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Enhancing Biodegradability of Lignocellulosic Residues for Organic Fertilizer Production through Hydrothermal Treatment

（有機肥料製造のための水熱処理によるリグノセルロース残渣の生物分解性の向上）

A dissertation
submitted in partial fulfillment
of the requirements for the Degree

of

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by

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Summary

The large volumes of lignocellulosic residues which could have been converted into organic fertilizers are burnt in field or regarded as waste each season everywhere in the world. However, it is the nature of lignocellulose to degrade slowly, because microbial access to cellulose, a major biodegradable component of lignocellulose, is inhibited by hemicellulose-lignin association during the composting process. Overall objective of this research was to investigate the hydrothermal treatment (HTT) technology as a pretreatment step in enhancing biodegradability of lignocellulosic residues for organic fertilizer production.

Because many lignocellulose components (e.g. cellulose and lignin) act as carbon sources as well as air-flow supporting materials during composting process, pretreatment which results in total solubilization or collapse of lignocellulose cell wall structure should be avoided. Therefore, initially, the effects of HTT on physicochemical properties and subsequent aerobic degradation of lignocellulose (date palm residue) was study. The results showed that 180 °C was the most effective HTT temperature for subsequent aerobic biodegradation by solubilizing the largest portion of hemicellulose (86.5%) within the cell wall, which had two consequences: 1) it supplied additional readily bioavailable form of carbon, which in turn promoted rapid microbial activities in the early stage of decomposition; and 2) it created pores and cavities within the cell wall, which permitted rapid bacterial penetration and cellulose degradation. As a consequence, biodegradation of the residue treated under this reaction temperature proceeded rapidly and stability phase was reached within 21 days, compared to 63 days of continued degradation for the untreated residue.

Composting of lignocellulosic materials can be associated with high rate of NH_3 loss into atmosphere, i.e. loss of essential nutrient. A simple solution that could prevent NH_3 loss effectively is to add simpler sugars, e.g., the forms of carbon that is readily available for rapid microbial immobilization of nitrogen. Previously, HTT with mild reaction temperature (180°C and 1.0 MPa) was found very effective in solubilization of hemicellulose polysaccharides into simpler sugars (i.e. xyloses and glucose). Therefore, attention was directed to examine the effectiveness of HTT (180°C, 1.0 MPa, 30 min) on reducing NH_3 loss during bench-scale composting of lignocellulosic residue. As results, the HTT was very effective for reducing NH_3 loss by solubilizing the largest portion of hemicellulose, which supported microbial immobilization to suppress NH_3 loss in earlier stage of composting, and improved susceptibility of cellulose particles to microbial attack, which supported immobilization to suppress NH_3 loss in later stages of composting. Effect of HTT was also expressed by high nitrogen content: 3.4% in treated residue compared to 2.3% in untreated residue.

The stability and maturity of the final compost product is very important for successful agricultural application. Unstable and immature compost can cause soil nitrogen rubbing, induce oxygen deficiency for plant roots /seeds, create toxicity due to high concentrations of ammonia or various organic acids and promote growth of certain soil pathogens and diseases. In order to allow comprehensive approach for assessing maturity, bin-scale (90L) composting of rice straw as a model of lignocellulosic residues was performed following the pilot-scale (200L) of HTT. The results showed that the rice straw compost product with HTT after 6 weeks of composting was adequate to be considered a stable and mature, as expressed by C/N ratio of 12.5, microbial stability of < 8.05 mg CO_2 g^{-1} OM d^{-1}, NH_4^--N content of 93.8 mg kg^{-1} OM, pH-8.4, EC-2.9 mS cm^{-1} and finally by germination index of >83%. As for rice straw compost product without HTT, the high microbial activity (> 12 mg CO_2 g^{-1} OM d^{-1}) even after 14 weeks of composting suggests that the residue has not stabilized yet and is far away from maturation phase.

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Table of Contents

Summary……...…….ii
Acknowledgement……..……iv
Table of Contents……..v
List of Figures…….vii
List of Tables……..…x

1. General Introduction……1

1.1. Motivation of the Research …….……..……...1
1.2. Nutrients Equivalent of Lignocellulosic Residues…….3
1.3. Principle of Aerobic Composting……...……4
1.4. Biodegradation of Lignocellulose Cell Wall…….…….5
1.4.1. Cellulose……...……6
1.4.2. Hemicellulose……...……7
1.4.3. Lignin……8
1.5. Selecting Pretreatment Technology: Hydrothermal Treatment…….9
1.6. Objective of the Study…….10
1.7. Thesis Contents and Chapters Outline……10

2. Effects of Hydrothermal Treatment on the Physicochemical Properties and Subsequent Aerobic Degradation Rate of Date Palm Lignocellulosic Residue.....19

Abstract……...19
2.1 Introduction……20
2.2 Material and Experimental……..21
2.2.1 Material……21
2.2.2 HTT Experiment……22
2.2.3 Proximate Analyses of CW Constituents…….24
2.2.4 HPLC Analyses of Condensed Steams……24
2.2.5 Aerobic Degradation Test……26
2.2.6 Validation of the Test System and the Quality of Compost Medium……..26
2.2.7 Microbial Stability Assessment……...27
2.3 Results and Discussions……..29
2.3.1 Effect of HTT on CW Solubilization……...29
2.3.2 Loss of Macronutrients (N,P,K,S) during HTT Process……..32
2.3.3 Distribution of N in Solid Residues……...33
2.3.4 Aerobic Biodegradability……34
2.3.5 Untreated CW Residue……34
2.3.6 Effect of HTT……..34

2.4 Conclusions……37
3. Effect of HTT on Ammonia Volatilization Reduction During Bench-scale Composting of Date Palm Lignocellulosic Residues……44
Abstract……...44
3.1 Introduction……45
3.2 Materials and Methods……46
3.2.1 Material and Hydrothermal Treatment……46
3.2.2 Aerobic Composting……46
3.2.3 Analyses……..47
3.3 Results and Discussions……..48
3.3.1 Solubilization of Hemicellulose Polysaccharides……48
3.3.2 Ammonia Volatilization……..……48
3.3.2.1 Residue without HTT……..49
3.3.2.2 Residue with HTT……...50
3.4 Conclusions……52
4. Evaluation of Stability and Maturity of Compost during Bin-Scale Composting of Rice Straw with and without Hydrothermal Treatment……57
Abstract……...57
4.1 Introduction……58
4.2 Experimental……60
4.2.1 Material and Hydrothermal Treatment……60
4.2.2 Substrate Preparation……...63
4.2.3 Microbial Inoculums Preparation……63
4.2.4 Composting Setup……...63
4.2.5 Sample Collection……...65
4.2.6 Analytical Methods……65
4.2.7 Microbial Activity Test……66
4.2.8 Phytotoxicity Test……66
4.2.9 Evaluation of N Losses……67
4.3 Results and Discussions……68
4.3.1 Evolution of Composting Temperature……...68
4.3.2 Variations in pH and EC……..69
4.3.2.1 pH……70
4.3.2.2 EC……70
4.3.3 Evolutions of NH_4^+-N and NO_3^--N……..72
4.3.3.1 NH_4^+-N……72
4.3.3.2 NO_3^--N……73

4.3.4 Variations in OM content…….75
4.3.5 Changes in TN Content……...76
4.3.6 Variations in C/N Ratio……...77
4.3.7 Evolutions of Carbon Dioxide…….79
4.3.8 Germination Index……...80
4.3.9 Evaluation of Stability and Maturity of Compost End Product……..81
4.3.10 Correlation between Stability and Maturity Parameters…….83
4.3.11 The losses of N……86
4.4 Conclusions……88
5. Conclusions and Recommendations……96
5.1 Conclusions……96
5.2 Recommendations……...98

List of Figures

Fig.1.1. World population and cereal crops production (Source of data: FAOSTAT)……...….1

Fig.1.2. World fertilizers import, in US$ (Source of data: FAOSTAT)……...1

Fig.1.3. Energy (natural gas and crude oil) and fertilizer (nitrogen, phosphate and potassium) prices (Source: Mueller et al., 2011)……..……..2

Fig.1.4. World ethanol production (Source: Berg, 2004)……2

Fig.1.5. Schematic description of lignocellulosic residue composting and the key factors affecting the speed of this process……..5

Fig.1.6. Structure of single cellulose molecule (Source: Harsen et al., 2013)……7

Fig.1.7. A schematic representation of the hemicelluloses backbone of arborescent plants (Source: Harsen et al., 2013)…….……..8

Fig.1.8. P-coumaryl- , coniferyl- and sinapyl alcohol: dominant building blocks of the three-dimensional polymer lignin (Source: Harsen et al., 2013)……..……8

Fig.1.9. Model structure of spruce lignin (Source: Harsen et al., 2013)……9

Fig.2.1. Schematic diagram of the small-scale HTT reactor……23

Fig.2.2. Temperature and pressure profile for HTT experiments (1-160 °C; 2-180 °C; 3-200 °C; 4-220°C. Pressure profiles are drawn according to the steam table)……23

Fig.2.3. Flow-diagram of CW constituents analyses in the untreated and the residues from HTT of date palm trunk woodchips……...……25

Fig.2.4. Biometer jar for simulating aerobic digestion of the test materials……..28

Fig.2.5. Mineralization of cellulose (as a reference material) and the cumulative CO_2 production of compost (blank). The results represent the mean of three replications…….28

Fig.2.6. Soluble carbohydrates in the water-soluble fraction of the untreated and the residues from HTT of date palm CW…….30

Fig.2.7. The HPLC spectra of the condensed `flash steam` collected during HTT of date palm CW residue (Peak`s legends: 1-Formic acid, 2-Levulinic acid, 3-5•HMF, 4-Furfural)……30

Fig.2.8. Distribution of N in solid fraction of the untreated and residues from HTT……..33

Fig.2.9. Biodegradation behavior of the untreated and the residues from HTT of date palm cell wall during 63 days of incubation (a -untreated CW; treated at b-160; c-180; d -200 and e-220°C reaction temperature. All results represent the mean of three replications. Mineralization is expressed as percentage of biodegradable fraction of the untreated CW. The broken line represents the threshold stability line, which is set to 90 % mineralization)……..……36

Fig.3.1. Biometer jar for simulating ammonia volatilization during composting process……..47

Fig.3.2. Cell wall constituents of date palm residues before and after HTT (180 °C, 1.0 MPa)…….48

Fig.3.3 Ammonia volatilization and carbon dioxide production rates during aerobic composting of date palm residue: a-untreated residue, b-residue after HTT and c-compost medium

(horizontal bars represent standard errors calculated based on three replications)……51
Fig.4.1. Schematic view of the pilot-scale HTT facility……..60
Fig.4.2. Snapshot of the HTT facility……...61
Fig.4.3. Temperature and Pressure profile during one of HTT batches……..61
Fig.4.4. Diagram of the composting reactor……64
Fig.4.5. The snapshot of the compost reactors……64
Fig.4.6. Steam distillation setup for measuring NH^+_4-N and NO^-_3-N during the experiment…….65
Fig.4.7. Germination test: a) - the Komatsuna seeds are being treated with distilled water and compost extracts; b) - germinated seeds after 4-day incubation; c) - the root lengths are being measured....67
Fig.4.8. Evolution of temperature during composting process of rice straw residue with and without HTT (The air temperature in the room was maintained between 23 and 25°C and therefore, 24°C was taken as a mean)……69
Fig.4.9. Flies and pests reaching the downstream air-supply tubes and acid traps during composting of rice straw with (left) and without (right) HTT……69
Fig.4.10. Variations in pH (a) and EC (b) during composting process of rice straw residue with and without HTT (The values are the means of three replications. Standard deviations are provided in Table 4.3)……...……..71
Fig.4.11. Variations in NH^+_4-N (a) and NO^-_3-N (b) contents during composting process of rice straw with and without HTT (The values are the means of three replications. Standard deviations are provided in Table 4.3)……...……74
Fig.4.12. The loss of OM content during composting of rice straw with and without HTT (The values are the means of three replications. Standard deviations are provided in Table 4.3)……76
Fig.4.13. Changes in TN contents during composting of rice straw with and without HTT (The values are the means of three replications. Standard deviations are provided in Table 4.3)……..….77
Fig.4.14. Variations in the C/N ratio during the composting process of rice straw with and without HTT (The values are the means of three replications. Standard deviations are provided in Table 4.3)……..78
Fig.4.15. Changes in microbial activities (as the CO_2 evolution rate) during composting of rice straw with and without HTT (The values are the means of three replications. Standard deviations are provided in Table 4.3)……..79
Fig.4.16. Changes in Komatsuna seeds germination indices during composting of rice straw with and without HTT (The values are the means of three replications. Standard deviations are provided in Table 4.3)……..80
Fig.4.17. Stability and maturity parameters of rice straw compost products with (a) and without (b) HTT plotted in octagonal diagram after 6 and 14 weeks of processing……82
Fig.4.18. The net loss of N (a) and cumulative NH_3 loss (b) during composting of rice straw with and without HTT……87

List of Tables

TABLE 1.1. World production of lignocellulose crop residue and nutrients equivalents (in 1998)…4

TABLE 1.2. Typical lignocellulose cell wall compositions……...6

TABLE 2.1. Properties of date palm trunk residues……... 22

TABLE 2.2. Chemical properties of untreated and residues from HTT of date palm wood before aerobic digestion test……..……..27

TABLE 2.3. Proximate analyses results of the untreated and the residues from HTT of date palm wood……29

TABLE 2.4. Effect of HTT on date palm cell wall constituents (The original analyses results from Table 2.3 were normalized to 100%)……31

TABLE 2.5. Losses of Macronutrients during the HTT……32

TABLE 3.1. Properties of date palm trunk residue before and after HTT……46

TABLE 4.1. Chemical properties of the rice straw residue before and after HTT……62

TABLE 4.2. Thresholds for Maturity and Stability Parameters……83

TABLE 4.3. Variations in selected biochemical properties during composting of rice straw with and without HTT……..84

TABLE 4.4. Pearson correlation coefficient between measured parameters of rice straw compost with and without HTT……...85

Chapter 1

1. General Introduction

1.1. Motivation of the Research

According to Kivu (2012), since the 1960th the world population is experiencing an exponential increase. If the number of population was only 3.1 billion in 1961, it increased to 6.9 by 2010 (FAOSTAT). This increase in population and the economic growth have been considered as the major forces driving increased global food demand, crop production and fertilizers consumption (FAO, 2010). Fig. 1.1 and 1.2, respectively, demonstrates that indeed, there are correlative increase in the population growth, production of cereal crops and the amount of fertilizers imported by the nations (expressed in US$), particularly until 2000th.

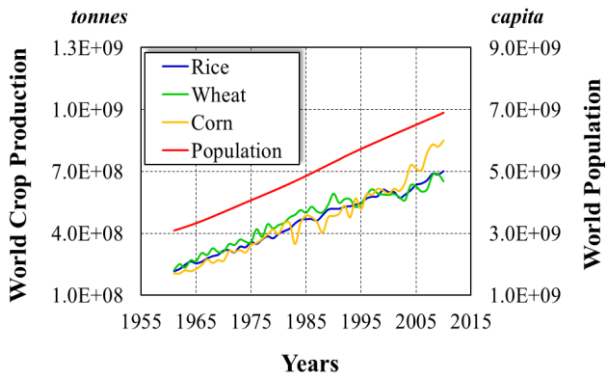

Fig.1.1. World population and cereal crops production (Source of data: FAOSTAT)

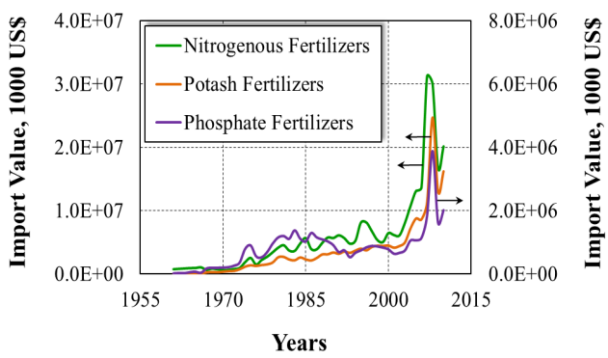

Fig.1.2. World fertilizers import, in US$ (Source of data: FAOSTAT)

It is widely known nitrogen is a key fertilizer, often most limiting to crop growth (Mikkelsen and Hartz, 2008). According to Maxwell (2004), about 97% of the world's nitrogenous fertilizers are derived from synthetically produced ammonia, specifically through well-known Haber-Bosch process with the nitrogen component derived from the air and the hydrogen component derived from the fossil fuels, particularly from natural gas (CH_4). The Haber-Bosch process is energy-intensive process. For example, on average, in the US, 51 MJ of energy is required in order to produce one kilogram of ammonia (Peterson et al., 2008). However, in recent years, the world price for energy increased rapidly and this, in turn, has pulled the world price of fertilizers up (Fig.1.3). In the same time, the high price of fossil fuels has provided strong incentives for ethanol production (Fig.1.4) as an alternative fuel source that in turn, boosted the demand for agricultural crops. According to Berg (2004), more than 95% of fuel ethanol is coming from the agricultural crops, especially from food crops such as corn, wheat, sugarcane, and soybeans. This hike in crops production subsequently, has led to further demand for fertilizers, raising its world price by 3-4 times over the past 10-12 years (Fig.1.3).

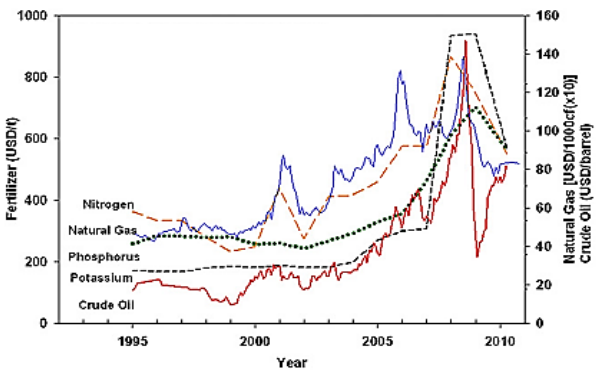

Fig.1.3. Energy (natural gas and crude oil) and fertilizer (nitrogen, phosphate and potassium) prices (Source: Mueller et al., 2011)

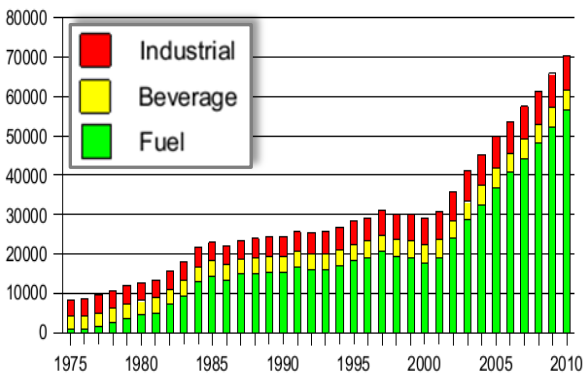

Fig.1.4. World ethanol production (Source: Berg, 2004)

Besides these current problems, the world population is expected to increase to more than 8 billion in 2025 (Kivu, 2012). According to Dayson (1999) and Rosengrant et al., (2001) world cereal production must rise by 135-140% in 2025 to meet the rise in food demand. Further, the global production of ethanol is expected to grow by 40% in 2022 (USDA, 2013). Bearing in mind these trends as well as the fact that phosphorus and potassium are finite resources, it is likely that these increases will be translated into another decade of increased crop production and the high price of fertilizers. Therefore, in order to reduce the cost of crop fertilizing in future, reuse and recycling of nutrients in lignocellulose crop residues is necessary.

1.2. World Lignocellulosic Residues Production and Nutrients Equivalents

It is widely realized that lignocellulosic residues represent an underutilized renewable resource which is available at large amounts (de Castro, 1994). According to Yadvinder-Singh et al. (2005), for example, on global basis, the seven major crops (Table 1) produced about 3000 million tons of lignocellulosic residues in 1998, which contained about 18.8 million tons of nitrogen (N), 2.9 tons of phosphor (P), and 24.0 tons of potassium (K). In other words, about 31, 26, and 154% of N, P, and K, respectively, of the fertilizer consumed in 1998 were found in these residues (Yadvinder-Singh et al., 2005). In addition, lignocellulosic residues contain other important micronutrients that are also essential for maintaining crop growth and sustainable production (Tirol-Padre et al., 2005). However, the large amount of these residues are burnt in the field or regarded as a solid waste each season almost everywhere in the world. For example, about 50% of the rice straw residue produced in Thailand is subjected to open-field burning, and in the Philippines it is 95% (Gadde et al., 2009). Indonesia produced about 60.1 million tons of rice straw residue in 2004, and of this, 37% was burnt in open-field (ICBC, 2004). Totally, 200 million tons of rice and wheat straw residues were generated in India in 2009, and from this total about 70 million tons was burnt on-farm (IARI, 2012). In China, depending on the provinces, approximately 25-48% of rice straw is burnt to prepare the fields for the next crop (Pan at el., 2011). In fact, the crop nutritional value of these residues has always been recognized by farmers and until the early 1950`s when chemical fertilizer gained rapidly in popularity because they were cheap and available, these materials served as the principle sources of plant nutrients (Parr and Colacicco, 1987). These lignocellulosic residues could have been used again, at least in part, as an alternative source of nutrients for reducing the cost of crop fertilizing. According to Hauck (1981, as cited by Parr and Volacicco, 1987), reintroduction and utilization of organic residues to improve crop productivity could contribute more than 50% of the increased food production, especially, in developing countries. However, lignocellulose agricultural residues contain significant amount of carbohydrates and because of certain agronomic issues such as temporary immobilization of

nutrients and associated crop yield reduction it is often required that the residues need to be biodegraded and sufficiently stabilized prior to soil application. Composting is widely considered as a low-cost and environmentally friendly technique for conversion of agricultural residues into stable and mature organic product that can be used as a valuable organic fertilizer (Arkhipchenko et al., 2005; Abdel-Rahman, 2009).

TABLE 1.1. World production of lignocellulose crop residue and nutrients equivalents (in 1998)

Crop	Residue	N	P	K
	Million tones			
Rice	1013.74	5.35	0.85	5.32
Wheat	946.73	5.65	0.66	7.76
Barley	208.23	1.52	0.22	2.37
Sugarcane	125.23	0.53	0.10	0.84
Cotton	6.80	0.07	0.01	0.07
Oats	51.60	0.33	0.08	0.85
Corn	604.01	5.39	0.98	6.82
Total	**2956.35**	**18.83**	**2.91**	**24.04**

Source: Yadvinder-Singh et al. (2005)

1.3. Principle of Aerobic Composting

According to Zucconi et al., (1986), composting is as a controlled bio-oxidative process leading to the production of carbon dioxide, water, minerals, and stabilized organic matter defined as ‘compost’. Schematic description of organic materials composting process is shown in Fig.1.4. The resident microbial community in compost consists of bacteria, actinomycetes and fungi (Tuomela et al., 2000). Microorganisms utilize available carbon, macronutrients such as nitrogen, phosphorous and potassium, and certain trace elements for their growth (Kluczek-Turpeinen, 2007). Part of carbon is converted into CO_2 gas, while another part of carbon is used for synthesis of new microbial cells (Knapp et al., 1983a-b). As composting activates, heat is generated due to metabolic activities of microorganism (Tiquia et al., 1996), which is partially given off to environment in the form of vapor. Composting is successful when organic materials are converted into stable organic matter that is rich in minerals and humus-like substances (Bernal et al., 1998). Nitrogen is a critical element for microorganisms because it is a component of the proteins, nucleic acids, aminoacids, enzymes and co-enzymes necessary for cell growth and functioning (Kluczek-Turpeinen, 2007). If nitrogen is a limiting factor during composting the degradation process will be slow (Jiang et al., 2011). In contrast, if there is excess nitrogen, it is often lost from the system as ammonia gas or

other nitrogen compounds (Prochnow et al., 1995).

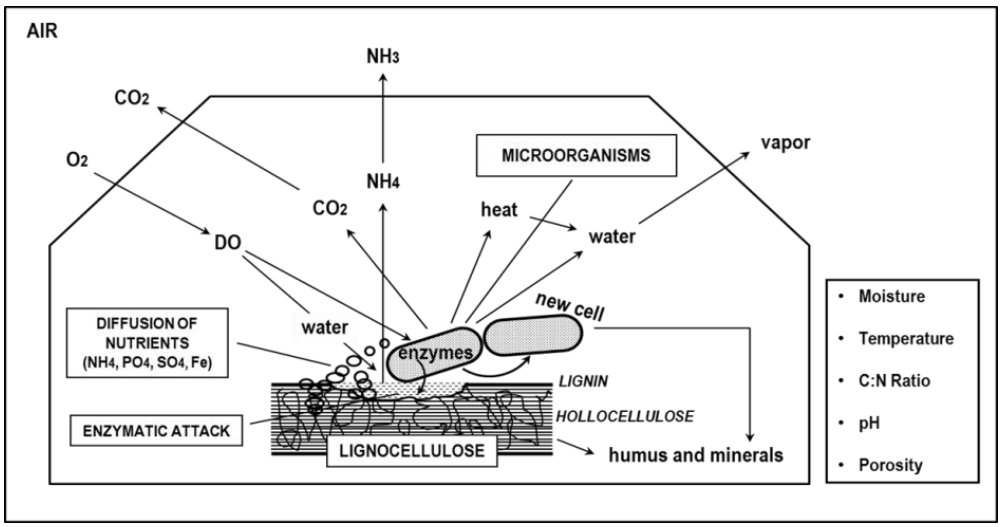

Fig.1.5. Schematic description of lignocellulosic residue composting and the key factors affecting the speed of this process

According to Kluczek-Turpeinen (2007), composting of waste materials proceeds in three phases: (i) the mesophilic phase, (ii) the thermophilic phase and (iii) the cooling and maturation phase. The key parameters of composting include C/N ratio, moisture content, particle size, airflow, oxygen concentration and temperature. Successful optimization of these parameters may significantly shorten the composting time and result in high quality of compost product (Jiang at el., 2011). Nevertheless, ordinary composting of lignocellulose may still be long in process. This is because microbial decomposition of cellulose (a major biodegradable component of lignocellulose) in crop residue is inhibited due to rapping effect of hemicellulose-lignin association in the crop cell wall structure. In native crop cell wall, lignin is intermeshed and chemically bonded with hemicellulose polysaccharides, which together form a barrier that become even more resistant to microbial degradation (Malherbe and Cloete, 2002). Therefore, to be rapidly composted into organic fertilizer, some pretreatment is necessary to disrupt cell wall hemicellulose-lignin association and improve the availability of cellulose particles for microbial attack.

