. coli* was even more affected following a prolonged holding time to 60min in which the reduction reaching 3.5 to 5.5logCFU/g was achieved while that for *S. senftenbergensis* showing a die-off range of only 1.1-1.8logCFU/g.



Results calculated as confidence Interval for log reduction at 95% confidence limit; n=5

Figure 6.1: Mean log reduction of *E. coli* and *S. senftenbergensis* following batch pasteurization at varied temperature-time regime.

Even at the longer retention of 24hrs, the highest reduction in *S. senftenbergensis* only reached 1.3-3.3log but not a complete elimination unlike in the case of *E. coli* which was completely inactivated during the 24hrs sample holding time at this minimum batch pasteurization temperature. Sample pasteurization at 55°C and 30min highly affected *E. coli* population as there was an average of only 2.3logCFU/g of the surviving cells. This demonstrated as >6.0log die-off equivalent to 99.999% inactivation which is an acceptable level conformity to the Regulation (EC) No. 208/2006 and DG ENV.C2/BZ/tb [117] as microbiologically safe in case the pasteurized material is to be used for purpose such as farmland and/or application on grass used for animal grazing. In other words, any biotreatment of biowaste contaminated with *E. coli* is capable at equally eliminate this enteric microorganism provided the temperature in the reactor is elevated to 55°C and

retained for at least 30min without interruption. Mitscherlich et al., [194] equally reported the survive time for *E. coli* to be less than 15min at 54.5°C.

Addition of temperature above 55°C confirmed the heat sensitivity of *E. coli* and there was no further resistance demonstrated or re-growth of *E. coli* in the other temperature-time combination further investigated. Pasteurization at 55°C for 30min and 60min though much reduced *S. senftenbergensis* up to the range of 3.6-5.3log and 3.8-6.4log respectively; there was still no complete elimination of this bacterium until when the pasteurization time was prolonged up to 24hrs. Major reduction in *S. senftenbergensis* was achieved following pasteurization at 60°C for 60 min in which the survived colony units were counted at 0.3log maximum in which 2 colony units were observed in one of the five samples pasteurized. Thereafter a 60°C pasteurization for 24hrs was equally enough to ensure a complete inactivation of *S. senftenbergensis*.

In this investigation, a pasteurization process at 65°C even at the lowest sample holding time of 30min proved to be sufficient for completely elimination of *S. senftenbergensis*. These results correlate well to the findings reported in Mitscherlich et al., [194] in which the survival time of *S. senftenberg*775W in milk following heat treatments took more than 60min at 54.4°C, but the time was reduced to 15min upon raising the temperature to 61.6°C, and even more shorter as 2min at 65.5°C.

6.8.3 Validation of Temperature-Time regime based on Enterobacteria

Validation of the temperature-time regime for inactivation of enteropathogens was done using the results derived from thermal reduction curves of *S. senftenbergensis*. Previously researches including Day et al., [121], Franke-Whittle and Insam [124] and Paluszak et al., [196] on the thermal death for common non-spore forming enteropathogen microorganisms and parasites (see Table 6-3) confirm that enteric bacteria in particular *S. senftenbergensis* are the most resistant specie to pasteurization temperature compared to other enteropathogens.

Table 6-3: Temperature-time regime for die-off in some common pathogens

Microorganism	Pasteurization and corresponding die-off time (min)			
	50°C	55°C	60°C	65°C
<i>Salmonella spp</i>	-	60min	15-20min	-
<i>S. thyphosa</i>	-	-	20min	-
<i>Escherichia coli</i>	-	60min	-	-
<i>Shigella spp</i>	-	60min	-	-
<i>Necator americanus</i>	50min	-	-	-
<i>S. senftenbergensis</i>	-	-	78min	≥ 30min

Source: Day et al., [121], Franke-Whittle and Insam [124], Paluszak et al., [196], Jay et al., [174], Angelotti et al., [197].

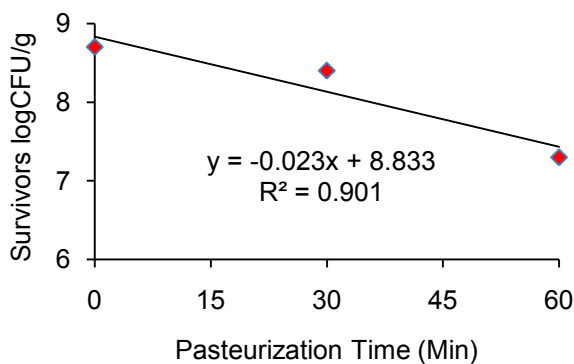
6.8.4 Decimal reduction Time of *S. senftenbergensis*

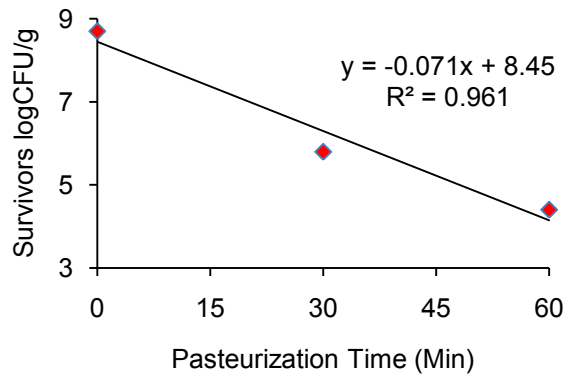
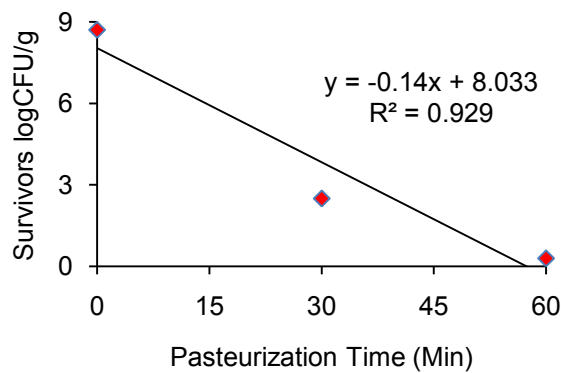
The results for decimal reduction time (D-value at 50°C, 55°C and 60°C) following a pasteurization of suspending menstruum of contaminated blended potato peels in water for traversing population of *S. senftenbergensis* by 1 log-cycle of viable cells from its original population given in Figure 6.1 above were used in deriving the validation parameters. Only results for *S. senftenbergensis* were used in this determination due to its heat resistance characteristics compared to *E. coli*. Therefore, the validation assumption was that the temperature-time combination capable to eliminate *S. senftenbergensis* automatically is considered sufficient for elimination of *E. coli*. Since the levels of bacteria die-off in the batch pasteurization vessels differed, the calculations were therefore based on the values of coefficient of variation (CV) of the survived *S. senftenbergensis* colony units based on the three lower pasteurization temperatures of 50°C, 55°C and 60°C as shown in Table 6-4. From the validation parameters, the calculated F_{max} was observed to be smaller than the critical values Hartley's F_{max0} for significance results at $\alpha = 0.05$. This confirmed the absence of statistical significant differences ($p < 0.05$) in the mean die-off of *S. senftenbergensis* within treatment. Having this inference therefore the null hypothesis was retained to be true.

Table 6-4: Validation parameters for *S. senftenbergensis* following batch pasteurization

Treatment	1			2			3		
Sample (n)	5	5	5	5	5	5	5	5	5
Temp (°C)	50	50	50	55	55	55	60	60	60
Holding time (min)	0	30	60	0	30	60	0	30	60
Bacterial density (logCFU/g)	8.7	8.4	7.3	8.7	5.8	4.4	8.7	2.5	0.3
Standard deviation	0.14	0.12	0.49	0.14	0.07	0.52	0.14	0.09	0.11
CV, %	1.6	1.4	6.7	1.6	1.2	11.8	1.6	3.6	36.7
F_{max} (calculated)		4.8			9.8			22.9	
F_{maxo} (n=5, df= 4, $\alpha = 0.05$)		25.2			25.2			25.2	

Variation existed on the compared coefficient of variation (CV %) of *S. senftenbergensis* reduction log (N_0/N) between treatments at any given temperature and on varied time of exposure. In this experiment, negative linear relationships observed between the colonies of *S. senftenbergensis* survived against time of pasteurization confirm a decreasing rate in bacteria population. High value of coefficient of determination ($R^2 > 0.90$) and coefficient of regression ($r > 0.95$) (see Figure 6.2) confirmed linearity of the method.

(a) $D_{50^\circ\text{C}}$ -value

(b) D_{55°C} -value(c) D_{60°C} -valueFigure 6.2: Decimal reduction time of *S. senftenbergensis* at varying temperature

Right from the minimum temperature of 50°C from the survivor curves above, it was observed that the D-value for *S. senftenbergensis* was independent and did not change depending on initial cells population. Linear regression equation and the corresponding D-Values (time in minutes) for *S. senftenbergensis* in suspended menstruum of potato peels in water at 50°C, 55°C and 60°C were calculated. In order for the population of *S. senftenbergensis* to traverse one-log cycle reduction in the population as shown in Table 6-5, pasteurization of contaminated potato peels at 50°C yielded a D_{50°C}-Value of 44min, while pasteurization at 55°C required 14min and at 60°C required 7min to achieve the same effect.

Table 6-5: Regression equations and the D-values for *S. senftenbergensis* in potato peels waste matrices at various pasteurization temperatures

Temperature	Regression Equation	r	D-Value (Min)
50°C	$y = -0.023x + 8.833$	0.95	44
55°C	$y = -0.071x + 8.45$	0.98	14
60°C	$y = -0.14x + 8.033$	0.96	7

This time frame required for 90% die-off in *S. senftenbergensis* at 60°C agrees well to the finding reported in Álvarez-Ordóñez [166] who reported $D_{63^{\circ}\text{C}}$ -value of 6.27min for *S. senftenbergensis* recalling that the pH value of potato peels waste used in this research presents an acidic range pH 5.7-6.3. While it requires 44 min at the temperature of 50°C for the population of *S. senftenbergensis* to be eliminated by 90%, in comparison with *E. coli*, a D-value graphically determined (see Figure 6.3) shows that at the same minimum temperature of 50°C, the time required calculated as negative inverse of the slope for *E. coli* to traverse 1 log-cycle of its original population is approximately 10min. This confirms that at this same temperature, *S. senftenbergensis* requires 5-folds more of the D-value of *E. coli* for the same 1 log-cycle reduction from its initial population. Therefore, based on these results, the validation process on thermal destruction (z-Value) was derived for *S. senftenbergensis* as the most heat resistant enteropathogen.

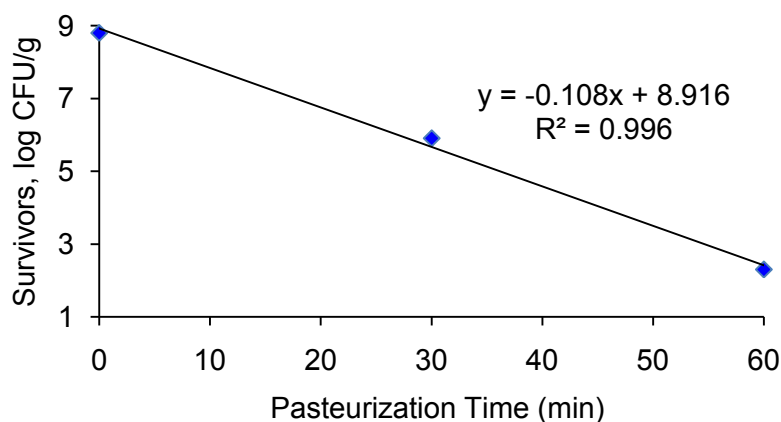


Figure 6.3: Decimal reduction time ($D_{50^{\circ}\text{C}}$ - value) of *E. coli*

6.8.5 Thermal destruction (z-Value) for *S. senftenbergensis*

z-Value refers to the temperature increase necessary to bring about a 1 log-cycle change in D-value during pasteurization [174]. This temperature is normally calculated as the negative reciprocal of the slope of the thermal death (D-value) against time graph as shown in the following Figure 6.4 that was obtained following batch pasteurization of potato peels waste artificially contaminated with *S. senftenbergensis*.

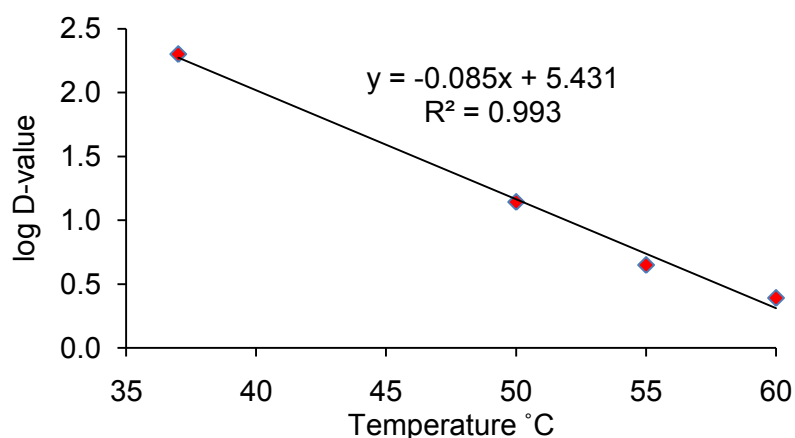


Figure 6.4: Thermal destruction z-Value for *S. senftenbergensis*

The log (D-value) against temperatures calculated from post hydrolysate samples revealed an increase in resistance of microorganism to the different heating doze applied. The z-Value result in the graph above determined as the negative inverse of the slope of the regression equation was therefore found to be 11°C. From the conducted batch pasteurization experiment, it took 6hrs for the process to achieve 90% reduction in *S. senftenbergensis* at a pasteurization temperature of 55°C. Although it was observed that heating the suspending menstruum samples of potato peels at 65°C for 30 min effectively eliminated both *E. coli* and *S. senftenbergensis*, pasteurization of the hydrolysate required even higher temperature of 70°C at 30min. Therefore, the z-value obtained is helpful in determination of the equivalent effectiveness of pasteurization in case various temperatures are to be assessed. In this case, since 300min pasteurization at 55°C gave 90% elimination of *S. senftenbergensis* in a batch pasteurization experiment, a z-value of 11°C calculated gave three possibilities, meaning that 3min at 75°C, 65°C at 30min or 300min at 55°C will effect the same level of 90% die-off in *S. senftenbergensis*.

The choice of temperature-time combination depends much on the desired goal of the respective biotreatment facility. The results from this experiment validates the 70°C for 60min temperature-time specification recommended in the Regulation (EC) No. 208/2006 for pasteurization by considering the non-spore forming bacteria and eggs of *Ascaris suum*. Yet, some studies [154,184] consider the 70°C for 60min temperature-time recommended not necessarily sufficient especially at inactivation of spore forming bacteria such as *Clostridium perfringens* [62,70]. Bagge [62] and Sahlström et al.,[154] observed no statistical difference $p>0.05$ in reduction for *C. perfringens* in samples pasteurized even at 70°C for 60min. Since pasteurization may be costly in case where external heating energy has to be used [198], this research hereby recommend based on the validated temperature-time regime to abide on the temperature-time combination of not less than 70°C for 30min the specification proposed in U.S- EPA/625/R-92/013[185] with exception to some places where there is scientific based evidence about the presence of more heat resistant microorganisms such as *Salmonella irumu* (more heat resistant spp of *salmonella* than *S. senftenbergensis*), *Bacilli spp* [13], Circoviruses, tobacco mosaic virus, bovine Parovirus [108] and many other pathogens which have been proven capable to pass the biotreatment process unaffected, then the temperature –time regime of 70°C or higher and at 60min holding time which is specified in Regulation (EC) No. 208/2006 should not be bypassed.

6.8.6 Enteropathogens die-off following post-biotreatment batch pasteurization

The inactivation results (log calculated as confidence interval \pm uncertainty for 95% confidence limits; n=3.) for post-biotreatment batch pasteurization of the two effluents (hydrolysate and methanogenic digestate) from two-phase anaerobic digestion of potato peels operated under mesophilic temperature range are presented below in Figure 6.5 and Figure 6.6 respectively. Regardless of the fact that equal volumes of bacteria suspension were added in both reactors, the pathogens density in hydrolysate was observed to be in the range of $\times 10^4$ CFU/g and $\times 10^5$ CFU/g for *S. senftenbergensis* and *E.coli* respectively. This level of contamination was lower compared to the population of $\times 10^6$ CFU/g observed in the methanogenic digestate for both *E. coli* and *S. senftenbergensis*.

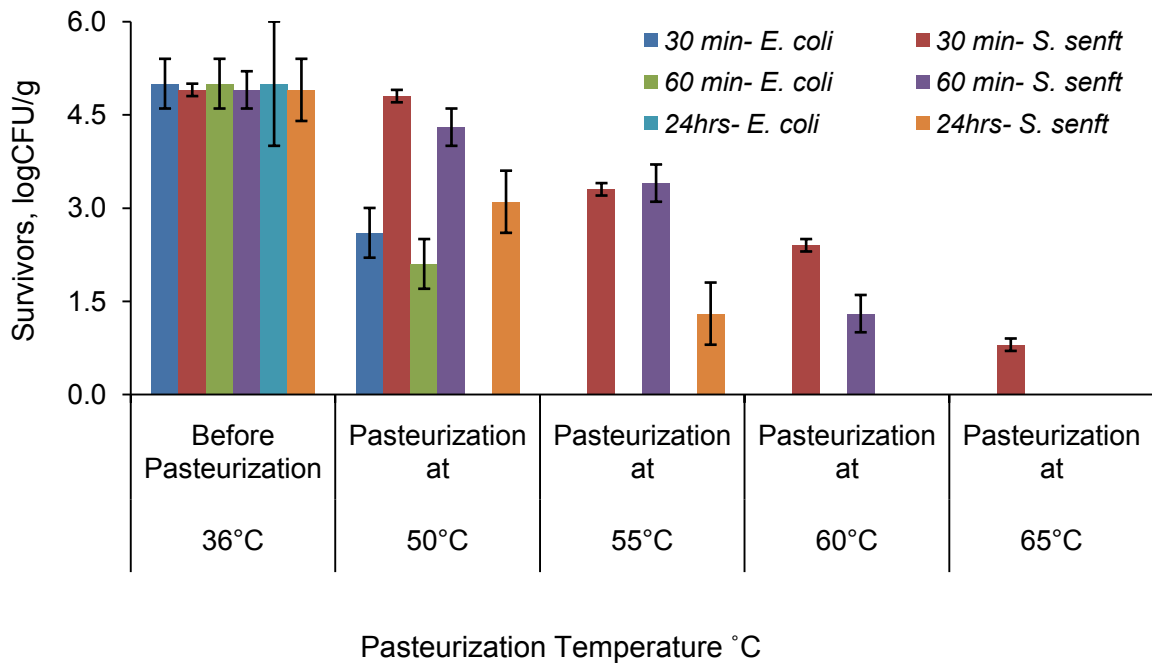


Figure 6.5: Mean log reduction of *E. coli* and *S. senftenbergensis* in acidogenic digestate of two-phase anaerobic digestion.

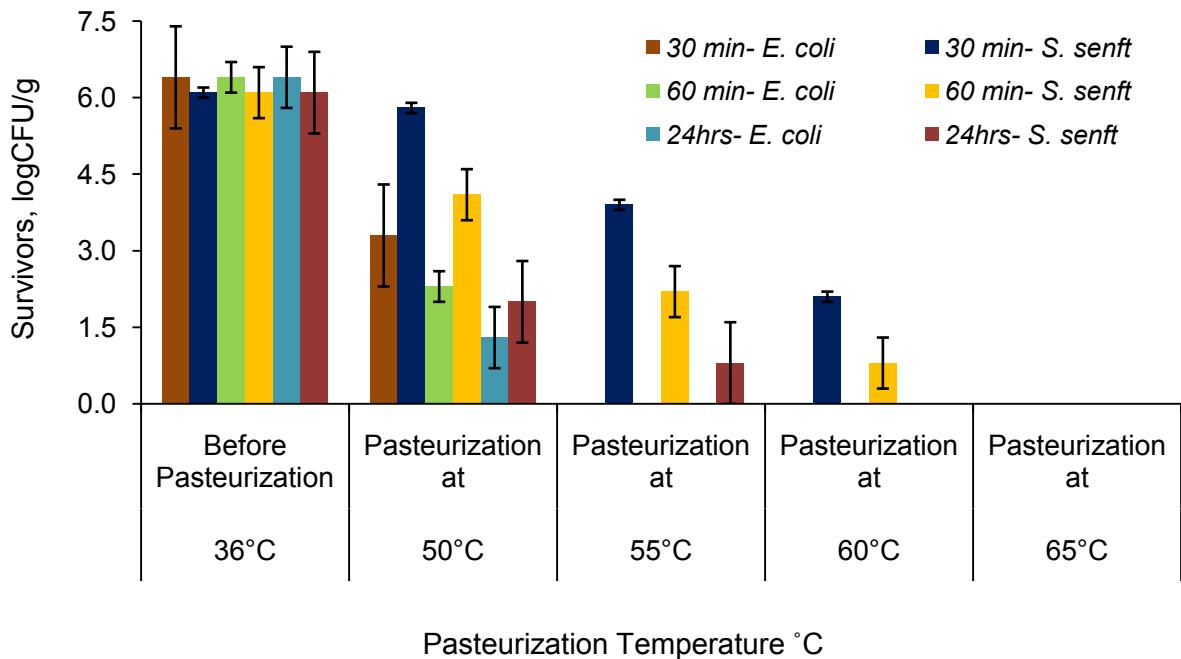


Figure 6.6: Mean log reduction of *E. coli* and *S. senftenbergensis* in methanogenic digestate of two-phase anaerobic digestion.

Several reasons could be responsible for the observed low population in the hydrogenesis reactor. Firstly it could be caused by acid nature of the suspended menstruum at low pH value of 4.7 following substrate fermentation. This pH is closer to the minimum reported pH for which the two enteropathogens can tolerate and regenerate, i.e. pH 4.4 for *E. coli* [199] and pH 4.0 for *S. senftenbergensis* [200]. Secondly could be as results of H₂S toxicity in the hydrolysis reactor which was measured above 5000ppm. Also the low C/N ratio in potato peel means higher nitrogen which during fermentation process results into more NH₃ which is equally toxic to the microbial community in the reactor. Other studies including Schieber and Saldaña [107] and Al-Weshahy and Rao [106] report the presence of natural bacteriostatic and bactericidal effect in potato peels. Therefore, the combination of these factors together with microbial antagonism might have contributed to the observed difference in the initial pathogens population.

Despite the observed differences in initial bacteria densities in the two effluents (hydrolysate and methanogenic digestate), the inactivation trends following batch pasteurization was very much similar. *S. senftenbergensis* continued to be the most resistant enteropathogen following this heat treatment similarly to the earlier observation in this research as well as of previous studies reported in Broennum [114]. During post-treatment pasteurization of the acidogenic digestate, no much of *S. senftenbergensis* inactivation was achieved at 50°C with sample residence at 30min as there was only 0.1log reduction from the initial population. A reduction of about 0.3logCFU/g was achieved at sample holding time of 60min and even under prolonged residence time of 24hrs there was only 1.8logCFU/g reduction. *E. coli* being highly sensitive to heat treatment was highly affected starting at 50°C for 30min as there was above 2.6log reduction that increased even more to above 3.0log reduction following 60min of sample holding time with no survivors after 24hrs. Pasteurization at 55°C and 60°C at 30min were much effective for *E. coli* but not for *S. senftenbergensis* as there was still up to 3.3log and 2.4log of the population in the acidogenic suspended menstruum. On increased pasteurization holding time to 60min, the massive reduction in *S. senftenbergensis* of above 3.6logcuf/g was achieved. While pasteurization temperature of 60°C for 60min was sufficient to completely assure biosafety of the digestate for the case of *E. coli*, surprisingly few population of about 20CFU/g *S. senftenbergensis* were observed after 55°C pasteurization for 24hrs and it was observed during the pasteurization of the

acidogens digestate that two petri-plates out of 5 incubated with sample from 65°C for 30min had 2 colony units each of *S. senftenbergensis* at 10⁻² - dilution. The possible explanation for this observation could be the increase in resistance of the *S. senftenbergensis* cells following their adaptation to low pH of the process waste similarly to what Álvarez-Ordóñez et al., [166] reported. In their article, Álvarez-Ordóñez et al., [166] expound that acid-adapted bacteria cells develop higher cyclic unsaturated fatty acid up to 2-folds of the ratio of unsaturated fatty acid to that of saturated fatty acids, this change in fatty acids composition in the pathogens cells cause low membrane fluidity which become a factor behind the increase acid-and thermal resistance in the bacteria cells. Another possibility was the fact that the hydrolysate was collected after 15 days of the anaerobic digestion process which means that some of the bacteria cells had reached stationary phase of growth. In this phase of growth, bacteria are reported to be more resistance to both low pH and thermal treatments [172,193,195,199,201]. The survived *S. senftenbergensis* cell validate the temperature-time regime set by the EU of 70°C with not less than 60min residence time for effectively elimination of thermo-tolerance pathogenic microorganisms for the assurance of proper hygienization and biosecurity in hydrolysate as to prevent contamination to the anaerobic digestion workers during the feeding of methanation reactor with contaminated hydrolysate.

Although the post-treatment batch pasteurization results for methanogenic digestate was quite similar to those obtained above during post-treatment batch pasteurization of acidogenic digestate, there was one exception. While the pasteurization temperature of 50°C for 24hrs showed to be effective at causing complete elimination of *E. coli*, the trend was not the same as in this experiment. There was about 1.3log CFU/g equivalent to 20CFU/g of *E. coli* in the suspending menstruum. Although the factor of induced tolerance could be the reason behind, the other possibility could be the growth of other bacteria species not *E. coli* that were present in the cow manure slurry used as seeding in the methanation reactor. Pasteurization at temperature above 50°C again was sufficient to full eradication of *E. coli* from the methanogenic digestate similar to the results observed in other experiment as well as reported in previous studies including Ceustermans [71].

Depending on the end use of the final digestate, post-pasteurization process is in fact more concerned with only the biosafety aspect of the digestate and does not necessarily

contribute into the biosecurity of facility workers or general microbiological safety in the biotreatment facility during the pre-anaerobic digestion processes. Although the volume of materials to be hygienized might have been reduced in the course of digestion and consumed organic total carbon that was changed to biogas, yet the agricultural based quality of the final digestate following post-pasteurization is reduced owing to the elimination of all microorganisms including the beneficial ones required to effect stabilization and maturation of the digestate prior to land application. The presence of beneficial microorganisms in the final digestate leaving the biotreatment facility to farmland is said to be of an addition benefit to plants by suppressing pathogenic microorganism and therefore eliminate plant diseases. Thus in cases where post-biotreatment handling of digestate does not allow recontamination, then pre-pasteurization is a feasible and economical worthwhile hygienization process for the pathogens contaminated substrate. Ward et al.,[184] referring to the U.S. EPA, 1994 norm advocates that pasteurization of organic fraction of MSW prior to mesophilic anaerobic digestion always produces digestate of class A. Under U.S EPA classification, class A digestate contains pathogens <1000 most probable number per gram (MPN/g) with the probability of detecting Salmonella below 3MPN/g.

6.9 Efficacy of chemical conditioning at effecting enteropathogens die-off

Literatures on pathogens elimination from contaminated materials confirm that changes in pH of the media [202], alteration of pathogens DNA following irradiation [203], heating of pathogens at lethal temperature above the survive range and moisture reduction through drying of the contaminated material [204,205] are the main means to effect pathogens die-off or inactivation. Other contributing factors to pathogens die-off but whose inactivation magnitude is unquantifiable include high concentration of evolved gas in particular H_2S >1000ppm and NH_3 >150ppm, presence of metals compounds, heavy metals, disinfectants, herbicides, insecticides and competition between indigenous useful microflora with the pathogens [59]. According to Smith et al., [171], chemical interaction and reactor configuration are equally contributing factors. Kaur et al., [206] and Shaw [207] consider high water activity (A_w) and absence of sugar and/or salt in the substrate/feedstock as the factors that render pathogens susceptible to die-off. Horan et al., [168] reveal also that pathogens die-off is a function of their initial population.

6.9.1 Acid conditioning

Microorganisms both indigenous and pathogens requires conducive environment for their activity for which pH is one among the important and limiting factors. In this research, Acid conditioning also referred to as biowaste stabilization using organic acids was initially studied at laboratory-scale as pre-test simulating acid-phase of anaerobic digester. But on the other hand this chemical method of hygienization has been proven sufficient at causing elimination of bacteria from biowaste. Presently in Europe MSW are used as base fuel in combustion of high risk Animal by-products of category 1 (ABP 1) specified in Regulation (EC) No. 1774/2002 for energy production [208]. A normal practice is that the mash of non-sanitized ABP 1 are ground to reduce the size less than 19mm and stabilized with formic acid producing the so called Biomal. Microbiological safety in both MSW and biomal during loading, transportation, off-loading as well as incineration is equally important to eliminate exposure to human and the environment. In this research, the performance of acetic acid at eliminating enteropathogens in the artificially contaminated biowaste was studied to present an alternative or substitute to formic acid. Albihn and Nyberg [73] report the efficiency of formic acid in Sweden at 1% concentration for inactivation and stabilization of *Salmonella* in slaughter house waste which are used as complementary fuel for heat generation in fluidized incinerators giving high heating value of 7.6-8.3 MJ/kg Biomal equally to using the conventional bio-fuel at 50% humidity.

6.9.2 Lime conditioning

For decades, the use of lime has been a practice in hygienization of sewage sludge [209] and industrial wastewater for stabilization of biosolids, adjust pH and elimination of pathogens leading to a high quality organic fertilizers for agricultural use [210]. Lime condition also has an added advantage of inhibiting trace metals immobilization into soil solution through the induced high pH which causes precipitation. Normally a known amount of slaked lime ($\text{Ca}(\text{OH})_2$) is added into sludge to effect change in pH of the media which eventually results into unfavorable condition to pathogens. Also in some treatments, CaO is used. The addition of quick lime besides changing pH of the media tends to equally induce exothermal reaction which generates heat as a result of reaction with organic matter, water and mineral salts in the sludge. Both changes in pH and/or the evolved heat affect pathogens survival and thus bring about hygienization. These two

chemical based experiments explore the potential of using organic acid i.e. acetic acid ($C_2H_4O_2$) for stabilizing and hygienization of biomass to be used as fuel with Biomal in place of formic acid and the possibility of stabilization of Biomal using lime $Ca(OH)_2$ thus widen diversification based on economic feasibility view point and choice on materials availability without compromising the biosafety of the final product. Also post-treatment of digestate with slaked lime $Ca(OH)_2$ focused at effecting hygienization of contaminated digestate after biotreatment of biowaste for safer use on arable lands and thereafter application in acid affected soil caused for instance by acid mine drainage both soil amendment and soil conditioner.

6.10 Materials and Methods

Acetic acid (99.8%) ANAR (Riedel-de Haen, Germany) having initial pH 0.6, salinity 0.0 and conductivity $0.2\mu S/cm$ was used for acid conditioning experiments. Calcium Hydroxide suspension (Schaefer Kalk GmbH, Germany) having initial pH 12.8, salinity 4.9 and conductivity $8.7mS/cm$ was used in alkali conditioning experiment. Both chemicals effect pathogens inactivation through changing the pH of the matrix far below (in case of acid) and far above (in case of alkali) the optimum surviving ranges of the microbial pathogens. Filters of size $0.45\mu m$ and $0.20\mu m$, syringe pipe and other glassware (UVW, Germany) were used during the experiment as received. Samples of Enteropathogens contaminated potato peels waste and unhygienized digestate from Methanogenesis reactor were used for this experiment. The conditioning of contaminated potato peels represents a pre-biotreatment hygienization while that of unhygienized digestate from anaerobic digestion of contaminated potato peels waste operated at mesophilic condition represents post-biotreatment hygienization process.

6.10.1 Acid and lime conditioning of contaminated potato peels waste

Exactly 25g of untreated potato peels waste sample (particle size $\leq 12mm$) was weighed and transferred into 500mL glass flasks. An addition of 247.5mL of sterilized ($121^\circ C$) water (*Leitungswasser*) having $514\mu S/cm$ conductivity and pH 7.4 ± 1.1 . The peels were subjected to constant agitation so as to stabilize pH of the peels/water mixture prior to addition of either Acetic Acid ($C_2H_4O_2$) or Calcium hydroxide $Ca(OH)_2$. To simulate the

contaminated biowaste, the enriched bacteria were spiked in the peel/water mixture at ratio 100:1 (Peels: bacteria suspension) to 8.7log *E. coli* (4.6×10^8 CFU/g) and 8.4log *S. senftenbergensis* (2.2×10^8 CFU/g) initial contamination levels denoted as (N_0) in that order. In this experiment, sum of 10 test samples were formulated (see Table 6-6) out of which four were conditioned with acetic acid while the rest 6 were conditioned with Calcium hydroxide.

Table 6-6: Adjustment of sample pH in potato peels for Acid/Lime conditioning

Pre-treatment hygienization of potato peels waste										
	Adjusted with Acetic Acid				Adjusted with Slaked lime					
Sample	1	2	3	4	5	6	7	8	9	10
pH-Target	3.5	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0
pH-Final	3.50	4.01	5.07	6.12	7.02	8.05	9.30	10.03	11.14	12.04

The conditioning reagents were separately added into the flasks containing contaminated potato peels suspending menstruum drop after the other with constant stirring (Magnetic stirrer Janke and Kunkel, Germany) as shown in Picture 6-5 while observing the targeted pH levels.



pH- adjustment



Sample agitation (130rpm)

Picture 6-5: Sample pH adjustment for Acid/Lime conditioning

The addition of acid or alkali in the respective flask stopped after reaching the targeted pH value. Ten pH targets were chosen starting with pH of 3.5 as minimum while the maximum reading was pH 12.0. Samples were aerobically treated at room temperature $23\pm 2^{\circ}\text{C}$ with constant agitation (Janke and Kunkel Ika labortechnik Ks 501 digital, Deutschland) at 130rpm so as to increase metabolic exchange between the bacteria cells and the liquid medium. Monitored of pH was done in all samples on daily basis, due to natural fermentation of the peels in some of samples adjusted with lime, pH re-adjustment was necessary to maintain the pre targeted pH throughout the experiment. Statistical analysis based on t-test was used to judge whether there exist significance difference in pathogens inactivation performance between acid and alkali conditioned samples.

