

COMPARISON OF WASTE DEGRADATION AND MICROBIAL COMMUNITY
PROFILES DURING COMPOSTING OF YARD WASTE, KITCHEN WASTE AND
COW MANURE

by

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*In loving memory of my father,
Mustafa ERDEM*

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COMPARISON OF WASTE DEGRADATION AND MICROBIAL COMMUNITY PROFILES DURING COMPOSTING OF YARD WASTE, KITCHEN WASTE AND COW MANURE

Composting process is an effective low-cost method that converts organic waste including animal manures, municipal biosolids, crop residues, food processing wastes to a valuable resource which can be used as fertilizer and soil conditioner. In this study, two experimental sets named Set1 (Y, K, YKM) and Set2 (YK, YM, KM) were carried out, where the moisture content was adjusted to 55 ± 5 % with the aid of sawdust. Analytical methods were applied to compare the quality of the end products with regulatory requirements to use as soil amendments and to control the process efficiency. Additionally, the bacterial community compositions at thermophilic stage were analyzed using next generation sequencing. Final C/N ratio, volatile solids content, electrical conductivity of all composting systems met with regulatory limitations. The pH in Y and YKM composts were found to be in the range of 5.5 to 8.5 stated in the regulations. Final moisture content was found higher than regulatory limits ($<30\%$) in all systems. The best performance of composting process was observed in Y, K and YK composting systems. *Firmicutes* and *Proteobacteria* were the most dominant phylum and the higher abundance of *Bacillus sp.* in all composting systems indicated very active thermophilic conditions during composting process.

BAHÇE ATIKLARI, MUTFAK ATIKLARI VE İNEK DİŞKİSİNİN KOMPOSTLAŞTIRILMASINDA ATIK AZALIMININ VE MİKROBİYAL KOMÜNİTE PROFİLLERİNİN KARŞILAŞTIRILMASI

Etkili ve düşük maliyetli bir yöntem olan kompostlaştırma hayvan dışkısı, biyobozunur belediye atıkları, hasat artıkları, gıda üretim atıklarının toprak düzenleyici ve gübre olarak kullanılabilen değerli bir kaynağa dönüştürülebilmesine olanak sağlamaktadır. Set1 (Y, K, YKM) ve Set2 (YK, YM, KM) adlı deney setlerinde nem içeriği talaş yardımıyla 55 ± 5 değerine ayarlanmıştır. Analitik metodlar, nihai ürünlerin toprak iyileştirici olarak kullanılabilirliği açısından yönetmelikler ile karşılaştırmak ve işlem verimliliğini kontrol etmek için uygulanmıştır. Buna ek olarak, tüm sistemlerde termofilik aşamadaki bakteri kompozisyonları, yeni nesil dizileme kullanılarak analiz edilmiştir. Bu çalışmada, tüm kompost sistemlerinde, elektriksel iletkenlik, nihai C / N oranı ve uçucu katı madde içeriği yönetmeliklerin sınır değerlerini karşılamıştır. Y ve YKM kompostlarındaki pH'ların düzenlemelerde belirtilen 5.5 ila 8.5 aralığında olduğu bulunmuştur. Nihai nem içeriği, yönetmeliklerde belirlenen sınırlardan daha yüksek (< 30) bulunmuştur. Y, K ve YK kompostu en iyi performansı göstermiştir. *Firmicutes* ve *Proteobacteria* kompostlama sürecinde en baskın şubeler olarak tespit edilmiştir ve tüm kompost sistemlerinde *Bacillus sp.* en yaygın olarak bulunan tür olmuştur. *Bacillus sp.* en yaygın tür olması aktif bir termofilik evrenin gerçekleştiğinin göstergesidir.

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LIST OF SYMBOLS/ABBREVIATIONS

Symbol	Explanation	Units Used
ARDRA	Amplified Ribosomal DNA Restriction Analysis	
ARISA	Automated Ribosomal Intergenic Spacer Analysis	
ASAE	American Society of Agricultural Engineers	
ASTM	American Society for Testing and Materials International	
ATP	Adenosine Triphosphate	
BMSW	Biodegradable Municipal Solid Waste	
C/N	Carbon-to-nitrogen Ratio	
CEC	Cation Exchange Capacity	
CFU	Colony Forming Unit	
CM	Cow Manure	
COD	Chemical Oxygen Demand	
DEFRA	Department for Environment Food and Rural Affairs	
DGGE	Denaturing Gradient Gel Electrophoresis	
DNA	Deoxyribonucleic Acid	
EC	Electrical Conductivity	dS/m
EDTA	Ethylenediaminetetraacetic Acid	
EU	European Union	
FAS	Free Air Space	
FS	Fresh Sample	
GC	Gas Chromatography	
H	Height	cm
IGS	Intergenic Spacer	
KW	Kitchen Waste	
L	Length	cm
MC	Moisture Content	%
mRNA	Messenger Ribonucleic Acid	
MSW	Municipal Solid Waste	
NGS	Next Generation Sequencing	
OTUs	Operational Taxonomic Unit	

PCR	Polymerase Chain Reaction	
PCR	Polymerase Chain Reaction	
PGM	Personal Genome Machine	
QIME	Quantitative Insights into Microbial Ecology	
RFLP	Restriction Fragment Length Polymorphism	
RISA	Ribosomal Intergenic Spacer Analysis	
RNA	Ribonucleic Acid	
rRNA	Ribosomal Ribonucleic Acid	
SD	Sawdust	
SSCP	Single Strand Conformation Polymorphisms	
TGGE	Temperature Gradient Gel Electrophoresis	
TKN	Total Kjeldahl Nitrogen	mg/kg
TOC	Total Organic Carbon	mg/kg
TS	Total Solids	g TS/g FS
TVS	Total Volatile Solids	g TVS/G TS
VOCs	Volatile Organic Compounds	
VS	Volatile Solids	g TVS/g TS
W	Width	cm
YW	Yard Waste	

1. INTRODUCTION

Over the last decades, the quantum of waste generated by industrial and agricultural activities increased significantly and led to various environmental problems. Municipal solid waste generated by households, institutions, commercial establishments and industries has major contribution to both atmospheric and hydrologic environmental problems. Municipal solid waste (MSW) is composed of solid waste including grass clippings, food scraps, newspapers, bottles, clothing product packaging, batteries, paint and appliances (Farrel and Jones, 2009). Biodegradable municipal solid waste (BMSW) including food waste, green waste, paper and cardboard waste is 60% and 70% of MSW depending on local conditions, industrialization degree, eating and drinking habits, and, climate (Garcia et al., 2005). According to Turkish Statistical Institute, the amount of municipal waste by disposal methods was 28.011 million ton at total in 2014 in Turkey and the production rate of MSW is approximately 2.77 kg MSW per person in Turkey (TUIK, 2015).

Disposal of wastes with high biodegradable content to landfills leads to decrease in available landfill space as well as production of gaseous effluents consisting of volatile organic compounds (VOCs), carbon dioxide and methane, and a leachate with high COD and TOC concentrations in the landfills (Park and Shin, 2001). In this regard, adapting an environmental-friendly, cost-effective and safe alternative to the disposal of MSW to landfills is profoundly important in order to reduce the amount of waste in landfills.

In many civilized countries, in the scope of integrated waste management, first priority has been prevention or minimization in generation and disposal of MSW to landfill has been considered most undesirable option (Taylor and Kosson, 1996; Sakai et al., 1997; Koufodimos and Samara, 2002). Furthermore, some countries advocate that any waste that does enter landfill must first be treated in order to minimize its impact on environment (Farrel and Jones, 2009). The European Union (EU) Landfill Directive requires by 16 July 2016, the reduction of biodegradable municipal solid waste (BMSW) entering the landfill to 35% of the total amount by weight of BMSW generated in 1995 (EU, 1999)-

Treatment of MSW includes physical, thermal, chemical and biological processes that aim to reduce the volume of waste or hazardous nature, facilitate the handling and enhance recovery by changing the characteristics of waste (DEFRA, 2005). The most usual management methods are composting, anaerobic digestion, incineration, thermolysis and gasification (Crowe et al., 2002).

Agricultural waste containing animal manure is another source of solid waste. The quantity of cattle manure produced in Turkey is around 52.9 million ton per year (TUIK, 2015; Berkes and Kışlaoğlu, 1993). Untreated animal manure results in odor, emissions such as ammonia, methane and nitrous oxide (Hörnig et al., 1999). Anaerobic digestion is most common treatment method for animal manure which provides biogas as a renewable energy source. However, as a biodegradable waste, composting of animal manure is also preferable option to reduce its environmental impacts (Guo et al., 2016).

Composting is an environmentally-friendly process which converts organic wastes including animal manures, municipal biosolids, crop residues, food processing wastes and biodegradable municipal solid waste to humus-like end product (Westerman et al., 2005). This process defined as the biological decomposition of organic matter under controlled aerobic conditions enables to obtain a stabilized end product by reducing bulk and odor, concentrating nutrients, killing pathogens (Farrell et al., 2009). The process is carried out by a diverse microbial population, which dynamics vary greatly both temporally and spatially, and generally involves the development of thermophilic temperature as a result of biologically produced heat (Swan et al., 2002). Moreover, as microbial dynamics are highly dependent on physicochemical conditions as well as organic wastes used as a compost product, microbial community structure at different stages of the composting process is barely known.

The interest in composting technologies at different levels of sophistication have been raised greatly in numbers for the disposal of MSW, sewage sludge, cow and pig manures in last 50 years (Haug, 1993). Basic scientific principles of composting system have been conducted in order to understand the process in many years. However, engineering designs depending on trial and error approach are limited to known substrates or previously tested process conditions (Cerenzio, 1987; Richard, 1997). The numerous studies relevant to food

waste composting in various composting systems have been conducted in last decade (Richard, 1997; Donahue et al., 1998; Nakasaki et al., 1998; Nakasaki and Ohtaki, 2002; Lemus and Lau, 2002; Das et al., 2003; Kwon and Lee, 2004; Nakasaki and Nagasaki, 2004; Seo et al., 2004; Cekmecelioglu et al., 2005; Komilis and Han, 2006; Chang et al., 2006). In addition, yard waste consisting of leaves, grass clippings, wood chips and prunings composting studies have gained considerable attention (Campbell et al., 1997; Benito et al., 2003; Kumar et al., 2010; Brown and Li, 2013).

Hence, in this study, comparison of yard waste, kitchen waste and cow manure in terms of waste degradation and bacterial community profiles was conducted in order to contribute the knowledge of composting process using different raw materials. Moreover, this study is a great example for local municipal waste management that reduces to organic wastes loads to landfills and also it contributes to sustainable development. Introducing new methods like the one that is investigated in the current study, would reduce the organic waste contribution to landfills and bring better local municipal waste management solutions.

2. LITERATURE SURVEY

2.1. Composting

Composting is a widely accepted technique which leads to economic and environmental attributions associated with municipal landfill capacity; costs related to landfilling and transportation of materials; reducing the use of commercial fertilizers (He et al., 1992, Otten, 2001, Hansen et al., 2006 and Zhang et al., 2006).

There is an extensively increased interest in composting in the last few decades as it transforms carbon-rich waste to a valuable resource. It is a cost-effective and environmentally sustainable method converting waste into stabilized, humus-like end product which can be used as fertilizer in agriculture, an animal feedstuff (Garcia et al., 2005), remediation of heavy metal contaminated sites and organically polluted sites (Farrell et al., 2009).

Composting process is conducted in anaerobic and also anaerobic conditions. Aerobic biological conversion process is utilizing aerobic bacteria in order to convert organic fraction of waste to humus like end product in the presence of O_2 . New cells, CO_2 , H_2O , NH_3 , SO_4^{-2} , heat, and compost are the end products of the composting process. Anaerobic biological conversion processes are utilizing anaerobic bacteria in conversion of organic materials to humus like end product in the absence of O_2 . New cells, CO_2 , CH_4 , NH_3 , H_2S , and compost are the end products of the composting process. The advantage of anaerobic composting is to achieve combustible gas, CH_4 which can be used as renewable energy source (Vigil, 1994).

Strict control of blast volume, high energy input and skilful operation of mechanical equipment are essential for biological decomposition of organic matter under aerobic conditions (Yu et al., 2015). In contrast to aerobic composting, maximum retention of sludge nutrients and lower power consumption are required while breakdown of solid organic fraction by microorganisms. Anaerobic composting has disadvantages including low stabilization degree, long period of the process, odor problem related to the formation

of H_2S and dissatisfactory maturity (Himanen and Hanninen, 2011; Banegas et al., 2007). Therefore, due to the problems facing in anaerobic composting, aerobic composting is widespread application comparing to anaerobic composting process.

2.2. Fundamentals of Aerobic Composting

Aerobic composting is conversion organic fraction of waste in the presence of O_2 and formation of end products consisting of new cells, CO_2 , H_2O , NH_3 , SO_4^{2-} , heat, and compost (Figure 2.1.).

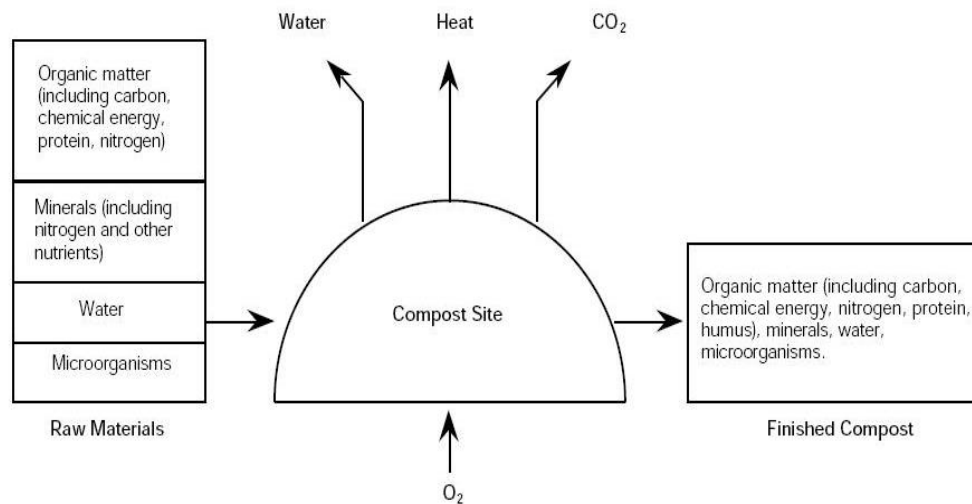


Figure 2.1. The composting process (Rynk et al., 1992).

Composting process is developed in two main phases including the biooxidative phase and the maturing phase also called curing phase (Bernal et al., 1996; Chen and Inbar, 1993). The biooxidative phase is divided into three stages (Keener et al., 2000) : an initial mesophilic phase (temperature range: $10\text{-}42^\circ\text{C}$), varying from a few hours to a couple of days where degradation of simple compounds such as sugars, amino acids, proteins, etc., is occurred via mesophilic bacteria and fungi; (ii) a thermophilic phase (temperature range: $45\text{-}70^\circ\text{C}$), lasting a few days, several weeks especially for food wastes, where thermophilic microorganisms degrade fats, cellulose, hemicellulose and some lignin, during this phase the degradation of organic content is at maximum while the destruction of pathogens occurs; (iii) cooling phase which mesophilic organisms have a role to recolonize the remaining substrates consisting of sugars, cellulose and hemicellulose and the microbial

activity is reduced relevant to the depletion of degradable organic matter (Tuomela et al, 2000; Insam de Bertoldi, 2003). During composting process, the organic compounds are converted to CO_2 and NH_3 , with the consumption of O_2 . Maturation phase ensures the stabilization and humification of organic matter and a mature with humic characteristics end product is acquired (Bernal et al., 2009).

The succession of the composting depends on various physical and chemical parameters needed to be adjusted appropriately to set convenient conditions for biotransformation of organic substrates (López-González et. al., 2015).

Chemical requirements for composting consist of carbon-to-nitrogen ratio (C/N), the balance between oxygen and moisture content, and pH. Compost physics is related to aeration, mechanism of heat loss, moisture, particle size and size of compost system. Variety of group of biological organisms including bacteria, actinomycetes, fungi, protozoa, invertebrates have the key role in decomposition process (Table 2.1.) (Ryckeboer et al., 2003).

The different steps of the process are defined as the temperature of the mass which lead to growth of the biological organisms involved in composting process occur according to the temperature of the mass, which defines the different steps of the process (Keener et al., 2000).

Predominance of bacteria occurs early stage of composting, fungi presenting during all composting process predominate whilst moisture content drops to the level below 35% and are not active at temperatures higher than 60°C . Fungi are able to degrade resistant polymers together with actinomycetes which predominate during stabilisation and curing (Bernai et al., 2009). The efficiency of the system is directly linked to these parameters associated with one another (Jayasree and Balan, 2012).

Table 2.1. Functional groups of organisms in composting process.

Tertiary Consumers	Secondary Consumers	Primary Consumers	Organic Residues
Centipedes	Springtails	Actinomycetes	Leaves
Predatory mites	Feather-winged beetles	Fungi	Grass clippings
Rove beetles	Some types of mites	Snails	Plant debris
Pseudoscorpions	Nematodes	Slugs	Food Scraps
	Protozoa	Millipedes	Fecal Matter
		Sowbugs	Soil invertebrates
		Some types of mites	
		Nematodes	
		Protozoa	

2.3. Environmental and Operational Factors Affecting Composting Process

2.3.1. Substrate

In composting process, substrate is the waste to be composted. It refers the organic material used as compost product. The origin of all degradable substrates is either of animal, plant, or microbial. Plant materials are involved in the largest fractions while animal tissues and microbial components are minor fractions of any mixture (Insam et al., 2010). The chemical and physical properties of the substrate is crucial in efficiency of the composting process in terms of course and rate like any other biological systems. The type of substrate is essential to balance the nutrients that dictate the feasibility of the process. Primary physical characteristics of the substrate are relevant to moisture content and particle size of compost product. Some of the most important chemical characteristics are associated with complexity, nature, molecular size and also elemental makeup of the molecules. The characteristics including the complexity and the nature of the molecular structure of the substrate link to the assimilability of the nutrients by the various microorganisms in the system (Diaz et al., 2007).

Lignin is a major component of plants and it may consist around 30% of wood. Degradation of lignin is a difficult task due to the extraordinary various of bonding among them. Fungi which primarily degrade lignin are often pathogens. After the degradation of lignin, cellulosic components are left behind. *Trametes versicolor* and *Streeum hirsutum* are known as lignin-degrading fungi (Insam et al., 2010).

Cellulose presented in almost every type of organic waste is the most abundant natural organic compound. When the cellulose is encrusted with lignin like in wood and straw, fungi are generally responsible for cellulose degradation. *Chaetomium*, *Fusarium*, and *Aspergillus* are well-known fungal cellulose degraders. The myxomycetes and relevant taxonomic groups of *Cytophaga*, *Polyangium*, and *Sorangium* are responsible for cellulose degradation. Moreover, *Pseudomonas* and related genera are found to be as cellulose degraders. As oxygen is limited in the process, mesophilic and thermophilic *Clostridia*, and also *Fibrobacter* species are important in cellulose utilization. Hemicellulosic compounds consisting of xylan, pectin and, starch is degraded by various fungi and also bacteria. *Xylanases* enzymes are produced to degrade xylan mostly found in straw, bagasse, and wood. *Pectinases* ensures pectin degradation and three groups of enzymes including *phosphorylases*, *amylo-1,6-glucosidase* and *alpha-amylase* have the key role in starch degradation (Insam et al., 2010).

2.3.2. Types and sources of nutrients

The macronutrients for microorganisms are carbon (C), nitrogen (N), phosphorus (P), and potassium (K). Manganese (Mn), cobalt (Co), copper (Cu), magnesium (Mg), and a number of other elements are the macronutrients for microbes.

The ensuing enzymatic makeup of individual microbe procures availability of nutrients. A certain number of microbes having enzymatic complex are not able to degrade and utilize the organic material found in a freshly generated waste; while the decomposition products are utilized by other microbes as a source of nutrients. The composting of a waste is the consequence of the dynamic activities of different groups of microorganisms in which one group compose the pathway for its successor group.

Materials including lignin and chitin are decomposed very slowly in spite of the fact that all other optimum environmental conditions are maintained as these organic materials are significantly resistant and refractory to microbial utilization. Nitrogen presented in peptide, proteinaceous, or amino acid form is easily available. However, it is difficultly available in chitin and lignin. Moreover, cellulose-C is readily available to fungi; whereas it is unavailable to the various of the microorganisms.

2.3.3. Carbon to nitrogen ratio (C/N)

Carbon to nitrogen ratio (C/N) of organic waste to be composted is the most important factor supporting the nutritional needs of the microorganism active in the system. The adequate conditions can be achieved when C/N ratio is in the range of 25-35 as the optimum microbial activity requires 30 parts of C per unit of N. Almost 30 parts of carbon for each part of nitrogen are utilized via living microorganisms in their metabolism. CO₂ (ATP) is formed by the oxidation of about 20 parts of carbon used for metabolic activities and 10 parts of carbon is utilized for the synthesis of protoplasm, cell or membrane and also storage products. Microorganisms consumes nitrogen to synthesize protoplasm. In fact, the average C/N in many bacteria varies between 9 to 10 (Diaz et al., 2007).

High C/N reduces biological activity in composting process leading to long operational time to decrease the C/N to a more suitable level (Golueke, 1977). The carbon bound in compounds such as chiefly lignin, some aromatics, and some physical forms of cellulose broken down formidably by biological activation wherefore carbon in microbes is formed slowly (Diaz et al., 2007).

The downside of having the amount of carbon over that of nitrogen lower than 20 is the loss of nitrogen through ammonia volatilization. High temperature and basic pH ranging 8 to 9 ensures this loss which actualizes at the beginning of the process during thermophilic stage. By reducing the nitrogen content of the end product, loss of ammonia limits the value of organic fertilizer. Compost with too low C/N can be phytotoxic to plant roots due to the ammonia released (Zucconi et al., 1981a,b).

C/N ratio should not be accepted as the only one factor to identify the nature of carbon in the wastes to be composted. The order of degradation of compounds is as follows: carbohydrates>hemicelluloses>cellulose>cellulose=chitin>lignin. Therefore, the rate at which organic materials are broken depends on the complexity of C compounds. On the contrary, organic wastes containing mostly simple carbohydrates such as fruit and vegetable are easily degradable while barks, trees, nutshells and leaves composed of cellulose, hemicellulose and lignin have lower decomposition rate (Epstein, 1997).

The biodegradable fraction of a substrate in terms of volatile solids (VS) can be calculated by following practical formula: biodegradable fraction = $0.83 - 0.028 \times \text{lignin content of VS}$ (Chandler et al., 1980). As there is no lignin-free plants, percentage of degradable fraction of VS cannot reached to 83%. Moreover, lignin is required as a precursor of the humic-like substances.

CO₂ concentration increases rapidly due to the microbial respiration reducing the C level in the mass. However, when the decrease in C content is higher than that of N, C/N ratio will be reduced in case of low ammonia volatilization and of negligible N content in leachate (Maheshwari, 2014). Therefore, C/N diminishes constantly when optimum conditions are procured for the composting process due to the biological mineralization of carbon compounds converting into (Diaz et al., 2007).

2.3.4. Hydrogen ion level (pH)

The pH reflecting the acid concentration is a function of the accumulated acid production and the decomposition of acids in order to produce CO₂ and heat (Sundberg, 2005). While the accumulation of acids occurs continuously, the pH decreases due to the higher rate of acid production than acid decomposition. As both rates of acid production and decomposition equal, the pH value reaches a minimum. The time for pH to reach a minimum is defined as acidification time which can be considered as an index for the composting rate.

The microbial formation and decomposition of organic acids are dependent on the oxygen level and temperature. According to previous studies, pH values varying between 7.4 to 8.8 during composting process indicate that optimum conditions relevant to microflora and oxygenation are achieved (Michel and Reddy, 1998; Eklind and Kirchmann, 2000; SánchezMonedero et al., 2001). Furthermore, Beck Friis et al. (2001) asserted that the pH of 6.7–9.0 supports good conditions for microbial activity during composting process. According to de Bertoldi et al. (1983) and Miller (1992) optimum values of pH are ranging from 5.5 to 8.0.

Higher oxygen concentration leads to lower maximum concentrations of organic acids in the compost and a faster decomposition of the acids, and thus a faster rise in pH (Beck-Friis et al., 2001). Degradation of organic matter in compost product results in the liberation of NH_4^+ leading to higher pH values at the beginning of composting, usually ranging from 7 to 9 (Tang et al., 2004).

It is reported that the microbial community is severely impeded to coincide with low pH in the matter (Day et al., 1998; Beck-Friis et al., 2001). The change from mesophilic to thermophilic conditions during the initial stage of composting related to a change in pH from acidic (pH=4.5–5.5) to alkaline (pH=8–9) (Beck-Friis et al., 2001).

2.3.5. Temperature

Temperature is widely accepted as an important environmental variable in composting efficiency (Namkoong and Hwang, 1997; Joshua et al., 1998). The energy released during the rapid microbial respiration procures heat which rises the temperature of the composting process (Farrel and Jones, 2009).

