Comparison of techniques to assess compost maturity and stability

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Declaration

By submitting this thesis, I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any other university for obtaining a degree.

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N.W. Cheung

Date: 19 November 2015

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Abstract

A number of physiochemical and microbiological stability parameters of compost were compared and their suitability in monitoring the composting process were evaluated. Two composting piles were constructed as open-windrow systems under local weather condition. A farm manure waste windrow (MA) was made from fresh feed-pad dairy manure and straw, spoiled hay, wood chips and poultry litter, while a green waste windrow (GW) consisted of municipally collected green waste. The changes observed in selected parameters, included the contents of C, N, organic matter (OM) and water soluble C (WSC), ^{13}C NMR spectroscopy, thermogravimetric analysis, microbial respiration, FDA hydrolysis activity, microbial biomass and nitrate and ammonium content, were investigated in samples collected from the four different phases of composting. The results showed OM transformations occurring during the composting process of both GW and MA windrows can be revealed from ^{13}C NMR spectroscopy and thermogravimetric analysis. In addition, this study again confirmed that WSC can act as a reliable measurement of the stability of compost regardless of the type of initial materials. The significant loss of OM (33.8% in MA windrow and 25.0% in GW windrow) and low values of WSC (2.46 and 2.02 mg per g of dried sample in MA and GW compost respectively after 175 days of composting) from both windrows were in agreement with the molecular, microbiological parameters and thermal analysis and confirmed that the end products from both windrows were fully stabilized. In light of the results obtained from the biochemical parameters, the reliability of using chemical strip tests for monitoring stability of compost on farm were evaluated by correlating the method results with standard chemical tests and validated by comparing with other stability parameters. The content of NO_3^--N and NH_3-N were estimated by the proposed Strip Test Methods and the methods were found to be strongly correlated with the standard chemical analysis (r= 0.967 & 0.977 for NO_3^--N and NH_3-N

respectively, $p<0.01$). In addition, an NH_3 strip test was found to have high r^2 value with WSC in both the GW (0.97, $p<0.01$) and MA windrows (0.93, $p<0.01$) indicating that both WSC and NH_3 strip test can be used as maturity indicators regardless of the substrate mixtures used in this study.

List of Figures

List of Tables

Table of Contents

CHAPTER THREE - EVOLUTION OF MICROBIOLOGICAL PARAMETERS DURING COMPOSTING OF GREENWASTE AND MANURE WASTE

CHAPTER FOUR – EVALUATING THE STABILITY OF COMPOST BY MEASURING THE INORGANIC NITROGEN CONTENT IN THE WATER EXTRACTION AND BY SIMPLE STRIP TESTS

CHAPTER ONE – LITERATURE REVIEW

1. Introduction

As the human population continues to grow, the demands for food and natural resources will only increase. However, at the same time, the reserves of natural resources such as quality soil which is essential for sustaining food production is being degraded by human activities such as excessive usage of chemicals in agriculture.

Composting, a traditional process to maintain soil fertility and crop yield, can provide an alternative way to utilize biomass waste, particularly plant materials and minimize environmental impacts caused by human activities (Pimentel and Wilson 2004; Pinero 2009). According to the U.S. Environmental Protection Agency (2014), compost manufactured from organic wastes can reduce the amount of waste sent to landfill, act as an alternative to chemical fertilizer, enhance soil fertility and prevent soil erosion. Composting can also be considered as a way to mitigate climate change. During aerobic decomposition in composting, C is more likely to be stabilized as humus-like substances for long term storage in soil. In addition an adequate composting allows the organic wastes to be decomposed in an aerobic condition and thus reduces the production of methane, which has a higher impact on greenhouse effect than CO_2, from anaerobic condition in landfill. (Zeman et al. 2002; Kreiling 2011).

Composting can also be considered as a controlled biodegradation process of a mixture of organic substrates that aims to produce a biological stabilized and partially humified organic matter (OM) that is beneficial to plant growth (Insam and Bertoldi 2007; Bernal et

al. 2009). Insam and Bertoldi (2007) outlined three reasons for transforming OM into compost: (1) to overcome the phytotoxicity of fresh non-stabilized organic matter; (2) to reduce the health risk constituted by remaining pathogens originating from the feedstock to animal and plants; and (3) to enhance soil properties by increasing the amount and variation of organic constituents including living microbial biomass. Several authors have reviewed the soil improvement achieved by the application of various types of compost (Hubbe et al. 2010; Richard 2004; Hargreaves et al. 2008). Application of compost can enhance physical, chemical and biological properties in soil. Compost affects the physical properties of soil by lowering the bulk density, increasing the porosity and permeability, enhancing water holding capacity and aggregate stability of the applied soil (Aggelides and Londra 2000; Celik et al. 2004). Richard (2004) also listed some physical and biochemical impacts on soil after compost application (Table 1.1). The same author suggested that compost can act as a C and N source in soil for plant and soil organisms, it further enhances soil fertility through the increase of N mineralization from increasing degradation of the existing soil OM by the promotion of microbial activity after application. This does not only provide an energy source to microbial activity but also increases the activity of specific enzymes and overall diversity of soil microflora. In addition some studies have shown that compost can be used in the remediation of metal pollution due to its binding and chelation capabilities (van Herwijnen et al. 2007; Cao et al. 2003; Gadepalle et al. 2008). Therefore the primary goals of composting are to enhance the properties of soil and to reduce the health risk constituted by the pathogen remained in the feedstock (Hubbe et al. 2010).

Table 1.1 : Result of Compost-Soil Interaction

SOURCE: *ADAPTED FROM RICHARD (2004)*

	Typical change
Physical	
Infiltration	Increases
Erosion	Decreases
Aggregate stability	Increases
Water-holding capacity	Increases
Total porosity	Increases
Permeability	Increases
Bulk density	Decreases
Chemical	
pH	Buffers near neutral
Cation exchange capacity	Increases
Electrical conductivity	Increases
Nutrient concentration	Increases
Nutrient availability	Varies
Trace elements and metals	Varies
Biological	
Microbial activity	Increases
Microbial biomass	Increases
Microbial diversity	Increases
Enzymatic activity	Increases
Phytotoxicity	Varies
Phytostimulation	Increases
Plant disease suppression	Varies

Even though numerous maturity and stability parameters have been proposed to monitor the quality of composting progress, most of the parameters used are not suitable in monitoring compost maturity and stability on-site especially for on-farm composting. In this review, the degree of compost maturity and stability indicated by the physical and biochemical characteristics during composting are discussed and the proposed characteristics from the parameters to indicate compost maturity and stability are listed. The limitations on routine assessments are also evaluated for the methods. The goal of this review is to better understand the role of measuring various maturity and stability parameters in monitoring composting on-site.

2. A Brief Description of the Composting Process

Although there is a large diversity of feedstocks (such as manure, greenwaste and municipal waste) and management practices (static pile, turned windrow, passive aerated windrow, forced aerated pile and enclosed composting) being employed in composting, it is generally accepted that an appropriate C/N ratio, adequate moisture and sufficient aeration are critical to allow proper development of the microbial population and maintain the degradation process during composting (De Bertoldi 1992; Richard 2004; Cooperband 2002). It is recommended that the C/N ratio of fresh feedstock mixture should range between 20 and 40 and the moisture should be maintained at about 50 to 60% during active composting in order to generate a stabilized product (Richard 2004; Cooperband 2002; Bernal et al. 2009). Four main stages occur during successful composting and these are: Mesophilic phase, Thermophilic phase, Cooling phase and the Maturation phase. These phases are distinguished from each other by the temperature and the microbial profile of the particular period during composting (Chen and Inbar 1993; Cooperband 2002). Since composting aims to manufacture a biologically stabilized and partially humified product, the phases also indicate the degree of the maturation of the compost. For example, substrate taken out from the thermophilic phase is considered as unstable and immature.

The Mesophilic phase is characterized as a gradual increase of temperature (between 25-40^{o}C / 77 -104^{o}F) during the first few days of composting. A feedstock which contains sufficient easily degradable compounds, like sugars and proteins is important to initiate the first stage of composting. These nutrients are first degraded by existing bacteria and fungi

in the pile and the increase in temperature indicates the steady increase of microbial respiratory activity in the first few days (Chen and Inbar 1993; Cooperband 2002).

As the decomposition continues, the mesophilic phase is followed by the thermophilic phase when there is a rapid rise in temperature to about 65^{o}C (150^{o}F) or higher (Richard 2004; Cooperband 2002). A sharp rise in temperature indicates the initial microbial population is replaced by another group – thermophilic organisms, which are adapted to the higher temperature of the composting process. As the thermophilic microbial community continues to grow and degrade the remaining readily degradable substrates, the activities of the thermophiles will continue to increase until the temperature of the pile reaches 70^{o}C (158^{o}F) when some of their activities are inhibited beyond this temperature (Insam and Bertoldi 2007; Diaz and Savage 2007). The great amount of heat produced as a result of the increased rate of respiration is responsible for destroying most of the pathogens, weed seeds and insect larvae (Richard 2004). In addition, antibiotics produced by actinobacteria during the thermophilic phase also play a role in the sanitization of the substrate (Bernal et al. 2009). However it is necessary to provide sufficient oxygen to support the microbial growth and the rate of microbial activity by either passive or mechanical aeration during the thermopilic phase. Both porosity and moisture of the pile are important factors for allowing sufficient oxygen to maintain microbial activity. Low porosity and excess moisture of the pile hinder air distribution among the pile and these may cause anaerobic respiration and generate odorous gas (Insam and Bertoldi 2007; Diaz and Savage 2007; Bernal et al. 2009).

After the thermophilic phase, the temperature of the composting pile begins to fall and the pile enters the cooling phase. During the cooling phase, there is a shortage of readily degradable substrates due to the extended degradation occurred in the thermophilic phase. Therefore a new group of mesophilic organisms starts to reproduce and replace the previous thermophilic community. This new group of mesophilic organisms is considered to be better adapted to the conditions since they are able to utilize the remaining starch, cellulose and hemicellulose in the composting pile. The cooling phase could last from a few weeks to several months and the temperature continues to decrease until it reaches ambient temperature. The composition of the microbial population keeps altering in the maturation phase while the quality of substrate keeps declining (Insam and Bertoldi 2007; Diaz and Savage 2007; Bernal et al. 2009). As humification of ligno-cellulose substrate continues during the long period of curing, polymerised organic compounds which are highly stable and much less degradable are formed. Finally, a matured compost containing certain humic characteristics is produced (Diaz and Savage 2007; Bernal et al. 2009; Chen and Inbar 1993). These phases are essential to the management and monitoring of compost during composting. It is important to ensure all stages occur so the end product is fully composted and stabilized (Figure 1.1).

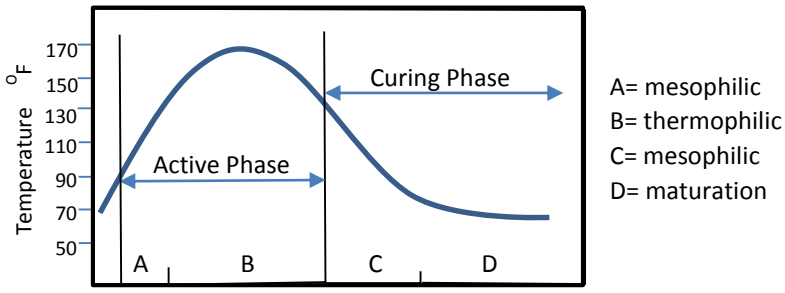


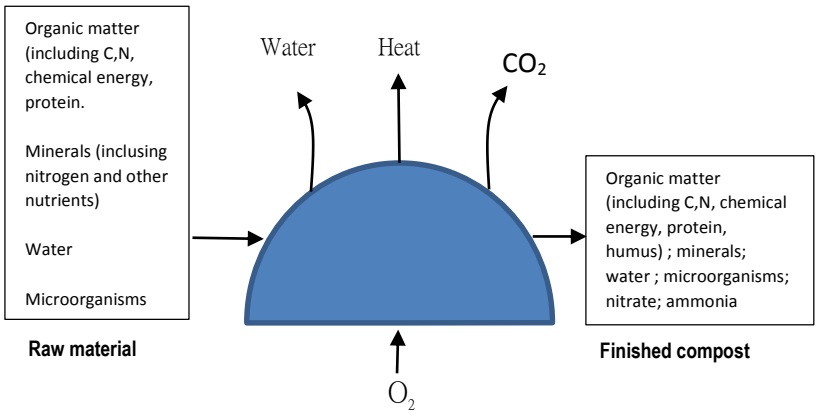
Figure 1.1: Temperature changes in an average compost pile
SOURCE: *ADAPTED FROM RICHARD (2004)*

3. Using C and N Dynamics to Examine Composting Phases and Maturity

Although temperature and microbial profile are used to characterize different phases during composting, C and N dynamics during composting provide an alternative way to determine these composting phases. During the early stage of composting easily degradable OM is first decomposed by microorganisms. An adequate supply of O_2 and water is essential to sustain the microbial activity at this biooxidative stage (Bernal et al. 2009; Pare et al. 1998). If the O_2 supply is sufficient, the mineralized C will be evolved as CO_2 and a decrease of C content is observed in the composting pile. This decomposition continues at high level in the thermophilic phase. Higher temperature conditions also encourage more decomposition and therefore C loss is found to be prominent during the thermophilic phase (Richard 2004; Rynk 1992). As composting continues, a gradual decrease of biodegradable nutrients and an increase of recalcitrant matter are found in the composting substrate, hence the rate of decomposition as well as the loss of C are reduced concurrently (Senesi 1989). Finally a gradual decomposition of highly recalcitrant materials such as lignin and formation of more stable humic substances, are found during the long curing period. The loss of C becomes minimal at this final stage due to the high resistance of the matured product to degradation (Senesi 1989).

During the OM decomposition, C content decreases through mineralization as CO_2 while N loss occurs via NH_3 volatilization, leaching and denitrification (Bernal et al. 2009; Tiquia and Tam 2000). When the OM is broken down by microbial organisms, NH_4^+ is formed through ammonification/mineralization of N in the organic substrates. As the microbial activity increases in the thermophilic phase, a high concentration of NH_4^+ ion is often expected in this stage. However the NH_4^+ formed in the thermopilic phase is readily

converted into NH_3 if the pH of the pile is sufficiently alkaline (pH >8.5) and the growth of nitrifying bacteria is inhibited under higher temperatures. This results in NH_3-volatilisation and contributes to N loss in the composting pile (Hubble et al. 2010; Barrington et al. 2002). When the temperature begins to decrease in the cooling phase, NH_4^+ can be converted to NO_3^- through nitrification, if sufficient O_2 is provided. Therefore, a gradual increase of NO_3^- is generally found in a composting pile during the curing period especially when the composting pile is made of large particles and the air flow is sufficient (Richard 2004; Castaldi et al. 2008). Pare (1998) observed a higher increase of NO_3^- than the decrease of NH_4^+ and so indicated that additional NO_3^- could be generated from other forms of N and the higher ratio of NO_3^- to NH_4^+ of the pile was usually found in the maturation phase. On the contrary, NO_3^- loss can occur through denitrification if there is not enough O_2 supply to the pile since the microorganisms are forced to utilize some weaker electron acceptors such as NO_3^- and SO_4^{2-} in anaerobic conditions (Senesi 1989). Significant loss of NO_3^- through leaching may also occur when composting is carried out outdoor and is exposed to rainfall. Due to the high solubility nature of NO_3^-, major loss of NO_3^- can be seen in coarse-textured permeable compost materials where water can percolate freely (Barrington et al. 2002; Richard 2004). Finally, an increase of NO_3^- content accompanied by the formation of stable N compounds by microbial organisms in the curing stage allows mature compost to act as a better N source for soil (Pare et al. 1998).



The C,N, chemical energy, protein and water in the finished compost is less than that in the raw materials. The finish compost has more humus. The volume of the finished compost is 50% or less of the volume of raw material.

Figure 1.2: The inputs and outputs of common composting pile
SOURCE: *ADAPTED FROM RYNK (1992)*

4. Current Compost Maturity and Stability Parameters

The terms "stability" and "maturity" are both widely used to describe the quality of compost products. However, these two terms can be distinguished by the processes of their determination. Compost "stability" is usually defined as the degree of bioavailability of OM in the compost. As composting proceeds, the composting substrate becomes harder to degrade by the microorganisms and the bioavailability of OM of the compost can be measured by either its biological activity or the chemical properties. The compost is classified as "stable" when it reaches a relatively low rate of microbial activity accompanied by an increased amount of humified OM (Changa et al. 2003; Wu et al. 2000; Spaccini and Piccolo 2007). Therefore, compost "stability" is a measurement of biochemical properties of the composting substrate. In order to determine the compost "maturity", the beneficial effects of compost on plant growth and its phytotoxicity are often the focuses. The "maturity" is often measured by germination index and plant assays (Aslam and Vander Gheynst et al. 2008). Therefore, "maturity" is a measurement of

beneficial biological response of a compost product. Since a high level of humification normally happens after a long period of curing and the humic characteristics of the cured compost can benefit plant growth, compost with high level of humification is often considered as matured (Mondini et al. 2003; Chefetz et al. 1996). This also explains why the terms "stability" and "maturity" are often used interchangeably to describe the quality of compost.

Even though many methods and parameters for assessing compost stability have been developed in the past, a universal assessment method for measuring the suitability of a compost amendment is still needed. This is because of the large diversity of substrate sources, processing conditions and biological activity among composts (Chen and Inbar 1993; Komilis et al. 2011). Many of the parameters also involve time consuming or costly chemical and biological measurements. In addition, in order to develop an effective management strategy to monitor the large diversity of composting piles, it is important to have a comprehensive understanding of the transformation of the physical and biochemical properties of compost using a wide range of analytical methods to closely evaluate the processes.

4.1 Traditional parameters

Some traditional indicators including temperature, odour, colour, pH and electrical conductivity (EC) have been used for determining the stages of a composing process (Bernal et al. 2009). During the initial mesophilic phase which usually has a higher moisture content and under temporary anaerobic condition, the pH of a composting pile often falls due to the production of organic acids. After a slight drop of pH, the pH of the

pile would gradually become alkaline as ammonia is liberated from the degradation of protein. Thereafter the pH value would decrease steadily to near-neutral (slightly higher than 7) during the maturation phase (Figure 1.3) (Paradelo et al. 2010; Hubbe et al. 2010). As the composting process continues, the size of the pile is reduced and more organic nitrogen is mineralized. Soluble salt content estimated by measuring the electric conductivity (EC) in aqueous extracts generally increases because of the accumulation of mineral ions. (Liu et al. 2011).

The colour of the compost, which is found to be well correlated to the amount of soluble organic C in water extracts of compost substrate, can be observed by eye or measured using a spectrophotometer across a range of wavelengths between 360nm to 750nm, and gradual darkening of the substrate and higher absorption is expected in matured compost (Chen and Inbar 1993; Iglesias Jiménez and Perez Garcia 1989; Paradelo et al. 2010). These parameters are considered to be the most direct and practical methods for assessing compost maturity in particular for composting processes handled by the general public such as farmers and gardeners. However, it is important to note that most of these indicators can only give rough information towards assessing compost maturity and stability. In addition, not all composting piles follow the same pattern as described above, due to the diversity of feedstock. For instance, feedstock with low nitrogen content may remain neutral (pH 7) throughout the composting process. The colour of the substrate is highly dependent on the type of feedstock used as some feedstock are darker than others and the EC is also highly varied among different composts. Finally, there is a need for more accurate monitoring parameters to ensure the quality of compost from large scale composting.

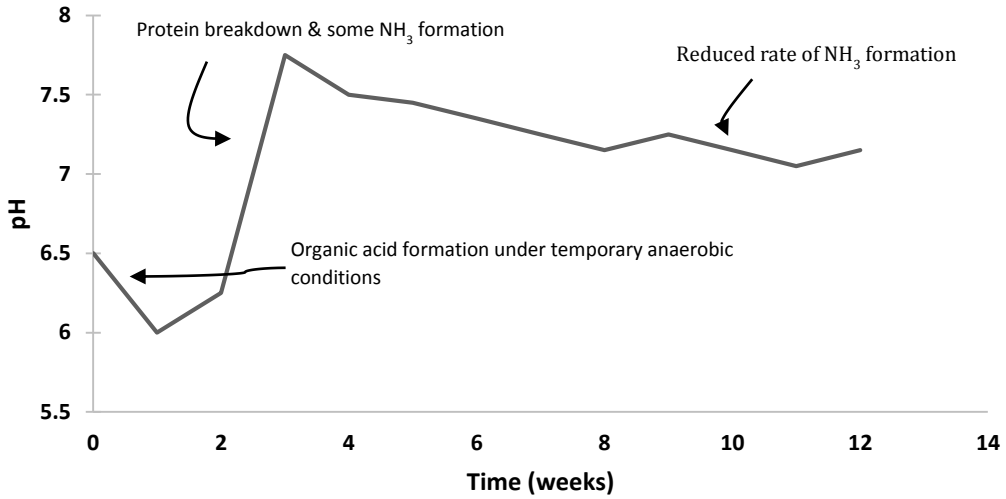


Figure 1.3: Variation of pH with time during a typical composting operation
SOURCE: *ADAPTED FROM FRAY AND BIDDLESTONE (1971)*

4.2 Biochemical parameters

Due to the close association between compost stability and chemical and microbiological characteristics of composting, many scientific studies have been undertaken to characterize the microbial activity as well as the change in OM at different phases of composting (Changa et al. 2003; Adani et al. 2003; Iannotti et al. 1994; Sadaka et al. 2006; Mondini et al. 2002; Tejada et al. 2009; Fourti et al. 2011). Although there are a large number of biochemical compost stability indicators in the literature, these indicators can be generally categorized into two main categories. (1) The biological characteristics of microbial organisms, (2) The transformation of C and N (especially NO_3^--N and NH_4^+-N) during composting.

4.2.1 The biological characteristics of microbial organisms

Biological characteristics of microorganisms during composting are often determined by one of, or a combination of three indicators: (i) Respiration, (ii) Enzyme activity and (iii) Microbial biomass.

(i) Respiration:

Compost respiration has been used to determine compost stability in the last few decades and early studies using electrolytic respirometers were conducted in order to develop an index of compost maturity where the compost was considered to be matured when the rate of O_2 consumption was smaller than 10mg O_2 per gram of compost during 7 days of incubation (Iglesias Jiménez and Perez Garcia 1989). These techniques have been used as a routine measurement for compost stability. Respirometric techniques work by determining microbial respiration activity by measuring either O_2 uptake or CO_2 production from the compost substrate. As the amount of carbon available for mineralization is directly related to the microbial activity in the substrate, low O_2 intake and CO_2 evolution indicate low microbial activity in the substrate (Changa et al. 2003; Adani et al. 2003). Static and dynamic O_2 uptake methods are two similar approaches where O_2 uptake is examined with (static) or without (dynamic) continuous air flow of the compost substrate (Komilis et al. 2011). Adani et al. (2003) reported a comparison of three respirometric indices, dynamic respiration index (DRI), static respiration index (SRI) and specific oxygen uptake rate (SOUR). These three indices were measured on 18 organic matrices and significant correlations were shown among indices and confirmed that all indices were able to reflect the trend of biological stability in the biological processes. Both methods are allowed to run directly on moistened solid sample or suspension of sample in

water. In the electrolytic respirometer, O_2 consumption can be detected by the electrolytic O_2 production cell which releases O_2 after the pressure is dropped. The drop in pressure is caused by the absorption of CO_2 (in strong base) evolved by microbial respiration (Sadaka et al. 2006; Sánchez Arias et al. 2012). Iannotti et al. (1994) indicated that O_2 respirometry could be used to determine the maturity of compost as it is highly correlated with ryegrass growth and reported matured MSW compost in the study with a mean O_2 uptake of 0.48 g O_2 kg^{-1} VS h^{-1}. In a study by Villaseñor et al. (2011), it was found that a proposed DRI value lower than 1 g O_2 kg^{-1} VS h^{-1} was required to achieve sufficient stability of the compost. O_2 respirometry provides the most accurate way to determine the stability of a compost sample. However the technique is not widely used for routine compost monitoring due to the extended time involved and the need for sophisticated equipment.

An alternative way for determining the rate of microbial respiration in compost is the examination of the rate of C mineralization which is indicated by the CO_2 production. Incubation methods (Figure 1.4) are usually applied where CO_2 is captured with strong base in a sealed vessel over a period of time at a set temperature. The amount of CO_2 evolved can be determined with back titration with acid after absorbed CO_2 is precipitated with barium chloride (Iannotti et al. 1994; Sadaka et al. 2006). The Pressure Sensor method (OxiTop method shown in Figure 1.4) can also be used to provide an alternative simple and effective way to measure CO_2 production during incubation. A compost sample is placed and incubated in a glass jar with 5-10 NaOH pellets (for CO_2 absorption) hanging below the stopper for 48h. The jar is closed with pressure sensor head with data logger attached to the top and the change of pressure is measured during the incubation. As CO_2 is produced by the microbial activity, a negative pressure builds up in the jar due to the

CO_2 being adsorbed by the strong base. The pressure drop reflects the uptake of O_2 from the sample (Sadaka et al. 2006; Grigatti et al. 2007). However, the rate of CO_2 evolution may not be accurately represented by these methods because part of the CO_2 released from microbial respiration may dissolve in the aqueous section of the sample and the CO_2 solubility is dependent on the pH of the sample (Barrena Gomez et al. 2006). CO_2 respirometry offers a more direct and easy to follow procedure when it is compared to O_2 respirometry. However it still requires certain highly trained skills to conduct the testing and evaluate the results.

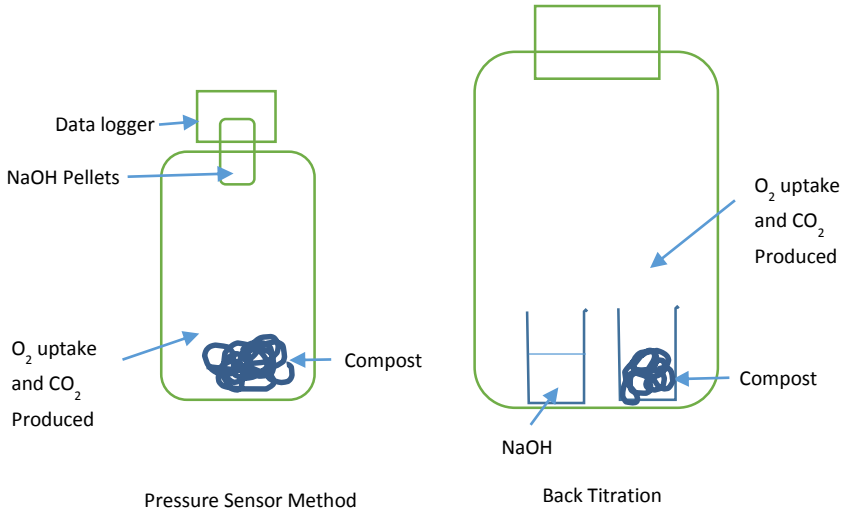


Figure 1.4: Schematic diagram of CO_2 respiratory experimental apparatus
SOURCE: *ADAPTED FROM SADAKA ET AL. (2006)*

Another approach to measuring the microbial activity relies on measuring the heat production by the compost sample under aerobic conditions. The Dewar self-heating test is one of the most common ways to measure compost stability involving measurement of heat production and has been widely used in the determination the compost stability in large scale of composting (Francou et al. 2005; Wagland et al. 2009). In this test, the highest temperature is recorded during a period of incubation (several days) in a vessel (Brinton et

al. 1995). Since the increase in temperature in the vessel is directly correlated to the rate of overall microbial respiration, higher temperature is reached when there are higher rates of decomposition during the incubation. Therefore the compost is determined to be stable when there is little change in temperature due to the low rate of decomposition. (Cabañas-Vargas et al. 2005). The Dewar self-heating test is a simple and quick technique to measure compost stability. However the test needs to be done over several days of incubation for reliable results, hence it may not be practical for a compost pile on a farm where the composter always has time constraints due to other duties. In addition a threshold value is very hard to develop for this technique as the result is highly related to the type of feedstock used and the management conditions.

