OPTIMISATION OF ANAEROBIC DIGESTION OF ORGANIC SOLID WASTE FOR THE PRODUCTION OF QUALITY COMPOST FOR SOIL AMENDMENT

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I certify that this is a true and accurate version of the thesis approved by the examiners.

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ABSTRACT

Organic Fractions of Municipal Solid waste (OFMSW) is rich in organic matter, which can be recycled through energy recovery and compost production. The move towards sustainable management of OFMSW is shifting from energy recovery alone to the integration of separation at source, feedstock selection and disposal/reuse of the residuals. The sustainable management of OFMSW presents a challenge due to policy shifts and increasing pressure on landfills. This research was proposed in this background to establish a suitable feedstock combination of source separated wastes for biogas and compost production. Little information is available on factors affecting the combined anaerobic/aerobic treatment of source separated OFMSW and the soil enhancing qualities of the resulting post treated digestate (anaerobic digestate compost). This study investigated suitability of laboratory simulated feedstock for biogas and compost production and the impacts of the compost produced on physical, chemical and biological properties of soil. The study was carried out in five stages. The first stage investigated the natural buffering capacity, biogas production and degradability of various proportions of food and green wastes in anaerobic cultures. The studies showed that a waste mixture of food waste: green waste: inoculum in the ratio 2:2:1 as optimal for biogas production, volatile solids destruction and pH stability. The study also showed that the choice of waste mixture can be useful for pH control during anaerobic digestion of OFMSW. The second stage, investigated the factors affecting aerobic post-treatment of the anaerobic digestate obtained from the first stage. Results showed increasing solids concentration and decreasing C:N ratio with increasing aerobic treatment. Generally, the results (TS, TVS, C: N) suggest increasing digestate stability with increasing aerobic post treatment. The third stage investigated the quality and stability of the post- anaerobically treated digestate using seed germination and plant growth tests. Results showed that non-digested model feedstock and anaerobic digestates collected at various times during anaerobic digestion showed low seed germination index. On the other hand digestates collected during aerobic post-treatment showed higher seed germination indexes with increasing aeration times. No seed germination inhibition (phytotoxicity) was observed in soils amended with aerobically post-treated digestate. Generally the results indicate increased digestates stability with increasing duration and intensity of anaerobic-aerobic treatments. The fourth stage investigated the soil enhancing qualities of anaerobic digestate and aerobically post-treated digestate. Results obtained showed that anaerobic digestate is not suitable soil for amendment of arable soils. However, post aerobically treated digestate (i.e. anaerobic digestate compost) improved the physical, chemical and biological qualities of amended soils. Soil analyses showed that the anaerobic digestate compost amendment improved water retention, water infiltration, carbon mineralization and assimilation and significantly increased the soil biomass and bioactivity. Plant growth tests showed that the anaerobic digestate compost significantly increased plant heights, number of leaves and dry matter contents. In the final stage microbial diversity was investigated in anaerobic digestate compost amended soils. The study revealed significant changes in the diversity of soil microbial populations. The results suggest greater variations in fungi indicating that anaerobic digestate compost amendments have greater impacts on soil fungal populations than bacterial communities. In general, this study demonstrated that depending on the raw waste composition and the nature and duration of biological treatment received, OFMSW could be associated with high biogas yield, soil fertility and productivity and can bring about changes in dynamics of soil populations and functions. Whilst the study indicates the suitability of anaerobic digestate compost for soil management applications, the holistic approach employed in this study if applied on a larger scale could lead to sustainability in management of OFMSW.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Acknowledgements

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I wish to acknowledge the University of Abertay Dundee for the M.Phil studentship award and the support of the management of Kaduna Polytechnic without which this study would have been impossible.

ADC- Anaerobic Digestate Compost

ATP – Adenosine Tri-Phosphate

BOD – Biological Oxygen Demand

Ca - Calcium

$Ca(OH)_2$ – Calcium Hydroxide

$CaCO_3$ – Calcium Carbonate

CH_4 - Methane

C: N – Carbon – Nitrogen ratio

COD – Chemical Oxygen Demand

CO_2 – Carbon dioxide

CSTR – Continuous Stirred Tank Reactor

Cr – Chromium

DGT – Anaerobic digestate

DC – Discovery Compost

EEC – European Economic Community.

EU – European Union

FW – Food Waste

FrW – Fresh Waste

GHG – Green House Gas

GI – Germination Index

GW – Green Waste

H – Hydrogen

H_2S – Hydrogen sulphide

HCO_3 – Bicarbonate ion

HGT- Height

HGV – Heavy Goods Vehicles

HRT – Hydraulic Retention Time

IWM – Integrated Waste Management

IEA – International Energy Agency

LCFA – Long Chain Fatty Acids

LLF – Length of Leaves

MSW – Municipal Solid Waste

M.CPT – Mature Compost

NAD^+ - Nicotinamide Adenine Dinucleotide (oxidised)

NADH – Nicotinamide Adenine Dinucleotide (reduced)

$NaHCO_3$ – Sodium Hydrogen carbonate

NH_3 - Ammonia

NLV – Number of Leaves

NO_X – Oxides of Nitrogen

O – Oxygen

OFMSW – Organic Fraction of Municipal Solid Waste

P – Phosphorus

PAYT – Pay-As-You-Throw

Pb – Lead

PCR – Polymerase Chain Reaction

PQ – Alkali Soluble Fraction

PNR – Potential Nitrification Rate

rRNA – ribosomal Ribose Nucleic Acid

rDNA – ribosomal Deoxy-Ribo Nucleic Acid

rpm – rotation per minute

SS – Suspended Solids

SSU – Single Stranded Unit

SCFA – Short Chain Fatty Acids

SIR – Substrate Induce Respiration

SNT – Supernatant

SO_X – Oxides of sulphate

SRT- Solids Retention Time

T-RFLP – Templete Restriction Fragment Length Polymorphism

TS – Total Solids

UNEP – United Nations Environmental Program

USEPA – United States Environmental Protection Agency

UASB – Upflow Anaerobic Sludge Blanket

VS – Volatile Solids

VFA – Volatile Fatty Acids

VSS – Volatile Suspended Solids

WHC – Water Holding Capacity

Zn – Zinc

Definitions

Anaerobic digestion: Microbial decomposition of carbon rich substrates in the absence of oxygen to produce biogas (carbon dioxide and methane) and digestate

Anaerobic digestate: Semi solid viscous material obtained at the end of anaerobic digestion of OFMSW

Anaerobic digestate compost (ADC): Compost produced from post-aerobic digestion of OFMSW

Biological treatments: Treatments techniques that employed micro organisms for the treatment of waste of organic origin (i.e. anaerobic digestion, composting)

Composting: Controlled aerobic process carried out by successive microbial populations combining both mesophilic and thermophilic activities, leading to the production of carbon dioxide, water, minerals and stabilized organic matter

Compost: solid particulate material that is the result of composting, that has been sanitised and stabilised and that confers beneficial effects when added to soil and/or used in conjunction with plants.

Discovery compost (DC): Aerobic compost produced from aerobic stabilization of green waste in Dundee

Feedstock: Carbon rich biodegradable organic substrates for biological treatments

Food waste (Kitchen waste): Waste arising from fresh or cooked materials intended for human consumption or originated as a consequence of preparation, have been surplus to consumption, or spoiled

Green waste: Waste of plant origin

Maturity: the degree of biodegradation at which composted material is not phytotoxic or exerts negligible phytotoxicity in any plant growing situation when used as directed

OFMSW: Organic fraction of municipal solid waste (i.e. biodegradable components of waste collected by local authorities)

Phytotoxicity: substances or conditions that is toxic to plants and causes symptoms such as delayed/reduced seed germination, root death, restricted root growth stunting, distortion, necrosis or chlorosis of shoots.

Soil amendment/conditioner: A material added to soil in situ to enhance its physical, chemical and/or biological properties

Stability: Readiness for use

Waste: Discarded unwanted materials

DEDICATION

To my father

Chapter One: Background

1.1 Introduction

Landfills are important in waste management as sites for storage, disposal or processing municipal solid waste (MSW). The use and operation of landfills has impacted negatively on land value, public perception and the local environment. In addition the high rate of generation of MSW has increased pressure on landfill sites by limiting their operation and expansion. This has necessitated the search for alternative management techniques. The EU Landfill Directive (99/31/EC) is a regulatory tool that sets challenging targets on member states for the diversion of biodegradable organic fraction of municipal solid waste (OFMSW) from landfills. This will be encouraged through waste minimisation and recycling technologies such as anaerobic digestion, composting and soil application. Although recycling of organic waste by household and local authorities is increasing, the use of landfill remains high and recovery of waste as energy is still low in the UK by European standards and according to Waste Strategy For England (WSFE), 2007 (DEFRA, 2007). This is because alternative technologies such as anaerobic digestion have not been fully established. The WSFE (DEFRA, 2007) also proposed the use of landfill tax escalator, private finance initiatives, and enhanced capital allowances to encourage the development of renewable energy sources. This is in addition to the banding system contained in the Energy White Paper, (DTI, 2007) for Renewable Obligation Certificates (ROCs) to encourage the development of alternative technologies. Under the banding system facilities producing energy from waste such as anaerobic digesters are eligible to twice (£43 per Megawatt) the support applicable to other renewable energy sources. Although the Department for Environment Food and Rural Affairs (DEFRA) has already recognised that anaerobic digestate, produced by anaerobic digestion, has a range of potential uses on land, including as a fertiliser or soil improver,

this potential has not been fully exploited. Thus DEFRA has asked Waste and Resources Action Programme (WRAP) and the Environment Agency to develop a standard protocol for anaerobic digestate to help build market confidence in its recovery on land (DEFRA, 2005). The protocol (BSI: PAS 110) is expected sometime in 2008. The UK Government's Energy White Paper, (DTI, 2007) has placed energy from waste in a wider energy policy context as a major contributor towards meeting renewable obligations (RO).

While the potential role of anaerobic digestion in waste management and energy recovery are well recognised (DTI, 2007; WSFE, 2007), the role of its residuals in soil management is not yet established. Major challenges associated with anaerobic digestion concern feedstock composition, quality and availability and treatment techniques that will address the key issues of energy from waste in addition to its potential role in waste and soil management. The Animal By-Product Regulations, (2003) is the major policy tool regulating feedstock composition and quality as well as the minimum standards required for biological treatment for the protection of public health and safety. The regulation is a preventive mechanism that controls the type of animal products allowed as substrates for biological treatments and sets criteria for sanitization, use and disposal of some of the arising waste products. Numerous studies (De-Baere, 2006; Ahrens and Weiland, 2004; Rulkens, 2004; Mata-Alvarez et al., 2000; Brummeler et al., 1992) have been carried out on energy recovery from waste especially in the area of anaerobic digestion of OFMSW however, none of these studies demonstrated the linkage between waste management, energy from waste and soil management applications. This linkage in Figure 1.1 is believed to be the key concept in present-day OFMSW management programmes. This project was proposed on this background and is aimed at integrating anaerobic and

aerobic treatments of OFMSW with soil application of stable organic soil fertilizer with the overall goal of achieving sustainability in the management of OFMSW. The use of combined anaerobic digestion and aerobic post-treatment processes as shown in Figure 1.1 is proposed in this study as a sustainable treatment method for source separated OFMSW for optimised biogas and compost production.

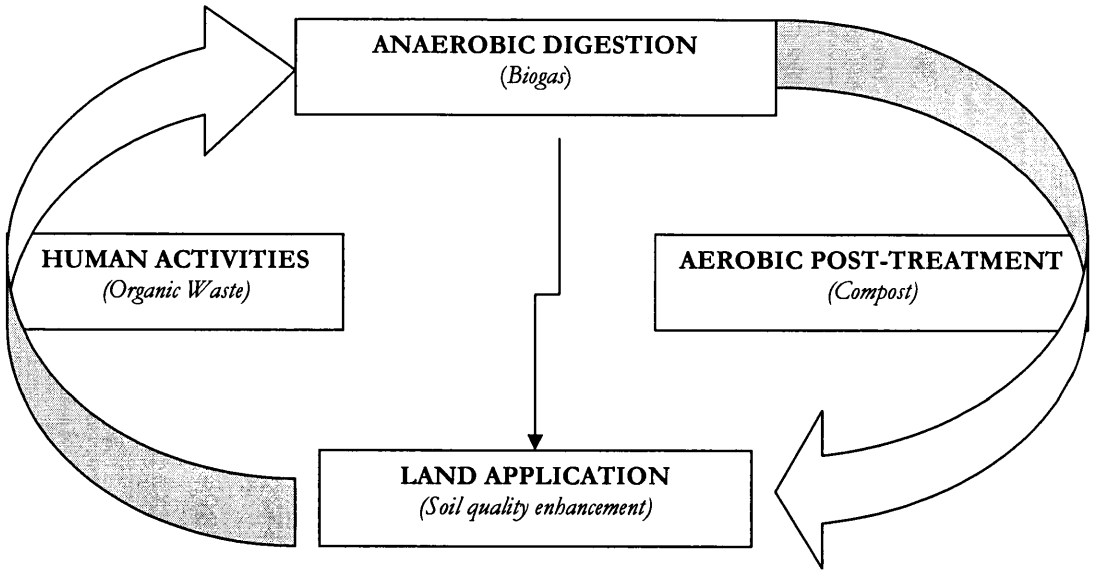


Figure 1.1 Integrated sustainable management of organic solid waste

According to Schjønning et al., (2004), lack of organic matter is a major cause of soil degradation which reduces soil vegetative cover and agricultural productivity, thus exposing the soil to erosion (Chambers et al., 2003). Soil carbon is a key factor in soil stability and sustained productivity (Lal, 2004). The demand for carbon and its transformation ensures that it is always in short supply in the soil. Addition of organic soil amendments is one of the ways used to manage this carbon deficiency and to improve interactions of physical, chemical and biological properties of soil (Sanchez-Mondero et al., 2004). This interaction is a key factor in the sustainability of soil fertility. Organic matter being the primary source of soil carbon is the most important component of soils

which influences soil structural development, aggregate stability, moisture supply, nutrient turn-over and availability, and natural fertility (Dick and Gregorich, 2004). Soil organic matter concentration determines the physical, chemical and biological qualities of the soil, which in turn determine the quality and quantity of plant products (Lal, 2004).

Organic feedstock's widely used in biological treatment and stabilization processes includes waste water from food and agro-allied industries (Franklin, 2001), sewage sludges (Braber, 1995) to OFMSW (Bolzonella et al., 2003; Cecchi et al., 2003; Hartmann and Ahring, 2006). Aerobic treatment of OFMSW utilizing co-mingled wastes to produce compost for soil application has the advantage of being cost efficient and faster with no benefit of energy recovery (McDougall et al., 2001). In contrast, anaerobic digestion is believed to be useful in the treatment of both co-mingled and source-separated OFMSW with the additional benefit of energy recovery producing digestate for soil application (Molner and Bartha, 1988). However, it has been widely reported that anaerobic digestate in its basic form is not a suitable soil conditioner due to its phytotoxicity (Poggi-Varaldo et al., 1997; McLachlan et al., 2004), viscosity, odour (Smet et al., 1998) and difficulty in handling (Tchobanoglous et al., 2002). Non-stabilized organic waste may be associated with bad odour which may lead to its rejection by farmers. Furthermore, fresh organic wastes used as soil amendments are injurious to plants (phytotoxicity) (Jimenez and Garcia, 1989). The phytotoxicity could be removed by aerobic stabilization (Tiquia and Tam, 1998; McLachlan et al., 2004). Anaerobic digestate therefore may require final 'polishing' to enhance its use as a soil conditioner. Aerobic 'polishing' removes moisture, odour as well as reduce carbon and pathogens (McDougall et al., 2001; Tchobanoglous et al., 2002), thereby enhancing the fertilizer value of organic wastes.

One of the barriers to large-scale use of composts and similar soil amendments made from organic waste resources is the need for a product of consistent and predictable quality (Levy and Taylor, 2003) and rate of application. Different varieties of the same product, as produced by different processes or manufacturers, must have essentially similar benefits for plant production if the amendment is to be used for agriculture or horticulture. Maintaining predictable compost quality and stability is a particular problem when the material is produced from sources such as OFMSW which are heterogeneous in nature.

Little information is available on anaerobic digestion and stabilization properties of OFMSW for the purpose of the production of compost. The impact of anaerobic compost on soils physical, chemical and biological properties and especially soil microbial populations is not well documented.

The overall aim of this research is to develop and optimise the OFMSW management strategy in Figure 1.1, which integrates waste management with energy and soil management techniques.

The specific objectives of the study are as follows:

1.2 Aims and objectives

I. To determine the effect of substrate composition on the digestibility, stability and biogas yield during anaerobic digestion of OFMSW.
II. To determine the effects of various degrees of aerobic post-treatment on the soil-enhancing properties of anaerobic digestate.

III. To evaluate feedstock composition and operating procedures for combined anaerobic digestion and aerobic stabilization for the production of anaerobic digestate compost.

IV. To evaluate changes in the characteristics of soil amended with aerobically treated anaerobic digestate (referred to as anaerobic digestate compost ADC).

V. To investigate the factors effecting the use of anaerobic digestate compost for plant growth.

VI. To evaluate changes in the diversity of microbial populations in the soil due to the application of anaerobic digestate compost.

VII. To provide guidelines on the use of anaerobic digestate compost for soil application.

1.3 Research methodology

The research methodology is summarized in Figure 1.2. Model OFMSW feed (or feedstock) was selected from different combinations of the main components i.e. food and green wastes. The experimental plan consisted of four stages namely anaerobic digestion, aerobic post-treatment, soil analyses and population dynamics. The quality of anaerobic digestate compost was assessed in bioassays and soil amendment studies, which investigated changes in physical, chemical and biological properties of soil. The impacts of ADC amendment on soil population was assessed using molecular tools as shown in the experimental plan.

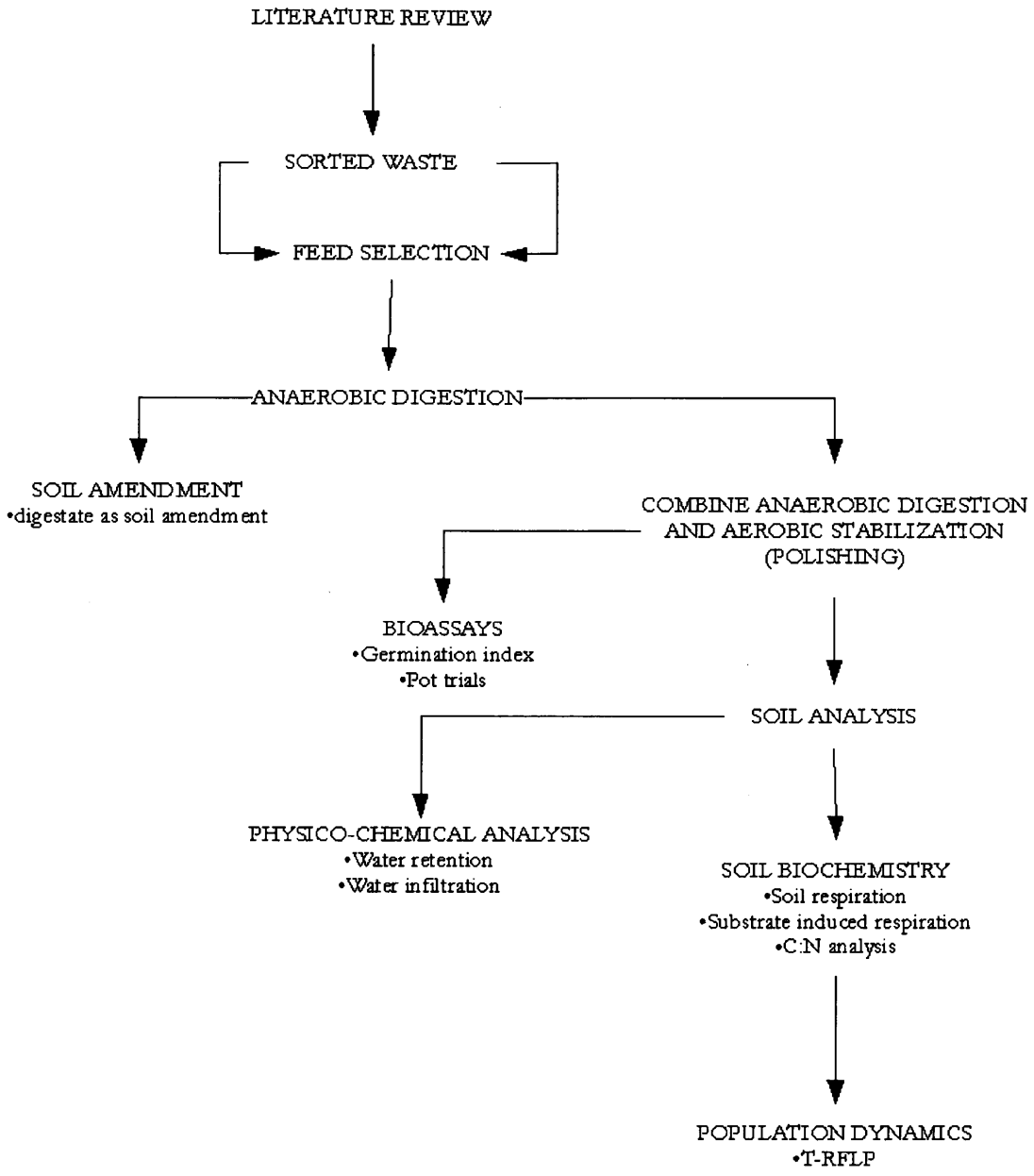


Figure 1.2 Experimental Plan

1.4 Major outputs

Abdullahi, Y.A. and Akunna, J.C. 2008. Investigating the aerobic post-treatment of anaerobically treated source-separated organic fraction of municipal solid Waste (OFMSW). Proceedings, *Postgraduate Researchers' Conference on "Meeting Environmental Challenges in the Coastal Region of Nigeria"*, University of Abertay Dundee, Dundee, United Kingdom, 28th – 29th September.

Abdullahi, Y.A. and Akunna, J.C., Ahmed, A.S., Sirajo, M.Z. and Bala, I.A. 2008. Developing an integrated sustainable municipal solid waste management strategy for a rapidly growing urban city: A case study of Abuja, Nigeria. Proceedings, *Postgraduate Researchers' Conference on "Meeting Environmental Challenges in the Coastal Region of Nigeria"*, University of Abertay Dundee, Dundee, United Kingdom, 28th – 29th September.

Abdullahi, Y.A. and Akunna, J.C., White, N.A., Hallett, P.D. and Wheatley, R. 2008. Evaluating the effects of anaerobic digestate compost on plant growth and development. Proceedings, *Postgraduate Researchers' Conference on "Meeting Environmental Challenges in the Coastal Region of Nigeria"*, University of Abertay Dundee, Dundee, United Kingdom, 28th – 29th September.

Abdullahi, Y.A., Akunna, J.C., White, N.A., Hallett, PD., Wheatley, R., 2008. Investigating the effects of anaerobic and aerobic post-treatment on the quality and stability of organic fraction of municipal solid waste as soil amendment, *Bioresour. Technol.*, 99 (18), 8631-8636

Akunna, J.C., Abdullahi, Y.A., Stewart, N.A., 2007. Anaerobic digestion of municipal solid wastes containing variable proportions of waste types. *Water Sci. Technol.* 56, (8), 143-151

Abdullahi, Y.A., and Akunna, J.C., 2006. Effect of waste type and proportions on pH control and regulation in high solids anaerobic digestion. Proceedings, *11th European Biosolids and Biowaste Conference*, Wakefield, UK, 13-15 November

1.5 Structure of thesis

The thesis structure as shown in Figure 1.3 elucidates the concepts, methodologies and experimental analyses to be carried out from the beginning to the end of the dissertation. Also included in the general outline are discussions on the obtained results and summary

of the major outcomes. Chapter one relates the past, current and likely future implications of waste and soil management. Chapter Two reviews the current waste management practices as it relates to biological treatment techniques. Chapter Three and Four are reviews of anaerobic digestion and composting outlining their potential roles in sustainable management of OFMSW. Chapter Five reviews the need for organic soil amendments in relation to the current waste and soil management practices. Chapter Six summarizes the literature reviews (Chapters 2, 3, 4 & 5) and provides a description of the experimental methods adopted to achieve the overall aim of the research project

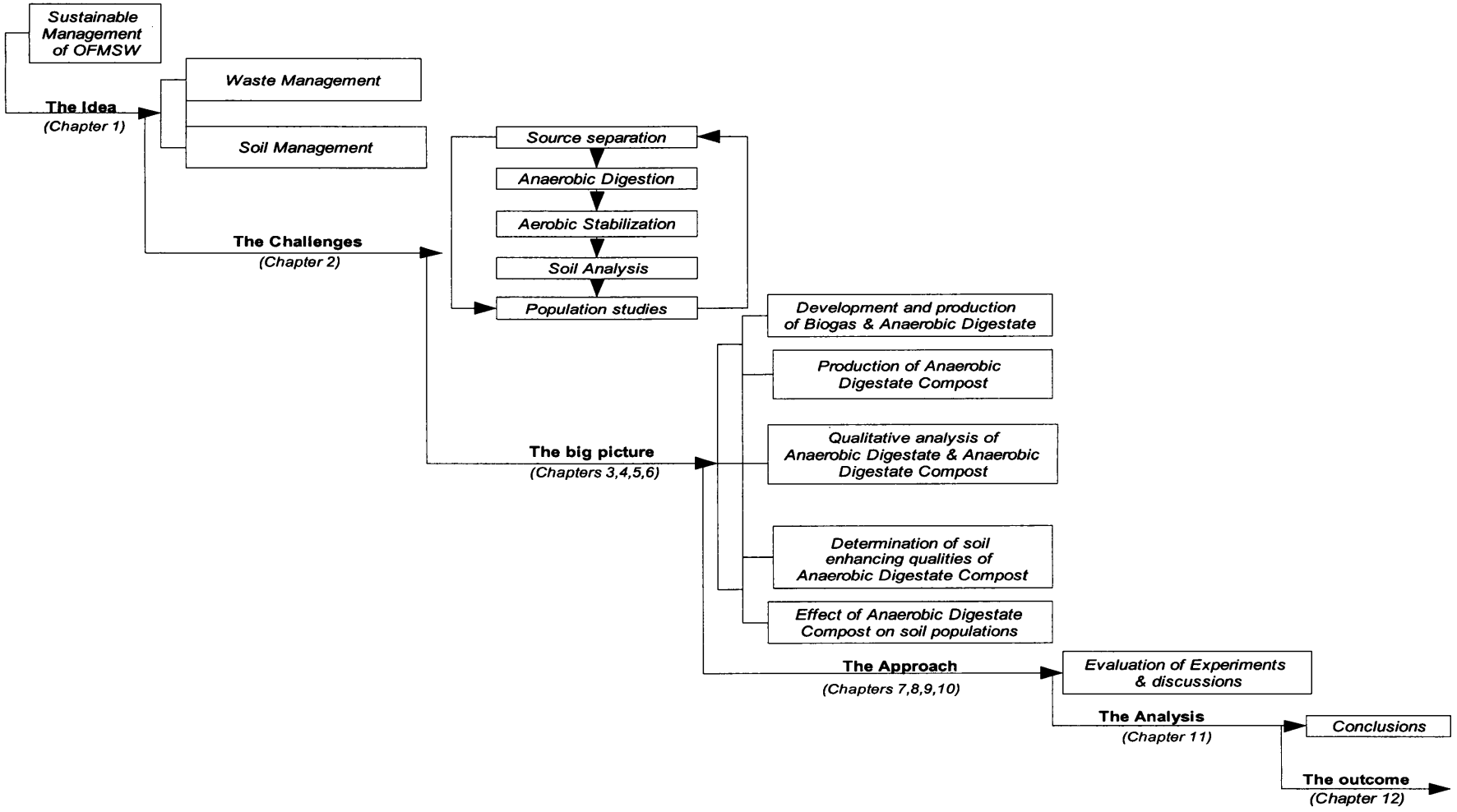


Figure 1.3 Structure of thesis

Chapter Two: Waste Management

2.1 The environment and biogeochemical cycling

The environment is the internal or external surrounding, which could be inhabited by living organisms. Microbial ecology is the study of interactions of micro-organisms between themselves and the environment, while environmental microbiology refers to the use of micro-organisms to benefit society (Maier and Pepper, 2000). As societies move towards adopting sustainable development as result of depleting resources and the need for improved productivity, environmental microbiology becomes more important to societal needs as micro-organisms will increasingly be harnessed being natural components of the environment. The activities of micro organisms are important in resource production, resource recovery and remediation of contaminated environments and bio-geo-chemical cycling of the essential elements of life. Large quantities of organic carbon are produced during photosynthesis, which are constantly consumed and degraded during respiration. This creates a biologically driven balance between biotic and abiotic factors. Carbon and other major elements are cycled the same way giving rise to biogeochemical cycles. One area where these cycles are critical is the sustainable management of organic fraction of municipal solid waste (OFMSW) and its integration with soil management applications. Micro-organisms are necessary in the biological conversion of organic waste to methane and compost. The use of compost as fertiliser relies on the nutrient cycling ability of indigenous soil communities for plant growth and development. In addition to nutrient enrichment, organic amendments may also introduce new organisms which could influence the population dynamics of soil communities.

2.2 Waste

The United Nations Environmental Program (UNEP) defines waste as objects which the owner does not want, need or use any longer, which requires treatment or disposal (Tchobanoglous et al., 2002). According to the Organisation for Economic Co-operation and Development (OECD), waste is a non-avoidable material for which there is no currently or near future economic demand and for which treatment or disposal is required (McDougall et al, 2001). The European Waste Directive 75/442/EEC defines waste as any object which the holder disposes or is required to dispose pursuant to the provisions of national laws in force. This definition was later modified in the EU Directive 91/156/EEC, an amendment of the Waste Directive (75/442/EEC) in which waste is defined as substances or objects in the categories set out in Annex 1 which the owner discards, intends or is required to discard (Council of European Communities, 1991). The Annex 1 entitled 'categories of waste' contains the lists of different types of materials considered as wastes. The proportion of municipal solid waste (MSW) out of the total solid waste stream is usually small (McDougall et al., 2001) however its contact with the public and difficulty of treatment and disposal due to its heterogeneity ensures that it attracts attention more than other solid waste streams. MSW is defined as waste controlled and collected by local authority or municipality (McDougall et al., 2001). These authors consider MSW as the solid waste generated at residences, commercial establishments (retail shops, offices and restaurants), institutions (schools, hospitals and prisons) and some industrial sources. Included in this definition are durable, non-durable goods, containers and packaging, food scraps, yard trimmings and miscellaneous inorganic waste from residential and commercial institutions and industrial sources. Excluded from the definition however, are construction and demolition waste, municipal sludge, combustion ash and waste from industrial processes that might be disposed of in

municipal landfills or incinerators (Hill and Glenn, 2002). The definition of MSW by Tchobanoglous et al., (1993), includes all the wastes generated in a community, including demolition and construction wastes and municipal sludge with the exception of industrial process wastes and agricultural wastes. Biowaste is a similar term used to describe waste arising from vegetables, fruits and garden wastes (McDougall et al., 2001) and depending on locality may also include kitchen waste and sorted paper. Although in sustainable waste management waste is regarded as matter out of place (Moll, 2004) the definition in the amended European Directive on Waste (91/156/EEC) is the most widely acceptable for regulatory and legal reasons.

2.3 Waste Management: The United Kingdom Strategy

In the United Kingdom, England alone produces about 100 million tonnes of waste from households, commerce and industry of which 85% is landfilled (DEFRA, 2007) with the recycling rates increasing from 1997 to 2006 as indicated in Figure 2.1. Scotland generates 3 million tonnes of household waste of which 90% goes to landfills (SEPA, 2003) only. A small proportion of household wastes comprising of kitchen and garden wastes is managed through incineration and composting.

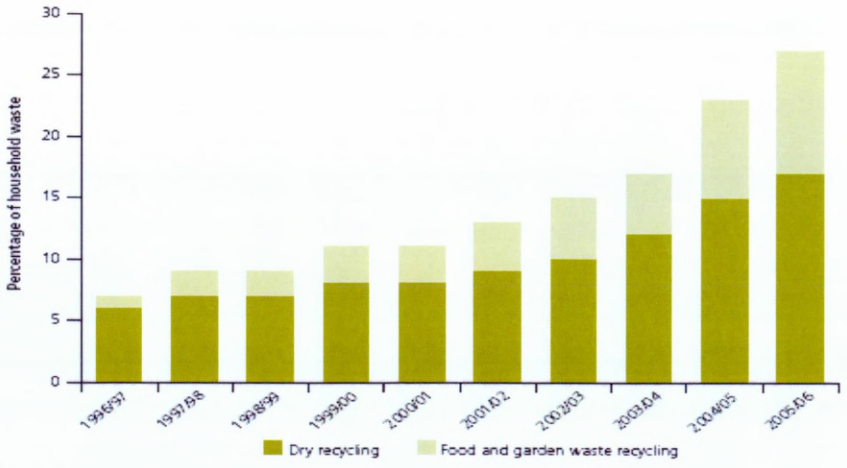


Figure 2.1 Household recycling rates in England (1996/97 – 2005/2006)
Source: DEFRA, 2007

The use of landfills for the management of biodegradable wastes (food and kitchen wastes) is not sustainable. This is because of problems associated with pollution of water courses by leachate, air pollution by gases such as methane, odour and vermin. The diversion of biodegradable waste from landfills is a legal requirement prescribed by the EU Landfill Directive (99/31/EC). The Landfill Directive has a major impact on the management of the organic fraction of municipal solid waste (OFMSW) as it set targets for the reduction of the quantity of biodegradable municipal waste which can be landfilled as well as promote the sustainable treatment of OFMSW. The targets as described by Environmental Agency EA, (2008) are as follows;

- 75% of 1995 levels by 2010
- 50% of 1995 levels by 2013
- 35% of 1995 levels by 2020.

The use of landfills in the United Kingdom for the management of MSW is one of the highest in the European Union as illustrated in Figure 2.2. Presently OFMSW is managed through small-scale household composting and much larger community based commercial composting schemes. OFMSW for these schemes are mostly garden wastes collected through the normal household waste or kerbside schemes with little or no source separation. Energy recovery is mostly through incineration with the bulk of the waste going to landfill. Kitchen waste collected from households and catering waste from institutions are landfilled due to hazards to health and safety and the potential for nuisance. Although the European Animal By-Products Regulation, APPR (2002/1774/EC) allowed the use catering waste for biological treatment, unfortunately, in

the UK, anaerobic digestion technology which recovers energy from food waste is not fully developed.

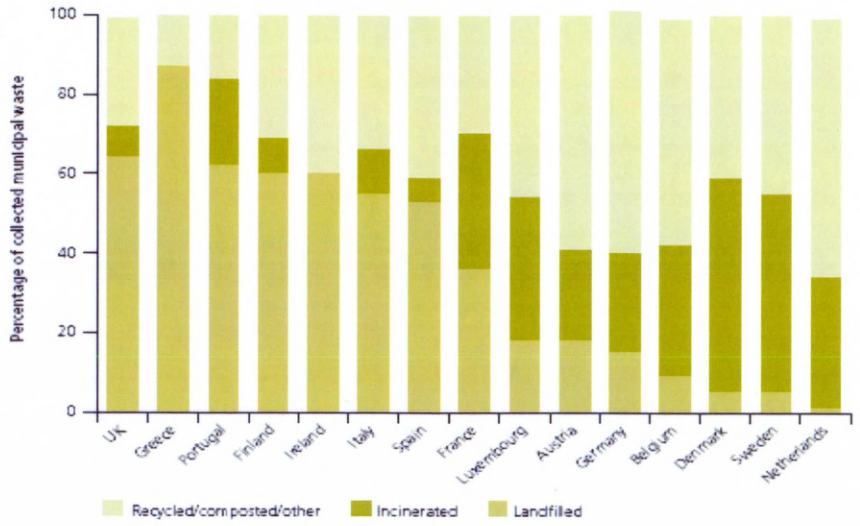


Figure 2.2 Management of municipal waste for the EU 15 (2005)
Source: DEFRA, 2007

Currently the choice of sustainable management techniques for OFMSW is guided by the Waste Strategy for England, (DEFRA, 2007) and National Waste Strategy, Scotland, (SEPA, 2003) based on best practice and local experience. These strategies have already identified biological treatment techniques such as composting and anaerobic digestion as sustainable treatment techniques for OFMSW. However, the waste type, quality and degree of biological treatment required for OFMSW is regulated by the EU Animal By-products Regulations, (2002/1774/EC) and in the UK specifically by the quality assurance mechanisms of the British Standard Institute's Publicly Available Specifications, (PAS), BSI:PAS 100 of 2005 and BSI:PAS 110 of 2008 for composting and anaerobic digestion respectively. The ABPR (2002/1774/EC) classified food waste as category 3 materials. These are substances derived from healthy animals for human consumption, not because of being rejected as unfit or due to commercial reasons. The regulation allowed the used of food waste for biogas production and contained the following:

- List of allowables for biotreatments
- Directives for collection, handling and treatment of wastes
- Approval of treatment of plants and allowable processing emissions
- Quality classes for compost and residues of biological treatment
- Control and analysis of end products
- Application standards for end products

The PAS 100 (BSI, 2005) is a specification that provides base-line standards for safety and consistency of compostable materials as well as sets limits for different end-use applications. The specification covers the production, storage labelling and traceability of compost. The PAS 110 (BSI, 2008) is a specification for anaerobic digestion and standards required for disposal and use of anaerobic digestate.

The need for efficient and sustainable treatments of OFMSW will continue to increase because the new targets for recycling and composting of household waste set in the Waste Strategy for England, (DEFRA, 2007) exceeded those prescribed by the Landfill Directive (i.e. at least 40% by 2010, 45% by 2015 and 50% by 2020). A major barrier to large scale application of waste products from biological treatments of OFMSW is lack of consistency and quality. Consistency, quality and sustainability in the treatment of OFMSW can only be achieved by integration of various aspect of waste management.

2.4 Sustainable waste management system: The big picture

Waste management systems could be described in three different strategies (McKinney and Schoch, 2003). These are *disperse and dilute*; *concentrate and contain*; and *resource recovery*. According to Moll (2004) the *disperse and dilute* strategy was used prior to 18th

century when wastes were treated by processes based on natural cycles. The large-scale uncontrolled pollution of water, air and soil due to industrial growth of the 19th and 20th centuries resulted in the development of *concentrate and contain* strategy. This technique requires high investments, large amount of land as well as permanent control of containment sites, thus leading to permanent loss of valuable resources. Although this strategy may be adequate for current waste management issues, it is not sustainable due to high costs, high consumption of material resources and its lack of consideration for future generations. The *resource recovery* strategy is more adequate for waste-resource management of present and future generations as it emulates natural cycles in resource management. Sustainable waste management system as proposed by World Business Council of Sustainable Development (De-Simone and Popoff, 1997) is a *resource recovery* based system that utilizes biological processes in waste management in a way that ensures eco-efficiency as well as protects the economic, social and environmental needs of present and future generations. Only when waste management systems evolve into optimised resource management systems can it be truly sustainable. Waste management therefore has to become an integral part of sustainable integrated resource management (ISWA, 2002).

In both England and Scotland, sustainable waste management is guided by waste management hierarchy (DEFRA, 2007; SEPA, 2003; SEPA, 1999). The hierarchy encourages the adoption of waste management options in order of priority as shown in Figures 2.3 and 2.4. The aim of the hierarchy is to encourage greater efficiency in the use of natural resources through waste minimisation, recycling and increased resource/value recovery from waste.

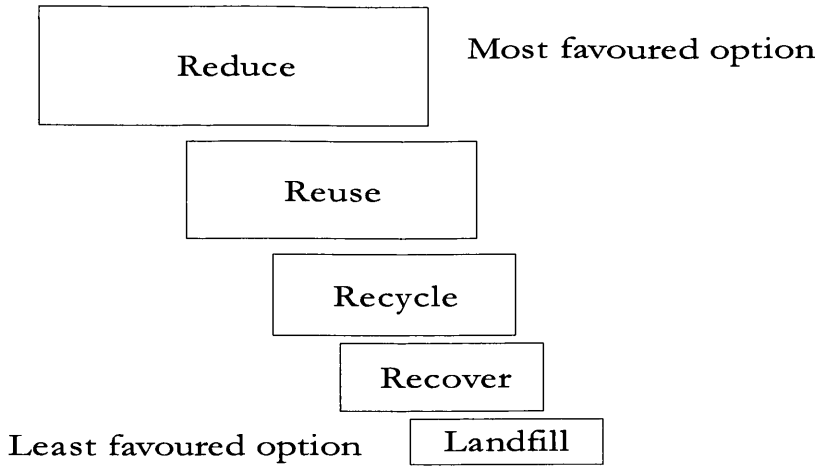


Figure 2.3 Waste Management hierarchy
Adopted from: Pert and Kinross Council, (2008)

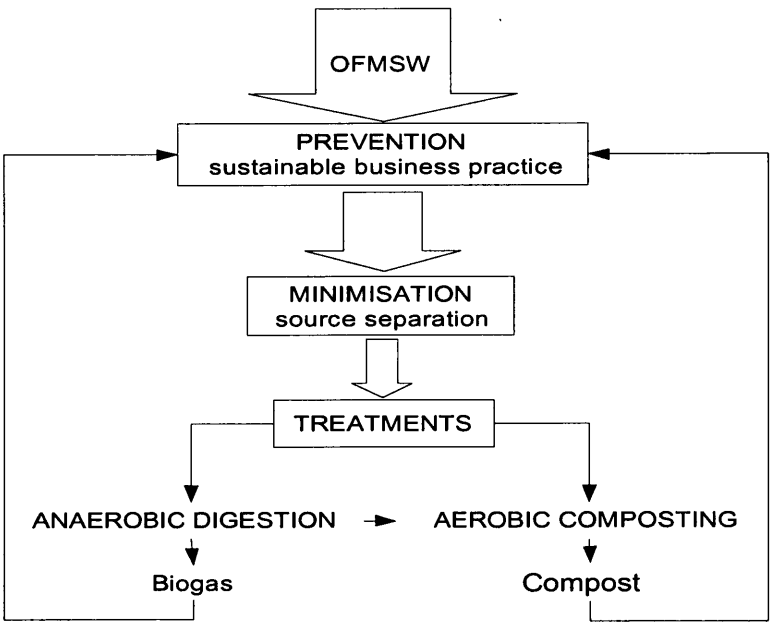


Figure 2.4 Sustainable waste management hierarchy of OFMSW

The National Waste Strategy Scotland (SEPA, 1999), defined a sustainable integrated waste management system (IWMS) as "the development and delivery of waste management systems and services, which, with a high degree of planned efficiency and at an acceptable balance of costs and benefit, are capable of minimising the level and hazard

of waste produced, maximising resource use efficiency and value recovery from wastes that are produced, whilst protecting the environment and human health". This definition indicates that the IWMS should take into account the principles of proximity self sufficiency and the principle of polluter pays in addition to precautionary and waste hierarchy principles. In practice no single disposal technique can deal with all wastes in a sustainable way. To achieve sustainability a combination of management options is required. The performance of any IWMS system will depend on environmental burdens, cost and acceptability by local communities (McDougall et al., 2001).

2.5 Waste classification and composition

The classification of waste within the European Union (EU) is based on the European Waste Catalogue (EWC) established by commission decision (2000/532/EC) which categorise waste according to composition and source. The EWC is a hierarchical guidance list of waste descriptions of which member states are required to integrate into domestic legislation for the protection of the environment through duty of care. In the UK a reference to EWC code is required on all duty of care transfer notes (EA, 2002). Generally, waste can be classified by its physical state (solid, liquid or gas) and within solid state by original use (packaging waste, food waste), by material (glass, paper), by physical properties (combustible, compostable, recyclable), by source (domestic, industrial, agricultural), or by safety (hazardous and non-hazardous waste). This waste lacks uniformity in material composition, thus making classification based on source most acceptable (Tchobanoglous et al., 2002). Based on sources waste can be classified into household, commercial and institutional wastes. A typical composition of household waste in England is illustrated in Figure 2.5

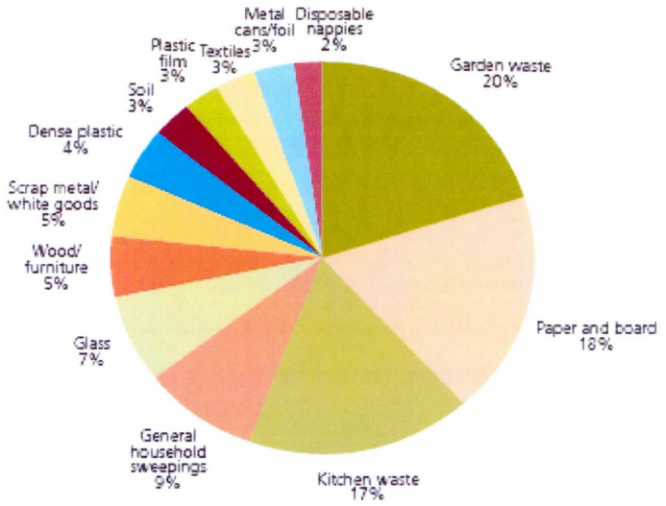


Figure 2.5 Household wastes composition England (2000/2001)
Source: DEFRA, 2007

The composition of MSW is important in developing a sustainable waste management system. Organic solid waste refers to organic-biodegradable-waste with moisture content of between 85-90% (Mata-Alvarez et al., 2000), with the most relevant being OFMSW. Generally developing countries produce higher levels of food and green wastes compared to developed countries (McDougall et al., 2001). This is due to high levels of scavenging and recycling of waste of non organic origin. The final quality of OFMSW depends on source separation efficiency and the nature of waste collection and transportation systems.

2.6 Separation, collection and transportation of OFMSW

OFMSW can be separated at source (households) or at civic amenity sites where pre-treatment may be carried out before final disposal to landfills. The separation of OFMSW is necessary for the efficiency of biological processes. Although waste producers will prefer wastes to be collected in a co-mingled state, collecting waste in this manner will limit the available waste management options. Public participation is therefore necessary for the success of any integrated waste management strategy (McDougall et al., 2001). The degree of household, commercial and institutional sorting depends on the abilities

and motivation of the waste producers. Waste producers could be motivated to sort their waste if they perceive it helps the environment or where they are required to sort it by law or more especially where they pay less for recycling more in pay-as-you-throw (PAYT) schemes (McDougall et al., 2001). Contamination of OFMSW is the major challenge of source separation. This may occur where a container intended for targeting one type of waste (e.g. food waste) turns out to contain non-targeted waste (e.g. metals).

