Determining Pathogen Survival and the Factors Affecting their Survival During the Bench-Scale Windrow Co-composting of Biosolids and Leaves

by

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DETERMINING PATHOGEN SURVIVAL AND THE FACTORS AFFECTING THEIR SURVIVAL DURING THE BENCH-SCALE WINDROW CO-COMPOSTING OF BIOSOLIDS AND LEAVES

BY

YAN ZHANG

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MASTER OF SCIENCE

ABSTRACT

Biosolids usually contain a large number of pathogens. This can cause public health problems if pathogens are not brought to certain levels before biosolids disposal. Pathogen destruction can be achieved by composting. The operating biosolids composting facilities have increased from 61 to 228 between 1983 and 1995 in the United States. Both the Canadian Council of Ministers of the Environment (CCME) and the Environmental Protection Agency (EPA) have published standards for final compost quality. Both U.S. EPA standards and CCME guidelines state that: "Using the windrow composting method, the solid waste must attain a temperature of 55°C or greater for at least 15 days during the composting period. Also, during the high temperature period, there will be a minimum of five turnings of the windrow (CCME 1995; U.S. EPA 1994). In their guidelines, pathogen destruction is an important criteria in quantifying finished compost. Coliform organisms have proven to be strong indicators of pathogen inactivation in finished compost.

The objective of this research was to study pathogen survival during bench-scale windrow co-composting of biosolids and leaves. Three experimental phases were done. Phase I was to find out the most suitable method for quantifying coliform organisms in compost. Currently, there are two standard methods available: the Multiple Tube Fermentation (MTF) method and Membrane Filter (MF) method. Time efficiencies, operating costs and the technical feasibility of these two methods were compared. It was found that the MTF method was more suitable. In Phase II, the inactivation of pathogens was studied through the first stage (high-rate phase) and the second stage (curing phase) under different operational parameters, such as temperature, C:N ratio, feedstock component, moisture content, and the ratio of recycled compost to biosolids to leaves. Bench scale (four litre) compost reactors were incubated in an environmental chamber to simulate the core zone of a windrow pile. The temperature in the core zone of a windrow is where temperatures will be at or above the critical temperature for pathogen inactivation (55°C). Under all experimental conditions, the final product pathogen criteria was easily met within a fourteen day retention time in the first stage and no regrowth occurred within the second stage. Once the pathogen level was under detectable limits, no regrowth was found during the second stage. For Phase III, the microbial activity and temperature were compared to see which plays a more substantial role on pathogen inactivation. Preliminary results suggest that temperature was the most important factor, and should therefore be carefully controlled during the windrow composting process.

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NOMENCLATURE AND ABBREVIATIONS

AAFC	--	Agriculture and Agri-Food Canada
BGB	--	Brilliant Green Broth 2%
BNQ	--	*Bureau de Normalisation du Quebec*
BVS	--	biodegradable volatile solids
°C	--	degree centigrade
CCME	--	Canadian Council of Ministers of Environment
cm	--	centimetre(s)
ds	--	dry solids
E.C.	--	*E. Coli*
FC	--	fecal coliform
g	--	gram(s)
hp	--	horse power
kg	--	kilogram(s)
kJ	--	kilojoule(s)
L	--	litre(s)
log	--	logarithm
m	--	metre(s)
MF	--	membrane filter
mg	--	milligram(s)
min	--	minute(s)
mL	--	millilitre(s)
MPN	--	Most Probable Number
MTF	--	multiple tube fermentation
N	--	normality
NBVS	--	nonbiodegragable volatile solids

PFU	--	plaque forming unit
psig	--	pounds per square inch gauge
R	--	bioreactor
sp.	--	species
T	--	temperature
TC	--	total coliform
TS	--	total solids
μm	--	micro metre(s)
U.S. EPA	--	U.S. Environmental Protection Agency
VS	--	volatile solids
W	--	watt
WEF	--	Water Environment Federation

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To My Parents

CHAPTER 1

INTRODUCTION

Biosolids is "a primarily organic, solid municipal wastewater treatment product that can be beneficially used" (WEF 1995). Biosolids usually contain large numbers of pathogenic bacteria, protozoa, parasites and viruses (Haug 1993). This can present public health problems if pathogens are not brought to certain levels before disposal. Pathogen destruction in biosolids can be achieved by the windrow composting process.

Both the Canadian Council of Ministers of the Environment (CCME) and the United States Environmental Protection Agency (U.S. EPA) require certain standards for the composting process and for the final compost product. In the finished compost, these agencies require that density of fecal coliform should be less than 1000 MPN g^{-1} dry solids $(ds)^{-1}$ or density of *Salmonella* sp. be less than 3 MPN $(4\ g)^{-1}$ ds^{-1} (CCME 1995; U.S. EPA 1992).

The purpose of this research is to develop the laboratory protocols and groundwork for the University of Manitoba laboratories to study pathogen destruction in windrow co-composting of biosolids and leaves.

Since I was conducting preliminary screening studies, small size reactors were used. To simulate a real windrow pile, the reactors were incubated in an environmental chamber to keep the reactor temperatures above 45°C during the composting process. The temperature in the core zone in a real windrow pile is above 55°C (Hay et al. 1985). Coliform have proven to be a good indicator for pathogen inactivation in previous studies (U.S. EPA 1985). In this study, coliform were also selected as indicator organisms.

There are three objectives in this research. The first objective was to find out the most suitable and efficient method for quantifying total and fecal coliform (TC and FC) in compost. The second objective was to demonstrate the pathogen destruction under different operational parameters. The third objective was to find which factors more responsible for pathogen destruction, temperature or microbial activity.

In order to achieve the three objectives, three experimental phases were done. In experimental Phase I, the Multiple Tube Fermentation (MTF) method and the Membrane Filter (MF) method were compared by the time efficiency, costs, and technical feasibility.

In experimental Phase II, pathogen inactivation was observed during bench-scale co-composting of biosolids and leaves under different operational parameters. These operational parameters include chamber temperature, compost mass, C:N ratio, moisture content and the ratio of recycled compost to biosolids to leaves.

In an effort to study the effect of microbial activity and high temperature on pathogen inactivation, in experimental Phase III, sterilized and non-sterilized recycled compost were used.

An overview of the history and science of composting is presented in Chapter 2. Factors affecting pathogen inactivation, methods of enumerating pathogen contents, and final compost quality guidelines are also discussed in Chapter 2. Experimental materials and methods are presented in Chapter 3. The experimental results and discussion are presented in Chapter 4 and 5, respectively. Conclusions and recommendations are presented in Chapter 6.

CHAPTER 2

COMPOSTING BIOSOLIDS AND PATHOGEN SURVIVAL

This chapter will first give a brief history of the composting process, and then will attempt to answer what biological factors are involved in pathogen inactivation during composting.

Pathogen inactivation in the composting process is a function of temperature and microbial activity. Temperature can be influenced by several parameters including moisture content, amount of recycled compost and aeration. These parameters will be discussed in detail.

2.1 History of Composting

Composting of organic material is a very old practice in the agricultural field and can be traced back several thousand years. However, the conversion of municipal residues into compost for later use in agriculture apparently occurred only in this century. Sir Albert Howard, in the twenties and thirties in India, developed the Indor-system of composting (Strauch 1983). In the decades following, the interest concentrated on composting of municipal solid wastes with the occasional addition of biosolids in smaller or larger amounts.

In the early seventies, the idea of composting, in general, had a new upswing, especially composting biosolids. Before that time, composting of municipal refuse with or without biosolids was stagnant. Since it is not possible to consider all the developments and the experiments done over the past two decades, please refer to the summarizing descriptions in the literature (Haug and Davies 1981; Haug 1993; MacGregor et al. 1981).

2.2 The Composting Process

There is no universally accepted definition for composting. In this thesis, the definition by Haug (1993) will be used since this definition is more related to this research. "Composting is the biological decomposition and stabilization of organic substrate, under conditions that allow development of thermophilic temperatures as a result of biologically produced heat, to produce a final product that is stable, free of pathogens and plant seeds, and can be beneficially applied to land" (Haug 1993, page 1).

The final product, known as compost, "is an organic soil conditioner 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" (Haug 1993, page 2).

For a material to reach stabilization, certain requirements should be met. For example, the C:N ratio should be less than 25:1, and the reduction of organic matter must be higher than 60%, by dry weight. A complete description can be found in "The Guidelines for Compost Quality" (CCME 1995). Two major objectives of any composting operation are stabilization of organic materials and destruction of pathogenic or disease-causing organisms.

According to Bishop and Chesbro (1982, page 25), the composting process can be characterized as follows: "1) a rise and fall in temperature; 2) the uptake of oxygen; 3) a change in appearance and odour of the material, i.e. the compost darkens and has an 'earthy odour'; 4) the carbon to nitrogen (C:N) ratio decreases; 5) the pH drops initially followed by an increase; and 6) ammonia is produced."

The temperature elevation is caused by the heat released from organic decomposition during the composting process. The volatile solids (VS) can be divided as biodegradable volatile solids (BVS) and nonbiodegradable volatile solids (NBVS). The BVS are those in the composting process that can be used by microorganisms. The NBVS will not be reduced during composting and will remain in the compost product. The heat will be released during BVS degradation. The heat released during the biodegradation of VS is approximately 23,260 kJ per kilogram volatile solids destroyed (WEF 1995).

Composting can occur either under aerobic or anaerobic conditions. With aerobic composting, the organic substrate decomposes in the presence of oxygen (air). The system is maintained by turning the material at periodic intervals, or by forcing air into the pile with blowers. The final products are carbon dioxide, water, ammonia and heat. The general aerobic transformation of organic substrate can be represented by the following equation (Finstein et al. 1986; Tchobanoglous et al. 1993):

$$\text{Organic matter} + O_2 + \text{nutrients} \overset{\text{bacteria}}{\Longrightarrow} \text{new cells} + \begin{matrix}\text{resistant}\\ \text{organic}\\ \text{matter}\end{matrix} + CO_2 + H_2O + NH_3 + SO_4^{2-} + \ldots + \text{heat} \qquad (2\text{-}1)$$

In the aerobic composting system, aerobic microorganisms require air for respiration. Therefore, materials to be composted must be structurally arranged to allow for air to pass through for oxygen replenishment (Higgin et al. 1981). Organic material with high moisture content needs structure conditioning to maintain enough porosity to assure adequate aeration. This can be accomplished by adding a bulking agent such as leaves, sawdust, and woodchips. Adding a bulking agent can also increase the strength of organic material. Adequate structural strength can provide good porosity and ensure that the feed does not collapse and squeeze out all the voids. Air can be supplied by turning the composting piles periodically.

In contrast, anaerobic composting is the decomposition of organic substrate in the absence of oxygen (air). Metabolic end products of anaerobic decomposition are methane, carbon dioxide, and numerous low molecular weight intermediates such as organic acids and alcohols. The general anaerobic transformation of organic substrate can be described by the following equation (Tchobanoglous et al. 1993):

$$\text{Organic matter} + H_2O + \text{nutrients} \xrightarrow{\text{bacteria}} \text{new cells} + \text{resistant organic matter} + CO_2 + CH_4 + NH_3 + H_2S + \text{heat} \qquad (2\text{-}2)$$

Compared to the anaerobic process, aerobic composting produces less odour since less odourants, such as ammonia and hydrogen sulphide, are produced. Furthermore, internal temperature levels seem to be sufficient for pathogen destruction. For these reasons, the aerobic composting process was chosen for this research. At present, there are three primary aerobic composting methods in use: the windrow method, the aerated static pile method, and the in-vessel method.

The windrow composting process is the oldest composting method. In this process, substrate is mixed with previously composted material and/or other bulking agents, such as leaves, sawdust, or woodchips, to lower the moisture content, adjust the C:N ratio, and increase the substrate strength. The material is then stacked in long, low parallel rows, typically 1.8 to 2.1 m high by 4.3 to 4.9 m wide. The piles are then turned frequently by mechanical equipment to provide needed aeration. The typical composting time for the windrow system is 3 to 4 weeks, during most of the year, however, this may need to be extended during periods of adverse weather, such as rain and cold temperatures. After the turning period, the compost is allowed to cure for an additional three to four weeks (without turning). During this curing period, residual decomposable organic materials are further reduced by fungi and actinomycetes (Benedict and Epstein 1987; Bishop and Chesbro 1982; Haug 1993; Iacoboni et al. 1984; Tchobanoglous et al. 1993; U.S. EPA 1985). The flow diagram of the windrow composting process is presented in Figure 2-1.

Compared to the aerated static pile and the in-vessel composting systems, the windrow composting system needs relatively low capital costs (U.S. EPA 1985). Materials required for windrow composting are a pad for the piles, a windrow machine for turning, and generally, a front-end loader. The other composting processes need much more equipment, such as blowers and water traps for the aerated static pile method, and reactors for the in-vessel method. One disadvantage of windrow composting method is that it requires relatively large amounts of land. But since in the prairie region, the population concentration is low and land is available, the windrow composting method was chosen as a case study for this research.

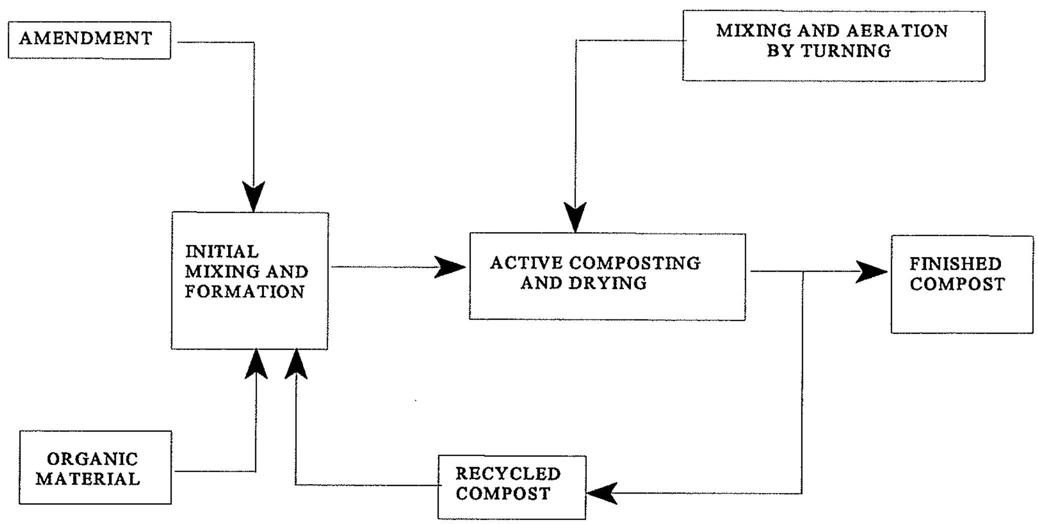

Figure 2-1. Flow Diagram of the Windrow Composting Process (Benedict and Epstein 1987)

Since the windrow composting system was chosen for this research, the other two methods, the aerated static pile and the in-vessel methods, will not be discussed here in detail. For more information about the aerated static pile and the in-vessel methods, please refer to the compilations issued here (Finstein 1980; Haug 1979; 1980; 1993; Tchobanoglous et al. 1993; U.S. EPA 1985; 1989a).

In windrow composting there are two distinct temperature stages. The first stage is the mesophilic stage, with a temperature range from ambient to 42°C (WEF 1995). In this stage, the mesophilic organisms are highly active and little pathogen reduction occurs. The heat produced by the organisms' metabolism causes an increase in the temperature of the compost pile. This rise in temperature stops the mesophilic stage, and the second stage, the thermophilic stage, occurs. In the thermophilic stage, the temperature range is from 42°C to 70°C (WEF 1995). When the temperature reaches the thermophilic range, organism diversity tends to decrease because the mesophilic organisms can no longer thrive. With this, the rate of decomposition of the organic matter decreases and less heat is released. The temperature drops to a more moderate level (around 55°C). Eventually, organism diversity that was lost during periods of elevated temperatures is reestablished (Finstein and Morris 1975).

2.3 Major Operational Parameters in the Windrow Composting Systems

Operational parameters should be selected to optimize conditions within the composting environment. This will insure the greatest degree of microbial activity and subsequent decomposition. Four major variables extrinsic to the microorganisms are: temperature, moisture content, carbon-to-nitrogen ratio (C:N), and aeration or oxygen availability (Bishop and Chesbro 1982; Haug 1993; Iacoboni et al. 1984; Tchobanoglous et al. 1993).

The optimum temperature for a composting system is, at best, a compromise between all the optimum temperatures for all the organisms involved. The activity of an organism is the highest when the temperature is within the optimum range specific to that organism. When temperatures exceed the organism's optimal growth temperature, the organism quickly goes under severe stress. Temperatures slightly below the optimum can generally be tolerated better than temperatures slightly above the optimum. Although thermophilic microorganisms are optimally adapted to grow at temperatures greater than 55°C to 60°C, they have been found to sacrifice some metabolic efficiency in order to do so (Allen and Brock 1968; Brock 1967; Brock T. and Brock M. 1968; McKinley and Vestal 1985). Thus, from a metabolic and efficiency of decomposition standpoint, it is probably not desirable to run any such biological system under such extreme conditions (Finstein 1975, 1980; McKinley and Vestal 1985).

For example, Jeris and Regan (1973) found that microbial activity dropped off sharply at temperatures exceeding 60°C. Subsequently, Suller and Finstein (1977) also found that microbial activity fell off sharply at temperatures greater than 60°C. Much higher microbial activity was found in the lower temperature regions of the compost pile (35°C to 45°C) than in the higher temperatures (65°C to 75°C) (McKinley and Vestal 1985).

It is noted that the cold weather can affect composting operations. Generally, cold weather inhibits microbial activity in the compost pile. It also increases the time necessary for a given pile to reach composting temperatures. But once high temperatures are reached, the cold weather has little impact on the composting process (Olver 1980). To counter the adverse effects of cold ambient temperatures on newly formed composting piles, several techniques have been developed. During the cold winter, the hot compost from a working pile can be used as a bulking agent, and has been found to increase the overall temperature of the compost (Olver 1980). And, having a lower initial moisture content in the feedstock will also be beneficial for initiating the process because high moisture can suppress the temperature rise (Kuhlman 1990).

Moisture influences, and is affected by, numerous aspects of the composting process. For example, water is both required and produced by microbial activity. Also, increase in water content decreases porosity and air diffusivity (less oxygen penetrates into composting piles). Water is removed as vapour by microbial heat generation (Finstein et al. 1987).

The moisture content for optimum microbial activity in a composting systems is between 45% to 65% (WEF 1995). Initial moisture contents above 65% can prevent initiation of microbiological reaction because of poor aeration (WEF 1995). Excess moisture can also cause many problems, such as malodous, clumping during mixing, and difficulties in handling finished product (U.S. EPA 1981). On the other hand, if initial moisture is below 45%, the rate of the microbiological reaction is significantly reduced (WEF 1995).

Nutrient requirements necessary for microbial activity must be present in assimilable forms within the compost material. The most important nutrient balance in the composting process is the carbon-to-nitrogen ratio (C:N). The optimum range for most organic wastes is from 20:1 to 35:1 (Haug 1993; Tchobanoglous et al. 1993; U.S. EPA 1985). If excess carbon is available (a higher C:N ratio), rapid microbial growth occurs. This, in turn, depletes the available nitrogen and temporarily slows down microbial growth. As microbes begin to die, their stored nitrogen becomes available to living microbes and the system is brought back into balance. The result, though, is a slower than optimum growth rate, a loss in heat, and a subsequent lower temperature. This reduces pathogen destruction. On the other hand, low C:N ratios result in a speedier composting process, accompanied by an increased loss of excess nitrogen as ammonia. Since plants depend on nitrogen in the soil for optimal growth, this loss of nitrogen reduces the compost's value as a soil conditioner.

Oxygen is a fundamental requirement for an aerobic composting system. The availability of oxygen to the organisms is crucial to the process. This largely depends on the degree of pore space in the material through which the oxygen can be transferred to the microbes. If the oxygen cannot penetrate the compost mass because of little pore space, anaerobic conditions will develop and aerobic organisms will be succeeded by anaerobes. The oxygen required to decompose compost material is influenced by such factors as temperature, moisture, size of the bacterial population and availability of nutrients, and is therefore difficult to quantify for a given composting system (Bishop and Chesbro 1982).

2.4 Windrow Composting of Biosolids

Biosolids is "a primarily organic, solid municipal wastewater treatment product that can be beneficially used" (WEF 1995). Biosolids contain considerable amounts of human pathogenic organisms. Since the wide variety of human pathogenic organisms contained in biosolids can pose a threat to public health, biosolids need to be treated before disposal. This can be done by biosolids composting. Composting biosolids offers several advantages (Duvoort-van and Coppola 1985; U.S. EPA 1981). These can be summarized as follows:

1. Decomposition by microbes oxidizes the organic material to a fairly stable state within a reasonably short incubation period. In this stable state, the compost produces less odour.

2. During decomposition, the heat produced destroys many of the human pathogens.

3. The moisture content of the biosolids is reduced, facilitating storage and handling operations.

4. Compost, a valuable product when used as a soil conditioner, is a source of macro and micronutrient for plants.

5. Composting uses very little external energy.

Biosolids usually have a low carbon to nitrogen (C:N) ratio. The C:N ratio of digested sludge is 15.7:1 (WEF 1995). On the other hand, the moisture content of biosolids is quite high (moisture content is 75-99%, typically being 80%; Tchobanoglous et al. 1993). Before composting, biosolids need both structure and nutrient conditioning. This can be done by adding organic amendments such as leaves, woodchips, and recycled compost. Leaves, which usually have a relatively high C:N ratio (C:N ratio is 40:1 to 80:1; Tchobanoglous et al. 1993) and a low moisture content (moisture content is 20-40%, and typically 30%; Tchobanoglous et al. 1993), can serve as a supplemental carbon source as well as a bulking agent.

2.5 Pathogen Destruction During Windrow Composting Process

Biosolids retained in the treatment plants are enriched with pathogens. Even anaerobic digestion does not eliminate all of these pathogenic organisms (U.S. EPA 1992). The most important pathogens, present in biosolids along with the diseases they cause, are shown in Table 2-1. As indicated in the table, these pathogens can be separated into four groups: bacteria, enteric viruses, protozoans, and the helminths or intestinal worms. The table is not presented as an exhaustive list of all potentially waterborne pathogens, but it does contain those of greater importance or with a higher frequency of occurrence.

Several species of fungi have been found to populate compost, a few of which can cause pneumonia or bronchial asthma (Burge et al. 1977; Kane and Mullins 1973; Olver 1979). *Aspergillus fumigatus* is one of the most prevalent fungi on earth and is commonly found in woodchips and biosolids at compost plants. Because *Aspergillus fumigatus* is beyond the scope of this research, it will not be studied. For more information about *Aspergillus fumigatus*, please refer to the literatures listed here (Burge et al. 1977; Fergus 1964; Finstein et al. 1987; Haug 1993; Kane and Mullins 1973; Millner et al. 1982; Olver 1979).

Table 2-1. Major Pathogens Found in Biosolids and the Diseases Associated With These Pathogens (Burge 1983; U.S. EPA 1989)

Organisms	Diseases/Symptoms
Bacteria	
Salmonella sp.	Salmonellosis (food poisoning), typhoid fever
Shigella sp.	Bacillary dysentery
Mycobacterium tuberculosis	Pulmonary tuberculosis
Vibrio cholera	Cholera
Protozoa	
Entamoeba histolytica	Amoebic dysentery
Giardia lamblia	Giardiasis (including diarrhea, abdominal cramps)
Enteric viruses	
Poliovirus	Poliomyelitis
Hepatitis A virus	Infectious hepatitis
Echovirus	Meningitis, paralysis, encephalitis, fever, common colds, diarrhea
Coxsackievirus	Meningitis, pneumonic, hepatitis
Helminthic parasites	
Ascaris lumbricoides	Digestive and nutritional disturbances, abdominal pain, vomiting, restlessness
Taenia saginata	Nervousness, insomnia, anorexia, abdominal pain, digestive disturbances
Trichuris trichiura	Abdominal pain, diarrhea, anemia, weight loss
Toxocara canis	Fever, abdominal discomfort, muscle aches, neurological symptoms

Pathogen destruction during the composting process can be brought about by different factors: the release of decomposition products having disinfecting properties (e.g., ammonia), competition with non-pathogenic microbes, depletion of nutrients, high temperatures attained and time (Finstein et al. 1987; Golueke 1982; Pereira-Neto et al. 1987; U.S. EPA 1980; Wiley and Westerberg 1969). "The intensity of the first three factors is presumably directly related to overall microbial activity" (Finstein et al. 1987, page 43).

In general, thermal inactivation is a time-temperature relationship. Such a time-temperature relationship for some common pathogens and parasites are presented in Table 2-2. As indicated by the table, a high temperature for a short period of time or a lower temperature for longer duration can be equally effective. During their research of biosolids composting, Ward and Brandon (1977) also found that over a period of 20 minutes, a temperature of 50°C provided almost no coliform bacteria destruction; a temperature of 52°C provided a reduction of about 1 $\log_{10}$; a temperature of 55°C provided a reduction of about 2 $\log_{10}$; and a temperature of 60°C provided a reduction of about 4 $\log_{10}$. For *Salmonella enteritidis*, a temperature of 50°C for 40 minutes provided a reduction of about 1 $\log_{10}$. A temperature of 52°C for 40 minutes provided a reduction of about 5 $\log_{10}$. During the windrow composting of biosolids, the Joint Water Pollution Control Plant (JWPCP) researchers found that at an internal windrow temperature of 50°C, total coliform can be reduced from above 1,000,000 MPN g^{-1} ds^{-1} to 10 MPN g^{-1} ds^{-1} after seven to twenty-five days (U.S. EPA 1985). At a temperature of 55°C, the required period of time is three to seventeen days.

Table 2-2. Temperature and Time Required for Pathogen Destruction (Gotaas 1956)

	Destruction Temperature and Time			
Organisms	Temp (°C)	Time (min)	Temp (°C)	Time (min)
Salmonella typhosa	55-60	30	60	20
Salmonella sp.	55	60	60	15-20
Shigella sp.	55	60		
Entamoeba histolytica cysts	45	few	55	few second
Taenia	55	few		
Trichinella spiralis larvae	55	quickly	60	few second
Brucella abortis	62.5	3	55	60
Micrococcus pyogenes	50	10		
Streptococcus pyogenes	54	10		
Mycobacterium tuberculosis	66	15-20	67	few
Corynebacterium diphtheriae	55	45		
Necator americanus	45	50		
Ascaris lumbricoides eggs	50	60		
Escherichia coli	55	60	60	15-20

Water also has a role in pathogen destruction. Most pathogens are destroyed at temperatures above 55°C. This destruction is apparently enhanced if the moisture content is sufficient, since moist heat is more lethal than dry heat (Haug 1993).

The best method in determining whether a process is successful in destroying pathogens would be to monitor all the pathogens themselves, but these procedures are too difficult for routine application (Pereira-Neto et al. 1987). To overcome this problem, analyses can be carried out for indicator organisms. These organisms are generally used as a model to express the behaviour of related pathogens under certain conditions.

As indicated by Pike (1983), indicator organisms should have the following properties. They should be:

1. present in composted material in high numbers.
2. a single species or, at least, closely related species with similar resistance.
3. easily detected and enumerated by using simple and reliable standardized methods with reasonable precision and accuracy.
4. slightly more resistant than the pathogens they model.
5. able to model the pathogen regrowth after composting.

These requirements show that indicator organisms must be carefully selected only after studying their survival properties in the composting process. They should be able to demonstrate that the relevant pathogens in composted material have been inactivated. Also, they should be able to demonstrate pathogen regrowth before applying the final product to agricultural land.

For monitoring pathogen reduction in the composting process, coliform organisms are a good indicator organism (U.S. EPA 1985). Researchers conducted several studies to examine the relationship between the survival of the various indicator organisms. Based on analysis of 142 finished compost samples, they found that *Salmonella* sp. are not a good indicator organism. At *Salmonella* sp. levels of higher than 100 MPN g^{-1} ds^{-1}, there is a poor correlation between *Ascaris* ova and *Salmonella* sp. survival. At levels of less than 100 MPN g^{-1} ds^{-1} of *Salmonella* sp., there is a good correlation between *Ascaris* ova and *Salmonella* sp. survival. However, there is still a relatively high probability (15 percent) that *Ascaris* ova will survival.