6.10.2 Lime conditioning of unhygienized methanogenic digestate

This post-treatment hygienization experiment was carried out using 6 samples of digestate drawn on June 22, 2015 from methanation reactor of two-phase mesophilic anaerobic digestion system digesting artificially contaminated potato peels waste with *E. coli* and *S. senftenbergensis*.

There was no addition of bacteria in the digestate instead; the pathogens population survived the treatment process that is $6.8\log$ *E. coli* equivalent to 6.3×10^6 CFU/g and $6.4\log$ *S. senftenbergensis* equivalent to 2.1×10^6 CFU/g respectively were used as initial population to be inactivated. A volume of exactly 300mL of enteropathogens contaminated methanogenic digestate was collected in bottles of 500mL (Picture 6-6).



Picture 6-6: Lime conditioning of digestate

The collected samples were conditioned following an addition of $\text{Ca}(\text{OH})_2$. In each bottle slaked lime was added percent-wise based on volume (%v/v) as shown in the following Table 6-7.

Table 6-7: Dosage of lime during inactivation of pathogens in un-hygienized digestate

Sample	Sample bottles					
	1	2	3	4	5	6
Added (%v/v)	0%	1%	3%	5%	8%	10%
Lime (mL)	0	3	9	15	24	30

The bottles with no addition lime were used as control. Samples were aerobically agitated (Janke and Kunkel Ika labortechnik Ks 501 digital, Deutschland) at 130rpm at 22±2°C throughout the experiment except during sample drawing for bacteriological analysis. Agitation increases metabolic exchange of materials between the bacteria cells to the surrounding medium and degassing of gases resulting from continuing natural fermentation of the digestate. Contrary to the previous experiment, there was no further adjustment of sample pH along the research after the first addition. Effectiveness and efficiency of alkali (lime) at inactivation of pathogens was monitored on the basis of pH. Sample electrical conductivity and salinity were also measured along with pH so as to comprehend whether or not the conditioned digestate will have effect on plants if used as organic fertilizer on arable land.

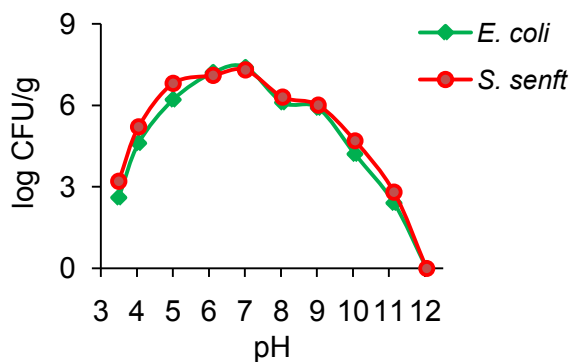
6.10.3 Efficacy of Acid/Lime conditioning on inactivating enteropathogens

The samples in both experiments, enteropathogens contaminated potato peels and unhygienized digestate were then serially 10-folds diluted in the dilutions range of 0 to 10⁻⁶. High viscosity in digestate made impossible for pipetting of the original sample for direct serial dilution, it was of the essence therefore to make dilution of the sample prior to undertake the decimal serial dilution. In each serially diluted sample including the original, a volume of 100µL of the appropriate dilutions made using sterilized water (*leitungs wasser*) was surface spread on petri-plates containing Endo Agar and modified BGA. The plates were incubated at 36°C for 24hr after which bacteria colony units were enumerated. The performance of acetic acid /slaked lime at completely inactivating *E. coli* and *S. senftenbergensis* in the potato peels waste and in digestate was then calculated and expressed in logCFU/g.

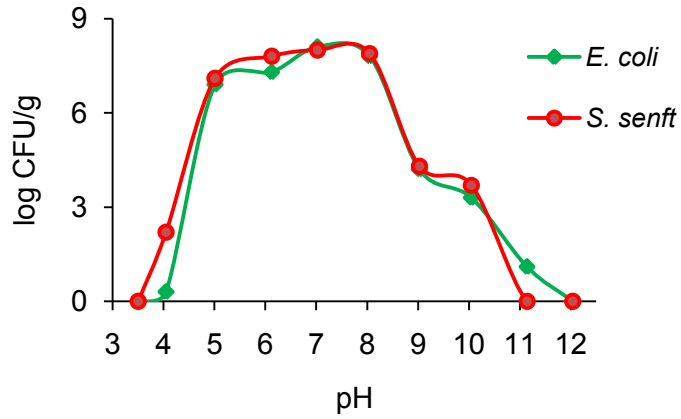
6.11 Results and Discussion

6.11.1 Low and high pH hygienization of potato peels waste

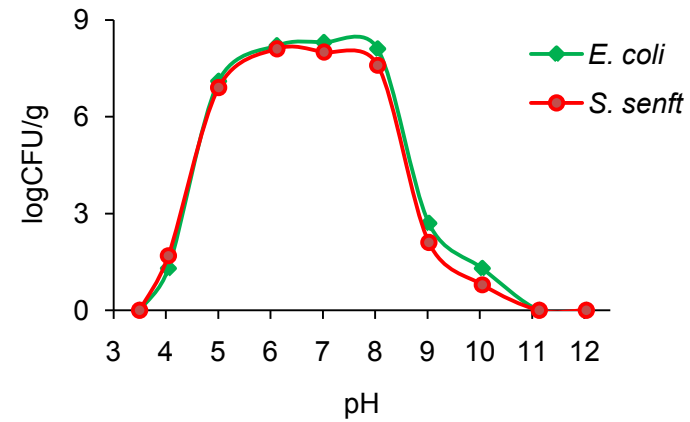
The summarized results for inactivation of *E. coli* and *S. senftenbergensis* at different pH following acid/lime conditioning of contaminated potato peels waste at different residence time are shown in Figure 6.7. The spiked concentration for *E. coli* was 2.2×10^8 CFU/g while that for *S. senftenbergensis* was 4.6×10^8 CFU/g. In other words, the viability of *S. senftenbergensis* following enrichment process in nutrient broth was 2-folds higher than that of *E. coli*. The amounts of cells population indicate that prior to conditioning potato peels contained about 45% *E. coli* and 55% *S. senftenbergensis* on the log-scale. At the end of the first 30min which was a minimum sample residence time under aerobic condition and room temperature of $23 \pm 2^\circ\text{C}$, both *S. senftenbergensis* and *E. coli* managed to survive pH 3.5 although theoretically the survive range of *Salmonella* in acidic media has been reported to be low as pH 3.8 [199] while that of *E. coli* being 4.4 [170] depending on the acidulant and residence time. Nevertheless, the observed acid-tolerance was not a new findings for enteric bacteria because naturally *Salmonella spp* and *E. coli* being enteric bacteria survive the extreme low pH value as 2.5 of the gastric acid in the stomach [211]. *Salmonella* are reported acid resistant to as low as pH 3.6 [207,212]. The graphical presentation of results hereunder confirms acid-tolerance of enteric bacteria validating the findings by Zhu et al., [213] and Zhao et al., [214] who observed the ability of *E. coli* to survive at pH value 3.5 and 3.6-4.0 respectively. At pH value 3.5 and 30min sample holding there was approximately 4.0×10^2 CFU/g of *E. coli* and about 1.6×10^3 CFU/g of *S. senftenbergensis* viable in the suspension.



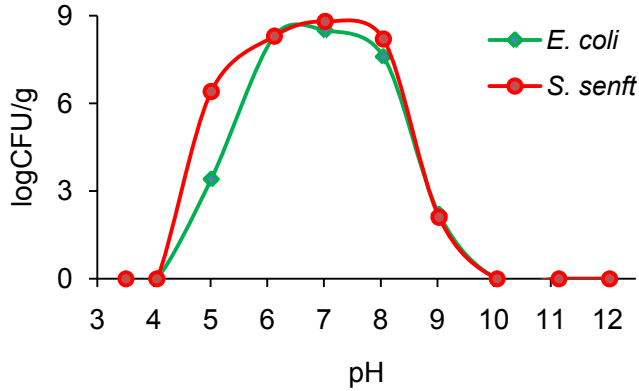
Inactivation curve after 30min of sample conditioning



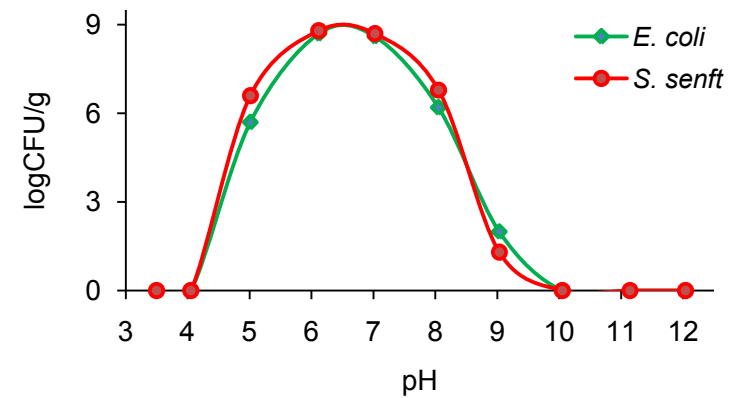
Inactivation curve after 60min of sample conditioning



Inactivation curve after 24hrs of sample conditioning



Inactivation curve after 5 days of sample conditioning



Inactivation curve after 10 days of sample conditioning

Figure 6.7: Patterns for enteropathogens inactivation using acid/lime conditioning at various residence times

Although both *E. coli* and *S. senftenbergensis* survived under this low pH, *S. senftenbergensis* was observed to be more tolerant than *E. coli* similar to the findings by Horan et al., [168] who observed *S. senftenbergensis* as more resistance than *E. coli* when treated under the same pH. It was hypothesized along this experiment that the enteropathogens die-off was independent of the initial population spiked, the observed results agree well to the observation by Álvarez et al., [195] who concluded that the microbial inactivation is independent of the initial cell concentration. Under prolonged residence time both bacteria were severely affected and at the onset of 24hrs there was no bacterial cell survived at this low pH. In the samples conditioned at pH 4.01 for 60min both *E. coli* and *S. senftenbergensis* survived though *E. coli* was observed to be much affected. Surprisingly, to the reasons not known after 24hrs, there were an increased number of *E. coli* colony units in the same matrix up to 1.3log equivalent to 20CFU/g while those of *S. senftenbergensis* maintained at 1.7log equivalent to 50CFU/g. In other sample matrices from pH 5.01 to pH 9.03 both bacteria survived the treatment with a peak proliferation observed at pH 7 for which there was 2.5×10^7 CFU/g of *E. coli* and 2.0×10^7 CFU/g of *S. senftenbergensis* in the sample after the first 30min of conditioning.

A small reduction observed from the spiked population amounting to 0.9log *E. coli* and 1.4log *S. senftenbergensis* in the sample matrix treated to pH 7.02 could be as a result of either acclimatization to new environment, antagonistic between the spiked cells with the indigenous microorganism (coliforms) present in the potato peels as explained in previous experiments on microbial quality of the potato peels waste or inhibition caused by a small amount of $\text{Ca}(\text{OH})_2$ added in the suspension while adjusting the pH of potato peels to A neutral zone. After 5 days of sample holding, pH 10.05 was detrimental to both bacteria cells and none could survive the treatment. Strong alkaline condition at pH11.14 was much lethal to both pathogens on which the population to both enteropathogens was forced below 3log with *E. coli* being much affected compared to *S. senftenbergensis*. pH value 12.04 was much stronger to both pathogens to the extent that no bacteria cell was able to grown on petri-plates spread with sample from this pH level even at 30min of residence in conformity to U.S EPA regulation (EPA 833-R-95-001) [215]. *Salmonella spp* and *E. coli* are reported to exhibit shorter doubling time estimated between 20min and 40min [89,115,116] when growing at $25 \pm 5^\circ\text{C}$. The 60min residence time for samples was therefore selected to confirm the first double increase in population of the pathogens in

respective conditioned samples. It was observed that both bacteria could no longer survive pH 3.5 and there was no longer bacteria growth in matrix treated to this low pH after a sample holding time of 60min. An interesting observation associated with this low pH and that at pH 12.04 was that, in both cases there was a clear separation between water and the peels with no sign of fermentation even after prolonged sample retention with continual agitation (see Picture 6-7).



pH 3.5



pH 7



pH 10



pH 12

Picture 6-7: Sample matrix after 10 days conditioning of potato peels at various pH

Different from other bottles from pH 5 to 10, fermentation was observed with gas production and marked hydrolysis of the starch in potato peels as an indication for ongoing fermentation activity by the consortium indigenous acidogenic micro-flora. This natural fermentation phenomenon necessitated occasional pH adjustments especially in alkaline due to pH drop. Under normal condition, pH 4.05 is still very low for survive to both *S. senftenbergensis* and *E. coli* that is why sample residence for 30 min at this pH

resulted into 3.7log reduction equivalent to 99.98% in *E. coli* and about 3.5log reduction equivalent to 99.96% in *S. senftenbergensis*. After 1hour to 1day exposure, there was more than 6.0log (99.999%) reduction in both *E. coli* and *S. senftenbergensis* spiked on prolonged residence to 5days and above, no viable cell of either *E. coli* or *S. senftenbergensis* survived in the sample matrix at pH 4.05 thereafter. This brought an interesting conclusion that the use of formic acid in Biomal could possibly be interchanged with acetic acid for the similar hygienization effect provided the conditioning is done at pH value 3.5 for not less than 1hr holding time or at pH value 4 for 2-5days for a complete die-off in enteropathogens. Although acetic acid took longer time than formic acid as reported in Vinnerås et al.,[208] who treated Biomal samples using formic acid at 3% (pH of 4.5) and observed complete inactivation of enteropathogens and enveloped viruses within 24hrs of sample conditioning. The choice for using formic acid and acetic acid would therefore depend much on the availability, cost and user preference among the acidulants. Acetic acid together with achieving the microbial quality expected in the final products, it prevents potential adverse health conditions including eyes irritation, sore throat, coughing, chest tightness and headache which are the commonly reported health side effects associated with long time exposure to formic acid. The findings from this experiment provided further a clue that acid phase in anaerobic digester could be effective at eliminating enteropathogens if the pH would be maintained not above pH value 4.

To some of the treatments, the final recorded matrix pH (see Table 6-8) slightly differed from the first adjusted final value given earlier in Table 6-6. This caused by either the buffering characteristic observed in potato peels or as result of microbial fermentation of the peels that lowered matrices pH by the increase in acidic which necessitated a small pH adjustment using lime. Taking a neutral pH 7.02 as reference point see Table 6-8, there was an equal increase in bacteria population by 0.7log to each enteropathogen after 60min of sample holding time. Since the optimum growing pH for *E. coli* as reported in literature is between 5.5-8.5 with a minimum of pH 4.4 and maximum pH 9.0 while that of *Salmonella spp* is between 6.5-8.5 with the minimum pH 3.8 and maximum pH 9.5 [199], no much bacteria reduction was observed in samples with pH adjusted to 5, 6, 7, 8 and 9 throughout 10 days of sample conditioning apart from the normal slow bacteria growth exhibited during the lag phase of their growth curve normally attributed to acclimatization to both new environmental conditions and new substrate.

Table 6-8: Bacteria counts (logCFU/g) in potato peels under pH – time aerobic – mesophilic conditioning

Time		Initial counts		30min		60min		Day1		Day5		Day10	
pH Treatment		<i>E. coli</i>	<i>S. senft-</i>	<i>E. coli</i>	<i>S. senft-</i>	<i>E. coli</i>	<i>S. senft-</i>	<i>E. coli</i>	<i>S. senft-</i>	<i>E. coli</i>	<i>S. senft-</i>	<i>E. coli</i>	<i>S. senft-</i>
Target pH	Final pH	Bacteria enumeration given in logCFU/g											
3.5	3.50	8.3	8.7	2.6	3.2	∅	∅	∅	∅	∅	∅	∅	∅
4.0	4.05	8.3	8.7	4.6	5.2	0.3	2.2	1.3	1.7	∅	∅	∅	∅
5.0	5.01	8.3	8.7	6.2	6.8	6.9	7.1	7.1	6.9	3.4	6.4	5.7	6.6
6.0	6.12	8.3	8.7	7.2	7.1	7.3	7.8	8.2	8.1	8.3	8.3	8.7	8.8
7.0	7.02	8.3	8.7	7.4	7.3	8.1	8.0	8.3	8.0	8.5	8.8	8.6	8.7
8.0	8.05	8.3	8.7	6.1	6.3	7.8	7.9	8.1	7.6	7.6	8.2	6.2	6.8
9.0	9.03	8.3	8.7	5.9	6.0	4.2	4.3	2.7	2.1	2.2	2.1	2.0	1.3
10.0	10.05	8.3	8.7	4.2	4.7	3.3	3.7	1.3	0.8	∅	∅	∅	∅
11.0	11.14	8.3	8.7	2.4	2.8	1.1	∅	∅	∅	∅	∅	∅	∅
12.0	12.04	8.3	8.7	∅	∅	∅	∅	∅	∅	∅	∅	∅	∅

Note: ∅ = no growth of bacteria colony was observed on spread petri-plate

Contrary to the theoretical maximum alkaline pH for *E. coli* and *S. senftenbergensis*, it was observed that *E. coli* was more resistant to alkaline condition compared to *S. senftenbergensis* as there were more cells of *E. coli* in the alkaline matrices than *S. senftenbergensis* cells. The results on *E. coli* observed in this research contradict to the observation in Estrada et al.,[216] in which complete inactivation of *E. coli* required up to 4hrs of retention at pH 9.8. In this study, *E. coli* survived pH value 10.03 up to 1day of sample holding time. Lime treatment at pH value 11 completely inactivated *S. senftenbergensis* in 60min while there was still up to 1.1logCFU/g of *E. coli* surviving at the same pH though on prolonged residence at 1 day a complete die-off in both *E. coli* and *S. senftenbergensis* was achieved.

After acclimatization the log phase was rapid until day 5. Stationary phase was observed from day 5 to day 10 in which the bacteria populations reached 8.5-8.6logCFU/g for *E. coli* while that of *S. senftenbergensis* reaching 8.7-8.8logCFU/g in reference sample with pH adjustment to 7. *S. senftenbergensis* adapted much faster than *E. coli* and compete much higher. Lime conditioning at pH 9 showed *E. coli* below 3logCFU/g a population which is considered acceptable in Regulation (EC) No. 208/2006 and DG ENV.C2/BZ/tb [117] for *E. coli*. The same regulations demands for complete elimination of *Salmonella* in the sample before the sample is considered safe for any intended use thereof, but the presence of 2.1log CFU/g *S. senftenbergensis* in day 1 and 5 of conditioning and 1.3log CFU/g after 10 days of sample condition disqualified the resulting sample to be categorized as safe. Sample conditioning for 1 day at pH 10 was enough at reducing *E. coli* to less than 2.0log with even much lower as 0.8logCFU/g in *S. senftenbergensis*. Similarly the presence of *S. senftenbergensis* in the sample disqualified the sample to be categorized as microbiological safe based on the aforementioned regulations.

On prolonged holding time at the same pH for 5days and 10days non among the enteropathogen survived. Complete enterobacteria die-off was achieved at pH value 12 even at a very short time of 30min residence similar to the recommendation in Sahlström [69]. The results obtained in this study correlates with the findings reported in Arthurson [179], Benette et al.,[217] and Bina et al.,[218]. In those studies, a complete inactivation of salmonella (other than *S. senftenbergensis*) and *E. coli* was reported to be achieved in

2hrs at pH value 12. One challenge experienced while using lime as biowaste conditioner was that after 30min of retention with constant agitation, a drop in pH was observed in some matrices of conditioned samples. This was perceived as buffering nature of potato peels since fermentation was yet to commence. Nevertheless, when fermentation of the peels started after 24hrs the generated carbon dioxide gas and probably the volatile fatty acids formed as by-products of fermentation caused drop in pH as well. This phenomenon presents a caution when using slaked lime as a conditioning media because the drop in pH definitely lowered medium pH from the adjusted value and consequently reduces the expected effectiveness and efficiency of the treatment and therefore favors microbial proliferation by re-growing of the survived cells. Regular monitoring of the pH stability and pH adjustment is therefore highly recommended when using chemical based hygienization options so as to achieve total pathogens kill-off and at the efficiency desired.

Depending on the end use of the biowaste, acetic acid proves to be effective at lower pH provided the residence time is above 1hr. The option of using acid as hygienization media therefore relies much on its availability, handling options for the acidic effluents (wash-off) after hygienization process and cost-benefit estimation prior to taking final decision knowing that while using acid in hygienization process, more corrosion resistant containers which may increase cost need to be used [219]. It was experienced that just a small volume of $\text{Ca}(\text{OH})_2$ could impart a significant pH change in the sample than when the equivalent volume of $\text{C}_2\text{H}_4\text{O}_2$ was used. Literature search on the use of alkali on biowaste conditioning revealed that prior to biological treatments in particular anaerobic digestion, the use of alkali on biowaste have many added advantages such as stabilizing alkaline condition in the system [220,221] and enhancing solubilization of solidified carbohydrate portion as well as digestion of lignin, cellulose and hemicelluloses in substrate therefore result into an increase in bioenergy production [150]. In comparison to acid conditioning as means for biowaste hygienization, slaked lime is said to be relatively cheap and can be handled with less caution [219].

6.11.2 Lime conditioning of methanogenic digestate

The initial pH value of the methanogenic digestate for lime conditioning was 7.7. It was observed that the use of $\text{Ca}(\text{OH})_2$ did not cause changes in sample temperature but caused an effect on electrical conductivity in which 6.5 units decrease was observed in the sample in which there was 10% addition of $\text{Ca}(\text{OH})_2$. The decrease in electrical conductivity observed could have probably caused by chemical reaction between mineral salts in the treated digestate with $\text{Ca}(\text{OH})_2$.

6.11.3 Pathogens inactivation in slaked lime conditioned digestate

The results obtained on reduction and/or die-off in microbial pathogens following conditioning of digestate with $\text{Ca}(\text{OH})_2$ is shown in Table 6.9. The findings shows a noticeable increase in pH for 1.1 units from the initial pH value following the addition of the initial doze 3mL equivalent to 1% of $\text{Ca}(\text{OH})_2$. Even at this small change in pH value in the methanogenic digested, there was reduction in pathogens right from the initial sample holding time in the order of 0.2log (37%), 1.7log (98%), 1.1log (92.1%) in *S. senftenbergensis* for 6 hrs, 1day and 2 days respectively.

Table 6-9: Pathogens reduction (logCFU/g) using slaked lime in methanogenic digestate

Ca(OH) ₂ (%v/v)	Sample °C	pH	Conductivity mS/cm	Residence Time					
				6hrs		24hrs		48hrs	
				S. <i>Senft-</i>	E. <i>coli</i>	S. <i>Senft-</i>	E. <i>coli</i>	S. <i>Senft-</i>	E. <i>coli</i>
0	26.3	7.7	13.1	6.8	6.3	6.8	6.3	6.8	6.3
1	24.9	8.8	11.6	6.6	4.5	5.1	4.0	4.0	5.2
3	25.2	9.1	10.3	3.6	3.3	2.3	3.4	2.9	3.7
5	24.4	9.5	10.2	3.2	3.1	2.1	1.7	2.2	1.3
8	24.8	10.8	7.3	2.1	∅	∅	∅	∅	∅
10	26.3	11.1	6.6	∅	∅	∅	∅	∅	∅

∅ =<LoQ (below limit of quantification); no viable bacteria cell was seen on petri-plate

Equally, reductions of about 1.8log (98.4%), 2.3log (99.5%) and 3.1log (99.92%) in *E. coli* were realized for the same 6 hrs, 1day and 2 days respectively of digestate retention. Though it is difficult to rule out the die-off trend, higher die-off in *E. coli* marks this bacterium strain as sensitive enteropathogen to higher pH values compared to *S. senftenbergensis*. Sample holding for 1 day at pH 9.5 brought a big reduction in both pathogens, with *E. coli* reaching 1.3log (2.0×10^1 CFU/g) thus conforms to the Regulation (EC) No. 208/2006 and DG ENV.C2/BZ/tb [117] specifying 1000CFU/g and $<5 \times 10^3$ CFU/g (wet wt.) as maximum population for *E. coli* in the sample tested, a result which was attained after 2 days of sample conditioning.

Maximum pathogens reduction was achieved at pH value 10.8 which was a result of 8% addition of $\text{Ca}(\text{OH})_2$ resulting into removal of 4.7log in *S. senftenbergensis* within 6hrs of sample retention. In this pH, sample retention for 1day was enough to completely eliminate both *E. coli* and *S. senftenbergensis*. With exception of *S. senftenbergensis* which even at the maximum doze of 10% $\text{Ca}(\text{OH})_2$, still demonstrated 1.3×10^2 CFU/g cells in the digestate retained for 6hrs. It was interesting to note that the two enteropathogens could no longer withstand a prolonged residence time beyond 1day of sample conditioning in slaked lime at pH value 10.8. The observed complete die-off in both *E. coli* and *S. senftenbergensis* results using 10% doze from this experiment which resulted into pH 11.1 coincides much to the observation reported in Bina et al.,[218] and Jimenez et al.,[222]. In their report, *Salmonella spp* other than *S. senftenbergensis* was completely eliminated from solid sludge after 2hrs holding time at alkaline pH values of between 11 and 12.

The findings from lime conditioning of the methanogenic digestate effluents in this investigation show that the performance on pathogens inactivation was promising starting with 1% addition of Calcium hydroxide. Citing Straub et al.,[118] and Arthurson [198] both explaining the mechanism on how alkaline condition in the media works, they revealed that at higher pH values, the NH_4^+ present in the digestate becomes deprotonated to ammonia gas (NH_3) which is a bactericidal as the gas formed diffuses in the bacteria cells as toxic substance causing cell die-off. Furthermore, in the same review work, Arthurson[198] quoted some researchers indicating possibility of eliminating enteropathogens at pH value 10 within 24hrs in dried sludge samples; the argument which

is somewhat in conformity with the results in this research in which at 8% addition of slaked lime that gave pH 10.8, *E. coli* was completely eliminated and *S. senftenbergensis* was much reduced $>4.5\log\text{CFU/g}$ within 6hrs and beyond this time none of the enteropathogens survived. As for the digested which has been pre-conditioned with lime, several advantages have been reported on the use of such digestate as soil conditioner in acid affected agricultural lands. Christie et al., [223] using biosolids conditioned with lime in spring barley (*Hordeum vulgare* L.) for instance obtained good yield results comparable to the use of inorganic fertilizers P or K . The high pH value in biosolids facilitated slower release of P and K making them available to plants in the similar way like the inorganic fertilizers. It was worth noting as well that slaked lime effectively reduced electrical conductivity of the methanogenic digestate from its original value of 13.1mS/cm to 6.6mS/cm conformity to the range of 4-8mS/cm specified as maximum applicable range acceptable in agricultural land even to sensitive plants [120]. This electrical conductivity attained in the methanogenic digestate conform to the grade of soil amendment [224] .

6.11.4 Decimal reduction time (D-value) for slaked lime conditioned digestate

Using the obtained data on logarithm survival for *E. coli* and *S. senftenbergensis* in Table 6-9 above, linear regression analysis was performed based on the first smaller values of lime addition in the methanogenic digestate that comprised of 1%, 3% and 5%. In these low alkalis conditioning of the digestate, both *E. coli* and *S. senftenbergensis* indicated die-off resistance to various degrees. The decimal reduction time (D-values) results was therefore calculated as the negative reciprocal of the slope for the best fit line for cell survived against the time taken in the conditioning process. The following Table 6-10 presents the results as time in hours. In both *E. coli* and *S. senftenbergensis*, the ability to survive as pH value increases from pH 7.7 to 9.5 following 0% to 5% addition of $\text{Ca}(\text{OH})_2$ demonstrated a decreasing order of magnitude in all cases. Despite having similar fatty acid membrane as reported in Álvarez-Ordóñez et al.,[166], theoretically it was expected that *E. coli* and *S. senftenbergensis* would have been affected in the similar way following exposure to pH stress.

Table 6-10: Decimal reduction time (hrs) for *E. coli* and *S. senftenbergensis* in slaked lime conditioned digestate

Ca(OH) ₂ (%v/v)	pH	D-values (Hours)	
		<i>S. senft-</i>	<i>E. coli</i>
1	8.8	16	83
3	9.1	15	30
5	9.5	14	12

On contrary more time was required by *E. coli* to traverse 1 log-cycle inactivation for pH values below 9.1 compared to *S. senftenbergensis*. But since the maximum survive pH for *E. coli* is pH value 9.0 [170] which is lower compared to pH value 9.5 of *S. senftenbergensis* [225], that was the reason to why above pH 9.0, a shorter time of 12hrs was required to inactivate 90% of the *E. coli* from its initial population. Despite differences in experimental sample matrix used, these results agree with those reported in Nyberg et al., [226] in which it required 26.4hrs for *E. coli* in the sample to traverse 1 log-cycle of its original population in soil conditioned with 0.5% Ca(OH)₂ giving stable pH value 9.0.

6.12 Efficacy of Ultraviolet radiation at effecting enteropathogens die-off in contaminated biowaste

6.12.1 Application of Ultraviolet radiation

Ultraviolet radiation (UV) is part of the electromagnetic spectrum comprising of four regions namely UVV, UVA, UVB and UVC demarcated based on their wave length as described in Bintsis et al., [227] and Guerrero-Beltrán and Barbosa-Cánovas [228].

- UVV (UV Vacuum) occupy wave length between 100 and 200nm UVA (long wave)
- UVA (long wave) occupy wave length between 320nm and 400nm and is responsible in changing in human skin (tanning or aging).
- UVB (medium wave) occupy wave length between 280nm and 320nm and is responsible in skin burn (cancer)

- UVC (short wave) occupy wave length between 200nm and 280nm and is responsible in DNA destruction through the germicidal radiations.

Of interest to hygienization process is the UVC light. The use of short- wave UVC has been a promising alternative to conventional thermal treatment method at ensuring elimination or reduction of pathogens and other food-borne contamination in many processes. In food processing facilities for example, UVC radiation have been applied in both liquid and solid food materials during food processing to ensure microbiological safety [179,229,230]. And for quite some time, the germicidal UVC radiations have been applied and proven effective at inactivating pathogens in environmental samples including air, water and wastewater [177,228,230]. Compared to chemicals and thermal treatments hygienization, UVC is confirmed to be safe, economical and even effective at inactivating more resistant microbes including large protozoa cryptosporidium and giardia, germs, molds and viruses [177,231–233] which in most disinfecting chemicals [191] and temperature-time regimes these pathogenic microorganisms have been capable to pass through the treatment process unaffected [62].

Koivunen and Heinonen-Tanski [234] expound that unlike disinfectant and chemical treatment, the germicidal UVC light efficiently induces hygienization without producing chemical residues. Nevertheless, the pathogens inactivation performance of the UVC radiation much depends on quality condition such as turbidity, type and clumping of microorganisms to be inactivated and the presence or absence of particulate matter in the sample to be irradiated [228]. UVC interaction mechanism on materials to be disinfected is in the form of absorption, reflection, refraction and scattering. The latter interactions potentially reduce the hygienization efficacy of the UVC system as the absorbed light will no longer be available for hygienization purpose even though changing the UVC light rays into other direction leaves the light with its germicidal efficient should in case fall on the cell of microorganisms within the media to be disinfected, but the chance of the rays missing the targeted cells in this case is great and therefore reduce the efficacy of the process.

6.12.2 Working principle of UVC radiation

In general, UVC radiations at wave length between 220-300nm are known as germicidal [228]. Short-wave UVC radiations at wavelength (λ) 254nm are considered more lethal and strongly microbiocidal germicidal effect when in contact with microbial cells on the surface targeted to be hygienized [227]. In principle, radiations on microbial cells interact with thymine and cystine nucleoside bases (see Figure 6.8), by so doing, the light damages the microbial DNA helix, causes the formation of thymine dimer which destructs the biological code (pyrimidine molecules i.e. cystocine, thymine and uracil) of the microbial cell, induces mutation, resulting into a microorganism that can neither regenerate nor infect and thus become destroyed and/or inactivated [235,236] U.S-EPA, 1999)⁹.

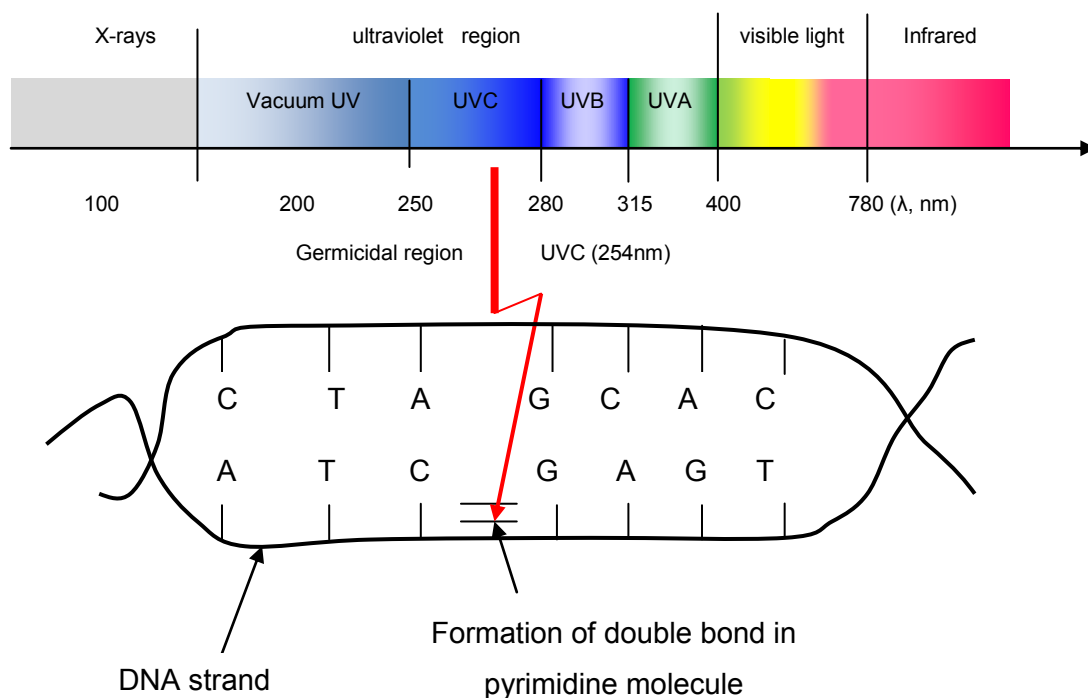


Figure 6.8: Spectrum and mechanism of germicidal UVC on DNA of microbial cell

⁹ http://www.epa.gov/safewater/mdbp/pdf/alter/chapt_8.pdf. Retrieved on July 16, 2015

With exception to colloids food, liquid food (milk, juice and water) and wastewaters, to the best knowledge of the researcher, no retrievable research has reported the use of UVC radiation for inactivation of pathogens in biowaste. In this research therefore, based on EU Commission proposal through SANCO/2632 and 2634 that encourages replacing thermal treatment (70°C for 60min) with validated processes capable at reducing at least 3logCFU/g of pathogens initial population, the goal was therefore set to investigate the ability of UVC radiations at effecting die-off in enteropathogens artificially spiked in acidogenic digestate in the hydrolysis reactor of the two-phase anaerobic digestion system treating potato peels waste and operated at mesophilic range (37°C) so as to produce safe substrate that is to be fed in the methanation reactor and eventually results into microbiologically safe methanogenic digestate (liquid biofertilizer) that can be safely discharged and used in farmland as organic liquid fertilizer having no more threats of transmitting the pathogens.