The use of co-mingled waste as feedstock for biological treatments is greatly discouraged. According to Bolzonella et al., (2006), anaerobic digesters processing source separated waste are more cost efficient compared to those handling co-mingled wastes. The digestion of co-mingled or mechanically sorted feedstock lowers quantity and quality of biogas and may result in compost contamination by heavy metal and animal products. The digestion of source separated OFMSW can produce up to three times more biogas yield than from mechanically sorted or co-mingled OFMSW (Bolzonella et al., 2006). Hartman and Ahring, (2006) reported that the ratio of energy output to energy input is highest for the anaerobic digestion source separated OFMSW.

The focal point of any integrated waste management system is the method of waste collection. This in conjunction with sorting efficiency determines factors such as waste quality, management options and quantity and quality of the final waste products. Waste collection system is critical in the management of biodegradable wastes as it affects the characteristics of OFMSW, the quantity and quality of biogas and final disposal routes of residuals that is, soil application, landfill or incineration (Bolzonella et al., 2006). Waste collection is the point of contact between waste producers and the waste management system. McDougall et al., (2001) believed that the waste producer-collector link should

be a customer-supplier relationship in a total quality sense. This is because the waste producer needs his waste to be collected with minimum inconvenience, whilst the waste collector needs to receive the waste in a form compatible with planned treatments methods.

Basically there are two waste collection methods for MSW namely, the "bring" and the "kerbside" system. In the "bring" system the waste producer is required to transport recyclable materials to a local point. Under the "kerbside" system the waste producer deposit recovered items in a bin bag or a container placed in a specified location on a specific day outside the property for collection. In the UK, biowaste (including vegetable, fruit and garden waste) is collected through "kerbside" collection and a central "bring" system popularly known as the civic amenity site. The "bring" system is where the householder uses his own vehicle for taking waste to local collection point (McDougall et al., 2001). In a food waste collection trials, the kerbside services were found to be more effective than the "bring" system in the collection of food waste from multi-occupancy dwellings (WRAP, 2008). Generally, household waste is collected mixed, but where source separation is practiced waste streams are collected differently in the same or different vehicles. Where food and catering wastes are intended for use in biological treatment, collection bins are usually laced with moisture absorbing materials such as waste paper and wood chips in order to minimise nuisance and loss of energy potential through uncontrolled decomposition process. Loss of energy potential can be minimised by increased collection frequency or refrigeration prior to treatment for health and safety reasons.

An integrated collection system is essential to the success of any IWMS and it could be a combination of "bring" (materials banks, local drop-off centres, civic amenity sites) and kerbside (for recyclables, biowaste and forest waste) collection systems.

Waste transportation is critical to the success of any waste management strategy. Sorted wastes are usually transported separately and different wastes types are transported individually within the boundaries of the collection system. Bulky materials may require special collection in bigger vehicles. The standard Heavy Goods Vehicle (HGV) is utilised by most waste contractors for the transportation of kerbside collections (McDougall et al., 2001). Recently, Aluminium Bodied Utility Vehicles (ABUV) has been developed specifically for the transportation of food and green waste. Vehicles may be compartmentalised to accommodate sorted wastes or a single vehicle is used to collect only one type of waste.

2.7 Pre-treatment

The pre-treatment of OFMSW prior to biological treatment is mandatory under the Landfill Directive (99/31/EC). It is generally carried out to serve two basic functions namely, the separation of organic materials from other waste fractions and the preparation of organic material for biological processing. Pre-treatment may also be carried out for size reduction, homogenisation and moisture control. Size reduction of OFMSW is necessary for homogeneity and faster chemical and biological reaction rate. The size reduction may be performed using screw cutting, milling, drumming, pulping or shredding. Where source separation of organic waste is well established, the requirement for pre-treatment is minimal.

Pre-treated organic waste may require storage prior to biological treatment depending on the loading rate and retention time of the biological system. For anaerobic conversion of OFMSW lack of storage may result in the loss of energy potential due to uncontrolled decomposition induced by environmental conditions (Mata-Alvarez, 2003b; Stewart et al, 2005). Storage may be in the form of cold rooms, refrigerators or deep freezers depending on the requirements of the system.

2.8 Treatment techniques

Solid wastes can be treated by physical, chemical or biological processes. These processes can be integrated together to manage waste in a way that is cost effective, environmentally friendly and socially acceptable. Biological waste treatment processes rely on aerobic or anaerobic microbial decomposition of organic waste to recover biogas and soil conditioners that can be recycled back to the environment. Although aerobic processes have a much wider application in the management of OFMSW, energy recovery from such processes is not well established.

The choice of what proportion of organic waste goes through anaerobic digestion, composting or both depends on waste volume, season, efficiency of source separation and demand for biogas or compost. The use of anaerobic digestion and composting for the treatment of OFMSW is guided by several factors. These factors are reviewed and discussed extensively in Chapters Three (3) and Four (4).

Chapter Three: Anaerobic Digestion of OFMSW

3.1 Introduction

Anaerobic digestion is a reliable technology for the treatment of organic fraction of municipal solid waste (OFMSW) with about one million tonnes (wet weight) per year being digested worldwide (Verstraete et al., 2000). These wastes are converted to biogas and stabilised residual matter. Anaerobic digestion takes place in enclosed dry or wet systems in closely controlled single or double-staged reactors. In single stage systems the reactions occur in one reactor, whereas in two stage systems, the reactions take place in two separate reactors. Among biological treatments techniques anaerobic digestion is believed to be the most cost effective due to high energy recovery and limited environmental impacts (Mata-Alvarez et al., 2000). The reductions in carbon dioxide emissions in addition to post-composting processes are responsible for its limited impact on the environment (Bolzonella et al., 2003).

Major challenges of anaerobic digestion of MSW are associated with source or mechanical separation of different waste fractions (Stroot et al., 2001), dewatering and disposal of residuals (Mata-Alvarez, 2003a). The variable nature of OFMSW may also be a challenge to all year round operation of anaerobic digesters. Mata-Alverez et al, (2000), reported that the performance of anaerobic digesters could be enhanced by pre-treatment of solid wastes. These pre-treatments could be in the form of pre-composting, size reduction or solubilisation. Although pre-treatment may improve biogas yield (De-Baere, 2006) its application in anaerobic digestion of OFMSW is limited due to cost implications.

The performance of anaerobic systems in general is controlled by process kinetics and composition of the feedstock. These are discussed in the following sections below.

3.2 Principles of anaerobic digestion

Anaerobic digestion occurs in three major biochemical stages namely; hydrolysis, acidogenesis and methanogenesis (Garcia-Heras, 1999). Other processes that may also occur are acidogenic respiration, nitrate and sulphate reduction (Mata-Alvarez and Macé, 2004). Hydrolysis is the first step in anaerobic digestion of organic solid waste. It involves the breakdown of complex polymeric solid substrates into smaller soluble molecules. The process is aided by size reduction and solubilisation of organic substrates. Hydrolysis is achieved by the action of extra-cellular enzymes such as cellululases, amylases and proteases on solid substrates. These enzymes split carbohydrates, proteins and lipids into simple sugars, organic acids, alcohols, amino acids and long chain fatty acids (McDougall et al, 2001). Generally only 50% of organic matter is converted during anaerobic digestion of OFMSW, the rest remained undegraded either due to lack of appropriate enzyme systems or due to limited enzyme permeability within the solid matrix (Bolzonella et al., 2006). The reaction sequences of anaerobic digestion are described in Figure 3.1

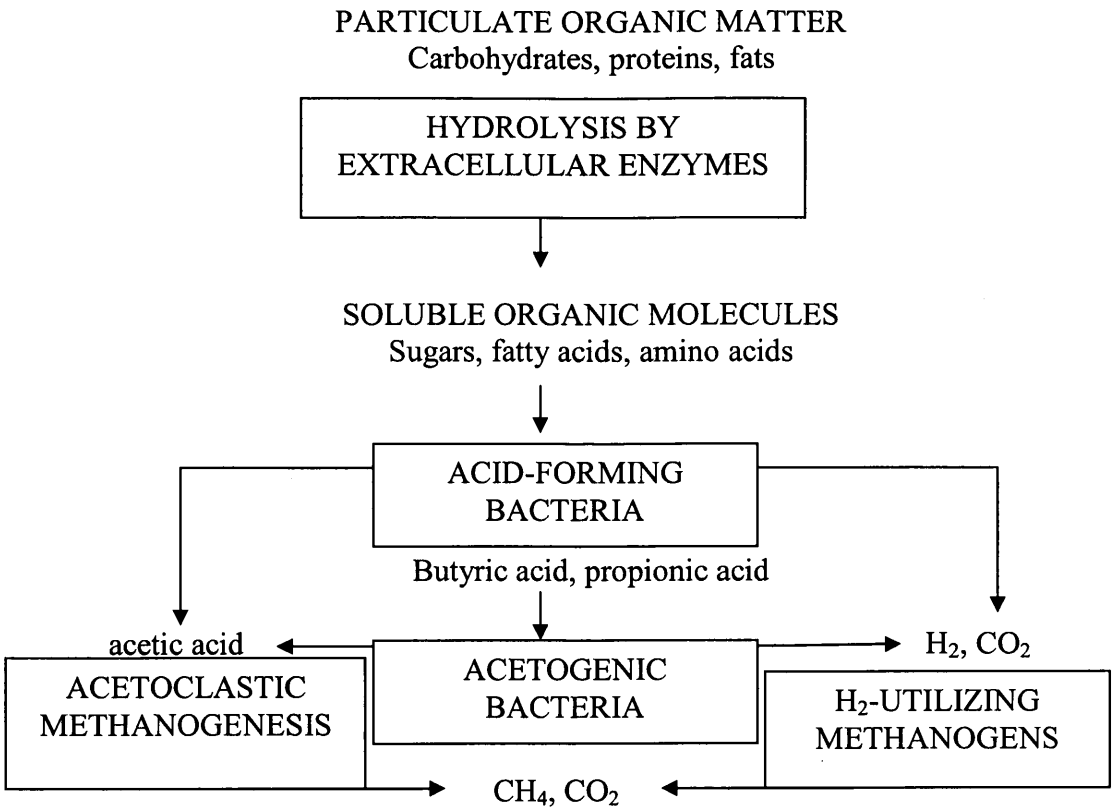


Figure 3.1 Reaction sequences of anaerobic digestion process
Source: Malina, (1992).

Hydrolysis is relatively slow and being the rate limiting step in the overall anaerobic digestion process is inhibited by high propionate concentrations as result of feed inhibition (Mata-Alvarez et al., 2003). The speed of hydrolytic reaction depends on size and surface to volume ratio of organic particles. According to Mata-Alvarez et al, (2000) the totality of all hydrolytic processes are first order reactions depending on the concentration of one substrate only. Hydrolysis is also affected by factors such as biochemical composition of waste, nutrients, toxic materials and temperature (Mata-Alvarez and Macé, 2004). Hydrolysis is also affected by temperature Veeken and Hammelers (1999), found that rates of hydrolysis of six components of biowaste comprising whole wheat bread, leaves, bark, straw, orange peelings and grass vary with temperature with hydrolysis constants ranging from 0.003-0.15 at 20^0C to 0.24-0.47 at 40^0C. Hydrolysis can be enhanced by mechanical maceration (Angelidaki and Ahring,

1999), thermo-chemical pre-treatment at 140^0C using sodium hydroxide at pH 12 for 30 minutes (Delgenes et al., 1999) and co-digestion with manure or sewage sludge (Mata-Alvarez et al., 1990). The following sections describe the hydrolysis of major biochemical components of OFMSW.

3.2.1 Starch

Starch is the most readily degradable component of OFMSW. It occurs in two natural forms namely, amylose and amylopectin. Amylose is the unbranched polymer of glucose joined together by 1, 4 $-\alpha$ linkages. In contrast amylopectin is a branched polymer of glucose with one 1, 6 $-\alpha$ linkage per 30 1, 4 $-\alpha$ linkages (Bhattacharyya and Banerjee, 2007). Starch is broken down by three enzymes comprising α-amylase, β-amylase and glucoamylase which are all abundant in a wide variety of micro-organisms. The α-amylase cleaves the 1, 4 $-\alpha$ bonds and β-amylase which break off maltose, a disaccharide of glucose from the non-reducing end of amylose and amylopectin. Glucoamylase is responsible for removing of glucose units from the non reducing end of amylolytic chain. Some of the microbes found to degrade starch in anaerobic digesters include *Streptococcus bovis*, *Bacteroides amylophilus*, *Selenomonas ruminatium*, *Succinomonas amylolytica*, *Bacillus ruminocola* and a variety of *Lactobacillus spp* (Palmisano and Barlaz, 1996). In the study of carbohydrate degradation, Noike et al, (1985), found that degradability decreased in the order of: glucose>soluble starch>acetic acid>cellulose, indicating that glucose is the most degradable with cellulose being the most resistant.

3.2.2 Ligno-cellulose

Ligno-cellulose refers to three major components of plant tissues, namely cellulose, hemicellulose and lignin. Generally processed OFMSW contains 40-50% cellulose, 12%

hemicellulose and 10-15% lignin by dry weight (Prescott et al., 2005). Major sources of these components are paper, cardboards, garden wastes and food wastes. Although cellulose and hemicellulose account for over 90% of the biochemical methane potential of OFMSW, not all are available for anaerobic digestion (Buffiere et al., 2006). This is because cellulose in the cell wall of plants is embedded in a heavily lignified material known as the middle lamellae. The middle lamellae which, is made up of 70% lignin and 30% hemicellulose, being non permeable, acts as a barrier limiting the diffusion and penetration of cellulose hydrolysing enzymes. Several researchers have reported that the rate of the hydrolysis of cellulose is the rate-limiting step in overall anaerobic digestion process. This may be overcome by size reduction, thermo-chemical pre-treatment, pre-composting or solubilising the substrates prior to anaerobic digestion (Mata-Alvarez et al., 2000).

Cellulose is a linear homopolymer of D-glucose units linked by β 1, 4 glucosidic linkages and is degraded by the enzymes hydrolases into a soluble disaccharide called cellobiose. Anaerobic bacteria known to produce cellulose hydrolysing enzymes (cellulases) include *Bacteroides succinogenes*, *Clostridium lochhadii*, *Clostridium cellobioporus*, *Ruminococcus albus*, *Clostridium thermocellum*, *Butyrivibrio fibrisolvens* and *Micromonospora bispora* (Prescott et al., 2005; Palmisano and Barlaz, 1996). Anaerobic bacteria may also produce phosphorylases specific for cellobiose which upon hydrolysis produce glucose-1-P in addition to D-glucose.

Hemicellulose is a linear or branched heteropolymer of D-xylose, L-arabinose, D-mannose, D-glucose, D-galactose and D-glucuronic acid (Prescott et al., 2005). Most of these sugars are linked by β 1, 4 linkages except in galactose based hemicellulose where

the linkages are 1, 3 β-linkages. Hemicellulose is more readily degraded than cellulose and its monomers are easily assimilated by micro organisms. The rumen bacteria known for their degradation of hemicellulose are *Bacteroides ruminocola*, *B. fibrisolvens*, *R. flavifaciens* and *R.albus*.

Lignin is a highly branched aromatic polymer of phenylpropane units linked by a variety of carbon-carbon and ether bonds (Prescott et al., 2005). Lignin is usually hydrolysed to homocyclic aromatic compounds. Although a variety of anaerobic bacteria can metabolise the aromatic compounds, their capacity for hydrolysing lignin is limited.

3.2.3 Proteins

Proteins are hydrolysed in anaerobic digesters to amino acids, ammonia and carbon dioxide by a variety of proteolytic enzymes. These enzymes are secreted by a group of specialized anaerobic organisms comprising *Clostridium perfringens*, *C. bifermentans*, *C. histolyticum* and *C. sporogenes* (Palmisano and Barlaz, 1996). The ammonia produced during hydrolysis is the main nitrogen source for biomass growth (Bolzonella et al., 2006).

3.2.4 Lipids

The major components of lipids in OFMSW are fatty acids (triglycerides). Complex lipids such as phospholipids and glycolipids may also be found sometimes in association with nitrogen, sulphur or small carbon molecules such as sugars, choline, ethanolamine and serine (Prescott et al., 2005). Lipid content of plant tissues is in the range of 1-25% (dry weight) (Cecchi et al., 2003). Large amounts of lipids may be present in the tissues of seeds as food reserve materials. Lipids are hydrolysed by a group of enzymes known as

esterases, these enzymes breakdown lipids into fatty acids and glycerol. Fatty acids and glycerol are oxidised by a wide range of organisms into organic acids especially acetate and propionate. Palmisano and Barlaz, (1996), reported that *Anaerovibrio lipolytica* and *Synthropomonas wofei* are the major lipid solubilising bacteria in anaerobic digesters.

3.3 Acidogenesis

Acidogenesis occurs either through fermentation or by anaerobic respiration and is mediated by both facultative and obligate anaerobes. Fermentation is an energy yielding process where organic molecules serve as both electron donors and electron acceptors. Anaerobic respiration is an energy yielding process where the terminal electron acceptor in the electron transport chain is an oxidized inorganic molecule other than oxygen. The major electron acceptors are nitrate, sulphate, carbon dioxide and metals such as iron (Mata-Alvarez, 2003b). Acidogenesis produces volatile fatty acids (VFAs) such as acetate, propionate and butyrate from degradation of simple sugars. Ammonia is also produced from degradation of amino acids (Garcia-Heras, 2003). Both long chain fatty acids (LCFA) and VFAs are degraded to hydrogen and carbon dioxide by obligate hydrogen producing acetogens (OHPA). The energy yield of the process is very low due to the use of organic molecules as both electron donors and acceptors.

Whilst VFAs acts as electron donors during the production of CO_2 and as electron acceptors in the transformation of H^+ to H_2, CO_2 is an electron acceptor being converted to methane with hydrogen by hydrogenotrophic methanogenic bacteria. This indicates that both acetogenesis and hydrogenotrophic methanogenesis can take place simultaneously (Garcia-Heras, 2003).

Acid-forming bacteria are generally facultative, although some are strict anaerobes and represent diverse microbial consortia. The acid-formers are relatively tolerant to changes in pH and temperature and they grow more rapidly than methane-forming bacteria (Mata-Alvarez, 2000). Facultative organisms prefer molecular oxygen (dissolved oxygen) for their metabolism. By consuming oxygen these microbes protect strictly anaerobic bacteria from small amounts of dissolved oxygen that may be introduced into the digestion system with the feedstock.

The major intermediates produced from fermentation and anaerobic respirations are volatile acids such as acetate, propionate, butyrate and formate. The accumulation of these acids may reduce the pH to a level at which the methane-forming bacteria are inhibited (Malina, 1992). These acids are the substrate used by methane-forming bacteria to generate methane and carbon dioxide (biogas).

In a mixed culture fermentation of OFMSW, a variety of micro organisms act on the end product of hydrolysis (sugars) either in concert or individually to produce different intermediates as described in Figure 3.2.

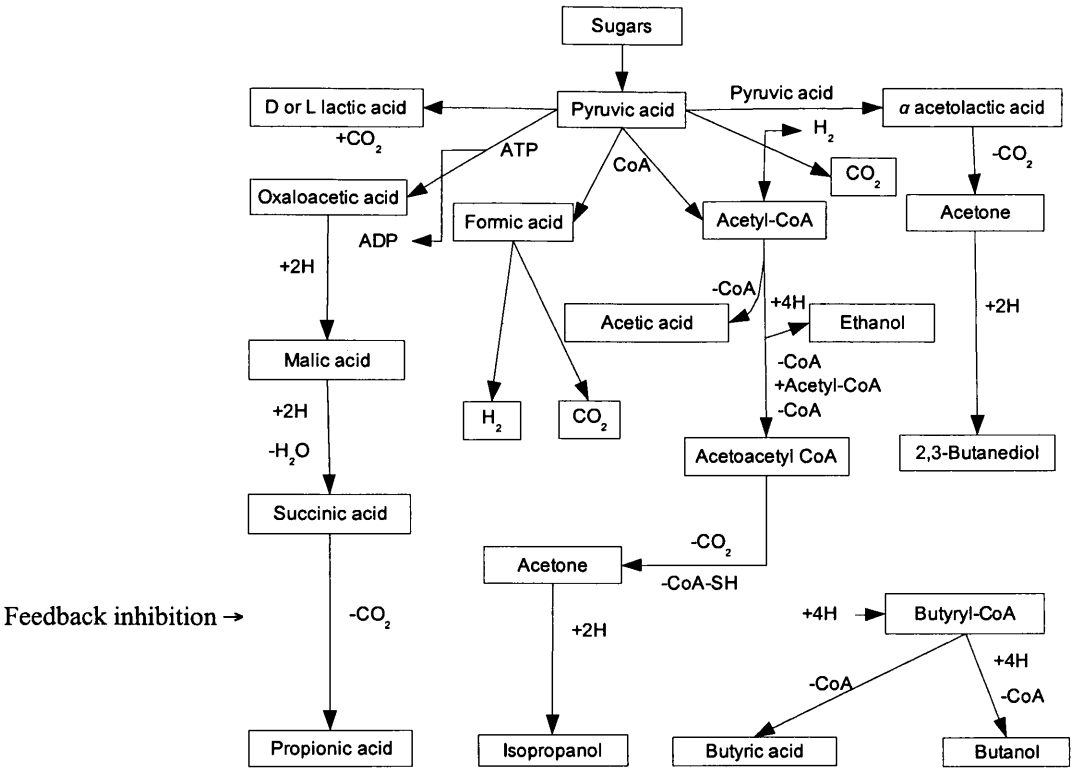


Figure 3.2 Major products of bacterial fermentation during anaerobic digestion of OFMSW
Source: Bhattacharyya and Banerjee, 2007.

3.4 Methanogenesis

Methanogenesis is a form of anaerobic respiration carried out by methane forming bacteria (methanogens). Methanogens are strict anaerobes, with each species fermenting a relatively restricted group of VFAs to methane, CO_2 and water. The group as a whole uses CO_2 as terminal electron in the metabolism of substrates such as formate, methanol, propionate, butyrate or acetate to produce methane or a combination of methane and CO_2 (See equations 3.1-3.4). Methanogens are sensitive to high and low pH. Where process deterioration occurs and the methanogens are unable to utilize hydrogen, accumulation of VFAs may lead to pH reduction and increased risk of process failure (Mata-Alvarez, 2003a; Vogels et al., 1988). Table 3.1 adopted from Prescott et al (2005) illustrates the different groups of methanogenic bacteria in anaerobic digesters.

Table 3.1 Selected groups of methanogens		
Genus	**Morphology**	**Methanogenic substrates**
Order 1 *Methanobacteriales* *Methanobacterium* *Methanothermus*	Long rods or filaments straight or slightly curved rods	H_2 + CO_2, formate
Order 2 *Methanococcales* *Methanococcus*	Irregular cocci	H_2 + CO_2
Order 3 *Methanomicrobiales* *Methanomicrobium* *Methanogenium* *Methanospirillum*	Short curved rods Irregular cocci Curved rods or spirilla	H_2 + CO_2, formate H_2 + CO_2, formate H_2 + CO_2, formate
Order 4 *Methanosarcinales* *Methanosarcina*	Irregular cocci packets.	H_2, CO_2, methanol methylamines and acetate.

Two different species of bacteria are required for the complete fermentation of propionic acid as indicated in equations 3.1 and 3.2.

$4CH_3CH_2COOH + 2H_2O \rightarrow 2CH_3COOH + 5CO_2 + 3CH_4$ ..... (3.1)

$4CH_3COOH \rightarrow 4CH_4 + 4CO_2$ ..... (3.2)

Butyrate is similarly decomposed in the following stages and shown in equations 3.3 and 3.4

$2CH_{32}CH_2CH_2COOH + 2H_2O \rightarrow CH_3COOH + 7CO_2 + 3CH_4$ ..... (3.3)

$4CH_3COOH \rightarrow 4CH_4 + 4CO_2$ ..... (3.4)

Other long chain organic acids are first biodegraded to either butyrate or propionate and then to acetic acid and methane and carbon dioxide (Prescott et al, 2005; Vogels et al., 1988).

Gas production is the most direct sensitive measure of anaerobic digestion, a decrease in production being the first indication of the process instability. Apart from a trace amount

of water vapour, hydrogen sulphide, hydrogen, nitrogen, unsaturated hydrocarbons and other gases, biogas is essentially a mixture of carbon dioxide and methane. Methane concentration in biogas mixtures varies between 55% and 65% depending on the substrate and fermentation technique (Ahrens and Weiland, 2004). A kilogram of organic matter can yield up to 600 litres of methane or $0.375m^3/kg$ of total dry solids from organic wastes (Prescott et al., 2005; Palmisano and Barlaz, 1996). The pathway for methane synthesis involves a series of co-enzymes and several unique co-factors. These are tetramethanopterin (H_4MPT), methanofuran (MFR), co-enzyme M (2-mercaptoethanesulfonic acid), co-enzyme F_{420} and co-enzyme F_{430}. The first three co-factors bear the C_1 unit when CO_2 is reduced to CH_4. F_{420} carries electrons and hydrogen, while the F_{430} serve as a co-factor for the enzyme methyl-COM-methyl reductase (Prescott et al., 2005). The ATP is generated through a combination of electron transport chain, proton pumping and chemo-osmotic mechanism.

The two major pathways of methanogenesis consist of the biological decomposition of acetic acid to CO_2 and CH_4 by acetoclastic methanogenesis (see equation 3.1), about two thirds of methane from anaerobiosis comes from acetate. The other pathway is through microbial reduction of CO_2 as shown in equation 3.5 and 3.6 below;

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad \text{..... (3.5)}$$

$$CO_2 + 8H^- \rightarrow CH_4 + 2H_2O \quad \text{..... (3.6)}$$

Methane is produced by two types of bacteria namely; acetoclastic methanogens which converts acetic acid to carbon-dioxide and methane and hydrogen oxidizing methanogens which reduces CO_2 to methane. Methanogens are the largest group of archeabacteria with three orders and twenty five genera with the major groups being *Methanosarcinaceae* and

Methanosaetaceae. They differ in shape, 16S rRNA sequence, cell wall structure and chemistry and membrane lipids. Figure 3.3 describes the sequence reactions involve in methanogenesis in the rumen of plants subsisting animals and a variety of anaerobic digesters.

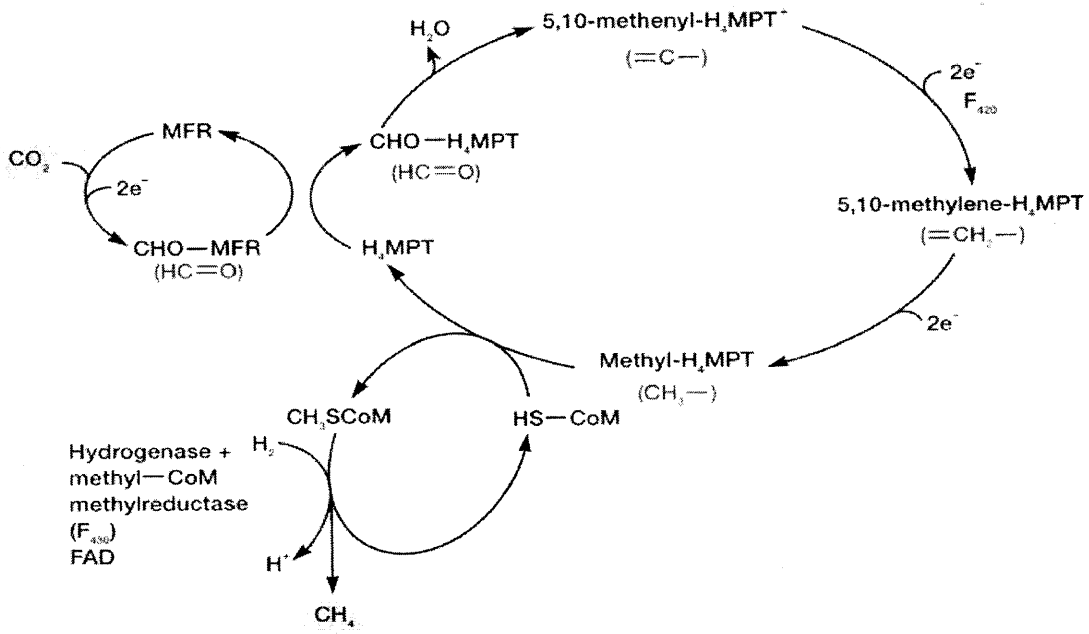


Figure 3.3 Pathway for CH_4 synthesis from CO_2 in *M. thermoautotropicum*
Source: Prescott et al, 2005

Cofactor Abbreviations: methanopterin (MPT), methanofuran (MFR), and 2-mercaptoethanesulfonic acid or coenzyme M (CoM). The transformation of carbon-dioxide to methane is indicated by the blue colour. The conversion of H_2 to H^+ is carried out by Hydrogenase methyl-com methyl reductase (F_{430}) FAD.

3.5 Operational parameters for anaerobic digestion of OFMSW

The major operational process fundamentals in anaerobic digestion are loading, inoculum (seed) mixing, start-up, nutrients, pH, degradability, temperature and the presence and absence of inhibitory substances. These, in combination with the type of reactor, determine process performance (Mata-Alvarez, 2003a).

3.5.1 Waste type and biochemical composition

Waste type (food waste, garden waste, biowaste) and biochemical composition (carbohydrates, proteins, lipids) of feedstock are critical in the selection of the most appropriate route of management for OFMSW. Generally OFMSW comprises wastes arising from biowaste, food waste and soiled paper. The quantity and biochemical composition of these wastes may vary with season, origin and time. Generally, the resource value of wastes is determined by collection system and type and degree of separation at source (McDougall et al., 2001). Source separation provides OFMSW of the highest quality with little or no contamination. Mechanically sorted OFMSW may contain contaminants, which depending on their proportion may lead to handling problems and acceptability of the digestate/compost as soil conditioner. According to Hartmann and Ahring, (2006) biogas potential is determined by cellulose content. As nutrient recycling is the major advantage of anaerobic digestion over incineration, biochemical composition is a good indicator of degradability and methane potential of different waste types of OFMSW kinetics assessment (Buffiere et al., 2006) and digestate quality (Hartman and Ahring, 2006). Methane productivity depends on the relative abundance of carbohydrates, proteins and fats as they all have different methane potentials (Mata-Alvarez et al., 2000). The most degradable components of OFMSW are the soluble and the hemi-cellulose fractions, whilst cellulose and lignin are resistant to anaerobic decomposition (Buffiere et al., 2006). C: N is used to predict nutrient deficiency and ammonia inhibition, particle size is also important in degradation rate of the waste.

Biodegradability of solid waste is critical in the design of digestion systems (Mata-Alvarez, 2003b), biogas yield, methane potential (Buffiere at al., 2006) and digestate quality (Bhattacharyya and Banerjee, 2007). Waste biodegradability is evaluated by a

reduction in volatile solids, which is dependent on waste type and biochemical composition. The biodegradability of food waste, yard waste, and paper differ significantly (Hartmann and Ahring, 2006). According to Scherer et al., (2000), the most degradable part of organic matter is the soluble and hemicellulose fraction with cellulose and lignin being resistant to microbial attack. Although the ligno-cellulosic part of waste is resistant to hydrolysis, the overall biodegradability in anaerobic systems was reported to be directly proportional to the fibre content in the cell wall of biological organic matter (Hartmann et al., 2003). Buffiere et al., (2006) observed that biodegradability decreases with increase in ligno-cellulosic content of the waste. Lignin content and biodegradable fractions are expressed as percentage dry weight of volatile solids (VS). VS contents of food wastes and yard waste were reported to be 82% and 72% respectively (Kayhanian, 1999) and 60% for waste paper (Hartmann and Ahring, 20006). Generally food waste gives the highest biogas yield, which decreases with increasing amounts of garden waste and paper. The addition of paper during high solid digestion of biowaste was observed to increase biogas yield from 3.6 to 7.1 l/KgVS.d^{-1} and reduced ammonia concentration from 3.5 to 1.7 g NH_4^+-N/l (Vermeulen et al., 1993).

3.5.2 Loading

Loading rate is another key process parameter. It is usually expressed as weight of organic matter either as volatile solids (VS) or total solids (TS) per culture or bed volume of reactor per day (Fannin and Beljetina, 1987). The loading rate describes the precise reactor size required for a given feed rate, while retention time is the average dwelling time of micro-organisms in the system. Generally, in most anaerobic systems, the higher the operating temperature the lower the retention time of the feedstock. Substantial cellulose degradation was reported at retention times greater than 20 days in MSW - fed –

Continuous Stirred Tank Reactor (CSTR) (Vinzant et al., 1990). Cecchi et al., (1991) reported that HRT of eight days is adequate in semi-dry thermophilic digestion of OFMSW. These findings suggest that the longer the retention time, the more substrate conversion achieved. Feedstocks with TS concentration greater than 10% are considered high solids (Malina, 1992). High solids digestion, also known as dry digestion, has the advantages of higher loading rates, lower energy requirement and produces less water as a waste product (Owens and Chynoweth, 1993). The performance of dry reactors in comparison to other reactors is enhanced or limited by such factors as feed addition, material handling, mixing and effluent removal (Mata-Alvarez et al., 2000).

3.5.3 Inoculum type and characteristics

Micro organisms responsible for degradation of organic matter are usually present in animal wastes. The non methanogenic bacteria are more abundant and fastidious in MSW with the methanogens being less abundant, less fastidious and more sensitive to environmental changes (Bhattacharyya and Banerjee, 2007). Source separated OFMSW therefore being devoid of animal waste will not contain naturally occurring methanogens in sufficient numbers for adequate conversion, thus necessitating the addition of inoculum or seeding prior to biological treatment. Seeds are usually biologically treated residual obtained from actively operating digesters or composting sites, sewage, animal manure or compost (Bhattacharyya and Banerjee, 2007). Generally anaerobic digesters are started by seeding heavily (at least 10% of VS of inoculum) or by maintaining pH in the range of 6.8 and 7.2 (Bhattacharyya and Banerjee, 2007). The performance and stability of the digesters depends on the quantity and quality of inoculum in the feedstock especially the process of methanogenesis. This is because of the differential growth rates of acidogens and methanogens and the sensitivity of methanogens to changes in the environmental. In

dry anaerobic digesters, an inoculum – to – feed ratio of greater than 10 is recommended (Mata-Alvarez, 2003b). In batch and plug-flow reactors where the population of methanogens is usually low, inoculums must be added with feed for process stability increase the (Palmisano and Barlaz, 1996). Low inoculum may lead to the dominance of acidogens over methanogens and pH depression. When this occurs, digester recovery may be possible depending on the alkalinity of the system. Where alkalinity is low, chemical buffer may be added to the feed to avoid system failure (Llabres-Luengo and Mata-Alvarez, 1988). Inactivation of inoculum may occur as a result of exposure to toxic substances or environmental stress factors such as temperature and pH for which they are not adapted. Granular sludge from anaerobic reactors is widely used as inoculum because they are less sensitive to inhibition compared to non granular sludge (Morvai et al, 1992). Improved performance of anaerobic systems seeded with granular anaerobic sludge has been reported when subjected to process shocks, toxins and environmental stress (Schmidt and Ahring, 1996).

3.5.4 Nutrients requirements

Anaerobes require certain substrates, growth factors, trace elements and nutrients for optimal growth and development. The most important elements are carbon and nitrogen which in biological systems are expressed as Carbon: Nitrogen (C:N) ratio. The concentration of carbon and nitrogen is critical in the performance of anaerobic digestion processes, as one or the other usually constitutes the limiting factor. Although carbon constitutes the major energy source for the micro organisms, nitrogen is essential for microbial growth. Where the amount of nitrogen is limiting, microbial populations may remain small and may take longer time to decompose the available carbon. Generally, bacteria in anaerobic digestion processes use up carbon 30 to 35 times faster than the rate

at which they convert nitrogen. So, for optimal operation, the ratio of the carbon-to-nitrogen should be about 30:1 in the feedstock (Igoni et al., 2007). As nitrogen is the limiting nutrient in the anaerobic digestion of OFMSW, its availability can be enhanced by the addition of manure, biosolids, urea (Mata-Alverez, 2003a), while woodchips and paper are useful for carbon adjustments. The inability of many anaerobes to synthesize essential vitamins and amino acids may necessitate the supplementation of the culture medium with nutrients for optimal growth metabolism.

Inorganic compounds of nutritional value to micro organisms include gases such as carbon dioxide, carbon monoxide, nitrogen, hydrogen sulphide and elemental sulphur. The cations include ferric and ferrous ions, magnesium, calcium, sodium, potassium, cobalt, copper, manganese, vanadium, nickel, zinc, mercury, cadmium and lead. The anions of significance are phosphate, carbonate, bi-carbonate, borate, sulphide, sulphate, nitrate, nitrite, chlorate, chloride, fluoride, silicate, selenite, molybdate and arsenate (Molnar and Bartha, 1988; Cecchi et al., 2003). Most of these elements are required in trace amounts and may become strong inhibitors even at relatively low concentrations (Mata-Alvarez, 2003b).

3.5.5 Toxic compounds

Beyond the required concentrations, heavy metals in particular are toxic. Their toxicity is due to their ability to bind sulfhydryl (-SH) groups of essential microbial proteins and enzymes (Prescott et al., 2005). Factors that influence the toxicity of heavy metals are pH and organic matter concentration. Low pH mobilizes heavy metals while high pH may precipitate them with reduced toxicity (Molnar and Bartha, 1988; Bhattacharyya and Banerjee, 2007).

There are other chemical compounds, such as mineral ions and detergents that beyond a given threshold may inhibit normal growth of bacteria in anaerobic digesters. Below such thresholds however, these substances are easily assimilated and may even be nutritionally beneficial to the resident bacteria.

VFAs are intermediate by-products of fermentation of OFMSW (see Figure 3.1). The un-dissociated species are more easily diffused into the cellular spaces and more toxic (Mata-Alvarez, 2003a). VFA toxicity is dependent on pH and alkalinity with propionate and butyrate being the most inhibitory. Toxicity of VFAs being the most common occurs at high concentrations and may lead to process failure in the absence of adequate buffering capacity. Process failure has been reported at propionate concentration beyond 3000 mg/l (Mata-Alvarez, 2003a).

Ammonia toxicity is dependent on waste composition, pH, solubility and temperature. Ammonia is present in aqueous solutions either as ammonium ion (NH_4^+) or as ammonia (NH_3), as described in equation (3.7) by Wujcik and Jewel, (1980).

$$NH_4^+ \leftrightarrow NH_3 + H^+ \quad \text{..... (3.7)}$$

When the pH is above 7.5, the equilibrium shifts to the right and free ammonia predominates. The free ammonia form is much more toxic than the ammonium ion. During dry thermophilic anaerobic digestion of OFMSW Kayhanian, (1999), reported ammonia inhibition at concentration of 1200 mg/l of ammonia. Ammonia toxicity can be overcome by dilution or by C: N adjustment of the feedstock (Mata-Alvarez, 2003a). Both methods rely on decrease in nitrogen concentration as suggested by Kayhanian, (1999). Ammonia toxicity may also be dependent on the type of methanogen as CN^-

anion is toxic to acetoclastic methanogenic bacteria and non toxic to the hydrogenofil methanogenic bacteria (Stonach et al., 1986, in; Mata-Alvarez, 2003a).

Toxicity by hydrogen sulphide is dependent on pH and alkalinity and waste composition. Sulphate is biologically reduced under anaerobic conditions into sulphide, which can combine with hydrogen to form hydrogen sulphide (H_2S). According to Lettinga (1995), sulphide is necessary for optimal growth of methanogens below inhibitory concentration. Although some anaerobic bacteria can adapt to moderate sulphur concentrations, higher concentrations of H_2S are toxic to some microorganisms (Lens at al., 2000). H_2S toxicity has been reported at concentrations between 200-1500 mg/l (Mata-Alvarez, 2003a). In sulphate rich substrates, acidogenic and methanogenic bacteria compete with sulphate reducing bacteria for the available substrates as described in Figure 3.4. The sulphate reducing bacteria always out-compete acidogenic and methanogenic bacteria within the same media (Colleran and Pender, 2002; Lens et al., 2000).

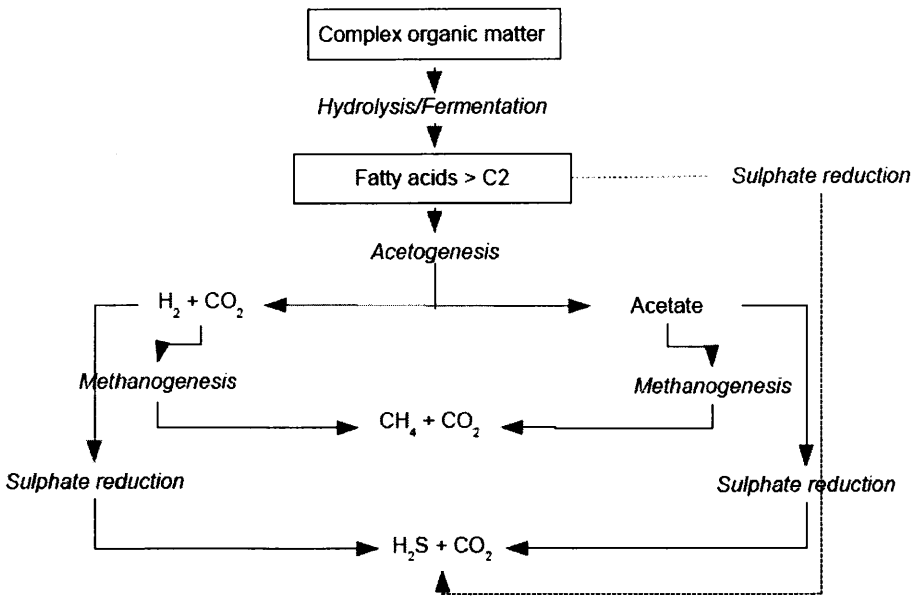


Figure 3.4 Mechanism of substrate competition between sulphate reducing bacteria, methanogenic bacteria and acidogenic bacteria
Source: Lens et al., 2000.

This competition may depend on waste characteristics, reactor configuration and retention time. Precipitation of sulphur by iron to iron sulphate (FeS) is one way of overcoming H_2S toxicity (Mata-Alvarez, 2003a).

3.5.6 pH and natural buffer systems

Feedstock degradability and process stability depend on pH stability in anaerobic digestion systems (Mata-Alvarez, 2003a). Anaerobic treatment processes operates best at near neutral pH. Deviations from this pH may lead to accumulation of acidic or alkaline end products such as VFAs or ammonia. pH may also affect solubility of organic and inorganic nutrients by influencing the reaction sequences. Under highly alkaline or acidic conditions some microbial components may be hydrolyzed and enzymes denatured. Certain microbes have been reported to tolerate extreme pH (Panikov, 1995). pH also affects the availability of substrates such as ammonium and phosphate, which limit microbial growth in many ecosystems and the mobility of heavy metals such as copper which are toxic to micro organisms (Cecchi et al., 2003). Low pH and excessive acid production and accumulation are more inhibiting to methanogens than to fermentative bacteria. The fermenters can continue to produce fatty acids even at low pH, thereby aggravating the environmental conditions for the methanogens (Prescott et al., 2005; Molner and Bertha, 1988). The optimum pH for methanogenic micro organisms are in the range of 6.8 to 7.5 (Williams, 1998). The effect manifests differently in various anaerobic consortia. *Methanosarcina barkeri* and *Methanosarcina vacuolata*, which are acid tolerant acetoclastic methanogens, grow well at low pH, with their optimum being a pH of 5 on hydrogen and methanol substrate (Pohland, 1992). However, no acetoclastic methanogens were found at extreme alkaline pH.

The bacteria involved in anaerobic digestion grow best at pH range of 6–8 with values close to 7 being the optimum (Mata-Alvarez, et al 2003a). In the initial phase of the process, the production of volatile fatty acids depresses the pH, but the reaction of CO_2, which is soluble in water, with hydroxyl ions to form HCO_3, tends to restore the neutrality of the process, thus giving rise to "self" or "natural" buffering capacity. When the rate of acid formation exceeds the rate of its breakdown to methane, process failure may result, leading to pH depression, reduced biogas yield and increased CO_2 content (Eckenfelder, 2000). pH control chemicals are widely used to prevent process failure in anaerobic digesters. According to Brummeler and Koster (1989) pH can be maintained above 6.5 with $NaHCO_3$ at buffer/substrate ratio of 0.06 kg/kg. Using Ca (OH) $_2$ at buffer/substrate ratio of 0.09 showed a depressed pH of 5.8 while $CaCO_3$ additions of 250 g/kg showed no buffering effect around neutral pH. Lime is frequently used for pH correction in anaerobic digestion systems (Eckenfelder, 2000; Bhattacharyya and Banerjee, 2007). The use of these chemicals is not only costly but may lead to precipitation of calcium carbonate where excess $CaCO_3$ is used. Chemical pH correction may result in toxic levels of cations which inhibit methane production. Additionally the time required by methanogenic bacteria to adapt to high concentration of pH control chemicals may limit the benefits of buffer addition in dry digestion of OFMSW (Mata-Alvarez, 2003a).

The natural buffering capacities and the pH of anaerobic digestion systems are determined by the combined concentrations of acetate, bicarbonate and ammonia. According to Garcia-Heras, (2003), ammonia–ammonium buffer system dominates within pH range of 5.5 to 8; the bicarbonate–carbon dioxide system dominates at medium pH values while VFAs are dominant at the lowest pH thresholds.

In general, viability and sustainability of any anaerobic system depends on feedstock biodegradability. pH stability will however depends on feedstock composition and the operating conditions of the reactor (Kayhanian 1995; Brummeler and Koster, 1989).

3.5.7 Temperature (mesophilic/thermophilic)

Biochemical processes are strongly temperature dependent, with reaction rates increasing with temperature up to 60^0C. Generally, anaerobic digestion processes are operated within two temperature ranges namely; mesophilic (near 35^0C) and the thermophilic (55-60^0C) (Panikov, 1995). There are two classes of microorganisms active in anaerobic digesters namely; the mesophilic bacteria which are active in the temperature range of 30-35^0C and the thermophilic bacteria active in the range of 45-65^0C (Bhattacharyya and Banerjee, 2007). Beyond 70^0C however, reaction rates have been reported to decrease even where substrates may be available for conversion (Mata-Alverez et al., 2000). In addition to increased reaction rates, thermophilic digesters reduce cooling costs and contamination problems (Metcalf and Eddy, 2003). Methane potential tests carried out on different food wastes showed that the rate of biodegradation doubled when temperature was increased from 35^0C to 55^0C (Di-Stephano et al., 2004). Anaerobic digesters operated at 55^0C have been reported to show greater process stability (Braber, 1995; Mata-Alvarez et al 2000; De-Baere, 2006; Bhattacharyya and Banerjee, 2007) and produced less offensive solids (Di-Stephano et al., 2004) and require less retention time compared to those operated at 35^0C. De-Baere, (2006) reported that the advantages of thermophilic digestion of OFMSW are greater in dry/batch systems than wet/continuous systems. This is because of the low requirement for heating once a thermophilic process commences as large amount of waste heat remain available for heating or drying purposes long after heating the feedstock to make thermophilic operation possible.