In another study, based on analysis of 306 finished compost samples, the JWPCP examined total coliform as an indicator that *Salmonella* sp. levels were higher than 1 MPN g^{-1} ds^{-1}. They found that total coliform is an excellent indicator of *Salmonella* sp. (U.S. EPA 1985). Based on analysis of 143 samples, the JWPCP also studied the total coliform versus the probability of *Ascaris* ova survival above 0.5 ova g^{-1} ds^{-1}. The data correlate well at 100,000 MPN g^{-1} ds^{-1} of total coliform, but not at levels above 100,000 MPN g^{-1} ds^{-1} of total coliform. However, the researchers found that when all coliform are killed, the probability of *Ascaris* ova surviving is extremely low (U.S. EPA 1985).

Figure 2-2 presents the results of analyses conducted of 307 compost samples. It shows the relationship between total coliform and the probability of pathogens (*Salmonella* sp., *Ascaris* ova, and virus) being present in a given sample above the limits recommended: < 1 MPN, < 0.5 ova, and < 0.1 PFU per gram dry solids for *Salmonella* sp., *Ascaris* ova, and virus, respectively (U.S. EPA 1985). This figure demonstrates a strong correlation between total coliform and pathogens. When the total coliform count is 500 MPN g^{-1} ds^{-1} or less, there is a better than 90 percent probability that the suggested limits are met. At a density of 10,000 coliform per gram dry solids, there is about 80 percent chance that most pathogens are inactivated. Total coliform counts above 100,000 would indicate a greater than 50% probability of pathogens above the limits. This research indicates that total coliform is a good indicator of overall disinfection. The laboratory tests for coliform yield results in a few days and can be performed inexpensively; therefore, the results can be used for daily quality control (Hay et al. 1985).

Although coliform is a good indicator, there is still a risk that pathogenic bacteria can survive during composting. The sufficient residual population increase in number once conditions become favourable (Bishop and Chesbro 1982).

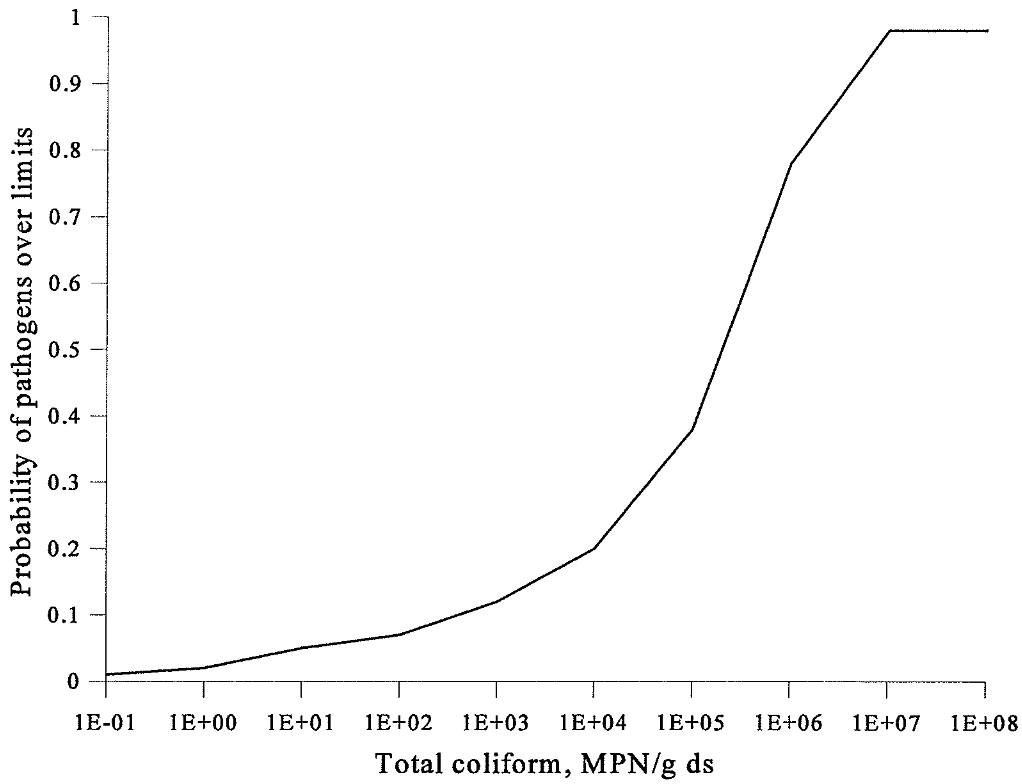

Figure 2-2. Total Coliform as an Indicator of Pathogen Inactivation in Finished Compost (U.S. EPA 1985)

Certain bacteria can regrow in compost once temperatures are reduced to sublethal levels, i.e., mesophilic stage. Poorly stabilized composts have high concentrations of soluble organic nutrients. These free nutrients support pathogen regrowth (Brodie et al. 1994; Hussong et al. 1985). The concentration of these available nutrients declines in well stabilized composts. Therefore, in properly stabilized composts, regrowth of nutrient dependent pathogens should not cause any problems (Inbar et al. 1990).

Hay et al. (1985) has researched on windrow composting of biosolids. Their research consisted of two stages. During stage one, small windrows were constructed and operated for three or four weeks. The windrow piles were turned three to four times per week during stage one. At the end of stage one, large windrows were formed by combining three of the small windrows from stage one. The large windrows were composted for another three weeks. Two kinds of feedstock were used. One of them contained biosolids and sawdust. The other contained biosolids and recycled compost. They found that the temperatures in the small windrows used in stage one did not exceed 55°C. During stage one, the temperatures were 5°C higher in the sawdust windrows than in the recycle windrows. Once the large windrows were formed, a significant temperature increase (above 55°C) occurred. Temperatures in the large sawdust windrows were just above 65°C for 13 days while temperatures in the large recycle windrows were just below 65°C for 13 days. They also studied pathogen inactivation. Coliform was chosen as the indicator organism. It was found that despite low temperatures (below 55°C) in step one, a marked coliform die-off occurred. The initial coliform counts were about 10^8 MPN g^{-1} ds^{-1}. At the of stage one, the coliform counts were reduced to less than 100 MPN g^{-1} ds^{-1}. A faster pathogen inactivation was observed in the small sawdust windrows while there was only a slight difference in temperature elevations and pathogen inactivation between the large sawdust and recycle windrows.

2.6 Standard Test for the Coliform Group

The coliform group consists of several genera of bacteria belonging to the family Enterobacteriaceae. Currently, the standard test for the coliform group may be carried out either by the multiple tube fermentation (MTF) technique, or by the membrane filter (MF) technique.

The multiple tube fermentation (MTF) method was instituted in the 1920s (Edburg S. and Edburg M. 1988) as a routine test for coliform in potable water. The test contains two phases: the presumption phase and the confirmed phase. In the presumption phase, a series of serial dilutions are made. A one mL sample from each of the serial dilutions is transferred to each of five fermentation tubes. These fermentation tubes contain lactose broth and an inverted gas collection tube. These tubes are then incubated at a temperature of 35 ± 0.5°C for 24 hours. The accumulation of gas, in the inverted gas collection tubes, after 24 hours, is considered to be a positive tube. In the confirmed phase, the positive tubes are subcultured into BGB broth and E.C. broth. Fermentation tubes with BGB broth are incubated at a temperature of 35 ± 0.5°C for 24 hours to test for total coliform. Fermentation tubes with E.C. broth are incubated in a water bath at a temperature of 44.5 ± 0.2°C for 24 hours to test for fecal coliform. Tubes with gas produced are considered positive tubes. Concentrations of coliform are reported in terms of the Most Probable Number (MPN) of organisms present. This number, based on certain probability formulas, is an estimate of the mean density of coliform in the sample (Standard Methods 1992). The MPN can be determined using the MPN table (Standard Methods 1992). To complete an entire MTF analysis now requires two to six days. Figure 2-3 (Standard Methods 1992) shows the flow chart for testing total and fecal coliform using the MTF method. In order to get an accurate result, a sample must be adequately shaken before the portions are removed. Shaking distributes the coliform evenly. Otherwise, clumping of bacterial cells can occur, and the MPN value will be an underestimate of the actual bacterial density (Standard Methods 1992).

The membrane filter (MF) technique can also be used to determine the number of coliform organisms. The membrane filter technique was developed in the 1950s (Shipe and Cameron 1954). In this procedure a known volume of water sample is passed through a membrane filter that has a very small pore size (0.45 μm). The bacteria are retained on the filter because they are larger than the pores (Standard Methods 1992). The membrane filter is then placed on the surface of a petri-dish containing Mendo Les agar to test for total coliform, or the M-FC media to test for fecal coliform. The petri dish with Mendo Les agar is incubated at a temperature of 35 $\pm$ 0.5°C for 24 hours. Total coliform shows up as green "sheen" colonies. The petri-dish with M-FC media is incubated in a water bath at a temperature of 44.5 $\pm$ 0.2°C for 24 hours. Fecal coliform shows up as blue colonies. For easy comparison, the flow chart for testing total and fecal coliform by MF technique is presented in Figure 2-4.

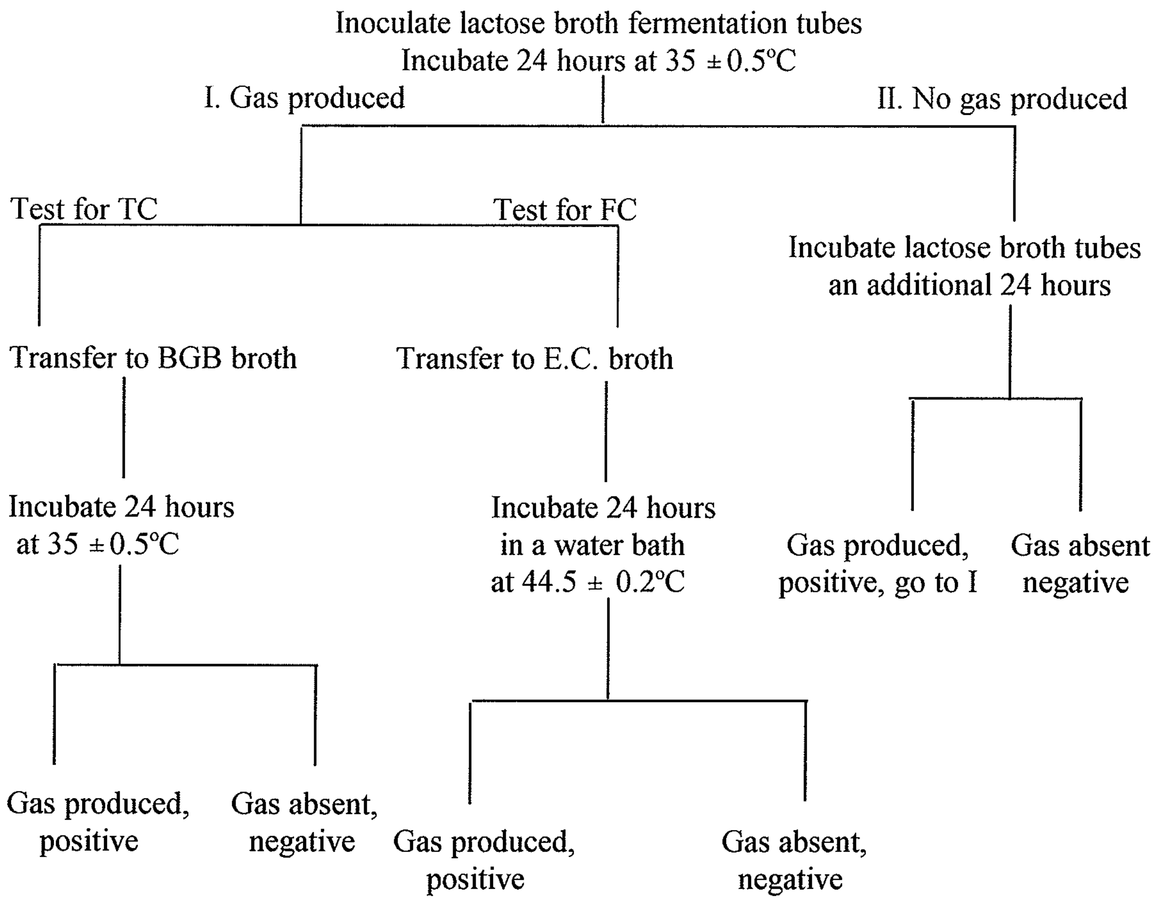

Figure 2-3. Flow Chart for TC/FC Using the MTF Method (Standard Methods 1992)

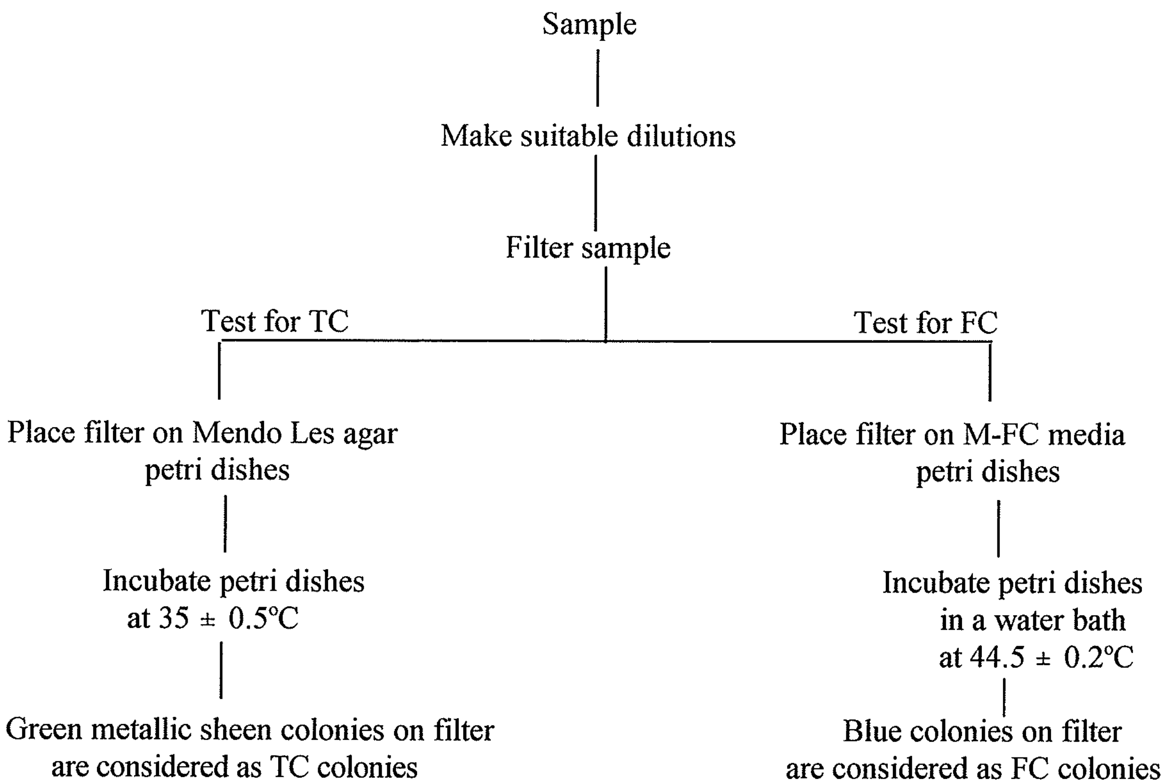

Figure 2-4. Flow Chart for TC/FC Using the MF Method

The membrane filter technique is highly reproducible, can be used to test relatively large volumes of sample, and yields numerical results more rapidly than the multiple tube fermentation procedure. The membrane filter technique is extremely useful in monitoring drinking water and a variety of natural waters. However, when testing waters with high turbidity, the MF technique does not work, since the material does not pass through the filter. In these cases, it would be best advised to use the MTF method (Standard Methods 1992).

2.7 The Composting Process and Final Product Criteria

Regulations restricting the use of biosolids due to pathogen concerns are becoming common worldwide. Recent regulations have been promulgated by the United States Environmental Protection Agency (U.S. EPA) which establish standards for composting (U.S. EPA 1993). In Canada, the Guidelines for Compost Quality is under development through a collaboration between *Bureau de normalisation du Quebec* (BNQ), Agriculture and Agri-Food Canada (AAFC) and the Canadian Council of Ministers of Environment (CCME 1995; Folliet-Hoyte et al. 1994).

There are two requirements in the CCME Guidelines (CCME 1995): the process requirement and the final product requirement. The process requirement for biosolids windrow composting states that: "Using the windrow composting method, the solid waste must attain a temperature of 55°C or greater for at least 15 days during the composting period. Also, during the high temperature period, there will be a minimum of five turnings of the windrow" (CCME 1995). This temperature standards are based on the work by Farrell (1979). Based on a literature review, Farrell (1979) concluded that maintenance of compost at 55°C in equilibrium with an atmosphere at near 100 percent relative humidity for 24 hours would destroy all pathogenic bacteria, viruses and parasites. Therefore, it is necessary to maintain composting temperatures at or above 55°C.

Pathogen destruction is also an important criteria for final compost quality. The final compost quality for indicator microorganisms and pathogens required by the U.S. EPA and CCME along with the recommended limits by Haug (1993) are listed in Table 2-3. The requirement for the final density of fecal coliform is less than 1000 MPN g^{-1} ds^{-1}. As shown in Figure 2-2, there is 90 percent chance that most pathogens are inactivated when total coliform count is 1000 MPN g^{-1} ds^{-1}.

2.8 Summary

Biosolids composting facilities have increased dramatically during the resent years. And Canadian Council of Minister of Environment has developed a new guidelines for composting in 1996. Temperature and pathogen inactivation are important criteria in this guidelines. Previous studies show that pathogen inactivation can be brought about either by temperature or by microbial activity in composting process. And coliform bacteria have proven a good indicator for pathogen inactivation in composting process.

Table 2-3. Comparison of Final Compost Quality Requirements for Indicator Microorganisms and Pathogens

Group/ Microorganism	U.S. EPA (1993) and CCME[1] (1995)	Haug (1993)
Fecal coliform, MPN g^{-1} ds^{-1}	< 1000 MPN g^{-1} ds^{-1}, or	a. Median of all samples < 10 MPN g^{-1} ds^{-1}. b. No more than 20% samples > 1000 MPN g^{-1} ds^{-1}. c. No sample > 10,000 MPN g^{-1} ds^{-1}.
Salmonella sp., MPN g^{-1} ds^{-1}	< 3 MPN $(4g)^{-1}$ ds^{-1}	a. Median of all samples < 1 MPN g^{-1} ds^{-1}. b. No more than 10% samples > 10 MPN g^{-1} ds^{-1}. c. No sample > 100 MPN g^{-1} ds^{-1}.
Ascaris Ova, ova g^{-1} ds^{-1}		No ova minimum detection limit of 0.5 ova g^{-1} ds^{-1}.
Virus, PFU g^{-1} ds^{-1}		No virus minimum detection limit of 0.1 to 0.25 PFU g^{-1} ds^{-1}.

[1]. CCME Guidelines for Category A.

CHAPTER 3

EXPERIMENTAL MATERIALS AND METHODS

This chapter gives a description of the experimental materials and methods. This experiment contained three phases. Phase I was completed to select the total and fecal coliform enumeration method by evaluating the time per analysis and the suitability of the multiple tube fermentation (MTF) method versus the membrane filter (MF) method. Phase II demonstrated the pathogen inactivation during the bench-scale windrow co-composting of biosolids and leaves under various operational parameters. Phase III studied the effect of microbial activity and temperature on pathogen inactivation. The materials and methods used in all three phases will be described separately.

3.1 Experimental Materials

3.1.1 Materials Used in Phase I:

The major materials used in Phase I were compost from a bench-scale breeder and apparatus for MTF and MF tests. The apparatus for MTF and MF tests are listed in Table 3-1.

Table 3-1. Experimental Apparatus for MTF and MF Tests

MTF test	MF test
Incubator (35°C ± 0.5°C)	Incubator (35°C ± 0.5°C)
Water bath (44.5°C ± 0.2°C)	Water bath (44.5°C ± 0.2°C)
Sterilized pipettes	Sterilized pipettes
Moisture balance	Moisture balance
Balance	Balance
Refrigerator	Refrigerator
Commercial blender	Commercial blender
Dilution bottle	Dilution bottle
2 L Mason jar	2 L Mason jar
pH meter	Petri dishes 47 × 12 mm with loose fitting lids
Magnetic stirrer	Petri dishes 47 × 12 mm with tight fitting lids
Durham tubes	Sterilized membrane filter unit (base and funnel)
Cotton ball	Manifold for holding filter unit
Test tube racks	Vacuum pump
Test tubes 18 × 150 mm	Sterilized absorbent pads
Autoclave oven	Forceps
Wooden applicator sticks	Burner
	Sterilized membrane filters (0.45 μm)
	Hot stirrer plate
	Flask

3.1.2 Materials Used in Phase II and Phase III

The major materials used in Phase II and III were experimental apparatus for MTF and MF tests, biosolids, leaves, recycled compost, breeder reactor, bench-scale reactor, and incubator chamber. In Phase III, sterilized recycled compost were used. The experimental apparatus for MTF and MF tests were the same as listed in Table 3-1.

The biosolids were collected from the City of Winnipeg's North End Wastewater Pollution Control Centre. The biosolids are combined primary and secondary biosolids which were anaerobically digested and dewatered (centrifuged). These biosolids were stored in 20 L plastic pails and refrigerated at 4°C to prevent biodegradation.

Leaves were collected from the King's Park's "Leaf It With Us" depot. The leaves were shredded using the Crary Bearcat Model 70530 3 hp gasoline chipper/shredder. Shredding reduced leaf volume by about one third (Hyrich 1995). The shredded leaves were easier to mix with the other components, as compared to unshredded leaves. The shredded leaves were then put into plastic garbage bags and stored at a temperature of 4°C. The recycled compost was fresh compost taken from the breeder reactor.

For Phases II and III, two kinds of reactors were used, a breeder reactor and bench-scale bioreactors. The breeder reactor was a 16 L plastic pail with diameters of 30 cm and 26 cm at the top and bottom, respectively, and a height of 34 cm. Inside the container, a metal screen was placed about 10 cm from the bottom. The screen allowed for added airflow and drainage. For additional airflow, three holes were drilled through the lid.

The windrow bench-scale reactors were 4 L clear plastic containers with lids, with diameters of 18 cm and 14 cm at the top and bottom, respectively, and 21 cm high (see Figure 3-1. For added airflow, four holes were drilled around the reactor, about 5 cm from the bottom. Three additional holes were drilled into the lid. To prevent contamination, the inside walls of the reactors and the lids were cleaned after each cycle. The breeder reactor was four times the size of the windrow bench scale reactor, and therefore the pile size was four times greater. The reactor was simulated to the core zone of a real windrow. The temperature in the core zone is above 55°C (Hay et al. 1985).

To better control the temperature, all bioreactors were placed inside an environmental chamber. A thermostatically controlled heater kept the chamber at the required operating temperature.

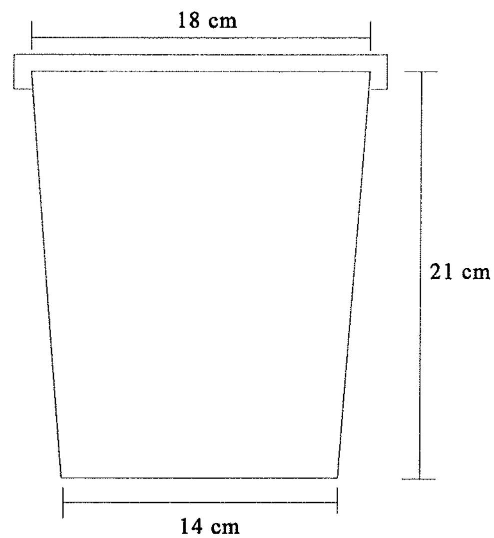

Figure 3-1. Windrow Bench-Scale Reactor

A Cole-Parmer Model 8402-00 Thermistor Thermometer equipped with a YSI reusable temperature probe accurate to ± 0.1°C was used to measure the compost temperature.

The moisture content of the compost mass was analyzed using the CSC Model N026680-1 Moisture Balance. The moisture balance utilizes a 5 gram torsion wire and a 125 W infrared bulb to heat material. Water was released as vapours. Therefore moisture content was calculated based on percent moisture lost. Calculations could be made within a ± 0.2% range. This procedure took approximately 15 minutes.

For sterilizing the recycled compost, a 2 L steel container and a tuttnauer Brinkmann 2540E autoclave oven were used.

3.2 Experimental Methods

3.2.1 Experimental Phase I

In general the MTF and MF tests for total coliform (TC) and fecal coliform (FC) were performed as described in Sections 9221 and 9222, respectively, in Standard Methods (1992), with the following modifications: to prepare the sample, one gram of dry weight compost was combined with 99 mL of buffered dilution water (see Table 3-2 for buffered dilution water preparation) to yield an approximate 10^{-2} dilution. The suspensions were then blended for one minute in a Waring commercial blender. Immediately after mixing, successive dilutions of 10^{-4} and 10^{-6} were made (see Table 3-3 for dilution preparation). Then, the samples were ready for inoculation.

The MTF method has two phases: (1) the presumption phase, and (2) the confirmed phase. For the presumption phase, 25 test tubes containing 10 mL sterilized lactose broth and inverted Durham tubes (see Table 3-3 for lactose broth preparation) were prepared. Then, the lactose broth test tubes were inoculated with diluted samples (10^{-3} to 10^{-7}) (see Table 3-5 for preparing series dilution with lactose broth). Each dilution (10^{-3} to 10^{-7}) was inoculated into five fermentation tubes. Finally, all the fermentation tubes were incubated at 35°C ± 0.5°C. After 24 hours, test tubes with gas collected in the Durham tubes were presumed to have coliform present. Those test tubes with no gas were reincubated and reexamined after another 24 hours. Tubes with gas collected were then considered positive tubes.

For the confirm phase, positive tubes from the presumption phase were subcultured with a wooden stick into sterilized Brilliant Green Broth 2% (BGB) and sterilized E. coli broth (E. C. broth) (see Table 3-4 for BGB broth and E.C. broth preparation). Durham tubes were again used to confirm the presence of coliform. The BGB tubes were incubated at 35°C ± 0.5°C for 24 hours, while the E.C. tubes were incubated in a water bath at 44.5°C ± 0.2°C for 24 hours. Then, the number of positive BGB and E.C. tubes were used to calculate the TC and FC counts, respectively (see Table 3-6 for results calculation). Both TC and FC were expressed as MPN g^{-1} ds^{-1}. Figure 3-2 shows the flow diagram of MTF method used in this research for testing TC and FC.

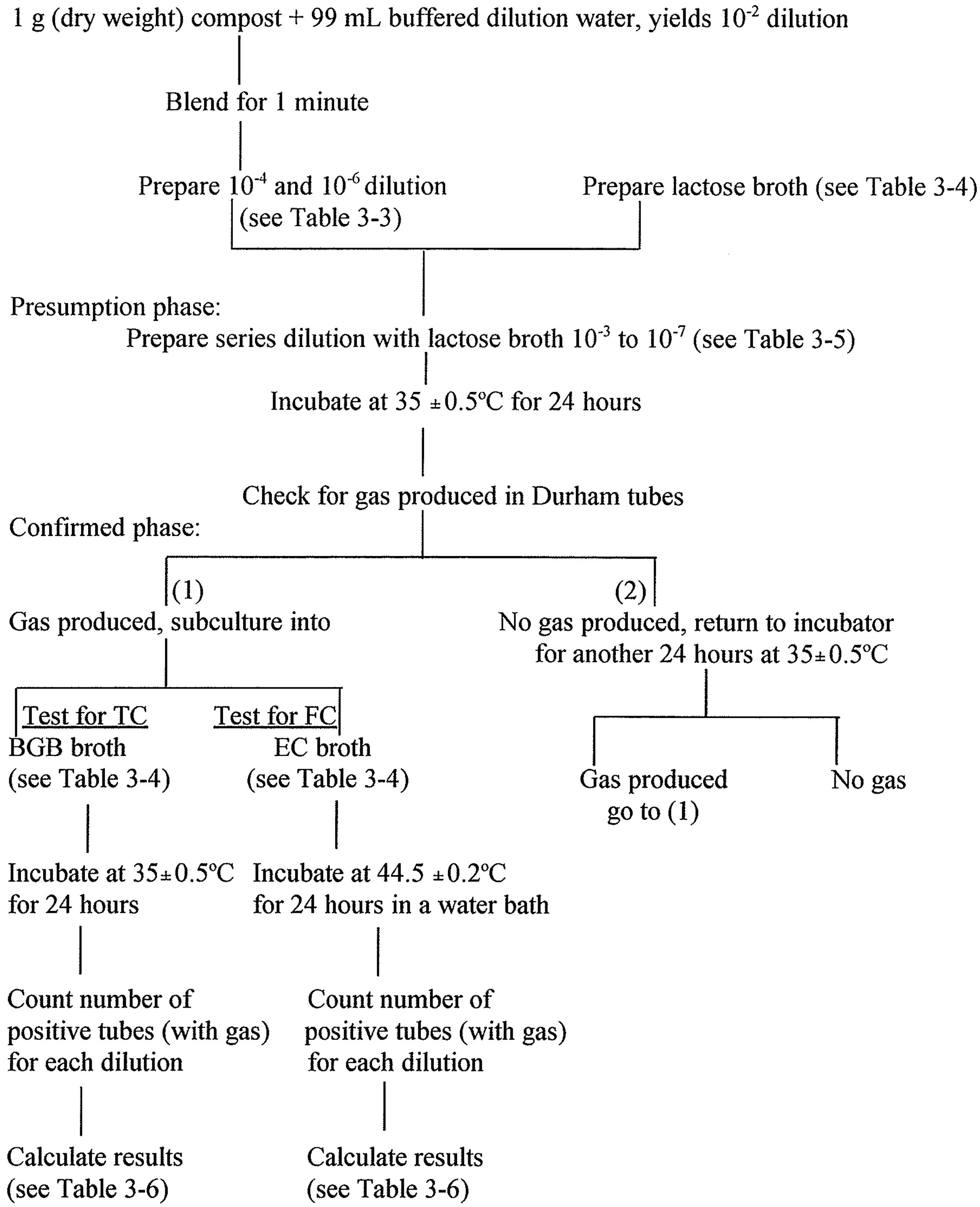

Figure 3-2. Flow Diagram of the MTF Method for Testing TC and FC

Table 3-2. Protocol for Preparing Sterile Buffered Dilution Water (Cadham 1991)

1. Prepare *stock phosphate buffer solution*:

   (i) Add 34.0 grams of potassium dihydrogenphosphate (KH_2PO_4) to 500 mL deionized water.