(ii) Enzyme activity

Microbial degradation of OM occurs throughout composting and the degree of biostability is often reflected by the groups of microbial communities present in the composting pile (Hubble et al. 2010). During composting, various hydrolytic enzymes with activities including amylasic, cellobiasic and proteolytic are responsible for controlling the degree of OM degradation at different stages (Raut et al. 2008; Iglesias Jiménez and Perez Garcia 1989). Therefore characterizing and quantifying specific enzyme activities during composting can reflect the quality and quantity of OM found in the composing substrate (Ros et al. 2006). Mondini et al. (2004) investigated the activity of a wide range of enzymes with increasing composting time and stated that a stable enzyme activity in air-dried samples could be used as a good indicator of compost stability. Tiquia (2005) found that the activity of dehydrogenase related well with humification parameters and provided an easy and rapid way to monitor compost stability. In that study, a dehydrogenase activity of

35 µg TPF g^{-1} was required (TPF, triphenylformazan, is a product of reduction of TTC, 2,3,5-triphenyltetrazolium), to be considered as associated with a mature compost.

Fluorescein diacetate (FDA) hydrolysis occurs from the reaction between a number of non-specific extracellular enzymes such as esterases, proteases and lipases and has been proposed to be a stability parameter for compost and used for prediction of suppressive capacity of compost on plant pathogens (Ceustermans et al. 2010; Craft and Nelson 1996). Furthermore, activities of protease, cellulase and xylanase, which are responsible for hydrolysis of protein, cellulose and hemi-cellulose respectively, have been used among different studies to evaluate the level of decomposition of the substrate at different stages of composting (Goyal et al. 2005; Lazcano et al. 2008; Tiquia at al. 2002; Liu et al. 2011; Charest et al. 2004). Among different microbial activity methods, enzyme activity offers a relatively cheap and rapid measurement of compost stability. However the enzyme activity at different stages of composing was found to be inconsistent as some enzymes may have high influence in decomposition of one compost but not the other. Threshold value is very difficult to be determined by the enzyme methods because of the large numbers of enzymes involved in composting. The main problem is that no agreement was found in using a specific enzymes for monitoring composting processes (Lazcano et al. 2008; Tiquia at al. 2002).

(iii) Microbial biomass

Tiquia et al. (2002) stated that not only the microbial activity but also the microbial biomass and numbers can be used as indicators to reveal the stage at which the composting process is at. These authors also found that microbial biomass correlated significantly with other

microbial activity indicators including O_2 consumption rate, ATP content and dehydrogenase activity (Tiquia 2005). The microbial biomass from a compost sample can be assessed directly by the counting of colony forming units (CFU) plated on suitable media after incubation at specific temperatures (Raut et al. 2008; Craft and Nelson 1996). The microbial biomass of compost can also be quantified by indirect methods such as the fumigation-extraction (FE) method or the lipid phosphate assay (Gattinger et al. 2004). The FE method has been used more often, due to its simplicity and easy to reproduce procedure, than the direct count method and lipid phosphate assay. The FE method is usually used to estimate C, N, P and S content of the soil microbial biomass but has also been used to estimate the microbial biomass in compost (Mondini et al. 2002; Tejada et al. 2009; Tiquia 2005; Fourti et al. 2011). During chloroform fumigation, the cells of microbial organisms lyse and part of the cytoplasm become extractable by various reagents such as potassium sulphate (K_2SO_4) and the microbial biomass can be calculated by the difference between a non-fumigated and fumigated samples (Vance et al. 1987). However Horwath and Elliott (1996) found that it was hard to relate microbial biomass measured by the FE method to the plate counting method because the viability of spores on agar media may not reflect the overall population of microorganisms especially the microbial community in the thermophilic phase. Examining the microbial biomass at different phases enhances our understanding of composting process but the method would not be practical for routine maturity testing as it is a time consuming technique even though the equipment required is relatively simple.

4.2.2 Transformation of C and N during composting

During composting, the microorganisms utilize biomolecules such as protein, cellulose and hemicellulose as C and N sources. Humic-like substances of increasing complexity are formed when the residual plant material is transformed along with the compounds of microbial original (Hubbe et al. 2010). Therefore, understanding the transformation that OM undergoes during composting provides a more complete picture of the composting process and hence can be used to determine the factors which effect the biostability of the substrate (Adani et al. 1999). OM transformations can be determined by many methods includes %OM, %C, %N, C/N ratio, spectroscopic and thermal analysis, humification index and C and N dynamics in aqueous extraction.

(i) %OM, %C, %N and C/N ratio

The availability of C and N are most important factors for manufacturing a high quality compost product. Microorganisms require sufficient C as an energy source for microbial respiration and N for maintaining the microbial activity (Bernal et al. 2009). Hence the C:N ratio is generally regarded as an important parameter to monitor in composting processes. Generally, a feedstock with a C/N ratio ranging from 25 to 35 is considered to be adequate for composting as 30 parts C per unit of N is assumed to be needed for supporting microbial activity (Bishop and Godfrey 1983). During the composting process the OM is decomposed by microorganisms and the rate of decomposition depends on the microbial activity at a particular stage of composting in the pile. Overall, the rate of the OM degradation decreases gradually and aligns with the reduction of biodegradable nutrients in the pile as composting continues (Pare et al 1998). The kinetics of C and N mineralization have been considered as first-order kinetics and can reflect the general dynamic of the OM mineralization.

Factors that determine the first order constant includes temperature, moisture, pH, lignin content, substrate particle size and C:N ratio (Powell et al. 1994; Mansoni et al. 2009).

C availability, overturning frequency and the size of the bulking agent are found to be the main factors for the contribution to the loss of N in the compost substrate (Barrington et al. 2002; Hansen et al. 1989). During OM decomposition, C content decreases through mineralization as CO_2 while N loss occurs via NH_3 volatilisation, mineralization to NO_3^- and subsequent, leaching and denitrification (Bernal et al. 2009). Only 30% to 40% of the C and about 60% of N with respect to the consumed OM contributes to the microbial biomass and the rest would be lost via mineralization (Barrington et al. 2002). Since the rate of N loss is often found to be smaller than C loss during composting, the %N (out of the total OM) of a composting pile generally increases. In addition to the reduction of the size and total OM of the pile, compost with C/N < 20 is generally considered to be stable. However, the C/N ratio does not always reflect the actual bioavailability of C and N in a composting pile and must be used with caution when determining the stability of compost (Puyuelo et al. 2011; Domeizel et al. 2004; Senesi 1989). The C/N ratio can even increase if the initial substrate has a very high N content (Tiquia 2002). Since the biochemical reactions mainly occur in the aqueous component of compost, an examination of the C and N content in an aqueous extract of the compost is considered to yield more reliable parameters than the C/N ratio (Senesi 1989). Nevertheless, %OM, %C and %N provide an indication of the change in composition during composting and are directly related to the degradation progress at different phases during composting.

(ii) Humification index and UV-visible spectroscopy

Humification is believed to be an essential process that enhances the quality of compost because humic-like substances are found to have a positive influence on plant growth and higher resistance to the mineralization process (Gonzfilez-Vila & Martin 1985). During composting the concentration of humic-like substances is expected to increase in the remaining OM. Humification parameters, based on the ratio between the extractable humic acid and non-humic organic C, have been used to evaluate the stability of compost (Dell'Abate et al. 1998; Hsu and Lo 1999). The extraction of humic acid (HA) is commonly used to refer to the precipitate formed from the soil or OM extract using diluted NaOH combined with $Na_4P_2O_7$ as extractant followed by acidification (Kuwatsuka et al. 1992). The non-humus fraction that remains soluble in the extract after acidification is generally referred as the fulvic acid fraction (FF) (Wu and Ma 2002). The change of concentration of FF and HA and the HA/FF ratio during composting have been used to assess the stability of compost but high variability is found among composts of different material sources, hence making it hard to develop a threshold value for such parameters (Wu and Ma 2002). Domeizel et al. (2004) reported that the ratio of humification (HR), the rate of extraction (TE), the humic acid to fulvic acid ratio and the index of polymerization (IP) are the most representative indices among maturity indices based on the monitoring of humic substances. UV-Visible Spectroscopy has been used to estimate the concentration of humic substances in compost (Domeizel et al. 2004). The absorptions corresponding to 664nm (E6), 472 nm (E4) and 280 nm (E2) of a compost extract solution are selected and the ratios between these wavelengths are found to be correlated to the amount of humic substances in compost. The ratio E2/E4 indicates the relative amounts of lignin at the beginning of humification. The ratio E2/E6 reviews the amount of non-humified and highly humified material. Finally,

the E4/E6 ratio is often used to reflect the degree of humification (Sellami et al. 2008). The humification index can be used to indicate the maturity of the compost as it is related to the formation of humic substances. However it normally requires a long extraction process and is not practical for routine monitoring of a compost pile. Even though UV-VIS offers a quicker analysis in the laboratory, more work needs to be undertaken to examine the correlation between different humification indices and for compost with different initial feedstocks and management conditions.

(iii) Spectroscopic and Thermal Analysis

Cross polarization magic angle spin (CP MAS) solid phase ^{13}C NMR and Fourier-Transform Infrared (FT-IR) spectroscopy can be used to examine the C and N transformations during composting. CP MAS ^{13}C NMR can be used to determine the ratio of aromatic C to aliphatic C in the compost sample, and also indicate the presence of lignin-like and melanin-like structures. Furthermore, the bonding found in lignin, cellulose and hemicelluloses can also be determined (Chen 2003). Fourier-Transform Infrared (FT-IR) can provide comprehensive information on the chemical composition of the compost sample. As decomposition occurs in the composting piles, the C signal indicating the level of carbohydrates in the NMR spectra is expected to decrease along with an increase in signals of alkyl C, aromatic C and carboxyl groups. A decrease in aliphatic C-H stretching and an increase in C=O are also expected found in FTIR spectra as composting continues due to the oxidation of easily degradable OM constituents such as cellulose and aliphatic chains. (Chen 2003; Castaldi et al. 2005).

Thermal analysis such as thermogravimetry (TG), differential thermal analysis (DTA) and differential scanning calorimetry (DSC) are useful techniques for examining the thermal properties of compost samples because of the rapid nature of these forms of analysis (Pietro and Paola 2004). The TG curve shows the percentage weight loss of the sample by programmed heating under a controlled atmosphere and the technique makes it possible to identify the weight loss for each sample across a temperature range during its combustion. Comparing samples with different degrees of stability, the thermal properties indicate the organic fraction characteristics. This is based on the assumption that combustion of OM with more ordered structure occurs at higher temperatures. (Otero et al. 2002; Pietro and Paola 2004; Gómez et al. 2007; Fernández et al. 2012; Som et al. 2009). The information reviewed by spectroscopic and thermal analysis has enhanced our understanding of C and N transformations during composting. However the required equipment is not available to most composters and the cost of the analysis is too high to have regular assessment of the composting pile.

(iv) C and N dynamics of water extracts

It is believed that most of the biochemical transformations of the OM in the composting reactions take place as microbial metabolism occurs in the soluble fraction (González-Vila et al. 1999; Hue and Liu 1995). As higher concentrations of C compounds that are available to microorganism (e.g. sugars, hemicellulose, organic and amino acids, proteins) are present in the initial stages of the decomposition, water soluble C (WSC) is expected to be detected in the initial and thermophilic stages (Wu et al. 2000). However WSC will gradually decrease with increasing curing time and a stable compost has been reported to contain less WSC than an immature compost (Hue and Liu 1995). Zmora-Nahum et al

(2005) proposed that compost with less than 4 mg g^{-1} dw (dry weight of compost) of WSC can be considered as a stabilized compost. Said-Pullicino et al. (2007) proposed that an increase in the ratio of hydrophobic water-extractable organic C to hydrophilic water-extractable organic C can be used as an indicator for the stability of compost when the water extract from a compost is divided into two fractions by eluting through amberlite XAD-8 resin. WSC is a reliable parameter to measure compost stability and also provides some stability threshold values to measure across different types of compost. More research is needed to investigate the nature and components of the water extractable OM in order to confirm its ability to determine compost stability across a wide range of composts.

Examination of N dynamics is commonly used to determine compost stability. The ratio of $N\text{-}NH_4^+$:$N\text{-}NO_3^-$ can be used to monitor the composting process as the production of NH_4^+ and NO_3^- is based on the type of dominant microbes present in different phases of composting. NH_4^+ is expected to have a higher rate of production during the thermophilic phase (around the second week of the composting period) while most of the nitrification occurs in the maturation phase. Therefore mature compost tends to have a low NH_4^+ to NO_3^- ratio (Raj and Antil 2011; Afifi et al. 2012). From a practical point of view, in larger scale composting pile, a quick qualitative test may be carried out by the use of specific nitrate and nitrite reagents with compost aqueous extracts.

The strip test is a simple chemical analytical tool used to perform semi-quantitative analysis of desired chemical. The principle of strip test kits are used in a variety of applications such a measuring pH and chlorine levels in swimming pool water, hardness and pH in aquarium water and glucose in blood, to give few examples. Each strip test consists of at least one

pad which contains reagents that will change colour when immersed in the test sample. The colour is compared with a colour chart that provides a semi-quantitative indication of the parameter being measured. Strip tests have been used to estimate the concentration of NO_3^--N and NH_4-N during composting (Iglesias Jiménez and Perez Garcia 1989). Itävaara et al. (2010) found a similar result of NO_3^--N/NH_4^+-N between strip test methods and chemical analysis methods and proposed that strip tests can be used to assess compost maturity of a large quantity of compost such as those from a composting plant. However, research on the examination of the reliability and correlation between strip testing and other parameters is lacking.

5. Conclusions

In conclusion, various indicators or parameters used in determining the degree of maturity of compost have been discussed in this review. The more stable and mature the compost is, the higher quality and value the compost products are. Therefore a combination of physical and biochemical parameters should be employed to determine the actual agricultural value of compost products manufactured with different initial materials and in different conditions. However, it is necessary to find a method that can provide simple and quick measurements for routine monitoring of compost stability for the composting pile, especially for non-industrial composters such as farmers and gardeners. Measurement of N dynamics during composting can be done using a simple strip test and it may provide a simple and quick alternative way for measuring compost stability and maturity.

6. Aims of this research

The aims of the study described in this thesis are as follows:

To monitor the composting process involving two distinct feed-stocks (manure based and greenwaste based) at the same location with the same management (moisture and frequency of turning) through the measurement of a range of biochemical compost stability parameters; and to correlate the findings with simple chemical strip tests that measure NH_3/NH_4^+ and NO_3^- in order to validate the reliability of applied strip tests for monitoring compost stability on-farm.

The research hypothesis is that simple chemical strip tests can be used to measure the contents of NH_3/NH_4^+ and NO_3^- in compost and the results can be used in monitoring open turned composting windrows with different origins of organic wastes. The project aimed to enhance our understanding of the biological characteristics and C and N transformations between the two composting piles under local weather conditions and established the viability of using strip tests in compost monitoring.

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CHAPTER TWO – EVALUATING THE STABILITY OF COMPOST USING A COMBINATION OF ANALYTICAL METHODS

1. Introduction

Depleted soil, polluted water and reduction of biodiversity originating from intensive industrial agriculture has caused a growing interest of sustainable agriculture which is believed to be able to preserve the resources that allow us to meet the needs of present and future generations. In addition, it is important to develop environmentally friendly waste management processes to reduce the impact on the environment from inappropriate disposal of organic wastes produced by intensive farming (Senesi 1989; Chapman et al. 2010). Composting of organic wastes is a widely used practice to generate stable products that can be used to improve soil quality and enhance plant growth by changing soil physical and biochemical properties through increased accumulation of OM, in particular humic-like substances (Dick and McCoy 1993; Hoitink and Fahy 1986). In order to maximize the benefits of compost application in the field, a fully stabilized composting product is desired and various physical, biological and chemical parameters have been developed over the years to determine compost stability and maturity (Bernal et al. 2009; Hubbe et al. 2010).

However it is not appropriate to determine compost maturity using just one parameter because of the complexity of the biological events and associated chemical changes, the large diversity of composting management practices and the mixture heterogeneity in feedstocks (Pietro et al. 2004). For example, when a composting pile is in the thermophilic phase, the pH of a composting pile is generally higher than 7 and is sometimes quite alkaline due to the loss of N as NH_3 and the C/N ratio generally decreases with increasing

composting time. However, studies have found that pH may stay close to neutral throughout composting when N loss is not significant in the thermophilic phase while the C/N ratio may increase instead of decrease as the feedstock loses significant amount of N in the same composting phase (Wang et al. 2004). Thus, if only one parameter is used to monitor the process, an incorrect judgment could be made and a high quality product is less likely to be manufactured from composting. At least two stability and maturity parameters are therefore usually required to monitor the composting pile prior to soil application (Paradelo et al. 2010; Mondini et al. 2006).

Two windrows consisting of domestic garden waste and farm manure wastes over a period of 175 days of composting were investigated in this study. Sample were collected twice in the first month and at the beginning and end of the curing stage and examined by various chemical analytical methods including OM, C (%C) and N (%N) content, C/N ratio, water soluble C (WSC), humification indices, solid state ^{13}C NMR spectroscopy and thermogravimetry. This chapter describes a study of the OM dynamics over time in two open windrow systems in which organic wastes were sourced locally. The understanding of the OM dynamics process of these specific feedstocks during composting can be used to determine the degree of stability of the final compost and also the chemical background. The information on chemical parameters will be compared with the proposed simple strip testing methods which will be described in Chapter 4 and the results can also be used to validate the strip testing methods.

2. Materials and Methods

2.1. Composting Pile Establishment

Two windrow systems were established by the former Department of Environment and Primary Industries (DEPI) in Victoria (now part of the Department of Economic Development, Jobs, Transport and Resources), at the DEPI compost research facility (Sneydes Road Werribee). The Farm Manure waste windrow (MA) was made from fresh feed-pad dairy manure and straw, spoiled hay, wood chips and poultry litter, while a Greenwaste windrow (GW) consisted of only greenwaste collected by the municipal collection services without mixing with other organic wastes or bulking agents. Both windrows were turned and moisture maintained by an operator (Dr. Kevin Wilkinson from DEPI). Two open air windrow systems with an estimated volume of $50m^3$ for MA windrow and $30m^3$ for GW windrow were established in a typical trapezoid shape with the height of around two meters and the base at about two to three meters wide. Both windrows were turned three times in the first week of composting and twice a week in the remaining first month. After the first month of composting, the windrows were then turned once every week until the mixtures were considered stabilized and then left for further maturation. The temperature of the windrows was monitored and recorded every hour throughout the whole composting process by the operator (data aimed to publish elsewhere). The moisture content was maintained within 50% to 60% for both windrows by regular water addition using soaker hose placed on top of the pile. The moisture of the compost was estimated with by squeezing the compost by hand. At about 50% to 60% moisture content, the compost will hold its form without water dripping off after squeezing, and resist crumbling. The sampling, storage and stability and some of the maturity tests including %C, %N, %OM, ammonium and nitrate contents of the compost were consistent with methodology

outlined in the Australian Standard for Composts, Soil Conditioners and Mulches (AS4454-2012). In this study, the maturity of the compost was evaluated through the examination of the transformation of the OM as well as the degree of humification during composting and with various chemical stability parameters including the changes in %OM, %C, %N, C/N ratio, concentration of WSC and HA and FF content. Solid phase ^{13}C NMR spectroscopy and thermal analysis (TGA and DSC) were also used to examine the molecular changes of the OM and mass changes during incremental heating respectively. Compost samples of different ages from both GW and MA windrows were collected after the windrows were turned on Day 2, 19, 77 and 175 (referred as GWd2, GWd19, GWd77, GWd175, MAd2, MAd19, MAd77 and MAd175 respectively). At each sampling period, samples were collected from four vertical cuts of the piles. Approximately 5 L of sample was collected from surface, middle and core of each cut and thoroughly mixed manually in a large container. The mixed samples were then separated into four equal portions (refer as R1, R2, R3 and R4) before transferring to air-tight plastic bags. The collected samples were delivered to the laboratory and stored in a freezer (-20^{o}C) before further sample preparation. The selected samples represented the four main stages of composting: mesophilic (day2), thermophilic (day19), cooling (day77) and maturation phases (day 175). These samples were temporarily frozen (for short term storage after sampling) until they were thawed, sieved (<9.5mm) and air dried for further analysis. Parts of the dried samples were ground to pass through a 400-µm sieve before performing molecular (%OM, CN analysis and solid phase ^{13}C NMR) and thermogravimetric analyses.

Since the substrates used contained large particles and were highly heterogeneous, and the sample size (less than 0.1g) analyzed by the molecular methods such as solid phase C^{13}

NMR, TGA and elemental analysis was relatively small, it was difficult to generate accurate quantitative results for all measured parameters even though standard procedures of sample preparation and analyses according to Australian Standard for Composts, Soil Conditioners and Mulches (AS4454-2012) were followed. Nevertheless, this work aims to evaluate the change in OM and the degree of humification during composting and evaluate the stability of the compost samples at the various stages.

2.2. Total Organic Matter and Elemental Analysis

Elemental analysis for total C (%C) and total N (%N) content were performed on the 2mg of compost samples using vario MICRO cube elemental analyser from Elementar Analysensysteme (n=4 replicates). The total organic matter content (%OM) was determined by combustion of a 3-5g of air dried sample at 550 ^{o}C for 8 h in a muffle furnace (analyzed in triplicate, n=4). Total OM and TN loss was then calculated according to the following equations:

$$\text{TOM loss (\%)} = 100 - 100\,[X_i TOM_f / X_f TOM_i] \quad (1)$$

$$\text{TN loss (\%)} = 100 - 100\,[X_i TN_f / X_f TN_i] \quad (2)$$

where X_i is %ash of day 2 ; X_f is %ash of the day measured and TOM_i is %OM of day2 ; TOM_f is %OM of the day measured ; TN_i is %N of day2 ; TN_f is %N of the day measured.

2.3. Water Soluble C and Humic Substances

A two-step extraction procedure was employed to separate the composts into different fractions and the procedure is described below (Wu and Ma 2002).

Step 1: To measure pH and water soluble carbon (WSC), for each time point: day 2, 19, 77 and 175, an 8 g air-dried compost sample (sieved < 9.5 mm and n = 4) was suspended in 80 mL of distilled water (1:10 w/v) and shaken for 2 h at 120 rpm on a horizontal shaker at room temperature followed by filtering through filter paper (Whatman no.6) according to the method of Wu and Ma (2002). The pH of the extract was then measured before further dilution for water soluble C measurement (20 times for day 2 &19 and 5 times for day 77 & 175). Water soluble C (known as WSC) is measured by the wet dichromate oxidation procedure (Walkley and Black 1934). Spectrophotometric method was then used to determine the amount of dichromate used for the oxidation of soluble C at 340nm. (Bolan et al.1996).

Step 2: A subsequent extraction was performed on the residue from step 1. The residue was extracted with 80 ml of 0.1 M NaOH solution with sonication (20W /L) for 3 h and left on the bench for 24h at room temperature. The suspension was centrifuged at 4000 rpm for 10 min and filtered through Whatman no.6 filter paper and the filtrate labelled as the alkaline soluble fraction. The humic acid was further separated from the fulvic acid by precipitation after acidification of the alkaline solution to pH < 2 with 6 M HCl. The C content of the alkaline soluble fraction (referred as $C_{alkaline}$) and the C content of fulvic acid fraction (referred as C_{FF}) were measured by the wet dichromate oxidation procedure. Then the used dichromate was measured spectrophotometrically (Walkley and Black 1934; Bolan et al.1996). The C content of HA (referred as C_{HA}) was calculated by subtracting the C_{FF} from $C_{alkaline}$.

2.4 Thermal Analysis

Thermogravimetry (TG) was used to analyze the thermal stability of the OM (ie the total compost mixture sample) at different stages of the composting process (Fernández et al. 2012; Som et al. 2009). Approximately 10 mg of the sample was placed in an aluminum crucible. The sample was subjected to air at a flow rate of 20 ml min^{-1} and loaded into Mettler Toledo DSC/TGA thermogravimetric analyser. An empty aluminum crucible was used as a reference material. A temperature-programmed test with starting temperature of 30 °C and a final temperature of 750 °C was used. The heating rate was at 10 °C min^{-1} and the thermoproperties of samples from different stages of composting were evaluated. (Fernández et al. 2012).

2.5. ^{13}C NMR spectroscopy

The solid-state cross polarization magic angle spinning (CPMAS) ^{13}C NMR spectra were obtained on a Bruker DPX300 NMR spectrometer (7.05 Tesla magnet) with a 5 mm Quad ^{1}H, ^{13}C, ^{19}F, ^{31}P switchable probe. NMR spectra were obtained by using the following parameters: frequency of 100.6 MHz, spin rate 10000 Hz, SW 50000 Hz, acquisition time 40 ms, 4096 data points and a 4 sec delay. Solid samples were packed into 4mm ZrO_2 rotors with a Kel-F cap and the spectrum was collected and processed using Brukers Topspin 2.1 program. Spectra were referenced to a glycine external reference. For quantification, the relative proportion of organic C functional groups was determined by integration of the signal intensity of each spectrum over given chemical shift regions (0-220ppm) (Spaccini and Piccolo 2007; Schaumann and Bertmer 2007).

The organic C functional groups were examined on days 19 and 175 of GW and MA compost samples. These groups were categorized by dividing the ^{13}C NMR spectrum of each sample into four regions: (1) alkyl C (0-50ppm) in waxes, fatty acid and aliphatic polymers; (2) O-alkyl C (50-110ppm) in polysaccharide, protein and methoxyl C in lignin; (3) aromatic C (110-160ppm) in lignin and protein; and (4) carbonyl C (160-220ppm) in aliphatic esters, amide and carboxyl group in aliphatic acids. The relative abundance of the different types of carbon in the compost sample was evaluated by integration of the intensity of each region (Zbytniewski et al. 2002; Veeken et al. 2001).

According to Veeken et al. (2001), the contribution of different biomolecules components (aliphatic, carbohydrate, protein and lignin) in OM of compost sample can be calculated from the relative %C contribution of each biomolecule component towards the four regions, alkyl C, O-alkyl, aromatic C and carbonyl C. The relative amount of aliphatic, carbohydrate, protein and lignin of compost samples was calculated as described below.

For each biomolecule component, the spectral distribution of signal intensity in four defined regions was determined by the known chemical shift of pure materials or representative samples as described in Nelson and Baldock (2005). The distribution of C (as % of total area) of the four types of biomolecules in spectral regions is summarized in Table 2.1. Four equation can then be derived by using the proposed percentage distributions of biomolecules in each region. According to equation 1-4, the relative intensity (I) of each region in the NMR spectrum is equaled to the sum of proportions of each biomolecule component multiply with its %C distribution in specified component:

$$I_{0\text{-}50} = 75.6\, f_A + 39.6\, f_P + 10.5 f_L \quad [1]$$

$$I_{50\text{-}110} = 13.5\, f_A + 99\, f_C + 24\, f_P + 34.9\, f_L \quad [2]$$

$$I_{110\text{-}160} = 4.3\, f_A + 1\, f_C + 10\, f_P + 50.1\, f_L \quad [3]$$

$$I_{160\text{-}220} = 6.6\, f_A + 26.4\, f_P + 4.6\, f_L \quad [4]$$

where I is the relative intensity of the NMR region (% of the total peak area), f is the % of C in component, A, C, P, L are aliphatic, carbohydrate, protein and lignin respectively. The relative amount of each biomolecule was calculated by solving the equations.