Thermophilic digestion of OFMSW is believed to be cost effective (Cecchi et al., 1991) and produces three times more biogas than mesophilic systems (Braber, 1995). Furthermore, it has been reported that thermophilic digesters require less time for sterilization and achieve greater pathogen reduction than mesophilic reactors (Sahlsröm, 2003). The sterilization effect is however more effective in thermophilic batch digesters than in continuously operated thermophilic digesters. Although biogas yield and kinetics favour thermophilic digestion, optimal conditions depend on substrate biodegradability and digester configuration.

3.5.8 Mixing

Mixing is required to optimize digestion because it enhances interaction between feed stock and inoculum (Mata-Alvarez, 2003b), promotes uniformity in terms of substrate concentration, temperature, and other environmental factors prevents scum formation and solid deposition. Mixing also facilitates the removal of inhibitory metabolic products from the cells. Mixing is commonly achieved by mechanical stirring or feedstock reuse of residuals. Rivard et al., (1995), reported that the energy requirements of low solids and high solids digesters are similar because in the low solids digesters higher mixing speeds are required to prevent settling of solids and scum formation. While comparing the effect of mixing on the performance of anaerobic digesters at different loading rates and solids levels, Stroot et al, (2001) observed that continuously stirred digesters were unstable at higher loading rates while minimally mixed digesters performed well at all loading rates. McMahon et al, (2001) reported that excessive mixing during digestion of MSW may inhibit the oxidation of volatile fatty acids resulting in reduced biogas yield. Slow mixing is therefore preferable however the degree and frequency will depend on waste composition.

3.6 Anaerobic digestion systems (dry/wet systems)

Anaerobic digestion processes can be divided into 'high-solids' and 'low-solids' content and it can be carried out in a 'single stage' or 'multiple stage' systems based on moisture content and hence total solids (TS) of the feedstock. Wet digestions are processes where the feedstock is less than 20% TS. In contrast, dry digestions are processes where the feedstock is greater than 20% TS while in high solids digestion the feedstock is greater than 30% TS (Hartmann and Ahring, 2006). Wet anaerobic systems are usually associated with high investment cost for large reactors, high running cost for heating, and high cost of dewatering and disposal of digested residues (Brummeler et al., 1991; Brummeler et al., 1992). Dry anaerobic digestion systems are increasingly attracting attention due to their simplicity, low investment and operational cost (Mata-Alvarez, 2003a). De-Baere, (2006) reported that of all full-scale thermophilic plants constructed worldwide in 2005, 95% were based on dry fermentation systems.

Wet anaerobic processes are usually single-stage systems. Due to the differential growth rates of acidogenic and methanogenic bacteria, pH fluctuations may render process optimisation difficult to achieve in a single reactor. This problem could be solved by a two-stage process in which hydrolysis and acidogenesis are stimulated in the first reactor at pH of about 6, while the second reactor is used for methanogenesis by keeping the pH between 7.5-8.2 (Mata-Alvarez, 2000).

Semi-solid and high solid OFMSW digestion can be carried out in a single-stage batch or continuous processes. Dry digestion systems require reduced volume, little or no moisture adjustment and are enhanced by thermophilic operation (McDougall et al., 2001). In comparison to wet systems, dry systems have the additional benefits of using wastes in

the form they are produced thereby reducing the requirement for water and problems associated with material handling. Dry systems also require little or no mixing equipment due to the absence of crust because of the solid nature of feedstock (Bolzonella et al., 2006) and require low amounts of chemicals for nutrient correction or pH control (De-Baere 1999). Because dry systems produce little or no liquid effluents these systems help conserve nutrients by immobilization. This eliminates the cost of digestate harvest by centrifugation, filtration or other similar processes.

3.7 Performance indicators

Several parameters have been used as indicators for process imbalance in anaerobic digestion systems. A good indicator should be easy to measure, should detect the imbalance at an early stage and should be able to reflect the metabolic state of the digester. It is important that the relative change in the indicator is compared to background fluctuations. Some of the most widely used indicators are listed in Table 3.2 and corroborated by (Cecchi et al., 2003; Mata-Alvarez et al., 2003a; Bhattacharyya and Banerjee, 2007.

Table 3.2 Indicators for process imbalance in anaerobic digestion (adopted from Poulsen, 2003)

Indicator	Principle
Gas production	Changes in specific gas production
Gas composition	Changes in the CH_4/CO_2 concentration ratio
pH	Drop in pH due to VFA accumulation
Alkalinity	Detects changes in buffer capacity
Total volatile fatty acids (VFA)	Changes in total concentration of VFA
Individual VFA	Accumulation of individual VFA
COD or volatile solids reduction	Changes in degradation rate

Chapter Four: Aerobic Stabilization of OFMSW

4.1 Introduction

Composting is a biological transformation process in which OFMSW is converted into a product, which is beneficial to soil as fertilizer or as a conditioner (Rulkens, 2004). Marshall et al., (2004), defined composting as decomposition of organic residues controlled by management of factors such as feedstock composition, moisture, oxygen and temperature. The manipulation of these factors usually favours a particular group of micro organisms that rapidly colonizes and decomposes the residues. As decomposition proceeds, organic matter undergoes significant changes of its physico-chemical properties resulting in stabilised compost. The resulting microbial community adapt to these changes and continues the decomposition process. Thus compost quality is not only determined by feedstock composition but also by the interactions of microbial community and process dynamics. Generally, the major objectives of composting are the transformation of unstable OFMSW into a stable useful product as well as to achieve reductions in volume, pathogens, toxic substances and weed seeds (Rulkens, 2004).

Composting has a wider application than anaerobic digestion as it produces less odour and can be used to stabilize large quantities of organic waste in a relatively short time. Compost is useful in wide range of human activities from improving soil fertility to pollution and erosion control. The use of compost in agriculture is well established especially in the areas of soil quality and productivity (Hargreaves et al., 2007; Ouedraogo et al, 2001; Shiralipour et al., 1992). Compost improves soil structure, porosity and density, water infiltration and permeability and water holding capacity, thus reducing water loss and leaching and erosion in sandy soils (Hoitink and Michel, 2004). Compost supplies significant quantities of organic matter and a variety of macro and

micronutrients to plants. Compost is useful in control and suppression of certain soil borne plant pathogens (Pharand et al., 2002). This is in addition to improving cation exchange capacities of soils and growing media (Schjønning et al, 2004), thus improving their ability to hold nutrients for plant use. Compost is a useful source of beneficial micro organisms to soil and growing media and it also stabilises soil pH and under favourable conditions can bind and degrade specific soil pollutants (Hoitink and Michel, 2004). Compost is also useful in bioremediation, weed and plant disease suppression and as a top soil for site restoration (Goyal et al., 2005) and breakdown of organic pollutants such as alkanes, aromatic and halogenated hydrocarbons (Prescott et al., 2005). Composting is therefore a sustainable method for management of OFMSW and soil quality.

Major barriers limiting large-scale application of compost are lack of uniformity and consistency of feedstock, contamination, stability and hygiene. Public acceptability and marketability may also be sources of concern where compost is associated with odour. The efficiency of composting and compost quality is determined by process and management factors described in the following sections.

4.2 Types of composting systems

The PAS:100 (BSI, 2005) recognised three types of large-scale composting systems, namely, the turned open air or housed windrow, in-vessel and aerated static piles. This is addition to small-scale household composting systems promoted to encourage resource recover and public participation. The choice of a composting technique is determine by criteria such as type and quantity of feedstock to be composted; time required for waste stabilization and pathogen inactivation; degree of composting to be employed; area and location of composting plant (Tchobanoglous et al, 2002).

Home composting is carried out by single housing units with gardens in small composting bin using green waste and food scraps as feed material. The objective is for the householder to actively use the composted material in his garden thereby minimising waste. The efficiency and sustained participation in home composting schemes depends on convenience factors such as time and effort as well as problems associated with flies, vermin and aesthetics (Tucker and Speirs, 2003)

Windrows are non-reactor agitated solids bed systems (Haug, 1993). Mixed feedstock materials are placed in rows and turned periodically. Height, width and shape of the windrows vary depending on feed material and turning equipment. Oxygen is supplied by natural ventilation or by gas exchange during turning. Turning renews microbial access to nutrients as well as disperses intermediate metabolites, whose accumulation may slow down the process. Turning frequency of 3 days is recommended by Diaz et al., (2002). Increasing the turning frequency is sometimes used to remedy water-logged conditions and compaction of the composting matter. Their major limitations are the requirement for land, the relatively long time required for waste stabilization and the possibility of inadequate aeration which may result in odour problems (McDougall et al., 2001). This is in addition to a greater risk of contamination and proliferation of vermin.

Aerated static piles are non-turned, non-agitated static solids bed systems formed over porous foundations or piped air distribution systems. The system is used for wet substrates such as sludge cake. The substrate is usually blended with a bulking agent such as wood chips and formed into a large pile. The bulking agent provides structural stability and maintains air voids without the need for periodic agitation. A limitation of this technique is that no mixing occurs once the pile is formed (Haug, 1993). Figure 4.1

summarizes the different composting systems as described by many Authors (Polprasert, 2007; Tchobanoglous et al., 2002; McDougall et al., 2001; Haug, 1993).

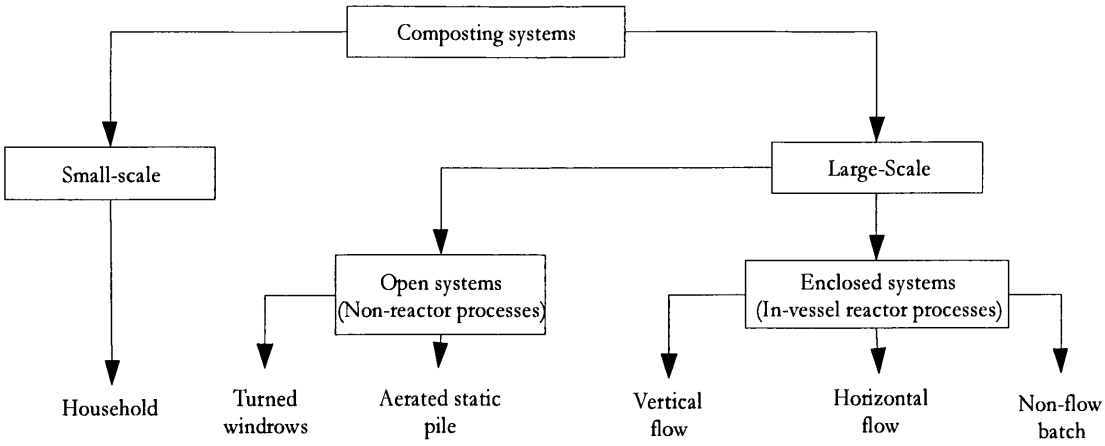


Figure 4.1 Composting systems

In-vessel processes are closed systems where the thermophilic phase is accelerated to shorten the time required for stabilization at the same time control odour, temperature, moisture, nutrient and aeration. The animal by-product regulation (2002/1774/EC/) prescribed the use of in-vessel composting for the stabilization of category 3 materials such as food waste. The major benefits of in-vessel systems are reduced requirement for land and low risk of contamination and vermin (Polprasert, 2007). Based on design in-vessel systems are classified into vertical flow, horizontal flow and non-flow batch processes (Haug, 1993).

Vertical flow systems are reactors with a series of trays, floors or cages vertically installed to allow passive aeration. Composting begins by loading the feed material at the top of the reactor where the temperature is high to accelerate microbial decomposition. In vertical moving packed reactors aeration and mixing occurs as the feed material moves

downwards. These reactors are widely use to stabilise sludge and can be fed on a continuous, intermittent or batch basis.

Horizontal and inclined flow systems can be tumbling, agitated or static solid bed reactors. The tumbling or rotating drum reactors use the dispersed or plug-flow systems to achieve stabilization, while agitation and a static solid bed are used in the agitated and static solid bed reactors respectively.

Non-flow batch processes uses compost boxes or tunnels where feed materials are loaded and remained enclosed for 1-2 weeks under controlled aeration.

4.3 Factors affecting aerobic stabilization of OFMSW

4.3.1 Feedstock/waste type/degradability

The feedstocks for composting are principally green wastes and food waste components of OFMSW. Green waste comprises of botanic residues from domestic, amenity, commercial and industrial sources. These include prunings, hedge and lawn clippings, spent bedding and pot plants, roots, tubers, corms and bulb, mature woody material, leaves and other organic debris from gardens, parks, green spaces, driveways and roadsides. Other substrates may include ornamental, edible and weed species as well as fungi and any soil that may be attached. Green waste may also include wood wastes originating from untreated wood or wood treated with preservatives such as sawdust. Wood wastes are the most consistent organic feedstock as they are principally made up of carbon in the form of lignin. These compounds are useful as bulking agents or support material. Each of these feedstock materials contributes different chemical and physical properties, both to the process and to the final end product (Haug, 1993; McDougall et al,

2001). Food wastes, kitchen or catering wastes are predominantly made up of materials of higher water and nitrogen content that decompose readily. These include all waste food including used cooking oil originating in restaurants, catering facilities and kitchens. Food wastes may also include former foodstuff such as waste from bakery and confectionery goods, food processing or manufacturing wastes not containing meat. Food wastes are easy to break down and provide material readily accessible for microbial decomposition and furnishing the end product with more readily available plant nutrients (Haug, 1993; McDougall et al, 2001).

Particulate size and surface to volume ratio are important in feedstock degradability and process optimisation. For optimum decomposition particulate size of 25-75mm is recommended (Tchobanoglous et al, 1993).

4.3.2 Nutrients

The principal nutrients required for growth of micro-organisms are carbon, nitrogen, phosphorus and potassium. Although bacteria also need trace amounts of sulphur, sodium, calcium, magnesium, iron and other substances, these elements are usually present in adequate quantities and do not limit bacterial activity (Hoornweg and Thomas, 1999). Carbon and nitrogen are the most important and the most limiting elements for microbial growth (occasionally phosphorus may also be limiting). When there is too little nitrogen, the microbial population may not develop to its optimum and the process may slow down. As nitrogen becomes a limiting factor, micro organisms are forced to go through additional cycles of carbon consumption, cell synthesis and decay to burn off the excess carbon as CO_2 (Haug, 1993). In contrast, high nitrogen concentration allows rapid microbial growth and accelerates decomposition, but this can create serious odour

problems due to formation of anaerobic environment as result of oxygen depletion. In addition, excess nitrogen is given off as ammonia gas which is responsible for the bad odour that may occur during composting.

Although nutrients such as phosphorus, potassium, and other trace elements may be lost through leaching, significant amount of nitrogen could be lost through volatilization as ammonia or other nitrogenous gases. These losses may have impact on the fertilizer value of the compost, thereby influencing crop yield with cost implications. The loss of nitrogen as ammonia is dependent on process parameters such as C: N ratio, pH, moisture, aeration, temperature, the chemical form of nitrogen in the feedstock, adsorptive capacity of the composting mixture, and mixing frequency with pH and temperature having the greatest effect on the ammonia NH_3 ammonium NH_4^+ equilibrium (Polprasert, 2007). High pH in the initial feedstock, especially where the feedstock is rich in ash (ash exhibits a pH of 10-11) enhances the release of ammonia. Lack of moisture may also increase the release of ammonia while excess moisture promotes its solubility. The loss of ammonia may increase with mixing, especially where the composting material is exposed to the atmosphere. Thus, a delicate balance is required for the regulation of pH, temperature, moisture, aeration and mixing frequency (McDougall et al., 2001) during composting operations.

4.3.3 Temperature

Temperature is a critical factor affecting the development of microbial communities during composting. Windrows prepared according to prescribed rules, that is, with adequate porosity, humidity, and C:N ratio, and exhibiting a minimal size to provide sufficient matrix for insulation will develop thermophilic temperatures independently of

ambient temperatures. This is because microbial activities generate heat during aerobic decomposition organic waste. As the temperature of the pile increases, different groups of organisms become active. Where the levels of oxygen, moisture, carbon, and nitrogen are adequate, compost piles can heat up to temperatures in excess of 65^0C. Failure of feedstock to heat up may serve as an indicator of process failure or that windrows are not properly formed. During aerobic decomposition in open windrows, organic piles can go through several temperature cycles. The groups of bacteria involved in the decomposition process could be a combination of psychrophilic (5-20^0C), mesophilic (20-50^0C) and thermophilic (50-70^0C) organisms. This diversity is necessary for the stepwise decomposition of the feedstock into stable compost. Although composting will also occur at lower temperatures, maintaining high temperatures is necessary for rapid composting as it destroys the thermo-sensitive human pathogens as well as weed seeds, insect larvae, and potential plant pathogens that may be present in the waste material. As the compost becomes mature and stable the temperature decreases and gradually approaches ambient temperature conditions.

Temperature is one of the key factors that need to be constantly monitored and controlled in composting processes (McDougall et al., 2001). The role of temperature in the aerobic stabilization of organic waste is not fully appreciated. Irvine, (2002), proposed the use of temperature for process monitoring of large scale commercial composting systems. The author described the different stages of microbial successions in an open windrow composting system as illustrated in Figure 4.2

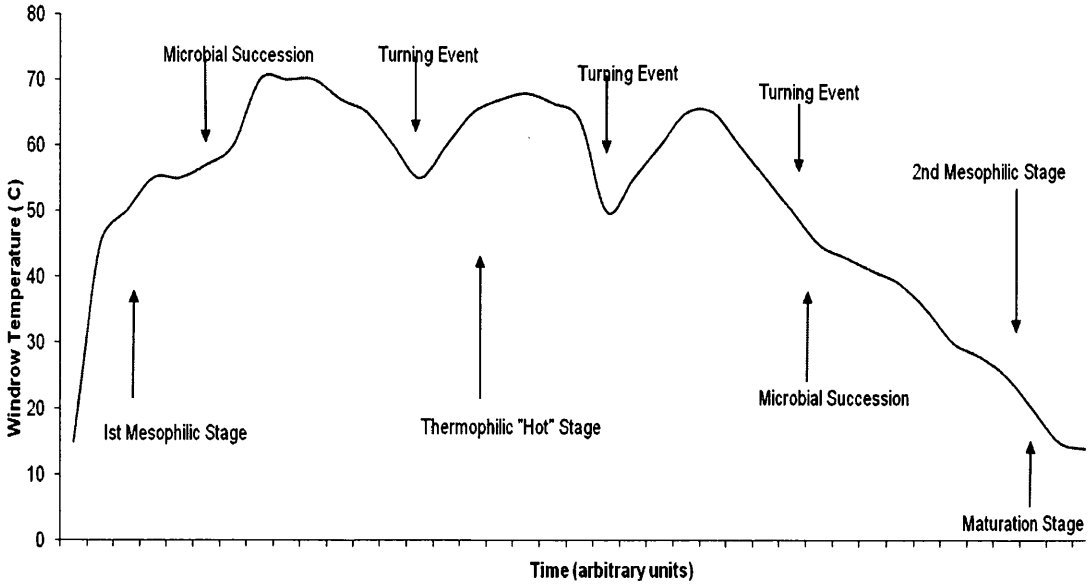


Figure 4.2 Generalized temperature trends observed in an idealised open windrow system over time, with major events

Adopted from: Irvine, 2002

The first mesophilic stage shows a rise in biologically produced heat to mesophilic level. This heat further accelerates microbial decomposition producing more heat which accumulates in the core of the windrow. Beyond 45^0C, the mesophilic decomposers are replaced by thermophilic communities which are better adapted to accelerated decomposition at higher temperatures. As nutrients and ventilation dwindle, the temperature also decreases only to rise again following each turning event. Eventually the thermopiles will exhaust the nutrients available for their biological activities leading to a gradual decline in temperature. As temperature approaches 45^0C and continues to dwindle, another microbial succession occurs with emergence of the second mesophilic stage. This group of decomposers further stabilize and cure the compost into maturity.

4.3.4 Moisture

Moisture content and size of particles are closely interrelated in terms of displacement of air in the pores by water, promotion of aggregation and lowering of the structural strength of the final end product. The surface area of organic particle exposed to micro organisms is critical in determining the rate of composting. Waste material that is shredded, chipped, or reduced in size is degraded more rapidly. This is critical especially with slow degradable woody materials. Maintaining adequate moisture content during composting is critical, as humidity is required by micro organisms for optimal degradation. Moisture also dissipates heat and serves as a medium for diffusion of nutrients. Moisture content of between 40 to 60 % by weight throughout feedstock with the peak being 55% is required for optimum microbial action (Polprasert, 2007; McDougall et al., 2001). Higher moisture levels may slow the decomposition process and promote anaerobic degradation as air spaces in the feedstock become filled with water to create anaerobic conditions. Moisture levels below 40% may slow microbial activities thereby prolonging the composting time.

4.3.5 Aeration

Oxygen required for composting is not only utilised for aerobic metabolism by micro organisms but also for oxidising various organic molecules present in the mass. Oxygen consumption during composting is proportional to microbial activity, thus creating a relationship between oxygen consumption, temperature and aeration. The greater the aeration rates the more rapid the rate of degradation. Aeration provides air required for rapid odourless decomposition and for destruction of pathogenic organisms by heat. The air contained in the interstitial spaces of the composting matter at the beginning of the microbial oxidative activity varies in composition. As the level of carbon dioxide

increases that of oxygen decreases and the oxygen level in the interstitial space falls below 10%, a condition that may indicate compost stability (McDougall et al., 2001).

The most common method for aerating the composting feedstock is by manual or mechanical turning (mixing). Active aeration refers to methods which actively blow air through the composting feedstock, while passive aeration relies on the natural diffusion of air through the composting feedstock. Passive aeration is enhanced by ventilation structures such as perforated pipes, openings in the walls of composting bins and size and structure of particles of the feedstock. Where air supply is limited, anaerobic conditions may set in, thus producing methane gas and malodorous compounds such as hydrogen sulphide and ammonia. The consumption of oxygen is greatest during the early stages of aerobic decomposition but gradually decreases with depletion of readily degradable substrates as the composting process proceeds to maturity.

Mixing and aeration usually occur simultaneously as both are critical to the survival of aerobic microbial communities responsible for stabilization of the compost. Marshall et al., (2004) reported lack of re-colonization by eukaryotic communities at lower oxygen levels in feedstock exposed to higher temperatures during mixing. This is believed to be due to high rate of aerobic decomposition which may results in significant differences in the composition of microbial communities.

4.4 Composting Process

The organisms involved in composting are grouped into six in decreasing order of abundance. These are bacteria, actinomycetes, fungi, protozoa, worms and some larvae (Tchobanoglous et al., 2002). During composting organic waste is first decomposed by

first level consumers such as bacteria, fungi and actinomycetes. The process is initiated by mesophilic (25 – 40^{0}C) bacteria, as the temperature rises the thermophilic (45 – 75^{0}C) bacteria gradually appear. Fungi usually grow after 5 – 10 days of composting on the exposed surfaces of windrows. As the compost matures, the temperature declines, leading to domination of the compost mass by actinomycetes. In the final phase the compost is cured by a combination of actinomycetes and moulds (Tchobanologous, et al., 1993; Sidhu et al., 2001). Where the concentration of the relevant microbes in each of the phases becomes inadequate, seeding the composting material with the appropriate inoculum may become necessary. Blending and seeding feedstock may reduce composting duration.

Organic substrates for composting vary in composition from the highly homogenous waste from food industries to the heterogeneous materials contained in MSW. The breakdown of these complex organic molecules may occur by different pathways acting independently or in concert to achieve stabilization. These pathways were summarized in the following pathways as described by McDougall et al., (2001):

- Proteins → Peptides → Amino acids → Ammonium compounds → Bacterial protoplasm and atmospheric N or NH_3
- Carbohydrates→ Simple sugars → Organic acids → CO_2 and Bacterial Protoplasm

In addition to hydrolysis, nitrification is also an important biochemical reaction in the composting process and its occurrence may indicate compost maturity. Two groups of nitrifying bacteria are responsible namely, the *Nitrosomonas*, which converts NH_4^+ to NO_2^-; and the *Nitrobacter*, which converts NO_2^- to NO_3^-. As NO_3^- associated with N-

mobilizing bacteria is the most available form of N for uptake by plants (Polprasert, 2007), the potential nitrification rates of composts is critical (Wheatley et al., 2001) especially where it is intended for use in agriculture. The nitrifying bacteria have a relatively slow growth rate and they become inactive at temperatures beyond 40°C (Polprasert, 2007). This temperature limitation ensures that they only become active in the final stages of waste decomposition.

4.5 Indicators of compost stability, maturity and quality

4.5.1 Odour

Odour is generated by the release of ammonia and other reduced compounds from poorly aerated feedstock. Odour can be reduced significantly by adequate aeration as air encourages ammonia stripping and transformation from nitrites to nitrates (De-Guardia et al., 2004). It has been recommended that aeration should be continued until ammonia odour disappears. The replacement of ammonia odour with "soil-like" or humic odour can be useful in the estimation of progress of the composting process. Generally, the use of odour as an indicator of process and compost stability is limited as current methods of its estimation are not well established (De-Guardia et al., 2004)

4.5.2 Temperature

Heat is generated during composting as result of microbiological activities. Where feedstock is rich in readily biodegradable matter and especially where aeration rate, moisture and temperature allow microbial growth, temperature will increase to between 60^0C to 70^0C and may remain constant at those values for several days. The consumption of organic matter by microbial activity will diminish available nutrients leading to a reduction in microbial activities and compost temperature. The variability of composted

matter combined with the fact that air may not be distributed equally within the composting matter indicates that temperature may be a less effective indicator of compost stability (Jimenez and Garcia, 1989).

4.5.3 pH

Organic feedstock with wide pH range of between 3 and 11 can be composted. However, optimum pH values for composting are between 5.5 and 8 for the feedstock and between 4 and 7 for the final end product (Haug, 1993). Although bacteria prefer a near neutral pH, fungi develop better in a slightly acidic environment. At the beginning of the composting process, the pH may drop to about 5 as organic acids are formed. However, the production of ammonia by microbial action will cause the pH to rise to the range of 8 to 8.5. Beyond pH of 8.5, nitrogen is lost in the form of ammonia (Haug, 1993; Polprasert, 2007). During maturation, when ammonium compounds are nitrified to nitrate, the pH gradually falls to below 8. Extremely high pH generally indicates lack of stability.

4.5.4 C: N ratio

The C: N ratio of composting substrates depends on the level of biological activity required. Optimum ratio is in the range of 25 - 50. C: N ratios lower than 15 may lead to the loss of nitrogen as ammonium (Diaz et al., 2002). At lower ratios ammonia may be released resulting in low level of biological activity and an extended composting time. Nitrogen is a limiting nutrient especially at higher ratios. The addition of nitrogenous waste such as green and food waste is used to lower the C: N ratio as the addition of carbon rich compounds such as paper, wood chips and hay is used to increase the ratio.

The C:N ratio of organic substrates may decrease during composting especially during fermentation (Benitez et al., 1999). A C: N ratio of about 12 is recommended for compost maturity (McDougall et al., 2001). The major barriers to the use of C: N ratio as an indicator for compost stability is that it accounts for both degradable and non-degradable carbon and nitrogen sources.

4.5.5 Respirometric activity

Respirometric activity is widely used as an indicator of compost stability as it provides valuable information on level of bioactivity and hence the stability of the composting matter. It relies on the measurement of microbial respiration during the breakdown of organic matter. Respirometric activity is measured through the incubation of compost samples for a few days at temperatures between 20- 37^{0}C (Iannotti et al., 1994; Rajbanshi et al., 1998). Generally, oxygen consumption and carbon dioxide production are directly proportional to microbial metabolic activities. Although respirometric techniques are a useful indicator of compost stability (De-Guardia et al., 2004), increased CO_2 flush could also be due to increased production of organic matter, lack or excess moisture, high temperature or insufficient aeration.

4.5.6 Germination Index (GI) Bioassay

Germination index bioassay is a seed germination test used to investigate compost stability and quality (Eustaùn et al., 1985; Tiquia and Tam, 1998; McLachlan et al, 2004). The test evaluates seed germination inhibition (phytotoxicity) of mature composts prior to soil application. Phytotoxicity may be caused by volatile fatty acids (Jimenez and Garcia, 1989; Hoekstra et al., 2002) usually abundant during active decomposition of organic matter. As optimum seed germination can only be achieved in the absence of volatile

fatty acids, high seed germination index therefore is an indication of the absence of readily degradable matter which signifies compost quality and stability.

Germination tests measure root length following 72 hours incubation at 25-28^{0}C of seeds placed on a filter paper previously moistened in aqueous extract of compost (De-Guardia et al., 2004). Although differences exist in number and type of seeds used, volume of aqueous extract and incubation time, all seeds growing tests measure the root length of germinated seeds which are compared to blanks obtained with seeds in distilled water (Blanco and Almendros, 1997). Germination index of each sample is determined using equation 4.1 (Zucconi et al, 1981).

GI = (No of Germinated Seeds/No of seeds) x (Total root length/Blank root length)

(4.1)

Compost is considered stable when the GI is above 50% (Zucconi et al., 1981; Rajbanshi et al, 1998).

Germination bioassays are carried out usually with variety of seedlings which are small and sensitive to toxic chemicals and capable of rapid germination (Zucconi et al., 1985). Cress (*Lepidium sativum*) was used by Murillo et al., (1995) and Levy and Taylor, (2003). Lettuce seedlings (*Lactuca sativa* L.) were used by Gariglio et al., (2002) while radish seeds (*Raphanus sativus* L.) were used by Tiquia and Tam, (1998), Hoekstra et al., (2002) and Levy and Taylor, (2003).

4.5.7 Pot trial bioassay

Pot trials are useful in describing the agronomic quality of composts (De-Guardia et al., 2004) in evaluating compost stability and maturity (Jimenez and Garcia, 1989), quality (Gariglio et al., 2002) and for the prediction of plant response to compost amendment (Shana et al., 2004). Germination index bioassay alone may not indicate the availability of nutrients in the compost for plant use; only subsequent pot seedling growth trial can provide this information. Pot trials are carried out in glass houses in pots containing soil or a variety of growth media where the plants are exposed to different regimes of light and darkness. Plants performance during the early growth stage will indicate if there are any phytotoxins within the media which could adversely affect the growth. Poor performance during later growth stages might reflect a lack of available plant nutrients for sustained growth (Shana et al., 2004).

4.5.8 Sanitary quality

Temperature control techniques such as pasteurization are widely used for sanitization of OFMSW, compost and anaerobic digestate. One of the requirements for sanitary quality of OFMSW during biological treatment is exposure to a minimum temperature of 55^0C for at least 24 hours at an average retention time of at least 20 days (PAS 100, BSI, 2005; Braun and Kirchmayr, 2004; Animal By-Product Regulation, 2002). Where this is not applied, the pre-treatment of fresh feedstock is required. That is, heating at 70^0C for one hour. Table 4.1 describes the requirements for sanitary quality in selected European countries as described by (Brinton, 2001).

Table 4.1 Selected compost hygiene standards (Brinton, 2001).		
Country	*Compost method*	*Temperature/time exposure*
Australia	All methods	>55^{0}C for at least 3 days, allowance for variation and lower temperatures
Germany	Open windrow Closed/in-vessel	>55^{0}C for 2 weeks or >65^{0}C for 1 week or >60^{0}C for 1 week Plus all new facilities: No presence in 25g of: *S. senftenberg* W775 No survival of added: Tobacco Mosaic Virus and *Plasmodiophora brassicae*
Austria	All composts	> 60^{0}C for 6 days, or >65^{0}C for 3 days, or > 65^{0}C 2 x 3 days
Switzerland		>55^{0}C for 3 weeks, or >60^{0}C for 1 week, or proven time temperature relationship
Denmark	All composts	>55^{0}C for 2 weeks

As pasteurization requires exposure to the temperature of 63^{0}C for one hour to achieve the destruction of human pathogens, temperatures below 63^{0}C, irrespective of exposure time, may not be enough for the achievement of sanitary quality. Stenbro-Olsen, (1998) reported that windrow temperatures of 55^{0}C are not sufficient for the elimination of mesophilic bacteria, regardless of exposure period. Thus, heating substrates at 70^{0}C for one hour before or after biological treatment is preferable.

4.6 Composting of anaerobic digestate

Anaerobic digestate is a potential source of organic soil fertilizer that has not been fully exploited. The digestate is usually a fibrous viscous liquor or sludge. The major problems with the use of anaerobic digestate as soil fertilizer are its high moisture content, low solids, pH and C:N ratio. Anaerobic digestates are typically rich in lignin which is resistant to anaerobic decomposition and high ammonia content which could induce

phytoxicity in the soil. However, the digestate is rich in plants nutrients which can be immobilized for soil application. In areas where there is a legal restriction on the application of liquid sludge to land, the use of anaerobic digestate as soil amendment will be severely affected. Anaerobic digestate may therefore require some aerobic post-treatment to render it more amenable to soil application. No standard protocol or literature is available on aerobic post-stabilization and bioconversion of anaerobic digestate into a stable soil fertilizer. One of the objectives of this research is to develop a protocol for aerobic post-treatment of anaerobic digestate into a stable soil fertilizer. The next chapter reviews factors and processes affecting the use of organic amendments in the management of soil quality

Chapter Five: Soil Quality Management

5.1 Soil

Soil is a natural non-renewable resource composed of minerals, organic compounds, air, water and living organisms interacting together to produce physical, chemical and biological processes (DEFRA, 2004). As sustainable management of soils is fundamental in sustainable development, the diversity and versatility of soils is the major factor shaping landscape and land use planning system in United Kingdom (DEFRA, 2004). Soils provide the platform for built development and protect and contextualise cultural heritage. In addition to being a growth medium for food, timber and other crops, soils also act as natural reservoirs of water, carbon, genes and biodiversity with the capacity to buffer and transform chemicals that could otherwise cause water or air pollution and possible contamination of agricultural products. Soil microorganisms play an important role in nutrient cycling of carbon, oxygen, nitrogen, sulphur, phosphorus and iron. These cycling activities are relevant in bioremediation, municipal waste disposal, and sustainable agriculture and mining (Maier and Pepper, 2000). In addition to the organisms which spend their full life cycle in soils, there are many for which a soil based phase is a crucial part of their life cycle. Soil biodiversity is essential in maintaining soil functions and sustainable systems as many of the key processes behind these functions are mediated by the soil organisms (DEFRA, 2004).

The need for soil amendment is as result of greater demand for improved soil productivity, changing agricultural practices, and land use policy and soil degradation. According to The First Soil Action Plan for England, (DEFRA, 2004) 18% of soil organic carbon present in the arable topsoil in 1990 was lost by 1995. In spite of the various management techniques developed for soil improvement, the loss of organic matter in

arable soils is still a major challenge in soil productivity. Some of these techniques include the addition of *Rhizobium sp* to enhance nitrogen fixation by legumes, soil inoculation with mycorrhizal fungi to enhance uptake of phosphorus by plants, inoculation with *Pharecheate sp* to encourage the decomposition of persistent organic pollutants. *Rhizobacteria* is also used to control soil-borne root infecting pathogens (Stotzky, 1997). Soil amendment with organic wastes or products derived from them is well documented (Adegbidi et al., 2003; Petersen et al., 2003; Mata-Alvarez, 2003a; Tchobanologous et al, 1993). The need to enrich soils with by-products of biological treatment of organic fraction of municipal solid waste (OFMSW) is due to the need to integrate improved soil productivity with waste recycling as contained in the Waste Management Strategies of Scotland, (SEPA, 2003); Waste Strategy for England (DEFRA, 2007)

The physical properties of soil are critical in determination of soil functions. The diameter of the soil particles differ from sand to clay as described by in Table 5.1

Table 5.1 Soil composition (Donahue et al., 1971)

Particles	Diameter (mm)
Sand	2.0-1.0
Coarse sand	1.0-0.5
Medium sand	0.5-0.25
Fine sand	0.25-0.1
Very fine sand	0.10-0.05
Silt	0.05-0.002
Clay	< 0.002

The relative availability of the soil particles determines the soil texture and processes. Soils with large amount of sand are sandy soils, those with high proportion of clay are clay soils and those that are neither clay nor sandy are loamy soils.

5.2 Management of soil quality

Soil quality is the capacity of soils to function within natural or managed ecosystems boundaries to sustain plant and animal productivity, maintain water and air quality and support human health habitation (Schjønning et al., 2004). Soil properties are grouped into physico-chemical and biochemical properties both of which are important in soil dynamics. The physico-chemical properties are defined by soil organic and inorganic mater, bulk density, soil texture, water retention, compression, aeration, aggregate stability and pH. The biochemical properties are soil respiration and biomass. Both properties can be useful indicators of soil quality and agricultural productivity.

As soil quality is defined by performance; critical soil functions such as fertility and health are determined by physical, chemical and biological indicators. For any parameter to be suitable as an indicator of biological soil quality, it should be able to account for spatial heterogeneity of soil environments (Ettema and Wardle, 2002) as well be sufficiently stable under non changing conditions. It is also necessary for biological indicators to be specific for different environmental factors and sensitive to agricultural management practices by showing changes in soil organic matter, nutrient cycling, soil structure or biological productivity. As no single parameter is adequate for indication of biological soil quality, the use of multiple indicators is desirable. According to Brussaard et al., (2004) soil bioactivity, biomass and the diversity of soil populations are suitable indicators of biological quality.

Organic matter is the most important component of soils, influencing soil structural development, aggregate stability, moisture supply, nutrient turn-over and availability and natural fertility (Smith et al., 1992).

Although soil biomass and soil respiration are good indicators of the state of soil microbial populations, a broader view of both active and less active populations can only be determined using molecular techniques such as Templete-restriction fragment length polymorphism (T-RFLP) (Marsh, 1999) and denaturing gradient gel electrophoresis (DGGE) or temperature gradient gel electrophoresis (TGGE) (Muyzer and Smalla, 1998).

Indicators such as germination index and plant growth bioassay techniques may also be considered as soil quality parameter especially where the intended soil function is in agricultural productivity (De-Guardia et al., 2004; Levy and Taylor et al., 2003; Hoekstra et al., 2002). Furthermore, soil organisms are responsible for cycling of carbon, nitrogen and phosphorus and also play a significant role in plant-pathogenic reactions as well as biological transformation of man-made chemical in the soil, their population dynamics is critical in understanding and predicting soil functions. The next sections review soil quality indicators and their importance in soil management

5.2.1 Physical Indicators

5.2.1.1 Water retention and water potential

Water retention affects soil quality due to its impact on plant growth and development by minimizing run-off and leaching. Water content can either be volumetric (by volume) or gravimetric (by weight) fraction of the total rock filled with liquid water and this may vary between 0 and 1 or 1% to 100% indicating the percentage porosity filled with water.

Soil water content depends on the quantity and quality of soil organic matter, soil texture and structure as well as aggregate strength and stability with soil aeration and soil compaction being more critical. Different organic amendments have been used in soil water management. These include biosolids/sewage sludge (Franco-Hernandez et al., 2003; Sanchez-Mondero et al., 2004), biowaste (Pascual et al., 1999) and compost (Aggelides and Londra, 2000; Debosz et al., 2002). Where water retention is high, plants may have to adapt to saturated conditions or drainage must be installed to prevent leaching, water logging and soil erosion. Although drainage improves soil aeration it may lower soil quality by increasing the degradation rate of soil organic matter (Karlen et al., 2004).

Soil water content is influenced by processes such as aeration, water potential and soil compaction. These can be quantitatively related in the least limiting water range (LLWR) concept (Van den Akker and Schjønning, 2004). The concept is based on the principle that the response of plants to varying water contents is least limited inside a range and most limited outside the same range. The soil water content limitations are expressed as functions of available water, aeration and mechanical stress.

Water potential measures the ability of water to move in a porous medium. The movement of soil water is influenced by such processes as infiltration, evaporation and plant uptake. Water potential is also influenced by adsorbtion of water to soil, dissolved solutes, elevation in gravitational field and pressure (Van den Akker and Schjønning, 2004). An increase in pressure results in decreasing water potential as result of increasing aggregate strength. Soil water potential is usually expressed in units of pressure (bars,

Pascals). Although there are many methods of measuring water retention, one of the acceptable techniques was described by Aggelides and Londra, (2000).

The moisture retention characteristics of soils are controlled by a number of physical properties which include pore space distribution, particle size, organic matter content, stone content and bulk density. According to Zhang et al., (2005), increasing soil organic matter produced corresponding increase in soil aggregate formation and aggregate stability, this in turn increased porosity and pore sizes resulting in improved water infiltration and retention. A decrease in soil organic matter may result in increasing soil bulk density, a condition that may decrease the amount of water available for plant growth. Aggelides and Londra, (2000) reported that compost amendments improved water content with the improvement being proportional to application rates. The application of non-stabilized organic fertilizers may induce water repellancy in the soil with soil water content being the most significant factor in its temporal variation (Zhang et al., 2004). Feeny et al., (2006), reported that the severity of water repellancy in substrates enriched soils vary with length of incubation.

Water potential is the potential energy of water relative to pure deionised water. It measures the tendency of water to move from one area to another due to osmosis, gravity, mechanical pressure, or matrix effects such as surface tension (suction). Water retention curve is the relationship between water content and soil water potential. The curve is unique for different soil types and is used to predict soil water content, water activity (field capacity) and aggregate stability. At potentials close to zero, a soil is close to saturation, and water is held in the soil primarily by capillary forces. At any given potential sandy soils will release most water at higher potentials, while clay soils with

greater adhesive and osmotic binding, will release water at lower (more negative) potentials. Generally, peaty soils will display higher water contents than clay soils, which would be expected to hold more water than sandy soils. By measuring soil water content at different suctions (negative water potentials) and incubation times, water retention curve could be obtained which can be used to quantify the moisture available for plant growth (Aggelides and Londra, 2000).

5.2.1.2 Water infiltration and sorptivity

Water infiltration rate is the measure of water absorption in soil usually expressed in millimetres per hour. Water infiltration decreases as the soil becomes saturated regardless of the types or values of forces or gradients (Kirkham, 2005). Hydraulic properties of soil are important in soil fertility and productivity as they determine the amount of water available for plant growth and microbial action. One way of improving soil hydraulic conductivity is by application of organic matter (Kirkham, 2005). Soil hydraulic conductivity depends on organic matter content and size and distribution of soil particles. Soil hydraulic conductivity is also a function of water potential of the soils and is useful in the prediction of direction and rate of soil water movement. Conductivity measures the ease of water movement through soil. As water content decreases, the hydraulic conductivity also decreases. The decrease in conductivity as soil dries may be due to displacement of water by air. As air moves in, the pathways for water flow between soil particles becomes smaller making flow more difficult. One of the methods used in determination of soil hydraulic properties is sorptivity (Zhang, 1997; Minasny and McBratney, 2000; Gerke and Köhne, 2002). Sorptivity is a measure of the capacity of a medium to absorb or desorb liquid by capillarity (Minasny and McBratney, 2000). Sorptivity is determined from data obtained from early-infiltration rates as the early stage

infiltration is dominated by capillary forces within the soil. It is assumed that increased sorptivity will translate into increased water content and higher soil productivity. As soil structural stability changes after tillage with irrigation or rainfall, these changes may significantly alter soil hydraulic properties (Lado et al., 2004). Although infiltration rate may increase with increasing aggregate size (Lado et al., 2004), soil organic matter can also bind the primary particles in aggregates physically and chemically, which in turn may increase permeability and prevent soil erosion.

5.2.2 Chemical Indicators

5.2.2.1 Carbon nitrogen dynamics

The addition of organic matter to arable soils is used to replenish the annual C losses and for improving soil physical, chemical and biological properties (Goyal et al., 1999). The availability and quality of carbon-nitrogen content of organic substrates affects microbial function and diversity (Pennanen et al., 2004). Soil organisms are important in transformations of carbon and nitrogen cycles as these elements are essential for growth and development. The C:N dynamics in compost enriched agricultural soils depends on rate of microbial decomposition, mineralization and immobilization. This is based on the assumption that the rate of decomposition of any substrate is proportional to the growth rate of its decomposers. C:N ratio in the soil is relatively constant at around 12; however, in decomposing plant residues it is highly variable and may increase with maturity. The C:N ratio is lower in micro organisms, usually around 8 (Fierer and Schimel, 2002). One of the effects of C:N ratio on the rate of decomposition of soil organic matter is that where N is limited, microbes will utilise the inorganic N in the soil to satisfy their N requirement, thereby competing with plants for the available N. Carbon-nitrogen

dynamics may provide information which could be used to predict N availability and the structure of soil microbial community (Wheatley et al., 2001, Cookson et al., 2005).

The management of organic C and N from compost amendments requires understanding of processes that influence the rate and conversion of these elements to forms that are available for plant uptake or loss to the environment (Burgos et al., 2006). Gilmour and Skinner, (1999) defined plant available N as the sum of initial nitrate-nitrogen (NO_3-N) and ammonium-nitrogen (NH_4-N) content in soils in addition to organic N mineralized. During mineralization, micro-organisms transform organic matter to inorganic forms (CO_2, NO_3-N, and NH_4-N) and other smaller intermediates (e.g. volatile fatty acids), simultaneously with a process of immobilization of inorganic C and N in the synthesis of proteins by micro-organisms (Brady, 1990). This dynamic system is described as mineralization-immobilization turn-over (MIT) by Jansson and Persson, (1982).

The C:N ratio of organic soil amendments has been shown to be inversely proportional to net N mineralization (Appel and Mengel, 1990). The rate of decomposition of added organic compounds decreases in order: soluble C (sugars and amino acids) > protein > cellulose > lignin (Smith et al., 1992). C:N ratio is also affected by soil texture as high clay content protects organic matter from mineralization and microbial attack (Stenger et al., (1995); Scott et al., (1996). Major environmental factors affecting C:N dynamics are moisture and temperature. Paul and Clark (1996), reported that the optimum water potential for organic matter decomposition in soil is between soil water potential of -0.03 and -0.1 MPa while the optimum soil temperature for organic matter decomposition is 30 – 35^0 C. Drying and rewetting cycles have been reported to influence microbial C: N dynamics with impacts lasting for more than a month after the last stress (Fierrer and

Schimel, 2002). This indicates that where soil is frequently exposed to mechanical stress, investigation of spatial and temporal changes in C:N dynamics is essential.