   (ii) Adjust to pH 7.2 with 1 N NaOH.

   (iii) Dilute to 1 litre with deionized water.

2. Prepare *stock magnesium chloride solution*: Add 81.1 grams of magnesium chloride ($MgCl_2$-$6H_2O$) to 12 litre of deionized water.

3. Prepare *buffered dilution water*: Add 1.25 mL of stock phosphate buffer solution and 5.0 mL of stock magnesium chloride solution to 1 litre of deionized water.

4. Autoclave buffered dilution water for 15 minutes at 121°C under 15 psig of pressure.

Table 3-3. Protocol for Preparing Series Diluted Samples (10^{-2}, 10^{-4}, and 10^{-6})

1. Prepare 10^{-2} dilution: Add 1 g of compost (dry weight) to 99 mL of buffered dilution water.

2. Prepare 10^{-4} dilution: Add 1 mL of 10^{-2} dilution to 99 ml of buffered dilution water.

3. Prepare 10^{-6} dilution: Add 1 mL of 10^{-4} dilution to 99 ml of buffered dilution water.

Table 3-4. Protocol for Preparing Mediums to Test Coliform (TC and FC) Using the MTF Method (Cadham 1991)

Lactose broth (Tests for presence of coliform in the presumption phase):

1. Add 13 g of lactose powder to 1 L of buffered dilution water and mix well.
2. Dispense 10 mL of broth into 18 × 150 mm test tubes with inverted Durham tubes. For one sample, use 25 test tubes, five test tubes per dilution (10^{-3} to 10^{-7}).
3. Close tubes with a cotton ball and sterilize in a autoclave at 121°C for 15 min. at 15 psig of pressure. The pH after sterilization should be 6.9 ± 0.2.

BGB 2% broth (Tests for TC in the confirm phase):

1. Add 40 g of BGB into 1 L of buffered dilution water and mix well.
2. Dispense 10 mL of broth into 18 × 150 mm test tubes with inverted Durham tubes.
3. Close tubes with a cotton ball and sterilize in a autoclave at 121°C for 15 min. at 15 psig of pressure. The pH after sterilization should be 7.2 ± 0.2.

E.C. broth (Tests for FC in the confirm phase):

1. Add 37 g of E.C. broth to 1 L of buffered dilution water and mix well.
2. Dispense 10 mL of broth into 18 × 150 mm test tubes with inverted Durham tubes.
3. Close tubes with a cotton ball and sterilize in a autoclave at 121°C for 15 min. at 15 psig of pressure. The pH after sterilization should be 6.9 ± 0.1.

Table 3-5. Protocol for Preparing Series Dilutions With Lactose Broth (10^{-3} to 10^{-7}) (Cadham 1991)

1. Prepare 25 test tubes containing 10 mL lactose broth (see Table 3-4 for lactose broth preparation).
2. 10^{-3} dilution with lactose broth

   Take five test tubes containing 10 mL lactose broth and pipette 0.1 mL of 10^{-2} dilution sample into each test tube.
3. 10^{-4} dilution with lactose broth

   Take five test tubes containing 10 mL lactose broth and pipette 1 mL of 10^{-4} dilution sample into each test tube.
4. 10^{-5} dilution with lactose broth

   Take five test tubes containing 10 mL lactose broth and pipette 0.1 mL of 10^{-4} dilution sample into each test tube.
5. 10^{-6} dilution with lactose broth

   Take five test tubes containing 10 mL lactose broth and pipette 1 mL of 10^{-6} dilution sample into each test tube.
6. 10^{-7} dilution with lactose broth

   Take five test tubes containing 10 mL lactose broth and pipette 0.1 mL of 10^{-6} dilution sample into each test tube.

Table 3-6. Estimating Coliform Density (MTF Method)

1. Count the number of positive BGB and E.C. test tubes for each dilution (10^{-3} to 10^{-7}).
2. Choose the greatest dilution with the highest positive result and then include the two succeeding dilutions. Please refer to page 9-49 in Standard Methods (1992).
3. Use the results of these three dilutions to estimate the MPN number according to the MPN table (Standard Methods, 1992, page 9-50).

Unlike the MTF method, the MF method has only one phase. Mendo Les agar and M-FC medium (see Table 3-7 for medium preparation) were used to confirm the presence of TC and FC, respectively. The details of the MF procedure used in this research are listed in Table 3-8. All organisms that produced a red colony with metallic sheen in Mendo Les agar were considered members of the TC group. And the organisms that produced a blue colony in M-FC medium were considered members of the FC group. Only the counts between 20 to 300 were considered. Outside this range, the results were considered invalid (Cadham 1991). The coliform density can be computed using Equation 3-1 (Standard Methods 1992). Both TC and FC are expressed as TC or FC colonies g^{-1} ds^{-1}.

$$\text{Coliform colonies } g^{-1}\ ds^{-1} = \frac{\text{coliform colonies counted} \times 100}{\text{g ds sample filtered}} \qquad (3\text{-}1)$$

Table 3-7. Protocol for Preparing Mediums to Test Coliform (TC and FC) Using the MF Method (Cadham 1991)

Mendo Les agar (Test for TC):

1. Suspend 50 g of Mendo Les powder in 1 L of buffered dilution water containing 20 ml of 95% ethanol, and mix well.
2. Heat and frequently agitate until the medium boils.
3. Cool to 45 to 50°C.
4. Dispense 3.5 mL to 47 mm petri dishes.

M-FC medium (Test for FC):

1. Prepare 0.2 N sodium hydroxide (NaOH).
2. Add 1 g of rosolic acid to 100 mL of 0.2 N NaOH, and mix vigorously to dissolve.
3. Suspend 3.7 g of M-FC broth powder in 100 mL of distilled water containing 1 mL of 1% rosolic acid in 0.2 mL NaOH (0.2 N).
4. Heat and gently agitate until the medium boils.
5. Cool to 45 to 50°C.
6. Dispense 0.2 ml of M-FC broth into petri dishes with tight fitting lids, each containing a sterile absorbent pad.

Table 3-8. Procedure for MF Method

Prepare petri dishes:

1. Prepare Mendo Les agar (see Table 3-6) and dispense 3.5 mL into 15 petri dishes.
2. Prepare M-FC medium (see Table 3-6) and dispense 0.2 mL into 15 petri dishes with tight fitting lids, each containing a sterile absorbent pad.
3. Divide each set (Mendo Les agar and M-FC medium) of 15 petri dishes into groups of three (to yield three petri dishes per dilution-10^{-3} to 10^{-7})

Prepare filtration system (see Figure 3-3):

1. Place a membrane filter (0.45 μM) on filter base.
2. Fill funnel three quarters full with buffered dilution water (for example: for a 250 mL size funnel dispense 175 mL of buffered dilution water).

Filter sample through filtration system:

1. Pipette sample from only one of the dilutions (10^{-3} to 10^{-7}) into funnel with water.
   a. For 10^{-3} dilution, pipette 0.1 mL of 10^{-2} diluted sample.
   b. For 10^{-4} dilution, pipette 1 mL of 10^{-4} diluted sample.
   c. For 10^{-5} dilution, pipette 0.1 mL of 10^{-4} diluted sample.
   d. For 10^{-6} dilution, pipette 1 mL of 10^{-6} diluted sample.
   e. For 10^{-7} dilution, pipette 0.1 mL of 10^{-6} diluted sample.
2. Turn filtration system on and wait for the water to run through the system.
3. Turn the system off, remove membrane filter and place it on a petri dish.
4. Clean filtration system with buffered dilution water.
5. Repeat five more times for each dilution for a total of six samples per each dilution-three for the Mendo Les agar and three for the M-FC medium.

Incubate petri dishes and calculate results:

1. (a) Incubate the petri dishes containing Mendo Les agar at 35 ± 0.5°C for 24 hours. (b) Incubate the tightly closed petri dishes (with sterile absorbent pad) containing M-FC medium at 44.5 ± 0.2°C for 24 hours.
2. Count the number of red colonies with a metallic sheen in the Mendo Les agar and count the blue colonies in M-FC medium. Calculate TC and FC using Equation 3-1.

Figure 3-3. Filtration System for MF Method

To evaluate the time needed to implement the MTF and MF methods, records were kept of the time required to: (1) prepare media, (2) do the test, (3) read the results, and (4) clean the test equipment. All tests were done manually.

3.2.2 Experimental Phase II

Biosolids are high in moisture (about 80%) (Tchobanoglous et al. 1993) and bulk density (Haug 1993). All void spaces in the biosolids are occupied by water. Biosolids also have a low carbon to nitrogen ratio (about 15.7:1). Therefore, it requires conditioning before composting. Conditioning was achieved by adding and thoroughly mixing leaves and recycled compost into the biosolids. Thus feedstock was prepared from biosolids, leaves and recycled compost.

Because of the high plasticity and moisture content of the biosolids, and the extremely low bulk density of the leaves, they were very difficult to thoroughly mix together. An electric drill (with an attachment designed for plaster) was used to mix the biosolids, leaves and recycled compost together. This also prevented the biosolids from clumping.

The amount of each feedstock component to be added to the reactor was calculated according to the following equations (Equations 3-2 and 3-3, Haug 1993; Equations 3-4 through 3-6 were derived from Eq. 3-2 and 3-3):

$$W_t M_t = W_b M_b + W_l M_l + W_r M_r \qquad (3\text{-}2)$$

$$R = \frac{W_r}{W_b + W_l} \tag{3-3}$$

where W_t, W_b, W_l and W_r is the total weight (wet weight) of the feedstock, biosolids, leaves and recycled compost, respectively; M_f, M_b, M_l and M_r is the fractional moisture content of the feedstock, biosolids, leaves and recycled compost, respectively; and R is the ratio of recycled compost to biosolids and leaves.

Combining Equations 3-2 and 3-3:

$$W_r = \frac{R\,W_t}{1 + R} \tag{3-4}$$

$$W_l = \frac{W_t (M_b - M_t) + R\,W_t (M_r - M_t)}{(1 + R)(M_b - M_l)} \tag{3-5}$$

$$W_b = W_t - W_r - W_l \quad (3\text{-}6)$$

For example, assume I want to prepare 3 kg of feedstock (W_t) at a moisture content (M_t) of 65%. Given the moisture contents of recycled compost (M_r), biosolids (M_b), and leaves (M_l) were 54%, 80%, and 27%, respectively. The ratio of recycled compost to biosolids and leaves (R) is 0.5:1 (wet weight basis). Using Equation 3-4, we can calculate the wet weight of recycled compost W_r. Using Equation 3-5, we can calculate the wet weight of leaves W_l. After calculating W_r and W_l, we can get the wet weight using Equation 3-6 (for the more detailed calculation, please see Appendix B).

The feedstock in the breeder was kept for 14 days in a wooden chamber at 45°C with a moisture content of 55%. The ratio of biosolids to leaves to recycled compost was 0.46:1.45:1 (dry weight basis). The breeder was turned on day one to ensure no biosolids clumps were remaining. After that, the breeder was turned every three days by hand (with gloves).

Two different chamber temperatures were used. Early research done by McKinley and Vestal (1985) found that much higher microbial activity occurred at temperatures between 35°C to 45°C in the composting pile. While pathogen destruction will occur at temperature above 55°C (WEF 1995). So, one of the chamber temperatures I chose was 52°C, which is slightly below 55°C. The other chamber temperature was 45°C.

In this study, three different moisture contents were selected. According to WEF (1995), the optimum moisture content of composting material is in the range of 45% to 65%. The moisture content in the three reactors was 65% (R65), 55% (R55), and 45% (R45).

When sterilizing the recycled compost, the container filled with recycled compost was placed for 15 minutes inside an autoclave oven (121°C) under 15 psig of pressure (Burton 1995, personal communication). The container was then left at room temperature for one week and autoclaved again for 15 min. at 121°C under 15 psig of pressure. The second autoclaving killed pathogens that may have emerged from spores or cysts.

Three composting cycles were done in this study. In cycle 1, three bench-scale reactors R65-1, R55-1 and R45-1 were fed and operated. The chamber temperature was 52°C. The ratio of biosolids to leaves to recycled compost for R65-1, R55-1 and R45-1 was 0.75:0.57:1, 0.46:1.45:1, and 0.21:2.38:1 (dry weight), respectively.

In cycle 2, three reactors R65-2, R55-2 and R45-2 were operated. The chamber temperature was set at 45°C. The feedstock components and the ratio of biosolids to leaves to recycled compost of R65-2, R55-2 and R45-2 were the same as in cycle 1. The theoretical

C:N ratio of R65-2, R55-2, and R45-2 were 24:1, 34:1, and 43:1 (dry weight basis), respectively.

In cycle 3, only two reactors R55-31 and R55-32 were used. The chamber temperature was 45°C. The ratio of recycled compost to biosolids to leaves of R55-31 and R55-32 was 0.46:1.45:1 and 0.31:0.88:1, respectively.

The composting cycle consisted of two stages: the first stage (high-rate phase) with a fourteen day retention time in the chamber, and the second stage (curing phase) with a ten day retention time at room temperature. The mixtures were turned at day one to reduce the clumping of the biosolids, and after that they were turned every three days for aeration. The densities of TC and FC were measured every two days. The methods quantifying TC and FC were the same as in Phase I.

The temperature of the compost mass was measured daily. The thermometer probe was inserted from the top of the reactor at three different points in the pile and allowed to equilibrate for 5 minutes before reading. The average of the three points was the value used for the compost mass temperature.

The moisture content of the compost mass was measured daily and adjusted if necessary. This was done by adding water when the moisture content was too low according to Equation 3-7:

$$V_{H_2O} = \frac{W_i M_f - W_i M_i}{(1 - M_f)\, r_{H_2O}} \qquad (3\text{-}7)$$

where V_{H_2O} is the volume of water added; W_i is the compost mass (wet weight) before adding water; M_i and M_f are the fractional moisture contents before and after adding water; and r_{H_2O} is the density of water.

Subjective observations of odour intensity were recorded during Phase II-Cycle 2. A descriptive scale of odour intensity from one to ten was prepared, with one being barely noticeable and a value of ten equalling to the odours during the feedstock preparation. The odour intensity was ranked during each mixing event.

A sample for testing moisture content, total solids and volatile solids was collected in the following manner. Immediately after the windrow pile was turned, three samples were randomly grabbed from top, centre and bottom of the pile. These samples were then mixed together, and a representative sample was obtained from this mixture.

The sampling plan is very important when evaluating the biological parameters of compost (Johnson et al. 1993). Sampling for microorganism analyses was similar to solids sampling. Special care was taken during sampling to obtain an uncontaminated sample for microorganism analysis. Plastic bags were first turned inside out, hands were then placed inside the bag, the sample was grabbed and the bag was reversed.

3.2.3 Experimental Phase III

In order to study the influence of microbial activity and temperature on pathogen inactivation, two sets of bioreactors were used. Each set had two reactors. The first set (set 1) was kept at 55% moisture. Reactor RN-55 contained non-sterilized recycled compost while reactor RS-55 contained sterilized recycled compost. The second set (set 2) had 45% moisture. Reactor RN-45 contained non-sterilized recycled compost and reactor RS-45 contained sterilized recycled compost. The theoretical C:N ratio of RN,S-55 and RN,S-45 were 34:1, and 43:1 (dry weight basis), respectively.

Composting is based on the phenomenon of spontaneous (self-initiating) biological self-heating (Haug 1993). The heat, generated from the microbial metabolism of organic material, accumulates in the material, resulting in an overall temperature increase. Due to small windrow piles in the reactors, self-insulation could not be achieved. To minimize heat loss, our laboratory composters were insulated with fibre glass pink insulation. The reactors were kept at room temperature for six days. The four reactors were then transferred to an incubator (45°C) for three days. The mixtures were turned every three days. Moisture content, temperature, and the FC density were monitored daily. Moisture content was calibrated daily. Total solids and volatile solids were also measured at day 0, day 6 and day 9.

Volatile solids (VS) are widely used as a rough measure of organic matter (Standard Methods 1992). Aerobic biological activity decrease the volatile solids content by converting organic carbon to carbon dioxide. For this reason, volatile solids was used to measure the rate of microbial activity during the composting process. The procedure for analyzing the solid samples was taken from Sections 2540B and 2540E in Standard Methods (1992).

CHAPTER 4

EXPERIMENTAL RESULTS

The experimental programs consisted of three phases. All experimental data is contained in the Appendix A. All tables and figures in this chapter were constructed from these results. All phases were conducted using biosolids, shredded leaves and recycled compost. In review, Phase I was a screening experiment, which looked at the two enumeration methods, MTF and MF, for quantifying coliform (TC and FC) in compost. Phase II was to demonstrate pathogen inactivation under various operational parameters during the bench-scale windrow co-composting of biosolids and leaves. Phase III investigated the relative pathogen inactivation potentials of microbial activity and temperature in compost.

4.1 Experimental Results for Phase I

Currently, a standard test for coliform can be carried out either by the multiple-tube fermentation (MTF) technique, or by the membrane filter (MF) technique. In Phase I, I compared the MTF method with the MF method for costs and time efficiency. The costs for MTF and MF methods are presented in Table 4-1. The costs contained the costs for reusable materials and the costs for disposable materials.

Table 4-1. Costs for MTF and MF Methods

MTF Method (per 100 samples)		MF Method (per 100 samples)	
1. Costs for Reusable Materials			
a. Fermentation test tubes	$ 255	a. Forceps	$ 1
b. Durham vials	$ 149	b. Petri dish racks	$ 128
c. Test tube racks	$ 113		
Total:	$ 517	Total:	$ 129
2. Costs for Disposable Materials			
a. Lactose broth	$ 76	a. Mendo Les agar	$ 44
b. BGB broth	$ 119	b. M-FC medium	$ 39
c. E.C. broth	$ 57	c. Petri dish with loose fitting lid	$ 105
d. Cotton ball	$ 20	d. Petri dish with tight fitting lid	$ 98
e. Applicator stick	$ 4	e. Membrane filter	$ 101
		f. Absorbent pads	$ 73
Total:	$ 276	Total:	$ 460

The time it took to complete each procedure was recorded. The results are presented as number of samples per day per person. The working hours per day is eight hours. For MTF and MF methods, I could do six and four samples per day, respectively. All the tests were done manually. The results may be different if part of the tests were done automatically, such as using an automatic media dispenser. In that case, I probably could do ten and eight to nine samples per day, respectively.

4.2 Experimental Results for Phase II

The research of Phase II was to demonstrate the pathogen destruction under various operational parameters during the bench-scale windrow co-composting biosolids and leaves. These parameters were temperature, compost mass, moisture content in compost mass, and the ratio of biosolids to leaves to recycled compost. Three composting cycles were performed in this study. All the compost piles were monitored for temperature, moisture content and density of coliform (both TC and FC). Odour emission was also observed during cycle 2.

4.2.1 Experimental Results for Phase II-Cycle 1

In cycle 1, three reactors R65-1, R55-1, and R45-1 were fed and operated. The ratio of biosolids to leaves to recycled compost of R65-1, R55-1, and R45-1 was 0.71:0.57:1, 0.46:1.45:1, and 0.21:2.38:1 (dry weight basis), respectively. They were incubated at a temperature of 52°C. The temperature and moisture content of the reactors were monitored and recorded daily. The average reactor temperatures of R65-1, R55-1, and R45-1 were

52.8°C, 52.6°C, and 52.5°C, respectively. The results for R65-1, R55-1, and R45-1 are presented in Figures 4-1, 4-2, and 4-3, respectively.

Total and fecal coliform were analyzed as indicator organisms. They were tested every three days. The initial feedstock total coliform counts of R65-1, R55-1, and R45-1 were 1.6×10^7, 8.8×10^6, and 1.6×10^6 MPN g^{-1} ds^{-1}, respectively. At day three, total coliform counts of all three reactors were reduced to less than 200 MPN g^{-1} ds^{-1}. This level of total coliform (< 200 MPN g^{-1} ds^{-1}) was kept to the end of the composting cycle.

The initial feedstock fecal coliform concentrations of R65-1, R55-1, and R45-1 were 1.22×10^6, 7×10^5, and 2.3×10^5 MPN g^{-1} ds^{-1}, respectively. At day three, fecal coliform counts of all three reactors were reduced to less than 200 MPN g^{-1} ds^{-1}. This level of fecal coliform (< 200 MPN g^{-1} ds^{-1}) was kept to the end of the composting cycle.

Regrowth studies of total and fecal coliform was conducted at different moisture contents during the curing stage. The compost mass with moisture contents 65%, 55%, and 45% were routinely analyzed for TC and FC every three days. No coliform (both TC and FC) regrowth occurred regardless of moisture content. The coliform densities in the three reactors were the same. The final concentrations of both TC and FC were less than 200 MPN g^{-1} ds^{-1}.

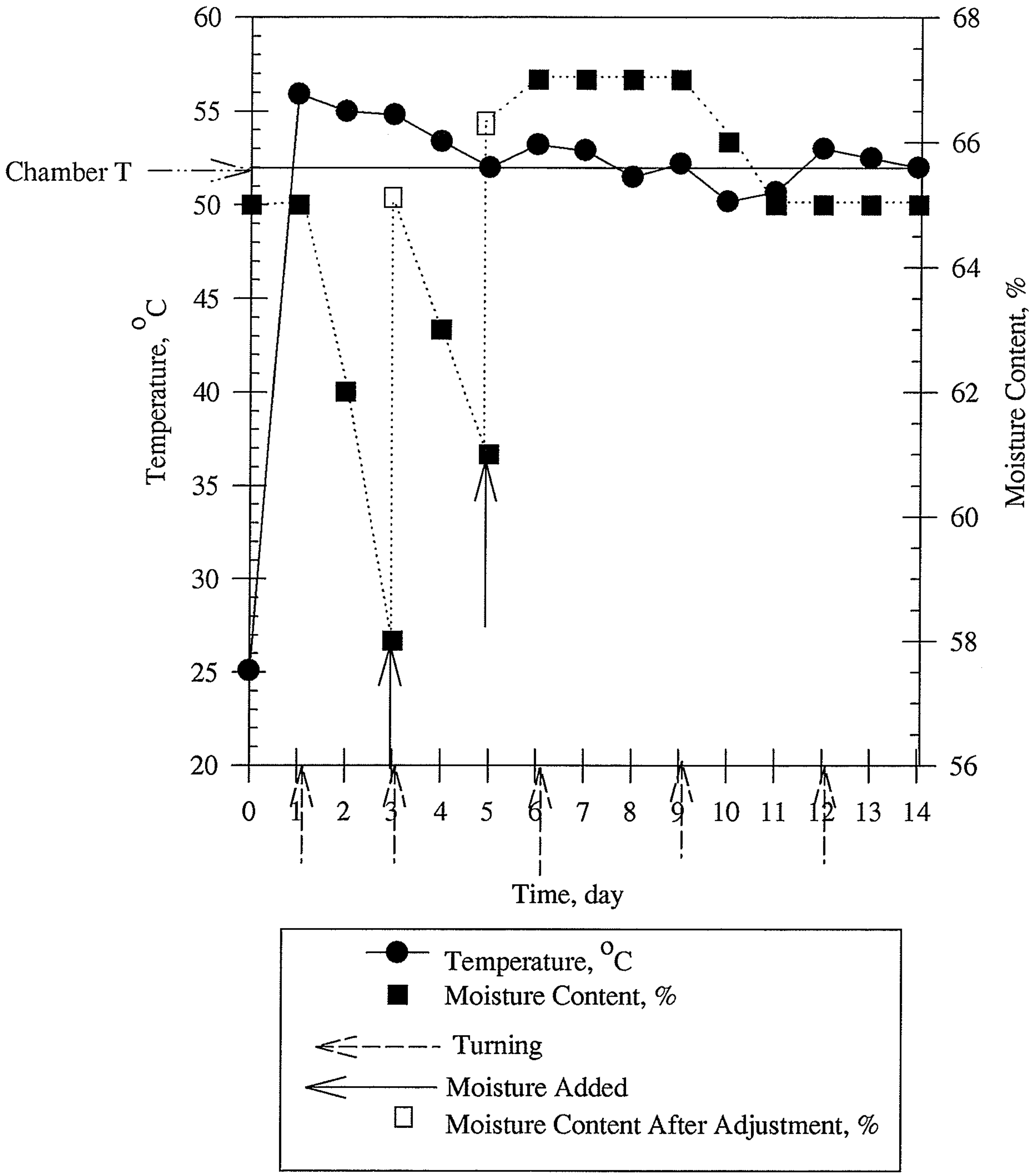

Figure 4-1. Phase II-Cycle 1: Effect of Turning and Moisture Content on Temperature of R65-1

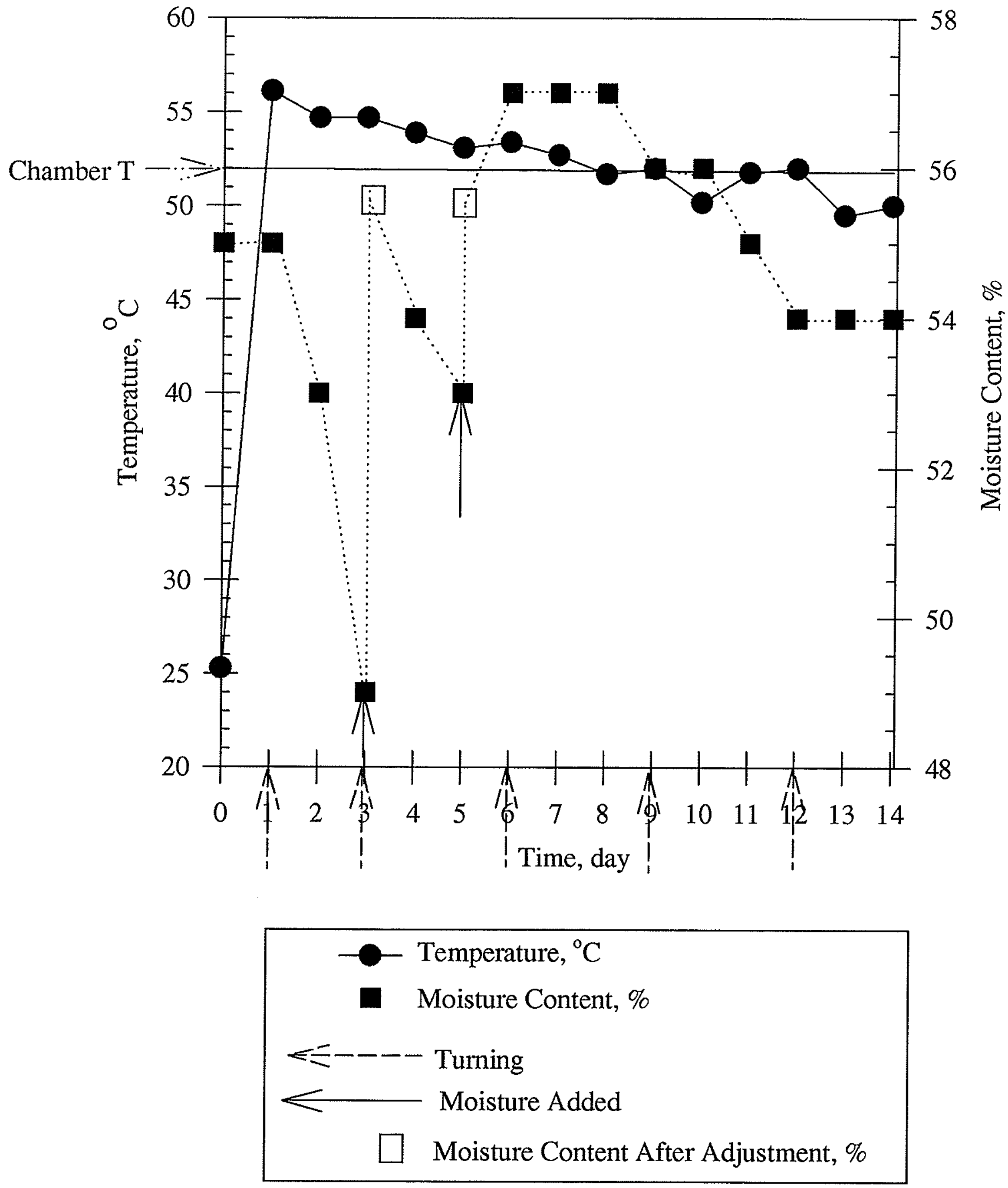

Figure 4-2. Phase II-Cycle 1: Effect of Turning and Moisture Content on Temperature of R55-1

