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USING THE ORGANIC CARBON FRACTIONS OF THE VAN SOEST METHOD TO DETERMINE COMPOUNDS RESPONSIBLE FOR C AND N MINERALIZATION FROM SLUDGE AMENDED SOILS

By

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Submitted in partial fulfillment of the requirements for the degree

MSc. (Agric.) Soil Science

In the Faculty of Natural and Agricultural Sciences

University of Pretoria

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November 2014

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DECLARATION

I Malobane M Elvis hereby certify that this thesis I am submitting to the University of Pretoria for the degree, MSc. (Agric.) Soil Science, is entirely my own work, except where duly acknowledged. I also certify that this thesis has never been submitted to any other tertiary institution for any degree.

Signature _____ Date: _____

ACKNOWLEDGEMENTS

> I know that You can do all things, and that no thought or purpose of Yours can be restrained or thwarted. (Job 42:2)
>
> Many are the plans in a person's heart, but it is the LORD's purpose that prevails. (Proverbs 19:21)

The author wish to sincerely acknowledge the following for their essential role in this study:

My Lord and Saviour Jesus Christ, for providing me His salvation, love, knowledge, wisdom, courage, patient and comfort to complete this degree.

Dr. Eyob. H Tesfamariam and Mr P. Chris de Jager for their love, care and supportive supervision and preparation of this manuscript.

Water Research Commission (WRC), East Rand Water Care Company (ERWAT) and National Research Foundation (NRF) for funding this study which without the study was have been possible.

My family, church mates, colleagues and friends for their love, support and prayers throughout the study.

Mr Charl D Hertzog for helping with laboratory training.

Mr M Lucas Nonyane for help with sample collection.

ABSTRACT

The composition of sludge organic matter is mainly influenced by the origin of wastewater. The biochemical composition of sludge could, however, alter from post wastewater treatment drying techniques. Such changes have a direct effect on the N fertilizer value of sludge because the biochemical composition of sludge dictates its decomposition rate. Therefore, proper understanding of the effect of wastewater treatment methods and post treatment drying techniques on a) the C and N contents as well as organic matter composition of sludge, and b) C decomposition and N mineralization is crucial for N management in sludge amended agricultural lands. The aim of this study was to investigate the effect of selected wastewater treatment and post treatment drying techniques on a) the N, C, and organic matter composition, as well as b) C decomposition and N mineralization of municipal sewage sludge. The study also investigated the compounds responsible for C and N release during a decomposition process. Two consecutive incubation studies (100 days each) were conducted to determine N release, C decomposition and sludge organic matter decomposition using different sludge types namely: Thermally hydrolysed sludge (THS), Activated sludge (Activated), and anaerobically digested sludge. The anaerobically digested sludges were collected from two different drying techniques (concrete beds and earth paddy) as well as two sludge drying depths on beds (≤10 cm and ≥25 cm). Inorganic N was determined by the steam distillation method. Sludge organic matter composition was analysed using Van Soest method. Total N and total organic C were determined using Carlo-Erba method. A parallel sludge alone incubation study was conducted from which organic compounds degradation and transformation analysis were conducted. The

anaerobically digested sludge dried in thin layers of ≤10 cm (ADS3) had higher total N (4.95%) than similar sludge types dried in thick layers of ≥25 cm in concrete beds (ADS1= 2.81%N) and in earth paddy (ADS2 = 2.83%N). Large fraction (>60%) of the organic C in all sludge types was in soluble compounds form (SOL). Thermally hydrolyzed sludge (THS) had the highest (90%) SOL. ADS3 and Activated had lower lignin fraction (<3%) than THS, ADS1 and ADS2. Net N mineralization per kg C applied was highest for activated (77 g kg^{-1} C) and was lowest for ADS2 (23 g kg^{-1} C). Despite having highest SOL, THS had relatively lower net N mineralization per kg organic N applied (350 g kg^{-1} organic N) than Activated sludge (449 g kg^{-1} organic N) mainly due to the relatively higher lignin fraction. It was apparent from this study that the soluble compounds were responsible for about 90% of the organic matter decomposition. The Van Soest method was also successfully used to determine residual compounds at intervals during the incubation study from the sludge alone study but not with the soil-sludge mixture.

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CHAPTER 1: GENERAL INTRODUCTION

Municipal sewage sludge is a biological waste which is made up of both organic and inorganic materials. Beneficial agricultural use of municipal sludge is a well-known practice around the globe. Sludge applied on agricultural lands serves as source of macro and micro nutrients (Muse et al., 1991; Ekama, 1993), source of organic matter, (Munn et al., 2000) and minimize soil erosion (Muse et al., 1991). It is well documented that sludge applied to food and feed crops improves crop yield (Cogger et al., 2001; Lavado et al., 2007; Tesfamariam, 2009).

Generally sewage sludge has significant amounts of N and P and can play a significant role as inorganic fertilizer substitute for these major plant nutrients (Hall, 1986). A large fraction of the N in sewage sludge is in organic form (Trindade et al., 2001) and its availability for crop uptake is dependent on its mineralization rate and other losses through volatilization, denitrification, and leaching (Tesfamariam et al., 2013). Therefore, quantitative understanding of N dynamics in sludge amended soils is critical to maximize crop production and minimize nitrate leaching.

Nitrogen in sewage sludge exists both in organic and inorganic forms. The inorganic N fraction mostly exists in the form of ammonium (NH_4^+) and nitrate (NO_3^-) (USEPA, 1995). The organic N fraction is made up of various organic compounds that decompose at different rates (Lerch et al., 1992; Serna and Pomares, 1992; Parnaudeau et al., 2004; Smith and Tibbett, 2004). Sludge composition is mainly influenced by the source. Nevertheless, wastewater treatment and post treatment dewatering/drying techniques can significantly alter the organic matter composition

(Banegas et al., 2007; Fernandez et al., 2007) as well as C and N mineralization of sludge (Ubierna et al., 2013; Mattana et al., 2014). However, there is still need for more detailed quantitative information on the combined effect of wastewater treatment and post treatment sludge drying techniques on C and N decomposition, the contribution of various organic compounds to C and N release as a function of time. There is little or no information at all on the effect of sludge drying time (duration) and sludge depth (thickness) in drying beds on sludge C and N content as well as decomposition.

The aim of this study was to investigate the effect of selected wastewater treatment and post treatment drying techniques on a) the nitrogen, carbon, and organic matter composition, as well as b) carbon decomposition and nitrogen mineralization of sewage sludge. The study also investigated the compounds responsible for the release of C and N using the Van Soest method.

The thesis is organized in the following order:

Chapter 2 is the literature review. In this chapter background information on benefits of sewage sludge application in agricultural lands is provided. In addition, detailed information on major N transformation processes such as mineralization, immobilization and nitrification are presented. The chapter further illustrates the most commonly used methods for the prediction of organic matter decomposition dynamics (biological and chemical extractants).

Chapter 3 deals with the effect of wastewater treatment and post treatment techniques on the carbon, nitrogen and Van Soest method extractable organic compounds. This

chapter presents the effect of wastewater treatment techniques (anaerobic digestion, activated sludge, and thermal hydrolyses) and post treatment dewatering/drying techniques (drying in concrete beds in thin sludge layers of <100 mm depth vs. thick layers of 250 mm as well as drying in soil paddies) on sludge total N, C, and organic matter composition.

Chapter 4 presents the effect of wastewater treatment and post treatment drying techniques on nitrogen and carbon decomposition of sludges. The effect of wastewater treatment and post treatment drying techniques on sludge N mineralization and C decomposition are discussed in detail. Chapter 5 is about the effect of wastewater treatment and post treatment drying techniques on dynamics of selected organic compounds. In this chapter the influence of wastewater treatment and post treatment drying techniques on the dynamics of chemical compounds during decomposition process is investigated.

Chapter 6 presents the general conclusion of the whole study and recommendations for future studies.

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CHAPTER 2: LITERATURE REVIEW

2. 1 Benefits of using sludge in agricultural lands

2.1. 1 Soil physical and chemical property improvement

The use of sewage sludge in agricultural lands was reported to improve soil organic matter (Navas et al., 1998; Munn et al., 2000; Marx et al., 2004), which lowers soil bulk density, enhances aggregation and aggregate stability, and improves soil water holding capacity (Chaudri et al., 2001; Ojeda et al., 2003). Sludge acts as a soil conditioner to increase water retention and improve soil workability (Ekama, 1993). According to Metzer and Yaron (1987), both the amount and composition of sludge organic matter added to the soil affects the extent to which the soil physical properties are modified. Sewage sludge application decreases soil pH (Epstein et al., 1976; Navas et al., 1998; Keramati et al., 2010). In other cases it was reported that sewage sludge application increased soil pH (Gallardo et al., 2007; Nakatani et al., 2012). Such variation in the soil pH is a function of the sludge treatment process in wastewater treatment plants such as the application of calcium carbonates (Sommers, 1977).

2.1. 2 Fertilizer values of sewage sludge

According to Finck (1995), soil fertility is a complex term and encompasses a combination of components such as soil texture, soil reaction, soil depth, nutrient content, soil microbial activity, organic matter content and composition, content or

absence of potentially toxic substances, etc. Generally sewage sludge has significant amounts of N and P and can play a significant role as inorganic fertilizer substitute for these major plant nutrients (Hall, 1986). Therefore, sludge can be considered as a low-grade fertilizer. In addition to the well-known macronutrients (N and P), sewage sludge also contains essential plant micronutrients such as Cu, Zn, Mn and B (Ekama, 1993; Snyman & Van der Waals, 2004). The mean macronutrient content of South African sewage sludges are presented in Table 2.1. According to these data South African sludge has appreciable amount of organic matter, which is vital for the amendment of the highly degraded South African soils. South African soils are highly degraded due to various reasons among which is the warm temperature which speeds up the mineralization of soil organic matter leaving the soil with low organic matter content (Kirschbaum, 1995).

Table 2. 1Typical nutrient content of dry South African sludge (Snyman and Herselman, 2006).

Nutrients	Range (%)
Total N	3.2-4.5
Total P	1.5-1.7
Total K	0.2-0.3
Organic content	40-70

2.2 Organic C and N mineralization

2. 2.1 Decomposition of organic matter

When organic materials are added to the soil, the various compounds start to decompose to release simpler organic and inorganic compounds (Brady and Weil, 2008). The biological breakdown of organic compounds is called decomposition (Juma, 1999; Brady and Weil, 2008). Different organic compounds decompose at differing rates (Hattori & Mukai, 1986; Lerch et al., 1992). Organic compounds are usually categorized into rapidly decomposing (sugars, starches, proteins), slowly decomposing (hemicellulose, cellulose, fats, waxes, resins and lipids), and lignified or recalcitrant compounds depending on their chemical structure (Alexandra and José, 2005; Brady and Weil, 2008). The rate at which different organic material decompose is influenced by type and chemical composition of organic material added, biological and environmental properties of the soil (Brussaard, 1994; Brady and Weil, 2008).

Soil organisms use organic matter from different organic materials added to the soil as source of food and energy during which nutrients (N, S and P) are also released to the soil (Alexandra and José, 2005; Benbi and Richter, 2002; Brady and Weil, 2008). The transformation of organic matter into inorganic components is called mineralization (Benbi and Richter, 2002; Alexandra and José, 2005; Brady and Weil, 2008; Sekoma, 2008). The opposite of mineralization can also be named net immobilization (Benbi and Richter, 2002; Sekoma, 2008) and the difference between the two is named as net mineralization. Net mineralization is a quantitative indication of the plant available nutrients (Benbi and Richter, 2002).

2.2.2 Nitrogen mineralization and immobilization

The mineralization of organic N (a) comprises of two major processes: ammonification (b) and nitrification (c1 and c2) (Brady and Weil, 2008; Sekoma, 2008).

$R\text{-}NH_2$ **+ $2H_2O$**→ OH^- + R-OH + NH_4^+ **+ O_2**→$4H^+$ + energy + NO_2^- + **$1/2O_2$** → Δ + NO_3^-, (Brady and Weil, 2008) (a)

Ammonification

Ammonification is a process whereby the organic N compounds are hydrolyzed with the aid of microorganisms to release NH_4^+ (Brady and Weil, 2008; Sekoma, 2008).

$$R\text{-}NH_2 + 2H_2O \rightarrow OH^- + R\text{-}OH + NH_4^+ \quad \text{(Brady and Weil, 2008)} \quad \text{(b)}$$

Nitrification

Nitrification is a process whereby NH_4^+ from ammonification is oxidized to nitrite with the aid of nitrosomonas bacteria (c1) then to nitrate by nitrobacter (c2) (Brady and Weil, 2008; Benbi and Richter, 2002; Sekoma, 2008).

$$NH_4^+ + O_2 \rightarrow 4H^+ + \text{energy} + NO_2^- \quad \text{(Brady and Weil, 2008).} \quad \text{(c1)}$$

$NO_2^- + 1/2O_2 \rightarrow$ energy + NO_3^- (Brady and Weil, 2008). (c2)

Nitrogen immobilization (d) takes place by biological as well as abiotic processes (Brady and Weil, 2008). Biological immobilization takes place when microorganisms that decompose organic N compounds transform inorganic N to organic form when there is not enough inorganic N to build the microbial biomass (Brady and Weil, 2008; Sekoma, 2008).

Energy + NO_3^- **– 1/2O_2**→ NO_2^- →$4H^+$ + energy + NO_2 **– O_2**→ OH^- + R-OH + NH_4^+ **-** $2H_2O$→ $R\text{-}NH_2$, (Brady and Weil, 2008). (d)

2.3 Methods for predicting the kinetics of organic compound decomposition

2.3.1 Biological methods

Biological methods predict potentially mineralisable N through incubation studies (Castellanos & Pratt, 1981; King, 1984; Chae & Tabatabai, 1986; Bitzer & Sims; 1988; Qafoku et al., 2001; Booth et al., 2005). Different sludges have different N mineralization rates (Parnaudeau et al., 2004; Parker and Sommers, 1983; Serna & Pomares, 1992; Smith and Tibbett, 2004). Previous studies have shown that aerobically

treated sludges have higher mineralization rates than anaerobically treated sludges (Serna & Pomares, 1992; Hernandez et al., 2002). Anaerobic digestion process reduces organic N by converting a substantial amount of organic N into ammoniacal N (NH_3 and NH_4^+) (Hue, 1995), thus reducing the organic N substantially relative to aerobic digestion (Parker and Sommers, 1983).

Nitrogen mineralization from similar sludge types could also differ when incubated in varying soil types (Terry et al., 1979; Ajwa and Tabatabai, 1994). This is because decomposition rate and N mineralization are affected by different soil texture, pH, soil organisms, soil temperature and soil moisture (Terry et al., 1979; Clark & Gilmour, 1983; Gilmour & Clark, 1988; Doublet et al., 2010).

Previous studies on N mineralization from sludge amended soils showed huge variation as could be seen from reports by Parnaudeau et al. (2004) (74 to 428 mg N/kg soil) and Serna & Pomares (1992) (-3.3 to 120 g N/kg soil). This wide variation is typical indication of the effect of sludge type on N mineralization and the need for individual parameterization of sludges (Parnaudeau et al., 2004).

Most incubation studies use a period of 16 weeks and more to predict potentially available N. Such long turnover rate to get results has increased interests in developing fast but accurate methods such as chemical extractants tests and infrared spectroscopy techniques (Magdoff & Amadon, 1980; Castellanos & Pratt, 1981; Parker and Sommers, 1983). Although biological methods are the standard methods against which other methods are calibrated (King, 1973; Magdoff & Amadon, 1980; Parker & Sommers,

1993), they are not practical for routine laboratory analysis because they are time consuming.

During N mineralization studies through incubation, C is released in the form of CO_2, and therefore often both C and N mineralization are studied together (Hart et al., 1994). Usually C mineralization was reported to be well correlated with gross N mineralization (Hart et al., 1994; Bengtsson et al., 2003) but Parfitt et al. (2003) reported a positive correlation between C mineralization and net N mineralization. Such variation is attributed to the potential influence of C:N ratio (Craine et al., 2007; Murphy et al., 2003) and composition of organic materials (Morvan et al. 2001) as well as the environmental factors (Doublet et al., 2010).

2.3. 2 Chemical extractants

The use of chemical extractants to fractionate organic compounds from soil and forage litter has been studied for many years by now. Van Soest (1963) developed a method to determine different organic compounds in forage litter. More details about the method were reported by Goering and Van Soest (1970). This method fractionates organic matter into soluble compounds (fats, protein, starch, organic acids and sugar), hemicellulose, cellulose and lignified fraction (Mottet et al., 2010). The method involves three different analyses namely: neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) (Goering and Van Soest, 1970; Van Soest, 1994).

Soluble compounds fraction is the fraction of compounds that dissolve when the sample is treated with NDF solution for 1 hour at 100 ^{o}C (Van Soest, 1994). The residues that remain after the NDF treatment consists mainly of hemicellulose, cellulose, lignin and minerals (Goering and Van Soest, 1970; Van Soest and Robertson, 1980; Van Soest, 1994). The fats fraction which forms part of the soluble compounds fraction can be separated by extracting with petroleum ether before the sample is treated with NDF solution (Van Soest, 1994). The ADF uses acid detergent solution which consists of 1 N sulfuric acid (H_2SO_4) to extract all the compounds which are soluble in NDF solution and hemicellulose, leaving cellulose, lignin and mineral in the residues (Van Soest 1963; Goering and Van Soest, 1970; Van Soest and Robertson, 1980; Van Soest, 1994). Acid detergent fiber is pre-treatment for ADL (Goering and Van Soest, 1970). For ADL, the residues which are left after ADF are treated with 72% H_2SO_4 for 3 hours at room temperature and the remaining residues are ashed (Goering and Van Soest, 1970; Van Soest and Robertson, 1980; Van Soest, 1994). The 72% H_2SO_4 digest cellulose which is left after ADF treatment leaving lignified fraction and minerals in the residues while ashing burn lignified fraction leaving minerals (Van Soest, 1994). Hemicellulose is estimated as the difference between NDF and ADF and cellulose as the different between and ADF and ADL (Van Soest and Robertson, 1980).

