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Optimization of In-Vessel Food Waste Composting: Enzyme Activity and Microbial Dynamics

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Cell and Molecular Biology

by

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This thesis is approved for recommendation to the Graduate Council.

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Abstract

A series of greenhouse-based, rotary-drum bioreactor experiments was designed to study microbial dynamics and enzyme activity during optimization of food waste composting. This work aims to optimize food waste composting by defining the best food waste-to-bulking agent proportion controlling conditions and by evaluating the food waste composting process when inoculated by a bacterial inoculant product compared to uninoculated compost. Three experiments were run in total. The two first experiments were conducted for 48 days with sampling at each step of composting, while the third experiment last 50 days and included one extra sampling date. In the first experiment, 50:50, 65:35 and 80:20 (mass:mass) food waste-to-woodchips proportions were evaluated and proportions of 65:35 and 80:20 were retained and evaluated again in experiment 2. The last experiment evaluated the effect of a commercial *Bacillus* sp. inoculant product on food waste composting and utilized the 80:20 proportion. Proportions of food waste-to-bulking agent in the first two experiments affected water content and enzyme activity. Generally, water content decreased over composting and enzyme activity was greater in the 80:20 compared to 65:35 food waste-to-woodchips proportion compost. The *Bacillus* treatment increased thermophilic bacteria, but not enzyme activity and enzyme activity increased throughout composting such that it was greatest in the curing stage. Observationally, 80:20 compared to 65:35 food waste-to-woodchips proportion retained composting in the thermophilic and higher temperatures and enzyme activity into the curing stage, which may facilitate decomposition. Inoculation facilitated faster increase into and prolonged retention within thermophilic stage. These effects should be investigated more thoroughly in future research.

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Chapter 1 - Literature Review

1. Scale and significance of food waste and benefits of composting

Every year food is spoiled, which in developed countries around the world costs the equivalent of billions of US dollars (Melikoglu et al., 2015). In the United States, food waste accounts for 30 to 40 % of the food supply (USDA, 2018). Food loss of 31% at the retail and consumer levels equates to approximately 60.3 billion kg and \$161 billion in the United States (USDA, 2018). Food waste at the retail and consumption levels equaled in 2012 approximately 31.75 million metrics tons (USEPA, 2018b). Wasted food often ends up in the landfill and contributes to environmental pollution. Food waste is the largest constituent of urban solid waste streams after renewable materials have been recovered (Yu and Huang 2009; Getahun et al. 2014). Also, according to the United States Environmental Protection Agency (USEPA, 2018a), 20% of what is thrown in landfills consists of food.

Environmental pollution is a critical issue. In landfills, organic wastes are converted into greenhouse gases, called landfill gases, typically composed of about 50% methane (CH_4) and 50% carbon dioxide (CO_2) with a small amount of volatile organic compounds (USEPA, 2018a). Conversely, organic wastes may be present in leachate, liquid that drains through a landfill carrying dissolved and suspended matter in it. Methane is 28 to 36 times more effective than CO_2 at trapping heat in the atmosphere over a 100-year period (USEPA, 2018a). While approximately 60-90% of the CH_4 emitted from landfills can be captured, dependent upon the system and its effectiveness, about 95% of the landfill gas needs to be captured to prevent such damage compared to release of CO_2 from the same organic material if decomposed aerobically (USEPA, 2018a).

Food waste can be a relevant resource for recycling (Mi-Hung and Jung-Wok, 2010). One method to recycle food waste is to convert it into compost. Composting is receiving greater consideration since it is a substitute for food waste treatment (Smårs et al., 2002; Quirós et al., 2014). Composting is also a relatively simple, sustainable, and affordable technology for diminishing and stabilizing biodegradable waste (Crowe et al., 2002). Compost is defined as the biological decomposition and stabilization of organic substrates under the condition of thermophilic temperatures as a result of biologically produced heat (Iyengar & Bhave, 2006). According to the United States Composting Council (2018), the recent definition of compost is the product manufactured through the controlled aerobic, biological decomposition of biodegradable materials. During composting, activities of successive microbial communities produce an amendment that can enhance soil quality (Gajdos, 1992; Kopicic et al., 2014). The product has been altered under mesophilic, thermophilic, followed by mesophilic temperatures, which significantly reduce the viability of pathogens and weed seeds and stabilize the carbon such that it is beneficial to plant growth. Compost is typically used as a soil amendment and may contribute to plant nutrients (United States Composting Council, 2018). Although composting has been used by farmers for centuries, food waste composting in the US has seen significant progress in development as a technology during the past decade in states such as California, Delaware, and Texas (Gbaconq, 2015).

2. Composting process

During the composting process, characteristic microbial communities work in succession, in adaptation to the environment and as nutritional levels change. Initial raw material is a consideration as microorganisms acclimatize to the environmental conditions and bio-accessibility of vital nutrients (Ryckeboer et al., 2003). It is not uncommon

for feedstock used in composting to vary widely in composition and thus in C and N contents, pH, and electrical conductivity (EC). General recommendations for initial conditions are the following: pH of approximately 7 - 8, and an initial carbon-to-nitrogen (C:N) ratio of the feedstock of 20 to 40:1, ambient temperature between 10 and 25 °C, 40 – 60% moisture on a volumetric basis, and at least 10% oxygen (O₂), which may require frequent turning in order to maintain aeration (Hue, 2011).

3. What can be composted?

Fruits and vegetables, eggshells, coffee grounds and filters, tea bags, nut shells, shredded newspaper, paper and cardboard, yard trimmings including grass, leaves, branches, and twigs, houseplants, hay and straw, sawdust, woodchips, cotton and wool rags, dryer and vacuum cleaner lint, hair and fur, and fireplace ashes can be composted (University of Georgia Extension, n.d.). According to the USEPA (n.d.), a balance of "greens" and "browns" is needed for proper composting to take place. Greens are nitrogen-rich materials, and include items such as grass clippings, and fruit and vegetable wastes. Browns are the carbon-rich yard clippings, such as dead leaves, branches and twigs, and woodchips.

4. Feedstock (raw or primary mixed food waste) inputs and bulking agents

Food waste can be markedly variable depending on its source. Waste collection and categorization systems affect food waste composition, the composting process, and the final compost product (Cerde et al., 2018). Food waste may contain a large percentage of inert materials such as metals, plastics, or glass, which should be removed before grinding. The heterogeneous mix of remaining biodegradable waste should then be reduced to a size of roughly 2.54 to 7.62-cm pieces (Hue, 2011). The size reduction of materials allows microbes to decompose the feedstock more easily.

A bulking agent is often added to the organic waste, which generates the final feedstock for composting. The bulking agent is added to help aerate the compost and, depending on the source, may alter C: N of the final feedstock. Examples of bulking agents include wood chips, wood shavings, shredded paper, shredded cardboard, sawdust, dry leaves, shredded landscape waste, shredded cardboard, or animal bedding.

5. Particle size

The particle size of feedstock inputs plays a significant role in the composting process. Shredding or grinding raw materials is valuable, particularly when composting materials rich in fibers such as woody plants, leaves, or corn stalks (Washington State University, n.d.). Shredding the bulking agent increases the surface area:volume ratio, which facilitates bacterial invasion and shortens decomposition time. Large pieces of wood packed together do not degrade quickly in a limited amount of time because of their size and structure. Shredding material increases bulk density and decreases pore space and increases variety of pore sizes, simultaneously promoting pile aeration and moisture retention, which are important for the aerobic microbial activity and growth necessary to obtain a uniform compost (Washington State University, n.d.). The best particle size for bulking agents appears to be approximately 5 cm in diameter to facilitate decomposition (Washington State University, n.d.).

Grinding can be done before composting and at the end or when compost has reached maturity. Regrinding near the end of the composting process re-activates the decomposition process and serves as a “turning” for the compost pile to increase aeration and promote compost stability. Haug (1993) recommended woodchips from 25.4 to 50.8 mm in size as the most used bulking agents. A study conducted by Raichura and McCartney (2006) tested if woodchip particle size would impact the performance of operations including compost pile temperature. They

adopted three treatments investigating coarse, medium, or fine woodchips with respective characteristic particle sizes of 40, 13, and 5.2 mm. They found that finer particle sizes resulted in thermophilic temperatures being reached sooner and sustained for a longer time and also that compost processing recovered rapidly after rainfall events. While the medium and fine material provided similar results (50.3 ± 13.4 °C and 51.3 ± 13.4 °C, respectively), the coarse material resulted in significantly ($\alpha = 0.05$) lower temperatures (42.0 ± 14.8 °C) (Raichura & McCartney, 2006). The moisture content of the coarse material decreased towards the end of composting compared to moisture content using fine material. Moisture contents were 34.8 ± 5.9 , 29.9 ± 3.9 , and $25.9 \pm 3.8\%$ (weight basis) from compost piles utilizing fine, medium, and coarse woodchip particles, respectively.

6. The microbial community, temperature, and composting process

There are three stages in the composting process: initial mesophilic, thermophilic, and final mesophilic or the curing stage. A large variety of mesophilic, thermotolerant, and thermophilic, aerobic microorganisms, including bacteria and actinomycetes, and yeasts and various other fungi have been extensively reported in composts (Antunes et al., 2016; Franke-Whittle et al., 2014; Hassen et al., 2001; Ishii and Takii, 2003; López-González et al., 2015). Using high-throughput sequencing, Wang et al. (2017) reported that the predominant bacterial phyla in batch-fed composting were Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria. Actinomycetes are bacteria tolerant of thermophilic temperatures and include genera such as *Nocardia*, *Streptomyces*, *Thermoactinomyces*, and *Micromonospora* as representative forms isolated from compost (Waksman et al., 1939; Strom, 1985). These genera have been identified as important agents of ligno-cellulose decomposition. *Trichoderma* sp. is a common fungus in soil (Leandro et al., 2007) and known to degrade hemicelluloses, facilitating

compost stabilization and which accelerates the composting process of the waste material (Singh and Sharma, 2002; Pramanik et al., 2007, 2009).

A recent study conducted at Johnson County Community College (JCCC) in Kansas explored the genomic analysis of the JCCC campus compost system (Hanson, 2019). With the goal to establish a baseline for microbial composition throughout the composting process, sampling was conducted throughout different stages the composting process. Their composting process had different stages: pre-compost (mixed food in dining hall), in vessel, layered (layering vessel compost with uncomposted food waste from JCCC), an unlayered bay, mid-young, and oldest farm-ready piles. Firmicutes and Actinobacteria were the most prevalent microbes during composting. The majority of Firmicutes have Gram-positive cell walls and produce endospores that help them to survive in harsh conditions (Firmicutes, 2019), perhaps contributing to the domination of Firmicutes in many compost microbial analyses (Fumiticuties, 2019). In the composts at JCCC, Firmicutes were the predominant phylum in the vessel (71.56%), layered (55.00%), non-layered (86.18%), and mid-young composts (59.69%). Actinobacteria, the second most common bacterial phylum represented in the JCCC composting system, is a common phylum that has a vital function in decomposition of organic matter (Anandan et al., 2016).

Actinobacteria was present in each stage of the JCCC composting process at percentages greater than 1 %, except pre-vessel, and it was the phylum comprising the largest proportion in the oldest farm-ready stage (34.01%). Proteobacteria is a phylum of diverse, Gram-negative bacteria that include decomposers, pathogens, and/or nitrogen-fixing bacteria (Overview of Proteobacteria, 2019). Proteobacteria were detected most frequently during the pre-vessel stage accounting for 81.95% of the community, but they comprised 23.00% of the community in vessel, 38.44% in the layered bay, 5.76% in the non-layered bay, 11.05% in mid-young, farm-

ready, and 23.41% in oldest, farm-ready compost. *Salmonella enteritica*, a common human pathogen (Porwollik et al., 2004), represented 2.01% of total bacteria in the pre-vessel stage and were not detected during any composting stage. Bacteroidetes are a phylum that colonize many ecosystems and are considered to be competitive at degrading proteins and carbohydrates (Thomas et al., 2011). In the JCCC compost system, Bacteroidetes displayed a trend of increasing abundance with maturing compost, where they comprised 10.2 % of oldest, farm-ready compost, 9.2% in the mid-young, farm-ready compost, and only 1.9 % in the non-layered bay, and 1.2 % in the pre-vessel stage.

Shaini & Jayasree (2016) characterized and isolated lipase-producing bacteria from windrow compost of garden and kitchen wastes. Solid waste and cow dung inocula at the ratio 10:1 were spread in successive layers. Of 73 bacterial colonies screened, 24 bacterial strains were identified, which biochemically identified lipase producers (Tembhurkar et al., 2012). Shaini & Jayasree (2016) identified the 24 strains of lipase-producing bacteria as *Pseudomonas aerogenosa*, *Staphylococcus saprophyticus*, *Streptococcus* sp., *Actinobacillus scotiae*, *Klebsiella pneumonia*, *Bacillus niacini*, *Yersinia pseudotuberculosis*, *Bacillus siamensis*, *Pseudomonas otidis*, *Bacillus neizhouensis*, *Lysine bacillus massiliensis*, *Paenibacillus polymyxa*, *Buttiauxella ferrugutiae*, *Obesumbasterum proteus*, *Baccillus horneckiae*, *Viridibacillus neidei*, *Bacillus acidicer*, *Corynebacterium pseudodiphtheria*, *Corynebacterium matruchotii*, *Avibacterium gallinarum*, *Allivibrio fischeri*, *Bacillus vietnamensis*, *Aneurinibacillus aneurinilyticus*, and *Lactobacillus ceti*. Of the 24, the isolates WCS1C2 (*Stapylococcus saprophyticus*), WCS3C2 (*Pseudomonas otidis*), WCS5C1 (*Corynebacterium xerosis*), WCS5C3 (*Corynebacterium matruchotii*) and WCS6C4 (*Lactobacillus ceti*) have been the greatest lipase producers. Two

exceptional lipase producers were further characterized by using 16S rDNA technology and recognized as *Staphylococcus saprophyticus* and *Pseudomonas otidis*.

Temperature is the primary factor altering the microbial community during composting, while multiple parameters influence the adaptability of microorganisms and the substrate specific metabolic pathways of decomposition (Vargas-García et al., 2010). After initiation of composting, the initial decomposition commences, carried out by mesophilic microorganisms. The mesophilic phase (20 to 40 °C) is brief and lasts only a few days, generally a maximum of 6 days (Cornell Composting, n.d.). Having sufficient carbon impacts the increase of the temperature during the thermophilic phase (Ajay and Kazmi, 2009). Microbial activity causes the temperature of the compost pile to increase quickly to approximately 55 – 65 °C (Hue, 2011), which is the second, thermophilic stage. The mesophilic bacteria are displaced by the thermophilic bacteria as the compost heats. This 55 – 65°C temperature range destroys most pathogens and weed seeds. In biological terms, the operating temperatures are as per the following: 55°C to maximize sanitation, 45–55°C to extend the biodegradation rate, and 35–40°C to amplify microbial diversity (Stentiford et al., 1996). The thermophilic phase is when high temperatures accelerate the degradation of proteins, fats, and complex carbohydrates such as hemicellulose and cellulose. Aeration techniques and frequency are important during the thermophilic stage to balance maintenance of sufficient aeration without decreasing temperature and/or decreasing moisture.

As the reservoir of these complex energy molecules are depleted, the compost pile temperature decreases and the mesophilic microorganisms take over once more, moving composting into the final mesophilic, or the curing, phase. During curing, the compost cools down, decomposition slows, and the remaining organic matter stabilizes, or reaches maturity,

forming a final humus-like material. The final temperature of the mature compost hovers around 30 °C. Greater diversity of bacteria have been detected in the final maturation stage compared to the beginning of the food waste composting process (Awasthi et al., 2018).