The microorganisms are not precisely effective in converting and utilizing the chemical energy bound in the substrate. Heat energy is generated by energy not used. The amount of heat rises within the activity of microbial population (Diaz et al., 2007). Therefore, the temperature profile indicates the microbial activity and the occurrence of the composting process (Bernal et al., 2009). Due to the degradation of easily decomposable components of the waste including sugars, starch and simple proteins, the rise in

temperature is exponential. During this phase, the exponential microbial growth is observed. Whilst the readily decomposable material is limited, bacterial activity diminishes and as a result temperature drops.

Temperature increase within composting material depends on the initial temperature of the system, metabolic heat evolution and the conservation of heat (Miller, 1992). In lag period, temperature rises gradually. Thereafter, in appropriate conditions, temperature increases exponentially with time until it begins to plateau at around 65 to 70°C. The thermophilic period depending on the system and the organic wastes used in the system persists for 1 to 3 weeks. Then, temperature begins to decrease gradually until temperature of the system has descended to ambient air temperature (Diaz et al., 2007).

Microbial metabolism and also the population dynamics of microorganisms are highly affected by temperature. High rates of decomposition is acquired by the achievement of minimum temperature levels which is essential to an effective composting process (Finstein and Morris, 1975; Finstein et al., 1986). A study conducted by Mosher and Anderson (1977) has demonstrated that temperatures of composting products below 20°C substantially incline to slow or even stop process. Temperatures in excess of 60°C have been shown to decrease the microbial activity, and above this temperature, the activity of microbial population diminishes due to excessive number of thermophilic microorganisms (Milller, 1992). Furthermore, several researchers have indicated that the stagnation in the microbial activity occurs if the temperatures reach to 82°C (Neil and Wiechers, 1978; Finstein et al., 1986; Fermor et al., 1989). The optimum composting temperatures were found to be in the range of 52-60 °C by MacGregor et al. (1981). However, Miller et al., (1989, 1990) have demonstrated that in order to produce high quality compost, the temperatures in this range are not essential. In addition, some researchers have found that lower temperatures may rise the activity of microorganisms (Suler and Finstein, 1977; McKinley et al., 1986).

2.3.6. Moisture content

Moisture content is an important variable which ensures a medium for the transport of dissolved nutrients needed for the physiological and metabolic activities of

microorganisms (Stentiford, 1996; McCartney and Tingley, 1998). The optimum moisture content for composting process varies with the type of composting product, physical state and the size of the particles. The moisture content in the starting material generally should be in the range of 50% to 60% (Tiqua et al., 1998; McKinley et al., 1986; Suler and Finstein, 1977).

Excessive water higher than 60% in the starting material tends to favor anaerobic conditions due to lessening of the space available for air and water. Excessive moisture could prevent and arrest the ongoing composting activities, resulting in a slower process and low quality final product (Diaz et al., 2007). Moreover, very low moisture content would result in early dehydration during composting, which will halt the biological process, thus giving physically stable but biologically unstable end products (Bertoldi et al., 1983). However, the maintenance of suitable conditions for moisture content will be based on the nature of feedstocks in the compost mixture (Agnew and Leonard, 2003). Composting of vegetable wastes with an initial water content of excess in 82% was conducted successfully by Vallini and Pera (1989). Liao et al. (1993) reported that composting of swine manure solids mixed with sawdust in a 5:1 ratio by weight with a 71% moisture content was feasible as long as sufficient air in the compost pile to maintain oxygen needed for microorganisms was supported.

Permissible moisture content and oxygen availability are strongly interrelated. The oxygen supply to microorganisms is the ambient air and also the interstitial air. Whilst the rate of diffusion of ambient air into the mass reaches inadequate levels, the air trapped within the interstices of composting products becomes the major source of oxygen. Consequently, high moisture content displacing most of the air from the interstices develops anaerobic conditions (Diaz et al, 2007).

The maximum permissible water content that is a function of particles of composting products indicates to the degree of the resistance of individual particles to compression. The permissible moisture content increases with the structural strength of the material to be composted. The materials including woodchips, rice hulls and corn stover have up to 75-80% permissible moisture content. However, amorphous materials which have little or no structural strength such as paper, fruit wastes, sludges, and animal manures have an upper

permissible moisture content in the range of 55-60%. In order to compost those materials, it is required to add “bulking” agent having a high degree of structural strength to the system (Diaz et al., 2007). The most commonly used bulking agents in the recent studies are cornstalks, rice straw, cotton waste, peanut shells, sawdust and woodchips (Yang et al, 2013; Shao et al., 2014; Zhou et al., 2014). Leonard et al. (1997) studied the efficiency of composting process when the initial moisture content was excess in recommended limit of 60% and the structural strength of compost material was extremely low. The mixture of a sludge-straw-sawdust with a moisture content of 80% used for composting process and the structure of the mixture collapsed. In order to restore porosity, the addition of straw was required. Therefore, optimum conditions for composting process are obtained with an initial moisture content which provides adequate water for microbial activity while maintaining sufficient oxygen supply and structure (Agnew and Leonard, 2003).

During composting, evaporation of a large quantity of water can be acquired, in order to sustain appropriate conditions for microbial activity being affected by decrease in the rate of decomposition, rewetting of the system is required (Bernal et al., 2009). Based on work using a laboratory composter, it is determined by Viel et al (1987) water lost through evaporation was lower than water released through microbial activities. The changes of moisture content during composting process depend on the method of composting, the bulking agent, and the feedstock used in the system (Epstein, 1997). At the end of the process, the moisture content of the final product should be approximately at 30% level in order to avoid any further biological activity in the stabilized material (Diaz et al., 2007).

2.3.7. Aeration

In composting, system designs have been improved for the provision of oxygen to the composting mass. Entrapped air in the composting mass, during the microbial activities, varies in composition. While carbon dioxide concentration is rising progressively, oxygen content reduces significantly. The sum of carbon dioxide and oxygen level in the content is approximately 20 %. Carbon dioxide concentration is varying from 0.5 to 5 % and oxygen from 15 to 20 %. When the level of the oxygen drops off this range, anaerobic conditions occurs due to excessive number of anaerobic microorganisms over aerobic ones. In order to maintain aerobic conditions, a constant oxygen supply is required. Otherwise, anaerobic

respiration and fermentation processes predominate the system leading to altered microbial activities (Diaz et al., 2007).

2.4. Microbiology of the Composting Process

Wild array of microorganisms is involved in composting process. Compost microbes are composed of useful microorganisms performing the recycling of organic waste and other that are mainly detrimental for environment, plant, animal and human. However, the upside of the process is the 'inactivation' of the harmful microbes and providing beneficial microbial community growth with the help of favorable conditions of oxygen, moisture content and C/N ratio (Fuchs, 2010). Bacteria, actinomycetes, fungi, and possibly also protozoa and algae are responsible for the process.

During the various composting stages, changes in the population of bacteria, fungi, and actinomycetes are observed, each of which being adapted to the current environment. Therefore, the variations of microbial communities predominant during the process. Domain organisms utilizing the initial substrates provide a physico-chemical environment appropriate for secondary decomposers by creating metabolites for the other.

Temperature fluctuations at the beginning of the process results in a rapid transition from mesophilic to thermophilic microflora (Fig. 2.2.). The high temperature inhibits the initial mesophilic populations while the thermophilic microflora has not yet growth due to the insufficient temperature level. At temperatures above 60 °C, the optimum conditions for thermophiles are generated and thermophilic phase concludes when the heat dissipation is higher than the heat production as a result of the depletion of easily degradable substrates. During cooling phase, decrease in microbial activity and heat output are observed due to the limited nutrients in the system. During the maturation phase, hardly degradable compounds such as lignin humus complexes become limiting factor for the microbial activity (Ryckeboer et al., 2003).

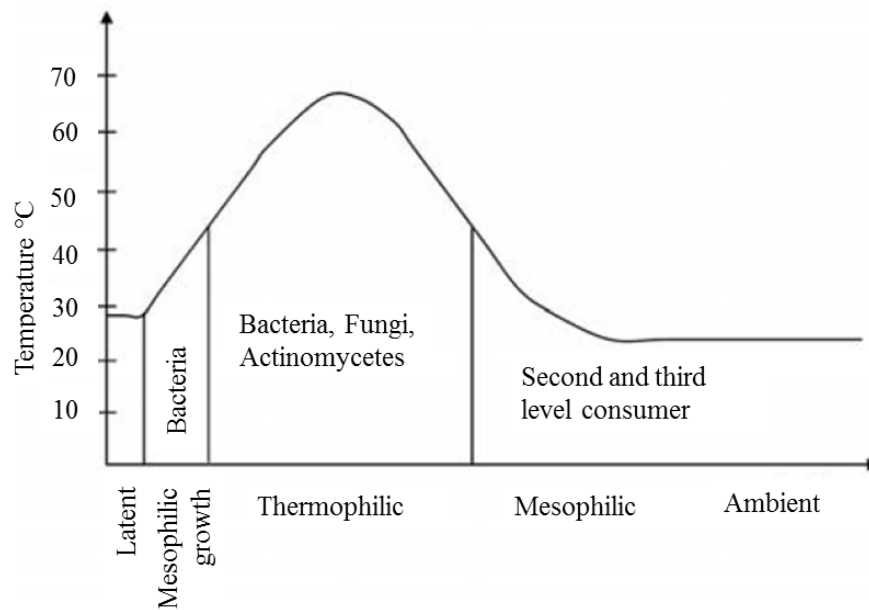


Figure 2.2. Changes in microbial diversity during composting process (Bhatia et al., 2015).

2.4.1. Microbial population

In initial phase of the composting process, easily degradable materials consisting of sugars and proteins are consumed by mesophilic and thermotolerant fungi and bacteria referred as primary decomposers (20-40 °C). During this phase, the competition between fungi and bacteria occurs and subsequently fungi is outcompeted within a few hours or days due to increase in pH resulted from ammonification. Bacteria are nutritionally domain group of organisms in composting process using wide range of enzymes for the degradation of various organic matters. Moreover, the growth rate of fungi is slower than bacteria which results in a competitive disadvantage during those phases of the process consisting of rapid changes in substrate availability and environmental parameters. As a result, the decomposition and heat generation actualize mostly by bacteria during the initial step of the composting process (Ryckeboer et al., 2003; Insam et al., 2010).

In thermophilic stage, mesophilic microorganisms are inactivated and thermophilic and/or thermotolerant bacteria, actinomycetes and fungi species diversity escalate (Beffa et al., 1996b). In early thermophilic stage of composting, proteins are metabolized and subsequently the substrates become more alkaline as a result of liberation of ammonium (Thambirajah et al., 1995). As temperature increases above 30 °C, actinomycetes,

peculiarly streptomycetes causing the characteristic earthy smell of soil and compost, are in an endeavour. After degradation of easily decomposable organic content by fungi and bacteria, actinomycetes consume natural polymers and may be responsible for inhibition of microbial growth by generation of antibiotics, lytic enzymes or even by parasitism (Ryckeboer et al., 2003). C and N sources for actinomycetes are cellulose and hemicellulose from plant material, chitin from fungus and soil fauna, lignin and humus. (Lacey, 1973; Hardy and Sivasithamparam, 1989; Beffa *et al.*, 1996a).

At temperatures varying between 50 to 60 °C endospore-forming bacteria like *Bacillus spp.* dominates (Herrmann and Shann, 1997; Ryckeboer et al., 2003). When temperature increases above 65 °C, thermophilic bacteria like *B. stearothermophilus* are responsible for the degradation process. At temperature ranging between 40 to 80 °C, with optimum growth from 65 to 75 °C, non-spore forming bacteria *Thermus/Deinococcus* group oxidizing sulfur and hydrogen play a major role in decomposition (Beffa et al., 1996b). In thermophilic stage, temperature may exceed 80 °C and such high temperatures lead to destruction of human and plant pathogens, demolition of weed seed and insect larvae (Insam et al., 2010). However, it is determined by Millner et al (1987) the suppression of *Salmonella spp.* was maintained more efficiently in composts at 55 °C comparing to in those at 70 °C. Moreover, temperatures exceeding 70 °C may result in delay in regrowth of mesophilic populations and appropriate reinoculation schemes can overcome this problem (Insam et al., 2010). In recent researches, *Firmicutes*, *Proteobacteria*, *Bacteroidetes* and *Actinobacter* are found to be dominant phyla at thermophilic stage of composting process (Partanen et al., 2010; Zhang et al., 2015b, 2016). Zhang et al. (2015a) reported that the high abundance of *Firmicutes* might come from its thermotolerance. Moreover, the abundance of *Proteobacteria* and *Bacteroidetes* might be applied to the lower temperature (Zhang et al., 2015b).

The growth of thermophilic fungi occurs when the temperatures are up to 55 °C and the substrate rich in cellulose and lignin are available in the system. Toumela et al. (2000) reported that a few Basidiomycota such as the white-rot fungus *Phanerochaete chrysosporium* (anamorph *Sporotrichum pulverulentum*) grow efficiently at elevated temperatures. The optimum growth was observed at temperatures around 36 to 40 °C and even at 46 to 49 °C. Temporary anoxic conditions may originate in thermophilic stage and

as oxygen supply is a limiting factor for fungal generation, their population in the process becomes negligible (Insam et al., 2010). Humification process of compost materials correlated with the degradation of lignin is maintained mostly by fungi if the optimum conditions are settled in thermophilic phase. Otherwise, bacterial species including *Flavobacterium*, *Pseudoxanthomonas*, *Sphingobacterium composti* (Karadag et al, 2013), *Bacillus* (Tian et al, 2013), *Ureibacillus* (Ting et al., 2013) and *Streptomyces* (Lu et al., 2013) are responsible for the lignin degradation. It is also reported that Bacilli and Clostridium group also play an important role in hydrolisation of cellulose under thermophilic and anoxic conditions (Lv et al, 2015; Maeda et al., 2010).

Whilst the activity of the thermophilic diminishes due to exhaustion of substrates, the temperature starts to decline. Recolonization of the substrate is maintained by mesophilic organisms originating from surviving spores, through spread from protected microniches, or from external inoculation. In second mesophilic stage, the characteristic of domain organisms is the ability to degrade starch or cellulose. Bacteria such as *Cellulomonas*, *Clostridium*, and *Nocardia* and fungi of the genera including *Aspergillus*, *Fusarium*, and *Paecilomyces* are cellulose degraders in this phase of composting process (Ryckeboer et al., 2003; Insam et al., 2010).

In maturation phase, the quality of the substrates decreases and the compounds comprised of lignin-humus complexes become predominant. During this phase, the fungal population overcomes bacterial communities due to the competitive advantages of fungi under lower water content and poorer substrate availability (Insam et al., 2010). Bacteria belonged to genus *Arthrobacter* are domain natural bacterial flora of soils and their presence and numbers could be used as indicator of mature compost. Hence, species diversity seems to be associated with compost maturity evaluation and also stability of the end product (Beffa et al., 1996a).

2.4.2. The techniques to assess microbial diversity during composting

Bacterial cultivation technique ensures the identification of only a small fraction of microorganisms (0.01-10%) in natural environments. Accurate molecular methods consisting of DNA extraction, followed by PCR amplification and ensuing 16s rRNA

genes developed to minimise the limitation relevant to culture dependent approaches. However, it is considered that cultivation techniques may also give information about bias (Bhatia et al, 2013).

Wide range of methods including DGGE (Ishii et al, 2000; Steger et al, 2007) (denaturing gradient gel electrophesis), SSCP (Peters et al, 2000) (single strand conformation polymorphisms), ARISA (Schloss and Handelsman, 2003) (automated ribosomal intergenic spacer analysis), cultivation (Beffa et al, 1996b; Ryckeboer et al, 2003), restriction analysis and sequencing (Dees and Ghiorse, 2001), and microarrays (Franke-Whittle et al, 2009), have been conducted to analyze compost microbiota to investigate which microorganisms are present during different stages of the process (Fig 2.3.).

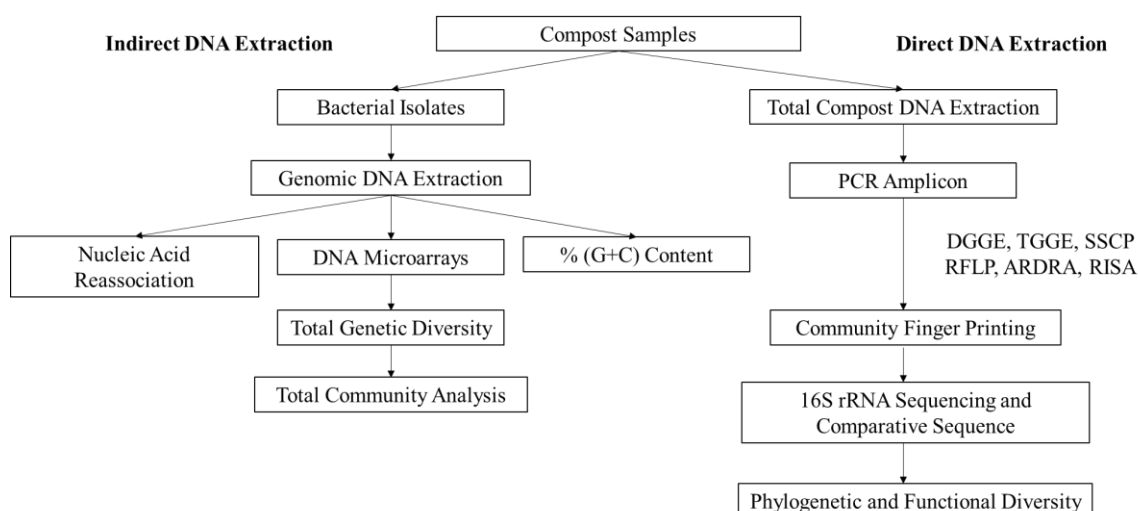


Figure 2.3. Molecular approach for analyzing microbial diversity (Agrawal et al., 2015).

2.4.2.1. Cultivation techniques. Isolation and enumerations of microorganisms from compost have been so far conducted on rich organic complex media to detect microbial diversity during composting process and the traditional plating techniques such as pour plate, spread plate and straking methods have been performed by various researches (Kane and Mullins, 1973; Finstein and Morris, 1975; Nakasaki *et al.*, 1985a, 1985b; Strom 1985a, 1985b; Hardy and Sivasithamparam, 1989; Davis *et al.*, 1991; Beffa *et al.*, 1996a; Choi and Park, 1998; Ryckeboer *et al.*, 2003; Van Gestel *et al.*, 2003). The number of microbes/mL or microbes/g in a sample can be found using the pour plate technique in

which 0.1 to 1.0 ml of diluted sample is pipetted into a sterile Petri plate and then melted agar is added and mixed with the sample. Colonies, each of which represents a colony forming unit (CFU) spread on the surface as well as throughout the agar. The preferable range of CFU varies between 30 to 300 colonies/plate for an accurate count. The drawback of this method is that the embedded colonies are smaller in contrast to the ones on the surface and, excessive attention is needed to know all colonies are overlooked. Adding that, obligate aerobes may develop if deeply imbedded in the agar. In the spread method, the diluted sample is spread over an agar plate and colonies are formed on the surface. A streak plate method ensures the isolation a single species from a mixed species population (Bhatia et al, 2013).

2.4.2.2. Molecular techniques. Conventional culture techniques yield only a limited fraction of all microbes present in a sample. Molecular techniques provide an information about the microbial community in its full diversity by analyzing of single cells. In order to investigate population structures and dynamics in terms of richness, evenness and composition, genetic fingerprinting methods are required (Fakruddin, 2003).

Denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) are similar analysis which examine microbial genetic diversity. DGGE/TGGE have been preferable methods in environmental microbiology to isolate bacteria, analyse enrichment cultures, compare DNA extraction techniques, study community complexity, monitor population shifts, investigate sequence heterogeneity of 16S RNA/18S RNA genes, detect PCR and cloning biases, and screen clone libraries (Muyzer and Smalla, 1998; Nicolaisen and Ramsing, 2002). These techniques are based on the extraction of DNA from soil sample, followed by the PCR amplification of 16S or 18S rRNA genes using universal primers. As separation on a polyacrylamide gel with a gradient of increasing concentration of denaturants including formadide and urea will occur relied on melting behaviour of the double-stranded DNA, the 5'-end of forward primer includes a 35 to 40 base pair GC clamp to provide that at least some part of DNA remains double stranded. On denaturation, melting of DNA being sequence specific ensues in domains, and DNA migrates differentially through the polyacrylamide gel (Muyzer and Smalla, 1998). After denaturation step, the visualisation of DNA bands in DGGE/TGGE profiles can be conducted using ethidium bromide, SYBR Green I, or silver staining

(Agrawal et al., 2015). Sequence variations in PCR-amplified DNA fragments of identical length are utilized and resolved based on differences in mobility in polyacrylamide gels consisting of gradient of a denaturing agent (Muyzer et al., 1993). Movement of fragments is dependent on the molecular weight. However, whilst they progress into higher denaturing conditions and each reaches a melting point depending on its sequence composition. In contrast to DGGE, in TGGE approach, a uniform concentration of denaturant in the gel is utilized and rise in temperature occurs uniformly with time throughout the electrophoresis leading to more easily reproducible separations (Agrawal et al., 2015). PCR biases and laborious sample handling limits DGGE/TGGE having an influence on the microbial community and variable DNA extraction efficiency (Theron and Cloete, 2000). Different sequence variations in PCR-amplified DNA fragments may have shown similar mobility characteristics in the polyacrylamide gel. Thus, one bacterial species may represent multiple bands due to multiple 16S rRNA genes with slightly different sequences and one band may not belong to one species (Gelsomino et al., 1999; Maarit-Niemi et al., 2001).

Single-strand conformation polymorphism (SSCP) is a molecular method used to analyse microbial diversity based on DNA polymorphisms which refers that after separating the amplification products on gels, the banding patterns distinguish organisms according to the absence or presence of bands (Agrawal et al., 2015). In this method, single-stranded DNA is separated on a polyacrylamide gel according to their mobility characteristics resulted from their folded secondary structure (Lee et al., 1996). SSCP involves all the same limitations of DGGE. Adding that, as some single-stranded DNA may generate multiple stable conformation, one species may give rise to more than one band on the gel. However, a GC clamp and the construction of gradient gels are not required (Peters et al., 2000).

Amplified ribosomal DNA restriction analysis (ARDRA) also known as restriction fragment length polymorphism (RFLP) is relied on PCR amplification of 16S rRNA genes, followed by digestion of the PCR product with tetracutter restriction endonucleases and resolving restricted fragments on agarose or polyacrylamide gels. Liu et al. (1997) reported that ARDRA provides analysis of microbial structural changes in microbial communities but not give information about the microbial diversity or specific phylogenetic groups.

ARDRA-ITS ensures the analysis of fungi without prior knowledge of their genome organization using the universal primers ITS 1 and ITS 4 (White et al., 1990) which anneal to the evolutionary stable 18S and 28 rRNA genes.

Ribosomal intergenic spacer analysis (RISA) and automated intergenic spacer analysis (ARISA) are relied on fingerprinting of the microbial community. PCR amplification provides the intergenic spacer (IGS) region between 16S and 23S ribosomal subunits that later denatured and separated on polyacrylamide gel under denaturing conditions. In RISA analysis, a community specific profile is obtained and each band represents at least one organism. ARISA analysis includes a fluorescence-labelled forward primer compare to RISA (Agrawal et al., 2015).

Next generation high-through put DNA sequencing techniques gives enormous opportunities in the life sciences including genome-wide characterisation and profiling of mRNAs, small RNAs, transcription factor regions, structure of chromatin and DNA methylation patterns, microbiology and metagenomics (Ansorge, 2009). Comparing to conventional techniques, next generation sequencing (NGS) technologies provide the inexpensive production of massive number of sequence data (Metzker, 2009). Furthermore, inherent limitations of conventional methods aimed the researchers to the detection of dominant microorganisms in complex microbial consortia compositions. NGS makes it possible to determine relatively low abundant microorganisms in microbial communities (Samarajeewa et. al, 2015). Applied Biosystems (SOLiD system), Roche (454) and Illumina (HiSeq control system, the Genome Analyzer, Ion Personal Genome Machine) are the three sequencing systems which perform better performance, and have their own advantages relevant to read length, accuracy, applications, consumables, informatics infrastructure and also man power requirement. The Ion Torrent sequencing technique (Personal Genome Machine) released at the end of 2010, is based on semiconductor chip and hydrogen ion sensor, in order to detect H^+ release during hydrolysis of the triphosphate moiety due to the incorporated position of nucleotides into DNA (Loman et al., 2012; Rothberg et al., 2011). The novelty of the Ion Torrent PGM is being the first commercial sequencing machine which does not require fluorescence and camera scanning, leading to higher speed, lower cost and smaller instrument size (Liu et al., 2012).

2.5. Compost Quality Attributes, Measurements, and Variability

Composting final product is humus-like and stabilized material which can be used as a soil amendment. The term “stabilization” indicates to the oxidation of organic matter and also the conversion of organic content to a more refractory form. Maturity refers to lower level of soluble organic carbon and yet higher levels of humified substances. It is a function of the organo-chemical properties of the compost implying the presence or lack of phytotoxic organic acids (Haug, 1993; Epstein, 1997; Farrel and Jones, 2009).