Ryan et al. (1990) modified part of Goering and Van Soest (1970) method to determine proximate carbon fractions of forest litter. The method fractionates organic matter into extractives (Hemicelluloses plus other labile compounds), cellulose and lignin (Ryan et al., 1990). Extractives fraction is the difference between the original sample mass and ADF (Ryan et al. (1990). The ADF in this method differ from the one outlined by Goering

and Van Soest (1970) because it omitted the use of decahydronaphthalene (Ryan et al., 1990).

Beyer et al. (1993) outlined a simple wet chemical extraction method to determine different organic compounds in soil organic matter (SOM). The method is made from a combination of the litter compound (sugar, starch, hemicellulose and cellulose) and humic compound (fulvic and humic acids) analysis. The fats fraction is extracted with a solution made up of ethanol/benzene (1:1) at 80 ^{o}C, sugar and starch with 0.025 M H_2SO_4 at 21 ^{o}C, hemicellulose with 0.63 M HCl at 100 ^{o}C and cellulose with 13.5 M H_2SO_4 at 100 ^{o}C, (Beyer et al., 1993). The litter compound analysis part was reported to produce better results (Beyer et al., 1993).

Ping et al. (2001) combined the method described by Malcolm (1992) for XAD resin, the forage litter method by Goering and Van Soest (1970) and the method from Beyer et al. (1993), to form a sequential SOM fractionation method. The fats fraction is extracted with a solution made up of toluene/ethanol (1:2.3) (Ping et al., 2001). The soluble compounds fraction (fraction that is soluble in NDF solution), hemicelloluse and cellulose in this method are extracted using solvent as described by Goering and Van Soest (1970). The method was reported to allow a complete characterization of SOM from different ecosystems (Ping et al., 2001). Both Ping et al. (2001) and Beyer et al. (1993) used 0.1 NaOH to extract humic substances (humic and fluvic acids). The use of 0.1 NaOH does not only extract humic substances but it also include other nonhumic substances such as low molecular weight acids and saccharides (simple sugar,

oligosaccharides, polysaccharides, amino sugars and sugar acids) (Thurman and Malcolm, 1989) and it can results in under estimation of other compounds.

According to Ping et al. (2001), methods for characterizing SOM are normally adopted from wood fiber analytical method by Ryan et al. (1990) or from forage analytical method (Goering and Van Soest, 1970) and feed analytical method (Van Cleve, 1974). This study will use the Goering and Van Soest, (1970) forage analysis method to characterize the sludge materials during and before incubation. The ADF and ADL of this method are recognized as part of Association of Official Analytical Chemist (AOAC) (Jung, 1997). The method was successfully used to characterize sewage sludge (Doublet et al., 2010; Mottet et al., 2010 and Zhao et al., 2011) and sludge composts and its decomposition (Jouraiphy et al., 2005).

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CHAPTER 3: The change in carbon, nitrogen and Van Soest method extractable organic fractions of municipal sewage sludge as influenced by wastewater treatment and post treatment drying techniques

Abstract

The biochemical composition of sludge is influenced by pre and post wastewater treatment techniques as well as the origin of the wastewater. The aim of this study was to investigate the effect of different sludge treatment techniques and post treatment drying techniques on the nitrogen, carbon, and the fraction of organic compounds in municipal sewage sludge. The candidate sludge materials were: activated sludge (Activated), Thermal hydrolysis sludge (THS), and three anaerobically digested sludges (ADS1, ADS2, and ADS3). The Activated and ADS3 sludge were dried in concrete beds in thin layers of less than 100 mm depth on average for 7 days. ADS1 and ADS2 were dried in thicker layers of 250mm with the former in concrete beds and the later in paddies on average for 21 days in summer and double in winter. Total N (TN) and C (TC) were determined using the Carlo Erba method and the inorganic N through steam distillation method. The principal organic compounds in the sludge were determined through fractionation using the Van Soest method. The ADS3 and Activated sludges, which were dried in thin layers for shorter period of time, had the highest TN (4.95% – 5.2%), indicating a higher N fertilizer value than THS (3.13%), ADS1 (2.81% N), and ADS2 (2.8% N). The inorganic N accounted for 10 – 11% of the total N for THS and ADS1 respectively, and less than 3% for the others. A large fraction (>60%) of the organic C in all sludge types was soluble compounds with the THS sludge having the

highest (90%). The ADS3 and Activated sludges had the smallest lignified fractions (<3% of the total organic C) but the largest hemicelluloses fraction (>20%). Slow drying of sludge in thick layers seems to reduce the N content while increasing the lignified fraction. This was expected to decrease their fertilizer value of sludge.

Keywords: Sludge, Lignified fraction, cellulose, hemicellulose, lipids, soluble compounds, N and C

3.1 Introduction

The biochemical composition of sludge is influenced by wastewater treatment and post treatment dewatering/ drying techniques (Ucisik and Henze, 2008). Thus it is of great importance to identify the effect of pre and post wastewater treatment techniques on the sludge composition. This is because the biochemical composition of sewage sludge dictates the rates of C decomposition and N mineralization (Seneviratne, 2000; Parnaudeau et al., 2004; Smith and Tibbett, 2004).

Anaerobic and aerobic digestion of sludge are the most commonly employed wastewater treatment techniques which produce a stable sludge (Chan et al., 2009) while activated sludge treatment process produce unstable sludge. The most commonly used sludge dewatering/drying techniques include: cake filtration, centrifugation, bed drying, thermal drying, and belt pressing (Dirkzwager & Hermite, 1988; Matsuoka et al., 2006). In South Africa the dominant sludge drying technique used is drying beds (Snyman et al., 2004). Drying beds are relativly cost effective compared to mechanical and thermal drying techniques (WEF, 2009). Post wastewater treatment sludge drying techniques are vital for agricultural use as this significantly influences the transport costs of sludge from wastewater treatment plants to receiving soils (Kouloumbos et al., 2008). The drying techniques could, however, influence the quantity and plant availability of N in the sludge.

The aim of this section of the study is to investigate the effect of selected wastewater treatment techniques (anaerobic digestion, activated sludge, and thermal sludge hydrolyses) and post treatment drying techniques (drying on concrete beds at 10 cm vs.

25 cm drying depth and drying on earth paddy) on the N, C, and organic compounds composition of municipal sewage sludge.

3.2 Materials and Methods

3.2.1 Sludge materials

The sludge materials investigated in the study were obtained from different wastewater treatment plants and were chosen to represent different wastewater treatment processes and post treatment drying method. The actual sewage treatment processes and dewatering/drying methods evaluated in this experimental work are shown in Table 3.1 while selected chemical properties of the sludge materials are presented in Table 3.2.

Table 3. 1 Description of sewage sludge used

		Post treatment drying process and duration		
Sludge acronym	Sludge treatment process	Drying method	Sludge layer depth during drying	Duration of drying process
Activated	Activate sludge	Concrete beds	< 100mm	About 7 days
ADS1	Anaerobically digested	Concrete beds	250mm	About 21 days in summer & 42 days in winter
ADS2	Anaerobically digested	Paddy	250mm	About 21 days in summer & 42 days in winter
ADS3	Anaerobically digested	Concrete beds	<100mm	About 7 days
THS	Thermally hydrolyzed	NA	NA	NA

Table 3. 2 Selected chemical properties of sludges investigated

Property	Activated	ADS1	ADS2	ADS3	THS
pH (H_2O)	6.24	5.86	6.34	6.58	6.78
EC (mS m^{-1})	264	236	77	211	317
Total C (%)	29.7	17	24.8	31.9	18.1
Total N (%)	4.95	2.81	2.83	5.47	3.13
*Organic N (%)	4.83	2.50	2.78	5.08	2.83
NH_4^+ (mg kg^{-1})	1008	2640	250	1449	2697
NO_3^- and NO_2^- (mg kg^{-1})	166	508	254	75.4	325
Total P (%)	2.44	3.02	2.34	2.00	2.70
K (%)	0.59	0.27	0.26	0.46	0.08
Na (%)	0.11	0.15	0.07	0.1	0.06
Ca (%)	1.96	2.86	2.76	1.83	3.38
Mg (%)	0.53	0.39	0.37	0.42	0.15
Zn (mg kg^{-1})	1166	2886	3065	1121	523
Mn (mg kg^{-1})	175	880	625	600	291
Al (%)	0.74	2.17	2.03	0.77	0.81
Fe (%)	1.21		2.15	1.12	

*Calculated as the difference between total N and total inorganic N (NH_4^+, and NO_3^- and NO_2^-).

All sludge materials were air dried and milled to pass through a 1 mm sieve. The air dried and milled sludge was extracted with petroleum ether using Soxtec™ 2043 Fat Extraction System to determine the fraction of lipids in the sludge. The lipids extraction was followed by estimation of the Neutral Detergent Fiber (NDF) as described by Goering and Van Soest (1970) and Van Soest (1994). The NDF solution was made up of sodium lauryl sulphate, disodium ethylenediaminetetraacetate (EDTA) dehydrate, sodium borate decahydrate, disodium hydrogen phosphate anhydrous, 2-ethoxyethanol (ethylene glycol monoethyl ether) purified grade and distilled water. A 0.3 g of air dried sample milled to pass 1 mm sieve, was placed in 50 ml crucible and boiled for 1 hour with 100 ml NDF solution at 100 ^{o}C. After boiling, the extract was filtered through the crucible and the residues that remained in the crucible were oven dried at 60 ^{o}C and not ashed. Extraction of NDF was conducted using Fibertec™ 2010 Auto Fibre Analysis System. The NDF extraction was followed by Acid Detergent Fiber (ADF) extraction as described by Goering and Van Soest (1970) and Van Soest (1994). The ADF solution was made up of Cetyl trimethyl ammonium bromide (CTAB) and 1 N H_2SO_4. A 0.3 g air dried sample milled to pass 1 mm sieve, was placed in 50 ml crucible and boiled for 1 hour with 100 ml ADF solution at 100 ^{o}C. After boiling, the solvent was filtered through the crucible and the residues that remain in the crucible were oven dried at 60 ^{o}C and not ashed. Extraction of ADF was conducted using Fibertec™ 2010 Auto Analysis System. Finally the acid detergent lignin (ADL) was determined as reported by Goering and Van Soest (1970) and Van Soest (1994). This procedure had two steps. The first step was to run ADF. The dried residues from ADF were treated with 72 % sulfuric acid for 3 hours at room temperature. Afterwards it was filtered through the crucible and the residues that remained in the crucible were oven

dried at 60 ^{o}C. In the second step, the dried residues from the 72 % sulfuric acid treatment were ashed. Similar to the NDF and ADF, extraction of ADL was conducted using Fibertec^{TM} 2010 Auto Analysis System.

Fractions of the neutral detergent soluble fraction, hemicelluloses, cellulose and lignified fraction were calculated as follows:

Soluble compounds fraction = original mass – (NDF+ fats fraction),

Hemicellulose = NDF- ADF,

Cellulose = ADF- 72 % H_2SO_4 extraction,

Lignified fraction = 72 % H_2SO_4 extraction – Ash.

The inorganic N (NO_3-N + NO_2^- and NH_4-N) was determined by the steam distillation method as described by Mulvaney (1996). Total N (TN) and C (TC) were determined using the Carlo Erba method. The EC and pH of the sludge materials were determined using 1:2.5 sludge to water ratio. Total P and all heavy metals were determined using Inductively Couple Plasma Optical Emission Spectrometer (ICP-OES) (Spectroflame Modula; Spectro; Kleve Germany) after wet acid digestion.

3.3 Results and discussion

3.3.1 Results

3.3.1.1Effect of wastewater treatment process and sludge drying techniques on total, organic and inorganic fractions of N in sludge

Sludge N content varied significantly (P≤0.005) between sludges that were dried in thin layers for short period (ADS3 and Activated) and sludges that were dried in thick layers for relatively longer time (Fig.3.1).

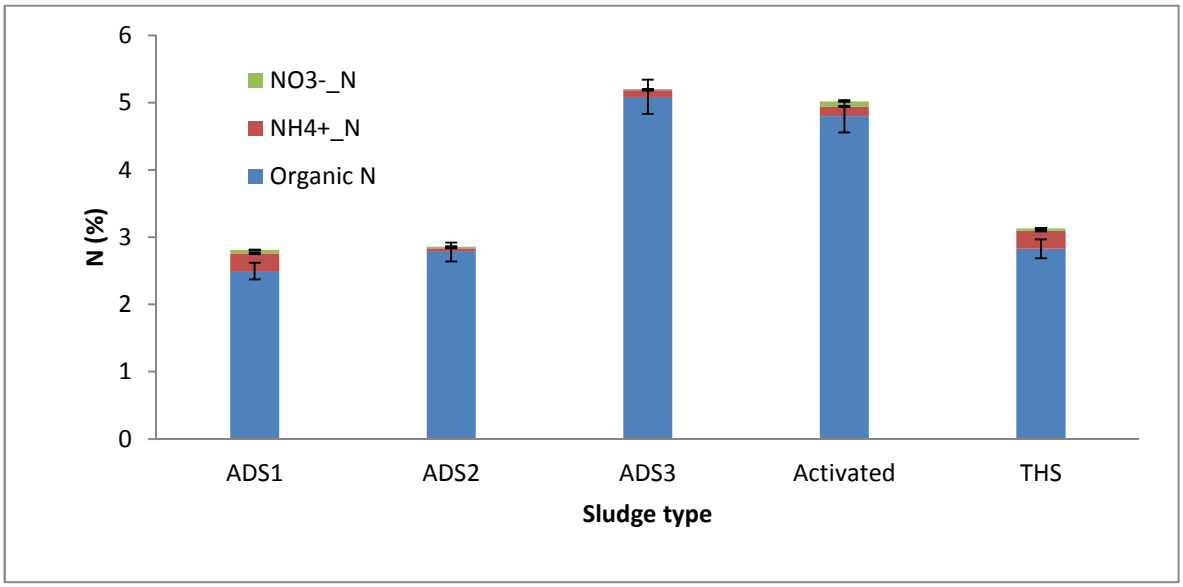


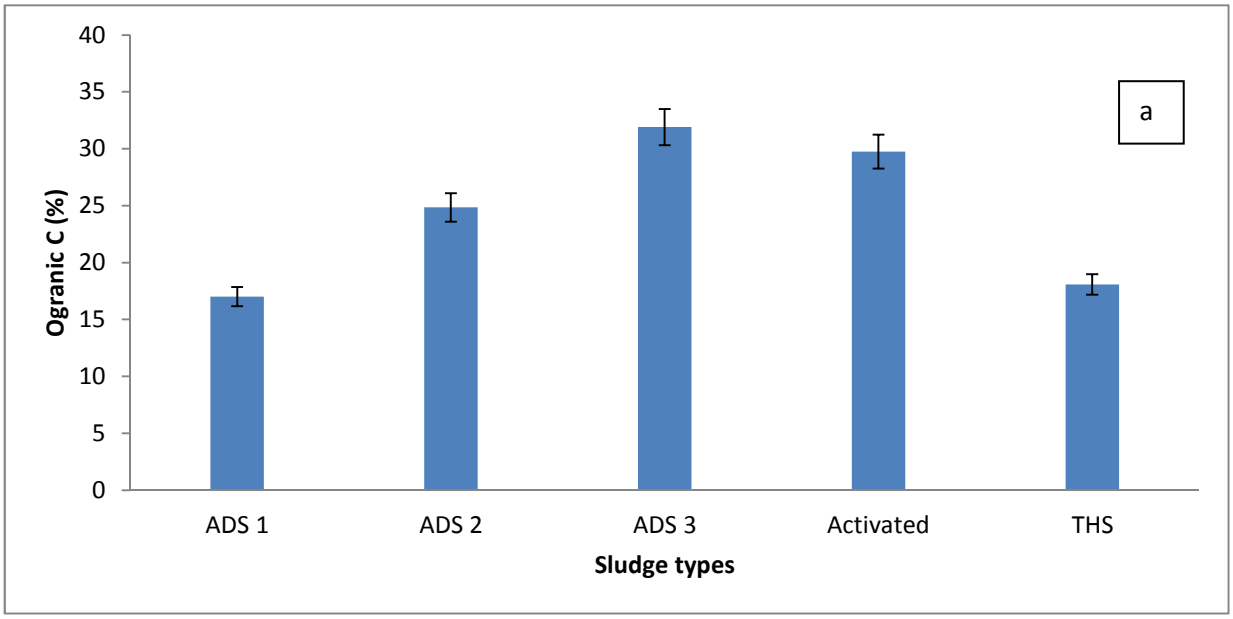
Figure 3.1Total organic and inorganic fraction of N in sludge as affected by wastewater treatment process and drying methods (anaerobically digested dried in concrete beds in layers of 250 mm (ADS1); anaerobically digested dried in paddies in layers of 250 mm (ADS2); anaerobically digested dried on concrete slab in layers of less than 100 mm (ADS3); activated sludge dried in concrete slab in layers of less than 100 mm (Activated); Thermal hydrolysis sludge (THS).