Microbial composition dynamics play a key role in the degradation of the organic matter because microorganisms operate within different tolerance limits for physico-chemical conditions such as pH, osmolarity, temperature, moisture, aeration, and C:N ratio of the feedstock. Hassen et al. (2001) conducted a study in a semi-industrial, pilot-plant, municipal solid waste composting system that had a capacity of 5 tons. *Staphylococci* spp. were dominant during the initial mesophilic and early in the thermophilic phase and *Bacillus* spp. predominated for the duration of the remainder of the composting cycle (Hassen et al., 2001). Similar results were found by Shaini et al. (2016) where *Bacillus* spp. and *Lactobacillus* spp. were predominant. When compost reached 55 to 60 °C, *Escherichia coli* and fecal *Streptococcus* spp. populations decreased from 2×10^7 to 3.1×10^3 and 10^7 to 1.5×10^3 cells/g waste dry weight (WDW), respectively (Hassen et al., 2001). Mesophilic bacteria decreased from 5.8×10^9 to 1.8×10^7 bacteria/g WDW; filamentous fungi and yeasts decreased from 4.5×10^6 to 2.6×10^3 CFU/g, and *Salmonella* spp. were not detected in compost after the twenty-fifth day, which was when the temperature reached 60°C (Hassen et al., 2001). Bacterial spore diversity increased initially; however, after the third week of composting, diversity diminished. After sonication of compost for three min to inactivate gram-negative bacteria, Hassen et al. (2001) found that gram-positive bacteria, particularly micrococcus, spores of bacilli, and fungal propagules survived, and reached excessive concentrations in the compost (Hassen et al., 2001).

Awasthi et al. (2018) examined changes in microbial groups and specific enzymes, such as amylase, cellulase, and protease activities during composting of post-consumption food waste

(PCFW) inoculated with either an oil-degrading bacterial consortium (treatment 2, T2) or a consortium containing two different bacteria (treatment 2, T3) compared to an uninoculated control (treatment 1, T1). They adopted these three treatments using a 130-L bench scale PVC reactor where PCFW was mixed with sawdust at a 4:1 ratio and 10% zeolite was added as a neutralizing agent. Although there was a succession in the microbial community during composting, the microbial community was composed of four groups of bacteria: cellulosic, proteolytic, amylolytic, and oil-degrading bacteria (Awasthi et al., 2018). Total aerobic bacteria were initially 1.5, 1.7, 1.5 on a log 10 CFU/g basis in T1, T2, and T3, respectively, and increased gradually during the thermophilic phase to 5.25 and 5.83 log 10 CFU/g in the treatment inoculated with oil-degrading bacteria (T2) and the treatment inoculated with two bacteria (T3), respectively, on day 14, followed by 4.10 log 10 CFU/g on day 28 in both treatments (Awasthi et al., 2018). In contrast, aerobic bacteria peaked at 3.4 log 10 CFU/g in the uninoculated control (T1) (Awasthi et al., 2018). After day 28, the concentration of aerobic bacteria decreased significantly for the treatments and the control. The maximum total aerobic amylolytic bacteria increased on day 14 to a greater extent in T1 (control) and T2 compared to T3 (Awasthi et al., 2018). In contrast to the total aerobic amylolytic bacteria, the composition of total aerobic proteolytic bacteria in treatment T3 was greater than in T1 and T2.

7. Influence of moisture and aeration on compost process, maturity and stability, and odor

Aeration plays a critical role in the quality and processing of food waste composting. Low moisture content, while promoting gas exchange, can significantly diminish decomposition because of substrate and waste diffusion constraints. Adequate aeration and moisture allow microorganisms to access substrate and nutrients while facilitating gas exchange (Linn and Doran, 1984). Excess water will limit gas exchange and cause the environment to go anaerobic if

activity exceeds the rate of gas exchange in pores. The ideal water content in compost ranges between 45– 60% by volume (Cooperband, 2002).

If aeration is not maintained for aerobic activity, the final compost will be of poor quality. The most common aeration method when composting is the physical turning of the material (Ajay and Kazmi, 2009). Previous research has focused on the effects of turning frequency on compost stability and its chemical characteristics (Ajay and Kazmi, 2009). Aeration during the early mesophilic phase of composting increases the activity of microorganisms (Haug, 1993; Bari & Koenig, 2001). Adequate aeration rates, ranging from 0.2 to 0.6 L min⁻¹ kg⁻¹ OM, show significant improvements in NH₃ lost and odor production, C:N ratio reduction, and compost maturity (Zhang and Sun, 2016). Compost matured in 22 days with two-stage optimized conditions rather than in 90–270 days typically required for traditional composting (Zhang and Sun, 2016). The two-stage composting of green waste was optimal with the addition of 0.15% β -cyclodextrin (β -cyclodextrin was added at rates of 0, 0.15, or 0.25%) (Zhang and Sun, 2016). On the other hand, excess aeration and turning generated a loss of moisture and ammonia (NH₃) in manure composting (Parkinson et al., 2004). However, Parkinson et al. (2004) stated that developing appropriate turning regimes resulted in pathogen kill but retained nitrogen in the manure heap and therefore reduced nitrogen losses, decreasing composting costs and environmental pollution. Their research confirmed that proper turning of manure stacks to aid composting can reduce NH₃-N losses during cattle manure composting.

Guo et al. (2012) showed that a low aeration rate (<0.2 L min⁻¹ kg⁻¹ OM) slowed degradation rate, moisture and heat loss, reduced overall NH₃ generation, and significantly decreased temperature and decreased microbial diversity. Inadequate aeration affected the composting maturation process by changing the extent of decomposition and generating a

product that was not stable. Zhang et al. (2016) and Zhang and Sun (2016) concluded that aeration is the main factor affecting compost stability, whereas the C:N ratio influenced compost maturity. The stability of the compost has been defined as a state when the compost is not undergoing rapid degradation and when the nutrients are gradually released into the soil when compost is added as a soil amendment. Maturity is defined as the level of completeness of the composting process. It is crucial to optimize the turning frequency to kill pathogens and generate a stable and valuable final product that will be mature.

In composting, odor noticeably contributes to the environmental effect of composting sites and instigates public concern, which in many cases results in limiting actions or facility closures (Colon et al., 2012). Odors are intrinsic by-products of composting related to the process conditions and the initial organic materials. Many types of gases are released during composting, including volatile organic acids (VOA) and NH_3 . During composting of five different organic wastes, NH_3 emissions increased exponentially in the thermophilic stage of composting, whereas NH_3 emissions and temperature were related linearly during the curing stage of composting (Pagans et al., 2006).

The production of VOA prior to and during composting imparts an “objectionable garbage or spoiled food-like odor, which frequently results in nuisance complaints” (Brinton, 1998). The VOA can be responsible for phytotoxicity when the compost process is incomplete and unfinished compost product is used for growing plants (Brinton, 1998). The VOA is generated under anaerobic conditions. Stronger odors have emanated from acidic (pH 5) compared to alkaline or neutral pH compost (Sundberg et al., 2013). Odor concentration in samples was greater at low pH as samples with pH values <6.0 had odor concentrations more than 70,000 odor units per m^3 air (ouE/m^3), while samples with pH values >6.6 had odor

concentrations $<41,000$ ouE/m³ (Sundberg et al., 2013). Clostridia and lactic acid bacteria, which characteristically produce odorous compounds, were detected in the high-odor samples.

8. Compost maturity

A critical method to evaluate compost health is the evaluation of composting maturity (Fu 2004; Zhang et al. 2009). To evaluate maturity entails a cumulative assessment method that uses multiple factors such as oxygen used, respiration rate, organic material release and reheating to illustrate particular metabolism levels and to disclose the compost qualities (Komine and Shiiba, 2004; Tognetti et al., 2007). Nearly all of the parameters that affect the compost process, including feedstocks, C:N, moisture, aeration, pH, and temperature, are potential influencers of compost maturity (Nair et al., 2006; Saha et al., 2013; Yu et al., 2010). In previous studies, pH has been used frequently as an index for evaluating the maturity of compost, where it tends to be alkaline in a mature compost (Song et al., 2014).

9. Effective Microorganisms (EM)

Effective microorganisms (EM) are mixed cultures of beneficial, naturally occurring microorganisms that can be applied as inoculants to increase the microbial diversity of an ecosystem. There is evidence that EM inoculation to soil can improve the quality of soil, plant growth and yield (Kengo and Hui-lian, 2000). The EM is also defined as an unrevealed mixture of naturally occurring microorganisms that supposedly have beneficial properties in a wide range of applications (Higa, 2002). Effective microorganisms were developed originally by professor Tiruo Higa of the University of Ryukyu, Okinawa, Japan and EM is sold as a commercial product. The precise composition of EM has not been disclosed (Formowitz et al., 2007). A study conducted by the Kyan et al. (1999) indicated that the EM product mainly contained populations of lactic acid bacteria, photosynthetic bacteria, yeast, and actinomycetes. Daly and

Steward (1999) determined that EM contains species such as *Streptococcus albus*, *Propionibacterium freudenreichii*, *Streptococcus lactis*, *Aspergillus oryzae*, *Mucor hiemalis*, *Saccharomyces cerevisiae*, and *Candida utilis*, plus a range of unspecified number of *Lactobacillus* sp., *Rhodopseudomonas* sp. and *Streptomyces griseus*.

Because of the reputed benefits of EM, EM has been used as inoculum in composting. Kamaruddin et al. (2018) adopted the following for feedstock: 2000 g of food wastes, 150 ml of inoculant of EM and 1000 g of brown layer garden and yard wastes. Every 4 days, they sprinkled water on the compost to maintain the moisture content. The inoculated compost and control compost had a moisture content of 56.5% and 50.18%, respectively, which should not be limiting for activity. The pH of compost decreased during the initial phase due to microbial decomposition of the food wastes and pH increased towards the end of the compost process after decomposition of acidic compounds. During the composting, the C:N ratio decreased due to the mineralization of organic matter to values in the range considered valuable for the product to act as fertilizer. The compost assisted by inoculation achieved 75% germination during testing, indicating that final product was stable and mature for plant growth, which compared to 25% germination in the presence of the control compost (Kamaruddin et al., 2018).

Microorganisms that are dominant during composting are hypothesized to drive the composting process, and the rationale behind inoculation is to add the microorganisms that are to become dominant in order to facilitate a consistent compost processing and product development. Inoculation should thereby eliminate inconsistency in the process that results from variable food waste feedstock inputs. Some researchers have investigated use of a microbial consortium of bacteria that degrade organic acids (MCDOA). The MCDOA are “EM” of the following taxa: *Dysgonomonas* sp., *Pseudomonas caeni* strain, *Aeribacillus pallidus* strain,

Pseudomonas sp., *Lactobacillus salivarius* strain, *Bacillus thuringiensis* strain, and a *Bacillus cereus* strain (Song et al., 2018). Song et al. (2018) adopted wheat bran as a bulking agent and MCDOA was added at the level of 1.25 mL kg⁻¹ dry composting mix where concentrations of the strains were approximately 19 x 10⁸ CFU mL⁻¹. Composting efficiency was greater in the inoculated treatment compared to other treatments (Song et al., 2018). The MCDOA eliminated the initial lag phase of the temperature rise that resulted from excessive acidification and effectively shortened the composting period.

Other microbial consortium have been created based on microorganisms isolated from composted soil, such as *Bacillus casei*, *Lactobacillus buchnei* and *Candida rugopelliculosa* and ligno-cellulolytic fungi (*Trichoderma* sp. and white-rot fungi) (Wei et al., 2007). Inoculation accelerated humification and maturation during municipal solid waste composting (Wei et al., 2007). On the other hand, a study conducted in Costa Rica concluded that addition of EM inoculated and “bokashi” (i.e. Japanese term describing fermented organic matter) banana residues (*Musa* sp.) was not different compared to uninoculated compost typically used in organic farming added to bokashi, except for an increased concentration of potassium in young banana leaves (Formowitz et al., 2007).

10. Composting systems and in-vessel composting

Composting serves to restore food waste into a valuable product and to sustain the environment. There are four fundamental approaches to composting: turned windrows, aerated static piles, enclosed channel systems, and in-vessel systems (UNFCCC, 2018). In-vessel composting occurs inside an enclosed container or vessel. The type of vessel could be a vertical tower, or horizontal rectangular, circular, or rotating tank. In-vessel composting systems can be designed as a plug-flow or agitated-bed system. In the plug-flow system, the relationship

between particles in the composting mass stays the same throughout the process, and the system works on the first-in, first-out principle. In the agitated-bed framework, the treated material is blended mechanically during the processing. Mechanical frameworks are intended to limit smell and process time by controlling ecological conditions, for example, wind stream, temperature, and oxygen concentration.

The vessel system in this research has been chosen in part to control odor emissions. Also, the vessel choice should allow increased precision in optimization of abiotic conditions during the composting process. Another advantage of in-vessel composting may be reduced labor cost (UNFCCC, 2018). In-vessel composting may reduce greenhouse gas emissions if the released air is filtered (UNFCCC, 2018). Fewer human resources are required to operate the system and staff may be less exposed to the composting material with use of an in-vessel system. The quality of the final product is expected to be more consistent and less bulking agent may be required than aerated static piles (UNFCCC, 2018). The compost process can be closely monitored with a vessel system. The last important consideration of an in-vessel system is that it requires less space or area compared to the other composting techniques (USEPA, 2018).

Iyengar et al. (2006) adopted five different type of reactors based on the maintained conditions: reactors differed in aeration, inclusion or exclusion of vegetables, and use of inoculants (compost, cow dung, and/or EM). Iyengar et al. (2006) found that the compost from a continually mixed, aerobic reactor that was continually loaded for two weeks generated humus that provided nutrients to plants and increased soil health. Thus, Iyengar et al. (2006) concluded that the aerobic, in-vessel composting of household wastes provided an efficient, eco-friendly, cost-effective, and nuisance-free solution for the management of household solid wastes and supplied a beneficial soil amendment.

Challenges of the in-vessel composting approach encountered by Iyengar et al. (2006) included that vessel systems can be expensive compared to other approaches such as windrows or static piles. The price of vessel systems varied from one region to the other; however, vessels were more expensive than a windrow if land was available because windrows require only an empty field to retain and mix the food waste and the bulking agent. For instance, a BW Organics Rotating Drum, model 405, a sophisticated vessel of 1.5 x 7.3 meters and a maximum capacity to process 3.0 m³ food scraps (FS)/day utilizing a 3-6 days retention time cost \$43,362 with a continuous flow system but also required additional curing compared to a “hot box” of 0.9 x 0.9 m² and a capacity of 0.76 m³. The “hot box” was priced from \$200 to \$400, depending on the lumber and PVC prices, but this vessel also required additional curing time and space and did not produce mature compost.

In addition to the potential of generating immature compost, vessels require regular maintenance and have associated operational costs if they are equipped with technological features that need to be checked and controlled. The other challenge of the in-vessel composting approach involves training of personnel to operate and maintain the vessels.

Research questions, objectives and hypotheses

1. How consistent is composting?

Does the current research literature provide consistent information to establish an in-vessel compost process for food waste without empirical experimentation to determine process conditions?

- Objective: Review of the existing research literature.
- Determine if there is consistency in existing research for specifically how to establish a vessel food waste composting system that will work efficiently without empirical experimentation to establish conditions.

2. What is the best proportion of post-consumer food waste and bulking agent to facilitate an optimized in-vessel composting process?

- Objective: Evaluate the impact of food waste:bulking agent ratios on the composting process.
- Hypothesis: A specific food waste:bulking agent ratio will be optimal to minimize initial mesophilic and maximize the duration and temperature range of the thermophilic stage during food waste composting, thereby increasing overall efficiency and differentiating compost properties.

3. Does inoculation of effective microorganisms affect composting?

- Hypothesis: Addition of effective microorganisms will optimize the composting process by shortening the time to reach thermophilic stage and maximizing thermophilic decomposition.