When the composting process progresses, the readily degradable organics in the substrate is utilized by the various microorganisms and gradually less degradable humus materials remain. At the end of the process, the remained materials are still degradable. However, the degradation rate is less in comparison to the feedstock. The aim of the composting process is to obtain an organic soil conditioner beneficial to plants. Stabilization can be defined as the impact of organic matters on plant growth. Therefore, a stabilized end product must be released from the nuisance potential and phytotoxic metabolites.

The degree of stabilization and evaluation of the compost product can be measured by several approaches explained in following:

1. decline in temperature at the end of batch composting or curing
2. a low level of self-heating in the end product
3. volatile solid content, chemical oxygen demand, carbon content, ash content or C/N ratio referring to organic content of the compost
4. oxygen uptake rate
5. the impact on seed germination and plant growth
6. the absence of particular components consisting of ammonia, sulfides, organic acids and starch and the presence of others such as nitrate
7. increase in the redox potential
8. characteristic changes in odor production during process and odor potential of the end product upon rewetting

9. lack of insect attraction or lack of insect larvae development in the end-product
10. experience of the operator (Haug, 1993).

Compost maturity and stability are immensely important for the use of final product as soil conditioner. Chemical, physical and biological parameters assist to evaluate the system and compost quality.

2.5.1. Chemical properties of composts

Organic matter concentration is a function of the total organic C of a compost. Measurement of total organic C is mostly conducted by two laboratory methods including combustion based on high temperature furnace oxidation and ensuing direct determination of C via an infrared detector and Walkley-Black (Schulte, 1988) method relied on partial chemical oxidation of total organic C. As the Walkley-Black test ensures an estimate of organic C, combustion method is more accurate and precise measurement. The Walkley-Black method calibrated for soil organic matter is not sufficient to compost organic matter. Moreover, due to the use of dichromate for analyze, it is non-environmentally friendly procedure.

The volatile solids in the content reduce during the composting process as a result of biodegradation of organic matter by various microorganisms. The volatile solids reduction varying 45 to 60% is dependent on feedstock used at the beginning of the process. The volatile solids methods determine the estimated organic and ash concentrations of composts. The lost portion in high-temperature (550 °C) approximates organic matter; the remained portion after combustion is ash.

Cation exchange capacity (CEC) is the holding capacity of exchangeable cations consisting of potassium (K), calcium (Ca), magnesium (Mg), and sodium (Na), to negatively charged surfaces. Dissociation of acidic functional groups in organic matter are the sources of negative charges in compost. The CEC rises with the increase in pH. During composting process, compost CEC increases due to the incremental humified organic

matter. Compost CEC tests is an indicator for compost maturity providing the evaluation of potting media for container plants.

The total N content of compost is highly dependent on feedstocks, the compost process, curing, and storage. Total Kjeldahl and combustion are two laboratory methods for measurement of total N. In contrast to Kjeldahl method, combustion method provides more accurate and precise result. In the Kjeldahl method, digestion of N present in lignin occurred insufficiently and samples consisting of large amount of lignin will have less N by Kjeldahl method than combustion which detects all N forms.

Inorganic forms of N including $\text{NH}_4\text{-N}$, ammonia-N ($\text{NH}_3\text{-N}$), and $\text{NO}_3\text{-N}$ are also the useful indicator of compost maturity. During composting, NH_4 concentration in the content decreases while NO_3 level increases. Immature or unstabilized composts mainly consist of substantial amount of $\text{NH}_4\text{-N}$. The estimation of plant-available N supplied with the compost is developed by inorganic N measurement of composts.

The pH of final product ranging from 6 to 8 can vary significantly based on the feedstock, processing conditions, and the addition of any amendments. Excessive alkalinity or acidity results in inhibition on plant growth and damage to plant roots. It is essential to comply with plant requirements before applying the compost to soil. In potting media, increase in pH can be conducted by the addition of lime, and pH can be decreased by elemental sulfur (S) addition which must be of very fine particle size (Marfa et al., 1998). Saturated paste and volume addition are the two methods to measure compost pH. The paste method is based on exceeding water-holding capacity of the compost sample by the addition of water. For volume method, a defined volume of water is mixing with a defined volume of sample in a predetermined ratio. Then, for both methods the pH is determined by immersing the pH electrode into the mixture. Usually, 0.1 to 0.3 higher pH units are obtained using volume method compared to saturated paste method.

Electrical conductivity (EC) of the compost used as a measurement of soil salt content is relevant to dissolved solutes content of soil (Brady and Weil, 1996). Measurement of EC is conducted by saturated paste and volume addition method like pH measurement. In saturated method, two composts with the identical electrical conductivity but differing in

water holding capacity will give a result showing that the compost with the higher water holding capacity has a lower EC. Moreover, EC correlates with other properties consisting of texture and bulk density (Agnew and Leonard, 2003). EC analyze representing the salinity of the compost does not specify the type of salts in the compost. Salts including chloride (Cl), sodium (Na), and boron (B) have a harmful impact on the plants at elevated concentrations. Cations or anions containing calcium (Ca), magnesium (Mg), sulfate-S ($\text{SO}_4\text{-S}$), and ($\text{NO}_3\text{-N}$) are also nutrients needed for plant growth. Soluble salt content in water extracts rises in value due to the release of organic acids and soluble salts in maturation phase (Avrimelech et al., 1996; Wu et al., 2000). Excessive saline compost has a detrimental influence on root health and seed germination. Some vegetable crops including onions (*Allium cepa* L.) and beans (*Phaseolus vulgaris* L.) have a low tolerance to salts. Composts consisting of B higher than 1 meq.L^{-1} of a saturated paste extract may have an effect on sensitive crops like beans, and Cl content in excess of 10 meq.L^{-1} of a saturated paste extract may inhibit grape (*Vitis spp.*) growth (Stofella and Kahn, 2001). A study conducted by Ringer (1997) indicated that manure compost with EC ranging from 0.7 to 1.5 mS cm^{-1} had no phytotoxic impacts on plant growth. Epstein (1997) suggested that the conductivity higher than 5 mS cm^{-1} may affect plant grow by conducting phytotoxicity.

2.5.2. Physical properties of composts

Moisture content assesses some understanding of processing and storage conditions. Moisture content lower than 30% prevent further microbial activity in the stabilized end-product. During the process, water content based on feedstock and processing may fluctuate widely (Stofella and Kahn, 2001). Widely used determination method of moisture content is the gravimetric method based on drying in convection or forced-air ovens. The mass of the water present in a sample is detected drying the material and recording the mass loss. For gravimetric method, it is assumed that only water is lost during drying process (Agnew and Leonard, 2003). Microwave techniques are also satisfactory method used in moisture determination for peat (Balascio, 1990, 1992) and forages (ASAE 2000) and also for compost (Cardenas, 1977; Ramer and Leonard, 1995). The accuracy of drying samples of yard waste and manure composts with commercially-available microwave oven was conducted by Ramer and Leonard (1995). The drying times ranging from 6 to 30

minutes was dependent on the sample size varying 20 to 100 g, the material and the moisture content. Results of microwaved samples which exposed to microwaves till reached a constant weight without charring were within 1.5% of those gained using conventional oven methods. Moreover, the soil moisture analysis consisting of tensiometers and neutron moisture probes have been investigated for compost samples to establish their suitability for use in compost (Arslan et al., 1997; Pojasok, 2000). The upside of the use of tensiometers is to monitor moisture content in real time in situ (Miller, 1989). Reliable and simple way of continuous moisture determination can be achieved by using tensiometers in composting facilities (Agnew and Leonard, 2003).

Bulking density expressed as the weight per unit volume of compost is influenced by ash content, moisture content, the degree of decomposition, and particle size distribution. While bulk density rises, interstitial air level decreases, and water holding capacity referring to the amount of water held in pores after gravitational loss for a specified time is increased (Stofella and Kahn, 2001). On the other and, lower values of bulking density imply excessive substrate aeration and, obliquely, a drop in the available water fraction (Nappi and Barberis, 1993). Measurement of bulking density mostly based on the simple mass per unit volume technique as described in the ASTM standards (He et al., 1995; Glancey and Hoffman, 1994). This procedure is based on filling sample in a beaker or container of known mass and volume. The material should be compacted to avoid large spaces. Then in order to calculate the bulking density, the weight of the sample and the container is determined.

Water holding capacity is the difference between the percentage of pore space and the percentage free air space. The water holding capacity of composts can be measured by adding water slowly onto a sample for approximately two hours then the sample is dried. The amount of the water held in the sample gives the water holding capacity of compost (Leege and Thompson, 1997).

The term “particle density” also known as absolute density (Wilson, 1983; Villar et al., 1993) is the mass of solids divided by the volume of the solids. The difficulty of the measurement of particle density is to eliminate air trapped from pore spaces and heterogeneous nature of the compost material results in interference to calculation (Agnew

and Leonard, 2003). Determination method is generally based on the measurement of air volume. Leege and Thomas (1997) measured particle density by adding water to a dried sample of compost until it is saturated completely. In this method, a wetting and draining procedure should be repeated for 4 times in order to fulfill all air voids. Additionally, it assumed that all air voids are filled with water which volume gives the volume of air spaces presented in the sample. Subtracting the volume of air from the total dry volume is the volume of the particles. Haug (1995) found indirect method based on organic material and ash content to measure bulk density. In this method, a specific gravity of 1.0 for organic matter and 2.5 for ash content.

The porosity of the material, or airspace and water filled voids governed by particle size and size gradation ensure the amount of water and air available to the microorganisms. Porosity indicates matrix structure but not specifically the potential of oxygen availability within the matrix due to the possibility of air-filled pores. On the other hand, free air space (FAS) is relevant to oxygen availability as it is the determination of air-filled pores. The reliable methods for determination of the porosity and free air space mentioned in the latest researches are the air pycnometer and water displacement techniques (Agnew and Leonard, 2003).

3. MATERIALS AND METHODS

3.1. Composting System Setup

Composting processes composed of different feed stocks including kitchen waste, yard waste and cow manure were conducted in two tumbler composting systems with a capacity of 1 ton/year. Each compost tumbler system had the dimensions of were 200cm L x 90 cm W x 150 H cm and both units were divided in two compartments. An additional mill unit was designed to increase the efficiency of the system by homogenizing the wastes and decreasing the in composting time (Fig 3.1b.).



Figure 1.4. Manual composting system (a) tumbler composting system (b) mill unit.

The composting systems were operated as a batch reactor in which compost products were added once, and then the systems were allowed to start composting process. Tumblers were turned manually three times a day throughout the process and the composting period lasted for 30 days.

Two experimental sets named Set 1 and Set 2 were carried out and sawdust was added to all the composting processes as a bulking agent to maintain moisture content at $55 \pm 5\%$ (Table 3.1.).

Table 3.1. Description of organic materials used for composting.

	System	Materials	Ratio (volume)
SET1	Y	YW	100
	K	KW	100
	YKM	YW:KW:CM	40:20:40
SET2	YK	YW:KW	50:50
	YM	YW:CM	50:50
	KM	KW:CM	50:50

Remark; YW = Yard waste KW = Kitchen waste CM = Cow manure

In the first set, yard waste (YW) and kitchen waste (KW) were used separately as a compost product and the last compartment was fed with a mixture of yard waste, kitchen waste and cow manure (CM) together. The first compartment (Y) was fed with 4 kg of sawdust and 15.5 kg yard waste. In the second compartment (K), 4 kg sawdust and 13.2 kg kitchen waste were added to the system. Mixture in the last compartment (YKM) was composed of 4 kg sawdust, 5 kg cow manure, 2.3 kg kitchen waste and 5 kg yard waste. Experiment was carried out in July 2015 and ambient air temperature was 27 ± 3 °C. Mixing sawdust as bulking agent with manure increased the total C but decreased total N content in the mixture above optimum C/N range while maintaining optimal moisture content. Therefore, in this study, manure composted with other feedstocks and the influence of manure on composting process was observed.

In the second set, compartments were fed with binary combinations of compost products consisting of yard plus kitchen waste, yard waste plus cow manure, and kitchen waste plus cow manure (in September 2015 at ambient air temperature 17 ± 3 °C) (Fig. 3.2.). In the first compartment (YK), 7kg sawdust, 12 kg kitchen waste and 12 kg yard waste were used as feedstocks. The second compartment (YM) was fed with 7 kg sawdust, 10 kg cow manure and 10 kg yard waste. 14 kg sawdust, 22 kg manure and 22 kg kitchen waste were added to the last compartment (KM). Maturity of compost was determined when temperature of the compost declines to ambient temperature and carbon-to-nitrogen (C/N) ratio became constant.

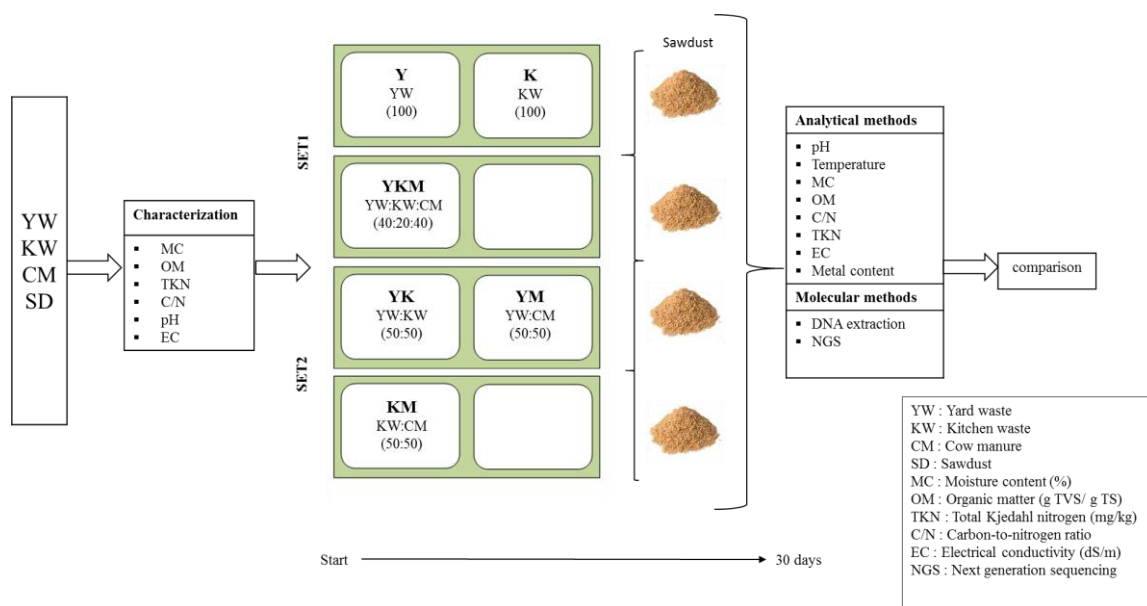


Figure 3.2. Illustration of composting systems' setup.

3.2. Characteristics of Compost Products

Fresh cow manure was obtained from the barn of Veterinary Faculty of Istanbul University, Istanbul, Turkey. Yard waste used during the study was clipping grass acquired from Boğaziçi University. Kitchen waste collected from student cafeteria of Boğaziçi University comprised of vegetable, meat and side dishes such as rice and pasta.

Characteristics of feed stocks used in composting processes are given Table 3.2.

3.3. Analytical Methods to Assess Waste Degradation

Compost samples were obtained from the tumbler system on day 0, 3, 5, 11, 18 and 30 after onset of composting. Three replicates were sampled to measure moisture content, organic content and dry solid analyses. Moisture content and dry solid were determined by drying samples at 105 °C for 24 h and organic matter content was detected by heating the sample at 550 °C for 5 h (Nelson and Sommerse, 1996).

Temperature fluctuations were detected during composting process with the help of a thermometer. For measuring pH and electrical conductivity, raw samples were mixed with deionized water at a weight ratio 1:10 (w/v) and shaken for half an hour. HANNA HI 221

Microprocessor pH meter was used to measure the pH value daily. For determining electrical conductivity, WTW LF 320 EC meter was used with 1:10 (w/v) water extract for characterization of end products (Tiquia et al., 2010).

C/N ratio was analyzed using ECS 4010 model Elemental Combustion System Costech CHNS-O (ABD) with dried samples at 105 °C for 24 h for characterization and determination of maturity. Metal content was analysed by Inductively Coupled Plasma optical emission spectrometry using Perkin Elmer ICP-OES for characterization of end products. Total Kjeldahl nitrogen (TKN) were measured according to Standard Methods for the Examination of Water and Wastewaters (APHA, AWWA-WEF, 1998) for characterization of raw materials and end products.

Table 3.2. Characteristics of compost products used during the study.

	Yard waste	Kitchen waste	Cow manure	Sawdust
TS, g TS/g FS	0.270±0.03	0.243±0.04	0.17±0.04	0.905±0.001
VS, g TVS/g TS	0.88±0.02	0.95±0.01	0.86±0.03	0.996±0.001
MC %	73.0±2.2	75.7±4.3	83±2.1	0.95±0.001
C%	35.57±2.4	35.5±9.9	37.65±0.63	39.16±0.63
N%	3.57±0.5	1.45±1.8	1.445±0.07	0.23±0.07
C/N	9.97:1±0.9	18.27:1±7.0	26.03:1±1.3	170.26:1±4.9
TKN, mg/kg	4252.9±24	3517.8±53	3069.0±18	2141.4±11
pH	6.07	5.45	8	4.73
EC, dS/m	0.576	1.454	0.19	0.107

3.4. Molecular Methods to Assess Microbial Community Structure

3.4.1. DNA extraction

Approximately 500 µL sample was added up to lysing matrix tubes along with 978 µL sodium phosphate and 122 µL MT buffer solution. The tubes contain mixture of ceramic and silica particles to lyse all microorganisms in sample. The lysing matrix tubes were

spinned in Ribolyser (Fast Prep TM FP120 Bio 101 Thermo Electron Corporation) for 45 seconds at speed of 6.5 m/s. The tubes were then centrifuged at 14000xg at 4°C for 5 minutes. After centrifugation, supernatants were transferred to clean 1.5 ml microfuge tubes and added 250 µL PPS reagent. To mix the composition the tubes were shaken for 30 seconds. After mixing, the tubes were centrifuged again at 14000xg for 5 minutes to pellet the precipitate. Supernatants were transferred to 15 mL conical tubes and 1 ml of binding matrix suspension was added to supernatant. The tubes were inverted for 3 minutes to allow binding of DNA to matrix. To settle the silica, matrix tubes were incubated at room temperature for 3 minutes. 500 µL of supernatant was removed carefully without disturbing settled silica matrix. Then the binding matrix was resuspended in the remaining supernatant. All mixture was filtered by centrifugation at 14000xg for 1 minute in filter spin tubes and filter was placed to a new tube. Filter was washed by 500 µL SEWS-M wash solution. After washing, filter was dried by centrifugation at 14000xg for 2 minutes. Filter was removed to a new tube and 50 µL DES (DNase/Pyrogen free water) was added. The filter with DES was then centrifuged at 14000xg for 2 minutes. Application-ready DNA was obtained in the tube. 1/10 and 1/100 diluted genomic DNA was run on the 1% (w/v) agarose gel, prestained with ethidium bromide (EtBr) in 1x Tris-acetate-EDTA (TAE) buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA; pH 8). Gel was visualized by using a gel documentation system, Mitsubishi 91.

3.4.2. Next generation sequencing (NGS)

NGS-based metagenomic sequencing approach was applied using Ion PGM™ protocol. V4 region of 16S rRNA gene was amplified from the extracted DNA samples using 515F and 806R 16S universal Eubacterial primers (Table 3.4.). PCR was operated with PCR HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA) and comprised of an initial denaturation step of 94 °C for 3 min, followed by 30 cycles of 94 °C for 30 s and 53 °C for 40s.

Table 3.2. Primer sequence used in amplification.

Primer	Sequence
515F	GTGCCAGCMGCCGCGGTAA
806R	GGACTACVSGGGTATCTAAT

The amplified PCR products were diluted to same concentration and purified using Agencourt Ampure beads kit (Agencourt Bioscience Corporation, MA, USA). Ion PGM methodology was conducted with supplied materials according to manufacturer's protocol. Sequencing data sets clarified from the data obtained without barcodes and shorter sequence (<200bp) were excluded. Furthermore, readings containing high homopolymer regions (<6bp) also were removed from the data sets. Averagely 41315 readings were obtained per sample from the total 413158 readings.

Operational taxonomic units (OTU) were determined based on maximum 97% similarity. OTUs were classified taxonomically using MSRN and BLASTn database (DeSantis et al., 2006). QIME (Quantative Insights Into Microbial Ecology) was operated in order to verify different taxonomic profiles between the samples and the categories.

4. RESULTS AND DISCUSSION

Composting processes composed of different organic wastes consisting of kitchen waste, yard waste and cow manure were operated in two tumbler composting system for 30 days. In Set 1, Y, K and YKM composts and in Set 2 YK, YM and KM composts were obtained. For all treatments, the process temperature and pH detected daily in order to control the composting systems. Moisture content and organic material were determined in sampling days (0.,3.,5.,11.,18.,30). C/N, TKN, EC and metal contents were determined as quality parameters. Characteristics of composts acquired from study are examined below.

4.1. Temperature

Temperature measurements are presented in Figure 4.1. In both set (Set 1 and Set2), the temperature increased rapidly during the first three days of composting process due to intense microbial activities favoured by the high concentration of easily decomposable organic matter. Temperature in K, YKM, YK and YM composts peaked on day 4, in Y peaked on day 3 and in KM had a peak on day 5. The maximum temperatures attained in composting processes were in K, Y and YK compost 62 °C, 70 °C and 72 °C, respectively. After peaking, the temperature in Y and YM compost began to drop gradually and reached to ambient air. The fluctuation in the temperature during thermophilic phase was observed in K, YKM, YK and KM composting processes.

Temperature is the main parameter which both affects and indicates the rate of biological reactions. It also defines the sanitation capacity of the composting process (Zhang and Sun, 2016). In some European countries and China, the thermophilic phase (55-60 °C) lasted longer than three days ensures the compliance with sanitation requirements related to absence of weed seeds and pathogens in compost (Sadaka and El-Taweel, 2003; Zhang et al., 2013). The thermophilic phases (55-60 °C) lasted longer than three days occurred in Y (4-5 days), K (3-4 days), YK (8-9 days) composting processes. This shows that the temperatures in Y, K and especially YK composts met the sanitation requirement.

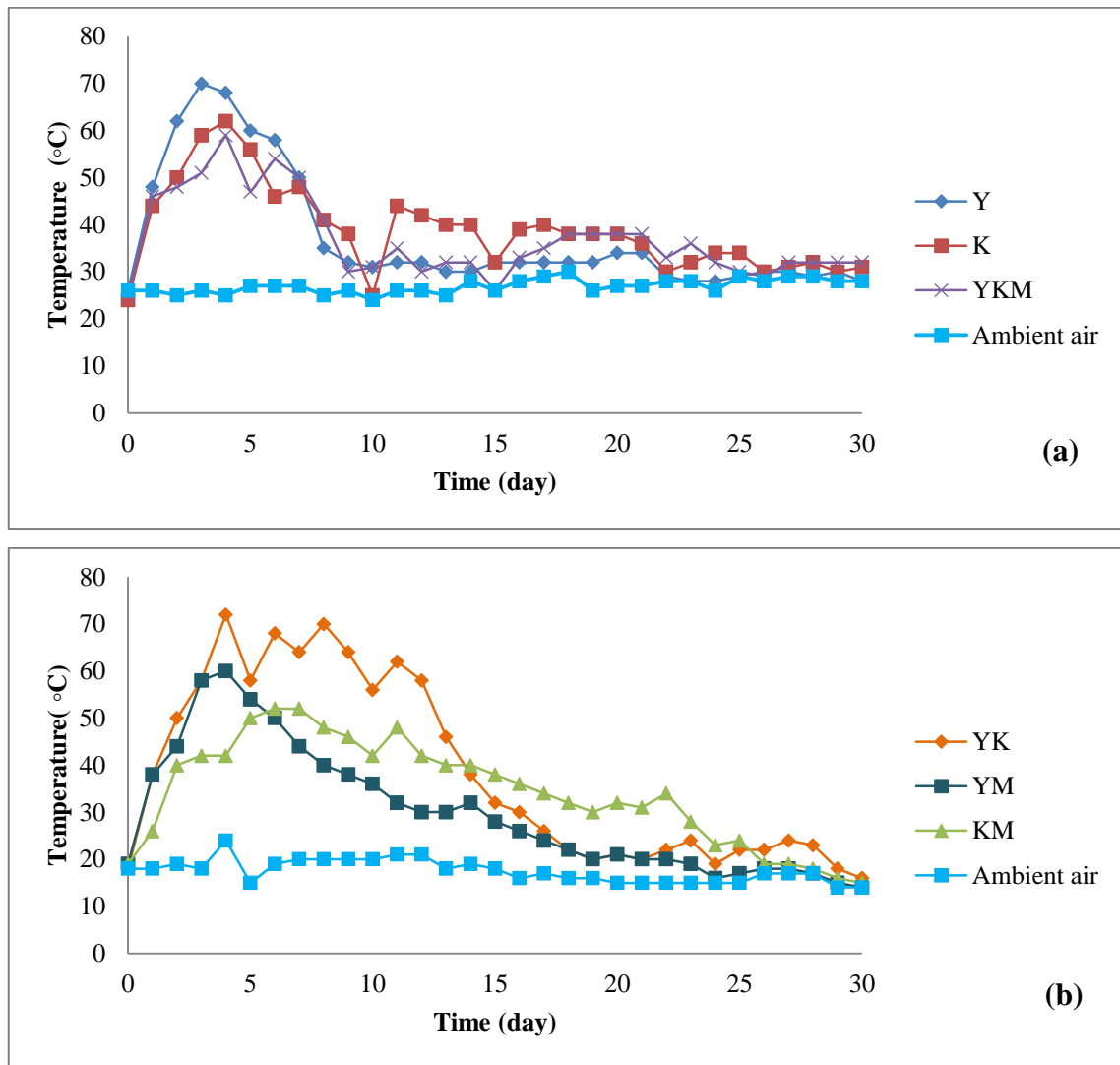


Figure 4.1. Temperature fluctuations during composting (a) SET 1 (b) SET 2.