1.4. Biodegradation of Lignocellulose Cell Wall

Lignocellulose is the term generally, used for residues from higher plants. The main components of the lignocellulosic materials are cellulose, hemicellulose and lignin. In addition, small amounts of proteins, minerals and other components can also be found in the lignocellulose as well (Alberts et al., 2002). Cellulose is polymer of C6 sugar (glucose) and is a major structural component of cell

walls, and it provides mechanical strength to plants. Hemicellulose is a copolymer of different C5 and C6 sugars that also exist in the plant cell wall. Lignin is polymer of aromatic compounds formed to give the plant structural support, impermeability, and resistance against microbial attack and oxidative stress (Hendriks and Zeeman, 2009). Hemicellulose, cellulose and lignin content in the most common crop residues is summarized in Table 1.2.

TABLE 1.2. Typical lignocellulose cell wall compositions

Lignocellulose	**Cell Walls Components, wt%**			**References**
	Hemicellulose	**Cellulose**	**Lignin**	
Rice straw	29.8	36.5	14.0	Present Study
Wheat straw	22.4	33.5	16.4	Thomsen et al., 2008
Barley straw	21.9	33.8	13.8	Mussatto and Teixeira, 2010
Sugarcane	29.0	40.0	13.0	Jackson, 1977
Cotton stalk	30.1	45.5	18.2	Tutus et al., 2010
Oats straw	27.4	39.4	17.5	Mussatto and Teixeira, 2010
Corn stalks	16.8	58.5	21.5	Mussatto and Teixeira, 2010
Sunflower stalks	20.2	33.8	17.3	Ruiz et al., 2008
Date palm trunks	33.1	34.2	22.5	Present Study

1.4.1. Cellulose

Cellulose is a linear polymer of glucose linked through α-1,4-linkages and is usually arranged in microcrystalline structures. Cellulose normally is also found in the amorphous structure. The structure of one chain of the cellulose polymer is presented in Fig.1.5. Many properties of cellulose depend on its degree of polymerization (DP), i.e. the number of glucose units that make up one polymer molecule. According to Malherbe and Cloete (2002), the DP of cellulose chains can range from 500 to 25 000. The combination of several cellulose polymer chains (20-300) leads to the formation of microfibrils, which in turn are united to form fibres. In this way cellulose can obtain a crystalline structure (Harsmen at al., 2013). This will also results in its low susceptibility to chemical and enzymatic attack (de Castro, 1994).

Biodegradation of cellulose requires the combined action of three protein enzymes: (1) endoglucanases to randomly cleave inter monomer bonds; (2) exoglucanases to remove mono- and dimers from the end of the glucose chain; and (3) β-glucosidase to hydrolyze glucose dimers (Malherbe and Cloete, 2002). However, despite these complexities in cellulose structure, the rate-limiting factor in biodegradation of crop residue cellulose during composting is its

susceptibility to microbial and enzymatic attack which is largely inhibited by complex association of lignin and hemicellulose carbohydrates (Hatfield et al., 1999).

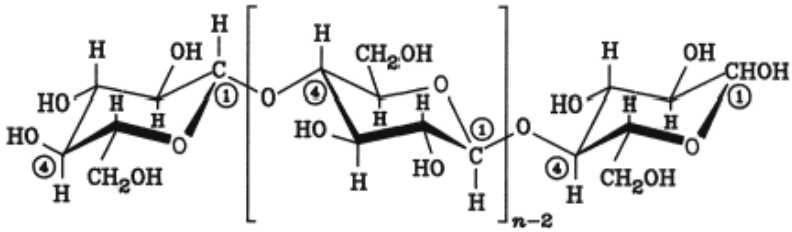

Fig.1.6. Structure of single cellulose molecule (Source: Harsen et al., 2013)

1.4.2. Hemicellulose

Hemicellulose is the second most abundant polymer in lignocellulose. Hemicellulose differs from cellulose in that it is heterogeneous and branched polymer of pentoses (xylose, arabinose), hexoses (mannose, glucose, galactose) and acetylated sugars (Fig.1.6). Hemicelluloses in hardwoods as well as agricultural residues like straw and grasses are composed mainly of xylan, while softwood hemicelluloses contain mainly of glucomannan (Jeffries, 1994; Laine, 2005). They have lower molecular weight compared to cellulose and branches with shorter chains (<200) and therefore, are easily hydrolyzed (Fengel and Wegener, 1984). Hemicellulose is intermeshed between the lignin and the cellulose fibers and gives the whole cellulose–hemicellulose–lignin network more rigidity (Laureano-Perez et al., 2005). Therefore, solubilization of hemicellulose creates additional pores and cavities within the structure of the substrate that is necessary for rapid penetration through and attack of enzymes on cellulose (Miron and Ben-Ghedalia, 1992).

Although hemicellulose polymers have lower molecular sizes than cellulose does, more enzymes and time are required for its complete biodegradation because of its greater heterogeneity (Malherbe and Cloete, 2002). In addition, hemicellulose degradation needs various accessory enzymes such as xylan esterases, ferulic and p-coumaric esterases, α-1-arabinofuranosidases, and α-4-O-methyl glucuronosidases, acting synergistically to efficiently hydrolyze xylans and mannans (Sanchez, 2009). Hemicelluloses within plant cell walls are thought to ‘coat’ cellulose-fibrils and it has been proposed that at least 50% of hemicellulose should be removed to significantly increase cellulose biodegradability (Agbor et al., 2011).

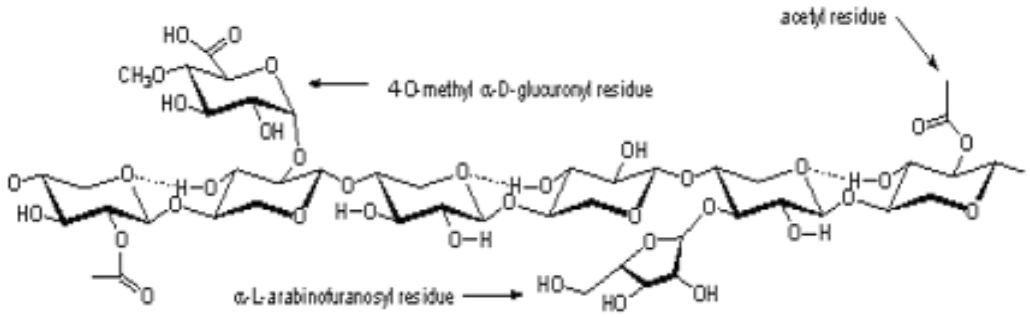

Fig.1.7. A schematic representation of the hemicelluloses backbone of arborescent plants (Source: Harsen et al., 2013)

1.4.3. Lignin

Lignin is the third most abundant polymers in lignocellulose. It is the most complex natural polymer consisting of three different phenylpropane units, i.e. p-coumaryl, coniferyl and sinapyl alcohol (Fig.1.7), that are held together by different kind of linkages (Fig.1.8). Lignin in softwoods principally consists of coniferyl alcohol unit, while in hardwoods it is composed of guaiacyl and syringyl units. Notably, the lignin in grass contains three units: guiacyl-, syringyl-, and p-hydroxyphenyl units (Palonen, 2004).

Biodegradation of lignin is an oxidative process (mainly, by fungi) and phenol oxidases, i.e. lignin peroxidases, manganese peroxi-dases and laccases appear to be the key enzymes (Miron and Ben-Ghedalia, 1992). Other enzymes that participate in the lignin degradation processes are H_2O_2-producing enzymes and oxidoreductases. According to Zhang et al. (2008), partial hydrolysis of lignin, may promote microbial enzyme activities important for the degradation of lignin and formation of humus.

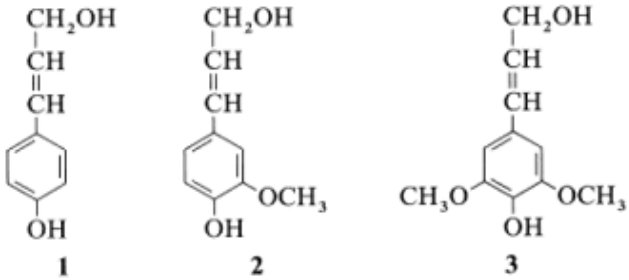

Fig.1.8. P-coumaryl- , coniferyl- and sinapyl alcohol: dominant building blocks of the three-dimensional polymer lignin (Source: Harsen et al., 2013)

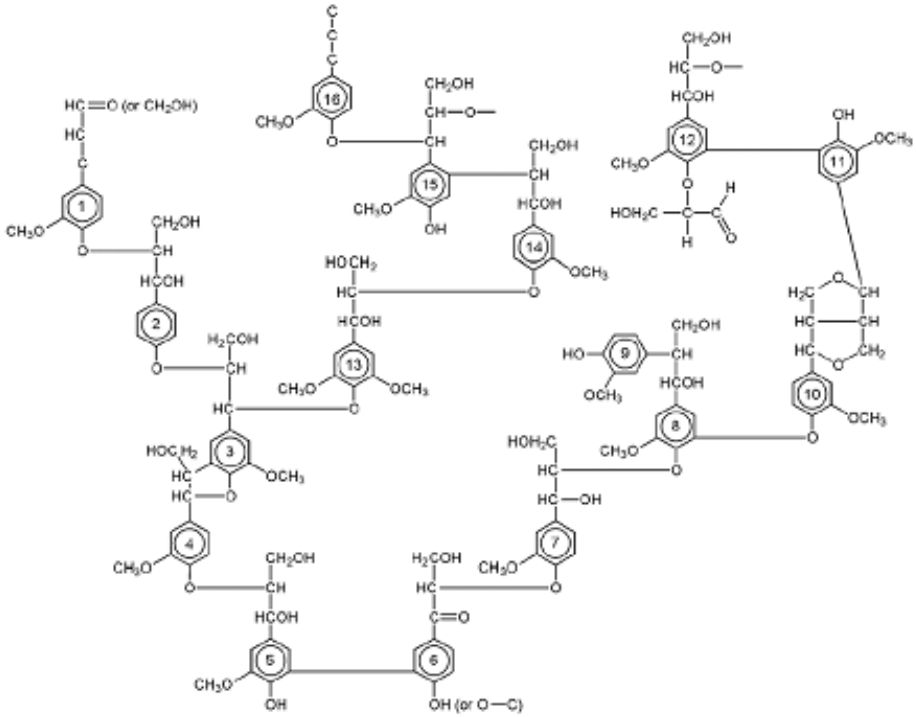

Fig.1.9. Model structure of spruce lignin (Source: Harsen et al., 2013)

1.5. Selecting Pretreatment Technology: Hydrothermal Treatment

The data presented above clearly indicate that lignocellulosic residues are underutilized and available on-spot resource that could be utilized as renewable sources of fertilizer nutrients. It was also shown that lignocellulose materials are poorly biodegradable and therefore development of efficient pretreatment, particularly practical/commercial-scale is necessary to disrupt its natural barriers thereby increasing its compostability. Moreover, all lignocellulose materials have structural similarities, meaning that selecting single or couple lignocellulosic materials as a model in the study would be sufficient and the developed pretreatment technology would successfully be applied to any kind of lignocellulosic materials.

Various pretreatment technologies are now available to improve the biodegradability of lignocellulose (Taherzadeh and Karimi, 2008; Hendriks and Zeeman, 2009), and their advantages and disadvantages fully described in Agbor (2011). The hydrothermal treatment (HTT) is already known as simple and an efficient pretreatment technology for solubilization of CW hemicellulose into readily bioavailable form of carbon (Thomsen et al., 2008), as well as effective in simultaneous conversion of biomass organic N into ammonia (Ren et al., 2006), the form that is

most preferred by microorganisms (Jansson, 1958). The main merits that favor the use of the HTT technology in compost feedstock pretreatment is the elimination of chemical use/disposal problems on-farm (Agbor, 2011) as well as reduction of offensive odors (Namioka et al., 2011).

The hydrothermal treatment HTT in general is a heat-treatment process that utilizes high-temperature and high-pressure water (i.e. near-subcritical, subcritical, and super-subcritical) as a reaction medium to upgrade the solid wastes into value-added products. The HTT technology developed in Yoshikawa Laboratory, Tokyo Institute of Technology employs near subcritical water, i.e. 160 °C < T < 220 °C, 0.6 MPa < P < 2.4 MPa. First, solid wastes are fed into the reactor, and then, saturated steam is injected into the reactor until the target temperature and pressure is reached (defined as heating time). The blades installed inside the reactor, then, begin to mix the waste for about 30-90 minutes (defined as holding time). After the treatment is complete, the reactor is decompressed by releasing the steam and treated solid are discharged. The steam is trapped by condenser, collected, treated and can be utilized as the boiler feed water again. The known HTT reaction mechanisms include many chemical reactions as such as hydrolysis, dehydration, decarboxylation, aromatization, polymerization, etc., and the temperature appears to be a key parameter in governing the rate of these reactions (Sakki et al, 1996; Funke and Ziegler, 2010; Hoekman et al., 2011). According to Prawisudha et al. (2012), the HTT technology developed at Yoshikawa Laboratory can be considered a self-sustained treatment system as part of treated residue (approximately, 1/4 of the energy content in the treated product) can be utilized as a source of energy required for generation of steam.

The HTT technology application in compost feedstock pretreatment may enjoy extra advantages, because the moisture content of treated materials is around 65-70%, which is considered optimal for the composting. In addition, there is no need for downstream water treatment system, as the condensed water is aerobically degradable, as well as it is rich in nutrients and can be incorporated into compost.

1.6. Objective of the Study

Overall objective of this research is to investigate HTT as a pretreatment step to enhance biodegradability of lignocellulosic residues for organic fertilizer production.

1.7. Thesis Contents and Chapters Outline

The contents of this thesis have been divided into five chapters as follows:

Chapter 1: Introduction

This Chapter first, discusses the relationship between food production, energy and fertilizers as well as their trends for near future. Next, lignocellulosic residues as renewable sources of nutrients and their potentials for organic fertilizers are presented. Study on cell wall structure and biodegradation of lignocellulose was conducted, which allowed identification of similarity in structure and major common barrier. Then, HTT was proposed based on the review of available pretreatment technologies. Finally, in order to meet the aim of the study, the objective of the study was set.

Chapter 2: Effects of HTT on the Physicochemical Properties and Subsequent Aerobic Degradation Rate of Date Palm Lignocellulosic Residue

In this Chapter, date palm (*Phoenix roebelenii*) trunk residues as model lignocellulose was subjected to HTT at mild reaction conditions (160 °C < T < 220 °C, 0.6 MPa < P < 2.4 MPa) for 30 min, and the effect of treatments on the CW solubilization, macronutrients and subsequent aerobic degradation rate (as CO_2 production) under controlled composting condition during 63 days of laboratory incubation (38 °C) was tested.

Chapter 3: Effect of HTT on ammonia volatilization reduction during bench-scale aerobic composting of date palm lignocellulosic residues

In our previous work (Chapter 2), HTT with mild reaction temperature (180 °C and 1.0 MPa) was found very effective in enhancing aerobic degradation of lignocellulosic residues and that this improved biodegradability was mainly attributed to solubilization of major portion of hemicellulose polysaccharides into simple sugars. Hence, it may also be possible to reduce AV effectively from compost through HTT of feedstock. Therefore, in this Chapter the attention was directed to examine the effectiveness of HTT (180 °C, 1.0 MPa, 30 min) on reducing AV rate during the 45 days of bench-scale aerobic composting of lignocellulosic residue.

Chapter 4: Evaluation of stability and maturity during composting of lignocellulose rice straw with and without HTT

In order to allow comprehensive approach for assessing stability and maturity of final product, bin-scale (90L) composting of rice straw was performed following the pilot-scale (200L) HTT. In the present Chapter, maturity of rice straw compost product with and without HTT taken at different time intervals (weeks 0, 2, 4, 6, 8 10, 12 and 14) was evaluated through analyzing

different parameter such C/N ratio, microbial stability test (as CO_2 evolution rate), NH^+_4-N content, NH^+_4-N / NO^-_3-N ratio, pH, electrical conductivity and finally, seed germination index.

Chapter 5: Conclusions and Recommendations

In this this Chapter, major results and findings of the study are summarized, including recommendations for reducing and retaining of N in final compost product.

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Chapter 2

2. Effects of Hydrothermal Treatment on the Physicochemical Properties and Subsequent Aerobic Degradation Rate of Date Palm Lignocellulosic Residue

Abstract

In this Chapter, date palm (*Phoenix roebelenii*) woodchip, a residue of palm tree plantation, was subjected to the hydrothermal treatment (HTT) at mild reaction conditions (160 °C < T < 220 °C, 0.6 MPa < P < 2.4 MPa) for 30 minutes, and the effect of treatments on the cell wall (CW) solubilization and subsequent aerobic degradation rate (as CO_2 production) under the controlled composting condition during 63 days of incubation (38 °C) was tested. HTT at 160 and 180 °C reaction temperatures notably solubilized hemicellulose, decreasing the fraction of this CW polymer from 34.1 % in the untreated material, to 9.5 and 4.6 % in the respective residues. However, treatment at 200 and 220 °C reaction temperatures rapidly liquefied the lignin, which apparently went into solution with hemicellulose and appeared to stabilize portion of this polysaccharides against hydrolysis. Consequently, the fraction of hemicellulose in 200 and 220 °C – treated residues gradually increased; the respective values were 5.8 and 9.4 %. The treatment temperature of 180 °C was the most effective HTT temperature for subsequent aerobic degradation by solubilizing the largest portion of hemicellulose within the CW, which had two consequences: 1) it supplied additional readily bioavailable form of carbon, which in turn promoted rapid microbial activities in the early stage of decomposition; and 2) it created pores and cavities within the CW, which permitted rapid bacterial penetration and CW degradation. As a consequence, biodegradation of the residue treated under this reaction temperature proceeded rapidly and the stability was reached within 21 days, compared to 63 days of continued degradation for the untreated CW. The enhanced biodegradability was also partially linked to the effect of 180 °C treatment temperature on solubilization of amorphous cellulose and partial hydrolysis of lignin. Based on the results, the HTT system can successfully be used as a pretreatment step to accelerate the aerobic digestion rate of date palm residues for the production of organic fertilizers.

2.1 Introduction

The growth of global food production, increased price of energy coupled with increased production of biofuels from food crops, have led to increased demand for fertilizers, raising its world price by four times in the past year (FAO UN, 2008). On the other hand, large volumes of lignocellulosic residues (LCR) (straw, woodchips) which could have been converted into valuable organic fertilizers are burnt in situ or regarded as unwanted by-product each season almost everywhere in the world. According to Ponnamperuma (1982), rice straw, for example, contains on average of about 0.6% N, 0.1% P, 0.1% S and 1.5% K. Assuming that the residues of rice amounts to 7 t ha^{-1} yr^{-1} on the average, subsequently, removing the residues will be equivalent to removing 42 kg N, 7 kg each of P and S, and 105 kg K from the soil. In fact, the nutritional value of these residues has always been recognized by farmers and historically their nutrients have partially been (and the practice continues to this day) recovered on-site through traditional techniques such as `ashing`, direct incorporation into the soil and surface retention. However, studies have shown that the benefit can be much smaller when it is compared to the loss which may bring application of these traditional techniques. Burning the residues on-site, for instance, causes nutrient loss into the atmosphere (Ponnamperuma 1982; Mandal et al., 2004) as well as destruction of the physical, chemical and biological properties of the soil (Raison, 1979; Biederbeck et al., 1980; White et al., 2011). In addition, burning on site emits air pollution and may bring up serious health issues (Hasegawa et al., 1999; Torigoe et al., 2000). Other studies have shown that direct incorporation of residue into the soil can lead to N deficiency and formation of various phytotoxic compounds in the soil (Raison, 1979; Sangatanan and Sangatanan, 1990). Residue retention, on one hand, protects the surface of the soil from erosion and increases the availability of organic nutrients on the surface, and on the other hand, it may act as a breeding site for harmful pests or can lead to machinery failures during land preparation for next cropping (Mandal et al., 2004).

Aerobic digestion (traditionally known as composting) is already a well-proven technique which can efficiently convert these nutrient-rich residues into a high-quality organic product that is beneficial for the plant, soil and natural environment (Drinkwater et al., 1995; Letourneau and Goldstein, 2001). However, ordinary composting in which LCR are directly subjected to microbial degradation is a too slow process for farmers (especially for those who have limited space or practice double or triple cropping in a year), because microbial access to cellulose, a major biodegradable component of lignocellulose, is known to be inhibited by hemicellulose-lignin association during the biodegradation process. In native lignocellulose, lignin is intermeshed and chemically bonded with hemicellulose, which together form a barrier that becomes even more resistant to microbial degradation (Jeffries, 1994; Malherbe and Cloete, 2002; Palonen, 2004). It

was previously shown that the solubilization of hemicellulose can create spaces within the CW structure, which is necessary for rapid bacterial penetration through and colonization on CW particles and subsequent degradation (Akin, 1975; Miron and Ben-Ghedalia, 1992.a-b). On the other hand, it was shown that the addition of readily available form of C and N can rapidly accelerate microbial decomposition of lignocellulose (Knapp et al. 1983.a-b). HTT is already known as simple and an efficient pretreatment technology for solubilization of CW hemicellulose into readily bioavailable form of C (Thomsen et al., 2008), as well as effective in simultaneous conversion of biomass organic N into ammonia (Ren et al., 2006), the form that is most preferred by microorganisms (Jansson, 1958). Various pretreatment technologies are now available to improve the biodegradability of LCR (Taherzadeh and Karimi, 2008; Hendriks and Zeeman, 2009). One of the merits that favor the use of the HTT technology in compost feedstock pretreatment is the elimination of chemical disposal problems (Agbor, 2011), especially in farms.

In order to accelerate the aerobic digestion rate and promote recovery of nutrients on-site, an innovative HTT technology with mild reaction conditions (160 °C < T < 220 °C, 0.6 MPa < P < 2.4 MPa) was selected as a pretreatment for solubilization of date palm hemicellulose before subsequent aerobic digestion. However, because many lignocellulose constituents act as carbon sources as well as air-flow supporting materials in composting (Richard, 1996), pretreatment which results in total solubilization or collapse of CW structure should be avoided. At the same time, losses and excessive decomposition of carbohydrates, as well as formation of microbial inhibitors (Thomsen et al., 2009; Tofighi et al., 2010; Ximenes et al., 2011), should be minimized. Therefore, a careful study of HTT process conditions is required. The objectives of this Chapter are to examine the effect of HTT on the solubilization of CW constituents and to measure the effect of these pretreatments on the biodegradation rate of date palm residue in compost environment.

2.2 Material and Experimental

2.2.1 Material

Date palm (*Phoenix roebelenii*) trunk residues in the form of woodchips with approximately 2 cm in length, was received from a palm plantation farm in Hachijo-Jima Island, which is about 250 km southeast of Tokyo, Japan. Date palm is considered to be a commercial plant (the fronds and leaves are sold for indoor decoration) and its plantation at present is a major agricultural activity in this remote island. However, after passing its economical age, the trunks (~ 1500 t/y) are left behind as a residue due to replanting. Date palm residue contains significant amounts of organic macro and micro nutrients, which provide added chemicals to its fertilizer values. Considering the

`uneconomical` price of chemical fertilizers nowadays, remoteness of the island associated with high cost of shipping, accelerated aerobic conversion of these residues into high-quality organic fertilizer, hence increasing the availability of renewable sources of nutrients on-the-spot, is the most viable treatment technology. The nutrient content along with other properties of the residue is provided in Table 2.1.

TABLE 2.1. Properties of date palm trunk residues

Properties	**Unit**	**Date Palm Wood**
Moisture Content	wt.%, a.r.	68.00
Volatile Solids	wt.%, d.b.	97.00
Bulk Density	kg/m^3, a.r.	354.20
	Elemental Analyses	
Total-C	wt.%, d.b.	43.40
Total-N	wt.%, d.b.	0.38
NH^+_4-N	wt.%, d.b	0.01
NO^-_3-N	wt.%, d.b	0.01
P	wt.%, d.b.	0.10
K	wt.%, d.b.	0.90
S	wt.%, d.b.	0.10

Note: wt.% - weight %, d.b.- dry base, a.r.- received base.