UV lamp produces intensity (E_A , W/m²) which is the total radiant power of all wave length (λ , nm) passing from all incident directions per treatment area[237]. The UVC lethal doze varies based on cell structures and depict the product of intensity with time (seconds), for instance with a conversion of (1W/m² =1000mW/m² =0.0001W/cm² =0.1mW/cm²). Citing few examples for instance, prokaryotic (bacteria) cells with non-protected DNA and simple plasma membrane, theoretically a low dosage equivalent to 1.5-6.0mWs/cm² is deemed enough to induce a complete inactivation [238]. Medium UVC doze equivalent to 6.0-10.0mWs/cm² is required for eukaryotic such as yeast and vegetative fungal cells having DNA in the nucleus, simple plasma membrane and comparatively large cellular body. A large application of radiation dosage from 8.0-1200.0mWs/cm² may be required for spores (such as fungal spore during survival stage) due to their thick cell wall and concentrated cytoplasm [232].

6.13 Materials and Methods

6.13.1 Sample collection and characterization

Sample turbidity, particulate matter and colour were the primary criteria for consideration prior to undertaking the laboratory scale UVC post-biotreatment hygienization study. Upon analysis, only sample of contaminated acidogenic digestate (process water) from hydrolysis reactor was used in this investigation. Sample characteristics including color, organic matter content and turbidity are the barriers to transmission of the UVC light within the medium and tend to reduce inactivation efficiency. Under similar mesophilic treatment temperature range, the hydrolysis of potato peels waste produces transparent pale yellow effluent with turbidity greater than 2000FAU whereby the methanation reactor, because of the cow slurry seeding, the resulting effluent (methanogenic digestate) is dark-brown in colour with higher turbidity value around 18000FAU. Based on literature findings in which Mahmoud and Ghaly [180] observed very poor UVC transmittance ~0% for cheese whey (4317 NTU), this became a reason for discarding the post-biotreatment of methanogenic digestate using UVC radiation.

The sample of acidogenic digestate was characterized for *E. coli* and *S. senftenbergensis* levels through total bacteria count on both Endo agar and modified BGA. Turbidity which is among the limiting factor to UVC treatment was measured in formazin attenuation unit (FAU) using (Hach-lange DR-2800). Due to high turbidity, the sample was diluted in the ratio: 1:20, 1:50, and 1:100 (sample to distilled water) prior to reading. Similarly, sample pH, salinity and conductivity were analyzed.

6.13.2 Laboratory irradiation of UVC on contaminated digestate

In this investigation, a UVC low pressure mercury lamp (LightTech Lamp Technology, Ltd; Hungary with 660mm length, 15mm diameter, 75W) was used. In principle, the UV lamp converts electric energy into UVC light and emits biocidal light intensity of short wave at 254 nm which is a resonant band of mercury at vapor pressure of 1.4×10^{-6} to 1.4×10^{-3} bars producing monochromatic average radiation intensity of 0.02 mW/cm^2 at an average of 85% efficiency. UVC irradiation and the expected possible interaction between radiations

and bacterial cells within the petri-plate following the experimental set-up are shown in Figure 6.9. The irradiation was applied across the surface area of the petri-plates containing hydrolysate contaminated with both *E. coli* and *S. senftenbergensis*.

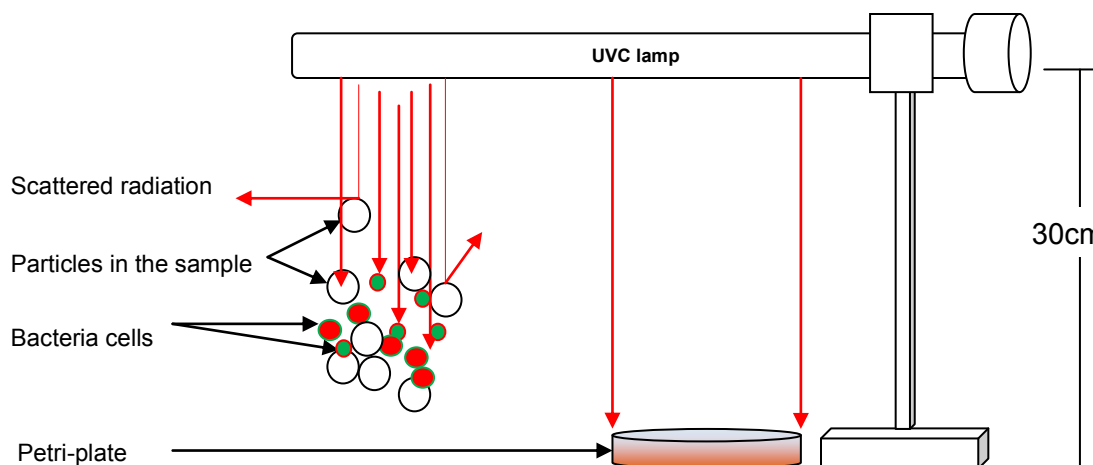


Figure 6.9: Illustration of Inactivation mechanism and set-up of UVC irradiation in turbid sample menstroom

In this investigation, the UVC lamp was mounted on an adjustable arm and installed in a class II safety cabinet of dimension 120 x 31.5 x 78cm and was held horizontally at an elevation of 30cm from the base of the cabinet.

6.13.3 Sample preparation and irradiation process

Sample for UVC irradiation (Picture 6-8) was prepared as described in Rice and Hoff [239] in which 25mL of process water (hydrolysate) from the hydrolysis reactor was firstly thoroughly agitated at 130rpm (Janke and Kunkel Ika labortechnik Ks 501 digital, Deutschland) for 30-60min to homogenize as well as cooling to room temperature. Thereafter the homogenized suspension was dispensed into sterile petri-plate of 90mm diameter covering a surface area of 141.37cm² making the sample thickness of approximate 3-4mm. The irradiation time was set based on the literature perusal including NESCS [232] in which the uncovered petri-plates were subjected to UVC radiations at sample residence time spread to cover 1hr of exposure in the following range of seconds:

60s, 120s, 180s, 300s, 480s, 600s, 900s, 1080s, 1200s, 1500s, 1800s, 2100s, 2400s, 2700s, 3000s, 3300s and 3600s. The fluence which is the product of the fluence rate (mW/cm^2) and exposure time (seconds) was therefore set for inactivation of both *E. coli* and *S. senftenbergensis*. The temperature in the irradiation cabinet on which the petri-plates were placed and exposed to the radiations was similar to room temperature $23\pm 2^\circ\text{C}$ adapting the routine use of the cabinet in the laboratory. From the specified UV lamp intensity, the dosage of irradiations for which the pathogens were subjected to were calculated as in the following equation (6.4) after Harris [237].

$$D = I (T) \quad (6.4)$$

Whereby:

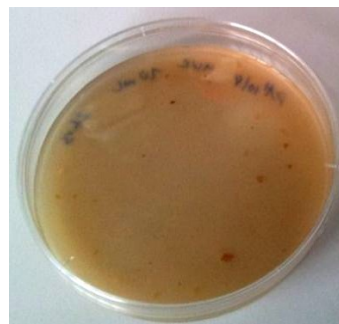
D = the dose expressed in mWs/cm^2 ,

I = the intensity of radiation expressed in mW/cm^2

T = exposure time, in seconds



Process water (Hydrolysate)



Hydrolysate ready for UV irradiation



Position of UVC lamp



UVC irradiation process

Picture 6-8: UVC treatment of enteropathogens contaminated hydrolysate

After reaching the specified exposure time, the UVC exposed samples were quickly transferred into test tubes for serial decimal dilution in which 0.5mL of the irradiated suspension was transferred into 4.5mL of sterilized water (*leitungs wasser*) followed by spread plating of 100µmL of the respective dilutions in culture media, Endo agar and modified BGA. The surface spread petri-plates were incubated at 36°C for 24hr after which the colony units of bacteria were counted and the performance reduction in bacteria calculated as colony forming units per gram (CFU/g) based on initial population counted (N_o) before irradiation and the final counts after UVC irradiation (N_t) that were converted into log scales for easy presentation. In order to model enteropathogens inactivation due to UVC exposure, the UV-Dvalue for both *E. coli* and *S. senftenbergensis* was determined as the negative inverse of the slope of best fit line for survival cells against the exposure time.

Pathogens inactivation rate was achieved in accordance with first-order kinetics model that described the survival enteropathogens to the UVC dose applied in accordance to Chick's Law:

$$\log\left(\frac{N_t}{N_o}\right) = -kD \quad (6.5)$$

Where by:

k = the rate constant,

N_t = the concentration of microorganisms after UVC treatment

N_o = is the initial population of microorganisms before UVC treatment

D = is the UVC dose used during treatment (mWs/cm²)

6.14 Results and Discussion

6.14.1 Efficiency of UVC at inactivation of enteropathogens

The physicochemical and microbiological attribute of acidogenic digestate (process water) from anaerobic digestion of contaminated potato peels collected on day 26 of the hydrolysis process operating at organic loading rate of 1.0kgoTS/m³.day are presented in

Table 6-11. It was observed that the population of *E. coli* was low by 10-fold in magnitude compared to that of *S. senftenbergensis*.

Table 6-11: Characteristics of process water (hydrolysate) from mesophilic reactor

Parameter	Process water	Units
Temperature	37.0	°C
<i>S. senftenbergensis</i>	3.7×10^4	CFU/g
<i>E. coli</i>	2.0×10^3	CFU/g
Turbidity	2.4×10^3	FAU
Salinity	1.5	‰
Conductivity	3.1	mS/cm
pH	4.5	-

E. coli was highly affected by UVC radiation and showed high die-off rate right from small exposure fluence. The low initial population of *E. coli* may be due to low pH sensitivity of the *E. coli* (min pH 4.4) to that of *S. senftenbergensis* (min pH 3.8)[114] and also the ability of *S. senftenbergensis* to produce more cyclic fatty acid following acid-adaptation in the hydrolysis phase of anaerobic digestate causing a reduced membrane fluidity[166]. The UVC inactivation behavior for *E. coli* and *S. senftenbergensis* treated at the same UVC dose and exposure time were considerably similar in higher UVC fluence probably due to the fact that UVC radiation affected the DNA configuration of the cells at the surface where the radiant were able to penetrate. The \log_{10} inactivation results for *E. coli* and *S. senftenbergensis* following UVC irradiation is presented in Figure 6.10 below. The cells underneath could not receive the required doze due to ether light scattering, refraction, reflection or shading by the particles in the acidogenic digestate. These unaffected cells therefore managed to regenerate and replaced the inactivated cells since the exposure time of 60min used was higher enough to even allow two doubling session to both *E. coli* and *S. senftenbergensis*.

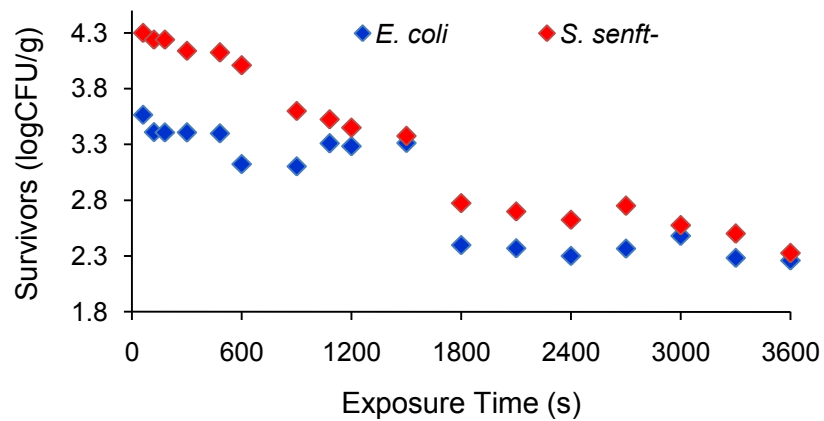


Figure 6.10: Logarithm inactivation of *E. coli* and *S. senftenbergensis* under UV radiation

Similar to Gayán et al., [235], the survival curves for both *E. coli* and *S. senftenbergensis* in this investigation comprised of a shoulder at the initial UVC treatment fluence followed by exponential die-off and ending with the tail on longer exposure time. Starting from the lower dosage, *E. coli* showed a high rate of die-off compared to *S. senftenbergensis* especially during the first 600 seconds of sample irradiation. The maximum inactivation rates were observed at UVC fluence between 10 and 35mWs/cm² as shown in Figure 6.11 following the sample exposure for about 600-2100s.

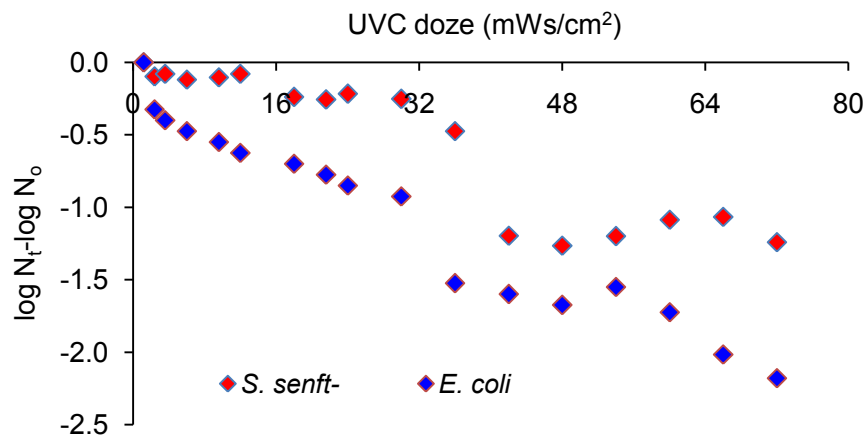


Figure 6.11: logCFU/g reduction of *E. coli* and *S. senftenbergensis* by UV light at varied exposure time

Most of the reported studies on UVC inactivation for *E. coli* for instance Gayán et al., [240] reported fluence of 7.60mWs/cm², Wong et al., [229] reported 12.0mWs/cm². While for *Salmonella spp*, Harris et al.,[237] observed that high doze was required to inactivate *S. faecalis* compared to *E. coli*. Coohill and Sagripanti [203] in the review reported 2.5-20.0mWs/cm² as the fluence required to achieve 4 log die-off in *Salmonella typhi*. Gayán et al., [235] reported 12.75J/mL for *S. enteritidis* and 18.03J/mL for *S. typhi*. Many other researchers including Faubert et al.,[177], Chang et al., [241] and Harris et al., [237] concur that the UVC lethal dose for pathogenic microorganisms including *E. coli* and *Salmonella spp* fall between 3.00mWs/cm² and 14.0mWs/cm² ranges which conform to the fluence observed in this research. American National Standards Institute and the National Sanitation Foundation specify 38mWs/cm² as the minimum fluence required to inactivate bacteria in water while Austria and Germany specify 40mW/cm² [177,231,242]. The high UVC fluence and longer exposure time required to render the enteropathogens inactive as observed in this investigation was a result of high turbidity in the sample. Guerrero-Beltrán and Barbosa-Cánovas [228] pointed out that the higher is the sample turbidity the greater is the absorption coefficient thus less light penetration in which sample agitation especially in the hydrogenesis stage could be the remedy due to the exposure of more bacterial cells to radiations through the produced turbulence prior to pumping of the hydrolysate into methanation reactor for methanogenesis.

6.14.2 Decimal reduction (UVC D-Values) of *S. senftenbergensis* and *E. coli*

Because of the diverse in fluence, estimation of the decimal reduction for die-off value to traverse one-log-cycle of the initial population, it was decided to use the survive curve at the shoulder until the exponential die-off phase which covers the fluence of 10-35mWs/cm² for determination of the UVC D-value in *E. coli* and *S. senftenbergensis* as the results showing in Figure 6.12. It was established that at the fluence of 10-35mWs/cm², the UVC D-value for inactivating *S. senftenbergensis* is about 24min while that for *E. coli* was about 17min. In simple words, in order to realize 90% reduction in *S. senftenbergensis* and *E. coli* about 28.8 and 20.4 UVC doze need to be absorbed by each of the enteropathogen cells respectively. It has been established that 30mWs/cm² is the lethal doze (LD99.9) for microbial disinfection in water while 40mWs/cm² produces a LD90

for microbial disinfection of highly turbidity water such as sewage water, water for domestic use and process water¹⁰, this minimum doze agree to the fluence obtained in this research for enteropathogens inactivation in highly turbid samples.

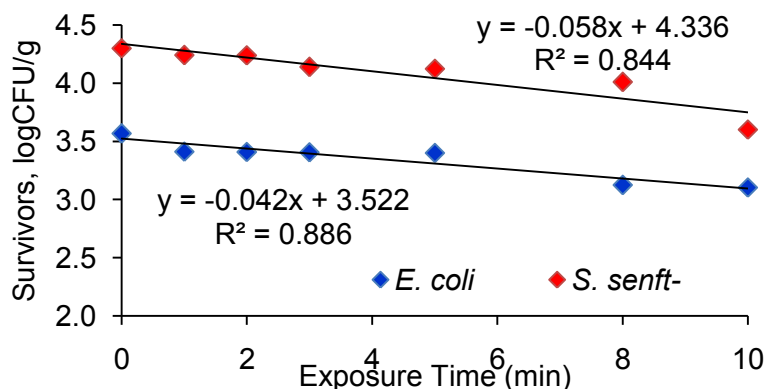


Figure 6.12: Decimal reduction (D-value) for *E. coli* and *S. senftenbergensis* after UVC irradiation

Although the UVC lamp may stay lit for many years, its germicidal effectiveness tends to diminish after 18 months of operation and even more diminished efficiency up to 85% during operation at prolonged time and therefore it is advisable to replace the UVC lamps prior to the attenuation in intensity due to longer service for effective results. In this investigation it was further observed that long time of sample exposure to the UVC radiation did not result into much effect on the inactivation rate but rather there was a slight increase in bacteria population observed indicating that the underneath bacteria cells where the beam of UVC light did not reach due to a blockage penetration, there was a possibility of cells regeneration and therefore caused an increase in the cell population replacing the inactivated ones. This concludes that UVC is not effective for treatment of more turbid suspension containing colour and other soluble organic matter as they tend to reduce hygienization efficacy. Guerrero-Beltrán and Barbosa-Cánovas [228] pointed out that the thickness for penetration of UVC light for the absorption of 90% in juice is 1mm. Achieving such small thickness layer may be impractical but with the use of turbulence as also recommended in Matak et al., [243] and Mahmoud and Galy [180] tends to increase

¹⁰ <http://www.purion.eu/areas/water/themes/sewage-water/>. Retrieved July 16, 2015

the die-off levels. For the higher turbid samples like process water (hydrolysate) used in this experiment, bacteria cells floating on top are the ones exposed to the UVC beam of light and are the ones easily to be inactivated. That is why higher bacteria inactivation rates were observed within the first 600s of sample exposure.

6.15 Passive solar heating

The use of solar energy in agriculture produce and food preservation is an advanced technology that existed and used for so many years [244–247]. Equally for decades now solar has been used in wastewater and sludge treatment process where the solar energy is used to dewater biosolids and in so doing eliminates pathogenic microorganisms in sewage sludge [204,248,249] for safer land application of the biosolids. Endowed with small investment capital (mainly do it yourself construction), low running costs, uncomplicated technical know-how, and independence from fuel supplies [244] as the most important raw materials for solar drying are solar radiation and air currents which are readily available everywhere in normal environment, passive solar heating (PSH) systems also conventionally known as natural-circulation solar drying bed [250] which includes solar tunnel dryer, stands to be commercially viable and ease to use method especially in tropical climates where sun shine is not a problem. Heat application on organic material removes moisture through evaporation which together with causes materials to dry and in tandem effect die-off to potentially infectious microorganisms present on the material being dried through the heat generated thereof and the created moisture stress all together leading to total hygienization[204].

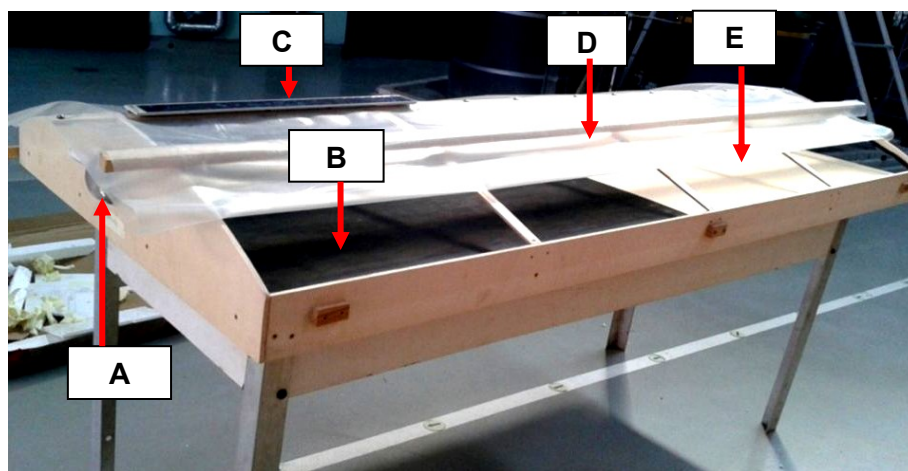
In this procedure normally temperature of about 80°C and above is applied to evaporate water content of the material from its state to less than 10%w/w causing microbial inactivation and assures complete hygienization unless the handling process allows for materials recontamination. In this thesis, the feasibility of using solar energy at effecting both pre-biotreatment hygienization of enteropathogens contaminated biowaste for safe use as substrate/feedstock as well post-biotreatment hygienization of byproducts from biotreatment (compost). In this experiment, passive solar drying and therefore hygienization of raw cow manure slurry was equally carried out aiming at eliminating

microorganisms that would be spread in case of application of untreated manure and thus endanger human health and the environment.

6.16 Materials and Methods

6.16.1 Features and assembly of solar tunnel dryer

This experiment was carried out during summer period (June – August, 2015) using the model of solar tunnel dryer of the type “Hohenheim”. Solar heating was carried out in a solar drying tunnel size 2mx1mx0.06m (length, width and height). Picture 6-9 describes the equipment. The proper functioning of the solar tunnel is a function of several components that include the black painted half base also called “the collector”, un-painted half base, plastic roof, solar panel, photovoltaic driven ventilator with protection from insect and dusts from the surroundings.



Key: A = Ventilator; B= Sunlight collector; C = Solar panel
D = Transparent foil roof; E = Unpainted base and drying base

Picture 6-9: Structure and working areas for solar tunnel dryer

6.16.2 Description of solar tunnel drier

The dryer is made of flat wood half painted in black colour used as collector of solar incident radiation while the other unpainted half is used as a drying point. On top of the

drier is the solar panel embedded for power generation making the drying process independent, energy self-sufficient and therefore environmentally friendly. The solar panel provides photovoltaic power which operates a photovoltaic driven ventilator so as to blow heated air from the collector to the materials to be dried. To maximize collection of solar radiation and facilitate loading and discharging of the material to be dried, the solar drying tunnel was positioned at an open space at 1.0m above the ground.

6.16.3 Solar drying process

Figure 6.13 describes the solar drying process. Depending on the sunlight strength on particular sample drying dates, the photovoltaic cell produces more power to run the ventilator (solar driven ventilator) and therefore making not possible for the formation of condensate on top of drying materials.

The sunlight passes through transparent plastic roof which also help to insulate the high temperature generated inside the solar tunnel from not escaping, resist UV radiation as well as protecting the material inside from dusts, insects and other falling contaminants.

The other end of the solar tunnel is mounted with wire meshed gable (mosquito net size) that prohibits insects while at the ventilator; the kitchen strainer is mounted for the same purpose.

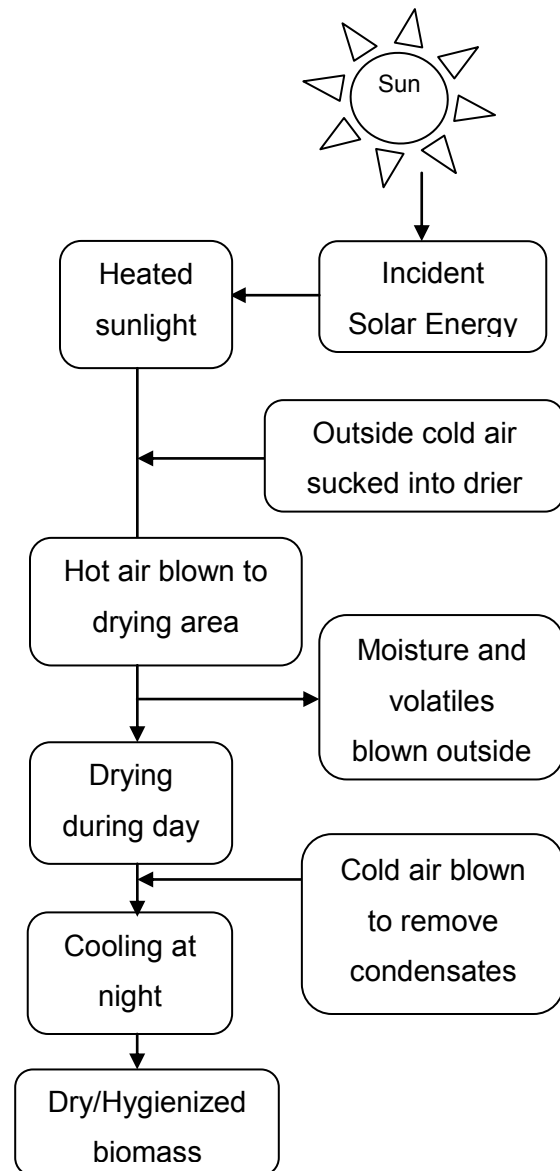


Figure 6.13: Layout of solar drying process

6.16.4 Working mechanism of solar drying tunnel

The heat generated on the collector area is blown by the ventilator placed to one end of the drier to the materials placed on the other unpainted half base to effect the drying process. Heat generated during the active sun shine is blown by the solar operated ventilator to the materials to be dried. Moisture evaporation from the material is a function of volumetric flow rate of inlet hot air that blows on top of the materials causing seasoning (Figure 6.14).

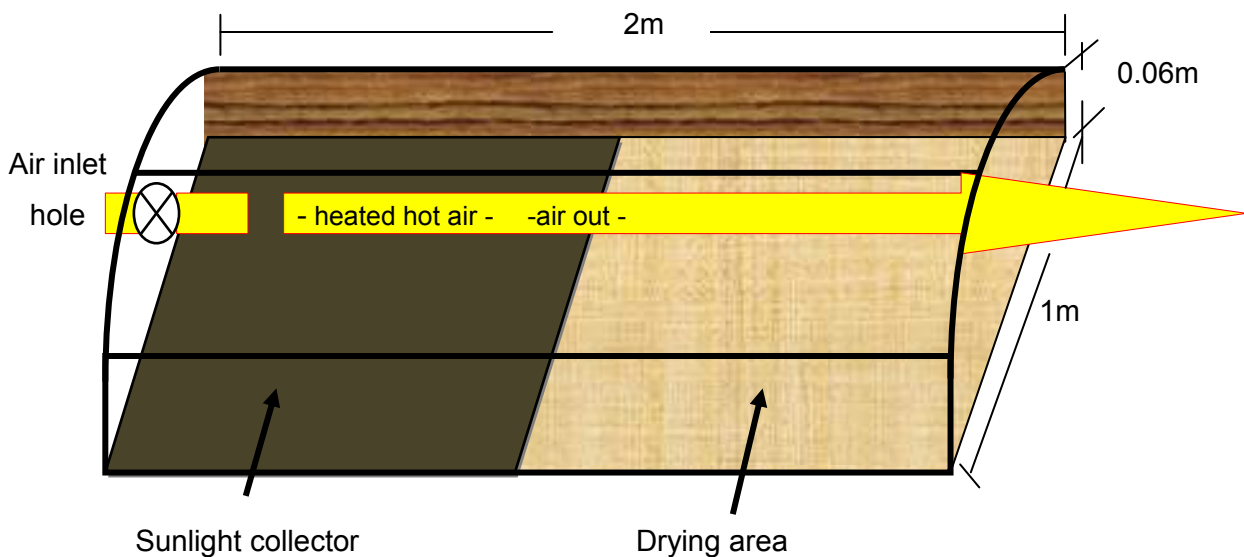


Figure 6.14: Working mechanism of solar drying tunnel

Air velocity entering the dryer and that leaving the dryer are to be calculated so as no pressure drop occurs in the drying chamber. The choice of ventilator therefore depends on the desired volumetric flow rate which is determined based on the size of the collector. Using the relation that $1\text{m}^3/\text{h} = 2.8 \times 10^{-4}\text{m}^3/\text{s}$ volumetric flow rate, the specified dryer base area used in this study was 2m^2 ; with this size, the recommended ideal air velocity at the dryer outlet is $V = 0.2\text{m/s}$ (specified by the manufacturer). The cross section area of wire meshed gable for keeping pressure constant in the dryer during both active sun-shine and at night and/or cool days was therefore calculated using the follows formula:

$$Area(m^2) = (a \times h) + \frac{(H-h)}{6a} [3(H-h)^2 + 4a^2] \quad (6.6)$$

6.16.5 Sample preparation for solar heating process

Hygienization of three samples consisting of artificially contaminated potato peels, unhygienized compost from aerobic composting experiment (one part of this research) and cow manure slurry that was used for seeding methanation reactor and contaminated with microorganisms including the identified *E. coli* (see Picture 6-10, Picture 6-11 and Picture 6-12 below) were studied under solar drying tunnel.

Sample 1:

Picture 6-10 presents blended potato peels at 10-12mm having moisture content of $77.3 \pm 0.6\%w/w$ that was used during solar drying experiment. The sample was artificially spiked with pathogens at a population of $7.4 \log E. coli$ (2.3×10^7 CFU/g) and $8.1 \log S. senftenbergensis$ (1.3×10^8 CFU/g) respectively.



Picture 6-10: Blended potato peels spiked with enteropathogens

Sample 2:

Picture 6-11 presents unhygienized compost from In-vessel composting of potato peels waste experiment that lasted for 16 days. The compost was analyzed for *E. coli* and *S. senftenbergensis* contamination and found to contain $5.2 \log E. coli$ (1.5×10^5 CFU/g) and $6.5 \log S. senftenbergensis$ (2.8×10^6 CFU/g) thus making a post-biotreatment hygienization process inevitable in order to eliminate the enteropathogens for safe land application.



Picture 6-11: Unhygienized compost from in-vessel composting

Sample 3:

Picture 6-12 presents a sample of Cow manure slurry that was used as seed for methanation reactor during anaerobic digestion. The slurry composed of 91.9% moisture, pH 7.6, and 8.1‰ salinity, Total enteropathogens population count on Endo Agar confirmed high *E. coli* contamination at 1.6×10^3 CFU/g (3.2log). Prior to solar drying process, the slurry was further spiked with pre-enrichment enteropathogens to a population of 6.1log *E. coli* (1.3×10^6 CFU/g) and 6.4log *S. Senftenbergensis* (2.4×10^6 CFU/g).



Picture 6-12: Enteropathogens contaminated cow manure slurry

6.16.6 Temperature and Moisture monitoring

Temperature and sample moisture reduction were the two parameters monitored during solar drying experiments. The sensor probes for temperature reading were calibrated using LAUDA eco Re.104 thermostat for a confidence temperature interval of $25.0 \pm 0.4^\circ\text{C}$. Seven sensor knobs each 30cm long were used for automatic temperature recording at the interval of 15min. The data were automatically recorded in computer using HP VEE-Lysi ME 300-4-2015 hp VEE computer programme. Six out of the seven calibrated knobs were directly inserted inside the solar drying tunnel while the other two were for external temperature monitoring (room and the outside temperatures) for process control. To avoid bacterial contamination on the heating bed, the samples were placed in trays with a sample volume ratio of 1:3:6 (Height: Width: Length). In all experiments the sample height was maintained at 5-10cm. Temperature attained by sample was measured by two sensor probes in which one sensor was inserted inside the sample to monitor the in-sample temperature and the other sensor was placed at the top of the sample holding container to record the up-sample temperature (temperature blown by the ventilator from the collector). Oven drier at 105°C (Haraeus 230V 50/60 Hz class II, Germany) was used for sample moisture determination according to *Bundergutgemeinschaft Kompost* of

1994 described in Burkhardt, [100] while total bacteria count technique was used for monitoring enteropathogens die-off.

6.16.7 Monitoring Pathogens inactivation after solar treatment

Both Endo agar and modified BGA were used for culturing and enumeration of bacteria colony units. As described in both Regulation (EC) No. 208/2006 and DG ENV.C2/BZ/tb [117], about 25g of solar dried contaminated potato peels, cow manure slurry or unhygienized compost was drawn and monitored for enteropathogens existence and survival after sample treatment. Sample portions drawn from the solar drying tunnel were suspended in sterilized water (*Leitungswasser*) in the ratio of 1g sample: 10mL water. The mixture was gently agitated at room temperature $23\pm 2^{\circ}\text{C}$ at 130rpm (Janke and Kunkel Ika labortechnik Ks 501 digital, Deutschland) for 15min which is just below both *E. coli* and *S. senftenbergensis* doubling time [251]. The resulting slurry was serially decimal diluted 10^{-1} to 10^{-6} and a volume equal to 100 μL of the appropriate dilutions made was surface spread on two petri-plates one with Endo Agar and the other with modified BGA.