4.2. pH

As illustrated in Fig.4.2a, in Y compost, the pH did not change markedly and varied between 6.61 to 7.47. The pH in K compost having initial pH at 4.36 increased significantly to 8.76. Stabilization with pH fluctuations was observed in YKM ranging from 7.37 to 8.12 during composting.

A fast pH increment during the first days of the process was detected in both composting systems including YK, YM and KM. Afterwards, the pH in YK and YM fluctuated slightly, from 6.52 to 8.67 and 7.02 to 8.56, respectively. The pH in KM

dropped to its minimum value 5.93 on day 5. After a rise in pH, another low pH was seen in KM compost. At the end of the process, pH in KM reached to 8.56 (Fig. 4.2.b).

Fluctuations in pH value during compost system may be due to volatilization of NH_4^+ . Moreover, the acidification can be also resulted from the production of organic acids. pH values, varying between 6.7 to 9.0 during the composting process are considered to lead to optimum aerobic conditions for the microbial activity (Beck Friis et al., 2001). In this study, pH evolution in compost systems showed that YKM (7.37 – 8.12), YM (7.23 – 8.82) and KM (7.02 – 8.56) compost process occurred in the optimum range indicating that they were subjected to good oxygenation conditions.

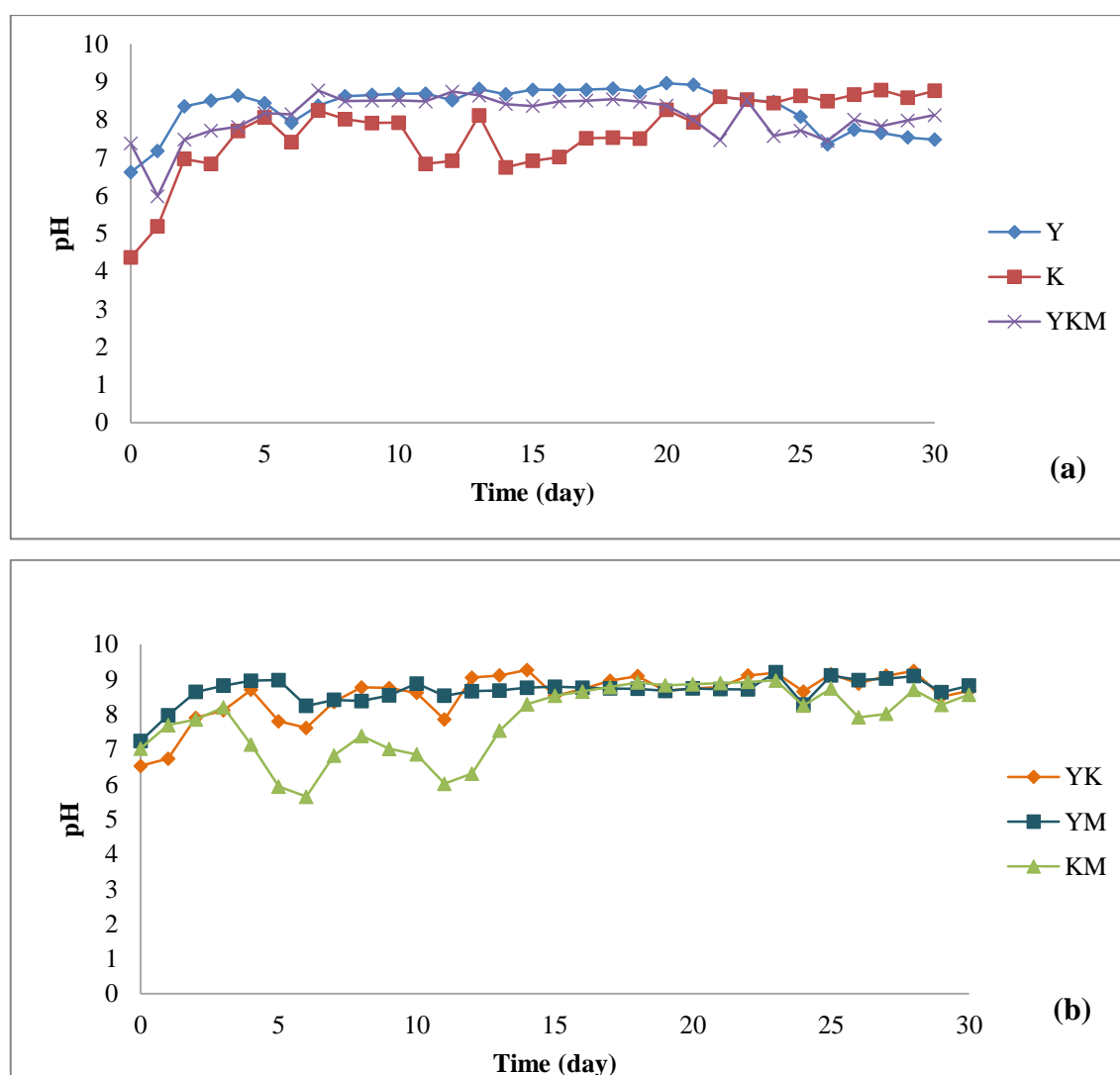


Figure 4.2. pH fluctuations during composting (a) SET 1 (b) SET 2.

4.3. Moisture Content

Fig. 4.3. and Fig. 4.4. illustrate the variation of moisture content (MC) during the composting period of SET 1 and SET 2 under the conditions of the same initial moisture content ($55\pm 5\%$). The temperature increased in Y (Fig. 4.3.a), K (Fig. 4.3.b), and YKM (Fig. 4.3.c) compost process thus diminished moisture content in both systems. MC of Y, K, and YKM compost declined from initial value of 55% to 45.28%, 49.5%, and 50.2%, respectively.

MC of YK (Fig. 4.4.a), YM (Fig. 4.4.b), and KM (Fig. 4.4.c) reduced from 55% to 48.3%, 50.22% and 53.8%, respectively. Variations of MC in SET 2 appeared to have a similar profile as Y, K and YKM. YK and Y compost possessing the highest temperature in thermophilic phase reached the lowest moisture content at the end of the process in comparison with other systems in their set.

The moisture content has a direct effect on the microbial activity, the compost temperature, and also the rate of decomposition (Kumar et al., 2010). The compost systems started at optimum range varying 50% to 60% (Tiqua et al., 1998; McKinley et al., 1986; Suler and Finstein, 1977).

In all compost processes, decrease in moisture content was observed. As the highest inner temperature maintained in Y, K and YK compost, higher moisture reduction was observed compared to other systems. In addition, as an amorphous material, manure may result in impediment of diffusion of interstitial air into the mass at adequate levels and have influence on moisture reduction in YKM, YM and YKM composting systems content.

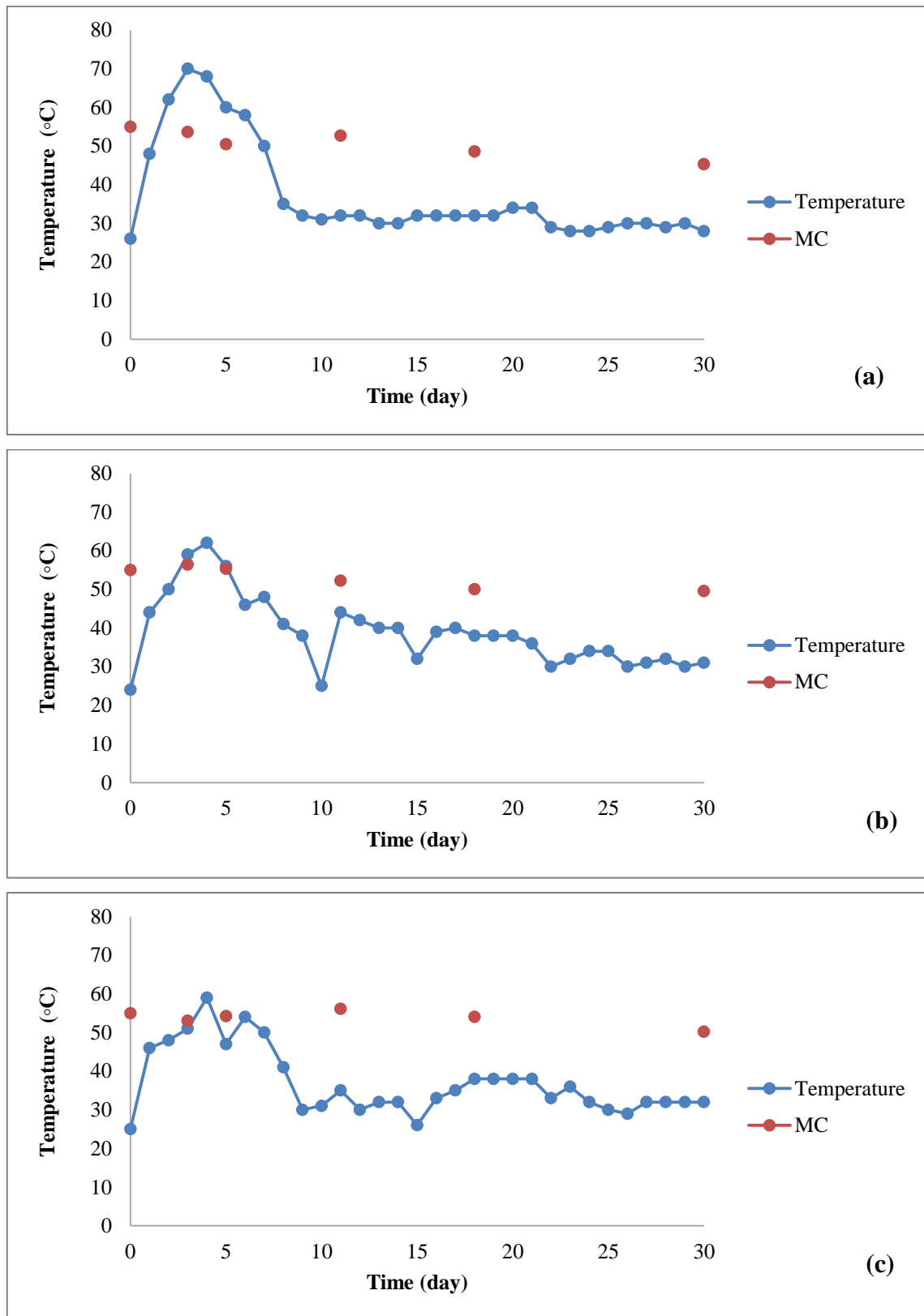


Figure 4.3. Profiles of moisture content (MC) and temperature during composting of SET 1
(a) Y (b) K (c) YKM.

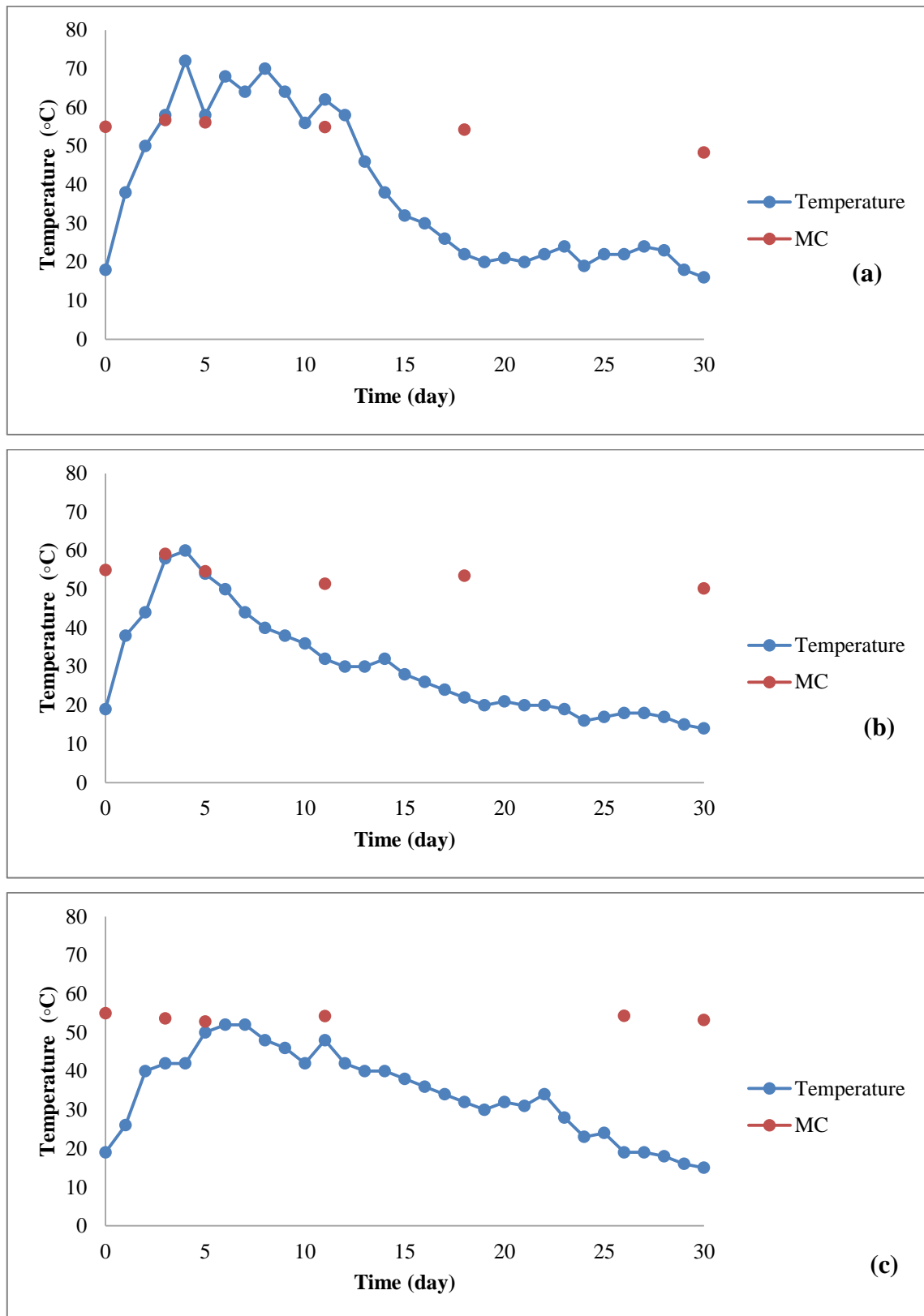


Figure 4.4. Profiles of moisture content (MC) and temperature during composting of SET 2

(a) YK (b) YM (c) KM

4.4. Organic Matter

The change in organic matter during composting process is given in Figure 4.5. Organic matter decreased rapidly in the first days of composting due to easily decomposable organic content in materials. The decrement in organic matter in subsequent phase was found to be slower related to hardly decomposable organic matter remained in the reactors.

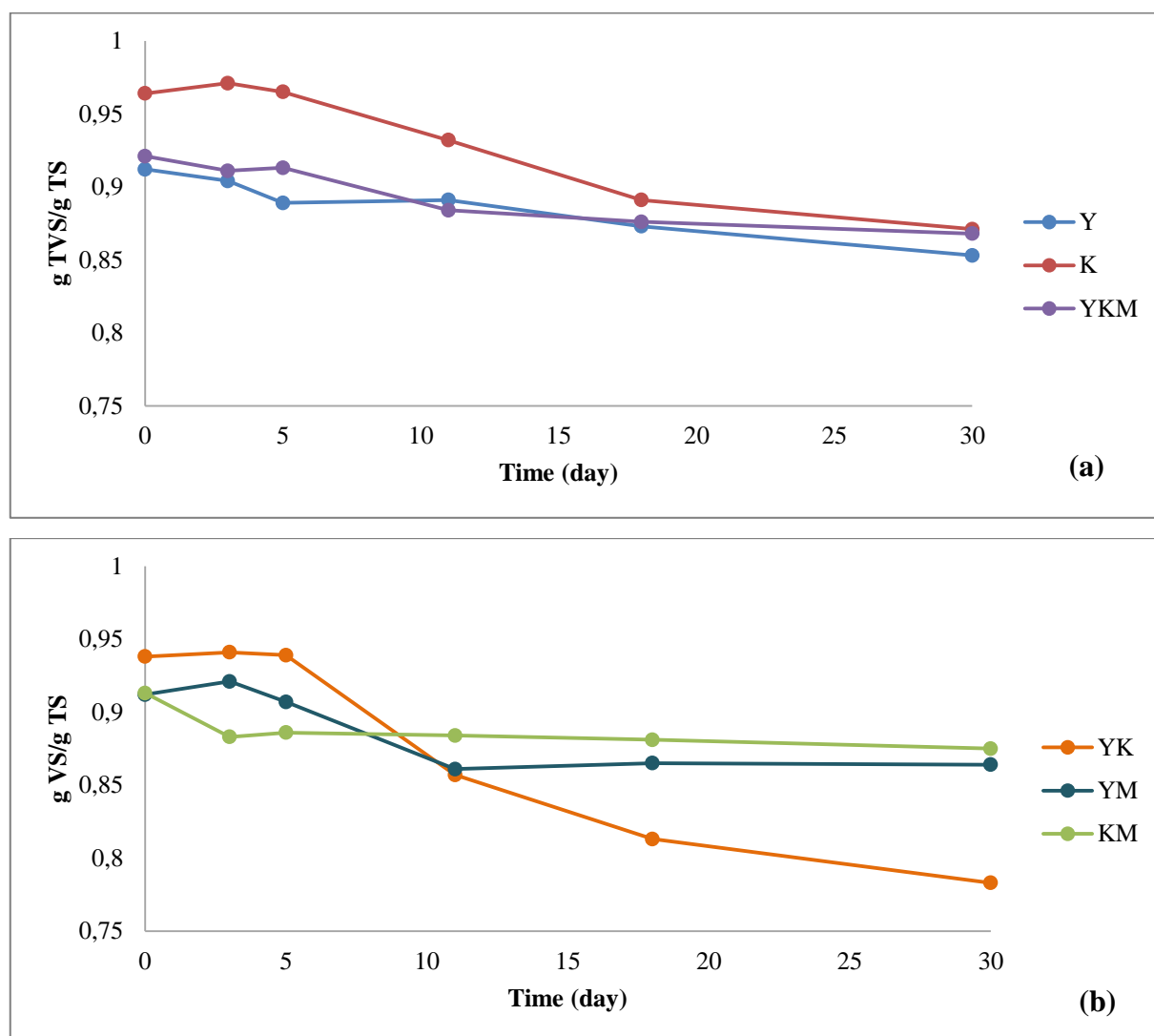


Figure 4.5. Profiles of organic matter during composting of (a) SET1 (b) SET2.

Initial organic content in Y, K and YKM (Fig.4.5a) compost was 0.912, 0.964 and 0.921 g TVS/g TS, respectively and at the end of process organic contents in the system were found to be 0.853 g TVS/g TS g in Y compost, 0.871 g TVS/g TS in K compost, and

0.868 g VS/g TS in YKM compost. Organic matter content of YK, YM, and KM declined from 0.938, 0.912, and 0.913 g TVS/g TS to 0.783, 0.864, and 0.875 g TVS/ g TS, respectively.

The total volatile solids (TVS) indicates the biodegradable organic matter and the decomposition of organic matter is asserted via the difference between the initial and final total volatile solids (Kwon and Lee, 2004). Maximum TVS reduction occurred in YK process with the value of 16.5 %.

4.5. Carbon to Nitrogen Ratio (C/N)

The initial and final C/N ratio of compost samples are shown in Figure 4.6. The initial C/N content was varying from 37.32 to 51.36 and C/N content of composting systems stabilized ranging between 23.2 to 28.7. In this study, due to excess C in treatments with high C/N ratios of composting systems, all nitrogen in the system might be immobilized and hinder nitrification (Eiland et al., 2001).

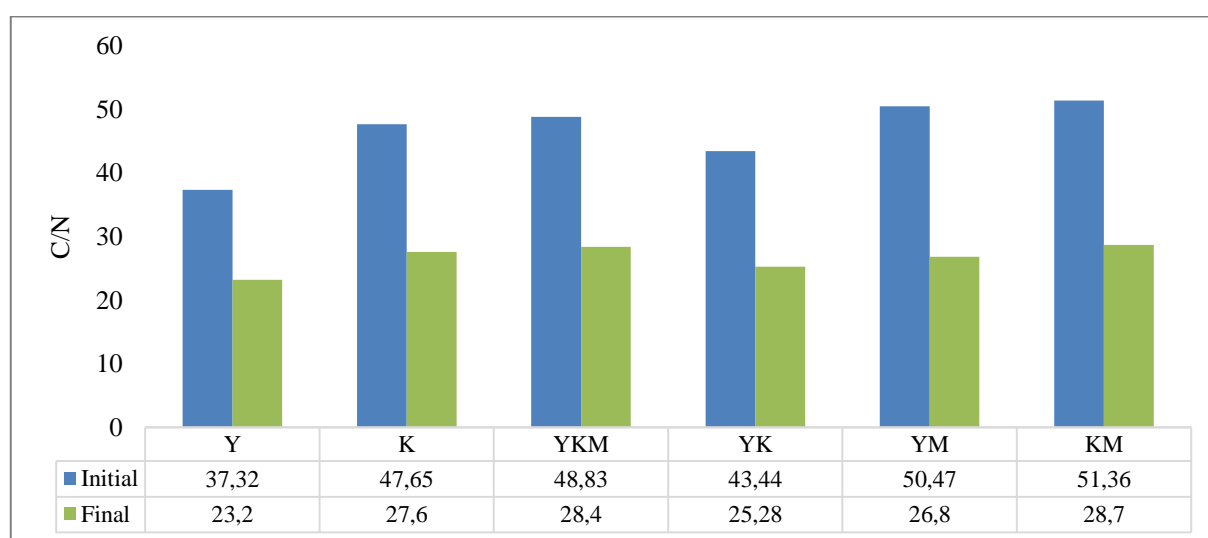


Figure 4.6. Initial and final C/N content in compost samples.

Eiland et al. (2001) examined the impact of initial C/N ratio (11, 35, 47, 50, 54) on chemical and microbial composition during long term composting of straw. Low initial C/N ratios resulted in fast degradation of fibers during the first phase of the process and lower fungal/bacterial ratio compared to the high C/N treatments. It is stated that high

degradation rate of easily degradable matters in treatments with low C/N ratio lead to less available material and poor quality substrate for microbial growth. Moreover, low C/N ratio resulted in high temperature over a relatively long time due to the high initial decomposition activity and less substrate remained for subsequent recolonization of fungi. In this study, thermophilic stage lasted longer in Y, K and YK composting systems that have lower C/N content compared to other composting systems.

Wang et al. (2017) investigated the effects of the feeding ratio of food waste on fed-batch aerobic composting and its microbial community. The duration of the study lasted 30 days. The initial C/N ratio and moisture content was approximately 38 and 62%, respectively. Feeding ratios of food waste to system were 0% as control, 5%, 10% and 15%. The C/N ratio of the control stabilized at 25 whilst the system temperature reached to ambient air. The final C/N ratios were found to be 15.7, 18.2 and 20.8 for the 5%, 10% and 15% systems, respectively. In this study, the C/N ratio reduction degrees of all composting systems were consistent to the levels of three treatments conducted by Wang et al. (2017).