2.2.2 HTT Experiment

In this Chapter, HTT was conducted in a 0.5 liter batch-type reactor of commercially available autoclave (MMJ-500, Japan) equipped with an automated stirrer, a pressure sensor and a temperature controller. The schematic diagram of the experimental set-up is shown in Fig. 2.1. The untreated date palm woodchip samples were introduced into the reactor without any pretreatment, except sieving to obtain natural CW vessels of 2-4 mm in length. The amount of sample and water mixture loaded into the reactor was about 60 g, corresponding to 1:3 mixing ratio. The reactor was then heated by the heating jacket to the target temperature (160, 180, 200 and 220 °C) at an average heating rate of 7.2 °C/min and a constant stirring speed of 200 rpm. In order to inhibit combustion during heating, the air

inside the reactor was evacuated with a stream of argon gas. The initial pressure inside the reactor was set to near atmospheric. After reaching the pre-set temperature, the mixture was further kept in the reactor for 30 minutes (Fig.2.2). Once the holding time was complete, the reactor was immediately decompressed by flashing the steam through the condenser and the wet residue was taken out promptly. The residue was then dried at the room temperature and preserved for the next experimental procedures.

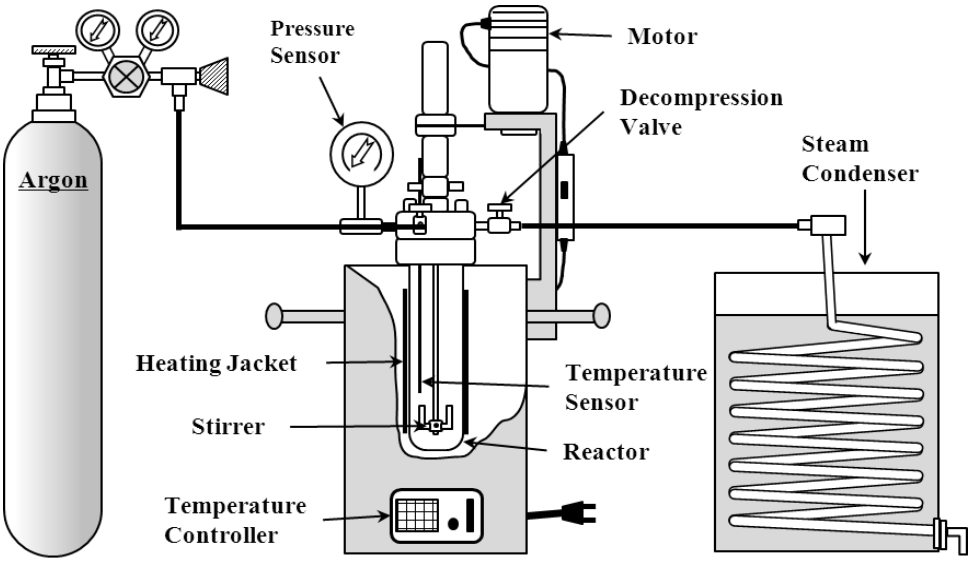

Fig. 2.1. Schematic diagram of the small-scale HTT reactor

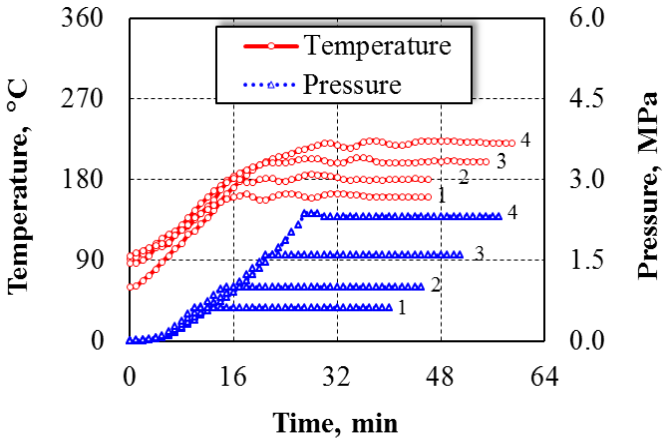

Fig. 2.2. Temperature and pressure profile for HTT experiments (1-160 °C; 2-180 °C; 3-200 °C; 4-220 °C. Pressure profiles are drawn according to the steam table)

2.2.3 Proximate Analyses of CW Constituents

The fraction of CW constituents in the untreated and the residues from HTT were determined according to the procedures described by Allen (1989). The flow diagram of these procedures is demonstrated in Fig. 2.3. Initially, raw and treated CW samples (milled to 0.3 mm) were subjected to hot water soluble extraction, carried out in a 1 liter boiling flask under reflux (100 °C, 2 h). After filtering the mixture through a Whatman 44 filter paper, the solid residues were air dried, and the weight lost (corrected for moisture content) after the extraction was recorded as (hot) water-soluble content in the samples. Sub-sample of each extract in this stage was also analyzed shortly for the total soluble carbohydrates (as xylose and glucose) content by color development method (anthrone reaction). Further, extractive-free residues were treated with a solution of sodium chlorite and acetic acid to obtain the hollocellulose (hemicellulose + cellulose). The available hollocellulose samples were then hydrolyzed with 24% KOH solution, and the resulting residues after filtration (Fine 2G-3) and oven drying (30 min, 105 °C) were weighed as the cellulose content in the samples. Subsequently, the differences between hollocelluloses and celluloses were taken as the hemicellulose fraction in the samples. The lignin content was attributed to the portion of extractive-free samples, which were resistant to strong acid hydrolysis. The hydrolysis was carried out with 72% sulfuric acid for 2 h at 20 °C, followed by reduction of acid strength to 3% (by adding pure water) and gentle boiling for 4 h under reflux. The resulting mixtures were then filtered through a pre-weighed crucible (Fine 2G-3), dried for 2 h at 105 °C and weighed as the amount of lignin. To ensure reproducibility of results, all experiments were conducted in duplicates and run in parallel.

2.2.4 HPLC Analyses of Condensed Steams

A HPLC system (LC-10Avp., Shimadzu) with PDA detector was used to qualitatively analyze the content of organic acids in condensates. Initially, the trapped flash steams generated during each HTT processes were filtered to pass through a 0.22μm membrane syringe filter. Then, samples were transferred to the volumetric flasks and pure water was added to bring them to the same volume. Analyses were carried out on sub-samples using an ion-exchange column (300x7.8 mm, Aminex, HPX-87H, Bio-Rad, USA). The column temperature, the flow rate and the concentration of H_2SO_4 mobile phase were 60 °C, 0.7 ml/min and 0.014 N, respectively. The HPLC-grade formic acid, levulinic acid, 5-HMF and furfural were purchased (WAKO, Japan) and used as a standard for identification of the peaks.

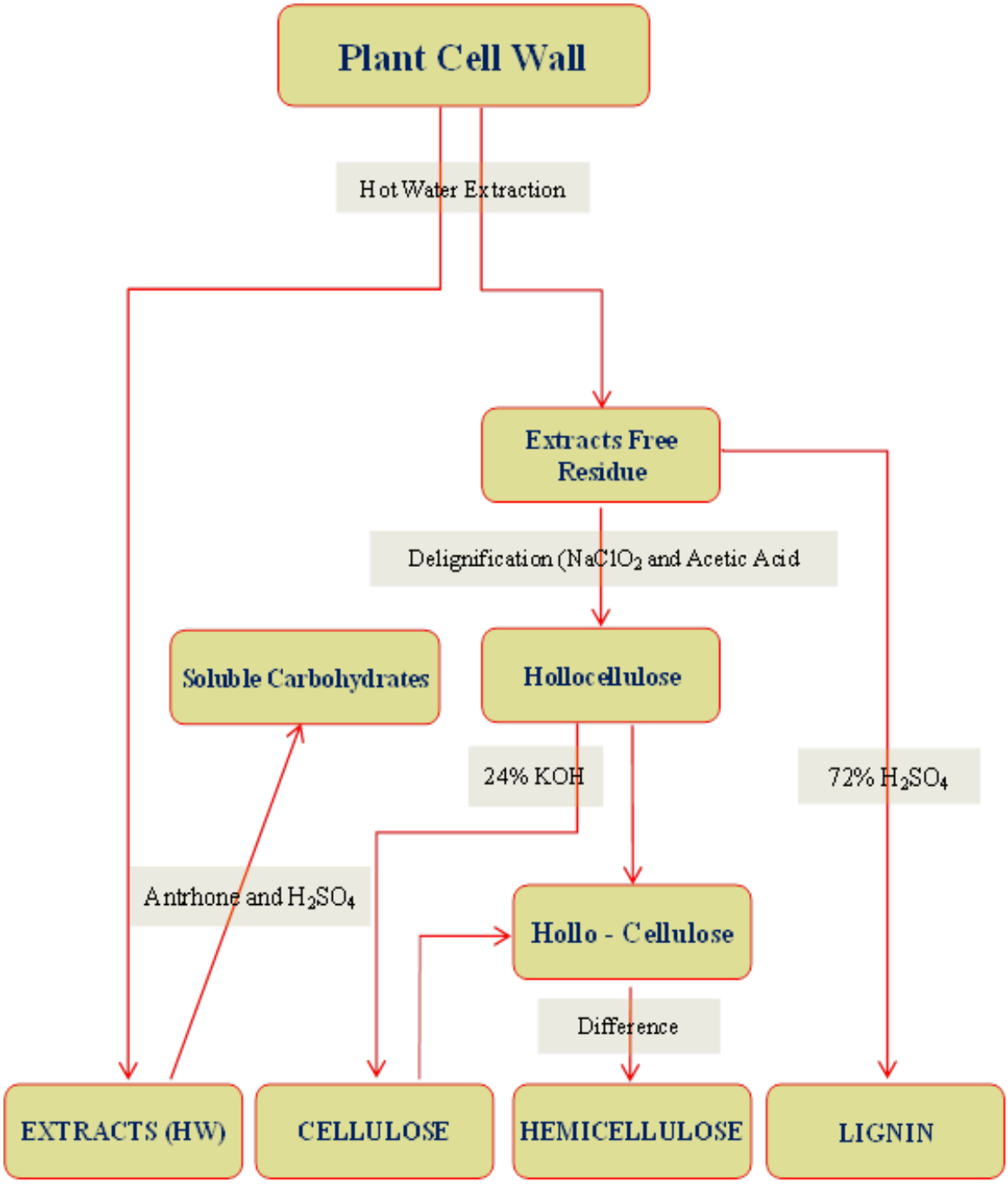

Fig. 2.3. Flow-diagram of CW constituents analyses in the untreated and the residues from HTT of date palm trunk woodchips

2.2.5 Aerobic Degradation Test

Aerobic degradation tests were carried out in 250 ml perforated polystyrene vessels in which pre-weighed amounts (12 g d.b.) of stable commercial compost with test material in the core were sandwiched (packed) between two porous layers of 5 g perlite (wetted with 15 mg pure water). Prior to use, compost was sieved (passed through 2 mm and retained on 1 mm mesh screen) and pre-incubated (38 °C) for 3 days with frequent mixing. This was done to guarantee favorable CO_2 emission from the compost medium, since limited CO_2 production was expected from the test materials. All test samples (1.1 g d.b.) were introduced into compost medium with uniform shape – puck, hence, uniform contact surface area with compost medium. Because the initial C/N ratios of all the test samples were higher than 30 (Table 2.2), nitrogen (as NH_4NO_3) was incorporated in the water used to optimize the moisture content of the samples (65%). Similar to the work of Chiellini et al. (2003), the perlite was used to ensure satisfactory aeration for compost medium, as well as to prevent possible over-drying during incubation. The cups were then put in 2 liter biometer jars (Fig.2.4) and incubated at 38 °C.

For trapping the CO_2 evolved from the vessels, each jar was mounted with a vial containing 15-20 ml of 1N NaOH solution. The trap was substituted at prefixed time (indicated by the markers in the biodegradation graphs Fig.2.5, 2.8.a-c) and back titrated with 1N HCl to a phenolphthalein endpoint, after adding excess $BaCl_2$. All measurements were done in three replications, including blank (compost medium only), control (untreated material) and cellulose powder (~20 μm, Aldrich, Germany) as positive reference sample materials. Aerobic digestion rate, i.e. mineralization rate, of each test material, was calculated according to expression (2.1) as follows:

$$\text{Mineralization rate}, \% = \frac{\text{mg CO}_{2\ \text{Sample}} - \text{mg CO}_{2\ \text{Blank}}}{\text{TheCO}_2} * 100\ \% \qquad (2.1)$$

where $CO_{2\ Sample}$ is the cumulative amount of CO_2 evolved in each vessel containing the test material; $CO_{2\ Blank}$ is the mean cumulative amount of CO_2 evolved in the blank vessels; $TheCO_2$ is the theoretical mass of CO_2 in the test sample. Positive/negative priming effects (Shen and Bartha, 1996; Hamer and Marschner, 2005) were not assumed in this experiment.

2.2.6 Validation of the Test System and the Quality of Compost Medium

In the present study, BS EN ISO (2004) criterion was adopted for the assessment of compost inoculum quality as well as the practicability of our test system. According to BS EN ISO (2004), the test is valid if the mineralization rate of reference material (cellulose) after 45 days of

incubation is more than 70% and the difference between the percentages of biodegradation at the end of the test in the replicate vessels is less than 20 %. Furthermore, the mean value of CO_2 produced by blanks after 10 days of incubation is in the range of 50 - 150 mg g^{-1} VS. The results from this experiment are demonstrated in Fig.2.5.

TABLE 2.2. Chemical properties of untreated and residues from HTT of date palm wood before aerobic digestion test

Parameters	Compost Medium	Untreated Sample	Residues from HTT			
			160 °C	180 °C	200 °C	220 °C
pH (water 1:5, v/v)	6.80	4.80	4.70	4.60	4.50	4.50
Ash: 550 °C (%)	35.00	3.00	3.00	4.00	6.00	10.00
Volatile Solids (%)	65.00	97.00	97.00	96.00	94.00	90.00
Total-C*	24.40	43.40	44.20	45.20	47.70	49.80
Total-N*	2.50	0.38	0.36	0.48	0.61	0.63
C/N ratio	9.60	114.2	122.8	71.80	78.20	103.80

* Determined by Perkin-Elmer CHNS/O Elemental Analyzer (four replications)

2.2.7 Microbial Stability Assessment

One of the important quality parameters in biomass conversion into organic fertilizer or compost is microbial stability and/or maturity of the final product. Generally, various physical, chemical and biological methods are available to test the stability/maturity of the compost (Wichuk and McCartney, 2010). However, those cited methods are not feasible for determining the stability of compost in such “cup-scale” experiments, since the amount of material recommended for those tests is relatively large. Therefore, in this study, the test material was regarded as stable if mineralization is ≥ 90 % of its initial biodegradable fraction (BDF), which is the same as the biodegradable fraction of the untreated material. It is assumed that there is no effect on BDF of the material due to the treatment. In this study, the BDF of the untreated material in compost environment is estimated according to the predictive model of Komilis and Ham (2003) which is expressed as follows:

$$BDF = 0.85 - 0.01 * L_i \quad (2.2)$$

with L_i being the initial lignin content (% VS) of lignocellulose solid substrate.

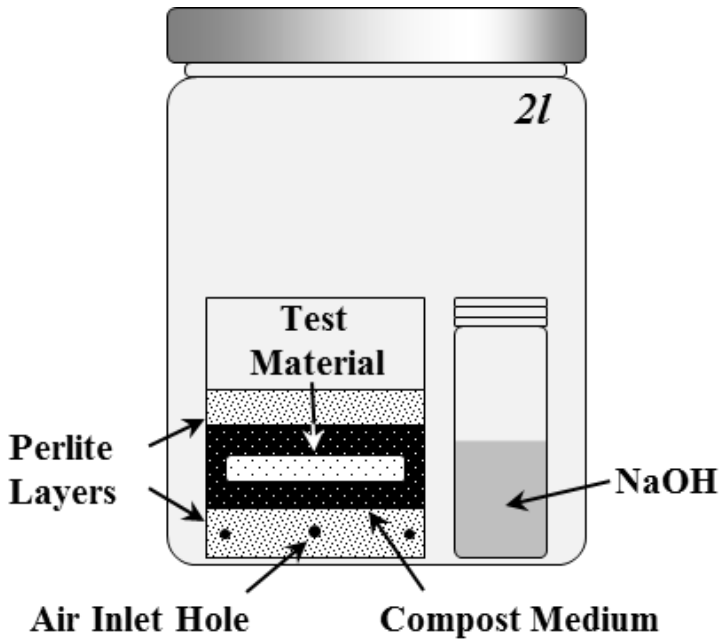

Fig. 2.4. Biometer jar for simulating aerobic digestion of the test materials

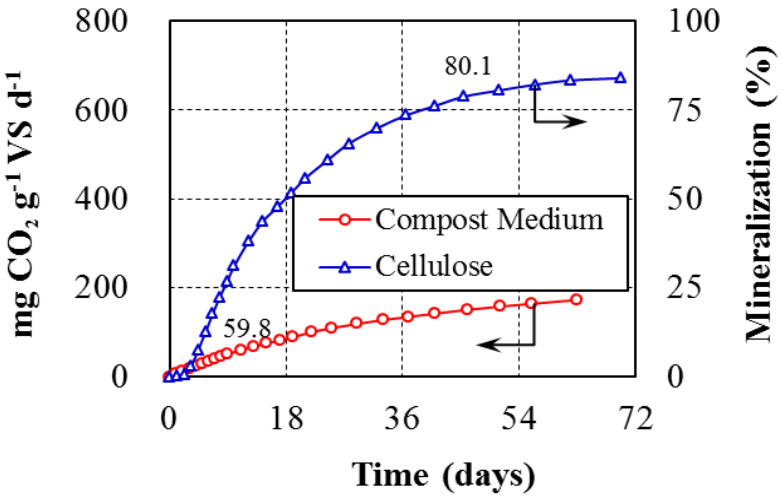

Fig.2.5 Mineralization of cellulose (as a reference material) and the cumulative CO_2 production of compost (blank). The results represent the mean of three replications.

2.3 Results and Discussions

2.3.1 Effect of HTT on CW Solubilization

The profile of CW constituents in the untreated date palm CW as well as in the residues obtained from HTT is shown in Table 2.3. After HTT, approximately, 60-90 wt. % of the original material was recovered as a solid residue enriched with either lignin or cellulose, or both. The HTT at 160 and 180 °C notably solubilized hemicellulose, decreasing the fraction of this CW polymer from 34.1 % in the raw material, to 9.5 and 4.6 % in the residues of 160 and 180 °C, respectively. This was also expressed by an increase of the soluble carbohydrate contents in corresponding water-soluble fractions (Fig. 2.6). However, under 200 and 220 °C reaction temperatures, hemicellulose started to increase gradually in the solid residues: the respective values were 5.8 and 9.4 %. This could have been due to rapid heating. The influence of heating rates (1 and 100 °C/min) on hemicellulose solubilization rate was clearly reported by Murakami et al. (2011). Furthermore, under 200 and 220 °C treatment temperatures, major portion of hemicellulose and cellulose-derived soluble carbohydrates excessively decomposed into various volatile organic compounds (Fig. 2.6), which partly escaped the reactor as a `flash steam` during the subsequent decompression process. According to the HPLC analyses results of condensed steam (Fig. 2.7), major compounds were furfural, 5-HMF, formic acid, levulinic and acetic acids. Consequently, the portion of lignin in the residues from 200 and 220 °C treatments increased: respective values were 31.7 and 39.4%.

TABLE 2.3. Proximate analyses results of the untreated and the residues from HTT of date palm wood

Cell Wall Fractions	Unit, d.b.	Untreated Residue	Residues from HTT			
			160 °C	180 °C	200 °C	220 °C
Other Water-soluble	wt.%	7.70	7.00	8.60	17.30	15.80
Glucose	wt.%	2.30	1.40	1.50	3.70	2.90
Xylose	wt.%	0.50	21.90	21.20	0.80	0.10
Hemicellulose	wt.%	34.10	9.50	4.60	5.80	9.40
Cellulose	wt.%	35.20	35.10	40.50	41.30	32.50
Lignin and Others	wt.%	23.20	27.50	24.90	31.70	39.40
Recovered solid residue	%, a.o.w.	-	91.00	80.30	65.70	57.10

Note: d.b. – dry base, a.o.w. – as original weight

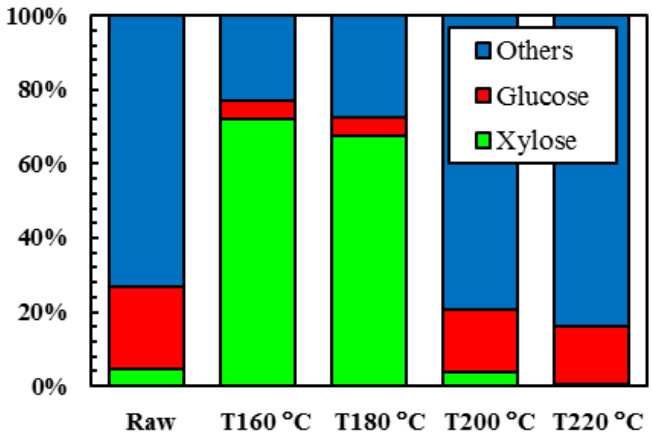

Fig. 2.6. Soluble carbohydrates in the water-soluble fraction of the untreated and the residues from HTT of date palm CW

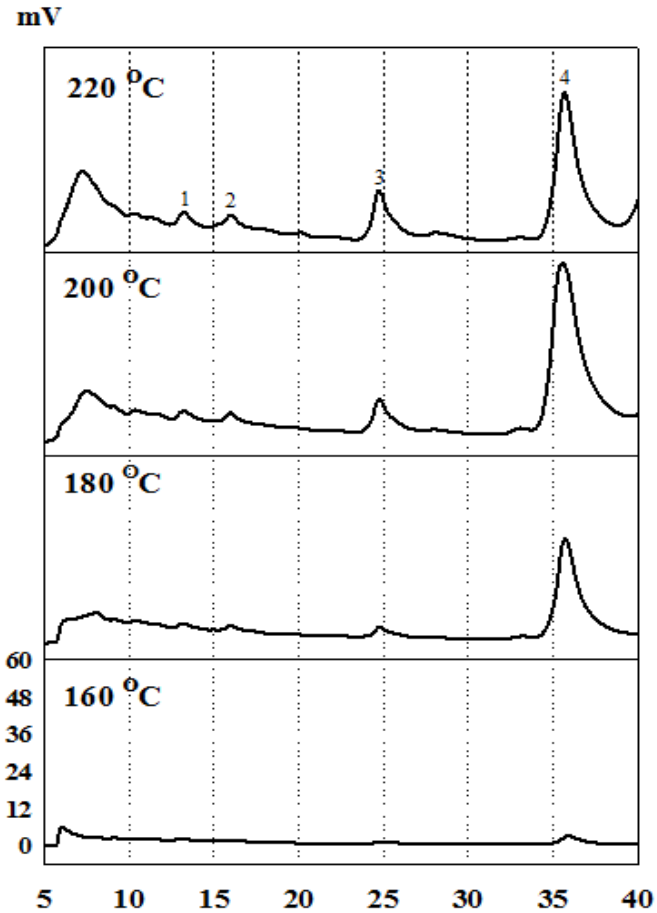

Fig. 2.7. The HPLC spectra of the condensed `flash steam` collected during HTT of date palm CW residue (Peak`s legends: 1-Formic acid, 2-Levulinic acid, 3-5•HMF, 4-Furfural)

However, because lignin and cellulose are important for supporting air flow during the composting process, the extent of the effect of HTT on these CW constituents should also be addressed. Excessive solubilization of these constituents may prove counterproductive to maintaining an aerobic condition during composting process (Richard, 1996). The dramatic decrease in solid residue recovery with the increase in treatment temperature (Table 2.3), suggests that portions of these constituents could have also been affected. In order to facilitate better evaluation, the original proximate analyses results from Table 2.3 were normalized to 100% and are summarized in Table 2.4. As can be seen from Table 2.4, HTT resulted in solubilization of other CW constituents, too. Cellulose solubilized slightly (2-3 wt. %) at 160 and 180 °C, and it is likely that only amorphous regions of cellulose were affected. Solubility of amorphous cellulose even at lower temperature (140 °C) was shown by Inaki et al. (2010) using combined FT-NIR and XRD analyses techniques. The effects of 200 and 220 °C treatment temperatures on cellulose, however, were significant, as reflected by low values of 27.0 and 18.5 % in respective residues relative to 34.2 % in the original material. Moreover, a little amount (~ 3 wt. %) of hydrolyzed portion of cellulose (glucose) was preserved in corresponding water-soluble fractions, pointing to excessive decomposition of cellulose, specifically into Formic acid and 5-HMF (Figure 2.7).

TABLE 2.4. Effect of HTT on date palm cell wall constituents (The original analyses results from Table 2.3 were normalized to 100%).