Viable bacterial colony enumerations and visual identification on the plate was done after incubation (Incubator Model 600, Memmert) of the petri-plates at 36°C for 24 hrs. The number of bacteria colony units enumerated was expressed as colony forming unit per gram (CFU/g). The original sample was also plated for assurance of complete inactivation of the pathogens. Verification of mould growth in the samples of solar treated potato peels was also conducted. In this experiment, a three drying-wetting cycles were conducted in which a sample of solar dried peels was rewetting at approximately 30-50% moisture, maintaining in dark closed vessel at room temperature ($18-23^{\circ}\text{C}$) for 24h, before drying in an open vessel for 14 days [252].

6.17 Results and Discussion

6.17.1 Temperature development and monitoring

The derived collector area for the solar drying tunnel had 2.0m^2 and the total cross sectional area of the gable was 0.148m^2 giving the required ventilation of 0.14m^2 that blow

hot air used at hygienization of enteropathogens contaminated samples. Since the UV radiations from the sun estimated to be 550-1075W/m² [253,254] are resisted by the transparent plastic roof, therefore pathogens reduction or die-off in solar drying tunnel is mainly caused by reduction in moisture and temperature. Reduction in moisture content causes an increase in dry matter and mineral concentration which lower water activity a parameter that support bacteria cells. For instance, *Salmonella spp* optimally grow at A_w 0.93 or higher [207] and *E. coli* requires A_w >0.950 [170,206]. Temperature higher above the pathogen cells survival ranges normally reported from 55°C for *E. coli* [121] and above 65°C for *S. senftenbergensis* [196] denature cell protein thus causing cell die-off.

6.17.2 Pre-hygenization of contaminated potato peels waste

The selected physicochemical and microbiological attributes of potato peels (simulated biowaste) and the monitoring results for temperature rise during pre-hygenization of potato peels waste are shown in Table 6-12 and Figure 6.15 respectively.

Table 6-12: Selected physicochemical and microbiological level of simulated biowaste

Parameter	Results	Unit
Moisture	77.3±0.6	% w/w
Temperature	26.0±0.5	°C
<i>E. coli</i>	2.3x10 ⁷	CFU/g
<i>S. senftenbergensis</i>	1.3x10 ⁸	CFU/g

Drying process and thus pathogens inactivation in potato peels waste took a total of 16 days. Within these days of drying, the maximum recorded temperature inside the solar drying tunnel was 79.1°C on days with good sunshine while the minimum was 44.3°C in cloudy and foggy days because of poor sunshine. The average maximum temperature from collecting zone that effected drying and bacteria die-off therefore was 68.3°C while the average temperature inside the potato peels was 56.7°C.

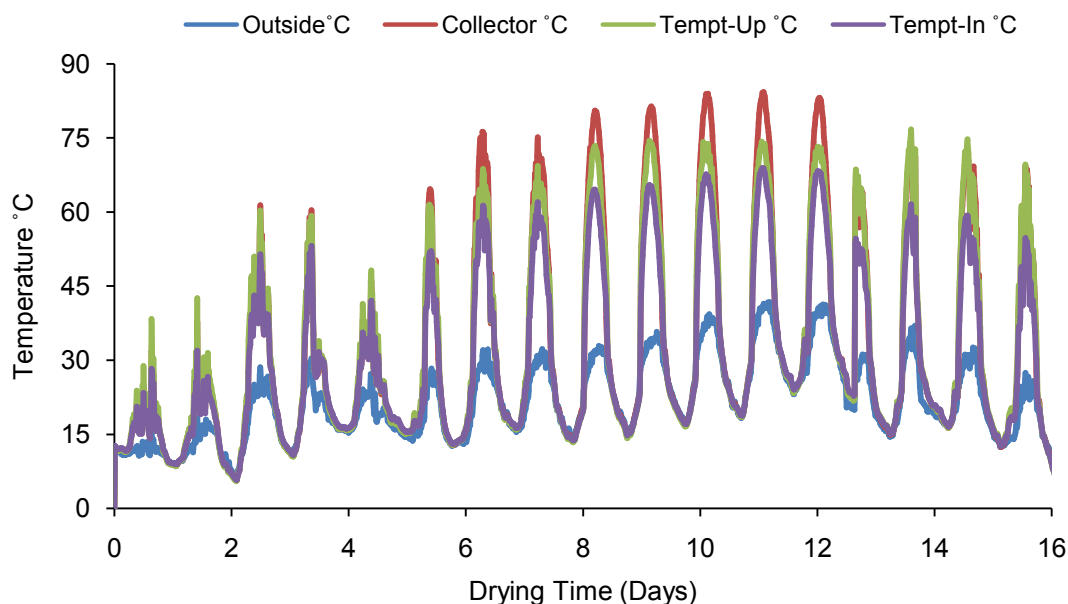


Figure 6.15: Temperature-Time patterns of passive solar heating of contaminated potato peels waste

The observed pathogen inactivation time during drying of the potato peels waste conforms to the time suggested in the Federal Biosolids Technical Regulations which recommends 55°C for 15 days. Denmark, The Netherlands specification and Germany biowaste Ordinance for compost pathogens equally recommend temperature-time combination of 55°C for 2 week to be sufficient at achieving complete pathogens inactivation [95,120].

6.17.3 Hygienization efficiency of passive solar heating

The pathogens die-off had similar patterns suggesting that moisture reduction and temperature were both affecting the pathogens in the similar way (see Figure 6.16). Bacteria like other living organisms need water as a media for transporting nutrients into their cells. In the first 3 and 4 days of solar heating, the population of *S. senftenbergensis* in the sample was surprisingly reduced at a higher rate than that of *E. coli* regardless of the proven heat resistance and the initial population of *S. senftenbergensis* spiked in the sample. This might be attributed to the presence of inhibitors such as protocatechuic acids, gallic, caffeine and chlorogenic which are bacteriostatic and bacteriocidal exhibited

in potato peels as earlier on extracted and reported in Schieber and Saldaña [107] and Al-Weshahy and Rao [106]. In the second week the generated heat during drying process induced stress to the microbial cells thus probably increased heat resistance especially to *S. senftenbergensis*. Also the reduced moisture content of the sample increased the mortality rate of the enteric pathogens as water requirement for their survive is estimated at 15% [204].

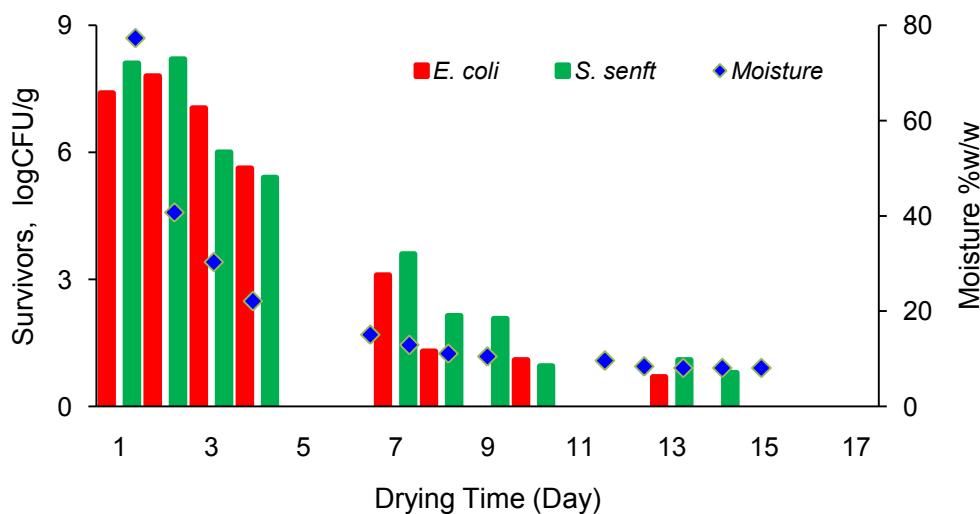


Figure 6.16: Pathogens inactivation from potato peels using solar drying tunnel

The average heating temperature in the solar drying tunnel was 56.7°C. This temperature corresponds well to the one recommended in other studies including Wichuk and McCartney[20] and Christensen et al., [98] as being effective at producing complete inactivation of pathogens in the final products. This temperature range managed to reduce moisture content in potato peels from its initial amount of 77.3% w/w down to 8.1% w/w. This efficiency of moisture reduction to <10%w/w in PSH has been equally reported in other studies by Mohanraj and Chandrasekar [255]. In their study they observed that the rate of moisture reduction from chill was from 72% to 9.1%. Similarly, Sarsavadia [256] reduced moisture content from 86% to 7% in onion using a solar-assisted forced convection dryer.

The drying time taken for inactivation of pathogen in this study compares well with the results reported in other investigations using different methods and matrix. For instance Déportes et al., [19] in their study, it took about 8-13 days to eliminate *Salmonella spp* at temperature more than 55°C. In our case, *S. Senftenbergensis* being the most heat resistant, definitely more time was required to ensure its complete inactivation given the changing weather conditions in the month of May 2015 which is the late spring period of the year. On the eighth-day of continual solar heating the moisture content in the sample dropped from 77.3%w/w to 12.9%w/w in which there was a maximum reduction in the enterobacteria. At this sample moisture content there was only 1.3log *E. coli* and 3.6log *S. senftenbergensis* demonstrating >6.0log reductions (99.999%) in both *E. coli* and *S. senftenbergensis*. But since Regulation (EC) No. 208/2006 and DG ENV.C2/BZ/tb [117] require a complete elimination of *Salmonella spp* in 25g of test sample; it was therefore of the essence to further investigating the lower dilutions including the undiluted original sample. However, there was no growth of either *E. coli* or *S. senftenbergensis* in solar dried potato peels waste in the sample tested on day 15, thus confirming the end of solar treatment with only 8.1% moisture remaining in the sample.

6.17.4 Decimal reduction (D-value) of enteropathogens following solar drying

Figure 6.17 shows the D-values at 28-69°C of enteropathogens following passive solar heating process. In this investigation, the decimal reduction time for achieving one-log cycle reduction of *E. coli* during solar drying of contaminated potato peels waste was found to be 1.92 days while that of *S. senftenbergensis* was determined to be 1.94 days. The D-values seem similar probably because two factors i.e. water stress to bacteria cells and the heat as stated by Chen et al., [125] simultaneously affected the bacteria cells and effected their die-off.

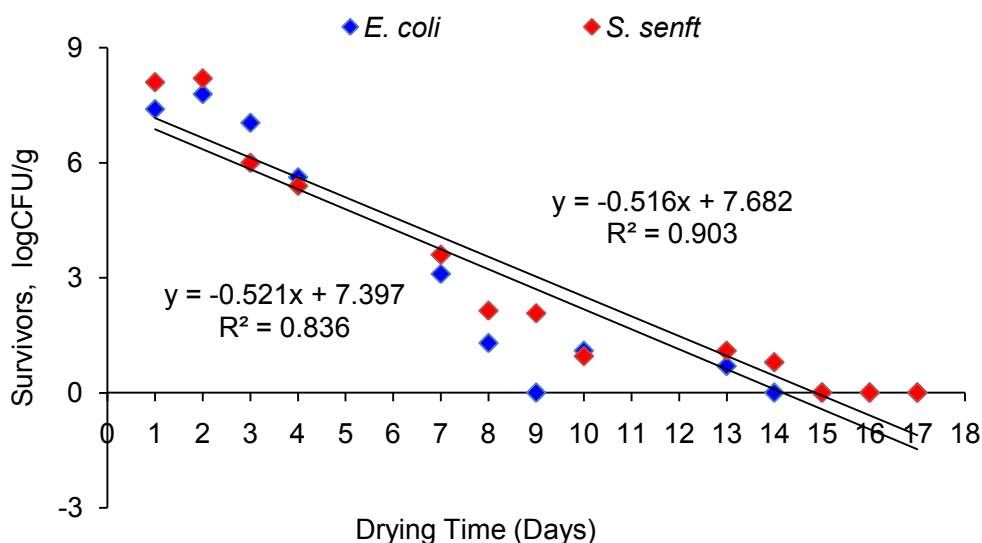



Figure 6.17: D-value of enteropathogens during solar drying

Since *E. coli* is not particularly heat-resistant when compared to *S. senftenbergensis*, the similar D-values obtained suggest that the pathogens die-off during passive solar heating does not depend on heating only but rather on other factors including water reduction. It is reported that reduction in water leads to an increase in mineral salts content in the sample. Yeager and Ward [204] confirm that reducing moisture content of the sample increases mortality rate of the enteric pathogens as water requirement for their survival is estimated at 15%.

6.17.5 Post-treatment attributes of biowaste

The physical, chemical and microbiological status of the solar dried potato peels waste used in this experiment are shown in Table 6-13 below. Except for physical appearance and moisture content of the solar-dried potato peels waste, solar drying did not affect the quality of the peels. Moisture content was reduced for nearly 10-folds thus causing about 4-folds elevation in the sample total solids.

Table 6-13: Characteristics of solar-dried potato peels

	Parameter	Before drying	After drying
	Moisture, %w/w	77.3	8.1
	pH	5.6	6.3*
	C/N ratio	18:1	23:1
	TS, %	22.7	91.9
	oTS (%TS) %	94.7	94.3
	Salinity, ‰	na	2.8*
	Conductivity, mS/cm	na	5.3*
	<i>E. coli</i> (CFU/g)	7.4log	< LoQ
	<i>S. senft-</i> (CFU/g)	8.1log	< LoQ
	Mould growth	na	Absence

* Values determined after dispensing solar-dried peels to water, 1g: 4mL

Upon water reconstitution 1:4 (solar-dried peels to water) as recommended in Deublein and Steinhauser [59], the peels showed improvement in pH 6.3, Salinity 2.8‰ and electrical conductivity of 5.3mS/cm. It is worth noting that, the solar drying of peels improved C/N ratio from the original value of 18-20:1 to 23:1. The observed units increase in the C/N ratio definitely comply to the loss of moisture together with some volatile compounds of nitrogen including ammonium–nitrogen ($\text{NH}_4^+\text{-N}$) and nitrate (NO_3^-) following high temperature heating days similar to the findings reported in O’Shaughnessy et al., [249]. The presence of pathogens at below detection limits conforming to Regulation (EC) No. 208/2006 signifying biosafety to biotreatment workers in both composting and anaerobic digestion plants whose daily works involve coming in contact with contaminated biowaste and also predestined safety of the compost and digestate that is to be produced from treated biowaste using passive solar heating.

6.17.6 Post-hygienization of unhygienized compost from In-vessel composting system

The physicochemical and microbiological levels in contaminated compost used in this experiment are given in Table 6-14 below with the corresponding results for temperature dynamics during post-hygienization the compost shown in Figure 6.18. The sample of unhygienized compost resulted from one of the investigation in this research following the In-vessel composting of potato peels.

Table 6-14: Selected physicochemical and microbiological level of contaminated compost

Parameter	Results	Unit
Moisture	67.9±1.3	% w/w
Temperature	28.0±0.5	°C
<i>E. coli</i>	1.5x10 ⁵	CFU/g
<i>S. senftenbergensis</i>	2.8x10 ⁶	CFU/g

The characteristics of compost presented moisture and temperature quite conducive for microbial survive and growth. As there was no addition of the pathogens, the survived *E. coli* and *S. senftenbergensis* population from the In-vessel composting experiment was used as initial pathogens population to be eliminated. The results indicated high proportion of *S. senftenbergensis* in the compost compared to the population of *E. coli* showing that the survive rate of *S. senftenbergensis* was much higher compared to that of *E. coli* during composting process since the lethal temperature dose to effect pathogens die-off during the process was not properly generated. The observed reduction in both *E. coli* and *S. senftenbergensis* could be attributed by the competition with the indigenous micro-flora in the composting vessels as suggested in Usda [257] and the inhibition caused by the observed anaerobic decomposition of the feedstock caused by high moisture content in the potato peels.

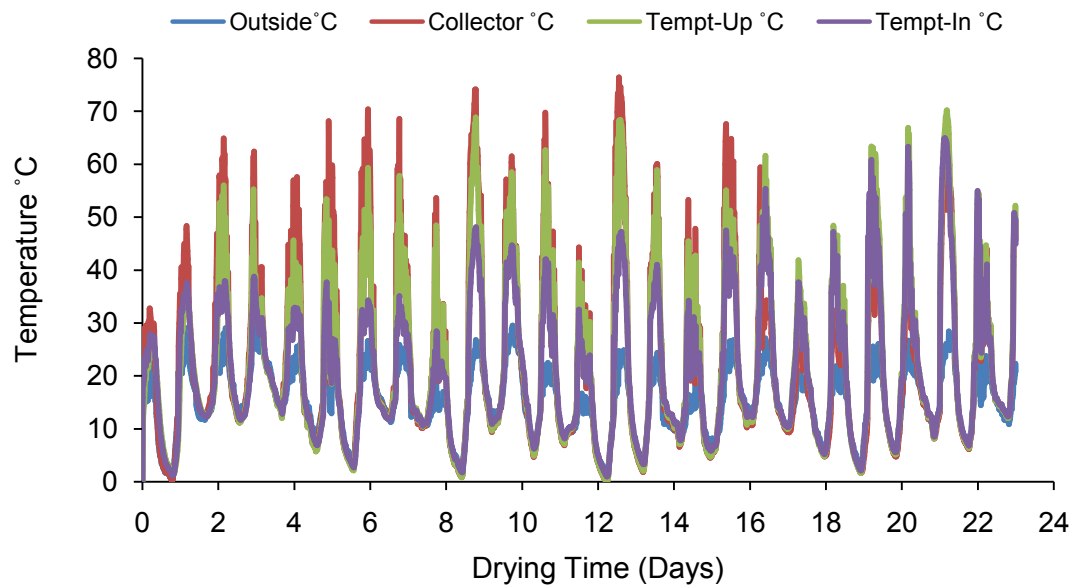


Figure 6.18: Passive solar heating of unhygienized compost

The contaminated compost was exposed to solar drying for 24 days. Within these days, the maximum recorded temperature in the solar drying tunnel was 76.3°C on days with good sunshine while the minimum temperature was 34.8°C in hazy days during mid and late spring period in the month of April and May 2015. The temperature rise caused sample dehydration as a result of evaporation. The inactivation process in unhygienized compost took longer time than the time taken to effect pathogens inactivation in potato peels waste. This could be attributed by the following reasons: firstly it could be that the generated heat during composting process might have induced stress to the microbial cells which then developed heat resistance. Also the reduced moisture and low pH in the composting vessel which operated at minimum oxygen supply and therefore created anaerobic condition is supposed to increase the heat resistance of the enteropathogens due to increased levels of unsaturated fatty acid per saturated fatty acids which consequently increase the level of cyclic fatty acid responsible for protecting the cell as earlier explained in Álvarez-Ordóñez [166]. Yeager and Ward [204] urge that, even a small variation of moisture content in the compost could be of great influence of the survive rates of the bacterial pathogens by causing increasing in heat resistance. The fact is that, moisture facilitates thermal breaking of the cell protein by enhancing easily

breaking of peptide bonds than when heating the cells on dry air. Ceustermans et al., [71] observed an increase in survive rate of *S. senftenbergensis* by 0.5log following 5% reduction in moisture of the composting feedstock.

6.17.7 Hygienization efficiency of PSH on contaminated compost

Figure 6.19 shows results for enteropathogens die-off during PSH. In the first week of drying at an average temperature of 56°C there was a reduction in moisture content to 46.7%. This moisture loss did not affect initial level of *E. coli* although an infinitesimal reduction 0.6log was observed in *S. senftenbergensis*. In the second week that was preceded by turning of the compost, a substantial drying was obtained that caused a moisture drop to 22.8% w/w.

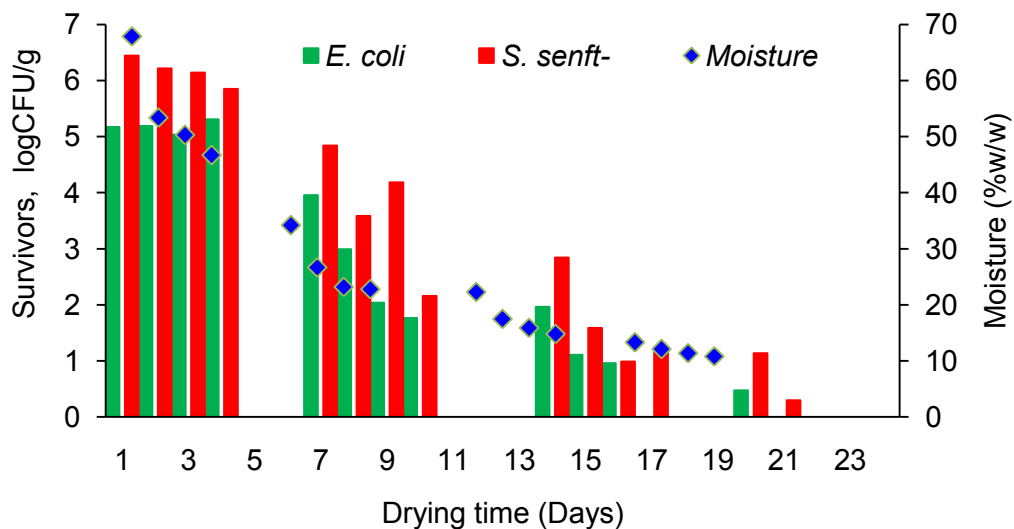


Figure 6.19: Pathogens inactivation from contaminated compost using passive solar heating

The temperature generated and this moisture drop induced pathogens die-off in which *E. coli* was reduced by 2.2log (99.4%) whereby there was equally substantial reduction in *S. senftenbergensis* by 2.7log (99.8%) in the second week of PSH. The temperature drop on day 13 as result of changes in weather condition (rainfall) could have attributed to the observed re-growth of both pathogens in the following day in which there was 0.2log

(37.2%) increase in *E. coli* where as 0.6log (79.5%) increase was observed in *S. senftenbergensis*. The presence of occasional rainfall during the drying period equally affected drying process as there was no reduction in moisture which remained at 22.3%. The presence of good sunshine on day 14 brought about higher increase in temperature of about 76.3°C. This temperature equally caused a drop in moisture to 17.5% which at the end of week it was as low as 14.8% which is below the minimum moisture estimate in which most of enteric pathogens could survive [204]. On day 21 in which the moisture content was reduced to 12.1%, *E. coli* was completely inactivated and there was no sign of regeneration at prolonged drying. Few cells of *S. senftenbergensis* were still resistant at this small moisture as there were still 0.3log of the surviving cells detected in the compost. The few surviving cells of *S. senftenbergensis* were completely inactivated when the moisture of the compost reached 11.4% on the 22 day of PSH and there were no cell growth on the next day 23 in which the final moisture in the compost was analyzed and determined to be 10.8%.

Figure 6.20 shows the D-values calculated at the heating temperature of 28-67°C for enteropathogens after PSH of unhygienized compost. In this investigation, based on PSH, the decimal reduction time of *E. coli* was calculated and found to be 77.9hrs (3.3 days) while that of *S. senftenbergensis* was found to be 90.6hrs (3.8 days).

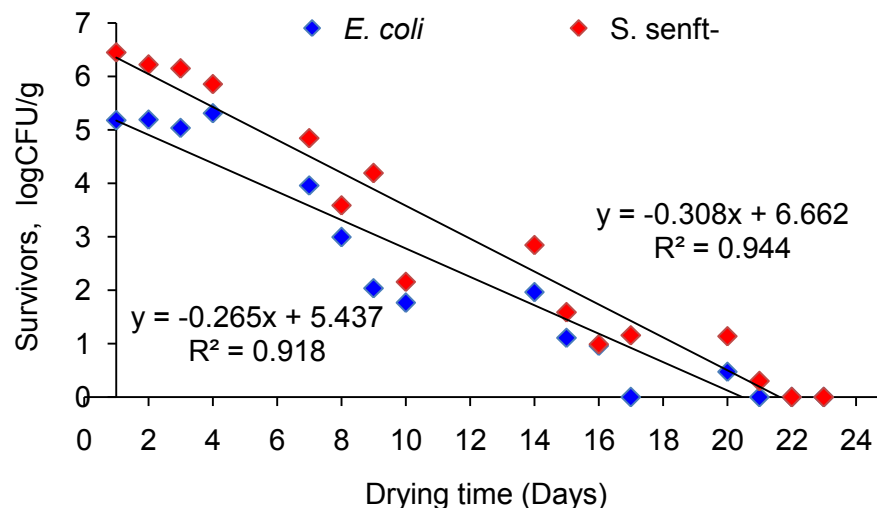



Figure 6.20: D-value of enteropathogens during solar drying of unhygienized compost

6.17.8 Post-treatment attributes of compost

Some of the selected physical, chemical and microbiological status of the post hygienized compost following drying in solar drying tunnel is presented in Table 6-15 below. Moisture content in the compost was reduced from its original for over 6-folds which caused water stress to the pathogens leading to a complete die-off in both *E. coli* and *S. senftenbergensis* that survived the composting process.

Table 6-15: Characteristics of solar dried compost

	Parameter	Before drying	After drying
	Moisture, %w/w	67.9	10.8
	pH	na	6.1*
	Salinity, ‰	na	2.8*
	Conductivity, mS/cm	na	5.3*
	<i>E. coli</i> (CFU/g)	5.2log	< LoQ
	<i>S. senft-</i> (CFU/g)	6.5log	< LoQ
	Mould growth	na	Absence

* Values determined after dispensing solar-dried compost to water, 1g: 10mL

The Spanish regulation APA/863/2008 as stated in Sánchez-Bascones et al., [112] on quality of compost specify 40% as the maximum moisture content in the final compost. High moisture in the compost make the product bulky and difficult to handle even though more dried compost can be dusty [125], therefore post handling quality of to the final compost including prevention from re-contamination is of paramount importance. Salinity and Electrical conductivity levels of less than 6 units in the final hygienized compost were within acceptable limits recommended as potting media amendment grade (compost for formulating growing media for potted crops) as specified in Rynk [224].

6.17.9 Hygienization of contaminated cow manure slurry

The temperature monitoring results for solar drying of contaminated cow manure slurry (CMS) that was used for seeding the methanation anaerobic digestion reactor used in previously reported investigation in this research is as shown in Figure 6.21. Prior to be used for seeding purpose, the cow manure slurry was analyzed and found to contain 1.6×10^3 CFU/g of *E. coli* the level which according to Regulation (EC) No. 208/2006 is above the acceptable contamination range for the manure in case is to be used as fertilizer on croplands fields without composting or hygienization.

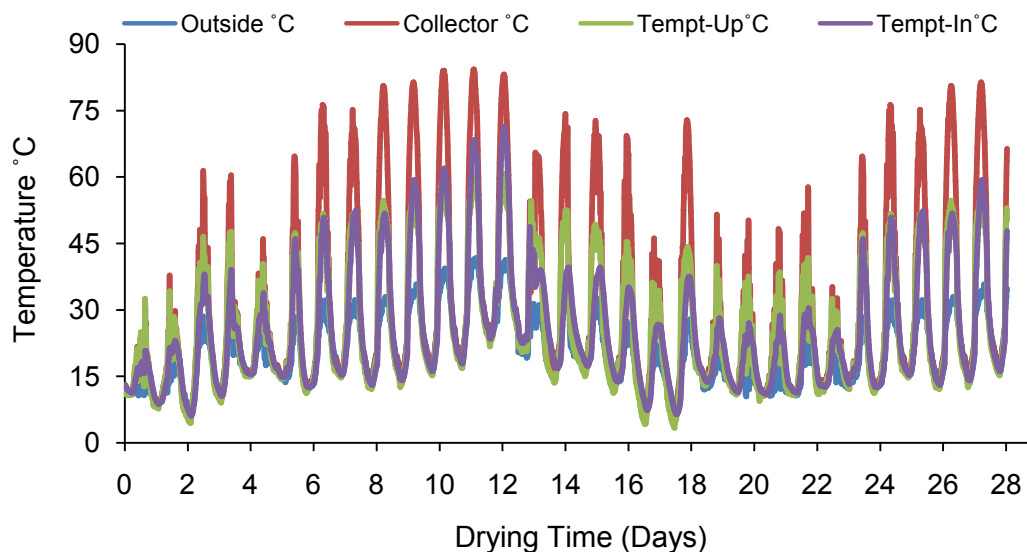


Figure 6.21: Passive solar heating of contaminated cow manure slurry

High moisture content determined to be 91.9% in the contaminated cow manure slurry plus the presence of mostly cloudy days on the month of May 2015 resulted into longer drying period summing to 28 days. During the days with strong good sunshine, the maximum temperature records in the solar tunnel dryer was 84.3°C while 34.1°C was the lowest temperature recorded. The temperature range inside the container with cow manure slurry was between 32.2°C and 58°C . The temperature high enough to cause inactivation of the bacterial cell was achieved only during the days with enough sunshine. In the incidences of most cloudy days, rainy days as well as during the night there was a

possibility for the partially destroyed bacterial cells and the cells that survived the heat during day time to recover and re-grow a phenomenon that brought about recurrent contamination of the partially hygienized cow manure slurry hence the observed prolonged hygienization period. Moisture reduction and die-off trend of *E. coli* and *S. senftenbergensis* in the cow manure slurry following solar drying is presented in Figure 6.22 below.

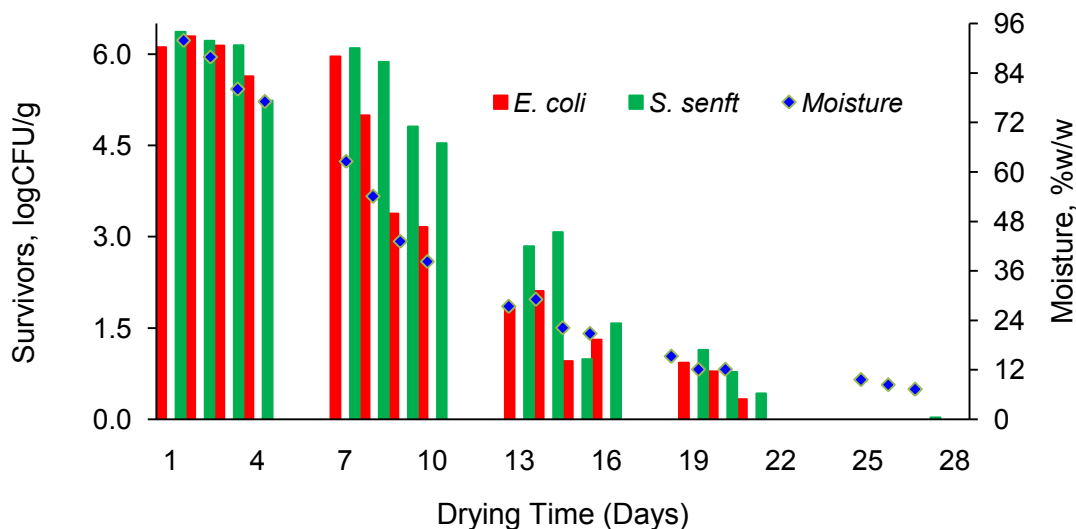


Figure 6.22: Pathogens inactivation in cow manure slurry using passive solar heating

At the beginning of drying period there was enough sunshine and the temperature recorded at the collector region of the solar drying tunnel on day 10 of the drying process was 84.3°C making both temperatures on top of the sample and inside the sample to heat almost in the similar magnitude i.e. 62.3°C and 62.1°C respectively. During this period, both *E. coli* and *S. senftenbergensis* were constantly reduced with *E. coli* which in this case the most heat sensitive seem to be the most affected regardless of the similar initial population levels. The moisture content in cow manure slurry during the first 13 days dropped to about 38.3% causing reduction in enteropathogens of 2.9log and 1.8log for *E. coli* and *S. senftenbergensis* in that order. During this period which is almost 2 weeks of drying process, there was a 99.9% reduction in *E. coli* and the population that was still in the sample already conformed to both Regulation (EC) No. 208/2006 and EC 2010


Working document: sludge and biowaste specifications for use on land application, but the level of *S. senftenbergensis* was still high 4.6logCFU/g making about 98.4% reduction and was supposed to be reduced to zero in 25g of sample to make the sample fully comply to the stated norms. That is why a prolonged sample drying time was inevitable.

From day 14, the weather changed and there was poor sunshine for about 7 days, this change in weather affected much the effective drying temperature in the sample which dropped to about 48°C and that on top of the sample dropped to 57.1°C. The sample moisture and pathogens inactivation during these days were in the same way affected. There was only 8.3% moisture removal from the sample while re-growth in pathogens were observed as there was an increase of 0.3logCFU/g (49.9%) *E. coli* while *S. senftenbergensis* increased by 0.2logCFU/g (36.9%). The minimum moisture of 15% for which enteropathogens could hardly survive according to Yeager and Ward [204] was reached on day 19 of the drying process, yet there was still 0.9logCFU/g of *E. coli* and 1.2logCFU/g of *S. senftenbergensis* in the sample of solar-dried cow manure slurry validating the observation in Ceustermans et al.,[71] that every 5% reduction in moisture of pathogens contaminated samples there is a corresponding increase in pathogens survival rate of about 0.5logCFU/g.

Immerging of good sunshine on day 20 of the drying process brought a sample moisture content to 12.1%, as a result, there was >5.0log reduction both in *E. coli* and *S. senftenbergensis* making almost 99.99% reduction. Microbiological analysis results on day 26 revealed sample moisture content of 7.3% and this low sample moisture together with the heat supplied were enough at resulting into *E. coli* and *S. senftenbergensis* free dried cow manure. On the following day one colony of *S. senftenbergensis* equivalent to 0.03logCFU in 25g of sample germinated, but on day 28 a complete elimination to both *E. coli* and *S. senftenbergensis* was confirmed. Upon completion of the hygienization experiment of the cow manure slurry, the pH, salinity and conductivity were as well determined following dispensing 20g of sample into 200mL of distilled water, and the results as shown in the same Table 6-16. With the final moisture content of 7.3%, the dried cow manure cake (otherwise known as patties) in India could alternatively be used as a source of energy for cooking with no threats of pathogens contamination and thus

complement the conventional sources, coal, charcoal and firewood massively used in developing countries by the rural poor [258].