4.6. Total Kjeldahl Nitrogen (TKN)

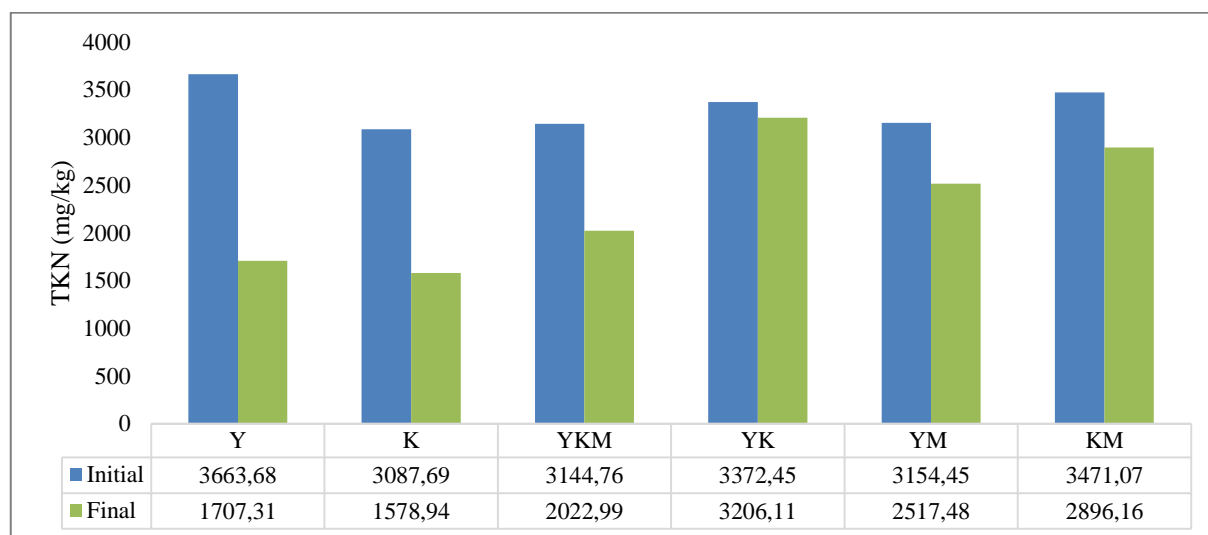


Figure 4.7. Initial and final TKN content in compost samples.

The initial and final TKN results illustrated in Figure 4.7. The initial TKN content of reactors ranging from 3150 to 3700 mg/kg declined to the levels varying from 1600 to 3200 mg/kg at the end of the process. The higher decrease in TKN in SET 1 could be due

to the immobilization of NH_4^+ ions via the carbonaceous materials (Ramaswamy et al., 2010).

4.7. Metal content

During composting process, degradation of heavy metals including Cr, Ni, Zn, Cd and Pb does not occur, and due to the microbial degradation and decline in water and carbon content, the heavy metals in the material consequently become more concentrated (Richard, 1992). The heavy metal contents differ from negligible background levels in composts such as source-separated food waste, to toxic levels in some mixed based composts. The sources of heavy metals are batteries, paints, electronics, plastics, ceramics and dyes of MSW which contribute to the metal levels in composts (Farrell and Jones, 2009).

Application of composts with high levels of heavy metals to soil poses a risk in terms of phytotoxicity of food crops (Papadimitrou et al., 2008). Repeated application to soil represents an obvious concern as rise in the heavy metal concentration occurs in land (Ramos and López-Acevedo, 2004).

The mechanisms such as complexation and sorption with organic matter, microbial mobilization and also oxidation bind metals and metal bioavailability decreases with length of the process (Greenway and Song, 2002). The water-soluble organic carbon content converting metals to non-bioavailable form follows descending trend during the process. Therefore, in order to decrease in metal availability, maturity phase needs to be concluded (Farrell and Jones, 2009). Comparison of the heavy metal concentrations in final products obtained in this study with regulatory limits is tabulated in Table 4.1. The results are found to be below the limits of regulations.

4.8. Electrical Conductivity

Electrical conductivity referred to the salt content of composts should be below 10 dS/m for the use of compost as a soil amendment according to the regulations. The composts obtained in this study have barely saline and agricultural soils EC varies from 0

to 4 dS/m (Hargreaves et al., 2008). Therefore, application on soil may not result in phototoxic effect on plants.

Table 4.1. Electrical conductivity results of final products.

	SET 1			SET 2		
	Y	K	YKM	YK	YM	KM
dS/m	2.0	1.4	1.2	1.8	1.4	1.6

4.9. Legal Requirements for the Use of Compost as a Soil Amendment

In this study, it is aimed to comply within regulation limits regarding the use of compost as a soil amendment. Hence, analytical results compared with legal requirements illustrated in Table 3.3. According to “Regulations Regarding the Production, Import, Marketing and Inspection of Organic, Organomineral Fertilizers and Soil Amendment Products and Other Products, Microbial and Enzyme Based Products” being published in the Official Gazette dated 29.03.2014 and numbered 28956 and, “Compost Regulation” being published in the Official Gazette dated 05.03.2015 and numbered 29286, C/N portion should be in the range of 10 to 30, volatile solid content should be at least 35 % of total solid, moisture content should be lower than 30%, and electrical conductivity should be lower than 10dS/cm. Furthermore, according to “Compost Regulation” pH should be in the range of 5.5 to 8.5.

In this study, final C/N ratio (23.2-28.7), volatile solids content (0.783 -0.875 g TVS/g TS) and electrical conductivity (1.18-2.07dS/m) in all composting systems were complied with the regulatory requirements. The final pH of Y and YKM compost met within the legal limitations. The pH in K, YK, YM and KM were found to be slightly above the limits with the value of 8.76, 8.67, 8.82 and 8.56, respectively. The moisture contents of compost were found to be in range of 45 % to 50 %. In order to comply with regulatory limitations, moisture content can be decreased via air ventilation or mechanical aeration.

Table 4.2. Metal contents of final products.

	SET 1			SET 2			Regulations
	Y	K	YKM	YK	YM	KM	
	mg/L						
Cr	5.0	12.3	22.3	9.3	7.0	4.00	350
Ni	4.7	8.7	12.3	6.7	5.7	3.7	120
Cu	6.7	11.0	13.0	10.7	9.7	10.0	450
Zn	50.3	180.3	80.7	169.0	311.7	92.7	1100
Cd	0.0	0.0	0.0	0.0	0.0	0.0	3
Pb	3.3	3.0	4.3	3.3	2.3	2.3	150

Table 4.3. Legal requirements in order to use compost as a soil amendment.

	Compost Regulation	Regulations Regarding the Production, Import, Marketing and Inspection of Organic, Organomineral Fertilizers and Soil Amendment Products And Other Products, Microbial And Enzyme Based Products
Cd	3	3
Cr	350	350
Cu	450	450
Ni	120	120
Pb	150	150
Zn	1100	1100
Moisture content	< %30	< %30
C/N ratio	10-30	10-30
Organic matter (in total solid)	> %35	> %35
pH	5.5-8.5	-
Electrical conductivity	<10 dS/cm	<10 dS/cm

4.10. Microbial community structure

NGS-based metagenomic sequencing approach was applied in order to analyse the microbial community structure in different compost treatments during the highest temperature and also to determine the most abundant bacteria species throughout the process.

The taxonomic distribution of the bacterial communities in each compost sample in thermophilic stage was illustrated at the class level in Fig. 4.8., and phylum level in Fig.

4.9. While comparing at phylum level, in all composting processes, *Firmicutes* and *Proteobacteria* were dominant. *Firmicutes* perform the initial degradation of organic contents into soluble matter by virtue of cellulolytic, hemi-cellulolytic, and proteolytic characterizations (Ariesyady et al., 2007; Valdez-Vazquez and Poggi-Varaldo, 2008). *Proteobacteria* known as humic-reducing microorganism like *Firmicutes* use the propionate, acetate and butyrate as a carbon source for their metabolic activities and contribute the biodegradation of recalcitrant pollutants (Xi et al., 2016; Gulhane et al., 2017). Heat-resistance capacity of *Firmicutes* might be the reason of its high abundance at thermophilic stage. *Firmicutes* was the most abundant phyla in the Y composting system which the highest temperature was observed during process (Fig. 4.8). In addition, lower temperature might contribute the subsequent dominance of *Proteobacteria* (Wang et al., 2017).

As illustrated in Fig.4.9., the most abundant class in Y compost were *Bacilli* (81.11%) and *Gammaproteobacteria* (8.95%) when the temperature reached at 70 °C. In treatment with K at 59 °C temperature bacterial community was dominated by *Gammaproteobacteria* (47.12%) along with *Bacilli* (38.69%). In YKM compost at 51 °C, higher abundance of *Gammaproteobacteria* (56.14%) and *Bacilli* (24.5%) were detected. In the last three composting system including YK at 58 °C, YM at 58 °C and KM at 50 °C, *Bacilli* was the dominant class with 42.88%, 39.61% and 71.98, respectively. In these systems *Bacilli* was followed by *Gammaproteobacteria* presented at a level of 40.93% in YK, 27.59% in YM, and 16.85% in KM compost. *Bacilli* were found to be a dominant class during composting process at thermophilic stage in the latest researches and the results indicated that this study overlaps with literature (Dees and Ghiorse, 2001; Takaku et al., 2006; Xi et al., 2016).

Bacilli emerged as the abundant class in all compost samples reached at thermophilic stage. In a study conducted by Maeda et al. (2010) demonstrated that under mesophilic conditions *Betaproteobacteria* and *Bacteroidetes* class were dominant during cow manure composting in the surface zones and it was reported that these class might have a role in nitrogen conversion. In another study by Maeda et al. (2010), the abundance of mesophilic *Gammaproteobacteria* in the initial step of the thermophilic process in surface zones of cattle manure compost was detected using DGGE of PCR-amplified 16S rRNA sequence.

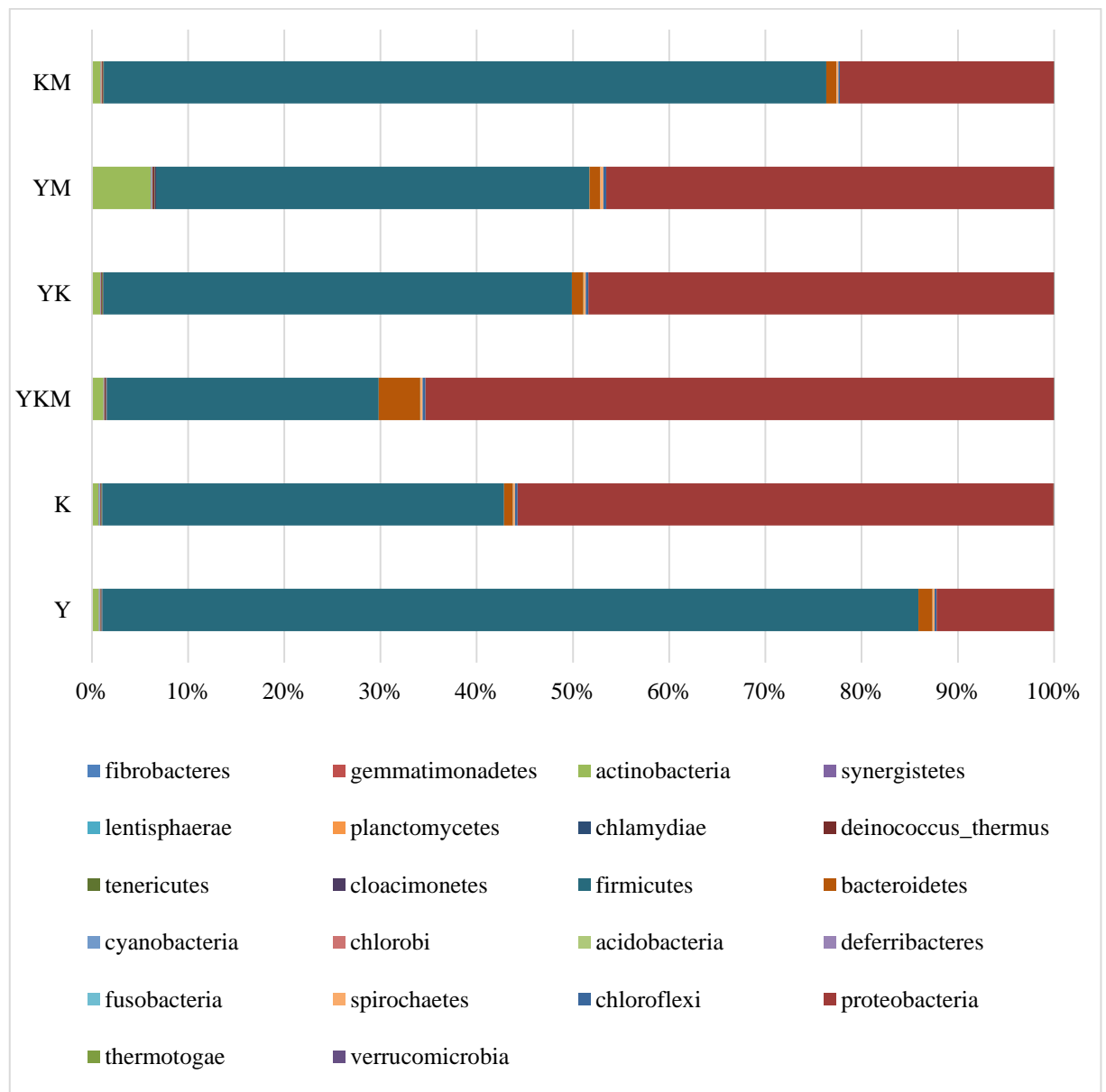


Figure 4.8. Taxonomic distribution of bacterial diversity at phylum level in thermophilic stage.

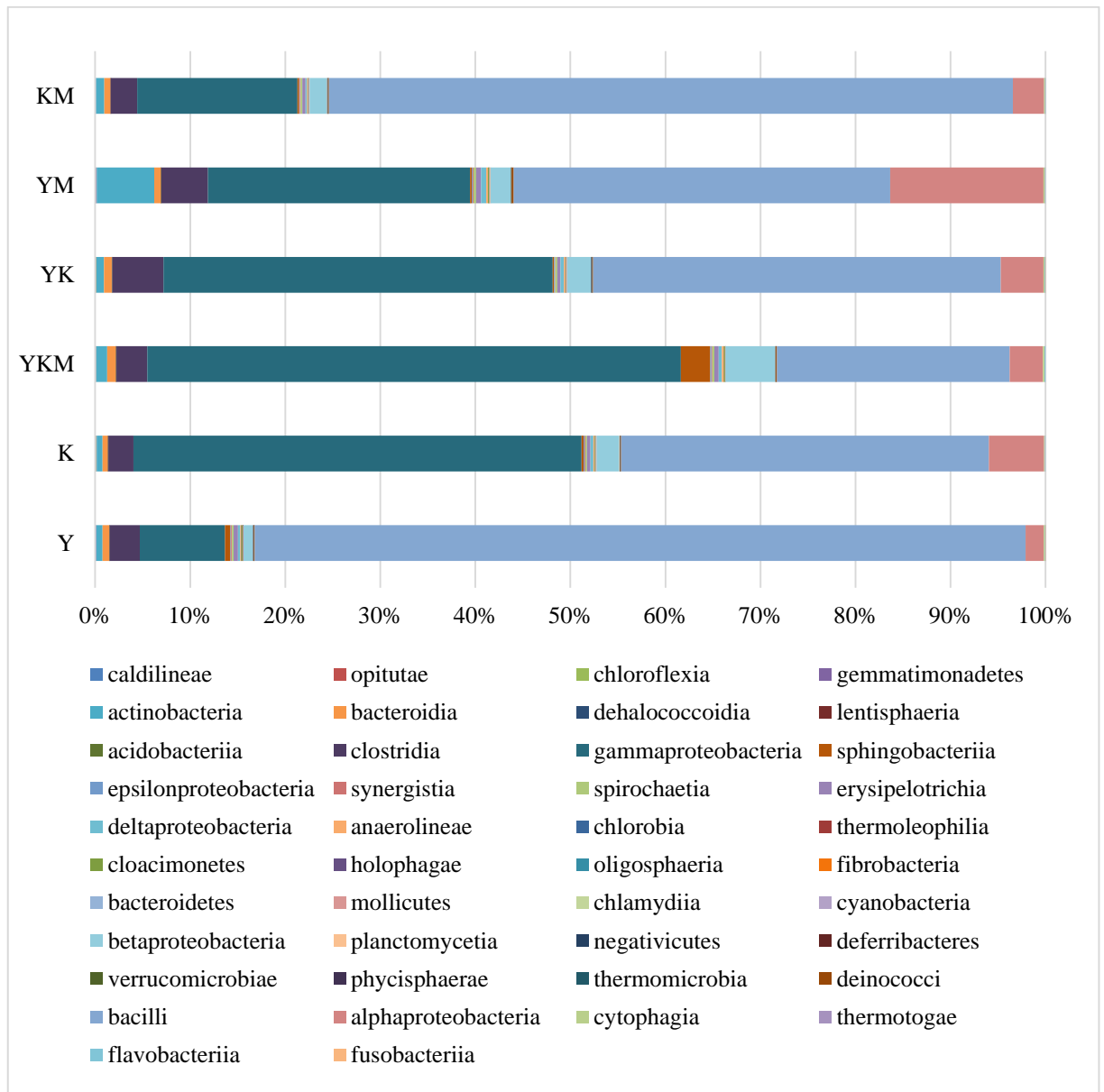


Figure 4.9. Taxonomic distribution of bacterial diversity at class level in thermophilic stage.

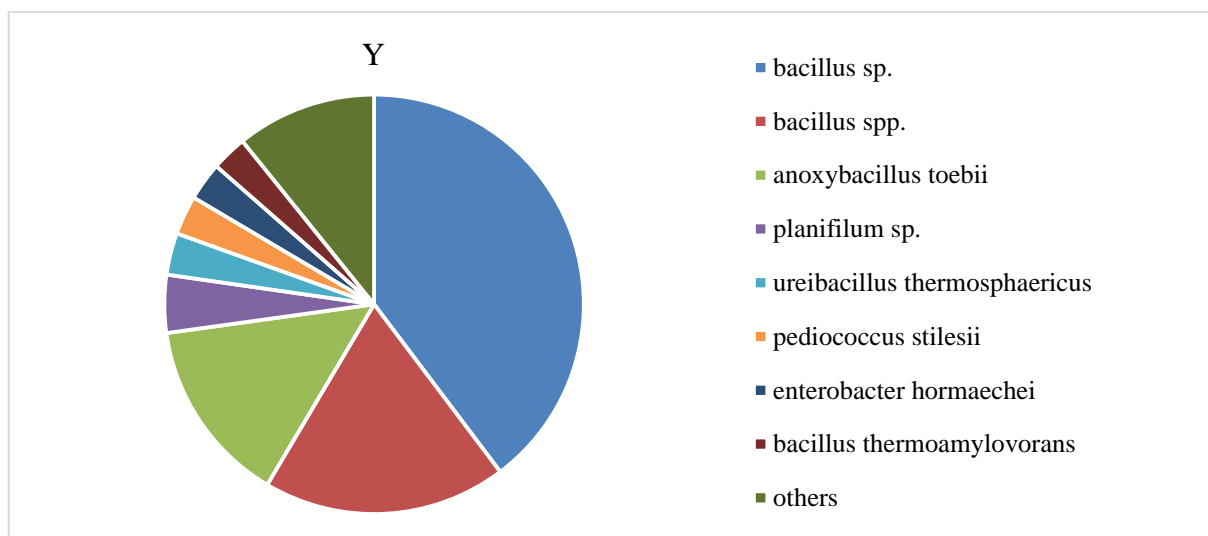


Figure 4.10. Taxonomic distribution of bacterial diversity at species level in Y compost with a proportion of at least 1% abundance.

In Y compost, *Bacillus sp.* (29.06%), *Bacillus spp.* (13.74%) and *Anoxybacillus toebii* (10.46 %) were abundant species in thermophilic phase during composting followed by *Planifilum sp.* (3.26%), *Ureibacillus thermosphaericus* (2.35%), *Pediococcus stilesii* (2.2%), *Enterobacter hormaechei* (2.14%) and *Bacillus thermoamylovorans* (2.01%) (Fig. 4.10).

Bacillus sp. and *Bacillus spp.* are endospore-forming bacteria and very active at temperatures around 50 - 60 °C (Ryckeboer et al. 2003). They were found to be the most dominant species at thermophilic stage during Y composting process. The high abundance of *Bacillus* species – the member of phylum *Firmicutes* showed that very active thermophilic conditions occurred during Y composting process (Jimenez et al., 2015).

As illustrated in Figure 4.11, *Enterobacter hormaechei* (18.57%), *Pseudoxanthomonas taiwanensis* (10.41%), and *Bacillus sp.* (8.83%) emerged as abundant species in thermophilic stage during K composting. The other domain species detected in K compost were *Salmonella enterica* (5.33%), *Bacillus thermoamylovorans* (5.08%), *Bacillus licheniformis* (4.95%), *Ureibacillus thermosphaericus* (4.36%), *Actinobacter sp.* (3.71%), *Sphingomonas spp.* (3.66%), and *Bacillus spp.* (3.45%).

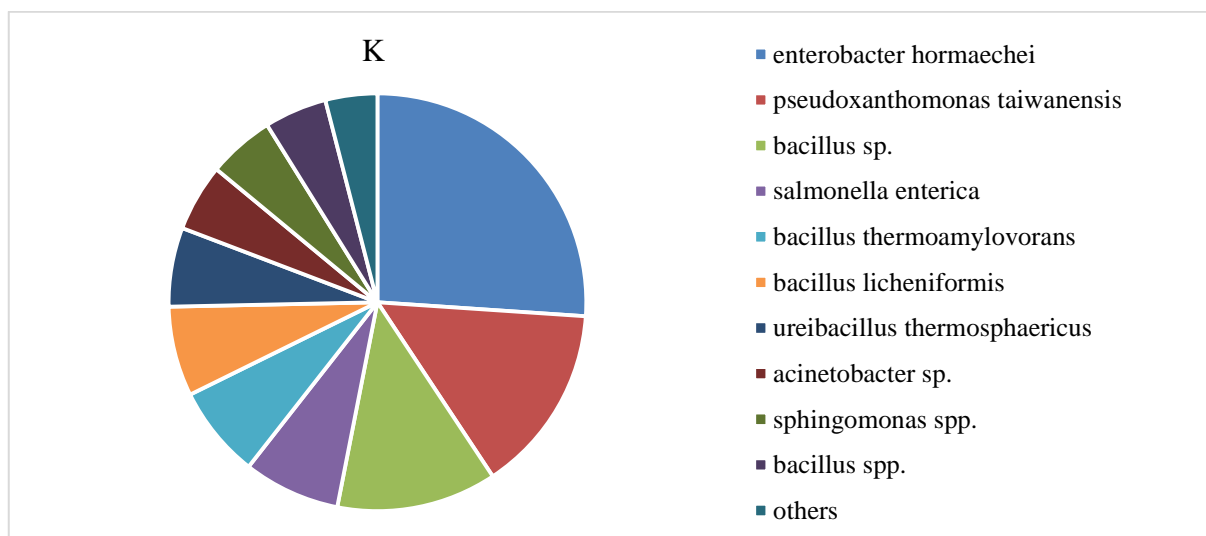


Figure 4.11. Taxonomic distribution of bacterial diversity at species level in K compost with a proportion of at least 1% abundance.

The most dominant species in K composting, *Enterobacter hormaechei* is non-spore-forming and aerobic bacteria belong to phylum *Proteobacteria*. Islam et al. (2011) investigated diversity of free-living nitrogen-fixing bacteria from paddy fields and the isolates were assigned to various species including *Enterobacter hormaechei*, *Bacillus sp.*, and *Sphingomonas sp.*. In another study conducted by Kim et al. (2008) *Enterobacter hormaechei* JH was screened from barnyard to discover thiosulfate-oxidizing microorganisms. Temperature and pH were detected to determine the optimal culture conditions for thiosulfate-oxidizing strain. The results indicated that *Enterobacter hormaechei* JH grew at temperature varying 25 to 60 °C and the optimum growth temperature was 30 °C. In addition, the growth of *Enterobacter hormaechei* JH was greater than well-known thiosulfate oxidizing microorganism *Thiobacillus delicatus* KCTC2851 (Kim et al., 2008).

Pseudoxanthomonas taiwanensis found in K composting process as abundant species is non-spore-forming, thermophilic and heterotrophic microorganisms that can convert nitrite to nitrogen. The high abundance of *Pseudoxanthomonas taiwanensis* and *Bacillus sp.* indicated active thermophilic conditions during composting of kitchen waste (Karadag et al., 2013).

Jimenez et al. (2015) investigated microbial composition in food waste composting in-vessel rotating compost system at thermophilic stage. The results indicated that the phylum *Proteobacteria* represented at level of 24%. The isolated species were *Acetobacter pasteurianus*, *Acinetobacter baumannii*, *E. coli*, *Paenaltcaligenes hominis*, *Proteus mirabilis*, *Pantoea* sp., *Paracoccus solventivorans*, *Acinetobacter* sp., *Klebsiella pneumoniae pneumoniae*, *Pseudomonas thermotolerans*, *Pseudoxanthomonas taiwanensis*, *Pusillimonas* sp., and *Enterobacter hormaechei hormaechei*. The results found in this study were consistent with previous researches.