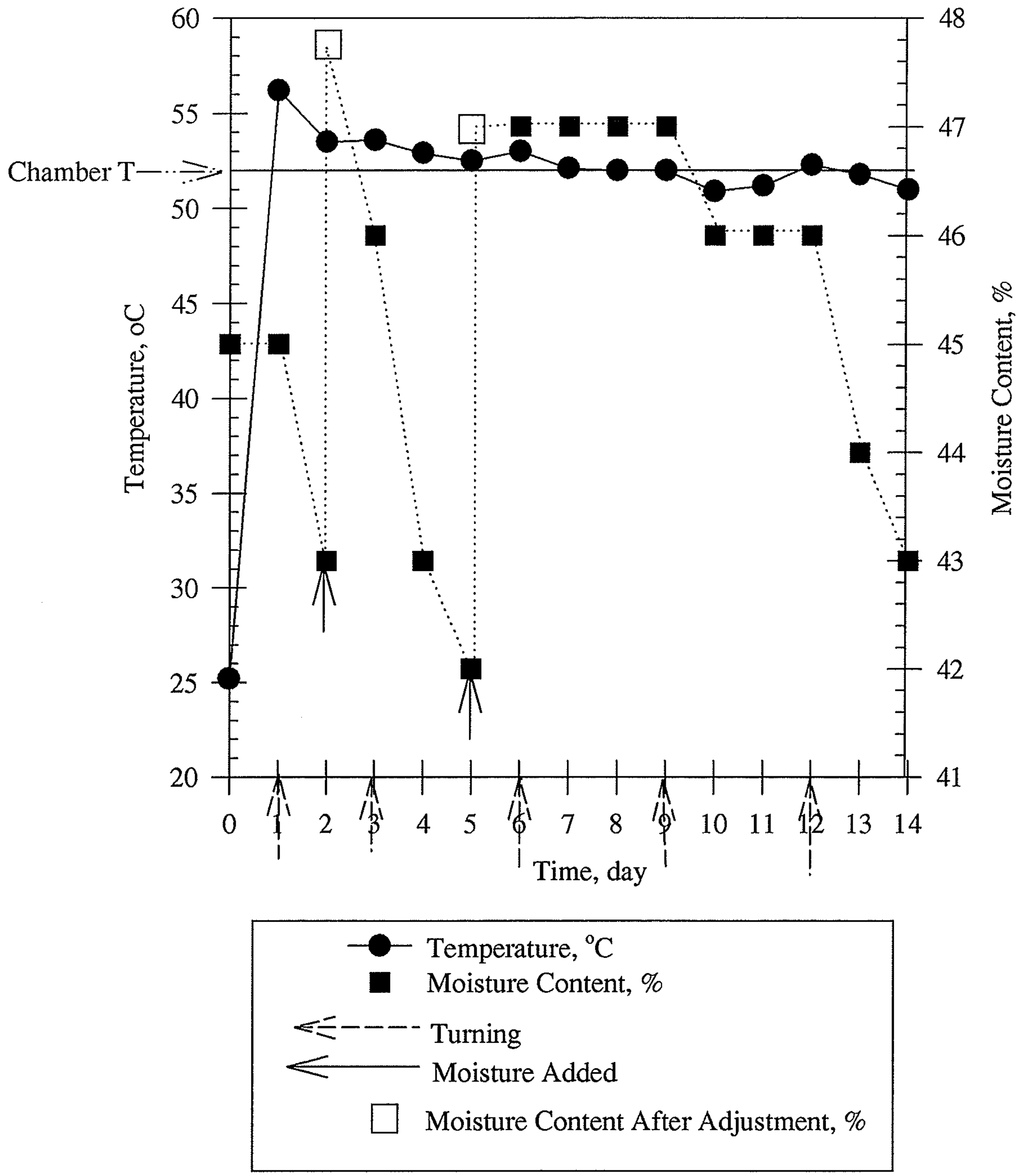

Figure 4-3. Phase II-Cycle 1: Effect of Turning and Moisture Content on Temperature of R45-1

4.2.2 Experimental Results for Phase II-Cycle 2

In cycle 2, three reactors R65-2, R55-2, and R45-2 were operated. The ratio of biosolids to leaves to recycled compost of R65-2, R55-2, and R45-2 was 0.71:0.57:1, 0.46:1.45:1, and 0.21:2.38:1 (dry weight basis), respectively. They were incubated at a temperature of 45°C. The theoretical C:N ratio of R65-2, R55-2, and R45-2 were 24:1, 34:1, and 43:1 (dry weight basis), respectively.

The average reactor temperature of R65-2, R55-2, and R45-2 was 48.9°C, 49.3°C, and 47.9°C, respectively. The initial BVS of R65-2, R55-2, and R45-2 were 0.26 kg, 0.32 kg, and 0.24 kg (dry weight basis), respectively. Coliform were analyzed every two days. The results of R65-2, R55-2, and R45-2 are presented in Figures 4-4, 4-5, and 4-6, respectively.

Based on my personal assessment, the odour emissions during cycle 2 were also observed. Odours from R65-2 were much higher than R55-2 and R45-2. R45-2 had the least odour among the three reactors. The highest odour emissions occurred during the early days of composting in all the three reactors. The odour emissions decreased with the composting time. Also odour emissions increased significantly immediately after turning, but declined to the original level within a few minutes following turning.

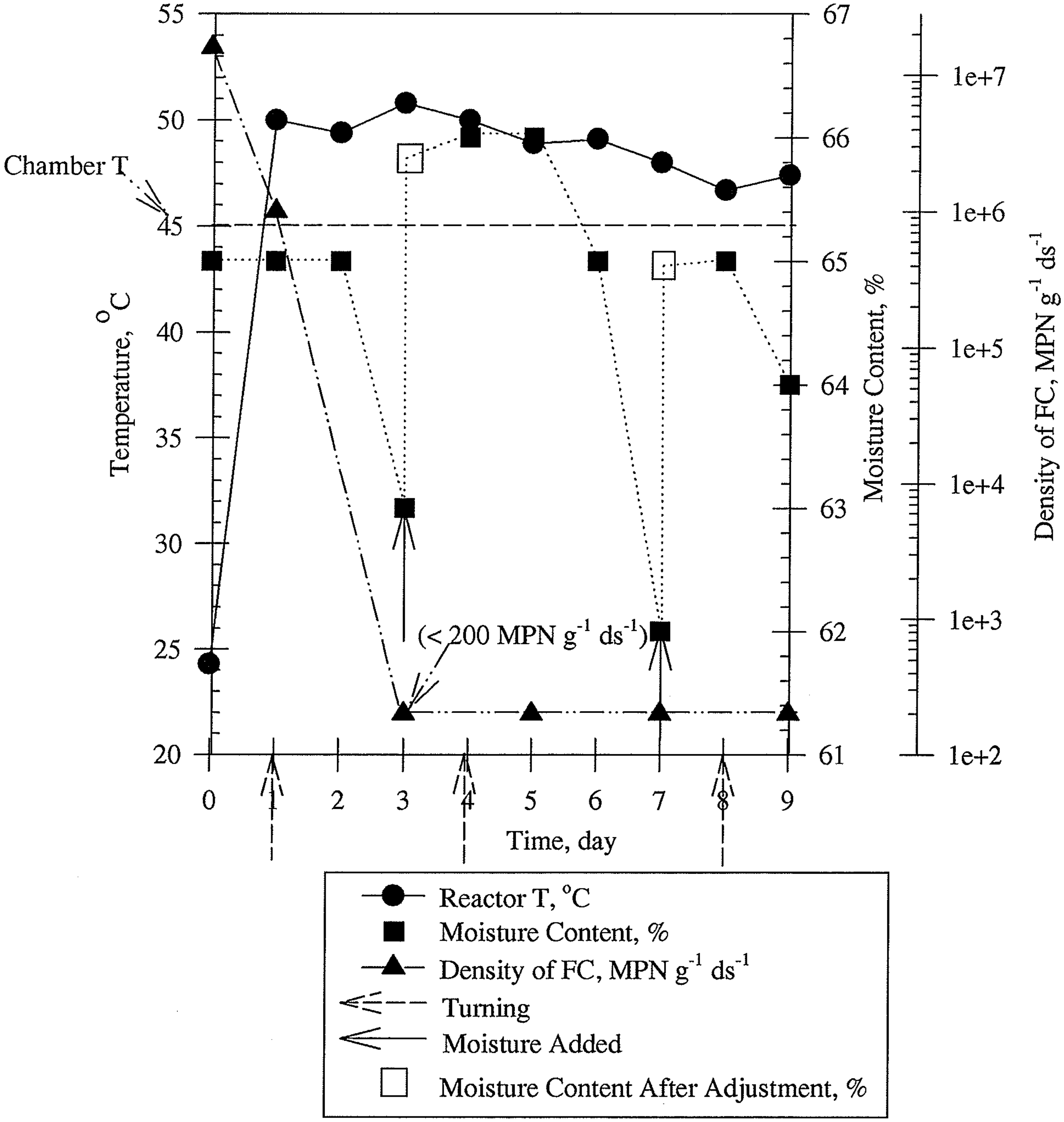

Figure 4-4. Phase II-Cycle 2: Temperature, Moisture Content, and FC Destruction for R65-2

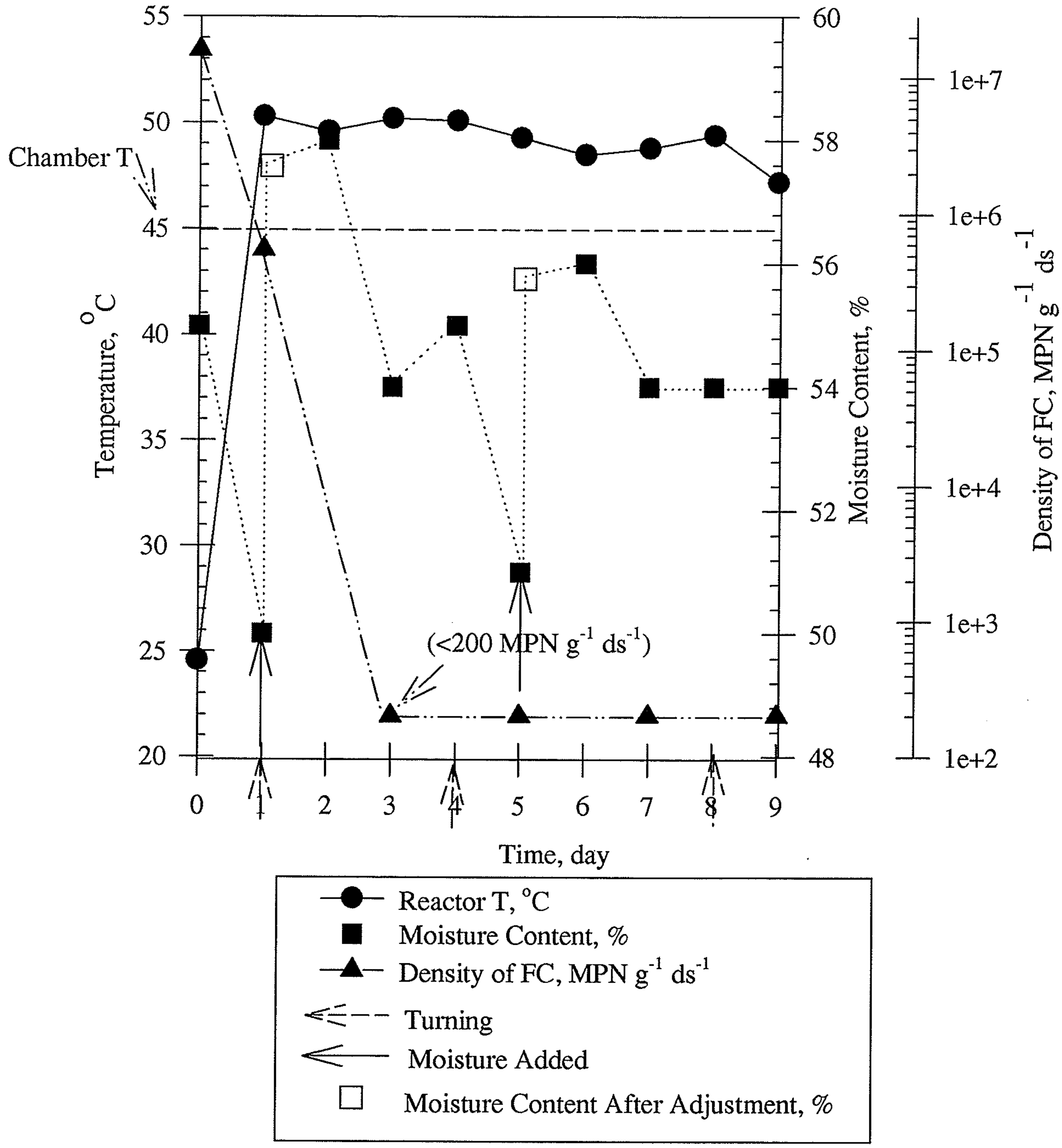

Figure 4-5. Phase II-Cycle 2: Temperature, Moisture Content, and FC Destruction for R55-2

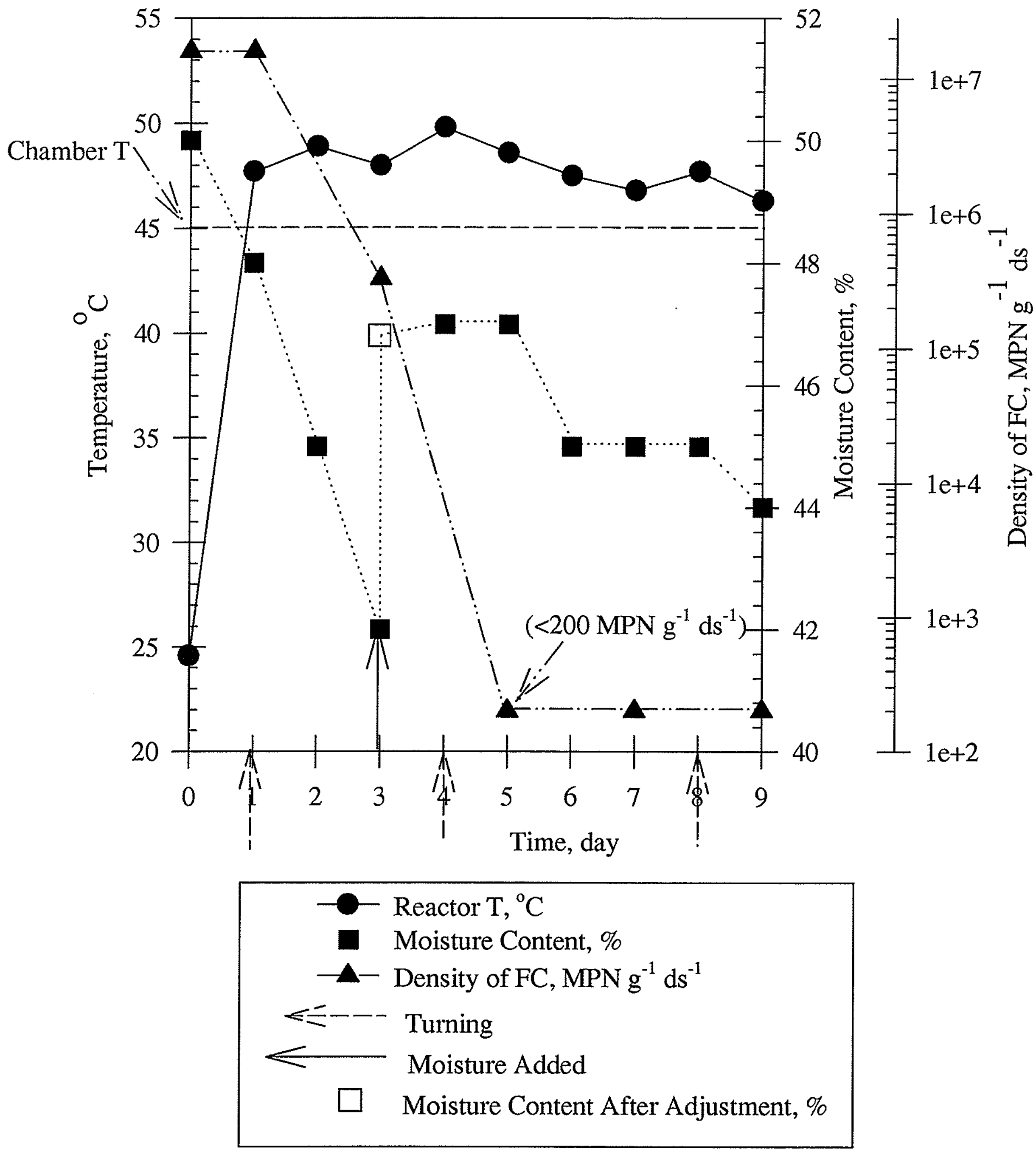

Figure 4-6. Phase II-Cycle 2: Temperature, Moisture Content, and FC Destruction for R45-2

4.2.3 Experimental Results for Phase II-Cycle 3

In cycle 3 two reactors R55-31 and R55-32 were operated. The initial moisture content of both reactors was 55%. The ratio of biosolids to leaves to recycled compost of R55-31 and R55-32 were 0.46:1.45:1 and 0.31:0.88:1 (dry weight), respectively. The initial BVS of R55-31 and R55-32 were 0.32 kg and 0.26 kg (dry weight), respectively. They were incubated at a temperature of 45°C. Temperature and moisture content were measured and recorded daily. The average reactor temperature of R55-31 and R55-32 was 49.3°C and 48.4°C, respectively. The results for R55-31 and R55-32 are presented in Figures 4-8 and 4-9, respectively.

Total and fecal coliform were monitored every two days. The initial feedstock total and fecal coliform counts of both reactors were 1.6×10^7 MPN g^{-1} ds^{-1}. At day one the density of total and fecal coliform in R55-31 was reduced to 1×10^6 MPN g^{-1} ds^{-1} and 5.5×10^5 MPN g^{-1} ds^{-1}, respectively. While the density of total and fecal coliform in R55-32 was kept at 1.6×10^7 MPN g^{-1} ds^{-1}. By day three, the total and fecal coliform concentrations in both reactors were reduced to less than 200 MPN g^{-1} ds^{-1}.

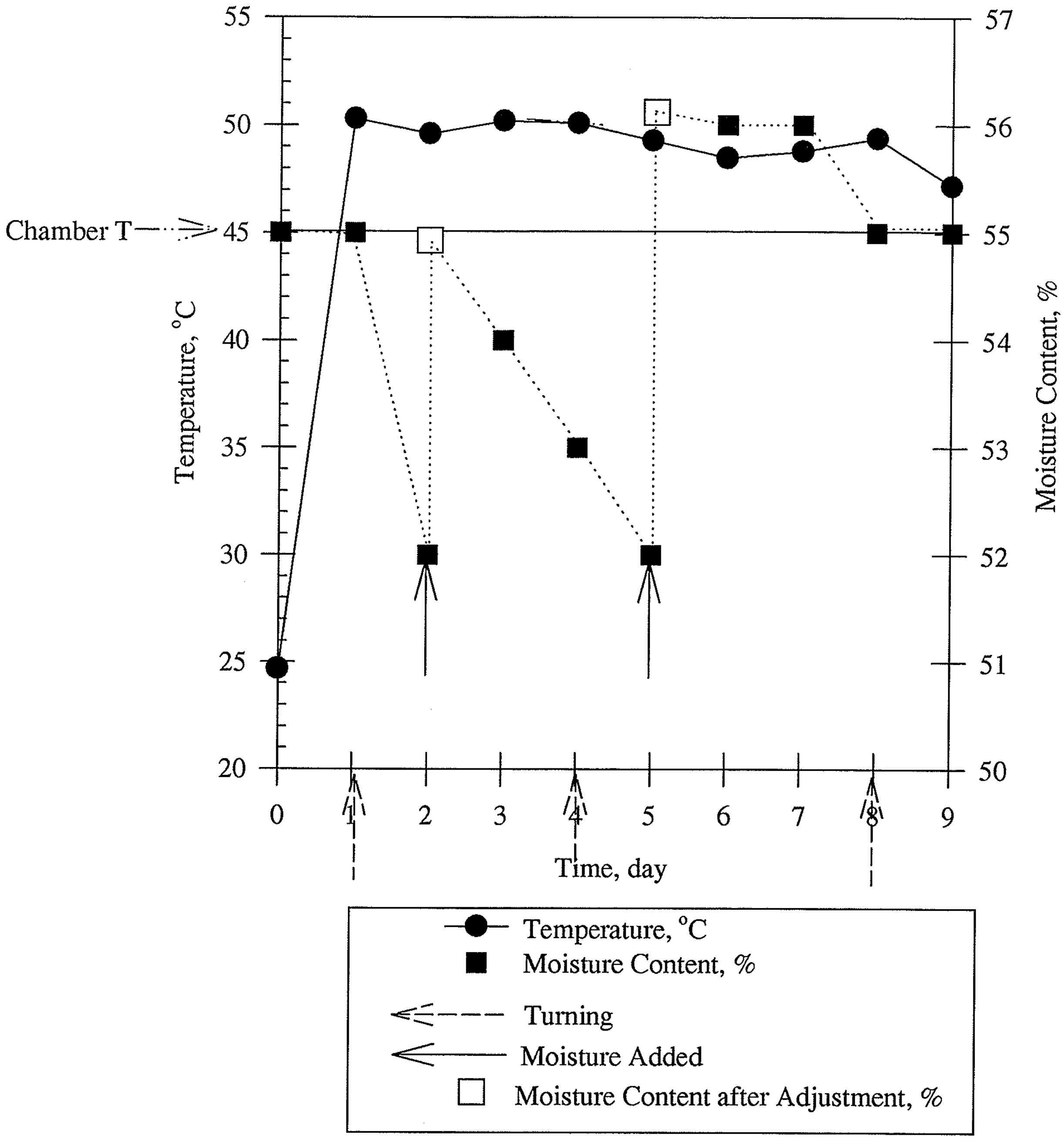

Figure 4-7. Phase II-Cycle 3: Effect of Recycle on Temperature of R55-31

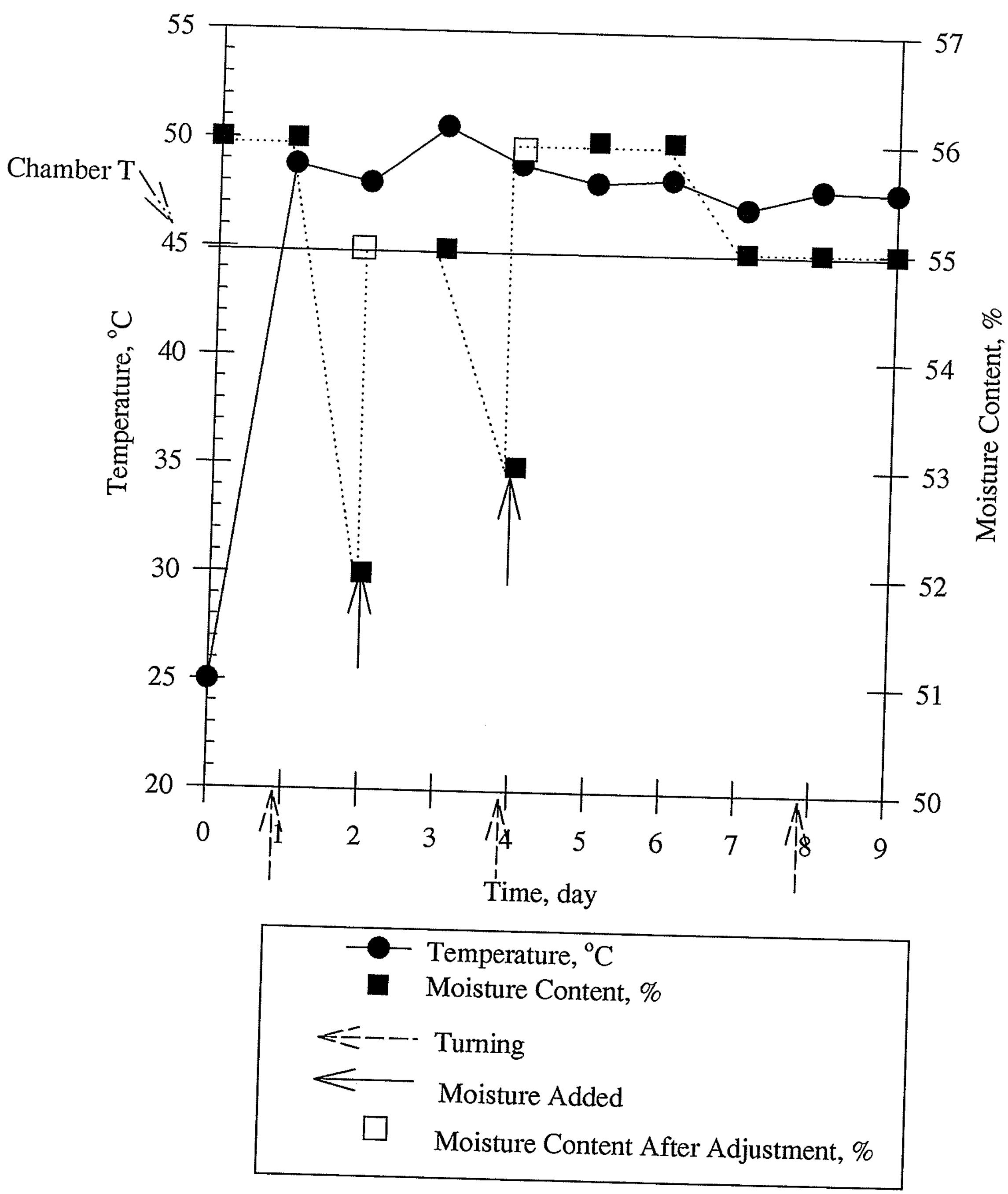

Figure 4-8. Phase II-Cycle 3: Effect of Recycle on Temperature of R55-32

4.3 Experimental Results for Phase III

The mechanisms which bring about the destruction of pathogenic organisms during the composting process are temperature and microbial activity (Finstein et al. 1983). The research in Phase III was done in order to study the effects of microbial activity and temperature on pathogen inactivation. Four reactors RS-55, RN-55, RS-45 and RN-45 were operated. The recycled compost in RS-55 and RS-45 was sterilized. While the recycled compost in RN-55 and RN-45 was non-sterilized. The moisture contents of RS-55 and RN-55 were 55%, while RS-45 and RN-45 both had a moisture content of 45%. The theoretical C:N ratio of RN,S-55 and RN,S-45 was 34:1 and 43:1, respectively. The recycled compost in RS-55 and RS-45 was sterilized compost, while the recycled compost in RN-55 and RN-45 was non-sterilized compost. The reactors were initially incubated at a temperature of 26°C for six days. From day seven, all the reactors were incubated at a temperature of 45°C.

The temperature, moisture content, and density of fecal coliform were measured and recorded daily. Volatile solids (VS) removal was used as a rough measure of the rate of the microbial activity in Phase III. The results of RS-55, RN-55, RS-45, and RN-45 were presented in Figures 4-9, 4-10, 4-11, and 4-12, respectively.