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Chapter 2. Review: In-vessel food waste composting is more efficient than open-system composting when optimized

1. Introduction

The global generation of municipal solid waste (MSW) was 267.8 million metric tons in 2017, approximately 5.7 million tons more than the amount generated in 2015 (USEPA, 2020a). Food waste constitutes the largest waste stream of municipal solid waste worldwide (USEPA, 2020b). In 2017, approximately 139.6 million tons of MSW were landfilled, and food was the largest component of MSW at about 22 percent. Wasted food often ends up in the landfill and contributes to environmental pollution. Previous studies stated that food waste is the largest constituent of urban solid waste streams after other renewable materials have been recovered (Yu and Huang 2009; Getahun et al. 2014). Consequently, there are public health and environmental concerns including greenhouse gas (GHG) emissions, waterborne pollutants, waste leachate, and soil and groundwater contamination, along with odors and vector-borne diseases in the surroundings of disposal sites.

Given these wastes and associated environmental concerns, composting has emerged as an appealing alternative for recycling food waste because of potential for recycling and final product benefits (Filippi et al., 2002; Das et al., 2003; Benito et al., 2006; Zenjari et al., 2006). Haug (1993) defines compost as “an organic material that has been stabilized to a humus-like product that is free of viable human and plant pathogens and plant seeds that does not attract insects or vectors, that can be handled and stored without nuisance, and that is beneficial to the growth of plants.” As explained by Haug (1993), compost is used as a soil amendment, mulch, and constituent of growing media. Compost is produced through accelerated aerobic decomposition through a period at thermophilic temperatures and back to mesophilic curing

stage as opposed to undergoing fermentation or anaerobic decomposition. Composting is produced using a variety of feedstocks - sewage sludge, manure, animal mortalities, food waste, yard waste, biosolids, industrial wastes, and military wastes – anything organic can be composted. Composting produces a useful bio-amendment agent, or organic fertilizer, as an end-product.

Therefore, composting allows organic material to be recycled, which significantly reduces mass and volume of waste material. During composting of food waste, there is a reduction of volume of the initial feedstock. Moisture loss during the composting process can be an indicator of decomposition, since heat generation that accompanies decomposition drives vaporization or moisture loss (Liao et al. 1996). The volume of compost produced is generally about half the original material being composted (Rynk, 1992).

Food waste has unique features as a raw compost agent. Food waste has a large water content, large organic-to-ash ratio, and lacks physical structure (Chang and Hsu, 2008), which requires mixing with a bulking agent in order to maintain adequate aeration within the material to allow aerobic decomposition to proceed in a timely manner. Furthermore, the conditions of composting, particularly as they apply to large-scale industrial systems, require that the process be controlled and managed (Epstein, 2011). Food waste, other feedstocks, and conditions of materials should be monitored thoroughly throughout the process to generate a stable, value-added end-product.

Alternative systems include four fundamental approaches to composting: turned windrows, aerated static piles, enclosed channel systems, and in-vessel systems (UNFCCC, 2018). The type of vessel could be a vertical tower, horizontal rectangular, circular tank, or circular, rotating tank. In-vessel composting system can be designed as a plug-flow or agitated

bed system. In the plug-flow system, the relationship between particles in the composting mass stays the same throughout the procedure, and the system works on the first-in, first-out principle. In the agitated bed framework, the treated material is blended mechanically during the processing. Mechanical frameworks are intended to limit odor and process time by controlling ecological conditions, for example, wind stream, temperature, and oxygen concentration. In-vessel technology allows for greater control of the environment and may enhance the process and decrease overall compost processing time (Kalamdhad & Kazmi, 2009). The aim of this review is to explore food-waste compost research to determine best practices for in-vessel food waste composting. Does the food-waste compost research provide the background to minimize time in a vessel compared to a windrow system for creating a stable, mature, consistent compost product?

2. Compost quality

Concepts of compost test standardization or compost quality were essentially unknown worldwide as recently as 1985 (Brinton, 2000). Standards and guidelines continue to be promulgated by different agencies and regulations differ by country; however, compost quality can be defined utilizing characteristics contributing to soil quality and plant health (Balis et al., 2002). Producing a consistently high-quality compost is especially important when the compost is for commercial purposes (Rynk, 1992). Quality has focused on eliminating potential human pathogens (USDA, 1980). Bess (2008) evaluated the microbiology of compost and stated that in order to imitate fertilizer analysis, traditional compost analysis has focused on nitrogen, phosphorus, potassium, and micronutrient contents; however, compost is more complex than fertilizer in that it has a critical microbiological component that affects how it performs as a plant suppressant or a soil inoculant. A standard analysis of compost microbiology is to determine the

concentrations of six functional groups of microorganisms: anaerobic bacteria, aerobic bacteria, fungi, pseudomonas, actinomycetes, and nitrogen-fixing bacteria (Table 1).

Compost maturity indicates the possibility of phytotoxicity caused by insufficient composting, while compost stability indicates the level of organic matter decomposition caused by (in)sufficient composting (Ma & Wu, 2001). Maturity and stability are two characteristics that are required for a safe compost to be incorporated into soil. They are related to the absence of phytotoxic compounds and plant or animal pathogens and the presence of stable organic matter (Bernal et al., 1998). Maturity is associated with plant-growth potential or lack of phytotoxicity (Iannotti et al., 1993), while stability is related to compost microbial activity. However, stability and maturity are closely associated since the microorganisms in any unstable compost produce the phytotoxic compounds. Bernal et al. (1998) stated that compost color, odor and temperature have been used to assess compost maturity.

The germination index (GI) is often utilized to evaluate compost maturity, where 50% or greater GI describes a phytotoxin-free product (Lvova and Nadporozhskaya, 2017). Compost stability is defined by the microbial degradation rate of the organic matter during composting (Haug, 1993). There are different types of stability according to Blok et al. (2008) - chemical, physical, and biological stability. Chemical stability is relative to the breakdown of certain carbohydrate structures, physical stability regards mechanical strength, and biological stability is associated with the microbiological activity. The stability and quality of compost is completely dependent on its raw materials (Ranalli et al. 2001; Benito et al. 2003; Wang et al. 2004). At the end of the composting process, various factors including the C:N ratio, pH, temperature, moisture content, and the presence of potential pathogens are used to assess the quality and stability of the compost (Erickson et al. 2009; Al-Turki 2010; Fourti et al. 2011; Sanmanee et al.

2011). Properly prepared compost has reduced soil-borne plant diseases without the use of extra chemical controls. Waste materials including vegetables, garden waste fruits are easily available and could be composted. Research show that compost made from those types of waste can be disease-suppressive (Blok et al., 2002; Postma & Kok, 2002).

Table 1. Criteria for evaluating microorganisms in finished compost, from Bess (2008)

| Functional group | Interpretation of compost bioassay | Function |
|----------------------------------|------------------------------------|---|
| Heterotrophic bacteria (Aerobic) | $10^8 - 10^{10}$ CFU/gdw | Decomposition, nutrient cycling, suppress plant diseases |
| Aerobic-to-anaerobic bacteria | $\geq 10:1$ | Eliminate compounds that might inhibit plant growth or seed germination |
| Yeasts and molds (Fungi) | $10^3 - 10^4$ CFU/gdw | Decomposition, nutrient cycling, stabilizing soil aggregates, and controlling plant disease |
| Actinomycetes | $10^6 - 10^8$ CFU/gdw | Decomposition and nutrient cycling of complex chemical substances such as chitin and cellulose, improving soil structure, reducing plant pathogen pressures, particularly efficient in alkaline soils |
| Pseudomonas | $10^3 - 10^6$ CFU/gdw | Nutrient cycling, assisting plants with phosphorus availability, links to biological control of plant pathogens |
| Nitrogen-fixing bacteria | $10^3 - 10^6$ CFU/gdw | Fix dinitrogen into ammonium form, inversely related to biologically available nitrogen |

Testing compost for pathogens is essential to determine if the product is safe as an amendment for the soil. Compost applied to areas growing food and feed crops should be tested for the presence of fecal coliform and *Salmonella* organisms as indicators of contamination. Fecal coliforms <1000 most probable number (MPN)/g and *Salmonella spp.* <3 MPN/4 g are recommended by the USEPA (n.d). The USEPA requires a process to further reduce pathogens (PFRP) prior to land application of composted biosolids (Hay, 1996). However, in the United

States there are no federal rules regarding food waste composts. In Europe, regulations vary between countries. In most cases compost temperature during thermophilic stage is used as an indicator of compost safety in all composting. Pathogens survive during the composting process up to 2 weeks, depending on the temperature of compost piles for compost that have lower temperatures (Vinnerås, 2007).

3. Conditions for composting

To produce quality compost, it becomes essential to optimize the process by managing the feedstock components and the process parameters such as temperature, moisture content, pH, bulk density, aeration (oxygen availability), matrix structure, and carbon-to-nitrogen (C:N) ratio. A range of conditions are considered acceptable for composting to proceed for a variety of inputs, or initial feedstocks, while a more narrow range of conditions promotes more rapid composting of organic materials (Table 2, Cundiff & Mankin, 2003). Overall, during composting, the C:N ratio decreases, the relative quantity of humus-like material increases, and the exchange capacity of the material increases (Rynk et al., 1992).

A wide range of food can be composted, including from agricultural industries, restaurants and food processing companies. Adequate aeration and moisture allow microorganisms to access substrate and nutrients while facilitating gas exchange (Linn and Doran, 1984). Excess moisture will limit gas exchange and cause the environment to go anaerobic if activity exceeds the rate of gas exchange in pores. The ideal moisture level in compost ranges between 45 – 60% by volume (Cooperband, 2002). Food waste does not have structural integrity to maintain pore spaces and aerobic conditions throughout the bulk of the material being composted. As composting is an aerobic process, with improper aeration compost often stalls, takes too long to be economically viable as an operation, and the final product is

unstable and immature. Thus, fermentation can become problematic during composting, producing organic acids and reducing pH. Waqas et al. (2017) investigated 85:15 (volume:volume) mixed and ground food waste-to-biochar compost produced at two temperatures in a bench-scale experiment (10 kg). Vessels filled at 70% capacity amended with biochar produced at 450 °C from lawn waste including grass clippings, date leaves, ornamental plant waste and coconut leaves increased to 60 °C.

Table 2. Recommended conditions for composting (Cundiff and Mankin, 2003):

| Condition | Reasonable range | Preferred range for optimal (rapid) composting |
|--|------------------|--|
| C:N ratio | 20:1 – 40:1 | 25:1 – 30:1 |
| Water content (% of wet mass) | 40 – 65% | 50 – 60% |
| Oxygen concentrations (mole fraction) | > 5% | > 5% |
| Particle size (diameter, mm) | 5 – 15 | 5 |
| pH | 5.5 – 9.0 | 6.5 – 8.0 |
| Temperature of thermophilic phase (°C) | 40 – 65 | 55 – 60 |

The quality of the final product was more consistent and less bulking agent may be required than aerated static piles (UNFCCC, 2018), but this assumes that the entire composting processes occurs in-vessel and does not apply to facilities that compost in-vessel initially and cure compost in windrows. In some cases, vessels are not used for the entire composting process; retention in drums may vary from 24 HR to seven days, depending on manufacturer specifications, with subsequent curing in windrow or aerated bay (Epstein, 2011). If vessels are not employed for the entire process, that complicates comparisons in approach between compost systems.

The most common aeration method is the physical turning of the material (Ajay et al., 2008). Previous research has focused on the effects of turning frequency on compost stability and its chemical characteristics (Ajay et al., 2008). Gases are released during composting, including volatile organic acids (VOA) and NH_3 , especially if conditions go anaerobic or carbon-to-nitrogen ratios are not well balanced (Cornell Composting, 2020). Problems with inadequate aeration include odor pollution, which can incite public concern and result in limiting actions or plant closures at compost sites (Colon et al., 2012).

The temperature changes during different stages of composting and reaching thermophilic stage, or $45\text{ }^{\circ}\text{C}$, and especially temperatures greater than $55\text{ }^{\circ}\text{C}$ are critical to eliminate pathogens. Reaching thermophilic stage is key in composting because it will allow different organisms to decompose the feedstock; it will also eliminate susceptible pathogens. During composting, the temperature is considered to mirror the metabolism of the microbial populations involved in the process (Harada et al., 1981). Microorganisms decomposes organic compounds progressively. In fact, decomposition progresses from simple molecules to complex compounds, which are either decomposed into simple molecules and respired as carbon dioxide (CO_2) or resynthesized into humus-like material and form compost. The temperature within a bioreactor or compost pile changes depending on the size, type, and activity of the resident microbial community (Monson and Murugappan, 2009). After the thermophilic stage, temperature cools to a final mesophilic curing stage during which decomposition and humification continue and the compost matures and stabilizes. Regulations in the U.S. require maintaining thermophilic temperatures in excess of $40\text{ }^{\circ}\text{C}$ for 5 days and $55\text{ }^{\circ}\text{C}$ for at least four hours to destroy pathogens within the compost (USEPA, 1994). The compost heats because during the accelerated bio-oxidation of the organic matter microorganisms liberate carbon

dioxide, heat, and water. A decrease in temperature during the thermophilic stage could mean that the materials need to be aerated or moistened (Gaur et al., 1982).

4. Vessel systems vs windrows

In-vessels systems may be best options for food waste composting. In fact, according to the USEPA (2020b), vessels may be a better option in terms of environmental conditions management. Kumar et al. (2010) adopted an aeration tube that was installed at the bottom of the reactor with an air supply at the rate of 10 L/min. They co-composted food waste and green waste and were able to achieve maturity in 12 days with maximum temperature at 70°C on day 5. The maturity test was a seed germination with 397/400 (germinated seed/total seed). Another study conducted by Chang et al. (2006) adopted a vessel system. They used a variable-speed, induced fan to provide oxygen to the compost. They recommended a range of 0.8 and 2.0 L/kg DS-min as an indicated range of air-suction rate. They were able to get matured compost after 96 hours.

Vessel composters can be managed with careful control or monitoring, often electronically, of the climate, which allows year-round use of this method (USEPA, 2020). Kumar (2010) adopted a 120-L bioreactor with thermocouple and computer-based acquisition data. Chang et al. (2006) adopted a system coupled with CO₂, O₂ analyzers, low speed electric motors to help with the turning process, agitators to mix compost, and condensers to monitor the composting system.

In-vessel composting can process large amounts of waste without taking up as much space as the windrow method. Vessels use much less land and manual labor than windrow composting. On the other hand, composting in pilot projects can take longer to get to maturity; it depends on the size of the pile and the composted material. In a pilot-scale composting study

conducted by Raichura and McCartney (2006) on municipal biosolids with woodchips as a bulking agent, compost was run for 98 days. The temperature of piles varied throughout the experiment, lost greater water, and were less controlled than vessels. Environmental factors such as rainfall and especially temperature caused decreases from 60 °C on day 21 to 40°C (fine woodchips) and even less to 26 °C on day 26 with coarse woodchips.

Zhou et al. (2018) investigated the microbial community of windrows from a commercial composting facility in New York and found that the age of the windrows varies. Some windrows were 1, 2, 3, 4, 6, 10 and even 24 months. They noticed that temperature piles differed with depth. The 15-cm depth temperature in a 4-month windrow was 35 ± 0.0 °C, while temperature at 60 cm was 42.7 ± 0.58 °C with a pH of 8.0 ± 0.21 . Temperature variability in windrows may be larger than vessels and therefore harder to achieve compared to a vessel where the temperature is constantly managed (Zhou et al. 2018). Zhou et al. (2018) used 16S rRNA gene sequencing to observe that the depth of the piles had an effect on bacterial community because of a lack of homogenization of the system. The 4 and 6-month-old windrows had genera that varied with depth. *Sphaerobacter*, *Geobacillus*, and *Ureibacillus* were impacted by depth in the 4-month-old samples, whereas *Thermaerobacter*, *Thermobifida*, *Rhodothermus*, and *Thermoactinomyces* were significantly decreased by depth in the 6-month-old samples.

Small reactor size may present problems for being able to reach and maintain thermophilic temperatures inside compost; however, small volume bioreactors have been utilized successfully, especially in research experiments. Guidoni et al. (2018) adopted a home composter and investigated variable proportions of food waste-to-bulking agent. Lashermes et al. (2011) investigated performance and reproducibility of composting in experiments where thermophilic condition of 69 ± 4 °C by self-heating was achieved in six laboratory-scale

bioreactors (<10 L), and water content decreased from 66 to $41 \pm 7\%$ (CV = 11%) of wet weight. Loss in total organic matter from the composting process was similar to large-scale vessels or windrows in on-site experiments. Initial C:N ratio of 15.7 decreased to 12.2 ± 1.5 after 83 days of composting. Lashermes et al. (2011) recommended use of a laboratory-scale model in experiments for scientific investigation of the initial feedstock mixtures because of the reproducibility of the final product.