Table 6-16: Characteristics of solar-dried cow manure slurry

	Parameter	Before drying	After drying
	Moisture, %w/w	91.9	7.3
	pH	7.6	8.1
	Salinity, ‰	8.1	6.3
	Conductivity, mS/cm	13.9	10.9
	TS, %	8.1	92.7
	<i>E. coli</i> (CFU/g)	6.1log	< LoQ
	<i>S. senft-</i> (CFU/g)	6.4log	< LoQ
	Mould growth	na	Observed

Dried cow manure had an alkaline pH acceptable for land application as specified by Cooperband [109]. Salinity and electrical conductivity levels of less than 20 units in the final hygienized cow manure were within acceptable limits recommended for soil amendment grade, improving agricultural soils and restoration of disturbed soil specified in Rynk [224]. Figure 6.23 shows the D-values calculated at the heating temperature of between 21-60°C for *E. coli* and *S. senftenbergensis* after passive solar heating of unhygienized compost. In this investigation, the decimal reduction time of *E. coli* was found to be 86.9hrs (3.6 days) while that of *S. senftenbergensis* was about 90.2hrs (3.8 days). Unstable weather condition and poor sunshine was a challenge in this experiment. More heat during this investigation was firstly required to evaporate water from the slurry and create moisture stress to the spiked enteropathogens thus resulted into more time of drying.

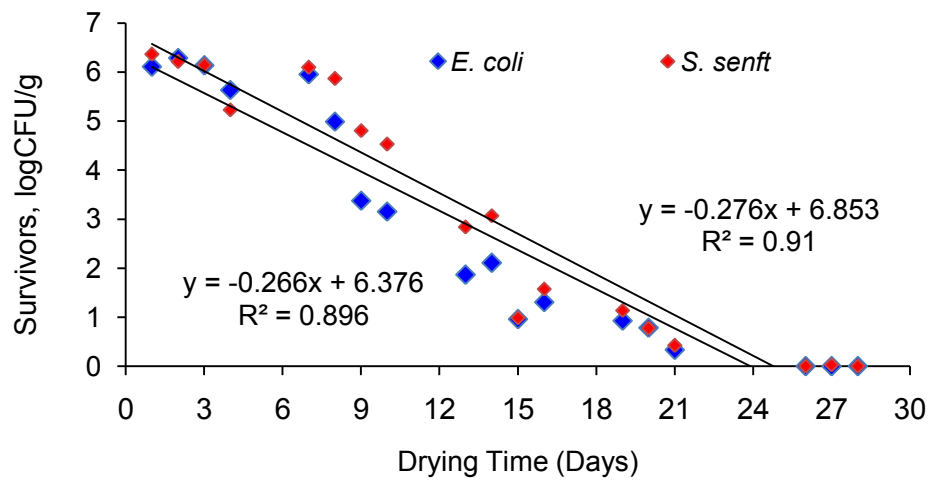


Figure 6.23: D-value of enteropathogens during solar drying of contaminated cow manure slurry

In this investigation it was further observed that two among the petri-plates spread with sample from day 28 of drying developed molds. These plates were further incubated for 3 more days, on the fourth day there was full grown white and black mould on the petri-plates. On the petri-plate with Endo agar three thallus of black mould germinated while on the other petri-plate containing modified BGA, one thalli of white mold emerged, see Picture 6-13 below.



Picture 6-13: Growth of fungi species of passive solar-dried cow manure slurry

The presence and survival of mold in both cow manure slurry and cow dung under reduced moisture and even after a mild temperature treatment has been reported by many researchers including McGranaghan et al., [252] and Fliegerova et al., [259] among others. Similar to what reported in Schnürer and Jarvis [164], formation of spores by mold under unfavorable condition makes it possible to survive even after heat or pasteurization process. This observation confirms that mold spore can survive under both water stressful of less than 10% moisture content and heat environment below 60°C with possibility to re-geminating when the growth condition turn favorable.

6.18 Conclusive summary

Safety of both the biowaste used as resource in the biotreatment process and consequently finished products which are directly applied to agricultural farms seems to have low consideration even to environmental management officials and policy makers and therefore depends solely on the discretion of the producers. To ensure safety during and after biotreatment under the existing heat energy constrains, introduction and use of alternative biowaste hygienization options has become a prudent decision provided the desired level of safety is achievable and confirmed through laboratory analytical procedures.

E. coli and *S. senftenbergensis* die-off take place in alkaline pH more than in acidic pH. Acetic acid treatment of enteropathogens contaminated biowaste at pH value 4 for 5days or lime treatment at pH value 11 to12 for 1day effectively caused a complete die-off in *E. coli* and *S. senftenbergensis*. The findings from these experiment reveal that there is a possibility of using acetic acid interchangeably with formic acid for the treatment of biomal and therefore escape the potential adverse health conditions effects including eyes irritation, sore throat, coughing, chest tightness and headache associated with exposure to toxic formic acid vapors. Pasteurization stands to be effective method for inactivation of microbiological pathogens during biotreatment process if applied at the right temperature and specified time. Temperature-time combination of 55°C for 300min, 65°C for 30min or 75°C for 3min are sufficient at effecting 1log cycle die-off of *S. senftenbergensis* with z-Value of 11°C. Partial low pH treatment of pathogens normally in the hydrolysis reactor

reduces the effectiveness of pasteurization due to induced heat resistance in acid-adapted bacteria. Although pasteurization time matters, this is the reason behind pathogens passing thermophilic anaerobic digestion reactor unaffected. Studies on efficacy of UVC radiation at elimination of *E. coli* and *S. senftenbergensis* though not very much convincing, yet the reduction of microbial at the fluence of 10-35mWs/cm², the UVC D-value for traversing 1 log cycle for *S. senftenbergensis* was about 24min while that for *E. coli* was about 17min. Absence of constant and mixing that could cause turbulence in suspension menstruum and expose the hidden cells behind particulate matter is considered the reason.

Moisture reduction below the range of 15%w/w which support enteropathogens growth, temperature above 55°C and exposure time were the factors behind efficacy of passive solar heating (PSH) at effecting pre-hygienization and post biotreatment hygienization of *E. coli* and *S. senftenbergensis* contaminated potato peels, unhygienized compost and cow manure slurry. The longer hygienization time taken while using PSH was attributed by the fluctuation in weather condition as the experiment commenced in Spring in which the sunshine is not that much reliable.

7 Potential of Potato Peels Waste for Co-composting and Biogasification

7.1 Co-composting of potato peels waste with yard-waste

High moisture content in potato peels disqualified its use as feedstock for aerobic composting process and resulted into observed low temperature-time patterns in the previous experiments and therefore poor and microbiological unsafe final compost. Surviving possibility of heat resistance pathogens such as protozoa and helminths [20], spore-forming pathogenic microorganisms, *Bacillus spp* and *Clostridium perfringes* [13,70] and many others in the final compost is of great concern as well as of public interest. In this case feasibility of undertake experiment on co-composting of potato peels waste with dry yard-waste was of the essence and for the following reasons:

- i. Adjusting moisture level in the peels even from the normal >77% to the acceptable composting moisture range of 45% to 60% w/w.
- ii. Reduce the amount of compost soil used as seeding during composting process
- iii. Improve composting conditions in the reactor vessel and hamper leachate formation.
- iv. Improve temperature-time patterns to as higher hygienization temperature ranges as possible for effecting pathogens die-off.

Singh et al., [260] recommended that together with feedstock size reduction and pH, C/N as well as type and population of composting micro-flora plays an important role in pathogens inactivation during composting process, in that case, as recommended elsewhere [261–264], co-composting of the peels with other dry waste portion having lower moisture content and that can supply more carbon was the best decision in optimizing the C/N ratio in the peels as well. The use of yard-waste in co-composting with potato peels aimed to improve the composting attributes in the feedstock [131]. The same In-vessel composting system used in the above other experiment was also used in this experiment. Feedstock moisture content being the critical parameter that governs both

micro-flora's activities and temperature dynamics during composting process was to be given its due priority after confirming that higher moisture contents in the potato peels was responsible with the poor hygienization results observed in the previous investigations.

7.2 Materials and Methods

7.2.1 Feedstock components for aerobic co-composting

The difference between this investigation and the one reported in previous experiments was that in this experiment yard-waste consisting of dried Australian grasses (*Poa labillardierei*) and autumn dried tree leaves from Linden (*Tilia cordata*) and Maple (*Acer griseum*) tree species was used. The fractions of yard-waste were collected in February, 2015 from the garden around Faculty 4, BTU Cottbus-Senftenberg and further dried under-shade for one month before starting the composting process. Readymade 10kg bag of inoculating compost (*ErdBlumen*) was bought from Netto Supermarket (Nordpark Str. 2 Cottbus, Germany) and used as composting starter. The individual co-composting components (potato peels, yard waste and soil compost) see Picture 7-1 were characterized for moisture content, pH, TS, Ash, organic matter and C/N ratio prior to mixing. The yard-waste was blended and added in the fresh blended potato peels along with seeding compost (starter). The mixture was spiked with *E. coli* 8.5log (3.2×10^8 CFU/g) and 8.9log *S. senftenbergensis* (7.9×10^8 CFU/g) to test the hygienization efficacy.



Fresh blended potato peels

Blended Yard-waste

Compost

Picture 7-1: Feedstock components used in co-composting process

7.2.2 Formulation of aerobic co-composting feedstock

The ratio of peels, yard-waste and seeding compost to be used for desired moisture were calculated using the equation (7.1) below suggested by Rynk [224]. The composting feedstock formulation was done in such a way that mixture of all components together makes 2.2kg of feedstock sample for each formulation.

$$Q_2 = \left(\frac{(Q_1 * G) + (Q_1 * M_1)}{M_2 * G} \right) * 100 \quad (7.1)$$

Whereby:

Q_2 = Mass of the Yard-waste to be used for co-composting process

Q_1 = Total mass of materials to be composted (kg)

G = Desired moisture goal (45-60%w/w)

M_1 = Specific moisture content of potato peels (77.9% w/w)

M_2 = Moisture content of the Yard-waste (12.9% w/w)

The result from the above equation was used as a guide towards estimating the number of feedstock samples to be produced (Table 7-1) for the co-composting experiment. Within the range of targeted moisture goal of 45-60%w/w, four samples mixtures were formulated in the order of decreasing potato peels waste and increasing yard-waste with the aim of optimizing the ratio in which the distasteful composting parameters (Moisture and C/N ratio) will no longer affect the process and thus achieve optimum bacteria activity in the composting vessels that will result in lethal temperature for maximum pathogens inactivation. The same amount of compost soil was added in each formulation assuming equivalent composting micro-flora in each composting vessel for running the decomposition process.

Table 7-1: Feedstock formulation for aerobic co-composting process

Feedstock	Peels (g)	Yard-waste (g)	Mixture (Ratio)	Compost starter (g)
Formulation 1	1500	500	3:1	200
Formulation 2	1600	400	4:1	200
Formulation 3	1700	300	6:1	200
Formulation 4	1800	200	9:1	200

7.2.3 Aerobic co-composting process

The four feedstock recipe formulated were each analyzed for physicochemical attributes. The selected physicochemical parameters analyzed include moisture, pH, TS and organic matter (OM). Thereafter, the feedstock were spiked with pathogens in the ratio of 100:1 (feedstock to pathogens suspension) giving 8.5 log *E. coli* (3.2×10^8 CFU/g) and 8.9 log *S. senftenbergensis* (7.9×10^8 CFU/g) simulating worst contamination situation for biowaste.

Like in the case of composting peels to compost soil, in this experiment, 2kg of the artificially pathogens contaminated feedstock mixture was weighed in the composting flasks after deducting the mass of empty flask. Air was forced into the composting flasks to supply aerobic condition. In order to avoid cooling effect in the reactors as result of forced air, monitoring of air supply was regularly checked on daily basis for the whole composting period. Sensory quality and the physicochemical attributes of the final resulted compost were analyzed in the same way as described earlier in the composting experiments conducted in this research. The full composting process was carried out as described in the following Figure 7.1.

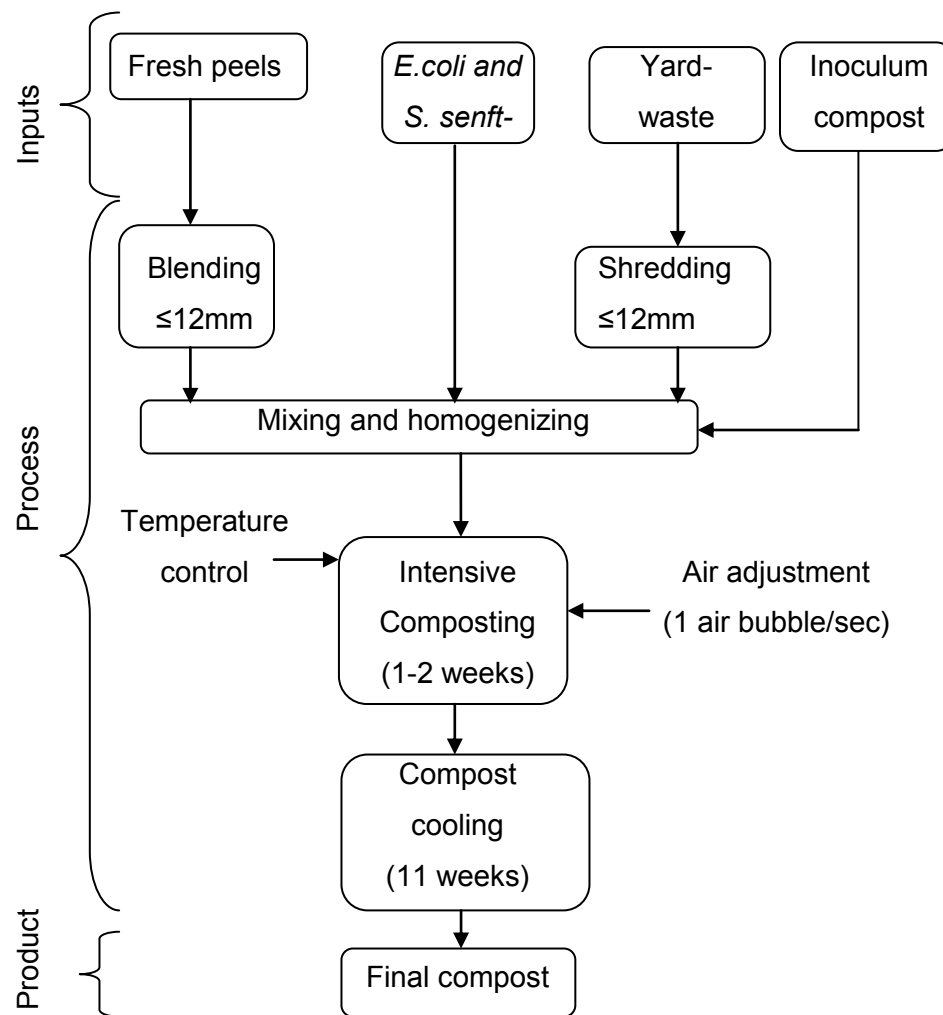


Figure 7.1: Schematic layout of feedstock formulation and aerobic co-composting process

7.3 Results and Discussion

7.3.1 Physicochemical attributes of feedstock used in co-composting

A summary of physicochemical characterization results of individual co-composting feedstock before mixing are presented in Table 7-2. The initial moisture content in yard-waste was good enough to adjust and optimize the high moisture in the potato peels.

Table 7-2: Characteristics of individual feed components before mixing

Composting feedstock	Mc	TS	Porosity	oTS	Ash (dry)	pH-
	values given in % w/w					H ₂ O
Potato peels	77.9	22.1	18	90.4	6.2	5.8
Yard-waste	12.9	87.1	43	89.3	9.7	7.1
Compost	40.2	59.8	27	47.7	52.3	7.6

Key: oTS(%TS) = Volatile organic matter Mc = Moisture content
 TS= Total solids

Porosity in blended yard-waste was higher than in the other two components. This was good news as it was of the essence to increase pore-space in potato peels and therefore prevent the onset of anaerobic condition observed in the previous experiments caused by blocked air veins due to oozing leachate. Although the pH for potato peels waste was slightly acidic, as the case to most food processing wastes [109], the observed pH in seeding compost and that of yard-waste was good enough to bring an adjustment and equally optimize the pH to the required level. Both peels and yard-waste presented higher organic matter with lower ash contents indicating higher potential for biodegradation.

Using the desired composting moisture goal of 55% in accordance with theoretical calculations stated in Rynk [224], the ideal ratio of Peels to Yard-waste for Recipe formulation was found to be 6:1. The physicochemical characteristics of the ideal formulation plus the other three formulations are given in Table 7-3. Moisture in all formulations was within acceptable composting range 45-60%w/w specified in Cooperband [109], Campbell [265], Darlington [111], Bidlinmaier et al., [108] and Dickson et al.,[48]. Optimum moisture content in composting feedstock is a critical factor as earlier on explained [97]. The higher the moisture content in the feedstock, the greater is the chance for the anaerobic decomposition to take place which is undesirable effect as it affect temperature build-up in the reactor during active composting process by reducing the composting activity of aerobic micro-flora.

Table 7-3: Selected attributes of feedstock recipe for co-composting

Formulation	Mc	TS	Porosity	oTS	Ash	C/N	pH-
	values given in %w/w						H ₂ O
3:1	45.3	54.7	39	78.7	21.3	45	6.8
4:1	50.4	49.6	26	79.3	19.7	36	6.5
6:1	56.8	43.2	24	76.8	18.2	27	6.3
9:1	58.9	41.1	22	76.5	13.5	26	6.1

Key: C/N = Ratio of Carbon to Nitrogen Mc = Moisture content
oTS(%TS) = Volatile organic matter TS= Total solids

All four formulated Recipes had moisture content, porosity and organic matter within the acceptable composting range. As pointed out in Ahmed et al., [54] and Cooperband [109], the pH values of the composting components ranging 6.0-7.5 provide an optimum composting range for bacteria while pH ranging 5.5-8.0 provide optimum composting range for actinomycetes and fungi. Similarly, the pH value for the formulated feedstock ranged from 6.1-6.8 which was within the acceptable optimum composting range for all major decomposer micro-flora. High amount of yard-waste in formulation 3:1 and 4:1 resulted into a slight elevation of C/N ratio above the recommended range in accordance to the values reported in Cooperband [109]. High C/N ratio in composting feed affect composting time as it takes long time for the composting aerobic micro-flora to complete the decomposition process.

7.3.2 Temperature-time patterns during co-composting process

According to the temperature-time patterns shown in Figure 7.2, the four stages of composting process including mesophilic, thermophilic, second mesophilic and cooling/maturation were observed in all feedstock formulation. The use of yard-waste did not only adjust moisture content but also increases carbon which prolonged the composting process to 77days. Similar composting period has been reported in Lemunier et al., [14] during co-composting of fruits and vegetables mixed with cardboards and

papers the process which lasted for 12 weeks demonstrating a maximum temperature of 75°C. Hariz et al., [266] in which the co-composting of rice straws and different animal manure lasted for 62 days with temperature maintained above 63°C.

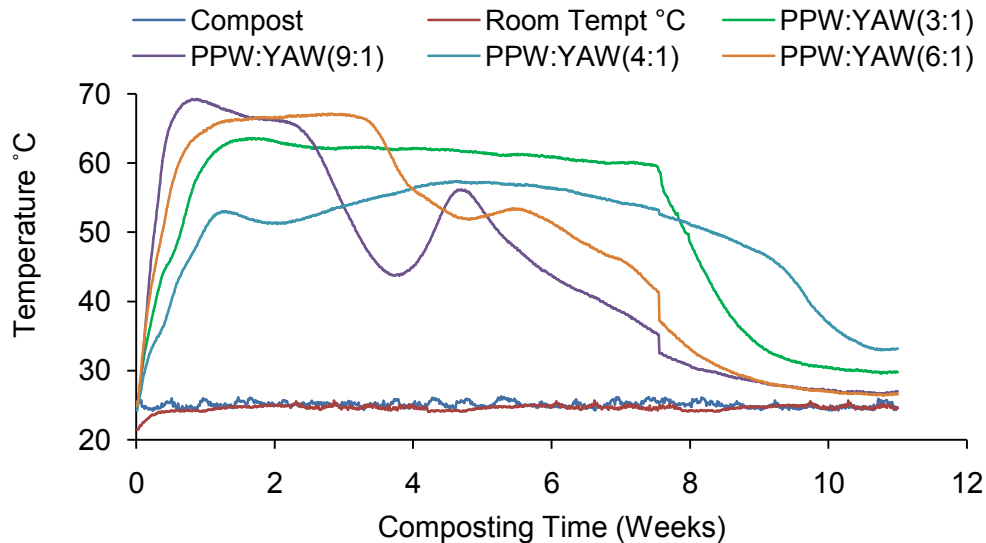


Figure 7.2: Temperature –time patterns of In-vessel co-composting of potato peels waste (PPW) with yard-waste (YAW) at various formulation ratios

Long composting period was based on the fact that the composting micro-flora had to use more time in oxidizing excess carbon in the yard-waste contrary to the previous two investigations which involved potato peels alone as the major feedstock in the Recipe. It was worth noting that during co-composting the strict temperature-time specification of 60-70°C was met. There was a temperature rise of between 55°C and 69.2°C in all four composting formulations. Increase in temperature during composting indicates good composting process in the sense of perfect microbial activity [98] thus corresponds to grade I of the compost described in *Bundesgutegemeinschaft Kompost e.V.*, 1994. In order to come up with good compost, favorable composting conditions that favour indigenous composting micro-flora yet destroy the pathogenic microorganism need to be met but with caution. de Bertoldi et al., [43] urged that higher temperature during composting that stay for long must be avoided otherwise the threat of eliminating the useful indigenous composting micro-flora becomes great and this phenomenon might lead

to longer time of composting process yet with poor quality of the final compost having partially decomposed materials as it was the case observed in the feedstock formulations 3:1 and 4:1.

7.3.3 Pathogens inactivation performance during co-composting process

The temperature generated 62°C in Formulation 3:1 was maintained un-interrupted for about 7 weeks followed by a sharp cooling phase. Initially, the temperature in Formulation 4:1 was 52°C that was retained for 2week, on the third week the heat increased to 55°C and was retained for 4 weeks. Formulation 1:6 got heated up to 67°C within the first 5 days. This temperature was maintained for 3 consecutive weeks before entering into second mesophilic phase. Therefore, while the compost produced in formulation 4:1 conforms to the set of composting criteria described for 55°C for 2 weeks, Recipe 6:1 and 9:1 attained the highest composting criteria of 65°C for 3 weeks while Formulation 9:1 was heating up to 68.9°C though dropped to 65°C and retained un-interrupted for 17 days before getting into second mesophilic phase of aerobic composting process. As described in Table 7-4, all four formulated co-composting Recipes demonstrated high percent of enteropathogens reduction thus hygienized compost that meets the requirements for quality and microbiological safe compost described in Brinton et al., [119], Lemunier at al., [14] and Hoggs et al., [120]. The temperature –time combination reached in all feedstock formulation during co-composting process conforms to as many regional and national regulatory bodies including Germany, Belgium and France.

Table 7-4: Efficacy of In-vessel composting on pathogens inactivation during co-composting

Recipe	Tempt °C	Duration Time	% Pathogens reduction	
			<i>E. coli</i>	<i>S. senft-</i>
3:1	62	7 weeks	99.69%	99.00%
4:1	55	4 weeks	99.96%	99.92%
6:1	67	3 weeks	99.69%	99.99%
9:1	65	2 weeks	99.99%	99.99%

was higher enough to cause evaporation of water vapor and other gases from the composting feed. Loss of water, decomposition of organic matter and mineralization resulted into decrease in weight of the final compost in the range of 24-58%w/w as well as decrease in organic matter in the final compost. The weight loss in the first two compost was below the range of 40-80% specified in Rynk [224]. The results shows a percentage reduction in organic matter after composting process which followed the following trend: In formulation 3:1 there was 46.5% reduction, formulation 4:1 had 53.4% reduction, and formulation 1:6 had 68.6% reduction while in formulation 9:1 there was 57.3% reduction. C/N ratios were high in formulation 3:1 and 4:1 as there was partial decomposition of the lignin from yard-waste as they were resistant to degradation. Electrical conductivity was found ranging 4.8-5.3mS/cm which is a closer characteristic of compost from yard trimming [137]. This range of conductivity presents a cut-off point for compost designated as top dressing grade and soil amendment recommended for improving agricultural soil, restoration of disturbed soil, establishment and maintenance of landscape [224].

7.3.5 Sensory characteristics of the final compost

In general all four final composts were free from objectionable odour, moist and had good and enough porosity indicating that aeration was sufficient during the whole composting process. The feed recipe with formulation 4:1 was a bit dry and with non composted straws of yard-waste. While the last formulations recipe 3:1 had more of un-composted materials and much dry. The moisture squeeze test as suggested by Dickson et al., [48] and Baldwin and Greenfield¹² confirmed the compost from Formulation 3:1 and 4:1 to be very dry since following squeezing of a handful of compost, there was no moisture as compared to the compost prepare from the other two formulations, ratio 1:6 and 1:9. Sensory attributes of finished compost presented in Picture 7-2 reflect extent of organic matter decomposition a product of micro-flora activity which in strict sense of words is responsible in the generation of heat higher enough to destroy the pathogenic microorganisms. It was observed in this investigation that two sample formulation ratio 9:1

¹² <http://www.farmsreach.com/welcome/wp-content/uploads/2014/11/CompostingOrganicFarms.pdf>
. Retrieved May, 2015

and 6:1 had good uniform in structure, good texture, brown/black colour with moldy-soil like smell confirming a proper and optimized aerobic composting process. In simple words, co-composting of fresh potato peels waste to a good quality and safe compost is possible at ratio 6 to 9:1 (peels to yard-waste). More addition of yard-waste results into poor decomposition with more dry byproducts while lower use of yard-waste will cause more moisture in the composting vessel that will consequently lead to the onset of undesirable anaerobic decomposition.



Potato peels: Yard-waste (9:1)



Potato peels: Yard-waste (6:1)



Potato peels: Yard-waste (4:1)



Potato peels: Yard-waste (3:1)

Picture 7-2: Attributes of compost from co-composting potato peels waste with yard-waste

7.4 Biogasification potential of potato peels waste

Value addition by converting potato peels waste into resource and substrate for biogas production and digestate as product with great potential at enhancing soil fertility to urban agriculture and horticultural practices is a worthwhile strategy towards utilization and proper management of the peels waste. As long as the concept of waste segregation at source in Tanzania is still at policy stage and therefore voluntarily practiced by mostly waste pickers who look for specific waste fraction which has direct economic advantage, introduction of anaerobic digestion of potato peels for Dar es Salaam city is of paramount importance in the following sense:

1. Provision of renewable and environment friendly energy source for household needs especially cooking and thus offer conservation to forest resources which are subjected to deforestation on high demand for wood based fuels (firewood and charcoal) as primary energy sources,
2. Reduction of the organic waste fraction from the MSW stream that would have to be handled by the municipal solid waste authorities,
3. Enhancement of environmental sanitation in the city by minimization of the challenging waste fraction threatening public health given the existing annually outbreaks of refuse based illnesses,
4. Provision of employment to biogas producers and the resulting digestate used to enhance soil fertility leading to an increase in urban horticulture produce, therefore promote the living situation of urban poor, and
5. Reduction or elimination of nuisance related to off-odour in streets and dumpsites given the high biodegradability of the peels under the hot climatic condition of the tropic city.

7.5 Potential of potato peels waste for biogas production

As specified in Khalid et al., [267], having C/N ratio of between 18-23:1 (value also obtained in all samples of potato peels waste analyzed in this research for this parameter) and high water content >77%w/w makes potato peels waste more useful resource/substrate for anaerobic digestion. While other waste fractions such as fruits waste and market wastes are seasonal and in most cases their availability is not reliable owing to the existing competition with livestock as animal feed, potato peels waste for Dar es Salaam is by far the best input. The peels are easily available, easy to handle during anaerobic digestion connected to the fact that prior to disposal the peels are always fresh and not mixed with other waste fractions making the waste segregation stage not necessary and thus an assurance of reduced biosecurity risks and threat of contracting infectious pathogens to the employers at the biotreatment facility and the environment thereafter.

7.6 Anaerobic digestion system and operation

This research was carried out along with the experiment set-up for investigation of performance of conventional two-phase mesophilic/thermophilic anaerobic digestion of potato peels on the reduction of enteropathogens. Mesophilic/Thermophilic two-phase anaerobic digestion operating as continuous stirred tank at 55°C was preferred in this investigation over the conventional mesophilic/mesophilic anaerobic digestion systems operating at 37°C due to the fact that thermophilic temperature induces hygienization effect to most non-spore forming bacteria, increase both biological and chemical reaction, increase removal of volatile solids and enhance solubility of organic compounds giving more biogas yield compared to the amount of biogas produced using mesophilic anaerobic digestion at the same organic loading rate [13,154,268].

7.6.1 Materials and Methods

Briefly, the same operated active volume of 50L in each reactor was used. The anaerobic digestion process operated as continuous stirred tank reactor (CSTR) was used (see Picture 7-3). The amount of substrate daily fed in both reactors was maintained until the

steady-state condition in which the OLR was increased step-wise. Following wet digestion principles, hydro-pulped potato peels waste having 5.2-5.7% total solids and 88.9% oTS as % of TS was the only substrate used.



CSTR -front view with gas meters



CSTR-rear view with biogas filled sacks

Picture 7-3: Set-up of the temperature co-phased anaerobic digestion

7.6.2 Operation procedure for the two-phase anaerobic digestion

The hydrolysis reactor was operated at mesophilic while the methanation reactor was operated at thermophilic temperature range. More emphasis in this investigation was on the potential of using the potato peels as substrate for anaerobic digestion process and the resulting biogas production per unit of input substrate. In this part of experiment, organic loading rate, daily units of biogas production, feasibility of biogas to complement if

not replace charcoal in Dar es Salaam and assessment of community's willingness to accept were investigated.

7.6.3 Analytical method and process control parameters

Mineral composition in potato peels waste was analyzed for determining suitability of the peels for anaerobic digestion including the threshold levels that can induce inhibition to the anaerobic digestion process. Other parameters for process control that were monitored on daily basis include: biogas generation rate, indoor pressure, gas temperature, reactor temperature and biogas composition (CH_4 , CO_2 , O_2 and H_2S). Gas composition was determined using Gasiotechnical Biogas instrument (Geotech, BM 11834 UK). The indoor pressure and gas temperature were important in converting biogas production rate to standard condition. Effluents (hydrolysate) from hydrolysis reactors and digestate from methanation reactor were sampled on daily basis for quality checks and efficiency determination of the anaerobic digestion process. Parameters monitored in the effluents include pH, salinity, conductivity and volatile solid (VS) removal. Samples for VS removal were collected and stored in refrigerator (4°C) and analyzed on weekly basis. All analysis were conducted according to standard methods and laboratory developed protocols while MS excel 2007 spreadsheet was used for data collection and analysis.

7.7 Results and Discussion

7.7.1 Mineral composition in potato peels waste for anaerobic digestion

Low concentration of minerals including sodium, potassium, calcium, magnesium and sulfur in the feedstock are vital for enhancing micro-flora during anaerobic digestion [86]. At high concentration, these minerals are among the substances mentioned of being capable of inducing toxicity in the anaerobic digestion system, causing inhibition to indigenous microorganisms, leading to upset in biogasification process and sometimes general system failure [59]. In this investigation the mineral content of the potato peels waste was determined and compared to the literature threshold concentration values (Table 7-6) specified by Zaher et al., [86] as detrimental to both acidogenic and methanogenic microorganisms.

Table 7-6: Minerals in potato peels and their corresponding limits in anaerobic digestion

Values from this study		Values from Literature*	
Mineral	Concentration mg/kg	Moderate inhibitory (mg/L)	Strong inhibitory (mg/L)
Ca	574.9	2500-4500	8000
Na	494.9	3500-5500	16000
K	2327	2500-4500	12000
Mg	1105	1000-1500	3000
P	2023	-	-

*Source for Literature values Zaher et al., [86] and Zupančič and Grilc[159]

The results of composition of essential nutrient in the peels waste were found within acceptable range anaerobic digestion as well as for land application of the resulting digestate thereafter the anaerobic digestion process and therefore relate well to the findings earlier reported in Zaher et al., [86]. Potato peels analyzed in this investigation shows the acceptable ranges to all minerals that would under normal condition never induce inhibition during anaerobic digestion process.

7.7.2 Substrate feeding regime in the temperature co-phased anaerobic digestion

Figure 7.3 presents the relationship between the substrate feeding regime and the behavior of HRT during digestion of potato peels using the mesophilic/thermophilic two-phase anaerobic digestion system. The reactors were operated for about 35 days with the initial OLR of 0.48kgTS/m³.day and gradually increased until 2.4kgTS/m³.day (a final OLR for this experiment with no observed sign of inhibition to both hydrolysis and methanation reactors) following a not less than 5 days lag prior to increasing the OLR. Until the last OLR, the biogas yield was still increasing and pH value in the methanation reactor was maintained at an acceptable operation range of 7.5-7.9 an indication that inhibition or overloading was not yet reached in compliance with recommendation by

Weiland [58] that a wet anaerobic digestion can operate up to a maximum of $4\text{kgO}_2/\text{m}^3\cdot\text{day}$.