Nitrate being the most preferred form of nitrogen for plant uptake is not stable and readily available in the soil. Its availability is influenced by leaching, denitrification, microbial interactions and concentration of soil organic matter. The rate of nitrate release from decomposing organic matter is determined as potential nitrification rate (PNR). Wheatley et al., (2001), reported increase in potential nitrification rate (PNR) in response to inputs of organic substrates into soil. An increase in PNR therefore could lead to an increase in available soil N with corresponding increase in soil productivity.

5.2.3 Biological Indicators

5.2.3.1 Soil respiration

The rate of respiration, that is, the amount of oxygen consumed and the amount of carbon dioxide produced determines the aeration requirement of the soil. The rate and spatial distribution of the process as well as its temporal variation depends on several factors. These factors include soil temperature, soil wetness, pH, organic matter content and composition and stability, crop growth stage and microbial activities and diversity of soil populations (Pennanen et al ., 2004; Van de Akker et al., 2004; Wheatley et al., 2001). Microbial respiration rate is a fundamental microbial property that affects soil quality (Godley, 2004). The higher the respiration rate the greater the bioactivity of soil populations. Respiration in soil is detected as CO_2 concentration in preconditioned incubated soil samples (Ritz and Wheatley, 1989). Soil respiration rates are affected by the nutritional status of soil microbial community. In the absence of fresh amendments microbes starve with their respiration rate rapidly declining; however additional

amendments may simulate further growth. In soil application of organic amendments, it is important to decide whether the impacts required are short-term or long-term. Readily biodegradable compounds such as glucose and non stabilized organic inputs only produced short term impacts, while input of compost and other stabilized organic materials stimulate long-term soil microbial bioactivity (Godley, 2004). Compost may therefore be a better soil amendment for sustained fertility and productivity. In assessing soil respiration rates it is important to consider the loading rates and length of incubation as both are critical in temporal and spatial analysis of the response of soil populations to organic inputs.

5.2.3.2 Soil biomass

Soil biomass being the amount of living matter is represented by soil populations. These populations could be mostly be grouped into microflora or microfauna. Microfauna comprises of worms, arthropods, nematodes and protozoa. The microfloras are predominantly algae, in addition to fungi, actinomycetes and bacteria which are in a group of their own (monera). As the soil is a highly competitive environment, the number of any individual species or population depends on aeration, moisture, nutrients, physical factors as well as temperature (Greenland and Hayes, 1981). Among soil populations bacteria and actinomycetes are the smallest in size and the most abundant. Majority of soil bacteria are heterotrophs and are important in mineralization of soil organic matter. Where the soil is enriched with nutrients, increased respiration may be accompanied with corresponding increased in soil biomass (Borken et al., 2002).

The procedure for substrate induced respiration for measuring soil microbial biomass (SMB) was first described by Anderson and Domsch (1978). The method evaluates the

response of the SMB to fresh addition of readily available substrates. SIR measures the response of only active soil microbial biomass to fresh addition of a readily available substrate (Bailey et al., 2002). Glucose is the most commonly used substrate because most soil populations can readily utilize it as carbon source (Ritz and Wheatley, 1989; Lin and Brookes, 1999b; Pennanen et al., 2004). According to Wheatley et al., (2001), the initial maximal respiration rate induced by glucose was proportional to the size of the original soil microbial biomass. The quantity of glucose added to achieve a maximal initial respiration rate may vary, depending on soil physical and chemical properties. The CO_2 flush (μl/g/soil) produced during a fixed incubation time is correlated to soil microbial biomass carbon (SMBC) (μg/g/soil) shown in equation 5.1 as described in Bailey et al., (2002).

$$SMBC = (40.04 \times CO_2) + 0.37 \qquad (5.1)$$

5.2.3.3 Diversity of soil populations

Soil being a living system has its processes and properties mediated by diverse microbial communities, which ultimately determines its quality. Soil microbial communities regulate the decomposition, dynamics and cycling of soil organic matter (Plassart et al., 2008). These communities are sensitive to changes in the environment especially soil management practices. Change in biodiversity gives early warning of long-term changes in soil organic matter, nutrient status and soil structure which may not be observed directly. Microbial diversity is responsible for the stability and resilience of the soil ecosystem especially where stress and management practices reduce the number of species (Brussaard et al., 2004). Changes in land use or management practices can lead to

changes in overall biodiversity which can impact on biologically mediated processes and soil functions (DEFRA, 2004).

The following sections describe molecular techniques used to investigate the diversity of soil populations

5.2.3.3.1 Gene probes and probing

Gene probing is a molecular technique based on the principle of nucleic acid hybridization as DNA can be denatured and reannealed. Probes are small pieces of DNA (oligonucleotides), which are complementary to the target sequence of interest that is marked in order to make them detectable. Probes are useful in genotype identification, examination of soil population diversity and testing virulent genes in sequences of suspected pathogens. In order to produce a probe, the DNA sequence of the gene of interest must be known. The gene of interest may be specific to a particular microbial species or may code for the production of an enzyme unique to some metabolic pathway (functional gene) (Brown, 1995).

5.2.3.3.2 Agarose Gel Electrophoresis

Agarose gel electrophoresis is a vital tool of nucleic acid studies. It is a simple method used for viewing and sizing DNA molecules contained in plasmids or DNA fragments. Voltage is applied to the gel, causing the DNA to migrate towards the anode as phosphates along the DNA backbone are negatively charged. The gel is stained with a dye usually ethidium bromide to allow the visualization of the DNA under UV light. The smaller DNA fragments migrate faster through the gel matrix, while the larger fragments migrate slowly. The molecular weight in base pairs (bp) of the DNA determines the rate

of migration through the gel and is estimated from the standards (ladder) of known size that are run parallel on the gel (Brown, 1995).

5.2.3.3.3 Polymerase Chain Reaction (PCR)

PCR is an in vitro technique used to synthesize large quantities of unique nucleotide sequences from small amounts of DNA (Prescott et al., 2005). PCR may amplify target DNA by up to 10^{-6} fold or more making detection very sensitive (Muyzer and Smalla, 1999). It is a simple reaction that uses DNA polymerase enzyme to copy target DNA sequence repeatedly during a series of 25-30 cycles. During each PCR cycle, the amount of target DNA is doubled, resulting in an exponential increase in the amount of the copied DNA. A PCR reaction requires the addition of two or more primers (short pieces of single stranded DNA) carefully chosen but which may be commercially available. The PCR products obtained are usually visualized by Agarose gel electrophoresis and the size estimated in comparison with DNA ladders.

A PCR cycle occurs in three stages: denaturation, primer annealing and DNA primer extension. In the first stage double stranded DNA unwind into single strands of template single stranded DNA. The second stage is characterized by hybridization of primers to the appropriate target sequence. In the final stage the enzyme DNA polymerase synthesize strands complementary to the original single stranded DNA. Generally PCR cycles are conducted in an automated self contained temperature cycler (thermocycler) in small microfuge tubes using commercially prepared enzymes, nucleotides and buffers (Brown, 1995).

As temperature is critical to PCR, denaturation of target sequences occurs at temperatures greater than the melting point of the DNA. For most PCR reactions the standards is 94^0C for 1.5 minutes. Primer annealing occur between 50-70^0C for 1 minute. The essential component of PCR reaction is the enzyme Taq polymerase used to add bases to primers. The enzyme obtained from *Thermus aquaticus* is heat stable withstanding temperatures of up to 98^0C and therefore could be reused for many cycles. The extension usually takes 1 minute and is performed at 72^0C. A typical PCR cycle normally takes about 3 hours, although for each primer, temperature, incubation time and concentration of reagents can be manipulated to optimise the process (Brown, 1995).

The choice of a primer is important for successful amplification of DNA sequence. The choice is guided by the objective of the study. Based on functionality, primers can either be conserved or universal. Conserved sequences are used where targeted DNA is restricted to similar but related bacterial species while universal sequences are employed where the target DNA is found in all known species of a genus. Most primers are between 17-30 base pairs (bp). The presence of PCR products is usually confirmed by Agarose gel electrophoresis or by gene probes (Brown, 1995).

5.2.3.3.4 Nested PCR

Nested polymerase chain reaction is a modification of polymerase chain reaction. The procedure is carried out to reduce contaminations of products due to the amplification of unexpected primer binding sites. Conventional PCR requires primers complementary to the terminals of the target DNA. A commonly occurring problem is primers binding to incorrect regions of the DNA, giving rise to unexpected products. Nested polymerase chain reaction involves two sets of primers, used in two successive runs of polymerase

chain reaction with the second run intended to amplify a secondary target within the first run products (Brown, 1995).

During the first PCR cycle the target DNA undergoes the first run of polymerase chain reaction with the first set of primers. The selection of alternative and similar primer binding sites gives a selection of products, only one containing the intended sequence. In the second PCR cycle the product from the first reaction undergoes another run with the second set of primers. It is very unlikely that any of the unwanted PCR products contain binding sites for both the new primers, ensuring the product from the second PCR has little contamination from unwanted products of primer mis-binding and alternative primer target sequences (Brown, 1995).

5.2.3.3.5 Terminal-Restriction Fragment Length Polymorphism Analysis

Terminal-restriction fragment length polymorphism (T-RFLP) is a culture-independent molecular method for fingerprinting microbial populations (Blackwood et al., 2003). T-RFLP has been used to show microbial diversity bacterial communities (Denaro et al., 2005), and soil fungal populations (Plassart, et al., 2008). One of the primers of a primer pair is labelled with a fluorescent dye and used to amplify a selected region of a gene of interest by PCR. In T-RFLP, the PCR products of the extracted DNA are digested with a restriction enzyme, which recognizes a specific DNA sequence of four base pairs and cut the DNA at every recognition site to produce restriction fragments (RFs). The resulting terminal restriction fragments are separated with an automated DNA analyzer. Microbial diversity in a community is estimated by analyzing the number and peak heights of terminal restriction fragments patterns (Marshall, et al; 2004; Liu et al., 1997). Thus it is possible to compare patterns between different populations.

Chapter Six: Summary of Literature Review

6.1 The concept of sustainable management of OFMSW

Sustainable development as proposed by the Bruntdland Commission (WCED, 1987) demands the application of integral initiatives on the society and the environment. These initiatives require the management of wastes as other material resources as the higher the consumption of material resources, the greater the waste produced. Although wastes have resource potentials, current production practices are not designed to exploit these opportunities. Sustainable management of OFMSW is a resource based strategy which utilizes treatment and disposal processes that imitate natural cycle patterns to optimise social and economic benefits with little or no negative impacts on the environment.

The resource properties of waste and especially that of OFMSW is gradually gaining acceptance. This is because waste can only be managed sustainably as a resource. Quality waste resources can only be obtained by complying with waste management hierarchy, source separation and the use of biological treatment processes. As only source separated OFMSW can be managed sustainably, anaerobic digestion and composting are biological treatment processes that can optimise the production of biogas and soil fertilizer. While organic wastes continue to increase in quantity and diversity, sustainability in the management of OFMSW can only be achieved by exploiting and optimising opportunities of energy from waste and simultaneously by integrating waste and soil management practices. Sustainable management of OFMSW therefore is aimed at managing OFMSW and soil resources in a way that will optimise biogas production and produce a soil fertilizer that will ensure the long-term productive potential of the soil and continuity of environmental functions.

6.2 The concept of combined anaerobic-aerobic treatments of OFMSW

Anaerobic digestion is a natural and biological treatment process used to recover biogas and stabilize OFMSW in the absence of oxygen, while composting is an aerobic process used to stabilise OFMSW for soil application. Both processes can be combined to optimise biogas and compost production. In spite of the benefits of anaerobic digestion, its large scale application in the UK is limited due to factors such as contamination and variability of OFMSW in terms of waste-type, composition and seasonal availability. This is in addition to problems associated with degradability and methane potential of different waste streams. The need to optimise biogas production and conversion of digestate into soil fertilizer is the major driver of research in fundamental aspects of anaerobic digestion of OFMSW especially in the areas of dewatering, reuse and disposal of residuals due to cost and impacts on the environment. A major challenge of anaerobic digestion is the differential growth and conversion rates of hydrolytic and methanogenic bacteria. Where the feedstock is rich in readily degradable matter rapid acidification may result leading to inhibition of methane production. The absence of standard protocols for the management of anaerobic digestate and the need to minimise pH, moisture and nutrient adjustments during treatment has been found to be critical in the application of anaerobic digestion within an integrated system for management of OFMSW.

A major barrier to large scale application of organic fertilizers in sustainable soil management practices is the lack of stability. Where the stability of the amendment is established, specific information on application rates and soil enhancing qualities of the product is frequently lacking. Knowledge on the impacts of the application of organic fertilizers on the diversity of soil populations is also very limited. These are the major

challenges that this project will aim to meet. To achieve these objectives a series of experiments will be conducted as described in the next chapters.

6.3 Sustainable Management of OFMSW: the Experimental Approaches

The first experiments will be designed to investigate the effects of various proportions of OFMSW on digestibility, biogas and digestate quality during anaerobic digestion. The anaerobic digestion will be high solids at 55^0C to optimise digestate quality and biogas production. A major goal of these studies is to identify OFMSW mixtures most appropriate for combined biogas and compost production.

Once identified, larger quantities of the digestate of the culture will be produced for aerobic post-treatment. As no standard protocol exist for stabilizing anaerobic digestate into soil fertilizer, the study will utilize selected process and operational parameters to aerobically stabilize the anaerobic digestate into organic soil fertilizer. The factors affecting the stability and maturity of the anaerobic digestate during aerobic post-treatment will be examined and an attempt will be made to establish suitable performance indicators.

Finally fresh wastes, anaerobic digestate and aerobically treated anaerobic digestate will be used to carry out studies aimed at investigating their impacts on soil quality, plant growth and soil populations. It is believed that this research project will provide valuable information on the impacts of OFMSW composition on biogas yield, digestate and compost quality as well as on soil fertility and sustained productivity. The study aims to critically assess the benefits and possibility of integrating energy, waste and soil

management policies as well as show the prospects of attaining sustainability in the overall management of OFMSW.

Chapter Seven: Development and production of anaerobic digestate and anaerobic digestate compost

7.1 Introduction

OFMSW forms a significant proportion of wastes collected by local authorities. These wastes although they are readily available, their composition may vary with season, source, collection and transportation systems. The aim of this aspect of the study is develop a suitable feedstock of OFMSW for biogas and compost production. In order to achieve this aim, two experiments were carried out namely:

1. Feedstock selection: This experiment will investigate the effects of substrate composition on digestibility, biogas production and pH stability of OFMSW during anaerobic digestion
2. Aerobic post-treatment: In this section the effects of various degrees of aerobic post-treatment on the stability of anaerobically pre-treated OFMSW for use as soil conditioner will be investigated.

Model feedstocks were simulated based on the need to divert food waste, garden waste and grasses in particular, from the civic amenity site in Dundee to recover valuable products through the production of biogas and compost. Following literature review (Huang et al., 2004; Komilis et al., 2004; Suzuki et al., 2004; Vander-Gheynst et al., 1997; Kayhanian, 1995; Sinclair and Kelleher, 1995; Six and De-Baere, 1992), the composition of the model food and green wastes was adjusted to account for (i) the biodegradability and biogas potential of available substrates, (ii) the need to balance the C:N ratio and provide structural stability and (iii) the need to minimise subsequent

moisture adjustments, with the objective of optimising biogas production and compost quality. In-vessel composting is a requirement of the European Animal By-product regulation (2002/1774/EC) for stabilization of substrates containing products of animal origin.

7.2 Methodology

7.2.1 Development of Source Separated Feedstock

The laboratory OFMSW simulated feedstock was a mixture of food wastes, green wastes and seed sludge as described below.

7.2.2 Green waste (GW)

Model green waste was prepared by mixing freshly mown grass clippings with dried fine woodchips and water at a w/w ratio of 3:2:1, respectively. The mixture had a total solids (TS) level of 35%; total volatile solids (TVS) 34% and carbon to nitrogen (C:N) ratio of 34.8.

7.2.3 Food waste (FW)

Model food waste was composed of a mixture of the foods, comprising cooked pasta (22%), cooked meat (9%), lettuce (11%), carrots (3%), potato (44%) and milk (11%). It was cooked from fresh by boiling in water for 20 minutes, drained and mixed in a food processor for 30 seconds to a smooth paste. The mixture had TS 20.8%, TVS 20.4% and C: N ratio of 13.

7.2.4 Seed sludge

The seed sludge consisted of digestate biomass taken from a continuous flow thermophilic anaerobic reactor treating a solid waste mixture of green and food wastes. The seed sludge had TS 23.6%, TVS 13.1% and C: N ratio 23.7.

7.2.5 Preparation of batch anaerobic digestion cultures

Approximately 500 g each of five duplicate cultures of food waste, green waste and inoculum namely A (FW 80%, AS 20%); B (FW 40%, GW 40%, AS 20%); C (FW 60%, GW20%, AS 20%); D (GW 60%, FW 20%, AS 20%) and E (GW 80% and AS 20%) as described in Table 7.1 were prepared in batch cultures (A, B, C, D and E). The batch digesters were three litre capacity bottles. The batch cultures A_1, B_1, C_1, D_1 and E_1 were buffered with $NaHCO_3$. The chemical buffering agent was added to the cultures as 0.06% of total solids (TS), as recommended by Brummeler and Koster, (1989). Duplicates cultures A_2, B_2, C_2, D_2, and E_2 did not receive $NaHCO_3$ buffer. The total solids content of the cultures at the start of digestion were 19.1, 37.4, 31.0, 44.6 and 53.0% respectively for cultures A, B, C, D and E. Batch cultures were subjected to 15 days anaerobic digestion at 55^0C. Parameters monitored were pH, biogas, TS and TVS.

Table 7.1 Waste Compositions of batch cultures					
Batch digester	**Food waste (% by weight)**	**Green waste (% by weight)**	**Anaerobic sludge seed (% by weight)**	**Initial TVS (%)**	**Buffer agent: $NaHCO_3$ (g)**
A_1*	80	None	20	19.3	24.69
A_2	80	None	20	19.3	None
B_1	40	40	20	37.43	20.43
B_2	40	40	20	37.43	None
C_1	60	20	20	30.97	20.79
C_2	60	20	20	30.97	None
D_1	20	60	20	44.57	21.28
D_2	20	60	20	44.57	None
E_1	None	80	20	52.98	21.28
E_2	None	80	20	52.98	None
NB: *Where the subscript 1 indicates buffered and 2 non-buffered cultures.					

A 10 litre plastic keg was used to construct an experimental anaerobic digester and aerobic reactor. The reactor was a single rectangular open container with the cover adapted to feedstock loading and gas collection as shown in Figure 7.1. Biogas was collected in bags attached to the reactor cover. Heating for anaerobic digestion to 55^0C and during aerobic treatment to 55, 35 and 25^0C was provided by placing the whole reactor inside an incubator. Table 7.2 is a summary of design characteristics of the batch anaerobic-aerobic reactor.

Table 7.2 Design characteristics of anaerobic batch /aerobic reactor	
Parameter	Value
Reactor dimensions (mm)	300 * 180 * 200 (L * W * H)
Working volume (litres)	10
Digestate sampling point	1
Gas collection point	1
Depth of feed in reactor (mm)	160

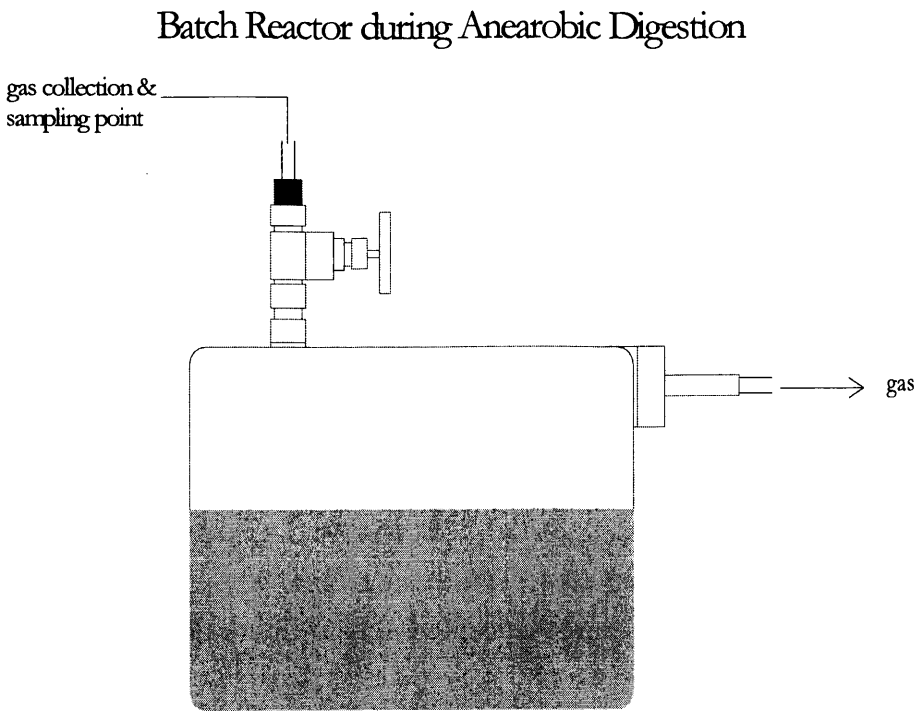


Figure 7.1 The batch reactor during anaerobic digestion (Not to scale)

7.2.6 Set-up for anaerobic digestion process

Approximately 8 kg of the model feedstock containing equal proportion of food waste and green waste (the B culture in table 7.1) was prepared as described previously and blended using a portable food processor. The pH of the feedstock was adjusted to 6.87 using a combination of calcium carbonate and calcium hydroxide. The blended feed was inoculated with thermophilic digestate seed at the ratio of 4:1. To avoid nutrient deficiency nutrients solution (see Appendix I) was added to the feedstock as recommended by Sanders et al., (2003). The feedstock was fed into the reactor through the inlet. The reactor was then sealed, mixed by shaking and allowed to digest naturally for 15 days at 55^0C. Figure 7.1 shows a sketch of the digester with the simulated feedstock. Figure 7.2 (A) shows the digester inside an incubator set at 55^0C and (B) outside the incubator during digestate and biogas sampling. The parameters monitored during digestion were pH, TS, VS, biogas, ammonia and C: N ratio

7.2.7 Aerobic post-treatment set-up

The aerobic reactor is the same anaerobic reactor above but modified for the aerobic post-treatment as shown in Figure 7.3 after the anaerobic digestion stage. The anaerobic digestion digestate was aerated in the reactor by pumping air intermittently for 5 hours daily for ten weeks. The aeration rate was 0.6 litre/min/kg during the first five weeks and reduced to 0.15 litre/min/kg in the last five weeks. The reactor temperature in the first 5 weeks was maintained at 55^0C, the following three weeks at 35^0C and the last two weeks at 25^0C. Mixing was carried out thrice weekly during the first 5 weeks and reduced to twice weekly in the last 5 weeks. The resulting anaerobic digestate compost harvested after 10 weeks was used in the subsequent studies. Figure 7.4 shows the reactor during the aeration process.

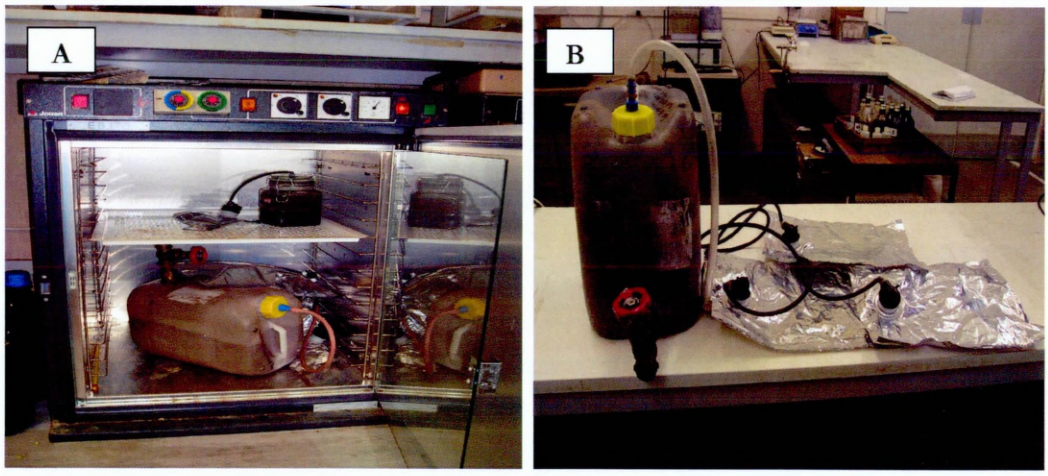


Figure 7.2 Batch reactor during anaerobic digestion (A) and biogas sampling (B)

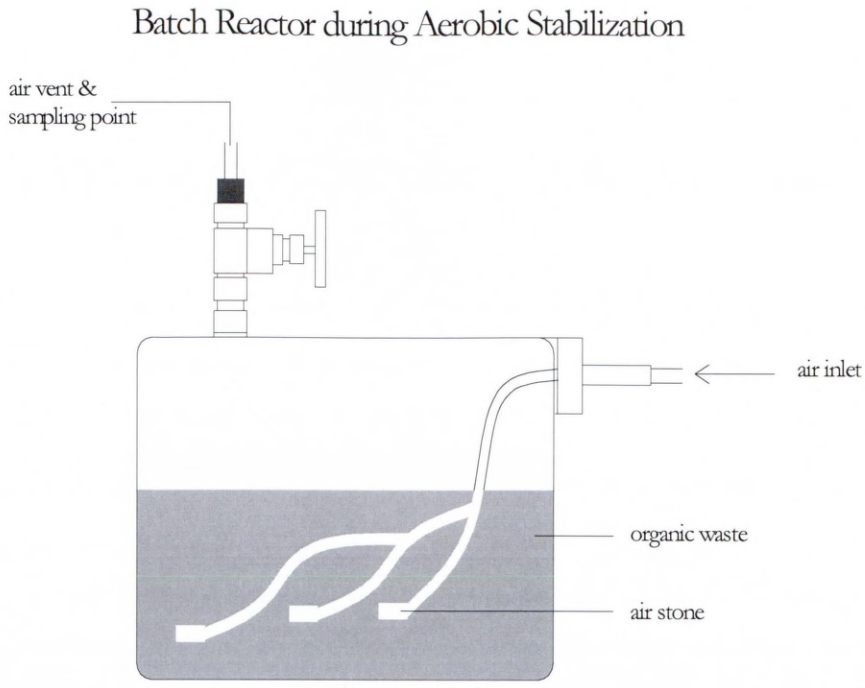


Figure 7.3 Batch reactor during aerobic post-treatment (Not to scale)

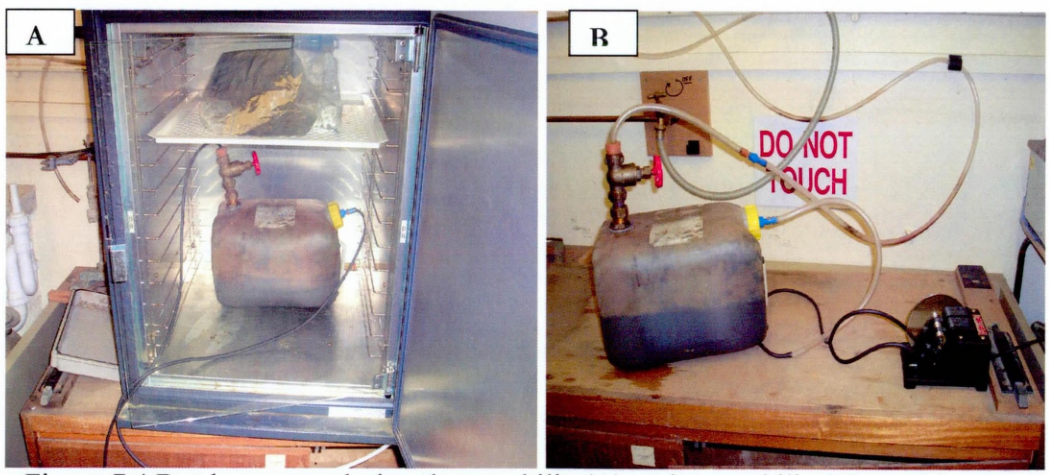


Figure 7.4 Batch reactors during thermophilic (A) and mesophilic (B) aerobic post-treatment

7.2.8 Methods of analysis

pH was measured using 10:1 water-sample (w/w) ratio for slurry of digestate/compost to distilled water using a METLER TOLEDO MP-230 pH meter (Suzuki et al., 2004).

Moisture content was measured gravimetrically by drying the samples at 103°C for 24 hours. Total volatile solids were measured gravimetrically following incineration at 430°C for 4 hours according to Standard Methods of American Public Health Association (APHA, 1992). Biogas volume was determined by displacement. Ammonia–nitrogen was determined by using a Russell 640 pH meter ammonia electrode model 95-5129. Total carbon and total nitrogen for C: N ratios were determined by drying samples at 55^0C and samples analysed using a mass spectrophotometer. For this test, samples were finely ground in a ball mill and approximately 1 mg samples were weighed into 6 x 4 mm tin cups for analysis by continuous flow Dumas combustion using a Europa Scientific (Crewe, UK) sample converter. Nitrogen and carbon were selectively detected (as N_2 and CO_2) using a Europa Scientific ANCASL mass spectrophotometer 2020, model PE2400 series II CHN analyser, Perkins Elmer, Inc., Wellesley MA, USA as described by Scrimgeour and Robinson (2003). Aeration during aerobic treatment was achieved by pumping air using an air/vacuum Charles Austen pump model DA7C. Anaerobic digestate-soil amendment studies were investigated using respirometric analysis to determine bioactivity. Biogas composition and CO_2 evolution were determined using gas chromatography (GC model Hewlett Packard, hp 5890A). Weight reduction of the reactor was monitored using an electronic weighting balance model ae-ADAM (CBC-30)

7.3 Results and discussion

7.3.1 Feedstock selection

7.3.1.1 pH variation during digestion

The pH profile in Figure 7.5 shows that only cultures A recorded pH values that are lower than the minimum pH of 6 recommended for good digestion. It can also be seen that pH

resilience was greater in cultures composed mainly of GW. Where FW dominates as in culture A rapid acidification was observed causing excessive accumulation of volatile fatty acids (VFAs) and hence low pH, a condition that may have inhibited methanogenic activity. In contrast cultures with high GW content showed slow acidification, which may be due to the higher proportion of less readily degradable substrates at the end of the 15 days digestion.

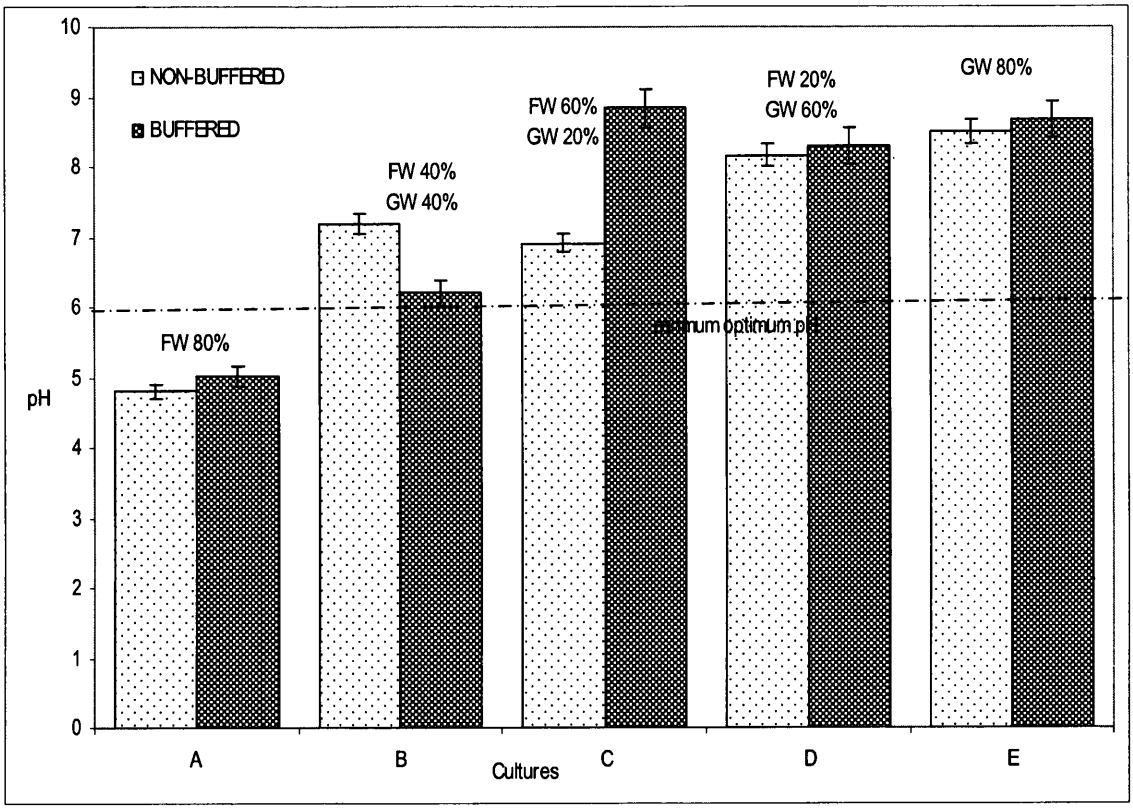


Figure 7.5 pH in buffered and non-buffered cultures. Dotted lines represent the minimum optimum pH. Error bars are calculated as the standard deviation of individual data points (n=3).

The pH profile thus indicates that while FW is readily biodegradable and GW is less readily biodegradable, the need for chemical pH correction decreases with increase in the GW proportion of the feedstock. Hence, FW alone may not be suitable substrate for a self-regulating (pH) anaerobic digestion. However, combining FW and GW in suitable

proportions may increase pH stability as demonstrated in cultures B, C, D and E. the high pH observed in buffered cultures C, D and E is believed to be due to accumulation of free ammonia as result of the absence of readily degradable matter. The result suggests that waste composition may be a useful tool for pH regulation in anaerobic digestion processes.

7.3.1.2 Solids reduction and digestate quality

Figure 7.6 shows that the total volatile solid (TVS) reductions occurred in both buffered and non-buffered cultures. Both buffered and non buffered culture B recorded the highest solid reduction of about 60% compared to less than 20% in E cultures. No significant differences in VS reductions were observed in A cultures. Figure 7.6 also shows that the higher the GW content the lower the TVS reduction during the experimental period. This may be due to the low degradability of GW coupled perhaps by toxicity due to free ammonia. The low TVS reduction observed in culture A was believed to be due to low pH values in those cultures as shown in Figure 7.5. The pH values of about 5 recorded in both buffered and non-buffered can adversely affect the digestion process as also observed by Garcia-Heras, (2003).

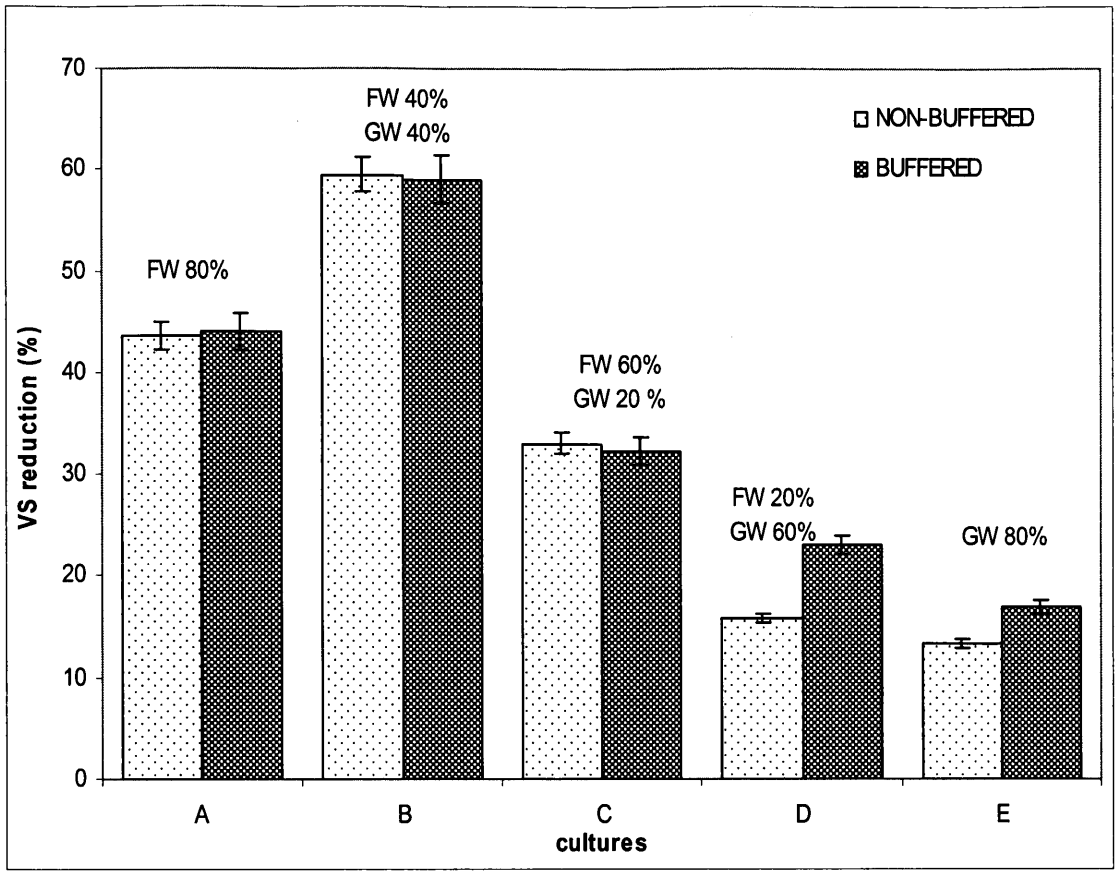


Figure 7.6 Total volatile solids reduction in buffered and non-buffered cultures. Error bars are calculated as the standard deviation of individual data points (n=3).

7.3.1.3 Biogas yield

Figure 7.7 shows biogas yield in the cultures. A gradual decrease in biogas recovery was observed from cultures A to E. Generally, the higher the FW content of the feedstock the greater the volume of biogas recovered per organic solids removed. No significant difference in biogas production was observed between buffered and non-buffered cultures. A cultures recorded significant increase in biogas production in first five days of the digestion beyond which no gas production was recorded. The stoppage of gas production was attributed to low pH of the cultures that resulted after five days of digestion. Buffered and non-buffered cultures B and C produced significant amount of biogas over the two weeks period of the study.

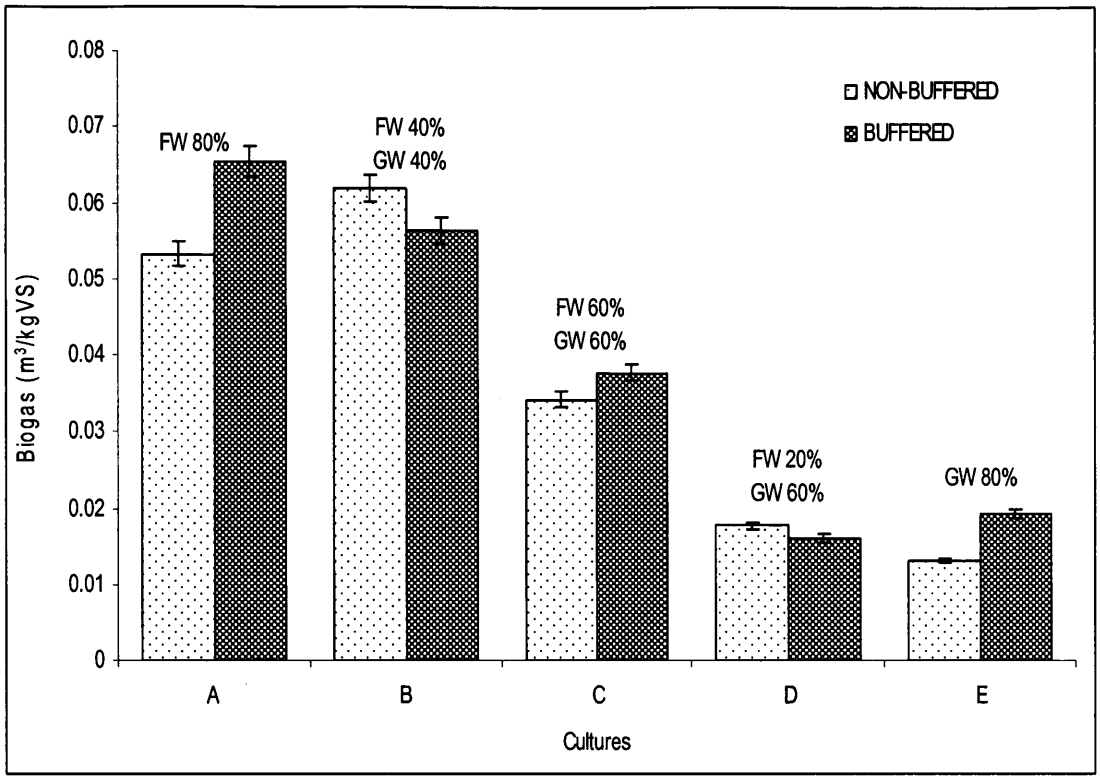


Figure 7.7 Biogas in buffered and non-buffered samples. Error bars are calculated as the standard deviation of individual data points (n=3).

7.3.1.4 Partial conclusion

The results of the batch culture studies indicated that the culture B containing FW: GW: seed in the ratio 2: 2: 1 was the most suitable of all the cultures studied for combine anaerobic digestion and aerobic treatment due to its natural pH stability, degradability, biogas yield and solids reduction.

The B culture was consequently adopted as feedstock for subsequent studies

7.3.2 Anaerobic digestion of OFMSW (Lab-scale batch reactor)

The B culture was used as feedstock for this studies and the digestate obtained was subsequently used as feed material for aerobic post-treatment.

7.3.2.1 Biogas yield and composition

Figure 7.8 shows biogas production during the digestion process started on the first day and ceases completely on the eleventh day. Total biogas production was 0.13 m^3/kgVS with the highest recovery of 0.08 m^3/ kgVS on day four of the digestion. The composition of the biogas obtained was about 50% CO_2 and 50% CH_4. The high amount of biogas recovered on day four of anaerobic digestion was attributed to the most readily degradable component of the model feedstock, which was the food waste. The small but steady amount of biogas recovered between days 5 and 10 of the digestion seemed to come predominantly from the slower degrading green waste. As observed earlier, these results indicate that food wastes have greater impact on biogas yield than green waste. From these results it is believed that anaerobic digestion of 7 to 10 days should be adequate for the feedstock.

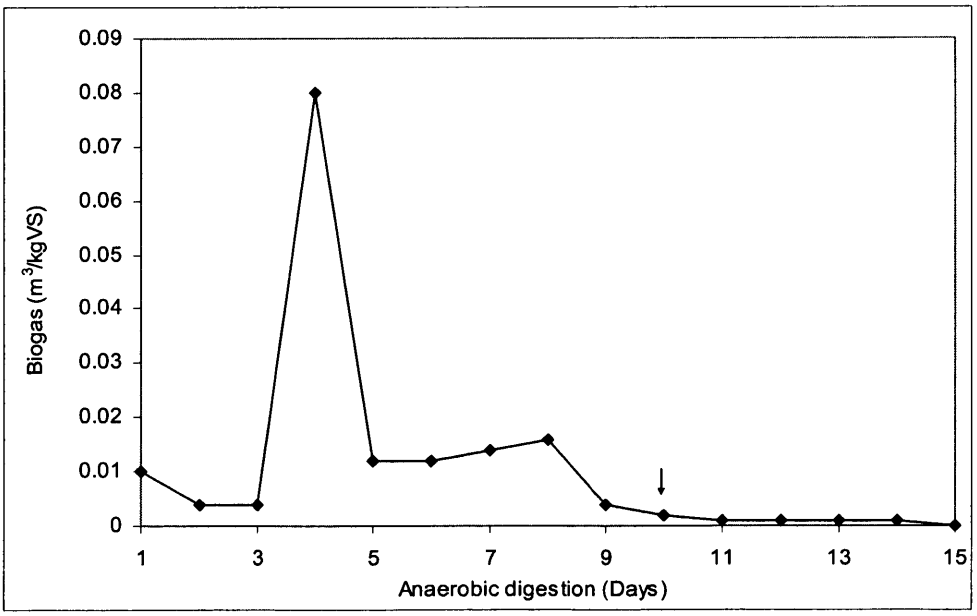


Figure 7.8 Biogas during anaerobic digestion. Arrow indicates the optimal cessation time for this experiment. Error bars not shown as n=1.

7.3.2.2 Solids reduction and pH variation

Figure 7.9 shows that significant volatile solids reduction was achieved during anaerobic digestion. This is believed to be mainly from the decomposition of food waste as green waste decomposes slowly. The depletion of the readily degradable matter in the first ten days of the digestion is believed to be responsible for the absence of biogas beyond the tenth day as shown in Figure 7.8. The absence of significant reduction in TVS beyond day 10 of digestion signifies that a ten day digestion is adequate for biogas production.

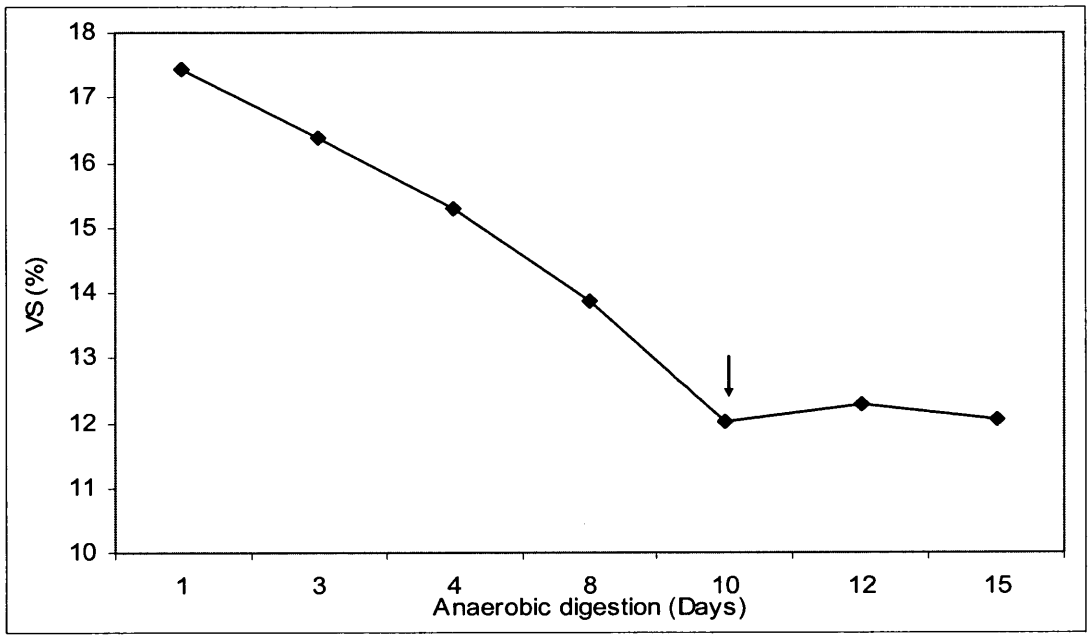


Figure 7.9 Total volatile solids reduction during anaerobic digestion. Arrow indicates the optimal cessation time for this experiment. Error bars not shown as n=1.