Table 2.1: Distribution of C (as % of total area) of four types of biomolecules in spectral regions adopted from Nelson and Baldock (2005)

Chemical shift region (ppm)	Aliphatics	Polysaccharides	Protein	Lignin
0-50	75.6	0.0	39.6	10.5
50-110	13.5	99.0	24.0	34.9
110-160	4.3	1.0	50.1	50.1
160-220	6.6	0.0	4.6	4.6

2.6. Statistical analysis

Statistical analyses were carried out with SPSS Version 22 (IBM Corporation) for Windows. Analysis of variance (ANOVA) was used to determine if there were significant effects of time on chemical parameters, including OM, C, N, WSC, C_{FF} and C_{HA} contents and C_{FF}/C_{HA} ratio of compost samples. Data that were determined to be heterogenous after the examination of the residual plots were transformed (log base-10) before further ANOVA analysis. These variables included C and WSC contents and C_{FF}/C_{HA} ratio. All statistical tests were evaluated at the 95% confidence level. Where the P-values were significant, the F-value was checked at a 95% confidence level using orthogonal contrasts. Pairwise comparisons were undertaken using least significant differences (LSDs).

3. Results and Discussion

3.1 Total C, Total N, Total OM and Water Soluble C

The average value and the standard deviation of the loss of ignition (%OM), total C (%C), total N (%N) and WSC content over time for each windrow are shown in Table 2.2. Total C (%C) contents of raw materials of GW and MA windrows were 25.0 % (s.d. 1.3%) and 33.8 % (s.d. 1.8%) respectively. A total decrease of 13.4 % of %OM was found in GW windrow while a decrease of 19.8 % was found in MA windrows over the composing period. In the analyzed period it was observed that the average %OM significantly decreased ($p<0.05$) between each of the stages during composting of GW substrates. However the %OM showed no significant difference ($p=0.14$) after 77 days of composting in MA windrow (Table 2.2).

Table 2.2: Evolution of main chemical parameters including %Organic Matter (OM), total C, total N, water soluble carbon (WSC) and carbon content of fulvic acid fraction (C_{FF}) and humic acid (C_{HA}) during the composting process of GW and MA windows (n=4). Mass of compost is expressed as a dry weight basis (mg per g of dw)

Windrow	Sampling Day	OM	C	N	WSC	C_{FF}	C_{HA}	C_{HA}/C_{FF}
		%			mg per g			
GW windrow	2	46.28±1.1a	25.20±1.34a	1.52±0.05a	5.47±0.37a	6.13±0.67a	14.29±1.98a	2.3±0.3a
	19	41.05±1.0b	23.36±1.75a	1.69±0.14a	3.45±0.20b	3.85±0.34b	12.36±1.43a	3.2±0.4a
	77	35.73±0.3c	21.93±1.61a	1.71±0.11a	2.02±0.15c	3.68±0.38b	11.96±6.34a	3.2±1.7a
	175	32.90±1.0d	20.38±4.0 a	1.88±0.30a	2.02±0.13c	3.06±0.42b	12.96±6.85a	4.2±2.3a
MA window	2	69.97±0.8a	33.81±1.79a	2.04±0.09a	10.01±0.85a	6.81±0.18a	9.27±0.90a	1.4±0.13a
	19	60.90±0.4b	32.06±1.06a,b	1.48±0.06b	5.58±0.36b	4.80±0.39b	9.41±1.06a	2.0±0.4a
	77	52.63±1.9c	26.76±2.04b	1.46±0.05b	2.23±0.47c	4.64±0.27b	9.18±2.18a	2.0±0.5a
	175	50.17±2.2c	31.47±4.61a,b	1.76±0.17a,b	2.46±0.36c	4.95±0.28b	10.05±1.65a	2.0±0.4a

† HA, humic acid. organic matter insoluble at pH<2.
‡ FF, fulvic fraction (fulvic acids - organic matter soluble at pH<2 + non-humified fraction).
Values in the same column and subtable not sharing the same subscript are significantly different at p< .05

The OM in MA windrow declined rapidly between day 2 and 19 and a total of OM loss was 33.2% (s.d. 1.2%). However a much slower rate of degradation was observed over the rest of the composting period to 56.7% of total OM loss after 175 days of composting. Total N content (%N) of MA windrow during composting dropped slightly from 2.0 % to 1.8%. The opposite was recorded for the total N content in GW windrow, which increased by 0.4% during the process from 1.5% to 1.9%. The increase in total N content and decreasing trends of C/N ratio showed in the GW windrow indicated the C had been consumed faster than the N with increasing composting time and these findings are in line with values reported in the literature (Bernal et al. 2009).

The decreasing trend of both C/N ratio and %OM in 175 days of composting reflected high level of stabilization had occurred in the GW windrow. There was a total of 43.2% OM loss after 175 days of composting in the GW windrow. In addition, the C/N ratio (11) of the final GW product was found to be within the threshold value (10-15) for mature compost. This suggested that that the GW windrow had reached maturity (Chefetz et al. 1996, Iglesias Jiménez and García 1992).

However a different pattern was observed in the MA windrow. The C/N ratio increased from 16.6 to 21.7 from day 2 to 19 then decreased gradually to the end of composting (Table 2.2). This observation can be explained by the likelihood that there was large amount of easily degradable N containing OM in the initial MA substrate which caused a higher N loss (44.3% of TON loss) than C loss (27.2% of TOC loss) between day 2 and 19 of composting. The high amount of N loss was confirmed with the observed pH values (about 8.6) of the water extracts of MA windrow in the thermophilic phase. The increase of C/N ratio during the thermophilic phase was also reported in several studies using feedstocks with relatively high N content such as chicken litter (Tiquia 2002; Larney et al. 2008; Wang et al. 2004).

The C/N ratio (18) of final MA product was found to be a slightly higher than those suggested threshold value. Since the MA windrows were constructed with a variety of raw materials and consisted of lignocellulose materials with a wide range of lignin to cellulose ratio, C/N ratio of the final product might not be an appropriate indicator for the windrow in this study. When composting separated pig manure, Nolan et al. (2011) found a lower decomposition (higher C/N ratio) of the compost substrate when using bulking materials rich in lignin compared to composting with straw (lower lignin content). In this study, the initial raw material of the MA windrow consisted of a relatively high amount of wood chip (~50% in volume) that was high in lignin and subjected to a lesser degree of decomposition. Therefore the final product with a relatively higher C/N ratio observed in MA was identified (Chefetz et al. 1996, Jiménez and García 1992). As C/N cannot be used to reflect maturity for certain composting substrates, the analysis of C in the water-soluble fraction (WSC) was suggested to be a better parameter instead of using the total C content (Wu et al. 2000; Hue and Liu 1995; Mondini et al. 2006). A comparison between C/N and WSC/N ratio of both windrows is shown in figure 2.1. A decreasing trend of WSC/N was observed in both windrows whereas C/N showed no particular trend in the MA windrow. The ratio of WSC to %N had been considered a better approach compared to C/N ratio to determine the stability of compost from different types of initial feedstock (Senesi 1989; Puyuelo et al. 2011).

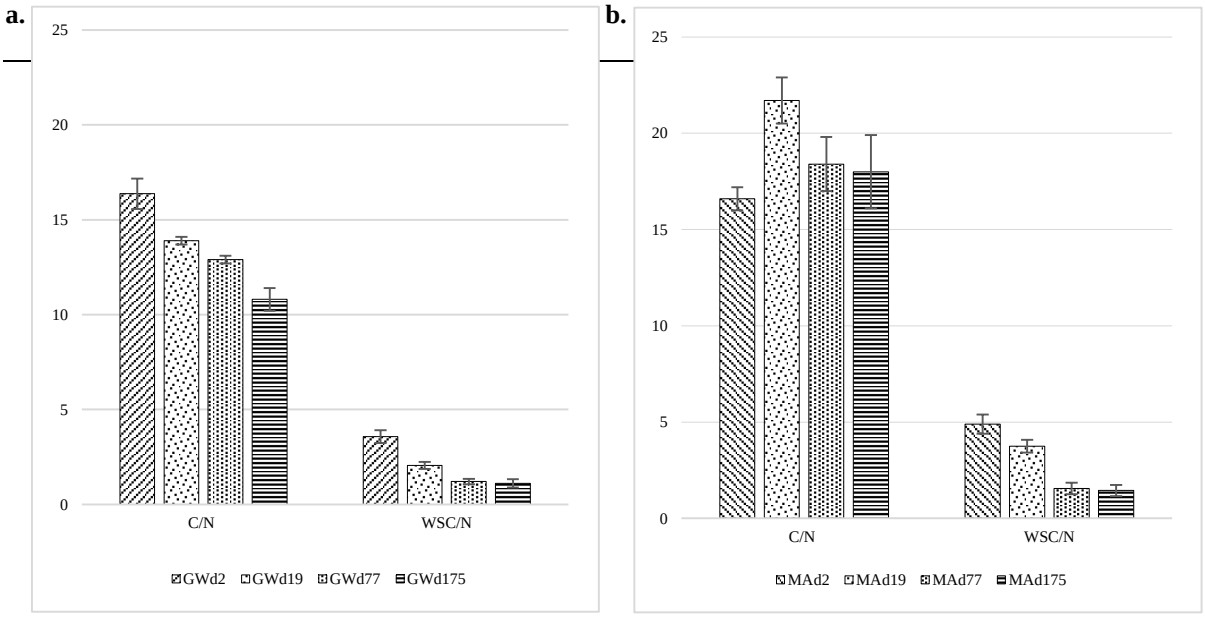

Figure 2.1: C/N ratio and WSC/N ratio in GW and MA compost samples at different stages of composting. (a) GW windrow; (b) MA windrow

Results from both windrows showed a significant decrease ($p<0.05$) of WSC content over days 2, 19 and 77 of the composting irrespective of the type of initial substrate. The initial WSC content of day 2 of GW (GWd2) and MA (MAd2) windrows were 5.5 and 10.0 mg g^{-1} dw respectively. The WSC decreased from 5.5 mg g^{-1} dw at day 2 in the GW windrow to 2.0 mg g^{-1} dw at day 77 and remained steady and no statistical difference to the end of the process ($p=1$). The WSC content of GW d175 and MA d175 (Table 2.2) were both below the threshold WSC content of 4 mg g^{-1} dw suggested by Zmora-Mahum et al. (2005). The relative content of WSC as a percentage of total extractable C at different stages of composting are shown in figure 2.2. The total extractable OM (sum of WSC, C_{FF} and C_{HA}), WSC contributed 21.2% (s.d. 1.2%) at day 2 and decreased to 12.8% (s.d. 0.6%) in final matured GW (GWd175). Similarly, a decreasing trend was observed in the MA composts with a larger reduction of percentage composition of WSC in total extractable OM from 38.4% (s.d. 1.4%) to 14.1% (s.d. 0.5%). Therefore the concentration of WSC of final compost products (day 175) from both windrows decreased by a factor between 2 to 4 compared to the initial concentration of WSC content of day 2 samples. Nevertheless, the

significant decrease of %OM during 175 days of composting and low values of WSC in the final products indicated the compost of the two windrows was mature and highly stabilized.

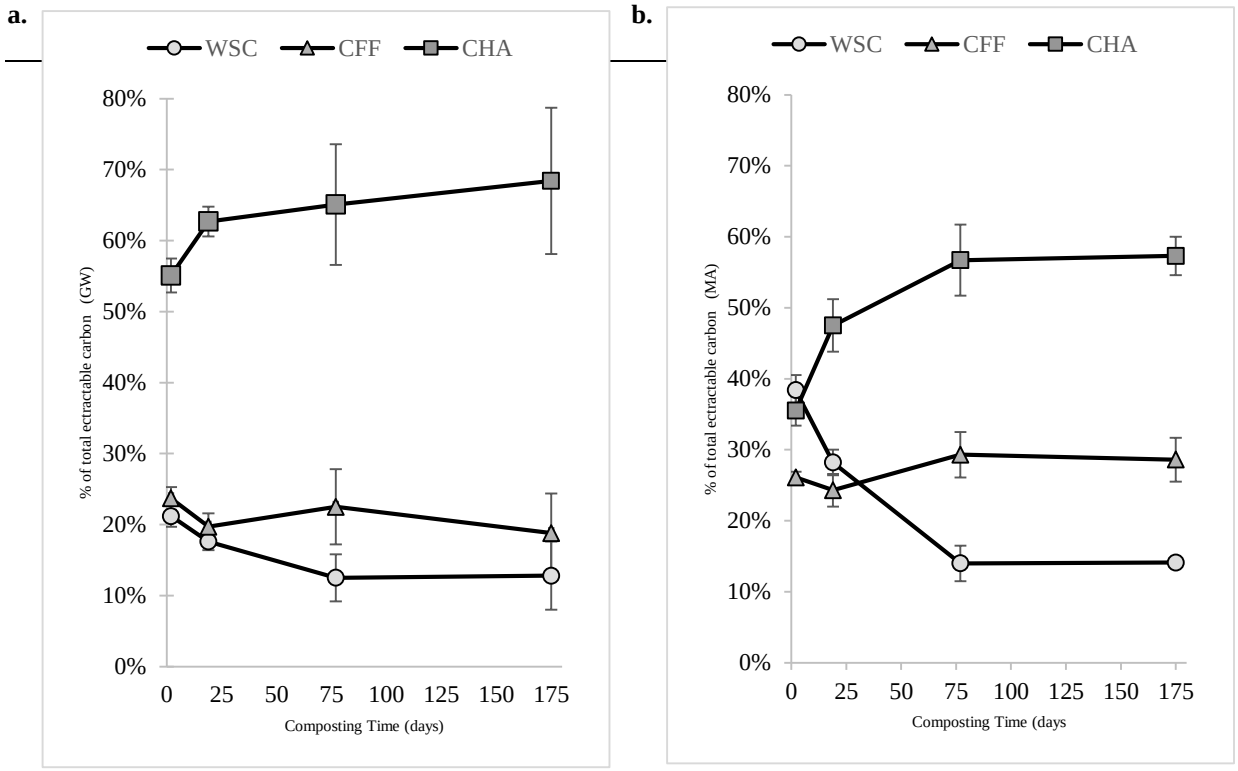

Figure 2.2: Relative content of WSC, Fulvic acid Fraction C (C_{FF}) and Humic Acid C (C_{HA}) as a percentage of total extractable C at different stages of composting. (a) GW windrow; (b) MA windrow

3.2 Humic Substance Content

The C content of the humic acid (C_{HA}) and fulvic acid fraction (C_{FF}) in the alkali soluble fraction and the ratio of the C content between the two fractions are presented in Table 2.2. A significant decrease of C_{FF} content ($p<0.001$) from 6.1 to 3.1 mg g^{-1} dw was observed in the GW windrow while a significant decrease ($p<0.001$) of 1.86 mg g^{-1} dw of C_{FF} from 6.81 to 4.95 mg g^{-1} dw was found in the MA windrow. Humic substances are one of the most important fractions of OM because of their unique properties, such as the capacity to interact with metal ions and the ability to act as a potential source of nutrients for plants. NaOH-extracted humic substance from composts

can be separated into a humic acid (HA) fraction and fulvic acid fraction (FF) which includes both fulvic acid and non-humified fraction (Chen and Inbar 1993). The relative %C content of humic acid (HA), fulvic acid fraction (FF) and WSC of GW and MA windrows to total extractable C during composing is presented in Figure 2.2.

In general, fresh composts contain a lower proportion of C_{HA} and the proportion increases gradually over the composting period (Inbar et al. 1990 ; Ciavatta et al. 1993; Chefetz et al. 1996), a trend that was also shown in this study. However a relatively large amount of HA was found present in the day 2 samples of the MA windrow, which indicated the initial substrate mixture already contained a high level of alkali soluble substances given that a low microbial activity was expected in the first two days of composting. The C_{HA} increased in GW windrow from 55.1% of the total extractable OM (sum of WSC, C_{FA} and C_{HA}) in the initial stage (day 2) to 68.4% (day 175) in the end product. The C_{HA} in MA windrow increased from 35.5% (day 2) to 57.3% (day 175). The ratio of humification measured the ratio between the C content HA and FA was commonly used to analyze the humic fraction (Iglesias Jiménez and Perez Garcia 1989; Chefetz et al. 1996). The ratio of humification was calculated from the C content in the alkaline extraction in this study and no significant difference (Table 2.2) was observed among all samples from the same windrow. Humic content was found between compost samples with high experimental errors. This could be explained by the fact that the total amount of humified substance was not taken into account in this study when the C_{FF} and C_{HA} were measured after the samples were extracted with water. Therefore the ratio of humification generated in this study were considered not suitable to reflect the degree of humification in the compost pile. However a consistent increasing trend was still found when comparing the ratio of C_{HA} to WSC during the

composting. The ratio increased from 2.6 to 5.3 and 0.92 to 4.06 in GW and MA windrow respectively. The increasing trend in the ratio of C_{HA} to WSC found in MA windrow during composting revealed that the water extracted contained more biodegradable OM than the water extracted in GW and contributed a higher decomposition in the MA windrow during the thermophilic phase.

3.3. ^{13}C NMR Spectroscopy

The examination of the change of proportions between the different forms of C studied by ^{13}C NMR spectroscopy allows the monitoring of C transformations occurring during composting (Zbytniewski et al. 2002). Figure 2.3 shows the solid state ^{13}C NMR spectra of day 19 and day 175 compost samples during the composting period. The transformations of the biomolecule components in both GW and MA windrows were analyzed by comparing the spectra from final stage (day175) to the thermophilic stage (day 19) of the same windrow. The relative distribution of signal intensity in the ^{13}C NMR spectra in different regions of the NMR of the two composts at days are given in Table 2.3. The chemical shift range between 0-50 ppm is assigned to alkyl groups including methylene groups in aliphatic rings or chains (Vane et al. 2006). The signal at 56 ppm typically associated with O-CH_3 groups in lignin (phenolmethoxyl of coniferyl and sinapyl moieties) and in hemicellulose (glucoronic acid in xylan) (Gómez et al. 2007). The 50-110 ppm region that includes a peak at 72 ppm is assigned to the overlapping resonances of C2, C3 and C5 in the pyranoside structure of cellulose and hemicellulose (Spaccini and Piccolo 2007).

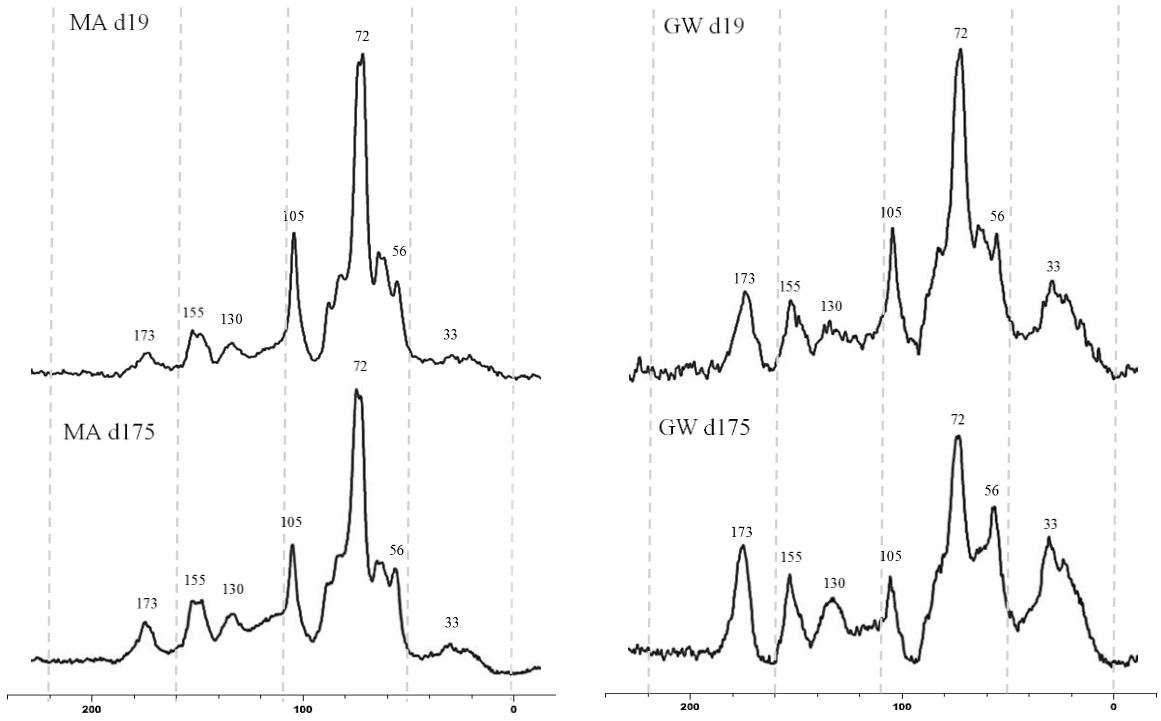

Figure 2.3: The ^{13}C CP/MAS NMR spectrum of the compost samples from day 19 and day 175 of GW and MA windrows.

Table 2.3: Relative distribution of signal intensity in the ^{13}C NMR spectra of MA and GW composts

Sample	Alkyl C 0-50 ppm	O-alkyl C 50-110 ppm	Aromatic C 110-160 ppm	Carbonyl C 160-220 ppm	aromaticity [110-160 ppm / 0-160 ppm]
GWd19	18.7%	53.4%	17.1%	10.8%	19.2%
GWd175	24.0%	45.4%	18.0%	12.6%	20.5%
MAd19	10.9%	61.1%	17.8%	10.2%	19.8%
MAd175	7.1%	57.4%	23.9%	11.5%	27.1%

The region also includes oxygenated alkyl carbons associated with lignin. The 100-110 ppm region is where anomeric carbon of carbohydrate units are typically found and a strong peak at 105 ppm is typical of the anomeric C1 of glucose units (Martinez-Sabater et al.

2009). The weak broad band centered around 130 ppm was characteristic of unsubstituted aromatic C, including C_1 quaternary carbons of syringyl and guaiacyl lignin units and the C_6 carbon of guaiacyl (Keeler et al. 2006; Martinez-Sabater et al. 2009). The 150-160 ppm region is assigned to oxygen substituted aromatic C, including both C-OCH_3 and C-OH groups (Keeler et al. 2006; Inbar 1992). This region can also be assigned to C_4 carbons of guaiacyl units involved in ether linkages to C_β of adjacent lignin units (Inbar 1992). The region between 160 and 200 ppm in the spectra is assigned to carbonyl/carboxyl carbons of carboxylic acids, esters, aldehydes and amide groups (Inbar 1992).

A decrease in the region of O-alkyl groups corresponding to the degradation of carbohydrates during stabilization was observed between thermophilic (day 19) and maturation stages (day 175) in both windrows (8.0% and 3.7% in GW and MA windrows respectively). The increase of aromatic C intensity (6.1%) and aromaticity (7.21%) in the MA windrow suggested that there was an accumulation of modified lignin which is rich in aromatic and phenolic rings after the high rate of carbohydrate decomposition (Fialho et al. 2010). Chen (1989) suggested the increase in % aromaticity might be attributed to a higher level of modified lignin found in the stabilized compost. The peak intensity of carbonyl C increases (1.8% and 1.4% in GW and MA windrows respectively) after maturation indicated the oxygen supply was sufficient to support aerobic degradation of OM in compost of both windrows. An increase of aliphatic C observed in GW windrow with increasing composting time was in agreement with the assumption that simple alkyl groups were generated during the microbial degradation of polysaccharides and an increase could be caused by the accumulation of aliphatic material due to its high resistance to degradation (Chen 2003). On the contrary there was a decrease of aliphatic compounds with respect to

a net decrease of O-alkyl C in the MA windrow. A possible explanation is that there was a significant amount of O-alkyl C present (mainly carbohydrate) in the initial stage (day 2) being transformed even in the early stage of MA window. The new aliphatic C generated by the decomposition was shown in the day 19 spectrum. Even though there were simple alkyl group produced by the microbial activity which utilizes and transforms the polysaccharides during the early stage of composting, the newly formed and existing alkyl groups might have been consumed by microbial organisms to support the high rate of microbial activity found in the MA windrow (Albrecht et al. 2008; Tang et al. 2006). Therefore it may have been better to conduct the ^{13}C NMR analysis on day 2 sample in order to investigate the relationship between the change of the alkyl C and O-alkyl C during the initial stage of composting in MA windrow.

A decrease in the proportion of carbohydrate and an increase in relative contribution of protein in total OM were observed in both windrows. An increase proportion of the protein in the OM during composting can be explained by the microbial enrichment during composting (Martinez-Sabater et al. 2009). An increase in the relative intensity of the lignin signal region in the MA compost contributed by the decrease of alkyl C and O-alkly C during composting. This indicated that the original lignin present in the initial substrate was hardly degraded and the lignin component in the windrow was transformed without loss in aromaticity and accumulated over time. Overall, the stability of the GW compost was indicated by a larger decrease in O-alkyl C and an overall relative increase in aliphatic C while the stability of MA compost was indicated by an increase of both aromaticity and the accumulation of lignin. An increase in relative intensity of the carbonyl C signal region

was consistent with an increase in the content of humic substances with increasing composting time.

3.4 Thermal analysis

Thermogravimetric analysis (TGA) and Differential Scanning Calorimetry (DSC) analysis were used to determine the mass loss profiles and the heat difference of a total of eight compost samples (GW d2, d9, d77 & d77 and MA d2, d19, d77 & d175). The TGA thermograms and DSC curves obtained are shown in Figure 2.4, while summary information of the percentage loss is included in Table 2.4. In DSC, temperature was plotted against the difference in amount of heat required to heat up the sample and the reference to the same temperature. An endothermic dehydration reaction was first observed at a lower temperature range (up to 105^{o}C) in DSC curve. A typical bimodal distribution (two distinct exothermic regions) was also found between 200^{o}C to 550^{o}C in most samples from both windrows. The first noticeable thermal oxidation generally occurred between 200^{o}C and 400^{o}C in the GW samples with a peak at about 340^{o}C and was followed by the second exothermic signal with higher intensity that was observed between 400^{o}C and 550^{o}C with a peak at about 475^{o}C. In MA sample similar patterns were also noticed in the day 2 and day 19 samples with two peaks at about 320^{o}C and 475^{o}C in the first and second exothermic regions respectively.

However three distinct regions corresponding to three exothermic reactions were observed in the day 77 and 175 of MA samples. The DSC curve from MA d77 sample had two exothermic reaction signals with peaks at 326^{o}C and 423^{o}C followed by a less intense reaction with a peak at 482^{o}C. Even though the first and third peaks of MA d175 sample

had shown a similar pattern with MA d77, the second peak was not clearly noticed. The TGA thermogram of GW samples showed similar weight losses pattern regardless the time of sampling. The temperature ranges in which the weight loss occurred are shown in Table 2.4 and the thermal behaviour of GW composts can be comprehended as followed : (1) The OM in the GW compost samples oxidized in the range between 150^{o}C and 400^{o}C corresponded to a less condensed component such as polysaccharide and amino acids. This first region was dominant throughout the composting process with the highest values of weight loss in the compost sample from day 2 (61.1%) followed by day 19 (59.3%), day 77 (57.1%) and finally day 177 (56.7%). (2) The weight losses occurred in the second exothermic region from GW compost samples with respect to more condensed OM such as the aromatic components of lignin and humified materials maintained around 36-38% throughout the composting period. (3) The weight losses that occurred above 550^{o}C from GW compost samples (OM with highest stability containing highly condensed aromatic substances) contributed to a small proportion of the overall OM content with the highest mass losses (5.4%) for day 175 followed by day 77 (4.2%), day 19 (3.6%) and day 2 (3.2%).