Activated sludge and anaerobically digested sludge dried as thin layers on concrete slabs (ADS3) had the highest total N compared with ADS 1 & 2 and THS. It was interesting to note that the two sludge types, ADS1 and ADS2, which were all anaerobically digested and dried in concrete beds and paddy in thicker sludge layers (250mm), had similar total N content (2.81 and 2.83%, respectively). However, the NH_4 content of ADS1 was almost an order of a magnitude greater than for ADS2 (2640 mg/kg for ADS 1 versus 250 for ADS2)

Sludge treated through thermal hydrolyses also had similar total N content (3%) to ADS1 and ADS2. The inorganic N fraction (NO_3 and NH_4) accounted only 2-3% of the total N in ADS2, ADS3 and Activated sludges and 10-11% of the ADS1 and THS.

3.3.1.2 Effect of wastewater treatment process and drying time on sludge organic matter content and composition

The total organic C content and composition of sludge varied significantly among the wastewater treatment processes and drying time. The study showed that ADS3 and activated sludge had highest total organic C followed by ADS2 and ADS1. Unlike the total N, however, ADS2 had relatively higher total organic C than ADS1 (Fig. 3.2a). Similar to the total N, the organic C content of THS remained similar to that of ADS1 but lower than ADS2. It was evident that the soluble compounds comprised of 62 – 88% of the total organic matter decomposition of all sludge types under investigation (Fig. 3.2b)



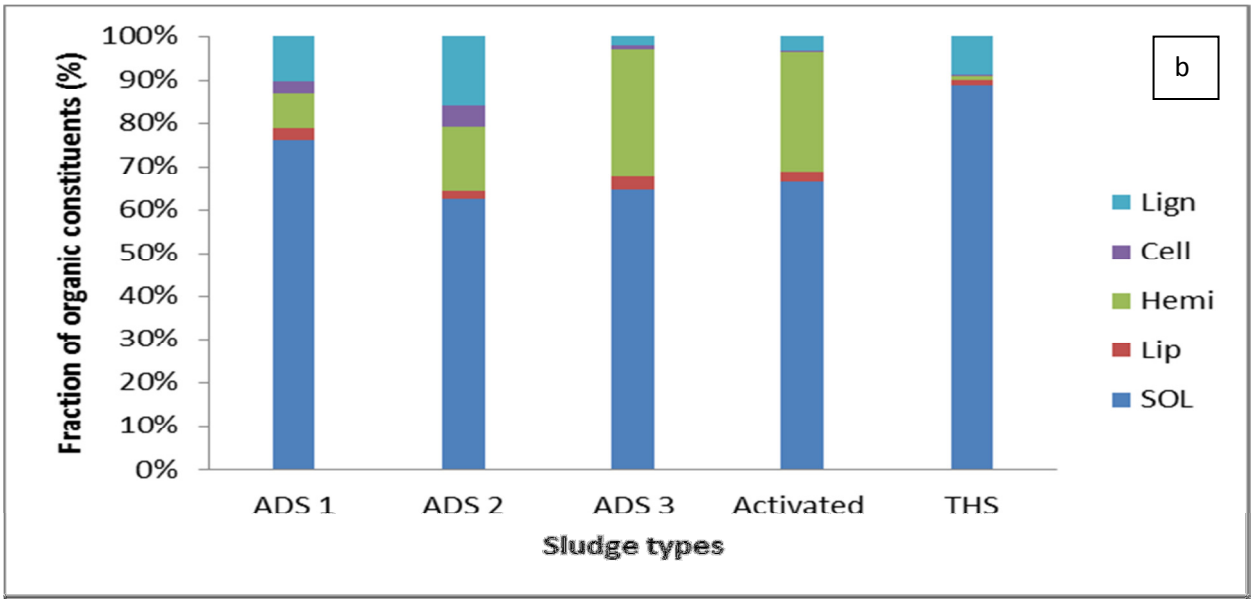


Figure 3.2 Total organic C contents (a) and percentage distribution of organic constituents (b) for five sludge types (anaerobically digested dried in concrete beds in layers of 250 mm depth (ADS1); anaerobically digested dried in paddy in layers of 250 mm depth (ADS2); anaerobically digested dried on concrete slab in layers of less than 100 mm depth (ADS3); activated sludge dried in concrete slab in layers of less than 100 mm depth (Activated) Thermal hydrolysis sludge (THS). NB. Lip = Lipids, SOL = Soluble organic compounds, Hemi = Hemicellulose, Cell = Cellulose and Lign = Lignified fraction

The other major differences among the sludge types were: hemicellulose for ADS3 and activated sludges; lignin for ADS1, ADS2 and THS sludges. Lipids and cellulose together constituted less than 7% of the total organic matter of all the sludge types considered in this study. Generally, the soluble compounds constituted 13 – 22% by mass of the sludge solid fraction (Fig. 3.3). Hemicellulose was the next dominant organic compound (2 – 10%) followed by Lignin (1 – 4%). In contrast, lipids and cellulose constituted <2%.

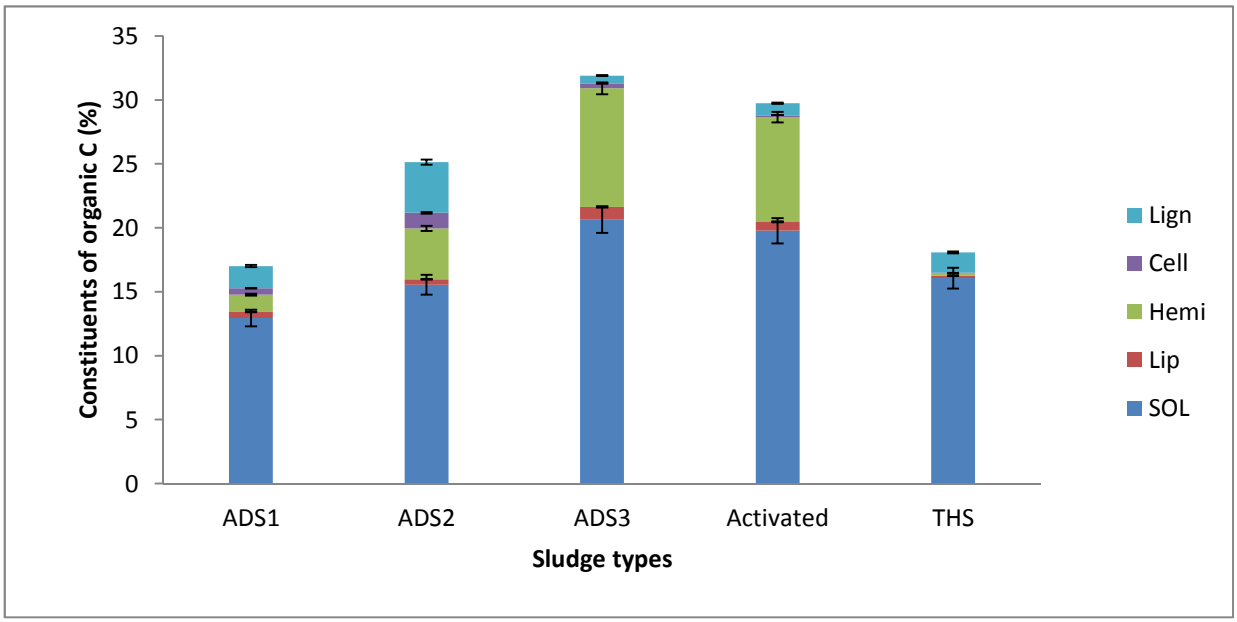


Figure 3.3 Percentage by mass of organic constituents in selected sludge types (anaerobically digested dried in concrete beds in 250 mm depth sludge layers (ADS1); anaerobically digested dried in paddy in 250 mm depth sludge layers (ADS2); anaerobically digested dried on concrete slab in layers of less than 100 mm depth (ADS3); activated sludge dried in concrete slab in layers of less than 100 mm depth (Activated); Thermal hydrolysis sludge (THS) NB. Lip = Lipids, SOL = Soluble organic compounds, Hemi = Hemicellulose, Cell = Cellulose and Lign = Lignified fraction

3.3.2 Discussion

3.3.2.1 Effect of wastewater treatment process and sludge drying techniques on total, organic and inorganic fractions of N in sludge

ADS1 sludge was anaerobically digested and dried in concrete beds. Similarly, ADS2 was anaerobically digested but was dried in paddy. Both sludges were dried on the beds in thick layers of 250 mm during the drying process. The similarity in the total N content of ADS1 and ADS2 seems to indicate that drying method (concrete or paddy) did not have significant influence on the total N content of anaerobically digested sludges as long as the sludge depth during drying and duration of the drying process remains similar. It was also interesting to note that there was similarity between the total N content of the stable thermally hydrolysed sludge and ADS1 as well as ADS2 sludges.

In contrast, the total N content of ADS3 was 1.8 times higher than ADS1 and ADS2. This is despite the similarity in the wastewater treatment processes (anaerobic digestion). This is most probably attributed to the sludge depth during drying on beds and the drying time (Pescod, 1971) which influence decomposition and volatilization significantly. According to the wastewater treatment plant managers, where sludge ADS3 originated from, this sludge was dried in thin layers of <100 mm depth and often dries within 7 days. While the drying process of ADS1 and ADS2 lasted for 21 days on average during summer and double the time during winter. The activated sludge was also collected from the same wastewater treatment plant as the ADS3 and was dried in drying beds in thin layers of <100 mm depth within approximately 7 days on average. Despite the differences in the sludge treatment process between ADS3 (anaerobically digested) and Activated (activated sludge), there was no

significant total N difference between the sludges. This is in contrast to the difference observed between ADS3 and ADS1 as well as between ADS3 and ADS2. This indicates that the depth of sludge in drying beds and the duration of drying process play a significant role in determining the N content of sludges.

As expected, ammonium was the dominant inorganic N species in all sludge types as all sludges were anaerobically digested. This is in line with previous findings of many authors who reported NH_4^+ as the dominant inorganic N species in anaerobically digested slduges (Wong et al., 2000; Adegbidi and Briggs 2003; Doublet et al., 2010; Zarabi and Jalali, 2013). The inorganic N fraction from this study accounted for <10% of each sludge total N content which was similar to the findings of Lasa et al., (1997; Vieira et al., (2005) and Cogger et al. (2001) from an anaerobically digested sludge.

3.3.2.2 Effect of wastewater treatment process and post treatment drying techniques on sludge organic matter content and composition

Despite the differences in the total organic C contents between ADS1 and ADS2, both sludge types had higher lignin fraction than ADS3, THS and activated sludge. This indicates that both ADS1 and ADS2 are more stable and are expected to have relatively lower N mineralization (Mottet et al., 2010). The difference in the total organic C content between ADS1 and ADS2 despite their similarities in the total N content has implications on N mineralization (Lashermes et al., 2010). This is due to their differences in the C:N ratio which affects the microbial activity (Pansu and Thuriés, 2003). Organic matter decomposition, however, is not only influenced by the

C:N ratio or the total N (Lashermes et al., 2010) and C content and therefore is inadequate for predicting decomposition kinetics (Thuriés et al., 2001).

Previous studies indicated a strong negative correlation between initial lignin content and N mineralization (Mubarak et al. 2010; Trinsoutrot et al., 2001; Palm et al., 2001) as well as cellulose and N mineralization (Rahn et al., 2003) from organic materials. Consequently, ADS2 is expected to have lowest cumulative N mineralization than the other sludge types due to its highest lignin:N ratio (Table 3.3) (Parnaudeau et al., 2004) and therefore lower fertilizer value. This is despite the similarity in total N content with ADS1 because an increase in lignin content turns to restrict the decomposition of organic matter (Baddi et al., 2004).

Table 3. 3 C:N ratio and lignin:N ratio of anaerobically digested sludges dried on concrete beds (ADS1 and ADS3) and paddy dried (ADS2), activated sludge dried in concrete drying beds (Activated), as well as Thermal hydrolysis sludge (THS).

	ADS1	ADS2	ADS3	Activated	THS
C:N ratio	6.05	8.78	6.44	5.72	5.77
Lignin:N ratio	0.61	1.40	0.12	0.18	0.50

Similar to previous findings (Morvan et al., 2006; Mottet et al., 2010; Parnaudeau et al., 2004; Doublet et al., 2010; Zhao et al., 2011), large fraction of the organic matter was made up of soluble compounds. These soluble compounds are the main sources of N mineralization and C decomposition during the first few days of organic matter breakdown (Sánchez-Monedero et al., 1999; Trinsoutrot et al., 2001; Alexandra and José, 2005; Brady and Weil, 2008). This show that higher percentage

of organic matter in sewage sludge forms part of the NDF soluble fraction, as it was stated by Parnaudeau and Dignac (2007).

3.4 Conclusion

Sludge N and C concentration decreased significantly when sludges were dried in thick layers of more than 250mm for longer duration of more than 20 days. There was, however, little or no difference in total N concentration between anaerobic digestion and activated sludge when both are exposed to similar procedures (sludge layer thickness and duration). The total organic C content, however, seemed to vary across drying methods in addition to the variation observed across wastewater treatment processes. Large fraction of the organic matter in all sludges types was accounted for soluble compounds. The lignified fraction was, however, relatively higher for the sludges that were dried in thick layers for longer time regardless of whether dried in concrete slabs or paddies. Sludges dried in thin layers within shorter period of time (7 days) were characterized by higher N, C, and low lignin fraction and were dominated by soluble compounds and hemicellulose indicating higher potential N fertilizer value than those dried in thick layers for longer duration.

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CHAPTER 4: The effect of wastewater treatment and post treatment drying techniques on N and C mineralization

Abstract

It is well documented that carbon and nitrogen mineralization of organic matter in sludge amended soils is a function of the treatment process the sludge underwent in wastewater care works. However, there is little information on the combined effect of sludge treatment and post treatment drying processes on C and N mineralization of sludge. The aim of this study was to test the hypothesis that a) thermally hydrolyzed sludge (THS) have higher mineralization per unit organic N applied than anaerobically digested sludge with similar initial total and organic N contents because of the low lignin content, b) Activated sludge (Activated) dried in thin layers of <100 mm in concrete beds have a higher fertilizer value than thermally hydrolyzed and anaerobically digested sludges due to higher initial N and relatively lower lignin fraction. Two consecutive incubation studies (each for 100 days) were conducted using four sludge types (THS, Activated, and anaerobically digested sludge dried in concrete beds (ADS1) and dried in paddy (ADS2)). The sludge was incorporated into a 100 g sandy clay loam soil to meet a 300 kg N ha^{-1} irrigated maize N requirements and KNO_3 was added to all but ADS1 to raise the initial inorganic N content of the soil-sludge mix to 60 mg kg^{-1}. Net N mineralization per kg C applied after 100 days of incubation was highest from activated sludge (77 g kg^{-1} organic carbon applied) compared with ADS1 (73 g kg^{-1}), THS (57 g kg^{-1}), and ADS2 (23 g kg^{-1}). Net N mineralization per kg N applied was, however, highest for ADS1 (492 g kg^{-1} organic N applied) followed by Activated (449 g kg^{-1}) and was lowest for ADS2 (179 g kg^{-1}). Carbon loss through decomposition accounted 59%, 36%, and 19% of the total C

applied from the Activated, THS, and ADS2 sludges, respectively. The N fertilizer value of Activated is higher than the other three sludge types investigated due to a higher initial total N and decomposable organic N.

Keywords: C decomposition, N mineralization, sewage sludge and incubation.

4.1 Introduction

Different sludge types have different C and N mineralization rates which are attributed to the differences in their composition (Parker and Sommers, 1983; Serna & Pomares, 1992; Parnaudeau et al., 2004; Smith and Tibbett, 2004). Previous studies have shown that sludge treatment processes could influence C and N mineralization of sludges (Epstein et al., 1976; Parker and Sommers, 1983; Serna and Pomares, 1992; Ubierna et al., 2013; Mattana et al., 2014).

According to Parker and Sommers (1983), activated sludge had the highest mineralizable N (40%), followed by raw and primary sludges (25%), anaerobically digested sludge (15%), and composted sludge (8%) of the total N applied during a 16 week incubation study. Other studies conducted by Serna and Pomares (1992) as well as Hernandez et al. (2002) also showed that aerobically digested sludges gave higher N mineralization than anaerobically digested sludges. In contrast, activated sludge was reported to give much higher mineralization than digested sludge due to its high active organic carbon and less stable compounds (Hsieh et al., 1981).

Recent methods of wastewater treatment such as thermal hydrolysis at temperatures ranging from 40 to 180ºC break the cell walls of biological organic materials in sludge improving degradability (Neyens and Baeyens, 2003; Wei et al., 2003). Studies conducted by Rigby et al., (2009) showed that thermally dried mesophilic anaerobically digested biosolids had larger mineralizable pool of N than dewatered mesophilic anaerobically digested biosolid, despite its lower total and mineral N content. Similar findings were reported by Smith and Durham (2002), Matsuoka et

al., (2006) and Fernández et al., (2007). According to the latter authors, thermal drying increased the easily mineralizable fraction of N and C in the sludge.

Little is known about the combined effect of wastewater treatment methods and sludge drying techniques on C and N mineralization of sludge. In addition, the effect of sludge depth on drying beds and duration of drying during the drying process on C and N mineralization needs investigation.

The aim of this section of the study was to test the hypothesis that a) thermally hydrolyzed sludge (THS) have higher mineralization per unit organic N applied than anaerobically digested sludge with similar initial total and organic N contents because of its low lignin content b) Activated sludge (Activated) dried in thin layers of <100 mm in concrete beds have a higher fertilizer value than thermally hydrolyzed and anaerobically digested sludges due to higher initial N and relatively lower lignin fraction.