Cell Wall Fractions	Unit	Untreated Residue	Residues from HTT			
			160 °C	180 °C	200 °C	220 °C
Lost fraction*	wt.%	0.00	9.00	19.70	34.30	42.90
Other Water-soluble	wt.%	7.50	6.20	6.80	11.30	9.00
Glucose	wt.%	2.20	1.20	1.20	2.40	1.70
Xylose	wt.%	0.50	19.50	16.80	0.50	0.06
Hemicellulose	wt.%	33.10	8.40	3.70	3.80	5.40
Cellulose	wt.%	34.20	31.20	32.10	27.00	18.50
Lignin	wt.%	22.50	24.40	19.70	20.70	22.50
SUMMATION	wt.%	100	100	1000	100	100

* Lost fraction = 100 % - Recovered solid residue, %

As for lining, it was hydrolyzed partially (~ 3 wt. %) under the treatment temperature of 180 °C, and apparently some of it was leached out. In contrast, the 200 and 220 °C treatment temperatures rapidly liquefied the lignin, which apparently went into solution with hemicellulose and appeared

to stabilize portion of these polysaccharides against hydrolysis (Mok and Antal, 1992). The fact of retaining lignin in the 200 and 220 °C residues supports the concept that the lignin degradation fragments under such conditions possess a high reactivity and quickly forms insoluble precipitates by interacting among other biomass components (Bobleter, 1994).

2.3.2 Loss of Macronutrients (N,P,K,S) during HTT process

As the main plant macronutrients, nitrogen, phosphorus, potassium and sulfur are important for production of high-value organic fertilizer (Tittarelli et al., 2007). Normally, biomass undergoes many chemical reactions under the HTT condition (Funke and Ziegler, 2010). Subsequently, the nutrients can partly be dissolved into aqueous phase and/or be further hydrolyzed to form various volatile forms (Ren et al., 2006), which can be lost along with steam during depressurization process of the reactor. Therefore, the loss of valuable materials during pretreatments process should be evaluated. The fractions of macronutrients nutrients (in % of Total initial value), i.e. N, P, K, S, found in solid residues as well as in `flashed` steams, trapped after each HTT process, are presented in Table 2.5.

TABLE 2.5. Losses of Macronutrients during the HTT

Residue	Total N (% of initial weight)		Total P (% of initial weight)		Total K (% of initial weight)		Total S (% of initial weight)	
	in solid	in flashed steam	in solid	in flashed steam	in solid	in flashed steam	in solid	in flashed steam
Raw	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00
160°C	94.70	5.30	99.95	0.05	99.93	0.07	99.59	0.41
180°C	94.30	5.70	99.98	0.02	99.95	0.05	99.08	0.92
200°C	79.60	20.40	99.75	0.25	99.92	0.08	99.08	1.92
220°C	50.70	49.30	99.71	0.29	99.95	0.05	95.94	4.06

As can be seen, the loss of P, K and S with flashed steam under all hydrothermal treatment reactions was not significant. In general, the mineral acids of P and S are known to be very reactive with lignin to form precipitates (Ibrahim et al., 2004). Therefore, it is likely that P and S went into reaction with hydrolysis lignin and remained in the solid fractions. However, the loss of N became apparent when it comes to 200 and 220°C, which may be associated with the higher pressure inside the reactor, since the higher pressure can promote not only `flash out` of steam but solid particles as well. The losses of N with the steam were 20.4 and 49.3% (of total added N) for 200 and 220°C

reaction temperatures, respectively.

2.3.3 Distribution of N in solid residues

Examining the form or distribution of nitrogen in compost feedstock is important. A high NO^-_3-N content in feedstock can be subjected to loss due to leaching (Alva et al., 1999). Although the NH^+_4-N is the most preferred form of nitrogen for most microorganisms (Jansson, 1958) its high content may cause rapid volatilization into atmosphere (Martins Dewes, 1992.). The presence of nitrogen in native lignocellulose is mainly associated with cell wall proteins (Alberts et al., 2002), which can be hydrolyzed into NH^+_4-N and CO_2 under the HTT condition. Fig. 2.8 shows the nitrogen distribution in date palm trunk residues as a function of the HTT reaction temperature. The NH^+_4-N in the untreated residue initially, were about 3.56% and this value increased to 7.59% with the HTT reaction temperature of 160 °C. This could mean that hydrolysis of organic nitrogen (proteins) was promoted at 160 °C. However, as the temperature increased to 180 and 200 °C, the amount of NH^+_4-N found in the residues dropped to 5.62 and 3.28%, respectively. According to Forostyan and Kovalchuk (1971), the lignin compounds under the high temperature can react with ammonia and form two forms of `aminated` lignin or aminophenol with 15-20% water-soluble and 80-85% water-insoluble. In this study, the start of hydrolysis of lignin was obvious from 180°C (Table 2.4), and therefore this study supports this point of view. The increase of NH^+_4-N in the residue from 220 °C reaction temperature was probably, due to partial degradation of ligneous compounds and subsequent release of ammonia. The low nitrate contents were detected in the residues because the oxygen in the reactor was previously replaced by argon gas.

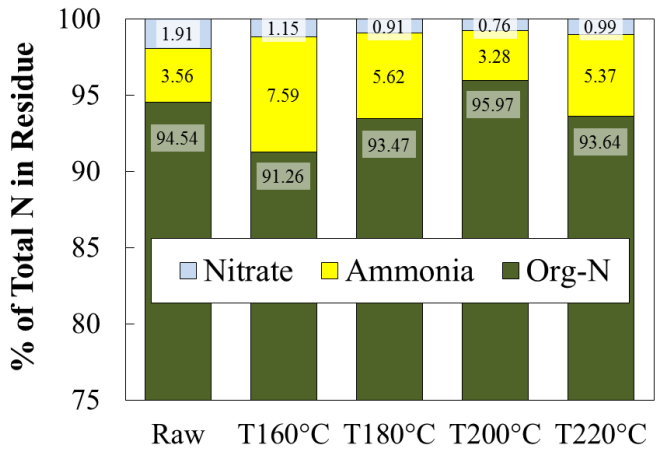

Fig.2.8. Distribution of N in solid fraction of the untreated and residues from HTT

2.3.4 Aerobic Biodegradability

In order to examine the effect of HTT on the aerobic degradation rate of date palm residue, a mesophilic (38°C) biodegradation test under controlled composting conditions was run. After compiling all the measurement data and performing the side calculations, the graph demonstrating the CO_2 evolution rate and biodegradation behavior (as mineralization of C) for each test material, was plotted (Fig. 2.9 a-e). By comparing the biodegradation rate, the major attention was drawn to three phases of biodegradation curve, which are lag, biodegradation and plateau phases (BS EN ISO, 2004).

2.3.5 Untreated CW Residue

The aerobic biodegradation behavior of the untreated CW residue is shown in Fig. 2.8a. As expected, slow biodegradation pattern was observed. The biodegradation curve obviously appeared with 1-2 days lag time and two-stage degradation phase - the fast and slow rate, and the plateau phase was not observed. The first fast stage apparently was stimulated by the decomposition of water-soluble fraction in the untreated CW (Fig. 2.6). This can also be induced from its CO_2 evolution rate profile, which peaks (137 mg CO_2 g^{-1} VS d^{-1}) on the second day of incubation. The second slow stage of degradation phase conceptually, could be assumed as the plateau phase but still the stability threshold line (≥ 90% mineralization of its BDF) was not reached throughout the incubation period, pointing to richness of substrate in the biodegradable fraction. The relatively high CO_2 release during this phase (3.6 mg CO_2 g^{-1} VS d^{-1}) could support this view. If the test was assumed to have continued under the same condition (assuming the same degradation rate) the stability threshold could be achieved but not earlier than 75 days. Michel et al. (2004) investigated the composting process of dairy manure amended with sawdust and straw in farm-scale composting facility and reported that both types of compost reached stability after 100 days.

2.3.6 Effect of HTT

The HTT resulted in an overall increase in the biodegradation of the CW residues, with specific reaction temperatures of 160 and 180 °C. The reaction temperature of 180 °C was the most effective pretreatment temperature for subsequent aerobic degradation by solubilizing the largest portion of hemicellulose (Fig.2.9c). Release of hemicellulose sugars promoted rapid increase in microbial biomass, as expressed by the highest rate of CO_2 evolution (226 mg CO_2 g^{-1} VS d^{-1}) in the early stage of decomposition (Reinertsen et al., 1984). According to the work of Miron and Ben-Ghedalia

(1992.a-b), dissolution of hemicellulose creates cavities and pores within CW, thus microbial accessibility to cellulose is also increased. As a result, biodegradation of the residue started sharply without an apparent lag time, and the plateau corresponding to 90% mineralization of its initial BDF (stability threshold line) was reached shortly within 21 days of incubation, indicating that composting is complete. The low level of CO_2 release during the plateau phase (1.1 mg CO_2 g^{-1} VS d^{-1}) also indicates the microbial stability of this test material. This is an apparent support to the view that biodegradation of organic residue can be speeded up by the addition of the readily bioavailable form of C, if the N is also immediately available (Knapp et al., 1984.a-b; Libmond and Savoie, 1993). When it is compared with the biodegradation of the untreated CW, notable acceleration can be observed: stability in 21 days, compared to 63 days of prolonged degradation. In fact, this enhanced biodegradability can also be linked to the effect of 180 °C reaction temperature on lignin and cellulose. It is assumed that solubilization of amorphous cellulose releases a new terminal end in microfibrils, which are necessary for microbial and enzymatic attack of cellulose (Kluczek-Turpeinen, 2007). Partial hydrolysis of lignin, as shown by Zhang et al. (2008), enhances microbial enzyme activities important for the degradation of lignin.

A similar biodegradation pattern was observed for the residue from the treatment temperature of 160 °C (Fig.2.9b). The high content of hemicellulose-derived sugars in the water-soluble fraction resulted in sharp rise in microbial activity (220 mg CO_2 g^{-1} VS d^{-1}), which in turn led to rapid mineralization of the residue. Biodegradation of this residue started with a short lag time and ended with the plateau phase in which no significant CO_2 emission (1.6 mg g^{-1} VS d^{-1}) was recorded afterward. However, a stability threshold was reached only after 46 days of incubation. This was probably due to the retained portion of hemicellulose (8.4 wt. %) within the CW structure (Table 2.4). It might also be attributed to the wrapping effect of lignin, which was not affected at 160 °C. The fact that the difference of soluble carbohydrate contents in water-soluble fractions of 160 and 180 °C residues (Fig. 2.6) was not proportionally expressed in CO_2 emission peaks (220 and 225 mg CO_2 g^{-1} VS d^{-1}, respectively), implies that the part of soluble non-carbohydrates products in 180 °C treated CW residue were bioavailable, contributing to its CO_2 yield.

On the contrary, the effect of 200 and 220 °C treatment temperatures on the biodegradability of cell wall residues appeared counterproductive (Fig. 2.9d-e). In the biodegradation curves of CW treated at 200 and 220 °C, there was a lag time of 1-2 days before degradation had started, and since the residues have been enriched with ligneous products, fairly low mineralization levels were achieved: 73.8 and 59.8 %, respectively. The influence of lignin content on biodegradation and mineralization rate of other types of LCR was also observed previously by Vikman et al. (2002) and Komilis

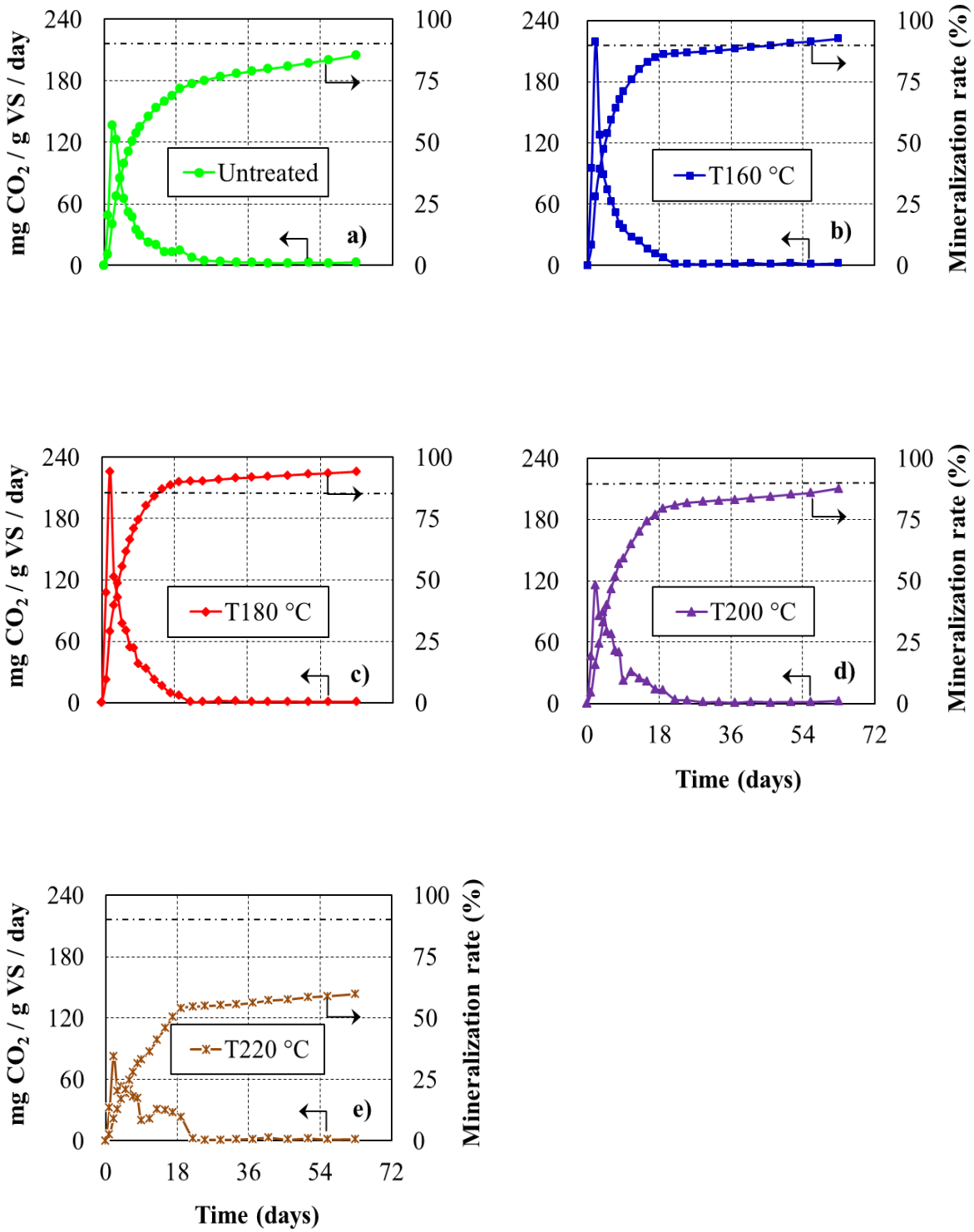

Fig. 2.9a-e. Biodegradation behavior of the untreated and the residues from HTT of date palm cell wall during 63 days of incubation (a -untreated CW; treated at b-160; c-180; d -200 and e-220 °C reaction temperature. All results represent the mean of three replications. Mineralization is expressed as percentage of biodegradable fraction of the untreated CW. The broken line represents the threshold stability line, which is set to 90 % mineralization).

and Ham (2003). In addition, only small part of water-soluble fractions in the residues was readily available for microbial degradation, as expressed by relatively low CO_2 emission peaks (116 and 82 mg CO_2 g^{-1} VS d^{-1}, respectively) on the second day of incubation. The appearance of turbulent-like CO_2 production curves, especially during the first 10 days, might reflect the toxicity or inhibitory effect of lignin degradation products, which are known as the strong deactivators for cellulotic enzymes (Ximenes et al., 2011). These results suggest that the use of 200 or 220 °C reaction temperatures in HTT of date palm cell wall residues for composting will be inefficient. Such high reaction temperature could be effective for the production of soil conditioner – biochar, especially, when longer holding time (7-12 hours) is employed (Rillig et al., 2010).

2.4 Conclusions

In this study, the effects of HTT on date palm cell wall solubilization and subsequent aerobic biodegradation rate were examined. The treatment temperature of 180 °C (with 30 min holding time) was the most effective pretreatment temperature for subsequent aerobic degradation by solubilizing the largest portion of hemicellulose polysaccharides within the CW structure, which had two consequences: 1) it supplied additional readily bioavailable form of carbon, which in turn promoted rapid microbial activities in the early stage of decomposition; and 2) it created cavities and pores within the CW, which permitted rapid bacterial penetration and CW degradation. As a consequence, biodegradation of the residue treated under this reaction temperature proceeded rapidly and the stability was reached within 21 days, compared to 63 days of continued degradation for the untreated CW. This enhanced degradability was also partially linked to the effect of 180 °C treatment temperature on solubilization of amorphous cellulose and partial disruption of lignin. To sum up, HTT can successfully be used as a pretreatment step to accelerate the aerobic digestion rate of date palm residues for the production of organic fertilizers. However, stability assessments based on CO_2 production does not always reflect stability/maturity of the compost or low phytotoxicity (Wu et al., 2000). Maturity and quality assessment based on 2 or more parameters is necessary. Therefore, pilot-scale HTT followed by bin-scale composting is identified as the future work of this study.

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Chapter 3

3. Effect of HTT on Ammonia Volatilization Reduction During Bench-scale Composting of Date Palm Lignocellulosic Residues

Abstract

Aerobic composting can be associated with high rate of ammonia volatilization (AV) into atmosphere, which not only causes environmental pollution but also the loss of essential nutrient as well. Various practices have been found to affect AV during composting, including optimizing composting parameters (e.g. C/N ratio, particle size, process temperature, moisture, pH, aeration regime) as well as addition of various mineral adsorbents and acidifying agents to the compost mixture. In this study, a new strategy was tested. Date palm woodchips, a residue of palm tree plantation, were initially subjected to hydrothermal treatment (HTT) at a mild reaction condition (180°C, 1.0 MPa, 30 min) and the effect of the treatment on the AV rate reduction during 45 days of bench-scale composting experiment was examined. The AV rate was evaluated by direct measurement of volatilized ammonia through absorption in acid trap. HTT was very effective for reducing the AV rate during composting by solubilizing the major fraction of hemicellulose polysaccharides within the lignocellulose cell wall structure, which had two concomitant consequences: 1) it added simple sugars, which supported immobilization to suppress AV in earlier stage of composting; and 2) it improved susceptibility of cellulose particles to microbial attack, which supported immobilization to suppress AV in later stages of composting. Based on the results, pretreating substrate with HTT can effectively reduce the AV rate and enhance agronomic quality of final compost product.

3.1 Introduction

Aerobic composting is considered as one of the most suitable techniques for recovery and recycling of plant nutrients in agricultural residues. However, agricultural residues contain considerable amount of lignocellulose and wrapping of cellulose with hemicellulose-lignin association makes lignocellulose very resistant to biodegradation (Jeffries 1994; Malherbe & Cloete 2002; Palonen 2004). Since most organic nitrogen compounds in agricultural residues are easily hydrolysable compared to organic carbon (Bacharach 1957; Beline *et al.* 1998), mineralization of nitrogen often goes beyond the needs of the microorganisms (Boucher *et al.* 1999). When more nitrogen is available than necessary for organisms to use carbon, large quantities of ammonia and other volatile forms of nitrogen are given off and lost (WCU, 2012). It is well established that ammonia volatilization (AV) is the major pathway for nitrogen loss during composting. Substantial losses of ammonia, sometimes as high as 40 to 80% of the initial nitrogen, have been reported previously in the literature (Kirchmann & Witter 1989; Martins & Dewes 1992; Kithome *et al.* 1999; Lee *et al.* 2009). Such massive AV not only creates onsite odor and environmental pollution problem but can also significantly reduce the agronomic value of final compost product.

The initial C/N ratio (Morisaki *et al.* 1989), the process temperature (Pagans *et al.* 2006; Eklind *et al.* 2007), pH (Nakasaki *et al.* 1993), aeration (de Guardia *et al.* 2008) and the moisture regime (Bueno *et al.* 2008; Jiang *et al.* 2011) have been found to influence the degree of AV from compost. Although, optimizing these parameters under the practical composting conditions is not always easy, the loss remains significant (24-60%). Notable reductions of ammonia volatilization and subsequent retention of nitrogen in final compost product could have been achieved after addition of various mineral absorbents and acidifying salts to the compost (Bernal *et al.* 1993; Termeer & Warman 1993; Mahimairaja *et al.* 1994; Prochnow *et al.* 1995; Kithome *et al.* 1999; Boucher *et al.* 1999; Jeong & Kim 2001; Zhang & Lau 2007; Alipour & Torkashvand 2009; Li *et al.* 2011). Unfortunately, mineral absorbents are not widely available and application of acidifiers requires special precaution to avoid overdosing, which may conversely slow down the activities of the microorganism and/or cause salinity problem (Zhang & Lau 2007; Lee *et al.* 2009).

A simple solution that could be used to prevent AV effectively is to add the simpler sugars, e.g., the forms of carbon that is readily available for rapid microbial immobilization of nitrogen and subsequent suppression of AV. Notable reduction in AV was shown when the fresh hog manure was supplemented with glucose sugar (Subair *et al.* 1995). Molasses, rich normally in sucrose sugar, was applied to compost mixture and AV was remarkably suppressed, which was caused by immobilization (Lian *et al.* 2006). It is said that when nitrogen is incorporated into microbial

cellular substances, it became relatively stable (Marumoto *et al.* 1977; Bengtson & Bengtson 2005; Shindo & Nishio 2005; Nishida *et al.* 2008). However, in order to reduce the AV effectively, a large amount of sugars (> 11% of DM) must be added and recently, application of the sucrose-rich residues is becoming unreliable due to its growing market as a bio-ethanol production feedstock.

In Chapter 2, HTT with a mild reaction temperature (180 °C and 1.0 MPa) was found very effective in enhancing aerobic composting of lignocellulosic residues and that this improved biodegradability was mainly attributed to solubilization of major portion of hemicellulose polysaccharides into simpler sugars (Nakhshiniev *et al., 2012)*. Hence, it may also be possible to reduce AV effectively from compost through HTT of feedstock. Therefore, the objective of this Chapter was to examine the effectiveness of HTT on reducing the AV rate during the bench-scale aerobic composting of lignocellulosic agricultural residue.

3.2 Materials and Methods

3.2.1 Material and Hydrothermal Treatment

The lignocellulose material and the HTT facility used in this chapter are the same with those in Chapter 2. However, the date palm residue received again from the palm plantation in Hachijo-Jima Island was relatively fresher and therefore, it was characterized with higher nitrogen content. After air drying and sieving to attain the natural CW particles with approximately 2 mm in length, the residue was subjected to 30 min HTT under the mild reaction condition of 180°C and 1.0MPa. Properties of the residue before and after HTT are summarized in Table 3.1.

TABLE 3.1. Properties of date palm trunk residue before and after HTT

Parameters	Untreated Residue	Residue after HTT: 180°C
pH (water 1:5, v/v)	4.80	4.60
Ash, % : 550 °C	3.00	4.00
Volatile Solids (OM), %	97.0	96.0
Total-C†, %	46.1	47.8
Total-N†, %	0.74	0.78

†Measured by Perkin-Elmer CHN analyzer (four replications)

3.2.2 Aerobic Composting

Composting experiments were carried out using the similar procedure described in Chapter 2,

except that 5.0g (dry basis) test samples were used. This was done to allow sufficiently detectable production of ammonia during the test period. After adjusting the C/N ratio (25), the samples were introduced into compost medium and were incubated for 45 days. In order to set up our bench-scale experiment as close to the conditions of the practical-scale composting facility, incubation temperature was set as follows: 38°C during day 1; 48°C during day 2; 56°C during days 3 and 4; 48 °C during day 5; and then back to 38°C and kept until the end of the test. For trapping the NH_3 gas volatilized from the vessels, each jar was mounted with a vial containing 20 ml of (20 g/l) boric acid solution (Fig.3.1). In order to monitor the microbial activities (i.e. nitrogen immobilization) a vial containing 20 ml solution of sodium hydroxide (1N) for trapping evolved CO_2 was also placed. The traps were substituted at prefixed times and are indicated by the markers in the biodegradation graphs (Fig.3a-c). All tests were run in three replications, including blank (compost only).

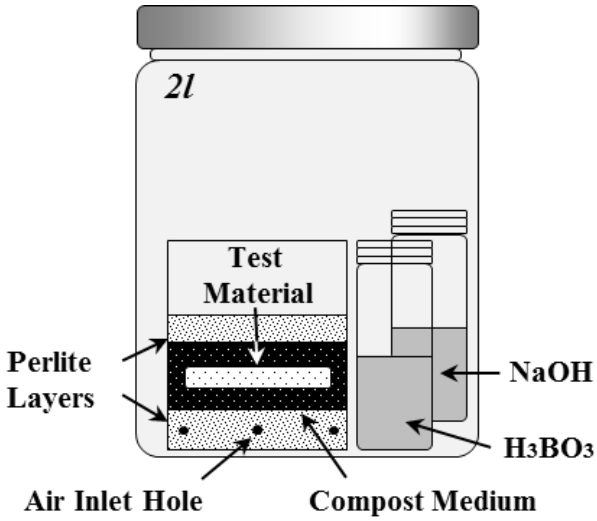

Fig.3.1. Biometer jar for simulating ammonia volatilization during composting process

3.2.3 Analyses

Proximate constituents (simple sugars, hemicellulose, cellulose, and lignin) analyses of date palm residue before and after HTT were determined according to the procedures described in Chapter 2. The results are incorporated into Fig. 3.2. The NH_3 collected in boric acid solution was determined by colorimeter after adding Nessler`s solution (APHA 1992). The net NH_3 volatilization rate from each test materials was calculated according to the equation (1):

$$mg\ NH_3\ (kg\ DM / day) = mg\ NH_{3\ (Sample + Compost)} - mg\ NH_{3\ (Compost)} \qquad (1)$$

The positive values obtained by this equation were considered to represent the amount of NH_3 volatilized from test samples, while the negative values were considered to represent the amount of

NH_3 biofiltrated (from compost medium) by test materials. The CO_2 collected in sodium hydroxide solution was measured by the back titration with 1N HCl to a phenolphthalein endpoint after adding excess $BaCl_2$. The CO_2 production rate from each test material was calculated similar to equation (1). Priming effects (Shen & Bartha 1996; Hamer & Marschner 2005) were not assumed in this experiment.