Figure 7.10 shows that a decrease in pH was observed in the first 3 days of anaerobic digestion. This was attributed to rapid and excessive accumulation of volatile fatty acids. For the remaining 12 days, the pH was stable and above 6, which was suitable for anaerobic digestion (Mata-Alvarez et al., 2003a).

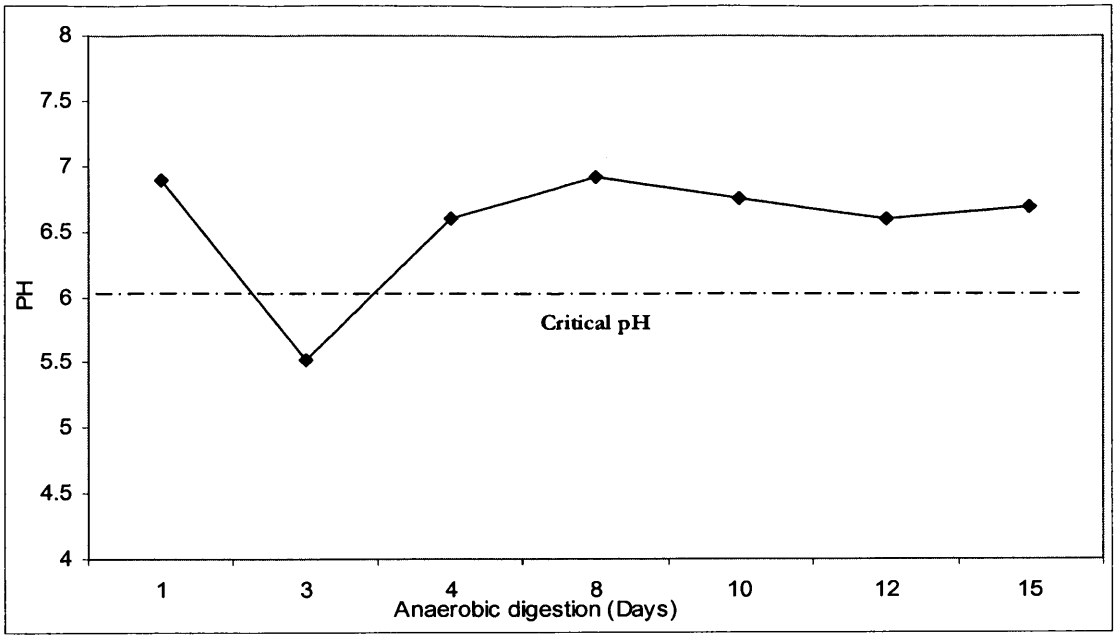


Figure 7.10 pH during anaerobic digestion. Dotted line indicates lowest possible pH below which anaerobic digestion becomes less efficient. Error bars not shown as n=1.

7.3.2.3 Ammonia

Figure 7.11 showed a significant increase in ammonia concentration beyond day four of the digestion. It is believed that the increase in ammonia concentration was due to GW degradation and was responsible for the pH stability of the system. As the concentration of ammonia nitrogen depends on acid-base equilibrium in the feedstock as illustrated in equation 7.1;

$$NH_3 + H_2O \leftrightarrow NH_4^+ + OH^- \quad (7.1)$$

Ammonium could have reacted with dissolved carbon dioxide (carbonic acid) leading to the build up of natural buffer systems by forming ammonium hydrogen carbonate as described by (Cecchi et al., 2003) and illustrated in equations 7.1 and 7.2 and 7.4;

$$3CO_2 + H_2O \leftrightarrow 2H_2CO_3 + HCO_3^- + H^+ \quad (7.2)$$

$$H_2CO_3 + OH^- \leftrightarrow HCO_3^- + H_2O \quad (7.3)$$

$NH_4^+ + HCO_3^- \leftrightarrow NH_4CO_3$ ..... (7.4)

The low concentration of ammonia in the first 4 days of the digestion corresponded to the degradation of FW and to the period of pH decrease below the minimum optimum pH (Figure 7.10). It also thought that the absence of readily biodegradable substrates beyond the fourth day and the slow but gradual decomposition of GW were responsible for the gradual restoration of pH stability which helped maintained the natural buffer required for the remaining period of the digestion.

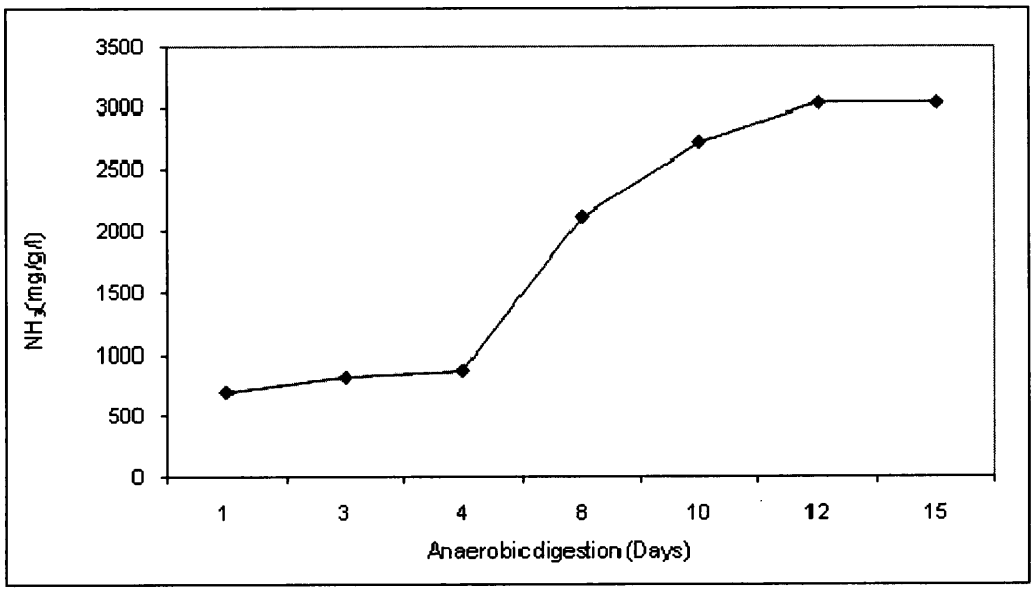


Figure 7.11 Ammonia during anaerobic digestion. Error bars not shown as n=1.

7.3.2.4 Feedstock degradability and digestate characteristics

The results in Table 7.3 show the characteristics of raw feedstock and that of the residual anaerobic digestate produced after 15 days of digestion. Anaerobic digestion resulted in TVS reduction of 33% was achieved with an average biogas yield of 0.13 m^3/kgTVS.

Table 7.3 Model feedstock and anaerobic digestate characteristics		
Parameter	**Feedstock**	**Digestate**
Total solids (%)	22.12	19.05
Total volatile solids (%)	17.50	11.70
C:N ratio	28.81	23.72
pH	6.887	6.7

The residual anaerobic digestate obtained showed a pH of 6.7, a decreased C: N ratio from 28.81 to 23.72 and TVS of 11.7%. It also seems that food waste had a greater impact on biogas production while the green wastes showed greater impact on pH digestion stability and quality of the residual anaerobic digestate.

The next section presents the results of aerobic post-treatment of the anaerobic digestate produced in section 7.3.2

7.3.3 Aerobic Post-treatment of anaerobically digested OFMSW

7.3.3.1 pH

Figure 7.12 shows pH changes during aerobic post-treatment. The changes in pH values may be as result of successional microbial activities necessary for stabilization and maturation of the digestate. The highest pH change was observed between days 20 and 40 and between day 40 and 70 suggesting that for the digestate characteristics and the operational strategy adopted, a stabilisation period of twenty days or less was adequate.

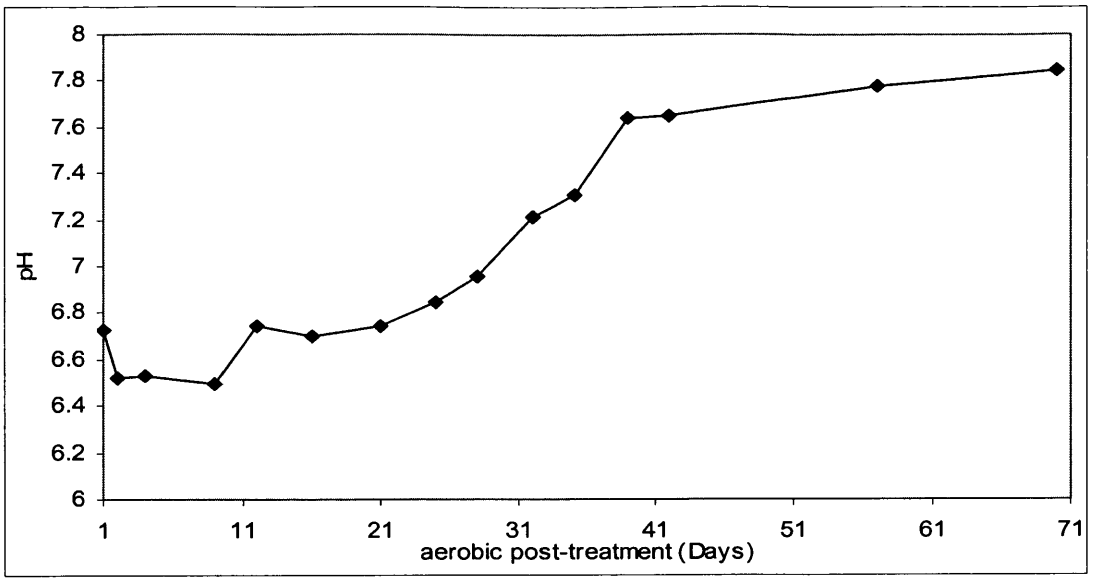


Figure 7.12 pH of digestate during aerobic post-treatment. Error bars not shown as n=1.

7.3.3.2 Solids reduction, degradability and stability

Figure 7.13 shows that solids (TS and TVS) increased with aerobic treatment. The increase in solids may have been due to nutrient precipitation out of solution, evaporation, volatilization and denitrification and biomass growth. Having removed the more readily degradable substrates during anaerobic digestion, the residual digestate may have contained only recalcitrant substrates resistant to anaerobic decomposition. The high temperature and aeration rate maintained during the first five weeks of aerobic treatment was to encourage the emergence of thermophilic aerobes for rapid stabilization of the digestate. This is because thermophilic micro organisms are more capable of rapid and efficient breakdown of different types of organic polymers (Stenbro-Olsen, 1998) than mesophiles and psychrophiles. As the solids stabilize between 25 to 30 days due to probably nutrient depletion, the temperature was reduced from 55 to 35^{0}C in the following three weeks to encourage the development of mesophilic communities and further stabilization of the substrate. As no change in solids was observed for three weeks, the temperature was further lowered to 25^{0}C to encourage fungal growth and possible maturation. The increase in solids observed in the last ten days of aerobic treatment may be due to fungi as they are more

nutritionally versatile than bacteria (Plassart et al., 2008; Anderson and Cairny, 2004). As little change in solids was observed beyond the 25th day of aerobic treatment, aerobic post-treatment of twenty five days or lower may be adequate for digestate stability. It is believed that improved aeration frequency could make the stability time even shorter.

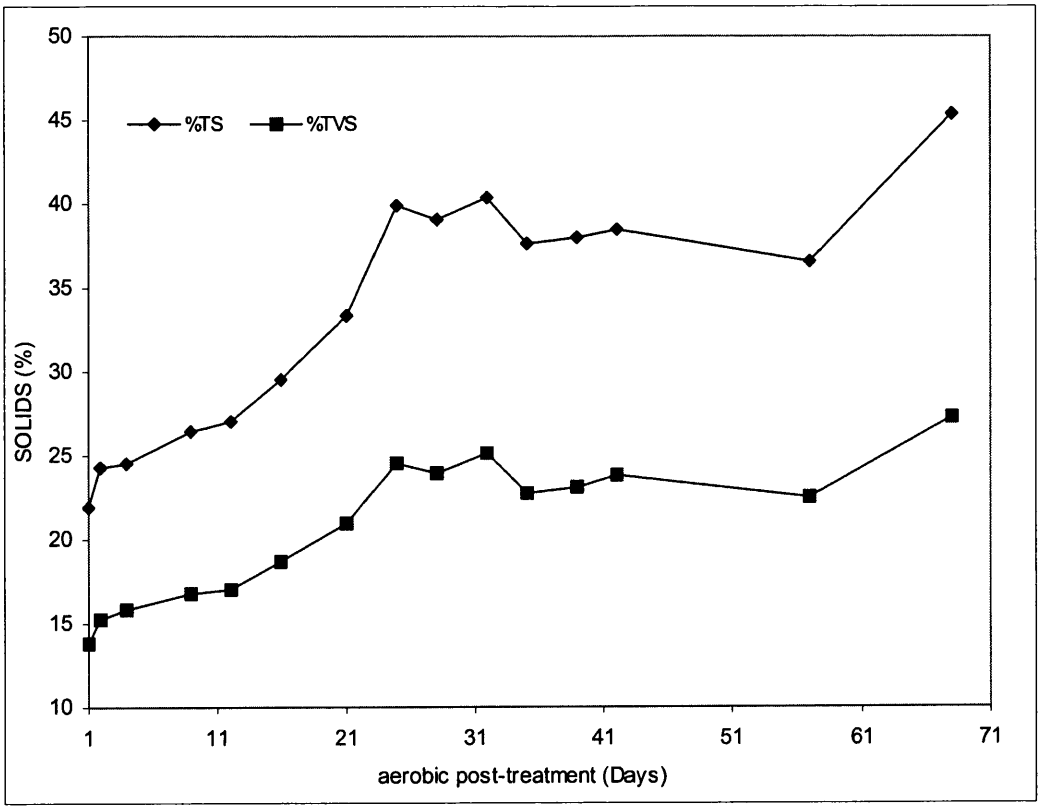


Figure 7.13 TVS and TS during aerobic post-treatment. Error bars not shown as n=1.

7.3.3.3 C: N ratio

Figure 7.14 showed that the C: N ratio decreases with increasing aerobic post-treatment. Beyond the 40th day, the C: N ratio became more or less stable, suggesting the complete breakdown of readily degradable organic compounds. This study thus indicates that C: N ratio can be a useful indicator of compost stability. The C: N ratio of 12.7 of the stabilized anaerobic digestate compost was within the values suggested by several authors (WRAP, 2003; McDougall et al., 2001; Jimenez et al., 1989) as suitable for soil amendment.

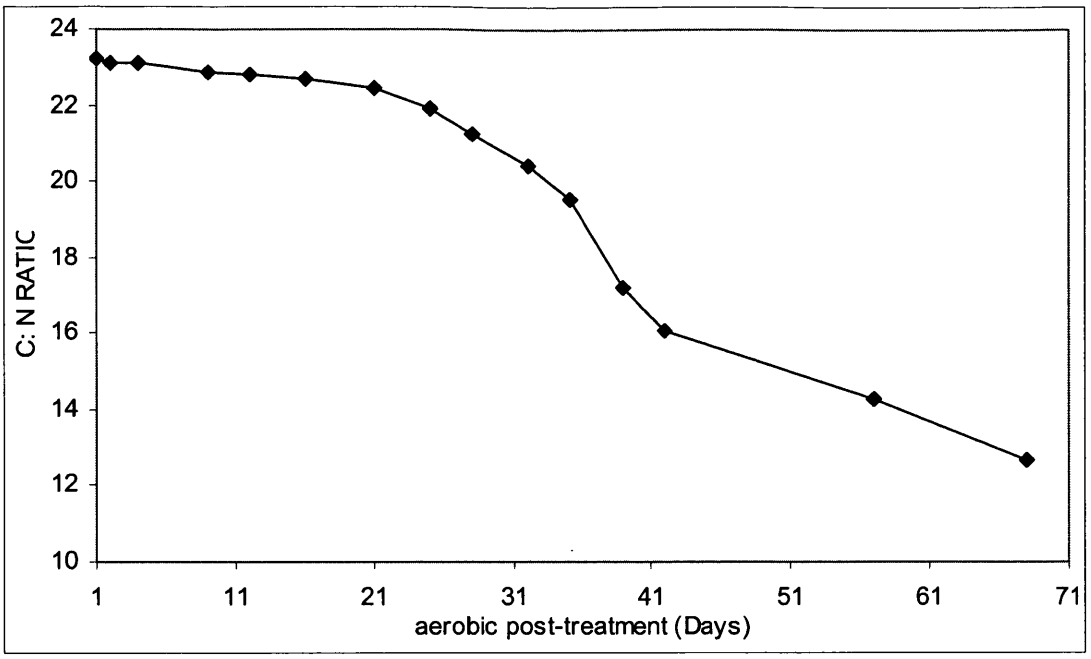


Figure 7.14 C: N ratio during aerobic post-treatment. Error bars not shown as n=1.

7.4 Conclusion and recommendations

The results from the first experiment showed that FW has a greater impact than GW on feedstock biodegradability and biogas potential, while GW has a greater impact than FW on pH stability. The solid content of anaerobic digestates increased with increase in green waste content of the raw feedstock. Consequently, the need for post digestion digestate dewatering that may be required for certain final disposal routes such as land application, may be reduced by combining FW and GW in suitable proportions. These observations are significant in rural, highly agricultural or holiday resorts where the production of GW and FW vary with seasons. FW and GW could also be useful in addressing the demand for biogas and compost which may vary with the availability of substrates. The studies also revealed increasing pH stability with increasing GW content indicating that GW could be used to enhance the natural buffering capacity of OFMSW during anaerobic digestion. Thus, careful selection of the feedstock composition for anaerobic digestion can minimise the need for chemical buffer addition and lower operational cost as well as

lower the cost for post treatment and final disposal of the digestate. Whilst high FW content may lead to rapid acidification and low pH, excessively high GW content may lead to high pH and ammonia toxicity. These pH extremes will slow down the digestion, increasing the possibility of process failure. For optimum biogas recovery, natural buffering capacity, and digestate quality a balanced proportion of FW and GW is essential. Thus, the studies show that OFMSW feedstock containing FW, GW and anaerobic sludge in relative ratio of 2:2:1 is appropriate for high solids anaerobic digestion.

From the literature, anaerobic digestate is not considered fully stable for soil application. During aerobic post-treatment of anaerobic digestate, stability increased with increasing aeration time. The results showed that pH and C:N ratio can be used as indicators for the resulting compost (anaerobic digestate compost ADC) stability and maturity.

The ADC was subsequently subjected to various quality tests in order to ascertain its suitability as soil conditioner and organic fertilizer. These studies were reported in the next chapters.

Chapter Eight: Investigating the quality and stability of anaerobic digestate & anaerobic digestate compost as soil amendments

8.1 Introduction

The quality and stability of organic soil fertilizers depends on the presence or absence of toxic compounds, which may inhibit seed germination and plant growth. Phytotoxic compounds are by-products of decomposition of organic matter which when accumulated beyond a certain threshold can cause phytotoxicity (De-Guardia et al., 2004; Levy and Taylor, 2003; Murillo et al., 1995). Phytotoxicity depends not only on compost stability but also on seed and plant sensitivity, soil conditions and environmental factors such as temperature and light. The most widely used techniques for investigating compost stability and phytotoxicity are a combination of respirometric, seed germination and plant growth tests as described by De-Guardia et al., (2004), Hoekstra et al., (2002) and Levy and Taylor, (2003) respectively.

This section comprises two separate experiments namely;

- Seed germination test: To investigate phytotoxicity effects of both anaerobic digestates and aerobically treated digestates (ADC) obtained from experiments carried out in the previous chapter (chapter 7).
- Plant growth tests: To examine the quality and stability of ADC as soil amendment

The experiments were carried out at the Scottish Crop Research Institute (SCRI) where the resources for the techniques are available.

8.2 Methodology

8.2.1 Anaerobic digestate and compost preparation

The anaerobic digestate and anaerobic digestate compost (i.e. aerobically post-treated anaerobic digestate) used in this study were obtained from the previous chapter (section 7.3.2 and 7.3.3)

8.2.2 Preparation of soil microcosms (cores)

The soil cores used through out this study were made from plastic pipes (40mm height x 50mm dia) with a volume of 78.6 cm^3. The base of each core was covered with fine net strong enough to prevent soil drop, but which would not obstruct air and water movement. Soil for the study was a loamy arable soil collected from an experimental plot located at the lower Pilmore of the Scottish Crop Research Institute (SCRI), Dundee, Scotland (GPS 56^0 27" 15' N, 03^0 04" 45' W, No 3348629620). The soil was passed through a 4 mm sieve after which the pH was determined to be 5.9. Water content of the sieved soil was adjusted to Water Holding Capacity (WHC) and allowed to stand for twenty four hours prior to mixing with soil amendments.

8.2.3 Soil-anaerobic digestate mixture for respirometric analysis

Three duplicates cores were prepared and labelled A, B and C. A_1 and A_2 were packed with non-digestate amended soil (i.e. controls), B_1 and B_2 were packed with a mixture of equal proportion (w/w) of soil and anaerobic digestate while C_1 and C_2 were packed with anaerobic digestate only. All the cores were placed in Kilner jars and incubated at 15^0C for eight weeks and monitored for CO_2 evolution twice weekly. Moisture adjustments and

aeration of the soil-anaerobic digestate mixtures were usually carried out a day or two prior to headspace sampling for CO_2.

8.2.4 Preparation of soil-anaerobic digestate compost (ADC) amendments

The experimental soil from section 8.2.2 was divided into 5 fractions. The first fraction was the blank, without ADC amendment, whilst the 2nd, 3rd, 4th, and 5th, fractions were amended with ADC at the rate of 10, 20, 50 and 100 tonnes/hectare (t ha^{-1}) respectively. Table 8.1 is a summary of the soil-ADC mixtures.

The amount of anaerobic digestate compost for soil application mixture was calculated based on equation 8.1 and mixed as summarized in Table 8.1.

$$\text{Dry loading rate (ADC (g)/soil (kg))} = [(1 + \text{ADC})/ (1 + \text{wet soil})] \quad (8.1)$$

Table 8.1 Anaerobic digestate compost loading rates and soil mixtures

Soil segment	Loading rate	Comment
I	0 tha-1	No ADC amendment (control)
II	10 tha-1	3.85 g of ADC per kg soil
III	20 tha-1	7.70 g of ADC per kg soil
IV	50 tha-1	19.25 g of ADC per kg soil
V	100 t ha-1	38.5 g of ADC per kg soil

Each core was packed with 114 g of prepared amended soils and controls to achieve a density of 1.3 g cm^{-3}, which was the normal density of soil in the field.

A total of 165 soil-ADC amended cores comprising 35 replicates from each amendment were prepared for soil analysis. 75 cores were retained for water retention tests, sorptivity tests and C:N analysis. 60 cores were used for seed germination tests while 30 cores were used for soil respiration and biomass analysis and the study of population dynamics. The sequence of the soil experiments is summarized in Figure 8.1. For incubated cores, equal proportions of cores consisting of the different ADC amendments were removed after week 1, 2, 3 and 12 of incubation for analysis. Removed cores were stored at 4^0C prior to each analysis. Incubation was carried out at 15^0C

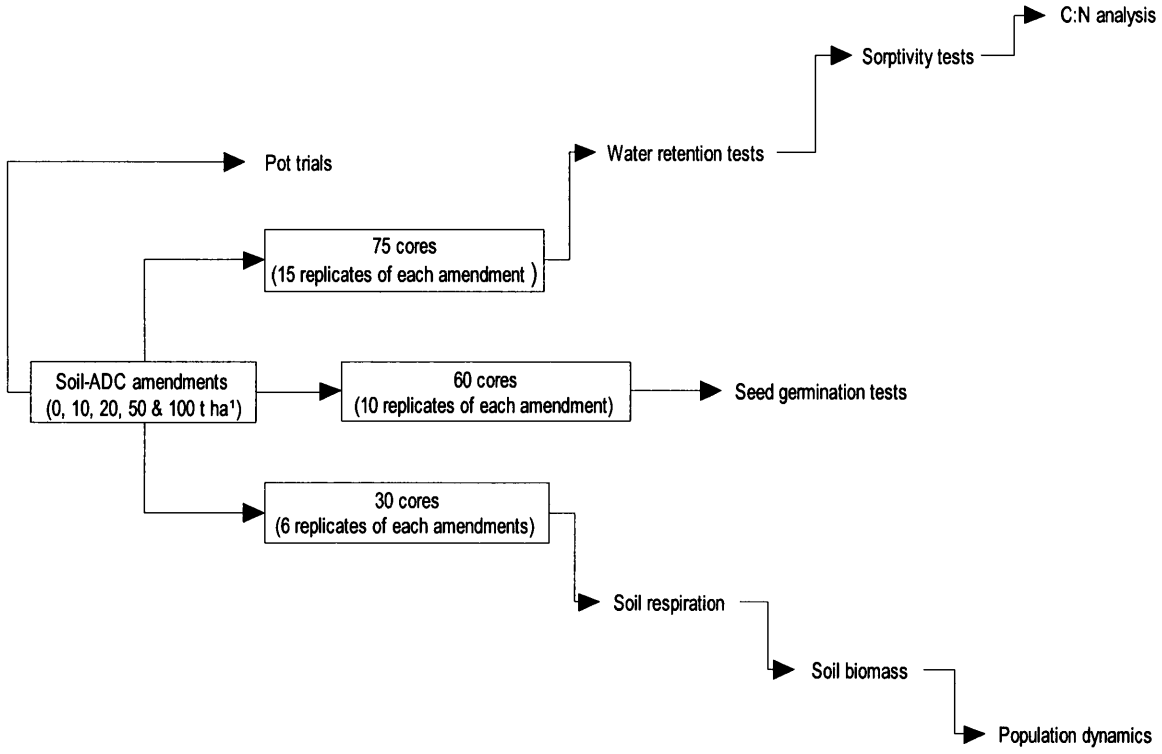


Figure 8.1 Sequence of soil analyses with soil-ADC amendments

8.2.5 Seed germination test (Germination index bioassay)

Water-soluble extracts from the following sources were obtained:

- Raw waste (non digested feedstock)
- Anaerobic digestate collected at various digestion period
- Digestates collected at different intervals of aerobic post-treatment
- Anaerobic digestate compost (ADC)
- Incubated and non-incubated ADC amended soils (see section 8.2.4)

The extracts were obtained by mixing 50 g of sample with 100 ml of distilled water at 15^0C. The sample-water mixture was shaken for 6 hours and then centrifuged at 3000 *g* using a Sigma 4-15k centrifuge for 20 min at 20^0C (Hoekstra et al, 2002). For each of the extract 50% and 25% dilutions were prepared for parallel tests. The undiluted extract, 50% and 25% diluted concentrations represented GI 100, GI 50 and GI 25 respectively. For the germination test, each Petri dish (10 cm diameter) was lined with Whatmans No 2 filter paper and received 5 ml of specific sample extracts; controls received only 5 ml of ultra-pure water. Ten seeds of radish (*Raphanus sativus* L.) were sown on each dish with 5 replicates per treatment. Seeded dishes were incubated at 25^0C for 72 hours in the dark after which germinated seeds were counted (G) and the radicle length (L) measured as described by Tiquia and Tam (1998) and Hoekstra et al., (2002).

8.2.6 Pot trials

Pot trials were carried out using the tomato plant (*Lycopersicon esculentum* L.; i.e. "Money Maker") as a test plant. The pot trials were carried out in pots with a volume of 500 cm^3 containing soils amended with different loadings rates (i.e. 0, 10, 20, 50 and 100 t ha^{-1}) of anaerobic digestate compost. Parallel tests were set up with pots containing soils

amended with commercially obtained compost made from green wastes (Discovery Compost, DC) for comparison. The commercial compost was made in Dundee from green waste composted by the open windrow technique. All tomato plants from both ADC and DC amended soils and controls were grown for 12 weeks, watered daily and given nitrogen supplements (See Appendix II) three times every two weeks in the last six weeks of the experiment. The plants were grown in a chamber maintained at 20^0C with alternating light and dark cycles of 16 and 8 hours respectively. Light in the chamber was provided by sodium lights operated on time switch.

8.2.7 Method of analysis

pH was measured using 10:1 water- sample (w/w) ratio for slurry of digestate/compost to distil water using a METLER TOLEDO MP-230 pH meter (Suzuki et al., 2004). Respirometric activity was determined as described by Wheatley et al., (2001). Seed germination index was expressed as a percentage of control. A 5 mm primary root elongation was adopted as the operational definition of seed germination as described by American Public Health Association (APHA, 1992). The percentages of Relative Seed Germination (RSG), Relative Root Elongation (RRG) and Germination Index (GI) were calculated as described in equations 8.2, 8.3, and 8.4 (Tiquia and Tam, 1998).

Relative Seed Germination, RSG (%)

= (No of seeds germinated in extract / No of seeds germinated in control) x 100 (8.2)

Relative Root Growth, RRG (%)

= (Mean root length in extract / Mean root length in control) x 100 (8.3)

Germination Index, GI (GI)

= (% seed germination) x (% root growth) / 100 (8.4)

Data obtained were analysed using descriptive statistics. Plant growth during pot trials were assessed using parameters as heights, number of leaves, length of the longest leaves and the weight of dried organic matter as used by many authors (Murillo et al., 1995; Hoekstra et al., 2002; Levy and Taylor, 2003).

8.3 Results

8.3.1 Respirometric test

Figure 8.2 illustrates the results of the respirometric analysis. In control soils (A), CO_2 flush increased from 0.001 μgC/g/h in the first week to 0.007 μgC/g/h following eight weeks of incubation. The CO_2 flush from the anaerobic digestate amended soils (B) increased from 0.004 μgC/g/h in the first week to 0.07 μgC/g/h after eight weeks of incubation. And the cores containing anaerobic digestate only (C) showed increased CO_2 flush from 0.001 μgC/g/h in the first week to 0.2 μgC/g/h at the eight week.

The non-amended soil (cores A) showed little change in CO_2 evolution throughout the eight weeks of incubation. This may be due to the absence of degradable organic matter enrichment as they contained no anaerobic digestate amendment. The anaerobic digestate amended soil (cores B) showed increasing CO_2 evolution during the first 3 weeks of incubation. However, the CO_2 evolution declined at week 4 at which hydrophobicity (water repellancy) was suspected. The distortion of soil diversity was believed to be responsible for the low CO_2 evolution. No significant increase in bioactivity was observed in the remaining period of incubation.

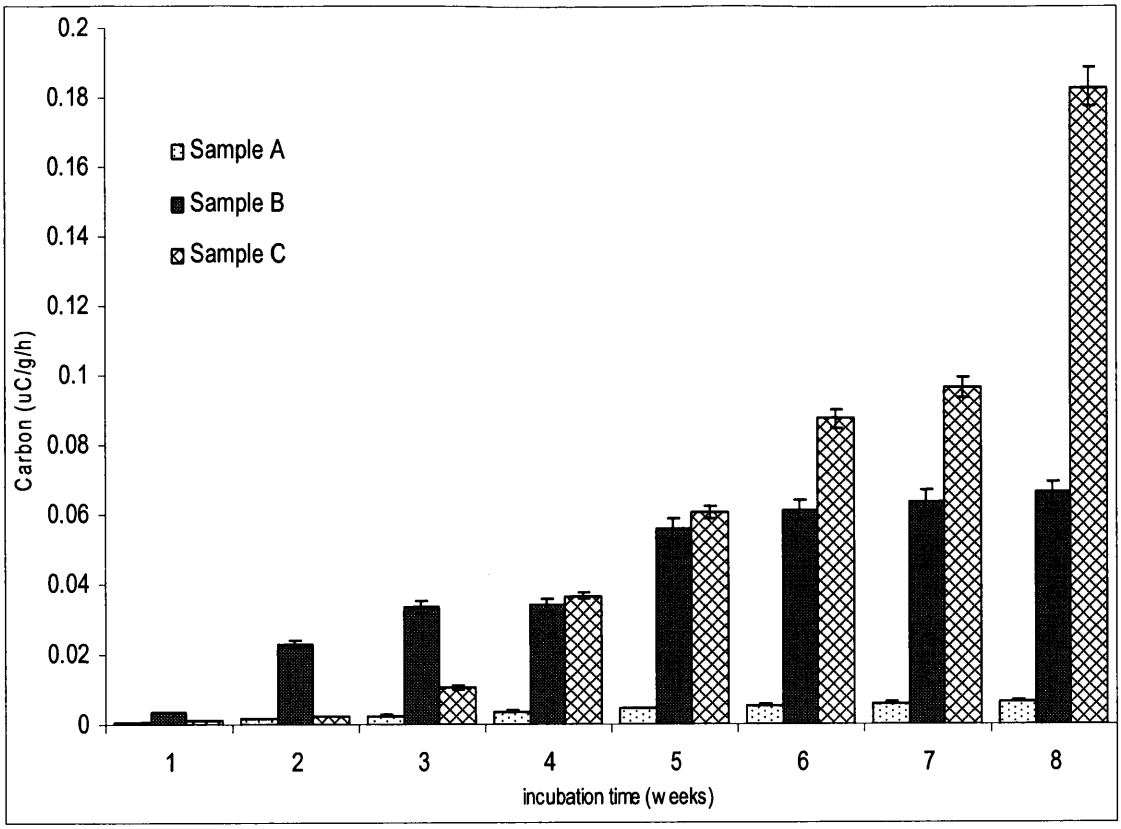


Figure 8.2 Cumulative CO_2 evolution in non-amended (A); anaerobic digestate amended soil (B); digestate only (C) cores. Error bars were calculated as the standard deviation of individual data points (n=3).

The digestate containing microcosms (C) showed lower bioactivity in the first two weeks of incubation but showed significantly higher CO_2 evolution and bioactivity in the remaining period of incubation. CO_2 was released as a result of bioactivity from the breakdown of readily degradable matter. The increasing CO_2 flush in C showed that the anaerobic digestate used was unstable, and thus unsuitable in its present form, for the enrichment of arable soils. The implication of these observations is that the anaerobic digestate may require further treatment to render it amenable for soil application.

Although the feedstock for anaerobic digestion was relatively high in solids content, the digestate was viscous due to solids breakdown. There was also high emission of undesirable odour (due to fatty acids, H_2S and mercaptans) normally associated with

anaerobic processes. It is believed that the strong odour and viscosity of anaerobic digestate may constitute a setback in its use as an alternative soil conditioner as reported by other authors (Poggi-Varaldo et al., 1997; Braber, 1995; Molnar and Bartha, 1989).

8.3.2 Seed germination with fresh feedstock and digestates obtained during anaerobic digestion

Germination tests were carried out for 72 hours at 25^0C. Figure 8.3 shows the results obtained using soluble extracts of fresh waste and digestates at various stages of the 15 day anaerobic digestion. Undiluted extracts of fresh raw waste and anaerobic digestates showed no seed germination (GI 100) during the incubation period. 25% dilution of extracts of the fresh waste (GI 25) showed a germination index of 1.4%. Increasing seed germination indexes were observed in 50% and 25% concentrations with increasing length of digestion period.

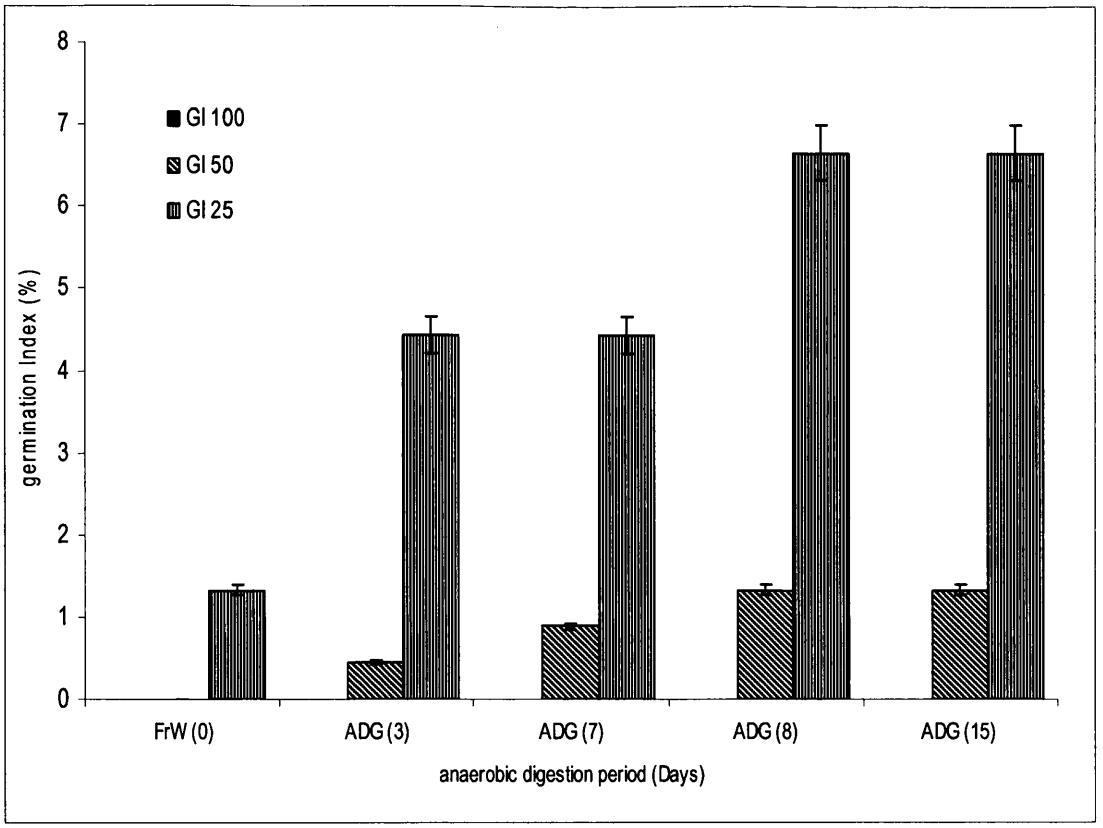


Figure 8.3 Seed germination indexes of fresh waste and anaerobic digestates (FrW= Fresh waste; ADG=Anaerobic Digestate) No germination was recorded in undiluted extracts (GI 100) hence, the absence of a bar for GI 100. Error bars were calculated as the standard deviation of individual data points (n = 3).

According to McLachlan et al. (2004), seed germination index (GI) greater than 70% is considered non-phytotoxic, while a GI of less than 70% is considered phytotoxic. Hence, Figure 8.3 suggests that both fresh raw waste and anaerobic digestates used in the study were phytotoxic, with phytotoxicity decreasing with increase in the duration of digestion or in biological stability (i.e. reduction of organic biodegradable organic matter).

8.3.3 Seed germination with organic matter obtained during aerobic post-treatment

Figure 8.4 shows the result of seed germination tests of anaerobic digestates waste obtained during aerobic post-treatment. This test was also carried out for 72 hours at 25^0C. No seed germination was observed in the undiluted extract (GI 100) from digestate obtained in the first eight weeks of aerobic treatment. The diluted extracts of 50 and 25%

concentrations (i.e. GI 50 and GI 25) showed seed germination index of less than 4. Undiluted extract from samples obtained at the end of aerobic treatment (i.e. after 10 weeks of aeration) showed germination index of 12.5. Whilst those with 50 and 25% diluted extracts showed higher seed germination indexes of 55.3 and 80% respectively.

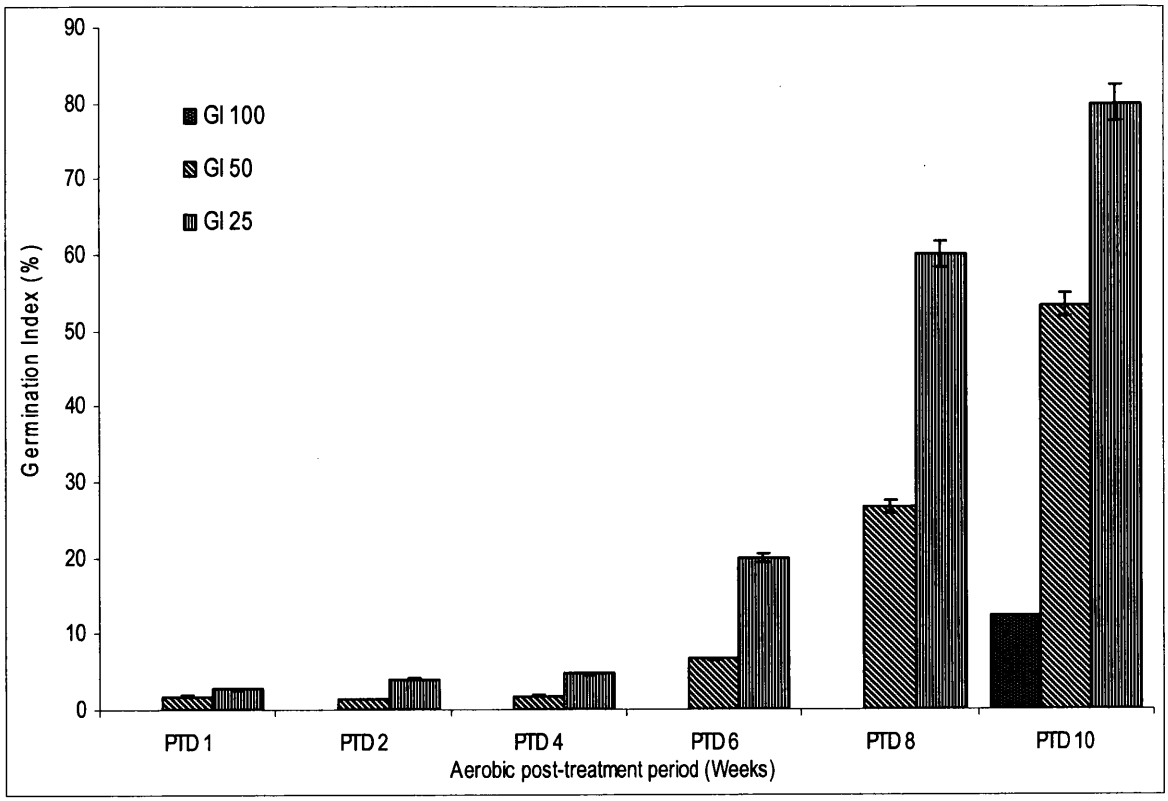


Figure 8.4 Seed germination of digestates during aerobic post-treatment (PTD=post-treated digestate; ADC=anaerobic digestate compost). Error bars are calculated as the standard deviation of individual data points (n = 3).

The relatively high germination indexes of the diluted extracts suggest that anaerobic digestate compost (i.e. aerobically treated anaerobic digestate) could be a useful amendment in arable soils, with its benefits increasing with increase in dilution and duration of aerobic treatment.

8.3.4 Seed germination tests using anaerobic digestate compost amended soils

Figure 8.5 shows the results of seed germination tests using liquid extracts from soils amended with variable loading rates of anaerobic digestate compost (ADC) at different incubation times. It is worth noting that ADC was obtained after 10 weeks of aerobic post-treatment when it was considered to be stable. The non-incubated soil samples amended with ADC load of 100 t ha^{-1} recorded the lowest germination index of 52% which subsequently increased to 124% after twelve weeks of incubation. Soils amended with ADC loading of 10 t ha^{-1} showed the highest increase in seed germination after 12 weeks of incubation. Generally, all ADC amended soils performed better than control (which was soil unamended with ADC). The results suggest that incubation seemed to reduce phytotoxicity through the reduction of available biodegradable matter in the compost.

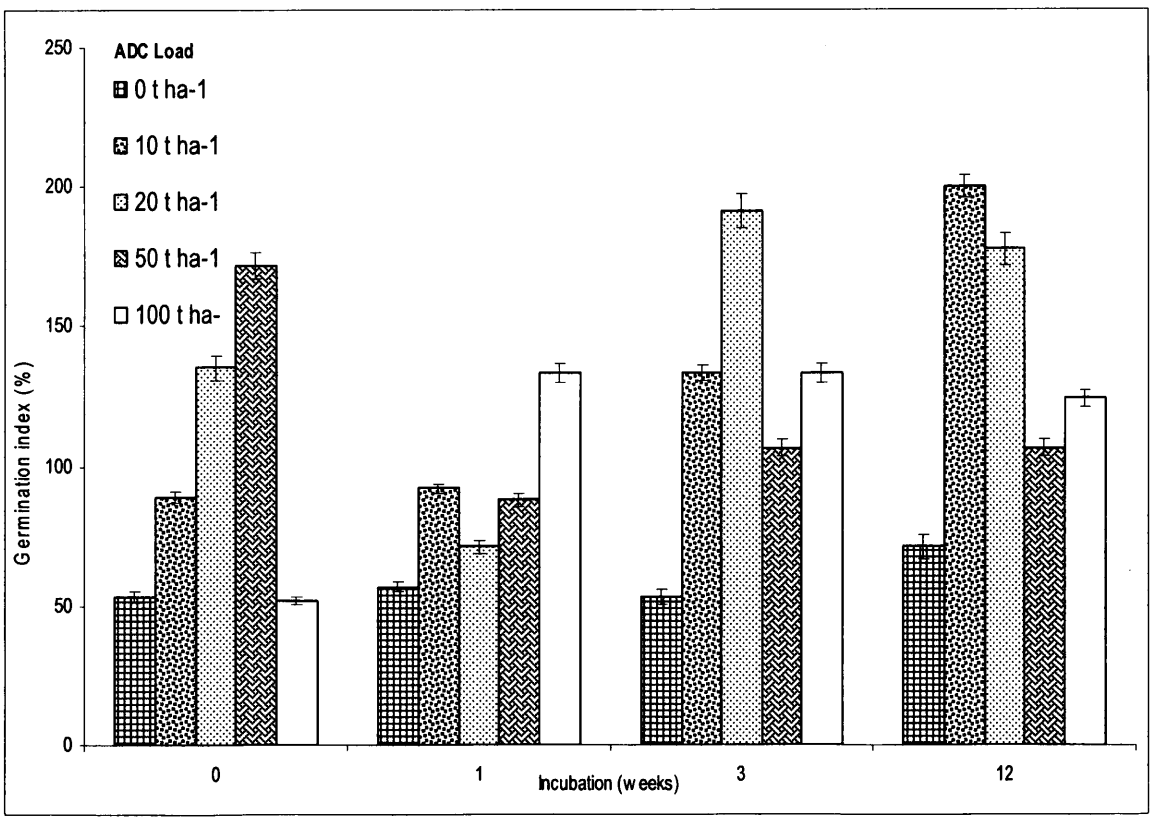


Figure 8.5 Seed germination of soluble extracts of ADC amended soils. Error bars are calculated as the standard deviation of individual data points (n = 3).