4.2 Material and method

4.2.1 Sludge materials

Four sludge materials were selected for this study. Detailed characteristics of these sludge materials are presented in Tables 3.1, 3.2, 4.1, and 4.2. All sludge materials were milled to pass 1 mm sieve.

Table 4. 1 Biochemical composition of sludge materials: Activated sludge (Activated) anaerobically digested dried in concrete beds (ADS1), anaerobically digested dried in paddy (ADS2) and Thermal hydrolysis sludge (THS)

	Organic constituents				
Sludge type	Lipids	Soluble compounds	Hemicellulose	Cellulose	Lignin
	%				
Activated	2.33	66.47	27.51	0.48	3.21
ADS1	2.79	76.19	7.9	3	10.14
ADS2	1.74	62.55	14.84	4.91	15.96
THS	1.07	88.88	1.12	0.23	8.7

Table 4. 2 C:N ratio and lignin:N ratio of sludge materials

Parameter	Sludge type			
	ADS1	ADS2	Activated	THS
C:N ratio	6.05	8.78	5.72	5.77
Lignin:N ratio	0.61	1.40	0.18	0.50

4.2.2 Soil

A soil sample for the incubation study was collected from a long term field study trial at East Rand Water Care Works (ERWAT), near Johannesburg, South Africa. The site from which the soil sample was collected has never received inorganic fertilizer or sludge. The soil is a red clay loam Hutton soil (Soil Classification Working Group, 1991). Selected properties of the soil used in the incubation study are presented on Table 4.3.

Table 4. 3 Selected properties of the soil used for the incubation study

	Units	
Total N	%	0.09
Organic C	%	1.7
Organic N*	%	0.084
C:N		20
NH_4^+	mg/kg	33.964
NO_3^-	mg/kg	20.384
pH		6.1
EC	mS m^{-1}	52
Silt	%	24
Clay	%	26
Sand	%	46

*Calculated as the difference between total N and total inorganic N (NH_4^+, and NO_3^- and NO_2^-).

4.2.3 Method

4.2.3.1 Incubation

Two consecutive incubation experiments were carried out at the Soil Science Laboratory of the University of Pretoria. The incubation was conducted in room

insulted with air conditioner to maintain room temperature of 25 ±1 ºC. The incubation for N mineralization was conducted in 1163 cm^3 volume airtight containers where 100 g soil was mixed with the candidate sludge to meet a 300 kg N ha^{-1} application rate. Inorganic N in the form of KNO_3 was added during the second incubation study to increase the soil NO_3^-–N level to 60 mg kg^{-1} (Parnaudeau *et al*., 2004) in order to minimize N immobilization. The soil moisture was maintained at field capacity by adding water based on mass difference.

The C decomposition study was conducted in airtight 2000 cm^3 volume desiccators where 100 g soil was also mixed with the candidate sludge to meet 300 kg N ha^{-1}. During the first incubation, only one sludge type (anaerobically digested dried in concrete beds (ADS1)) was used. In the second incubation, however, three sludge types (ADS2, Activated and THS) were investigated. Similar to the N mineralization studies, KNO_3 was added in the second incubation study to increase the initial inorganic N content of the soil-sludge mix to 60 mg kg^{-1}. A zero control treatment, which did not receive sludge, was included for each incubation study.

The sludge, soil and inorganic fertilizer were thoroughly mixed at the commencement of the study. Both CO_2 evolution and inorganic N release analysis were conducted at similar time intervals during the incubation study. The analyses intervals were set at days (d) 0, 1, 3, 7, 15, 30, 65, and 100. Both N mineralization and CO_2 evolution experiments were aerated during sampling intervals for the first seven days of incubation. After day seven, the N-mineralization experiment was opened and weighed once a week and at sampling time to replenish water to field capacity while CO_2 evolution set up was opened only at sampling time.

4.2.3.2 Chemical analyses methods

Inorganic N determination

Inorganic N (NO_3-N and NH_4-N) release from sludge amended soils and control were determined by the steam distillation method described by Mulvaney (1996). A soil sample of 10 g on dry mass base was extracted with 100 ml 1 *M* KCl after 1 hour of shaking time. After shaking, the extracts were filtered through Whatman No. 42 filter papers and analyzed for inorganic N using Eq. 1.

A titre of 1 cm^3 0.0025 mol dm^{-3} H_2SO_4 equals to 35 µg N and 10 g soil was extracted with 100 cm^3 KCl, therefore:

$$Inorganic\ N\ (mg\ kg^{-1}) = \frac{A \times 100 \times 35}{C \times 10} \quad \text{Eq.1}$$

Where A = volume (mL) of 0.0025 M H_2SO_4 needed for titration

C = volume (ml) of aliquot sample used for distillation

The cumulative amount of net N mineralized from sludge was calculated using Eq. 2.

$$Net\ N\ mineralized = N(sludge\ amended\ soil) - [N(control) - N(initially\ in\ sludge)] \quad \text{Eq.2}$$

Where N is inorganic nitrogen at each sampling time.

The net-N mineralized was calculated to net-N mineralized per organic C applied (g kg^{-1}) and net-N mineralized per organic N applied (g kg^{-1}) using Eqs. 3 and 4, respectively.

$$NetN\ (per\ organic\ C\ applied) = \frac{Net\ N\ mineralized}{Organic\ C\ added\ from\ sludge\ per\ kg\ soil} \quad \text{Eq. 3}$$

$$NetN\ (per\ organic\ N\ applied) = \frac{Net\ N\ mineralized}{Organic\ N\ added\ from\ sludge\ per\ kg\ soil} \quad \text{Eq. 4}$$

CO_2 evolution measurement

A 20 ml of 1*M* NaOH was used as a carbon dioxide trap placed together with the incubated samples in air tight desiccators. A 100 ml beaker having 20 ml of distilled water was also placed in the dissectors to minimize drying out of the samples. The amount of CO_2 respired was measured by titration with 0.5 *M* HCl after the addition of 4 ml of 1 *M* barium chloride ($BaCl_2$) and 2 ml of phenolphthalein. CO_2 flux was estimated from the titration using Eq. 5. The CO_2 flux was computed according the equation of Anderson (1982) per mass of soil incubated.

$$CO_2 flux\ (mg/kg\ soil) = (B - V)\ \times NE\ /\text{m} \qquad \text{Eq. 5}$$

Where B = volume (mL) of 0.5 M HCl needed to titrate the NaOH in the control,

V = volume (mL) of 0.5 M HCl needed to titrate the NaOH in the sample,

N = molarity of the HCl (0.5M),

E = equivalent weight; to express as milligrams of CO_2, (22)

m = mass of soil (kg)

The decomposed C was converted to decomposed C per organic C applied using Eq. 6.

$$Decomposed\ C\ per\ organic\ C\ applied\ (g\ kg^{-1}) = \frac{Decomposed\ C}{organic\ C\ applied\ from\ sludge\ per\ kg\ soil} \qquad \text{Eq. 6}$$

4.3 Results and discussion

4.3.1 Results

4.3.1.1 N mineralization

Nitrogen mineralization varied significantly across sludge types (Fig. 4.1). The amount of N mineralized per kg C applied remained lowest for ADS2. While net N mineralization from the Activated sludge remained highest after day 30. The net N mineralization from ADS2 remained lowest at most of the time in the study period.

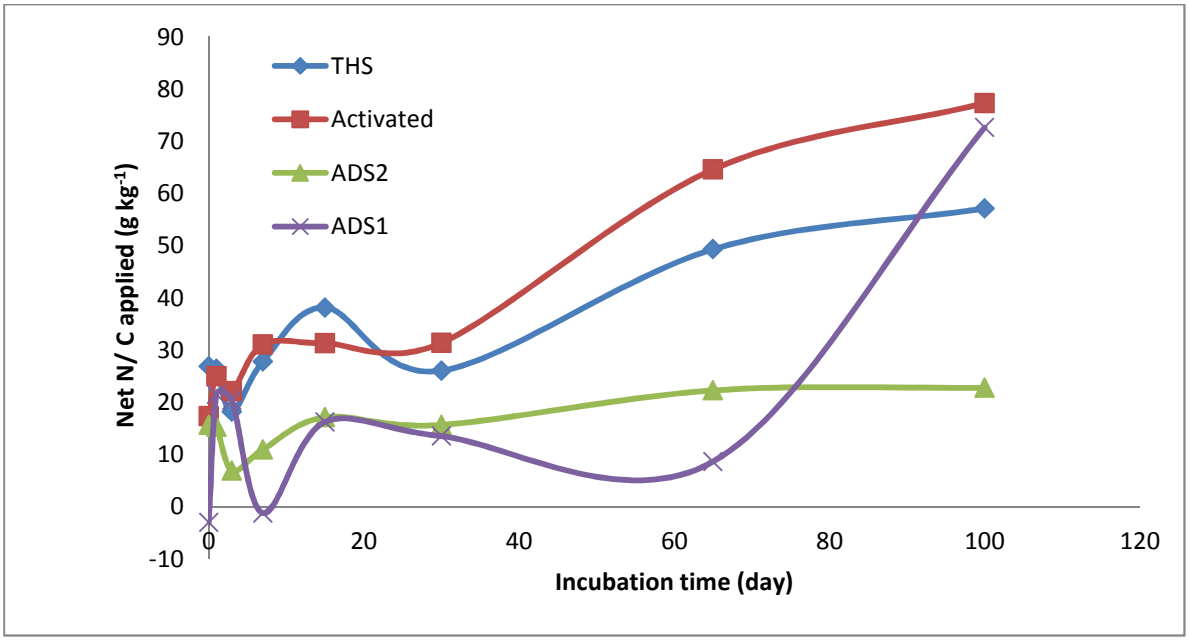

Figure 4.1 N mineralization from anaerobically digested dried in concrete beds in thick layers of 250 mm (ADS1) dried in paddy in thick layers of 250 mm (ADS2), Activated sludge dried in thin layers of less than 100 mm (Activated) and Thermal hydrolysis sludge (THS) (KNO_3 added at commencement of study only for ADS2, Activated & THS)

There were visible N immobilization events on days 1, 3, and 30 for THS, day 3 for Activated, days 1, 3, and 30 for ADS2, and days 3, 7, 30, and 65 for ADS1 (Fig.1). This was despite the addition of KNO_3 to all but ADS1. However, the amount and

intensity of immobilization was lower for the sludge treatments that received KNO_3. The first incubation study, which was conducted using anaerobically digested sludge dried on concrete beds without the addition of KNO_3 (ADS1), showed more immobilization events than the second batch incubation (Fig. 4.1). This is despite the similarity in the total N contents as well as the sludge treatment processes between ADS1 and ADS2, which were both anaerobically digested (Table 4.2).

The net N mineralization per kg organic N applied was highest for ADS1 (491.89 g kg^{-1}) and lowest for ADS2 at day 100 (Fig. 4.2). For most of the study period, however, ADS1 had the lowest net N mineralization.

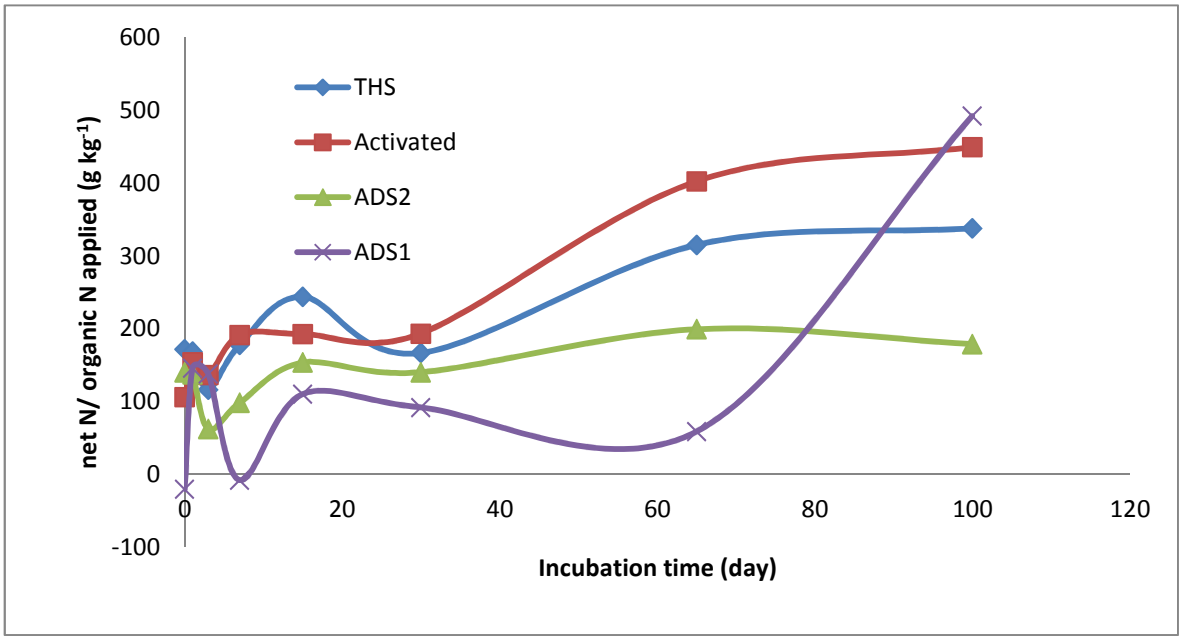

Figure 4.2 N mineralized per unit kg organic N applied from anaerobically digested dried in concrete beds in thick layers of 250 mm (ADS1) dried in paddy in thick layers of 250 mm (ADS2), activated sludge dried in thin layers of less than 100 mm (Activated) and Thermal hydrolysis sludge (THS) (KNO_3 added at commencement of study only for ADS2, Activated & THS)

4.3.1.2 Carbon decomposition

Generally cumulative C decomposition per kg organic C applied was highest for activated sludge and lowest for ADS2 (Fig. 4.3). Carbon decomposition rate was very high during the first seven days for ADS2, Activated, and THS sludges and during the first three days for ADS1 sludge (Fig. 4.3). Cumulative C decomposition at day 100 was highest from Activated (587 g kg^{-1} organic C applied), followed by THS (362 g kg^{-1} C applied) and was lowest for ADS2 (191 g kg^{-1} C applied).

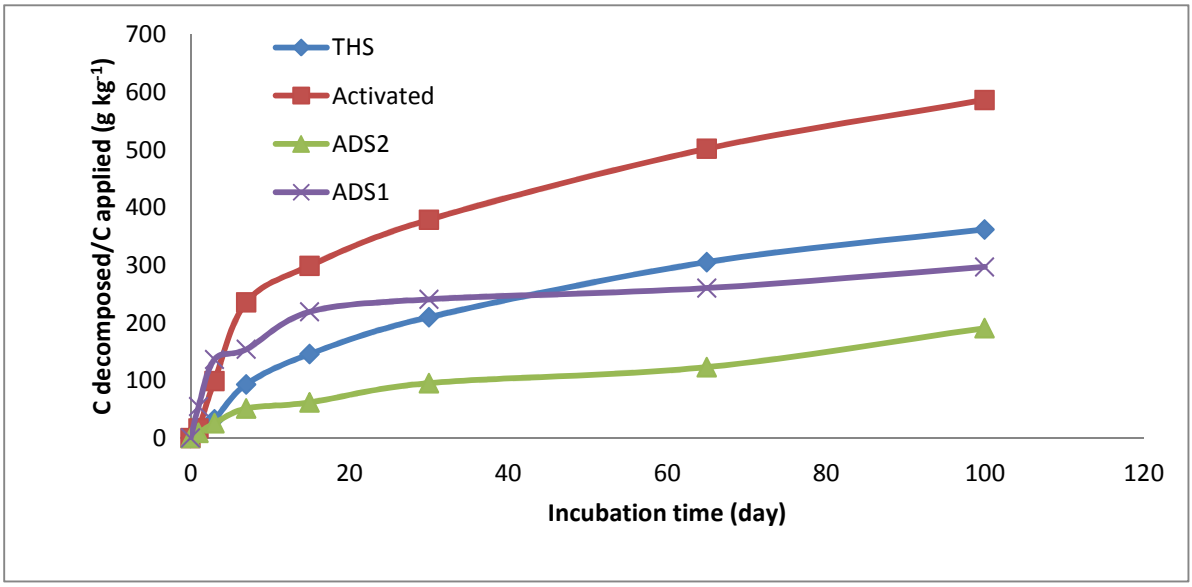

Figure 4.3 Carbon decomposition of anaerobically digested sludge dried in concrete paddies in thick layer of 250 mm (ADS2), activated sludge dried in thin layers of less than 100 mm (Activated) and Thermal hydrolysis sludge (THS) during a 100 day incubation study (KNO_3 added at commencement of study only for ADS2, Activated & THS)

The C decomposition rate of the fast cycling organic C fraction for the first three days was highest from ADS1 (44.80 g kg^{-1}) followed by Activated (34.64 g kg^{-1}), THS (13.02 g kg^{-1}), and was lowest for ADS2 (7.24 g kg^{-1}) (Fig. 4.4).