3.3 Results and Discussions

3.3.1 Solubilization of hemicellulose polysaccharides

The profile of proximate constituents in date palm residue before and after HTT is shown in Fig. 3.2. As shown, HTT notably solubilized hemicellulose polysaccharides. The fraction of this cell wall polymer decreased from 33.21% in the untreated residue to 4.9% in the residue from HTT. This was also expressed by an increase of simple sugars contents (as glucose and xylose equivalent) in treated residue: 21.5% compared to 3.1% in the untreated residue. However, due to the hydrothermal reaction, hemicellulose partially underwent extensive decomposition into various volatile organic compounds, which apparently were lost as a “flash steam” during the subsequent decompression of the reactor.

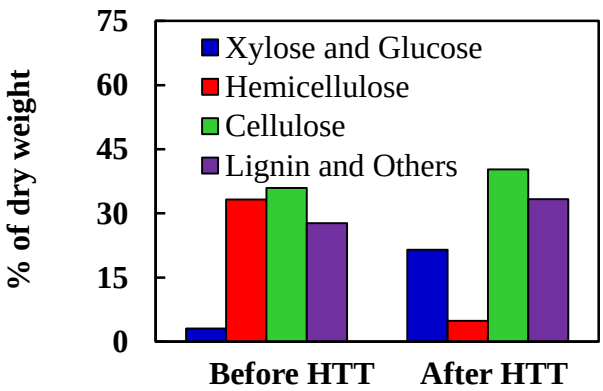

Fig.3.2. Cell wall constituents of date palm residues before and after HTT (180 °C, 1.0 MPa)

3.3.2 Ammonia volatilization

The rates of AV from the compost medium, untreated and the residue from HTT of date palm residues are shown in Fig. 3.3a-b. In order to facilitate the better demonstration of the effect of carbon availability on the AV rate, the plot of the daily CO_2 production (i.e. microbial activities) from each test materials was also incorporated into corresponding figures.

3.3.2.1 Residue without HTT

As expected, AV occurred during composting of the untreated residue. During day 1, the microorganisms utilized the low amount of readily biodegradable carbohydrates to immobilize available mineral nitrogen in substrate and subsequently AV was negligible (Fig. 3.3b). The native date palm cell wall residues may normally contain various readily biodegradable extracts such as simple carbohydrates, proteins and pectin (FAO 1993). The residue used in our test, contained about 3.0% glucose (Fig. 3.2). Therefore, it is likely that the presence of these readily bioavailable carbons facilitate microbial immobilization of nitrogen, which in turn controlled AV at this early stage. This can be observed from the CO_2 production rate, which peaked at 58 g CO_2 kg^{-1} DM day^{-1} on day 1. As these carbon sources were depleted, microbial activities started to reduce during days 2 - 4, leading to the low value of CO_2 production rates (below 16 g CO_2 kg^{-1} DM day^{-1}) on day 4. As a result, more ammonium became accumulated, which along with the high incubation temperature (56°C) favored the increase of its volatilization rate, reaching the maximum value of 14.0 mg NH_3 kg^{-1} DM day^{-1} on day 4. The effect of a high process temperature on the AV rate as well as microbial activity is often reported by various authors (Finstein & Miller 1984; Sundberg *et al.* 2004; Eklind *et al.* 2007). The AV rate then sharply decreased (to about 3 mg NH_3 kg^{-1} DM day^{-1}) during days 5-7, showing again inverse relationship with sharp increase of the microbial activity, which peaked at about 39 g CO_2 kg^{-1} DM day^{-1} on day 7. This was apparently favored by the relatively low process temperature (48°C) as well as the decomposition of organic carbons which were slower than glucose in biodegradation. The fraction of hemicellulose in date palm residue was as high as about 34% of DM (Fig. 3.2). Therefore, this sharp increase in microbial increase could be due to `flash` release of hemicellulose polysaccharides into simple sugars, although the release appeared to be not high enough to completely prevent ammonia loss. After day 7, the AV rate gradually decreased and no more loss was observed from day 20 until the end of the test. Relatively stable CO_2 production rate during this period indicates that the release of some carbon supported microbial activities which in turn controlled AV. Considering the fact that cellulose in native lignocellulosic residues requires a longer incubation period because of the protective effect of hemicellulose-lignin association, as well as its long chain structure of glucose molecules, the release of carbon at this period could be attributed to the decomposition of this cell well polysaccharide. The negative values in the AV rate observed after day 24 is likely to indicate the lack of available inorganic nitrogen for microorganisms to utilize the available carbon in the substrate.

3.3.2.2 Residue with HTT

The hydrothermally treated residue showed the AV rate pattern that in which no loss was observed (Fig.3c). The large amount of hemicellulose sugars (~22% of dry matter) released after HTT of residue (Fig. 3.2), promoted rapid increase of microbial activity which soon caused complete immobilization of ammonia at the early stage of composting. The high CO_2 production rates of 77 and 96 g kg^{-1} DM day^{-1} on day 1 and day 2, respectively, can reflect this activity. Subsequently, AV was suppressed and nearly no ammonia loss was observed during day 1 and day 2. The high initial microbial activity then sharply decreased during days 3-6 to reach a low level of 16 g CO_2 kg^{-1} DM day^{-1} on day 6, which was probably due to the depletion of readily decomposable form of carbons. This could also be due to the effect of relatively high (56°C) process temperature since it is often found to inhibit microbial activities (Eklind *et al.* 2007). Nevertheless, no AV was observed during this period**.** Rather, as expressed by negative values in the AV rate plot, hydrothermally treated residue acted as a bio-filer substrate, absorbing the ammonia gas that was volatilizing from the compost medium. Although, as shown in Fig.3c, the ammonia volatilization favored probably by higher process temperature was observed in the jar containing only the compost medium. This could be explained by the microorganisms being nitrogen-limited in abundant available carbon substrate, since previously immobilized nitrogen is not available in short term (Shindo & Nishio 2005; Nishida *et al.* 2008). After day 6, ammonia loss might have occurred but increase in microbial activity (reaching 45 g CO_2 kg^{-1} DM day^{-1}) observed during days 7-12 caused further AV suppression. It was shown that the solubilization of hemicellulose matrix polysaccharides can create pores and spaces within the lignocellulose cell wall structure, which is necessary for rapid bacterial colonization of cellulose particles and subsequent degradation (Miron & Ben-Ghedalia, 1992). Therefore, the rapid growth during this period might be attributed to the microbial attack on cellulose particles, since significant portion of hemicellulose polysaccharides after HT treatment was solubilized (Fig.3.2). Microbial activities then started to gradually decrease after day 12 and stabilized at a low level of around 6-7 g CO_2 kg^{-1} DM day^{-1} from day 26 to day 45 but it appeared sufficient to prevent any ammonia loss. These results are, in fact, in line with the work of Subair *et al.* (1995) in which combination of glucose and saw dust (i.e. cellulose) was found to be more effective in reducing AV compared to glucose alone. It is suggested that addition of glucose promotes immediate immobilization of nitrogen in the early stage of composting, and after all liable carbon are consumed, the moderately liable carbon (e.g. cellulose) may become available and continues supporting microbial activities in the later stage of composting.

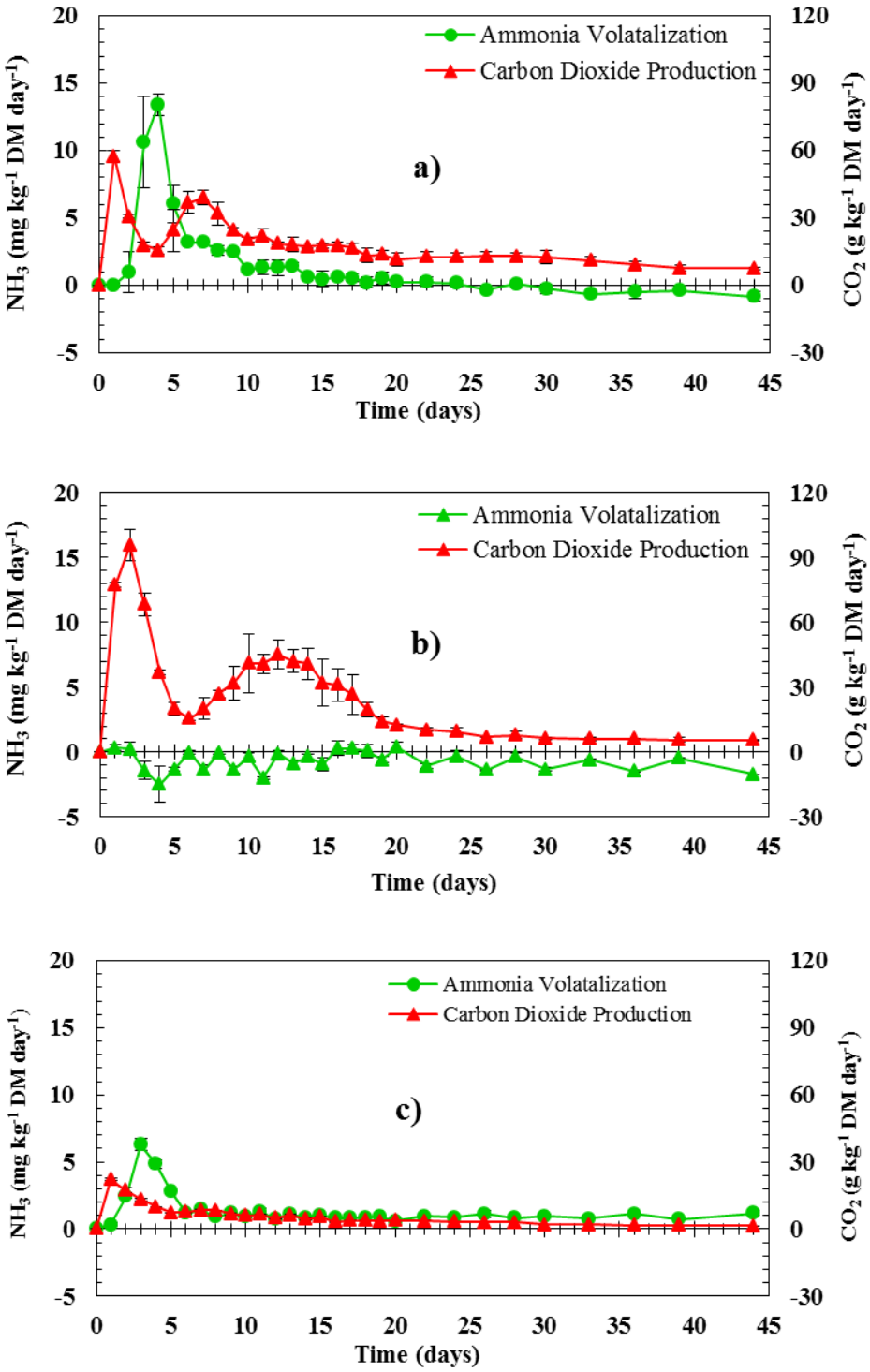

Fig.3.3a-c. Ammonia volatilization and carbon dioxide production rates during aerobic composting of date palm residue: a-untreated residue, b-residue after HTT and c-compost medium (horizontal bars represent standard errors calculated based on three replications)

3.4 Conclusions

In this Chapter, a new strategy for reducing ammonia volatilization from composting of lignocellulosic residue was evaluated. The hydrothermal treatment (HTT), which solubilized hemicellulose polysaccharides within lignocellulose structure into simpler sugars, was very effective in suppressing ammonia volatilization, which had two consequences: 1) it supplied additional simpler sugars, which in turn suppressed ammonia volatilization in the early stage of composting; and 2) it improved bioavailability of cellulose particles which supported microbial activity to suppress ammonia volatilization in longer term. The obtained results suggest that HTT can be effective in reducing ammonia volatilization during aerobic composting of lignocellulosic residues. The effect of HTT was also expressed by high nitrogen content: 3.4% in treated residue compared to 2.3% in untreated residue. However, in this bench-scale study, 56°C was set as the highest composting temperature, while in practical composting scale it may reach 65-70°C, thus higher and different volatilization pattern may occur. Therefore, effect of HTT on AV reduction during a larger-scale composting process should be considered as a future work.

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Chapter 4

4. Evaluation of Stability and Maturity of Compost during Bin-Scale Composting of Rice Straw with and without Hydrothermal Treatment

Abstract

In order to evaluate the hydrothermal treatment (HTT) in enhancing compost stability and maturity of lignocellulose agricultural residues, bin-scale (90L) composting of rice straw following the pilot-scale (200L) HTT was performed. Stability and maturity of rice straw compost product with and without HTT taken at different time intervals (weeks 0, 2, 4, 6, 8, 10, 12 and 14) was evaluated through analyzing different parameter such as pH, the electrical conductivity (EC), the C/N ratio, NH^{+}_{4}-N and NO^{-}_{3}-N contents, the microbial activity (as the CO_2 evolution rate), and finally the seed germination index (GI). The results showed that the rice straw compost product with HTT after 6 weeks of composting was adequate to be considered as stable and mature. Stability and maturity after 6 weeks of composting was expressed by pH of 8.41, EC of 2.96 mS cm^{-1}, the NH^{+}_{4}-N content of 93.75 mg kg^{-1} DM, the C/N ratio of 12.5, the microbial activity of 8.05 mg CO_2 g^{-1} OM d^{-1}, and finally by GI of >83%. As for the rice straw compost product without HTT, the high microbial activity (> 12.28 mg CO_2 g^{-1} OM d^{-1}) even after 14 weeks of composting suggested that the residue had not stabilized yet and was far away from maturation phase, although higher GI (>100%) was observed. In addition, the rice straw compost product with HTT was likely free of pests and pathogens because of `sterilization` during the pretreatment process. Furthermore, it appeared (after 6 weeks) granular, dark gray in color and free of offensive odor. As regards the loss of N, no net loss was observed during composting of rice straw with HTT, while during the composting of rice straw without HTT it could reach 40.3% of the initial value. HTT was also effective in N loss reduction through the denitrification process. Finally, the HTT technology is recommended for enhancing biodegradability of lignocellulosic residue for high-quality organic fertilizer production.

4.1 Introduction

Compost products offer many potential benefits to plants, soil and natural environment. Compost final product can supply nutrients for plant growth (Arkhipchenko et al., 2005), organic amendment for soil health (Abdel-Rahman, 2009) and natural agents for controlling of soil-borne plant diseases and pests (Arkhipchenko et al., 2005; Bonanomi et al., 2007). However, despite many above-mentioned benefits, there can also be undoubtedly negative effects from compost product, especially, when unstable and immature compost is applied to the soil. As demonstrated by Brinton and Evans (2002), application of unstable and immature compost into soil may induce higher microbial activity, leading to increased oxygen consumption and thus severe subsequent oxygen deficiency for plant roots/seeds. In the field study carried out by Fuchs et al. (2011), the incorporation of unstable compost into soil caused nitrogen immobilization rather than supplying it and consequently the growth of plant (maize) was inhibited. Similar effects were exhibited on lettuce and Amaranthus plants when less-aged and immature composts were applied (Akhtar et al., 2010). A greenhouse tomato pot experiment was conducted by Griffin and Hutchinson (2007) to evaluate the effect of 13 composts on plant growth and several of the most immature composts were clearly phytotoxic, significantly reducing tomato seedling growth after adding the composts into soil. It is also reported that unstable compost can conversely enhance disease severity because unstable organic matter may provide the substrate for growth of pathogens (Sugahara and Katoh, 1992) or may release phytotoxic compounds that could damage plant roots and predispose them to pathogens attack (Bonanomi et al., 2007). For these reasons, to be considered beneficial and safe for agricultural application, compost final product must sufficiently be stable and mature.

Nevertheless being important compost quality properties, there are no universally accepted definitions for compost stability or maturity. Although, many different definitions have been proposed by different researchers, a clear distinction between these two properties can still be differentiated. As extensive review of literature (offering uncited definitions for compost stability and maturity) by ADAS Consulting Ltd. (2005) has shown, stability is defined mostly as ***`microbial stability or respiration rate`***, whereas maturity is defined mostly as ***`no adverse effects on plants or no phytotoxicity`***.

A large variety of physical, chemical and biological parameters have been proposed for the determination of compost stability and/or maturity (Mathur et al., 1993; Bernal et al., 2009; Wichuk and McCartney, 2010). Microbial stability evaluated by respirometric measurements based either on the CO_2 evolution rate, O_2 uptake or release of heat has been frequently applied in compost stability determination (Gomez et al., 2006). Of the three techniques proposed, the respiration rate

based on the CO_2 production is considered the most direct and accurate evaluation of compost stability, because its production directly correlates to aerobic respiration of a compost microbial population (ADAS Consulting Ltd., 2005). Furthermore, the approach of measuring CO_2 production is simple and can be undertaken at relatively low cost.

Compost maturity, on the other hand, which implies no toxicity to plants upon immediate application of compost, still is best determined using the plant phytotoxicity tests, such as seeds germination and/or plant growth bioassays (Wang et al., 2004). The germination index, which is a measure of phytotoxicity based on relative seed germination and relative root elongation (Zucconi et al., 1981), is one of the indices that has gained widespread acceptance in compost maturity determination. It is reported that combination of the germination and the stability indices can provide satisfactory evaluation of maturity if C/N ratio of compost product is less than or equal to 25 (CCQC, 2001). In practice, phytotoxicity can also be induced by other factors such as high amounts of free ammonia, excess soluble salts or certain organic acids. Therefore, the change in chemical parameters during the composting process such as pH, the electrical conductivity (EC), the C/N ratio, NH^-_4 formation and the ratio of NH^-_4 to NO^-_3 have been found as the useful indicators in compost maturity determination (Wang et al., 2004; Gomez-Brandon et al., 2008). Integrated use of these parameters and indices has been shown to give valuable information on the dynamics of the compost process, thus better evaluation of compost stability and maturity (Mondini et al., 2003).

Previously (in Chapter 2), we investigated a novel hydrothermal treatment (HTT) technology with mild reaction conditions (160°C < T < 220°C, 0.6 MPa < P < 2.4 MPa) as a pretreatment step to enhance composting process (thus stability and maturity) of lignocellulosic residue for organic fertilizer production. As a result, the reaction temperature of 180°C (30 min, 1.0 MPa) was found to be most effective: biodegradation of the treated residue proceeded rapidly under small-scale controlled composting condition and microbial stability (as CO_2 evolution rate) was reached within 21 days, compared to 63 days of continued degradation for the untreated residue. However, microbial stability or low CO_2 production rate alone does not necessary reflect the stability and maturity of the compost end product (Wu et al., 2000; Itavaara et al., 2002). Therefore, the stability and maturity of compost are best assessed by measuring two or more parameters (CCQC, 2001).

In order to evaluate HTT in enhancing compost stability and maturity of lignocellulose agricultural residues based on an integrated approach, a bin-scale (90L) composting of rice straw following the pilot-scale (200L) HTT was performed. The objective of this work was to study the physical, chemical and biological parameters during the bin-scale composting of lignocellulose rice straw

residue with and without HTT, and evaluate the stability and maturity of the compost products by combining physical, chemical and microbiological parameters and indices. Since, the design of the experimental facility allowed measuring the rate of volatilized ammonia during composting process, the effect of HTT on ammonia and total nitrogen loss reduction was also assessed.

4.2 Experimental

4.2.1 Material and Hydrothermal Treatment

In this study, lignocellulose rice straw residue is used as the main material. It was purchased from the local gardening store. Since, the residue was already cut into the pieces ranging from 2-4 cm no additional cutting was required. In order to obtain sufficient amount of material for subsequent bin-scale composting experiment, a pilot-scale HTT facility with the reactor capacity of 200 L was employed in this study. The schematic diagram as well as the snapshot of the HTT facility is shown in Fig. 4.1 and 4.2, respectively. First, the rice straw (about 6 kg in dry weight) was fed into the reactor, and then, saturated steam supplied from the boiler was injected into the reactor until the pre-set hydrothermal conditions (180°C, 1.0 MPa) were reached. The blades installed inside the reactor then started to mix the residue for about 30 minutes. After the treatment is complete, the reactor was decompressed immediately by flashing the steam through the condenser and the moist (about 68%) treated residue was discharged by rotating the bladders, which also act as a screw conveyer. Four batches were performed. The temperature and pressure profile during one of the HTT batches is shown in Fig. 4.3. The treated products cooled down, mixed and were preserved (10°C) until the next experimental procedure. The chemical properties of the residue with and without HTT are shown in Table 1.

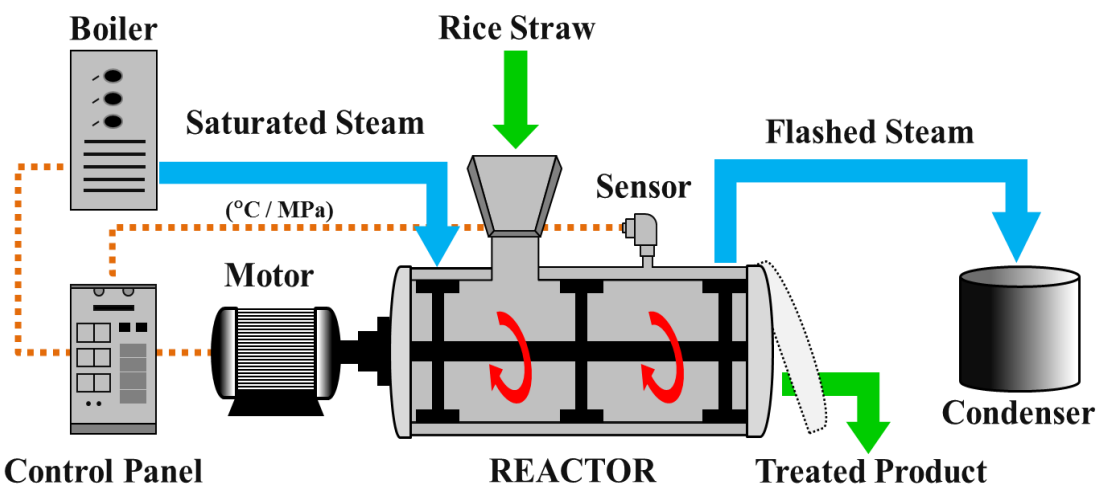


Fig.4.1. Schematic view of the pilot-scale HTT facility

Fig.4.2. Snapshot of the HTT facility

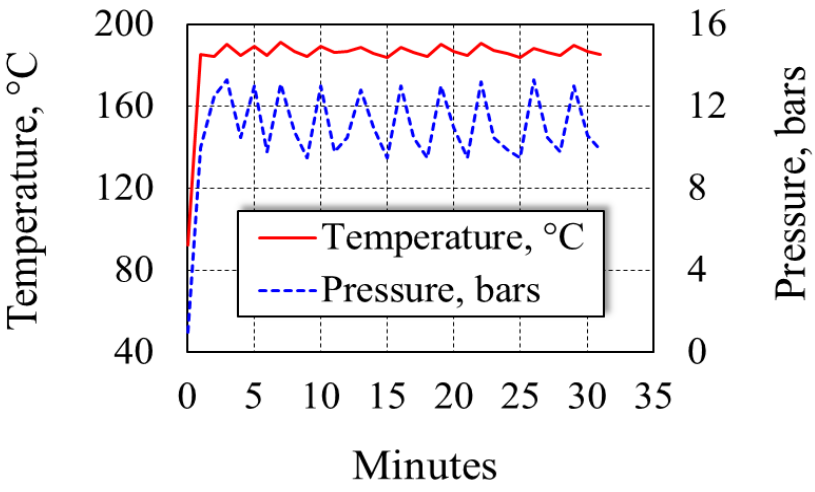


Fig.4.3. Temperature and Pressure profile during one of HTT batches

TABLE 4.1. Chemical properties of the rice straw residue before and after HTT

Parameters	**Before HTT**	**After HTT**
Hemicellulose, %	29.78	7.35
Cellulose, %	36.46	43.45
Lignin, %	13.97	19.91
Ash (550 °C, 2 h), %	9.67	11.29
Moisture Content, %	14.00	68.00
OM (100%-Ash), %	90.33	88.71
pH	6.81	3.86
EC, mS cm^{-1}	2.20	4.75
TC,%	42.49	45.25
TN, %	0.85	0.98
C/N Ratio	49.99	46.17
P†, %	0.17	0.19
K†,%	0.52	0.59
S†, g kg^{-1} DM	1.01	1.17
Mg†, g kg^{-1} DM	2.18	2.45
Ca†, g kg^{-1} DM	3.89	4.46
Na†, g kg^{-1} DM	1.83	2.24
Fe†, g kg^{-1} DM	0.36	0.63
Al†, g kg^{-1} DM	0.18	0.50
Cu‡, g kg^{-1} DM	0.01	0.011
Mn‡, g kg^{-1} DM	0.08	0.11
Mo‡, g kg^{-1} DM	0.01	0.01
Co‡, g kg^{-1} DM	0.01	0.01

† Measure by ICPE after HNO_3 and $HClO_2$ pretreatment

‡ Measured by ICPS-8100 after HCl and HNO3 pretreatment

4.2.2 Substrate Preparation

The rice straw residues, with and without HTT, were then spread on separate blue-plastic sheets and microbial inoculums were applied using a bottle with water spray nozzle. Since, the residue obtained after HTT had already moisture content of 68%, no correction of the moisture content was required. As for the untreated residue, however, it was initially soaked in the water for 36 hours to constitute the moisture content of 68%. Because the initial C/N ratios of both substrates were higher than the range considered to be optimum (25-30) for starting composting process (Diaz and Savage, 2007), necessary amount of nitrogen as urea was added to the suspensions (right before applying) used to inoculate the substrates. All preparation was conducted outdoor when an ambient temperature was below 15 °C, so no special care was taken to prevent the water or nitrogen loss. The amount of substrates was then loaded in each composting reactor, which was about 18 kg (wet basis). While the substrate was being loaded into each reactor, original samples were withdrawn (bottom, middle, top) immediately for subsequent analyses, and the composting process was begun; the composting time was noted as week zero (Week 0).