Vessel systems may offer the benefit of protection from austere weather conditions; however, insulation may be needed to compensate for heat loss in high surface area-to-volume ratio bioreactors (Adhikari et al., 2008; Lashermes et al., 2011; Smårs et al., 2000). At a capacity less than 30 L, insulation may not be enough to allow a proper self-heating of the material mass if the substrate is not easily decomposed (Campbell et al., 1990). Wong et al. (2009) evaluated coal fly ash (CFA) on the decomposition efficiency of food waste and synthetic food waste in a 20-L insulated laboratory-scale reactor where temperature was monitored continuously by computer.

Water provides the medium of nutrient transport, participates in chemical reactions, and facilitates transport of microorganisms throughout the compost. Feed material, added water, and product water (final moisture content) are three considerations for the water balance in composting. Added water in particular may require an adjustment from a design viewpoint (Epstein, 2011). In practice, compost material should be maintained at a moisture content ranging from 40% and 65% on a volumetric basis (Rynk et al., 1992). Others have found that decomposition proceeded optimally at 50 to 70% volumetric water content (Monson and Murugappan, 2009). Thus, while aeration is critical, variability in feedstock materials means water content may vary between 40 to 70%.

To sustain active microbial populations, some researchers suggested adding water to the feedstock. Chang and Hsu (2009) in a food waste composting study used 2.87 kg (dry basis) carbohydrate, protein, and fat bulked with 9 kg of rice husks and added water to maintain a water content up to 55%.

Carbon and nitrogen are two essential nutrients for microbial activity and growth. Carbon serves primarily as a food source and nitrogen is an important element in proteins. Monson and Murugappan (2009) explored the optimal combinations of bulking agents in an in-vessel composting system of vegetable food waste and determined that C:N ratio should be maintained between 20 to 40:1 and preferably 30 to 35:1. Bulking agents including sawdust, dry leaves, wood shavings, paddy straw, or paddy husk sugarcane bagasse provide carbon, reduce excess moisture, and increase porosity and integrity of organic solid matrices (Monson and Murugappan, 2009).

The ideal pH range is around of 6.5-8.0 for finished compost, but the natural buffering capacity of the organic matter makes it possible to perform composting at a wider pH range. Composting may proceed effectively at pH levels between 5.5 and 9 (Rynk et al., 1992). Because of the range of organisms involved in composting, composting is fairly indifferent to pH and pH changes throughout the process; however, ammonia loss and alkalinity become concerns at a high pH (above 8.5). Decreased pH is characteristic of organic acids released in the initial stage of food waste composting and an increase in pH later in the process might be due to the production of ammonia (Rynk et al., 1992). Chang and Hsu (2009) determined using quadratic regression models that including greater amounts of protein in food waste will result in greater final pH in the compost product. Sundberg (2005) also determined that accumulated acids decomposed later in the composting process producing carbon dioxide and heat. In-vessel

composting may reduce greenhouse gases emissions if the released air is filtered (UNFCCC, 2018), and some vessels are coupled with computers to allow close monitoring of properties (Lashermes et al., 2011; Wong et al., 2009).

5. Additions and inoculations

Co-composting of food waste has been efficient, and several researchers have tested the addition of yeast and bacterial cultures (Sundberg and Jösön, 2005). Co-composting is employing multiple feedstocks and inoculation adds microorganisms or enzymes to initiate or promote microbial growth and activity. The idea with both approaches is to optimize conditions to promote microbial activity to facilitate decomposition.

Coal fly ash (CFA, 5-10%) and 1.88% lime may enhance the rate and efficiency of decomposition (Wong et al., 2009) that produces acids and reduces the pH of the composting mass decreasing decomposition efficiency (Nakasaki et al., 1998). Wong et al. (2009) found that the addition of alkaline materials (coal fly ash and lime) successfully buffered against the development of low pH conditions during food waste composting process and maintained a more consistent composting duration of approximately 28 days.

Zeolite, aluminum and silicon minerals used as ion exchangers and absorption materials, can reduce compost salinity and antibiotic gene resistance and heavy metals (Soudejani et al., 2019) and ammonia emissions during food waste composting (Bernal et al., 1993). An average 53 g zeolite/kg has resulted in 80% less N emitted from compost (Bernal et al., 1993).

While there are ranges within conditions allowing composting, the variability inherent in food waste, microbial communities, and across space and time may require that multiple modifications may be needed to optimize composts. Awashi et al. (2018) composted a consortium of 20 bacteria isolated from food waste compost and kitchen sink waste grown on

modified basal salt liquid medium in a small-scale in-vessel composter with food waste (mainly consisting of steamed rice, wheat meal, soy, fish, meat and lard mixed with sawdust at the ratio of 4:1 (g:g)).

Effective microorganisms (EM) are a consortium of active bacteria used in food waste composting. They are mixed cultures of beneficial, naturally occurring microorganisms that can be applied as inoculants to increase the microbial diversity of an ecosystem. There is evidence that EM inoculation to soil can improve the quality of soil, plant growth and yield (Kengo and Hui-lian, 2000). The EM is defined as an unrevealed mixture of naturally occurring microorganisms that have beneficial properties in a wide range of applications (Higa, 2002). As a commercial product, the precise composition of EM has not been disclosed (Formowitz et al., 2007). Kyan et al. (1999) indicated that the EM product mainly contained populations of lactic acid bacteria, photosynthetic bacteria, yeast, and actinomycetes, while Daly and Steward (1999) determined that EM contains species such as *Streptococcus albus*, *Propionibacterium freudenreichii*, *Streptococcus lactis*, *Aspergillus oryzae*, *Mucor hiemalis*, *Saccharomyces cerevisiae*, and *Candida utilis*, plus a range of unspecified number of *Lactobacillus* sp., *Rhodopseudomonas* sp. and *Streptomyces griseus*.

Because of the reputed benefits of EM, EM has been used as inoculum in composting. Kamaruddin et al. (2018) adopted the following for feedstock: 2000 g of food wastes, 150 ml of inoculant of EM and 1000 g garden and yard wastes. Every 4 days, they sprinkled water to maintain the inoculated compost and control compost moisture contents at 56.5% and 50.18%, respectively. The pH changes, microbial decomposition, and the C:N ratio were in the range considered valuable for the product to act as fertilizer. The final compost assisted by inoculation

achieved 75% germination compared to 25% germination in the presence of the control compost (Kamaruddin et al., 2018).

Microorganisms that are dominant during composting are hypothesized to drive the composting process, and the rationale behind inoculation is to add the microorganisms to facilitate a consistent compost processing and product development. Inoculation should thereby eliminate inconsistency in the process that results from variable food waste feedstock inputs. Some researchers have investigated use of a microbial consortium that degrade organic acids (MCDOA). The MCDOA of the following taxa (19×10^8 CFU mL⁻¹) have been used: *Dysgonomonas* sp., *Pseudomonas caeni* strain, *Aeribacillus pallidus* strain, *Pseudomonas* sp., *Lactobacillus salivarius* strain, *Bacillus thuringiensis* strain, and a *Bacillus cereus* strain (Song et al., 2018). Song et al. (2018) added wheat bran as a bulking agent and 1.25 mL MCDOA kg⁻¹ dry compost. The MCDOA inoculation eliminated the initial lag phase of the temperature rise that resulted from excessive acidification and effectively shortened the composting period.

Other consortium of isolates have come from compost and soil, such as *Bacillus casei*, *Lactobacillus buchnei* and *Candida rugopelliculosa* and ligno-cellulolytic fungi (*Trichoderma* sp. and white-rot fungi) (Wei et al., 2007). Inoculation accelerated humification and maturation during municipal solid waste composting (Wei et al., 2007). On the other hand, a study conducted in Costa Rica concluded that addition of EM inoculated and “bokashi” (i.e. Japanese term describing fermented organic matter) banana residues (*Musa* sp.) was not different compared to uninoculated compost typically used in organic farming added to bokashi, except for an increased concentration of potassium in young banana leaves (Formowitz et al., 2007).

6. Conclusion

Composting is a natural process of recycling organic material. It is a biological process, carried out under aerobic conditions to convert biodegradable organic waste, such as food waste, into a valuable organic product. While it is a process that is robust to a range of conditions, the duration, process, and the quality of the final compost depend on optimizing the process conditions of the composting system, including the water content, organic matter and feedstock inputs and structure, oxygenation, temperature, pH, and the microbial activity. Inoculation of bacteria combined with optimization of abiotic parameters facilitates composting, and that optimization of abiotic parameters may be achieved by addition of substances, coal fly ash, biochar, or zeolite to ameliorate suboptimal physical and chemical properties. Vessels stand out as a best option when it comes to composting. Even though they might be of a higher cost they have notable advantages such as rapid composting process, better nuisance control and better control of the environment.

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Chapter 3. Experimental studies

1. Introduction

Having sufficient carbon impacts the increase of the temperature to thermophilic phase (Ajay and Kazmi, 2008). Vessels offer greater degree of control of environmental conditions during composting, and conditions such as aeration are critical to facilitate rapid decomposition of food waste and to generate stable and mature final compost. However, while proportion of food waste:bulking agent ratio is critical, there has not been a consistent food waste:bulking agent ratio that has been established as optimal for food waste composting. Furthermore, people are inconsistent in applying volumetric and gravimetric (mass-based) ratios in determining appropriate food waste:bulking agent ratios for composting. Lack of consistency in parameterization may arise from variability in food waste inputs, bulking agent inputs, type of composting system being employed, size of composting system, and compost product demands, or any combination of these variables, and possibly from other factors.

A study conducted in Brazil investigated how the proportions of organic household waste and bulking agent can affect the results of the composting process (Guidoni et al., 2018). Three treatments varying the ratio of vegetable leftovers and raw fruits to rice husks were used in investigating a pilot-scale, home-composting system. The proportions of food waste:bulking agent were 70:30, 50:50, and 30:70 on a mass:mass basis. The proportion of the materials used in composting affected the decomposition of the organic matter, compost toxicity, and plant germination. The 70:30 proportion of food waste:bulking agent promoted microbial growth, increased concentration in mineral matter, and resulted in a C:N ratio that was better than other treatments for composting (Guidoni et al., 2018).

In another study using a rotary drum composter of 250 L capacity in India, mature compost was generated in less than three weeks utilizing cattle manure (25 kg), mixed green vegetables (20 kg), and sawdust (10 kg) mixed at a 2.5:2:1 ratio on a wet mass basis resulting in an initial 22:1 ratio for C:N (Ajay and Kazmi, 2008). Compost was aerated at a turning frequency of once per day (Ajay and Kazmi, 2008).

Composting in these experiments were conducted with food waste from local restaurants and a locally obtained bulking agent of wood chips. Woods chips were adopted as the bulking agent to balance the C:N ratio and to help to provide pore space for bacterial degradation. Experiments were carried out to measure conditions during the composting process in vessels using pre-determined feedstock ratios in order to evaluate initial optimal ratios and the effect of a microbial inoculant in order to optimize vessel food waste composting. Specific objectives included evaluating the impact of food waste:bulking agent ratios on the composting process, and evaluating the effectiveness of a microbial inoculant on food waste composting. It was hypothesized that composting would be optimized at a specific food waste:bulking agent ratio and that would result in enhanced thermophilic abundances during the increased temperature stage. Increased enzyme activity was expected to differentiate compost treatments. A bacterial inoculant was hypothesized to increase decomposition and facilitate the increase in temperature to thermophilic and maintenance within thermophilic conditions, enhancing the composting process.

2. Methods

2.1. Experimental approach

The in-vessel food waste experiments were run in a greenhouse located at the University of Arkansas System Milo Shult Main Agricultural Experiment and Extension Center in

Fayetteville, AR (36° N, 94° W). Temperature in the greenhouse was measured throughout the second and third experiments. The vessel or 208-L barrels were kept on a table and treatments were randomized on each side of the table. This thesis research consisted of three experiments. The first experiment had three treatments with two replicates. The second and third experiments had two treatments and three replicates. Experiments 1 and 2 were run for 48 days and evaluated proportions food waste:bulking agent ratios of 50:50, 65:35, and 80:20 (mass:mass) and 65:35, and 80:20 (mass:mass) basis, respectively. These proportions were chosen after an initial pilot test at proportions investigating 70:30, 75:25, and 80:20 (mass:mass) basis (Appendix 1). This final experiment was run for a total of 50 days on compost established at a 80:20 (mass:mass) food waste:bulking agent ratio and evaluated the effect of a microbial inoculant on the composting process.

Layers of food waste and bulking agent were piled to make the initial compost inside each vessel. For each experiment, the barrels were filled to a capacity of 70%. With a total capacity of 208.2 kg, 70% capacity equaled 154 kg. At a 80:20 ratio, 123.2 kg food waste was added to 31.8 kg of bulking agent. The equivalent mass weighed for the 65:35 ratio equated to 100.1 kg food waste and 53.9 kg bulking agent. Finally, the proportions of food waste and bulking agent at the 50:50 ratio equaled 77 kg of food waste and 77 kg bulking agent. The air temperature in the greenhouse was collected by a static thermometer that was placed inside the greenhouse throughout the entire experiment. Side doors on each barrel were constantly closed unless during sampling and after sampling for exactly 5 min. Six rotations were provided every day of each experiment to aerate compost.

2.2. Bioreactor: Rotary drum

The three main experiments were conducted in pilot-scale, rotary drum vessels constructed from 208.2-L (55-gallon) drums with food-grade, lined interior coating (Figure 1). The original bench-scale test was conducted 19-L vessels described in Appendix 1. Rotary drum vessels (208.2 L) had a diameter of 58.4 cm on the outside edge and were 90.2 cm tall. The drums were constructed from 4-mm thick metal. Each drum was designed with two half-size openings at the ends to facilitate loading and unloading and aeration after rotations. The side of each drum had two groups of four 1-cm diameter holes covered by four layers of screen made of PVC-coated galvanized steel with a mesh size 2.5 cm by 2.5 cm. The holes in the mesh screening drained leachate from the vessel while retaining composting particles. An additional hole was drilled at the side to insert a temperature probe that did not get used in the experiments. Each composting vessel was positioned on its side on wheels with a 453.6-kg load capacity in order to facilitate rotation of the drum.



Figure 1. Barrel (208-L) during the construction to make modifications for composting (e.g. drainage holes for leachate). Barrel is shown on its side on the platform used to facilitate turning in order to maintain aeration.

2.3. Food waste and bulking agent

Food waste was obtained from a local start-up company, Food Loops, located in Benton County (<https://foodloops.net/>) whose original mission was to develop efficient and consistent in-vessel composting of food waste. Food Loops collected food waste from local restaurants and institutions in the Northwest Arkansas region and delivered those wastes to their base in Rogers, AR where the food was ground into 3 to 4-cm length particles. The food was brought to the greenhouse prior to each experiment. The food waste varied with each delivery and consisted of vegetables, fruits, such as lettuce, spinach, carrot, cabbage, peppers, potatoes, citrus and other fruits, rice, beans, and meats, such as fish, and chicken (Figure 2).

Bulking agent (wood chips) was provided by a local company Monster Tree service (<https://www.whymonster.com/northwest-arkansas/>) located in Springdale, AR. Food waste and bulking agent were delivered to the University of Arkansas in separate containers to allow control and alteration of feedstock ratios as needed for different experiments. The wood chips were stored at the greenhouse and were used whenever needed. (The same wood chips source was used throughout the experiment to ensure the consistency of the experiment). The food waste was mixed with the bulking agent prior to the start of each experiment according to the proportions described in the experimental design.