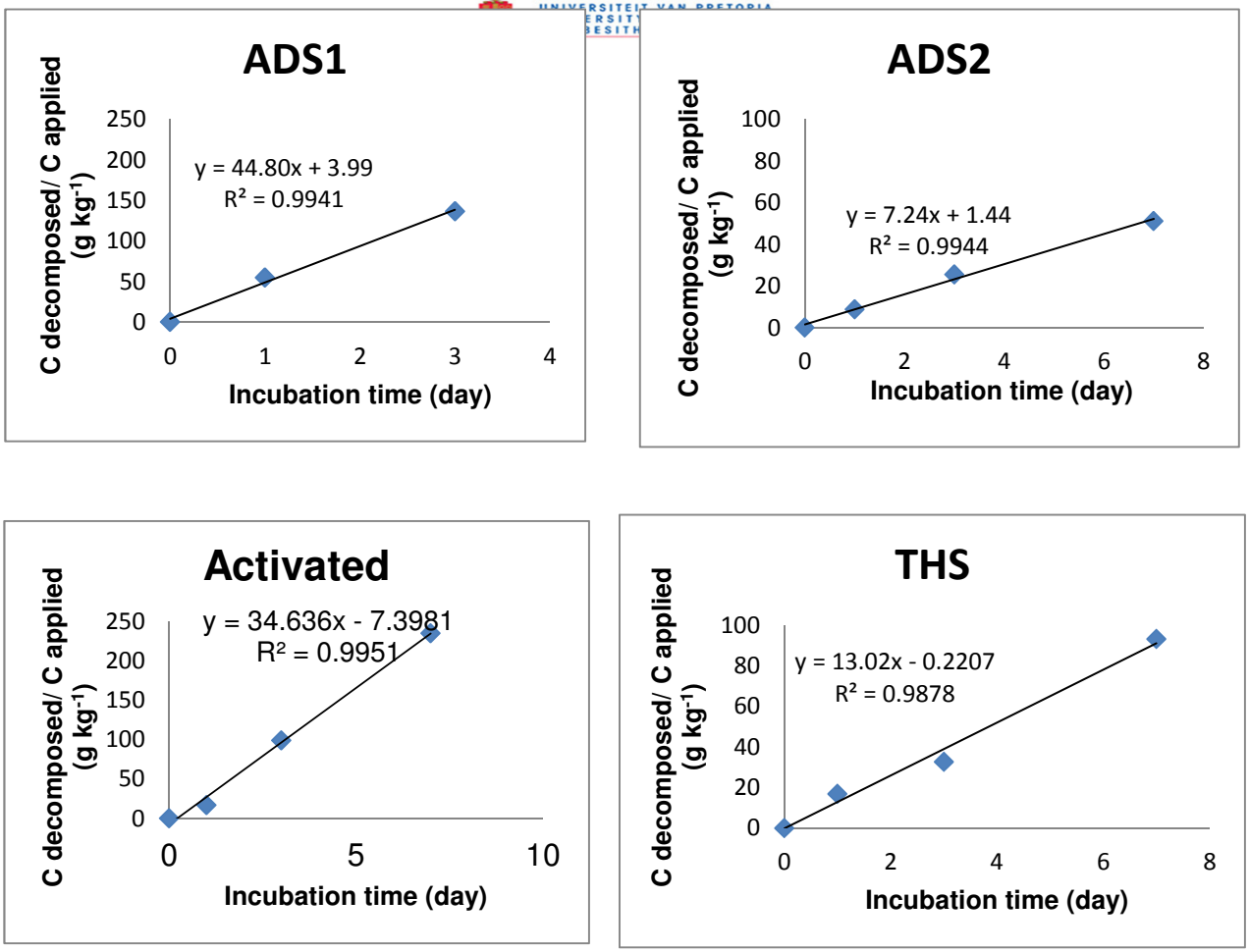

Figure 4.4 Carbon decomposition rate (g kg^{-1}) of the fast cycling fraction of an anaerobically digested sludge dried on paddy in thick layers of 250 mm (ADS1) dried on concrete beds in thick layers of 250 mm (ADS2), activated sludge dried in thin layers of less than 100 mm (Activated) and Thermal hydrolysis sludge (THS)

The second set of C decomposition data which can be categorized under the same slope (moderately slow pool) with high r^2 (>98%) was between days 3 and 15 (ADS1) and days 7 and 30 (ADS2, Activated, & THS) (Fig. 4.5). The decomposition rate of the relatively slow organic C fraction was also highest for ADS1 (7.06 g kg^{-1}) followed by Activated (6.12 g kg^{-1}), THS (4.96 g kg^{-1}) and was lowest for ADS2 (1.96 g kg^{-1}).

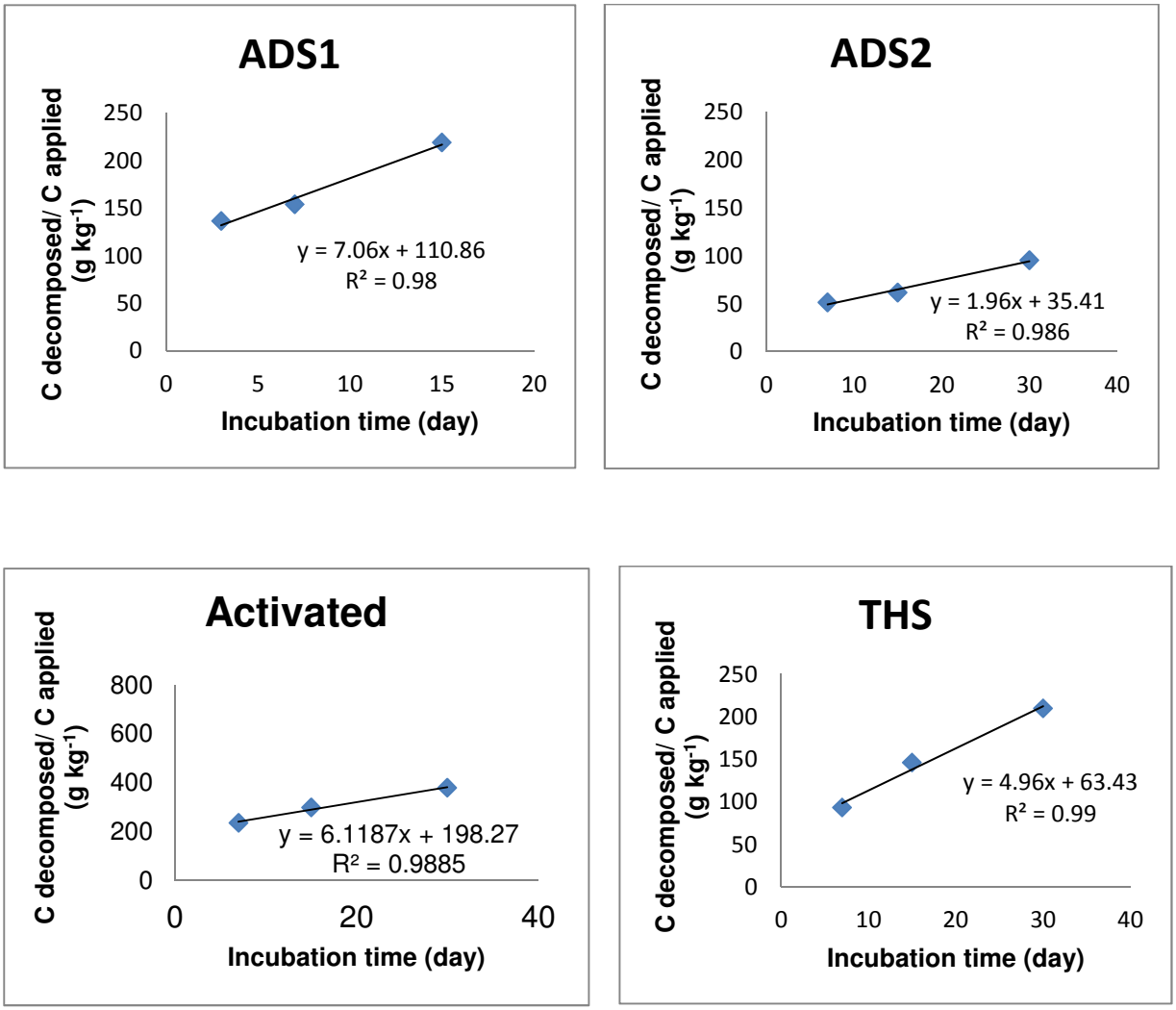

Figure 4.5 Carbon decomposition rate (g kg^{-1}) of the moderately slow cycling fraction of an anaerobically digested sludge dried on paddy in thick layers of 250mm (ADS1) dried on concrete beds in thick layers of 250 mm (ADS2), activated sludge dried in thin layers of less than 100 mm (Activated) and Thermal hydrolysis sludge (THS)

The third set of C decomposition data (slow pool) which was categorized under the same slope with high r^2 (>95%) was between days 15 and 100 (ADS1) and days 30 and 100 for ADS2, Activated, and THS (Fig. 6). Unlike the decomposition rate of fast and moderately slow, the decomposition rate of slow organic C pool was highest for Activated (2.97 g kg^{-1}) and lowest for ADS1 (0.86 g kg^{-1}).

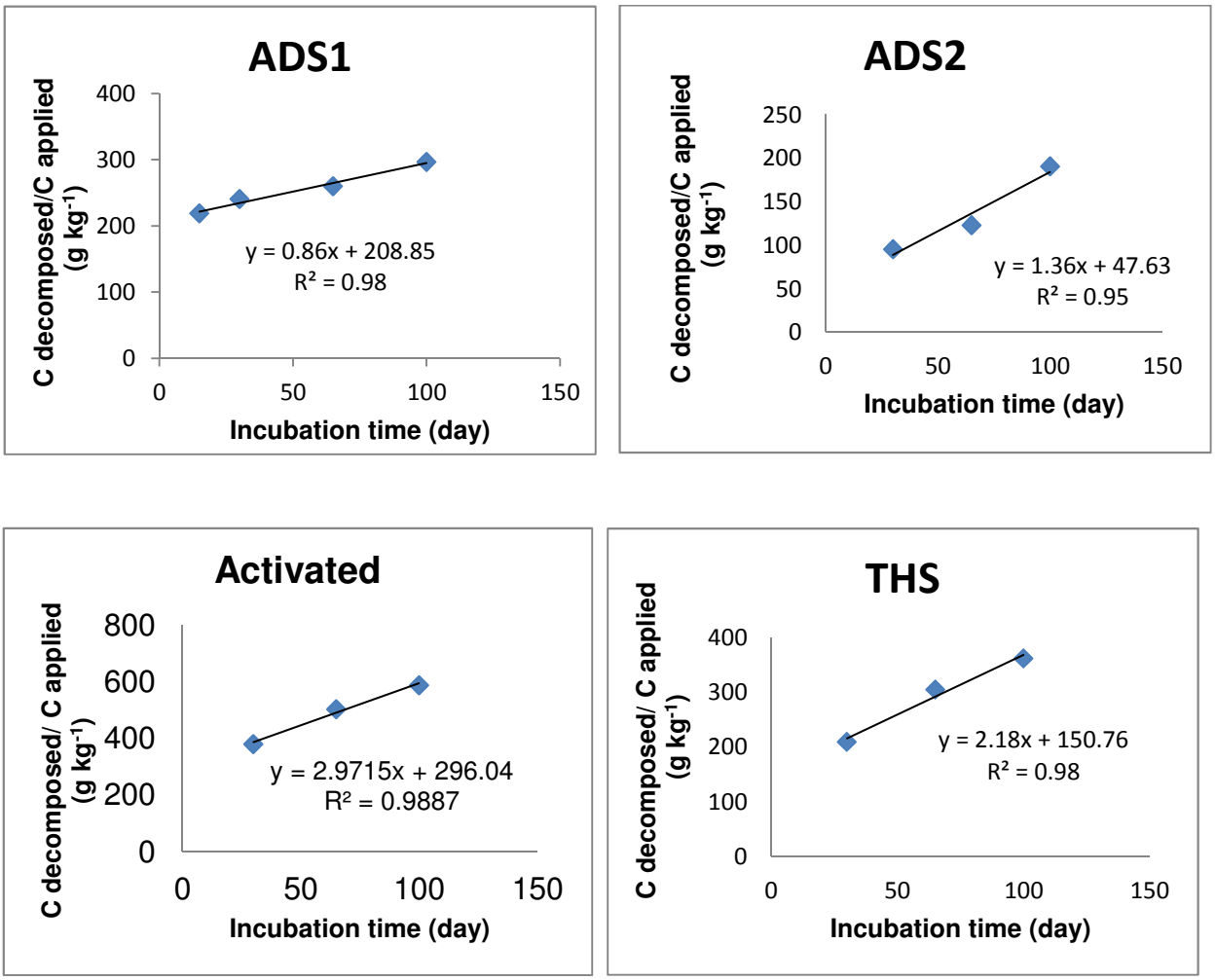

Figure 4.6 Carbon decomposition rate (g kg^{-1}) of the slow cycling fraction of an anaerobically digested sludge dried on paddy in thick layers of 250 mm (ADS1) dried on concrete beds in thick layers of 250 mm (ADS2), activated sludge dried in thin layers of less than 100 mm (Activated) and Thermal hydrolysis sludge (THS)

Similar to N mineralization (Fig. 4.1), ADS2 had the lowest and Activated the highest C decomposition per kg mass of organic C added during a 100 day incubation study.

4.3.2 Discussion

4.3.2.1 N mineralization and C decomposition

The initial N content and C:N ratio are the first litter chemistry that highlight the potential decomposition rate of a biological organic material (Tian et al., 1993; Valenzuela-Solano and Crohn, 2006; Lashermes et al., 2010). Previous studies with plant litter have shown that low C:N ratio materials are characterized by rapid decomposition and less immobilization than materials with higher C:N ratios (Muhammad et al., 2011). It is also well documented that organic materials which are rich in lignin, cellulose and hemicellulose are characterized by low decomposition rates (Trofymow et al., 2002; Santiago, 2007; Baddi et al., 2004; Parnaudeau et al., 2004).

Cumulative N mineralization varied among sludge types significantly. This is in agreement with previous studies which reported that N mineralization from sludge is a function of sludge treatment processes (Parker and Sommers, 1983; Serna & Pomares, 1992; Smith and Tibbett, 2004; Zarabi and Jalali, 2013). Generally net N mineralization from the anaerobically digested sludges (ADS1 and ADS2) was higher than mineralization from similar sludge types reported by Parnaudeau et al. (2004). This is because of the relatively higher initial N content (Table 3.2) and lower C:N ratio (Table 4.2) of the sludges from our study compared to that of Parnaudeau et al. (2004).

Net N mineralization per kg C applied after 100 days of incubation was highest from activated sludge than the other three (ADS1, ADS2, and THS). This was mainly attributed to the higher initial N content of the sludge (4.95%) compared with the

other three which ranged between 2.81 and 3.13% (Table 3.2) because the initial N concentration influences the amount of N that can be mineralized (Valenzuela-solano and Crohn, 2006; Lashermes et al., 2010). In addition Activated sludge had the lowest lignin fraction (3.21%) compared with the other three, which ranged between 8.7 and 15.96% by mass of the total organic matter. Previous studies have shown that lignin affects N mineralization negatively (Baddi et al., 2004). On the other hand, cumulative net N mineralization per kg organic N applied at the end of 100 day incubation was highest for ADS1.

Despite a 9.6% difference in the initial total N content between THS (3.13%) and ADS2 (2.83%), N mineralization from THS (57.11 g N kg^{-1} C applied) on day 100 was 61% higher than ADS2 (22.27% g N kg^{-1} C applied). Such a difference is most probably attributed to the higher lignin fraction (15.96%), lignin:N ratio (1.40), and C:N ratio (8.78) of ADS2 compared with THS (lignin=8.7%, lignin:N ratio=0.50, C:N ratio=5.77). It is well documented that higher Lignin:N ratio results in low net N mineralization due to N immobilization (Baddi et al., 2004; Parnaudeau et al., 2004).

Cumulative net N mineralized on day 100 from ADS1 was three times higher than ADS2. This is despite a very small total N (0.7%) and organic N (10%) differences. Carbon concentration of ADS2 sludge (24.84%) was, however, 32% higher than ADS1 (17%). Thus, the C:N ratio of ADS2 (8.78) was 2.73 times higher than ADS1 (6.05). In addition, ADS2 had about 18% lower soluble compounds by mass (62.55%) than ADS1 (76.19%). It was also apparent that the lignin (15.96%), cellulose (4.91%), and hemicellulose (14.84%) fractions for ADS2 were 37%, 39%, and 47% higher than that of ADS1, respectively. Therefore, the main reason for the higher cumulative N mineralization from ADS1 relative to ADS2 was the lower C:N

ratio, lower lignin, cellulose, and hemicellulose as well as relatively higher soluble compounds fraction composition of ADS1.

The C decomposition pattern from this study fits well into three phase (pool) system and has similarity to the findings of Parnaudeau et al. (2004). It was interesting to note that the cumulative organic C decomposed per kg organic C applied at day 100 from anaerobically digested sludges (ADS1 and ADS2) from this study were within the same ranges to the findings by Parnaudeau et al. (2004) for anaerobically digested limed and thickened anaerobically digested centrifuged sludges.

Carbon decomposition from ADS1 during the first three days was 6, 1.3, and 3.4 times higher than ADS2, Activated, and THS, respectively. The net N mineralization during those three days, however, remained similar for all sludge types. Similarly, C decomposition rate of the moderately slow carbon fraction was highest for ADS1 and was 4, 1.2, and 1.4 times higher than ADS2, Activated, and THS, respectively. Net N mineralization during the same time period was, however, lowest for ADS1. This is most probably attributed to immobilization because ADS1 unlike other treatments did not receive KNO_3 at the beginning of the incubation. Carbon decomposition rate during the last 70 to 85 days, however, was highest from Activated and lowest from ADS1. The net N mineralization from ADS1 between days 7 and 65 remained lowest but turned to be the highest in day 100. The sudden flush of net N release on day 100 despite the lowest C decomposition could probably be attributed to the cell lysis due to excessive turgur pressure caused by influx of water through cell membrane (Mikha et al., 2005), or transport of intercellular solutes out of cells which results in the release of the cell solutes, such as amino acids, ammonium compounds, and glycerol (Kieft et al., 1987).