4.2.3 Microbial Inoculums Preparation

Compost microbial inoculum was prepared by means of shaking a 1:20 (w/v) compost/water mixture for 15 min in a warm water bath (32°C). Compost was commercially produced (Wakayama Organic Productive Union, Japan) and prior shaking, it was pre-incubated at 32°C for 3 days in air-tight collapsible container in order to active the microbes. Produced gas was released and compost was mixed once daily. Because the hydrothermally treated residue had undergone a `sterilization` process, microbial inoculums from untreated rice straw (rice rinse water) was also prepared in similar way to include `native` microbes presented naturally in the untreated residue. The prepared suspensions were then mixed and diluted with 5 part of pure water before it was applied as a compost inoculum.

4.2.4 Composting Setup

Plastic dust bins with the volume of 90L (Fig. 4.4) were modified and used as composting reactors. The vessels were externally insulated with two layers of glass wool and aluminum foil thermal insulators to minimize the convective heat loss. A removable air-tight lid was put on the top of each reactor to facilitate intermittent mixing and sampling of substrate during the course of composting. The lids however were insulated from the inner side (foam rubber) in order to minimize the occurrence of the reflux condition. One thermocouple (K-Type) connected to the data logger (TM-947SD) was fitted at the center of each reactor to record the temperature of the composts with

the interval of 30 minutes. The temperature in the laboratory was maintained at 23±1.5°C. In order to have the air flow uniformly distributed throughout the compost substrate, the material was placed on the stainless punch plate installed at the bottom of the reactors. The air was supplied continuously by a compressor pump and the flow rate was fixed at 0.48 L kg-1 DM min-1 in every reactors (Jiang et al., 2011). The exhaust gas from the reactor passed through a condenser and an acid trap (1N H2SO4) connected in series to trap water condensate and volatilized ammonia, respectively. The snapshot of the composting reactors can be seen in Fig.4.5. The compost was mixed manually at 1- or 2-weeks interval throughout of the composting reaction.

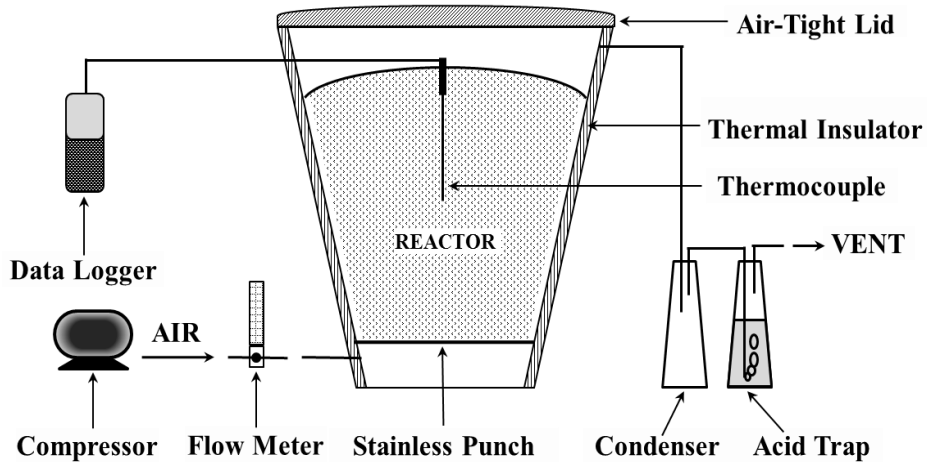


Fig. 4.4. Diagram of the composting reactor

Fig. 4.5. The snapshot of the compost reactors

4.2.5 Sample Collection

Besides the samples taken at the beginning of composting (Week 0), seven more solid samples (about 250 g) were taken from the each reactor throughout the composting process, specifically during the turning at Weeks 2, 4, 6, 8, 10, 12 and 14. Samples were taken from three parts (top, middle, bottom) of the reactor and after homogenization (considering the MC) were divided into two parts; with one part kept fresh at 4 °C, and another part air-dried and grounded to pass through 0.25 mm sieve. The air-dried and ground samples were used to analyze the total carbon (TC), the total nitrogen (TN), ash and organic matter (OM) contents. The fresh samples were used to analyze the dry matter (DM), pH, the electric conductivity (EC), ammonium nitrogen (NH+3-N) and nitrate (NO-3-N). The fresh samples were used as well in microbial stability and phytotoxicity tests.

4.2.6 Analytical Methods

The TC and TN were determined using an automatic high sensitive NC-analyzer (Sumigraph NC-220F, SCAS, Japan). The DM content was assessed by oven-drying at 105 °C for 24 hours and the ash was determined by calcination of 3 g oven-dried sample at 550 °C for 2 hours in a muffle furnace. Subsequently, the difference between DM and ash was considered as OM or volatile solids (VS). The pH and EC were analyzed in a 1:10 (w/v) compost/water (distilled) solution following a 30-min shaking in the water bath (25 °C) shaker. The NH_4-N and NO_3-N were extracted with 0.5 M K_2SO_4 (1:10 w/v) solution and analyzed by steam distillation (Fig.4.6) using MgO-Devarda's alloy followed by back titration of the boric acid distillates using 0.0025M H_2SO_4 solution (Bremner and Keeney, 1965). Organic nitrogen (Org.-N) was then calculated by the difference between TN, NH^+_3-N and NO^-_3-N.

Fig.4.6. Steam distillation setup for measuring NH^+_4-N and NO^-_3-N during the experiment

4.2.7 Microbial Activity Test

Microbial activity stability index was evaluated by the microbial respiration test based on CO_2 evolution rate (mg CO_2 g^{-1} OM d^{-1}), which is similar to the procedure described in CCQC (2001). Approximately 10 g of sample conditioned at 60% moisture content was sealed in a 3.0 L vessel along with a vial containing a known volume of 1M NaOH solution. The vessel was sealed and incubated at 37°C for 4 days. The amount of CO_2 trapped by NaOH was determined daily over a 4-day period by back titration with 1M HCl to phenolphthalein endpoint, after adding excess amount of $BaCl_2$. All measurements were performed in three replications, including a jar without compost as a blank.

Microbial respiration rate (MR) was calculated using the expressions (1), (2) and (3):

$$\text{mg } CO_2 \text{ evolved per day} = [(B - S) \times 44.2] / 2 \quad (1)$$

$$\text{Total mg } CO_2 = \text{Sum of mg } CO_2 \text{ evolved over 4 days} \quad (2)$$

$$\text{mg } CO_2\ g^{-1}\text{OM } d^{-1} = [\text{Total mg } CO_2] / [\text{Dry Weight of Sample} \times VS \times 4] \quad (3)$$

where B and S is the volume of 1M HCl used for titration of NaOH trap from the blank and sample jar, respectively (ml).

4.2.8 Phytotoxicity Test

A phytotoxicity test employing seed germination index (GI) was used to evaluate compost maturity in this study (Zucconi et al., 1981). Distilled water was added to the fresh sample to attain a 1:10 (w/v) compost/water mixture and after shaking 6 hours at 25 °C, the aqueous extracts were obtained by centrifugation (8000 rpm, 20 min) and filtration through a 0.45 μm membrane filter. A Tanepita germination test sheet (FHK, Japan) pasted orderly with 50 seeds of Komatsuna (*Brassica rapa* var. *peruviridis*), was placed inside a UV-sterilized Petri dish and was wetted with 5 ml of compost extract. Komatsuna seeds have been widely used in Japan in germination tests (Hase and Kawamura, 2012). The Petri dishes were then incubated for 4 days at 25°C in the dark. After 4-days incubation, the germination was stopped (by adding 5 ml of ethanol) and germinated seeds were counted and the root length was measured (Fig. 4.7). Three replicates (with total seeds number of 150) were set out for each treatment, including distilled water that was used as a control. The GI was then calculated according to the expression GI(%) = $(A \times C)/(B \times D) \times 100\%$ in which, A and C represent the number of seeds germinated in extract-treated and control dishes (distilled

water), respectively; and B and D represent the average root length of extract-treated and control seeds, respectively (Zucconi et al., 1981).

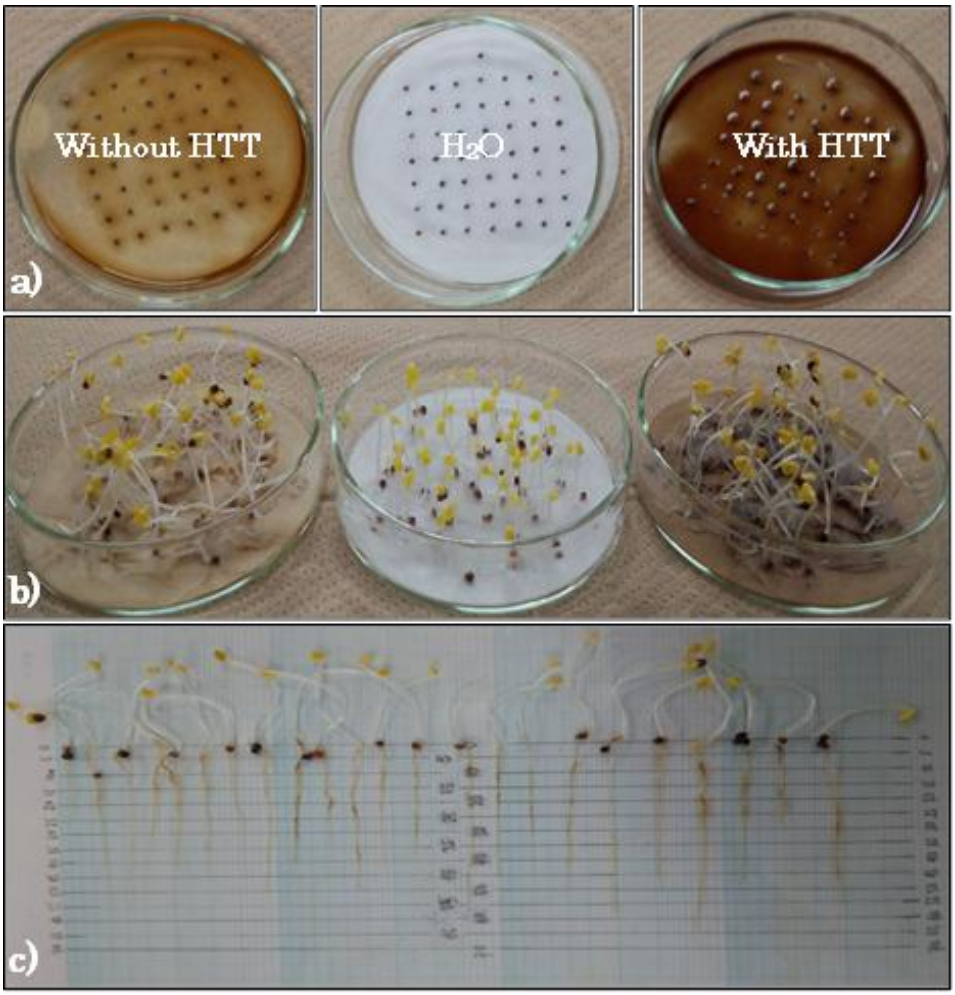


Fig.4.7. Germination test: a) - the Komatsuna seeds are being treated with distilled water and compost extracts; b) - germinated seeds after 4-day incubation; c) - the root length are being measured

4.2.9 Evaluation of N Losses

The losses of (total) N over the composting period were calculated using the ash contents as internal standards. Because ash in the rice straw principally consists of variety of inorganic materials (Table 1), it is generally unaffected by biological action, meaning that it should pass through the composting process unaltered. Thus, as organic matters or N will be lost, the ash content should proportionally increase. The ash content at the beginning ($A_{Week\text{-}0}$) and the ash at any

particular time during composting (A_{Week-t}) were used to estimate the loss of N (%) during the composting process according to the formula N loss (%) = 100 – 100 [(A_{Week-0}/ N_{Week-t}) / (A_{Week-t}/ N_{Week-0})] as proposed by Paredes et al. (2001). The amount of NH_3 loss was attributed to the amount of NH_3 gas absorbed in acid traps. The differences between the total N losses and the losses of NH_3 were then assumed the loss through the denitrification process.

4.3 Results and Discussions

4.3.1 Evolution of Composting Temperature

Monitoring of compost temperature is important for the stability and maturity determination (Gao et al., 2010). Evolution of temperature has been found to strongly reflect metabolic activity of microorganisms during the composting process (Tiquia et al., 1996). The final decrease in temperature to reach an ambient without reheating upon a mixing may indicate the maturation phase (Tang et al., 2007). According to Insam and de Bertoldi (2007), if the temperature of the compost is more than 8°C higher than the ambient air, the compost is still fairly unstable. In addition, compost must experience the temperature exciding 55 °C for at least 3 days in order to fulfill pathogens and weeds elimination requirement (Brinton, 2000). Fig. 4.8 demonstrates the evolutions of the temperature during composting process of the rice straw with and without HTT.

During the composting experiment the room temperature fluctuated between 23 (night) and 25 °C (day), therefore 24 °C was assumed as a mean. The temperature in the compost reactors began to rise soon after establishing the composting conditions as well as after the each turning during the active stages (seen as the sharp drops in temperature curves). The compost temperature of the residue with HTT reached a maximum of 63 °C (on day 9) and remained above 55 °C for more than 15 days. Then, the temperature decreased rapidly and remained between 34 and 37 °C until Week 6. Afterward, the temperature diminished and become close to the room temperature, and thus composting entered a maturation phase. However, the compost temperature of the residue without HTT only reached a maximum of 51 °C (on day 10) and requirement for germs, pathogens and pests elimination was not fulfilled (refer to Fig.4.9). The temperature then decreased and was maintained at the lower levels between 35 and 40 °C until Week 4. After Week 4, a further slight decrease led the compost temperature to the range between 30 and 35 °C until Week 10. Then, it dropped and stayed close to the room temperature until the end of the composting process.

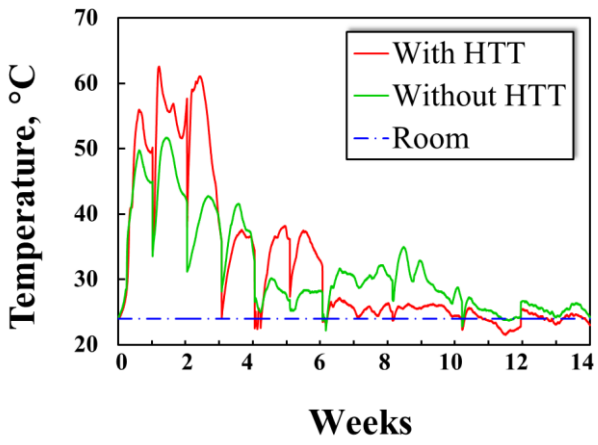


Fig.4.8. Evolution of temperature during composting process of rice straw residue with and without HTT (The air temperature in the room was maintained between 23 and 25°C and therefore, 24°C was taken as a mean)

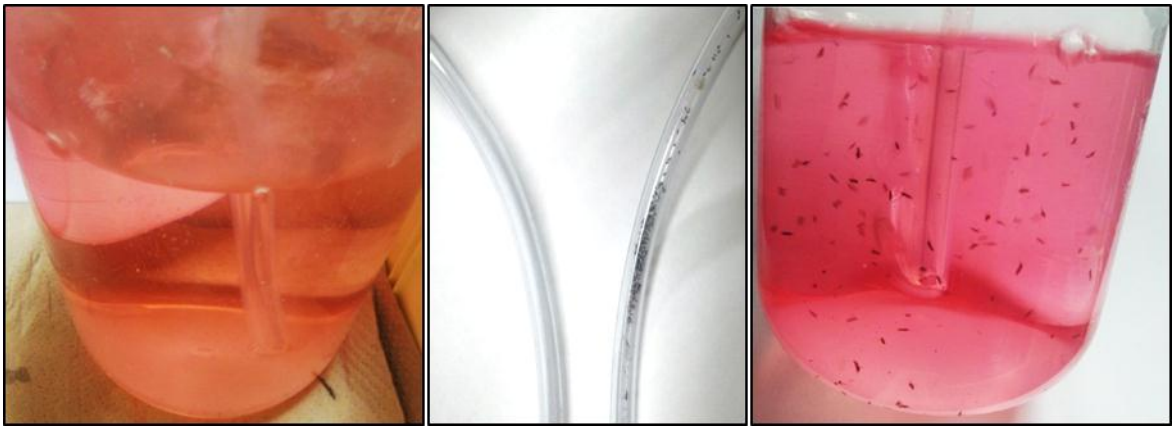


Fig.4.9. Flies and pests reaching the downstream air-supply tubes and acid traps during composting of rice straw with (left) and without (right) HTT

4.3.2 Variations in pH and EC

Variations in pH and EC are useful parameters for monitoring the composting process (Hosseini and Aziz, 2013). In addition, pH and EC are considered as important compost parameters because they can affect the quality and suitability of the final product for plant growth. The compost pH value ranging from 5.5 to 8.5 is considered to be acceptable and EC exceeding the 4.0 mS cm^{-1} value is identified as a critical for seed germination (Allison, 1973; Garcia et al., 1992). The variations of pH and EC during composting process of the rice straw residue with and without HTT are shown in Fig. 4.10a and 4.10b, respectively.

4.3.2.1 pH

The pH value of the rice straw compost with HTT at the start of composting (Week 0) was about three units lower than the rice straw compost without HTT (Fig. 4.10a). This pH difference can be attributed to the presence of organic acids formed normally during HTT of lignocellulosic residues. Nevertheless, the pH of rice straw compost with HTT increased rapidly and alkaline value of 8.2 was observed on Week 2. This could be explained both by decomposition of organic acids by microorganisms as well as release of ammonia (Fig. 4.11a) from mineralization of organic nitrogen. Then, a further slight increase led the pH to reach the highest value of 8.4 on Week 6. From Week 6 onwards, the pH gradually but steadily decreased and the final value detected was 7.8. A gradual decrease in pH coincided well with nitrate formation, and was probably caused by hydrogen ions release during nitrification (Fig. 4.11b). According to Eklind (1998, as cited by Wu et al., 2000), the pH increase in early stage of composting then followed by slow and steady decrease might suggest that the compost has entered a curing and maturation phase from that point. The pH variation in rice straw composting without HTT, followed a similar pattern; in particular, an increase to around 8.2 on Week 2 and the final value of around 7.8. A natural pH drop typical in early stage of composting processes is not seen, probably because the conversion of OM to acidic compounds occurred sooner than sampling the compost on Week 2.

4.3.2.2 EC

Unlike pH, the initial EC value in rice straw compost with HTT was higher than the EC value in rice straw compost without HTT: the respective values were 4.75 and 2.2 (mS cm^{-1}) (Fig.4.10b). This difference was apparently due to presence of soluble organic compounds. As these soluble organic compounds were consumed by microorganisms, the EC reduced to 2.9 (mS cm^{-1}) on Week 4. Afterwards, the EC stabilized and the final value was 3.01 (mS cm^{-1}). The overall behavior of EC in rice straw compost with HTT could indicate the process of decomposition and stabilization. As for the rice straw compost without HTT, a different EC behavior was observed during composting. A slight increase from the initial value of 2.2 to 2.4 (mS cm^{-1}) on Week 2 was probably due to the release of soluble salts such as ammonium from the decomposition of urea. As the composting process progressed nitrification of ammonia occurred (Fig.4.11b), which could be the possible reason for decrease of EC to 1.6 (mS cm^{-1}) on Week 6. But thereafter, the EC profile followed gradual increase and the initial value of 2.2 (mS cm^{-1}) was restored at the end of composting.

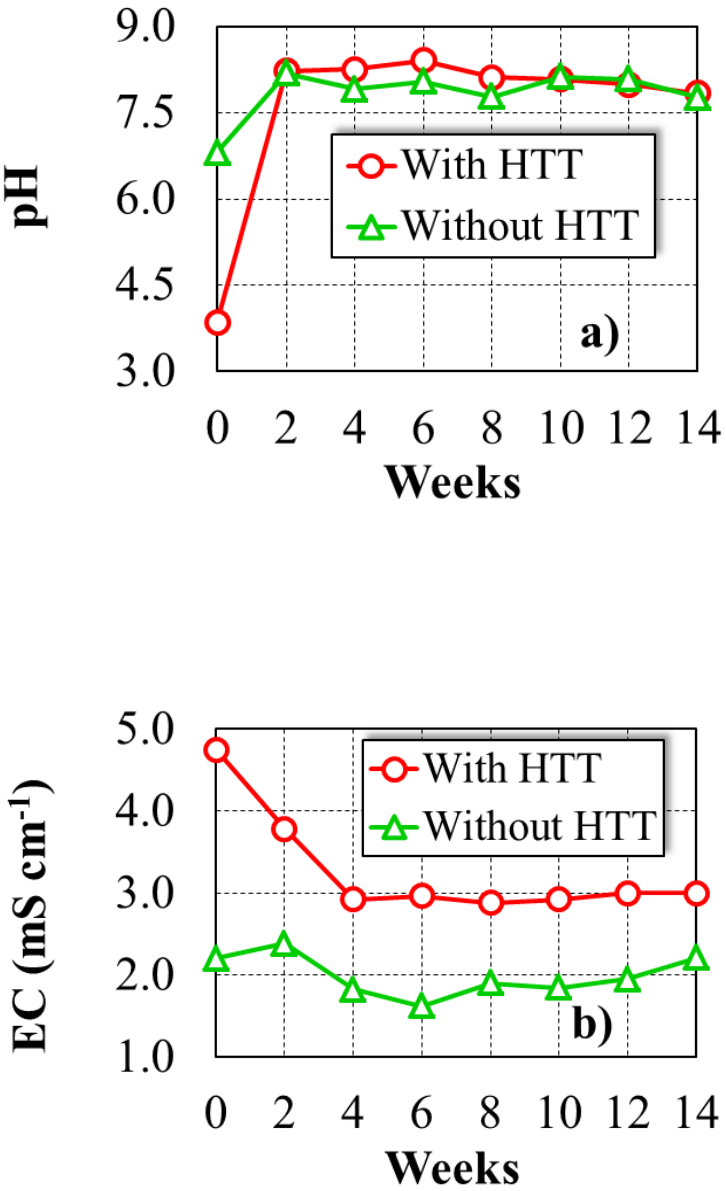


Fig. 4. 10. Variations in pH (a) and EC (b) during composting process of rice straw residue with and without HTT (The values are the means of three replications. Standard deviations are provided in Table 4.3)

4.3.3 Evolutions of NH_4^+–N and NO_3^-–N

During the composting process, organic nitrogen is usually decomposed during active phase of composting resulting in the release of relatively high levels of NH_4^+–N; as composting process continues and maturation phase is reached, ammonia is eventually reduced through volatilization or nitrification to form NO_3^-–N (Haug, 1993). Therefore, high NH_4^+–N concentrations in compost have often been used as indicators of instability, and the presence of NO_3^--N or the low ratio of NH_4^+–N / NO_3^-–N have been used as indicators of compost maturity (Wichuk and McCartney, 2010). According to CCQC (2001), mature compost should not have the NH_4^+–N content of more than 500 mg kg^{-1}, or the NH_4^+–N / NO_3^-–N ratio of >3. The variations of NH_4^+–N and NO_3^-–N during composting of rice straw with and without HTT are shown in Fig.4.11a and 4.11b, respectively.

4.3.3.1 NH_4^+–N

The initial NH_4^+–N content in the rice straw compost with HTT was only 135.1 mg kg^{-1} DM, but soon it reached 1167.0 mg kg^{-1} DM (or 4.9% of TN in compost) on Week 2. This was probably the cause of rapid increase of pH on Week 2. As composting proceeded, immobilization of NH_4^+–N by microorganisms occurred and the NH_4^+–N content in compost product decreased to reach the lower level of 93.8 mg kg^{-1} DM on Week 6. This decrease in NH_4^+–N was related to microbial immobilization because no significant nitrification (Fig.4.11b) or NH_3 volatilization (Fig.4.18b) was observed during this period. After Week 6, the NH_4^+–N content continued to decrease and only 16.7 mg kg^{-1} DM was measured at the end of the experiment.