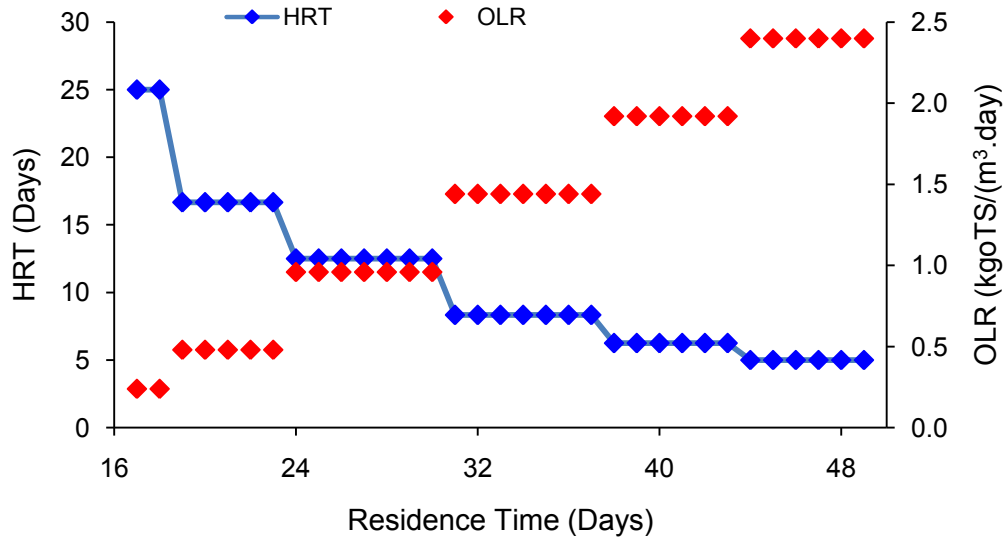


Figure 7.3: Substrate loading during thermophilic Two-phase anaerobic digestion

As a measure of preventing organic loading shock in the methanation reactor and therefore prevent inhibition, the ratio for O_2S in the substrate to that in the inoculum (cow manure slurry) was maintained at 0.299 which is below 0.5 a value recommended by VDI 4630 [157]. The increase in OLR means more addition of substrate in the reactor, this brought about a reduction in HRT from 25days operated in the mesophilic/mesophilic anaerobic digestion to almost 5days at the end of this experiment. Although it was intentionally performed for the purpose of checking process hygienization performance, the results of two-phase mesophilic/mesophilic anaerobic digestion of potato peels was not considered here firstly due to the shorter digestion period used and the low OLR used below the minimum recommended amount in VID 4630 (2006). Yet the process parameters revealed biogas productivity of $35.4\text{ l}_\text{N}/\text{l}\cdot\text{d}$, CH_4 productivity of $0.44\text{ l}_\text{N}/\text{l}\cdot\text{d}$, and methane content reaching 62.5%v/v within 10 days of operation at HRT of 25days.

7.7.3 Attributes of hydrolysate for methanation

In this research, the anaerobic digestion received five organic loading that were increased stepwise in the order of 0.48goTS/m³.day, 0.96goTS/m³.day, 1.44goTS/m³.day, 1.92goTS/m³.day and 2.40goTS/m³.day. The average pH, electrical conductivity and salinity in acidogenic digestate in relation to the organic loading rate that was fed to methanation reactor are as specified in following Table 7-7.

Table 7-7: Quality of acidogenic digestate from hydrolysis reactor for feeding methanation reactor

Parameter	Mean values from several similar substrate feeding rate				
	Digestion residence (Days)	5	7	7	6
Salinity (‰)	1.5	1.9	2.9	3.2	2.9
Conductivity (mS/cm)	3.0	3.7	5.3	5.8	5.7
pH	4.7	4.5	4.4	4.3	4.2
OLR (kgoTS/m ³ .day)	0.44	0.96	1.44	1.92	2.40

The general trend observed was that, the decrease in pH in the acidogenic digestate as a result of fermentation of organic matter in the potato peels was inversely proportional to both salinity and electrical conductivity (see Figure 7.4). Such low pH was attained following addition of process water from other hydrolysis anaerobic digester as reported earlier in this research. The advantage of attaining low pH during hydrolysis stage of anaerobic digestion is that the fermentation process facilitates release of CO₂ which freely escape into air and when the pH values reaches 4, all the CO₂ in the hydrolysate exist as free molecules [59].

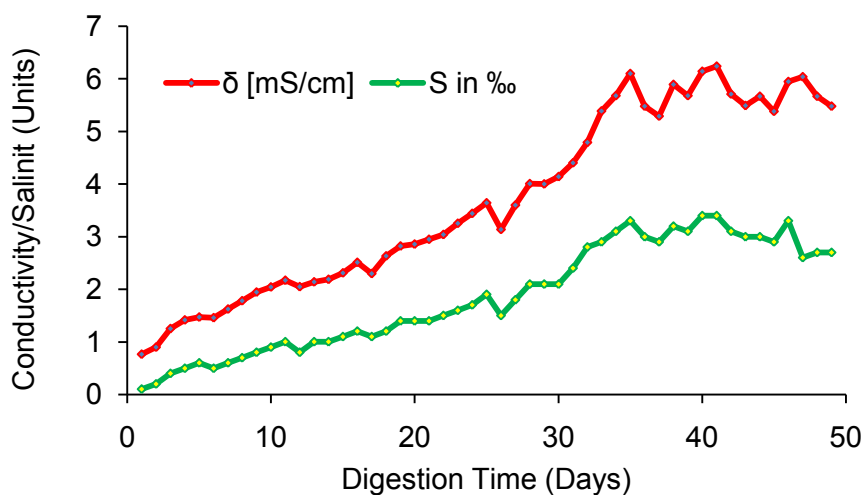


Figure 7.4 : Electrical conductivity and Salinity patterns in hydrolysis reactor

The possible explanation for this increasing trend was the fact that under acidic condition, there is a high tendency for metals and mineral salts bound in the biomass cells to solubilize and therefore causing their concentration to be high in the solution. The concentration of the mineral salts in the biomass then determine the concentration and if it is too high there is always a tendency of inducing inhibition to the whole anaerobic digestion process due to the effect these salts and mineral salts have on both useful micro-flora and even the pathogenic microorganisms.

7.7.4 Patterns of Electrical conductivity and salinity in methanogenic digestate

The selected parameters reflecting quality of methanogenic digestate are presented in Table 7-8 below. The initial electrical conductivity and salinity of the seeding (cow manure slurry) was 13.9 mS/cm and 8.1‰ respectively. At the onset of anaerobic digestion process, both values were observed to increase. On day 8 which was the end for hungry operation of the methanation reactor, electrical conductivity reached 14.9 mS/cm while that of salinity was elevated to 8.8 ‰. Increase in OLR was observed to have no influence on pH but rather on salinity and conductivity, this indicates the stability of methanation process and optimal processing condition with no sign of inhibition.

Table 7-8: Attributes of digestate from methanation reactor

Parameter	Mean values from several similar feeding rate (kg/day)				
	5	7	7	6	6
Digestate residence (Days)	5	7	7	6	6
Salinity (‰)	7.8	7.1	6.4	6.1	5.6
Conductivity (mS/cm)	13.2	11.9	11.0	10.4	9.6
pH	7.5	7.9	7.9	7.9	7.7
OLR (kgoTS/m ³ .day)	0.44	0.96	1.44	1.92	2.40

The pattern results for electrical conductivity and salinity in the digestate from methanation reactor are presented in Figure 7.5. From day 9 in which the substrate (acidogenic digestate) from hydrolysis was added in the reactor, both electrical conductivity and salinity started decreasing until the end of the anaerobic digestion process the value for electrical conductivity was 9.6mS/cm and that for salinity was 5.6‰.

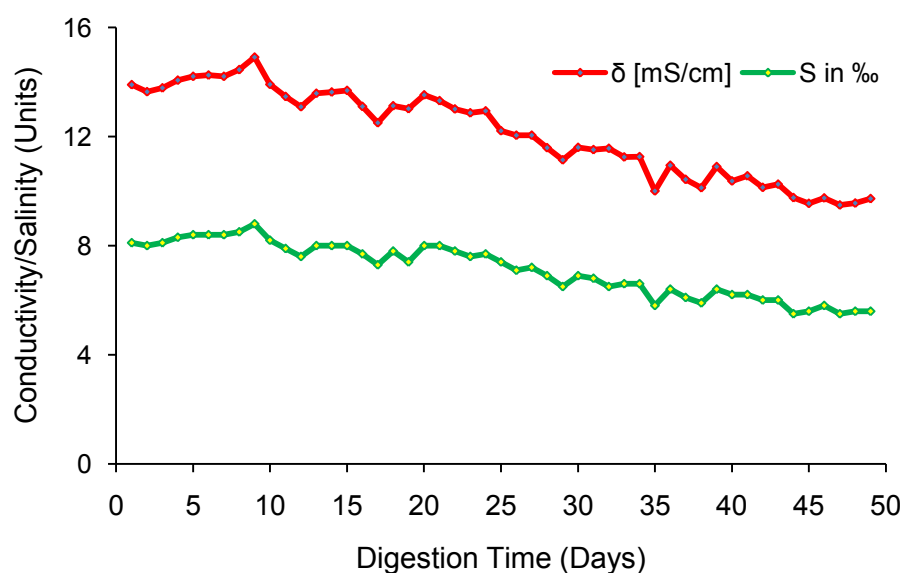


Figure 7.5 : Electrical conductivity and salinity patterns in methanogenic digestate

The decrease in salinity and electrical conductivity besides the decomposition of organic matter in potato peels waste signify the potential of alkali pH in the digestate which up to the end of experiment was at slightly alkali range of pH value 7.7. At such alkaline pH range, mineral salts and metal ions which are responsible with the increase in electrical conductivity precipitate and are sorbed in the digestate particles and do not solubilize and therefore become of little effect especially in the anaerobic digestion system.

The observed conductivity was within acceptable range of salt concentration in the final digestate that is meant to be used as soil amendment [224] designated for improvement of derelict agricultural soils, restoration of disturbed soil and maintenance of the landscape but not for agricultural soil because the requirement of conductivity level as pointed out by Hargreaves et al.,[128] is between 0 and 4mS/cm that means the digested discharged from the methanation reactor contains between 2 and 3 folds of the required level. If the resulting digestate is used in this form there is a possibility of disturbing the soil salt content and therefore consideration on application doze of the digestate to both soil and types of crops is of paramount.

7.7.5 Processing parameters and yield in two-phase mesophilic/thermophilic biogasification of potato peels

The results for biogas production and process parameters following anaerobic digestion of potato peels waste are shown in Table 7-9. The feeding regime that required daily opening of the methanation reactor during substrate feeding introduced a small amount of oxygen in the system, as results oxygen content in the biogas was measured up to 0.78% at the start of the process. Control measures including faster feeding and the use of air-tighten reactors helped in decreasing the amount of oxygen in the system by 5-folds. It can be seen from the table that reactor pH was stable throughout the digestion process regardless of the steady increase in OLR. Similarly, after 5days the temperature was stable at thermophilic range.

Table 7-9: Biogas production of potato peels waste on two-phase anaerobic digestion

Parameter	Mean values from several similar substrate				
	feeding rate				
Digestion period (Days)	5	7	7	6	6
OLR kgoTS/m ³ .day	0.48	0.96	1.44	1.92	2.40
Temperature °C	47.2	54.8	53.7	55.2	55.0
pH	7.8	7.8	7.9	7.9	7.5
oTS in substrate (kg FM)	0.04	0.08	0.11	0.15	0.19
oTS in substrate [g/kgTS]	24.2	48.5	72.7	96.9	121.1
Biogas yield Y [l _N biogas/kg oTS]	314.8	625.8	504.0	598.5	602.1
Biogas yield Y [l _N biogas/kg FM]	26.0	47.6	38.3	45.5	45.8
Biogas productivity [l _N biogas/l.d]	0.26	0.95	1.15	1.82	2.29
Methane yield [l _N CH ₄ /kgo TS]	131.6	352.2	288.0	335.3	341.2
Methane yield [l _N CH ₄ /kg FM]	10.0	26.8	21.9	25.5	25.9
Methane productivity [l _N CH ₄ /l.d]	0.11	0.54	0.66	1.02	1.30
Methane content (%v/v)	59.7	56.2	58.2	56.4	55.9
Carbon dioxide (%)	35.7	37.4	33.1	34.7	35.9
Oxygen (%)	0.78	0.57	0.22	0.25	0.18
H ₂ S [ppm] in Hydrolysis reactor	>LoQ	>LoQ	>LoQ	>LoQ	>LoQ
H ₂ S [ppm] in Methanation reactor	>LoQ	>LoQ	1264	252	223

The observed biogas yield in this research compares well to the biogas yield from other previous research. For instance, Linke [269] in the research following anaerobic digestion of potato processing waste using CSTR at 55°C and OLR of 0.8-3.4kgoTS/m³.day reported biogas yield of 0.65-0.85m³/kgVS, Nayono [147] during anaerobic digestion of biowaste reported biogas yield of 0.590m³/kgVS input while in the other study, Bouallagui et al., [270] reported biogas yield of 0.730m³/kg VS using abattoir wastewater mixed with fruits and vegetable waste as substrate.

Figure 7.6 presents the results on biogas yield (I_N /day) and methane rate in relation to OLR following mesophilic/thermophilic two-phase anaerobic digestion process of potato peels waste that lasted for 31 days. At the beginning the drop in both biogas yield, methane rate and methane concentration in the measurements was observed. This was as a result of thermal-shock to the methanogens bacteria (*Methanosaeta* and *Methanosarcina*) that were adapted to mesophilic temperature range after running the mesophilic/mesophilic anaerobic digestion for about 10 days.

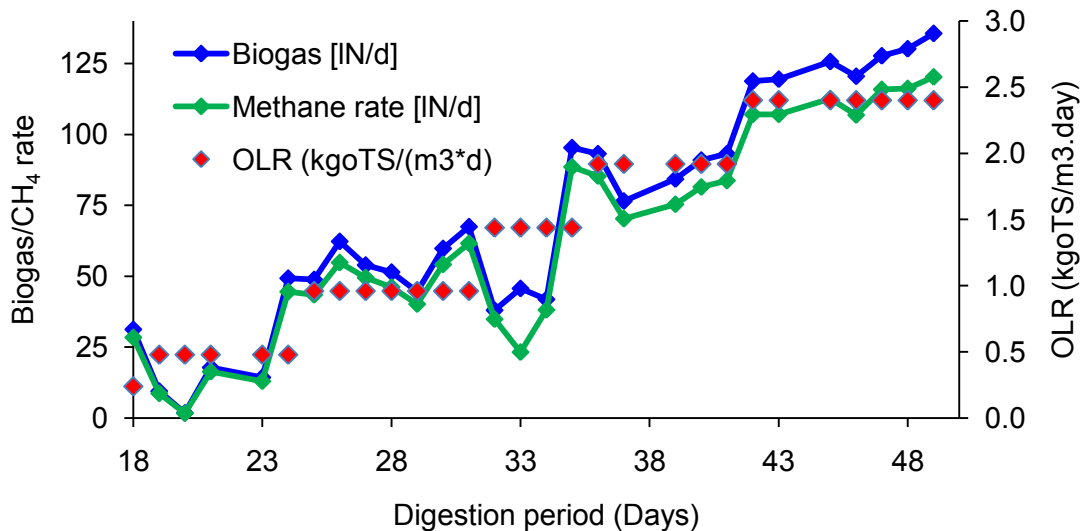


Figure 7.6: Biogas yield and methane rate from potato peels waste in two-phase mesophilic/thermophilic anaerobic digestion

Elevation of temperature in the methanation reactor that was set at $55 \pm 1^\circ\text{C}$ instantly enabled the thermophilic micro-flora to take over the digestion process. Zupančič and Grilc [159] explain that under normal environment, the micro-flora responsible with methanation process requires between 5-16 days to regenerate compared to acidogenic and acetogenic bacteria which requires shorter time of less than 36hrs and 80-90hrs respectively. Thereafter there was an increasing production of biogas and methane correspondingly to the increase in OLR. The reason behind the observed drop in the biogas yield on day 33 of operation was the low oTS in the hydrolysate fed into the methanation reactor following the organic shock loading that reduced the fermentation efficiency of useful indigenous acidogenic bacteria. Addition of the process water from

another running reactor brought a remedy. Until the end of the experiment at the feeding rate of $2.4 \text{ kgTS/m}^3 \cdot \text{day}$; both biogas and methane were increasing in generation with no sign of having process inhibition. The average biogas yield, methane yield, biogas productivity and methane productivity following gasification of potato peels under mesophilic/ thermophilic temperature two-phase anaerobic digestion in this research was increasing proportionally to the organic loading rate (see Figure 7.7 and Figure 7.8).

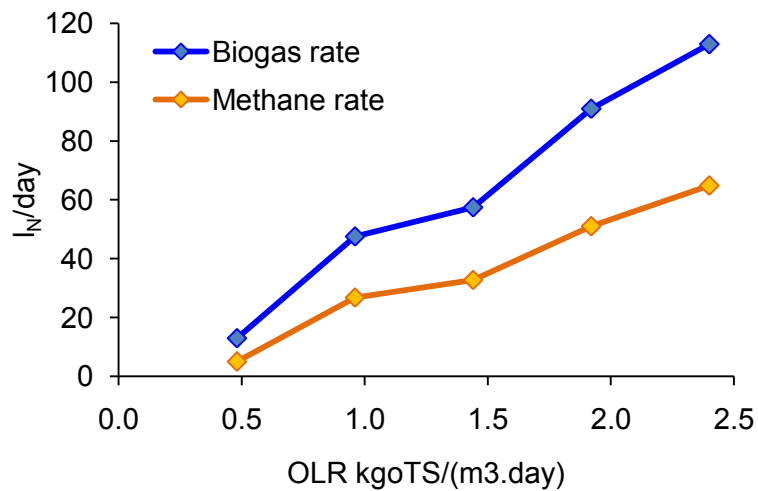


Figure 7.7: Mean biogas and methane rate (I_N/day) during biogasification of potato peels

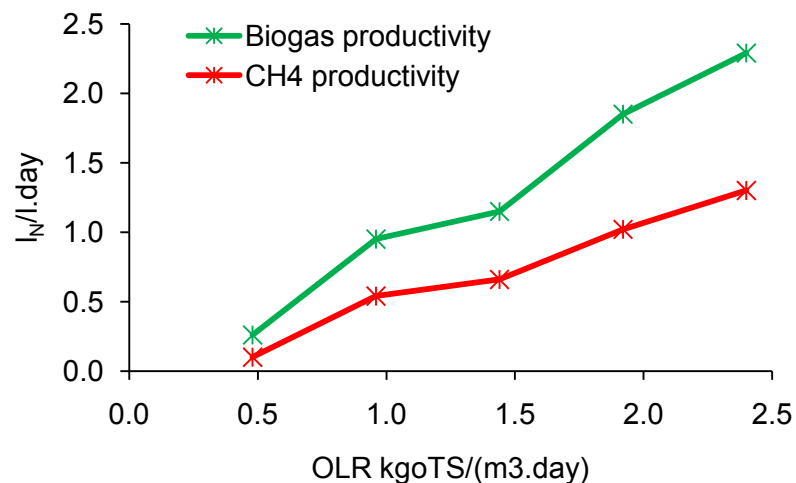


Figure 7.8: Mean biogas and methane productivity during biogasification of potato peels

It can be clearly observed that up to the maximum of 2.5kg of fresh potato peels equivalent to oTS of 0.19kg of the input and OLR of 2.4kgoTS/(m³.day), still the anaerobic digestion process was stable in operation with no signs of inhibition. This is an indication that the maximum organic loading rate for the digestion process was not yet reached, thus conforming the recommendation in Deublein and Steinhauser [59] and Weiland [58] that the typical ideal OLR for wet digestion of biowaste reaches between 2 and 4kgoTS/m³.day. This means that by the end of this investigation there was still a possibility for the anaerobic digestion process to accommodate additional 1.6kgoTS/m³.day without threat of experiencing inhibition or having system failure.

Figure 7.9 below gives the percent of methane concentration (CH₄ (%)) proportional to the changing organic loading rate (OLR). The biogas quality in terms of percent methane composition was found well corresponding to other results obtained in various investigation on biogas produced from other organic fraction of MSW. This quality meets basic criteria for energy required for normal cooking [110,142,271]. It can be seen from the figure that mesophilic /thermophilic two-phase anaerobic digestion process effectively produced good quality biogas containing between 56.2 and 64.9% methane on average.

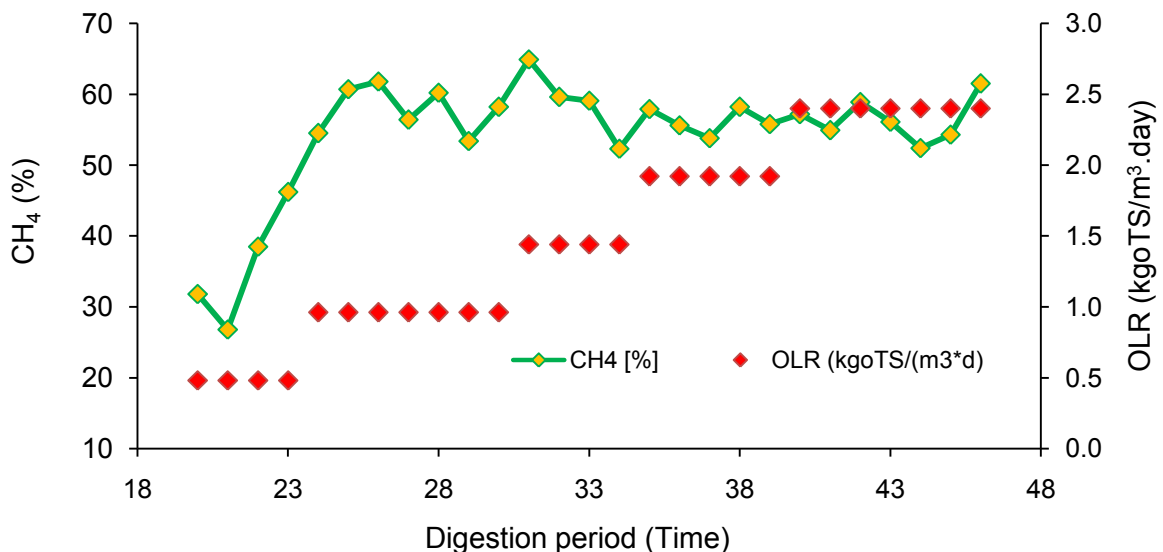


Figure 7.9: Methane concentration for two-phase mesophilic/thermophilic anaerobic digestion of potato peels waste

Generally, the results for H₂S [ppm] in Hydrolysis reactor using this set up was above the limits of quantification though in the methanation reactor the concentration was reduced from H₂S above 5000ppm to 1688ppm. Although this final concentration was still higher and capable of causing process inhibition and toxicity to the indigenous acidogens microflora, Deublein and Steinhauser [59] urge that maintaining mesophilic temperature in the hydrolysis stage help at mitigating H₂S toxicity which is more pronounced with rise in temperature in case the hydrolysis reactor is operated at high temperatures for instance at thermophilic range. Therefore operating hydrolysis at mesophilic range and methanation at thermophilic range which was the reactor set-up used in this research is recommended to overcome H₂S toxicity since much of the gas is removed during hydrolysis stage and at low temperature. The amount of H₂S concentration remained in the hydrolysate was later on decreased to about 223ppm in the methanation reactor irrespective of the increase in OLR. This biogas containing H₂S level to this amount was reported and recommended in Busch et al.,[56] as quality of biogas good enough for many application especially heat energy for household cooking even without carrying further purification thereafter.

The results on methane yield from potato peels were compared to the methane yield reported in other research (see Table 7-10) and also to the results documented by Muhammad et al., [221] in their review article that combined anaerobic digestion processes carried out on other biodegradable substrates. In this comparison, temperature in the methanation reactor was equally taken into account for the reason of having process diversification. It was observed that methane yield (m³/kg oTS input) produced using potato peels waste as substrate compares well to the methane yield from other OFMSW as well as the Cow manure. Volatile solid (VS) removal and methane composition (%) were even higher in potato peels than in some of the comparable substrates. This marked a high potential of potato peels as substrate for anaerobic digestion and thus a prudent strategy for diverting the peels waste from entering the MSW stream.

Table 7-10: Comparability of quality and methane yield from potato peels and other substrates

Research source	Methanation temperature	Substrate used	Methane yield (m³/kg oTS input)	Methane (%)	VS removal (%)
This investigation	Thermophilic	¹³ PPW	0.13-0.35	58	81
Zupančič et al., [272]	Mesophilic	¹⁴ OW + sludge	0.39-0.6	n/a	n/a
Gallert and Winter	Thermophilic	¹⁵ OFMSW	0.35	59	65
Zhang et al.,[273]	Thermophilic	Food waste	0.348; 0.435	73	81
Davidsson [274]	Thermophilic	¹⁶ SS- OFMSW	0.28-0.41	62	81
Gunaseelan [275]	Thermophilic	OFMSW	0.128-0.319	n/a	50
Riuji [142]	Mesophilic	Food waste	0.57	57	85
Riuji [142]	Mesophilic	Market waste	0.63	66	73
Bolzonella et al.,[276]	Thermophilic	OFMSW	0.23	68	n/a
Babae and Shayegan [277]	Mesophilic	¹⁷ FWW	0.12-0.4	64	88
Fernandez et al., [278]	Mesophilic	OFMSW	0.3	58	73
Nayono[147]	Mesophilic	Potato sludge	0.40	56	65
Ferrer et al.,[279]	Mesophilic	Cow manure	0.35	65	n/a

¹³ PPW= Potato peels waste

¹⁴ OW= Organic waste

¹⁵ OFMSW=Organic fraction of municipal solid waste

¹⁶ SS= Source separated

¹⁷ FWW=Fruits and vegetable waste

7.7.6 Potential contribution of biogas to household energy-mix

At the end of experiment which reached maximum substrate feeding of 2.5kgFM per day having 23%TS and 94.7% oTS as % of TS, it was established that an average of 0.098m³ biogas is produced in each kg of fresh potato peels waste (wet weight) fed in the methanation reactor. On average, the quality of biogas generated composed of 58% CH₄, 35% CO₂, 0.3% O₂, less than 0.03% H₂S and the remained less than 6.9% being other impurities component such as trace of other gases and water vapour. Although by the end of the experiment the maximum organic loading rate was not yet reached, the interim results (Figure 7.10) reveal that an increase in 0.5kg of potato peels fresh mass input in the hydrolysis reactor and equally transfer of the same amount into methanation reactor brought an increase of about 0.049m³ of biogas generation that can be used as fuel for household cooking directly without a need of undertaking further purification.

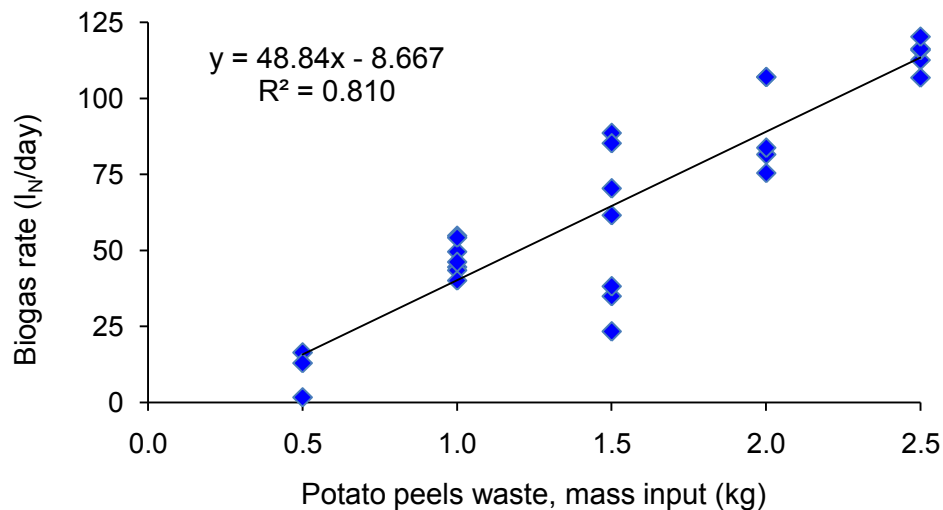


Figure 7.10: Daily biogas production rate in relation to mass of fresh substrate input

The disparities in biogas production rate during 1.5kg of Potato peels fresh matter addition which affected much results precision falls within the days on which there was low substrate fermentation rate in the hydrolysis reactor due to organic shock loading of the indigenous fermentative micro-flora a phenomenon that was resolved by adding process

water (hydrolysate) from another reactor that rectified the fermentation process. Therefore the amount of biogas estimated from the 85 tons of potato peels waste quantified and qualified in Muhondwa et al., [97] for biogas production in Dar es Salaam city and other associated parameters are as summarized in the following Table 7-11. With the estimated family cooking time of 5 hrs per day, it is possible to rescue approximately 34.2 tons of fuel-wood daily from the energy-mix.

Table 7-11: Estimated biogas generation from potato peels waste and its potential in Dar es Salaam

Parameter	Value	Unit
Potato peels generation per day	85,000	kg
Average OLR	1.74	kgoTS/m ³ .day
Assumed HRT	25	days
Biogas yield from potato peels waste	0.530	m ³ /kgoTS
Daily biogas production (volume)	9,769.7	m ³
Reactor Volume	13,280	m ³
Biogas burning rate per stove	0.2	m ³ /h
Estimated cooking time per day	24,425	hours

7.7.7 Post-biotreatment hygienization of digestate

The results on selected chemical and bacteriological characteristics of digestate from methanation reactor of the two-phase mesophilic anaerobic digestion system (MMAD) treating artificially contaminated potato peels after 8 days of anaerobic digestion process are shown in Table 7-12. Prior to conditioning, the system showed a good reduction of volatile solid within the acceptable range of 50-65% demonstrating a health anaerobic digester. Optimum pH and temperature in the methanation reactor and the fact that the reactor operation required a daily discharge of equal digestate as effluent from methanation reactor similar to the hydrolysate added from hydrolysis reactor was the reason for the observed large bacteria population in the digestate. Shorter substrate residence time (≤ 24 hrs) in the hydrolysis reactor due to the fill and draw mode of

operation was the reason behind adding more bacteria in the methanation reactor as it was possible for some live bacteria cells that was added on the previous day to possibly pass the hydrolysis process unaffected.

Table 7-12: Selected chemical attributes and microbial levels in digestate

Parameter	Value	Unit
pH	7.7	-
Temperature	36.8	°C
VS reduction	59.4	%
<i>E. coli</i>	6.8x10 ⁶	CFU/g
<i>S. senftenbergensis</i>	2.1x10 ⁶	CFU/g

The observed small amount in *E. coli* and *S. senftenbergensis* reduction from the original population could be attributed by intrinsic factors in the reactor such as competition between the spiked bacteria as well as between the indigenous micro-flora. Another factor could be H₂S toxicity as was determined above LoQ. Deubleins and Steinhauser [59] earlier expounded that even at concentration above 50ppm, H₂S inhibit growth of microorganisms during anaerobic digestion process through its high dissociation potential in the digestate as weak acid and become direct poison to bacteria cells.

7.7.8 Removal of Volatile solids

As one of the basic purposes of anaerobic digestion of potato peels was to offer proper means that will manage this waste fraction from the MSW stream, the performance of anaerobic digestion at performing solid reduction was evaluated. The results for removal of volatile solids in relation to organic loading rates for the MTAD are given in Figure 7.11. Until the end of the experiment, there was up to 81.3% removal of volatile solids in the methanation reactor. This high value reflect the performance of the digestion system and also good degradability of potato peels, good proportion of C/N ration that made easier for the fermentative microorganism to digest and well assimilate the volatile organic solids.

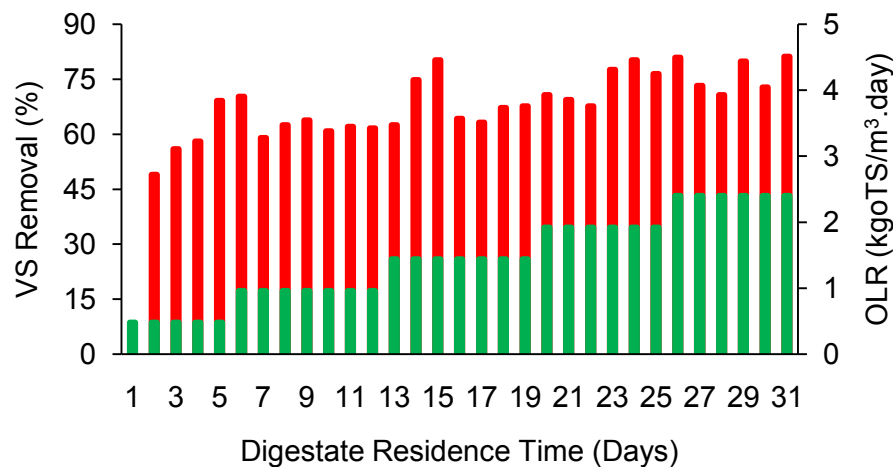


Figure 7.11: Volatile solid removal following anaerobic digestion of potato peels waste

7.8 Conclusive summary

Potato peels waste demonstrated to have potential as feedstock for aerobic co-composting with yard-waste and/or substrate for anaerobic digestion. Co-composting of fresh potato peels waste with yard-waste has shown the best results in terms of hygienization performance as well as physico-chemical quality and sensory attributes of the finished compost. This therefore presents the best option for recycling potato peels waste or any other biowaste with high moisture content above 75%w/w. Co-composting process equally elevate C/N ratio in the peels to the recommendable composting range, eliminate leachate formation and inhibit the onset of anaerobic digestion process by enhancing aeration in the composting flasks. Achievement of such higher temperature as the one observed in this experiment gives a preliminarily assurance of optimized composting parameters and therefore possibility of having hygienized and quality compost. Similarly, the pilot-scale experiment on two-phase anaerobic digestion of potato peels waste for biogas production was demonstrated feasible and would be the best option in reducing the amount of peels waste that would require extra management attention from the municipal solid waste authorities in terms of collection, handling and disposal. In a nutshell, the two waste management strategies applied on potato peels waste apart from reducing waste from the MSW stream, they in the same way sustainably supporting the management of forest resources and biodiversity.

8 Feasibility and Strategies for Managing Potato Peels Waste in Dar es Salaam through Biological Treatments

8.1 Introduction

Generally, waste management and environmental sanitation in Dar es Salaam city has been a long time challenge [33,40–42,280]. Potato peels like any other biowaste from food processing facilities, food vendors and households present a fraction of organic waste in municipal solid waste (MSW) stream that poses environmental menace in Dar es Salaam city given the existing haphazardly dumping done by the mushrooming potato chips processors. Of late, potato peels waste have increased share of MSW in Dar es Salaam that requires attention from the MSW Authority. Population growth, urbanization and rising per capita income as part of economic growth a common phenomenon occurring to many other cities of developing nations has triggered changes in food consumption habits among the community in favor of potatoes and potato based products [281]. Although other waste fraction such as food leftovers/residuals from household's kitchen, hotels, institutions and market waste could be used as feedstock during biotreatment process and produce biogas, the major reasons attached to the failure in implementation of biogas production from those organic rich wastes lie on the reliability and sustainability of collecting and handling the waste.