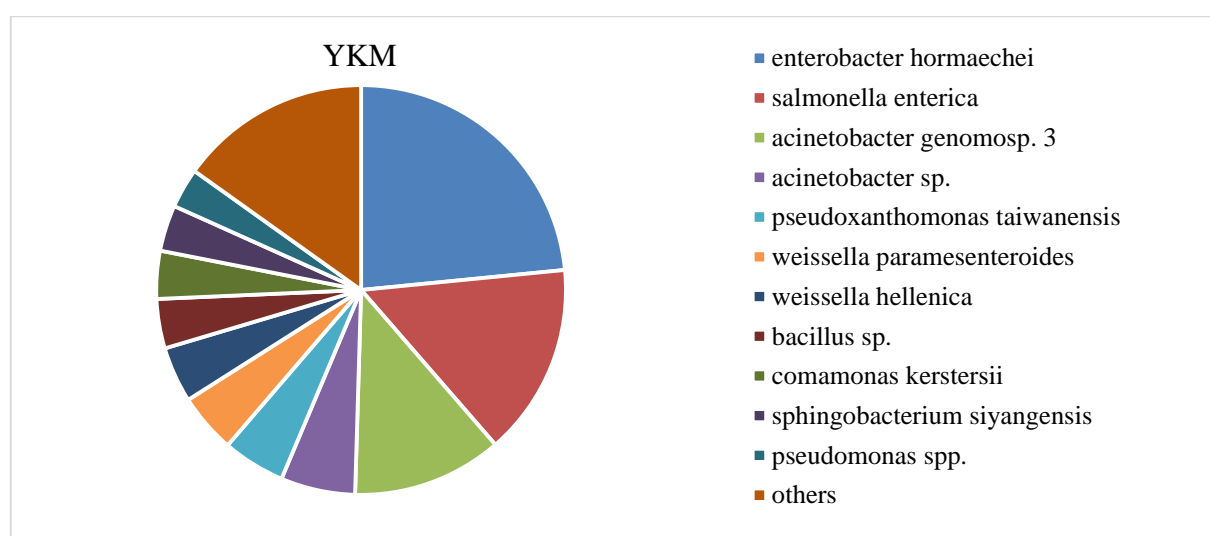


Figure 4.12. Taxonomic distribution of bacterial diversity at species level in YKM compost with a proportion of at least 1% abundance.

In YKM compost, higher abundance of *Enterobacter hormaechei* (15.63%), *Samonella enterica* (10.12%), and *Actinobacter genomsp. 3* (7.09%) were detected in thermophilic stage of composting. Thereafter, *Actinobacter* sp. (3.92%), *Pseudoxanthomonas taiwanensis* (3.29%), *Weissella paramesentreoides* (3.13%), *Weissella hellenica* (2.93%), *Bacillus* sp. (2.60%), *Comamonas kerstersii* (2.52%), *Sphingobacterium siyangensis* (2.42), and *Pseudomonas* spp. (2.10%) were presented as dominant species during this phase (Fig. 4.12).

N-fixing bacteria *Enterobacter hormaechei* was the most dominant species in YKM composting system at thermophilic stage followed by an enteric bacteria *Salmonella enterica*. *Salmonella enterica* may be presented in YKM compost due to manure

application to feedstocks (Natvig et al., 2002). Bronikowski et al. (2001) investigated effects of temperature on growth rate in natural isolates *Esheria coli* and *Salmonella enterica* from different thermal environment. The results indicated that the temperature range over which 75% of maximum growth rate occurred for *Salmonella enterica* was varying 27.7 to 39.8 °C. Therefore, determination of *Salmonella enterica* DNA may not indicate current activity at thermophilic stage during YKM composting (Hultman et al., 2010).

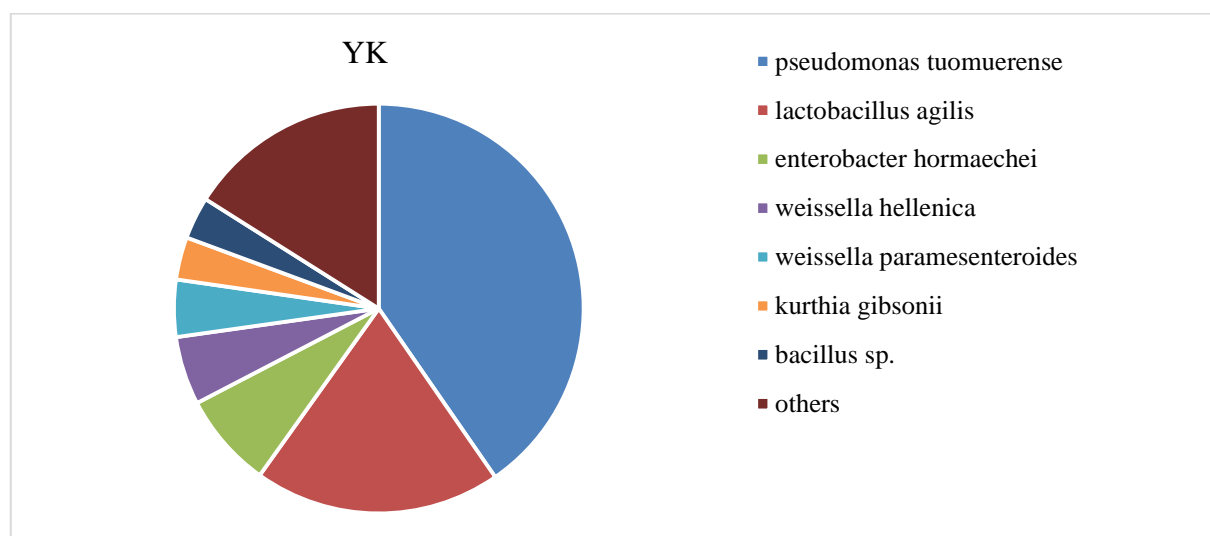


Figure 4.13. Taxonomic distribution of bacterial diversity at species level in YK compost with a proportion of at least 1% abundance.

Figure 4.13 illustrates taxonomic distribution of bacterial diversity at species level in YK composting. *Pseudomonas tuomuerense* (27.53%), and *Lactobacillus agilis* (13.29%) had higher abundance followed by *Enterobacter hormaechei* (5.11%), *Weissella hellenica* (3.68%), *Weissella paramesenteroides* (3.07%), *Kurthia Gibsonii* (2.29%), and *Bacillus sp.* (2.27%) at species level in thermophilic stage of YK.

Pseudomonas tuomuerense has also been known by the names *Serpens flexibilis* and *Pseudomonas flexibilis* (Shin et al., 2015) is non-spore-forming bacteria which optimal temperature range is between 28 to 33 °C. *Pseudomonas tuomuerense* restrictedly uses lactate as energy and carbon sources (Hespell, 1977). *Pseudomonas tuomuerense* was seldom reported in previous composting studies. The role of *Pseudomonas*

tuomuerense during composting is necessary to investigate for future studies (Song et al., 2015).

Lactobacillus agilis known as lactic acid bacteria is non-spore-forming facultative anaerobic species that ferments sugar primarily into lactic acid (Coeuret et al., 2003). The anaerobic conditions may exist in part of compost mixture favouring the growth of anaerobic microorganisms. The presence of *Lactobacillus agilis* may be resulted from the lack of air in some part of the compost mixture of YK (Yamada and Kawase, 2005).

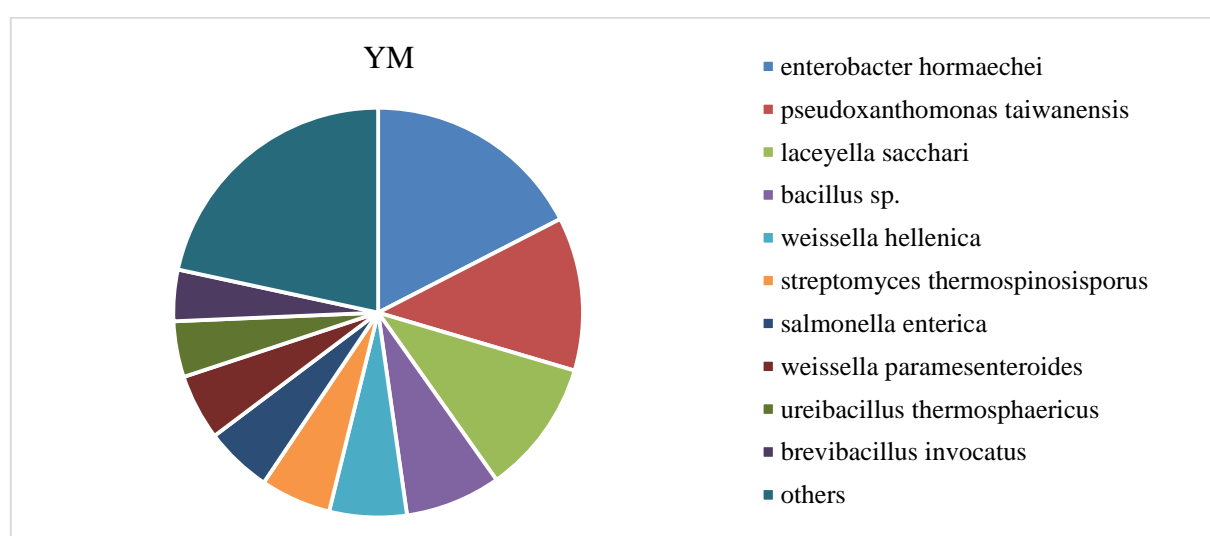


Figure 4.14. Taxonomic distribution of bacterial diversity at species level in YK compost with a proportion of at least 1% abundance.

Domain species were belonged to *Enterobacter hormaechei* (9.95%), *Pseudoxanthomonas taiwanensis* (6.92%), and *Laceyella sacchari* (6.06%) in YM compost. *Bacillus sp.* (4.30%), *Weissella hellenica* (3.48%), *Streptomyces thermospinosporus* (3.18%), *Salmonella enterica* (3.02%), *Weissella paramesenteroides* (2.96%), *Ureibacillus thermosphaericus* (2.51%), and *Brevibacillus invocatus* (2.31%) were also presented as abundant species in thermophilic stage (Fig. 4.14).

The dominant species found in YM composting system at thermophilic stage resembled with K composting system. N-fixing bacteria, *Enterobacter hormaechei* and *Pseudoxanthomonas taiwanensis* were the most abundant species in yard waste and manure compost system.

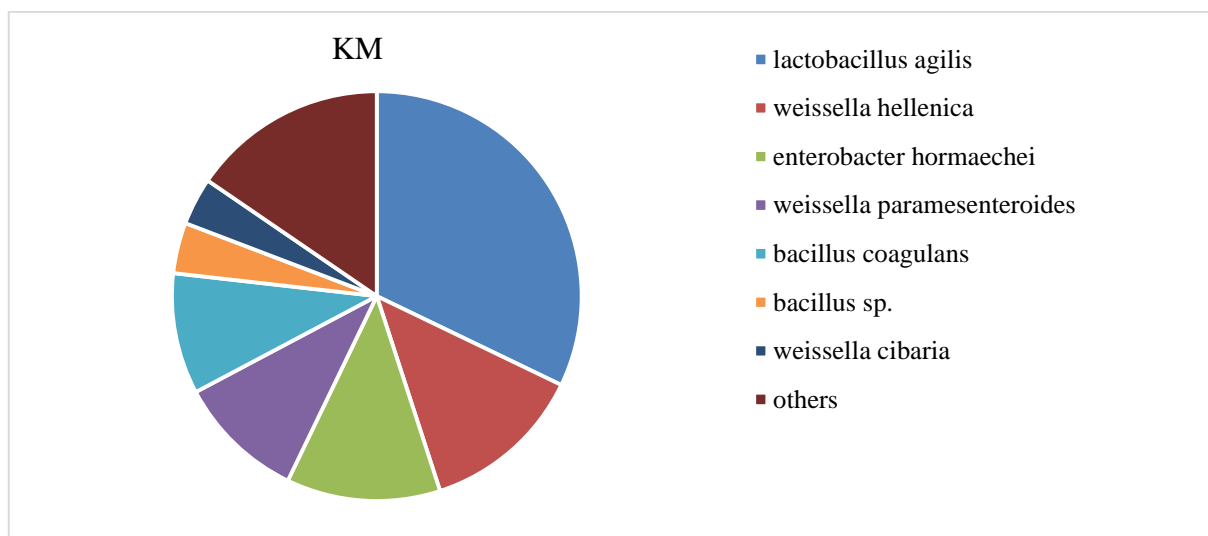


Figure 4.15. Taxonomic distribution of bacterial diversity at species level in KM compost with a proportion of at least 1% abundance.

Bacteria belonged to species *Lactobacillus agilis* (23.39%), *Weissella hellenica* (9.35%), *Enterobacter hormaechei* (8.84%), *Weissella paramesenteroides* (7.34%), and *Bacillus coagulans* (6.95%) were found to be dominant in thermophilic stage during KM composting followed by *Bacillus sp.* (2.91%), and *Weissella cibaria* (2.72%) (Fig. 4.15).

Lactic acid bacteria *Lactobacillus agilis* and *Weissella hellenica* found the most dominant species in KM composting process. *Weissella paramesenteroides*, and *Weissella hellenica* produce bacteriocins that is a member of natural antimicrobial agents by forming lactic acid as a main end-product of fermentation (Woraprayote et al., 2015).

Enterobacter hormaechei (Phylum: Proteobacteria) and *Bacillus sp.* (Phylum: Firmicutes) were found at every compost sample. While some species including *Weissella paramesenteroides*, and *Weissella hellenica* detected in almost every sample. Various studies indicated that predominant genera present throughout the composting process was belonged to *Bacillus sp.* that is typical bacteria of thermophilic phase. *Bacillus sp.* has the ability to survive in the compost pile by forming the endospores during thermophilic stage (Chandna et al., 2013; Tuomela et al., 2000). Therefore, the high abundance of *Bacillus* might be due to its capacity for heat resistance in contrast to other bacteria. In current study, the abundance of *Bacillus sp.* reflected satisfactory conditions for thermophilic stage during composting process (Wang et al., 2017).

All in all, heterogeneity of microbiota of compost is dependent on combination of materials, operating conditions, and surrounding environment. Therefore, enormous studies were conducted relevant to different microbiota composition presented in composting process (López-González et al., 2015). Moreover, microbial DNA determination is not a sign of the presence of live or intact microbes because of the persistence of DNA within or released from cells that once live in interested samples (Hultman et al., 2010).

5. CONCLUSIONS

Several composting processes were investigated with the current study to find the best end product that would fulfil the official regulatory limits in order to be used as soil amendment. Traditional methods to follow physiochemical composition and abiotic factors were applied together with innovative methods such as NGS that were used to understand the biotic players behind the composting process.

In this study, two sets of composting processes, in which yard waste, kitchen waste, and cow manure were used as feedstocks, were operated in two tumbler composting systems for a month. In all composting systems, initial moisture content was adjusted to 55 ± 5 % with the aid of sawdust as bulking agent. Maturity of compost was determined when temperature of the compost declines to ambient temperature and carbon-to-nitrogen (C/N) ratio became constant. The experimental sets named Set1 (Y, K, YKM) and Set2 (YK, YM, KM) were carried out and temperature, pH, moisture content and total volatile solids were detected throughout the process to control the composting efficiency. Analytical methods were conducted for end products to compare the results with regulatory limitations to use compost as a soil amendment.

According to “Regulations Regarding the Production, Import, Marketing and Inspection of Organic, Organomineral Fertilizers and Soil Amendment Products and Other Products, Microbial and Enzyme Based Products” and “Compost Regulation”, C/N portion should be in the range of 10 to 30, volatile solids content should be at least 35% of total solids, moisture content should be lower than 30%, and electrical conductivity should not exceed 10 dS/cm. Additionally, according to “Compost Regulation” pH should be varying between 5.5 to 8.5. In this study, the results indicated that final C/N ratio, volatile solids content, electrical conductivity of all composting systems met with regulatory requirements. The pH in Y (7.47) and YKM (8.12) compost were found to be in the range stated in regulations. Other compost products had pH that was slightly higher than the limitations (K:8.76, YK: 8.67, YM: 8.82, KM: 8.56). Final moisture content was found higher than regulatory limits (<30%). In consideration this result, air ventilation can be apply to maintain the appropriate moisture conten

Moreover, the thermophilic phases (55-60 °C) occurred in Y compost for 4-5 days, in K compost for 3-4 days and in YK compost for 8-9 days. The thermophilic phases lasted longer than three days ensured that Y, K and YK composts met with sanitation requirement relevant to the absence of weed seeds and pathogens in compost.

Firmicutes and *Proteobacteria* – the humic-reducing microorganisms were the most abundant phyla in which *Bacilli* (Y:81%, K:39%, YKM:24%, YK:43%, YM:40%, KM:72%) and *Gammaproteobacteria* (Y:9%, K:47%, YKM:56%, YK:41%, YM:28%, KM:17%) dominated at class level in all composting processes at thermophilic stage varying from 58 to 70°C. Species such as *Enterobacter hormaechei* and *Bacillus sp.* detected in all compost systems at thermophilic stage. The high abundance of Firmicutes pointed active thermophilic conditions during the process.

The results demonstrated that the best performance of composting process was demonstrated by Y, K and YK compost as they met with sanitation requirements. After application of air ventilation, these composts can be used as soil amendment according to regulations. However, the efficiency of the composting process decreased with the aid of manure in the system. The results indicated that thermophilic stage lasted less than three days in the system that contained manure as a compost product. Bulking agent that has lower C/N ratio rather than sawdust can be used in composting process to achieve optimal initial C/N ratio for manure. Additionally, manure samples can be dried using a freeze dryer to decrease water content while minimizing loss of volatile solids before composting system proceeds.

Composting process ensures the conversion of organic wastes to a valuable resource that can be used as a soil amendment. The current study is applicable for local waste management strategies. Composting innovations can significantly decrease the load of organic wastes sent to landfills and maintain the creation of sustainable living conditions.

6. FURTHER RECOMMENDATIONS

In this study, NGS method were conducted to detect bacterial diversity in the samples which highest temperature observed during sampling days of composting. NGS results indicated that pathogenic bacteria were presented in this stage. For further investigations, NGS studies can be extended to target abundant bacterial populations in maturation phase of composting. Moreover, a future study can be carried out to investigate pathogens in rRNA level for understanding their activity in the last stage.

The compost systems composed of yard and kitchen waste that were obtained from Boğaziçi University demonstrated the best performance. In Boğaziçi University, approximately 1200 - 1400 kg yard waste and 500 - 600 kg kitchen waste depending on winter and spring term are generated in a month. Therefore, in the scope of green campus, composting of yard and kitchen waste applications can be used.

In this study, comparison of yard waste, kitchen waste and cow manure in terms of waste degradation and bacterial diversity was conducted to contribute the knowledge of composting process using different raw materials. Investigation of such innovative methods to reduce load of organic waste contributing to the landfills would improve local municipal waste management and support to the sustainable development.

REFERENCES

Agnew, J.M., Leonard, J.J., 2003. The physical properties of compost. *Compost Science and Utilization*, 11, 238-264.

Turkish Statistical Institute News Report Animal Production Statistics Home Page. <http://www.tuik.gov.tr/PreHaberBultenleri.do?id=18852>. (accessed January 2015).

Ansorge, W.J., 2009. Next-generation DNA sequencing techniques. *New Biology*, 25, 195-203.

Ariesyady, H.D., Ito, T., Okabe, S., 2007. Functional bacterial and archaeal community structures of major trophic groups in a full-scale anaerobic sludge digester. *Water Research*, 41, 1554-68.

Arslan, A., Razzouk, A.K., Al-Ain, F., 1997. The performance and radiation exposure of some neutron probes in measuring the water content of the topsoil layer. *Australian Journal of Soil Research*, 35, 1397-1407.

ASAE. 2000. Moisture measurement-forages. ASAE S358.2. ASAE Standards 2000. American Society of Agricultural Engineers. St Joseph, Michigan.

ASTM. 1994a. Standard test methods for moisture, ash and organic matter of peat and other organic soils. D 2974-87. Annual Book of ASTM Standards. Section 4. Construction; Volume 04.08 Soil and Rock. ASTM, Philadelphia, Pennsylvania.

ASTM. 1994b. Standard test methods for bulk density of peat and peat products. D 4531-86. Annual Book of ASTM Standards. Section 4. Construction; Volume 04.08 Soil and Rock. ASTM, Philadelphia, PA.

Avvimelech, Y., Bruner, M., Ezrony, I., Sela, R., Kochba, M., 1996. Stability index for municipal solid wastes compost. *Compost Science and Utilization*, 4, 13-20.

Balascio, C.C., 1990. Precision of peat moss water contents measured by microwave drying. *Applied Engineering in Agriculture*, 6, 592-596.

Balascio, C.C., 1992. Calibration of microwave-oven drying techniques versus air-oven methods for measurement of peat moss moisture content. *Applied Engineering in Agriculture*, 8, 197-200.

Banegas, V., Moreno, J.L., Moreno, J.I., Garcia, C., Leon, G., Hernandez, T., 2007. Composting anaerobic and aerobic sewage sludges using two proportions of sawdust. *Waste Management*, 27, 1317-1327.

Beck-Friis, B., Smars, S., Jonsson, H., Kirchmann, H., 2001. Gaseous CO_2 emissions of carbon dioxide, ammonia and nitrous oxide from organic household waste in a compost reactor under different temperature regimes. *Journal of Agricultural Engineering Research*, 78, 423-430.

Beffa, T., Blanc, M., Marilley, L., Fischer J.L., Lyon P.-F., Aragno M., 1996a. Taxonomic and metabolic microbial diversity during composting. In: de Bertoldi M., Sequi P., Lemmes B., Papi T., Eds, *The Science of Composting. Part 1*, Chapman & Hall, London, pp. 149-161.

Beffa, T., Blanc, M., Lyon, P. F., Vogt, G., Marchiani, M., 1996b. Isolation of *Thermus* strains from hot composts (60 to 80 degrees C), *Applied and Environmental Microbiology*, 62, 1723-1727.

Benito, M., Masaguer, A., Moliner, A., Arrigo, N., Palma, R.M., 2003. Chemical and microbiological parameters for the characterisation of the stability and maturity of pruning waste compost. *Biology and Fertility of Soils*, 37, 184-189. DOI: 10.1007/s003-0584-7.

Berkes, F., Kışlalioğlu M.B., 1993. *Çevre ve Ekoloji*, 4. Edition, Remzi Kitabevi, Istanbul.

Bernal, M.P., Albuquerque, J.A., Moral, R., 2009. Composting of animal manures and chemical criteria for compost maturity assessment. A review. *Bioresource Technology*, 100, 5444-5453.

Bertoldi, M.D., Vallini, G., Pera, A., 1983. The biology of composting. *Waste Management and Research*, 1, 157–176.

Bhatia, A., Rajpal, A., Madan, S., Kazmi, A.A., 2013. Techniques to analyze microbial diversity during composting- A mini review. *Indian Journal of Biotechnology*, 14, 19-25.

Brady, N., Weil, R., 1996. *The Nature and Properties of Soils*, 12th ed. Prentice, New Jersey, USA, pp. 385- 495.

Bronikowski, A.M., Bennet, A.F., Lenski, R.E., 2001. Evolutionary adaptation to temperature. VIII. Effects of temperature on growth rate in natural isolates of *Escherichia coli* and *Salmonella enterica* from different thermal environment. *Evolution*, 55, 33-40.

Brown, D., Li, Y., 2013. Solid state anaerobic co-digestion of yard waste and food waste for biogas production. *Bioresource Technology*, 127, 275-280.

Cardenas, R.R., 1977. Rapid moisture determination in compost for process control. *Compost Science*, 18, 14-15.

Cekmecelioglu, D., Demirci, A., Graves, R.E., Davitt, N.H., 2005. Applicability of optimized in-vessel food waste composting for windrow systems. *Biosystems Engineering*, 91, 479–486.

Campbell, A.G., Folk, R.L., Tripepi, R.R., 1997. Wood ash as an amendment in municipal sludge and yard waste composting processes. *Compost Science and Utilization*, 1, 62-73.

Cerenzio, P.F., 1987. Turnaround in Sussex county. *BioCycle*, 28, 26–28.

Chanda, P., Nain, L., Singh, S., Kuhad, R.C., 2013. Assessment of bacterial diversity during composting of agricultural byproducts. *BMC Microbiology*, 13, 1-14.

Chandler, J.A., Jewell, W.J., Gossett, J.M., Soest, P.J., Van, Robertson J.B., 1979. Predicting methane fermentation biodegradability. *Biotechnology Bioengineering Symposium* (16th edn.) 10, 93-107.

Chang, J.I., Tsai, J.J., Wu, K.H., 2006. Thermophilic composting of food waste. *Bioresource Technology*, 97, 116-122.

Chang, J.I., Hsu, T., 2008. Effects of compositions on food waste composting. *Bioresource Technology*, 99, 8068-8074.

Chen, Y., Inbar, Y., 1993. Chemical and spectroscopical analyses of organic matter transformation during composting in relation to compost maturity. In: Hoitink, H.A.J., Keener, H.M. (Eds.), *Science and Engineering of Composting: Design, Environmental, Microbiological and Utilization Aspects*. Renaissance Publications, Ohio, pp. 551–600.

Choi, M.H., Park, Y.-H., 1998. The influence of yeast on thermophilic composting of food waste. *Letters in Applied Microbiology*, 26, 175-178.

Compost Regulation. 2015. Official Gazette of Republic of Turkey, 29286, 5 March 2015.

Coeuret, V., Dubernet, S., Bernardeau, M., Gueguen, M., Vernoux, J.P., 2003. Isolation, characterization and identification of lactobacilli focusing mainly on cheeses and other dairy products. *Le Lait*, INRA Editions, 83, 269-306. DOI: 10.1051/lait:2003019.