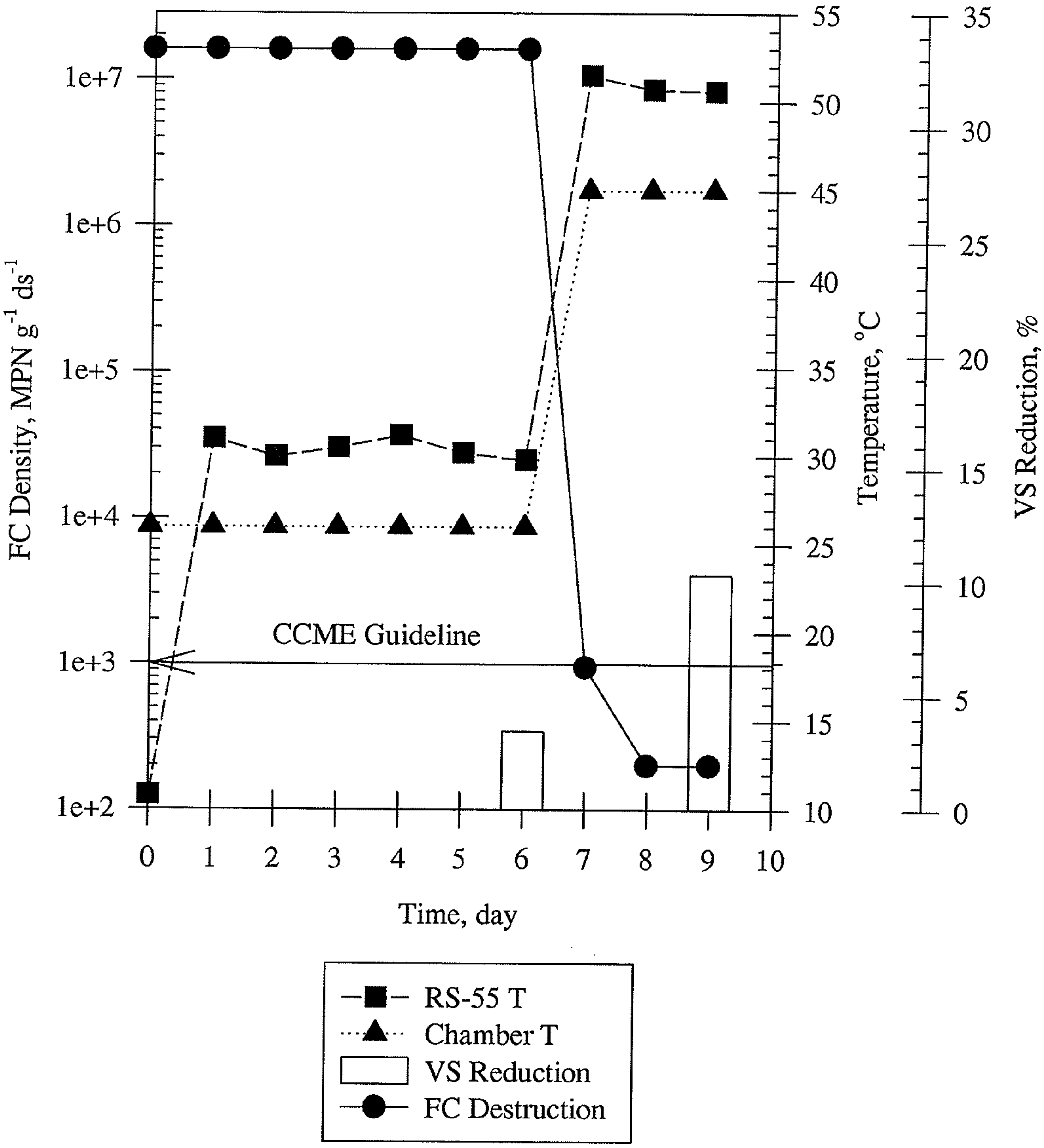

Figure 4-9. Phase III-RS-55: Effect of Chamber Temperature on FC Inactivation

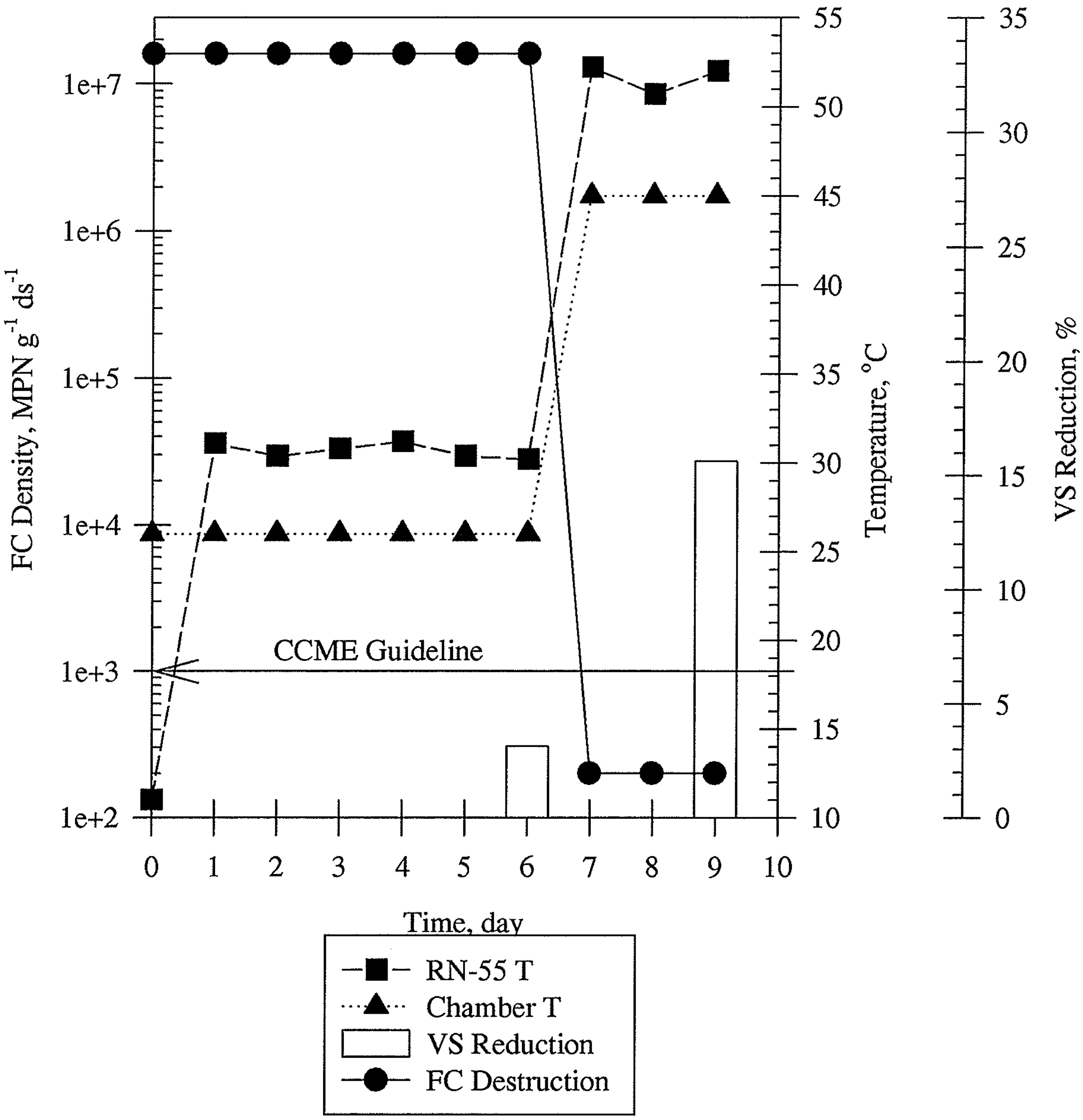

Figure 4-10. Phase III-RN-55: Effect of Chamber Temperature on FC Inactivation

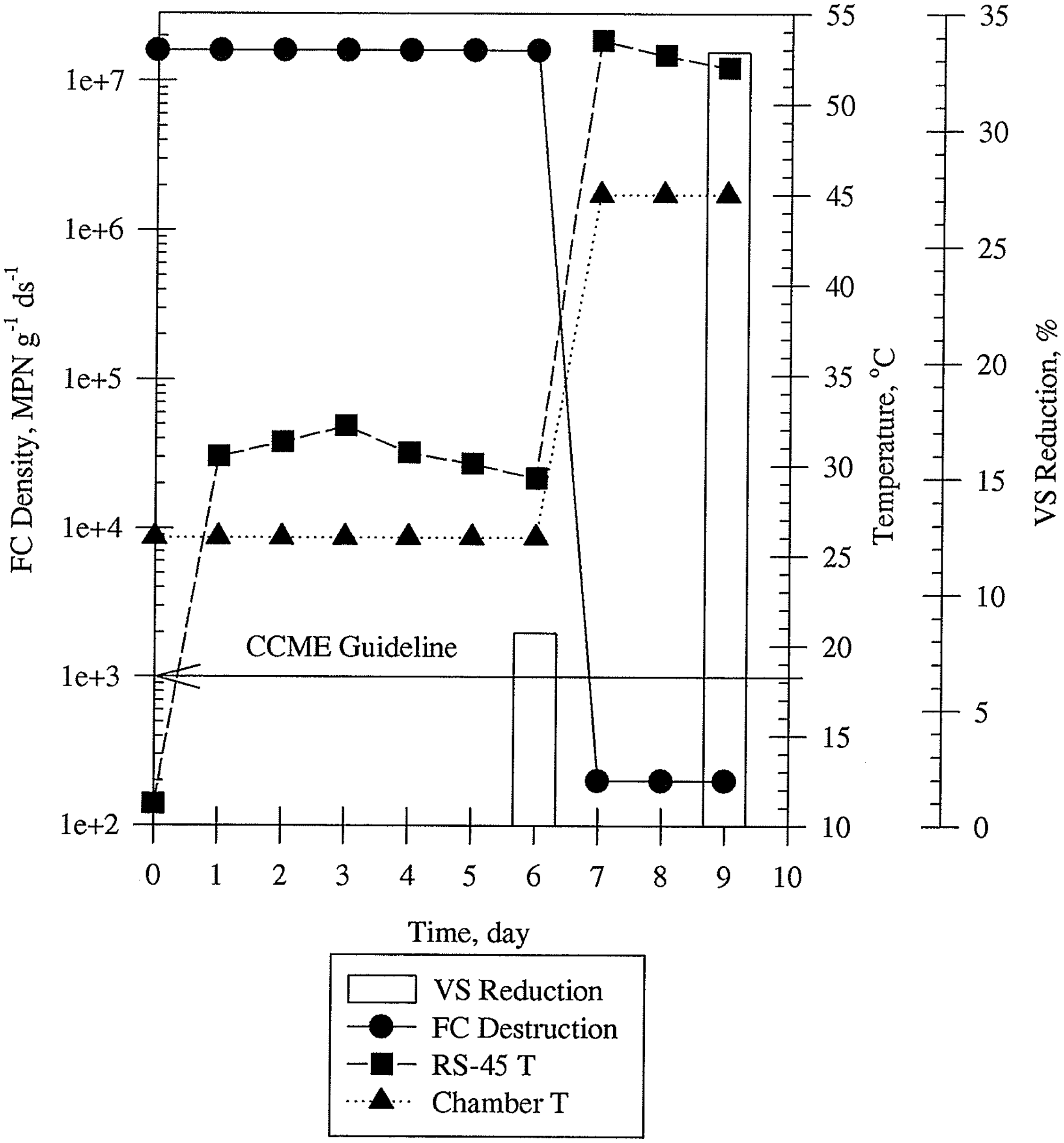

Figure 4-11. Phase III-RS-45: Effect of Chamber Temperature on FC Inactivation

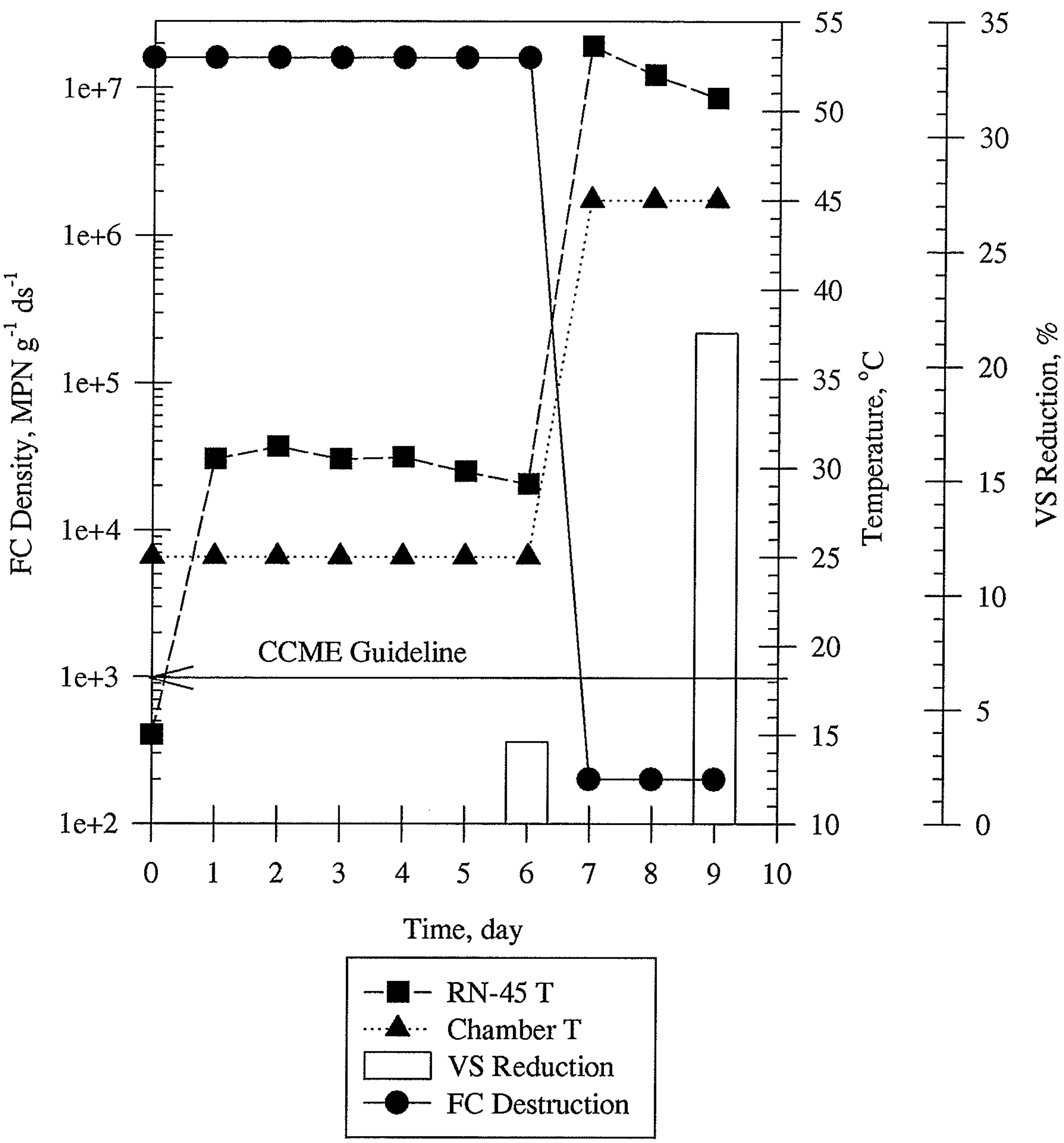

Figure 4-12. Phase III-RN-45: Effect of Chamber Temperature on FC Inactivation

The reactor temperatures were measured daily. The temperatures in all four reactors did not change dramatically the first six days (see Figures 4-9 through 4-12). At day seven, the reactors were incubated at a temperature of 45°C and the reactors' temperatures immediately increased to approximately 55°C. During the first six days, the average temperature of RN-55, RS-55, RN-45, and RS-45 was 30.7°C, 30.5°C, 30.3°C, and 30.7°C, respectively. From day seven to day nine, the average temperature of RN-55, RS-55, RN-45, and RS-45 was 51.6°C, 50.9°C, 52.1°C, and 52.7°C, respectively. The influence of initial BVS on reactor temperatures and VS destruction at two different environmental temperature are presented in Table 4-2.

Fecal coliform is used as the indicator organism for the pathogen kill in this phase. From Figures 4-11 through 4-14, we see that regardless of different initial total mass, C:N ratio, moisture contents and type of recycled compost, the density of FC did not change when the reactors were incubated at a temperature of 26°C for the first six days. Once the reactors were incubated at a temperature of 45°C, the density of FC of all reactors decreased immediately below the recommended limits (1000 MPN g^{-1} ds^{-1}). Table 4-3 shows the relationship of average VS destruction, reactor temperature, and FC inactivation.

Table 4-2. Phase III: Effect of Initial BVS and VS Destruction on Temperature

		Environmental Chamber Temperature			
	Initial BVS	26°C		45°C	
Reactor	kg	Δ VS, kg d^{-1}	Average T, °C	Δ VS, kg d^{-1}	Average T, °C
RN-55	0.17	0.002	30.7	0.01	51.6
RS-55	0.14	0.002	30.5	0.007	50.9
RN-45	0.16	0.002	30.3	0.02	52.1
RS-45	0.18	0.003	30.7	0.03	52.7

Table 4-3. Phase III: Effect of VS Destruction and Reactor Temperature on FC Inactivation

Reactor	Δ VS, %	Average Reactor Temperature, °C	Δ $\log_{10}$ FC
RN-55	3.13	30.7	0
	15.63	52.2	4.9
RS-55	3.45	30.5	0
	10.35	51.5	4.9
RN-45	3.57	30.3	0
	21.43	53.6	4.9
RS-45	8.33	30.7	0
	33.33	53.5	4.9

4.4 Summary

4.4.1 Summary for Experimental Results for Phase I

The MTF and MF methods were compared with the costs, time efficiency, and technical feasibility. The costs for reusable materials for MTF method was \$ 388 more than for MF method. While the costs for disposable materials for MTF method was \$ 184 less than for MF method. Also the MTF method needed less time to be completed.

4.4.2 Summary for Experimental Results for Phase II

In cycle 1, when the chamber temperature was 52°C, the average reactor temperature of R65-1, R55-1, and R45-1 was 52.8°C, 52.6°C, and 52.5°C, respectively. And despite the different initial concentrations of coliform, the coliform counts were reduced to less than 200 MPN g^{-1} ds^{-1} in all three reactors at day three. This level of coliform (< 200 MPN g^{-1} ds^{-1}) was kept to the end of the composting cycle. Also no coliform regrowth occurred regardless of moisture content during the curing stage.

In cycle 2, the chamber temperature was 45°C. The average temperature of R65-2, R55-2, and R45-2 was 48.9°C, 49.3°C, and 47.9°C, respectively. The initial BVS of R65-2, R55-2, and R45-2 was 0.26 kg, 0.32 kg, and 0.24 kg. With the lowest initial BVS, R45-2 had the lowest average temperature. Also coliform removal rate is R45-2 was the lowest. The coliform removal rate in R45-2 was almost one third as in R65-2 and a quarter as in R55-2.

Odour emissions were also observed. Odours in R65-2 were the highest among the three reactors. And odour emissions decreased with composting time. Odours were also increased after turning.

In cycle 3, the chamber temperature was 45°C. Compared to R55-31, R55-32 had more recycled compost and less initial BVS. The average temperature in R55-31 and R55-32 were 49.3°C and 48.4°C, respectively. The initial coliform counts of both reactors were 1.6 $\times 10^7$ MPN g^{-1} ds^{-1}. At day one the density of FC in R55-31 was reduced to 5.5 $\times 10^5$ MPN g^{-1} ds^{-1}. No FC destruction was observed in R55-32. By day three the coliform in both reactors were reduced to less than 200 MPN g^{-1} ds^{-1}.

4.4.3 Summary for Experimental Results for Phase III

With a chamber temperature of 26°C, the average temperature of RN-55, RS-55, RN-45, and RS-45 was 30.7°C, 30.5°C, 30.3°C, and 30.7°C, respectively. The VS removal at day six for these reactors was 3.13%, 3.45%, 3.57%, and 8.33%, respectively. Once the reactors were incubated at a temperature of 45°C, the average temperature of RN-55, RS-55, RN-45, and RS-45 increased to 51.6°C, 50.9°C, 52.1°C, and 52.7°C, respectively. And the VS removal was also increased to 15.63%, 10.35%, 21.43%, and 33.33%, respectively. No coliform inactivation was observed during the first six days. Once the reactor temperatures increased above 51°C, a significant coliform destruction occurred.

CHAPTER 5

DISCUSSION OF EXPERIMENTAL RESULTS

5.1 Discussion of Experiment Phase I

The objective of Phase I was to compare the two coliform enumeration methods, MTF and MF, for quantifying coliform (TC and FC) in compost. The capital costs for reusable materials for MTF method was higher than for MF method while the costs for disposable materials were less than for MF method (Table 4-1). The MTF method needed less time to complete a similar number of samples. Using the MTF method, two more samples could be done per day per person than using the MF method.

The MF method was not technically feasible. It was found that, when using the MF method, either the filter was plugged by the samples with dilutions below 10^{-3} or the bacterial count was below normal (less than 20) at dilutions above 10^{-3}. As described in Standard Methods (1992), the MF technique is extremely useful in monitoring drinking water and a variety of natural waters, where a filter is not a problem. However, the MF technique has limitations (because of the filter), particularly when testing highly turbid waters. For the above reasons, the MTF method was chosen to enumerate total and fecal coliform for the rest of this research.

5.2 Discussion of Experiment Phase II

The objective of experiment Phase II was to demonstrate pathogen reduction under various operational conditions during the bench-scale windrow co-composting of biosolids and leaves. These operational conditions were the chamber temperature, feedstock component, C:N ratio, moisture content of compost mass, and the ratio of recycled compost to biosolids to leaves. Three cycles were done in Phase II.

5.2.1 Phase II-Cycle 1

Three reactors R65-1, R55-1, and R45-1 were operated in cycle 1. They were incubated at a temperature of 52°C. The temperatures in all the three reactors reached the maximum at day one (see Figures 4-1 to 4-3). At day five to six, the temperatures dropped to around 52°C, and hovered around this level (52°C) for the rest of the composting cycle. The average temperatures of R65-1, R55-1, and R45-1 were 52.8°C, 52.6°C, and 52.5°C, respectively. There was no significant difference in reactors' temperature between the three reactors. It was also observed that some temperatures were below the environmental chamber temperature of 52°C. When measuring the temperatures, the reactors were taken out of the environmental chamber. Some of the heat in the reactors may have been lost to the room environment. So, during the rest of the experiments, the reactor temperatures were measured inside the environmental chamber.

Theoretically, turning can cause the temperature to drop in the reactor. When the warm compost surface is exposed to the cooler ambient air during the turning, a rapid surface temperature drop may occurred because of radiative, conductive, and convective heat lost to the outside ambient air. From Figures 4-1 through 4-3, we see that the temperatures in the reactors did not seem to be affected by turning. This was maybe a result of when the temperatures were measured. As measurements were taken before the reactor contents were turned, therefore any temperature changes would not have been detected.

Meanwhile, the moisture contents in all three reactors dropped significantly from day two to day five, because heat of the compost turned the moisture into vapour and in turn dried the remaining material. After day five, there was no significant moisture lost in all three reactors. The reason why no moisture loss occurred after day five could not be explained. No changes were nade to the operating procedures.

Total and fecal coliform were analyzed as indicator organisms in this research. Despite of different initial total coliform counts in the feedstock in R65-1, R55-1, and R45-1, the total coliform concentrations were less than 200 MPN g^{-1} ds^{-1} at day three. According to Figure 2-2, there was a greater than 90 % chance that most pathogens were inactivated. But there was still 10% chance that pathogens survived. The survived pathogens can regrow once the conditions become favourable. Also the densities of fecal coliform in all three reactors were reduced to less than CCME Guidelines (1000 MPN g^{-1} ds^{-1}) after three days regardless of different feedstock component, C:N ratio and moisture content. Since the coliform were tested every three days, the results did not show us any difference between the three reactors on pathogen destruction. The coliform bacteria could be destroyed at any time with the three days. So in cycle 2, I decided to test the coliform at day one and every two days for the rest of the composting cycle.

Concern has been voiced regarding regrowth of bacteria in finished compost. The compost mass with moisture contents 65%, 55% and 45% were routinely analyzed for TC and FC. No coliform (TC and FC) regrowth occurred regardless of moisture content. The final concentrations of the TC and FC were both less than 200 MPN g^{-1} ds^{-1} at the end of the curing stage. These results were under the recommended limits of less than 1000 MPN g^{-1} ds^{-1} (CCME 1995).

No regrowth of TC and FC, regardless of moisture content, was probably due to the controled management of the reactors which resulted in a stabilized compost after stage one. In a stabilized compost, concentrations of soluble organic nutrients required by pathogen growth are not available (Inbar et al. 1990). Therefore, in stabilized composts, regrowth of pathogens should not occur (Inbar et al. 1990).

5.2.2 Phase II-Cycle 2

Three reactors R65-2, R55-2, and R45-2 were operated in cycle 2. The ratio of biosolids to leaves to recycled compost of R65-2, R55-2, and R45-2 was 0.71:0.57:1, 0.46:1.45:1, and 0.21:2.38:1, respectively. The theoretical C:N ratio of R65-2, R55-2, and R45-2 was 24:1, 34:1, and 43:1, respectively. They were incubated at a temperature of 45°C. Figure 5-1 presents the comparison of reactor temperatures in R65-2, R55-2, and R45-2. Compared to R65-2 and R55-2, the temperatures in R45-2 were the lowest except at day eight (see Figures 5-1). This may have been due to the less initial BVS of R45-2.

The initial BVS of R65-2, R55-2, and R45-2 was 0.26 kg, 0.32 kg, and 0.24 kg (dry weight basis), respectively. Among these three reactore R45-2 had the least initial BVS. As we know, the temperature elevation is caused by the heat released from organic decomposition during the composting process. The BVS are those in the composting process that can be used by microorganisms. And the heat is released during BVS degradation. So more BVS produces more heat, and in turn higher temperature.