An intensive NH_4^+–N formation was observed in the rice straw compost without HTT. This can be examined from the high NH_4^+ –N content of 1746.5 mg kg^{-1} DM (or 10.8% of TN) on Week 0. According to Ndegwa et al. (2008), urea can be converted to NH_3 in a period of hours to few days by the extracellular urease enzyme, which is found in many microorganisms during the compost process (Saviozzi et al., 2004). In this experiment, the rice straw residue initially, was soaked in water for 48 hours and it is likely that this pre-treatment promoted development of the urease activity of the microorganisms (naturally present in the residue), which in turn, promoted rapid decomposition of urea at such an early stage of the composting process. The NH_4^+–N content then increased again and reached 3964.9 mg kg^{-1} DM (17.5% of TN) on Week 2. After this peak, the NH_4^+–N content in the substrate decreased rapidly and only 66.2 mg kg^{-1} DM was measured on Week 6, which was apparently due to transformation to NO_3^-–N (Fig.4.11b) as well as

volatilization of NH_3 (Fig.4.18b). After Week 6, the NH_4^+–N content changed slightly and the value measured at the end of experiment was 88.9 mg kg^{-1} DM.

4.3.3.2 NO_3^-–N

The contents of NO_3^-–N in both compost substrates were very low at an early stage of composting (Fig. 4.11b). In the rice straw compost with HTT, the NO_3^-–N content was about 87.7 mg kg^{-1} DM on Week 0 and the content did not show significant change until Week 6. Probably, relatively high temperature (over 55 °C) during active phase of composting suppressed the growth of nitrifying bacteria. After Week 6, when temperature became close to the room temperature, the NO_3^-–N contents started to increase slowly but consistently. The reason for such slow rate in the NO_3^-–N formation could be attributed to a slow rate of organic nitrogen re-mineralization into NH_4^+–N that had been immobilized by microorganism during the active phase. It is said that when nitrogen is incorporated into microbial cellular substances, it becomes relatively slow in release (Marumoto et al. 1977; Bengtson & Bengtson 2005; Shindo & Nishio 2005; Nishida et al. 2008). In practice, the leaching of NO_3^-–N and NH_4^+–N from compost-amended topsoil, with subsequent groundwater contamination, was observed (Daliparthy et al., 1995; Li et al., 1997). In the experiment of Leclerc et al. (1995), nitrogen leaching with compost fertilization amounted to 28 kg N ha^{-1} yr^{-1}. Therefore, the slow nitrogen release pattern revealed in compost product with HTT could be considered as an added quality or another merit of the use of HTT technology in compost production from lignocellulosic residues.

The NO_3^-–N contents in the rice straw compost without HTT however, were highly dynamic. If the NO_3^-–N contents were about 31.7 and 17.9 mg kg^{-1} DM on respective Week 0 and Week 2, relatively high content was detected on Week 4 (979.8 mg kg^{-1} DM). This could be favored by presence of high ammonia as well as temperature drop in compost to around 40 °C. The later one could be affected by the coarse structure of the untreated substrate, which might permit `good` aeration of compost. The available NO_3^-–N was then immediately lost through denitrification process as relatively low content of the NO_3^-–N (44.4 mg kg^{-1} DM) was measured on Week 6. This was concluded because there was a still large net loss in TN (Fig.4.18a) although no leaching (from the bottom of the reactors) or ammonia volatilization occurred during this period. In the following weeks, the NO_3^-–N contents increased but fluctuated between 220-550 mg kg^{-1} DM.

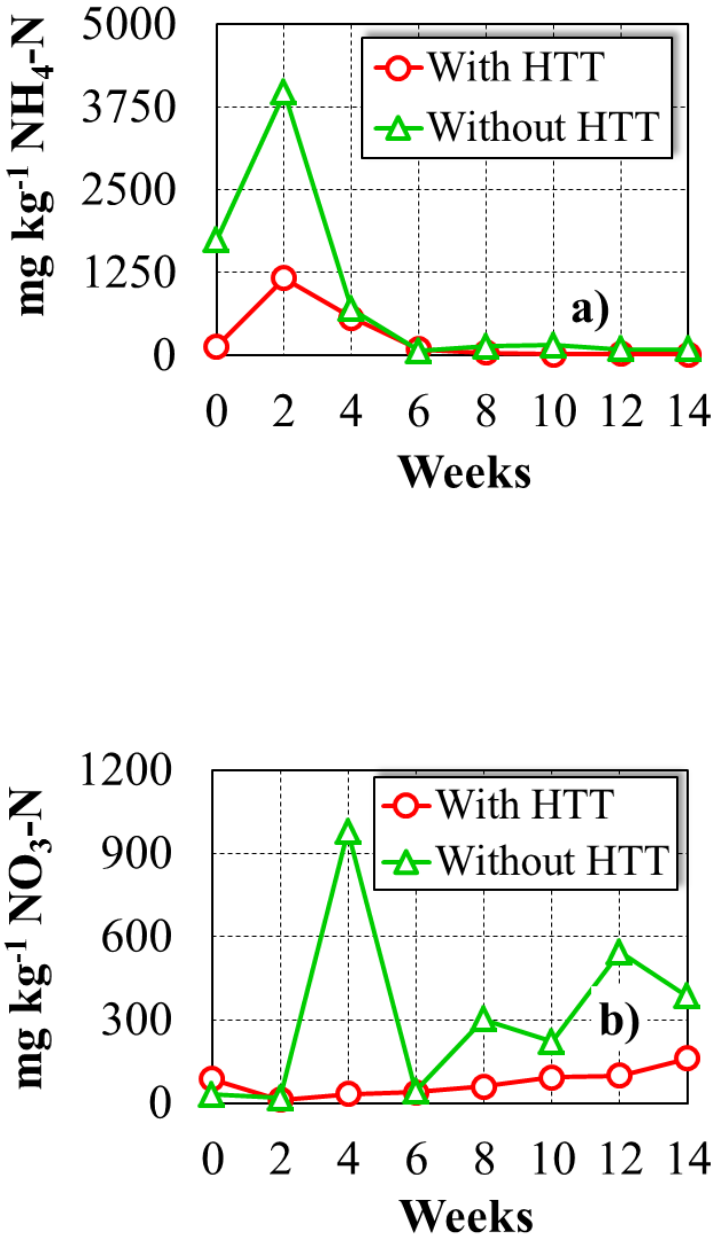


Fig.4.11. Variations in NH_4^+-N (a) and NO_3^--N (b) contents during composting process of rice straw with and without HTT (The values are the means of three replications. Standard deviations are provided in Table 4.3)

From the NH_4^+–N / NO_3^-–N ratio, the compost products in both reactors should have entered maturation phase on Week 6, since from this point, the ratio for both cases did not exceed the established value (i.e. >3). However, the NH_4^+–N / NO_3^-–N ratio cannot be used as an indicator of maturity in this research because the NH_4^+–N and NO_3^-–N contents in either composts were not sufficiently high, i.e. 250 mg kg^{-1} DM (CCQC, 2001).

4.3.4 Variations in OM content

During the composting process, part of OM in the substrate is converted to CO_2, H_2O and energy, while the remainder is eventually converted to stable organic compounds (Insam and de Bertoldi, 2007). Therefore, the content of OM should reduce during composting and this reduction or loss is often monitored to evaluate the extent of decomposition and the stability of the end product. The losses of OM content during composting of the rice straw with and without HTT are illustrated in Fig. 4.12.

The initial OM contents were approximately equal in both compost reactors. The contents decreased during the course of composting from 88.54 to 76.24% in the rice straw compost with HTT, and from 90.19 to 70.11 % in the rice straw compost without HTT. The loss of OM in the rice straw compost with HTT occurred predominantly during the active stage, specifically, between Week 0 and Week 6, when the temperature (Fig. 4.10) and the microbial activity (Fig.4.15) were high. This can be explained that most of OM in the substrate was depleted by microorganism at this stage. Subsequently, after Week 6, the loss of OM in the rice straw with HTT slowed down and became fairly stable. This behavior in OM loss coincided well with evolution of the C/N ratio (Fig. 4.13) and the microbial activity (Fig.4.15), which may suggest that the compost have indeed reached stability. However, the loss of OM in the rice straw compost without HTT occurred throughout the composting process, reflecting the high rate of biodegradation in the substrate. The high rate of degradation was also reflected by high microbial activity in the compost (Fig.4.15), although a greater loss in OM content was achieved at the end of the process (20.18 compared to 12.29% in the rice straw compost with HTT).

In this experiment, the rice straw residue during the HTT processes was enriched in lignin fraction (Table 1) because of overshooting the pre-set conditions (Fig. 4.3) and subsequent losses of hemicellulose fraction. According to Vikman et al. (2002) and Komilis & Ham (2003), a strong linear relationship exists between the lignin content and biodegradable fraction of the OM in the lignocellulosic residues. This is probably due to the known fact that only a smaller portion of lignin is mineralized into CO_2 (and lost) during the composting process, while the major portion are

transformed into relatively stable lignin and humus-like substances (Bernal et. al., 1998a-b; Tuomela et al., 2000). Therefore, the difference in OM mineralization in this study is attributed to the higher content of lignin in the rice straw compost with HTT (Bernal et. al., 1998a-b; Komilis and Ham, 2003).

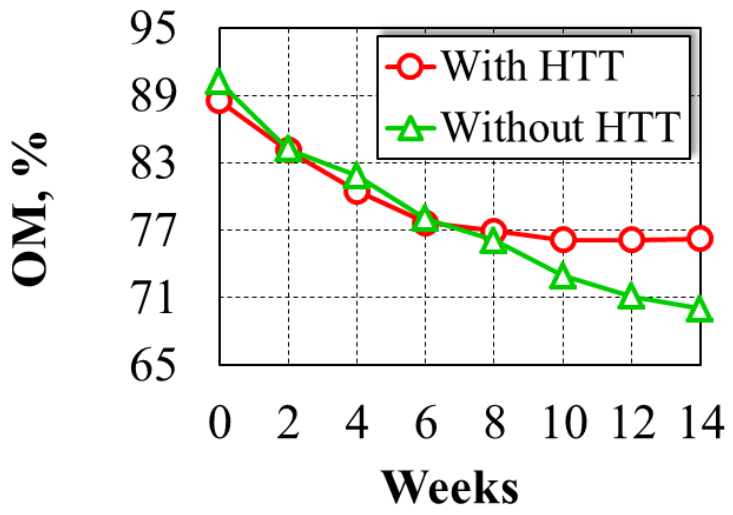


Fig.4.12. The loss of OM content during composting of rice straw with and without HTT (The values are the means of three replications. Standard deviations are provided in Table 4.3)

4.3.5 Changes in TN content

The TN content is not considered as an indicator of compost stability and maturity. However, the TN content is important parameter for evaluating the agronomic value of compost product since it is the plant nutrient that is often most limiting the crop growth and profitable crop production (Mikkelsen and Hartz, 2008). It is also important parameter for determination of compost application rate to soil (e.g. kg per hectare). Therefore, measuring its evolution during the composting process is important from the agronomic point of view. Normally, the TN content is expected to increase because of the greater loss of organic carbon than nitrogen during composting. Although, the TN content may also increase partially, through microbial (atmospheric) nitrogen fixation during the composting process (Nuntagij et al., 1989). Evolution of the TN content during composting of rice straw with and without HTT is illustrated in Fig. 4.13.

The staring values of the TN content in both composts were approximately equal. There was a rapid increase in the TN content of the rice straw compost with HTT, specifically from 1.59 on Week 0 shortly to 3.27% on Week 6. In the following weeks, the TN content followed a gradual

increase and the value measured at the end of the composting process (Week 14) was 3.42%. The major increase in the TN content of rice straw residue with HTT occurred when the OM loss (Fig. 4.12) and the microbial activity (Fig. 4.15) in the compost were relatively high. Therefore, it is apparent that this increase was mainly due to the net loss of organic carbon as CO_2 during active phase of composting. However, significantly different pattern was observed in the TN content profile of the rice straw compost without HTT. There was initially, an increase from 1.67 on Week 0 to 2.27% on Week 2 in the TN content. Then it slowed down and stayed around 2.3% until Week 6. Such lag period in the TN content profile corresponded well with the peaks of the NH_4^+–N (Fig. 4.11a) and the NO_3^-–N (Fig.4.11b) contents in the rice straw compost without HTT. Therefore, this slowdown probably, was caused by nitrogen losses through ammonia volatilization and denitrification process. After Week 6 onwards, the TN content started to increase again and the final value observed at the end of composting (Week 14) was 3.02%.

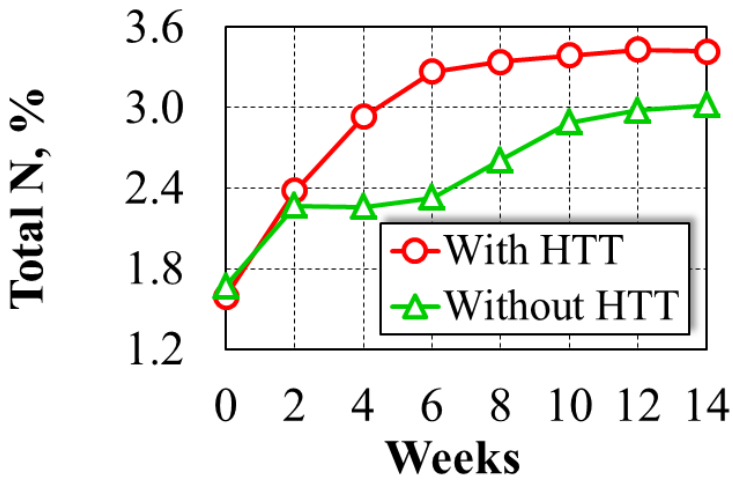


Fig.4.13. Changes in TN contents during composting of rice straw with and without HTT (The values are the means of three replications. Standard deviations are provided in Table 4.3)

4.3.6 Variations in C/N ratio

The C/N ratio is an important agronomic parameter of final compost product as it was found to affect immobilization and release of nitrogen and other important crop nutrients in the soil (Ahmad et al., 1969). Therefore, the C/N ratio has traditionally been used to evaluate the compost stability and maturity (Wichuk and McCartney, 2010). Because of higher loss of carbon compounds generally, decreasing trend in the C/N ratio is expected. The C/N ratio smaller than 25 is indicative of an acceptable maturity (CCQC, 2001), a ratio of 15 or even less being most preferable (Jimenez and Garcia, 1989). The trends in the C/N ratio of the rice straw composts with and without HTT are

illustrated in Fig.4.14.

The major reduction in the C/N ratio of the rice straw compost with HTT occurred during the active phase of decomposition, i.e. Week 0 and Week 6. The C/N ratio value at Week 0 and Week 6 was 27.4 and 12.5, respectively. This can suggest that biodegradability of the rice straw was enhanced with HTT. After Week 6, the C/N ratio leveled off and stabilized at 12, which could indicate the stability phase of the compost. Indeed, the compost could be stable from this point, since this value is very close to the C/N ratio of many humic substances found in many stable soil organic matters (Yonebayashi and Hattori, 1988; Piccolo et al., 1992; Xuebin and Yuping, 1997).

As for the C/N ratio of the rice straw compost without HTT, considerable decrease from the initial value of 25.7 to 17.2 occurred between Week 0 and Week 2. One of the possible reasons for this rapid drop could be decomposition of easily available carbons by microorganisms. Then, the C/N ratio remained unchanged until Week 4, implying that the loss of nitrogen from the system was also substantial during this period. After Week 4, the C/N ratio started to decrease again and the compost had a C/N ratio of about 11.2 on Week 14. However, continuous decrease in the profile of the C/N ratio suggests that the substrate was rich in some carbon materials that yet to be degraded. Therefore, as stated by Jimenez and Garcia (1989), the C/N ratio cannot be considered as indicative of compost maturity until stability in the C/N ratio change is not seen.

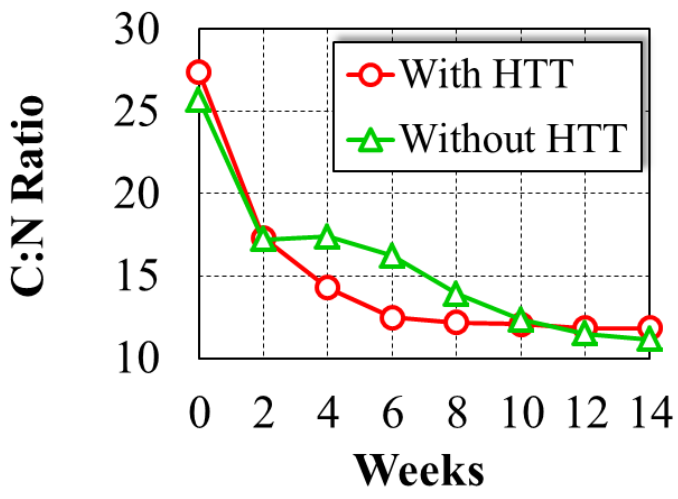


Fig.4.14. Variations in the C/N ratio during the composting process of rice straw with and without HTT (The values are the means of three replications. Standard deviations are provided in Table 4.3)

4.3.7 Evolutions of carbon dioxide

As compost approaches stability, the microbial activity decreases and the CO_2 evolution rate is expected to decline (Wichuk and McCartney, 2010). Therefore, the CO_2 production has been frequently applied in compost stability and maturity determination (Gomez et al., 2006). It is also one of the most reliable and recommended stability index (ADAS Consulting Ltd., 2005). According to CCQC (2001), a compost product with microbial activity of ≤ 8.0 mg CO_2 g^{-1} OM d^{-1} is stable and mature. Fig.4.15 shows the evolution of CO_2 as an indication of stability during the composting of rice straw with and without HTT.

In the early stages of the process, the CO_2 evolution rate for the rice straw compost with HTT was about 1.7 times higher than for the rice straw compost without HTT because of the enhancement of the rice straw digestibility after pretreatment (Thomsen et al., 2008). The CO_2 evolution rate then decreased rapidly and the thresholds stability value of 8.0 mg CO_2 g^{-1} OM d^{-1} was reached on Week 6. This result corresponds well with the change of other measured parameters and further confirms that the rice straw compost with HTT reached obvious stabilization phase on Week 6.

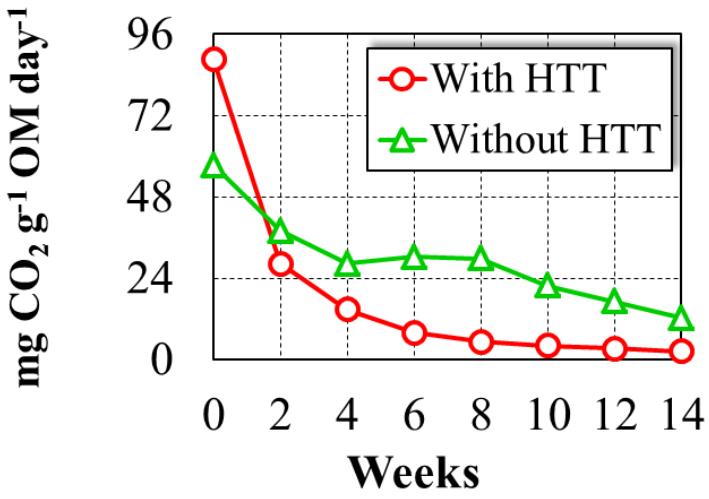


Fig.4.15. Changes in microbial activities (as the CO_2 evolution rate) during composting of rice straw with and without HTT (The values are the means of three replications. Standard deviations are provided in Table 4.3)

However, there was no clear trend in the CO_2 evolution rate for the rice straw compost without HTT. Initially, the CO_2 evolution rate decreased to reach a value of 28.25 mg CO_2 g^{-1} OM d^{-1} on Week 4, and then stayed almost unchanged until Week 8. It is like that the microbial activity during Week 4 and Week 8 was supported by release of carbon from cellulose that was largely unavailable

until Week 4 due to protective effect of hemicellulose-lignin association (Malherbe and Cloete, 2002.). In the following weeks, the CO_2 evolution rate started to decrease again and the value measured at the end of experiment (Week 14) however, was still high (12.28 mg CO_2 g^{-1} OM d^{-1}). Such a high microbial activity could suggest that the residue had not stabilized yet and was far away from the maturation phase.

4.3.8 Germination index

While organic materials decompose, a variety of metabolic compounds are released during composting and these compounds can be toxic to plants (Zucconi et al., 1985). The germination index (GI) was a sensitive indicator and its increase corresponded with the decreases in concentrations of phytotoxic compounds as compost aged (Tiquia and Tam, 1998). Compost with GI of ≥80% is considered phytotoxic-free and adequately matured (CCQC, 2001). The changes in GI percentages of Komatsuna seeds during composting of rice straw with and without HTT are shown in Fig. 4.16.

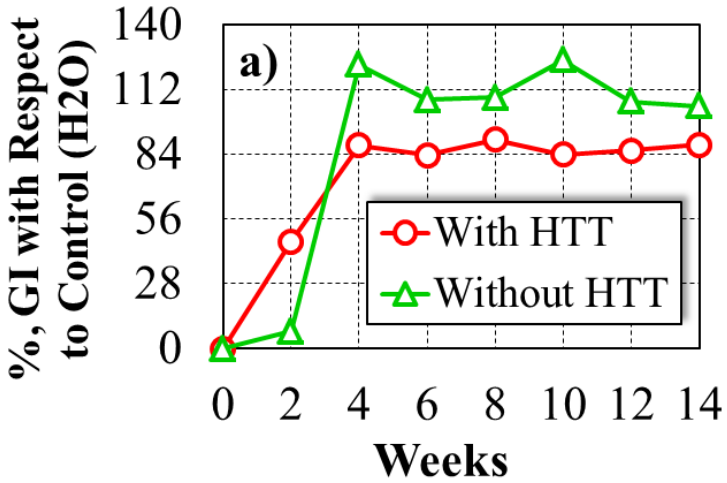


Fig.4.16. Changes in Komatsuna seeds germination indices during composting of rice straw with and without HTT (The values are the means of three replications. Standard deviations are provided in Table 4.3)

The initial GI was 0% for both compost products. Then, soon both composts overcame the threshold value (≥80%) and yielded high GI (87.7-119.3%) on Week 4, which may indicate the disappearance of phytotoxic compounds (Tiquia and Tam, 1998). The GI value in rice straw

compost with HTT on Week 0 and Week 2 could be controlled by high EC value, since increase in GI corroborates well with decrease of EC during this period. The low GI in rice straw compost without HTT probably was due to phytotoxic effect of urea and associated NH_4^+–N release during the early stage of composting (Fig. 4.11a). The fluctuations observed for both compost products after Week 4 onwards, are in agreement with the finding of Zucconi and de Bertoldi (1987). The higher GI (108-126%) found after Week 4 for the rice straw compost without HTT suggests that the high content of NO_3^-–N (Fig. 4.11b) exerted a positive influence on GI (Tiquia and Tam, 1998). However, these results demonstrated that the compost would not have any phytotoxic effects, even if the microbial activity was still high and that stability and maturity are different compost properties.

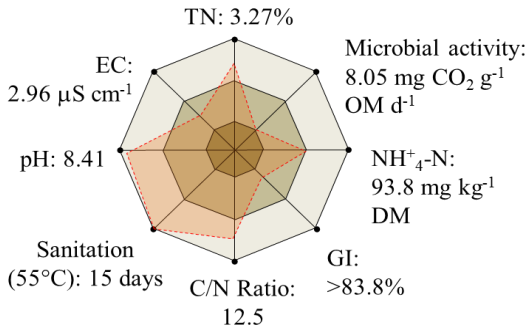
4.3.9 Evaluation of stability and maturity of compost end product

Compost feedstock is extremely heterogeneous in nature and in view of this, there is no universally accepted method exists to evaluate stability and maturity. In order to facilitate a better evaluation, the measured parameters and indices of the stability and maturity of rice straw compost products with and without HTT after 6 and 14 weeks of composting were plotted in octagonal diagram with three rating zones (e.g. Very Mature/Very Stable, Mature/Stable and Immature/Unstable) (Fig. 4.17). The thresholds of the stability and maturity parameters for each rating zone are given in the Table 4.2.

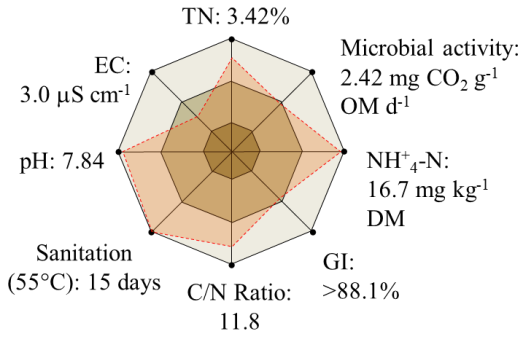
As can be seen from the diagram, the area of distribution of stability and maturity parameters in rice straw compost products with HTT after 6 and 14 weeks of processing occupied broader span, with all measured values placed predominantly, in Mature or Very Mature Rating Zone (Fig. 4.17a). Therefore, it seems reasonable to suggest that the rice straw compost product with HTT after 6 weeks of composting can be considered safe for agronomic use. As for the rice straw compost product without HTT, the area of distribution of stability and maturity parameters was rather narrow even after 14 weeks of composting (Fig. 4.17b). Since some observed values (i.e. microbial activity and sanitation or experiencing temperature of >55°C for at least 3 continuous days) rested in the Rating Zone reserved for not matured compost, the large span of Immature Zone was left uncovered, and thus compost cannot be considered sufficiently stable and mature.