Crowe, M., Nolan, K., Collins, C., Carty, G., Donlon, B., 2002. Biodegradable Municipal Waste Management in Europe. Part 1. Strategies and Instruments. European Environment Agency, Copenhagen, pp. 6–24.

Das, K.C., Tollner, E.W., Eiteman, M.A., 2003. Comparison of synthetic and natural bulking agents in food waste composting. *Compost Science and Utilization*, 11, 27–35.

Day, M., Krzymien, M., Shaw, K., Zaremba, L., Wilson, W.R., Botden, C., Thomas, B., 1998. An investigation of the chemical and physical changes occurring during commercial composting. *Compost Science and Utilization*, 6, 44-66.

Davis C.L., Hinch S.A., Donkin C.J., Germishuizen P. 1991. Changes in microbial population numbers during the composting of pine bark. *Bioresource Technology*, 39, 85-92.

de Bertoldi, M., Vallini, G., Pera, A., 1983. The biology of composting: a review. *Waste Management Resource* 1, 157-176.

Dees, P.M, Ghiorse, W.C, 2001. Microbial diversity in hot synthetic compost as revealed by PCR-amplified rRNA sequences from cultivated isolates and extracted DNA, *FEMS Microbiology Ecology*, 35, 207-216.

DEFRA, 2005. Government interpretation of the landfill (England and Wales) regulations 2002. Department for Environment Food and Rural Affairs, HMSO, London.

DeSantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K., Huber, T., Dalevi, D., Hu, P., Andersen, G.L., 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied and Environmental Microbiology*, 72, 5069–5072.

Diaz, L.F., de Bertoldi, M., Bidlingmaier, W., Stentiford, E., 2007. *Compost Science and Technology*. Elsevier.

Donahue, D.W., Chalmers, J.A., Stores, J.A., 1998. Evaluation of in-vessel composting of university postconsumer food wastes. *Compost Science and Utilization*, 6, 75-81.

Edwards, U. Rogall, T., Bölcker, H., Emde, M., Böttger, E.C., 1988. Isolation and complete nucleotide determination of entire genes characterisation of a gene coding for 16S ribosomal RNA. *Nucleic Acids Research*, 17, 7843-7853.

Eiland., F., Klammer, M., Lind, A.-M., Leth, M., Bååth, 2001. Influence of initial C/N ratio on chemical and microbial composition during long term composting of straw. *Microbial Ecology*, 41:272-280. DOI: 10.1007/s0024880000071.

Eklind, Y., Kirchmann, H., 2000. Composting and storage of organic household waste with different litter amendments. II: nitrogen turnover and losses. *Bioresource Technology*, 74, 125-33.

Epstein, E., 1997. *The Science of Composting*, Technomic Publishing Co., Inc., Lancaster, Basel.

European Union, 1999. Council Directive 1999/31/EC on the landfill of waste. *Official Journal of the European Communities*. L 182, 16/07/ 1999, 0001–0019.

Fakruddin, M., Shahnewaj, K., Mannan, B., 2013. Methods for analyzing diversity of microbial communities in natural environments. *Ceylon Journal of Science (Biological Sciences)*, 42, 19-33.

Farrel, M., Jones, D.L., 2009. Critical evaluation of municipal solid waste composting and potential compost markets, *Bioresource Technology* 100, 4301- 4310.

Fermor, T.R., Wood, D.A., Lynch, J.M., 1989. Microbiological processes in compost. In: *Int. Symp. Compost Prod. and Use*. San Michele AllAdige, Italy, pp. 282–300.

Finstein, M.S., Morris, M.I., 1975. Microbiology of municipal solid waste composting. In: *The influence of temperature and moisture contents regimes on the aerobic microbial activity of a biosolids composting blend*, (Liang, C., Das, K.C., McClendon, R.W.), 2002. *Bioresource Technology*, pp:132.

Finstein, M.S., Miller, F.C., Strom, P.F., 1986. Waste treatment composting as a controlled system, *Biotechnology*, 8, 396-443.

Finstein, M.S, Morris, M.L, 1975. Microbiology of municipal solid waste composting, *Advances in Applied Microbiology*, 19, 113-151.

Franke-Whittle, I.H., Knapp, B.A, Fuchs, J., Kaufmann, R., Insam, H., 2009. Application of COMPOCHIP microarray to investigate the bacterial communities of different composts, *Microbiology Ecology*, 57, 510-521.

Fuch, J.G., 2010. Interactions between beneficial and harmful microorganisms: From the composting process to compost application. In: Insam, H., Franke-Whittle, I., Goberna, M., (eds) *Microbes at work*. Springer, Berlin.

Garcia, A.J., Esteban, M.B., Marquez, M.C., Ramos, P., 2005. Biodegradable municipal solid waste: characterization and potential use as animal feedstuffs. *Waste Management*, 25, 780-787.

Gelsomino, A., Kejzer-Wolters, A.C., Cacco, G., van Alsas, J.D., 1999. Assessment of bacterial community structure in soil by polymerase chain reaction and denaturing gradient gel electrophoresis, *Journal of Microbiological Methods*, 38, 1-15.

Glancey, J.L., Hoffman, S.C. 1994. Physical properties of solid waste materials. Paper no. 94-1592. American Society of Agricultural Engineering, Atlanta, Georgia.

Golueke, C.G., 1977. *Biological Reclamation of Solid Waste*. Rodale Press, Inc., Emmaus, Pennsylvania, USA.

Greenway, G.M., Song, Q.J., 2002. Heavy metal speciation in the composting process. *Journal of Environmental Monitoring*, 4, 300-305.

Gulhane, M., Pandit, P., Khardenavis, A., Singh, D., Purohit H., 2017. Study of microbial community plasticity for anaerobic digestion of vegetable waste in Anaerobic Baffled Reactor, *Renewable Energy*, 101, 59-66.

Guo, L., Wu, G., Li, C., Meng, J., Liu, H., Yu, X., Jiang G., 2016. Effects of cattle manure compost combined with chemical fertilizer on topsoil organic matter, bulk density and earthworm activity in a wheat–maize rotation system in Eastern China, *Soil and Tillage Research*, 156, 140-147. DOI:10.1016/j.still.2015.10.010.

Hansen, T., Bhandar, G., Christensen, T., Bruun, S., Jensen, L., 2006. Life cycle modeling of environmental impacts of application of processed organic municipal solid waste on agricultural land (EASEWASTE). *Waste Management and Research*, 124, 153-166.

Hardy, G.E.S.J., Sivasithamparam, K., 1989. Microbial, chemical and physical changes during composting of a eucalyptus (*Eucalyptus calophylla* and *Eucalyptus diversicolor*) bark mix. *Biology and Fertility of Soils*, 8, 260-270.

Hargreaves, J.C., Adl, M.S., Warman, P.R., 2008. A review of the use of composted municipal solid waste in agriculture. *Agriculture, Ecosystems and Environment* 123, 1-14.

Haug, R.T., 1993. *The Principal Handbook of Compost Engineering*. Lewis Publishers, Boca Raton, FL, USA.

Haug, R.T., 1995. *The Practical Handbook of Compost Engineering*. Lewis Publishers, Boca Raton, Florida.

He, X., Traina, S., Logan, T., 1992. Chemical properties of municipal solid waste compost. *Journal of Environmental Quality*, 21, 318–329.

He, X.T., T.J. Logan, Traina, S.J., 1995. Physical and chemical characteristics of selected U.S. municipal solid waste composts. *Journal of Environmental Quality*, 24, 543-552.

Herrmann, R.F., Shann, J.F., 1997. Microbial community changes during the composting of municipal solid waste. *Microbiology Ecology*, 33, 78-85.

Hespell, R.B., *Serpens flexibilis* gen. nov., sp. nov., an unusually flexible, lactate-oxidizing bacterium. *International Journal of Systematic Bacteriology*, 4, 371-381.

Himanen, M., Hanninen, K., 2011. Composting of bio-waste, aerobic and anaerobic sludges-effect of feedstock on the process and quality of compost. *Bioresource Technology*, 102, 2842-2852.

Hörnig, G., Türk, M., Wanka, U., 1999. Slurry Covers to reduce Ammonia Emission and Odour Nuisance. *Journal of Agricultural Engineering Research*, 73, 151-157.

Hultman, J., Kurola, J., Rainisalo, A., Kontro, M., Romantschuk, M., 2010. Utility of molecular tools in monitoring large scale composting. *Microbes at Work* (Insam H Franke-Whittle I Goberna M, eds), pp. 135–151. Springer, Berlin, Heidelberg.

Insam, H., de Bertoldi, M., 2003. Microbiology of the composting process. In: Golueke C., Bidlingmaier W., de Bertoldi M., Diaz L., Eds, *Compost Science and Technology*, Elsevier Science Ltd.

Insam, H., Franke-Whittle, I.H., Goberna, M., 2010. Microbes in aerobic and anaerobic waste treatment. In: Insam H., Franke-Whittle I.H., Goberna M., editors. *Microbes at Work From Wastes to Resources*. Springer-Verlag; Heidelberg, Germany: pp. 1–36.

Ishii, K., Fukui, M., Takii, S., 2000. Microbial succession during a composting process as evaluated by denaturing gradient gel electrophoresis analysis, *Journal of Applied Microbiology*, 89, 768-777.

Islam, R., M., Sultana, T., Cho, J-C., Joe, M., M., Sa, T., M., 2012. Diversity of free-living nitrogen-fixing bacteria associated with Korean paddy fields. *Annals of Microbiology*, 62:1643-1650. DOI: 10.1007/s13213-012-0421-z.

Jayasree, S., Balan, R., 2012. Characterization and bacterial succession studies of garden waste during the windrow composting process at Mercy College Campus. *Biochemical and Cellular Archives*, 2, 403-413.

Joshua, R.S., Macauley, B.J., Mitchell, H.J., 1998. Characterization of temperature and oxygen profiles in windrow processing systems. *Compost Science and Utilization*, 6, 15-28.

Kane, B.E, Mullins, J.T, 1973. Thermophilic Fungi in a municipal waste compost system, *Mycologia*, 65, 1087-1100.

Karadag, D., Özkaya, B., Ölmez, E., Nissila, M.E., Çakmakçı, M., Yıldız, S., Puhakka, J.A., 2013. Profiling of bacterial community in a full-scale aerobic composting plant. *Int. Biodeterioration Biodegradation*, 77, 85-90.

Keener, H.M., Dick, W.A., Hoitink, H.A.J., 2000. Composting and beneficial utilization of composted by-product materials. In: Dick, W.A. (Ed.), *Land Application of Agricultural, Industrial, and Municipal By-Products*. Soil Science Society of America, Inc., Madison.

Kim, S-H., Kim, I.H., Lee, W.J., Lee, J-H, 2008. Characterization of thiosulfate-oxidizing *Enterobacter hormaechei* JH isolated from barnyard manure. *Korean Journal of Chemical Engineering*, 25, 1131-1135.

Komilis, D.P., Han, R.K., 2006. Carbon dioxide and ammonia emissions during composting of mixed paper, yard waste and food waste. *Waste Management*, 26, 62-70.

Koufodimos, G., Samara, Z., 2002. Waste management options in southern Europe using field and experimental data. *Waste Management*, 22, 47-59.

Kumar, M., Ou, Y.L., Lin, J.G., 2010. Co-composting of green waste and food waste at low C/N. *Waste Management*, 30, 602-609.

Kwon, S.H., Lee, D.H., 2004. Evaluation of Korean food waste composting with fedbatch operations I: using water extractable total organic carbon contents (TOCw). *Process Biochemistry*, 39, 1183-1194.

Lacey J., 1973. Actinomycetes in soils, composts and fodders. In: Sykes G., Skinner F.A., Eds, Actinomycetales: Characteristics and Practical Importance. Academic Press, New York.

Lee, D.H., Zo, Y.G., Kim, S.J., 1996. Nonradioactive method to study genetic profiles of natural bacterial communities by PCR-single-strand-conformation polymorphism, *Applied and Environmental Microbiology*, 62, 3112-3120.

Leege, P.B., Thompson, W.H., (Eds.) 1997. Test methods for the physical examination of compost and composting. In: Test Methods for the Examination of Composting and Compost, U.S. Composting Council.

Lemus, G.R., Lau, A.K., 2002. Biodegradation of lipidic compounds in synthetic food wastes during composting. *Journal of Biosystems Engineering*, 44, 33-39.

Leonard, J.J., Mu, R., McGill, W., 1997. Composting of industrial bio-oxidation sludge. Proceedings of the 7th Annual Composting Council Conference, Sherbrooke, PQ.

Liao, P.H., Vizcarra, A.T., Chen, A., Lo, K.V., 1993. Composting of separated solid swine manure. *Journal of Environmental Science and Health*, 28, 1889-1901.

Liu, W.T, Marsh, T.L, Cheng, H., Forney, L.J., 1997. Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. *Applied and Environmental Microbiology*, 63, 4516-4522.

Liu, L., Li, Y., Li, S., Hu, N., He, Y., Pong, R., Lin, D., Lu, L., Law, M., 2012. Comparison of next-generation sequencing systems. *Journal of Biomedicine and Biotechnology*, 251364. DOI: 10.1155/2012/251364.

López-González J. A., Suárez-Estrella F., Vargas-García M. C., López M. J., Jurado M. M., Moreno J., 2015. Dynamics of bacterial microbiota during lignocellulosic waste composting: Studies upon its structure, functionality and bio diversity. *Bioresource Technology*, 175, 406-416.

Loman, N.J., Constantinidou, C., Chan, J.Z.M., Halachev, M., Sergeant, M., Penn, C.W., Robinson, E.R., Pallen, M.J., 2012. High-throughput bacterial genome sequencing: an embarrassment of choice, a world of opportunity. *Nature Reviews Microbiology*, 10, 599-606.

Lu, L., Zeng, G., Fan, C., Ren, X., Wang, C., Zhao, Q., Zhang, J., Chen, M., Chen, A., Jiang, M., 2013. Characterization of a laccase-like multicopper oxidase from newly isolated *Streptomyces* sp. C1 in agricultural waste compost and enzymatic decolorization of azo dyes. *Biochemical Engineering Journal*, 72, 70-76. DOI: 10.1016/j.bej.2013.01.004.

Lv, B., Xing, M., Yang, J., Zhang, L., 2015. Pyrosequencing reveals bacterial community differences in composting and vermicomposting on the stabilization of mixed sewage sludge and cattle dung. *Applied Microbiology and Biotechnology*, 99, 10703-10712.

Maarit-Niemi, R., Heiskanen, I., Wallenius, K., Lindstrom, K., 2001. Extraction and purification of DNA in rhizosphere soil samples for PCR-DGGE analysis of bacterial consortia. *Journal of Microbiological Methods*, 45, 155-165.

MacGregor, S.T., Miller, F.C., Psarianos, K.M., Finstein, M.S., 1981. Composting process control based on interaction between microbial heat output and temperature. *Applied and Environmental Microbiology*, 41, 1321-1330.

Madae, K., Hanajima, D., Morioka, R., Osadz, T., 2010. Characterization and spatial distribution of bacterial communities within passively aerated cattle manure composting piles. *Bioresource Technology*, 24, 9631-9637.

Maeda, K., Toyoda, S., Shimojima, R., Osada, T., Hanajima, D., Morioka, R., Yoshida, N., 2010. Source of nitrous oxide emissions during the cow manure composting process as revealed by isotopomer analysis of and *amoA* abundance in betaproteobacterial ammonia-oxidizing bacteria. *Applied and Environmental Microbiology*, 76, 1532-1555.

Marfa, O., J.M. Tort, C. Olivella, Caceres, R., 1998. Cattle manure compost as substrate II- Conditioning and formulation of growing media for cur flower cultures. *Acta Horticulture* 469, 305-312.

Michel, F.C., Reddy, C.A., 1998. Effect of oxygenation level on yard trimmings composting rate, odor production, and compost quality in bench-scale reactors. *Compost Science and Utilization*, 6, 6-14.

McCartney, D., Tingley, J., 1998. Development of a rapid moisture content method for compost materials. *Compost Science and Utilization*, 6, 14-25.

McKinley, V.L., Vestal, J.R., 1984. Biokinetic analysis of adaptation and succession: microbial activity in composting municipal sewage sludge. *Applied and Environmental Microbiology*, 47, 933-941.

McKinley, V.L., Vestal, J.R., Eralp, A.E., 1986. Microbial activity in composting. In: *The biocycle guide to in-vessel composting*. JG Press Inc., Emmaus, Pennsylvania, pp. 171–181.

Metzker, M.L., 2010. Sequencing technologies-the next generation. *Nature Reviews*, 11, 31-46.

Miller, F.C., Harper, E.R., Macauley, B.J., 1989. Field experiments of temperature and oxygen relationships in mushroom compost stacks-consideration of stack oxygenation based on utilization and supply. *Aust. J. Exp. Agric.* 29, 741–750. Miller, F.C., Harper, E.R., Macauley, B.J., Gulliver, A., 1990. Composting based on moderately thermophilic and aerobic conditions for the production of commercial mushroom growing compost. *Australian Journal of Experimental Agriculture*, 30, 287-296.

Miller, F.C., 1989. Matric water potential as an ecological determinant in compost, a substrate dense system. *Microbial Ecology*, 18, 59-71.

Miller, F.C., 1992. Composting as a process based on the control of ecologically selective factors. In: Metting, F.B., Jr. (Ed.), *Soil Microbial Ecology, Applications in Agricultural and Environmental Management*. Marcel Dekker, Inc., New York.

Millner, P.D., Powers, K.E., Enkiri, N.K., Burge, W.D., 1987. Microbially mediated growth suppression and death of *Salmonella* in composted sewage sludge. *Microbial Ecology* 14, 255-165.

Mosher, D., Anderson, R.K., 1977. Composting sewage sludge by high-rate suction aeration techniques—the process as conducted at Bangor, ME, and some guidelines of general applicability. Interim Report Number SW-614d. US Government Printing Office, Washington, DC.

Muyzer, G., Dewall, E.C., Uitterlinden, A.G., 1993. Profiling of complex bacterial population by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl Environmental Microbiology*, 59, 695-700.

Muyzer, G., Smalla, K., 1998. Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. *Antonie Leeuwenhoek*.

Nakasaki, K., Sasaki, M., Shoda, M., Kubota, H., 1985a. Change in microbial numbers during thermophilic composting of sewage sludge with reference to CO₂ evolution rate. *Appl. Environmental Microbiology*, 49, 37-41.

Nakasaki, K., Shoda, M., Kubota, H., 1985b. Effect of temperature on composting of sewage sludge. *Applied and Environmental Microbiology*, 50, 1526-1530.

Namkoong, W., Hwang, E.Y., 1997. Operational parameters for composting night. *Compost Science*, 5, 46-51.

Nakasaki, K., Akakura, N., Atsumi, K., Takemoto, M., 1998. Degradation patterns of organic materials in batch and fedbatch composting operations. *Waste Management Resource* 16, 484-489.

Nakasaki, H., Ohtaki, A., 2002. A simple numerical model for predicting organic matter decomposition in a fed-batch composting operation. *Journal of Environmental Quality*, 31, 997-1003.

Nakasaki, K., Nagasaki, K., 2004. Degradation of fats during thermophilic composting of organic waste. *Waste Management Resource*, 22, 276-282.

Natvig, E.E., Ingham, S.C., Ingham, B.H., Cooperband, L.R., Roper, T.R., 2002. *Salmonella enterica* Serovar Typhimurium and *Escherichia coli* contamination of root and leaf vegetables grown in soils with incorporated bovine manure. *Applied and Environmental Microbiology*, 6, 2737-2744. DOI: 10.1128/AEM.68.6.2737-2744.2002.

Nell, J.H., Wiechers, S.G., 1978. High temperature composting. *Water Science in Africa*, 4, 203-212.

Nelson, D.W., Sommers, L.E., 1996. Total carbon, organic carbon, and organic matter. p. 961-1010. In D.L Sparks et al. (eds.) *Methods of soil analysis. Part 3. Chemical Methods*. SSSA Book Series No. 5, SSSA and ASA, Madison, WI.

Nicolaisen, M.H., Ramsing, N.B., 2002. Denaturing gradient gel electrophoresis (DGGE) approaches to study the diversity of ammonia-oxidizing bacteria. *Journal of Microbiological Methods* 50, 189-203.

Otten, L., 2001. Wet–dry composting of organic municipal solid waste: current status in Canada. *Canadian Journal of Civil Engineering*, 28, 124-130.

Papadimitrou, E.K., Barton, J.R., Stentiford, E.I., 2008. Sources and levels of potentially toxic elements in the biodegradable fraction of autoclaved nonsegregated household waste and its compost/digestate. *Waste Management Resource*, 26, 419-430.

Partanen, P., Hultman, J., Paulin, L., Auvinen, P., Romantschuk, M., 2010. Bacterial diversity at different stages of the composting process. *BMC Microbiology*, 10, 1-11.

Peters, S., Koschinsky, S., Schweiger, F., Tebble, C.C., 2000. Succession of microbial communities during hot composting as detected by PCR-single-strand-conformation polymorphism- based genetic profiles of small-subunit rRNA genes, *Applied and Environmental Microbiology*, 66, 930-936.

Pojasok, T., 2000. A field method of measuring compost maturity. *Proceedings of the 10th Annual Composting Council Conference*, Edmonton, AB.

Pinto, F., André, R.N., Carolino, C., Miranda, M., Abelha, P., Direito, D., Perdikaris, N., Boukis, I., 2014. Gasification improvement of a poor quality solid recovered fuel (SRF). Effect of using natural minerals and biomass wastes blends. *Fuel*, 117, 1034-1044.

Ramaswamy, J., Prasher, S., O., Patel, M., P., Hussain, S., A., Barrington, S., F., 2010. The effect of composting on the degradation of a veterinary pharmaceutical. *Bio-Resource*, 10, 2294-2299.

Ramer, S., Leonard, J.J., 1995. Moisture determination of compost by microwave drying. Paper no. 956637. *American Society of Agricultural Engineering*, Chicago, Illinois.

Ramos, M.C., López-Acevedo, M., 2004. Zinc levels in vineyard soils from the alt penedès-anoia region (ne spain) after compost application. *Advanced in Environmental Research*, 8, 687–696.

Regulations Regarding the Production, Import, Marketing and Inspection of Organic, Organomineral Fertilizers and Soil Amendment Products And Other Products, Microbial And Enzyme Based Products, 2014. *Offical Gazette of Rebuplic of Turkey*, 28956, 29 March 2014.

Richard, T.L., 1992. Municipal solid waste composting: physical and biological processing. *Biomass and Bioenergy*, 3,163–180.

Richard, T.L., 1997. The kinetics of solid state aerobic biodegradation, Ph.D. dissertation, Cornell University, Ithica, NY, USA.

Ringer, C.E., 1997. Suppression of seedling damping-off disease in potting mix containing animal manure composts. *Compost Science and Utilization*, 5, 6-15.

Rothberg, J.M., Hinz, W., Rearick, T.M., Schultz, J., Mileski, W., Davey, M., Leamon, J.H., Johnson, K., Milgrew, M.J., Edwards, M., Hoon, J., Simons, J.F., Marran, D., Myers, J.W., Davidson, J.F., Branting, A., Nobile, J.R., Puc, B.P., Light, D., Clark, T.A., Huber, M., Branciforte, J.T., Stoner, I.B., Cawley, S.E., Lyons, M., Fu, Y., Homer, N., Sedova, M., Miao, X., Reed, B., Sabina, J., Feierstein, E., Schorn, M., Alanjary, M., Dimalanta, E., Dressman, D., Kasinskas, R., Sokolsky, T., Fidanza, J.A., Namsaraev, E., McKernan, K.J., Williams, A., Roth, J.T., Bustillo, J., 2011. An integrated semiconductor device enabling non723 optical genome sequencing. *Nature* 475, 348–352.

Ryckeboer, J., Mergaert, J., Coosemans J, Deprins K, Swings J, 2003. Microbiological aspects of biowaste during composting in a monitored compost bin, *Journal of Applied Microbiology*, 94, 127-137.

Ryckeboer, J., Mergaert, J., Vaes, K., Klammer, S., De Clercq, D., Coosemans, J., Insam, H., Swings, J., 2003. A survey of bacteria and fungi occurring during composting and self-heating processes. *Annals of Microbiology*, 53, 349-410.

Rynk R., Kamp, M., Richard, T., Klega, J., Gouin, F., 1992. *On Farm Composting Handbook*. Northeast Regional Agricultural Engineering Service, 152 Rile- Robb Hall, Cooperative Extension, Ithaca, N. Y. 14853-5701, USA.

Sánchez-Monedero, M.A., Roig, A., Paredes, C., Bernal, M.P., 2001. Nitrogen transformation during organic waste composting by the Rutgers system and its effects on pH, EC and maturity of the composting mixtures. *Bioresource Technology*, 78, 301–8.

Sadaka, S., El-Taweel, A., 2003. Effects of aeration and C: N ratio on household waste composting in Egypt. *Compost Science and Utilization*, 11, 36-41.

Sakai, S., Sawell, S.E., Chandler, A.J., Eighmy, T.T., Kosson, D.S., Vehlow, J., 1997. World trends in municipal solid waste management. *Waste Management*, 16, 341-350.