2.4. Sampling and analysis

Compost temperature was measured daily throughout experiment using a hand-held VWR traceable digital thermometer. Temperature was measured at both doors of the barrels and a daily average for the barrel was calculated. Compost temperature in experiment 1 was used to determine sampling dates (sampling occurred in each compost stage) and the same dates were used in subsequent experiments. During experiment 1, a T-PRO K-Type Sheathed Thermocouple

0-1000°C, R1/8 screw thread, Thermocouple Plug with 2M/6.6Ft that can record temperature every hour was tried as the temperature monitoring device; however, continuous monitoring could not be maintained in the greenhouse environment. Thus, the hand-held thermometer had to be used to monitor temperature.



Figure 2. Food waste delivered in barrels for one of the experiments that was used to construct initial compost mixtures.

Samples were collected during each stage of the composting process, i.e. during the mesophilic, thermophilic and the final mesophilic stages. Samples were collected on the following days: 0 (initiation), 11 (thermophilic), 33 (late thermophilic/early curing), and 48 (mature curing, experiments 1 and 2) or day 50 (in experiment 3). An additional sampling date was added in experiment 3 between day 11 and day 50 and samples were collected on day 25 as well. Compost was composited from the two sampling ports (approximately 10 g each) of the

bioreactor for each sample collected. Samples were collected by spoon after mixing the feedstock. Sampling was performed at each end of the barrel, using the half-doors as access points. Compost samples were stored at 4 °C in the refrigerator and then divided as described below. For each compost collected, one subsample was used for chemical and physical analysis, one subsample was frozen at -80 °C for future molecular analysis, and the remainder was stored 4 °C in the refrigerator after immediate processing for microbial analysis. The enzyme activity was conducted within one month of sampling in the first experiment and within one week of sampling during the second and third experiments.

Water content was measured gravimetrically after oven-drying to a constant weight (105 °C for 24 hour) and was calculated from the difference in wet mass and dry mass divided by the original wet mass. The pH and electrical conductivity (EC) were each measured by probe and calibrated meter at a 1:2 (wt:vol) compost:deionized water ratio. The pH and EC were measured by VWR*Symphony*B20PI Benchtop Multi Parameter Meter. The carbon and nitrogen content were measured by combustion on a Carlo Erba instrument on initial samples and at the conclusion of composting. The C:N ratio represents the relative proportion of the two elements. Compost stability and maturity were assessed on final compost by measuring C:N ratios, EC, and pH.

Cultivation-based microbial community assessments were made after initially blending 10 g of feedstock with 90 ml of 0.1% buffered peptone water (BPW) for 60 seconds on a vortex mixer. A ten-fold serial dilution to 10^{-9} was prepared using tubes containing a total of 10 mL in the dilution series, and 100 µL of each dilution was plated onto 0.1X tryptic soy agar (TSA) and 1 mL onto Petrifilm aerobic count plates. The initial mesophilic bacteria were incubated at 32.5°C for 48 h and plated on TSA. Thermophilic bacteria were incubated at 55°C for 24 h.

Colonies were counted and colony forming units (CFU) were calculated based on the appropriate dilution factor and reported per g dry compost following the formula below:

$$\text{CFU / g compost} = (\text{no. of colonies} / \text{dilution factor}) / \text{g dry compost}$$

Enzyme activity was measured colorimetrically for β -glucosidase expressed as $\mu\text{mol } p\text{-nitrophenol g}^{-1} \text{ h}^{-1}$ on a dry weight basis following the method described by Tabatabai (1994). This method is based on the hydrolysis of the *p*-nitrophenyl- β -D-glucopyranoside (25 mM pNPG in MUB-HCl buffer, pH 6) after incubation at 37 °C for 1 h. Measurements of potential enzyme activity were performed on a spectrophotometer at a wavelength of 405 nm.

2.5. Data analysis

Experiments adopted a randomized block design. Each experiment was analyzed separately, and cross-experiment comparisons were evaluated descriptively. Differences in temperature among stages within an experiment or across experiments were evaluated descriptively. The CFU/g was log transformed for analysis. Days and treatments were assumed to follow a gamma distribution, and water content was changed to a proportion and was assumed to follow a beta distribution with proportions between 0 and 1. Compost properties were analyzed by split-plot ANOVA for statistical differences and one-way ANOVA at the end of composting to compare treatments. Treatments were considered significant at an alpha of 0.05 or 0.1 and when applicable treatments were separated based on least significant difference (LSD). Data were graphed using software R version 3.6.3.

3. Results

3.1. Experiment 1

Leachate leaked out of barrels for 18 days after the experiment started but volume was not measured. The effect of treatment on temperature depended on when during the composting

processing temperature was measured (Figure 3). During the initial mesophilic phase, the 80:20 food waste:bulking agent treatment had the lowest temperatures and then during the late thermophilic and early curing phase 80:20 food waste:bulking agent treatment was greater in temperature than the 50:50 food waste:bulking agent treatment. Regardless of changes in temperature between treatments, temperature in all three treatments followed the expected pattern for composting and increased from mesophilic into thermophilic followed by a decrease into a final mesophilic curing stage.

The temperature increased from 17°C to 37°C during the initial phase and increased into the thermophilic phase and stayed above 40 °C days for more than 9 days (Figure 3). Temperature averaged 17, 17, and 18 °C when the experiment began (on day 0) in the 50:50, 65:35, and 80:20 food waste:bulking agent treatments, respectfully. In this experiment, temperature numerically peaked on day 9, started to decrease in all barrels around day 14 and was more pronounced on day 20 and dropped in all barrels between day 20 and day 30. From day 39 until the end of the experiment at day 48, temperature was not different among treatments and remained steady between 23 and 26 °C. No greenhouse temperature was recorded, but observationally, as the greenhouse temperature was not actively maintained and did fluctuate in relationship to outside temperature, this experiment was likely the most affected by weather among the three experiments in this thesis. There does appear to be an interaction between time and the different treatments for temperature, where the relationship of temperature in a treatment changed with time; however, the three treatments changed together and maintained the expected composting process overall.

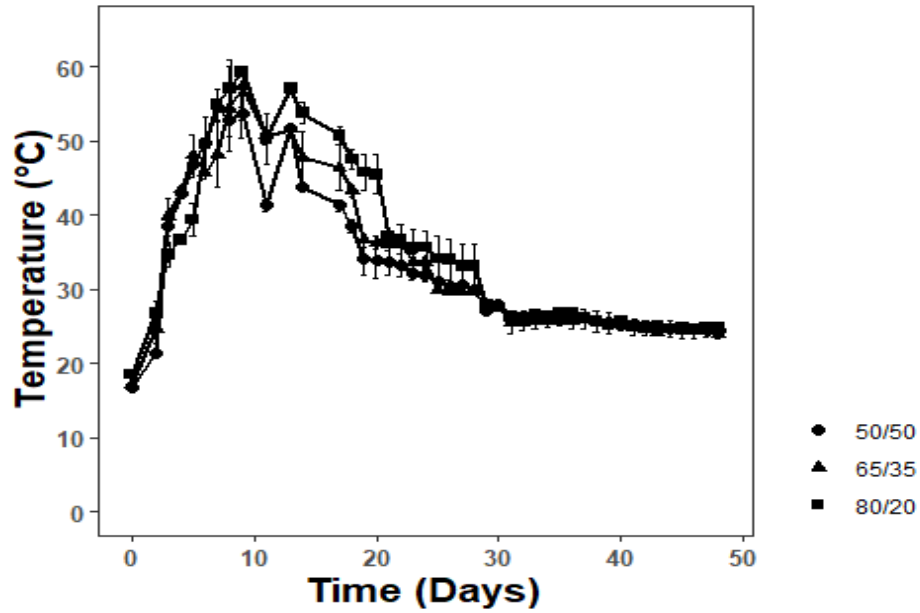


Figure 3. Mean daily (\pm standard error) temperature profile over time in a 48-day in-vessel composting experiment evaluating 50:50, 65:35, and 80:20 post-consumer food waste:wood chip bulking agent (mass:mass) treatments ($n = 2$) in food-grade lined steel (208-L) barrels maintained in a greenhouse and filled at 70% capacity rotated and aerated daily.

Table 3. Analysis of variance (ANOVA) summary of the effects of food waste to bulking agent treatments, sampling time, and their interactions on compost properties

| Property | Time | Treatment | Time x Treatment |
|-------------------------|---------|-----------|------------------|
| pH | <0.0001 | 0.5603 | 0.5489 |
| Electrical conductivity | 0.1460 | 0.3163 | 0.2770 |
| Thermophile | 0.0018 | 0.1787 | 0.5384 |
| Mesophile | <0.0001 | 0.1902 | 0.1766 |
| Glucosidase | 0.0096 | 0.1800 | 0.0971 |
| Water content | 0.0007 | 0.2762 | <0.0001 |
| C:N | 0.4788 | 0.9908 | 0.3016 |

Compost proceeded as expected with a predominance of mesophilic bacteria regardless of treatment (Table 3, Figure 4); numbers decreased during the thermophilic phase of composting. While there would be an expected decrease in the number of thermophilic bacteria (Figure 5) during the second mesophilic stage (or the final composting stage), the final stage was characterized by an increase in mesophiles, such that mesophilic bacteria recovered in abundance by the end of composting (Figure 4).

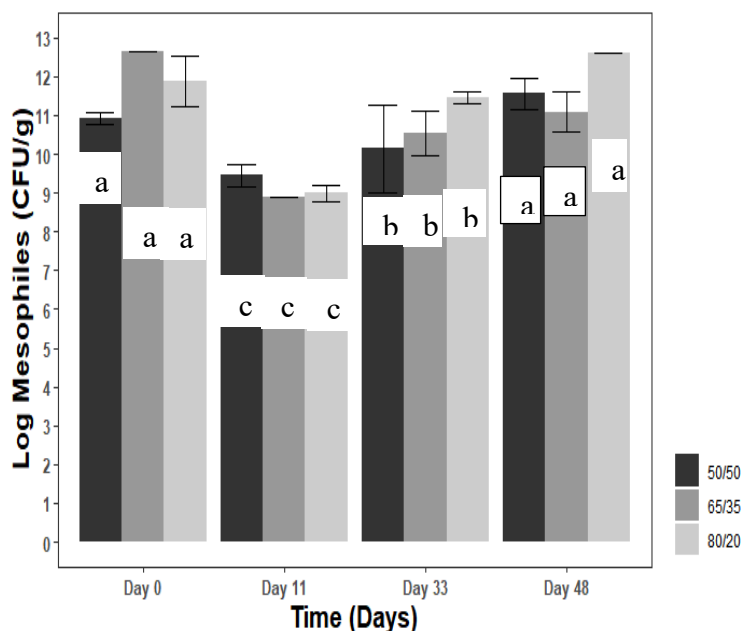


Figure 4. Mesophilic bacteria (mean \pm standard error) over time in a 48-day in-vessel composting experiment evaluating 50:50, 65:35, and 80:20 post-consumer food waste:wood chip bulking agent (mass:mass) treatments ($n = 2$) in food-grade lined steel (208-L) barrels maintained in a greenhouse and filled at 70% capacity rotated and aerated daily. Time and treatments followed by the same letter are not significantly different ($P < 0.1$).

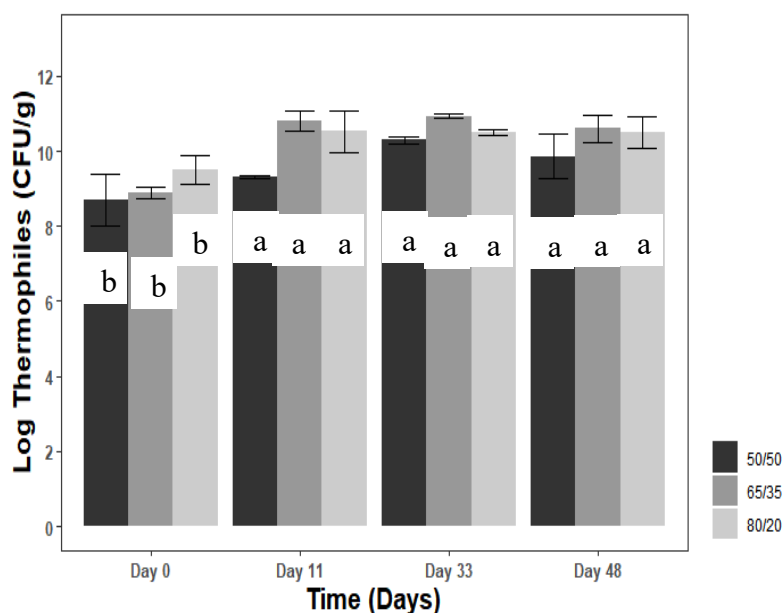


Figure 5. Thermophilic bacteria (mean \pm standard error) over time in a 48-day in-vessel composting experiment evaluating 50:50, 65:35, and 80:20 post-consumer food waste:wood chip bulking agent (mass:mass) treatments ($n = 2$) in food-grade lined steel (208-L) barrels maintained in a greenhouse and filled at 70% capacity rotated and aerated daily. Time and treatments followed by the same letter are not significantly different ($P < 0.1$).

Enzyme activity and water content both changed during composting based on treatment and sampling time (Table 3). The β -glucosidase activity showed an overall decrease with time; however, there was no difference in any sampling date in the 50:50 treatment, while the 65:35 and the 80:20 showed that the end of composting was lower than the beginning despite variability at different sampling times (Figure 6).

The water content was lower than the other two treatments on day 11 (thermophilic stage) in the 80:20 food waste:bulking agent treatment and lower in the 65:35 food waste:bulking agent treatment on day 33 (Figure 7). Water content initially (day 0) ranged between 69 and 72 % for the three treatments and really did not decrease overall during the experiment except for the noted two exceptions. The EC was < 2, 2.6, and 3.5 S/cm for 50:50, 65:35, and 80:20 food waste: wood chip bulking agent treatments, respectfully.

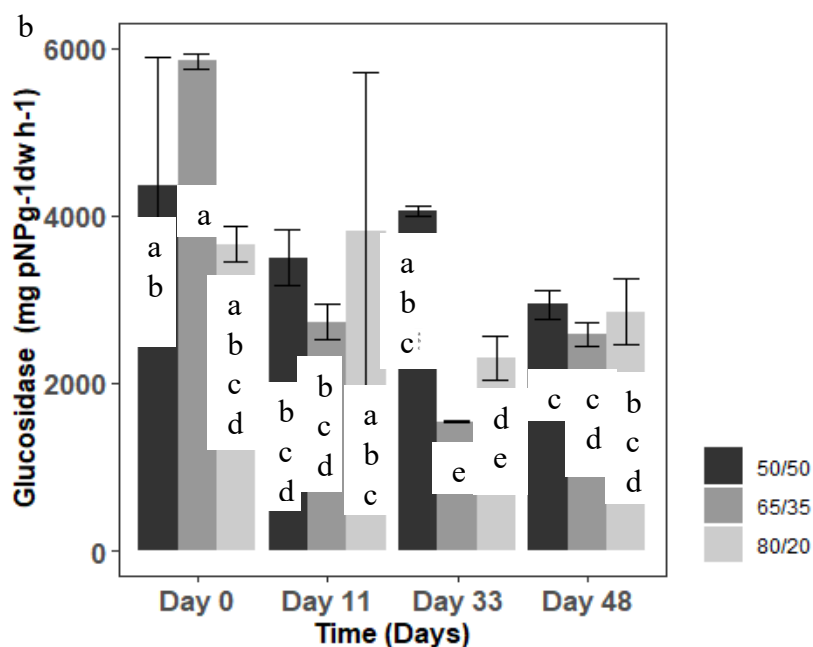


Figure 6. β -Glucosidase activity (mean \pm standard error) over time in a 48-day in-vessel composting experiment evaluating 50:50, 65:35, and 80:20 post-consumer food waste:wood chip bulking agent (mass:mass) treatments ($n = 2$) in food-grade lined steel (208-L) barrels maintained in a greenhouse and filled at 70% capacity rotated and aerated daily. Treatments at each time followed by the same letter are not significantly different ($P < 0.1$).