Total and fecal coliform were also analyzed as indicator organisms in cycle 2. The initial coliform counts in feedstock in R65-2, R55-2, and R45-2 were 1.6×10^7 MPN g^{-1} ds^{-1} (95% confidence limits is between 6×10^6 MPN g^{-1} ds^{-1} and 5.3×10^7 MPN g^{-1} ds^{-1}). At the end of the composting cycle, the final total coliform concentrations were typically less than

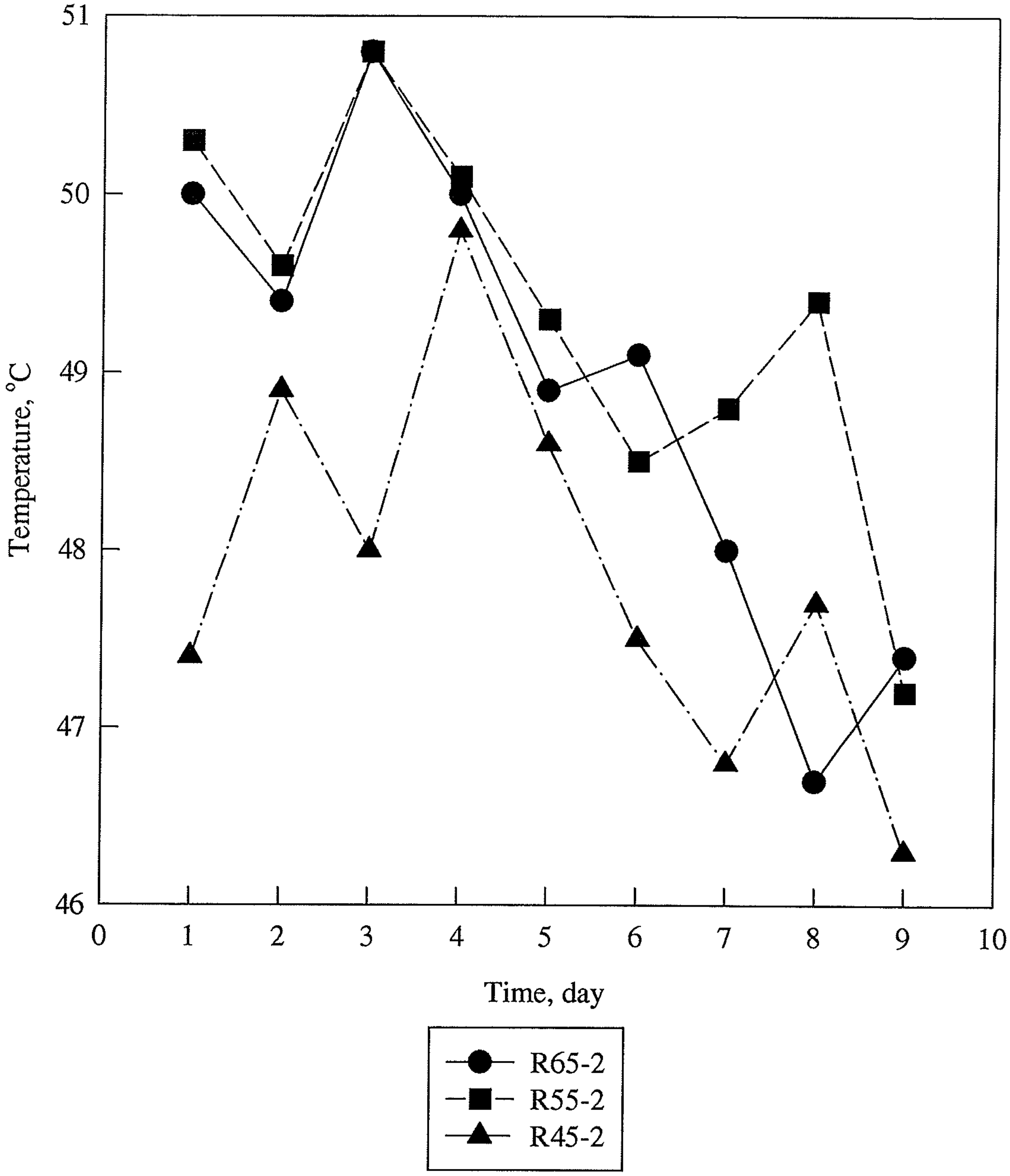

Figure 5-1. Phase II-Cycle 2: Reactor Temperatures During Cycle

200 MPN g^{-1} ds^{-1}, regardless of different moisture contents. This indicates that there was a greater than 90% chance that most pathogens were inactivated (see Figure 2-2). The final fecal coliform density in all the three reactors were also reduced to below 200 MPN g^{-1} ds^{-1}, which were under the CCME Guidelines (1000 MPN g^{-1} ds^{-1}) (see Figures 4-4 to 4-6).

Table 5-1 shows the coliform inactivation rates in the three reactors. The actual rates in T65-2 and R55-2 from day 1 to day 3 and in R45-2 from day 3 to day 5 may have been higher than the measured rates. The dilution range used was from 10^{-3} to 10^{-7}. The coliform concentration may be lower than 200 MPN g^{-1} ds^{-1} if the dilution is higher than 10^{-7}. R65-2 and R55-2 took three days to achieve the CCME Guidelines, while R45-2 took five days to achieve it. This may be due to the relatively lower temperature in R45-2 (see Figure 5-1). It may also partially due to the lower moisture content in R45-2. As mentioned by Haug (1993), moist heat is more lethal than dry heat to those organisms exposed to it. Therefore, it took a longer time for R45-2 to achieve pathogen destruction than for R65-2 and R55-2.

Table 5-1. Phase II-Cycle 2: Heat Inactivation of Coliform Bacteria

Time Period (days)	Δ $\log_{10}$ TC d^{-1}			Δ $\log_{10}$ FC d^{-1}		
	R65-2	R55-2	R45-2	R65-2	R55-2	R45-2
0-1	0.86	1.18	0	1.20	1.46	0
1-3	≥ 4.04	≥ 3.72	1.39	≥ 3.70	≥ 3.44	1.68
3-5			≥ 3.51			≥ 3.22

Also from Table 5-1 we see that the coliform inactivation rate ($\Delta \log_{10}$ TC d^{-1} and $\Delta \log_{10}$ FC d^{-1}) in R65-2 was 73% and 82%, respectively as in R55-2 during day zero to day one. R45-2, which reached a temperature of 47.4°C by day one, showed no measurable coliform reductions. From day one to day three, the average temperature achieved in R65-2, R55-2, and R45-2 were 50.1°C, 50.2°C, and 48°C, respectively. And the coliform inactivation rate ($\Delta \log_{10}$ TC d^{-1} and $\Delta \log_{10}$ FC d^{-1}) in R45-2 was significant lower than in R65-2 and R55-2. This may be due to the lower temperature and moisture in R45-2. Ward and Brandon (1977) also found that over a period of 20 minutes, a temperature of 50°C provided almost no coliform bacteria destruction; while a temperature of 52°C provided a reduction of about 1 $\log_{10}$. If the time period is extended from 20 minutes to 24 hours, the difference on coliform inactivation will be very significant. Coliform destruction was observed when the temperature was slightly above 50°C. While Ward and Brandon (1977) found almost no coliform destruction at a temperature of 50°C.

Subjective odour observations were also recorded during cycle 2. Odour from R65-2 was much higher than R55-2 and R45-2. Reactor R45-2 had the lowest odours. These results were as expected. The high moisture content and higher mass of biosolids in R65-2 may have caused the stronger odours.

The highest odour emissions occurred during the early days of composting because of the less stabilized material and more active composting. Odour emissions, however increased significantly immediately after turning probably due to the release of the odorous gases within the pore spaces closer to the bottom of the reactors. Compared to the top of the reactor, there was probably less oxygen in the bottom of the reactor. The anaerobic condition can be developed in the bottom and the reduced gaseous compounds (H_2S and NH_3) were not oxidized to less odorous compounds.

5.2.3 Phase II-Cycle 3

The objective of cycle 3 was to try to observe the pathogen destruction under the same moisture content but different feedstock component. Two reactors R55-31 and R55-32 were operated. The moisture content of R55-31 and R55-32 was 55%. The ratio of biosolids to leaves to recycled compost of R55-31 and R55-32 were 0.46:1.45:1 and 0.31:0.88:1, respectively. They were incubated at a temperature of 45°C. Total and fecal coliform were monitored every two days. A comparison of reactor temperatures is presented in Figure 5-2.

Compared to reacctor R55-32, reactor R55-31 had higher temperatures during the cycle except at day three and day nine (see Figure 5-2). It maybe because R55-31 had more biodegradable volatile solids than R55-32. Recycling of compost product does not add new

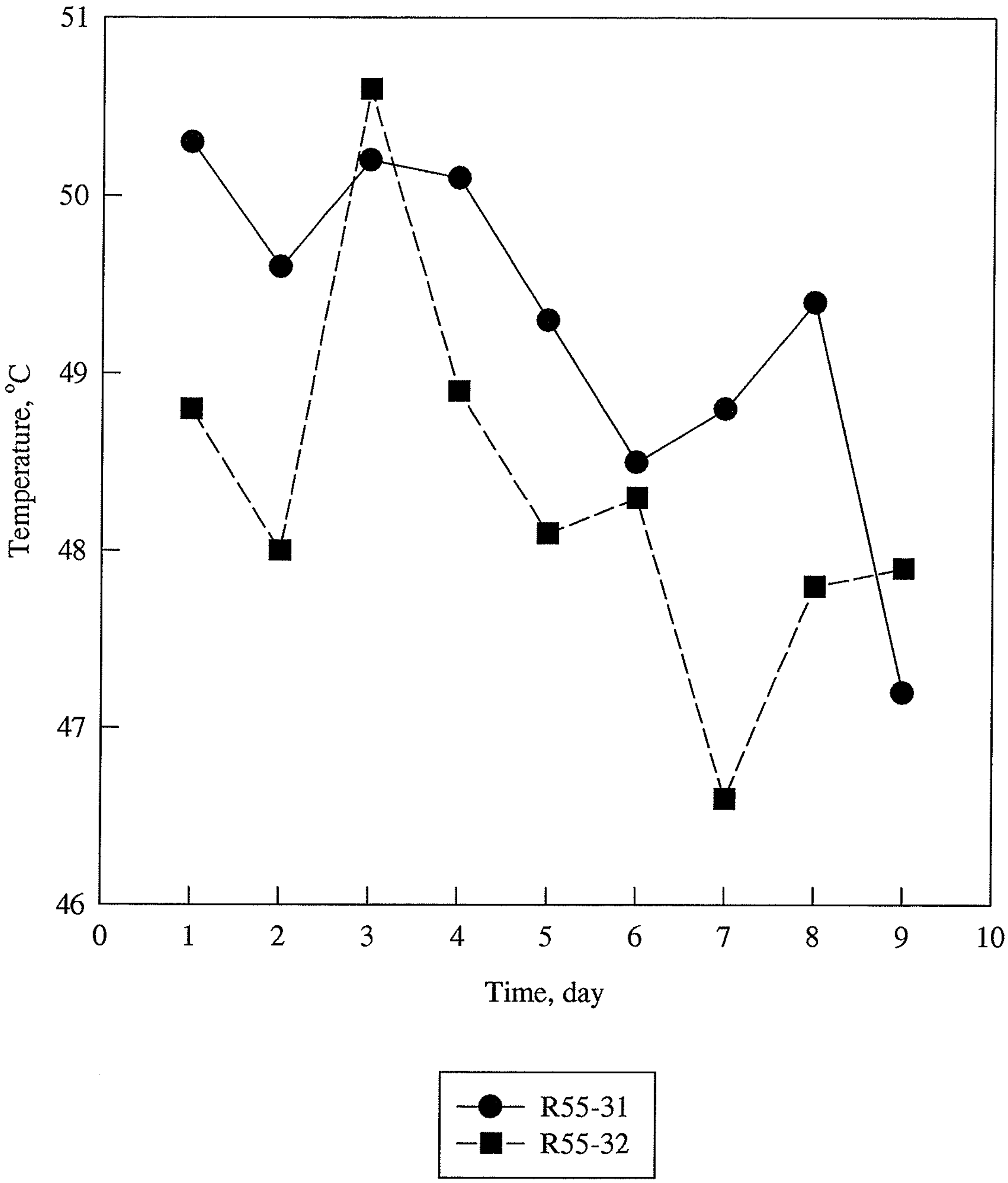

Figure 5-2. Phase II-Cycle 3. Reactor Temperatures During Cycle

biodegradable volatile solids to the feedstock (Haug 1993). Meanwhile, the moisture content in both reactors decreased sharply during the first five days. The moisture content was adjusted at day two to three and day five. Again effects of turning on temperature were not measured.

The initial feedstock coliform counts of both reactors were the same (1.6×10^7 MPN g^{-1} ds^{-1}). At day one the coliform density in R55-31 was reduced to 1×10^6 MPN g^{-1} ds^{-1}, while the coliform density in R55-32 was kept at 1.6×10^7 MPN g^{-1} ds^{-1}. At day three, the coliform concentrations in both reactors were reduced to less than 200 MPN g^{-1} ds^{-1}. This may be due to the higher temperature in R55-31.

Since recycled product did not add any BVS to the feedstock, high recycle ratio would decrease the BVS. Thus it would affect the reactor temperature, and in turn the pathogen destruction. The results showed us that an increased recycle ratio did not improve the pathogen reduction performance in the bench-scale windrow.

5.3 Discussion of Experiment Phase III

The objective of Experiment Phase III was to study the effects of microbial activity, recycle and temperature on pathogen inactivation. Four reactors RS-55, RN-55, RS-45 and RN-45 were operated. The reactors were initially incubated at a temperature of 26°C for six days. From day seven, all the reactors were incubated at a temperature of 45°C. The temperature, moisture content, and density of fecal coliform were measured and recorded daily. Volatile solids (VS) removal was used as a rough measure of the rate of the microbial activity.

Within the first six days, with a chamber temperature of 26°C, little VS reduction occurred (see Figures 4-11 to 4-14). These results told us that the microbial activity was very slow. After day six, when the reactors were incubated at a temperature of 45°C, a significant VS reduction occurred within three days, which showed us that a high rate of composting had occurred. The VS destruction per day was almost ten times more at 45°C than at 26°C (see Table 4-3). This may be due to the low feedstock temperature and the small size of the reactors. For this phase, all materials were stored in the refrigerator before mixing. The initial temperatures of all feedstock were about 10°C to 11°C. With a low feedstock temperature, a low rate of biological activity occurred because low temperature inhibits microorganism activity (Haug 1993). And, when the reactors were kept for six days at 26°C, it was still lower to initialize the biological reaction. Also, the heat produced by the microbial activity was more easily lost to the cold ambient air due to the small reactors. Since small piles could not achieve the self insulation. When the reactors were incubated at a temperature of 45°C, the microorganisms were quite active since the microbial activity was the greatest in composting piles with temperatures ranging between 35°C to 50°C (McKinley et al. 1985).

Also we see that with 55% moisture content, VS reduction in RN-55 was higher than in RS-55. While with 45% moisture content, VS reduction in RS-45 was higher than in RN-45 (see Figures 4-11 to 4-14). The data show conflicting results of microbial concentration on VS reduction. Since recycled compost contains a variety of active microorganisms, recycling compost during the composting process should increase the microbial concentration, thus the microbial activity. But the research conflicted with this. In fact, the impact of microbial concentration on the composting rate has been a subject of controversy for many years. As reported by Haug (1993), using continuous composters operated on refuse materials, Regan and Jeris examined the effect of recycle rates. The other environmental factors, such as moisture, temperature, free air space, and aeration, were kept the same. They observed that the maximum oxygen consumption rates were 25, 80 and 99% for recycle rates of 25, 50, and 75%, respectively. Therefore, the observed results can probably be ascribed to the increased microbial concentration (Haug 1993). On the other hand, McGauhey and Gotass examined the effects of adding product recycle, soil, and up to 30% horse manure into refuse. They found that none had any measurable effect on the composting rate.

The temperatures of the reactors were measured daily. The temperatures in all four reactors did not change dramatically the first six days (see Figures 4-11 through 4-14). The average temperatures inside the reactors were around 30.5°C when the chamber temperature was 26°C (see Table 4-6). There was 4.5°C difference between the average temperatures and the environmental chamber temperature. At day seven, the reactors were incubated at 45°C and the average reactors' temperatures increased above 51°C (see Table 4-3). The difference of the average temperatures and the environmental chamber temperature was 6°C. The average reactor temperatures above the environmental temperature was 1.5°C higher at 45°C than at 26°C. This may due to low microbial activity in the first six days, since a rise in temperature is caused by the heat produced by the microbiological reaction. As indicated in Table 4-3, VS destruction was much higher with the average reactor temperatures of 51°C.

During the experiment Phase II, I found that when fecal coliform were reduced to less than 200 MPN g^{-1} ds^{-1}, total coliform were also reduced to less than 200 MPN g^{-1} ds^{-1}. This occurred under various operational conditions. The results showed that fecal and total coliform had a good correlation. In this phase fecal coliform was used as the only indicator organism for the pathogen kill. Although there were VS reductions occurred within the reactors, no FC destruction was observed at a temperature around 31°C (see Table 4-4). Once the reactor temperatures increased to above 51.5°C, the density of FC of all reactors decreased immediately below the recommended limits (1000 MPN g^{-1} ds^{-1}). This occurred whether the feedstock was inoculated with non-sterilized or sterilized recycled compost. It is clear that temperature was the main factor in pathogen destruction and not microbial activity.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

The purpose of this project was to demonstrate the potential for pathogen inactivation during co-composting of biosolids and leaves in bench-scale reactors. Based on the results of this investigation, the following conclusions can be drawn:

1. Multiple Tube Fermentation (MTF) method should be chosen when quantifying total and fecal coliform (TC and FC) in compost. The MF method was not technically feasible due to problems with finding an appropriate dilution factor. A dilution below 10^{-3} will clog the filter, while a dilution above 10^{-3} results in low bacterial count.

2. Maintaining a high temperature (above 50°C) is critical for pathogen destruction. It took a longer time to inactivate pathogens at temperatures a few degrees lower than 50°C (around 48.5°C). As indicated in Figure 5-1, compared to R65-2, R55-2, and R45-2, R-45 had relatively low temperatures. R45-2 took five days to achieve pathogen inactivation while R65-2 and R55-2 took three days. Because of the low temperatures in Manitoba, this is a significant concern.

3. Low temperature composting (with ceiling temperature of 60°C) produces stabilized compost, and as such reduces pathogen regrowth. Using coliform as indicator, no

pathogen regrowth were found during curing stage regardless the moisture content. The most reactor temperatures during bench-scale composting were below 55°C.

4. Temperature seems to be a more important factor in pathogen destruction than microbial activity. As shown in Phase III, although some VS reductions occurred, no pathogen destruction was observed at low temperatures (around 31°C) regardless of different recycle ratios, but as soon as the reactor temperatures were increased to above 52°C, a significant pathogen destruction occurred immediately.

Further studies are needed to elucidate the impact of the moisture content, C:N ratio on pathogen inactivation and the impact of compost recycle on pathogen reduction. For this reason, the following suggestions for further research are made:

1. To investigate the effects of different moisture content on pathogen destruction. I suggest using moisture contents of 65%, 55% and 45%. The other operational conditions, such as C:N ratio and the ratio of recycled compost to biosolids to leaves (dry weight) should be kept the same.

2. To investigate the effects of different carbon to nitrogen ratios on pathogen inactivation. Keeping the other operational parameters the same, one could investigate the effects of various C:N ratios. Since the optimum C:N ratio during the

composting process is between 20:1 to 30:1 (U.S. EPA, 1985), I suggest using C:N ratios of 15:1, 20:1, 25:1, 30:1, and 35:1.

3. To study the impact of recycled compost on pathogen reduction. We can use two reactors: one reactor containing only biosolids and leaves and another one containing biosolids, leaves and recycled compost. Keeping the other operational conditions the same, we can study the impact of recycled compost on pathogen reduction.

4. To investigate the effect of low ambient temperatures on pathogen inactivation in a full-scale windrow co-composting of biosolids and leaves in Manitoba.

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APPENDIX A.

EXPERIMENTAL RAW DATA

Phase II-Cycle 1: Reactor Temperature, C

Day	Breeder	R65-1	R55-1	R45-1
1	58	55.9	56.1	56.2
2	55.3	55	54.7	53.5
3	57.2	54.8	54.7	53.6
4	56.1	53.4	53.9	52.9
5	56.1	52	53.1	52.5
6	56.6	53.2	53.4	53
7	55.8	52.9	52.7	52.1
8	56.7	51.5	51.7	52
9	55	52.2	52	52
10	55.1	50.2	50.2	50.9
11	55.7	50.7	51.8	51.2
12	55.5	53	52	52.3
13	54.9	52.5	49.5	51.8
14	54	52	50	51

Phase II-Cycle 1: Moisture Content and Turning

Day	Breeder	R65-1	R55-1	R45-1	Turning
1	56%	65%	55%	45%	Turning
2	55%	62%	53%	3% (48%)	
3	55%	3% (65%)	)% (56%)	46%	Turning
4	55%	63%	54%	43%	
5	3% (55%)	1% (66%)	3% (56%)	2% (47%)	
6	55%	67%	57%	47%	Turning
7	55%	67%	57%	47%	
8	55%	67%	57%	47%	
9	54%	67%	56%	47%	Turning
10	54%	66%	56%	46%	
11	2% (55%)	65%	55%	46%	
12	55%	65%	54%	46%	Turning
13	55%	65%	54%	44%	
14	54%	65%	54%	43%	

Phase II-Cycle 1: Coliform Density in R65-1

Day	Sample	Medium	Dilution 10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
Day 0							
	Sample 1	Lactose	5	5	5	5	3
			5	5	5	5	4
		BGB	5	5	5	5	4
			5	5	5	5	5
		EC	5	5	3	5	0
			5	5	4	4	0
	Sample 2	Lactose	5	5	5	5	3
			5	5	5	5	4
		BGB	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	4	3	0
			5	5	3	3	0
Day 3	Sample 1	Lactose	5	5	1	0	0
			5	5	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	4	1	0	0
			5	5	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0

Day	Sample	Medium	10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
			Dilution				
Day 6							
	Sample 1	Lactose	5	5	2	0	0
			5	5	2	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	5	0	0	0
			5	5	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
Day 9	Sample 1	Lactose	5	5	0	0	0
			5	4	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	4	0	0	0
			5	4	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0

Day	Sample	Medium	Dilution 10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
Day 12	Sample 1	Lactose	5	4	0	0	0
			4	4	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	3	0	0	0
			4	3	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
Day 14	Sample 1	Lactose	4	3	0	0	0
			5	3	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	3	0	0	0
			4	4	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0

Phase II-Cycle 1: Coliform Density in R55-1

Day	Sample	Medium	Dilution 10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
Day 0							
	Sample 1	Lactose	5	5	5	5	0
			5	5	5	1	1
		BGB	5	5	5	5	5
			5	5	5	5	0
		EC	5	5	5	2	0
			5	5	5	3	0
	Sample 2	Lactose	5	5	5	5	5
			5	5	5	5	4
		BGB	5	5	5	5	4
			5	5	5	5	0
		EC	5	5	5	3	0
			5	5	5	2	0
Day 3	Sample 1	Lactose	5	3	0	0	0
			5	0	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	2	1	0	0
			5	3	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0

Day	Sample	Medium	Dilution				
Day 6			10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
	Sample 1	Lactose	5	2	0	0	0
			5	2	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	3	0	0	0
			4	3	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
Day 9	Sample 1	Lactose	5	0	0	0	0
			4	0	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	4	1	0	0	0
			5	0	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0

Day	Sample	Medium	Dilution				
Day 12			10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
	Sample 1	Lactose	4	0	0	0	0
			4	0	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	4	0	0	0	0
			4	0	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
Day 14	Sample 1	Lactose	3	0	0	0	0
			4	0	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	4	0	0	0	0
			4	0	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0

Phase II-Cycle 1: Coliform Density in R45-1

Day	Sample	Medium	Dilution				
			10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
Day 0							
	Sample 1	Lactose	5	5	5	2	0
			5	5	5	2	0
		BGB	5	5	5	5	0
			5	5	5	5	0
		EC	5	5	5	0	0
			5	5	5	0	0
	Sample 2	Lactose	5	5	5	2	1
			5	5	5	2	0
		BGB	5	5	5	5	0
			5	5	5	5	0
		EC	5	5	5	0	0
			5	5	5	0	0
Day 3	Sample 1	Lactose	4	1	0	0	0
			4	1	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	0	0	0	0
			5	2	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0

Day	Sample	Medium	Dilution				
Day 6			10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
	Sample 1	Lactose	5	3	0	0	0
			5	4	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	3	0	0	0
			5	3	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
Day 9	Sample 1	Lactose	5	0	0	0	0
			4	0	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	0	0	0	0
			5	0	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0

Day	Sample	Medium	Dilution 10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
Day 12							
	Sample 1	Lactose	4	0	0	0	0
			3	0	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	4	0	0	0	0
			3	0	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
Day 14	Sample 1	Lactose	3	1	0	0	0
			4	0	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	4	1	0	0	0
			5	0	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0

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Phase II-Cycle 1: Coliform Density in R65-1 during Curing Stage

Day	Sample	Medium	Dilution 10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
Day 0	Sample 1	Lactose	5	2	0	0	0
			5	1	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	0	0	0	0
			5	0	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
Day 3	Sample 1	Lactose	5	3	1	0	0
			5	4	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	3	0	0	0
			5	3	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0

Day	Sample	Medium	Dilution				
			10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
Day 6	Sample 1	Lactose	5	1	2	0	0
			5	1	2	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	2	0	0	0
			5	2	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
Day 9	Sample 1	Lactose	5	2	0	0	0
			5	2	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	2	0	0	0
			5	2	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0

Day	Sample	Medium	Dilution				
Day 10			10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
	Sample 1	Lactose	5	2	1	0	0
			5	2	1	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	2	0	0	0
			5	2	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0

Phase II-Cycle 1: Coliform Density in R55-1 during Curing Stage

Day	Sample	Medium	Dilution				
Day 0			10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
	Sample 1	Lactose	5	4	1	0	0
			5	3	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	1	0	0	0
			5	0	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
Day 3	Sample 1	Lactose	5	3	1	0	0
			5	3	1	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	1	2	0	0
			5	2	1	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0

Day	Sample	Medium	Dilution				
Day 6			10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
	Sample 1	Lactose	5	4	1	0	0
			5	4	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	3	1	0	0
			5	4	1	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
Day 9	Sample 1	Lactose	5	1	1	0	0
			5	2	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	2	0	0	0
			5	1	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0

Day	Sample	Medium	Dilution				
Day 10			10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
	Sample 1	Lactose	5	3	1	0	0
			5	3	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	3	1	0	0
			5	3	1	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0

Phase II-Cycle 1: Coliform Density in R45-1 during Curing Stage

Day	Sample	Medium	Dilution				
Day 0			10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
	Sample 1	Lactose	5	2	0	0	0
			5	1	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	2	0	0	0
			5	2	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
Day 3	Sample 1	Lactose	5	4	1	0	0
			5	4	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	3	0	0	0
			5	3	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0

Day	Sample	Medium	Dilution				
			10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
Day 6	Sample 1	Lactose	5	2	0	0	0
			5	1	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	2	0	0	0
			5	2	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
Day 9	Sample 1	Lactose	5	1	0	0	0
			5	0	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	1	0	0	0
			5	1	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0

Day	Sample	Medium	Dilution				
Day 10			10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
	Sample 1	Lactose	5	2	0	0	0
			5	2	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	1	0	0	0
			5	1	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0

Phase II-Cycle 2: Reactor Temperature, C

Day	Breeder	R65-2	R55-2	R45-2
1	53.6	50	50.3	47.4
2	52	49.4	49.6	48.9
3	52.2	50.8	50.8	48
4	51	50	50.1	49.8
5	50.2	48.9	49.3	48.6
6	49.8	49.1	48.5	47.5
7	49	48	48.8	46.8
8	48.9	46.7	49.4	47.7
9	48.7	47.4	47.2	46.3

Phase II-Cycle 2: Moisture Content and Turning

Day	Breeder	R65-2	R55-2	R45-2	Turning
1	55%	65%	)% (58%)	48%	Turning
2	55%	65%	58%	45%	
3	55%	3% (66%)	54%	2% (47%)	
4	3% (58%)	66%	55%	47%	Turning
5	58%	66%	1% (56%)	47%	
6	56%	65%	56%	45%	
7	56%	2% (65%)	54%	45%	
8	54%	65%	54%	45%	Turning
9	55%	64%	54%	44%	

Phase II-Cycle 2: Coliform Density in R65-2

Day	Sample	Medium	Dilution 10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
Day 0							
	Sample 1	Lactose	5	5	5	5	5
			5	5	5	5	5
		BGB	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
	Sample 2	Lactose	5	5	5	5	5
			5	5	5	5	5
		BGB	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
Day 1	Sample 1	Lactose	5	5	5	3	0
			5	5	5	3	0
		BGB	5	5	5	5	0
			5	5	5	5	0
		EC	5	5	5	3	0
			5	5	4	4	0
	Sample 2	Lactose	5	5	5	4	1
			5	5	5	3	2
		BGB	5	5	5	4	1
			5	5	5	5	0
		EC	5	5	5	3	0
			5	5	4	3	0

Day	Sample	Medium	Dilution				
Day 3			10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
	Sample 1	Lactose	5	5	3	0	0
			5	5	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	5	2	2	0
			5	5	4	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
Day 5	Sample 1	Lactose	5	5	5	0	0
			5	5	5	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	5	2	3	0
			5	5	2	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0

Day	Sample	Medium	Dilution				
Day 7			10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
	Sample 1	Lactose	5	5	3	0	0
			5	5	3	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	5	3	1	0
			5	5	2	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
Day 9	Sample 1	Lactose	5	5	3	0	0
			5	5	3	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	5	0	0	0
			5	2	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0

Phase II-Cycle 2: Coliform Density in R55-2

Day	Sample	Medium	Dilution				
Day 0			10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
	Sample 1	Lactose	5	5	5	5	5
			5	5	5	5	5
		BGB	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
	Sample 2	Lactose	5	5	5	5	5
			5	5	5	5	5
		BGB	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
Day 1	Sample 1	Lactose	5	5	5	3	0
			5	5	5	3	0
		BGB	5	5	5	5	0
			5	5	5	5	0
		EC	5	5	3	3	0
			5	5	4	3	0
	Sample 2	Lactose	5	5	5	4	1
			5	5	5	3	2
		BGB	5	5	5	4	1
			5	5	5	5	0
		EC	5	5	4	2	0
			5	5	4	3	0

Day	Sample	Medium	Dilution				
Day 3			10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
	Sample 1	Lactose	5	4	1	0	0
			5	2	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	5	0	0	0
			5	3	2	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
Day 5	Sample 1	Lactose	5	5	4	0	0
			5	5	4	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	5	2	3	0
			5	5	3	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0