The increasing mass losses found as the composting process advanced can be explained by the increased of complexity of OM. Since organic C was utilized as main energy source through respiration and released as CO_2 during composting (Bernal et al. 2009), higher mass loss in the region of 550^{o} C above indicated less organic C can be utilized. An overall 2.2% increase (from 3.2% to 5.4%) within the range of 400^{o}C-550^{o}C indicated an increase of thermal stability of OM with increasing composting time in GW window.

Substrates from the MA windrow were more heterogeneous compared to the GW window, the thermal behaviour of MA samples from more advanced stages (day 77 and 175) were quite different from those of day 2 and 19. The thermal behavior of MA composts can be comprehended as followed: (1) The first exothermic peak corresponding to readily oxidized materials such as small carbohydrate molecules progressively moved to a higher temperature at the later stages of composting of the MA substrate. (2) A much more noticeable weight loss occurred between 375^{o}C and 450^{o}C in both the day 77 (23.1%) and 175 (21.4%) MA compost samples compared to samples from day 2 (15.7%) and 19 (16.9%). (3) Much less intense peaks between 450^{o}C and 550^{o}C in DSC curve were observed in the more advanced composting stages.

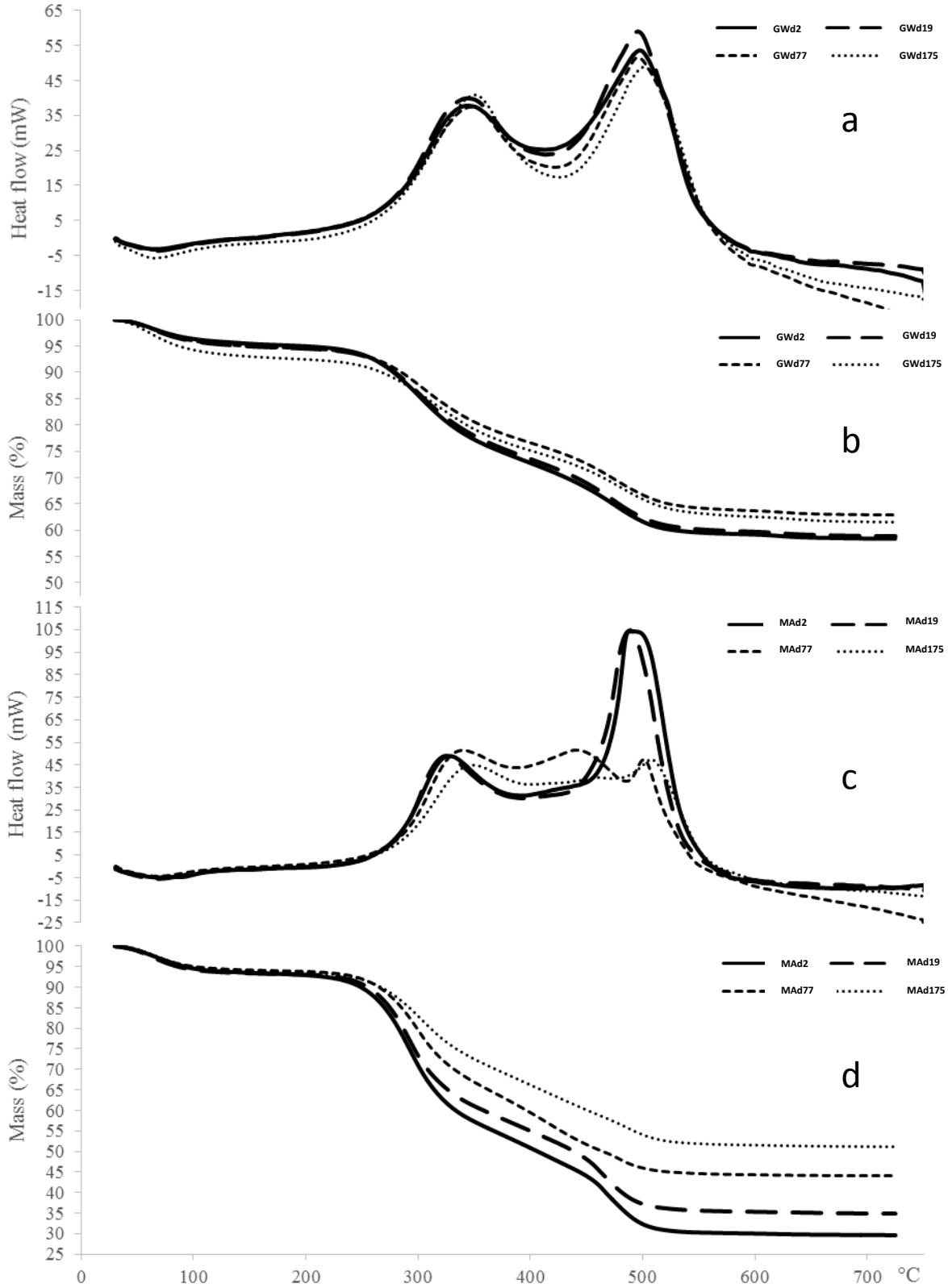

Figure 2.4: TGA Thermograms and DSC curves of the samples collected at different times of the composting process. (a) DSC curve of GW windrow; (b) TGA Thermograms of GW windrow; (c) DSC curve of MA windrow; (d) TGA Thermograms of MA windrow.

Table 2.4: Weight losses (% of air dried mass and % of OM in bracket) of the compost samples corresponding to the main peaks shown in the thermogram and the temperature ranges (°C) in which they occurred.

Windrow	Temperature ranges (°C)	Sampling day 2	19	77	175
		%			
GW windrow	0-150	4.5	5.1	5.0	6.9
	150-400	22.7 (61.1*)	21.4 (59.3*)	18.4 (57.1*)	17.9 (56.7*)
	400-550	13.2 (35.7*)	13.4 (37.0*)	12.4 (38.7*)	11.9 (37.9*)
	550-750	1.2 (3.2*)	1.3 (3.6*)	1.4 (4.2*)	1.7 (5.4*)
	Ash (TG)	58.4	58.8	62.9	61.6
MA window	0-150	6.5	6.6	5.9	6.1
	150-375	39.5 (61.7*)	35.4 (60.5*)	30.9 (61.6*)	24.6 (57.5*)
	375-450	10.0 (15.7*)	9.9 (16.9*)	11.6 (23.1*)	9.2 (21.4*)
	450-550	13.8 (21.5*)	12.5 (21.4*)	7.1 (14.2*)	8.3 (19.3*)
	550-750	0.7 (1.0*)	0.7 (1.2*)	0.6 (1.1*)	0.7 (1.7*)
	Ash (TG)	29.6	34.9	44.0	51.1

*percentage out of total %OM which is equal to [100-ash (TG)]

The observed differences in the MA compost sample with increasing composting time suggested an intense OM transformation as composting continued. During the composting process, the progressive reduction of carbohydrates accompanied with the increase in intensity between 375°C-450°C in DSC curve indicated organic polymers were generated from the stabilization process of the MA substrate (Gomez et al. 2007). The drop (from 21.4% to 14.2%) from the range of 450°C-550°C found between day 19 and 77 of the MA samples might indicate that the consumption of more resistant OM occurred in the MA windrow in between the thermophilic and cooling phase. However, it was also noted that the increase (from 14.2% to 19.3%) within the range of 450°C-550°C between day 77 and 175 of MA compost samples might reflect the process of humification that occurs in the maturation phase (Otero et al. 2002). The different thermal behaviour between the

windrows suggested that the transformation of OM in the windrows was highly dependent on the types of input materials. According to the ^{13}C NMR spectra and the relative signal intensity (Table 2.3), an increase of 6.1% aromatic C and decrease of 3.7% of O-alkyl were found in MA windrow during composting whereas only 0.9% increase of aromatic C and decrease of 8% of O-alkyl were found in GW windrow. This indicates the chemical difference between the two input materials which explained the distinct thermal behavior. A reduction of O-alkyl C signal intensity observed in ^{13}C NMR spectra and a decrease of amount of OM corresponding to the intensity about 300^{o}C in DSC curve indicated the gradual decrease of carbohydrate content in the substrates. In addition to the degradation of easily degradable OM such as aliphatics, cellulose, hemicellulose and protein in the early stage of composting shown by ^{13}C NMR spectra, TG and DSC analysis, optimum composting conditions were assumed to occur in both windrows during the composting periods. Lignin degradation was considered to be very low in both windrows as was shown in ^{13}C NMR spectra. The chemical nature suggested by ^{13}C NMR spectra and the thermal behavior of both the GW and MA composts showed the change of composition of OM between different phases and also indicated final products from both windrows had been stabilized by the OM transformation.

4. Conclusion

This study demonstrated that chemical methods can be used to monitor OM transformations occurring during the composting process of open windrow systems. Indicators includes a significant loss of OM, low values of WSC, reduction of O-alkyl C signals intensity in ^{13}C NMR and the decreased intensity at about 300^{o}C in DSC curve suggested a continued degradation of organic C particularly carbohydrates during the 175 days of composting. In particular, the analysis of WSC in aqueous extractions with increasing composting time provided a powerful tool to monitor compost stability and generated similar results irrespective of the type of initial feedstock. The OM transformations and the WSC change in the aqueous extract indicated that there was a substantial change of OM yielding highly stabilized and more thermally resistant humic-like substances over the composting time. Both the final compost products as described in this study were confirmed to be highly stabilized and matured at 175 days of composting under the local weather conditions. In conclusion, this study not only enhanced the understanding of composting processes of open windrow systems with GW and MA wastes but also confirmed that WSC can act as a reliable measure of stability. The OM transformation can be followed by ^{13}C NMR and thermal analysis regardless of initial organic inputs in the open windrow systems.

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CHAPTER THREE – EVOLUTION OF MICROBIOLOGICAL PARAMETERS DURING COMPOSTING OF GREENWASTE AND MANURE WASTE

1. Introduction

Soil provides habitats for soil organisms and plays an important role in the recycling system of nutrients and organic matter. It stores water and nutrients for biological activity and provides natural environment for seed germination and root growth. Food security is under pressure when the quality of soil around the world continues to decline while the human population continues to increase. Soil organic matter is considered as a major component for governing the nutrient cycling and maintaining soil fertility. Applying composts manufactured from organic wastes to depleted agricultural soil can help to restore fertility in soil (Senesi 1989). Composting is a controlled, microorganism facilitated process and of all the stability and maturity parameters available, methods which assess microbial activity during composting seem to be the most acceptable methods. (Fourti et al. 2011; Mondini et al. 2002; Wang et al. 2004; Goyal et al 2005; Liu et al 2011). A large number of techniques have been developed to examine microbial profile and activity at different phases of composting (Horwath et al. 1996; Mondini et al. 1997). In this chapter, the microbiological properties of the two open turned windrow systems (GW and MA) previously described, were examined. The determination of microbial respiration by measuring CO_2 production with the Solvita ® maturity test, provides a simple and practical approach for monitoring respiration (Wang et al. 2004; Barrena Gómez et al. 2006; Changa et al. 2003). This method is simple and efficient. It involves using a colour system to indicate the level of CO_2 released per unit volume (Solvita 2014). The hydrolysis of fluorescein diacetate (FDA) to monitor the change of microbial activity and the change of

microbial biomass during composting have been used to determine compost stability in various studies. (Horwath et al. 1996; Mondini et al. 1997; Charest et al. 2004; Ayed et al. 2007; Fourti et al. 2011; Ryckeboer et al. 2003; Cayuela et al. 2008). Instead of examining individual enzyme activity, measurement of the rate of fluorescein diacetate (FDA) hydrolysis has been used frequently to evaluate total enzymatic activity of the microbial populations present in soil (Schnurer and Rosswall 1982; Craft and Nelson 1996; Haynes 1999; Cayuela et al. 2008). The FDA hydrolysis activity has been suggested as a valid parameter for measuring the degree of stability of the composting material and correlated with important stability indices (García-Gómez et al. 2003; Ryckeboer et al. 2003).

Since there are no reports in the literature that compared the Solvita ® respiration test to the FDA hydrolysis activity and microbial biomass C and N among different stages of composting, the Solvita ® respiration test was compared with the activity of FDA hydrolysis and changes in microbial biomass C and N. This study aimed to evaluate the microbial activity at different stages of composting by determining the rate of FDA hydrolysis and the microbial biomass C and N. The results were correlated with the Solvita® respiration test. We aimed to provide a useful evaluation of using the Solvita® maturity test for future applications for on-farm composting conducted in Western Victoria, Australia.

2. Materials and Methods

All biological stability parameters were determined on the air dried compost samples from day 2, 19, 77 and 175 of composting. A total of 11 samples (day 0, 2, 5, 12, 19, 26, 40, 54, 77, 105 and 175) with the same preparation methods were examined with Solvita ® maturity test. Details of the preparation of the sample can be found in Chapter 2. All results are expressed on an air-dried basis and represent the mean of 4 replicates.

2.1. Microbial Biomass

One of the frequently used methods for determining the size of microbial biomass community is the fumigation-extraction (FE) method (Vance et al. 1987). In the FE method, extracted biomass C (E_C) is measured by subtracting extracted organic C (with 0.5M K_2SO_4) of a non-fumigated sample from extracted organic C of a 24h fumigated similar sample (Cai et al. 2011). The biomass C (B_C) and the biomass N (B_N) can then be calculated by multiplying the E_C and E_N with K_E which refers to the proportion of the microbial extracted (Mondini et al. 1997; Brookes et al. 1985; Horwath and Elliott 1996). Briefly 5g of wet compost (50% moisture) was fumigated with ethanol-free chloroform ($CHCl_3$) for 24h at ambient temperature in a desiccator. Organic carbon was extracted from the fumigated and non-fumigated compost extracts after mixing them using a reciprocal shaker with 40ml of 0.5M K_2SO_4 for 20min. After centrifugation, the supernatants were passed through Whatman no. 6 filter papers. The extracts were frozen until C and N were quantified by dichromate digestion, (Walkley Black method), and ninhydrin-reactive N methods respectively (Joergensen and Bookes 1990). The K_E values used were 2.64 and 2.22 for B_C and B_N, respectively (Brookes 2001).

2.2. Microbial Activity

Microbial activity was monitored by measuring the rate of fluorescein diacetate hydrolysis (FDA_{HY}) with a procedure adopted from methods described by Craft et al. (1996), Aryantha et al. (2000) and Inbar et al. (1991). The compost sample equivalent to 0.5g dw (dry weight) was placed in a plastic vial. To the vial containing the compost, 19ml of sodium phosphate buffer (60 mM, pH 7.6) was added, followed by 1ml of FDA solution (400µg/ml in acetone). The mixture was incubated on a horizontal shaker (120 rpm) at 22ºC for 1h. 20mL of acetone was then added to stop the hydrolysis. Samples were centrifuged for 2 min at 4,000rpm to remove particulates. The absorbance of all the solutions, after being filtered with Whatman No.1 filter paper, was determined at a wavelength of 490nm using a spectrophotometer. To compensate for background absorbance from soluble sample components, absorbance blanks that consisted of a buffer extract (0.5g of compost sample in 20ml of 60 mM sodium phosphate buffer, pH 7.6) from each compost type prepared as described above but without the addition of FDA were used. A standard curve was also prepared for each compost to which 0, 25, 50, 100, 150, and 200µg of FDA was added with 19ml of buffer. The volume was adjusted to 20mL by topping up with buffer and placed in a boiling water bath for 60 minutes. After cooling to room temperature, 0.5g of compost sample and 20mL of acetone were then added to each standard. Samples were centrifuged for 2 min at 4,000rpm to remove particulates. The absorbency of all the solutions, after being filtered with Whatman No1 filter paper, was determined at a wavelength of 490nm using a spectrophotometer. Any samples with an absorbance reading out of the linear range were diluted and measured again. The microbial activity ($FDA_{HY,}$ µg fluorescein per g of compost per hour) was calculated by the

application of the following equation [1]. The value of *q*FDA, FDA hydrolysis activity per unit (mg C) of biomass carbon was calculated by dividing FDA_{HY} by B_C.

$A^o490 = a + b*[FDA]$ [1]

Where A^o490 and [FDA] are referred to the absorbance at 490nm and the concentration of fluorescein produced respectively. Then *q*FDA, FDA hydrolysis activity per unit (mg C) of biomass carbon, was calculated by dividing FDA_{HY} by B_C.

2.3. Solvita® Maturity Test

The Solvita® test measures CO_2 evolution and NH_3 emissions simultaneously. Two individual gel-paddles (CO_2 evolution and NH_3 emission) are inserted into moist compost sample (around 50% WHC) which is first allowed to equilibrate at room temperature in a sealed jar and incubated for 4 hours. The color change of the gel-paddle is observed after the incubation and the relative amount of CO_2 evolution and NH_3 emission are determined by photometer designed for the gel-paddle (Changa et al. 2003 ; Solvita 2012). The amount of CO_2 evolution was displayed in the meter as (1) $\%CO_2$ in the jar and (2) a colour scale ranges from yellow to purple.

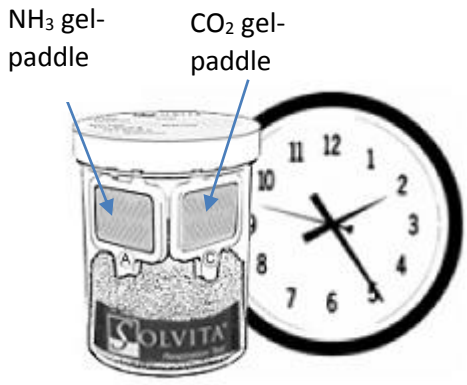


Figure 3.1: Solvita maturity tests. Source : Solvita (2012).

The colour of CO_2 paddle ranged from yellow to purple on a scale of 1 to 8 (where purple or no.8 referred to the lowest CO_2 content measured in the clear container). The colour of NH_3 paddle ranged from dark blue to yellow on a scale of 1 to 5 respectively (where yellow or no.5 referred to lowest volatile NH_3 concentration measured in the clear container). Finally the two values collected from the photometer were used to determine the maturity index using the table of compost maturity index calculator table in the Solvita® kit manual. A compost is considered to be matured when the average value is between 6 and 8 from the compost maturity index (Figure 3.2). Two charts are shown below for determining the Solvita maturation index and status of the composting process. (Figure 3.2 & 3.3)

			SOLVITA® CARBON DIOXIDE TEST RESULTS							
			1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
SOLVITA® AMMONIA TEST RESULT	**5**	Very Low / No NH_3	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
	4	Low NH_3	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
	3	Medium NH_3	**1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
	2	High NH_3	**1**	**1**	**1**	**2**	**3**	**4**	**5**	**6**
	1	Very High NH_3	**1**	**1**	**1**	**1**	**1**	**2**	**3**	**4**

Figure 3.2: Solvita maturation index calculator (adapted from the Solvita 2012).

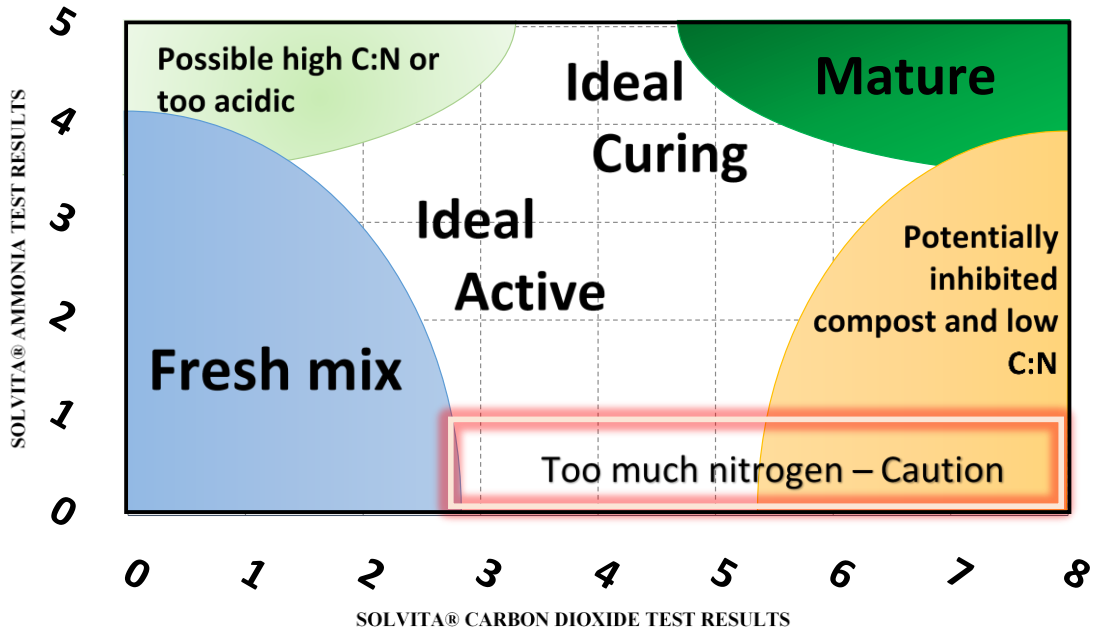


Figure 3.3: Status of composting process (adapted from the Solvita 2012). Compost is matured when it has a CO_2 value of 6-8 and an Ammonia value of 4-5.

2.4. Statistical analysis

Statistical analyses were carried out with SPSS Version 22 (IBM Corporation) for Windows. One-way independent analysis of variance (ANOVA) was used to investigate the impact of composing time on the effects of each of the biological parameters, including B_C, B_N, FDA_{HY}, *q*FDA, and CO_2 evolution of the compost samples. Data that were determined as heterogenous after the examination of the residual plots was transformed (log base-10) before further ANOVA analysis. These variables included B_C, B_N, FDA_{HY}, *q*FDA, and CO_2 evolution. All statistical tests were evaluated at the 95% confidence level. Where the P-values were significant, the F-value was checked at a 95% confidence level using orthogonal contrasts. Pairwise comparisons were undertaken using least significant differences (LSDs). The relationships between stability parameters including B_C, B_N, FDA_{HY} and CO_2 evolution were tested using spearman rank correlation.

3. Results

3.1. Microbial Biomass and Microbial Activity

The ANOVA revealed a significant impact of composting time on each of the microbial biomass C, microbial biomass N and FDA hydrolysis in both MA [B_C ($F(3,12) = 65.17$, $p<0.0001$), B_N ($F(3,12) = 56.24$, $p<0.0001$) & FDA_{HY} ($F(3,12) = 66.37$, $p<0.0001$)] and GW windrows [B_C ($F(3,12) = 14.67$, $p<0.0001$), B_N ($F(3,12) = 16.19$, $p<0.0001$) & FDA_{HY} ($F(3,12) = 69.44$, $p<0.0001$)]. Microbial biomass (B_C and B_N) evolution, FDA hydrolysis activity (FDA_{HY}) and *q*FDA index are displayed in Figure 3.4. Microbial biomass C (B_C) content of the samples with increasing composting time started from 3.7 mg C g^{-1} dw (day 2) to 0.5 mg C g^{-1} dw (day 175) in GW windrow and started from 12.2 mg C g^{-1} dw (day 2) and reduced to 2.8 mg C g^{-1} dw (day 77) in MA windrow. B_C was observed to be highest on day 2 samples from both windrows with 4.0 mg C g^{-1} dw in GW windrow and 13.2 mg C g^{-1} dw in MA windrow. B_C decreased during the 175 days of composting in both the GW and MA piles to values of 14.4% and 31.5% of the initial values respectively. Also the B_C to %C ratio, as a measure for the relative microbial colonization, decreased from 0.15 to 0.03 and 0.36 to 0.13 in GW and MA windrows respectively during composting. A similar trend was observed in the microbial biomass N (B_N) contents; B_N declined to less than 0.1 mg N g^{-1} dw in the GW windrow after 77 days of composting and was found to have no further significant change until the end of composting ($p<0.11$). B_N was highest in day 2 samples from both windrows with 0.28 mg N g^{-1} dw and 1.12 mg N g^{-1} dw in the GW and MA piles respectively. B_N decreased during the 175 days of composting to a value which is 26.3% of the initial values of the GW windrow and 19.2% in the MA windrow. In both the GW and MA windrows the FDA_{HY} did not change significantly during composting within the early stages (day 2 and day 19)

and within the curing stage (day 77 and day 175). However significant decreases were observed in hydrolysis activity in both windrows, between day 19 and 77. FDA_{HY} measured from day 2 samples showed maximum values of 100.7 and 119.7 µg FDA g^{-1} h^{-1} in the GW and MA windrows respectively. FDA hydrolysis activity also indicated that no further significant change (p=0.63) occurred until the end of composting after day 77 in both windrows. For *q*FDA, the highest value was found in the GW d175 with 68.1µg FDA g^{-1} $h^{-1}$and lowest value was 5.0 µg FDA g^{-1} h^{-1} in MA d175. Comparing the two composting windrows, the lower *q*FDA was observed in the MA windrow in all sample days.