Generally, ADC amended soils showed higher seed germination than unamended soil, however seed germination increased with decreasing dilution and increasing incubation time as further illustrated in Figures 8.6 and 8.7. The non ADC amended soil controls showed no significant change in seed germination with increasing dilution or incubation time. The high seed germination indexes observed in ADC amended soils and especially in 50 and 25% concentrations of soluble extracts which in all cases was higher than the controls is an indication of the potential long-term benefits of the addition of ADC to arable soils.

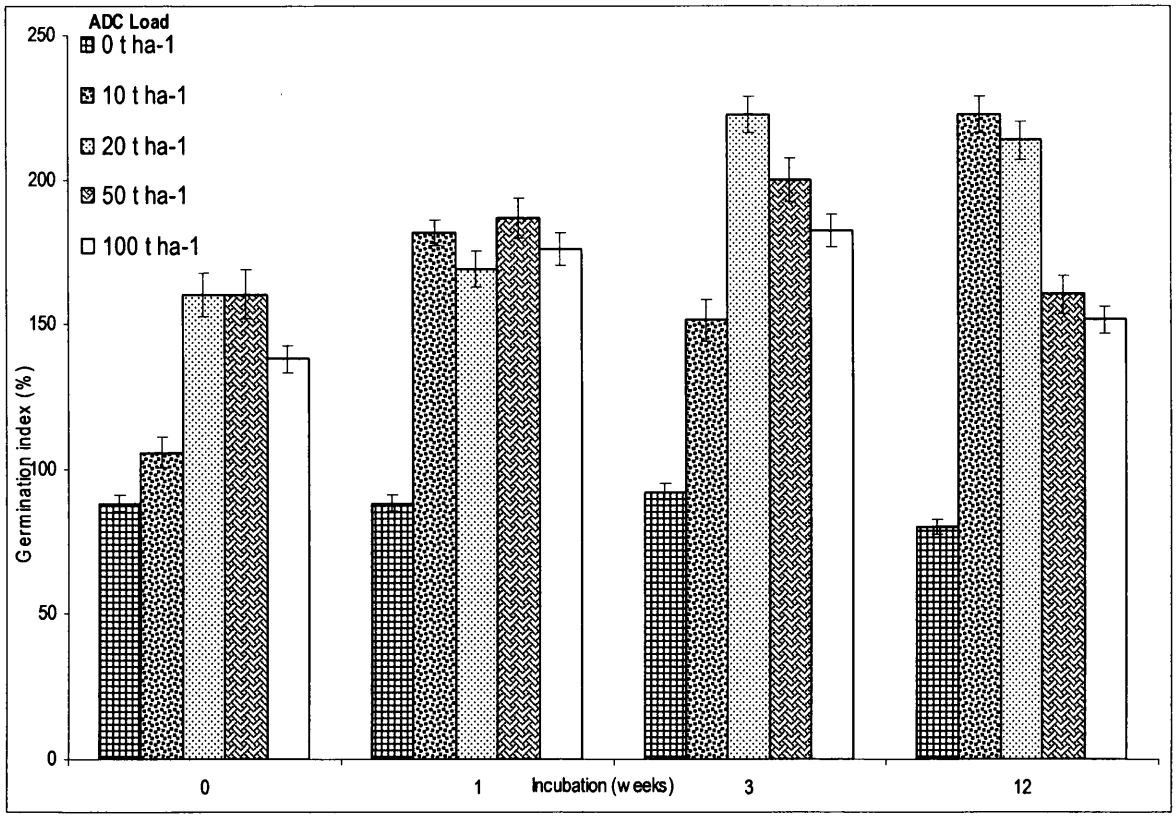


Figure 8.6 Seed germination in 50% (GI 50) concentration of soluble extracts of ADC amended soils. Error bars are calculated as the standard deviation of individual data points (n = 3).

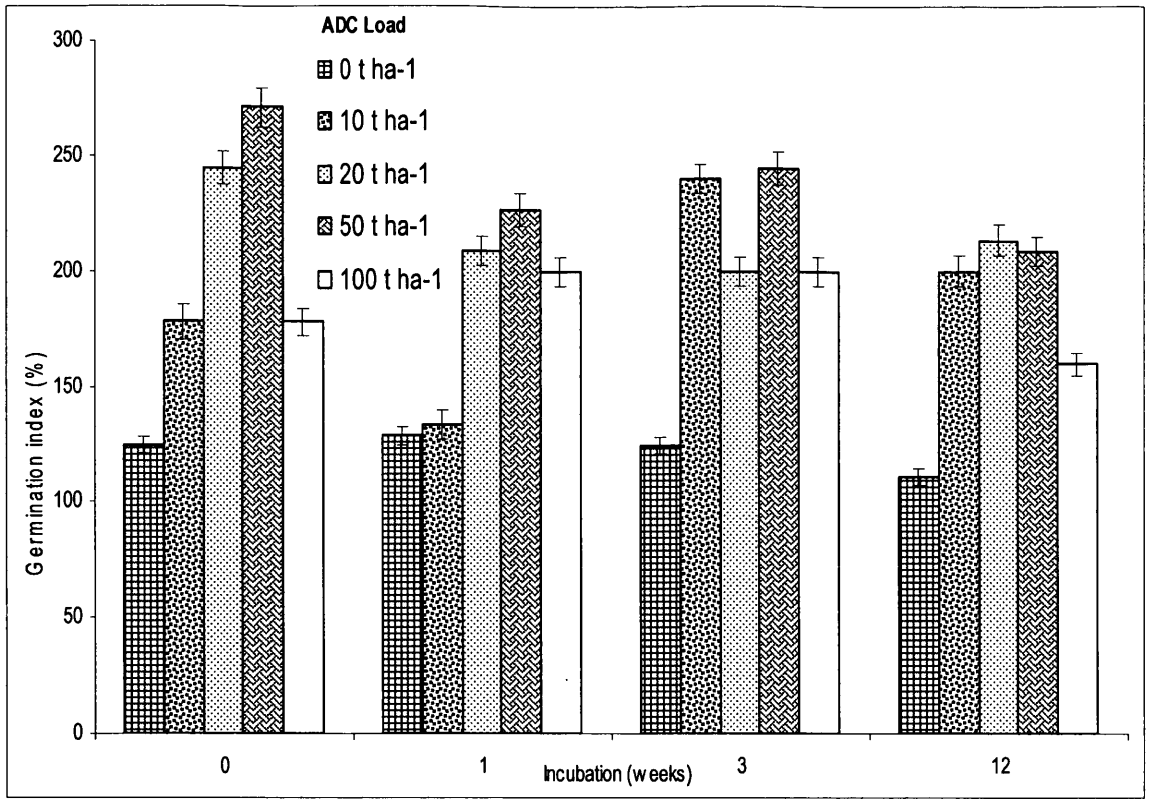


Figure 8.7 Seed germination in 25% (GI 25) concentration of soluble extracts of ADC amended soils. Error bars are calculated as the standard deviation of individual data points (n = 3).

8.3.5 Pot trials

The pot trials lasted for 12 weeks. Figure 8.8 show the weight of shoots of harvested tomato plants grown in both ADC and DC (Discovery Compost-commercially available compost made from green waste) amended soils at the end of the trial. Generally the weight of tomato shoots increased with increasing compost amendments. The growth of tomato shoots seems to be higher in ADC and DC amended soils than in non-amended controls, indicating that DC amendments may be more beneficial to plant growth than ADC at low loading rates.

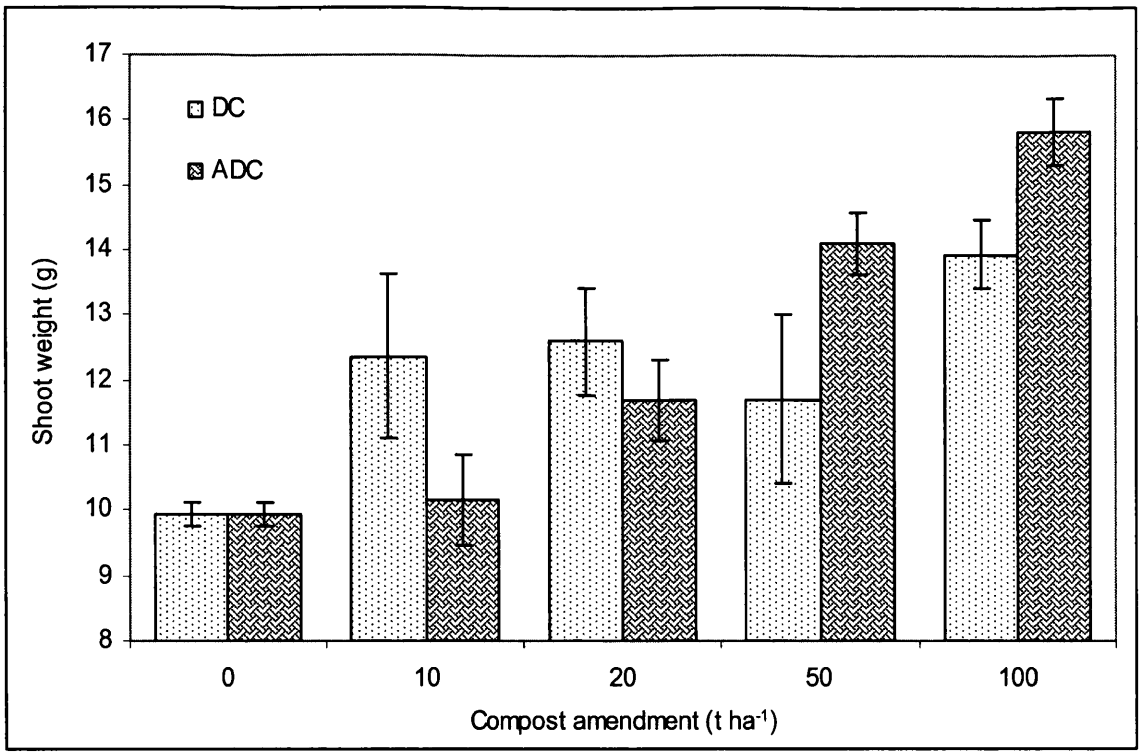


Figure 8.8 Weights of shoots of tomato plants in ADC and DC amended soils. Same replicates were used as controls (0) for both ADC and DC amended plants. Error bars were calculated as the standard deviation of individual data points (n = 3).

The correlation between shoot weight and compost amendment after the 12 weeks of plant growth was higher in ADC amended plants ($R^2 = 0.94$) than in those amended with DC ($R^2=0.55$), suggesting that ADC has greater impact on shoot growth and development.

Figure 8.9 shows the weights of harvested tomato roots in ADC and DC amended plants. It seemed that the weight of roots increased with increasing compost amendment. However, the strength of relationship between root weight and compost amendments was higher in DC ($R^2 = 0.69$) than in ADC ($R^2 = 0.46$) amended plants indicating that DC had greater impact on root development.

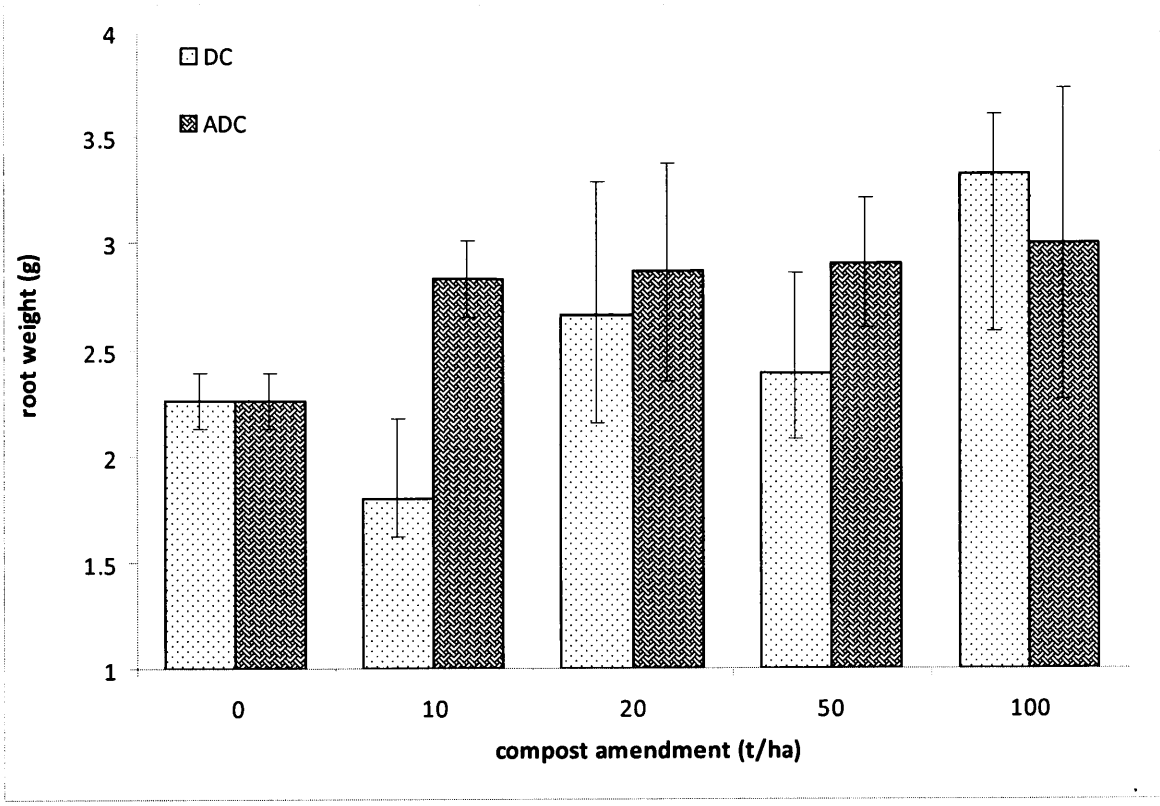


Figure 8.9 Weight of roots in tomato plants in ADC and DC amended soils. Same replicates were used as controls (0) for both ADC and DC amended plants. Error bars were calculated as the standard deviation of individual data points (n = 3).

Figure 8.10 and 8.11 illustrate the heights of tomato plants in ADC and DC amended soils showing increased heights with increasing compost amendments. The heights increased from 38.3 cm in non amended control soils to 49.3 cm and 52.2 cm in soils amended with 100 t ha^{-1} of DC and ADC respectively. Although the increases in height were significant with increasing compost amendments, no significant differences were observed between ADC and DC plants of similar compost amendments for low loading rates. Figure 8.10 seems to suggest that ADC is slightly more beneficial at high loading rates. The correlation between plant height and compost amendments was slightly higher in ADC (R^2=0.83) than in DC (R^2=0.81) amended plants suggesting that both ADC and DC may have had somewhat equal impacts on plant growth and development.

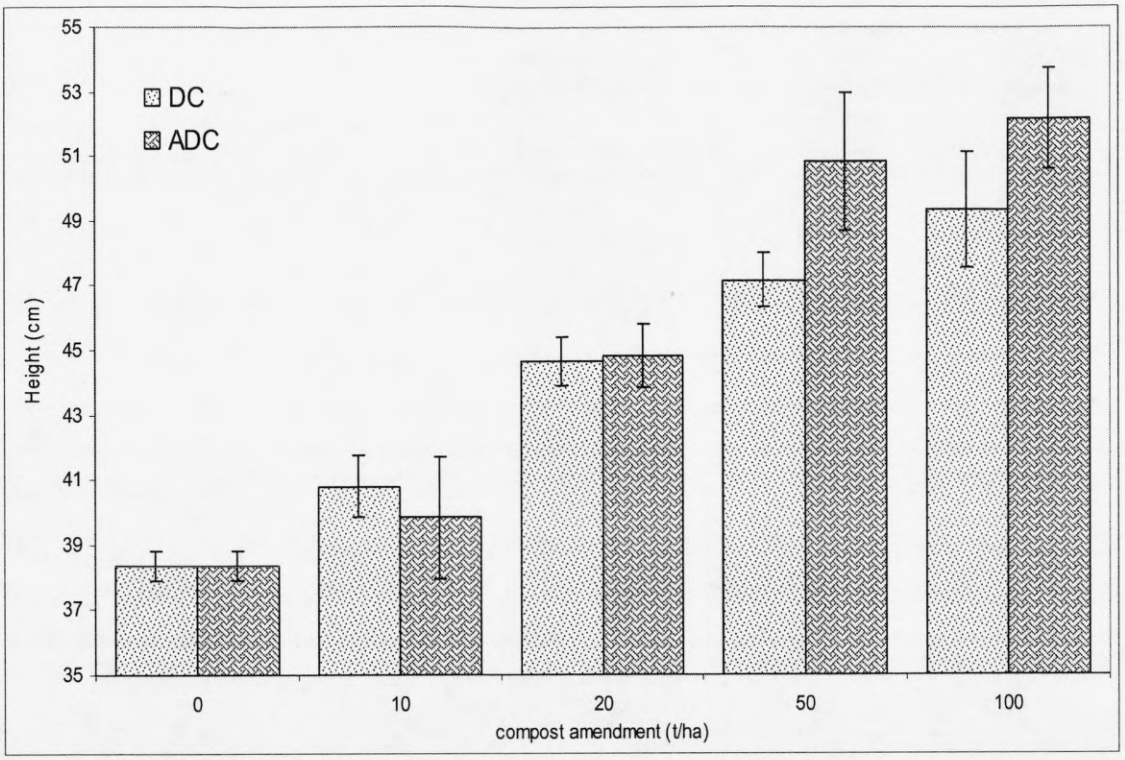


Figure 8.10 Heights (HGT) of tomato plants in ADC and DC amended soils. Same replicates were used as controls (0) for both ADC and DC amended plants. Error bars were calculated as the standard deviation of individual data points (n = 3).

Figures 8.11 and 8.12 shows the appearance of tomato plants in ADC and DC amended soil at week 8. The plants showed increasing heights with increasing compost amendments.

Figure 8.11 Tomato growth in ADC amended soils after 8 weeks (Left to right are pots amended loading rates of 0 t ha^{-1}; 10 t ha^{-1}; 20 t ha^{-1}; 50 t ha^{-1} and 100 t ha^{-1}).

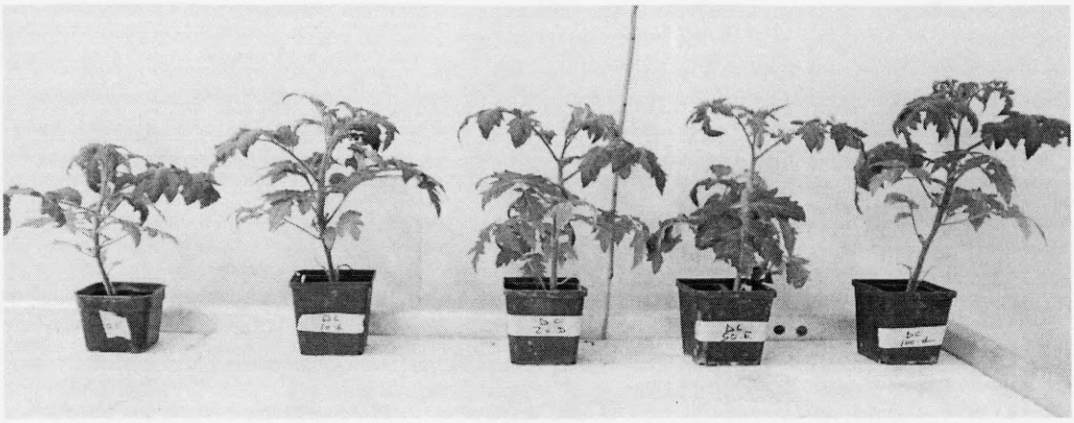


Figure 8.12 Tomato growth in DC amended soils after 8 weeks (Left to right are pots amended loading rates of 0 t ha^{-1}; 10 t ha^{-1}; 20 t ha^{-1}; 50 t ha^{-1} and 100 t ha^{-1})

Figure 8.13 illustrates the lengths of the longest leaves (LLF) of harvested tomato plants in ADC and DC amended soils at the end of the 12 weeks experimental period. The length of the longest leaves increased from 84 mm in non-amended control soils to 113 mm and 108 mm in soil amended with 100 t ha^{-1} of DC and ADC respectively. As shown in the Figure (8.13) no significant differences in LLF were observed between ADC and DC plants of similar amendments. However, a stronger correlation was observed between compost amendment and length of leaves for DC amended plants (R^2=0.78) than to those for ADC amended plants (R^2=0.55).

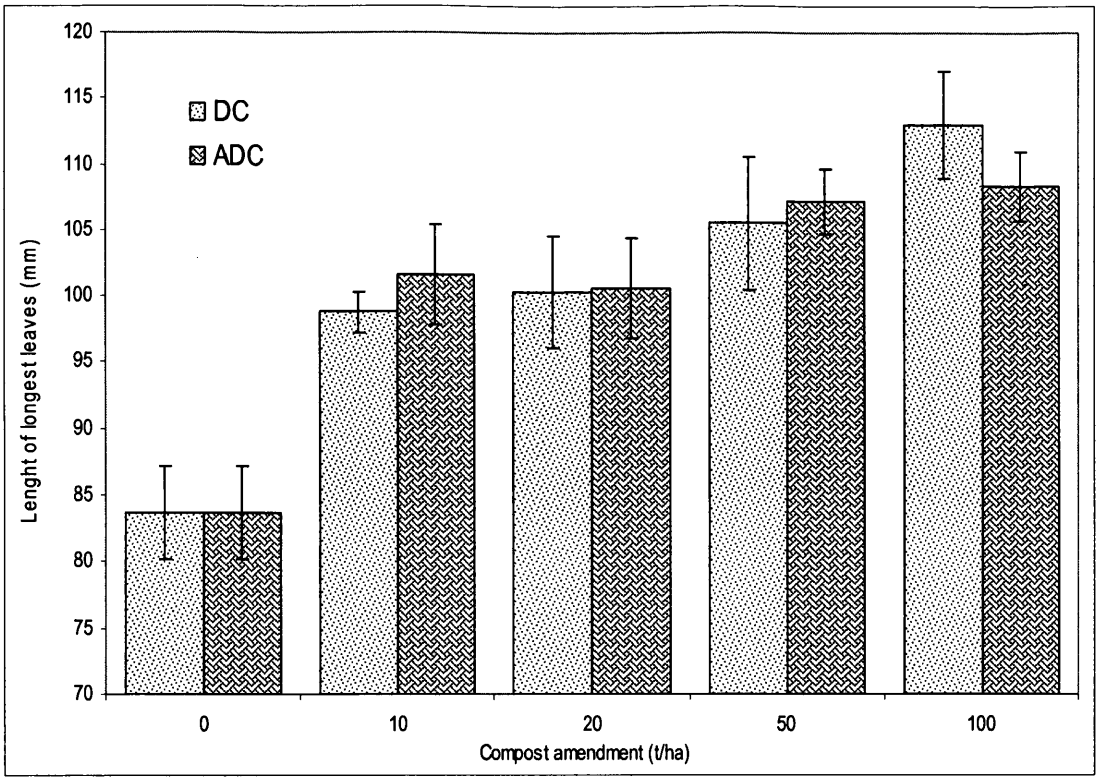


Figure 8.13 Length of the longest leaves (LLF) of tomato plants in ADC and DC amended soils. Same replicates were used as controls (0) for both ADC and DC amended plants. Error bars were calculated as the standard deviation of individual data points (n = 3).

Figure 8.14 shows the number of leaves (NLF) of harvested tomato plants in ADC and DC amended soils. The number of leaves increased from 167 in non amended soil controls to 301 and 318 in plants amended with 100 t ha^{-1} of DC and ADC respectively. No significant difference in number of leaves was observed between ADC and DC amended plants of similar compost loadings.

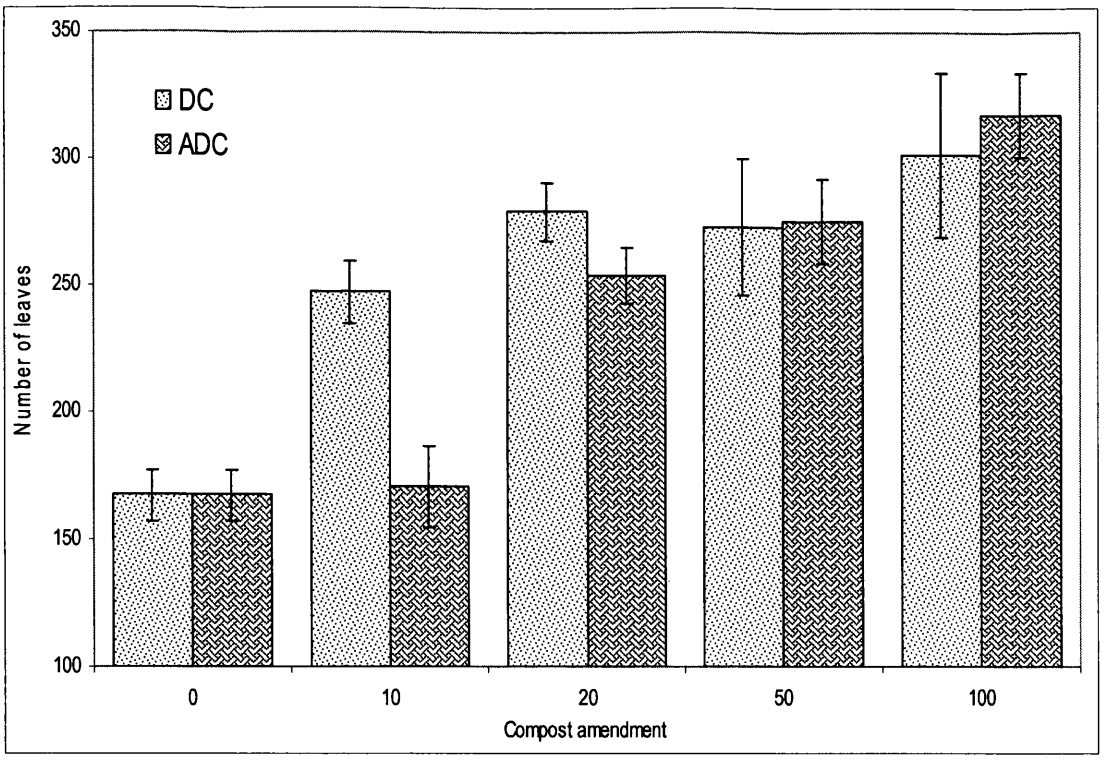


Figure 8.14 Number of leaves (NLF) of tomato plants in ADC and DC amended soils. Same replicates were used as controls (0) for both ADC and DC amended plants. Error bars were calculated as the standard deviation of individual data points (n = 3).

Figure 8.15 shows root growth and development prior to analysis, suggesting that the growth of trial plants might have been constrained by the limited space available for growth. As result, the use of larger quantity of trial soils is recommended in future similar studies.

Figure 8.15 Roots of tomato plants amended with 50 t ha^{-1} of ADC during harvest (i.e. Week 12).

The pot trials in general show that anaerobic digestate compost and the commercial compost have similar beneficial effects on plant growth, which increases with increasing compost load. This study demonstrates the suitability of ADC for use as organic soil fertilizers in arable soils. Yellowing of leaves was observed at week six in both ADC and DC amended plants and this was believed to be due to nitrogen deficiency probably caused by uncontrolled denitrification. Consequently, nitrogen supplements may be required for improved benefits of the composts. This observation suggests that whilst organic fertilizers are good source of plants nutrients, the capacity of plants to harness these nutrients may depend on soil management practices and environmental conditions that affect the loss of nitrogen.

8.4 Conclusions

The study shows that fresh waste (raw waste feedstock) and anaerobic digestates may be phytotoxic, with phytotoxicity decreasing with decrease in the amount of easily biodegradable organics in the waste. Increasing digestion period and subsequent aerobic post-treatment can bring about a decrease in the amount of easily biodegradable

components of the waste and as consequence, decrease the phytotoxic effects of the resulting soil amendment. Seed germination has also been found to increase with dilution and incubation time, suggesting that lower application rates and longer lag periods between application of ADC and planting can significantly reduce the amount of biodegradable organics in the ADC and thus reduce the occurrence of phytotoxicity and as consequence increase the beneficial effects on soil.

It has been demonstrated that ADC can compare favourably with commercially available composts as organic fertilizers. The study shows that whilst organic fertilizers are reservoirs of plants nutrients, the capacity of plants to absorb these nutrients may also depend on soil management practices and environmental conditions that may influence the loss of these nutrients from the soil.

The next chapter will investigate the effect of the addition of anaerobic digestate on soil quality parameters.

Chapter Nine: Effects of ADC addition on soil quality

9.1 Introduction

The aim of this study was to determine the effects of biological treatments of OFMSW on soil quality parameters. As no single is experiment is sufficient to investigate the soil enhancing qualities of ADC, a combination of physical, chemical and biological soil quality analyses were developed towards this objective. The quality parameters were as follows:

- Water retention tests (for changes in soil water content)
- Sorptivity analysis (for changes in hydraulic properties of soils)
- Carbon-Nitrogen dynamics
- Soil respiration (to determine bioactivity of soil populations)
- Soil microbial biomass (to quantify soil microbial populations)

Techniques adopted for the analyses were based on literature and the availability of resources and expertise on these methods at SCRI.

9.2 Methodology and analysis

9.2.1 Soil-ADC amendments

Soil-ADC amendments were prepared as in section 8.2.4. For the physico-chemical studies 75 soil-ADC amendment cores were first used for water retention analysis after which they were dried for sorptivity tests. C:N analysis was subsequently carried out on the same cores at the end of the sorptivity tests. For the biochemical studies 30 soil-ADC

amendments cores were utilized in the soil respiration tests for 12 weeks after which the soils were sampled for analyses of soil microbial biomass and population dynamics.

9.2.2 Water retention

Water retention test was carried out based on the method described by Aggelides and Londra, (2000). Of the 75 soil cores, 15 (i.e. 3 from each amendment) were not incubated and the rest incubated at 15^0C. During incubation, 15 cores (i.e. 3 from each amendment) were removed from the incubator after 1, 2, 3 and 12 weeks of incubation. Water loss in each core during incubation was adjusted by addition of distilled water in proportion to weight loss. Cores removed from incubator were stored at 4^0C for the water retention test. Water retention was determined by first adjusting the water content of all the 75 soil-ADC amendments to water holding capacity and by carefully placing each core afterwards on tension plates where their water retention were determine by suction at 0, -1, -2, -5, -10, -20, -50 and -100 kPa.

Figure 9.1 shows the soil cores on a tension plate (A) and tension plates and suction plates attached to suction pumps (B).

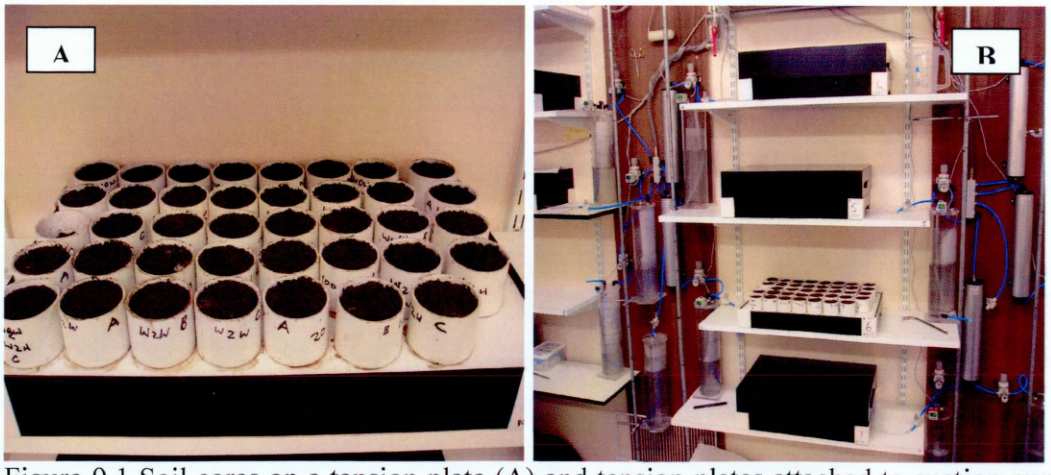


Figure 9.1 Soil cores on a tension plate (A) and tension plates attached to suction pumps (B)

9.2.3 Sorptivity

The 75 cores from water retention tests were dried at 40^0C for 48 hours. Water infiltration test was carried out on each soil core using infiltrometer as shown in Figure 9.2. Water infiltration was determined by placing each core on a perforated disc containing filter paper and quartz sand connected to a reservoir (bottle). Water levels in the disc and water bottle were adjusted after each test to same level by means of a ruler. A thin layer of sand provided a good contact with soil cores. An electronic balance accurate to 1 mg was used to measure water uptake by soil samples. Electronic recordings of 5 seconds upon contact with soil samples were taken and were repeated every 5 seconds for approximately 5 minutes.

Sorptivity, S ($mms^{-1/2}$) was determined by means of the rate of liquid infiltration I (ms^{-1}), into the soil using the relationship described in equation 9.1:

$$I = S\sqrt{T} \qquad (9.1)$$

Where I = cumulative infiltration; S = sorptivity and T = time (Zhang et al., 2004)

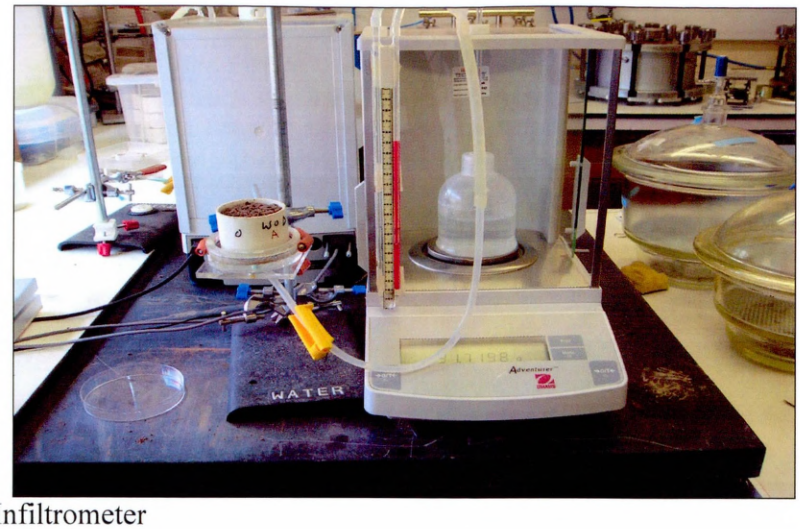


Figure 9.2 Infiltrometer

9.2.4 Carbon nitrogen dynamics

The 75 soil-ADC cores used for sorptivity test were dried immediately at 40^0C for three days and sampled for carbon and nitrogen. Carbon and nitrogen were determined using a mass spectrophotometer. Samples were finely ground in a ball mill and approximately 1 mg of compost and 5 mg of soil samples were each weighed into 6 x 4 mm tin capsules for analysis by continuous flow Dumas combustion using a Europa Scientific ^{TM} (Crew UK) ANCASL sample converter. Nitrogen and carbon were selectively detected as N_2 and CO_2 using a Europa Scientific Mass Spectrometer. The C:N ratio of soil samples were quantified relative to a leucine/citric acid mixture of known carbon and nitrogen content. Reference gravimetric samples were used for quality control.

9.2.5 Soil respiration

A total of 30 soil-ADC amendments cores (6 replicates from each amendment and for non-amended controls as described in section 8.2.3 and 8.2.4) were used for respiration studies. Each soil core was placed inside a sealed 500 ml Kilner jar as shown in Figure 9.3 and incubated for 12 weeks at 15^0C.

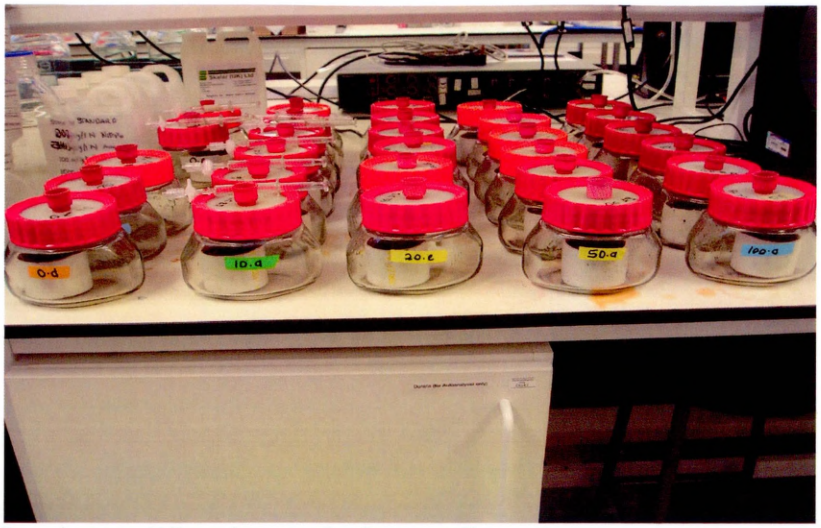


Figure 9.3 Respiratory Kilner jars ready for sampling.

Soil respiration and substrate induced respiration were investigated by analysis of CO_2 evolution as described by Ritz and Wheatley, (1989). CO_2 evolution was determined by extracting 2 ml of gas from the jar headspace with a syringe and feeding it into a gas chromatograph for CO_2 analysis. Results obtained were analysed using Analysis of variance (ANOVA) in Microsoft Excel

9.2.6 Soil microbial biomass

Soil biomass estimation was carried out by Substrate Induced Respiration (SIR) as described by Pennanen et al., (2004). 30 soil-ADC amendments were sampled for biomass analysis following respiration studies. 10 g of soil from each core were taken and transferred to McCartney bottles. The moisture of the samples were adjusted to 2 X WHC (water holding capacity) and transferred to another set of 30 ml McCartney bottles sealed with No 25 sub seal. Samples were each enriched with glucose solution (3 ml each of

10%w/v glucose) and incubated on a roller bed at 20^0C. Head space CO_2 was taken from each bottle at 2, 4 and 6 hours intervals and analysed by gas chromatography

9.3 Results and Discussion

9.3.1 Water retention

Figure 9.4 shows the result of the water retention test before incubation (week 0). Results generally indicate that at low water potential soil amended with 100 t ha^{-1} of ADC showed water content of 0.49 m^3/m^3 compared to 0.39 m^3/m^3 in non-amended controls. Similarly, at high water potentials soils amended with 100 t ha^{-1} of ADC showed water content of 0.28 m^3/m^3 compared to 0.24 m^3/m^3 in non-amended controls.

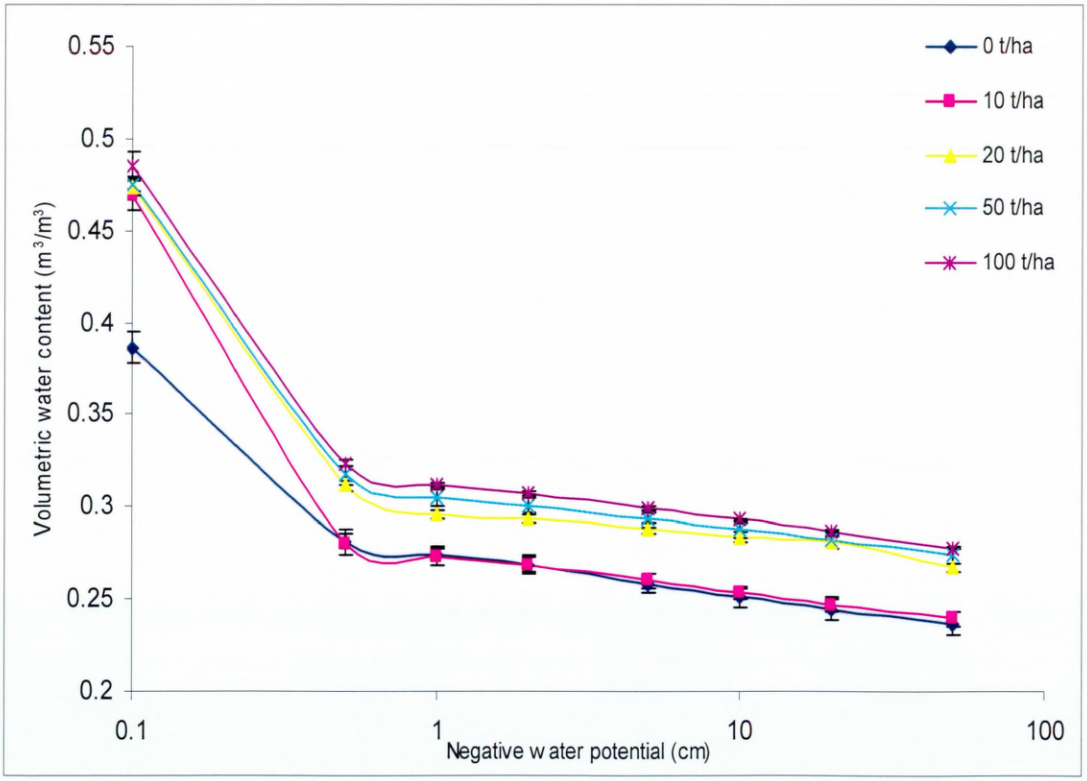


Figure 9.4 Water retention curves of ADC amended soil before incubation (week 0). Error bars were calculated as the standard deviation of individual data points (n = 3).

Figures 9.5, 9.6, 9.7 and 9.8 show water retention curves carried out after 1, 2, 3 and 12 weeks incubation respectively. The generally show similar trends as in Figure 9.4 that is, decreasing water content with increased water potential and increasing water content with increasing ADC amendment. The results also show decreasing water content with increasing length of incubation. The high water contents observed in soils amended with 50 t ha^{-1} of ADC after 3 weeks of incubation as shown Figure 9.7 may be due to experimental error as the trend was not observed in other figures.

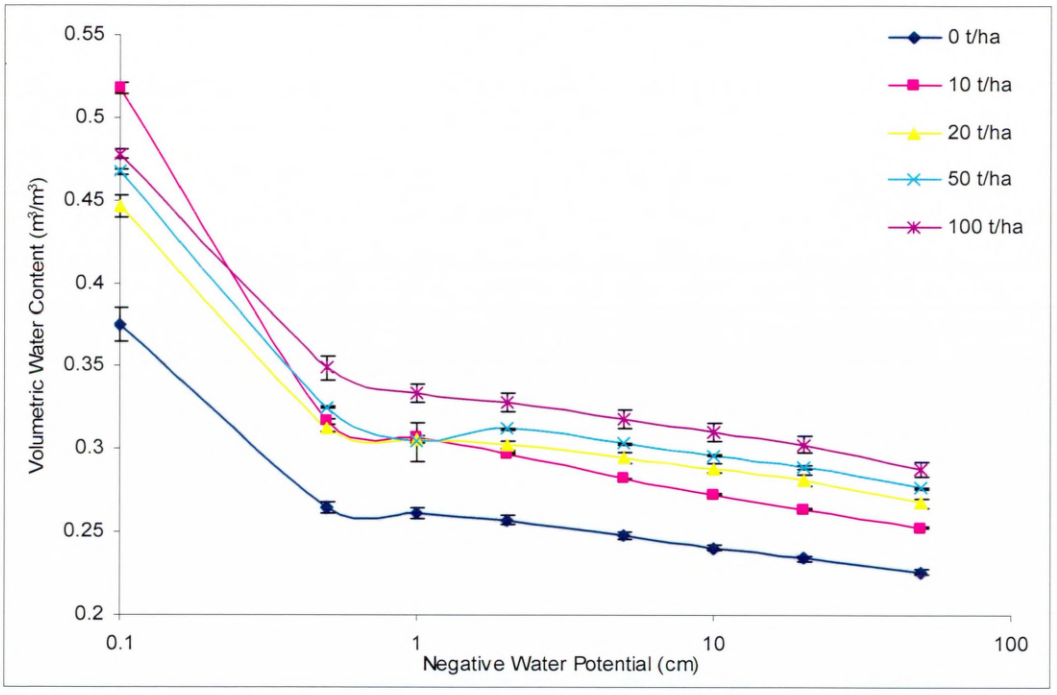


Figure 9.5 Water retention curves of ADC amended soils (week 1). Error bars were calculated as the standard deviation of individual data points (n = 3).

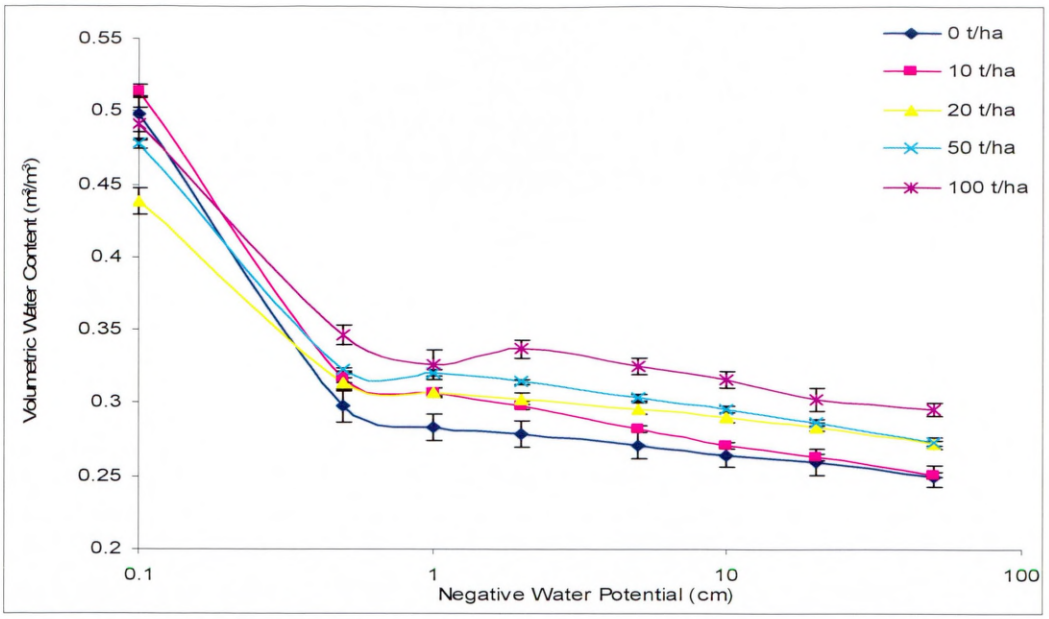


Figure 9.6 Water retention curves of ADC amended soils (week 2). Error bars were calculated as the standard deviation of individual data points (n = 3).