Two reasons account for this, first is the fact that the concept of source-segregation of waste which is a pre-requisite step towards quality of the feedstock is yet to be implemented while the post-generation segregation of waste is obvious tedious and unfriendly especially in mechanization challenged countries [282]. The second reason is that food remains and food preparation wastes hardly find their way into normal waste dumping system as they are collected for feeding non-ruminant animals including pets, pigs and free range indigenous fowls. Like in many other countries of developing countries, combating hunger and ensuring food availability at all time is a primary priority. Therefore the use of the so called energy crops such as maize, sorghum, wheat, etc for biogas becomes out of context as they constitute staple food crops. Waste fraction like potato peels presents the waste recourse with substitution potential to feedstock and/or

substrate for aerobic and anaerobic decomposition processes without creating pressure to food security in the course of producing bioenergy.

8.2 Sustainability of potato peels for biogas production in Dar es Salaam

8.2.1 Supply and possible availability of the potatoes for peels generation

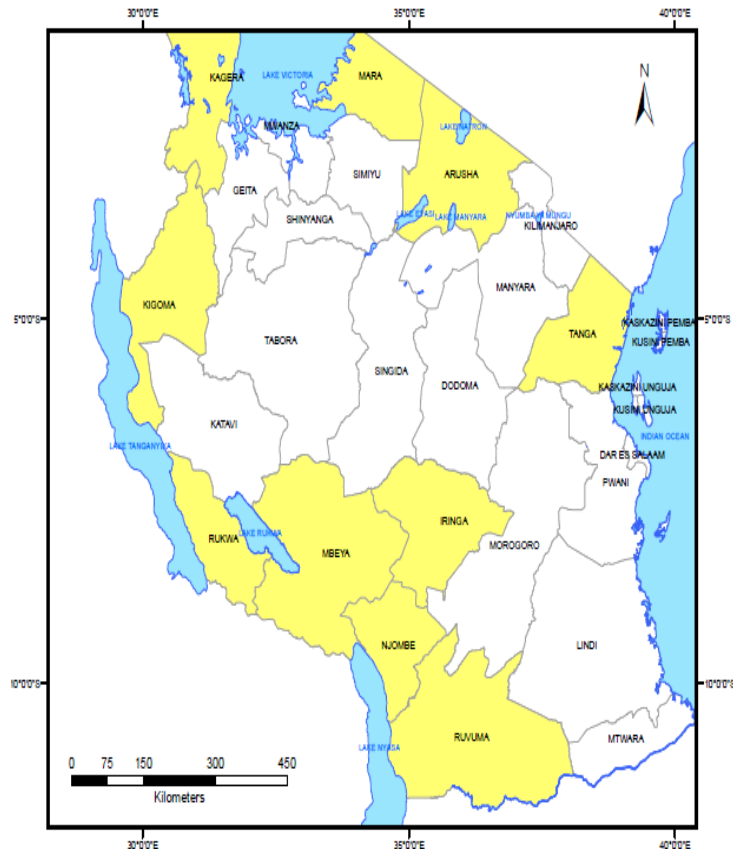
Together with the presence of small shops trivially known as ‘*vibanda*’ from where potatoes are available and can be cheaply bought, the city contains a total of 59 traditional open markets selling potatoes meeting customers demand (Picture 8-1). During the field survey, Ilala was the leading municipality having 24 markets followed by Kinondoni 18 and Temeke had 17 markets. Among these, the main markets where potatoes customers (whole sellers and retailer) purchase potatoes are Ilala and Buguruni markets in Ilala Municipality, Mbagala market in Temeke Municipality and Urafiki-Mabibo market in Kinondoni Municipality.



Picture 8-1: Marketing of potatoes, Mabibo-Urafiki Market, Kinondoni Municipality

Other popular traditional open markets for potatoes include Kariakoo, Tegeta, Tazara and Temeke Stereo. Potatoes usually packed in bags of approximately 115-120kg locally known as *lumbesa* are transported by trucks to Dar es Salaam mainly from Regions known to be the major producers of potatoes in Tanzania including the Southern Highlands, in particular Iringa, Njombe and Mbeya regions [283]. Other regions include Arusha, Tanga and Kagera. Similarly, areas that were previously referred to as minor

producers regions including Mara, Kigoma, Rukwa, Ruvuma and possible elsewhere (see Figure 8.1) have been reported to increase and/or start production [283,284] and all together consider Dar es Salaam as their potential market.



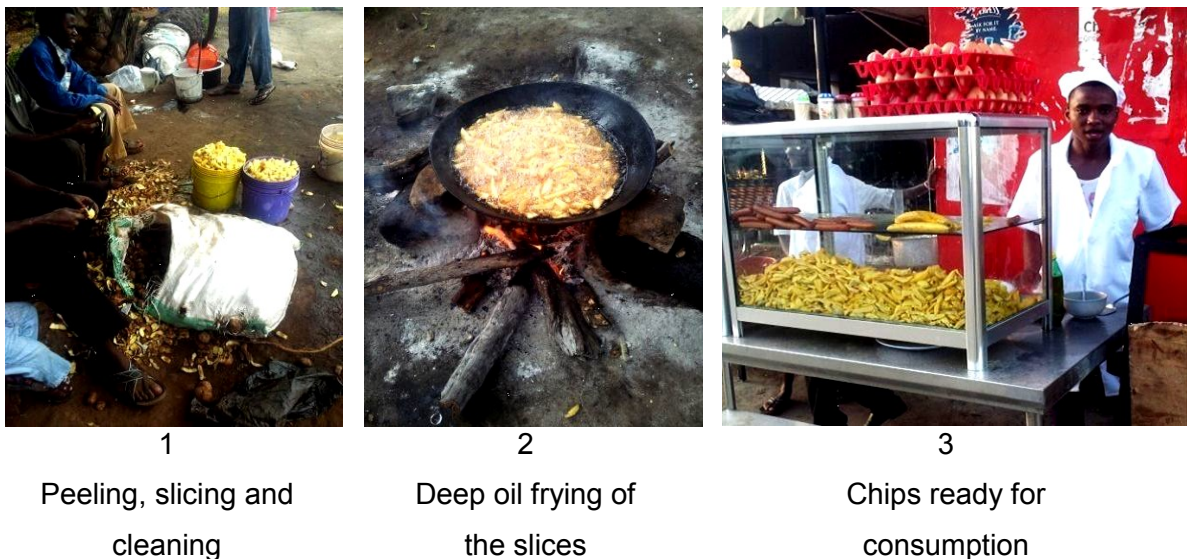
Yellow mark= Potato producing regions in Tanzania

Figure 8.1: Major regions growing and supplying potatoes in Dar-es Salaam markets

8.2.2 Potatoes processing in Dar es Salaam and peels waste generation

Processing of potatoes into fast food and take-away in Dar es Salaam city is a growing business that attracts low wages employees and unemployed occupants. Nowadays, daily meal recipes in the city are complemented with mashed potatoes or chips in both local and continental dishes such as *mtori*, *mchemsho*, *kachori*, *sambusa*, *pilau* potato soup, Croquette, Bangers and mash French fries, Hash brown, mashed potatoes, crisps and

other value-added fast food like kachori and samosa etc. This has proportionally causing an increase in the peels waste generated in the city day by day [97]. About 80% of all potatoes marketed in the city are processed into chips (French fries) (food made by deep oil frying of sliced round potato tubers) [97]. Changes in society's eating habit in favor of readymade fast foods fueled by globalization, urbanization and growth of tourism sector are the reasons behind the growing demand value-added products from potatoes [283]. Chips is more preferred and have become a popular fast food due to other factors such as shorter preparation time with simple processing steps (Picture 8-2) that involve peeling of potato tubers, slicing of the tubers, washing of the slices and finally deep oil frying.



Picture 8-2: Main steps in chips making process

8.3 Management challenges of potato peels in Dar es Salaam

Proliferation of chips processing kiosks in the city have resulted into massive daily generation of potato peels waste. Chips vendors with low knowledge, poor attitude and practice towards environment have resulted into poor management of the peels which are haphazardly disposed of in commingled with other waste fractions, see Picture 8-3. The dumped peels together with other waste harbor disease vectors (mosquitoes, flies, cockroaches, etc) and offer shelter to small animals such as rats and mouse which in a

nutshell have greater impact on public health, environment and its components and contribute much into dilapidation of infrastructure. It is estimated that refuse based diseases in the city accounts for more than 70% causing mortality and morbidity especially during every rain season [33].



i. Potato peeling process



ii. Fresh potato peels disposed of



iii. Decomposing potato peels



iv. Decomposed potato peels

Picture 8-3: Wasting of potato peels waste resource in Dar es Salaam

On the other scenario, long transportation routes of potato tubers from production regions to Dar es Salaam city and sometimes higher supply than the actual market demands as well as poor marketing systems that necessitates longer staying of potatoes at the market place affects much potatoes keeping quality. As such, potatoes tubers being perishable

get spoiled easily. This has also resulted into many sacks of spoiled tubers to be abandoned either at the market place, along the road, riverbanks, dumped at open spaces, in storm water channels, under the bridges, along the pedestrian pathways or many others being dumped along the road as it can be observed in Picture 8-4.



i. Sack of spoiled potatoes
openly dumped



ii. Spoiled potatoes dumped
along the road

Picture 8-4: Abandoned sacks of spoiled potatoes and peels along the roads

8.4 Energy situation and the role would biogas play in Dar es Salaam

Dar es Salaam city shelters more than 10% of all Tanzanians estimated to be 45 million [285]. Charcoal demand in the city is reported to have doubled from the 2.5 million tons estimates of the year 2009 to 5.2 million tons for the year 2015 [286] proportionally to the increase in population and so far is still the primary cooking fuel to more than 85% of the city dwellers [45]. Mwampamba [287] and Kilahama [288] estimate charcoal consumption of between 75-140kg/capita/annum. The existing use of traditional kilns (see Picture 8-5) whose conversion efficiency of wet-wood to charcoal is estimated at 22% [289] make a great deforestation to forest resource thus remove soil covers and carbon dioxide sink and on the other hand create pressure to biodiversity and forest depending organisms. Unreliability in supply and price instability of charcoal and firewood caused by many factors such as an increasing awareness among community on the importance of trees to ecosystems, the distribution difficulties of fuelwood and charcoal particularly during rain seasons, existence of stringent environmental regulations on forest resources and

shortage of trees due to unsustainable exploitation practiced are pushing Dar es Salaam residents to diversify charcoal with other commercial energy sources such as coal, electricity, kerosene, biogas and LPG. Yet only 1% of the residents are reported to afford while others use as complement to charcoal [290]. Msuya et al., [286] based on these facts estimated that even if other factors remain constant, the transition pace from dependence on charcoal in Dar es Salaam city is very slow and will likely take longer time unless otherwise.



Picture 8-5: Traditional charcoal processing, charcoal kilns and marketing in Dar es Salaam

8.5 Materials and Methodology

8.5.1 Estimating potato peels production in Dar es Salaam

The field survey was scheduled to last for a period of six months. But because of limited financial resources, it was compressed to three months (June - August, 2014). Personal observations and semi-structured interviews were the two methods used to collect field data. The author was a main researcher and was responsible at ensuring quality of interview data collected along with other 8 semi-trained researchers from Ardhi University (ARU) and Sokoine University of Agriculture (SUA) who played a role of enumerators. The quantity of potatoes entered Dar es Salaam city in the year 2013 was used in estimating consumption rate proportional to the current city population and population growth rate (r). Based on the estimated average potato consumption of a person per year and using a ratio of weight of potato tuber to weight of peels, the peels generation G_R (kg/day) was

derived using equation 8.1 below based on the mostly applied manual peeling of the potato tubers among the chips vendors.

$$\text{Peels generation (tons/yr)} = \sum_{t=0}^n G_R P_0 (1+r)^t \times 10^{-3} \times 365 \quad (8.1)$$

Whereby:

G_R = peels generation rate,

r = population growth rate,

t = time (year),

P_0 = population of Dar es Salaam during the survey

8.5.2 Assessment of existing energy-mix and uses in Dar es Salaam

Initially the available energy sources options for Dar es Salaam were identified through desk study. Participants were asked along with other questions (see Appendix 1) on the type of primary energy source used for cooking and the alternative energy (if any). The frequency of responses were then collected, tallied and analyzed using SPSS for the percent of users of particular energy source and the reason for choosing that particular energy source.

Based on the observed biogas generation potential of potato peels (see Chapter 7 above), it was of the essence to exactly describe the would be benefits upon household implementation of centralized anaerobic digestion system for potato peels in Dar es Salaam in terms of adding alternative energy source that would complement to the traditional wood fuel based energy sources. In this case, the city was subdivided into strata that allowed easy administration of the questionnaire by having a cumulative population in each stratum (see Figure 8.2). At least 50 respondents out of the stratum population were randomly selected and participated in the semi-structures interview.

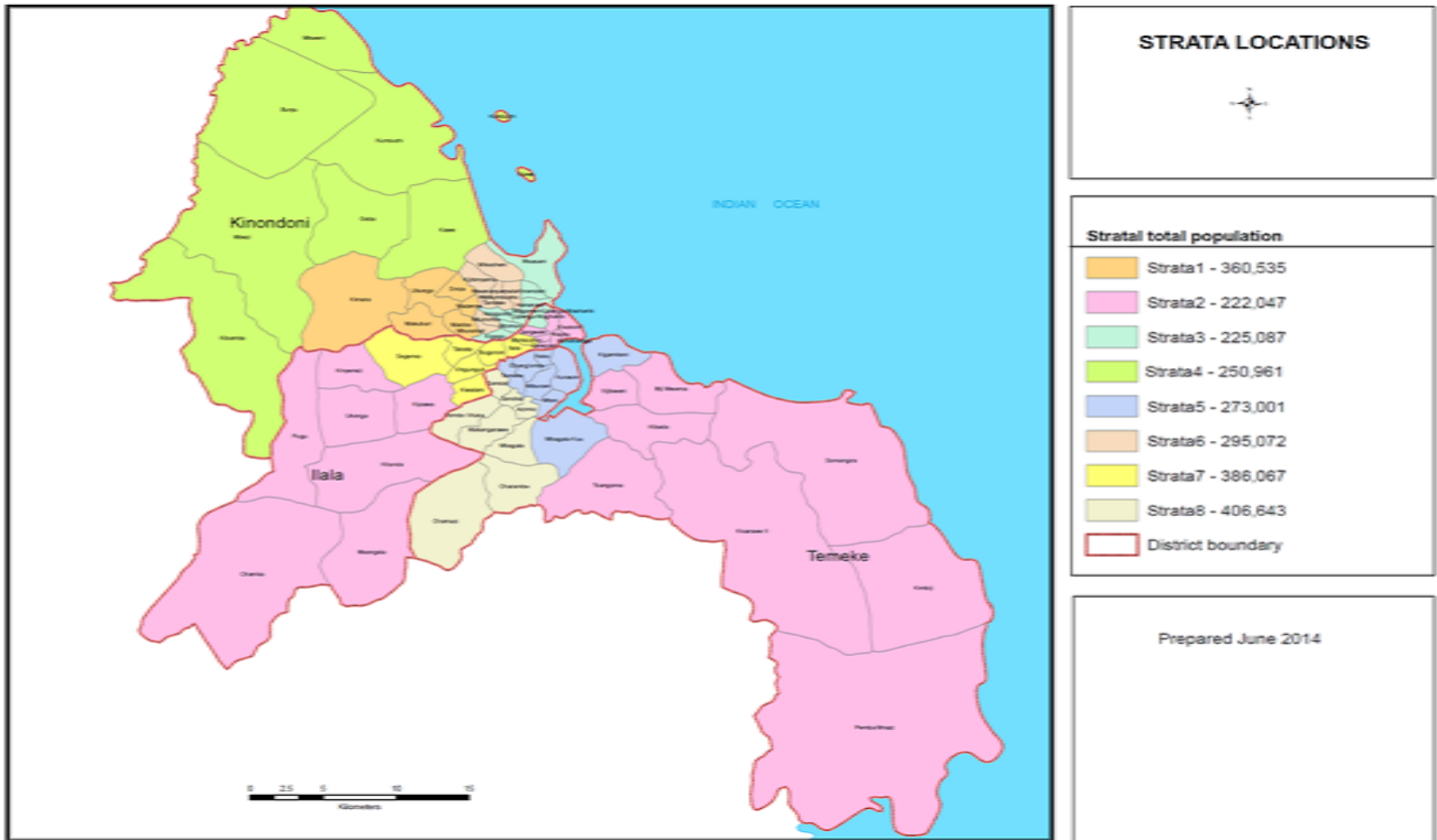


Figure 8.2: Wards based strata for Dar es Salaam City used during the field survey on available energy and uses

8.6 Results and Discussion

8.6.1 State and use of alternative sources of energy in Dar es Salaam

Results from bench study indicates that although hydropower and solar energy are the matured technology extensively existed and studied in Tanzania for over forty years [290,291], they are the least in exploitation to less than 1% [290] while biogas since its inception in Tanzania 65 years ago, with many external supports from such organization like the *Deutsche Gesellschaft für Internationale Zusammenarbeit* (GIZ) by then GTZ, no expansion has been yet in place and mainly the process is biased at institution levels [271] and focused on animal manure as the solitary substrate. However, spoonful initiatives were done to introduce small-scale decentralized biogas systems based on ARTI (Appropriate Rural Technology Institute) Indian invented bioreactor technology capable at digesting 1-2 kg of kitchen waste per day, yet the technology is confirmed less efficiency with daily biogas loss untapped amounting to 22% to the atmosphere [271] due to the fact that the reactors used are non-gastight in operation.

8.6.2 Energy mix used in Dar es Salaam for cooking

Respondents were asked on the type of energy source that is usually used for cooking food at home. The results in Figure 8.3 shows the types of energy mix used in Dar es Salaam for cooking with the corresponding population based on the field survey. Among the 327 respondents interviewed and in most cases physical observations at the houses where the questionnaires were administered face to face, it was revealed that 71% of the city residents depend on charcoal as source of energy for cooking. High cost of electricity and petroleum based fuels coupled with uncertainty in supply are the reasons behind high demands for charcoal in the city.

The average income estimate for Dar es Salaam residents according to the field survey conducted in June 2014 (see Appendix 1 and 2) was estimated to be Tsh. 5,647,706.42 per annum (equivalent to € 2,801.44/year) giving an average of Tsh. 470,642.2 per month (equivalent to € 233.45/month). As most of the residents live in rented houses with an

average number of 4 members in a house [285], then charcoal becomes the best primary energy resort (see Appendix 5 for detailed information). One bag of charcoal (50-60 kg) sold at Tsh. 35,000 to 40,000/= was reported to last for a maximum of 2 weeks thus compounding an expenditure of between Tsh.70, 000/= to 80,000/= for energy per month. Even though firewood fetch lower price compared to charcoal, firewood is not much used in Dar es Salaam although in the order of magnitude shown in Figure 8.3 it ranks the second in consumption (9% of respondents) after charcoal. Firewood availability, difficulties in keeping the woods indoor as firewood requires large space and the dirty involved (soot) on both house and cooking utensils makes firewood unfriendly to most of the city occupants, and in most cases therefore it is used as an emergence alternative just in case of charcoal shortfall. Other sources used as alternative were identified to be saw dusts, motopoa (bio-ethanol gel from molasses) and briquettes from agriculture waste. Biogas, LPG and kerosene equally fall under alternative sources simply because they complement charcoal in cooking certain types of food due to cost involved in getting them or low production that do not guarantee a constant supply.

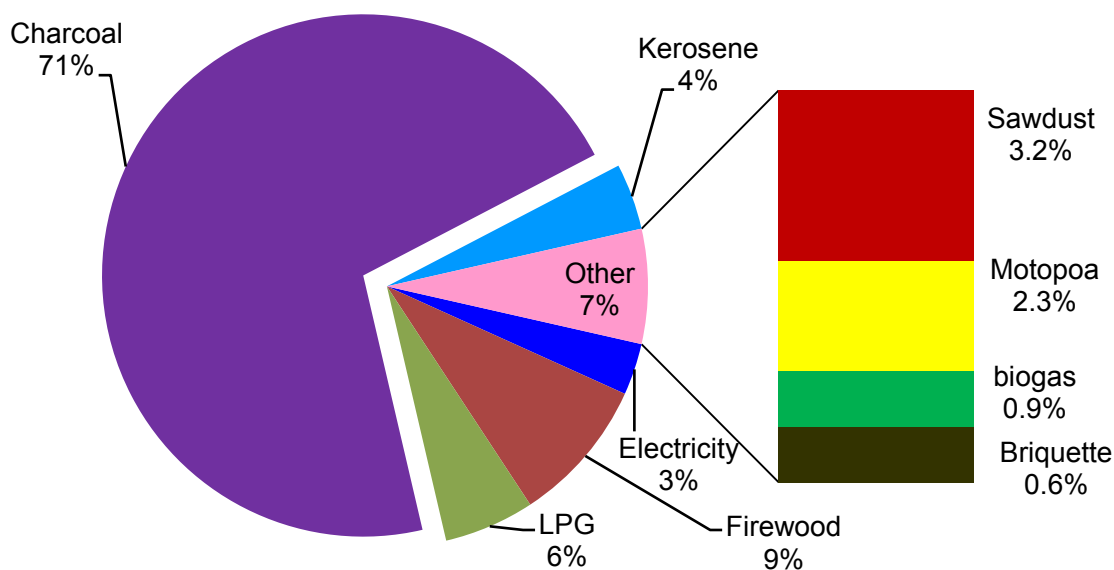


Figure 8.3: Energy –mix used in Dar es Salaam for cooking

Some participants complained that apart from being expensive, gas based fuels are not suitable for cooking some dishes giving examples of beans and rice as they consume

much time and adding that dishes cooked on gas fuels do not taste good. Interview with LPG dealers confirmed that the price and products availability seem to be stable in both rainy and dry seasons compared to charcoal which change according to weather but yet the installation cost of LPG stove and its associated gadget seem unaffordable to majority of the city dwellers. The findings further revealed that 2.4% of the respondents participated in the survey have never heard about biogas. The use-satisfaction of existing energy-mix among the Dar es Salaam inhabitants was assessed and generally there were respondent declared to be satisfied on using any of the existing energy source (Appendix 6) rather most of the respondent showed to have no feasible alternative or substitute. The main reasons for not satisfied being more consumption of cooking time to some of the energy sources, involved health risks, un-reliability in supply, price fluctuation and mostly not affordable in terms of price as summarized in the following Figure 8.4.

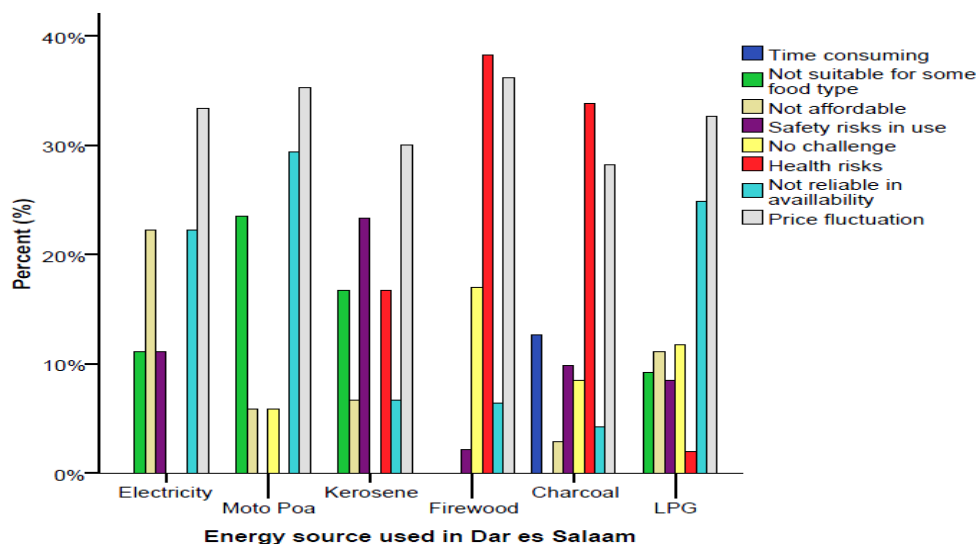


Figure 8.4: Respondents satisfaction on using commonly existing energy-mix

Most of the respondents during the field survey were enthusiastic about biogas production, substrate to be used, cost of installation and payback period. Analysis on the energy challenges facing Dar es Salaam revealed that 64.6% of the residence interviewed are ready to shift from their conventional energy currently in use to biogas while 30.7% were ready to include biogas as alternative source of energy and the remained 4.7% remained undecided with hope of making decision after seeing, tested and undertaking

comparative analysis between the energy source currently in use and the biogas more of the responses can be equally seen in Appendix 7.

8.6.3 Analysis of peels generation, handling and use in Dar es Salaam

Results from field survey in five tradition open market in Dar es Salaam city reveals that, on average 2960 Sacks of potatoes are sold in Dar es Salaam city per day. This amount is equivalent to 340 tons of potatoes consumed per day. A total of 310 potato customers were interviewed at tradition open market in which 80% of them were identified to be chips vendors, 10% claimed to purchase potatoes for making *mchemsho* (food recipe prepared by boiling meat with round potatoes), 8.4% of the customers bought potatoes for their small markets (retailers) while about 1.0% claimed to purchase potatoes for crisps making, and 2 customers just came to buy for social ceremonies. With the stated city population of 4.4million people [285], a consumption rate was derived to be 28.5kg/person/year amount which is higher compared to 10-15kg/person/year estimation by Statistics division of the Food and Agriculture Organization of the United Nations (FAOSTAT) cited in Rahko [292]. It was therefore estimated in this study that potato peels contribute about 31,033 tons/year equivalent to 2.5% of the total MSW generated in Dar es Salaam city [97]. Out of 486 chips vendors visited in the city to identify major producers of the peels, only 37 chips vendors' equivalent to 7.6% claimed to use the peels for feeding animals (cattle and pigs). However, the digestion difficulty of the peels that necessitates treatment prior to feeding was the major concern. Similarly, about 89 chips vendors equivalent to 18.4% claimed to have people who occasionally collect the peels for feeding their animals.

Therefore, with the pre-destined criteria of processing between 2-4 sacks of potatoes per day, sum of 259 chips vendors were identified as major producers of potato peels waste. Applying the established ration of 4:1 (weight of fresh potato tubers to weight of peels), a total of 22.5 tons of potato peels per day were estimated for which 43.6% tons were estimated to come from 114 vendors in Kinondoni, 36% tons from 92 vendors in Ilala and 20.4% tons from 53 vendors in Temeke. If well organized these chips vendors could form an initial collection points (cells) for the peels to be used for biogas or compost production.

Given the present *status quo* of waste management in Tanzania and Dar es Salaam city in particular, this promising huge additional waste calls for immediate attention from the community, environmental scientists and more the policy makers. Among the 259 chips vendors interviewed, 75% claimed to send the peels to the transfer station to be collected by the waste management authority. Other means used as handling of the produced peels and spoiled potato tubers reported during the field survey and their derived proportions are as presented in Figure 8.5 here under and more information see Appendix 3.

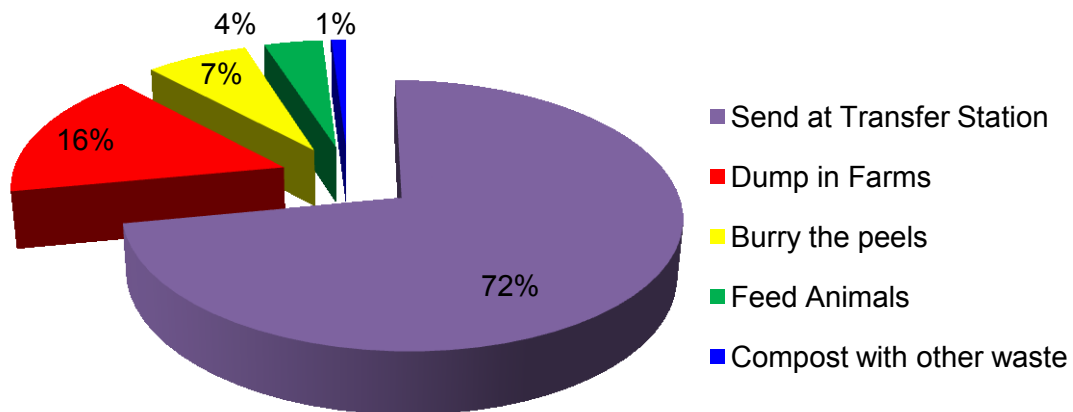


Figure 8.5: Handling potato peels and spoiled potato tubers in Dar es Salaam

Observation on the general management of the peels waste and any existing possible alternative use of the peels waste revealed that among those vendors who claimed to send the peels at transfer station are the ones mostly end up throwing the peels waste haphazardly. The long distance between the designated waste collection point from their business place, the time for which the business close which is usually very late and the poor means used to store and carry the peels are the reasons behind throwing the peels or spoiled potato tubers anyhow. The abandoned rotting potato tubers and the haphazardly thrown peels are source of nuisance and discomfort to the general public given the oozing leachate and off-odours produced as byproduct of aerobic decomposition of these waste given the temperature of the city averaged to 29°C mean annual temperature leading to attracting flies and other insect vectors altogether pose risks to public health due to the threat of outbreak of refuse based diseases which in Dar es

Salaam have become common realities especially during rainy seasons which are normally accompanied by recurring floods.

8.6.4 Knowledge, attitude and practice on peels waste management

Assessment results for potato chips processors based on knowledge, attitude and practices towards proper management of the potato peels waste after chips processing are given in Figure 8.6 and detailed in Appendix 3.

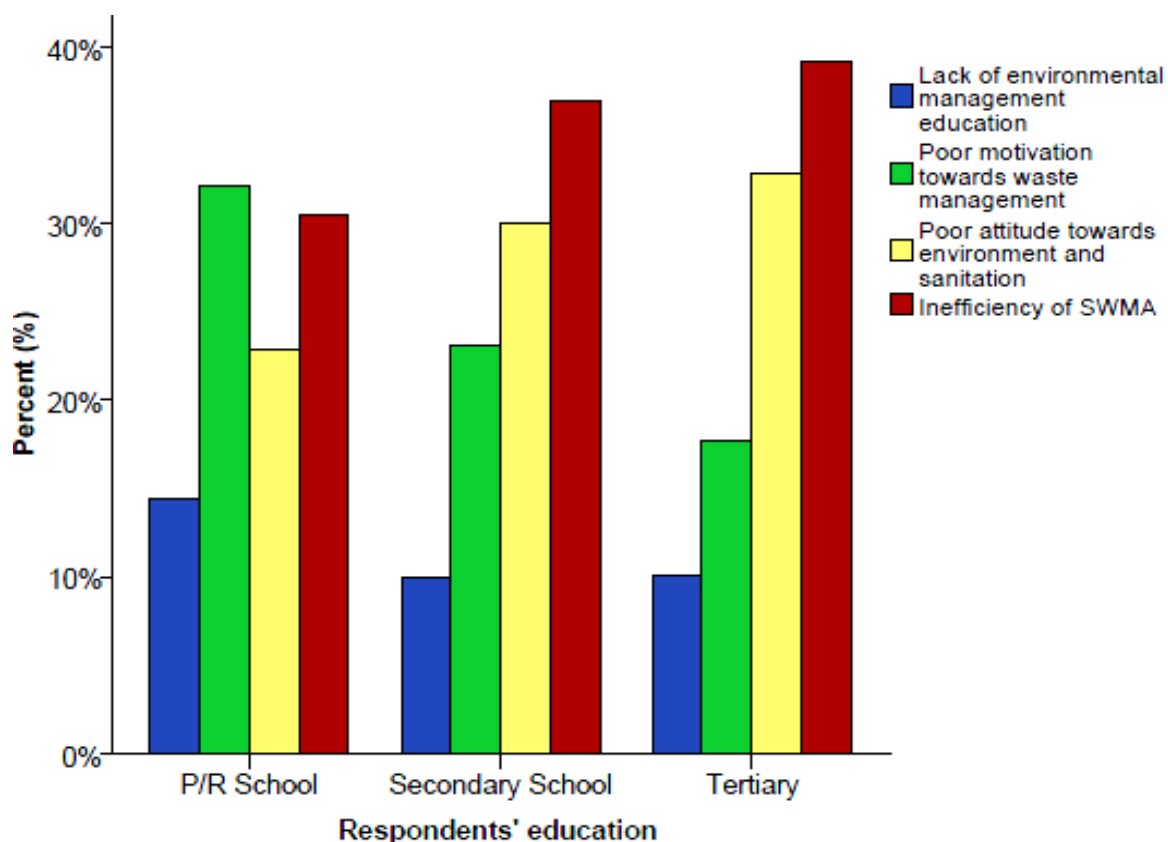


Figure 8.6: Reasons for poor management of potato peels waste in Dar es Salaam

It was worth noting that all respondents had formal education and attended school with the majority 39.7% claimed to have attained secondary school level, 36.1% passed Primary School, 24.2 holding tertiary education and about 9.2% still students at various levels. Education level of the respondent in relation to environmental management was even more reflected when testing whether lack of environmental management knowledge

is the factor behind general poor waste management in the city. More knowledgeable respondent saw it as not a factor behind existing poor sanitation and it was decreasing with an increase in education level. This may be explained by the fact that those attended school up to Secondary education stands a better chance to understand environmental related issues than those with only primary education.

Similar trend was observed on whether or not the community needs motivation in order to participate in waste management programmes. Among the respondents attained primary school level of education, poor motivation and commitment towards waste management was highly rated by 33% followed by inefficiency of MSWA 30%. To this group of respondents, poor attitudes and personal initiative towards environmental management and sanitation was moderately rated while 14% of respondents claim lack of environmental management education as the major reason for poor waste management in the city. An increasing trend was observed among the respondents on who exactly should manage the waste?. similarly to what Kassim and Ali [42] reported, the mid- and higher educated levels respondents pointed more to the solid waste management authority (SWMA). It was observed that 38% and 40% of secondary school level and tertiary level respondents sees that MSWA inefficiency towards collection of MSW is a major reason.