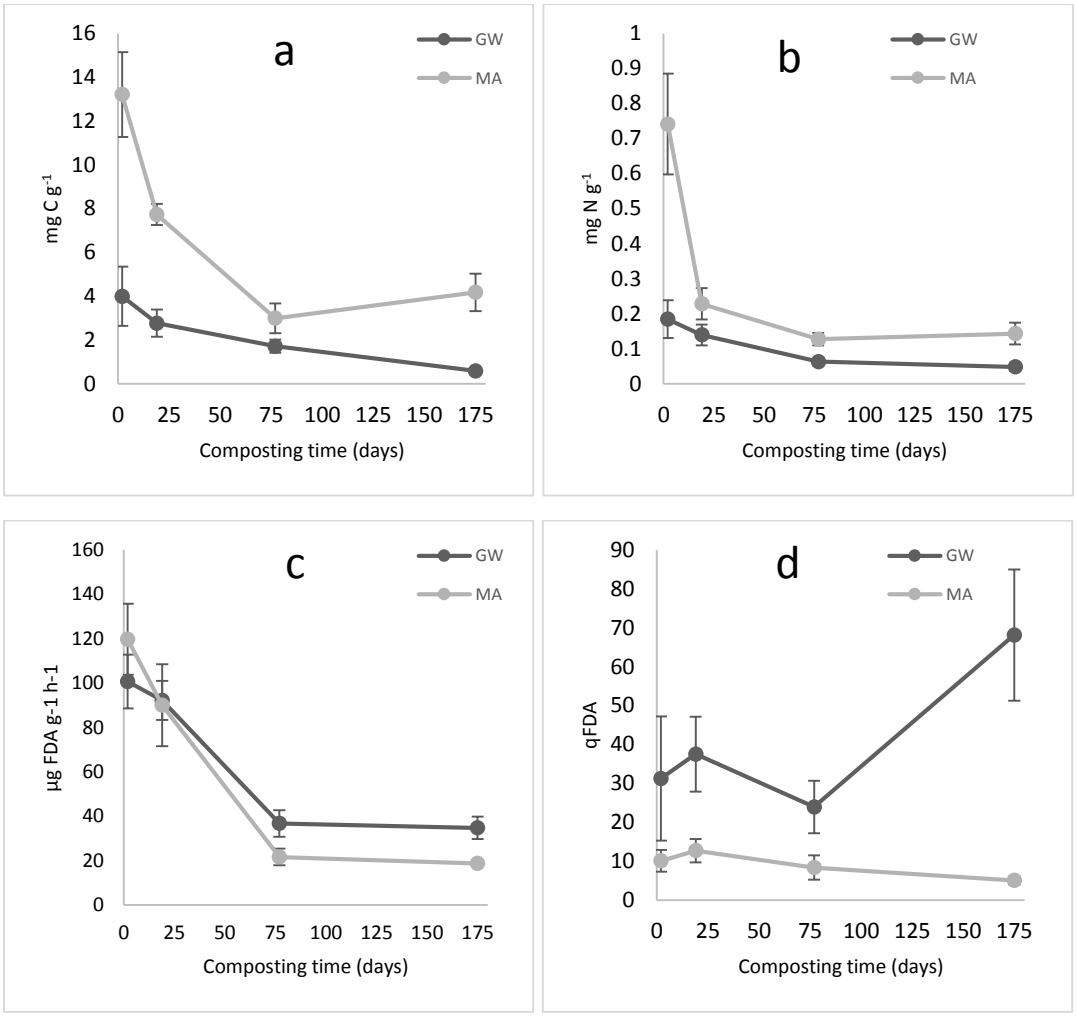


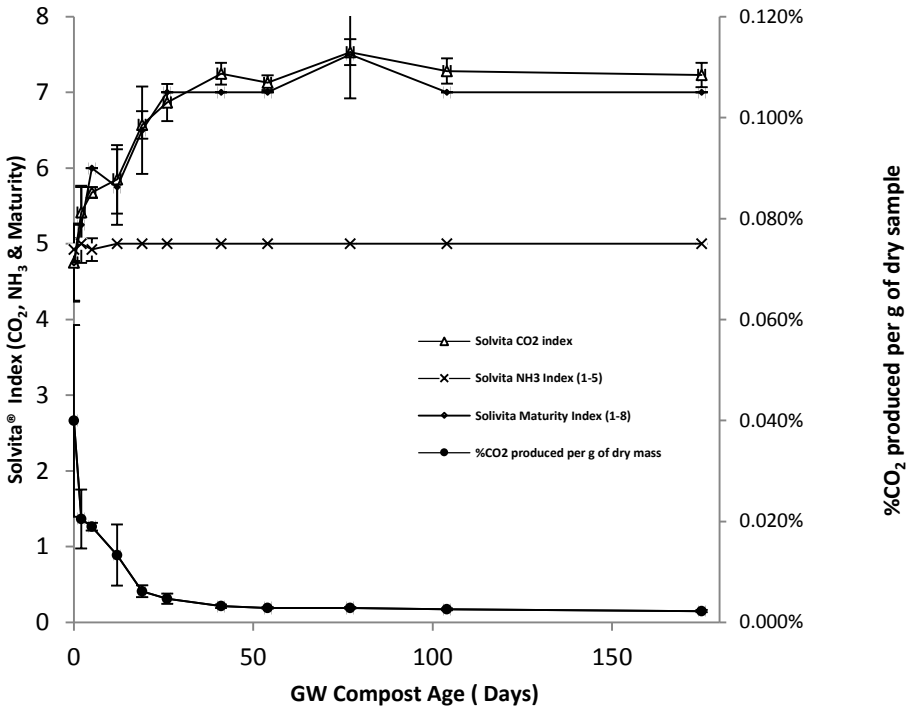
Figure 3.4: (a) Microbial biomass C (B_C) ; (b) Microbial biomass N (B_N) ; (c) FDA hydrolysis activity and (d) *q*FDA index during the composting process

3.2. Solvita® Maturity Test

The $\%CO_2$ emission during the 4 h incubation was first converted to $\%CO_2$ per g dried weight in the given volume (space in the container) by dividing the $\%CO_2$ emission by the dry weight of the sample. $\%CO_2$ released decreased from 0.0822% g^{-1} dw (Solvita ® CO_2 scale = 4.2) to 0.0048% g^{-1} dw (Solvita® CO_2 scale = 6.9) and from 0.0399% g^{-1} dw (Solvita® CO_2 scale = 4.8) to 0.0022% g^{-1} dw (Solvita® CO_2 scale = 7.2) in the MA and GW compost respectively after 175 days of composting. Test values between 4 and 6 indicated the microbial activity were moderately active while values above 7 indicated there was minimal microbial activity present in the samples.

According to the Solvita® NH_3 index for ammonia found in Figure 3.2, little NH_3 was emitted by the manure waste substrate with average values of 4.31, 4.30 and 4.54 from day 0, 2 and 5 respectively. All average values were above 4 showed that very low NH_3 was detected. In addition, all the samples from GW windows after day 12 and samples from MA windrow after day 41 has scored 5 in the Solvita® NH_3 test. These showed NH_3 emission from these samples were too low for the test paddle to be detected. The Solvita® maturity Index (1-8) was calculated using the calculator table provided in the manual (Figure 3.2). The maturity index showed similar patterns for both windrows ranging from 3 to 5 in the initial substrate to 6 to 7.5 from day 26 samples onwards. The maturity values between 3 and 5 indicated the substrates were at active stages while values between 6 and 7.5 indicated the substrate were at curing stages. The Solvita indexes including CO_2, NH_3 and maturity were shown in Figure 3.5.

a.



b.

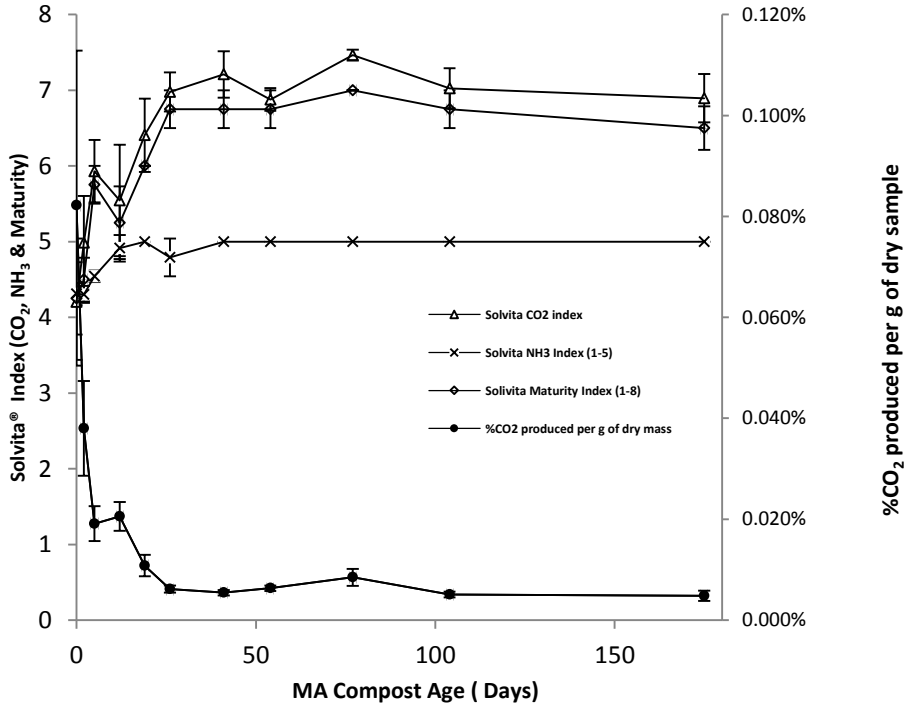


Figure 3.5: Average (n=4) trends in Solvita® CO_2, NH_3, Maturity index and %CO_2 produced per g of dry mass during 4 h incubation windrows for day2,5,12,19,26,41,54,77,104 and 175 samples in a. GW windrow and b. MA windrow. Each value represents the mean ± one standard deviation.

4. Discussion

Air dry samples were used to determine the evolution of microbial activity during composting in this study for the two following reasons. Firstly, using dried samples is important because they enhance the applicability of the method. The low water found in the air dried sample is also in the moisture content range of most compost samples after a long period of curing (Mondini et al. 2002). In addition the use of air dried samples increases the versatility of the methods used for measuring microbial activity, since it is not always practical to measure biological parameters right after sampling (Wu and Ma 2001).

A decrease in respiration accompanied with a lower rate of FDA hydrolysis was observed in the later stages of composting and this indicated a decline of the OM quality and its high level of resistance to be degraded by the microorganisms. A progressive decrease in the rate of FDA hydrolysis observed during the composting period suggested a significant decrease of overall microbial activity between the thermophilic (day 19) and curing phases (day 77). The values of FDA activity in this study were similar to the values found by Gattinger et al. (2004) (40-160 μg FDA g^{-1} h^{-1}) and the final products from both windrows had values below the limit (50 μg FDA g^{-1} h^{-1}) proposed by Komilis (2011) et al. who correlated FDA hydrolysis activity with various respiration indices using 13 compost samples including manure and green waste based composts.

Figure 3.4 shows that the B_C in the rewetted samples collected at the four stages of composting showed that there was a significant decrease in B_C of GW ($p<0.01$) and MA

($p<0.01$) windrows from 4.0 ± 2.7 (day 2) to 2.8 ± 1.2 (day 19) mg C g^{-1} and from 13.2 ± 3.9 (day 2) to 7.7 ± 1.0 (day 19) mg C g^{-1} respectively. However, no further significant change was found until day 175. This result followed the same trend as described by Mondini et al. (1997). Even though similar patterns were also observed in CO_2 production and FDA hydrolysis activity, the declines in the GW windrow were less pronounced. It could be explained by the relatively high ash content (less % of OM in GW compost samples shown in Chapter 2) and lower amount of readily degradable materials (less mass change occurred in GW compost samples in TGA shown in chapter 2) in the initial substrate of GW windrow. The results also indicated that the microbial activity and biomass were highly dependent on the chemical composition of the initial substrate.

According to the Solivita® test kit manual (Solvita 2012), the Solvita® maturity index values of the final products (day 175) of MA and GW windrows (Index 6-7) indicated that the composts were matured after 77 days and thus suitable for agricultural use. Low respiration rates were observed from day 41 onwards after a rapid drop during the mesophilic and thermophilic stages (composting up to day 19) and suggested a rapid decomposition of OM had occurred in the early stage of composting. Further evidence such as significant decreases of %OM and the FDA hydrolysis activity between day 19 and 77 in both windrows also suggested ideal conditions for composting were established. Lower content of easily degradable OM content (as shown in WSC) as well as the low respiration rate indicated that the substrates were low in microbial activity after 77 days of composting and that the substrates in the composting piles would have sufficient time for maturation during the curing period. No NH_3 was detected with the gel paddle (Index 5) from any samples after days 12 and 41 for the GW and MA composts respectively. This suggests

that the potential phytotoxicity from ammonia was very low. The unexpected low ammonia emissions in Solvita test from the MA samples in the first week (day 0, 2 and 5) suggested that the ammonia was lost as a result of volatilization in the drying process during the sample preparation. However the first week of composting is generally expected to have high NH_3 content releases in manure waste composting piles (Bernal et al. 2009) and relatively high content of NH_3 were still detected by analyzing the aqueous extractions of day 2 compost samples with chemical analysis methods (discussed further in the next chapter). This may suggest that Solvita ® ammonia test was not appropriate to test stability of dry compost samples as it failed to detect the initial N-transformation of the pile. Solvita ® tests are designed for samples collected directly form the composting tile without delay and therefore may produce a misleading result when the compost product is tested without maintaining an adequate moisture. However the results from Solvita ® maturity tests were still able to show that both GW and MA windrows remained relatively biologically active in the first 41 days of composting.

In this study, both FDA_{HY} and B_C were observed to be strongly correlated to water soluble C (WSC) ($r=0.91$ for FDA_{HY} and 0.961 for B_C, $p<0.01$) (Table3.1). Therefore it can be concluded that both FDA_{HY} and B_C can both be considered as reliable methods for measuring compost stability.

Table 3.1. Correlation between Solvita® CO_2 with microbial biomass C (B_C), microbial biomass N (B_N), FDA hydrolysis activity (FDA_{HY}) and water soluble C (WSC) during the composting process (n=32).

	B_C	B_N	FDA_{HY}	*Solvita® CO_2*	*WSC*
B_C	1	.942**	.876**	.729**	.961**
B_N		1	.774**	.796**	.927**
FDA_{HY}			1	.645**	.910**
Solvita® CO_2				1	.843**
WSC					1

**. Correlation is significant at the 0.01 level (2-tailed).

Further analysis of the data collected from FDA_{HY} and B_C can be used to calculate the index of *q*FDA, FDA hydrolysis activity per unit of biomass carbon (mg C). The index has been used to evaluate soil disturbance and stress due to the presence of xenobiotics (Perucci et al. 2000). The evaluation of the index of *q*FDA may help us to examine the degradability of OM present in the composting substrate and enhance our knowledge of the composting process. However literature regarding the use of *q*FDA to evaluate the response of microorganisms to the rapidly changing conditions between different composting phases is scarce. The metabolic quotient, an index of the rate of respiration per unit of biomass, had been used to determine compost stability by Islam et al. (2012). This study showed a matured compost generally had a low metabolic quotient and vice versa. Horwath and Elliott (1996) also showed that microorganisms in the higher temperature phase (50^{o}C) had a lower efficiency of using OM in substrate than the treatment with lower temperature (25^{o}C) in composting of straw. Therefore the greater metabolic quotient indicates more energy is required to spend on decomposition per unit biomass.

Since FDA hydrolysis activity and CO_2 production were found to be strongly correlated as shown by Gattinger et al. (2004), so *q*FDA may be used in the same manner. A decreasing trend of *q*FDA in MA windrow in this study could indicate the presence of better adapted microbial population and their ability to better utilize C source especially at the maturation stage of composting (Anderson and Domsch 1990). However an irregular fluctuation of *q*FDA in GW windrow had shown a different results. An irregular result was observed in the GW substrate with increasing time of composting and the highest *q*FDA value was found in the d175 sample. The high value observed in the later stage of composting may be due to the increase of importance of functions mediated by extracellular enzymes such as esterases, proteases and lipases in GW mature compost (Gattinger et al. 2004; Sánchez-Monedero 2008). Schwab et al. (1994) showed FDA activity was not correlated well for fungi and actinomycetes during the composting of organic waste. Therefore, in the GW compost where fungi may have been the dominant microorganism in the later stage of composting, higher FDA activity than in the MA was expected and observed in the GW (García-Gómez et al. 2003) windrow. Furthermore, lower values of *q*FDA accompanied by relatively high microbial biomass were found in all the MA samples when compared to the GW sample from the same composting stage. The observed high microbial population found in the MA compost might be due to the presence of microorganisms which were derived from the animal digestive tract (Gattinger et al. 2004).

To evaluate the ability of the Solvita® respiration test to monitor the composting process in the GW and MA open turned windrows, correlations were made between Solvita® CO_2 and B_C, B_N and FDA_{HY}. The Solvita® CO_2 test was found to be moderately correlated at a high probability level to both microbial biomass C and N and FDA hydrolysis results (GW:

r=0.752 with FDA_{HY}, r=0.863 with B_N and MA: r=0.796 with B_N and r=0.729 with B_C , $p<0.01$ shown in Table 3.2). The results indicate that even though the Solvita® CO_2 test may not be strongly correlated to other stability parameters during composting, it still provides a good estimation of compost stability. The test can be used for monitoring the composting conducted on-farm given that it is quick, relatively cheap and easy to use.

Table 3.2. Correlation between Solvita® CO_2 with microbial biomass C (B_C), microbial biomass N (B_N), FDA hydrolysis activity (FDA_{HY}) and water soluble C (WSC) during the composting process of GW and MA windows (n=16).

Windrow	B_C	B_N	FDA_{HY}	WSC
GW	0.632**	0.863**	0.752**	0.900**
MA	0.863**	0.752**	0.900**	0.632**

**. Correlation is significant at the 0.01 level (2-tailed).

5. Conclusion

In this part of the study, comparison between two different organic waste composts in open turned windrows was made. This study showed that microbial biomass C and N and FDA hydrolysis activity were valid biological parameters to determine the stability of compost in two composting piles with OM of different origins. The *q*FDA index failed to indicate the stability of the composts, however, it may be possible to use the index to determine the efficiency of C utilization in compost piles. The Solvita® CO_2 test was found to be moderately correlated to microbial biomass and FDA hydrolysis results and can be used to provide a moderate estimation of compost stability for both MA and GW composting piles.

Taking into account all measurement from the FDA hydrolysis activity, microbial biomass and Solvita ® maturity test, it was found that biological stability had been reached after 77 days of composting. The extended period of curing in both GW and MA windrows allowed sufficient time for the final maturation process to occur and generate matured and partially humified products which are known to enhance soil properties and benefit plant growth. Results from the biological parameters used in this chapter along with the chemical and thermal stability measurement in the previous chapter confirmed that the final compost products were mature.

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CHAPTER FOUR – EVALUATING THE STABILITY OF COMPOST BY MEASURING THE INORGANIC NITROGEN CONTENT IN THE WATER EXTRACTION AND BY SIMPLE STRIP TESTS

1. Introduction

On-farm composting is likely to provide a way to a sustainable farming system that allows better utilization of farm waste by recycling nutrients and building up soil organic matter (Cooperband 2002; Puglisi et al. 2010). According to the Department of Food and Agriculture (DFA) in United States:

"*The idea of on-farm composting is to allow farmers to participate in resolving organic waste management issues while producing compost product that enhances soil management practices and reduces environmental risks associated with agricultural nutrient management practices*" (Barriuso et al. 1997; Piccolo et al. 2004).

Even though on-farm composting has been encouraged by governments around the world, it is not easy to conduct on-farm composting as it is a labour intensive practice for farmers who might have limited time available (Riggle 1994). Furthermore, the skill of monitoring the composting process requires significant effort and time to learn and develop. Each farm needs to find its own way to fit composting into their setting in order to make the practice sustainable. Milk is one of the major agricultural products in Victoria, and there are over 1500 dairies in Western Victoria (Dairy Australia 2009). In a dairy farm system, it is essential to optimize the value of the animal manure. Composting the manure waste allows dairy farmers to have compost made out of their own waste and be able to use it to

enhance both structure and fertility of the soil. Furthermore, applying compost can reduce the usage of synthetic fertilizers, as well as improving soil health by promoting desired soil organisms (Grobe 1997; Senesi 1989; Richard 2004).

As interest in on-farm composting grows, the need for reliable methods to determine if the compost is mature also increases. However, most methods can only be carried out in laboratory conditions, which is generally not feasible for a famer who requires reliable information at short notice. A quick and reliable on-farm test is necessary in order to allow farmers to manage their compost pile on-site, and allowing them to make an informed decision as to when the compost is ready to distribute in the field (Riggle 1994; Changa et al. 2003). Quick test kits such as the Solvita ® maturity test, which measure the CO_2 and ammonia release with gel-paddle test have been developed to allow farmers and composters to closely monitor their piles. However, the colorimetric paddle for the ammonia test in the Solvita® testing kit can only measure volatile ammonia, while the amount of nitrate in the samples cannot be detected (Change et al. 2003; Seekins 1996).

Nitrate represents a major form of inorganic N in the compost and its concentration is directly related to N-dynamics in the windrows. The ratio of nitrate to ammonium has been widely used to monitor the composting process and is related to the degree of degradation and OM transformation in composting (Tiquia et al. 2002; Huang et al. 2004; Wang et al. 2004). N transformation starts at the initial stage of composting when protein is first degraded. An excess amount of ammonium is produced during protein degradation and in some situations, N can be lost as ammonia gas (Huang et al. 2004; Leconte et al. 2009). Therefore compost which has a low C/N ratio often contains phytotoxic concentrations of

ammonia N and is not suitable as a growing medium for plants (Hubble et al 2010;Wang et al. 2004). On the other hand, an increased content of nitrate-N during the curing stage due to the increase of nitrification can be correlated to the maturity of compost (Castalsi et al. 2008; Zmora-Nahum et al. 2005). Low nitrification is found in the initial stage because the nitrifying activity is often hindered by higher temperatures and high competition for oxygen within the compost pile (Larney et al. 2008; Tiquia 2002; Bernal et al 2009). Consequently the small ratio of NO_3^- to NH_4^+ (high content of ammonia and low nitrate production in the initial stage) can indicate the instability of the composting piles while a high ratio of NO_3^- to NH_4^+ (low content of ammonia and high rate of nitrification) of a compost sample indicates a highly stabilized (matured) product. This behaviour could potentially be used as basis for developing a quick and meaningful test for compost maturity, if the information can be obtained from the compost pile. From environmental and agricultural points of view, N management for compost production can maintain the quality of the compost product by ensuring a successful degradation progress (Cooperband 2002; Puglisi et al. 2010).

Even though the change of concentration of ammonium/ammonia and nitrate content in water extracts of compost can be used as reliable parameters, these indicators are not widely used by farmers and composters due to the cost and time involved in sending compost for laboratory analysis. Estimation of nitrate and ammonia/ammonium content by test strips has been proposed for monitoring composting process in a few studies but are not often used by composters in monitoring the pile (Iglesias Jiménez and Perez Garcia 1989; Itävaara et al. 2010). As these test strips are already commercially available for water testing from aquarium shops, it is believed that these tests can easily be adopted by famers

and composters with minimal training. However, there was a need to develop and validate a simple method of obtaining an aqueous extract from the maturing compost, in order to apply the test strips to measure nitrate and ammonium in the aqueous extracts.

The Azo dye method was employed in the nitrate test strip while Salicylate chemistry was employed in measuring ammonia in the ammonia test strip. The Azo dye method in nitrate (NO_3^-) strip test measures the NO_3^- present in the sample by first reducing NO_3^- to NO_2^- with a reducing agent. The NO_2^- is then converted to nitrous acid in the presence of an acidic buffer. An aromatic amine is diazontized by the nitrous acid and couple with N-(1-naphthyl)-ethylenediamine to form a red-violet azo dye (Apps Laboratories). The amount of the red dye produced is used to estimate the concentration of nitrate. Salicylate chemistry employed in the ammonia (NH_3) test strip measures the free NH_3 by reacting with the NH_3 with hypochlorite to form chloramine which then reacts with salicylate to form a green-colored complex (5-aminosalicylate) in the presence of sodium nitro-ferricyanide (Krom 1980). The procedure for running these tests is simple and generally only takes a few minutes to finish after extraction with water. Therefore it seemed pertinent to investigate the possibility of using strip testing of nitrate and ammonia content in the water extract of compost to monitor the composting process.

Although these methods might be suitable for on-farm compost monitoring, the reliability and suitability of such methods for monitoring compost stability over different stages of composting process has not been determined. Evaluation of the method was conducted in this investigation by comparing the results collected from strip tests alongside standard laboratory methods and the results were correlated with a commercial maturity test kit

(Solvita) and other maturity parameters including water soluble C to N ratio (WSC/ N) and microbial biomass to N ratio (B_C/N).

The present study aimed to determine the compost stability by measuring nitrate and ammonia/ammonium content of water extracts from the four main phases of composting. Standard laboratory methods for measuring NH_4^+-N and NO_3^--N by analysing the KCl extracts spectrophotometrically (Doane and Horwáth 2003) and chemical strip test methods mentioned above were used to analyze the same samples simultaneously. The correlation between the ammonia and nitrate methods was examined in order to evaluate the reliability of using strip tests to measure nitrate and ammonia/ammonium content in compost piles. In addition, the study aimed to examine the ease of using the strip test on aqueous compost extracts and identify limitations of these tests.

2. *Materials and Methods*

2.1 The Strip Test method

In this study, the "Strip Test" method terminology is used with reference to using nitrate and ammonia strip tests for determining compost maturation. The "Strip Test" method aims to evaluate and validate the reliability of readily available home aquarium indicator "test strips" in monitoring compost stability by systematic experimental procedures.

2.1.1 Nitrate Determination

Nitrate can be measured using the Water Works ™ nitrate test strips. The compost sample is extracted with distilled water with 1:10 m/v (8g of 50% moisture content compost in 80ml water). After shaking the mixture for 30 min on a horizontal shaker, the suspension was filtered through Whatman no.6 filter paper. The aqueous extracts were then tested without dilution as directed by the packet instructions (Appendix 15). Results were expressed in ppm (mg/L) nitrate- nitrogen, NO_3^--N and all tests were conducted in triplicate.

2.1.2 Ammonia determination

The measurement of ammonia was undertaken using an Aquaria Test ™ 1 ammonia test strips. Further dilution is required to determine the ammonia content because of the high sensitivity of the strip test (between 0-5ppm). The same aqueous extract as used in the nitrate test strip (see 2.1.1: Nitrate Determination) was further diluted by a factor of 16 and 8 for the MA and GW extracts respectively with distilled water before testing as directed by the packet instructions (Appendix 15).

All NH_3 tests were conducted in triplicate and the results are expressed in mg NH_3 in 100 g of dried sample (mg /100g). Since the extracts were diluted 16 and 8 times for MA and GW water extracts respectively, the ammonia strip test reading was multiplied by 16 for the MA windrow and 8 for the GW windrow (the value is first adjusted for dilution and then expressed as dry mass) in order to determine the NH_3 concentration in mg per 100 g of dried sample.

In order to validate the results more precisely, ammonia and nitrate standards were prepared and tested with the corresponding test strips instead of using the reference chart provided. The standards prepared by diluting 30% NH_3 solution and 1000ppm standard NO_3^- solution were: ammonia (0, 0.2, 0.5, 1, 2, 3.5, 5, 6, 7.5 and 10 ppm as NH_3), and nitrate (0, 5, 10, 20, 30, 40, 50, 60, 80 and 100 ppm as N). The concentration of NO_3^--N was determined by comparing the colour of the test strip with the results generated by the standards (Figure 4.1). A similar procedure was applied to measure NH_3-N in each compost sample. However, instead of monitoring the colour change of the pad attached on the strip, the content of ammonia measured with the ammonia strip test was determined by comparing the colour of solution of the water extracts after reacting with the released chemicals from the pads and compared with the results generated by the NH_3 standards (Figure 4.1). The procedure from the commercial strip test package can be found in Appendix 15. As a result of this the water extracts produced coloured (yellow to green) solutions and therefore the analyte generated in the ammonia strip test was suitable for colorimetric analysis (Figure 4.1). A calibration curve of the prepared standards of ammonia solution measured at a wavelength of 650nm was constructed. The concentration of ammonia in the analyte, measured with the ammonia test strip, was also determined

spectrophotometrically. The results from strip test method were compared with the Australian Standard for nitrate and ammonium tests in the same water extracts (Doane and Horwáth 2003; Bower and Holm-Hansen 1980) to determine the reliability and accuracy of the tests.

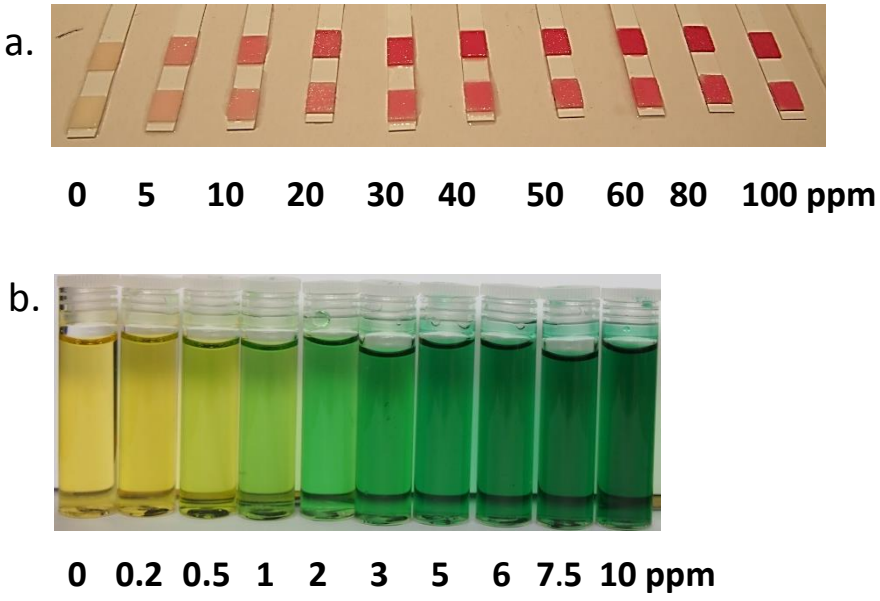


Figure 4.1: (a) Chemical strip tests results of the (a) nitrate (0, 5, 10, 20, 30, 40, 50, 60, 80 and 100 ppm as N) and (b) ammonia standards (0, 0.2, 0.5, 1, 2, 3.5, 5, 6, 7.5 and 10 ppm NH_3), and Source: photo taken by the author

2.2. Determining the Compost Stability by other Chemical Methods

Homogenised air dried composted material (2 g) was extracted with 40 ml of 2M potassium chloride solution in the ratio of sample (dw) to KCl of 1:20 (m/v). The mixture was shaken for 1 hour at room temperature and filtered through a Whatman no 6 filter paper. The concentrations of both NH_4^+-N and NO_3^--N were determined by analysing the KCl extracts spectrophotometrically following the method described by Doane and Horwáth (2003). The NO_3^--N was determined by reduction of the nitrate to nitrite by vanadium (III) ion in acidic pH combined with detection by the Griess reaction where nitrite is diazotized with

sulphanilamide and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride. A highly coloured azo dye is formed and the solution is measured colorimetrically at 520 nm. Similar to the strip test method, the salicylate method (Bower and Holm-Hansen 1980) was used to determine the ammonia and ammonium ion in the compost samples. However the 1000 ppm ammonium standard solution instead of diluting ammonia solution was used in the calibration in this standard method. The free ammonia reacts with the hypochlorite ions and with sodium salicylate at about pH 12 in the presence of sodium nitroprusside to form a coloured compound ranging from light green to dark blue which is measured at 650nm. Tartrate added to the salicylate solution prevents precipitation.