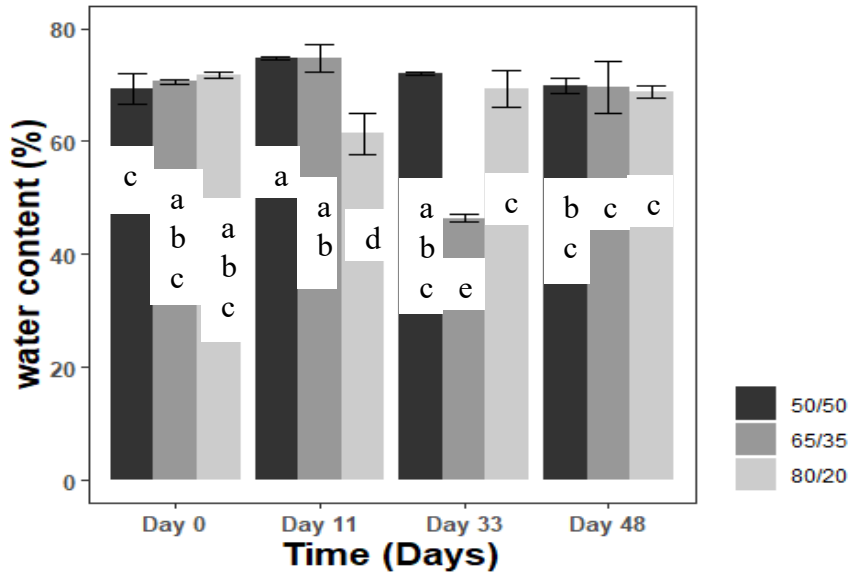


Figure 7. Water content (mean \pm standard error) over time in a 48-day in-vessel composting experiment evaluating 50:50, 65:35, and 80:20 post-consumer food waste:wood chip bulking agent (mass:mass) treatments ($n = 2$) in food-grade lined steel (208-L) barrels maintained in a greenhouse and filled at 70% capacity rotated and aerated daily. Treatments at each time followed by the same letter are not significantly different ($P < 0.1$).

During the first stage of composting, pH values were at their minimum (Figure 8). The pH was initially (day 0) 4.6, 4.5, and 4.4 for the treatment 50:50, 65:35, 80:20 treatments. The pH increased at every sampling period and was not significantly different between day 33 and 48 but was not different between treatment at any sampling time.

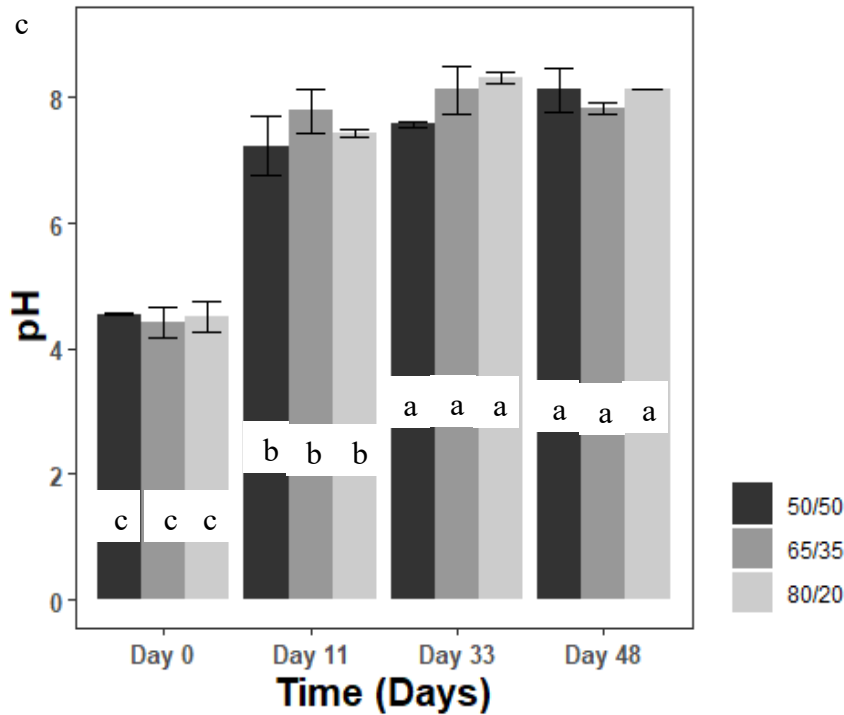


Figure 8. The pH (mean \pm standard error) over time in a 48-day in-vessel composting experiment evaluating 50:50, 65:35, and 80:20 post-consumer food waste:wood chip bulking agent (mass:mass) treatments ($n = 2$) in food-grade lined steel (208-L) barrels maintained in a greenhouse and filled at 70% capacity rotated and aerated daily. Time and treatments followed by the same letter are not significantly different ($P < 0.1$).

The carbon and nitrogen content were measured at the beginning and at the end of the experiment and the C:N was calculated, and there were no differences between treatments. In 50:50, the C:N ratio was on average 24:1 while in 80:20 treatment the C:N ratio was on average at 18:1 and 29:1 in 50:50 treatment (Figure 9).

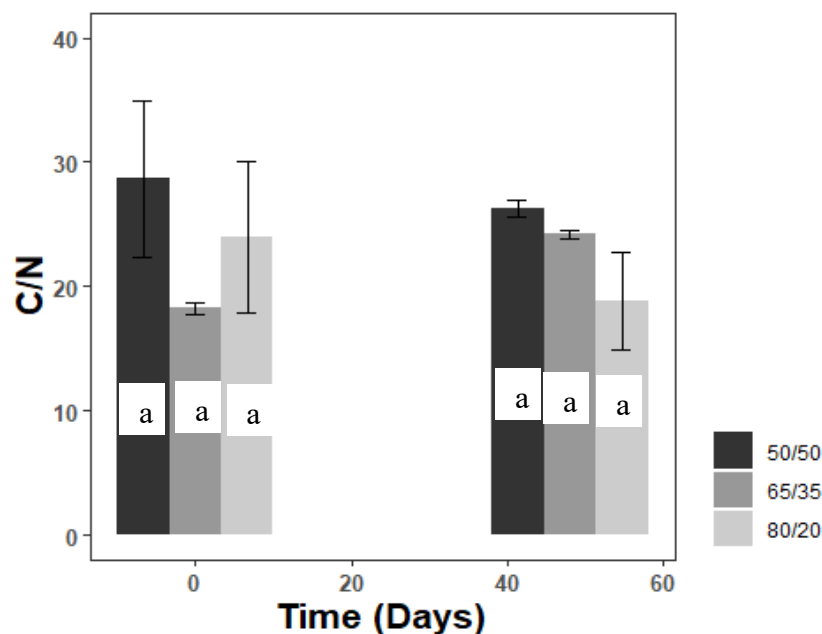


Figure 9. Carbon-to-nitrogen ratio (mean \pm standard error) at the beginning (day 0) and end (day 48) in a 48-day in-vessel composting experiment evaluating 50:50, 65:35, and 80:20 post-consumer food waste:wood chip bulking agent (mass:mass) treatments ($n = 2$) in food-grade lined steel (208-L) barrels maintained in a greenhouse and filled at 70% capacity rotated and aerated daily. Time and treatments followed by the same letter are not significantly different ($P < 0.1$).

3.2. Experiment 2

Experiment 2 was similar to the first experiment but was run with three replications, which required deletion of a treatment. Thus, treatments included 65:35 and 80:20 food waste: bulking agent treatments. Composting proceeded through the three expected stages of the composting process: initial mesophilic, thermophilic, and final curing stage. General observations in the greenhouse were that leachate leaked out of the barrel for 15 days after the start of the experiment. Temperature during the experiment 2 was on average at 17 and 18 °C at day 0 in the 65:35 and 80:20 food waste:bulking agent treatments, respectively (Table 4, Figure 10). During the initial phase, temperature increased until day 7. Temperature increased above 40 °C in all barrels and kept increasing to between 56 and 63 °C on day 13. Temperature remained

above 55°C for more than 9 days. The temperature started decreasing until day 39 and was stable during the curing stage until day 48. The greenhouse temperature was fairly constant throughout the experiment hovering about 28 °C.

Table 4: Analysis of variance (ANOVA) summary of the effects in a 48-day in-vessel composting experiment evaluating 65:35 and 80:20 post-consumer food waste:wood chip bulking agent (mass:mass) treatments (n = 3), sampling time (n = 4), and their interactions on compost properties in food-grade lined steel (208-kg) barrels maintained in a greenhouse and filled at 70% capacity rotated and aerated daily.

| Property | Time | Treatment | Time x Treatment |
|-------------------------|---------|-----------|------------------|
| pH | <0.0001 | 0.2092 | 0.0300 |
| Electrical conductivity | <0.0001 | 0.6222 | 0.2254 |
| Thermophiles | <0.0001 | 0.1124 | 0.8621 |
| Mesophiles | <0.0001 | 0.5631 | 0.1720 |
| Glucosidase | 0.0002 | 0.0016 | 0.4263 |
| Water content | <0.0001 | <0.0001 | 0.0065 |
| C:N ratio | 0.9033 | 0.0288 | 0.8523 |

Similar to experiment 1, treatment did not impact mesophilic or thermophilic bacterial abundances. Mesophilic bacterial abundance clearly decreased during thermophilic stage at day 11 and thermophilic abundance clearly increased and the reverse in abundances occurred at day 33 and decreased further at day 48 (Figures 12 and 13, respectfully). During the second mesophilic phase (day 33 and 48), as temperature drops, mesophilic bacteria increase in abundance (Figure 12) since the temperature was favorable for their growth while thermophilic bacteria decrease (Figure 13). There is also a significant interaction between time and treatment for C:N ratio.

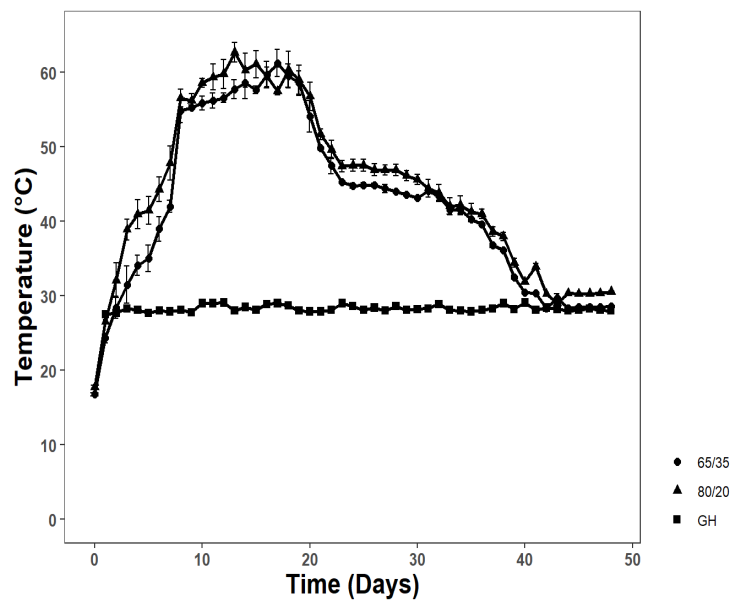


Figure 10. Temperature profile (mean \pm standard error) over time in a 48-day in-vessel composting experiment evaluating 65:35 and 80:20 post-consumer food waste:wood chip bulking agent (mass:mass) treatments ($n = 3$) in food-grade lined steel (208-L) barrels maintained in a greenhouse and filled at 70% capacity rotated and aerated daily.

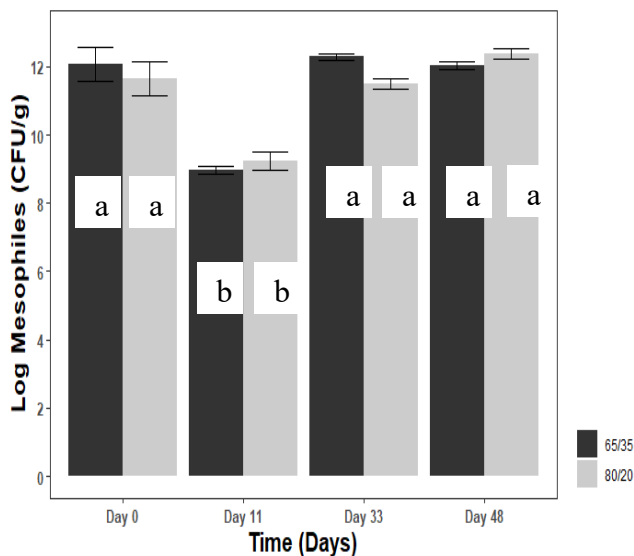


Figure 11. Mesophilic bacteria (mean \pm standard error) over time in a 48-day in-vessel composting experiment evaluating 65:35, and 80:20 post-consumer food waste:wood chip bulking agent (mass:mass) treatments ($n = 3$) in food-grade lined steel (208-L) barrels maintained in a greenhouse and filled at 70% capacity rotated and aerated daily. Time and treatments followed by the same letter are not significantly different ($P < 0.1$).

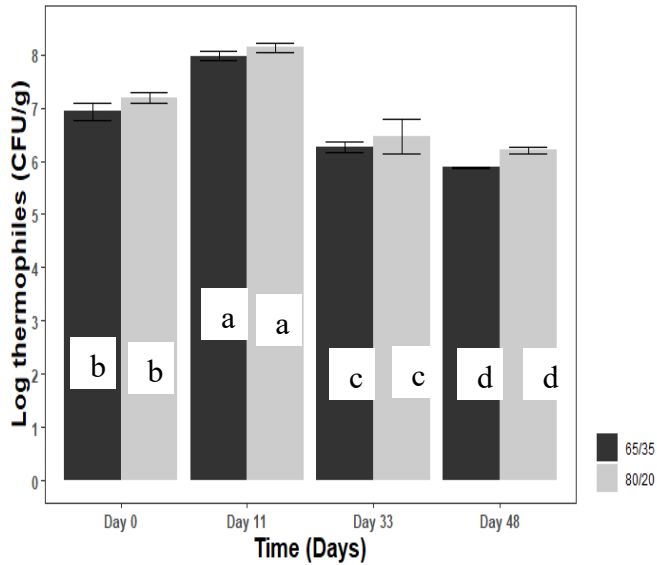


Figure 12. Thermophilic bacteria (mean \pm standard error) over time in a 48-day in-vessel composting experiment evaluating 65:35, and 80:20 post-consumer food waste:wood chip bulking agent (mass:mass) treatments ($n = 3$) in food-grade lined steel (208-L) barrels maintained in a greenhouse and filled at 70% capacity rotated and aerated daily. Time and treatments followed by the same letter are not significantly different ($P < 0.1$).

At the beginning of the experiment, in the early mesophilic and thermophilic phase, there was less β -glucosidase activity (Figure 14). On days 33 and 44, β -glucosidase activity increased indicating the potential for greater decomposition of small organic compounds after the effect of high temperature. Furthermore, treatment significantly impacted β -glucosidase activity where enzyme activity was greater in the 80:20 compared to the 65:35 post-consumer food waste:wood chip bulking agent (mass:mass) treatment.

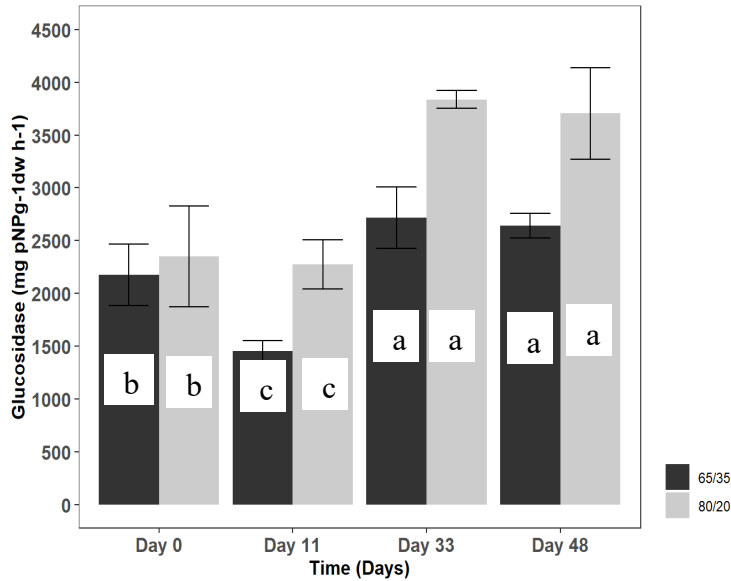


Figure 13. β -Glucosidase activity (mean \pm standard error) over time in a 48-day in-vessel composting experiment evaluating 65:35 and 80:20 post-consumer food waste:wood chip bulking agent (mass:mass) treatments ($n = 3$) in food-grade lined steel (208-L) barrels maintained in a greenhouse and filled at 70% capacity rotated and aerated daily. Time and treatments followed by the same letter are not significantly different ($P < 0.1$).