Samarajeewa, A.D., Hammad, A., Masson, L., Khan, L.U.H., Scroggins, R., Beaudette, L.A., 2015. Comparative assessment of next-generation sequencing, denaturing gradient gel electrophoresis, clonal restriction fragment length polymorphism and cloning-sequencing as methods for characterizing commercial microbial consortia. *Journal of Microbiological Methods*, 108, 103-111.

Schloss, P.D., Handelsman, J., 2003. Biotechnological prospects from metagenomics, *Current Opinion in Biotechnology*, 14, 303-310.

Schulte, E.E. 1988. Recommended soil organic matter tests, p. 29-31. In: W.C. Dahnke (ed.). Recommended Chemical Soil Test Procedures for North Central Region. North Dakota State University, Fargo. North Dakota Agricultural Experiment Station Bulletin 499 (revised).

Seo, J.Y., Heo, J.S., Kim, T.H., Joo, W.H., Crohn, D.M., 2004. Effect of vermiculite addition on compost produced from Korean food wastes. *Waste Management*, 24, 981-987.

Shao, L.-M., Zhang, C.-Y., Wu, D., Lü, F., Li, T.-S., He, P.-J., 2014. Effects of bulking agent addition on odorous compounds emissions during composting of OFMSW. *Waste Management*, 34, 1381-1390.

Shin, S.K., Hwang, C.Y., Cho, Y.J., Yi, H., 2015. Reclassification of *Serpens flexibilis* Hespell 1977 as *Pseudomonas flexibilis* comb. Nov., with *Pseudomonas tuomuerensis* Xin et al. 2009 as later heterotypic synonym. *Systematic and Applied Microbiology*, 38, 563-6. DOI: 10.1016/j.syapm.2015.09.007.

Song, C., Li, M., Xi, B., Wei, Z., Zhao, Z., Jia, X., Qi, H., Zhu, C., 2015. Characterization of dissolved organic matter extracted from the bio-oxidative phase of co-composting of biogas residues and livestock manure using spectroscopic techniques. *International Biodeterioration and Biodegradation*, 103, 38-50.

Stoffella, P.J., Kahn, B.A., eds. 2001. Compost Utilization in Horticultural Cropping Systems, Boca Raton, Florida: Lewis Publishers.

Streger, K., Sjogren, A.M., Jaris, A., Jansson, J.K., Sundh, I., 2007. Development of compost maturity and Actinobacteria populations during full-scale composting of organic household waste, *Journal of Applied Microbiology*, 103, 487-498.

Strom, P.F., 1985a. Effect of temperature on bacterial species diversity in thermophilic solid-waste composting. *Applied and Environmental Microbiology*, 50, 899-905.

Strom, P.F., 1985b. Identification of thermophilic bacteria in solid-waste composting. *Applied and Environmental Microbiology*, 50, 906-913.

Suler, D.J., Finstein, M.S., 1977. Effect of temperature, aeration, and moisture on CO₂ formation in bench-scale, continuously thermophilic composting of solid waste. *Applied and Environmental Microbiology*, 33, 345-350.

Sundberg, C., 2005. Improving compost process efficiency by controlling aeration, temperature and pH. Doctoral thesis, Swedish University of Agricultural Sciences, Uppsala.

Stentiford, E.I., 1996. Composting control: principles and practice. In: de Bertoldi, M., Sequi, P., Lemmes, B., Papi, T. (Eds.), *The Science of Composting: Part I*. Chapman and Hall Inc., London.

Swan, J.R.M., Crook, B., Gilbert, E.J., 2002. Microbial emissions from composting sites. *Issues in Science and Technology*, 18, 73-101.

Stoffella, P.J., Kahn, B.A., eds. 2001. Compost Utilization in Horticultural Cropping Systems, Boca Raton, Florida: Lewis Publishers.

Takaku, H., Kodaira, S., Kimoto, A., Nashimoto, M., Takagi, M., 2006. Microbial communities in the garbage composting with rice hull as an amendment revealed by

culture-dependent and independent approaches. *Journal of Bioscience and Bioengineering*, 101, 42-50.

Tang, J.C., Kanamori, T., Inoue, Y., Yasuta, T., Yoshida, S., Katayama, A., 2004. Changes in the microbial community structure during thermophilic composting of manure as detected by the quinone profile method. *Process Biochemistry*, 39, 1999-2006.

Taylor, T., Kosson, D.S., 1996. USA. National overview on waste management. *Waste Management*, 16, 361-366.

Thambirajah, J.J., Zulkali, M.D., Hashim, M.A., 1995. Microbiological and biochemical changes during the composting of oil palm empty-fruit-bunches. Effect of nitrogen supplementation on the substrate. *Bioresource Technology*, 52, 133-144.

Theron, J., Cloete, T.E., 2000. Molecular techniques for determining microbial diversity and community structure in natural environments, *Critical Reviews in Microbiology*, 26, 37-57.

Tian, W., Sun, Q., Xu, D., Zhang, Z., Chen, D., Li, C., Shen, Q., Shen, B., 2013. Succession of bacterial communities during composting process as detected by 16S rRNA clone libraries analysis. *International Biodeterioration and Biodegradation*, 78, 58-66.

Ting, A.S.Y., Tay, H., Peh, K.L., Tan, W.S., Tee, C.S., 2013. Novel isolation of thermophilic *Ureibacillus terrenus* from compost of empty fruit bunches (EFB) of oil palm and its enzymatic activities. *Biocatalysis and Agricultural Biotechnology*, 2, 162-164.

Tiquia, S.M., Tam, N.F.Y., Hodgkiss, I.J., 1998. Changes in chemical properties during composting of spent pig litter at different moisture contents. *Agriculture, Ecosystems and Environment*, 67, 79-89.

Tuomela, M., Vikman, M., Hatakka, A., Itävaara, M., 2000. Biodegradation of lignin in a compost environment: *Bioresource Technology*, 72, 169-183.

Turkish Statistical Institute, 2015. “Amount of Municipal waste by disposal methods”, <http://www.turkstat.gov.tr>. (accessed January 2015)

Wang, X., Pan, S., Zhang, Z., Lin, Z., Zhang, Y., Chen, S., 2017. Effects of the feeding ratio of food waste on fed-batch aerobic composting and its microbial community. *Bioresource Technology*, 224, 397-404.

Westerman, P.W., Bicudo, J.R., 2005. Management considerations for organic waste use in agriculture, *Bioresource Technology*, 96, 215-221.

Woraprayote, W., Pumpuang, L., Tosukhowong, A., Roytrakul, S., Perez, R.H., Zendo, T., Sonomoto, K., Benjakul, S., Viessanguan, W., 2015. Two putatively novel bacteriocins active against Gram-negative food borne pathogens produced by *Weissella hellenica* BCC 7293. *Food Control*, 55:176-184, 10.1016/j.foodcont.2015.02.036.

Wu L., Ma L.Q., Martinez G.A., 2000. Comparison of methods for evaluating stability and maturity of biosolids composts. *Journal of Environmental Quality*, 29, 424-429.

Xi B., Zhao X., He X., Huang C., Tan W., Gao R., Zhang H., li D., 2016. Successions and diversity of humic-reducing microorganisms and their association with physical-chemical parameters during composting. *Bioresource Technology*, 219, 204-211.

Valdez-Vazquez, I., Poggi-Varaldo, H.M., 2003. Hydrogen production by fermentative consortia, *Renewable and Sustainable Energy Reviews*, 13:1000-1013, DOI: 10.1016/j.rser.2008.03.003.

Vallini, G., Pera, A., 1989. Green compost production from vegetable waste separately collected in metropolitan garden-produce markets. *Biological Wastes*, 29, 33-41.

Van Gestel, K., Mergaert, J., Swings, J., Coosemans, J., Ryckeboer, J. 2003. Bioremediation of diesel oil-contaminated soil by composting with biowaste. *Environmental Pollution*, 125, 361-368.

Viel, M., D. Sayag, and L. Andre. 1987. Optimization of agricultural industrial wastes management through in-vessel composting, pp. 231-237. In M. De Bertoldi et. Al. (ed.). *Compost: Production Quality and Us*. Elsevier Applied Science, London.

Vigil, S.A. 1994. Comparison of the environmental effects of aerobic and anaerobic composting technologies. Air and Waste Management Association, 94-WP100.05.

Yamada, Y., Kawase, Y., 2005. Aerobic composting of waste activated sludge: Kinetic analysis for microbiological reaction and oxygen consumption. *Waste Management*, 1, 49-61.

Yang, F., Xue Li, G., Yuan Yang, Q., Hai Luo, W., 2013. Effect of bulking agents on maturity and gaseous emissions during kitchen waste composting. *Chemosphere* 93, 1393-1399.

Yu, H., Jiang, J., Zhao, Q., Wang, K., Zhang, Y., Zheng, Z., Hao, X., 2015. Bioelectrochemically –assited anaerobic composting process enhancing compost maturity of dewatered sludge with synchronous electricity generation, *Bioresource Technology* 193, 1-7.

Zhang, M., Heaney, D., Henriquez, B., Solberg, E., Bittner, E., 2006. A four year study on influence of biosolids/MSW cocompost application in less productive soils in Alberta: nutrient dynamics. *Compost Science and Utilization*, 14, 68-80.

Zhang, L., Sun, X.Y., Tian, Y., Gong, X.Q., 2013. Effects of brown sugar and calcium superphosphate on the secondary fermentation of green waste. *Bioresource Technology*, 131:68–75.

Zhang, L.L., Ma, H.X., Zhang, H.Q., Xun, L.Y., Chen, G.J., Wang, L.S., 2015a. *Thermomyces lanuginosus* is the dominant fungus in maize straw composts. *Bioresource Technology*, 197, 266-275.

Zhang, L.L., Zhang, H.Q., Wang, Z.H., Chen, G.J., Wang, L.S., 2015b. Dynamic changes of the dominant functioning microbial community in the compost of a 90m³ aerobic solid state fermentor revealed by integrated meta-omics. *Bioresource Technology*, 203, 1-10.

Zhang, L.L., Jia, Y.Y., Zhang, X.M., Feng, X.H., Wu, J.J., Wang, L.S., Chen, G.J., 2016. Wheat straw: an inefficient substrate for rapid natural lignocellulosic composting. *Bioresource Technology*, 209, 402-406.

Zhang L., Sun X., 2016. Influence of bulking agents on physical, chemical, and microbiological properties during the two-stage composting of green waste. *Waste Management* DOI: 10.1016/j.wasman.2015.11.032

Zhou, H.B., Ma, C., Gao, D., Chen, T.B., Zheng, G.D., Chen, J., Pan, T.H., 2014. Application of a recyclable plastic bulking agent for sewage sludge composting. *Bioresource Technology*, 152, 329-336.

Zucconi, F., Pera, A., Forte, M., de Bertoldi, M., 1981a. Evaluating toxicity of immature compost. *Biocycle*, 22, 54-57.

Zucconi, F., Forte, M., Monaco, A., de Bertoldi, M., 1981b. Biological evaluation of compost maturity. *Biocycle*, 22, 27-29.

APPENDIX A. EVOLUTION OF COMPOST PRODUCTS ACCORDING TO SAMPLING DAYS

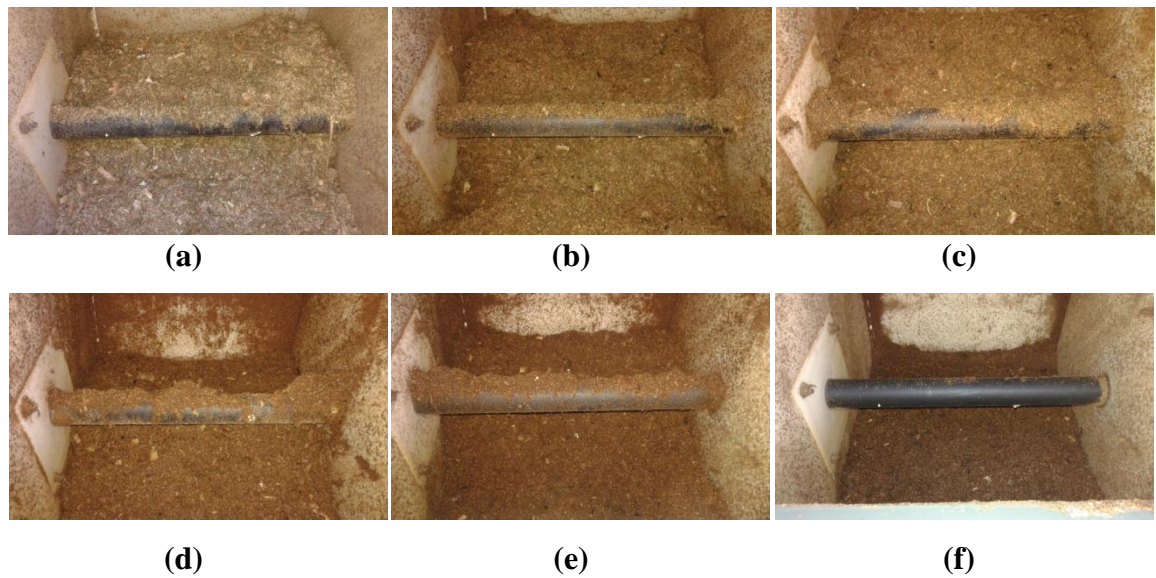


Figure A.1. Yard waste evolution during composting process (a) day 0 (b) day 3 (c) day 5 (d) day 11 (e) day 18 (f) day 30.

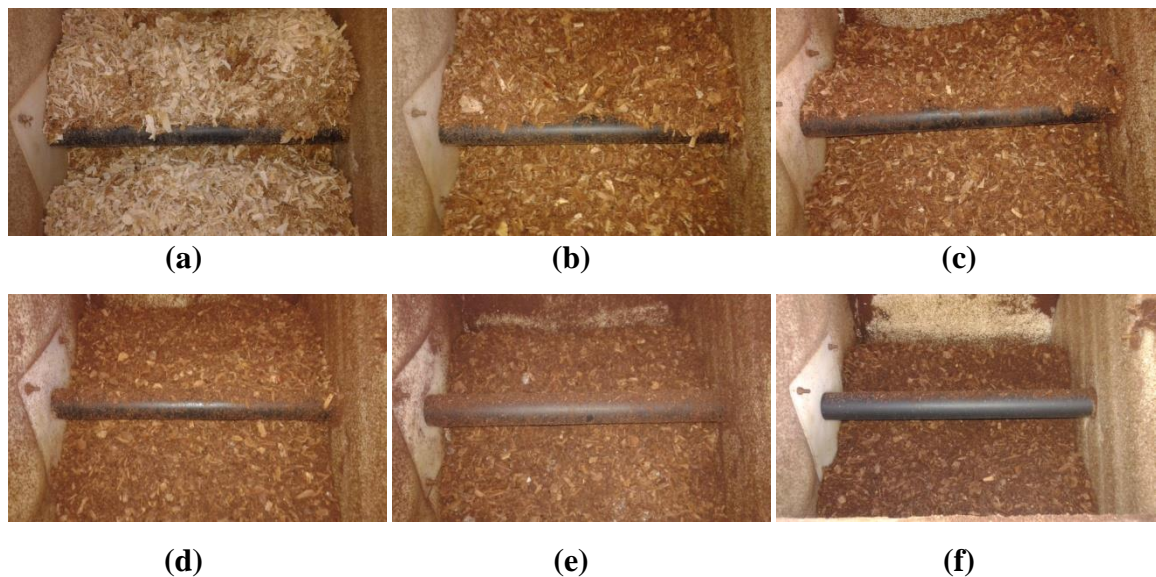


Figure A.2. Kitchen waste evolution during composting process (a) day 0 (b) day 3 (c) day 5 (d) day 11 (e) day 18 (f) day 30.

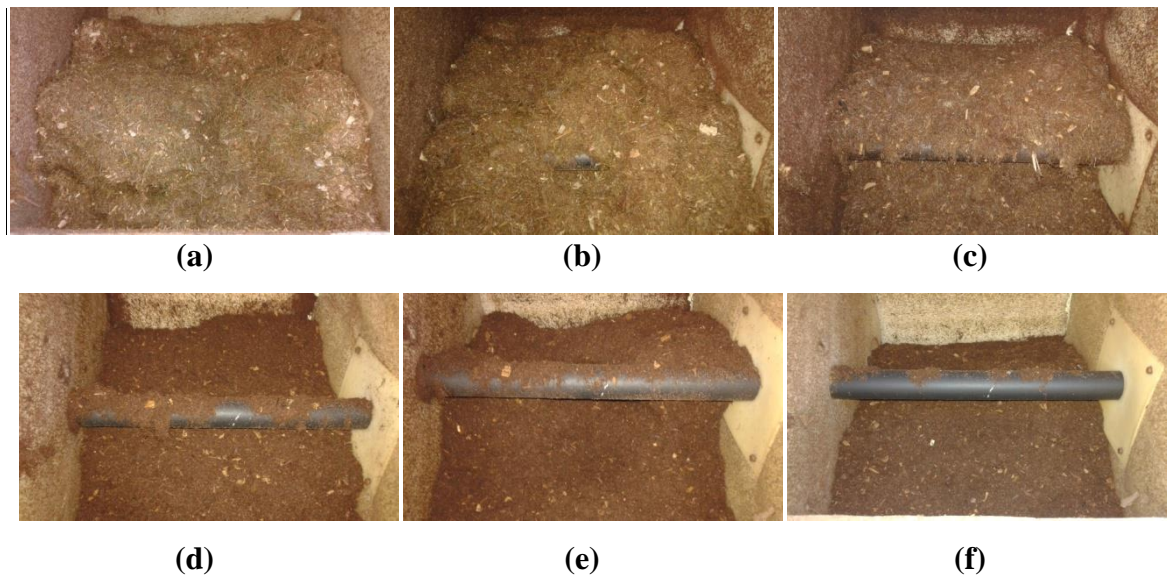


Figure A.3. Yard waste, kitchen waste and cow manure combined compost evolution during composting process (a) day 0 (b) day 3 (c) day 5 (d) day 11 (e) day 18 (f) day 30.

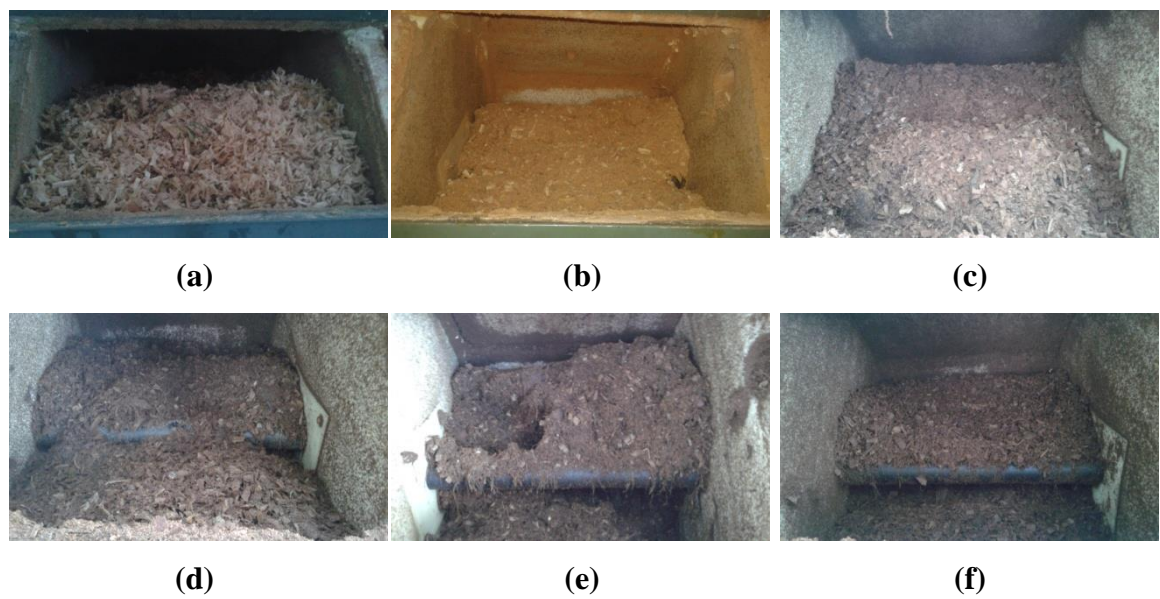


Figure A.4. Yard waste plus kitchen waste compost evolution during composting process (a) day 0 (b) day 3 (c) day 5 (d) day 11 (e) day 18 (f) day 30.

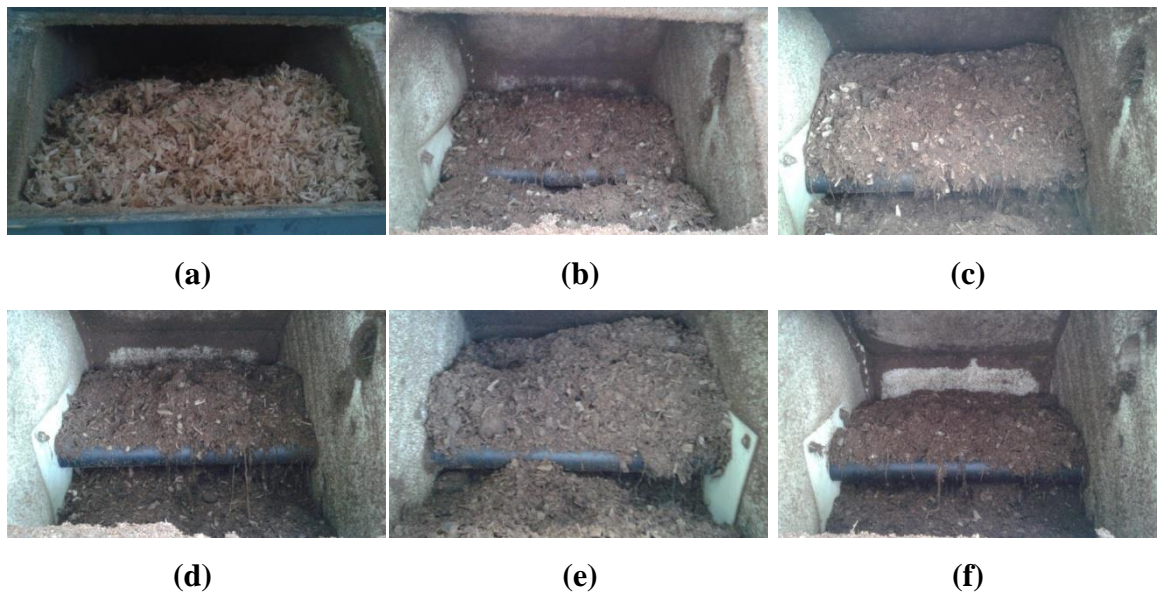


Figure A.5. Yard waste plus cow manure compost evolution during composting process (a) day 0 (b) day 3 (c) day 5 (d) day 11 (e) day 18 (f) day 30.

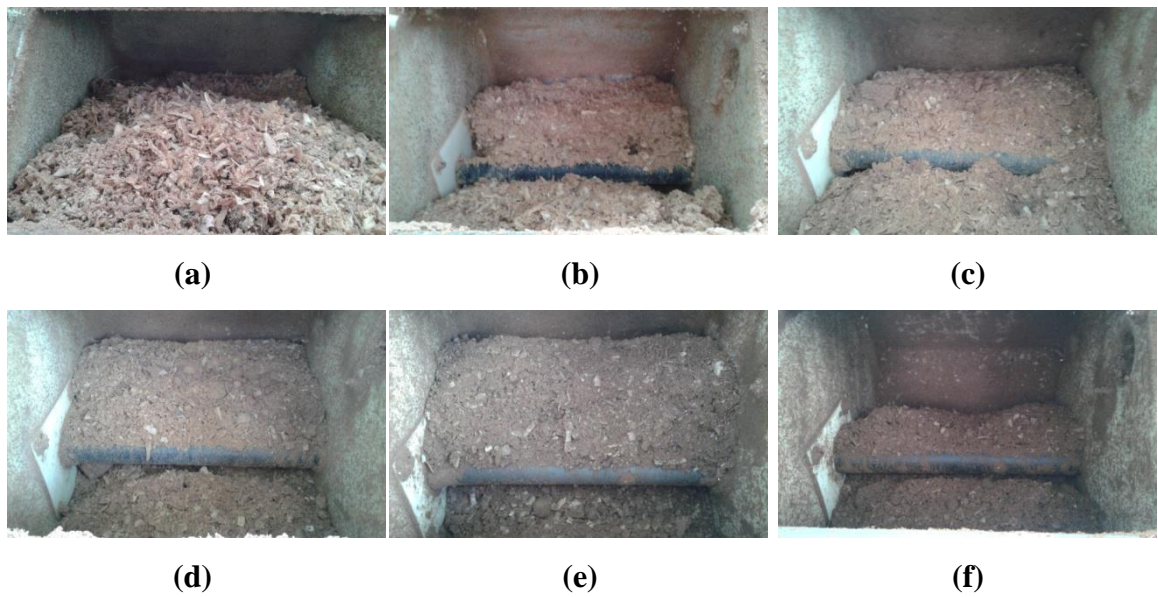


Figure A.6. Kitchen waste plus cow manure compost evolution during composting process (a) day 0 (b) day 3 (c) day 5 (d) day 11 (e) day 18 (f) day 30.