A positive trend was observed on the poor community attitudes towards environment and sanitation. Among the respondents, 23% of those with primary education, 30% of those having secondary school level and 35% of respondents with tertiary levels of education accepted that poor attitude towards environment and sanitation is a challenge that needs to be addressed for the existing poor waste management in Dar es Salaam. All the groups agree on the importance of motivation towards sensitizing the community on proper management of the generated waste. Environmental management knowledge though was not given a due weight in comparisons to the other three factors, yet the community concurred on the need of having this knowledge so as to foster proper management of waste and recovery of useful resources. Studies on possibility of using fresh potato peels as feed for non-ruminant animals in particular pigs proved abortive due to high water and fibre content in the peels which impairs digestibility in the stomach. Some of the livestock keepers have tried to use the peels to feed their animals, but there has been one reason

or the other that hinders many livestock keepers from using potato peels as feed to their flocks. Some of the portrayed reasons during the field survey were as follows:

- (i) Potato peel wastes are perishable and must be used quickly and within a close proximity due to both microbial (yeast, mould and bacteria) and enzymatic spoilage while livestock are kept at the peri-urban (Oral information: Mrs. Ngopi, 2014/06/13; Ms. Violet, 2014/07/24).
- (ii) The peels consumption level in animals is limited due to lack of processing know-how and absence of reliable energy source to cook them before feeding to animals especially pigs (Oral information: Mungure and Veronica 2014/06/21; Mama Mapunda, 2014/07/17).
- (iii) Some livestock keepers claim that poor handling of the peels at source induces worms when raw fed to animal, also causes blotting and diarrhea. This makes cooking of the peels before feeding to animals an important but tedious and time consuming process hence unfriendly to most of livestock keepers (Oral information: Mama Leah, 2014/06/21; Elias and Nnkuu, 2014/08/08).
- (iv) Fresh potato peels are not palatable to animal and therefore only small portion is consumed if mixed with other concentrates such as maize bran and roughages. High water content in the peels affect feed intake causing impaired growth among piglets (Oral information: Mungure, 2014/06/21; Mapunda, 2014/07/17; Yusuph, 2014/07/14 and Mshana, 2014/07/14).
- (v) Mr. Ngaiza and Ms. Grace Maagi, 2014/07/23 explained that high sun-light in the activate production and accumulation of green-like pigments (scientifically known as glycoalkaloids toxins) on peels. Similar information relate to the findings by Cantwell [293] who further expound that feeding animals with peels accumulated with glycoalkaloids toxins as such induce health risks to animals due to poison.

8.6.5 Expected contribution of biogas generated from potato peels to energy –mix in Dar es Salaam

The population distribution and housing census of 2012 for Dar es Salaam stand at 4 people in each house [285]. Table 8-1 shows the results on estimated daily demand for wood fuels (charcoal and firewood) in the city which is supplied from exploitation of the forest resource to provide the cooking energy among the city dwellers.

Table 8-1: Daily consumption of wood fuels (Tons) for cooking in Dar es Salaam

Stratum	Household ¹⁸	Wood fuels		Forest loss (Tons/day)
		Consumption (Tons)		
		Charcoal ¹⁹	Firewood ²⁰	
1	90134	127.9	21.4	603.1
2	55512	78.8	13.1	371.4
3	56272	79.9	13.3	376.5
4	62740	89.0	14.9	419.8
5	68250	96.9	16.2	456.7
6	73768	104.7	17.5	493.6
7	96517	137.0	22.9	645.9
8	101661	144.3	24.1	680.3
Total	604,854	858.9	143.7	4047.8

Considering an average percentage conversion rate of 22% for wood to charcoal based on wood carbonization in both traditional unimproved and semi improved kilns [286,289], it is established that approximately 4.1 kilotons of the forest undergo deforestation every day being turned into wood fuel as energy source. This estimated value of forests loss correspond well to the value of 4.4 kilotons reported in Hoffmann et al., [294] and 5.2 kilotons estimated in Msuya et al., [286] reported as forests that are being cleared

¹⁸ Results estimated from Wards population based on the rate of 4 people per household

¹⁹ Results calculated based on 71% of City Residents using charcoal as energy source

²⁰ Results calculated based on 9% of City Residents using firewood as energy source

every day for making charcoal used in Dar es Salaam city. Biogas produced from potato peels has a potential of bridging this existing households energy-challenge and therefore reduce the rate of deforestation. From the results on biogas generation from potato peels given in clause 7.7.6 of Chapter 7 and Table 7-11 in this thesis, it was estimated that 10.2kg (wet weight) of the potato peels waste is required to produce 1.0m³ of biogas. Voegeli et al.,[271] and Riuji [142] estimated that 0.2m³ of biogas with above 56% CH₄ content burns for about 45 -60min, and according to Vögeli et al.,[110], 1.0m³ of biogas with such methane quality produces calorific value of 6kWh which is equivalent to 21.6MJ of energy. FAO through FAO/TCP/NET/4415-T (Nepal) estimates that 1.0m³ of biogas generated from biowaste produce heat energy equivalent to that generated from burning 3.5kg of firewood. This means that anaerobic digestion of about 3kg of potato peels waste can produce similar calorific values as burning 1kg of well seasoned firewood.

The quantity of peels waste entering MSW stream in Dar es Salaam equally reported in Muhondwa et al., [97], is estimated at 32kilotons per annum averaged to 85tons production per day. In order to replace the fuelwood as cooking energy to a total of 604,854 households in Dar es Salaam city therefore, a family size decentralized anaerobic reactors of size 5-8m³ digesting potato peels waste at the HRT of 20-30days and produces up to 2.0m³ of biogas per day that is estimated sufficient to produce 5hrs daily cooking time using efficient biogas stoves. The biogas produced is enough to forego almost 7.0kg of fuel-wood in a day which is equivalent to 2.6tons, estimated to more than half of the forest trees consumed per annum in Dar es Salaam city alone as fuel for cooking. Given the endowed aforementioned advantages of anaerobic digestion of potato peels for Dar es Salaam and the fact that together with causing environmental tenacity, potato waste presently have no economic use value in the Dar es Salaam, it is therefore feasible to invest in biogas project which will among other things reduce the volume of MSW to be handled by the municipal solid waste authorities and at the same time recycle vital plants nutrients in the agricultural lands through the application of digestate.

8.7 Conclusive summary

Processing of potato into chips and other value added products in Dar es Salaam city is a growing business. This assures constant availability of feedstock and substrate that would be required during biological treatment process. In recognition of the environmental degradation, outbreaks of refuse based diseases and dilapidated infrastructures associated with poor management of MSW in the city, implementation of biotreatment of potato peels waste in Dar es Salaam city is considered a prudent and sustainable decision. Specifically, a number of significant benefits are expected. These include among others:

- (i) Reduction in the volume of MSW fraction thus minimize costs associated with solid waste management
- (ii) Minimization of environmental pollution including soil, surface and ground waters through leachate and off-odours,
- (iii) Minimization of CH₄ and CO₂ emissions resulting from decomposition of the peels which lead to greenhouse effect and subsequently global warming,
- (iv) Minimization of environmental health problems such as diarrhea and many other refuse related diseases,
- (v) Enhancement of nutrients recycling into soil through the use resulting compost and digestate, and
- (vi) Minimization of costs of buying electricity for lighting and cooking purpose as the biogas would be sustainable contrary to the existing hydroelectric power and complement wood-fuel based energy used for cooking and therefore play an important role in mitigating deforestation and destruction to biodiversity.

It is therefore upon the decision making authorities to encourage, promote, implement and acknowledge the scientific finding reported in this research for the realization of the potential of potato peels as resource not to be wasted.

9 General conclusions and Recommendations

9.1 Conclusions

One most important task of our time is to ensure total protection of public health by preventing environmental contamination from all forms of contaminants and in particular the refuse based portions. Unless hygienization processes are applied, there exist human health risks due to microbial contamination while handling biowaste along the waste management chain. Prevalence of microorganism pathogens in biowaste has raised concern on the biosecurity aspect while using biowaste as feed during biological treatment and also during handling of the final byproducts (compost and digestate) if unhygienized. The finding from this research on the presence of high population of *Enterobacteriaceae* in biowaste, soil and leachate samples justifies the problem and therefore the need for serious application of hygienization process for safer biotreatment and the use of the organic waste.

In-vessel composting system is confirmed technically not capable at effecting fully inactivation of microorganism pathogens, especially if moisture content in the biowaste is higher above the required composting range making co-composting of the feedstock inevitable. This leaves one message to the compost producers and agriculture practitioners that not every heap of rotten garbage means compost. Similarly, mesophilic/mesophilic and mesophilic/thermophilic two-phase system of anaerobic digesters operated as continuous stirred tank reactors proved to be technically not feasible to fully produce pathogens free digestate unless the suspension menstruum residence in the respective reactor is not less than 72hrs and 48hrs respectively. As a matter of balancing energy use and system stability to biogas production, most of the biotreatment facilities prefer mesophilic anaerobic systems which run continuously. In this kind of situation, external hygienization aimed to ensure biosecurity becomes a unit not to be bypassed. In this research, existing and alternatives pre and/or post-biotreatment hygienization options proved promising at effecting complete pathogens die-off in biowaste before biological treatment process or in the resulting byproducts after the biological treatment though at various levels of efficacy.

The use of UVC light at reducing enteropathogens population in hydrolysate reactor of two-phase anaerobic digestion process is possible. Installation of UVC system in a mixing tank of the process water prior to feeding into the methanation reactor would increase pathogens inactivation due to the turbulence created during mixing which would in principal expose most if not all pathogens to the radiation and become deactivated. On the other hand, to energy challenged places but with unlimited sun shine, passive solar heating (PSH) proved technically effective as pre and post- biotreatment hygienization option of contaminated biowaste and could be used during the compost curing phase which goes together with the maturation process. Even though most of these experiments have been performed under laboratory scale, the described Hygienization concepts, techniques and output can be proportionally transferred into pilot and large scale commercial composting and biogas production plants.

Feasibility of managing potato peels as fraction of biodegradable waste from the MSW stream in Dar es Salaam is possible. Co-composting of potato peels waste with yard-waste MSW stream resulted into enhanced temperature-time pattern of the composting process thus ensures microbiological safe compost with good quality in terms of physico-chemical and sensory attributes. Higher moisture of the potato peels waste and the presence of optimum physico-chemical attributes make this waste an ideal substrate for anaerobic digestion. Mesophilic/thermophilic two-phase anaerobic digestion of potato peels waste even at 2.40 OLR $\text{kgTS}/\text{m}^3\cdot\text{day}$ showed no sign of inhibition and was stable demonstrating methane generation rate of 0.13-0.35 m^3/kgTS input with up to 81% volatile solid removal. Production of biogas as cooking energy source play a conservation role to forest resource up to 4047.8 tons per day which are highly exploited because of the demand for wood-fuels in Dar es Salaam. Other intertwined benefits from biotreatment of potato peels include: reduction in income poverty through self employment as the biogasification process would provide employment to the individuals as biogas producers within the community, reduction in budget allocated for solid waste management, reduction of GHGs emissions, elimination of bad odour from decomposing peels and avoid the foreseen risks to the environment and health problems to the immediate community and the whole country at large.

In this study as it can be observed, both academic attributes and practical application of the findings have been presented. It is therefore anticipated that microbiologists, environmental engineers and solid waste managers will find the findings in this research useful for safe anaerobic digestion and safe production of compost and digestate without compromising on the quality of environmental components, biodiversity and human health. Although potato peels waste was the only biowaste fraction used in all experiments in this research, yet most of the procedures and methods on specific experiment presented or used in the study could be possibly adapted to other source-segregated biowaste as a whole or applied with minor validation. It is therefore supposed that the findings in this study if implemented could eliminate biosecurity risks associated with haphazardly disposal of organic waste thus improve living situation to the general public at large. However the study has the following set of recommendations:

9.2 Recommendations

- i. As a strategy to curb emergence of pathogens and solve the global health threats, there should be hygienization based legislations that are harmonized on safe production and application of digestate and/or compost so that to effects that desirable outcomes on pathogens elimination for safer world thus solve the global health problems regardless of regional differences.
- ii. In order to avoid spreading of disease causing agents to the environment that passed partially or totally unaffected from the waste treatment facilities, there should be recommended process control in biological waste treatment facilities. Among others the traceability of raw waste resource from collection source, transportation, treatment until final disposal of the finished product need to be under control and clearly stated to attest biosafety of the compost or digestate .
- iii. Environmental laboratories should be considered an integral part of the hygienization process of biowaste before and after composting and/or anaerobic digestion. Safety of the byproducts (compost and digestate) should be assured before the products can be used for land application.

- iv. All investigated hygienization options reported in this research can be used only with prior knowledge on types of pathogens and the aspect of economic applicability of each option to enable producers make correct decisions regarding which hygienization options to use according to their choice and the level of pathogens die-off desired
- v. More research is required on the repeatable use of acetic acid in terms of robustness of the method at conditioning of biowaste without compromise on its hygienization efficacy. Equally, a full scale use of acetic acid on biomas (real sample) is required to confirm its full use in the long run application.
- vi. Exploitation of potato peels waste for biogas production would be the best option in reducing the amount of waste that needs to be collected and disposed of as well managing ecology, diversity and forest resource from degradation. In Tanzania, for instance, environmental degradation caused by removal of CO₂ sink and emission of greenhouse gases mostly from poorly managed organic waste fractions are the key reasons behind the experienced weather variability and climate change impacts.
- vii. There should be in place adequate litter bins to stop littering. A policy accompanied with By-laws governing Dar es Salaam community and Tanzania at large on the importance and advantages of source separation of waste. In this aspect, intervention from Government/NGO/Private sector/Partnerships and cooperation on provision of waste collection bins enough for the process is of paramount importance.
- viii. Decision makers and the Dar es Salaam city Councils should embark and invest on waste recycling and recovery of resource from the biodegradable fractions of MSW. In that doing, biomedical waste including animal by-products waste should strictly not be dumped in commingled with other OFMSW. Additionally, the Municipalities should also seek upgrading of the Pugu dump to an engineered landfill so that to prevent the ongoing environmental pollution given the *status quo*.

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Appendices

Appendix 1: Semi-structured interview questions

Knowledge Attitude and Practices towards management of potato peel waste (Potato chips/crisps Vendor²¹)

1. Gender of respondent (*please tick*) **Female** () **Male** ()
2. Age of respondent (*please tick the corresponding age group*)
 15-24 25-34 35-44 45-54 55-64 65-Above
3. Name of the area/Ward where the respondent reside -----
4. How many sacks (*gunia*) of potatoes do you process in a day? -----
5. How do you handle potato peels after chips/crisps making? (*please rank the option*)
 - Throw them openly for the SWMA to collect them ()
 - Collect and dump at specified dumpsite ()
 - Burry them while fresh in farms ()
 - Collect and feed to animals ()
 - Composting with other wastes and use the compost for agriculture ()
 - Other options, please describe -----
6. Of the following options, which is your preferred way of managing potato peels waste as part of MSW? (*You can choose more than one option*).
5 = most preferred option, **4** = a preferred option, **3** = Neutral, **2** = not preferred option, **1** = not a preferred option at all.
 - Burry the waste in farm ()
 - Collecting and dump at specified dumpsite ()
 - Collect and dump the peels at specified dumps ()
 - Composting of waste for agriculture use ()
 - Anaerobic digestion for biogas production ()
7. Are you satisfied with the way potato peels waste are handled at your premises? ()
5 = very satisfied, **4** = satisfied, **3** = Neutral, **2** = dissatisfied, **1** = very dissatisfied.
8. What is the challenge about management of potato peels waste?

²¹ Vendor include large, small and medium enterprise (SMEs) dealing with potato chips/ crisps processing.

- Spoil quickly ()
 - Unplanned collection schedule ()
 - Poor city infrastructure for collectors articulation ()
 - No ample land to bury the waste ()
 - No animals to feed the peels ()
9. How do you handle the spoiled potatoes (*please choose only one*) ()
- 1= collect and feed to animals, 2= burry in farms, 3= through them for SWMA to collect, 4= compose for land application, 5= give to private collector to dispose
10. What do you consider the reason for poor waste management in Dar es Salaam city?
- Lack of environmental management education? ()
 - Poverty ()
 - Inefficiency of SWMA ()
 - Poor attitude towards environment sanitation ()

2. Energy mix, use and satisfaction (*General Public Survey*)

Demographic and Socio-economic characteristics of respondents

11. Gender of respondent (*please tick*) Female () Male ()
12. Age of respondent (*please tick the corresponding age group*)
- 15-24 25-34 35-44 45-54 55-64 65-Above
13. Name of the area/Ward where the respondent reside -----
14. Position of respondent in household (*please tick*) 1=Father () 2= Mother ()
- 3= Child () 4= Other, please explain -----
15. What is education level of the respondent (*please choose*) ()
- 1= None, 2= P/R School, 3= Secondary School, 4= Tertiary
16. Work or business status: (*please tick*) 1= Student () 2= Self employed 3= Employee () 4= Retired ()
17. What is the collective monthly income (in Tsh. x1000) of the household (*please tick only one*)
- 200 or less 300 – 600 700 – 1000 Above 1000
18. How many people (children and adults) live in the household? -----

19. What kind of energy source do you use for cooking at home? (*you may tick more than one source*)

Firewood Charcoal Kerosene LPG Electricity Moto-Poa

Others, specify -----

20. How much do you pay per **month** for that kind of energy? ----- Tsh

21. Are you satisfied with that kind of energy? **Yes** () **No** () **No alternative** ()

22. Is there challenge(s) you face before getting the energy source? **Yes** () **No** ()

23. If the answer above is **Yes**, what challenge(s) -----

24. What challenges do you face while using such type of energy source for cooking? --

25. What energy source do you prefer as alternative for cooking?

Firewood Charcoal Kerosene LPG Electricity Moto-Poa

Others, specify -----

26. How much do you pay per month for that alternative source? -----

27. Have you heard of biogas, as energy source? **Yes** () **No** ()

28. If biogas is made available, are you willing to shift from the energy source you are using to biogas? **Yes** () **No** ()

29. If the answer above was **No** please explain why? -----

30. If the answer was yes, will you be willing to pay the same amount of money for biogas similar to what you are currently paying for energy? **Yes** () **No** ()

31. If the answer was No, please explain why not: -----

32. In case additional costs associated with biogas arise, how much are you willing to pay at maximum (*please tick*)

10% 20% 30% 40% 50% > 50%

3: Potatoes Business information (*potato stakeholders at TOM²²*)

33. Gender of respondent (*please tick*) Female () Male ()

²² Stakeholders includes but not limited to: Whole sellers at TOM, retailers, potato peelers, chips and crisps processors, chips and crisps buyers, etc

TOM = tradition open market

34. Age of respondent (*please tick the corresponding age group*)

15-24 25-34 35-44 45-54 55-64 65-Above

35. Name of the area/Ward where the respondent reside -----

36. How many tons of potatoes do you receive in the tradition open market per day? ---

37. How many sacks of potatoes do you sell per day? -----

38. In case of a bad market, how do you handle spoiled potato tubers?

- Collecting and dump at specified dumpsite ()
- Burry them in farms ()
- Wait for private waste collectors ()

4: Expert opinions based on customers' willingness to pay (WTP) for LPG

39. Gender of respondent (*please tick*) Female () Male ()

40. Age of respondent (*please tick the corresponding age group*)

15-24 25-34 35-44 45-54 55-64 65-Above

41. Name of the area/Ward where the respondent reside -----

42. How much do people pay for LPG? ----- Tsh

43. Which size of the LPG cylinder customers prefer the most? (*please tick only one*) 1 kg (), 6 kg (), 15 kg (), 38 kg ()

44. Have you noticed any changes in customer's purchase of LPG based on seasonal variability? **Yes** (), **No** ()

45. Does the price of LPG change in the market? **Yes** (), **No** () (If yes *please specify*)
During rainfall () or during dry season ()

*** Thank you for your time and cooperation..! ***

Appendix 2: Respondent's cumulative monthly income by gender (n=327)

Respondent's Monthly income*		Respondent's Gender		Total
		Male	Female	
Tsh. 200,000 or less	Count	23	42	65
	% within Respondent's Monthly income	35.4%	64.6%	100.0%
	% within Respondent's Gender	16.8%	22.1%	19.9%
	% of Total	7.0%	12.8%	19.9%
Tsh. 300,000-600,000	Count	70	114	184
	% within Respondent's Monthly income	38.0%	62.0%	100.0%
	% within Respondent's Gender	51.1%	60.0%	56.3%
	% of Total	21.4%	34.9%	56.3%
Tsh. 700,000-1,000,00	Count	33	26	59
	% within Respondent's Monthly income	55.9%	44.1%	100.0%
	% within Respondent's Gender	24.1%	13.7%	18.0%
	% of Total	10.1%	8.0%	18.0%
>Tsh. 1,000,000	Count	11	8	19
	% within Respondent's Monthly income	57.9%	42.1%	100.0%
	% within Respondent's Gender	8.0%	4.2%	5.8%
	% of Total	3.4%	2.4%	5.8%
Total	Count	137	190	327
	% within Respondent's Monthly income	41.9%	58.1%	100.0%
	% within Respondent's Gender	100.0%	100.0%	100.0%
	% of Total	41.9%	58.1%	100.0%

* € 1 was equivalent to Tsh. 2016 applicable exchange rate for August 17, 2014 (www.crdbbank.com)

Appendix 3: Dar es Salaam Chips vendors satisfaction on management of potato peels waste (n =259)

Vendor satisfaction with management of PPW		Respondent's gender		Total
		Male	Female	
Satisfied	Count	6	1	7
	% within Vendor satisfaction with management of PPW	85.7%	14.3%	100.0%
	% within Respondent's gender	2.4%	16.7%	2.7%
	% of Total	2.3%	.4%	2.7%
Neutral	Count	37	0	37
	% within Vendor satisfaction with management of PPW	100.0%	.0%	100.0%
	% within Respondent's gender	14.6%	.0%	14.3%
	% of Total	14.3%	.0%	14.3%
Dissatisfied	Count	146	3	149
	% within Vendor satisfaction with management of PPW	98.0%	2.0%	100.0%
	% within Respondent's gender	57.7%	50.0%	57.5%
	% of Total	56.4%	1.2%	57.5%
Very dissatisfied	Count	64	2	66
	% within Vendor satisfaction with management of PPW	97.0%	3.0%	100.0%
	% within Respondent's gender	25.3%	33.3%	25.5%
	% of Total	24.7%	.8%	25.5%
Total	Count	253	6	259
	% within Vendor satisfaction with management of PPW	97.7%	2.3%	100.0%
	% within Respondent's gender	100.0%	100.0%	100.0%
	% of Total	97.7%	2.3%	100.0%

Appendix 4: Challenges respondent face while using energy and possibility to shift to Biogas

List of challenges respondent face while using the energy		If the respondent is ready to shift from current energy to Biogas		Total
		No	Yes	
No challenge	% within List of challenges respondent face while using the energy	29.4%	70.6%	100.0%
	% within If the respondent is ready to shift from current energy to Biogas	5.9%	5.0%	5.2%
	% of Total	1.5%	3.7%	5.2%
Quality fluctuation	% within List of challenges respondent face while using the energy	28.9%	71.1%	100.0%
	% within If the respondent is ready to shift from current energy to Biogas	41.2%	35.5%	37.0%
	% of Total	10.7%	26.3%	37.0%
Time consuming in operation	% within List of challenges respondent face while using the energy	22.5%	77.5%	100.0%
	% within If the respondent is ready to shift from current energy to Biogas	21.2%	25.6%	24.5%
	% of Total	5.5%	19.0%	24.5%
Not suitable for certain food type	% within List of challenges respondent face while using the energy	28.6%	71.4%	100.0%
	% within If the respondent is ready to shift from current energy to Biogas	4.7%	4.1%	4.3%
	% of Total	1.2%	3.1%	4.3%
Health risks	% within List of challenges respondent face while using the energy	28.6%	71.4%	100.0%

	% within If the respondent is ready to shift from current energy to Biogas	11.8%	10.3%	10.7%
	% of Total	3.1%	7.6%	10.7%
Far from Home	% within List of challenges respondent face while using the energy	28.0%	72.0%	100.0%
	% within If the respondent is ready to shift from current energy to Biogas	8.2%	7.4%	7.6%
	% of Total	2.1%	5.5%	7.6%
Safety risks	% within List of challenges respondent face while using the energy	22.2%	77.8%	100.0%
	% within If the respondent is ready to shift from current energy to Biogas	4.7%	5.8%	5.5%
	% of Total	1.2%	4.3%	5.5%
Not reliable	% within List of challenges respondent face while using the energy	11.8%	88.2%	100.0%
	% within If the respondent is ready to shift from current energy to Biogas	2.4%	6.2%	5.2%
	% of Total	.6%	4.6%	5.2%
Total	% within List of challenges respondent face while using the energy	26.0%	74.0%	100.0%
	% within If the respondent is ready to shift from current energy to Biogas	100.0%	100.0%	100.0%
	% of Total	26.0%	74.0%	100.0%

Appendix 5: Primary energy sources used in Dar es Salaam with respect to Household size

Number of people in the household		Primary energy source used in Dar es Salaam					Total	
		Firewood	Charcoal	Kerosene	LPG	Electricity		Moto-Poa
1	Count	0	3	0	1	0	1	5
	Expected Count	.5	3.4	.0	1.0	.1	.0	5.0
	% within Number of people in the household	.0%	60.0%	.0%	20.0%	.0%	20.0%	100.0%
	% within Primary energy source used in Dar es Salaam	.0%	1.4%	.0%	1.6%	.0%	50.0%	1.5%
2	Count	1	10	2	8	1	0	22
	Expected Count	2.2	14.9	.2	4.3	.3	.1	22.0
	% within Number of people in the household	4.5%	45.5%	9.1%	36.4%	4.5%	.0%	100.0%
	% within Primary energy source used in Dar es Salaam	3.1%	4.5%	66.7%	12.5%	25.0%	.0%	6.7%
3	Count	8	35	1	9	1	1	55
	Expected Count	5.4	37.3	.5	10.8	.7	.3	55.0
	% within Number of people in the household	14.5%	63.6%	1.8%	16.4%	1.8%	1.8%	100.0%

	% within Primary energy source used in Dar es Salaam	25.0%	15.8%	33.3%	14.1%	25.0%	50.0%	16.8%
4	Count	9	78	0	21	1	0	109
	Expected Count	10.7	74.0	1.0	21.3	1.3	.7	109.0
	% within Number of people in the household	8.3%	71.6%	.0%	19.3%	.9%	.0%	100.0%
	% within Primary energy source used in Dar es Salaam	28.1%	35.1%	.0%	32.8%	25.0%	.0%	33.3%
5	Count	9	51	0	18	1	0	79
	Expected Count	7.7	53.6	.7	15.5	1.0	.5	79.0
	% within Number of people in the household	11.4%	64.6%	.0%	22.8%	1.3%	.0%	100.0%
	% within Primary energy source used in Dar es Salaam	28.1%	23.0%	.0%	28.1%	25.0%	.0%	24.2%
6	Count	5	31	0	6	0	0	42
	Expected Count	4.1	28.5	.4	8.2	.5	.3	42.0
	% within Number of people in the household	11.9%	73.8%	.0%	14.3%	.0%	.0%	100.0%
	% within Primary energy source used in Dar es Salaam	15.6%	14.0%	.0%	9.4%	.0%	.0%	12.8%
7	Count	0	8	0	1	0	0	9
	Expected Count	.9	6.1	.1	1.8	.1	.1	9.0

	% within Number of people in the household	.0%	88.9%	.0%	11.1%	.0%	.0%	100.0%
	% within Primary energy source used in Dar es Salaam	.0%	3.6%	.0%	1.6%	.0%	.0%	2.8%
8	Count	0	5	0	0	0	0	5
	Expected Count	.5	3.4	.0	1.0	.1	.0	5.0
	% within Number of people in the household	.0%	100.0%	.0%	.0%	.0%	.0%	100.0%
	% within Primary energy source used in Dar es Salaam	.0%	2.3%	.0%	.0%	.0%	.0%	1.5%
9	Count	0	1	0	0	0	0	1
	Expected Count	.1	.7	.0	.2	.0	.0	1.0
	% within Number of people in the household	.0%	100.0%	.0%	.0%	.0%	.0%	100.0%
	% within Primary energy source used in Dar es Salaam	.0%	.5%	.0%	.0%	.0%	.0%	.3%
Total	Count	32	222	3	64	4	2	327
	Expected Count	32.0	222.0	3.0	64.0	4.0	2.0	327.0
	% within Number of people in the household	9.8%	67.9%	.9%	19.6%	1.2%	.6%	100.0%
	% within Primary energy source used in Dar es Salaam	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

Appendix 6: Challenges respondent face while using the primary energy source of their choice

Challenges respondent face while using the energy		Primary energy source used in Dar es Salaam						Total
		Firewood	Charcoal	Kerosene	LPG	Electricity	Moto-Poa	
No challenge	Count	0	6	0	11	0	0	17
	% within Challenges respondent face while using the energy	.0%	35.3%	.0%	64.7%	.0%	.0%	100.0%
	% within Primary energy source used in Dar es Salaam	.0%	2.7%	.0%	17.2%	.0%	.0%	5.2%
	% of Total	.0%	1.8%	.0%	3.4%	.0%	.0%	5.2%
Quality fluctuation	Count	0	121	0	0	0	0	121
	% within Challenges respondent face while using the energy	.0%	100.0%	.0%	.0%	.0%	.0%	100.0%
	% within Primary energy source used in Dar es Salaam	.0%	54.5%	.0%	.0%	.0%	.0%	37.0%
	% of Total	.0%	37.0%	.0%	.0%	.0%	.0%	37.0%
Time consuming in operation	Count	3	77	0	0	0	0	80
	% within Challenges respondent face while using the energy	3.8%	96.2%	.0%	.0%	.0%	.0%	100.0%

	% within Primary energy source used in Dar es Salaam	9.4%	34.7%	.0%	.0%	.0%	.0%	24.5%
	% of Total	.9%	23.5%	.0%	.0%	.0%	.0%	24.5%
Not suitable for certain food type	Count	0	0	0	14	0	0	14
	% within Challenges respondent face while using the energy	.0%	.0%	.0%	100.0%	.0%	.0%	100.0%
	% within Primary energy source used in Dar es Salaam	.0%	.0%	.0%	21.9%	.0%	.0%	4.3%
	% of Total	.0%	.0%	.0%	4.3%	.0%	.0%	4.3%
Health risks	Count	19	15	1	0	0	0	35
	% within Challenges respondent face while using the energy	54.3%	42.9%	2.9%	.0%	.0%	.0%	100.0%
	% within Primary energy source used in Dar es Salaam	59.4%	6.8%	33.3%	.0%	.0%	.0%	10.7%
	% of Total	5.8%	4.6%	.3%	.0%	.0%	.0%	10.7%
Far from Home	Count	10	0	0	13	0	2	25
	% within Challenges respondent face while using the energy	40.0%	.0%	.0%	52.0%	.0%	8.0%	100.0%
	% within Primary energy source used in Dar es Salaam	31.2%	.0%	.0%	20.3%	.0%	100.0%	7.6%
	% of Total	3.1%	.0%	.0%	4.0%	.0%	.6%	7.6%
Safety risks	Count	0	0	1	17	0	0	18

	% within Challenges respondent face while using the energy	.0%	.0%	5.6%	94.4%	.0%	.0%	100.0%
	% within Primary energy source used in Dar es Salaam	.0%	.0%	33.3%	26.6%	.0%	.0%	5.5%
	% of Total	.0%	.0%	.3%	5.2%	.0%	.0%	5.5%
Not reliable	Count	0	3	1	9	4	0	17
	% within Challenges respondent face while using the energy	.0%	17.6%	5.9%	52.9%	23.5%	.0%	100.0%
	% within Primary energy source used in Dar es Salaam	.0%	1.4%	33.3%	14.1%	100.0%	.0%	5.2%
	% of Total	.0%	.9%	.3%	2.8%	1.2%	.0%	5.2%
Total	Count	32	222	3	64	4	2	327
	% within Challenges respondent face while using the energy	9.8%	67.9%	.9%	19.6%	1.2%	.6%	100.0%
	% within Primary energy source used in Dar es Salaam	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
	% of Total	9.8%	67.9%	.9%	19.6%	1.2%	.6%	100.0%

Appendix 7: Respondent's willingness to shift from current energy source to Biogas

Primary energy source used in Dar es Salaam		If respondent is willing to shift from current energy to Biogas		Total
		No	Yes	
Firewood	Count	6	26	32
	Expected Count	8.5	23.5	32.0
	% within Primary energy source used in Dar es Salaam	18.8%	81.2%	100.0%
	% within If the respondent is ready to shift from in use energy to Biogas	6.9%	10.8%	9.8%
	% of Total	1.8%	8.0%	9.8%
Charcoal	Count	61	161	222
	Expected Count	59.1	162.9	222.0
	% within Primary energy source used in Dar es Salaam	27.5%	72.5%	100.0%
	% within If the respondent is ready to shift from in use energy to Biogas	70.1%	67.1%	67.9%
	% of Total	18.7%	49.2%	67.9%
Kerosene	Count	0	3	3
	Expected Count	.8	2.2	3.0
	% within Primary energy source used in Dar es Salaam	.0%	100.0%	100.0%
	% within If the respondent is ready to shift from in use energy to Biogas	.0%	1.2%	.9%
	% of Total	.0%	.9%	.9%
LPG	Count	18	46	64

	Expected Count	17.0	47.0	64.0
	% within Primary energy source used in Dar es Salaam	28.1%	71.9%	100.0%
	% within If the respondent is ready to shift from in use energy to Biogas	20.7%	19.2%	19.6%
	% of Total	5.5%	14.1%	19.6%
Electricity	Count	1	3	4
	Expected Count	1.1	2.9	4.0
	% within Primary energy source used in Dar es Salaam	25.0%	75.0%	100.0%
	% within If the respondent is ready to shift from in use energy to Biogas	1.1%	1.2%	1.2%
	% of Total	.3%	.9%	1.2%
Moto-Poa	Count	1	1	2
	Expected Count	.5	1.5	2.0
	% within Primary energy source used in Dar es Salaam	50.0%	50.0%	100.0%
	% within If the respondent is ready to shift from in use energy to Biogas	1.1%	.4%	.6%
	% of Total	.3%	.3%	.6%
Total	Count	87	240	327
	Expected Count	87.0	240.0	327.0
	% within Primary energy source used in Dar es Salaam	26.6%	73.4%	100.0%
	% within If the respondent is ready to shift from in use energy to Biogas	100.0%	100.0%	100.0%
	% of Total	26.6%	73.4%	100.0%