Water content was higher initially in the 80:20 compared to the 65:35 food waste:bulking agent treatment (Figure 15). Water content was at 64% compared to 61% on day 0 for the treatment 80:20 versus the 65:35 food waste:bulking agent treatment. Water content decreased in both treatments, and it was 56 and 55 % for the 80:20 and 65:35 food waste:bulking agent treatment, respectfully, at the end of the experiment and was different between treatments ($P < 0.05$). The pH (Figure 16) and EC (Figure 17) increased and leveled out during composting.

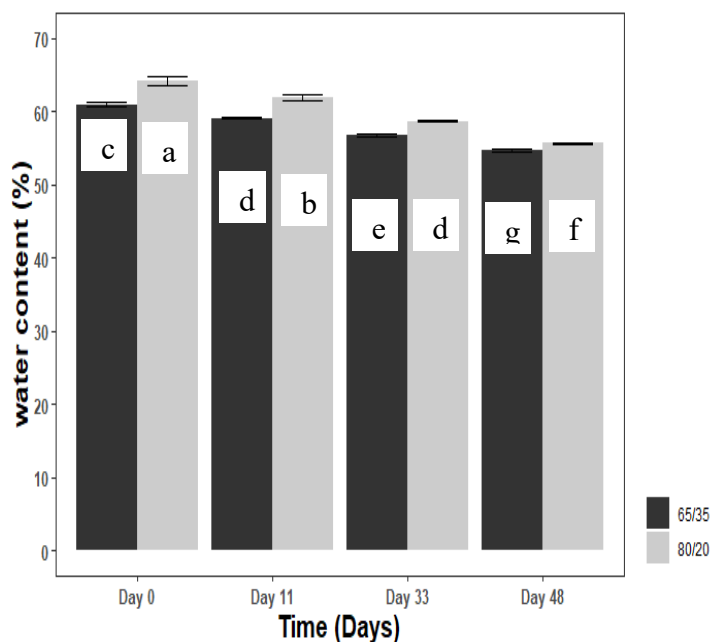


Figure 14. Water content (mean \pm standard error) over time in a 48-day in-vessel composting experiment evaluating 65:35 and 80:20 post-consumer food waste:wood chip bulking agent (mass:mass) treatments ($n = 3$) in food-grade lined steel (208-L) barrels maintained in a greenhouse and filled at 70% capacity rotated and aerated daily. Time and treatments followed by the same letter are not significantly different ($P < 0.1$).

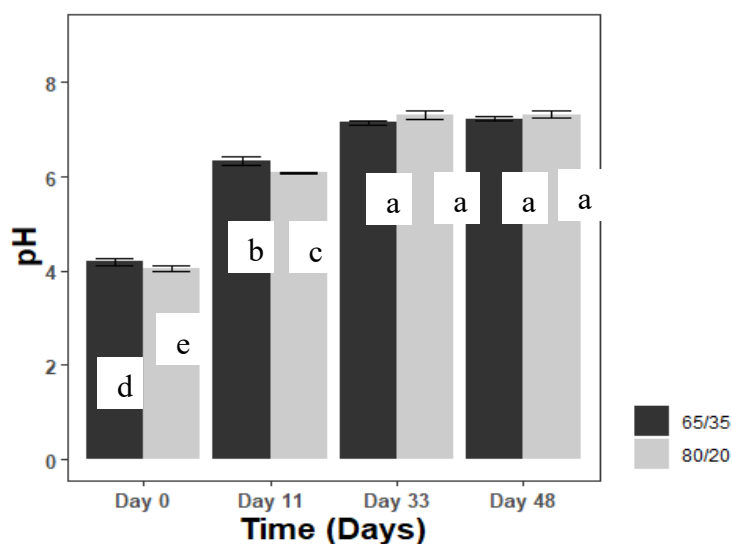


Figure 15. The pH (mean \pm standard error) over time in a 48-day in-vessel composting experiment evaluating 65:35 and 80:20 post-consumer food waste:wood chip bulking agent (mass:mass) treatments ($n = 3$) in food-grade lined steel (208-L) barrels maintained in a greenhouse and filled at 70% capacity rotated and aerated daily. Time and treatments followed by the same letter are not significantly different ($P < 0.1$).

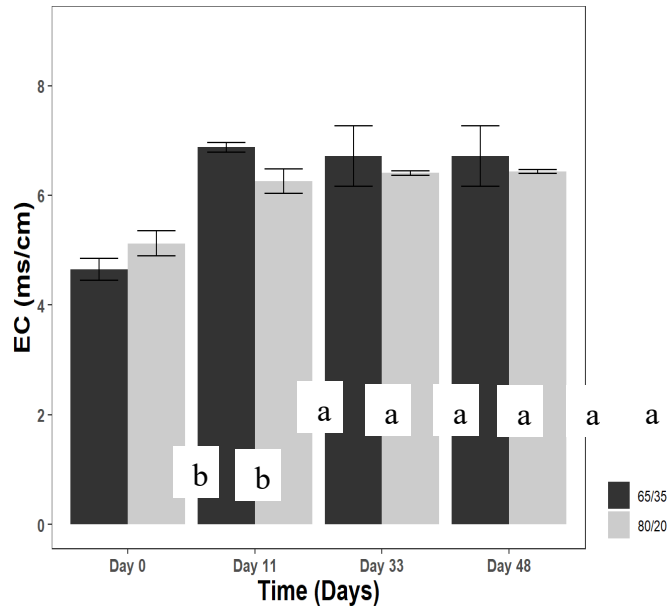


Figure 16. Electrical conductivity (EC) (mean \pm standard error) over time in a 48-day in-vessel composting experiment evaluating 65:35 and 80:20 post-consumer food waste:wood chip bulking agent (mass:mass) treatments ($n = 3$) in food-grade lined steel (208-kg) barrels maintained in a greenhouse and filled at 70% capacity rotated and aerated daily. Time and treatments followed by the same letter are not significantly different ($P < 0.1$).

3.3. Experiment 3

Inoculation with a commercial *Bacillus* species based product was tested to determine if it had a significant effect on the composting process and extended the thermophilic stage. Leachate was observed until day 14. Greenhouse temperature averaged 28 °C, similar to experiment 2 (Figure 18). Compost temperature in the barrels with inoculation reached thermophilic sooner than the control. On day 6, temperature was above 45 °C and was at 47 °C. Also, the temperature lasted longer in the thermophilic stage when inoculated with the *Bacillus* sp. containing product. Temperature stayed above 45 °C until day 23 of the experiment. Furthermore, this was the first experiment where treatment resulted in a significant increase in mesophilic (Table 5, Figure 19) and thermophilic abundances (Figure 20). Interestingly, β -

glucosidase activity was not affected by treatment (Figure 21); whereas, all properties were affected by compost stage as expected (Table 5).

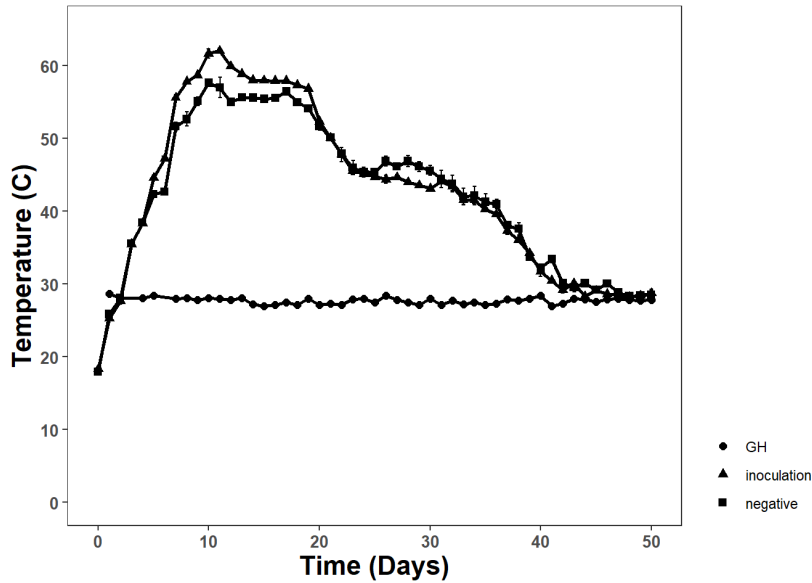


Figure 17. Temperature (mean \pm standard error) over time of a 50-day in-vessel composting experiment of 80:20 post-consumer food waste:wood chip bulking agent (mass:mass) (n = 3) evaluating the effect of inoculation using a commercial *Bacillus* sp. product.

Table 5. Analysis of variance (ANOVA) summary of the effects of treatments, sampling time and their interactions on compost properties of a 50-day in-vessel composting experiment of 80:20 post-consumer food waste:wood chip bulking agent (mass:mass) evaluating inoculation using a commercial *Bacillus* sp. product

| Compost properties | Time | Treatment | Treatment x Time |
|--------------------|---------|-----------|------------------|
| pH | <0.0001 | 0.6644 | 0.6856 |
| EC | <0.0001 | 0.3064 | 0.4744 |
| Thermophiles | <0.0001 | 0.0001 | 0.0294 |
| Mesophiles | <0.0001 | 0.0002 | 0.1143 |
| Glucosidase | <0.0001 | 0.6920 | 0.8196 |
| Water Content | <0.0001 | 0.6168 | 0.9654 |

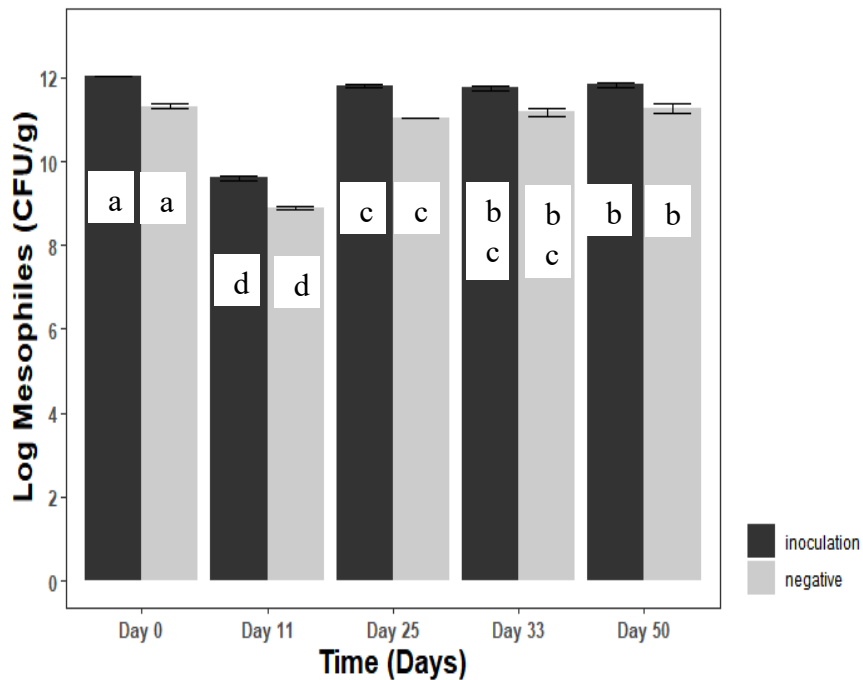


Figure 18. Mesophilic bacteria (mean \pm standard error) over time in a 50-day in-vessel composting experiment of 80:20 post-consumer food waste:wood chip bulking agent (mass:mass) (n = 3) evaluating the effect of inoculation using a commercial *Bacillus* sp. product. Time and treatments followed by the same letter are not significantly different (P < 0.1).

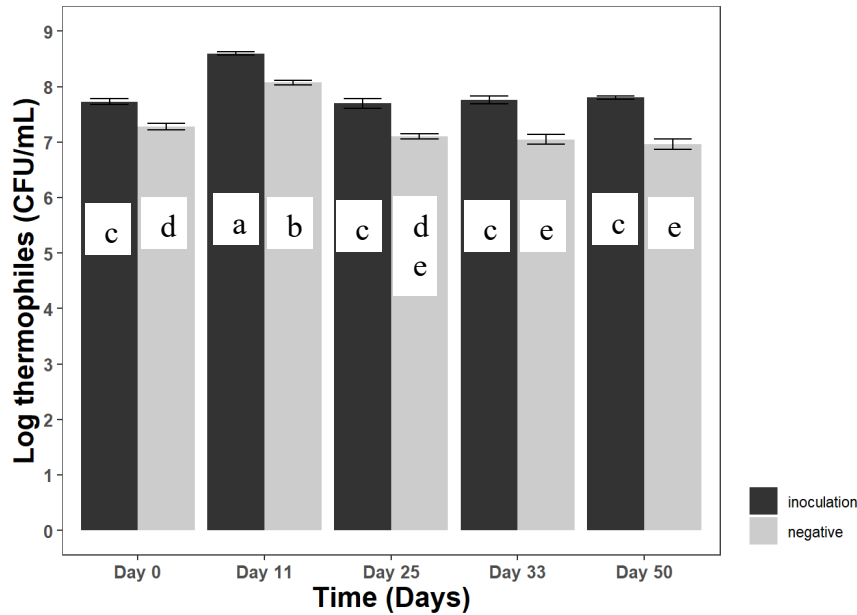


Figure 19. Thermophilic bacteria (mean \pm standard error) over time in a 50-day in-vessel composting experiment of 80:20 post-consumer food waste:wood chip bulking agent (mass:mass) (n = 3) evaluating the effect of inoculation using a commercial *Bacillus* sp. product. Time and treatments followed by the same letter are not significantly different (P < 0.1).

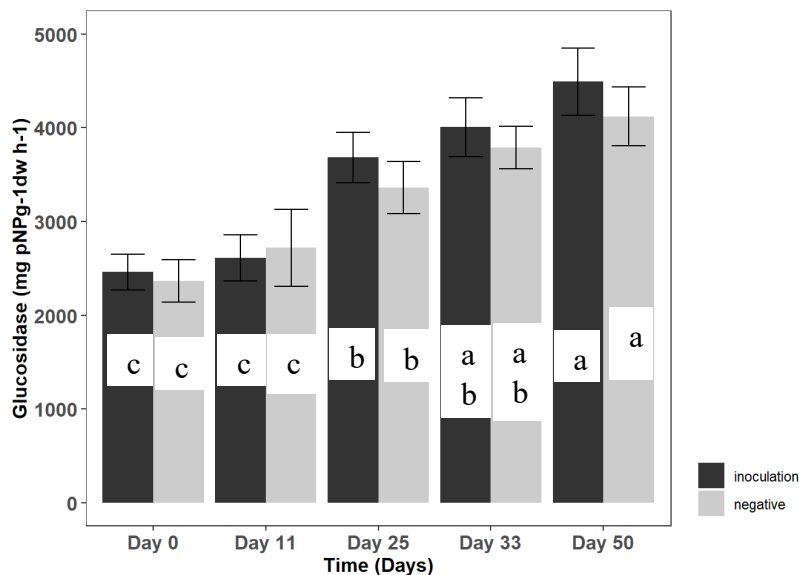


Figure 20. β -Glucosidase activity (mean \pm standard error) over time in a 50-day in-vessel composting experiment of 80:20 post-consumer food waste:wood chip bulking agent (mass:mass) (n = 3) evaluating the effect of inoculation using a commercial *Bacillus* sp. product. Time and treatments followed by the same letter are not significantly different (P < 0.1).