2.3. Statistical Analysis

Statistical analyses were carried out with SPSS Version 22 (IBM Corporation) for Windows. Analysis of variance (ANOVA) was used to determine if there were significant effects of time on chemical tests of nitrate and ammonia/ammonium concentration including NO_3^- *strip,* NH_3 *strip*, NH_3 *650nm*, NO_3^- *stad* and NH_4^+ *stad*. Data that was determined as heterogenous after the examination of the residual plots were transformed (log base-10) before further ANOVA analysis. These variables included NO_3^-*strip,* NH_3 *strip*, NH_3 *650nm*, NO_3^- *stad* and NH_4^+ *stad*. All statistical tests were evaluated at the 95% confidence level. Where the P-values were significant, the F-value was checked at a 95% confidence level using orthogonal contrasts. Pairwise comparisons were undertaken using least significant differences (LSDs).

3. Results and Discussion

3.1. Evaluation of compost stability by NH_4^+-N and NO_3^--N content by standard chemical analysis methods

The NH_4^+-N showed no significant differences ($F(3,12) = 0.27$, $p = 0.85$) with values ranging between 12.0 to 13.0 mg N $100g^{-1}$ dw (air dried) in all four stages of composting in the GW windrows whereas the NH_4^+-N in the MA windrows showed a high initial concentration (138 mg N 100 g^{-1} dw) then fell significantly ($p<0.0001$) between day 2 and day 19 and continued to drop before there was no further statistically significant change ($p=1$) in the curing stage. The concentration of NH_4^+-N of both final compost products were below the minimum ammonia content of 0.04% (40mg per 100g) of compost for agricultural purposes suggested by Hue and Liu (1995) and Leconte et al. (1990). The initial stage of both windrows appeared to have low NO_3^--N (Figure 4.1) but there was a significant increase ($p<0.0001$) in NO_3^- (from 13.4 to 155 mg N $100g^{-1}$ dw) during the thermophilic stage of the MA compost. Even though the total N content (Table 2.3-1, Chapter 2) in the GW windrow increased slightly from 1.5% to 1.9% during composting, a rapid significant increase ($p<0.0001$) of nitrate from 16.1 to 212 mg N 100 g^{-1} dw was observed during the maturation in the GW windrow. Greater differences were observed for NO_3^--N to NH_4^+-N ratio between the two composts after 175 days of composting. The concentration of NO_3^--N was nearly 18 times higher than NH_4^+-N found in the GW at day 175 while it was only 1.7 times higher in the MA at day 175. There was only a small difference in NH_4^+-N content between the GW and MA composts at day 175, however a large difference in NO_3^- in GW and MA composts was observed at day 175 (212 mg N $100g^{-1}$dw and 14.0 mg N 100^{-1}dw respectively). The higher ratio (higher NO_3^--N concentration) may reflect that the GW compost pile had a higher rate of nitrification

during the curing stage, as has been observed by others (Tiquia and Tam 2000; Larney et al. 2008; Khan et al. 2009).

Ammonium (NH_4^+) and nitrate (NO_3^-) are the forms of N most susceptible to change during composting as their evolution are affected by the decomposition of substrates and nitrification occurring during composting. The low NO_3^--N to NH_4^+-N ratio observed in the initial MA samples indicated a high rate of degradation of nitrogen containing OM such as protein while the biggest NO_3^--N ratio / NH_4^+-N (17.7:1) in the final GW compost sample indicated a high degree of nitrification in the curing stage of the GW windrow. The concentration of the NO_3^- and NH_4^+ examined by the chemical analysis method from both windrows were shown in Figure 4.2.

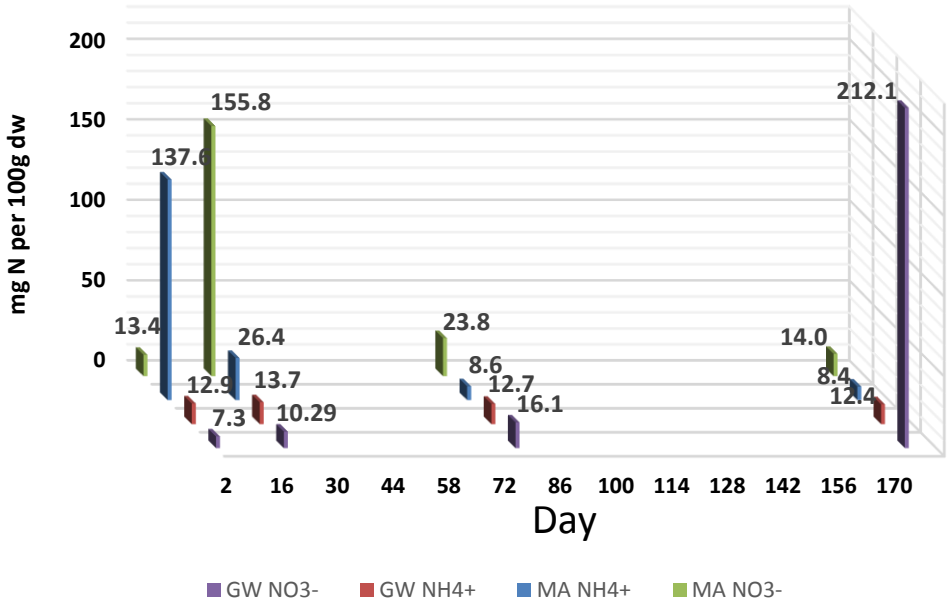


Figure 4.2: Nitrate and ammonia concentrations (mg N per 100g dw) over time measured by wet chemical methods

However, instead of an increasing trend of NO_3^--N as predicted in MA windrow, a decreasing trend of NO_3^--N concentration was observed. There was no evidence of denitrification occurring as no fermentation odour was detected in the MA compost during the curing period (Larney et al. 2008; Khan et al. 2009). The MA windrow was mainly in an aerobic condition as sufficient free air space was found in MA compost due to the presence of large particles such as woodchips and no more than 60% moisture content was measured throughout composting. Therefore, anaerobic respiration was minimal in the period. A possible explanation for this change is that the loss of nitrate was due to leaching and/or immobilization of N within the windrow (Bernal et al. 2009; Gómez-Brandón et al. 2008). As the windrows were constructed as open turned windrows, a high degree of leaching was likely with frequent rain. It was also suggested that it is best to avoid measuring soil NO_3^--N when the compost pile is exposed to rain, due to the problem of leaching and denitrification (Liang et al. 2006). Even though the GW windrow was set up under the same conditions, the MA pile may have experienced a higher degree of leaching because the texture of the MA windrow was found to be higher in porosity and coarse due to the high proportion of woodchips (Larney et al. 2004; Gómez-Brandón et al. 2008).

3.2. Evaluation of compost stability by NH_3/NH_4^+-N and NO_3^--N content with chemical strip test methods

The analysis of compost maturity using the strip test method is summarized in Table 4.1. Both NH_3-N and NO_3^--N concentration values obtained with the strip tests were generally lower than the value obtained from chemical analysis methods except NO_3^--N of the GW compost at day 77, which showed a slightly larger value. They ranged 1 to 60 ppm lower in NH_3-N measured by strip test method than NH_4^+-N measured by the chemical analysis

method while 1 to 50 ppm lower in NO_3^--N measured by strip test than the chemical analysis method. However the strip tests demonstrated a similar trend between stages of composting with the standard chemical analysis methods. A gradual decrease in NH_3-N measured by strip test method over the composting period showed a similar trend that was seen in NH_4^+-N from the chemical analysis method. Both NH_3-N strip test and NH_4^+-N chemical analysis methods were also in agreement with MAd2 (c.80 mg $100g^{-1}$dw) having the highest concentration and lowest on the MAd175 (c.4 mg $100g^{-1}$ dw). Significant increases in NO_3^--N from day 2 to 19 in the MA windrow and in the curing stage of GW windrow were also observed with the NO_3^--N strip test method. The mature compost products had an approximate 17:1 and 3:1 NO_3^--N to NH_4^+-N ratio in the GW and MA composts respectively, compared to about 18:1 and 2:1 as measured by standard chemical analysis methods.

Table 4.1: (a) Nitrate and ammonia concentrations measured by test strips and wet chemical methods

	Strip Test Method			Chemical Standard Method (references here)		
	NO_3^- *strip*	NH_3 *strip* (NH_3 *650nm**)	[NO_3^- *strip* /NH_3 *strip*] ratio	NO_3^- *stad*	NH_4^+ *stad*	[NO_3^- *stad*/NH_4^+ stad] ratio
	mg (as N) per 100g dw			mg (as N) per 100g dw		
GW d2	**c.0**	**c.12 (7.9*)**	**0**	**7.35$_a$**	**12.9$_a$**	**0.56**
GW d19	**c.10**	**c.8 (8.4*)**	**1.25**	**10.3$_a$**	**13.7$_a$**	**0.76**
GW d77	**c.20**	**c.6 (5.9*)**	**3.33**	**16.1$_a$**	**12.7$_a$**	**1.25**
GW	**c.>100**	**c.6 (5.2*)**	**>16.7**	**212 $_b$**	**12.4$_a$**	**17.7**
MA d2	**c.0**	**c.80 (67*)**	**0**	**13.4$_a$**	**138 $_a$**	**0.10**
MA d19	**c.>100**	**c.28 (18*)**	**>3.57**	**156 $_b$**	**26.4$_b$**	**7.20**
MA d77	**c.10**	**c.8 (4.8*)**	**1.25**	**23.8 $_a$**	**8.63$_c$**	**2.80**
MA d175	**c.10**	**c.4 (3.0*)**	**2.50**	**14.0 $_a$**	**8.42$_c$**	**1.69**

*Concentrations were determined spectrophotometrucally by measuring the absorbance of 650nm of the coloured solution generated by the ammonia strip tests.
Values in the same column and subtable not sharing the same subscript are significantly different at p< .05

3.3. Correlation between strip methods and standard wet chemical analysis methods and various other biochemical parameters

Table 4.2 shows the ratio of several selected biological and chemical parameters measured during the composting of the GW and the MA. The ratio of water soluble C to N (WSC/N) has been stated as a good indicator of compost maturity and it has been reported that compost sample values with WSC/N ratios under 0.5 are regarded as having a high level of stability (Mondini et al. 2006). The WSC/N ratio of both final compost products both had WSC/N ratios (0.12 and 0.31 in GW and MA windrows respectively) below is the value (Table 4.2). As the concentration of WSC was directly related to the availability of labile C for the consumption of microorganisms, a similar pattern in microbial biomass C to N ratio (B_C/N) was also found in this study (Table 4.2). The B_C/N ratio suggested that there was a higher microbial biomass per unit of N in the initial substrates and this gradually decreased over the time of composting. When the OM is found to be more easily degraded by the microbial organisms, N in OM is utilized and supports the growth of the microbial biomass. The higher ratio of microbial biomass per unit of N in the MA windrow was also observed throughout the composting when compared with the GW windrow. This may imply that the N (both organic and inorganic forms) can be better utilized by the microorganisms in the MA windrows than the GW windrows (Ayed et al. 2007; Mondini et al. 1997).

Table 4.2: Ratios of selected stability parameters and Solvita maturity index of GW and MA composts used in the study

Sample	WSC/ N	$[NO_3^-$-N/ NH_4^+-N/$]_{stad}$	B_C/N	Solvita maturity
GW d2	0.58	0.6	2.6	5.3
GW d19	0.31	0.8	1.6	6.5
GW d77	0.18	1.3	1.0	7.5
GW d175	0.12	17.7	0.3	7.0
MA d2	0.68	0.1	6.5	4.5
MA d19	0.58	7.2	5.2	6.0
MA d77	0.32	2.8	2.0	7.0
MA d175	0.31	1.7	2.4	6.5

In order to find out if the proposed strip test method could be used as an indicator for compost stability, the correlation coefficients (r) were calculated against several chemical parameters and some typical stability parameters including C/N, WSC/N and solvita® CO_2 & NH_3 index . Table 4.3 shows significant correlations found between the $NH_{3\ strip}$ with WSC/N (r=0.836) and Solvita® NH_3 (r=0.937). The value of correlation coefficient for nitrate strip test method ($NO_{3\ strip}^-$: $NO_{3\ stad}^-$, r = 0.967, p<0.01) of both the GW and MA composts with alternate chemical analysis methods also confirmed that the two methods were highly correlated to each other (Table 4.4).

Table 4.3: Pearson correlation (R) between biochemical parameters and ammonia chemical strip tests. (For all samples from MA and GW windrows)

	$NH_{4\ stad}^+$	Solvita® NH_3	WSC/ N	$NH_{3\ 650nm}$	$NH_{3\ strip}$
$NH_{4\ stad}^+$	1	0.972**	0.733**	0.967**	0.977**
Solvita® NH_3		1	0.653**	0.907**	0.937**
WSC/ N			1	0.767**	0.836**
$NH_{3\ 650nm}$				1	0.963**
$NH_{3\ strip}$					1

**. Correlation is significant at the 0.01 level (2-tailed).

Table 4.4: Pearson correlation (R) between Nitrate chemical analysis methods and chemical strip tests. (For all samples from MA and GW windrows)

	NO_3^--N_{strip}	NO_3^--N_{stad}
NO_3^--N_{strip}	1	0.967**
NO_3^--N_{stad}		1

**. Correlation is significant at the 0.01 level (2-tailed).

The correlation between biochemical parameters and chemical strip tests in the MA and GW windrows are shown in Tables 4.5a and 4.5b respectively. High correlations were observed between the NH_3 strip test and WSC/N ratio and Solvita® CO_2 index in both the MA and GW compost. Among the parameters, WSC/N values provided a reliable indicator for the stability of both the MA and GW composts and strong correlations (0.882 for the MA and 0.978 for the GW) suggested that the NH_3 strip test can be used as good maturity indicator for both MA and GW compost windrows. It was also noticed that the NH_3 strip test correlated well (r=0.817 for the MA; r=0.935 for the GW) with another quick stability test - Solvita ® CO_2 index. On the other hand, the NO_3^--N strip test correlated poorly with all measured parameters in the MA compost as no general increasing or decreasing trend was found during composting (the NO_3^- content increased significantly from day2 to day19 then decreased throughout the rest of the composting time). Therefore, the NO_3^--N strip test is not recommended for monitoring stability of MA windrow in this study. On the contrary, NO_3^--N strip test was found to provide a moderate estimation of stability for the GW windrow. Strong negative correlation (-0.835) and moderately negative correlation (-0.643) between C/N and WSC/N respectively were shown when the NO_3^--N strip test was applied to GW compost (Table 4.5). The C/N ratio as described earlier was a good stability indicator for GW compost (Chapter 2) and the strong negative correlation suggested an

increase of nitrification in the GW windrow during the curing phase partially contributed to the decrease of C/N ratio. Therefore the NO_3^--N strip test could be used to indicate compost stability for the windrow with no essential loss of NO_3^- through leaching.

Table 4.5: Pearson correlation (R) between biochemical parameters and chemical strip tests in each windrow. (a) MA windrow (b) GW windrow

a.	NH_3 *strip*	NO_3^- *strip*	C/N	WSC/ N	Solvita CO_2
NH_3 *strip*	1	-0.179	-0.360	0.882**	0.817**
NO_3^- *strip*		1	0.796**	0.227	-0.254
C/N			1	-0.003	-0.422
WSC/ N				1	0.770**
Solvita CO_2					1

b.	NH_3-N_{strip}	NO_3^--N_{strip}	C/N	WSC/ N	Solvita CO_2
NH_3 *strip*	1	-0.618*	0.915**	0.978**	0.935**
NO_3^- *strip*		1	-0.835**	-0.643**	0.554**
C/N			1	0.913**	0.876**
WSC/ N				1	0.891**
Solvita CO_2					1

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

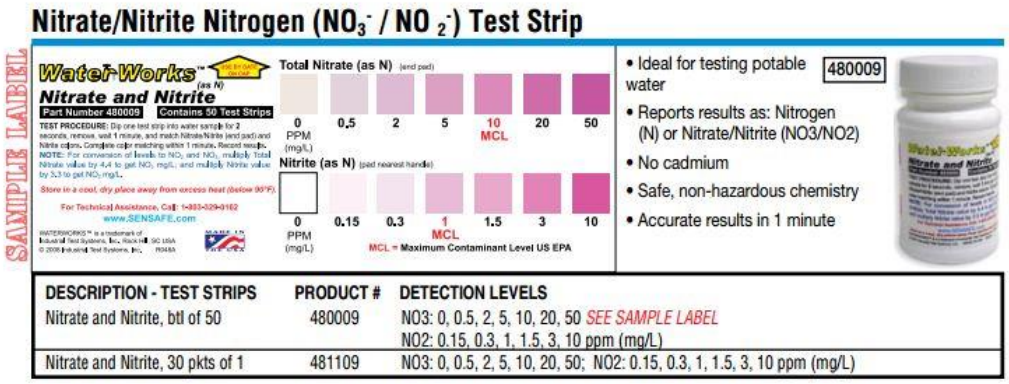
Nevertheless the strip tests for ammonia and nitrate successfully detected changes in inorganic N during composting. This method was able to show differences in N between the major stages of composting. Therefore the tested "Strip test method" in this project successfully determined the proper timing of administering the test during composting process and provides a guide of using these tests for determining stability of compost sample by the modified procedure. The validation of this method with independent chemical analysis methods (Australian Standard for available N) suggested the strip test correlates well with both nitrate ion and ammonia/ammonium ion. However the results of

the nitrate tests (both strip and chemical analysis) suggested there is no consistent trends between the two windrows for nitrate ion. Therefore the ammonia data is more reliable as an indicator of compost maturity for turned windrows with raw material inputs based on greenwaste or animal manure.

3.4. Ease and reliability of the using the Strip Tests as compost stability indicators

I. The strip tests used in the study were found to be fairly easy to use and the instructions on the manufacturer's package are clear and easy to follow (Figure 4.3a). However, the colour reference chart for the ammonia test tended to be darker than the actual results (Figure 4.3b). Sample preparation was considered to be the most time consuming part of the process, but it is straightforward and a number of tests can be run simultaneously.

a.



b.

Figure 4.3: Colour change of (a) Nitrate/Nitrite and (b) Ammonia test strip provided by the manufacturers.

II. Triplicate runs were conducted on each sample to determine if results could be reproduced. In general, consistent results were obtained for most of the samples (Figure 4.3-3 and 4.3-4). Furthermore, it was essential to homogenise the sample before conducting the test as the compost material was generally highly heterogeneous. If conducting the test on-farm it is suggested to thoroughly mix the collected sample for about 5 to 10 minutes before the testing. A comparison between the strip test results and the standard chemical methods gave strong correlation between data for the same measured parameter.

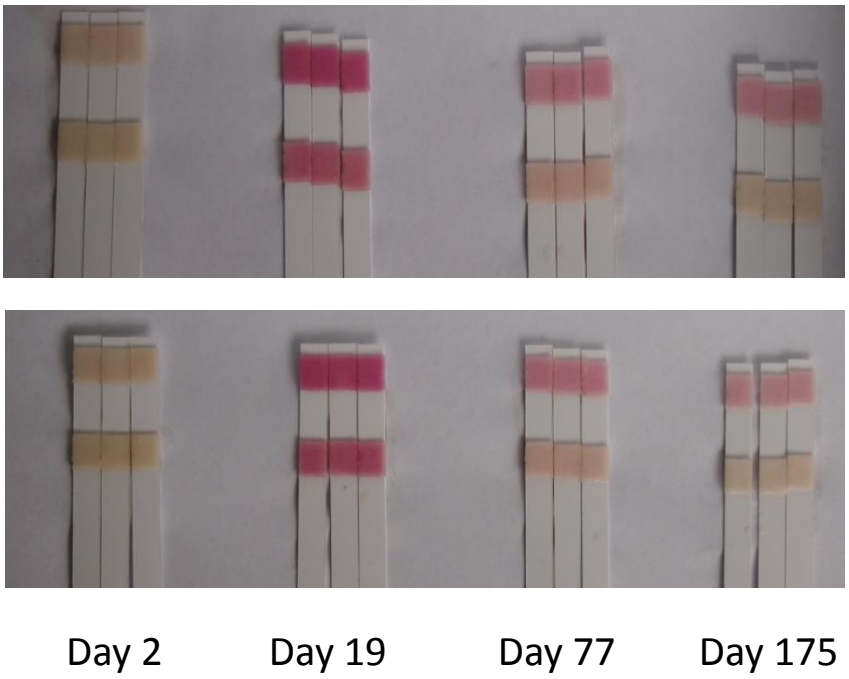


Figure 4.4: Nitrite/Nitrate strip tests results of water extract of MA compost (n=3)
Source: photo taken by the author

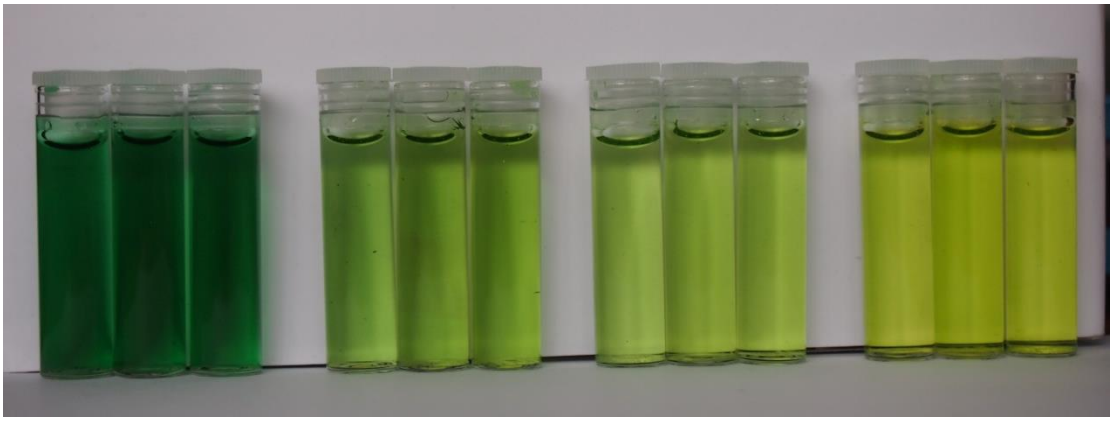


Figure 4.5 : Ammonia strip tests results of water extract of MA compost (n=3)
Source: photo taken by the author

III. The unexpected low concentration of nitrate found in the MA windrow during the curing stage indicated that the ratio of NH_4^+-N to NO_3^--N might not accurately represent the nitrate content in the pile if the composting is exposed to rain, hence leaching out of the NH_4^+-N and NO_3^--N. However the methods worked well with the GW windrow due to its lower porosity and finer texture. The ratio of NH_4^+-N to NO_3^--N of the GW samples was found to be very close to what was expected as reported in the literature (Khan et al. 2009) (Table 4.3-1).

3.5. Application for On-farm monitoring

One of the main objectives of the project was to evaluate the accuracy and reliability of existing strips tests for testing maturity for on-farm composting. The following provides discussion on a modified procedure to apply these tests to monitor composting maturity on farm (Appendix 16). It is assumed that the compost will be prepared and managed

according to the On-farm Composting guidelines available to farmers and other land-managers (Cooperband 2002; Rynk 1992).

1. When manure waste is used, it is important to conduct the ammonia strip test in the initial stage of composting (within the first week) to get a sense of available nitrogen for microorganism in the compost pile. The values obtained in the first week should be above 5ppm NH_3 (i.e. more than 100 mg NH_3 per 100g dw). The ammonia level of the manure compost pile should decrease significantly to safe levels of less than 0.2 ppm NH_3 as read from the colour chart. This level will not be reached for 3-4 months.
2. It is recommended that after applying the strip test method in the first week a further check is not required before two months have passed, since the ratio of Nitrate to Ammonia should be very small in the early stage of composting. The bigger ratio are expected in the later stage of composting as the compost becomes more mature. The land manager needs only to take closer notice of the ammonia levels and regular testing should be performed weekly after three months of composting.
3. According to the results from this project, the ratio between NO_3^- to NH_3 which is bigger than 3 should represent a stable compost due to a significant decrease of ammonia level (from above 5ppm to below 0.2 ppm NH_3 according to the colour chart).
4. When the ratio of nitrate to ammonia reaches above 10 and a significant increase of nitrate is observed in greenwaste windrow, it indicates the compost is stable.

4. *Conclusion*

It can be concluded that applying existing strip tests to monitor the content of NH_3/NH_4^+ and NO_3^- in composting piles allows a fairly good estimation of compost stability. Two different compost piles were used in this project and it was found that the ratio of NO_3^- to NH_3/NH_4^+ determined independently by standard chemical and Strip Test Methods generally increased during composting. However, the loss of NH_4^+-N and NO_3^--N through leaching after exposure to rain may lead to variable results. This was found to be the case for the MA compost windrow and was the result of the coarse texture of the composting pile which consisted of large proportion of woodchip as bulking agent. In order to minimize the environmental impact and generate more reliable results, it is suggested that various strategies, such as replacement of woodchip with other organic waste, covering the composting pile during the curing stage and recycling the leachate wherever possible, should be applied when using on-farm composting. Strip testing can provide a quick, reliable and cost effective way to monitor an open on-farm windrow system as the results correlated well in most instances with the standard chemical methods for several stability parameters including WSC/N, B_c/N and the Solvita® CO_2 index. However, the permeability and porosity of the compost pile may lead to leaching of ammonium and nitrate ions. Therefore, strip tests work better in monitoring maturity of compost windrows with less permeability such as the GW windrow of this study. It is important to note that the behavior of compost piles will vary due to the type of feedstock used, the management of the compost pile and the local weather conditions, in particular rainfall. Hence, it is recommended that with on-farm composting regular strip testing should be undertaken weekly after 3 months.

5. References

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CHAPTER FIVE – FUTURE SUGGESTION AND CONCLUSION

Suggestions for further work

The results from this study showed that compost derived from different organic wastes undergoes different N dynamics during composting and eventually possesses different spectroscopic and biochemical properties. Since little is known about the reliability of using chemical strip tests to determine compost stability and maturity for a variety of raw materials, further comparative studies using different composts with different origins should be conducted before establishing the usefulness of using chemical strip tests.

Furthermore, a better understanding of the N dynamics in the composting pile with different composting conditions would help to validate the accuracy and reliability of using chemical strip tests for monitoring compost stability. Further work should also focus on monitoring the N content changes in a composting pile with minimal leaching or recycling of the leachate.

Finally, it is recommended that a combination of quick monitoring methods that determine both the WSC content as well as the nitrate and ammonia content would provide more reliable monitoring of a compost pile. Measuring the WSC content in the compost can provide valuable information about the microbial activity of the compost sample, and combining the WSC test with the proposed strip test would allow on-farm composters to monitor the maturity and stability of compost with more assurance.