With HTT

After 6 Weeks

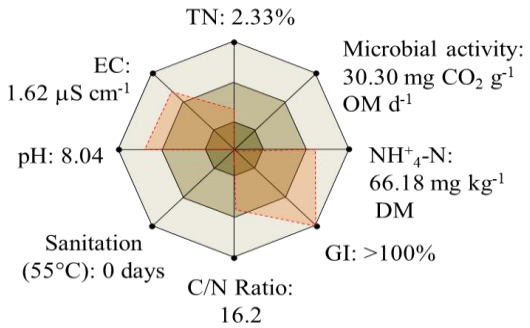


After 14 Weeks



Without HTT

After 6 Weeks



After 14 Weeks

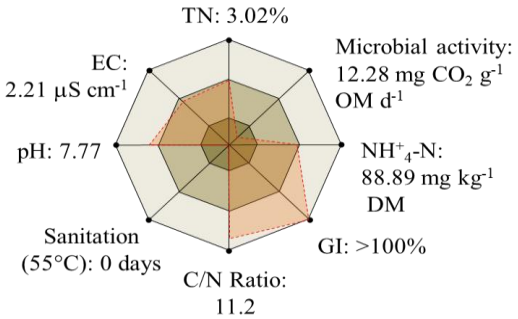


Fig.4.17. Stability and maturity parameters of rice straw compost products with (a) and without (b) HTT plotted in octagonal diagram after 6 and 14 weeks of processing

TABLE 4.2. Thresholds for Maturity and Stability Parameters

Stability and Maturity Parameters/Indices	Very Mature Very Stable	Mature Stable	Immature Unstable
	Rating Zones		
C/N Ratio	10-15	≤ 25†	> 25†
Microbial Stability (mg CO_2 g^{-1} OM d^{-1})	< 2†	2-8†	> 8†
NH^+_4-N (mg kg^{-1} DM)	< 75†	75-500†	> 500†
Germination Index, %	> 90†	80-90†	< 80†
Sanitation (55°C for at least 3 days)	> 3	> 3	< 3
EC (mS cm^{-1})	1-2	2-4	> 4
pH	~ 7.5	8.5-6.5	< 6.5
Total N	≥ 1	≥ 1	< 1

Notes: †CCQC (2001)

4.3.10 Correlation between stability and maturity parameters

The Pearson correlation coefficient (r) among measured parameters was calculated in order to find a simplest indicator(s) which could be used to assess the stability and maturity of the compost with and without HTT (Table 4.4). The results indicate that there was very strong statistical correlation (at $p < 0.01$) among various stability and maturity parameters of rice straw compost with HTT. Specifically, the CO_2 evolution had very strong positive correlation with the EC (0.968), OM (0.934) and C/N ratio (0.998). On the other hand, the GI had very strong but negative correlation with the EC (-0.997), OM (-0.943) and C/N ratio (-0.976). There was also very strong negative correlation between the GI and the CO_2 evolution (-0.969). According to these statistical results, the EC and C/N ratio (or OM) may be used to evaluate stability and maturity of rice straw compost with HTT. As for rice straw compost without HTT, the CO_2 evolution correlated well (at $p < 0.01$) with OM (0.926) and C/N ratio (0.960). A strong negative correlations (at $p < 0.05$) were also calculated between the GI and the EC (-0.753), OM (-0.772), C/N ratio (-0.736) and the CO_2 evolution (-0.797). However, it appears that the use of the parameters such as the EC, C/N ratio and OM is not sufficient for determination of both the stability and maturity of rice straw compost without HTT. For example, compost had the GI as high as an indicative of mature compost but resulted in the high CO_2 evolution, which is indicative of unstable compost. Therefore, conducting respirometric test is also necessary in order to ensure an adequate evaluation of stability and maturity of rice straw compost without HTT.

TABLE 4.3. Variations in selected biochemical properties during composting of rice straw with and without HTT

Parameters	Composting periods (weeks)							
	0	2	4	6	8	10	12	14
pH	3.86 ± 0.12	8.23 ± 0.25	8.26 ± 0.20	8.41 ± 0.16	8.11 ± 0.12	8.08 ± 0.16	7.99 ± 0.14	7.84 ± 0.08
	[6.81 ± 0.08]	[8.18 ± 0.23]	[7.91 ± 0.14]	[8.04 ± 0.17]	[7.78 ± 0.13]	[8.12 ± 0.09]	[8.08 ± 0.12]	[7.77 ± 0.10]
EC (mS cm^{-1})	4.75 ± 0.05	3.78 ± 0.03	2.92 ± 0.15	2.96 ± 0.07	2.88 ± 0.10	2.92 ± 0.07	3.02 ± 0.08	3.00 ± 0.05
	[2.20 ± 0.06]	[2.38 ± 0.17]	[1.82 ± 0.11]	[1.62 ± 0.20]	[1.90 ± 0.15]	[1.84 ± 0.11]	[1.95 ± 0.16]	[2.21 ± 0.18]
NH_4^+-N (mg kg^{-1} DM)	135.1 ± 9.00	1167 ± 21	565.2 ± 5.4	93.75 ± 5.51	39.77 ± 5.68	16.67 ± 3.06	22.22 ± 5.56	16.67 ± 5.56
	[1747 ± 12]	[3965 ± 44]	[700.0 ± 20.7]	[66.18 ± 3.90]	[137.5 ± 8.86]	[155.6 ± 6.1]	[88.89 ± 5.56]	[88.89 ± 5.00]
NO_3^--N (mg kg^{-1} DM)	87.80 ± 0.01	12.89 ± 5.37	32.85 ± 5.47	38.84 ± 0.04	60.36 ± 1.12	94.03 ± 8.9	98.45 ± 5.53	161.50 ± 2.20
	[31.71 ± 3.66]	[17.91 ± 5.37]	[979.9 ± 11.0]	[44.44 ± 5.56]	[302.9 ± 17.9]	[222.4 ± 6.6]	[544.3 ± 35.4]	[384.2 ± 0.01]
OM (%)	88.54 ± 0.02	84.12 ± 0.08	80.52 ± 0.10	77.66 ± 0.12	76.91 ± 0.15	76.16 ± 0.17	76.12 ± 0.07	76.24 ± 0.24
	[90.19 ± 0.15]	[84.23 ± 0.25]	[81.88 ± 0.07]	[78.03 ± 0.15]	[76.07 ± 0.08]	[72.96 ± 0.05]	[71.11 ± 0.05]	[70.11 ± 0.05]
Total C (%)	43.51 ± 0.40	41.25 ± 0.42	41.90 ± 0.01	40.83 ± 0.16	40.69 ± 0.02	40.89 ± 0.07	40.61 ± 0.05	40.47 ± 0.06
	[42.96 ± 0.06]	[39.10 ± 0.10]	[39.32 ± 0.10]	[37.82 ± 0.04]	[36.27 ± 0.03]	[35.68 ± 0.06]	[34.22 ± 0.01]	[33.71 ± 0.02]
Total N (%)	1.59 ± 0.002	2.38 ± 0.03	2.93 ± 0.01	3.27 ± 0.02	3.34 ± 0.02	3.39 ± 0.02	3.43 ± 0.01	3.42 ± 0.00
	[1.67 ± 0.01]	[2.27 ± 0.03]	[2.26 ± 0.01]	[2.33 ± 0.01]	[2.61 ± 0.01]	[2.89 ± 0.01]	[2.98 ± 0.00]	[3.02 ± 0.00]
C to N ratio	27.37 ± 0.25	17.33 ± 0.37	14.30 ± 0.03	12.49 ± 0.05	12.18 ± 0.07	12.06 ± 0.04	11.84 ± 0.01	11.83 ± 0.02
	[25.72 ± 0.18]	[17.23 ± 0.19]	[17.40 ± 0.08]	[16.23 ± 0.04]	[13.90 ± 0.06]	[12.35 ± 0.01]	[11.49 ± 0.01]	[11.16 ± 0.01]
CO_2 evolution (mg g^{-1} OM d^{-1})	88.59 ± 0.14	28.25 ± 0.62	14.83 ± 0.15	8.05 ± 0.10	5.19 ± 0.17	4.08 ± 0.05	3.33 ± 0.05	2.42 ± 0.16
	[57.35 ± 1.33]	[38.01 ± 0.37]	[28.25 ± 0.30]	[30.29 ± 0.48]	[29.81 ± 0.47]	[21.80 ± 0.31]	[17.19 ± 0.29]	[12.28 ± 0.10]
Germination Index (%)	0.00 ± 0.00	46.07 ± 1.10	87.66 ± 2.30	83.73 ± 2.30	92.05 ± 3.00	83.91 ± 2.00	85.47 ± 4.40	88.11 ± 2.40
	[0.00 ± 0.00]	[7.25 ± 0.80]	[122.8 ± 5.30]	[107.5 ± 7.00]	[108.6 ± 4.10]	[124.8 ± 1.10]	[106.7 ± 1.60]	[104.7 ± 1.50]

The results are the means of three replicates ± standard error. A [B], where A = with HTT and [B = without HTT].

TABLE 4.4. Pearson correlation coefficient between measured parameters of rice straw compost with and without HTT

	pH	EC	NH_4^+-N	NO_3^--N	OM	C/N ratio	CO_2 evolution	GI
pH	-	-0.882**	ns	ns	ns	ns	ns	0.872**
		[ns]	[ns]	[ns]	[ns]	[ns]	[ns]	[ns]
EC		-	ns	ns	0.937**	0.975**	0.968**	-0.997**
			[ns]	[ns]	[ns]	[ns]	[ns]	[-0.753*]
NH_4^+-N			-	ns	ns	ns	ns	ns
				[ns]	[ns]	[ns]	[ns]	[-0.849**]
NO_3^--N				-	ns	ns	ns	ns
					[ns]	[ns]	[ns]	[ns]
OM					-	0.954**	0.934**	-0.943**
						[0.940**]	[0.926**]	[-0.770*]
C/N ratio						-	0.998**	-0.976**
							[0.960**]	[-0.736*]
CO_2 evolution							-	-0.969**
								[-0.797*]
GI								-

ns, not significant; * Significant at $p<0.05$; ** Significant at $p<0.01$; A [B], where A = with HTT and [B = without HTT].

4.3.11 The losses of N

The high rate of N losses is a key concern in the aerobic composting because of air pollution problem and final nitrogen content of the compost (Li Y. et al., 2011). During the composting of animal excrements, for example, the loss of N through the NH_3 volatilization amounted 46.8-77.4% (Martins & Dewes, 1992). HTT was effective in reducing ammonia loss from aerobic composting of lignocellulosic residue (Chapter 3). However, the N loss may also occur through denitrification, a process by which nitrogen is finally reduced to N_2 gas during the process (Tiquia, 2002). Therefore, the evaluation of HTT in total N loss reduction during the composting process was necessary. The losses of N during composting of rice straw with and without HTT are shown in Fig. 4.18a. The plot of cumulative NH_3 losses during composting of rice straw with and without HTT was also constructed (Fig. 4.18b) in order to approximate the losses of N through the denitrification process.

The net loss of N during composting of rice straw with HTT was practically non-existent (Fig. 4.18a). Despite 2.1% of the loss of N that occurred in the form of NH_3 between Week 0 and Week 4 (Fig. 4.18b), recovery and gain in nitrogen content (equivalent to 8.8% of initial weight of nitrogen) took place simultaneously due to the activity of N_2-fixing bacteria (Nuntagij et al., 1989). A phenomenon of N_2-fixation often observed when lignocellulosic residues are composted (Sanchez-Monedero et al., 2001). Effectiveness of HTT on NH_3 loss reduction during composting of lignocellulose was previously discussed in Chapter 3. Although, it is worth noting that in the previous bench-scale experiment, 56°C (2 days) was set as the highest process temperature, while in this experiment it reached 63°C and stayed above 55 °C for more than two weeks. After Week 4, the loss of N (~4.6%) due to the activity of denitrifying organisms appeared to occur but the amount was not so high to result in the net loss of N during the process. The N loss at this period was attributed to the denitrification process because a cumulative amount of NH_3 loss as well as leaching of N during this period was almost negligible (Fig. 4.18b).

However, the loss of N was substantial during the composting process of the rice straw without HTT. The net loss of N at the end of composting amounted 40.3% of the initial value of nitrogen. Of this, 37.5% occurred predominantly between Week 0 and Week 6, which was mainly due to NH_3 loss, specifically between Week 0 and Week 4. The cumulative amount of NH_3 loss during this period was 18.9% (Fig. 4.18), which was probably favored by the alkaline pH (Fig. 4.10a) and the relatively high NH_4^--N content (Fig. 4.11a) of the compost. The loss of N (~11.8%) between Week 4 and Week 6 can be attributed to denitrification, since the loss through the leaching and NH_3 emission (Fig.4.18a) from the compost was insignificant during this period. The rapid drop in the NO_3^--N content of the compost on Week 6 (Fig. 4.11b) could also support this point of view. However, after Week 6 onwards, the rate of denitrification became slower and therefore, amounted at 2.8%. In general, denitrification is accepted as an anaerobic process. However, according to Insam and de Bertoldi (2007), there is always anaerobic niches present in compost and therefore, suggesting that denitrification may also occur in well-aerated compost.

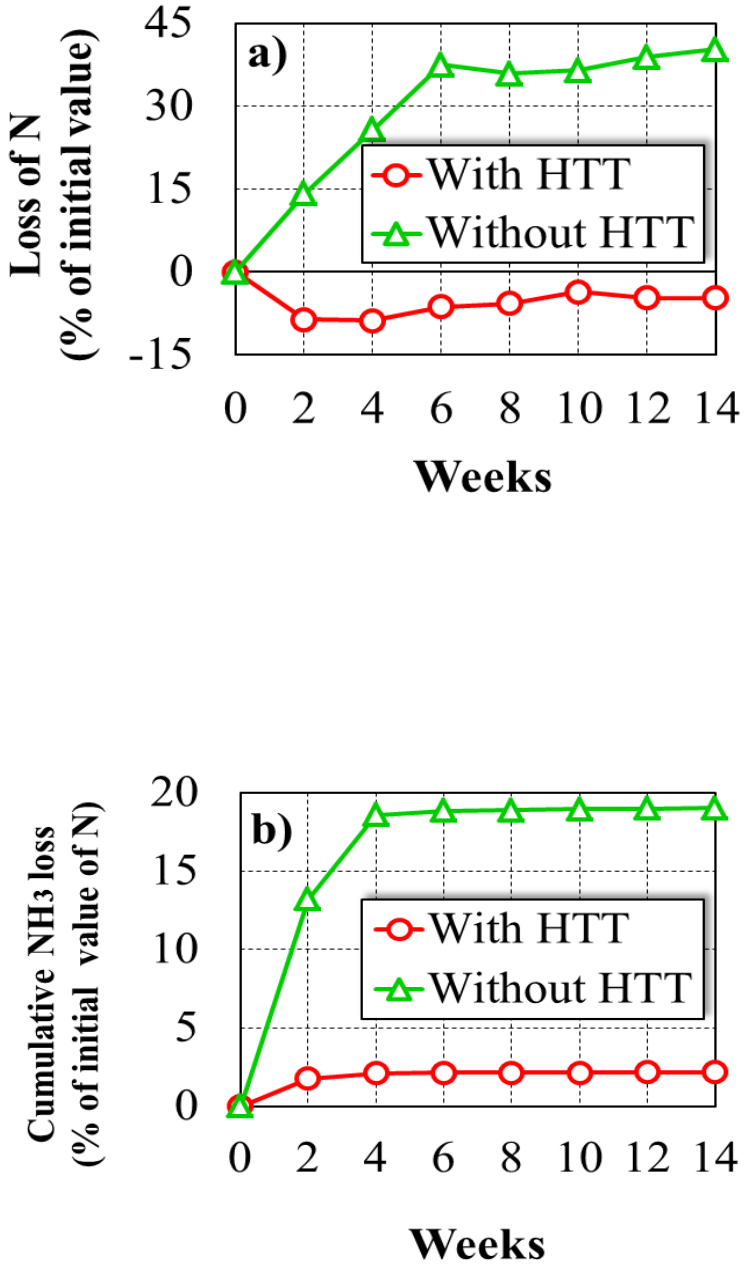


Fig. 4.18. The net loss of N (a) and cumulative NH_3 loss (b) during composting of rice straw with and without HTT

4.4 Conclusions

Bin-scale (90L) composting of rice straw with and without pilot-scale HTT was performed in order to adequately evaluate the novel HTT technology in enhancing compost stability and maturity of lignocellulose rice straw residue. According to the results, HTT (180°C, 1.0 MPa, 30 minutes) can efficiently enhance the rice straw compost stability and maturity. The compost product can be considered safe and adequate for agronomic use after 6 weeks of composting. Stability and maturity of compost product after 6 weeks of processing was expressed by the C/N ratio of 12.5, the microbial stability of 8.0 mg CO_2 g^{-1} OM d^{-1}, NH_4-N content of 93.75 mg kg^{-1} DM, pH-8.41, EC-2.96 mS cm^{-1} and finally by GI of >83%. As for the rice straw compost product without HTT, the high microbial stability (> 12.28 mg CO_2 g^{-1} OM d^{-1}) even after 14 weeks of composting suggests that the residue has not stabilized yet and is far from the maturation phase, although higher GI (> 100%) was observed. In addition, the rice straw compost product with HTT was likely free of pests and pathogens because of `sterilization` during the pretreatment process. Furthermore, it appeared (after 6 weeks) granular, dark gray in color and free of offensive odor. As regards the loss of N, no net loss was observed during composting of rice straw with HTT, while during the composting of rice straw without HTT it could reach 40.3% of the initial value. HTT was also effective in N loss reduction through the denitrification process. Finally, HTT technology is recommended for enhancing biodegradability of lignocellulosic residue for high-quality organic fertilizer production.

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Chapter 5

5. Conclusions and Recommendations

5.1 Conclusions

The large volumes of lignocellulosic residues which could have been composted into organic fertilizers are burnt in field or regarded as waste each season everywhere in the world. However, it is the nature of lignocellulose to degrade slowly during composting, because microbial access to cellulose, a major biodegradable component of lignocellulose, is inhibited by hemicellulose-lignin association during the composting process. In the present study, a novel HTT technology at mild reaction conditions (160 °C < T < 220 °C, 0.6 MPa < P < 2.4 MPa, 30 minutes), as pretreatment step in enhancing biodegradability of lignocellulose agricultural residues (date palm woodchips and rice straw as a model) for organic fertilizer production was investigated.

Based on the findings and observations of this investigation, the followings can be concluded:

- The HTT temperature of 180°C (1.0MPa, 30 minutes) was the most effective pretreatment temperature for subsequent aerobic degradation by solubilizing the largest portion of hemicellulose polysaccharides within the lignocellulose CW structure which had two effects: 1) it supplied readily bioavailable form of carbon, which in turn promoted rapid microbial activities in the early stage of decomposition; and 2) it created pores and cavities within the CW, which permitted rapid bacterial penetration and CW degradation;
- The enhanced degradability was also partially linked to the effect of 180 °C treatment temperature on solubilization of amorphous cellulose and partial hydrolysis of lignin. Solubilization of amorphous region of cellulose is important for releasing a new terminal end in microfibrils, which are necessary for microbial and enzymatic attack of cellulose. The partial hydrolyses of lignin is

important for banding the ammonia, especially during the active phase of degradation-ammonification when potential of its loss is high;

- The trapped `flashed` steam from HTT of lignocellulosic residues may contain organic compounds (such as acetic acid, furfural, 5-HMF) that are known very effective in controlling many soilborne plants pathogens, and therefore can be applied to soil as bio-agents for controlling plants diseases (hence, no downstream wastewater treatment unit is necessary). Therefore, this can be considered an added advantage of HTT technology use in compost feedstock pretreatment.
- HTT was also effective in reducing ammonia loss during the aerobic composting process of lignocellulosic residue. Nearly, no ammonia emission was observed during the controlled bench-scale composting of date palm lignocellulose ;
- The effect of HTT on ammonia loss reduction was expressed through solubilization of hemicellulose polysaccharides into simpler sugars which had two concomitant consequences: 1) it added simpler sugars, which in turn suppressed ammonia volatilization (via rapid immobilization of nitrogen) in the early stage of compositing; and 2) it improved bioavailability of cellulose particles which supported microbial immobilization of nitrogen to suppress ammonia volatilization in a longer term;
- The hydrothermally treated (~180°C, ~1.0MPa, ~30 minutes) lignocellulosic residues can efficiently be used as Bio-filter substrate for adsorbing ammonia gas;
- HTT was also effective in enhancing compost stability and maturity of (rice straw) lignocellulosic residue;
- The compost product with HTT was safe and adequate for agronomic use after 6 weeks of composting, while the high microbial activity after 14 weeks of processing suggested that the compost product without HTT is far from stability and maturity phase;
- No net loss of N was observed during composting of lignocellulose rice straw with HTT, while during the composting of rice straw without HTT it could reach 40.3% of the initial value.
- HTT appeared to be effective also in nitrogen loss reduction through the denitrification process. However, the effect was indirect, e.g. suppressing and inhibiting the growth of denitrifying bacteria through rapid temperature rise in compost (>50°C) during the early stage of composting;
- HTT of lignocellulose can promote higher nitrogen recovery/gain during composting, specifically through promoting the N2-fixation process. The net amount of nitrogen that was fixed through this process, in this study, was estimated to be 8.8% of the initial value of nitrogen added to the compost;

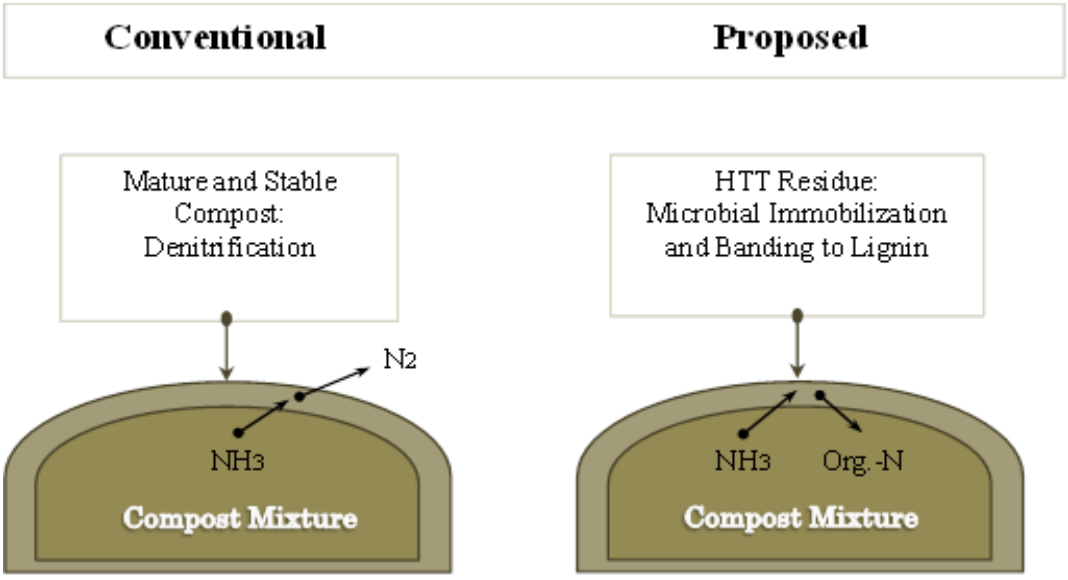
- The compost product with HTT was likely to be free of pests and pathogens because of `sterilization` during the HTT process. Moreover, it appeared (after 6 weeks) granular, dark gray in color and free of offensive odor;
- The compost product with HTT (after 6 weeks of composting) had higher nitrogen content (3.27% in compost product with HTT against to 2.33% in compost product without HTT);
- Finally, the HTT technology can efficiently be used in enhancing biodegradability of lignocellulosic residue for high-quality organic fertilizer production.

5.2 Recommendations

One of the key issues in composting is the high rate of nitrogen losses. As discussed before, the main passway of nitrogen loss are ammonia volatilization and the denitrification process. If the main goal of composting is to produce a high-quality final product, conservation of nitrogen is necessary.

Based on the findings and observations of this study, the following measures may help to control and retain the nitrogen in the compost end product:

- The potential of hydrothermally treated residues is high in adsorbing the ammonia gas. Therefore, as demonstrated by the figure bellow, if the compost pile is lined and covered with a layer of hydrothermally treated residues (without adjusting the C/N ratio), the ammonia emission may be significantly suppressed (via microbial immobilization or banding to lignin hydrolysis compounds). The advantage of this method, when compared to conventional techniques, is that nitrogen will finally retain in compost product. As in the case of conventional methods, stable and mature compost is normally used as a cover to reduce ammonia emission. However, it is used to reduce ammonia gas to N_2 before it is emitted to air, and therefore it doesn't contribute to agronomical value of end product;
- The loss of nitrogen through the denitrification process can also be significant during composting. Therefore, in order to eliminate such a loss, the temperature of the pile should be allowed to increase high enough (>50°C). The high process temperature is important at the early stage of composting (especially when ammonification potential is high) for suppressing denitrifying microorganisms. This can be achieved easily, for example, by increasing the size of the pile.



Covering compost pile with mature compost (conventional) and the residue from HTT (proposed by this study) for reducing the ammonia emission during composting