4. Discussion

Results from experiment 1 were not wholly unexpected but suggested that power in the experiment was low from small number of replications and that variability was large. For example, inconsistency in water content was large and resulted in difficulty in distinguishing differences between treatments. Therefore, the experiment was repeated but with three replications, which required dropping a treatment, or deletion of the 50:50 food waste:bulking agent. Overall, temperature profiles and other properties indicated that experiment 2 results were similar to experiment 1, but less variable. Composting proceeded through the three expected stages of the composting process: initial mesophilic, thermophilic, and final curing stages.

Temperature is one of the main indicators of performance of composting process. According to Kumar et al. (2010), temperature directly relates microbial activity with the decomposition of organic matter. The temperature results depicted clearly the three stages of composting during the experiments. During the experiments, temperature did not exceed 65 °C; which was important because temperature above 65°C is considered as too high for efficient composting and could alter, or limit, the process by reducing microbial activity (Valente et al., 2009). Comparable increases of temperature rise have been found in similar sludge-based mixtures in larger composting devices (de Guardia et al., 2008) and in full-scale industrial operations (Jouraiphy et al., 2005).

The raw material used in food waste composting is made of both complex and assimilable molecules. Labile compounds will be decomposed first and will drive the initial, rapid increase in temperature in the compost. Because compost is heaped, whether in a pile outside or in a vessel, the heat released during respiration is trapped within the physical pile and the compost increases in temperature. Mesophilic organisms are active at temperatures between

20 and 45 °C. When the temperature range exceeds 45 °C, then thermophilic organisms will take over and mesophilic organisms will be inhibited. In this second, thermophilic stage (45 – 65°C), most pathogens and weed seeds are destroyed at the elevated temperature range, if the temperature stays elevated for a long enough time period. Temperatures greater than 55°C are especially important to maximize sanitation. However, temperatures in the 45–55°C range helps to extend decomposition, which is important for stabilizing and maturing compost (Stentiford et al., 1996). While thermophilic decomposition is particularly important to accelerate the degradation of proteins, fats, and complex carbohydrates such as hemicellulose and cellulose, curing of the compost includes recolonization by mesophilic organisms after cooling and finalizes decomposition. Final, stable and mature compost has a pH of 6.5-8.0 because organic acids have been mineralized or incorporated with other compounds such as ammonia into humus (Rynk et al, 1992).

Effective composting requires controlled and specific conditions to be uniform. For that reason, the openings on the ends of the vessels remained closed unless during and after sampling for exactly 5 minutes in these experiments. Six rotations were provided every day to allow a considerable and consistent aeration, which was essential for the composting process. With regard to in-vessel composting (in our case a rotary composting), the key function of rotation was to allow the feedstock to breathe by providing oxygen or remain aerobic during respiration. These experiments appeared to be effective because the results were fairly consistent despite having low replicate number, high variability in food waste inputs, and uncontrolled seasonal temperature fluctuations that could influence compost performance.

The general recommendation (USEPA, 2020) for composting is to compile 3:1 nitrogen-rich: carbon-rich organic sources, or “greens-to-browns” as translated at a colloquial level. Given

the variability in potential inputs and conditions, there is much room for variability in results. Thus, in experimental or commercial purposes where more precision and control are desired, I sought to determine if the process was robust or needed to be specific in feedstock ratios given that food waste itself would always be variable in composition. All three experiments proceeded through the mesophilic, thermophilic, and late mesophilic stages in the same time frame and at similar temperatures. In experiment 1, the mesophilic stage started on day 0 persisted until day 7. The thermophilic stage took over and lasted until day 19 and followed with the second mesophilic stage. In experiment 2, thermophilic stage lasted longer; it went from day 7 for the 80:20 treatment and day 8 for 65:35 until day 23 for 65:35 and day 30 for the 80:20 treatment. The longer thermophilic stage resulted in use of the 80:20 treatment for the third inoculation experiment. The third experiment confirmed results and inoculation improved the thermophilic stage. This indicates that conditions are fairly robust and that the more experiments, the better precision with the composting process.

Inoculation significantly affected mesophilic and thermophilic bacteria, both of which likely participated in the decomposition of organic matter and stabilization of a matured and pathogen-free product (compost). To confirm the stability and maturity of the compost, tests of respiration and germination, at the very least, should be conducted on the final compost product. Furthermore, pathogens could be tested for using cultivation-based or PCR analyses.

The early mesophilic stage was minimized in the last experiment. The inoculated vessels had a shorter early mesophilic stage, which was 7 days for the uninoculated control and 6 days for inoculated vessels. Microbial activity likely influenced the temperature differences between experiments during initial establishment and time to thermophilic stage as both mesophilic and thermophilic abundances were increased by inoculation. The microbial abundances between the

inoculated treatment and uninoculated control corresponded to trapped heat released during respiration. Inoculation of microorganisms facilitates composting and enhances the metabolism. Others have found that *Bacillus* spp. contribute positively to composting performance. Hassen et al. (2001) conducted a study in a semi-industrial, pilot-plant, municipal solid waste composting system that had a capacity of 5 tons. *Staphylococci* spp. were dominant during the initial mesophilic and early in the thermophilic phase and *Bacillus* spp. predominated for the duration of the remainder of the composting cycle (Hassen et al., 2001).

Temperature was maintained in thermophilic phase longer in experiment 2 and with inoculation. The initial mixtures largely influence the temperature increase via their nutrient balance and content in easily biodegradable organic fractions (Haug, 1993). In these experiments, temperatures increased and stayed above 45°C for more than the three days recommended for pathogen inhibition (Baby et al., 2005). Tchobanoglous and Kreith (2002) stated that temperature above 55 °C for more than 4 h duration is considered sufficient to kill many pathogens. The US Environmental Protection Agency (USEPA, 2003) requires temperatures above 55 °C must be reached for 3 consecutive days.

While composts are fueled by organic compounds in food, labile compounds do not constitute the majority of compounds present in plants or animals, and thus in foods. Plant based foods such as fruits and vegetables are made up of carbohydrates and fibers while meats and proteins-based foods are made up of roughly 75% water, 20% protein, and 5% fat. Wood chip constituents are mainly carbon (45 to 50% of the total mass), oxygen (40-50%), hydrogen (about 6%), and nitrogen (less than 1%). The carbon comes from the woodchips and the nitrogen from food waste. Addition of the bulking agent is thus an opportunity to both alter and adjust porosity and aeration and adjust carbon and nitrogen contents and C:N of the feedstock to facilitate

processing. In these experiments, despite variability of food waste, the 80:20 food waste:wood chips proportion seemed to optimize enzymatic activity in the first two experiments and microbial abundances in the inoculated experiment.

Microbial decomposition of organic matter relies on various hydrolytic enzymes, and the most important of these are dehydrogenase, cellulase, β -glucosidase, protease, urease, and catalase (Nikaeen et al., 2015). Lignocellulose, one of the most recalcitrant compounds of plants, must be hydrolyzed by enzymes before microorganisms can release and utilize carbon and nitrogen from the compound. β -Glucosidases are enzymes involved in the pathway of decomposition of complex carbohydrates such as starch and glycogen into smaller molecules (monomers). Fungal and actinomycete species degrade lignocellulose fractions as a source of carbon for the production of sugars and other chemicals; however, cellulose has inherent complexity and heterogeneity. Efficient decomposition demands synergy of different types of hydrolytic enzymes after the depletion of easily biodegradable substances. β -Glucosidases are enzymes active in the pathway of cellulose polymer decomposition. Polymers have to be broken down outside of cells. Polymers are decomposed into oligomers, dimers and monomers and then taken into cells for respiration. The biodegradation profiles of the lignocellulosic fractions depend on the activity of the microorganisms present in the composting processes (Wei et al., 2019). Given that thermophiles are competitive for recalcitrant polymers, the thermophilic stage of composting could be particularly important for composting. It may be especially encouraging to measure increased β -glucosidase activity in the final curing stage of composting given that polymers would be decomposed, and the final curing stage is important for fueling microbes and stabilizing compounds that are partially decomposed. It was beyond the scope of the experiment

to relate enzyme activity to extracellular accumulation versus changes in the active and growing microbial community during the stages of composting.

Several studies have related compost stability with enzyme biotransformation on lignocellulosic fractions (Jurado et al., 2015; Villar et al., 2016). In that regard, enzyme dynamics during composting could also be considered as an indicator of and the maturity of final compost (Castaldi et al., 2008). The degradable cellulose content in the mixture may have stimulated microbial growth and enzyme synthesis during the thermophilic stage of composting, which was maintained toward the end of composting. The increased activity that hydrolyzed more organic matter could produce a stable and mature compost in a shorter time period and perhaps lead to more consistent product under repeated applications, explaining the decreased variability observed in results observed in experiments 2 and 3 compared to experiment 1.

Lessons learned from these experiments

There are some key considerations when establishing a vessel compost system. First, the size of the vessel was important. I ran an initial pilot experiment for 30 days using a 19-L vessel (Appendix 1). The maximum temperature reached was 44.7 °C (Table 6, Appendix 1). The thermophilic stage was not reached in the bench-scale vessel. In the 208-L rotary drums, thermophilic stage was reached in three experiments, which were run across seasons (late fall into winter, spring, and fall). Although the first experiment had the least time in thermophilic stage and was run in the winter (October to December), it was not qualitatively different than the other two in terms of results and the process. Thus, the process and end results appear fairly robust. However, the results also indicate that there is room for precision and improvement on the process that can make some subtle but important differences that may become important in a commercial or large-scale operation, or if the end-result depends on consistent properties.

The second lesson learned is the quality of the material used as the vessel matters for heat retention and continual, repeated use. Stainless steel is recommended to increase and maintain temperature. In these experiments, a rotary drum vessel was employed, and the vessel were lined on the interior with a food-grade coating. Temperature fluctuated the most during experiment 1, which was run during the fall into the winter, indicating that the environment could have influenced thermophilic phase. In experiments 2 and 3 that occurred during spring and autumn, temperatures were consistent, and the compost maintained thermophilic stage more consistently across experiments. Greenhouse temperature was not recorded in experiment 1; thus, speculation about the influence of room temperature influence on the vessel composting process would need more testing to verify.

Recommendations

Stability and maturity of compost from these experiments was not confirmed with respiration tests or germination experiments. These tests should be completed with compost from these experiments to verify that achieving a stable, mature compost was produced at the end of the vessel mesophilic stage (days 48-50) after prolonged thermophilic composting where pH increased to between 7-8, EC was stable, and C:N was within acceptable values.

A future experiment should continue to investigate if inoculants of enzymes or effective microorganisms could be used to optimize the thermophilic stage. One could apply a different cellulolytic microbial inoculum such as *Phanerochaete chrysosporium* or *Trichoderma reesei* to accelerate microbial decomposition. Another suggestion is to inoculate mature compost to investigate if compost itself will accelerate microbial degradation and therefore shorten the composting process. Inoculation of available mixtures that are marketed commercially as those have been adopted by commercial composting facilities could be investigated. Timing of

inoculation also could vary. For this experiment, I inoculated compost at initiation, but inoculation could also be done at the end of the mesophilic stage. Other feedstocks or bulking agents could be investigated. A future experiment could also vary the aeration rate. Automated turning of the barrels would improve consistency in the process, save time and labor, and maintain the vessels uniformly for homogenization purposes.

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Chapter 4. Conclusion

In vessel food waste could be composted successfully within 48 to 50 days regardless of the variability in the composition of the food waste. Stability and maturity tests need to be conducted on the final compost. Respiration tests are a common test evaluating overall aerobic activity that assesses stability. Compost release gasses, in particular carbon dioxide during respiration. Measurement of ammonia emission would provide an indication of maturity of the compost. A germination test would evaluate the toxicity risk of the final compost.

The proportions of 80:20 to 65:35 are acceptable proportions of food waste-to-bulking agent to conduct a successful composting experiment. With the proportions 80:20 food waste-to-bulking agent and inoculation, longer thermophilic stages and higher temperature were observed. Inoculation of commercial a *Bacillus* sp. product increased mesophilic and thermophilic bacterial abundances even though they did not have an effect on enzyme activity. Bacteria are, however, expected to create a favorable environment and facilitate decomposition. For future experiments, evaluating inoculation of compost with mature compost is proposed. Another experiment could investigate inoculation during the thermophilic stage as opposed to at initiation of the composting process.

Appendix

1. Initial Bioreactor

Initially, vessels were bench-scale size (19-L) bioreactors (Figure 22). Bench-scale bioreactors (n = 3) modified from parameters recommended for a forced-air vessel by Cornell (Cornell Composting, n.d.) were constructed from 19-L (5-gallon), enclosed, autoclavable Nalgene vessels. The diameter of each vessel was 29.8 cm. The lid contained a capped exhaust pipe and attached turning fork. Two sampling ports were placed along the side of the vessel and a bottom port drained excess leachate. There were three ports for continuous temperature monitoring by thermocouples (T-PRO K-Type Sheathed Thermocouple 0-1000°C, R1/8 screw thread, Thermocouple Plug with 2M/6.6Ft Compensating lead wire) inserted at different vertical intervals into the compost. The composter had an internal support system to elevate compost above any collection of leachates at the bottom of the vessel.

Initial testing determined that temperature did not rise as needed using vessels with a capacity of 19 L (Table 6). Therefore, rotary drum vessels were constructed from 208-L drums with food-grade, lined interior coating.

Table 6. Temperatures (recorded hourly) for compost in vessels of 19-L capacity for 30 days containing post-consumer food waste:wood chip bulking agent combined at a 4:1 ratio (mass basis). Three conditions were evaluated for the reactors, insulated, 19-L vessel housed within a secondary 250-L container, and a vessel without any insulation (n = 1).

| Reactor condition | Maximum temperature reached (°C, 30-d duration) | Minimum temperature (°C, 30-d duration) | 30-day average (°C) | Standard deviation for 30-day duration for individual hourly measurements |
|---------------------|---|---|---------------------|---|
| Insulated | 47.7 | 15.1 | 34.6 | 6.1 |
| Secondary container | 38.8 | 16.8 | 30.3 | 4.6 |
| Reactor only | 44.7 | 16.8 | 35.7 | 5.3 |

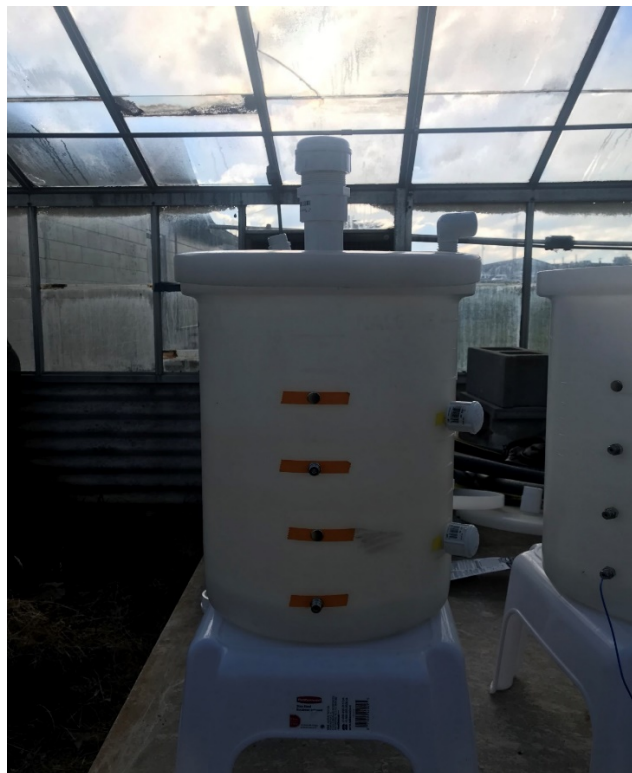


Figure 21. Initial 19-L bench-scale bioreactor.

2. Reference

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<http://compost.css.cornell.edu/microorg.html>