Conclusion

In conclusion, the goals of this project were to perform a comparative study of biochemical stability parameters during composting of green waste and manure waste with open turned windrow systems and to evaluate the potential use of quick and simple strip testing to determine the stability of the compost. Both the GW and MA compost products as described in this study were confirmed by the biochemical tests to be stabilized after 77 days of composting and extended long period of curing (up to 175 days) allowed the compost to be fully matured during the composting period. The strip tests were also found correlated with routine biochemical compost stability parameters. The Strip Test Method was successfully validated in this project to allow farmers to monitor the content of NH_3/NH_4^+ and NO_3^- in composting piles. The information of the ratio of NO_3^- to NH_3/NH_4^+ determined can indicate whether the composting pile has reached to certain stability for agricultural use. This study enhanced the knowledge of composting in an open turned windrow system under prevailing weather conditions. This knowledge will benefit local farmers when selecting appropriate feedstock to use in their compost and provide them with simple ways to monitor the on-farm composting process. In addition, the simple and quick application of strip testing can encourage farmers to practice on-farm composting by providing them with a cheap but effective way to monitor the quality of compost manufactured on their farms.

Appendix 1: Percentage Organic matter (OM)

ASHING %OM																									
		crucible no	mass of crucible	Weight wet + crucible	Weight 105 + Crucible	DRY MASS	Weight 550 + crucible	Ash mass	moisture	%OM		%ash				crucible no	mass of crucible	Weight wet + crucible	Weight 105 + Crucible	DRY MASS	Weight 550 + crucible	Ash mass	moisture	%OM		%ash
GWD2	R1	1	41.914	48.838	48.462	6.548	45.397	3.483	5.4%	46.8%	47.14%	53.2%		MAD2	R1	76	40.241	46.388	45.972	5.731	41.898	1.657	6.8%	71.1%	70.9%	28.9%
GWD2	R1	18	42.435	48.746	48.399	5.964	45.568	3.133	5.5%	47.5%		52.5%		MAD2	R1	112	40.382	46.4	45.994	5.612	42.031	1.649	6.7%	70.6%		29.4%
GWD2	R2	75	40.923	47.723	47.349	6.426	44.316	3.393	5.5%	47.2%	47.00%	52.8%		MAD2	R2	128	38.804	44.918	44.489	5.685	40.513	1.709	7.0%	69.9%	70.0%	30.1%
GWD2	R2	99	42.799	48.587	48.263	5.464	45.706	2.907	5.6%	46.8%		53.2%		MAD2	R2	131	42.185	48.456	48.014	5.829	43.927	1.742	7.0%	70.1%		29.9%
GWD2	R3	31	40.938	47.344	46.971	6.033	44.264	3.326	5.8%	44.9%	44.69%	55.1%		MAD2	R3	135	40.957	48.376	47.91	6.953	43.119	2.162	6.3%	68.9%	69.0%	31.1%
GWD2	R3	32	41.876	48.669	48.279	6.403	45.429	3.553	5.7%	44.5%		55.5%		MAD2	R3	219	39.466	46.924	46.455	6.989	41.621	2.155	6.3%	69.2%		30.8%
GWD2	R4	38	43.466	49.975	49.594	6.128	46.728	3.262	5.9%	46.8%	46.32%	53.2%		MAD2	R4	221	39.631	46.203	45.763	6.132	41.449	1.818	6.7%	70.4%	70.0%	29.6%
GWD2	R4	45	43.086	50.569	50.14	7.054	46.904	3.818	5.7%	45.9%		54.1%		MAD2	R4	232	36.166	43.619	43.125	6.959	38.273	2.107	6.6%	69.7%		30.3%
GWD19	R1	17	40.348	49.544	48.915	8.567	45.404	5.056	6.8%	41.0%	41.4%	59.0%		MAD19	R1	121	41.406	48.111	47.686	6.28	43.807	2.401	6.3%	61.8%	61.5%	38.2%
GWD19	R1	44	41.039	49.898	49.288	8.249	45.844	4.805	6.9%	41.8%		58.2%		MAD19	R1	130	41.379	47.921	47.506	6.127	43.757	2.378	6.3%	61.2%		38.8%
GWD19	R2	57	42.802	50.817	50.267	7.465	47.184	4.382	6.9%	41.3%	41.2%	58.7%		MAD19	R2	132	39.382	46.118	45.714	6.332	41.882	2.5	6.0%	60.5%	60.8%	39.5%
GWD19	R2	59	44.152	51.485	50.981	6.829	48.176	4.024	6.9%	41.1%		58.9%		MAD19	R2	207	40.296	46.764	46.376	6.08	42.662	2.366	6.0%	61.1%		38.9%
GWD19	R3	60	41.518	50.57	49.951	8.433	46.62	5.102	6.8%	39.5%	39.6%	60.5%		MAD19	R3	212	34.552	41.206	40.817	6.265	37.018	2.466	5.8%	60.6%	60.6%	39.4%
GWD19	R3	82	40.241	49.075	48.472	8.231	45.211	4.97	6.8%	39.6%		60.4%		MAD19	R3	222	41.941	48.962	48.551	6.61	44.543	2.602	5.9%	60.6%		39.4%
GWD19	R4	102	41.03	50.126	49.502	8.472	45.966	4.936	6.9%	41.7%	42.0%	58.3%		MAD19	R4	225	39.037	45.658	45.266	6.229	41.505	2.468	5.9%	60.4%	60.7%	39.6%
GWD19	R4	105	43.546	52.982	52.333	8.787	48.624	5.078	6.9%	42.2%		57.8%		MAD19	R4	234	39.017	45.66	45.263	6.246	41.456	2.439	6.0%	61.0%		39.0%
GWD75	R1	1	41.914	50.428	49.944	8.03	47.087	5.173	5.7%	35.6%	35.5%	64.4%		MAD75	R1	76	40.241	47.342	46.911	6.67	43.41	3.169	6.1%	52.5%	52.5%	47.5%

GWD75	R1	18	42.435	51.903	51.367	8.932	48.208	5.773	5.7%	35.4%		64.6%		MAD75	R1	112	40.382	46.292	45.935	5.553	43.015	2.633	6.0%	52.6%		47.4%
GWD75	R2	23	38.584	48.31	47.753	9.169	44.453	5.869	5.7%	36.0%	36.1%	64.0%		MAD75	R2	128	38.804	44.807	44.459	5.655	41.394	2.59	5.8%	54.2%	53.5%	45.8%
GWD75	R2	30	42.674	50.246	49.812	7.138	47.221	4.547	5.7%	36.3%		63.7%		MAD75	R2	131	42.185	48.272	47.923	5.738	44.889	2.704	5.7%	52.9%		47.1%
GWD75	R3	31	40.938	49.923	49.438	8.5	46.394	5.456	5.4%	35.8%	35.9%	64.2%		MAD75	R3	135	40.957	46.499	46.171	5.214	43.297	2.34	5.9%	55.1%	54.5%	44.9%
GWD75	R3	32	41.876	50.385	49.917	8.041	47.02	5.144	5.5%	36.0%		64.0%		MAD75	R3	219	39.466	45.473	45.128	5.662	42.079	2.613	5.7%	53.9%		46.1%
GWD75	R4	38	43.466	52.246	51.743	8.277	48.812	5.346	5.7%	35.4%	35.4%	64.6%		MAD75	R4	221	39.631	46.224	45.836	6.205	42.72	3.089	5.9%	50.2%	50.0%	49.8%
GWD75	R4	45	43.086	52.009	51.497	8.411	48.513	5.427	5.7%	35.5%		64.5%		MAD75	R4	232	36.166	42.585	42.204	6.038	39.202	3.036	5.9%	49.7%		50.3%
GWD175	R1	17	41.403	51.126	50.572	9.169	47.474	6.071	5.7%	33.8%	33.7%	66.2%		MAD175	R1	121	40.351	46.451	46.085	5.734	43.055	2.704	6.0%	52.8%	52.4%	47.2%
GWD175	R1	44	41.378	51.23	50.668	9.290	47.551	6.173	5.7%	33.6%		66.4%		MAD175	R1	130	41.04	47.669	47.285	6.245	44.034	2.994	5.8%	52.1%		47.9%
GWD175	R2	57	39.382	50.74	50.104	10.722	46.512	7.13	5.6%	33.5%	33.7%	66.5%		MAD175	R2	132	42.802	50.996	50.521	7.719	46.912	4.11	5.8%	46.8%	47.2%	53.2%
GWD175	R2	59	40.294	50.399	49.823	9.529	46.596	6.302	5.7%	33.9%		66.1%		MAD175	R2	207	44.146	50.797	50.405	6.259	47.42	3.274	5.9%	47.7%		52.3%
GWD175	R3	60	34.553	45.494	44.903	10.350	41.537	6.984	5.4%	32.5%	32.5%	67.5%		MAD175	R3	212	41.516	47.593	47.228	5.712	44.348	2.832	6.0%	50.4%	50.5%	49.6%
GWD175	R3	82	41.938	52.908	52.283	10.345	48.918	6.98	5.7%	32.5%		67.5%		MAD175	R3	222	40.237	47.493	47.058	6.821	43.614	3.377	6.0%	50.5%		49.5%
GWD175	R4	102	39.937	51.822	51.145	11.208	47.559	7.622	5.7%	32.0%	31.7%	68.0%		MAD175	R4	224	41.03	47.886	47.495	6.465	44.241	3.211	5.7%	50.3%	50.6%	49.7%
GWD175	R4	105	38.116	49.715	49.065	10.949	45.617	7.501	5.6%	31.5%		68.5%		MAD175	R4	225	43.546	50.747	50.329	6.783	46.873	3.327	5.8%	51.0%		49.0%

Appendix 2: Percentage C, N and H

	N%	C%	H%	C/N
GWD2R1	1.4	24.66	3.507	20.5
GWD2R2	1.48	23.86	3.45	18.8
GWD2R3	1.39	21.94	3.173	18.4
GWD2R3	1.47	24.63	3.534	19.5
GWD19R1	1.57	22.25	3.157	16.5
GWD19R2	1.58	21.67	3.106	16.0
GWD19R3	1.41	19.61	2.797	16.2
GWD19R4	1.71	23.49	3.353	16.0
GWD77R1	1.55	19.96	2.856	15.0
GWD77R2	1.49	19.03	2.692	14.9
GWD77R3	1.68	21.26	2.997	14.8
GWD77R4	1.72	22.53	3.188	15.3
GWD175R1	2.13	24.27	3.47	13.3
GWD175R2	1.75	19.51	2.78	13.0
GWD175R3	1.75	17.8	2.56	11.9
GWD175R4	1.45	15.32	2.2	12.3
MAD2R1	1.91	33.29	4.936	20.3
MAD2R2	1.82	32.3	4.73	20.7
MAD2R3	1.86	29.64	4.455	18.6
MAD2R3	2.03	30.97	4.646	17.8
MAD19R1	1.43	28.9	4.148	23.6
MAD19R2	1.4	31.26	4.47	26.0
MAD19R3	1.42	29.74	4.288	24.4
MAD19R4	1.32	30.61	4.401	27.1
MAD77R1	1.43	26.91	3.873	22.0
MAD77R2	1.31	25.23	3.654	22.5
MAD77R3	1.38	26.08	3.754	22.0
MAD77R4	1.38	22.51	3.325	19.0
MAD175R1	1.48	25.29	3.67	19.9
MAD175R2	1.82	35.32	5.03	22.6
MAD175R3	1.55	30.41	4.35	22.9
MAD175R4	1.76	27.46	3.99	18.2

Appendix 3: Total OM loss and Toal N loss

Xi = ash of day2Xf = OM of day2	TOMf=OM of the day TOMi= ash of the day	initial OM	initial ash%				initial %N		initial ash%
GW		46.29%	53.71	GW			1.52		53.71
	%OM	%ash	TOM loss (%)		%N		%ash		TN loss (%)
D2R1	47.14	52.86	-3.5	D2R1	1.48	79.52829191	52.86	80.3472	1.02
D2R2	47	53	-2.9	D2R2	1.57	84.20635593	53	80.56	-4.53
D2R3	44.69	55.31	6.2	D2R3	1.47	79.21156499	55.31	84.0712	5.78
D2R4	46.32	53.68	-0.1	D2R4	1.56	83.81496815	53.68	81.5936	-2.72
D19R1	41.4	58.6	18.1	D19R1	1.69	90.52571122	58.6	89.072	-1.63
D19R2	41.2	58.8	18.7	D19R2	1.70	91.15123523	58.8	89.376	-1.99
D19R3	39.6	60.4	24.0	D19R3	1.51	81.25654506	60.4	91.808	11.49
D19R4	42	58	16.0	D19R4	1.84	98.65102041	58	88.16	-11.90
D77R1	35.5	64.5	36.2	D77R1	1.64	88.2826087	64.5	98.04	9.95
D77R2	36.1	63.9	34.5	D77R2	1.58	84.86521739	63.9	97.128	12.63
D77R3	35.9	64.1	35.1	D77R3	1.78	95.43395029	64.1	97.432	2.05
D77R4	35.4	64.6	36.5	D77R4	1.82	97.96521739	64.6	98.192	0.23
D175R1	33.7	66.3	41.1	D175R1	2.26	121.3173913	66.3	100.776	-20.38
D175R2	33.7	66.3	41.1	D175R2	1.85	99.62109168	66.3	100.776	1.15
D175R3	32.5	67.5	44.2	D175R3	1.85	99.51561673	67.5	102.6	3.01
D175R4	31.7	68.3	46.2	D175R4	1.54	82.54319025	68.3	103.816	20.49
		initial OM	initial ash				initial %N		initial ash%
MA		69.975	30.025	MA			2.04		30.025
	%OM	%ash	TOM loss (%)				%ash		TN loss (%)
D2R1	70.9	29.1	-4.5	D2R1	2.05	61.49892761	29.1	59.364	-3.60
D2R2	70	30	-0.1	D2R2	1.96	58.75860215	30	61.2	3.99
D2R3	69	31	4.5	D2R3	1.99	59.60138741	31	63.24	5.75
D2R4	70	30	-0.1	D2R4	2.17	65.29271559	30	61.2	-6.69
D19R1	61.5	38.5	31.5	D19R1	1.53	45.82257204	38.5	78.54	37.69
D19R2	60.8	39.2	33.4	D19R2	1.49	44.71808511	39.2	79.968	40.27
D19R3	60.6	39.4	34.0	D19R3	1.51	45.28465215	39.4	80.376	39.82
D19R4	60.7	39.3	33.7	D19R4	1.40	42.14035088	39.3	80.172	43.86
D77R1	52.5	47.5	52.6	D77R1	1.52	45.70063864	47.5	96.9	49.63
D77R2	53.5	46.5	50.6	D77R2	1.39	41.73236074	46.5	94.86	53.01
D77R3	54.5	45.5	48.6	D77R3	1.46	43.98566879	45.5	92.82	49.39
D77R4	50	50	57.1	D77R4	1.47	44.03241233	50	102	53.89
D175R1	52.4	47.6	52.8	D175R1	1.57	47.22316684	47.6	97.104	48.06
D175R2	47.2	52.8	61.6	D175R2	1.93	58.04089219	52.8	107.712	42.45
D175R3	50.5	49.5	56.2	D175R3	1.65	49.50930851	49.5	100.98	47.63
D175R4	50.6	49.4	56.0	D175R4	1.87	56.06790451	49.4	100.776	40.58

Appendix 4: Water soluble C content

			mg / L	mg	g	mg C /g				mg / L	mg	g	mg C /g
	vol of water	after dilution	before dilution	C in total dry mass	dry mass	mg C in 1 g of sample		vol of water	after dilution	before dilution	C in total dry mass	dry mass	mg C in 1 g of sample
M2R1	80.00	29.90	298.9544	23.92	4.00	5.98	M2R1	80.00	50.24	502.4405	40.20	4.00	10.05
M2R2	80.00	25.90	259.0323	20.72	4.00	5.18	M2R2	80.00	45.23	452.3352	36.19	4.00	9.05
M2R3	80.00	27.50	275.0011	22.00	4.00	5.50	M2R3	80.00	55.56	555.6291	44.45	4.00	11.11
M2R4	80.00	26.12	261.2349	20.90	4.00	5.22	M2R4	80.00	49.19	491.9055	39.35	4.00	9.84
M19R1	80.00	31.52	157.5993	12.61	4.00	3.15	M19R1	80.00	25.58	255.7685	20.46	4.00	5.12
M19R2	80.00	36.17	180.8642	14.47	4.00	3.62	M19R2	80.00	27.89	278.894	22.31	4.00	5.58
M19R3	80.00	34.99	174.9448	14.00	4.00	3.50	M19R3	80.00	29.97	299.7069	23.98	4.00	5.99
M19R4	80.00	35.13	175.6331	14.05	4.00	3.51	M19R4	80.00	28.22	282.2343	22.58	4.00	5.64
M77R1	80.00	19.16	95.78881	7.66	4.00	1.92	M77R1	80.00	26.01	130.0683	10.41	4.00	2.60
M77R2	80.00	21.99	109.9681	8.80	4.00	2.20	M77R2	80.00	22.96	114.7798	9.18	4.00	2.30
M77R3	80.00	18.66	93.31089	7.46	4.00	1.87	M77R3	80.00	15.58	77.90745	6.23	4.00	1.56
M77R4	80.00	20.81	104.0486	8.32	4.00	2.08	M77R4	80.00	24.68	123.3876	9.87	4.00	2.47
M175R1	80.00	20.09	100.4693	8.04	4.00	2.01	M175R1	80.00	22.96	114.7798	9.18	4.00	2.30
M175R2	80.00	18.52	92.62258	7.41	4.00	1.85	M175R2	80.00	20.72	103.6025	8.29	4.00	2.07
M175R3	80.00	20.95	104.7369	8.38	4.00	2.09	M175R3	80.00	29.15	145.7423	11.66	4.00	2.91
M175R4	80.00	21.55	107.7655	8.62	4.00	2.16	M175R4	80.00	25.60	128.0127	10.24	4.00	2.56

Appendix 5a: Fulvic and humic acid C content (greenwaste)

		mg / L	mg	g	mg C /g					mg / L	mg	g	mg C /g	
vol of water	after dilution	before dilution	C in total dry mass	dry mass	mg C in 1 g of sample	vol of water	after dilution	before dilution	C in total dry mass	dry mass	mg C in 1 g of sample	naoh	gwff	GWhac
80.00	39.21	313.7107	25.10	4.00	6.27	80.00	55.47	1109.434	88.75	4.00	22.19	22.19	6.27	15.92
80.00	32.91	263.2505	21.06	4.00	5.27	80.00	50.66	1013.197	81.06	4.00	20.26	16.95	5.27	11.68
80.00	43.13	345.0532	27.60	4.00	6.90	80.00	51.88	1037.625	83.01	4.00	20.75	20.75	6.9	13.85
80.00	38.01	304.0757	24.33	4.00	6.08	80.00	54.49	1089.85	87.19	4.00	21.80	21.8	6.08	15.72
80.00	52.28	209.1055	16.73	4.00	4.18	80.00	37.64	752.7574	60.22	4.00	15.06	15.06	4.18	10.88
80.00	42.30	169.2134	13.54	4.00	3.38	80.00	37.00	739.9644	59.20	4.00	14.80	14.8	3.38	11.42
80.00	48.22	192.8821	15.43	4.00	3.86	80.00	39.43	788.6093	63.09	4.00	15.77	17.09	3.86	13.23
80.00	49.61	198.4232	15.87	4.00	3.97	80.00	39.27	785.4505	62.84	4.00	15.71	17.86	3.97	13.89
80.00	45.32	181.2857	14.50	4.00	3.63	80.00	61.03	1220.622	97.65	4.00	24.41	24.69	3.63	21.06
80.00	50.36	201.4508	16.12	4.00	4.03	80.00	37.60	751.9151	60.15	4.00	15.04	15.04	4.03	11.01
80.00	39.68	158.7024	12.70	4.00	3.17	80.00	24.32	486.4213	38.91	4.00	9.73	9.73	3.17	6.56
80.00	48.68	194.7291	15.58	4.00	3.89	80.00	32.73	654.5726	52.37	4.00	13.09	13.09	3.89	9.2
80.00	34.90	139.5846	11.17	4.00	2.79	80.00	57.22	1144.496	91.56	4.00	22.89	22.89	2.79	20.1
80.00	45.91	183.6279	14.69	4.00	3.67	80.00	50.33	1006.669	80.53	4.00	20.13	21.19	3.67	17.52
80.00	34.47	137.8899	11.03	4.00	2.76	80.00	26.21	524.1159	41.93	4.00	10.48	10.48	2.76	7.72
80.00	38.05	152.2092	12.18	4.00	3.04	80.00	23.88	477.682	38.21	4.00	9.55	9.55	3.04	6.51

Appendix 5b: Fulvic and humic acid C content (Manure waste)

			mg / L	mg	g	mg C /g				mg / L	mg	g	mg C /g		
	vol of water	after dilution	before dilution	C in total dry mass	dry mass	mg C in 1 g of sample		vol of water	after dilution	before dilution	C in total dry mass	dry mass	mg C in 1 g of sample	MA FFC	MA HAC
M2R1	80.00	43.19	345.5518	27.64	4.00	6.91	M2R1	80.00	35.05	876.2424	70.10	4.00	17.52	6.91	10.61
M2R2	80.00	42.38	339.0609	27.12	4.00	6.78	M2R2	80.00	31.51	787.6658	63.01	4.00	15.75	6.78	8.97
M2R3	80.00	43.60	348.7973	27.90	4.00	6.98	M2R3	80.00	31.44	785.906	62.87	4.00	15.72	6.98	8.74
M2R4	80.00	41.11	328.8608	26.31	4.00	6.58	M2R4	80.00	30.69	767.1348	61.37	4.00	15.34	6.58	8.77
M19R1	80.00	55.48	221.9218	17.75	4.00	4.44	M19R1	80.00	29.93	748.3636	59.87	4.00	14.97	4.44	10.53
M19R2	80.00	59.83	239.3083	19.14	4.00	4.79	M19R2	80.00	29.18	729.5924	58.37	4.00	14.59	4.79	9.81
M19R3	80.00	57.74	230.9627	18.48	4.00	4.62	M19R3	80.00	27.80	694.983	55.60	4.00	13.90	4.62	9.28
M19R4	80.00	66.72	266.8948	21.35	4.00	5.34	M19R4	80.00	26.70	667.4128	53.39	4.00	13.35	5.34	8.01
M77R1	80.00	59.89	239.5401	19.16	4.00	4.79	M77R1	80.00	24.07	601.7136	48.14	4.00	12.03	4.79	7.24
M77R2	80.00	57.57	230.2673	18.42	4.00	4.61	M77R2	80.00	33.45	836.3536	66.91	4.00	16.73	4.61	12.12
M77R3	80.00	53.45	213.8081	17.10	4.00	4.28	M77R3	80.00	24.26	606.4064	48.51	4.00	12.13	4.28	7.85
M77R4	80.00	60.93	243.7128	19.50	4.00	4.87	M77R4	80.00	28.78	719.6202	57.57	4.00	14.39	4.87	9.52
M175R1	80.00	66.03	264.113	21.13	4.00	5.28	M175R1	80.00	28.34	708.4748	56.68	4.00	14.17	5.28	8.89
M175R2	80.00	58.78	235.1355	18.81	4.00	4.70	M175R2	80.00	26.18	654.5076	52.36	4.00	13.09	4.70	8.39
M175R3	80.00	58.96	235.831	18.87	4.00	4.72	M175R3	80.00	32.87	821.6886	65.74	4.00	16.43	4.72	11.72
M175R4	80.00	63.48	253.9129	20.31	4.00	5.08	M175R4	80.00	32.56	814.0628	65.13	4.00	16.28	5.08	11.20

Appendix 6: Moisture content

Green waste		m(vial)	m(vial+sample)	m(vial+dried sample)	m(dried mass)	% moisture	Manure waste		m(vial)	m vial+sample)	m vial+dried sample)	m(dried mass)		
Day2	R1	13.206	19.463	16.246	3.04	51.4%	Day2	R1	13.495	20.782	17.122	3.627	50.2%	
	R1	13.478	19.251	16.093	2.615	54.7%		R1	13.148	19.796	16.643	3.495	47.4%	48.8%
	R2	13.512	20.056	16.632	3.12	52.3%		R2	13.145	19.972	16.81	3.665	46.3%	
	R2	13.33	18.591	15.749	2.419	54.0%		R2	13.171	19.297	16.31	3.139	48.8%	47.5%
	R3	12.999	19.687	15.475	2.476	63.0%		R3	13.497	20.089	16.734	3.237	50.9%	
	R3	13.214	17.938	15.459	2.245	52.5%		R3	13.34	19.722	16.553	3.213	49.7%	50.3%
	R4	13.414	20.174	16.716	3.302	51.2%		R4	13.444	19.94	16.834	3.39	47.8%	
	R4	13.107	18.672	15.721	2.614	53.0%		R4	13.232	20.38	16.61	3.378	52.7%	50.3%
day 19	R1	13.448	20.853	17.159	3.711	49.9%	day 19	R1	13.615	20.688	16.8	3.185	55.0%	
	R1	13.373	22.765	17.816	4.443	52.7%		R1	13.512	20.715	16.47	2.958	58.9%	57.0%
	R2	13.432	20.854	17.25	3.818	48.6%		R2	13.437	21.47	17.259	3.822	52.4%	
	R2	13.425	24.178	18.644	5.219	51.5%		R2	13.461	21.762	16.965	3.504	57.8%	55.1%
	R3	13.617	20.917	16.981	3.364	53.9%		R3	13.551	22.886	17.571	4.02	56.9%	
	R3	13.433	22.101	17.579	4.146	52.2%		R3	13.396	21.57	16.787	3.391	58.5%	57.7%
	R4	13.455	21.563	17.282	3.827	52.8%		R4	13.461	21.752	16.889	3.428	58.7%	
	R4	13.511	21.576	17.52	4.009	50.3%		R4	13.105	19.756	15.835	2.73	59.0%	58.8%
day 75	R1	13.512	21.763	17.665	4.153	49.7%	day 75	R1	13.608	21.778	17.092	3.484	57.4%	
	R1	13.249	20.982	17.173	3.924	49.3%		R1	13.226	21.168	16.638	3.412	57.0%	57.2%
	R2	13.538	23.937	18.718	5.18	50.2%		R2	13.567	21.096	16.753	3.186	57.7%	
	R2	13.488	21.098	17.224	3.736	50.9%		R2	13.386	20.855	16.41	3.024	59.5%	58.6%
	R3	13.259	19.565	16.338	3.079	51.2%		R3	13.514	20.816	16.632	3.118	57.3%	
	R3	13.48	23.04	18.42	4.94	48.3%		R3	13.31	18.98	15.657	2.347	58.6%	58.0%
	R4	13.53	22.293	17.749	4.219	51.9%		R4	13.623	21.126	17.045	3.422	54.4%	
	R4	13.516	23.225	18.231	4.715	51.4%		R4	13.468	20.331	16.414	2.946	57.1%	55.7%
day 175	R1	13.726	25.575	20.357	6.631	44.0%	day 175	R1	13.368	22.985	17.754	4.386	54.4%	
	R1	13.194	24.137	19.004	5.81	46.9%		R1	13.494	24.502	18.628	5.134	53.4%	53.9%
	R2	13.42	24.806	19.552	6.132	46.1%		R2	13.487	23.9	18.333	4.846	53.5%	
	R2	13.392	25.562	19.931	6.539	46.3%		R2	13.485	22.464	17.516	4.031	55.1%	54.3%
	R3	13.296	23.048	18.56	5.264	46.0%		R3	13.463	24.368	18.696	5.233	52.0%	
	R3	13.239	23.89	18.938	5.699	46.5%		R3	13.45	23.865	18.116	4.666	55.2%	53.6%
	R4	13.221	25.166	19.853	6.632	44.5%		R4	13.472	21.913	17.58	4.108	51.3%	
	R4	13.576	22.814	18.343	4.767	48.4%		R4	13.466	22.593	18.023	4.557	50.1%	50.7%