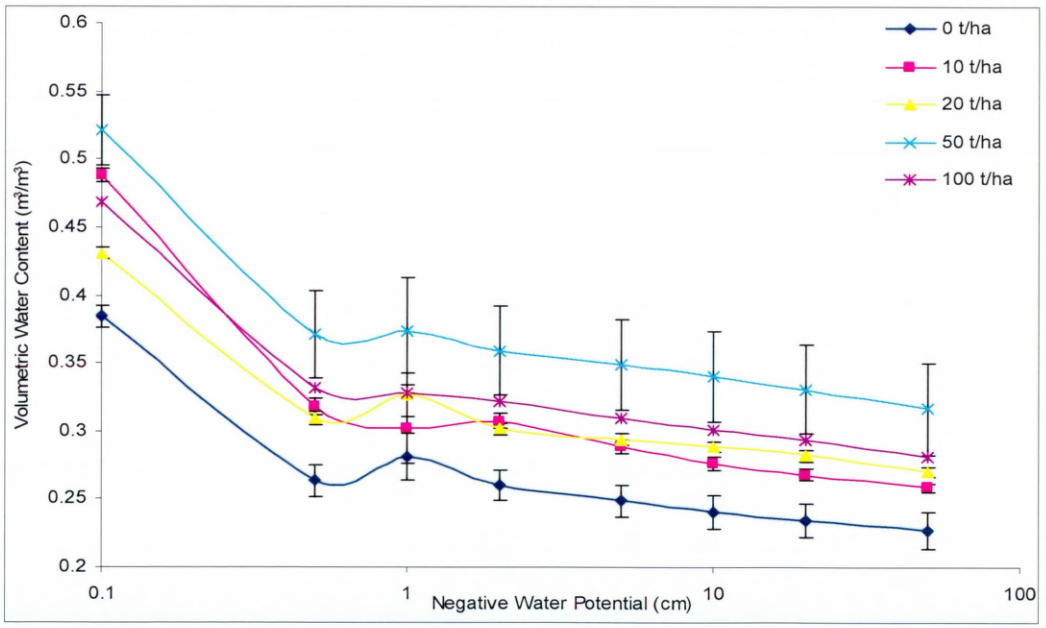


Figure 9.7 Water retention curves of ADC amended soil (week 3). Error bars were calculated as the standard deviation of individual data points (n = 3).

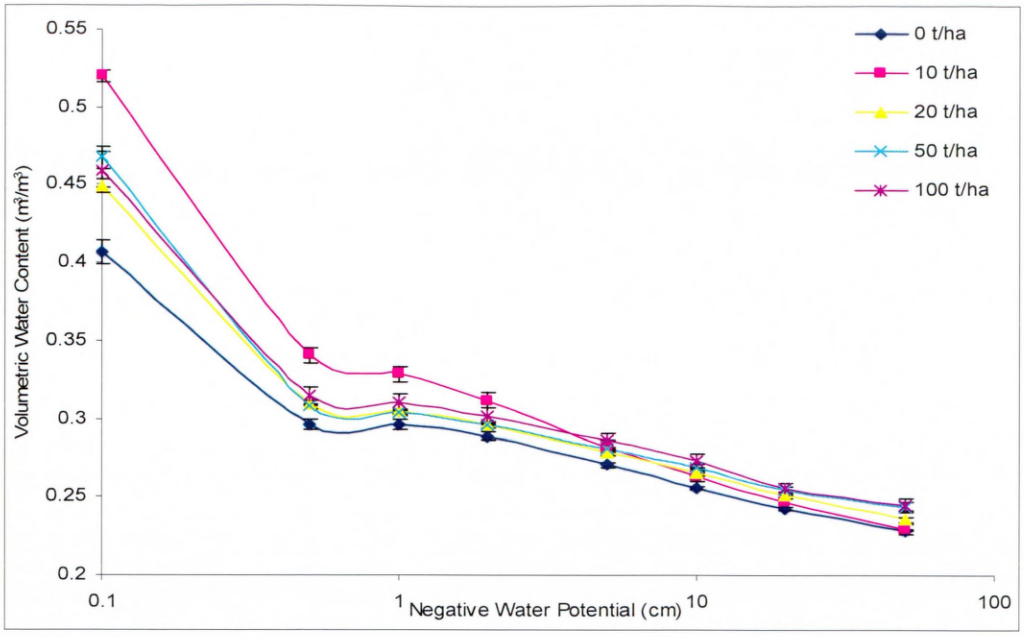


Figure 9.8 Water retention curves of ADC amended soil (week 12). Error bars were calculated as the standard deviation of individual data points (n = 3).

Generally, water content decreases with increasing water potential and increases with increasing ADC amendments but decreases with increasing length of incubation. The decrease in water content with increasing incubation time is believed to be due to the reduction of the organic matrix used for water storage as result of slow decomposition of organic matter. These observations confirm the suitability of ADC as a conditioner for improving water retention properties of arable soils. Similarly just like other organic fertilizers the capacity of ADC for soil improvement diminishes as it decomposes. Thus, there may be need for successive additional ADC amendments depending on soil functions.

9.3.2 Sorptivity tests

Figure 9.9 illustrates the results of the sorptivity tests. High sorptivity was observed in soils amended with 10 t ha^{-1} of ADC but decreased with increasing incubation time. This

was believed to be due to depletion of organic matter as result of microbial decomposition of soil organic matter. The low sorptivity recorded in soils amended with higher ADC loading rates may be due to water repellancy caused by intermediates of decomposition of soil organic matter. The results indicate that although high amendments of ADC may have temporary negative impacts on hydraulic properties of soil, mild application may be beneficial. As high sorptivity indicates greater hydraulic properties of soil, soils amended with high ADC loading rates may require some lag period prior to sowing to reduce the negative impacts of early intermediates of microbial decomposition of the applied organic matter.

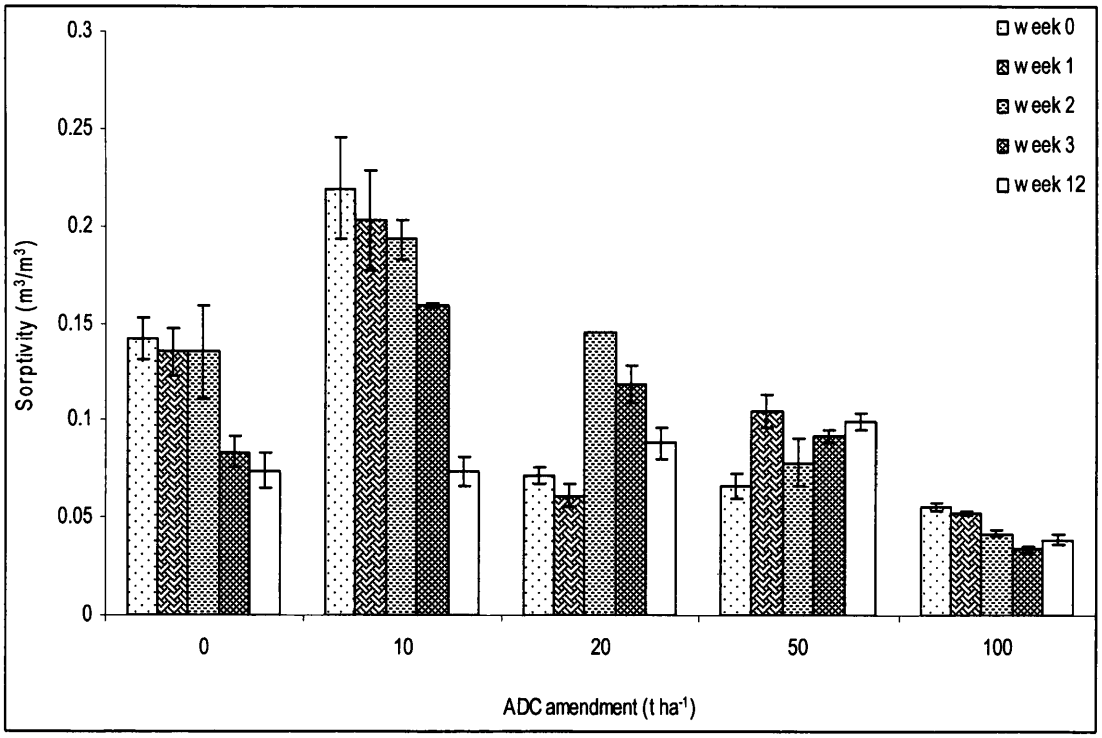


Figure 9.9 sorptivity in ADC amended soil. Error bars were calculated as the standard deviation of individual data points (n = 3).

9.3.3 *Carbon-nitrogen dynamics*

Figure 9.10 shows the dynamics of C:N ratio during incubation studies. The lowest C:N ratio of 11.78 was observed in non-incubated soils amended with 20 t ha^{-1} of ADC, while the highest ratio of 15.12 was observed in soils amended with 100 t ha^{-1} after 3 weeks of incubation. It is believed that the loss of inorganic N through denitrification may have been encouraged by increased nitrate and absorbable C in the soil-ADC amendments. The increased denitrification is probably responsible for the reduced N-immobilization resulting in lower than expected C:N ratios in soil-ADC amendments.

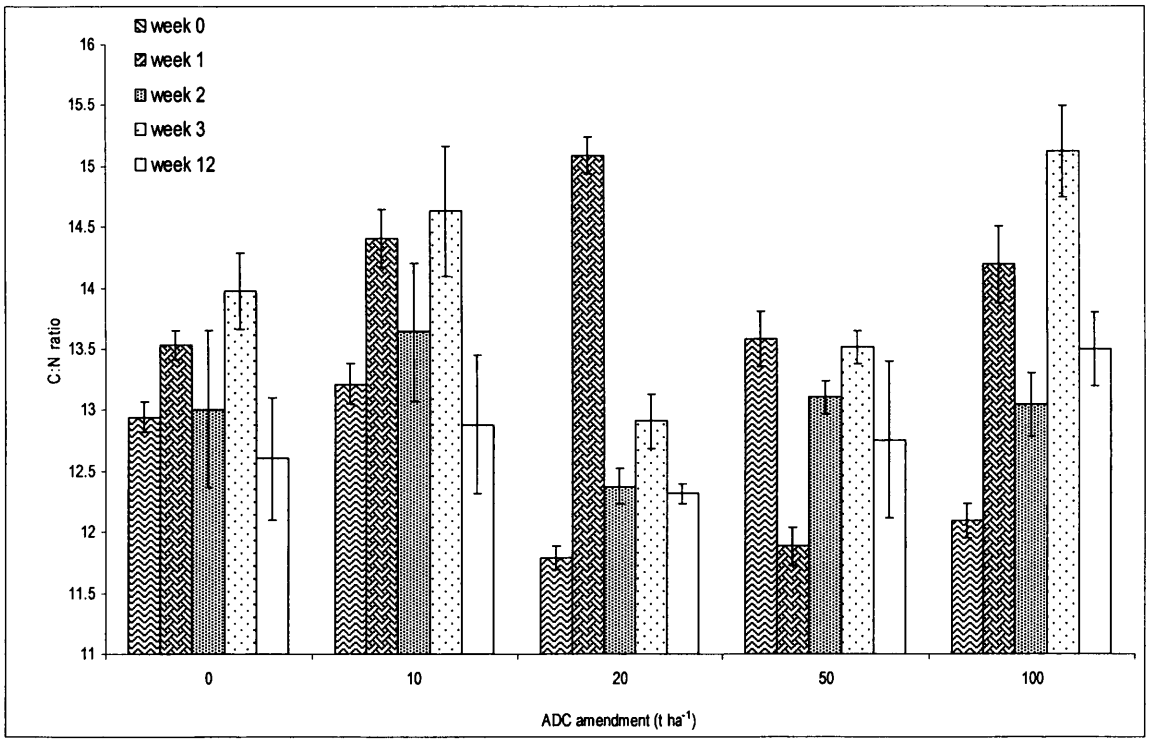


Figure 9.10 C:N dynamics in ADC amended soil. Error bars were calculated as the standard deviation of individual data points (n = 3).

Although significant differences in C:N ratio was observed in soils with different ADC amendments, no strong correlation was observed between C:N ratio and ADC amendments and between C:N ratio and the length of incubation. Lack of correlation may also be due to considerable N mineralization and loss of N through denitrification. Similar

findings were made by some authors (Calderón et al., 2004 and Bernal and Kirchmann, 1992).

9.3.4 Soil respiration

Figure 9.11 illustrates CO_2 evolution (bioactivity) during soil respiration studies. The figure indicates increased bioactivity of soil populations with increasing ADC amendments. The CO_2 evolution in non incubated soils increased from 0.01 μgC/g/h in non-amended control soils to 0.4 μgC/g/h in soils amended with 100 t ha^{-1} of ADC. The results also show that bioactivity increased with length of incubation especially in the first 30 days of incubation after which it subsided possibly due to reduced availability of assimilable carbon. For the non-incubated control soils bioactivity increased from 0.01 to 0.1 μgC/g/h after 80 days of incubation. The highest bioactivity was observed in soil amended with 100 t ha^{-1} of ADC where bioactivity increased from 0.4 μgC/g/h before incubation to 1.8 μgC/g/h after 80 days of incubation. The slow but continuous CO_2 evolution beyond 30 days was believed to be due to slow carbon release from the decomposing ADC. Slow decomposing organic fertilizers are considered stable and most suitable for sustainable soil fertility and increased productivity as reported by several authors (Calderon et al., 2007; Godley, 2004; Debosz et al., 2002).

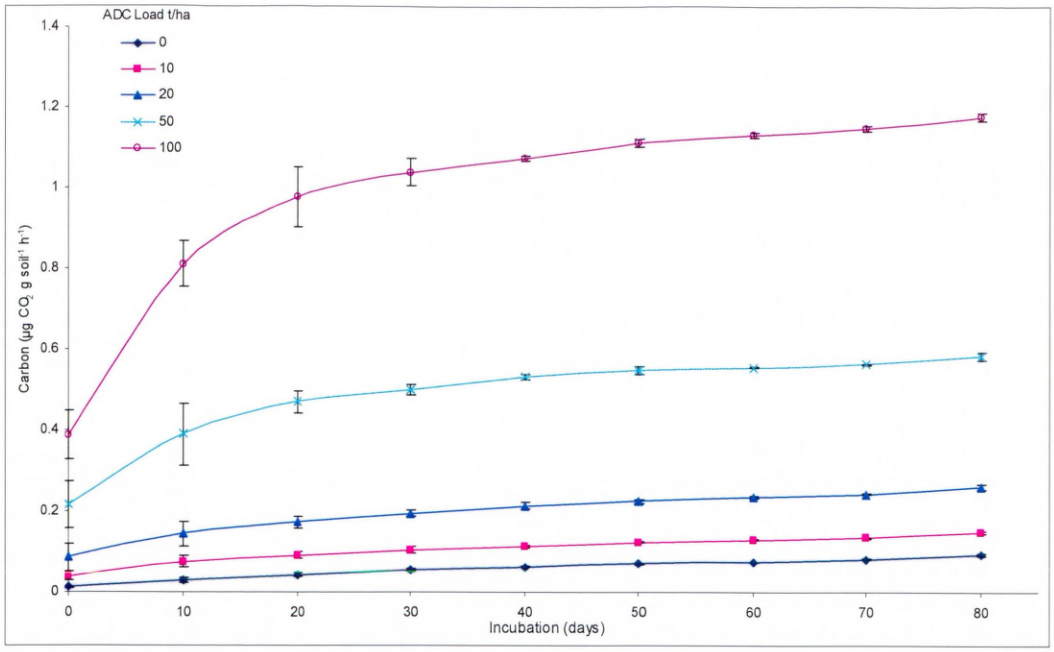


Figure 9.11 Cumulative respiration in ADC amended soils microcosms. Error bars were calculated as the standard deviation of individual data points (n = 3).

9.3.5 Soil microbial biomass

The result shown in Figure 9.12 illustrates soil microbial biomass after 2, 4 and 6 hours of incubation following glucose enrichment. These results show increasing SMBC with increasing ADC amendment. While correlation between soil microbial biomass carbon (SMBC) and ADC amendment was 0.6 (R^2=0.6) at 2 hours, the correlation at 6 hours was 0.9 (R^2=0.9). As SMBC is proportional to soil biomass, this signifies that soil biomass not only increases with increasing ADC amendments but also increases with increasing length of incubation. This is believed to be due to availability of readily degradable matter to most segments of soil populations. As soil populations metabolize different substrates at different rates, ADC amendments may have induced changes in the structure and diversity of soil populations. This is because as ADC decomposes nutrients may be released that will enhance microbial interaction and plant growth as well as substances that may also enhance other physical, chemical and biological properties of soil.

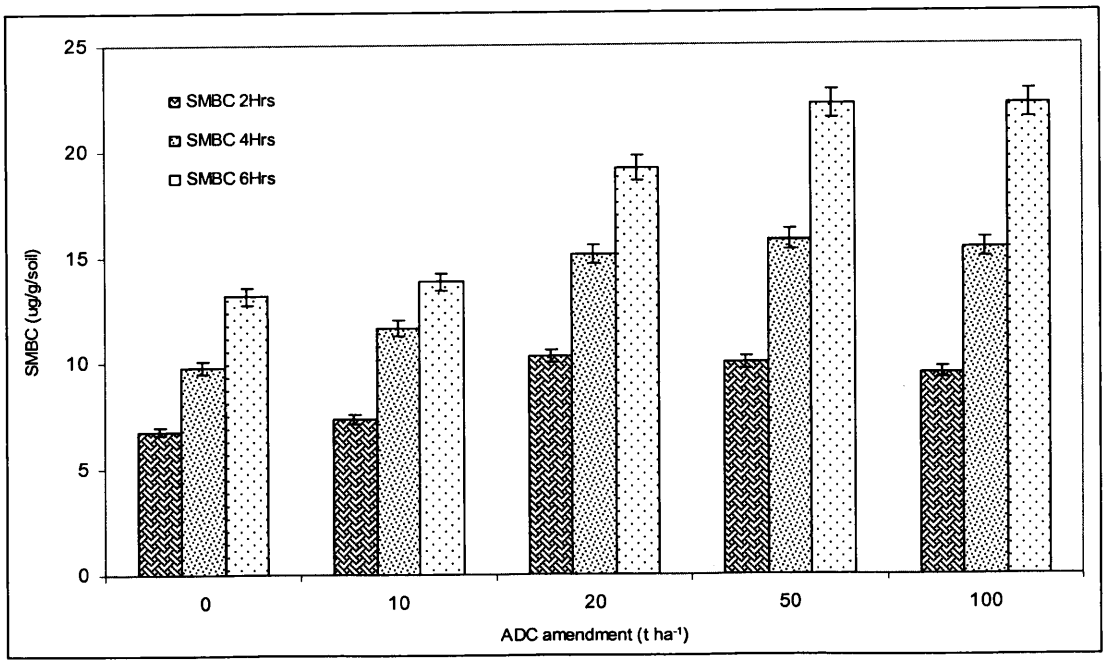


Figure 9.12 Soil microbial biomass carbon (SMBC) in ADC amended soils
Error bars were calculated as the standard deviation of individual data points (n = 3).

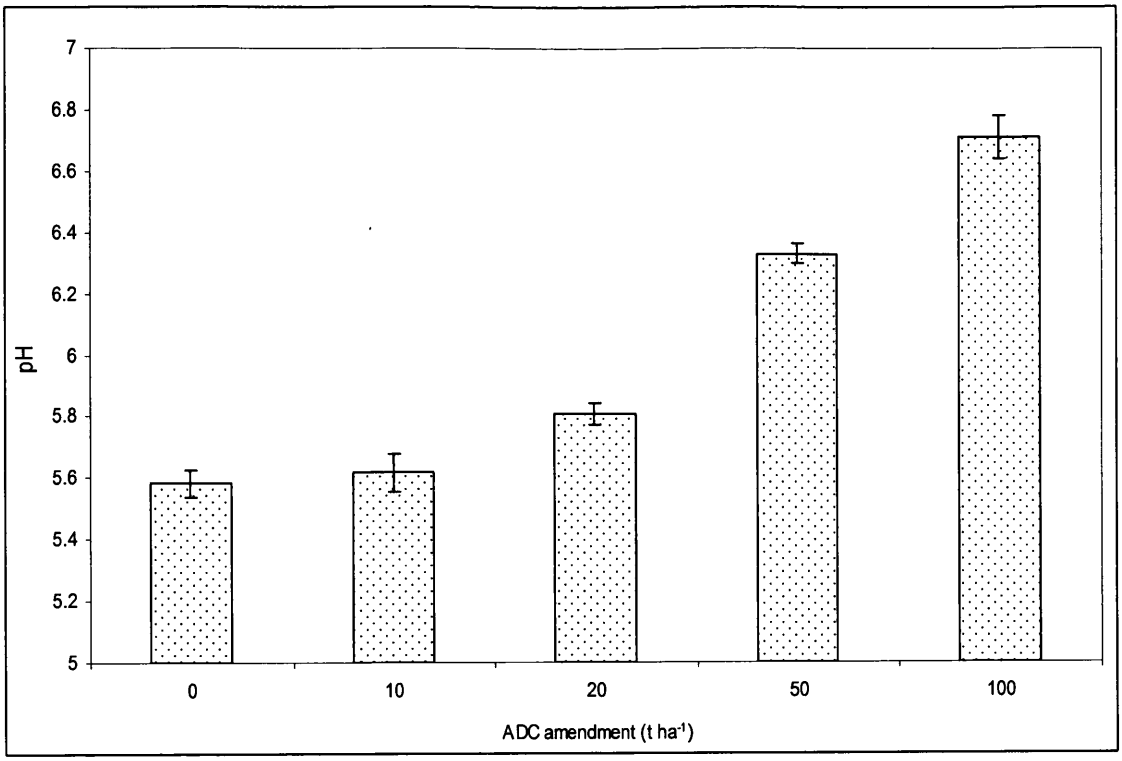


Figure 9.13 pH in ADC amended soils used in biomass analysis. Error bars were calculated as the standard deviation of individual data points (n = 3).

Figure 9.13 illustrates pH in soils used in the SIR tests showing increasing pH with increasing ADC amendments. pH is a critical factor in SIR as it promotes the dissolution of CO_2 where the pH is greater than 6.5 (Bailey et al., 2002) . This critical pH value was exceeded in soils amended with 100 t ha^{-1} of ADC where the average pH was 6.7. This was believed to be responsible for the similarity of soil biomass recorded between soils amended with 50 t ha^{-1} of ADC where the pH was 6.3 and those amended with 100 t ha^{-1} of ADC (Figure 9.12) as some of the produced CO_2 might have been dissolved in solution thereby reducing the quantity of CO_2 actually produced. In general, SMBC increases with ADC amendments and length of incubation. This increase in soil biomass is an indication of the stability and suitability of ADC organic fertilizer for application in arable soils.

9.4 Conclusions

Water retention analysis shows increased water content in ADC amended soils compared to non-amended soils and the higher the ADC amendment the greater the water content, which is beneficial to soil fertility. The sorptivity tests revealed that mild application of ADC could be beneficial, however higher ADC amendments may have temporary negative impacts on the hydraulic properties of soil. Although significant relationship was observed between ADC amendments and soil bioactivity, the C:N dynamics showed no correlation with ADC amendments or length of incubation. This was attributed to increased decomposition of soil organic matter and the loss of organic N through denitrification. It is possible that ADC addition may result in N enrichment to appropriate C:N ratio for optimum soil functioning. The soil respiration studies revealed that ADC amendments increased the bioactivity of soil populations. Slow but continuous release of CO_2 observed during the studies is an indication of the suitability of ADC for application in arable soils. Soil microbial biomass also increases with ADC amendments and the higher the ADC amendments the greater the soil biomass. As respiratory capacity of soils increases with improvement in soil fertility, the observed increases in bioactivity and soil biomass demonstrate the suitability of ADC for application in arable soils. Generally, the results of the soil quality analysis showed that ADC obtained from OFMSW has no negative impacts on soil functioning.

The incubation studies suggest that ADC amendment may have significant impacts on the diversity of soil populations. The next chapter will investigate the diversity of soil populations that may have been induced by ADC amendment.

Chapter Ten: Investigating the impact of anaerobic digestate compost amendments on soil microbial populations

10.1 Introduction

Microbial diversity is important in sustainability and productivity of agricultural soils (Hole et al, 2005). The complexity of soil microbial populations makes understanding of the dynamics of soil communities difficult as the majority cannot be isolated, cultivated or characterized (Marsh, 1999). The reconstruction of active microbial communities from a very small sample has resulted in the development of culture-independent molecular techniques for identification of demographic complexity of soil communities (Marsh, 1999). Molecular techniques provide unbiased information about microbial communities compared to traditional cultivating methods (LaMontague et al., 2002). These techniques use Deoxyribonucleic Acid (DNA) profiles or genetic markers to establish microbial diversity. The use of DNA profiling techniques with 16S rRNA (ribosomal Ribose Nucleic Acid) and 18S rRNA primers as phylogenetic markers have been shown to be fast and efficient methods of assessing microbial diversity (Mills et al., 2003), however, the most common profiling methods are based on restriction enzyme digestion of 16S rRNA and 18S rRNA genes. The digestion techniques could be Restriction Fragment Length Polymorphisms (RFLP) (Pukall et al., 1998), fluorescent Terminal Restriction Fragment Length Polymorphisms (T-RFLP) (Dunbar et al., 2000) or Denaturing Gradient Gel Electrophoresis (DGGE) (Vanbroekhoek et al., 2004). The distribution of restriction site sequences within the 16S rRNA and 18S rRNA genes have been found to show phylogenetic relationships at a number of taxonomic levels.

T-RFLP analysis is a reliable tool for investigating the composition and diversity of microbial populations (Mills et al., 2003; Marsh et al., 1999). The advantages of T-RFLP include the ease of direct reference to sequenced database and the fact that sequencing has a greater resolution compared to DGGE and Single Strand Conformation Polymorphism (SSCP). Additionally, T-RFLP analysis is faster and can produce digital output (Marsh, 1999).

The objectives of this study were

- To establish the influence of different levels of ADC amendments on the diversity of soil microbial populations
- To elucidate the efficacy of different restriction enzymes in the study of soil microbial diversity.

The methods of analysis adopted in this segment are based on the available resources and expertise at SCRI. In this study, soil microcosms containing different levels of anaerobic digestate compost amendments were set up and incubated for respiration (see section 9.3.4) and subsequent molecular analysis. Multivariate Analysis (MA) was applied to Polymerase Chain Reaction (PCR)-based studies of microbial communities to compare terminal restriction fragment length polymorphisms patterns of 16S rRNA and 18S rRNA genes using Genstat and Microsoft excel statistical packages.

10.2 Methodology and analytical methods

10.2.1 Preparation of soil microcosms (cores)

Soil microcosms were prepared as outlined in Section 8.2.4. Soil cores for population studies were the same microcosms used in respiration studies (see section 9.3.4). A total

of thirty soil cores (six for each treatment) were used in the respiration studies. These microcosms represent ADC treatments of 10, 20, 50, 100 and 0 t ha^{-1}. At the end of the respiration experiment, 1.0 g of soil was taken from each microcosm, frozen immediately in liquid nitrogen and stored at -80^{0}C awaiting nucleic acid extraction.

10.2.2 DNA extraction and purification

The stored soil samples were defrosted and 1 g of each of the samples was transferred into Falcon tubes ^{TM} containing 1 mm Di-Ethyl-Pyro-Carbonate (DEPC) treated glass beads. To each of the falcon tubes, 2 ml of DNA and RNA spiked 0.12M Na_2HPO_4 1% Sodium Dodecyl Sulphate (SDS) buffer were added. The tubes were placed in a bead beater at 30 speed for 1min 30 sec. The bead beating was repeated twice after which the tubes were centrifuged at 4000 *g* for 5 mins to release the nucleic acids. The supernatants were poured into new sets of clean tubes to which equal volume of phenol chloroform: IsoAmylAlcohol (IAA) ratio (25:24:1) were added. The solutions were gently mixed and centrifuged in microfuge (Refrigerated centrifuge Sigma 4k15c^{TM}) at 4000 *g* for 5 mins. The aqueous phase was transferred to a new set of tubes containing equal volume (450μl) of chloroform: IAA (ratio 24:1). The solutions were gently mixed and centrifuged at 4000 g for 1 min. To a new set of tubes, an equal volume (400μl) of 0.3M Isopropanal and sodium acetate was added to the aqueous phase. The tubes were frozen at -20^{0}C for 20 mins after which they were centrifuged at 4000 *g* for 5 mins to pellet the DNA. The supernatants were discarded and pellets washed in ice-cold 70% ethanol and centrifuged at 4000 *g* for 5 mins. Ethanol was removed by turning the tubes upside down on tissue for evaporation. The pellets were subsequently cleaned using PolyVinylPolyPyrrolidone (PVPP) and water to yield 50μl of sample (see Appendix III).

During DNA extraction, SDS was used to break the cell walls for the release cellular contents, while phenol was used as solvent for proteins and DNA. The Chloroform: IAA mixture was employed to remove traces of phenol as the PVPP, while ethanol to precipitate the extracted DNA.

10.2.3 PCR, AGE, and T-RFLP

Two rounds of PCR amplifications were carried out for each DNA sample using bacterial and fungal primers according to the protocol outlined in appendix III. The primer pair and PCR conditions were summarized in Table 10.1. PCR was carried out using PCR thermocycler model Bio-Rad DNA engine (Dyad) and Peltier thermal cycle with SCRI apparatus (ID 9320).

Table 10.1 Polymerase chain reaction conditions for first and second round amplifications targeting rRNA and rDNA region for bacteria and 18S rRNA and rDNA fungi

Domain	PCR round	Primer pair	PCR amplification conditions
Bacteria	1	16f27 1494r	(i) 4 mins at 94^{0}C (ii) 35 cycles of 30s at 94^{0}C, 30s at 51^{0}C & 1 min 30s at 68^{0}C, (iii) 10 mins at 68^{0}C
	2	63fFAM 1405rVIC	(i) 4 mins at 94^{0}C, (ii) 25 cycles of 30s at 94^{0}C, 30s at 51^{0}C & 1 min 30s at 68^{0}C, (iii) 10 mins at 68^{0}C
Fungi	1	EF3RCNL ITS4	(i) 4 mins at 94^{0}C, (ii) 35 cycles of 30s at 94^{0}C, 30s at 51^{0}C & 1 min 30s at 68^{0}C, (iii)10 mins at 72^{0}C
	2	ITS1fNED ITS4PET	(i) 4 mins at 94^{0}C, (ii) 35 cycles of 30s at 94^{0}C, 30s at 51^{0}C & 1 min 30s at 68^{0}C, (iii)10 mins at 72^{0}C

The PCR products from both the first and second round amplifications have been shown to give reproducible AGE fingerprints that reflect the fungal and bacterial sequence diversity of the microbial community (Pennanen et al., 2004). PCR products were separated by agarose gel electrophoresis using 10% acrylamide gels according to the manufacturer's instructions (DCode Universal Mutation Detection System, Bio-Rad).

Each of the PCR products were combined with loading dye (ratio 3:2) and loaded unto gels with 1 kbp (kilo base pair) and 500 bp (base pair) ladder occupying the first and the last well of the gel. The PCR products were separated and visualized by electrophoresis. The samples were run at room temperature, at 70 volts for 15 mins. Electrophoresis was carried out in gel plates by Scie-Plas, model No H1-set (max volts 200 volts) and serial identification 4072. Power to the gel plates was supplied by Bio-Rad power PAC 300 (SCRI apparatus Id 629). Digital imagery of DNA bands were taken by a camera model SYNgene produced by Synoptics limited and printed using Sony, Syngene digital graphic printer model UP-D890. Bands obtained are illustrated in Figures 10.1a, b, c and d.

Restriction enzymes digests of nested second round PCR products were prepared according to bacterial and fungal T-RFLP protocol (appendix III). Restriction enzymes used were AluI, DdeI and HincII for 16S rRNA while HinfI, RsaI and TaqI were used for 18S rRNA fungi. Restriction enzymes and associated buffers used were supplied by Promega (2006) as described in Table 10.2. The enzymes were used as recommended by manufacturer. Restriction fragments were sequenced and peaks obtained analysed statistically.

Table 10.2 Characteristics of restriction enzymes (Promega, 2006)			
Enzyme	**Source**	**Recognition sequence**	**Sequencing dye**
AluI	*Arthrobacter luteus*	5'AGCT 3'TCGA	Blue & green
DdeI	*Desulfovibrio desulfuricans*	5'CTNAG 3'GANTC	Blue & green
HincII	*Haemophilus influenzae Rc.*	5'GT(T/C)(AG)AC 3'CA(AG)(T/C)TG	Blue & green
HinfI	*Haemophilus influenzae Rf.*	5'GANTC 3'CTNAG	Red & yellow
RsaI	*Rhodopseudomonas sphaeroides*	5'GTAC 3'CATG	Red & yellow
TaqI	*Thermus aquaticus YTI*	5'TCGA 3'AGCT	Red & yellow

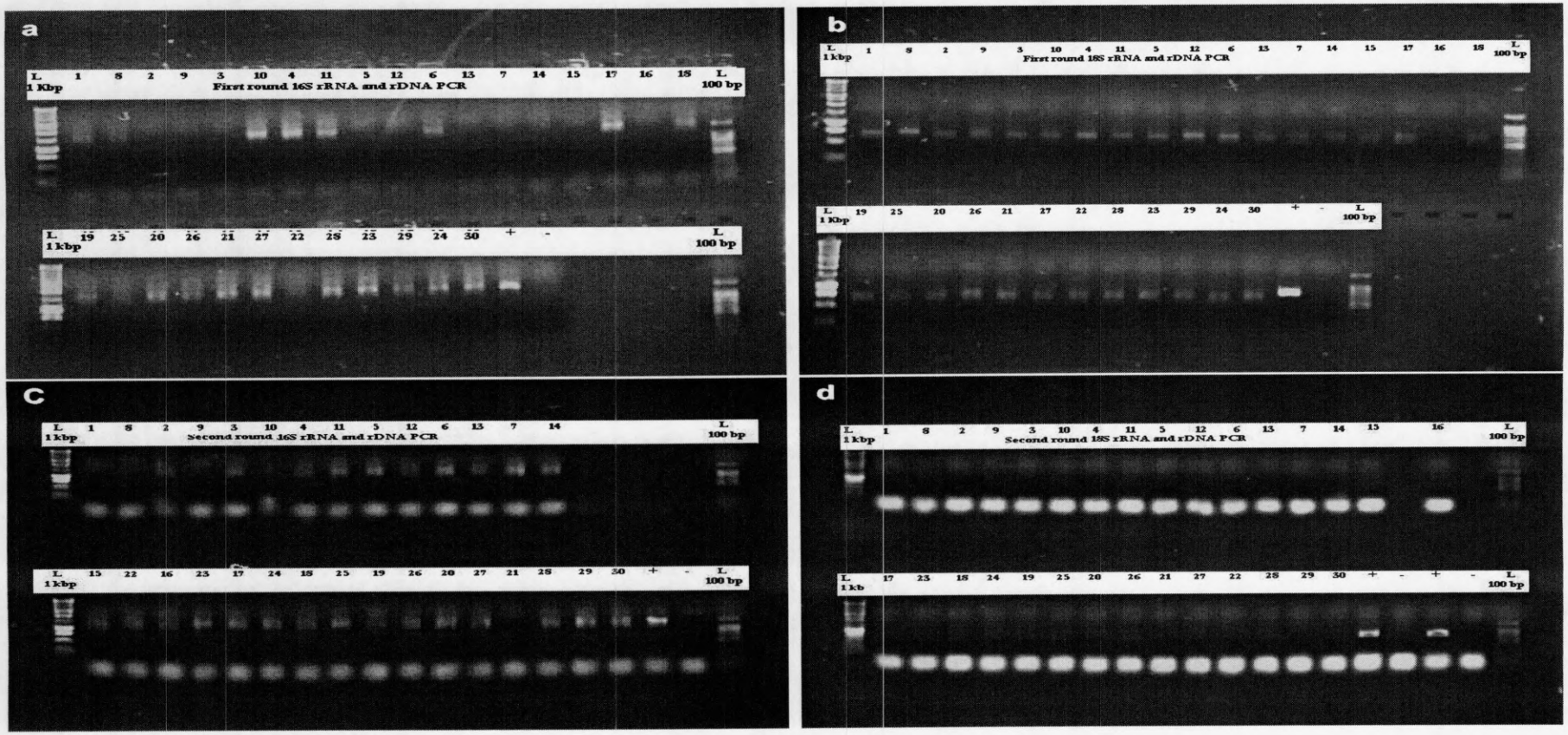


Figure 10.1 AGE images of (a) first round 16S rRNA and rDNA (b) first round 18S rRNA and rDNA (c) second round nested 16S rRNA and rDNA (d) second round nested 18S rRNA and rDNA PCR products from ADC amended soil microcosms where numbers refer to microcosm with ADC amendments as follows: 1 – 6, no compost; 7 – 12, 10 tha^{-1}; 13 – 18, 20 tha^{-1}; 19 – 24, 50 tha^{-1}; 25 – 30, 100 tha^{-1}. L, refer to ladder while + & - refer to positive and negative samples respectively.

10.2.4 Statistical analysis

Multivariate analysis (MA) was applied to PCR-based studies of microbial communities using GENSTAT statistical packages (McCaig et al., 2001), to compare terminal restriction fragment length polymorphisms (TRFLP; Liu et al., 1997) of DNA obtained from soil samples. The principal components obtained were subjected to analysis of variance (ANOVA) in Microsoft Excel to test for significant differences in soil samples.

10.3 Results and discussion

10.3.1 AGE images of microbial community

Figure 10.1 shows AGE images of first and second rounds PCR products of 16S and 18S rRNA and rDNA obtained from ADC amended and non-amended soils. Total community DNA was extracted from all soil microcosms to the required yields for population studies. PCR products were obtained from all the extracted 30 soil DNA samples, corresponding to the six treatments of 0, 10, 20, 50 and 100 t ha^{-1} of ADC amendments respectively. Fingerprints of the most dominant populations were obtained after separation of PCR products from first and second round of amplifications using bacterial and fungal primers in AGE. Banding patterns obtained in AGE from samples were used for preliminary comparison on the structural diversity of bacterial and fungal communities in the compost amended and non-amended soil microcosms. A comparison of 16S rRNA and 18S rRNA gene-based AGE fingerprints of amended and non-amended soils showed relative abundance and stability of the bacterial and fungal community profiles between treatments. It appeared that dominant bands in AGE appeared in all soils, with or without amendments. The AGE profiles shown in Figures 10a, b, c and d suggest that all soil microcosms were rich reservoirs of 16S and 18S rRNA and rDNA as bands were common in both ADC amended and non-amended soil samples. Banding appeared to be

more pronounced in the nested PCR products compared to the first round products. It appeared that the higher the ADC amendments the more pronounced the banding especially in the first round 16S rRNA and rDNA PCR products as illustrated in Figure 10.1a. Banding appeared to be more common in the first round 18S rRNA and rDNA PCR products, signifying that fungal communities may be less affected by ADC enrichment compared to bacterial groups. The AGE profiles showed that all the control positives and negatives remained unchanged during both the first and second round PCR cycles.

Figure 10.2 illustrates the principal coordinate scores of sequenced restriction fragments generated from digestion with restriction enzymes and primers specific for 16S rRNA /rDNA and 18S rRNA/rDNA. Generally the coordinates showed that microcosms with little or no diversity between their populations come together to form a cluster, the closer the microcosms the closer the populations however, the farther apart the clusters the greater the diversity between populations. Figure 10.2 also illustrates the diversity of sequenced 16S rRNA/rDNA fragments digested with the restriction enzyme AluI using blue and green dyes. The result showed significant changes in the diversity of bacterial populations in ADC amended microcosms compared to non-amended controls with a variation of between 16 to 37%. In general, the higher the ADC amendments the greater the diversity of the soil populations as microcosms with little or no ADC amendments (0, 10, 20 t ha^{-1}) appeared to have formed a cluster distinctively separate from microcosms with high ADC amendments (50, 100 t ha^{-1}).

Figure 10.3 shows the principal coordinate scores of sequenced fragments of 18S rRNA and rDNA cut by the restriction enzyme HinfI using red and yellow dyes. The result

showed changes in soil populations between ADC amended microcosms and non-amended controls, indicating significant changes in fungal populations. The clusters observed in Figure 10.3 are similar to those of Figure 10.2. The ADC amendments appeared to have a greater impact on the diversity of fungal communities compared to those of bacteria (Figure 10.2) with variation of between 22 to 50%. Little or no diversity was observed in microcosms with low ADC amendments (10, 20 t ha^{-1}) which came together to form cluster distinctively separate from another cluster formed by microcosms with higher ADC amendments (50, 100 t ha^{-1}). This signify that the higher the difference in ADC amendments between microcosms, the greater the diversity of their populations. Figures 10.4, 10.5 and 10.6 show principal coordinate scores of ADC amended and non-amended control soil microcosms treated with restriction enzymes DdeI, HincII, and TaqI showing fragments coming together to form big clusters, suggesting that the communities targeted by these enzymes are closely related with little or no diversity. The variations in the samples were 20-36% in DdeI, 15-25% in HincII and 12-79% in TaqI respectively. Generally, the 18S rRNA and rDNA fragments showed greater variations than those of 16S rRNA and rDNA, suggesting that ADC amendment has a greater impact on fungi than bacteria. This signifies that the restriction fragments produced by DdeI, HincII and TaqI were not only abundant in all the soil microcosms but were also less affected by ADC amendments. Thus, DdeI, HincII and TaqI may not be good markers for the detection soil microbial diversity in compost amended soils.

Figure 10.7 illustrates the principal coordinate scores of restriction fragments digested with RsaI showing significant diversity between ADC amended and non-amended microcosms of 18S rRNA and rDNA populations. The formation of a large multi-cluster by ADC amended microcosms independent of control, suggest close relationship between

the fragments of ADC amended microcosms and the diversity between the amended and the non-amended controls.

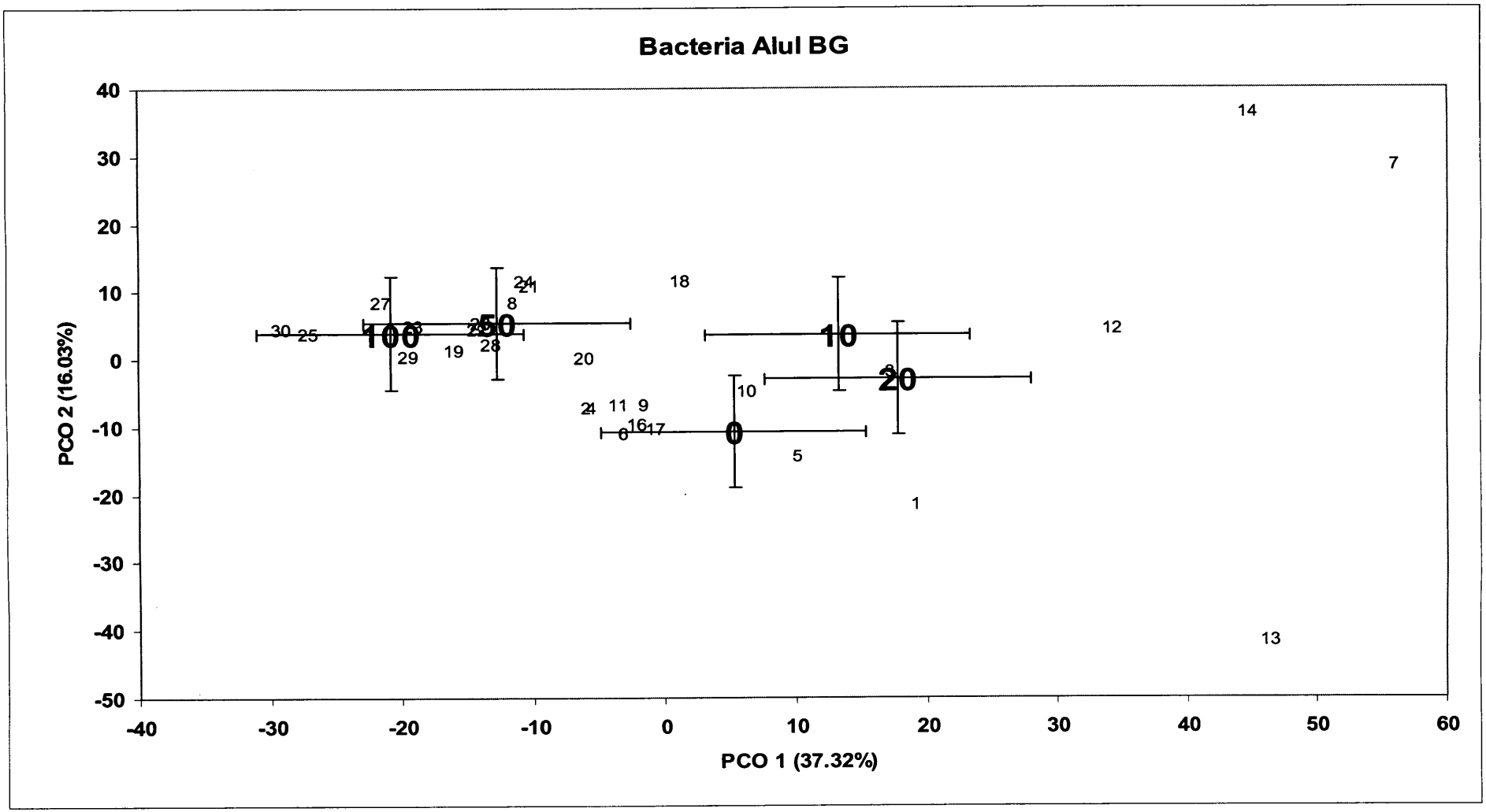


Figure 10.2 Principal co-ordinate scores of sequenced AluI restriction enzyme digest fragments where small numbers refer to microcosm with compost amendment as follows: 1 – 6, no compost; 7 – 12, 10 t ha^{-1}; 13 – 18, 20 t ha^{-1}; 19 – 24, 50 t ha^{-1}; 25 – 30, 100 t ha^{-1} the larger numbers refer to compost loading in t ha^{-1} Bars are calculated as the standard deviation of individual data points (n = 6).