4.4 Conclusion

Carbon decomposition and N mineralization are functions of sludge treatment methods and post treatment drying processes. Sludge treatment and drying processes affect C and N mineralization by altering the chemical composition of sludges. Similar to previous findings, sludge C and N decomposition was significantly influenced by the initial N concentration, C:N ratio, lignin content, and Lignin:N ratio of sludge. C and N mineralization per unit organic C applied was highest for activated sludge and was lowest from anaerobically digested paddy dried sludge. However, N mineralization per unit organic N applied was highest from anaerobically digested sludge dried in concrete beds. Considering the higher N content of activated sludge and higher N release rate per unit organic N applied, the fertilizer value of activated sludge is higher than the other sludge types investigated.

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CHAPTER 5: The effect of wastewater treatment and post treatment drying techniques on the dynamics of selected organic compounds

Abstract

Sewage sludge organic matter decomposition and mineralization is influenced by the composition of biochemical compounds within the sludge. Many studies were done on sludge composting to determine the change of biochemical compounds with time. There is, however, little information on the periodic transformation of dominant biochemical compounds (Soluble compounds fraction (SOL), Lipids (Lip), Hemicellulose (Hem), Cellulose (Cel) and Lignin (Lig)) from municipal sludge amended soils. The aim of this study was to investigate the biochemical compounds responsible for the periodic release of C and N. Two consecutive incubation studies (each for 100 days) were conducted using four sludge types (Thermal hydrolysis sludge (THS), activated sludge (Activated), and anaerobically digested sludge dried in concrete beds (ADS1) and dried in paddy (ADS2) incorporated into a 100 g of sandy clay loam soil. The sludge was applied to meet 300 kg N ha^{-1} requirements. During the first incubation ADS1 was incubated without the addition of KNO_3 while in the second batch KNO_3 was added to the soil-sludge mix to bring the soil inorganic N level to 60 mg kg^{-1}. The second batch included parallel incubation experiments using sludge alone (ADS2 and Activated). Total C% (TC) was determined using the Carlo Erba method. The selected biochemical compounds in the sludge, sludge-amended soil and controls were determined using the Van Soest fractionation method. The sludge-amended soil and control (soil) data was excluded due to high standard errors and unclear organic C decomposition patterns. The residual organic

C declined more in Activated as compared to ADS2 in sludge alone incubation. This supports studies which reported that the high initial Lig content restricted the organic matter decomposition. All compounds decreased during the study with the buildup of Lig content after day 30 to the end of the study. The SOL was found to be the main fraction contributing more to organic matter loss followed by Hem and Cel during the study.

Keywords: Sludge, Lignified fraction, cellulose, hemicellulose, lipids, soluble compounds, degradation and transformation.

5.1 Introduction

Sewage sludge is a biological waste which is made up of both organic and inorganic constituents. The organic fraction of sewage sludge is usually influenced by the origin of wastewater and the treatment process (Banegas et al., 2007). The biochemical composition of sewage sludge dictates the rate of C decomposition and N mineralization (Seneviratne, 2000; Parnaudeau et al., 2004; Smith and Tibbett, 2004). Thus understanding the rate of degradation of the dominant biochemical compounds in sludge is crucial to the understanding of C and N release differences between sludge types.

Previous studies conducted by Charest et al. (2004); Jouraiphy et al. (2005); Banegas et al. (2007); Francou et al. (2008); Doublet et al. (2010); Doublet et al., 2011; Mottet et al. (2010) and Zhao et al. (2011) investigated the transformation of sludge biochemical compounds during a compositing process. Other studies conducted by Chantigny et al. (2000) investigated the transformation and decay of de-inking paper sludge in agricultural soils. Unlike municipal sludge, which is dominated by soluble compounds (Parnaudeau and Dignac, 2007; > 60% by mass of organic matter), de-inking paper sludge is characterized by high hemicellulose, cellulose and lignin fraction (Chantingy et al., 2000). There is, however, little information on the transformation of dominant biochemical compounds from municipal sludge as a function of time.

The aim of this study was to investigate the dynamics of biochemical compounds in general and the compounds responsible for the periodic release of C and N during sludge decomposition in particular.

5.2 Material and method

5.2.1 Sludge materials

Four sludge materials were selected for this study. Detailed characteristics of these sludge materials are presented in Tables 3.1, 3.2, 4.1, and 4.2. All sludge materials were ground and milled to pass a 1 mm sieve after drying in oven at 40 ^{o}C.

5.2.2 Soil

Soil for the incubation study was collected from the experimental plots of the long term field study trial at East Rand Water Care Works (ERWAT), near Johannesburg, South Africa. Selected properties of the soil used for the incubation study are presented on Table 4.3.

5.2.3 Method

5.2.3.1 Incubation

Two consecutive incubation experiments were carried out at the Soil Science Laboratory of the University of Pretoria. The incubation was conducted at a constant room temperature of 25 ±1 ºC. The incubation was conducted in 1163 cm^3 volume airtight containers where 100 g soil was mixed with the candidate sludge to meet a 300 kg N ha^{-1} application rate. Unlike the first batch incubation, inorganic N in the form of KNO_3 was added during the second incubation study to increase the soil NO_3^-–N level to 60 $mgkg^{-1}$ (Parnaudeau et al., 2004) in order to minimize N

immobilization. The soil moisture was maintained at field capacity by adding water based on mass difference.

The treatments during the first batch include: ADS1 treated soil and a zero sludge control (soil alone) replicated three times. The second batch incubation treatments include: ADS2 amended soil, THS amended soil, Activated amended soil and a zero sludge control all replicated three times. During the second incubation a sludge alone incubation was conducted for sludges ADS2 and Activated without the addition of inorganic N. Results from the soil-sludge mix gave very high variation between replications (very big standard errors) and inconsistencies across time. Thus, this chapter will deal with results from the sludge alone incubation. Results of the soil-sludge mix incubation study are presented in the Appendix.

The sludge, soil, and inorganic fertilizer were thoroughly mixed at the commencement of the study. Analyses on residual organic compounds across time from the soil-sludge mix as well as sludge alone incubation study was done on days (d) 0, 1, 3, 7, 15, 30, 65, and 100. The experiments were aerated during sampling intervals for the first seven days of incubation. After day seven, the experiments were opened once a week and at sampling time to replenish water to field capacity.

All sludge amended soils, sludge alone and control were freeze dried before biochemical composition analyses using the Van Soest fractionation method (Goering and Van Soest, 1970; Van Soest, 1994)

5.2.3.2 Biochemical composition fractionation

Freeze dried samples were extracted with petroleum ether using Soxtec™ 2043 lipid extraction system to determine the fraction of lipids. The lipids extraction (lip) was followed by estimation of the Neutral Detergent Fiber (NDF) as described by Goering and Van Soest (1970). The NDF solution was made up of sodium lauryl sulphate, disodium ethylenediaminetetraacetate (EDTA) dehydrate, sodium borate decahydrate, disodium hydrogen phosphate anhydrous, 2-ethoxyethanol (ethylene glycol monoethyl ether) purified grade and distilled water. A freeze dried sample (1 g) was placed in a 50 ml crucible and boiled for 1 hour with 100 ml NDF solution at 100 ^{o}C. After boiling, the solvent was filtered through the crucible and the residues that remained in the crucible were oven dried at 60 ^{o}C and not ashed. Extraction of NDF was conducted using Fibertec™ 2010 Auto Fibre Analysis System.

The NDF extraction was followed by Acid Detergent Fiber (ADF) extraction as described by Goering and Van Soest (1970). The ADF solution was made up of cetyltrimethyl ammonium bromide (CTAB) and 1 N H_2SO_4. A 1 g of freeze dried sample milled to pass 1 mm sieve, was placed in 50 ml crucible and boiled for 1 hour with 100 ml ADF solution at 100 ^{o}C. After boiling, the solvent was filtered through the crucible and the residues that remain in the crucible were oven dried at 60 ^{o}C and not ashed. Extraction of ADF was conducted using Fibertec™ 2010 Auto Analysis System.

Finally the Acid detergent lignin (ADL) was determined as reported by Goering and Van Soest (1970). This procedure is a two-step process; initially the dried residue from the ADF extraction was treated with 72% sulphuric acid for 3 hours at room temperature. The 72% sulphuric acid digested sample was filtered through the

crucible and the residues that remained in the crucible were oven dried at 60 ^{o}C and weighed before ashing. This is followed by ashing of the dried residue. Similar to the NDF and ADF, extraction of ADL was conducted using Fibertec^{TM} 2010 Auto Analysis System.

Fractions of the neutral detergent soluble fraction, hemicelluloses, cellulose and lignified fraction were calculated as follows:

Soluble compounds fraction (SOL) = original mass – NDF- lipids fraction,

Hemicellulose (Hem) = NDF- ADF,

Cellulose (Cel) = ADF- 72 % H_2SO_4,

Lignified fraction (Lign) = 72 % H_2SO_4 – Ash.

Total C was determined using Carlo Erba method.

5.3 Results and discussion

5.3.1 Results

5.3.1.1 Sludge alone incubation

The residual carbon of both sludges (ADS2 and Activated) decreased with time during the 100 day incubation (Fig. 5.1a and c). The fraction of soluble compounds in the residue decreased as incubation time progressed for both sludge types. The second dominant residual compound, hemicellulose, showed a different pattern across time between sludge types. For sludge ADS2, the hemicellulose fraction increased gradually until day 7 but decreased thereafter as the time progressed (Fig. 5.1b). In contrast, with Activated sludge the hemicellulose fraction remained fairly similar until day 3 then suddenly decreased by 70% compared to the first three days (Fig. 5.1d). On day 15, however, the hemicellulose for Activated sludge was doubled compared with day 7 but gradually decreased with time (Figure 5.1d).

Lignin fraction, which accounted for 21 % of the total C in ADS2 at the beginning of study showed an alternate decreasing and increasing patterns between sampling times until day 65, which finally showed a triple increase on day 100 compared with day 65 (Fig. 5.1b). The pattern of lignin fraction on residual Activated sludge, however, differed from that of ADS2 (Fig. 5.1d). During the first three days, the lignin fraction of Activated sludge decreased gradually followed by a gradual increase for the next three days until day 15. It suddenly decreased on day 30 compared with day 15 followed by a gradual increase for the rest of the incubation study (Fig. 5.1d). The cellulose fraction remained low and with almost no distinct degradation pattern throughout the incubation study for Activated sludge (Fig. 5.1d). It, however, showed

a distinct decreasing pattern for ADS2 sludge after day 7 until end of incubation. The lipid fraction remained similar throughout the incubation period for ADS2 (Fig. 5.1b) but showed a sudden increase on day 7 for Activated (Fig. 5.1d).

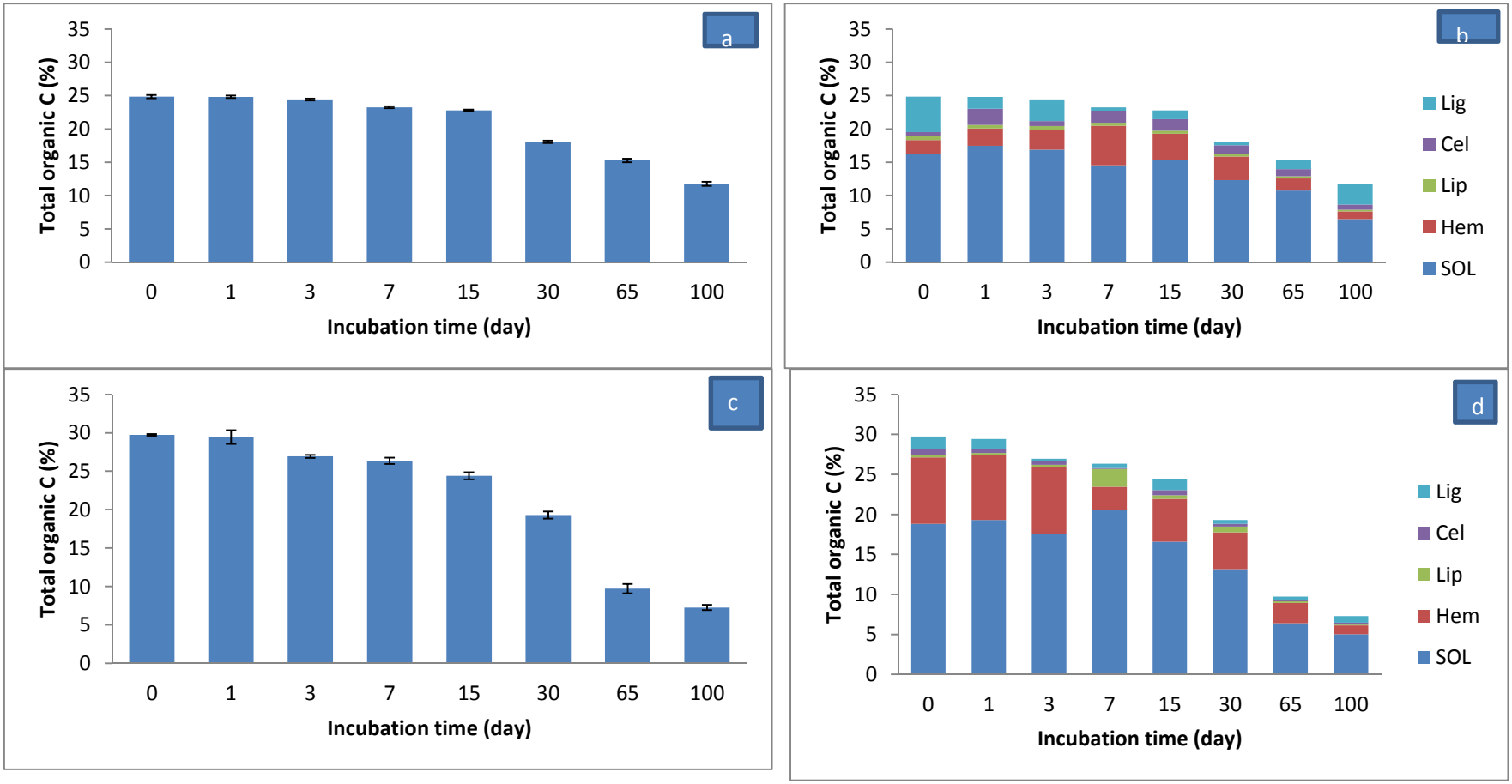

Figure 5. 1 Residual organic C (a and c) and transformation and degradation of organic compounds (b and d) during a 100 day incubation period of anaerobically digested dried in paddy in thick sludge layers of 250 mm (ADS2) (a and b) and activated sludge dried in concrete beds in thin layers of about 100 mm (Activated) (c and d)

5.3.1.2 Sludge soil mix incubation

There was very high variation between results for replicated samples leading to very high standard errors within and across time for the soil-sludge mix incubation study. Therefore results for the soil-sludge mix incubation study are excluded and are separately presented in the appendix.

5.3.2 Discussion

The sludge alone experiment (Fig. 5.1) was used in order to get a better resolution on the dynamics of organic compounds at higher resolution. This is because the soil used had very low organic matter (1.38%) compared to sludge (>20%). In addition, the organic matter fractionation method, Van Soest method, was intensively tested on sludge alone (Chantigny et al., 2000; Charest et al., 2004; Jouraiphy et al., 2005; Banegas et al., 2007; Francou et al., 2008; Doublet et al., 2010; Mottet et al., 2010; Zhao et al., 2011) than on sludge-soil mix.

At the end of 100 days of incubation, 60 and 51% of the organic C from the Activated and ADS2 sludges were decomposed, respectively. The relatively lower decomposition from ADS2 is most probably attributed to the relatively higher initial lignin content of the sludge. The presence of lignin in appreciable amount slows down organic matter degradation (Chantigny et al., 2000; Baddi et al., 2004; Parnaudeau et al., 2004).

The soluble compounds fraction of both sludges decreased by 23% during the first 30 days of incubation period. In contrast, 27% of the total carbon in the sludge was

decomposed during the same time period. Therefore, 23% of the decomposition could most probably have come from the soluble fraction. This is because soluble fraction is made up of organic compounds which are easily decomposable (Charest et al., 2004; Doublet et al., 2010; Zhao et al., 2011). The remaining 4% might have come from other compounds. Between days 30 and 65, 12% and 38% of the residual soluble fraction for ADS2 and Activated, respectively, was decomposed. It was also interesting to note that between days 65 and 100, 39% and 10% of the residual soluble compound fractions of the ADS2 and Activated sludges, respectively, disappeared. Higher soluble fraction decomposition from the ADS2 sludge took place at a later stage between days 65 and 100. This is in contrast to Activated sludge, whose most decomposition took place at earlier stage. This could be attributed to the higher initial lignin fraction of the ADS2 which slowed down the organic matter decomposition.

On day 7, the soluble organic carbon fraction of Activated sludge increased by about 3%. This is most probably attributed to the hydrolyses of the relatively stable fractions such as hemicellulose to soluble organic compounds (Francou et al., 2008; Sánchez-Monedero et al., 1999; Charest et al., 2004; Doublet et al., 2010; Zhao et al., 2011). One of the main reasons being the sudden decline of the hemicellulose fraction by half between days 3 and 7 (Fig 5.1d). The increase in the hemicellulose fraction observed between days 1 to 7 on ADS2 sludge as well on day 15 of Activated sludge are mostly attributed to the transformation of soluble compounds to more complex forms through synthesis during the decomposition process. It can be clearly observed from the figures that during those periods where an increase in hemicellulose was observed, the soluble fraction decreased.