Appendix 7a: Microbial biomass C content (manure waste)

		mg / L	mg	g	mg C /g		MBC mg per gram					
	vol of water	before dilution	C in total dry mass	dry mass	mg C in 1 g of sample		Bc=Ec X 2.22		Bc=Ec/0.35		Bc=Ec X 2.64	
M2R1	64.00	320.66	20.52	4.00	5.13		11.39		14.66		13.54	
M2R2	64.00	318.64	20.39	4.00	5.10		11.32		14.57		13.46	
M2R3	64.00	286.75	18.35	4.00	4.59		10.19		13.11		12.11	
M2R4	64.00	229.97	14.72	4.00	3.68		8.17		10.51		9.71	
M19R1	64.00	157.92	10.11	4.00	2.53		5.61		7.22		6.67	
M19R2	64.00	162.64	10.41	4.00	2.60		5.78		7.43		6.87	
M19R3	64.00	176.59	11.30	4.00	2.83		6.27		8.07		7.46	
M19R4	64.00	179.26	11.47	4.00	2.87		6.37		8.19		7.57	
M77R1	64.00	76.08	4.87	4.00	1.22		2.70		3.48		3.21	
M77R2	64.00	79.93	5.12	4.00	1.28		2.84		3.65		3.38	
M77R3	64.00	54.63	3.50	4.00	0.87		1.94		2.50		2.31	
M77R4	64.00	50.53	3.23	4.00	0.81		1.79		2.31		2.13	
M175R1	64.00	83.89	5.37	4.00	1.34		2.98		3.84		3.54	
M175R2	64.00	68.12	4.36	4.00	1.09		2.42		3.11		2.88	
M175R3	64.00	109.29	6.99	4.00	1.75		3.88		5.00		4.62	
M175R4	64.00	103.27	6.61	4.00	1.65		3.67		4.72		4.36	

Appendix 7b: Microbial biomass C content (Greenwaste)

		mg / L	mg	g	mg C /g		MBC mg C per gram			
	vol of water	before dilution	C in total dry mass	dry mass	mg C in 1 g of sample		Bc=Ec X 2.22		Bc=Ec/0.35	Bc=Ec X 2.64
G2R1	64.00	107.76	6.90	4.00	1.72		3.83		4.93	4.55
G2R2	64.00	103.26	6.61	4.00	1.65		3.67		4.72	4.36
G2R3	64.00	93.86	6.01	4.00	1.50		3.33		4.29	3.96
G2R4	64.00	43.71	2.80	4.00	0.70		1.55		2.00	1.85
G19R1	64.00	45.54	2.91	4.00	0.73		1.62		2.08	1.92
G19R2	64.00	62.44	4.00	4.00	1.00		2.22		2.85	2.64
G19R3	64.00	55.70	3.56	4.00	0.89		1.98		2.55	2.35
G19R4	64.00	77.96	4.99	4.00	1.25		2.77		3.56	3.29
G77R1	64.00	32.66	2.09	4.00	0.52		1.16		1.49	1.38
G77R2	64.00	43.18	2.76	4.00	0.69		1.53		1.97	1.82
G77R3	64.00	30.83	1.97	4.00	0.49		1.09		1.41	1.30
G77R4	64.00	43.09	2.76	4.00	0.69		1.53		1.97	1.82
G175R1	64.00	8.25	0.53	4.00	0.13		0.29		0.38	0.35
G175R2	64.00	13.80	0.88	4.00	0.22		0.49		0.63	0.58
G175R3	64.00	13.78	0.88	4.00	0.22		0.49		0.63	0.58
G175R4	64.00	14.39	0.92	4.00	0.23		0.66		0.66	0.61

Appendix 8a: Microbial biomass N content (manure waste)

	vol of water	before dilution	N in total dry mass	dry mass	µM/ g	µg /g	mg N /g		BN=En X 2.22
M2R1	64.00	2575.48	164.83	4.00	41.21	3093.46	0.577		1.281
M2R2	64.00	2323.85	148.73	4.00	37.18	2791.22	0.521		1.156
M2R3	64.00	2486.67	159.15	4.00	39.79	2986.79	0.557		1.237
M2R4	64.00	1613.37	103.26	4.00	25.81	1937.86	0.361		0.802
M19R1	64.00	625.37	40.02	4.00	10.01	751.14	0.140		0.311
M19R2	64.00	873.29	55.89	4.00	13.97	1048.93	0.196		0.434
M19R3	64.00	717.88	45.94	4.00	11.49	862.26	0.161		0.357
M19R4	64.00	555.06	35.52	4.00	8.88	666.69	0.124		0.276
M77R1	64.00	366.34	23.45	4.00	5.86	440.02	0.082		0.182
M77R2	64.00	458.85	29.37	4.00	7.34	551.13	0.103		0.228
M77R3	64.00	384.84	24.63	4.00	6.16	462.24	0.086		0.191
M77R4	64.00	325.64	20.84	4.00	5.21	391.13	0.073		0.162
M175R1	64.00	424.53	27.17	4.00	6.79	509.91	0.095		0.211
M175R2	64.00	347.34	22.23	4.00	5.56	417.20	0.078		0.173
M175R3	64.00	400.41	25.63	4.00	6.41	480.94	0.090		0.199
M175R4	64.00	564.43	36.12	4.00	9.03	677.95	0.13		0.281

Appendix 8b: Microbial biomass N content (greenwaste)

	vol of water	before dilution	N in total dry mass	dry mass	µM/ g	µg /g	mg N /g		BN=Enx2.22
G2R1	64.00	634.16	40.59	4.00	10.15	761.70	0.142		0.315
G2R2	64.00	420.31	26.90	4.00	6.72	504.85	0.094		0.209
G2R3	64.00	427.69	27.37	4.00	6.84	513.70	0.096		0.213
G2R4	64.00	752.14	48.14	4.00	12.03	903.41	0.168		0.374
G19R1	64.00	553.04	35.39	4.00	8.85	664.27	0.124		0.275
G19R2	64.00	412.94	26.43	4.00	6.61	495.99	0.092		0.205
G19R3	64.00	376.07	24.07	4.00	6.02	451.70	0.084		0.187
G19R4	64.00	346.57	22.18	4.00	5.55	416.28	0.078		0.172
G77R1	64.00	193.24	12.37	4.00	3.09	232.11	0.043		0.096
G77R2	64.00	164.76	10.54	4.00	2.64	197.90	0.037		0.082
G77R3	64.00	168.83	10.81	4.00	2.70	202.79	0.038		0.084
G77R4	64.00	235.96	15.10	4.00	3.78	283.41	0.053		0.117
G175R1	64.00	113.91	7.29	4.00	1.82	136.82	0.026		0.057
G175R2	64.00	193.24	12.37	4.00	3.09	232.11	0.043		0.096
G175R3	64.00	146.46	9.37	4.00	2.34	175.91	0.033		0.073
G175R4	64.00	117.98	7.55	4.00	1.89	141.71	0.026		0.059

Appendix 9: FDA Hydrolysis activity

		M2 R1			M2 R2			M19 R1			M19 R2	
60 mins		Calibration			Calibration			Calibration			Calibration	
µg/ml		y = 88.07x - 1.0313			y = 90.61x + 0.5256			y = 92.282x + 0.0663			y = 107.47x - 0.6617	
average of result		0.316667			0.345667			0.228			0.270333	
Result (1:4) dilution		26.88689			31.87666			20.974			28.3552	
Adjustment of concentration		107.5476			127.5066			83.89598			113.4208	
		M2 R3			M2 R4			M19 R3			M19 R4	
		Calibration			Calibration			Calibration			Calibration	
µg/ml		y = 101.24x - 0.0469			y = 123.16x + 5.613			y = 78.471x + 5.7059			y = 67.051x + 2.3576	
average of result		0.347			0.329			0.324			0.310	
Result (1:3) dilution		35.1			46.1			31.1			23.1	
Adjustment of concentration		105.3			138.4			93.4			69.4	
		M75 R1			M75 R2			M175 R1			M175 R2	
		Calibration			Calibration			Calibration			Calibration	
µg/ml		y = 105.01x - 2.5998			y = 105.15x + 1.0258			y = 100.62x + 0.16			y = 95.124x + 0.2985	
average of result		0.198667			0.170667			0.1935			0.184667	
no dilution		18.29719			19.00645			19.68028			17.89644	
		M75 R3			M75 R4			M175 R3			M175 R4	
		Calibration			Calibration			Calibration			Calibration	
µg/ml		y = 79.956x + 2.4749			y = 74.217x + 4.4069			y = 85.984x - 1.5535			y = 95.325x - 2.4309	
average of result		0.300			0.247			0.250			0.209	
no dilution		26.5			22.7			19.9			17.5	

		Gd2 r1			Gd2r2			Gd19r1			Gd19r2	
		Calibration			Calibration			Calibration			Calibration	
µg/ml		y = 151.69x - 3.4207			y = 149.54x - 6.7375			y = 161.42x - 8.3088			y = 113.91x - 1.2764	
average of result		0.391833			0.396			0.359333			0.389167	
Result (1:2) dilution		56.04178			52.48034			49.64098			43.03459	
Adjustment of concentration		112.0836			104.9607			99.28196			86.06918	
		G2 R3			G2 R4			G19 R3			G19 R4	
		Calibration			Calibration			Calibration			Calibration	
µg/ml		y = 50.348x + 0.3104			y = 51.853x + 1.6243			y = 54.967x + 0.0709			y = 68.143x - 2.0911	
average of result		0.548			0.623			0.503			0.521	
Result (1:3) dilution		27.9			33.9			27.7			33.4	
Adjustment of concentration		83.7			101.8			83.2			100.2	
		d75 r1			d75r2			d175r1			d175r2	
		Calibration			Calibration			Calibration			Calibration	
µg/ml		y = 155.87x - 4.8283			y = 113.78x - 2.7135			y = 112.2x - 0.1386			y = 133.85x - 0.0905	
average of result		0.226			0.222			0.190			0.151	
Result (2:3) dilution		30.4			22.5			21.2			20.1	
Adjustment of concentration		45.6			33.8			31.8			30.2	
		G75 R3			G75 R4			G175 R3			G175 R4	
		Calibration			Calibration			Calibration			Calibration	
µg/ml		y = 54.594x + 0.2848			y = 63.499x - 0.5815			y = 53.164x - 0.2789			y = 50.409x + 0.3947	
average of result		0.192			0.194			0.227			0.267	
Result (1:3) dilution		10.77			11.7			11.8			13.9	
Adjustment of concentration		32.3			35.2			35.4			41.6	

Appendix 10: Nitrate and ammonium N content (standard chemical analysis)

NITRATE		GW	GW	GW	GW	MA	MA	MA	MA
		day 2	day 19	day 77	day 175	day 2	day 19	day 77	day 175
mg in 80mL		0.14	0.26	0.40	4.20	0.28	3.15	0.57	0.28
		0.11	0.22	0.41	4.53	0.25	3.51	0.43	0.14
		0.20	0.17	0.27	4.53	0.26	2.60	0.49	0.33
		0.14	0.18	0.22	3.71	0.29	3.19	0.41	0.38
mg (as N) per 100g dw		7.0	12.9	19.8	209.9	13.9	157.7	28.6	13.9
		5.5	10.9	20.3	226.7	12.4	175.4	21.7	7.0
		9.9	8.4	13.4	226.7	12.9	130.1	24.7	16.3
		7.0	8.9	10.9	185.3	14.3	159.7	20.3	18.8

NH4-N		GW	GW	GW	GW	MA	MA	MA	MA
		day 2	day 19	day 77	day 175	day 2	day 19	day 77	day 175
mg in 80mL		0.23	0.25	0.27	0.27	2.66	0.69	0.25	0.24
		0.26	0.32	0.30	0.31	2.85	0.48	0.21	0.25
		0.30	0.27	0.24	0.24	2.88	0.68	0.22	0.23
		0.24	0.26	0.20	0.17	2.62	0.26	0.28	0.22
mg (as N) per 100g dw		11.6	12.3	13.6	13.4	132.9	34.6	12.4	12.0
		12.9	15.8	15.2	15.5	142.3	24.1	10.6	12.4
		15.2	13.4	11.9	12.0	144.2	33.8	10.8	11.3
		12.0	13.2	10.0	8.6	130.8	13.1	13.9	10.8

Appendix 11: Ammonia N content (strip test method)

NH3 - 650nm			mg / L	mg	g	mg N /g			mg / L	mg	g	mg N /g
		vol of water	before dilution	NH3 in total dry mass	dry mass	mg N in 100 g of sample		vol of water	before dilution	NH3 in total dry mass	dry mass	mg N in 100 g of sample
G2R1		80.00	5.56	0.44	4.00	8.64	M2R1	80.00	35.65	2.85	4.00	55.46
G2R2		80.00	5.59	0.45	4.00	8.69	M2R2	80.00	34.41	2.75	4.00	53.53
G2R3		80.00	5.13	0.41	4.00	7.98	M2R3	80.00	50.02	4.00	4.00	77.81
G2R4		80.00	4.09	0.33	4.00	6.36	M2R4	80.00	51.97	4.16	4.00	80.84
G19R1		80.00	5.42	0.43	4.00	8.44	M19R1	80.00	14.63	1.17	4.00	22.76
G19R2		80.00	5.59	0.45	4.00	8.69	M19R2	80.00	8.34	0.67	4.00	12.98
G19R3		80.00	5.15	0.41	4.00	8.01	M19R3	80.00	17.42	1.39	4.00	27.11
G19R4		80.00	5.54	0.44	4.00	8.62	M19R4	80.00	6.88	0.55	4.00	10.70
G77R1		80.00	3.50	0.28	4.00	5.45	M77R1	80.00	2.45	0.20	4.00	3.81
G77R2		80.00	3.58	0.29	4.00	5.57	M77R2	80.00	2.42	0.19	4.00	3.76
G77R3		80.00	4.38	0.35	4.00	6.82	M77R3	80.00	3.57	0.29	4.00	5.55
G77R4		80.00	3.75	0.30	4.00	5.84	M77R4	80.00	3.90	0.31	4.00	6.07
G175R1		80.00	2.86	0.23	4.00	4.44	M175R1	80.00	1.00	0.08	4.00	1.55
G175R2		80.00	3.82	0.31	4.00	5.94	M175R2	80.00	1.69	0.14	4.00	2.63
G175R3		80.00	3.30	0.26	4.00	5.14	M175R3	80.00	2.74	0.22	4.00	4.26
G175R4		80.00	3.39	0.27	4.00	5.27	M175R4	80.00	2.25	0.18	4.00	3.50

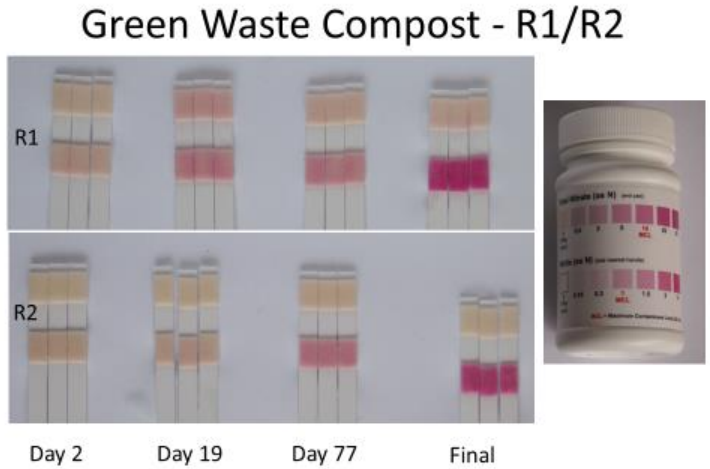
Appendix 12a: Summary of mean and standard deviation of various physiochemical and biological parameters (greenwaste)

			OM	Moisture	pH	%C	%N	WSC	FFC	HAC	MBC	MBN	FDA	SolNH3	SolCO2	NO3	NH4	strip650	StripNH3	stripNO3	MBCMBNratio	HAFArati o	WSCNrati o	CNratio
GW	2	Mean	.4628	.5400	7.0000	25.1975	1.5200	5.4700	6.1300	14.2925	3.9850	.18425	100.6500	0.0000	.020525	7.3500	12.9000	7.9175	12.0000	0.0000	24.0500	2.3400	3.5750	16.5750
		Std.	.011019	.02513	.19613	1.337787	.05228	.36860	.67196	1.97547	1.34988	.054206	12.09201	0.00000	.0058449	1.84120	1.61245	1.08758	0.0000	0.0000	11.56359	.27435	.34034	.80156
	19	Mean	.4105	.5145	7.2100	23.3600	1.6850	3.4450	3.8475	12.3550	2.7600	.13900	92.2000	0.0000	.006150	10.2750	13.6750	8.4400	8.0000	10.0000	21.0500	3.2275	2.0500	13.8500
		Std.	.010225	.01229	.14048	1.748081	.13528	.20404	.33876	1.43431	.62038	.030044	8.80568	0.00000	.0011619	2.05649	1.49527	.30540	0.0000	0.0000	8.09012	.42122	.19149	.19149
	77	Mean	.3573	.5033	7.3350	21.9325	1.7050	2.0175	3.6800	11.9575	1.7100	.06300	36.7250	0.0000	.002825	16.0750	12.6750	5.9200	6.0000	20.0000	27.5750	3.2425	1.2000	12.8500
		Std.	.003330	.00954	.14708	1.611196	.11358	.15108	.37824	6.33746	.30199	.010893	6.03400	0.00000	.0001708	4.64857	2.23514	.62177	0.0000	0.0000	6.02073	1.72622	.14142	.20817
	175	Mean	.3290	.4610	7.2625	20.3775	1.8750	2.0275	3.0650	12.9625	.5750	.04750	34.7500	0.0000	.002250	212.1500	12.3750	5.1975	6.0000	100.0000	12.4000	4.2275	1.1000	10.8250
		Std.	.009980	.00408	.14361	4.012448	.29535	.13326	.42241	6.85160	.13077	.012069	5.05800	0.00000	.0002380	19.57371	2.89871	.61473	0.0000	0.0000	3.31763	2.27490	.21602	.56789

Appendix 12b: Summary of mean and standard deviation of various physiochemical and biological parameters (manure waste)

MA	2	Mean	.6998	.4923	8.17775	33.8100	2.04225	10.0125	6.8125	9.2700	13.2125	.74100	119.6750	.2975	.038000	13.3750	137.5750	66.9100	40.0000	0.0000	18.0250	1.3600	4.9000	16.5750
		Std.	.00776	.01350	.13841	1.78585	.09287	.84894	.17576	.89937	1.93667	.14405 8	15.98674	.05737	.018 8119	.89582	6.70640	14.41043	0.0000	0.0000	1.70563	.12517	.49666	1.15866
	19	Mean	.6090	.5715	8.62225	32.0600	1.48225	5.5825	4.7975	9.4050	7.7275	.22825	90.0250	0.0000	.010825	155.7500	26.4250	18.3875	14.0000	100.0000	34.9500	1.9825	3.7500	21.6750
		Std.	.00408	.01555	.08808	1.06292	.05737	.35743	.38888	1.06196	.47514	.04 5361	18.44530	0.0000 0	.004 2058	18.78235	10.06193	7.82175	4.0000	0.0000	7.77410	.35976	.33166	1.39134
	77	Mean	.5263	.5738	8.5150	26.7550	1.4600	2.2325	4.6375	9.1825	2.9850	.12650	21.6250	0.0000	.008475	23.8000	11.9250	4.7975	6.0000	10.0000	23.5750	1.9800	1.5500	18.3500
		Std.	.01931	.01255	.29983	2.03837	.05354	.46486	.26196	2.18263	.67777	.01 8358	3.78010	0.0000 0	.003 3540	3.70675	1.54353	1.18843	0.0000	0.0000	3.94155	.47145	.30000	1.38203
	175	Mean	.5018	.5313	7.8400	31.4700	1.7550	2.4600	4.9450	10.0475	4.1675	.14300	18.7500	0.0000	.004800	13.9750	11.6250	2.9850	4.0000	10.0000	29.5000	2.0375	1.4500	17.9250
		Std.	.02167	.01642	.65518	4.60988	.17234	.36065	.28349	1.65119	.86098	.03 0659	1.22610	0.0000 0	.001 9900	5.07830	.71356	1.16563	0.0000	0.0000	5.67509	.37026	.28868	1.92072

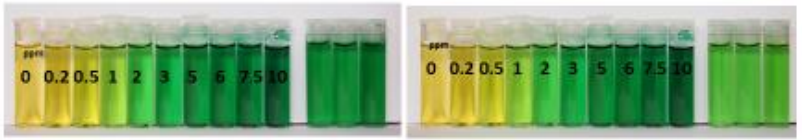
Appendix 13: Example of Chemical strip test results (greenwaste)



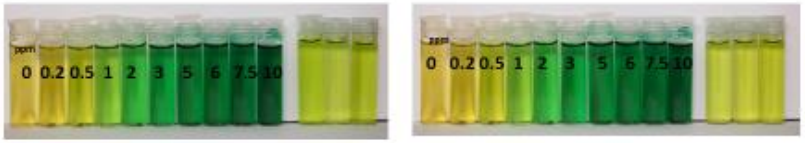
Appendix 14: Example of Chemical strip test results (manure waste)

Manure Compost – R3

- D2 R3 – 1ppm<D2<2ppm
- D19 R3 – 1ppm<D2<2ppm

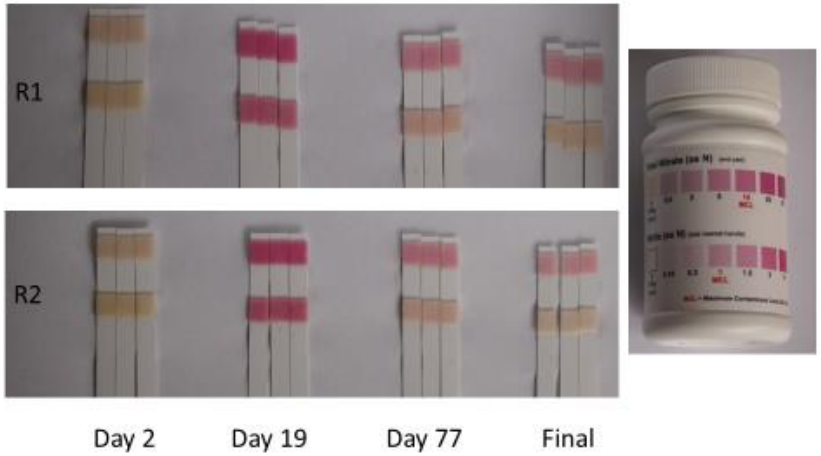


- D77R3- 0.5ppm<D2< 1ppm
- FinalR3-0.5ppm<D2<1ppm

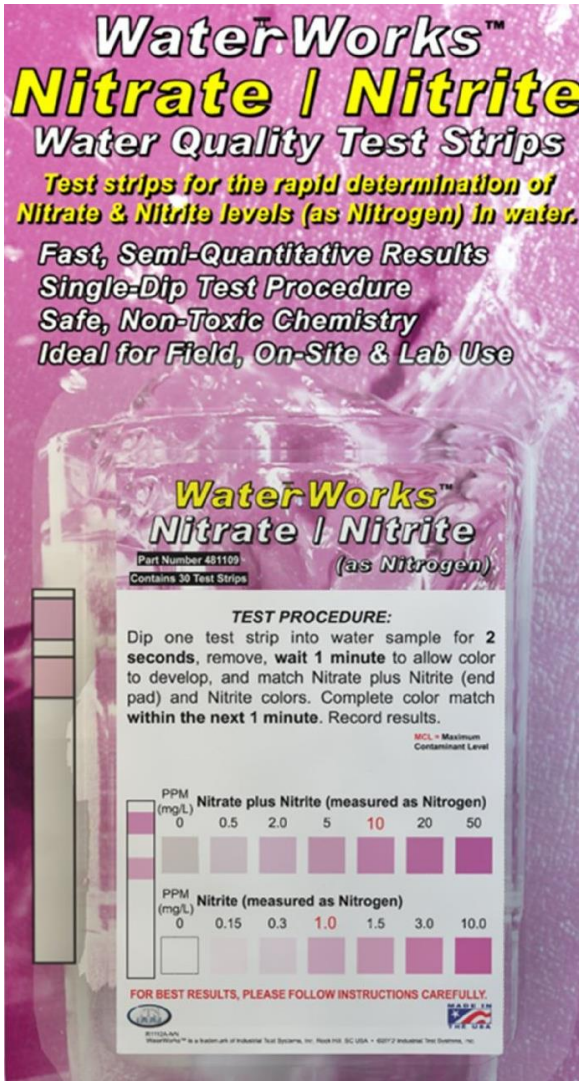


Manure Compost – R3

Manure Compost – R3/R4



Appendix 15: Nitrate and Ammonia strip test procedure



Ammonia test strip

1. Remove an Ammonia Test Strip from the bottle and replace the cap tightly. Fill the sample vial to top line with testing sample.
2. Dip the test strip into water sample. Move strip vigorously up and down in the water sample for 30 seconds. Make sure both pads are always submerged.
3. Wait 5 minutes for the color to develop in the water sample.
4. Read the test results by matching the test solution against the ammonia color chart.
5. The tube should be viewed against the white area beside the color chart. Color comparisons are best made in a well-lit area.
6. The closest match indicates the ppm (mg/L) of ammonia in the water sample.

Appendix 16: The Outline of the proposed Strip Test Method for monitoring on-farm Manure based composting pile

1. Conduct the ammonia and nitrate strip tests within the first week of composting:
    a) Take about 10 g of moist compost mixture and suspend the mixture in 100 ml of water. Vigorously shake the compost solution for at least 2 minutes (the longer the better) and filter the suspension with filter paper (or cheese cloth).
    b) For each testing, transfer 10ml of the filtrate into a small vial and it is recommended that each sample should be conducted in triplicate i.e three test strips for testing nitrate and another three test strips for testing ammonia
    c) Follow the test procedure on the packet (appendix 15) to measure the nitrate content directly in the aqueous extracts with nitrate test strips. (If the first filtrate is too dark and the result is not clear, run the strip test again after filtering the first filtrate with a better filter paper)
    d) A further dilution is required for undertaking the ammonia strip testing. Put 10 ml of the filtrate into a measuring cup and then add water to the 200 ml mark. Follow the test procedure on the packet (appendix 15) to measure the ammonia content from the diluted solution. (If the first filtrate is too dark and the result is not clear, run the strip test again after filtering the first filtrate with a better filter paper)
    e) Follow the steps shown below to calculate the nitrate and ammonia content (mg N per 100g of dry sample) in the pile
2. Repeat procedures 1a – e once every 3 to 4 weeks.

* Number of sample needed for each pile is determined by its size (rough idea can be found in Australian Standard for Composts, Soil Conditioners and Mulches (AS4454-2012).

Calculation : Assume the moist compost has 50% moisture and there is 5 g dry mass in each 10 g of compost sample

Reading from nitrate strip test	in 5 g dry mass	mg N in 100 g of dry mass sample
5ppm (5 mg per L)	0.5 mg	100 mg N in 100 g of sample

÷10 x 20

Reading from ammonia strip test	Before further dilution	in 5 g dry mass	mg N in 100 g of dry mass sample
1 ppm (1 mg per L)	20ppm (20 mg per L)	2 mg NH_3	33 mg N in 100 g of sample

x 20 ÷10 x 16.5

The ratio of NO_3^- : NH_3 is 100 ÷33 = 3.33