Day	Sample	Medium	Dilution				
			10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
Day 7	Sample 1	Lactose	5	5	3	0	0
			5	5	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	4	1	1	0
			5	5	1	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
Day 9	Sample 1	Lactose	5	5	3	0	0
			5	5	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	5	0	0	0
			5	3	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0

Phase II-Cycle 2: Coliform Density in R45-2

Day	Sample	Medium	Dilution 10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
Day 0	Sample 1	Lactose	5	5	5	5	5
			5	5	5	5	5
		BGB	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
	Sample 2	Lactose	5	5	5	5	5
			5	5	5	5	5
		BGB	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
Day 1	Sample 1	Lactose	5	5	4	4	5
			5	5	5	5	4
		BGB	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
	Sample 2	Lactose	5	5	5	4	3
			5	5	5	5	3
		BGB	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5

Day	Sample	Medium	Dilution 10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
Day 3	Sample 1	Lactose	5	5	5	5	1
			5	5	4	4	1
		BGB	5	5	5	1	2
			5	5	5	2	1
		EC	5	5	5	1	0
			5	5	0	2	0
	Sample 2	Lactose	5	5	5	5	0
			5	5	4	3	1
		BGB	5	5	5	3	0
			5	5	5	1	1
		EC	5	5	5	2	0
			5	5	5	0	0
Day 5	Sample 1	Lactose	5	2	1	0	0
			5	5	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	5	2	0	0
			5	5	2	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0

Day	Sample	Medium	Dilution				
Day 7			10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
	Sample 1	Lactose	5	5	1	0	0
			5	4	2	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	5	3	0	0
			5	5	3	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
Day 9	Sample 1	Lactose	5	5	1	0	0
			4	3	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	0	0	0	0
			5	2	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0

Phase II-Cycle 3: Reactor Temperature, C

Day	R55-31	R55-32
1	50.3	48.8
2	49.6	48
3	50.2	50.6
4	50.1	48.9
5	49.3	48.1
6	48.5	48.3
7	48.8	46.6
8	49.4	47.8
9	47.2	47.9

Phase II-Cycle 3: Moisture Content and Turning

Day	R55-31	R55-32	Turning
1	50% (58%)	56%	Turning
2	58%	52% (55%)	
3	54%	55%	
4	55%	53% (56%)	Turning
5	51% (56%)	56%	
6	56%	56%	
7	54%	55%	
8	54%	55%	Turning
9	54%	55%	

Phase II-Cycle 3: Coliform Density in R55-31

Day	Sample	Medium	Dilution				
Day 0			10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
	Sample 1	Lactose	5	5	5	5	5
			5	5	5	5	5
		BGB	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
	Sample 2	Lactose	5	5	5	5	5
			5	5	5	5	5
		BGB	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
Day 1	Sample 1	Lactose	5	5	5	3	0
			5	5	5	3	0
		BGB	5	5	5	5	0
			5	5	5	5	0
		EC	5	5	4	3	0
			5	5	3	3	0
	Sample 2	Lactose	5	5	5	4	1
			5	5	5	3	2
		BGB	5	5	5	4	1
			5	5	5	5	0
		EC	5	5	4	2	0
			5	5	4	3	0

Day	Sample	Medium	Dilution				
			10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
Day 3	Sample 1	Lactose	5	4	1	0	0
			5	2	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	5	0	0	0
			5	3	2	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
Day 5	Sample 1	Lactose	5	5	4	0	0
			5	5	4	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	5	2	3	0
			5	5	3	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0

Day	Sample	Medium	Dilution				
Day 7			10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
	Sample 1	Lactose	5	5	3	0	0
			5	5	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	4	1	1	0
			5	5	1	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
Day 9	Sample 1	Lactose	5	5	3	0	0
			5	5	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	5	0	0	0
			5	3	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0

Phase II-Cycle3: Coliform Density in R55-32

Day	Sample	Medium	Dilution				
Day 0			10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
	Sample 1	Lactose	5	5	5	5	5
			5	5	5	5	5
		BGB	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	4	5
			5	5	5	5	5
	Sample 2	Lactose	5	5	5	5	5
			5	5	5	5	5
		BGB	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
Day 1	Sample 1	Lactose	5	5	5	5	4
			5	5	5	4	4
		BGB	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
	Sample 2	Lactose	5	5	5	5	5
			5	5	5	5	5
		BGB	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5

Day	Sample	Medium	Dilution				
Day 3			10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
	Sample 1	Lactose	5	5	3	0	0
			5	5	1	1	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	5	4	0	0
			5	3	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
Day 5	Sample 1	Lactose	5	4	2	0	0
			5	5	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	5	0	0	0
			5	3	2	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0

Day	Sample	Medium	Dilution				
Day 7			10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
	Sample 1	Lactose	5	4	4	1	0
			5	5	3	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	5	2	0	0
			5	5	1	2	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
Day 9	Sample 1	Lactose	5	4	4	1	0
			5	5	0	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	3	0	0	0
			5	2	2	0	0
		BGB	0	0	0	0	0
			0	0	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0

Phase III: Reactor Temperature, C

Day	RN-55	RS-55	RN-45	RS-45	Chamber T
1	31.3	31	30.5	30.5	26.1
2	30.4	30	31.2	31.3	26.4
3	30.8	30.5	30.5	32.2	26.6
4	31.2	31.2	30.6	30.7	25.7
5	30.4	30.2	29.8	30.1	25.9
6	30.2	29.8	29.1	29.3	25.6
7	52.2	51.5	53.6	53.5	45
8	50.7	50.7	52	52.7	45
9	52	50.6	50.7	52	45

Phase III: Moisture Content and Turning

Day	RN-55	RS-55	RN-45	RS-45	Turning
1	57%	59%	46%	48%	Turning
2	57%	58%	46%	45%	
3	57%	56%	45%	43% (46%)	Turning
4	56%	56%	45%	45%	
5	56%	56%	45%	45%	
6	55%	55%	46%	46%	Turning
7	56%	56%	45%	45%	
8	56%	54% (56%)	46%	44% (46%)	
9	56%	56%	46%	45%	Turning

Phase III: Fecal coliform Density in RN-55

Day	Sample	Medium	Dilution				
Day 0			10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
	Sample 1	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
	Sample 2	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
Day 1	Sample 1	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
	Sample 2	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
Day 2	Sample 1	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
	Sample 2	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5

Day	Sample	Medium	Dilution				
			10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
Day 3	Sample 1	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
	Sample 2	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
Day 4	Sample 1	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
	Sample 2	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
Day 5	Sample 1	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
	Sample 2	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5

Day	Sample	Medium	Dilution				
			10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
Day 6	Sample 1	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
	Sample 2	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
Day 7	Sample 1	Lactose	5	4	4	1	1
			5	5	3	1	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	5	4	0	0
			5	4	4	1	0
		EC	0	0	0	0	0
			0	0	0	0	0
Day 8	Sample 1	Lactose	5	5	5	3	0
			5	5	4	2	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	5	4	0	0
			5	5	3	1	0
		EC	0	0	0	0	0
			0	0	0	0	0
Day 9	Sample 1	Lactose	5	5	2	0	0
			5	5	2	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	5	1	1	0
			5	5	1	1	0
		EC	0	0	0	0	0
			0	0	0	0	0

Phase III: Fecal coliform Density in RS-55

Day	Sample	Medium	Dilution				
Day 0			10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
	Sample 1	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
	Sample 2	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
Day 1	Sample 1	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
	Sample 2	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
Day 2	Sample 1	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
	Sample 2	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5

Day	Sample	Medium	Dilution				
			10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
Day 3	Sample 1	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
	Sample 2	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
Day 4	Sample 1	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
	Sample 2	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
Day 5	Sample 1	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
	Sample 2	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5

Day	Sample	Medium	Dilution				
			10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
Day 6	Sample 1	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
	Sample 2	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
Day 7	Sample 1	Lactose	5	5	5	1	1
			5	5	3	1	0
		EC	5	5	1	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	4	4	0	0
			5	5	2	1	1
		EC	1	1	0	0	0
			0	0	0	0	0
Day 8	Sample 1	Lactose	5	4	2	0	0
			5	5	3	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	5	2	1	0
			5	5	1	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
Day 9	Sample 1	Lactose	5	5	3	0	0
			5	4	4	1	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	5	1	0	0
			5	5	1	0	0
		EC	0	0	0	0	0
			0	0	0	0	0

Phase III: Fecal coliform Density in RN-45

Day	Sample	Medium	Dilution				
			10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
Day 0	Sample 1	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
	Sample 2	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
Day 1	Sample 1	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
	Sample 2	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
Day 2	Sample 1	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
	Sample 2	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5

Day	Sample	Medium	Dilution				
			10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
Day 3	Sample 1	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
	Sample 2	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
Day 4	Sample 1	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
	Sample 2	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
Day 5	Sample 1	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
	Sample 2	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5

Day	Sample	Medium	Dilution				
			10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
Day 6	Sample 1	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
	Sample 2	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
Day 7	Sample 1	Lactose	5	5	3	2	2
			5	5	3	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	5	4	0	0
			5	5	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
Day 8	Sample 1	Lactose	5	5	3	1	1
			5	5	4	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	5	2	0	0
			5	4	1	1	0
		EC	0	0	0	0	0
			0	0	0	0	0
Day 9	Sample 1	Lactose	5	5	3	0	0
			5	5	3	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	5	1	1	0
			5	4	3	0	0
		EC	0	0	0	0	0
			0	0	0	0	0

Phase III: Fecal coliform Density in RS-45

Day	Sample	Medium	Dilution				
Day 0			10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
	Sample 1	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
	Sample 2	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
Day 1	Sample 1	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
	Sample 2	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
Day 2	Sample 1	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
	Sample 2	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5

Day	Sample	Medium	Dilution				
			10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
Day 3	Sample 1	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
	Sample 2	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
Day 4	Sample 1	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
	Sample 2	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
Day 5	Sample 1	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
	Sample 2	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5

Day	Sample	Medium	Dilution 10 (-3)	10 (-4)	10 (-5)	10 (-6)	10 (-7)
Day 6	Sample 1	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
	Sample 2	Lactose	5	5	5	5	5
			5	5	5	5	5
		EC	5	5	5	5	5
			5	5	5	5	5
Day 7	Sample 1	Lactose	5	5	3	1	1
			5	5	4	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	4	4	1	0
			5	5	2	2	0
		EC	0	0	0	0	0
			0	0	0	0	0
Day 8	Sample 1	Lactose	5	5	1	1	1
			5	5	0	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	3	1	1	0
			5	5	3	1	0
		EC	0	0	0	0	0
			0	0	0	0	0
Day 9	Sample 1	Lactose	5	5	2	2	0
			5	5	3	0	0
		EC	0	0	0	0	0
			0	0	0	0	0
	Sample 2	Lactose	5	5	1	0	1
			5	5	1	0	0
		EC	0	0	0	0	0
			0	0	0	0	0

Phase III: TS and VS for RN-55

Day	Sample	No. of Crucible	Crucible	Crucible + Sample	After Burn	After Ignition
Day 0	Sample 1	76	89.6 g	110.99 g	99.3 g	93 g
	Sample 2	J-1	103.2 g	123.5 g	112.5 g	106.6 g
	Sample 3	J-4	91.3 g	108.1 g	99.3 g	94.1 g
Day 6	Sample 1	J-3	85.1 g	94.4 g	89.3 g	86.6 g
	Sample 2	56	90 g	102.9 g	96.1 g	92.6 g
	Sample 3	J-12	87.1 g	98.3 g	92.7 g	89 g
Day 9	Sample 1	50	90.7 g	100.5 g	95.5 g	92.4 g
	Sample 2	38	90.6 g	105.9 g	98.3 g	93.8 g
	Sample 3	2	93.1 g	103 g	97.7 g	94.8 g

Phase III: TS and VS for RS-55

Day	Sample	No. of Crucible	Crucible	Crucible + Sample	After Burn	After Ignition
Day 0	Sample 1	A-11	89.2 g	110.6 g	98.1 g	92.6 g
	Sample 2	1	90.3 g	111.9 g	99.5 g	93.6 g
	Sample 3	A-8	89.9 g	107.7 g	96.7 g	92.2 g
Day 6	Sample 1	45	83.1 g	95.6 g	88.5 g	85.5 g
	Sample 2	74	85.5 g	101.7 g	93.1 g	88.1 g
	Sample 3	17	86.9 g	98 g	91.4 g	88.6 g
Day 9	Sample 1	40	91.6 g	111.8 g	101.1 g	95.3 g
	Sample 2	25	92.2 g	119 g	105 g	97.6 g
	Sample 3	55	83.3 g	106 g	93.9 g	87.9 g

Phase III: TS and VS for RN-45

Day	Sample	No. of Crucible	Crucible	Crucible + Sample	After Burn	After Ignition
Day 0	Sample 1	2	93.1 g	101.1 g	97.5 g	94.1 g
	Sample 2	50	90.7 g	104.2 g	97.5 g	92.3 g
	Sample 3	38	90.6 g	100.4 g	96.3 g	92 g
Day 6	Sample 1	60	101.6 g	110.1 g	106.1 g	102.9 g
	Sample 2	7	89.3 g	96.9 g	93.7 g	90.1 g
	Sample 3	23	90.5 g	96.9 g	94 g	91.7 g
Day 9	Sample 1	A-11	89.1 g	99.6 g	95.3 g	90.7 g
	Sample 2	A-8	89.8 g	96.8 g	93.8 g	90.9 g
	Sample 3	A-15	90.3 g	95.4 g	93.2 g	91.1 g

Phase III: TS and VS for RS-45

Day	Sample	No. of Crucible	Crucible	Crucible + Sample	After Burn	After Ignition
Day 0	Sample 1	90	93.06 g	96.9 g	95.5 g	93.7 g
	Sample 2	37	90.61 g	94.8 g	93 g	91.3 g
	Sample 3	50	90.71 g	94.1 g	92.9 g	91.2 g
Day 6	Sample 1	J-6	89.1 g	95.7 g	93 g	90.1 g
	Sample 2	53	87.9 g	93.4 g	91.1 g	88.7 g
	Sample 3	51	81 g	92.3 g	87.3 g	82.9 g
Day 9	Sample 1	J-4	91.3 g	98.4 g	95.3 g	92.4 g
	Sample 2	J-17	91.7 g	97.6 g	94.8 g	92.9 g
	Sample 3	10	94.3 g	101.3 g	98.3 g	95.5 g

APPENDIX B.

CALCULATION OF FEEDSTOCK PREPARATION

Example 1:

If we want to prepare 3 kg of feedstock at a moisture content of 65%. The moisture content of recycled compost, biosolids, and leaves is 54%, 80%, and 27%, respectively. The ratio of recycled compost to biosolids and leaves is 0.5:1.

Solution:

$W_t = 3$ kg $M_t = 65\%$ $M_r = 54\%$ $M_b = 80\%$ $M_l = 27\%$ $R = 0.5:1$

1. Using Eq. 3-4:

$$W_r = \frac{R W_t}{1 + R} = \frac{0.5 \times 3}{1 + 0.5} = 1 \text{ kg}$$

2. Using Eq. 3-5:

$$W_l = \frac{W_t(M_b - M_t) + R W_t(M_r - M_t)}{(1 + R)(M_b - M_l)}$$

$$= \frac{3 \times (80\% - 65\%) + 0.5 \times 3 \times (54\% - 65\%)}{(1 + 0.5) \times (80\% - 27\%)}$$

$$= 0.36 \text{ kg}$$

3. Using Eq. 3-6:

$$W_b = W_t - W_r - W_l = 3 - 1 - 0.36 = 1.64 \text{ kg}$$

Example 2:

If we want to prepare 2 kg of feedstock at a moisture content of 55%. The moisture content of recycled compost, biosolids, and leaves is 54%, 80%, and 27%, respectively. The ratio of recycled compost to biosolids and leaves is 0.5:1.

Solution:

$W_t = 2$ kg $\quad M_t = 55\%$ $\quad M_r = 54\%$ $\quad M_b = 80\%$ $\quad M_l = 27\%$ $\quad R = 0.5:1$

1. Using Eq. 3-4:

$$W_r = \frac{R\,W_t}{1 + R} = \frac{0.5 \times 2}{1 + 0.5} = 0.67 \text{ kg}$$

2. Using Eq. 3-5:

$$W_l = \frac{W_t(M_b - M_t) + R\,W_t(M_r - M_t)}{(1 + R)(M_b - M_l)}$$

$$= \frac{2 \times (80\% - 55\%) + 0.5 \times 2 \times (54\% - 55\%)}{(1 + 0.5) \times (80\% - 27\%)}$$

$$= 0.62 \text{ kg}$$

3. Using Eq. 3-6:

$$W_b = W_t - W_r - W_l = 2 - 0.67 - 0.62 = 0.71 \text{ kg}$$

APPENDIX C.

FEEDING CONDITIONING OF SUBSTRATE USING AMENDMENT AND RECYCLED COMPOST

R45-1,2:

Biosolids:

$$BVS = 45\% \times 55\% \times 20\% \times 0.16 \text{ kg} = 0.0079 \text{ kg}$$

$$NBVS = (1 - 45\%) \times 55\% \times 20\% \times 0.16 \text{ kg} = 0.0097 \text{ kg}$$

$$ASH = (1 - 55\%) \times 20\% \times 0.16 \text{ kg} = 0.0144 \text{ kg}$$

$$WAT = 0.16 - 0.16 \times 20\% = 0.128 \text{ kg}$$

Leaves:

$$BVS = 71.5\% \times 88\% \times 73\% \times 0.51 \text{ kg} = 0.2343 \text{ kg}$$

$$NBVS = (1 - 71.5\%) \times 88\% \times 73\% \times 0.51 \text{ kg} = 0.0934 \text{ kg}$$

$$ASH = (1 - 88\%) \times 73\% \times 0.51 \text{ kg} = 0.0447 \text{ kg}$$

$$WAT = 0.51 - 0.51 \times 73\% = 0.1377 \text{ kg}$$

Recycled compost:

$$BVS = 0 \text{ kg}$$

$$NBVS = [(0.0097 + 0.0934)/(0.0097 + 0.0934 + 0.0144 + 0.0447)] \times 46\% \times 0.33 \text{ kg} = 0.0965 \text{ kg}$$

$$ASH = [(0.0144 + 0.0447)/(0.0097 + 0.0934 + 0.0144 + 0.0447)] \times 46\% \times 0.33 \text{ kg} = 0.0553 \text{ kg}$$

$$WAT = 0.33 - 0.33 \times 46\% = 0.1782 \text{ kg}$$

Mixture:

$$BVS = 0.0079 + 0.2343 = 0.2422 \text{ kg}$$

$$NBVS = 0.0097 + 0.0934 + 0.0965 = 0.1996 \text{ kg}$$

$$ASH = 0.0144 + 0.0447 + 0.0533 = 0.1144 \text{ kg}$$

$$WAT = 0.128 + 0.1377 + 0.1782 = 0.4439 \text{ kg}$$

R55-1,2, 31:

Biosolids:

$BVS = 45\% \times 55\% \times 20\% \times 0.72 \text{ kg} = 0.0356 \text{ kg}$

$NBVS = (1 - 45\%) \times 55\% \times 20\% \times 0.72 \text{ kg} = 0.0436 \text{ kg}$

$ASH = (1 - 55\%) \times 20\% \times 0.72 \text{ kg} = 0.0648 \text{ kg}$

$WAT = 0.72 - 0.72 \times 20\% = 0.576 \text{ kg}$

Leaves:

$BVS = 71.5\% \times 88\% \times 73\% \times 0.61 \text{ kg} = 0.2802 \text{ kg}$

$NBVS = (1 - 71.5\%) \times 88\% \times 73\% \times 0.61 \text{ kg} = 0.1117 \text{ kg}$

$ASH = (1 - 88\%) \times 73\% \times 0.61 \text{ kg} = 0.0534 \text{ kg}$

$WAT = 0.61 - 0.61 \times 73\% = 0.1634 \text{ kg}$

Recycled compost:

$BVS = 0 \text{ kg}$

$NBVS = [(0.0436 + 0.1117)/(0.0436 + 0.1117 + 0.0648 + 0.0534)] \times 46\% \times 0.67 \text{ kg}$
$= 0.1750 \text{ kg}$

$ASH = [(0.0648 + 0.0534)/(0.0436 + 0.1117 + 0.0648 + 0.0534)] \times 46\% \times 0.67 \text{ kg}$
$= 0.1332 \text{ kg}$

$WAT = 0.67 - 0.67 \times 46\% = 0.3618 \text{ kg}$

Mixture:

$BVS = 0.0356 + 0.2802 = 0.3158 \text{ kg}$

$NBVS = 0.0436 + 0.1117 + 0.1750 = 0.3303 \text{ kg}$

$ASH = 0.0648 + 0.0534 + 0.1332 = 0.2514 \text{ kg}$

$WAT = 0.576 + 0.1647 + 0.3618 = 1.1025 \text{ kg}$

R65-1,2:

Biosolids:

$$BVS = 45\% \times 55\% \times 20\% \times 1.60 \text{ kg} = 0.0792 \text{ kg}$$

$$NBVS = (1 - 45\%) \times 55\% \times 20\% \times 1.60 \text{ kg} = 0.0968 \text{ kg}$$

$$ASH = (1 - 55\%) \times 20\% \times 1.60 \text{ kg} = 0.1440 \text{ kg}$$

$$WAT = 1.60 - 1.60 \times 20\% = 1.2800 \text{ kg}$$

Leaves:

$$BVS = 71.5\% \times 88\% \times 73\% \times 0.40 \text{ kg} = 0.1837 \text{ kg}$$

$$NBVS = (1 - 71.5\%) \times 88\% \times 73\% \times 0.40 \text{ kg} = 0.0732 \text{ kg}$$

$$ASH = (1 - 88\%) \times 73\% \times 0.40 \text{ kg} = 0.0350 \text{ kg}$$

$$WAT = 0.40 - 0.40 \times 73\% = 0.1080 \text{ kg}$$

Recycled compost:

$$BVS = 0 \text{ kg}$$

$$NBVS = [(0.0968 + 0.732)/(0.0968 + 0.0732 + 0.1440 + 0.0350)] \times 46\% \times 1.00 \text{ kg}$$
$$= 0.2241 \text{ kg}$$

$$ASH = [(0.1440 + 0.0350)/(0.0968 + 0.0732 + 0.1440 + 0.0350)] \times 46\% \times 1.00 \text{ kg}$$
$$= 0.2359 \text{ kg}$$

$$WAT = 1.00 - 1.00 \times 46\% = 0.5400 \text{ kg}$$

Mixture:

$$BVS = 0.0792 + 0.1837 = 0.2629 \text{ kg}$$

$$NBVS = 0.0968 + 0.0732 + 0.2241 = 0.3941 \text{ kg}$$

$$ASH = 0.1440 + 0.0350 + 0.2359 = 0.4149 \text{ kg}$$

$$WAT = 1.2800 + 0.1080 + 0.5400 = 1.9280 \text{ kg}$$

R55-32:

Biosolids:

$$BVS = 45\% \times 55\% \times 20\% \times 0.62\text{ kg} = 0.0307\text{ kg}$$
$$NBVS = (1 - 45\%) \times 55\% \times 20\% \times 0.62\text{ kg} = 0.0375\text{ kg}$$
$$ASH = (1 - 55\%) \times 20\% \times 0.62\text{ kg} = 0.0558\text{ kg}$$
$$WAT = 0.62 - 0.62 \times 20\% = 0.496\text{ kg}$$

Leaves:

$$BVS = 71.5\% \times 88\% \times 73\% \times 0.49\text{ kg} = 0.2251\text{ kg}$$
$$NBVS = (1 - 71.5\%) \times 88\% \times 73\% \times 0.49\text{ kg} = 0.0897\text{ kg}$$
$$ASH = (1 - 88\%) \times 73\% \times 0.49\text{ kg} = 0.0429\text{ kg}$$
$$WAT = 0.49 - 0.49 \times 73\% = 0.1323\text{ kg}$$

Recycled compost:

$$BVS = 0\text{ kg}$$
$$NBVS = [(0.0375 + 0.897)/(0.0375 + 0.0897 + 0.0558 + 0.0429)] \times 46\% \times 0.89\text{ kg} = 0.2305\text{ kg}$$
$$ASH = [(0.0558 + 0.0429)/(0.0375 + 0.0897 + 0.0558 + 0.0429)] \times 46\% \times 0.89\text{ kg} = 0.1789\text{ kg}$$
$$WAT = 0.89 - 0.89 \times 46\% = 0.4806\text{ kg}$$

Mixture:

$$BVS = 0.0307 + 0.2251 = 0.2558\text{ kg}$$
$$NBVS = 0.0375 + 0.0897 + 0.2305 = 0.3577\text{ kg}$$
$$ASH = 0.0558 + 0.0429 + 0.1789 = 0.2776\text{ kg}$$
$$WAT = 0.496 + 0.1323 + 0.4806 = 1.1089\text{ kg}$$

RN-55:

Biosolids:

$BVS = 45\% \times 55\% \times 20\% \times 0.36 \text{ kg} = 0.0178 \text{ kg}$

$NBVS = (1 - 45\%) \times 55\% \times 20\% \times 0.36 \text{ kg} = 0.0218 \text{ kg}$

$ASH = (1 - 55\%) \times 20\% \times 0.36 \text{ kg} = 0.0324 \text{ kg}$

$WAT = 0.36 - 0.36 \times 20\% = 0.288 \text{ kg}$

Leaves:

$BVS = 71.5\% \times 79\% \times 75\% \times 0.35 \text{ kg} = 0.1483 \text{ kg}$

$NBVS = (1 - 71.5\%) \times 79\% \times 75\% \times 0.35 \text{ kg} = 0.0591 \text{ kg}$

$ASH = (1 - 79\%) \times 75\% \times 0.35 \text{ kg} = 0.0551 \text{ kg}$

$WAT = 0.35 - 0.35 \times 75\% = 0.0875 \text{ kg}$

Recycled compost:

$BVS = 0 \text{ kg}$

$NBVS = [(0.0218 + 0.0591)/(0.0218 + 0.0591 + 0.0324 + 0.0551)] \times 43\% \times 0.35 \text{ kg}$
$= 0.0723 \text{ kg}$

$ASH = [(0.0324 + 0.0551)/(0.0218 + 0.0591 + 0.0324 + 0.0551)] \times 43\% \times 0.35 \text{ kg}$
$= 0.0782 \text{ kg}$

$WAT = 0.35 - 0.35 \times 43\% = 0.1995 \text{ kg}$

Mixture:

$BVS = 0.0178 + 0.1483 = 0.1661 \text{ kg}$

$NBVS = 0.0218 + 0.0591 + 0.0723 = 0.1532 \text{ kg}$

$ASH = 0.0324 + 0.0551 + 0.0782 = 0.1657 \text{ kg}$

$WAT = 0.288 + 0.0875 + 0.1995 = 0.575 \text{ kg}$

RS-55:

Biosolids:

$$BVS = 45\% \times 55\% \times 20\% \times 0.48 \text{ kg} = 0.0238 \text{ kg}$$

$$NBVS = (1 - 45\%) \times 55\% \times 20\% \times 0.48 \text{ kg} = 0.0290 \text{ kg}$$

$$ASH = (1 - 55\%) \times 20\% \times 0.48 \text{ kg} = 0.0432 \text{ kg}$$

$$WAT = 0.48 - 0.48 \times 20\% = 0.384 \text{ kg}$$

Leaves:

$$BVS = 71.5\% \times 81\% \times 75\% \times 0.26 \text{ kg} = 0.1129 \text{ kg}$$

$$NBVS = (1 - 71.5\%) \times 81\% \times 75\% \times 0.26 \text{ kg} = 0.045 \text{ kg}$$

$$ASH = (1 - 81\%) \times 75\% \times 0.26 \text{ kg} = 0.0371 \text{ kg}$$

$$WAT = 0.26 - 0.26 \times 75\% = 0.065 \text{ kg}$$

Recycled compost:

$$BVS = 0 \text{ kg}$$

$$NBVS = [(0.029 + 0.045)/(0.029 + 0.045 + 0.0432 + 0.0371)] \times 45\% \times 0.37 \text{ kg} = 0.0799 \text{ kg}$$

$$ASH = [(0.0432 + 0.0371)/(0.029 + 0.045 + 0.0432 + 0.0371)] \times 45\% \times 0.37 \text{ kg} = 0.0866 \text{ kg}$$

$$WAT = 0.37 - 0.37 \times 45\% = 0.2035 \text{ kg}$$

Mixture:

$$BVS = 0.0238 + 0.1129 = 0.1367 \text{ kg}$$

$$NBVS = 0.029 + 0.045 + 0.0799 = 0.1539 \text{ kg}$$

$$ASH = 0.0432 + 0.0371 + 0.0866 = 0.1669 \text{ kg}$$

$$WAT = 0.384 + 0.065 + 0.2035 = 0.6525 \text{ kg}$$

RN-45:

Biosolids:

$$BVS = 45\% \times 55\% \times 20\% \times 0.12kg = 0.0059 \text{ kg}$$

$$NBVS = (1 - 45\%) \times 55\% \times 20\% \times 0.12 \text{ kg} = 0.0073 \text{ kg}$$

$$ASH = (1 - 55\%) \times 20\% \times 0.12 \text{ kg} = 0.0108 \text{ kg}$$

$$WAT = 0.12 - 0.12 \times 20\% = 0.096 \text{ kg}$$

Leaves:

$$BVS = 71.5\% \times 85\% \times 75\% \times 0.33 \text{ kg} = 0.1504 \text{ kg}$$

$$NBVS = (1 - 71.5\%) \times 85\% \times 75\% \times 0.33 \text{ kg} = 0.06 \text{ kg}$$

$$ASH = (1 - 85\%) \times 75\% \times 0.33 \text{ kg} = 0.0371 \text{ kg}$$

$$WAT = 0.33 - 0.33 \times 75\% = 0.0825 \text{ kg}$$

Recycled compost:

$$BVS = 0 \text{ kg}$$

$$NBVS = [(0.0073 + 0.06)/(0.0073 + 0.06 + 0.0108 + 0.0371)] \times 43\% \times 0.22 \text{ kg} = 0.0553 \text{ kg}$$

$$ASH = [(0.0108 + 0.0371)/(0.0073 + 0.06 + 0.0108 + 0.0371)] \times 43\% \times 0.22 \text{ kg} = 0.0393 \text{ kg}$$

$$WAT = 0.22 - 0.22 \times 43\% = 0.1254 \text{ kg}$$

Mixture:

$$BVS = 0.0059 + 0.1504 = 0.1563 \text{ kg}$$

$$NBVS = 0.0073 + 0.06 + 0.0553 = 0.1226 \text{ kg}$$

$$ASH = 0.0108 + 0.0371 + 0.0393 = 0.0872 \text{ kg}$$

$$WAT = 0.096 + 0.0825 + 0.1254 = 0.3039 \text{ kg}$$

RS-45:

Biosolids:

$$BVS = 45\% \times 55\% \times 20\% \times 0.04 \text{ kg} = 0.002 \text{ kg}$$

$$NBVS = (1 - 45\%) \times 55\% \times 20\% \times 0.04 \text{ kg} = 0.0024 \text{ kg}$$

$$ASH = (1 - 55\%) \times 20\% \times 0.04 \text{ kg} = 0.0036 \text{ kg}$$

$$WAT = 0.04 - 0.04 \times 20\% = 0.032 \text{ kg}$$

Leaves:

$$BVS = 71.5\% \times 82\% \times 75\% \times 0.41 \text{ kg} = 0.1803 \text{ kg}$$

$$NBVS = (1 - 71.5\%) \times 82\% \times 75\% \times 0.41 \text{ kg} = 0.0791 \text{ kg}$$

$$ASH = (1 - 82\%) \times 75\% \times 0.41 \text{ kg} = 0.0554 \text{ kg}$$

$$WAT = 0.41 - 0.41 \times 75\% = 0.1025 \text{ kg}$$

Recycled compost:

$$BVS = 0 \text{ kg}$$

$$NBVS = [(0.0024 + 0.0719)/(0.0024 + 0.0719 + 0.0036 + 0.0554)] \times 45\% \times 0.22 \text{ kg} = 0.0552 \text{ kg}$$

$$ASH = [(0.0036 + 0.0554)/(0.0024 + 0.0719 + 0.0036 + 0.0554)] \times 45\% \times 0.22 \text{ kg} = 0.0438 \text{ kg}$$

$$WAT = 0.22 - 0.22 \times 45\% = 0.121 \text{ kg}$$

Mixture:

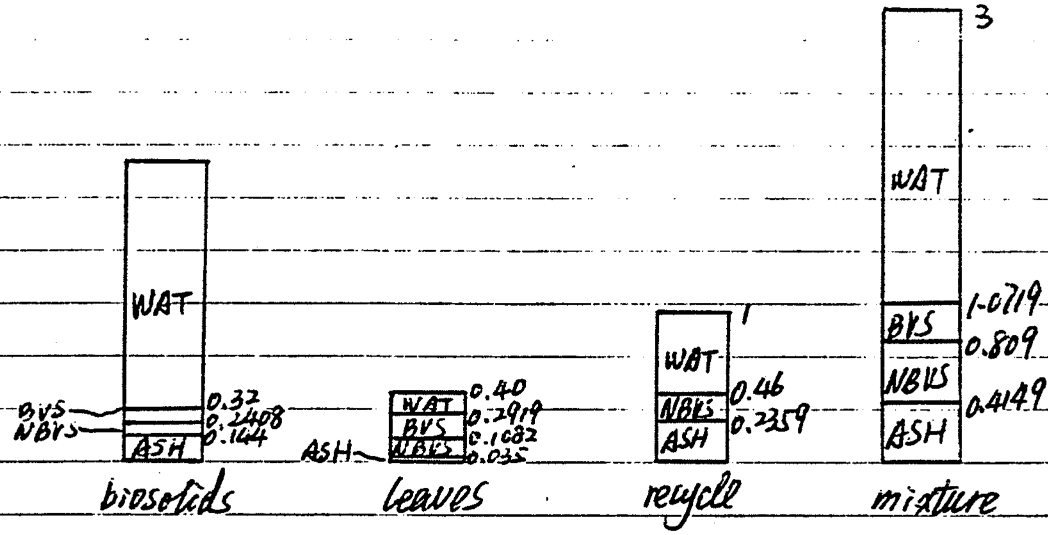
$$BVS = 0.002 + 0.1803 = 0.1823 \text{ kg}$$

$$NBVS = 0.0024 + 0.0719 + 0.0552 = 0.1295 \text{ kg}$$

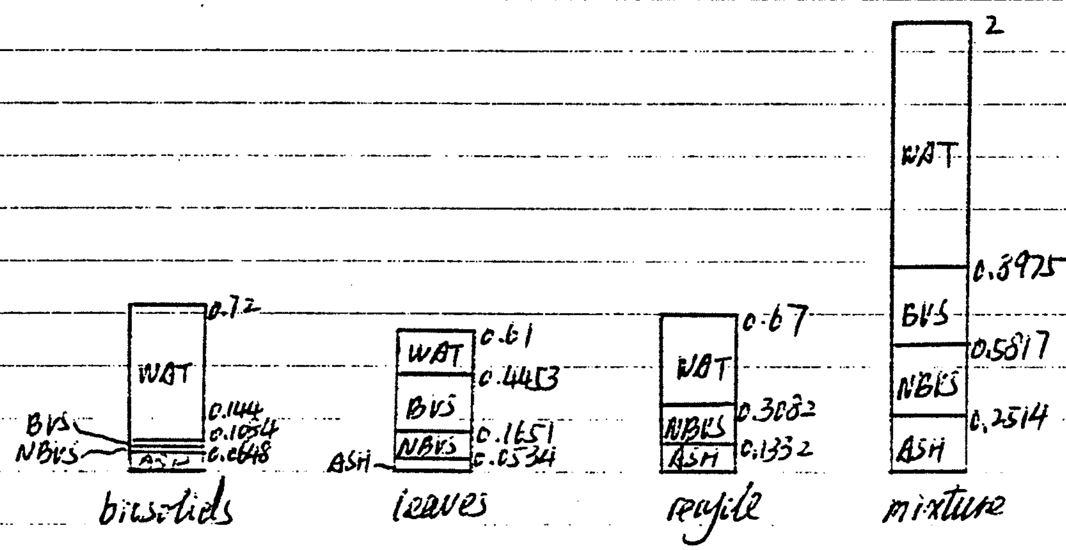
$$ASH = 0.0036 + 0.0438 + 0.0554 = 0.1028 \text{ kg}$$

$$WAT = 0.032 + 0.1025 + 0.121 = 0.2555 \text{ kg}$$

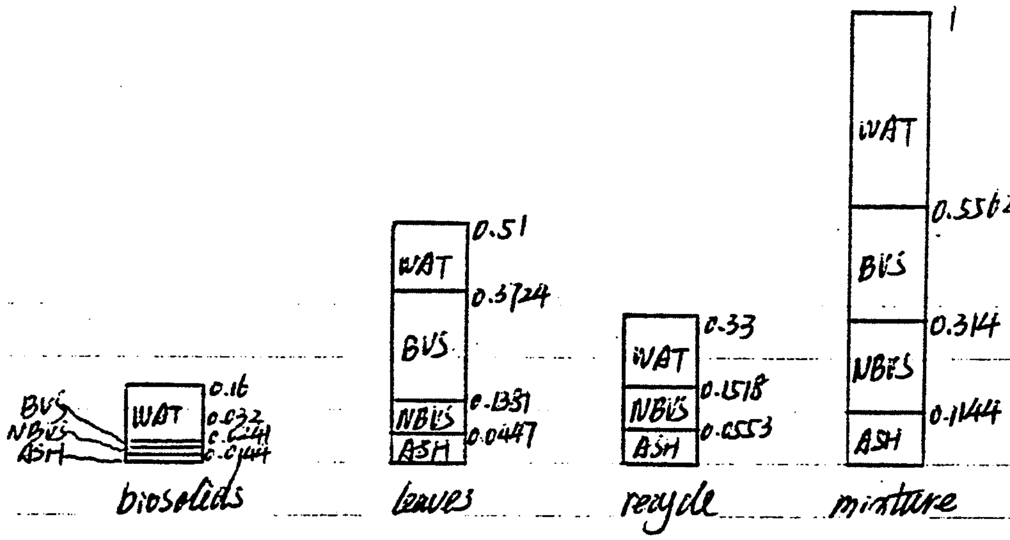
R65-1,2:


WAT
BVS
NBVS
ASH
0.32
0.2408
0.144
biosolids
WAT
BVS
NBVS
ASH
0.40
0.2919
0.1082
0.035
leaves
WAT
NBVS
ASH
1
0.46
0.2359
recycle
3
WAT
BVS
NBVS
ASH
1.0719
0.809
0.4149
mixture

R55-1,2:

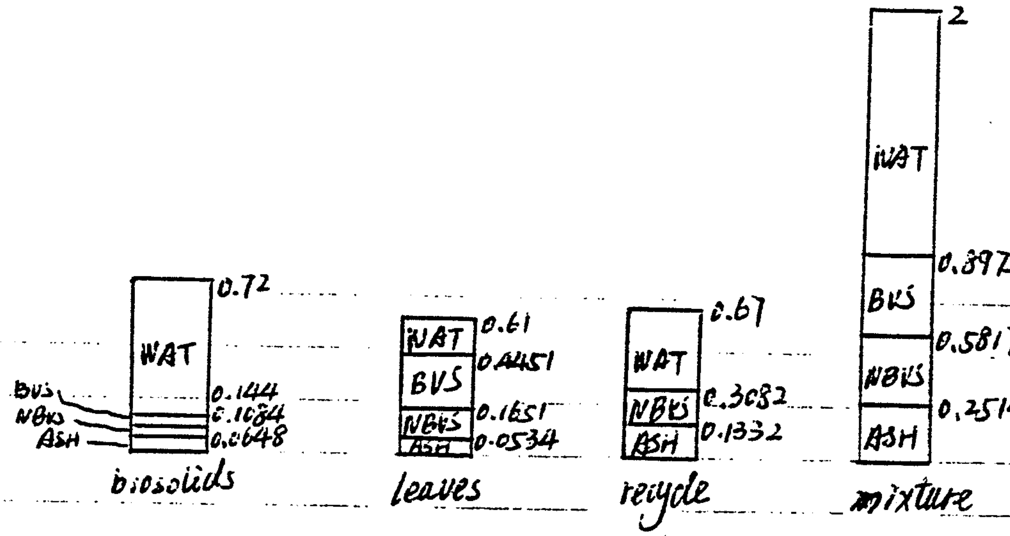

WAT
BVS
NBVS
ASH
0.72
0.144
0.1054
0.0648
biosolids
WAT
BVS
NBVS
ASH
0.61
0.4453
0.1651
0.0534
leaves
WAT
NBVS
ASH
0.67
0.3082
0.1332
recycle
2
WAT
BVS
NBVS
ASH
0.8975
0.5817
0.2514
mixture

R45-1,2:

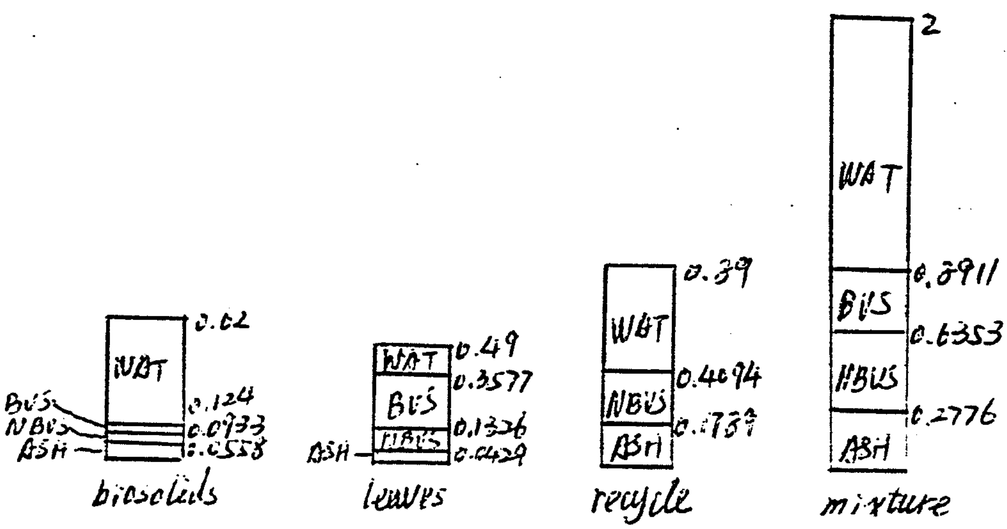


BVS
NBVS
ASH
WAT
0.16
0.032
0.0241
0.0144
biosolids
WAT
0.51
0.3724
BVS
0.1331
NBVS
0.0447
ASH
leaves
WAT
0.33
0.1518
NBVS
0.0553
ASH
recycle
1
WAT
0.5562
BVS
0.314
NBVS
0.1144
ASH
mixture

R55-31.

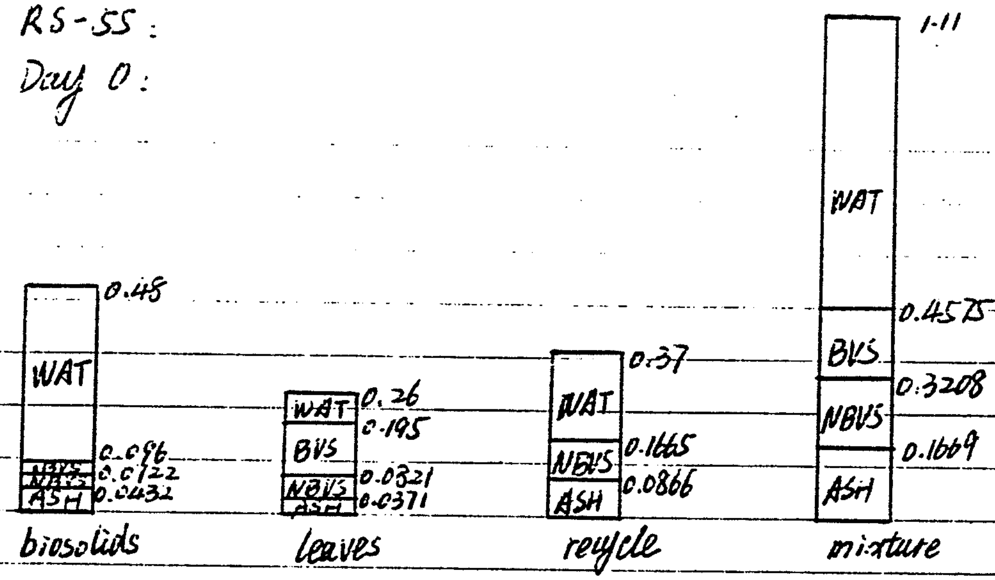

WAT
0.72
BVS
NBVS
ASH
0.144
0.1084
0.0648
biosolids
WAT
BVS
NBVS
ASH
0.61
0.4451
0.1651
0.0534
leaves
WAT
NBVS
ASH
0.67
0.3082
0.1332
recycle
2
WAT
BVS
NBVS
ASH
0.3975
0.5817
0.2514
mixture

R55-32.


WAT
0.62
BVS
NBVS
ASH
0.124
0.0733
0.0558
biosolids
WAT
BVS
NBVS
ASH
0.49
0.3577
0.1336
0.0429
leaves
WAT
NBVS
ASH
0.39
0.4094
recycle
2
WAT
BVS
NBVS
ASH
0.3911
0.6353
0.2776
mixture

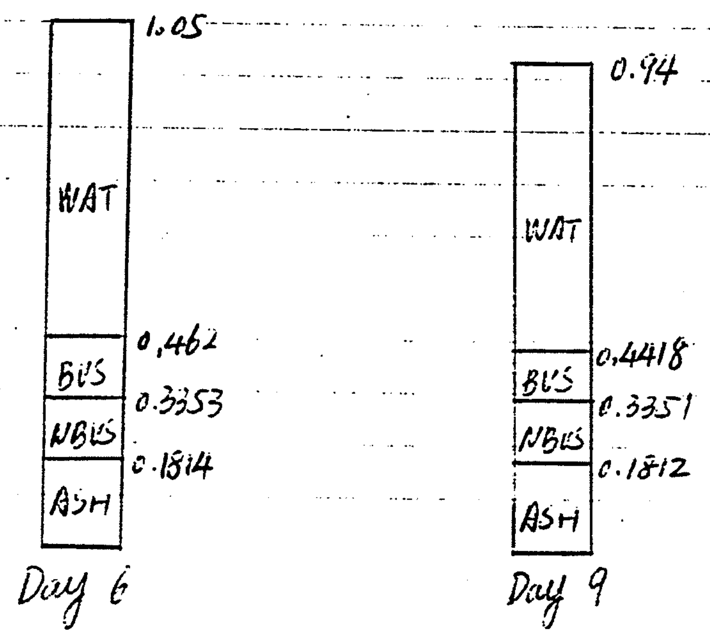
RS-55:

Day 0:



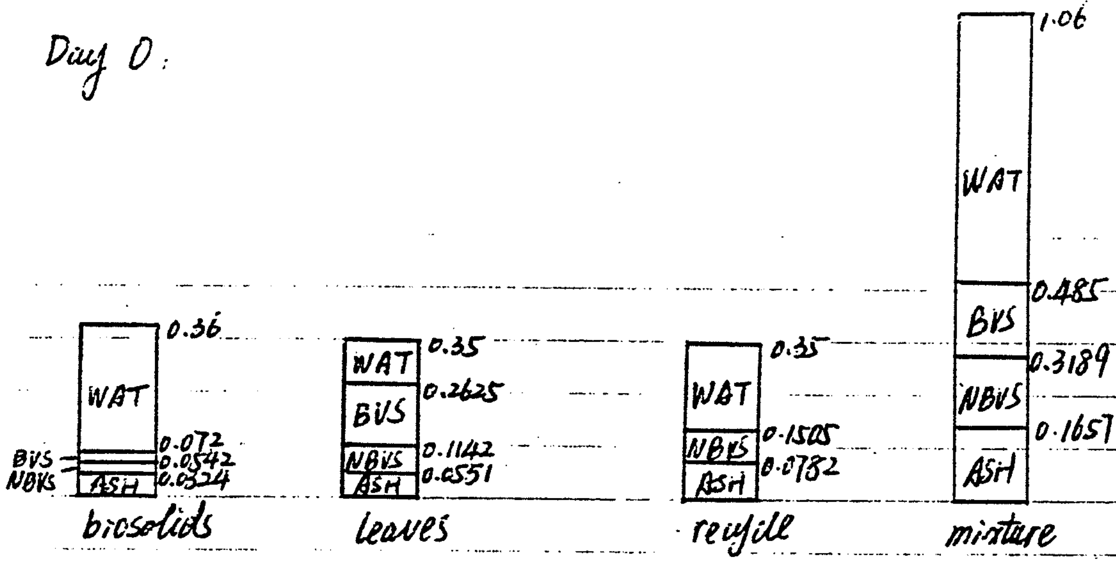
Day 6: NBVS = 0.1539 kg, WAT = 0.588 kg. BVS = 0.1267 kg
WAT = 0.1814 kg

Day 9: NBVS = 0.1539 kg, WAT = 0.4982 kg. BVS = 0.1067 kg
WAT = 0 1812 kg



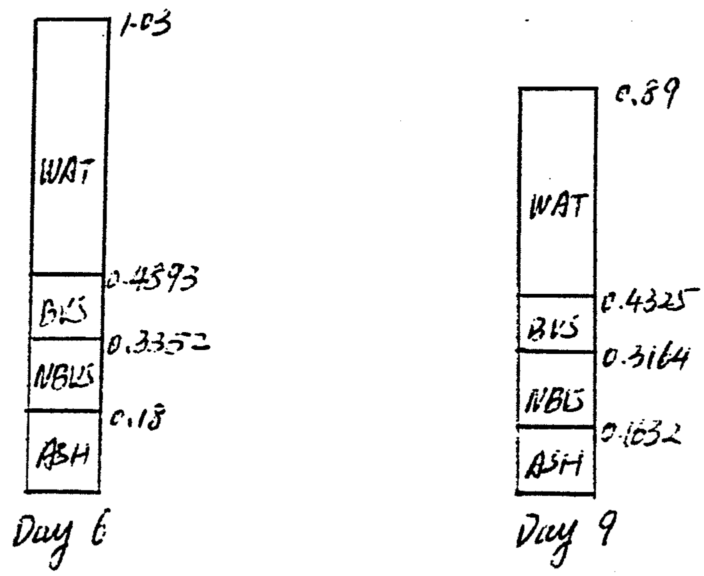
RN-55:

Day 0:

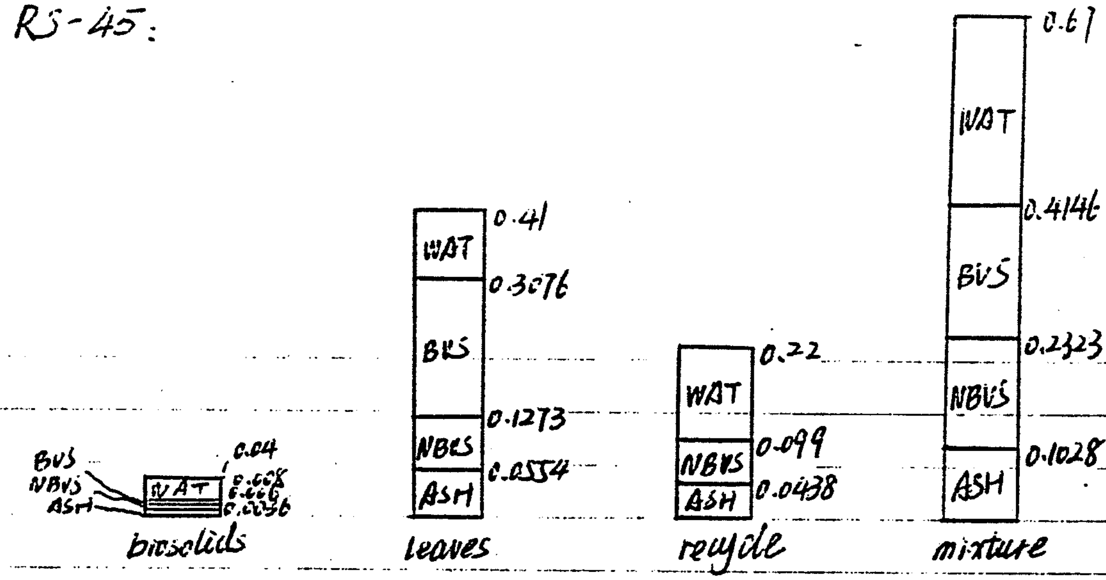


Day 6: NBVS=0.1532 Kg, WAT=0.5407 Kg. BVS=0.1561 Kg. ASH=0.18 Kg

Day 9: NBVS=0.1532 Kg. WAT=0.4575 Kg, BVS=0.1161 Kg. ASH=0.1632 Kg

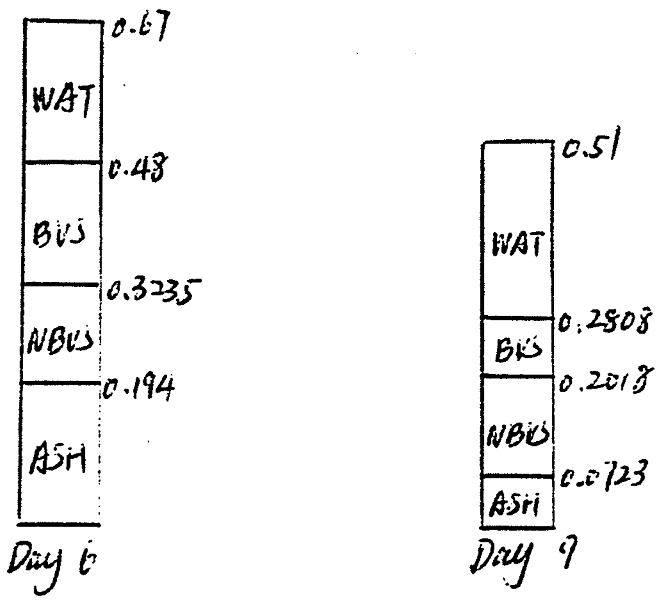


RS-45:



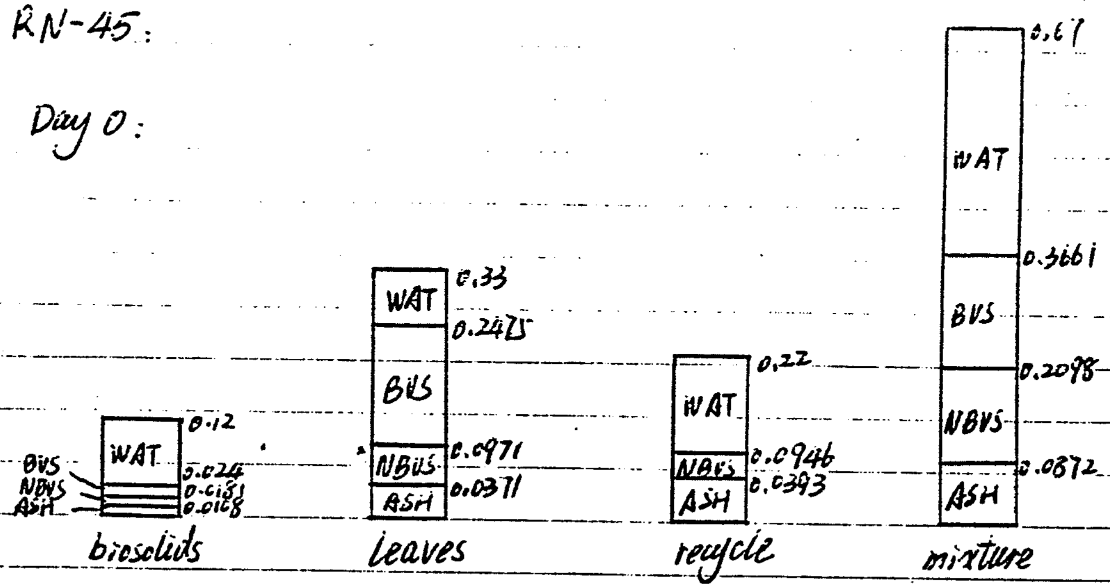
Day 6: NBVS = 0.1295, WAT = 0.19 kg, BVS = 0.1565 kg, ASH = 0.194 kg

Day 9: NBVS = 0.1295 kg, WAT = 0.2295 kg, BVS = 0.079 kg, ASH = 0.0723 kg



RN-45:

Day 0:



Day 6: NBVS=0.1226 kg, WAT=0.3039 kg, BVS=0.1463 kg, ASH=0.0972 kg

Day 9: NBVS=0.1226 kg. WAT=0.22 kg. BVS=0.0963 kg. ASH=0.0811 kg