General legend

1 – 6 = Non-amended soils (controls)

7 – 12 = soils amended with 10 t ha-1 of ADC

13 – 18 = soils amended with 20 t ha^{-1} of ADC

19 – 24 = soils amended with 50 t ha^{-1} of ADC

25 – 30 = soils amended with 100 t ha^{-1} of ADC

0, 10, 20, 50, 100 = ADC amendments in t ha^{-1}

 = Mean soil population distribution for each ADC amendments (ANOVA)

*PCO = principal components (Multivariate analysis)
**1 = dimensions (multivariate analysis)
*** (%) = percentage variation (Multivariate analysis)

BG = Blue and Green dyes (16S rRNA and rDNA)
RY = Red and Yellow dyes (18S rRNA and rDNA)

AluI, DdeI, HincII, TaqI, RsaI, HinfI = Restriction enzymes

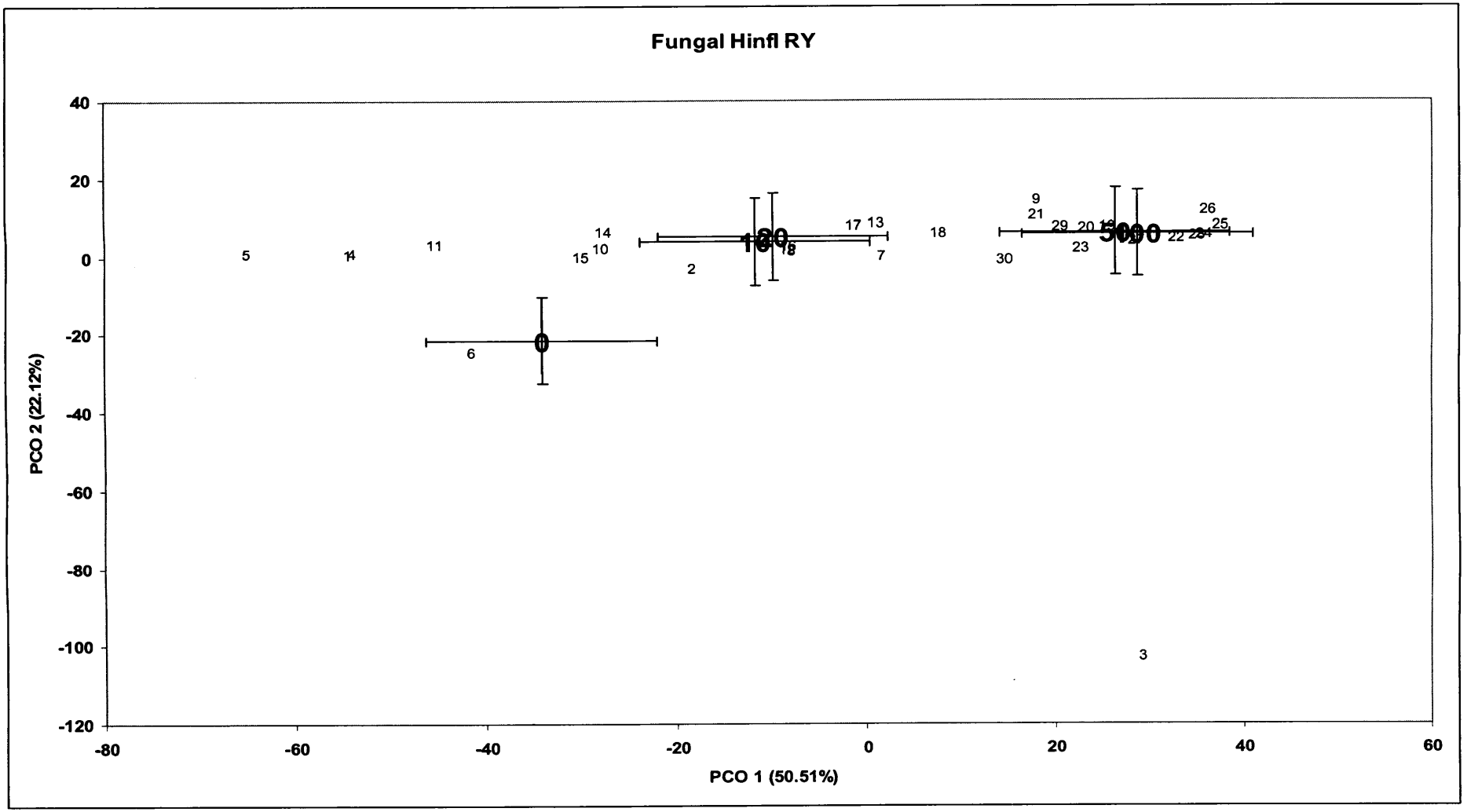


Figure 10.3 Principal co-ordinate scores of sequenced HinfI restriction enzyme digest fragments where small numbers refer to microcosm with compost amendment as follows: 1 – 6, no compost; 7 – 12, 10 t ha^{-1}; 13 – 18, 20 t ha^{-1}; 19 – 24, 50 t ha^{-1}; 25 – 30, 100 t ha^{-1} The larger numbers refer to compost loading in t ha^{-1} Bars are calculated as the standard deviation of individual data points (n = 6).

General legend

1 – 6 = Non-amended soils (controls)

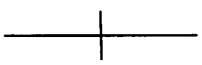
7 – 12 = soils amended with 10 t ha-1 of ADC

13 – 18 = soils amended with 20 t ha^{-1} of ADC

19 – 24 = soils amended with 50 t ha^{-1} of ADC

25 – 30 = soils amended with 100 t ha^{-1} of ADC

0, 10, 20, 50, 100 = ADC amendments in t ha^{-1}


= Mean soil population distribution for each ADC amendments (ANOVA)

*PCO = principal components (Multivariate analysis)
**1 = dimensions (multivariate analysis)
*** (%) = percentage variation (Multivariate analysis)

BG = Blue and Green dyes (16S rRNA and rDNA)
RY = Red and Yellow dyes (18S rRNA and rDNA)

AluI, DdeI, HincII, TaqI, RsaI, HinfI = Restriction enzymes

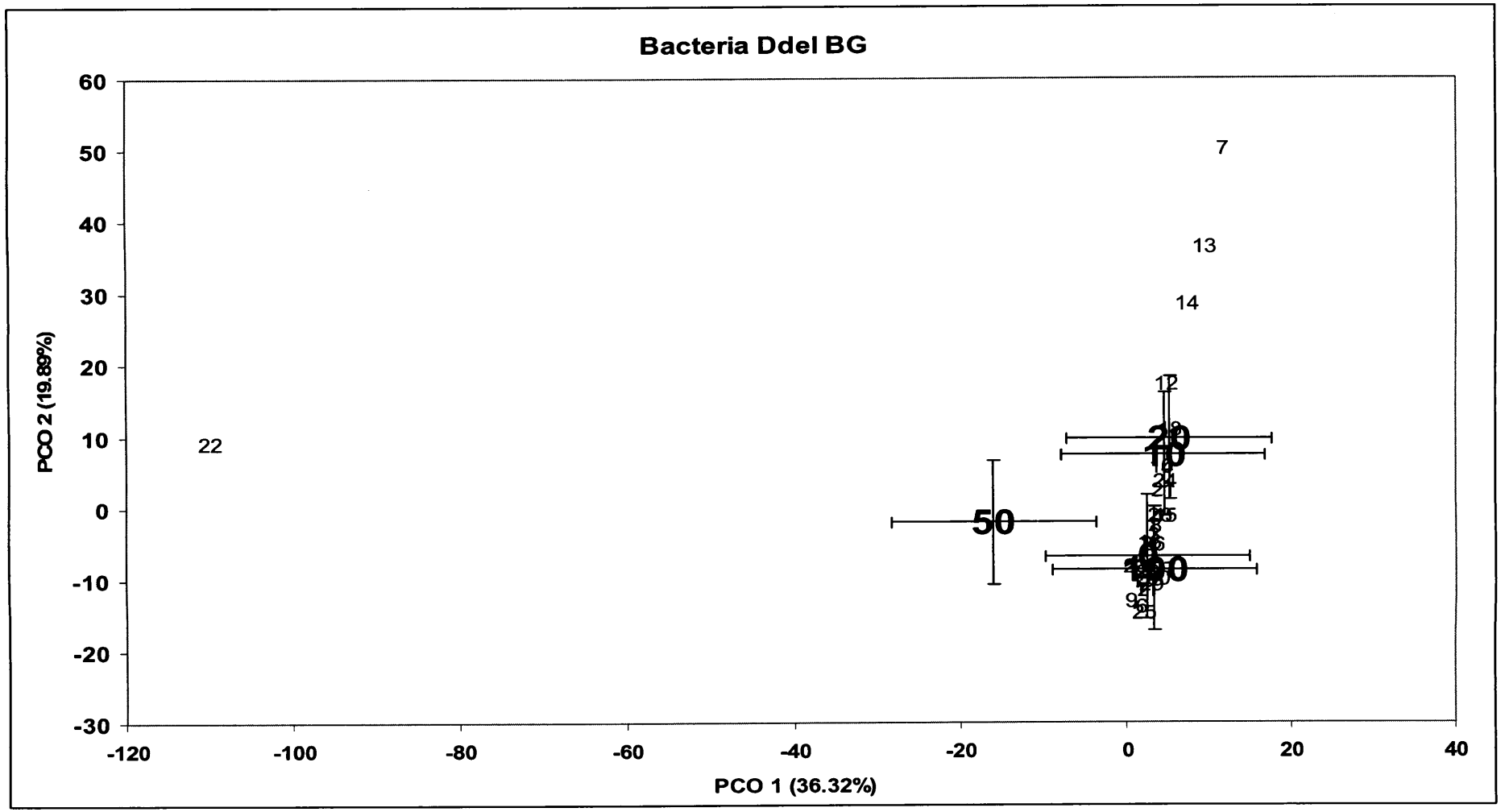


Figure 10.4 Principal co-ordinate scores of sequenced DdeI restriction enzyme digest fragments where small numbers refer to microcosm with compost amendment as follows: 1 – 6, no compost; 7 – 12, 10 t ha^{-1}; 13 – 18, 20 t ha^{-1}; 19 – 24, 50 t ha^{-1}; 25 – 30, 100 t ha^{-1} the larger numbers refer to compost loading in t ha^{-1} Bars are calculated as the standard deviation of individual data points (n = 6).

General legend

1 – 6 = Non-amended soils (controls)

7 – 12 = soils amended with 10 t ha-1 of ADC

13 – 18 = soils amended with 20 t ha^{-1} of ADC

19 – 24 = soils amended with 50 t ha^{-1} of ADC

25 – 30 = soils amended with 100 t ha^{-1} of ADC

0, 10, 20, 50, 100 = ADC amendments in t ha^{-1}

 = Mean soil population distribution for each ADC amendments (ANOVA)

*PCO = principal components (Multivariate analysis)
**1 = dimensions (multivariate analysis)
*** (%) = percentage variation (Multivariate analysis)

BG = Blue and Green dyes (16S rRNA and rDNA)
RY = Red and Yellow dyes (18S rRNA and rDNA)

AluI, DdeI, HincII, TaqI, RsaI, HinfI = Restriction enzymes

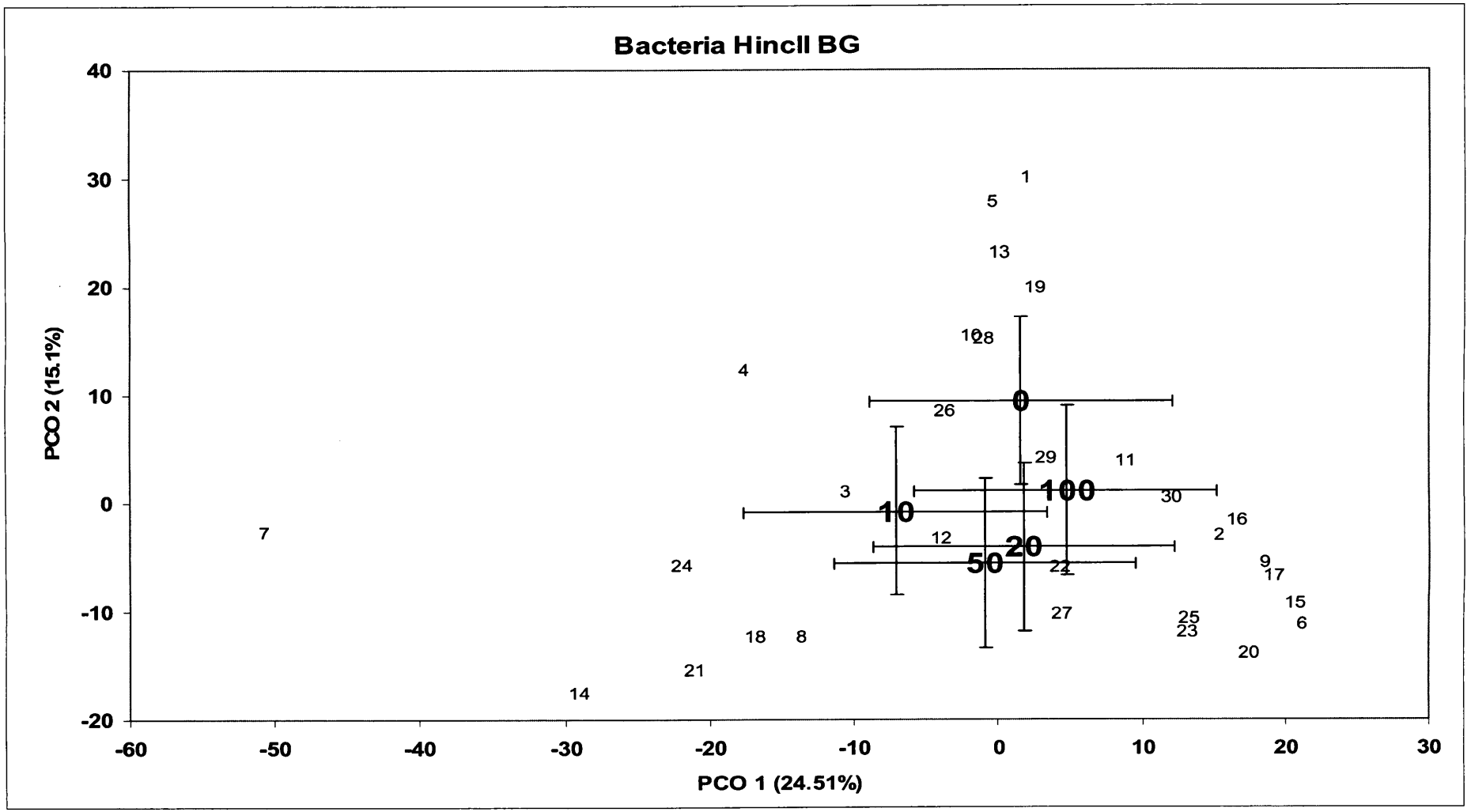


Figure 10.5 Principal co-ordinate scores of sequenced HincII restriction enzyme digest fragments where small numbers refer to microcosm with compost amendment as follows: 1 – 6, no compost; 7 – 12, 10 t ha^{-1}; 13 – 18, 20 t ha^{-1}; 19 – 24, 50 t ha^{-1}; 25 – 30, 100 t ha^{-1} the larger numbers refer to compost loading in t ha^{-1} Bars are calculated as the standard deviation of individual data points (n = 3).

General legend

1 – 6 = Non-amended soils (controls)

7 – 12 = soils amended with 10 t ha-1 of ADC

13 – 18 = soils amended with 20 t ha^{-1} of ADC

19 – 24 = soils amended with 50 t ha^{-1} of ADC

25 – 30 = soils amended with 100 t ha^{-1} of ADC

0, 10, 20, 50, 100 = ADC amendments in t ha^{-1}

 = Mean soil population distribution for each ADC amendments (ANOVA)

*PCO = principal components (Multivariate analysis)
**1 = dimensions (multivariate analysis)
*** (%) = percentage variation (Multivariate analysis)

BG = Blue and Green dyes (16S rRNA and rDNA)
RY = Red and Yellow dyes (18S rRNA and rDNA)

AluI, DdeI, HincII, TaqI, RsaI, HinfI = Restriction enzymes

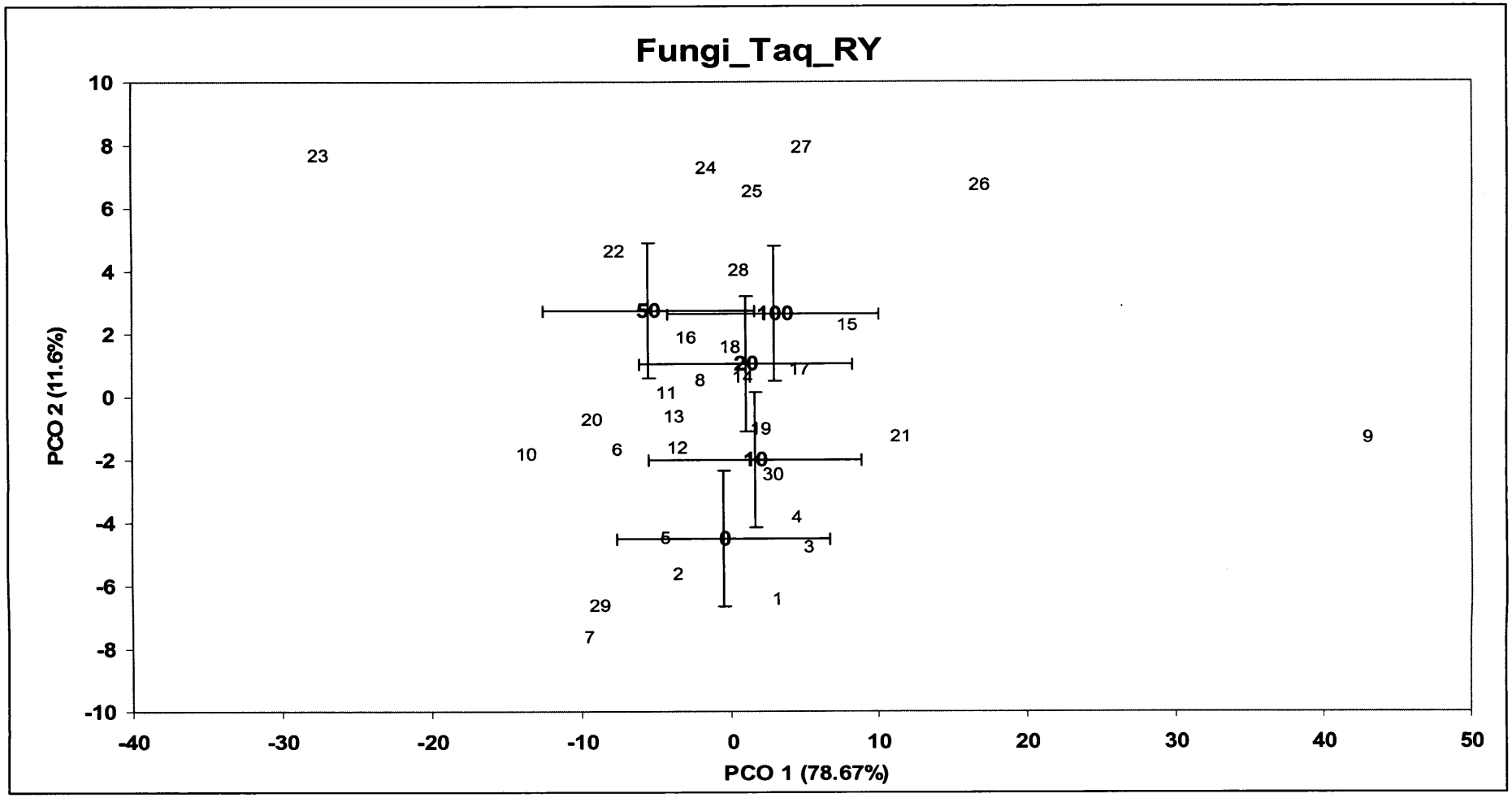


Figure 10.6 Principal co-ordinate scores of sequenced TaqI restriction enzyme digest fragments where small numbers refer to microcosm with compost amendment as follows: 1 – 6, no compost; 7 – 12, 10 t ha^{-1}; 13 – 18, 20 t ha^{-1}; 19 – 24, 50 t ha^{-1}; 25 – 30, 100 t ha^{-1} the larger numbers refer to compost loading in t ha^{-1} Bars are calculated as the standard deviation of individual data points (n = 6).

General legend

1 – 6 = Non-amended soils (controls)

7 – 12 = soils amended with 10 t ha-1 of ADC

13 – 18 = soils amended with 20 t ha^{-1} of ADC

19 – 24 = soils amended with 50 t ha^{-1} of ADC

25 – 30 = soils amended with 100 t ha^{-1} of ADC

0, 10, 20, 50, 100 = ADC amendments in t ha^{-1}

 = Mean soil population distribution for each ADC amendments (ANOVA)

*PCO = principal components (Multivariate analysis)
**1 = dimensions (multivariate analysis)
*** (%) = percentage variation (Multivariate analysis)

BG = Blue and Green dyes (16S rRNA and rDNA)
RY = Red and Yellow dyes (18S rRNA and rDNA)

AluI, DdeI, HincII, TaqI, RsaI, HinfI = Restriction enzymes

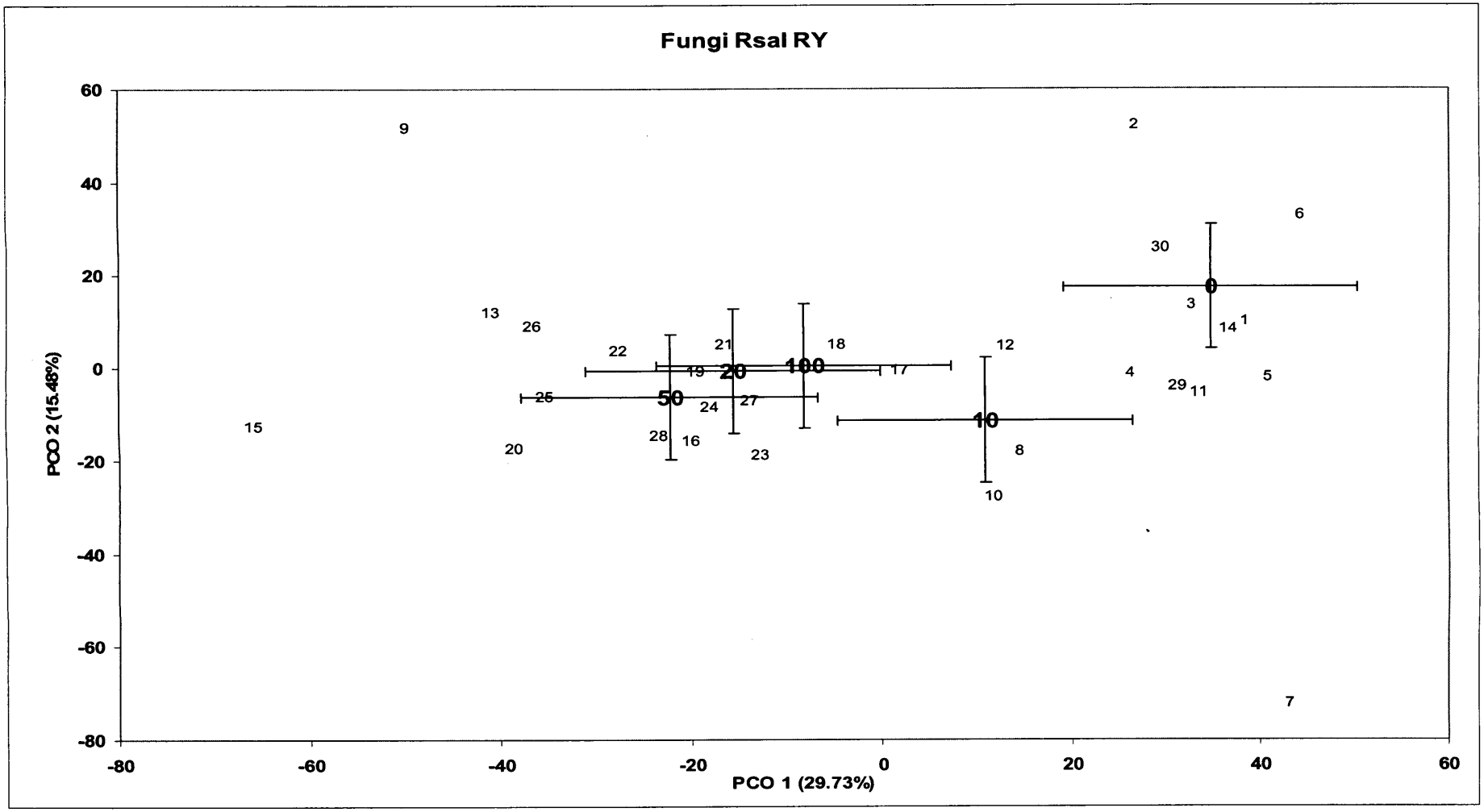


Figure 10.7 Principal co-ordinate scores of sequenced RsaI restriction enzyme digest fragments where small numbers refer to microcosm with compost amendment as follows: 1 – 6, no compost; 7 – 12, 10 t ha^{-1}; 13 – 18, 20 t ha^{-1}; 19 – 24, 50 t ha^{-1}; 25 – 30, 100 t ha^{-1} the larger numbers refer to compost loading in t ha^{-1} Bars are calculated as the standard deviation of individual data points (n = 6).

General legend

1 – 6 = Non-amended soils (controls)

7 – 12 = soils amended with 10 t ha-1 of ADC

13 – 18 = soils amended with 20 t ha^{-1} of ADC

19 – 24 = soils amended with 50 t ha^{-1} of ADC

25 – 30 = soils amended with 100 t ha^{-1} of ADC

0, 10, 20, 50, 100 = ADC amendments in t ha^{-1}

 = Mean soil population distribution for each ADC amendments (ANOVA)

*PCO = principal components (Multivariate analysis)
**1 = dimensions (multivariate analysis)
*** (%) = percentage variation (Multivariate analysis)

BG = Blue and Green dyes (16S rRNA and rDNA)
RY = Red and Yellow dyes (18S rRNA and rDNA)

AluI, DdeI, HincII, TaqI, RsaI, HinfI = Restriction enzymes

10.4 Conclusions

The population studies suggest that anaerobic digestate compost amendment induced significant changes in the diversity of soil microbial communities. The principal coordinate scores of sequenced restriction enzymes digests fragments suggest increased diversity of soil populations with increasing ADC amendments especially in digests fragments fragments with AluI, HinfI and RsaI. These enzymes may be more adequate markers for studies of population dynamics in soil populations than DdeI, HincII and TaqI. The high variations observed in templates digested with HinfI, RsaI and TaqI indicate greater impacts of ADC amendment on fungi than in bacteria, indicating that fungal populations were more strongly affected by ADC amendment than bacteria. The population studies have shown that ADC amendment not only result in increased bioactivity and higher soil microbial biomass but may also bring about significant changes in the population dynamics of soil. The diversity of soil population is critical in ecosystem functioning and in sustainable soil management practices where organic fertilizers such ADC are the principal source of nutrients and for optimum productivity.

Chapter Eleven: General discussion, conclusions and recommendations

11.1 Summary of the work carried out and discussions

This research was carried out to investigate the effects of biological treatments of Organic Fraction of Municipal Solid Wastes (OFMSW) on the production of biogas and the quality of Anaerobic Digestate Compost (ADC). The study investigated the impacts of the application of anaerobic digestate and anaerobic digestate compost (ADC) on the physical, chemical and biological properties of soil.

The study was carried out in five stages. The first stage investigated the pH stability, biogas production and degradability of various proportions of Food Waste (FW) and Green Waste (GW). In the second stage anaerobic digestion of the selected feedstock culture was repeated in large scale to obtain adequate quantities of digestate for aerobic post-treatment for the production of ADC. The third stage investigated the quality of ADC as soil amendment using respirometric, seed germination and plant growth tests and the fourth stage investigated soil enhancing qualities of ADC using physical, chemical and biological soil quality parameters. In the final stage changes in the dynamics of soil populations induced by ADC amendment were examined. The sections below summarized the findings in each stage.

The first stage of the studies investigated the digestibility and stability of OFMSW during anaerobic digestion. Batch cultures containing different waste types comprising Food Waste (FW) and Green Waste (GW) were subjected to high solids thermophilic anaerobic digestion at 55^0C for 15 days. Results showed that pH resilience was greater in cultures

composed mainly of GW. Where FW dominated the feed composition, rapid acidification was observed causing excessive accumulation of volatile fatty acids (VFAs) and hence low pH, thus creating a condition that may inhibit methanogenic activity. In contrast cultures with high GW content showed slow acidification. The variations in pH indicate that whilst FW is readily biodegradable GW is less readily biodegradable. Thus the need for chemical pH correction during anaerobic digestion of OFMSW decreases with increase in the GW proportion of the feedstock. Furthermore, for a relatively low seed sludge to feedstock ratio the need for chemical pH correction during start-up and digestion operation decreases with GW proportion of the waste. A high seed sludge feedstock ratio may be necessary to ensure pH stability without addition of chemical buffer. Results also showed that literature estimates of buffer quantity required for pH buffer may not necessarily achieve the desired results when digesting readily degradable waste. Before full scale application preliminary studies may be necessary to determine the correct dose for a given waste-type combination. The results also suggest that waste composition may be a useful tool for pH regulation in anaerobic digestion processes (Akunna et al., 2007).

It was also observed that the moisture content of the anaerobic digestates decreased with increase in GW content of the raw feedstock. Low digestate moisture content is beneficial for land application as it minimises dewatering costs. The study demonstrated that appropriate selection of raw waste composition can reduce the need for post digestion dewatering which may be necessary for the final disposal of the digestates, thus bringing about a significant reduction in the overall waste management costs.

In general, gas production followed the same pattern as volatile solids reduction, that is decreasing with increasing GW content of the feedstock. The higher the FW content of the feedstock the greater the volume of biogas recovered per organic solids removed.

Having selected a suitable waste mixture for biogas yield, the second stage evaluated factors affecting the aerobic post-treatment of anaerobic digestate. This was carried out by first repeating the digestion of the selected waste type/feedstock to obtain larger quantities of anaerobic digestate. The major objective of the aerobic post-treatment was to further stabilise the anaerobic digestate through reduction of carbon, moisture and odour in a controlled environment.

The final product following aerobic treatment was termed anaerobic digestate compost (ADC). During the aerobic treatment, an initial drop in pH was observed in the first few days followed by a rise. The pH change was attributed to the actions of different groups of microorganisms during the process. pH stability was found to be indicative of compost stabilisation. Both TS and VS increased with aeration time, due probably to one and/or a combination of precipitation out of solution, evaporation and microbial growth. It was also observed that C: N ratio decreased with increasing aerobic treatment, due probably to organic compound degradation. At the end of aerobic post-treatment the anaerobic digestate compost obtained had a total solid contents of 45%, pH of 7.8 and C:N ratio of 12:7, all within values recommended for good composts. C:N ratio and TS/VS were found to be less variable at compost stability, thus both, including pH (which is easier to measure) can be used as indicators to assess compost maturity and stability during aerobic post-treatment of anaerobic digestate.

The third stage of the studies examines the quality of anaerobic digestate and ADC using respirometric, seed and plant growth tests. The respirometric test showed that anaerobic digestate in its original form contains significant amounts of readily degradable organic matter. This organic matter is susceptible to aerobic decomposition during aeration. Additionally, its relatively high moisture content can create pockets of anaerobic environments in the soil-digestate mixture leading to termination of aerobic bioactivity in the soil for certain period of time.

Seed germination tests were carried out using fresh raw waste and digestates collected during anaerobic digestion and at various stages of aerobic post-treatment to investigate their effects on seed germination. Undiluted extracts of fresh raw waste and anaerobic digestates showed no seed germination and germination index increased with increasing rates of dilution of the extracts. Both fresh raw waste and anaerobic digestates were found to be phytotoxic. It was also found that phytotoxicity decreased with increase in biological stability (reduction in organic biodegradable matter, through in this case aeration). Thus both fresh raw waste and their anaerobic digestates are not sufficiently stable or suitable for direct soil application; aerobic treatment can render these suitable for soil amendment.

Tests with non-amended and ADC amended soils showed higher seed germination indexes in ADC amended soils than in non-amended controls. In general seed germination increased with dilution and increasing incubation times. The relatively high seed germination indexes observed in ADC amended soils is an indication of the suitability of ADC as an organic amendment in arable soils.

The pot trials revealed that ADC and the commercial compost have similar impacts on tomato plant growth. However, plants grown on soils amended with each of the two types of compost were found to be significantly superior in terms of growth characteristics to those grown on non-amended soils. Thus, increased plant productivity can be achieved with ADC amendment. Although the pot trials showed that ADC is suitable for long-term carbon supply for plant growth, its capacity for long term nitrogen supply is limited due to potential losses through denitrification.

In the fourth stage the study investigated the soil enhancing qualities of ADC using physical, chemical and biological soil quality parameters. Water content decreases with increasing water potential and increases with increasing proportions of ADC amendments but decreases with increasing length of incubation. The decrease in water content with increasing incubation period was attributed to diminishing soil organic matter as ADC decomposed with time. Thus water retention is directly proportional to the organic carbon content.

The water infiltration tests among the non-incubated soil samples showed increased sorptivity only in soils amended with 10 t ha^{-1} of ADC where the sorptivity increased by more than 30% due to addition of ADC. The remaining soil samples showed decreased sorptivity with increasing ADC amendments. As increased sorptivity translate into higher hydraulic conductivity and greater water potential, the results suggest that mild application of ADC may be beneficial to water infiltration properties of soil. High loading rates of ADC may require longer incubation times to produce significant increase in sorptivity.

The soil respiration and bioactivity tests showed increasing bioactivity with increased ADC amendments. In all ADC amended soils the increase in CO_2 flushes was rapid in the early period of the test and slowed down afterwards due seemingly to reduced ventilation and depletion of soil nitrogen. The increased bioactivity observed in ADC amended soils was attributed to increased metabolic activities caused by the decomposition of ADC. Both soil microbial biomass and bioactivity increased with ADC amendments. Population diversity was investigated using Templete-restriction fragment length polymorphism (T-RFLP). The analysis revealed significant changes in the diversity of soil populations with increasing ADC amendments with greater impacts on fungi than in bacteria. The impact on fungi is believed to be due to greater functional diversity and better adaptation to successional changes.

In general, the study has shown that anaerobic digestate, though unsuitable for soil application, is rich in nutrients and enhanced biodiversity which could be useful in remediation of Brownfield sites or as a top soil mix in landfills. The ADC being from OFMSW could also be of use in aquaculture as feed for fish. Being rich in stable organic matter, ADC could be beneficial as mulch for control of erosion in top soils exposed to high storm water flow or windstorms.

Figure 11.1 summarises the principal outcomes of this study

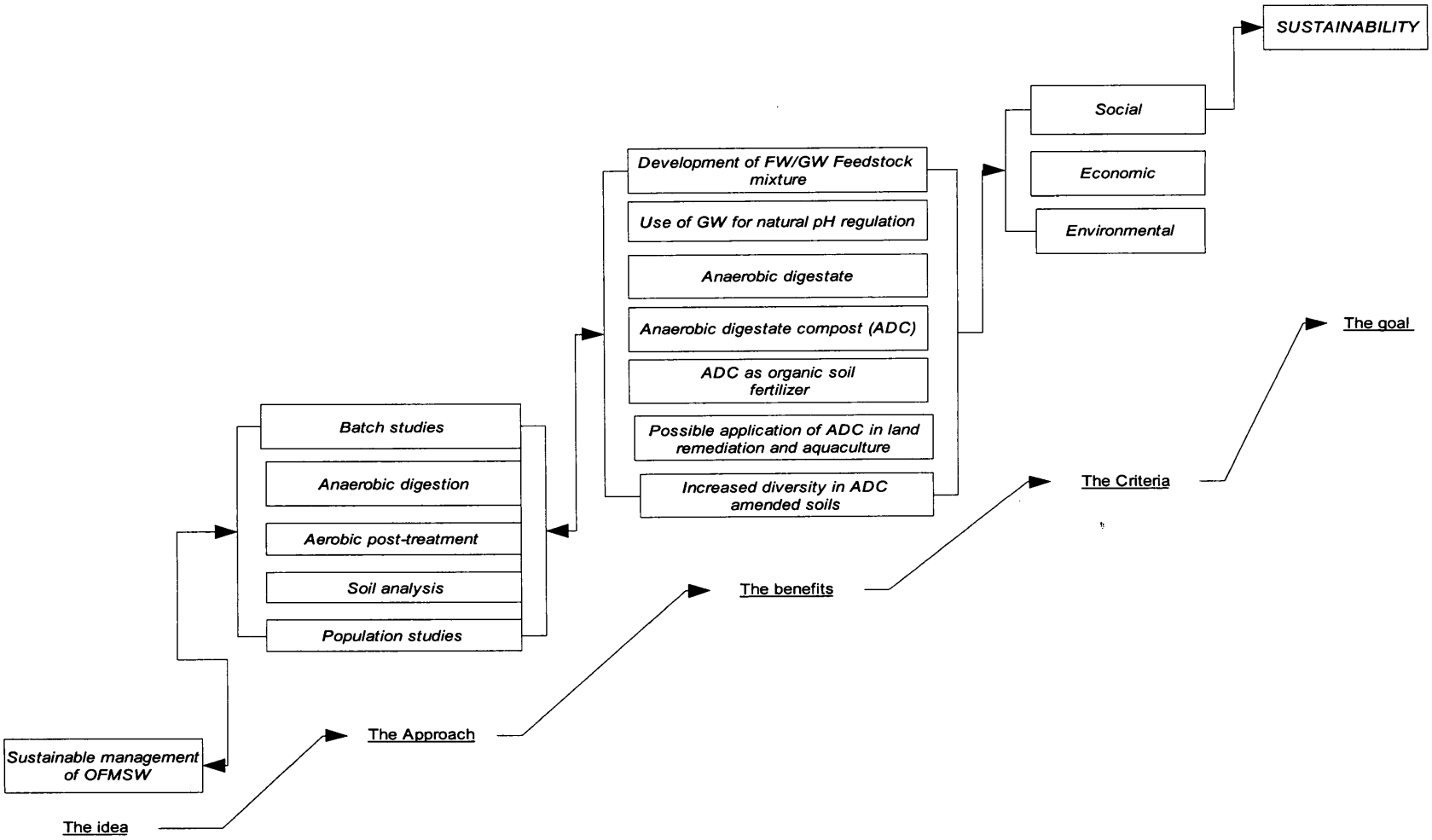


Figure 11.1 Summary of outcomes

11.2 Conclusions

Waste composition has been shown as being critical in digestibility and process stability during anaerobic digestion of OFMSW. The study also revealed that readily degradable substrates such as FW have greater impact on biogas production but could lead to process failure from rapid acidification of the system if not properly managed. In contrast GW has greater impact on pH stability and digestate quality, creating conditions that permit biogas recovery with minimum risk of rapid acidification and producing digestate with relatively little amount of wastewater. Combining FW, GW and seed sludge in various proportions can affect biogas production, digester performance and digestate quality. The proportion of GW in feedstock can thus be used for pH regulation, thus minimizing the need for pH control chemicals. The low moisture content of the digestate for GW enriched feedstock enhances aerobic post-treatment. The study has shown that ADC can be beneficial for plant growth and development and has positive impacts on microbial diversity and population development in the soil with higher variations in fungi than in bacterial populations. As increased bioactivity and soil biomass increase with increasing soil fertility, these findings indicate the suitability of ADC for soil application. It was also found that low loading rates of ADC may be more beneficial for agricultural application with higher loading rates for non-agricultural soil management practices.

11.3 Recommendations

Having addressed the key issues involved in sustainable management of OFMSW using bench-scale experiments, to corroborate the outcomes of the study, further field

research is required to investigate the long-term effects of ADC application on soil functioning.

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List of Appendices

1. Nutrients additions

2. Formulation of Nitrogen Feed given to plants

3. Protocol for large-scale DNA/RNA extraction

4. Refereed journal articles

Appendix I

Nutrient and trace elements

Stock solution was added at the rate of 10% to every batch

Stock solution	*g/l*
NH_4Cl	2.8g
K_2HPO_4	2.5g
$MgSO_4.7H_2O$	1.0g
$CaCl_2.2H_2O$	0.1g
Yeast extracts	1.0g

Plus 10ml of trace element solution

Trace element solution	*g/l*
$FeCl_2.4H_2O$	2.0g
H_3BO_3	0.03g
ZnCl	0.05g
$CuCl.2H_2O$	0.038g
$MnCl_2.4H_2O$	0.5g
$(NH_4)6Mo7O_2.4H_2O$	0.05g
$AlCl_3.6H_2O$	0.09g
$CoCl_2.6H_2O$	2.0g

(Sanders et al, 2003)

Appendix II

Formulation of Nitrogen Feed given to plants

Product Name: VITAX VITA FEED 111

Address: VITAX LIMITED COALVILLE, LEACESTER, LE67 3DE

NPK 1: 1: 1 (19: 19: 19) WITH TRACE ELEMENTS

• TOTAL NITROGEN	19%
OF WHICH:	
NTRIC NITROGEN	5.5%
AMMONICAL NITROGEN	3.8%
UREIC NITRGEN	9.7%
• PHOSPHORUS PENTOXIDE (P_2O_5)	
SOLUBLE IN NEUTRAL AMMONIUM CITRATE	19% (8.3% P)
AND IN WATEROF WHICH SOLUBLE IN WATER	19% (8.3% P)
• POTASIUM OXIDE (K2O) SOLUBLE IN WATER	19% (15.8% K)
• BORON (B) SOLUTION IN WATER	0.013%

• COPPER (Cu) SOLUTION IN WATER	0.025%
OF WHICH CHELTED BY EDTA	0.24%
• IRON (Fe) SOLUTION IN WATER	0.050%
OF WHICH CHELATED BY EDTA	0.048%
• MANGANESE (Mn) SOLUTION IN WATER	0.025%
OF WHICH CHELATED BY EDTA	0.024%

Source: Product Bag (SCRI, 2006)

Appendix III

Protocol for large-scale DNA/RNA extraction

1. weight 1 g of soil into falcon tube
2. add 1 mm DEPC treated glass beads into each tube using black tray
3. add DNA and RNA spike to 0.12M Na_2HPO_4 1% SDS buffer solution
4. Add 2 ml of 0.12M Na_2HPO_4 1% SDS solution to falcon tube
5. Add 1 ml of soil slurry slowly to the tubes using cut blue tips
6. Place in the bead beater at 30 speeds for 1 min 30 sec – do this 3 times. NB put on ice in between and turn round the boxes – see end of protocol for instructions in how to use the bead beater.
7. Spin at 5K rpm for 5 min. NB once start step 9 do not stop until finished step 18
8. Add approx 450 µl (an equal volume)of phenol/chloroform: IAA (ratio 25:24:1)to clean set of tubes
9. Pour the SNT into the Phenol/Chloroform: IAA
10. Gently mix by turning boxes upside down a couple of times
11. Spin 5K rpm for 5 min
12. Add approx 450 µl (an equal volume)of chloroform: IAA (ratio 24:1)to clean tubes
13. Transfer aqueous phase to chloroform: IAA using multi – channel set to lowest speed. Turn boxes upside down a couple of times to gently mix
14. Spin 5K rpm for 1 min
15. Add 400 µl of Isopropanal (equal volume) and final volume of 0.3 M Na Acetate (40 µl of 3 M stock)to clean tubes
16. Transfer aqueous phase to Isopropanal/ Na Acetate with multi – channel set to lowest speed

17. Put in the -20^{0}C freezer for 20 mins (can be left overnight)
18. Spin 5K for 5 min to pellet the DNA
19. Discard the SNT and turn upside down on tissue
20. Wash using ice cold 70% ethanol
21. Spin 5K for 5 min
22. Empty out ethanol and turn upside down on tissue to allow traces of ethanol to evaporate
23. Resuspend pellet in 50 µl TE or water

Clean up phase

Fill the filter plate 3 times with PVPP using the black plate

Add 200 µl of water to each well using multi – channel pipette vacuum

Add 100 µl of water to each well using multi – channel pipette vacuum

Carefully pour the DNA into the plate vacuum

Approx 50 µl in collection plate

Can split this into another collection plate so not to defrost lots of times

Information

SDS breaks open the cells walls to release the cell contents

Phenol is a good solvent of proteins but can also dissolve small quantities of DNA

Therefore use phenol/chloroform: IAA as DNA does not dissolve in this organic mix.

Chloroform: IAA then removes any traces of phenol

The PVPP removes traces of the phenolics also.

The DNA then precipitated using ethanol

Items required for 192 tubes

8 x blue boxes with 96 tubes

192 falcon tubes labelled

4 boxes Matrix tips autoclaved

2/3 boxes blue tips with ends cut off

2 RNA free breakers

400 ml of 0.12M Na_2HPO_4 1% SDS solution

Approx 90 ml phenol/chloroform: IAA (25:24:1)

Approx 90 ml phenol/chloroform: IAA (24:1)

Approx 80 ml Isopropanal

Approx 8 ml 3M sodium acetate

2 ml of 1 x 10^7 copies of both spikes in 400 ml buffer (changed from 1 x 10^6 copies/µl)

Clean up-

- PVPP
- Millipore filter (catalogue no MSHVN4550, Promega, 2006)and collection plate
- Sterile water
- Vacuum pump

Using the bead beater

Remove plastic lid from 96 welled boxes

Set the box into the white tray as the base and the black piece as the lid making sure fitting properly

NB the raised buttons need to be mirrored on each side of the box

Place the box into the bead beater with the raised buttons nearest the machine – the raised button should fit into the

Tighten the outer nut until hand tight

Use the tork wrench until it clicks

Hand tighten the inner long nut

Repeat with the other box

Set for time and speed

Once beating finished

Loosen the long inner nut first

Loosen the outer nut using hand only - do not use the wrench

Remove box

Turn box around so the tubes that were nearest the machine are now on the outside

400 ml of 0.12M Na_2HPO_4 1% SDS solution

If FW Na_2HPO_4 = 142 g

Need 6.816g Na_2HPO_4

4g SDS

Up to 400 ml DEPC treated water then autoclave

The content of pages 214- 226 have been removed due to copyright restrictions. The content comprised 2 published journal articles and the citations to these are given below.

Abdullahi, Y.A., Akunna, J.C., White, N.A., Hallett, P.D. and Wheatley, R. (2008) 'Investigating the effects of anaerobic and aerobic post-treatment on quality and stability of organic fraction of municipal solid waste as soil amendment', *Bioresource Technology,* 99(18), pp. 8631-8636. doi: 10.1016/j.biortech.2008.04.027

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