The lipid fraction remained more or less similar throughout the incubation period for ADS2 but showed a sudden increase on day 7 for Activated sludge. This indicates little contribution to C decomposition. The cellulose fraction showed some dynamics on sludge ADS2, which increased on day 1, most probably due to synthesis from simple soluble compounds by microbial activity. It then decreased on day 3 which could have either decomposed or transformed to much stable Lignin fraction. From day 7 onwards, however, the cellulose fraction decreased gradually and could be considered as a third contributor to C decomposition after hemicellulose. The lignin fraction trend was not as expected, to increase as time progressed, however, this is not the first time such events have been reported. Others studies by Sánchez-Monedero et al. (1999) and Jouraiphy et al. (2005) also reported a decline in lignin fraction.

5.4 Conclusion

The Van Soest method was successfully used to determine the periodic degradation and transformation of biochemical compounds in ADS2 and Activated sludges. Almost 90% of the total C decomposition came from the soluble organic carbon fraction indicating that the soluble organic fraction being the main source of C decomposition in municipal sludge. The next main contributor was found to be hemicellulose, which accounted to 5-7% of the total C decomposition.

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CHAPTER 6: GENERAL CONCLUSION AND RECOMMENDATION

Conclusion

Wastewater treatment methods as well as post treatment drying depth in beds and duration have significant implication on the fertilizer value of sludge. Anaerobically digested sludge dried in thin layers of ≤100 mm for less than 7 days (ADS3) had a higher total N (5.08%) content than that dried in thick layers of ≥250 mm for more than 21 days in concrete beds (ADS1; 2.81%) and in earth paddy (ADS2; 2.83%). Large fraction (>60%) of the organic C in all investigated sludge types was in soluble compounds with thermally hydrolysed sludge (THS) having the highest (90%). With regards to sludges dried in thin layers of ≤100 mm within short duration of about 7 days, ADS3 and Activated sludges, had lower lignin fraction (<3%) than THS, ADS1 and ADS2. Net N mineralization per kg C applied was highest from activated sludge (77 g kg^{-1} C applied) and lowest for ADS2 (23 g kg^{-1} C applied) mainly due to the low lignin:N ratio of activated sludge (0.18) than ADS1, ADS2, and THS which had a lignin:N ratio of 0.61, 1.40, and 0.50, respectively. Despite its highest soluble organic fraction, THS sludge, had relatively lower net N mineralization per kg organic N applied (350 g kg^{-1} organic N applied) compared with Activated sludge (449 g kg^{-1} organic N applied) mainly due to the relatively higher lignin fraction. It was also apparent from this study that the soluble compounds, as determined by the Van Soest method, were responsible for about 90% of the organic matter decomposition. The Van Soest method was also successfully used to determine residual compounds at intervals during the incubation study from the sludge alone study but not with the soil-sludge mixture.

Recommendations

1) Detailed study is required to identify the optimal drying depth which does not compromise the fertilizer value of sludge.
2) Further fractionation study is required to identify the components of the soluble fraction which make up the easily available fraction.
3) The effect of sludge drying thickness and duration on sludge C, N, and composition needs to be investigated using the same sludge material in order to isolate the effect of wastewater source.

APPENDIX

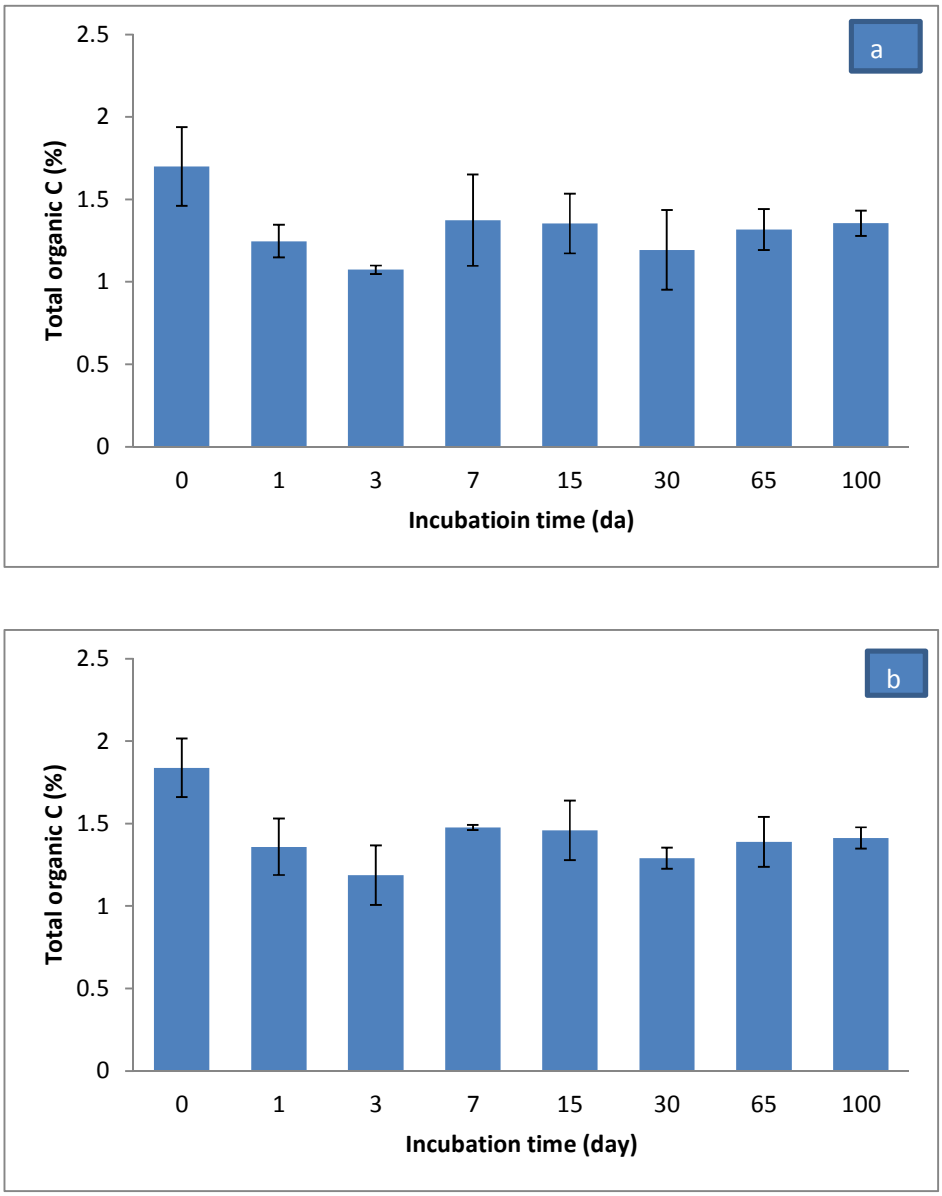

Figure A 1 Residual organic carbon during a 100 day incubation period from a) control (soil alone) and b) Anaerobically digested dried in concrete beds in thick layers of 250 mm (ADS1) without the addition of KNO_3.

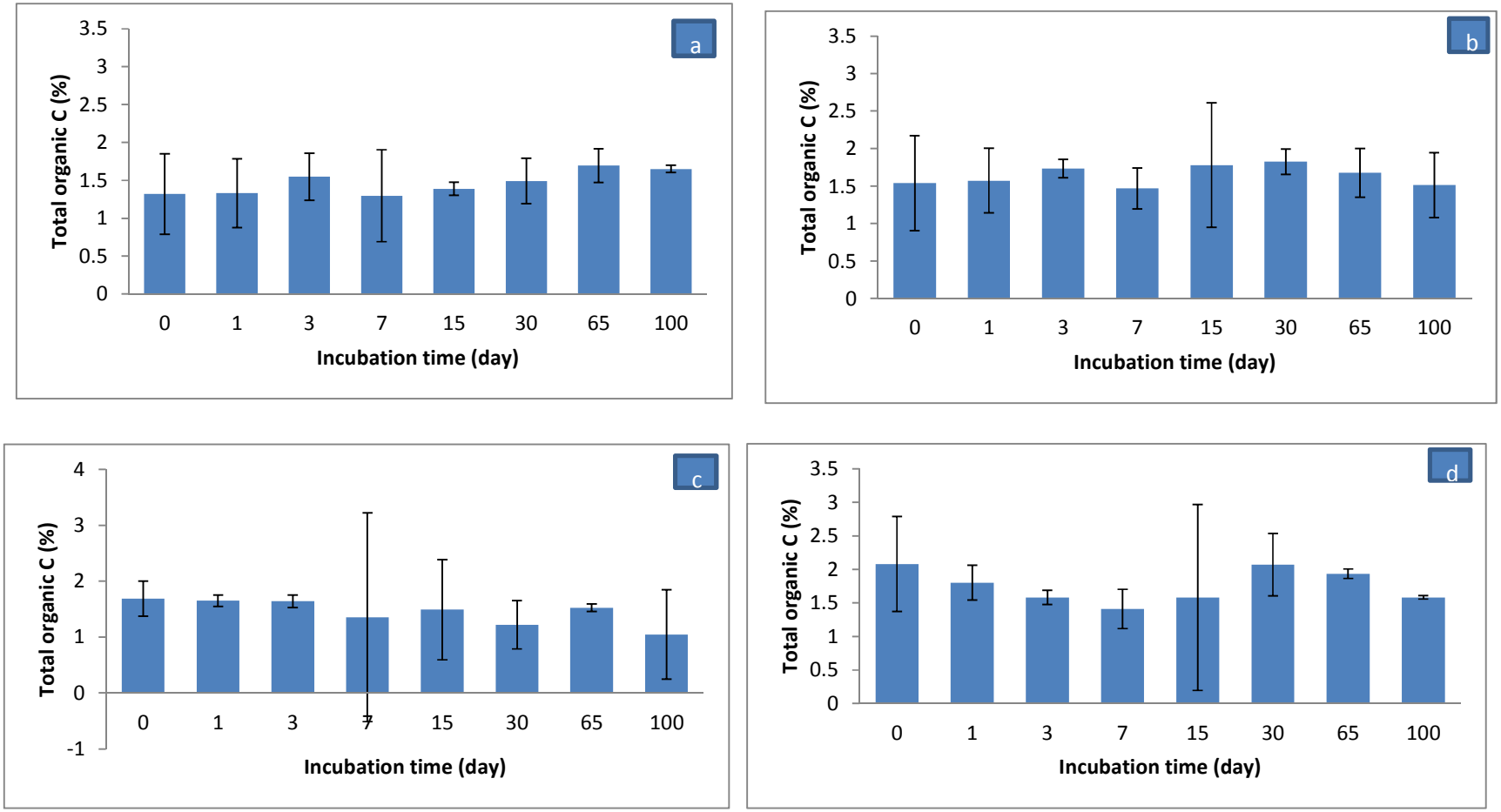

Figure A 2 Residual organic carbon (C) during a 100 day incubation period of a) control (soil alone) and b) Anaerobically digested dried in concrete beds in thick layers of 250 mm (ADS2) treated soil, c) Thermal hydrolyses (THS) treated soil, d) activated sludge dried on concrete beds in thin layers of 100 mm (Activated) treated soil. All treatments received KNO_3.

Table A 1 Compounds degradation and transformation during a 100 day incubation period from Anaerobically digested dried in concrete beds in thick layers of 250 mm (ADS1) treated soil and control (soil alone) without the addition of KNO_3.

ADS1-amended soil										
Day 0										
	R_1	**R_2**	**R_3**	**Ave**	**Std err (±)**	**Lips (%)**	**SOL (%)**	**Hem (%)**	**Cel (%)**	**Lig (%)**
Lips (%)	0.69	0.61	0.71	0.67	0.053	0.67	104.6	-14.8	1.70	7.82
NDF (g)	0.1288	0.1312	0.1389	0.1330	0.005					
ADF (g)	0.1421	0.1550	0.1725	0.1565	0.015					
ADL(H_2SO_4) (g)	0.1388	0.1528	0.1698	0.1538	0.015					
ADL (ASH) (g)	0.1264	0.1326	0.1652	0.1414	0.021					
Day 1										
Lips (%)	0.31	0.24	0.32	0.29	0.043	0.29	107.1	-16.8	1.51	7.94
NDF (g)	0.1284	0.1290	0.1318	0.1297	0.002					
ADF (g)	0.1401	0.1558	0.1734	0.1564	0.017					
ADL(H_2SO_4) (g)	0.1378	0.1534	0.1708	0.1540	0.016					
ADL (ASH) (g)	0.1264	0.1326	0.1652	0.1414	0.021					

Day 3										
Lips (%)	0.28	0.25	0.31	0.28	0.030	0.28	121.3	-33.9	1.01	11.3
NDF (g)	0.1452	0.0650	0.1115	0.1072	0.040					
ADF (g)	0.1786	0.1790	0.1255	0.1610	0.031					
ADL(H_2SO_4) (g)	0.1758	0.1773	0.1252	0.1594	0.030					
ADL (ASH) (g)	0.1264	0.1326	0.1652	0.1414	0.021					
Day 7										
Lips (%)	0.1	0.08	0.09	0.09	0.010	0.09	90.9	6.05	0.76	2.14
NDF (g)	0.1574	0.1424	0.1669	0.1556	0.012					
ADF (g)	0.1494	0.1347	0.1539	0.146	0.010					
ADL(H_2SO_4) (g)	0.1484	0.1335	0.1524	0.1448	0.010					
ADL (ASH) (g)	0.1264	0.1326	0.1652	0.1414	0.021					
Day 15										
Lips (%)	0.16	0.18	0.23	0.19	0.036	0.19	95.4	-4.98	1.89	7.69
NDF (g)	0.1440	0.1439	0.1582	0.1487	0.008					
ADF (g)	0.1591	0.1579	0.1527	0.1566	0.003					
ADL(H_2SO_4) (g)	0.1578	0.1512	0.1518	0.1536	0.004					
ADL (ASH) (g)	0.1264	0.1326	0.1652	0.1414	0.021					

Day 30										
Lips (%)	0.11	0.13	0.18	0.14	0.036	0.14	105.2	-11.54	0.50	6.68
NDF (g)	0.1295	0.1496	0.1245	0.1345	0.013					
ADF (g)	0.1542	0.1445	0.1597	0.1528	0.008					
ADL(H_2SO_4) (g)	0.1535	0.1436	0.1589	0.1520	0.008					
ADL (ASH) (g)	0.1264	0.1326	0.1652	0.1414	0.021					
Day 65										
Lips (%)	0.10	0.11	0.11	0.1067	0.006	0.11	105.1	-4.16	2.14	-3.15
NDF (g)	0.1454	0.1342	0.1199	0.1332	0.013					
ADF (g)	0.1386	0.1359	0.1450	0.1398	0.005					
ADL(H_2SO_4) (g)	0.1380	0.1352	0.1360	0.1364	0.001					
ADL (ASH) (g)	0.1264	0.1326	0.1652	0.1414	0.021					
Day 100										
Lips (%)	1.70	1.74	1.74	1.7267	0.023	1.73	99.9	-3.47	0.94	4.29
NDF (g)	0.1305	0.1415	0.1443	0.1388	0.007					
ADF (g)	0.1210	0.1545	0.1574	0.1443	0.020					
ADL(H_2SO_4) (g)	0.1192	0.1532	0.1561	0.1428	0.020					
ADL (ASH) (g)	0.1264	0.1326	0.1652	0.1414	0.021					

Control soil										
Day 0										
Lips (%)	0.29	0.49	0.37	0.3833	0.100	0.38	82.5	9.33	2.77	5.04
NDF (g)	0.1695	0.1757	0.1607	0.1686	0.007					
ADF (g)	0.1460	0.1636	0.1517	0.1538	0.010					
ADL(H_2SO_4) (g)	0.1398	0.1607	0.1478	0.1494	0.010					
ADL (ASH) (g)	0.1264	0.1326	0.1652	0.1414	0.021					
Day 1										
Lips (%)	0.42	0.32	0.40	0.38	0.053	0.38	87.9	3.78	1.13	6.81
NDF (g)	0.1300	0.1527	0.1974	0.1600	0.034					
ADF (g)	0.1416	0.1531	0.1674	0.1540	0.013					
ADL(H_2SO_4) (g)	0.1401	0.1508	0.1656	0.1522	0.013					
ADL (ASH) (g)	0.1264	0.1326	0.1652	0.1414	0.021					
Day 3										
Lips (%)	0.13	0.17	0.15	0.15	0.020	0.15	105.3	-8.95	0.69	2.77
NDF (g)	0.1217	0.1365	0.1399	0.1327	0.010					
ADF (g)	0.1498	0.1559	0.1349	0.1469	0.011					
ADL(H_2SO_4) (g)	0.1475	0.1559	0.1341	0.1458	0.011					
ADL (ASH) (g)	0.1264	0.1326	0.1652	0.1414	0.021					

Day 7										
Lips (%)	0.33	0.35	0.34	0.34	0.010	0.34	86.7	11.4	1.28	0.25
NDF (g)	0.1497	0.1610	0.1750	0.1619	0.013					
ADF (g)	0.1570	0.1258	0.1486	0.1438	0.016					
ADL(H_2SO_4) (g)	0.1550	0.1239	0.1464	0.1418	0.016					
ADL (ASH) (g)	0.1264	0.1326	0.1652	0.1414	0.021					
Day 15										
Lips (%)	0.45	0.56	0.73	0.58	0.141	0.58	104.6	-16.20	0.76	10.2
NDF (g)	0.1205	0.1357	0.1431	0.1331	0.011					
ADF (g)	0.1545	0.1579	0.1641	0.1588	0.005					
ADL(H_2SO_4) (g)	0.1533	0.1566	0.1630	0.1576	0.005					
ADL (ASH) (g)	0.1264	0.1326	0.1652	0.1414	0.021					
Day 30										
Lips (%)	0.11	0.16	0.15	0.14	0.026	0.14	106.6	-4.01	0.69	-3.34
NDF (g)	0.1413	0.1277	0.1431	0.1307	0.008					
ADF (g)	0.1300	0.1315	0.1500	0.1372	0.011					
ADL(H_2SO_4) (g)	0.1285	0.1306	0.1491	0.1361	0.011					
ADL (ASH) (g)	0.1264	0.1326	0.1652	0.1414	0.021					

Day 65										
Lips (%)	0.24	0.23	0.22	0.2300	0.010	0.23	90.8	10.4	0.94	-2.39
NDF (g)	0.1782	0.1580	0.1306	0.1556	0.024					
ADF (g)	0.1595	0.1400	0.1177	0.1391	0.021					
ADL(H_2SO_4) (g)	0.1586	0.1395	0.1148	0.1376	0.022					
ADL (ASH) (g)	0.1264	0.1326	0.1652	0.1414	0.021					
Day 100										
Lips (%)	0.37	0.39	0.40	0.3867	0.015	0.39	103.1	-2.33	1.83	-3.03
NDF (g)	0.1489	0.1389	0.1196	0.1358	0.015					
ADF (g)	0.1409	0.1396	0.1381	0.1395	0.001					
ADL(H_2SO_4) (g)	0.1386	0.1371	0.1341	0.1366	0.002					
ADL (ASH) (g)	0.1264	0.1326	0.1652	0.1414	0.021					

Table A 2 Compounds degradation and transformation during a 100 day incubation period Anaerobically digested dried in concrete beds in thick layers of 250 mm (ADS2) treated soil, Thermal hydrolyses (THS) treated soil, activated sludge dried on concrete beds in thin layers of 100 mm (Activated) treated soil and of control (soil alone). All treatments received KNO_3.

ADS2 amended soil										
Day 0										
	R_1	**R_2**	**R_3**	**Ave**	**Std err (±)**	**Lips (%)**	**SOL (%)**	**Hem (%)**	**Cel (%)**	**Lig (%)**
Lips (%)	1.08	1.01	1.06	1.0500	0.036	1.05	98.2	-3.48	1.36	2.89
NDF (g)	0.5052	0.5264	0.4355	0.4890	0.047					
ADF(g)	0.4829	0.552	0.4857	0.5069	0.039					
ADL(H_2SO_4) (g)	0.4816	0.5368	0.4812	0.4999	0.032					
ADL (ASH) (g)	0.3564	0.5223	0.5763	0.4850	0.115					
Day 1										
Lips (%)	0.95	1.01	0.95	0.9700	0.035	0.97	100.5	-9.46	4.12	3.86
NDF (g)	0.4526	0.4772	0.5023	0.4774	0.025					
ADF (g)	0.5185	0.4877	0.572	0.5261	0.043					
ADL(H_2SO_4) (g)	0.518	0.4827	0.5141	0.5049	0.019					
ADL (ASH) (g)	0.3564	0.5223	0.5763	0.4850	0.115					

Day 3										
Lips (%)	1.21	1.48	1.12	1.2700	0.187	1.27	79.2	-2.93	2.60	19.8
NDF (g)	0.681	0.5842	0.4914	0.5855	0.095					
ADF(g)	0.714	0.5717	0.5162	0.6006	0.102					
ADL(H_2SO_4) (g)	0.6918	0.5636	0.5062	0.5872	0.095					
ADL (ASH) (g)	0.3564	0.5223	0.5763	0.4850	0.115					
Day 7										
Lips (%)	1.03	1.00	1.15	1.0600	0.079	1.06	105.6	-15.6	11.0	-2.13
NDF (g)	0.4392	0.4533	0.4588	0.4504	0.010					
ADF (g)	0.655	0.4064	0.5315	0.5310	0.124					
ADL(H_2SO_4) (g)	0.5364	0.4035	0.4821	0.4740	0.067					
ADL (ASH) (g)	0.3564	0.5223	0.5763	0.4850	0.115					
Day 15										
Lips (%)	0.48	0.54	0.61	0.5400	0.065	0.54	81.6	-27.1	1.1	43.8
NDF(g)	0.4638	0.4936	0.7733	0.5769	0.170					
ADF(g)	0.7234	0.7043	0.7216	0.7164	0.010					
ADL(H_2SO_4) (g)	0.7220	0.6935	0.7168	0.7108	0.015					
ADL (ASH) (g)	0.3564	0.5223	0.5763	0.4850	0.115					

Day 30										
Lips (%)	0.27	0.21	0.19	0.2200	0.042	0.22	91.24	-43.73	2.35	50.13
NDF(g)	0.5442	0.5203	0.5258	0.5301	0.012					
ADF(g)	0.7086	0.7299	0.8273	0.7553	0.063					
ADL(H_2SO_4) (g)	0.7016	0.7107	0.8172	0.7432	0.064					
ADL (ASH) (g)	0.3564	0.5223	0.5763	0.4850	0.115					
Day 65										
Lips (%)	0.38	0.45	0.52	0.4500	0.07	0.45	96.6	-40.7	3.26	40.3
NDF (g)	0.5146	0.4799	0.5053	0.4999	0.018					
ADF(g)	0.8235	0.6181	0.6870	0.7095	0.104					
ADL(H_2SO_4) (g)	0.7939	0.6025	0.6816	0.6927	0.096					
ADL (ASH) (g)	0.3564	0.5223	0.5763	0.4850	0.115					
Day 100										
Lips (%)	0.29	0.29	0.41	0.3300	0.069	0.33	55.2	-5.86	0.76	49.6
NDF (g)	0.7576	0.6758	0.7186	0.7140	0.041					
ADF(g)	0.7251	0.7377	0.7697	0.7442	0.023					
ADL(H_2SO_4) (g)	0.7204	0.7327	0.7679	0.7403	0.025					
ADL (ASH) (g)	0.3564	0.5223	0.5763	0.4850	0.115					

Activated sludge amended soil										
Day 0										
Lips (%)	1.04	0.95	0.97	0.9900	0.047	0.99	100.8	-7.44	1.28	4.33
NDF (g)	0.4688	0.4688	0.4892	0.4756	0.012					
ADF(g)	0.5553	0.5294	0.4570	0.5139	0.051					
ADL(H_2SO_4) (g)	0.5425	0.5236	0.4557	0.5073	0.046					
ADL (ASH) (g)	0.3564	0.5223	0.5763	0.4850	0.115					
Day 1										
Lips (%)	1.11	1.02	1.00	1.043	0.058	1.04	102.3	-8.19	3.26	1.63
NDF (g)	0.5302	0.4917	0.3822	0.4680	0.077					
ADF (g)	0.5222	0.4738	0.5346	0.5102	0.032					
ADL(H_2SO_4) (g)	0.5031	0.4625	0.5146	0.4934	0.027					
ADL (ASH) (g)	0.3564	0.5223	0.5763	0.4850	0.115					
Day 3										
Lips (%)	1.34	1.09	1.16	1.2000	0.130	1.20	86.8	7.10	1.32	3.57
NDF (g)	0.5659	0.4943	0.5801	0.5468	0.046					
ADF (g)	0.5242	0.5407	0.4658	0.5102	0.039					
ADL(H_2SO_4) (g)	0.5127	0.5386	0.4588	0.5034	0.041					
ADL (ASH) (g)	0.3564	0.5223	0.5763	0.4850	0.115					

Day 7										
Lips (%)	1.01	1.06	1.09	1.0533	0.04	1.05	83.9	-31.2	3.84	42.4
NDF (g)	0.4902	0.6979	0.4994	0.5625	0.117					
ADF(g)	0.7282	0.7476	0.6931	0.7230	0.028					
ADL(H_2SO_4) (g)	0.7152	0.7244	0.6701	0.7032	0.029					
ADL (ASH) (g)	0.3564	0.5223	0.5763	0.4850	0.115					
Day 15										
Lips (%)	1.39	0.97	0.79	1.0500	0.308	1.05	108.9	-72.0	1.71	60.4
NDF (g)	0.4395	0.4444	0.4177	0.4339	0.014					
ADF (g)	0.7877	0.8125	0.8141	0.8048	0.015					
ADL(H_2SO_4) (g)	0.7862	0.7916	0.8101	0.7960	0.012					
ADL (ASH) (g)	0.3564	0.5223	0.5763	0.4850	0.115					
Day 30										
Lips (%)	0.65	0.56	0.40	0.5366	0.127	0.54	81.52	-23.6	1.42	40.1
NDF (g)	0.6204	0.4589	0.6529	0.5774	0.104					
ADF (g)	0.6879	0.7869	0.6220	0.6989	0.083					
ADL(H_2SO_4) (g)	0.6776	0.7771	0.6200	0.6916	0.079					
ADL (ASH) (g)	0.3564	0.5223	0.5763	0.4850	0.115					

Day 65										
Lips (%)	0.5	0.4	0.38	0.4267	0.064	0.43	92.2	-39.9	2.50	44.7
NDF (g)	0.5115	0.5292	0.5284	0.5230	0.010					
ADF (g)	0.7806	0.7132	0.6915	0.7284	0.046					
ADL(H_2SO_4) (g)	0.7664	0.7023	0.678	0.7155	0.045					
ADL (ASH) (g)	0.3564	0.5223	0.5763	0.4850	0.115					
Day 100										
Lips (%)	0.23	0.26	0.28	0.2567	0.025	0.26	45.4	12.8	4.06	37.5
NDF (g)	0.7892	0.7402	0.7655	0.7649	0.024					
ADF (g)	0.7479	0.7077	0.6411	0.6989	0.54					
ADL(H_2SO_4) (g)	0.7319	0.6713	0.6308	0.6780	0.051					
ADL (ASH) (g)	0.3564	0.5223	0.5763	0.4850	0.115					

THS sludge amended soil										
Day 0										
Lips (%)	1.03	1.02	1.02	1.0233	0.006	1.02	88.8	-26.6	1.01	35.7
NDF (g)	0.4518	0.5098	0.6500	0.5372	0.102					
ADF(g)	0.6994	0.6667	0.6569	0.6743	0.022					
ADL(H_2SO_4) (g)	0.6935	0.6618	0.6520	0.6691	0.022					
ADL (ASH) (g)	0.3564	0.5223	0.5763	0.4850	0.115					
Day 1										
Lips (%)	1.27	1.25	1.22	1.2467	0.025	1.25	103.3	-30.1	0.13	25.4
NDF (g)	0.4457	0.4747	0.4647	0.4617	0.015					
ADF (g)	0.6107	0.6256	0.6141	0.6168	0.008					
ADL(H_2SO_4) (g)	0.6102	0.6247	0.6134	0.6161	0.008					
ADL (ASH) (g)	0.3564	0.5223	0.5763	0.4850	0.115					
Day 3										
Lips (%)	1.42	1.23	1.61	1.4200	0.190	1.42	84.5	-16.5	0.35	30.2
NDF (g)	0.6027	0.4630	0.6063	0.5573	0.082					
ADF(g)	0.5587	0.6857	0.6824	0.6423	0.072					
ADL(H_2SO_4) (g)	0.5586	0.6848	0.6780	0.6405	0.071					
ADL (ASH) (g)	0.3564	0.5223	0.5763	0.4850	0.115					

Day 7										
Lips (%)	1.03	1.00	1.15	1.0600	0.079	1.06	105.6	-15.6	11.1	-2.13
NDF (g)	0.4392	0.4533	0.4588	0.4504	0.010					
ADF (g)	0.6550	0.4064	0.5315	0.5310	0.124					
ADL(H_2SO_4) (g)	0.5364	0.4035	0.4821	0.4740	0.067					
ADL (ASH) (g)	0.3564	0.5223	0.5763	0.4850	0.115					
Day 15										
Lips	0.58	1.02	1.31	0.9700	0.367	0.97	103.8	-44.5	1.63	38.1
NDF (g)	0.4576	0.4432	0.4806	0.4605	0.019					
ADF (g)	0.6368	0.7143	0.7182	0.6898	0.046					
ADL(H_2SO_4) (g)	0.6318	0.7046	0.7078	0.6814	0.043					
ADL (ASH) (g)	0.3564	0.5223	0.5763	0.4850	0.115					
Day 30										
Lips	0.68	0.57	0.50	0.5833	0.091	0.58	94.1	-54.2	1.53	58.0
NDF (g)	0.5332	0.5156	0.4888	0.5125	0.022					
ADF (g)	0.7834	0.8033	0.7888	0.7918	0.010					
ADL(H_2SO_4) (g)	0.7793	0.7935	0.7788	0.7839	0.008					
ADL (ASH) (g)	0.3564	0.5223	0.5763	0.4850	0.115					

Day 65										
Lips (%)	0.44	0.42	0.38	0.4133	0.030	0.41	96.5	-26.3	1.92	28.3
NDF (g)	0.4952	0.5113	0.4969	0.5011	0.009					
ADF(g)	0.6321	0.6583	0.6189	0.6364	0.020					
ADL(H_2SO_4) (g)	0.6310	0.6532	0.6090	0.6310	0.022					
ADL (ASH) (g)	0.3564	0.5223	0.5763	0.4850	0.115					
Day 100										
Lips (%)	0.37	0.33	0.21	0.3033	0.083	0.30	59.0	0.54	1.14	39.0
NDF (g)	0.7353	0.6784	0.6698	0.6945	0.035					
ADF (g)	0.7947	0.6267	0.6536	0.6917	0.090					
ADL(H_2SO_4) (g)	0.7890	0.6221	0.6462	0.6858	0.090					
ADL (ASH) (g)	0.3564	0.5223	0.5763	0.4850	0.115					

Control soil										
Day 0										
Lips (%)	0.69	0.87	0.75	0.7700	0.092	0.77	93.8	9.59	0.72	-4.85
NDF (g)	0.6448	0.4460	0.4485	0.5131	0.114					
ADF (g)	0.4374	0.4500	0.5036	0.4637	0.035					
ADL(H_2SO_4) (g)	0.4336	0.4452	0.5014	0.4600	0.036					
ADL (ASH) (g)	0.3564	0.5223	0.5763	0.4850	0.115					
Day 1										
Lips (%)	1.00	1.07	1.05	1.0400	0.036	1.04	89.3	-23.4	0.43	32.6
NDF (g)	0.4023	0.5361	0.6652	0.5345	0.131					
ADF (g)	0.6594	0.6386	0.6675	0.6552	0.015					
ADL(H_2SO_4) (g)	0.6570	0.6378	0.6643	0.6530	0.014					
ADL (ASH) (g)	0.3564	0.5223	0.5763	0.4850	0.115					
Day 3										
Lips (%)	1.11	1.29	1.17	1.1900	0.092	1.19	61.1	14.7	3.80	19.16
NDF (g)	0.7118	0.6924	0.6330	0.6790	0.041					
ADF (g)	0.6744	0.5031	0.6324	0.6033	0.089					
ADL(H_2SO_4) (g)	0.6236	0.5023	0.6253	0.5837	0.070					
ADL (ASH) (g)	0.3564	0.5223	0.5763	0.4850	0.115					

Day 7										
Lips (%)	1.28	1.25	1.18	1.2367	0.051	1.24	89.0	-33.9	2.33	41.3
NDF (g)	0.6125	0.4871	0.5056	0.5351	0.068					
ADF (g)	0.7267	0.6860	0.7157	0.7095	0.021					
ADL(H_2SO_4) (g)	0.7064	0.6787	0.7074	0.6975	0.016					
ADL (ASH) (g)	0.3564	0.5223	0.5763	0.4850	0.115					
Day 15										
Lips (%)	1.35	2.82	1.13	1.5867	0.919	1.59	84.3	-46.0	0.87	59.2
NDF (g)	0.5373	0.5582	0.5767	0.5574	0.020					
ADF (g)	0.8118	0.7816	0.7891	0.7942	0.016					
ADL(H_2SO_4) (g)	0.8113	0.7761	0.7818	0.7897	0.019					
ADL (ASH) (g)	0.3564	0.5223	0.5763	0.4850	0.115					
Day 30										
Lips (%)	0.26	0.24	0.19	0.2300	0.036	0.23	90.2	-33.2	2.1	40.7
NDF(g)	0.5852	0.5391	0.4780	0.5341	0.054					
ADF(g)	0.7240	0.7661	0.6259	0.7053	0.072					
ADL(H_2SO_4) (g)	0.7152	0.7512	0.6172	0.6945	0.069					
ADL (ASH) (g)	0.3564	0.5223	0.5763	0.4850	0.115					

Day 65										
Lips (%)	0.46	0.96	0.41	0.6100	0.304	0.61	97.5	-59.3	3.92	57.3
NDF (g)	0.5155	0.4656	0.5027	0.4946	0.026					
ADF (g)	0.7810	0.8048	0.8148	0.8002	0.017					
ADL(H_2SO_4) (g)	0.7666	0.783	0.7905	0.7800	0.012					
ADL (ASH) (g)	0.3564	0.5223	0.5763	0.4850	0.115					
Day 100										
Lips (%)	0.19	0.25	0.25	0.2300	0.035	0.23	98.4	-68.5	12.9	56.9
NDF (g)	0.5046	0.4942	0.4767	0.4918	0.014					
ADF (g)	0.8671	0.8548	0.8116	0.8445	0.029					
ADL(H_2SO_4) (g)	0.7808	0.7758	0.7778	0.7781	0.002					
ADL (ASH) (g)	0.3564	0.5223	0.5763	0.4850	0.115					