THE RELATIONSHIP BETWEEN TEMPERATURE REGIME AND PATHOGEN KILL IN COMPOSTING AND ASSESSING EXPOSURE OF COMPOST WORKERS TO AIRBORNE BIOHAZARDS

by

Xi Zhang

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF APPLIED SCIENCE

in

The Faculty of Graduate Studies
(Civil Engineering)

THE UNIVERSITY OF BRITISH COLUMBIA
(Vancouver)

October, 2008
© Xi Zhang, 2008
ABSTRACT

The study has been conducted in three parts: 1. Collect both temporal and spatial thermal data; 2. Quantify the number of organisms of interest in compost samples; 3. Collect and analyse bioaerosol samples to test the workers' exposure to organisms of concern.

The study obtained more detailed and extensive data of both spatial and temporal temperature distributions in real, operational compost piles than any previous studies. By using data loggers allocated at every part of the pile (approximately surrogate of each 0.5 m³ material) and continuously record temperatures from building the new pile till screening. A series of findings have been achieved from the intensive temperature monitoring, such as how differently material at different positions could behave in terms of temperature; how physical parameters (particle size, porosity etc.) of the material could influence the process and so on.

Furthermore, the study also correlated the time-temperature regime with level of pathogen-kill for a full-scale operational composting practice. Total coliforms, Thermo-tolerant coliforms, *E.coli* and *Enterococci* were confirmed and quantified. Significant destruction of microbial organisms was observed during composting based on the performance of *Enterococci*. Some limitations about the test methods have been found. Also, some other factors such as temperature fluctuation, occurrence of re-growth, interference of environmental organisms etc. all made the composting microbiology a complex issue.

Besides studying the bacteria in the pile, effort has also been given to investigating exposures of how the airborne bacteria from handling the compost to workers at the site. It has been found that the cab can well protect the workers from being exposed to the bioaerosols; precipitation could significantly reduce the number of airborne bacteria; turning and screening the pile do result in relatively high concentration of bioaerosols while the influence on upwind and downwind area from composting facility is negligible; also the presence of airborne bacteria does show some consistency with the performance of bacteria in the feedstock over time.

In addition, moisture content and nutrients (C, N) concentration analysis were determined on the compost samples. The weather conditions had been monitored continuously during the whole process.
TABLE OF CONTENTS

ABSTRACT ......................................................................................................................... ii
TABLE OF CONTENTS ........................................................................................................ iii
LIST OF TABLES ................................................................................................................... vi
LIST OF FIGURES ................................................................................................................. vii
ACKNOWLEDGEMENTS ...................................................................................................... ix
CHAPTER 1 INTRODUCTION ............................................................................................. 1

CHAPTER 2 LITERATURE REVIEW .................................................................................. 4
  2.1 Composting Principle ................................................................................................. 4
  2.2 Compost Feedstocks ................................................................................................. 5
  2.3 Compost Methods ..................................................................................................... 5
  2.4 Compost Regulations ............................................................................................... 6
    2.4.1 Trends and Comparison in Regulation All Over the World .................................. 8
  2.5 Composting Process ................................................................................................. 9
  2.6 Temperature of compost ........................................................................................ 10
    2.6.1 Debate on optimal temperature for composting ................................................. 10
    2.6.2 Temperature distribution .................................................................................... 11
  2.7 Moisture .................................................................................................................... 12
  2.8 Nutrient balance-carbon/nitrogen ratio .................................................................. 12
  2.8 pH ............................................................................................................................ 13
  2.9 Windrow size ............................................................................................................ 13
  2.10 Turning .................................................................................................................... 14
  2.11 Seasonal variability ................................................................................................. 15
  2.12 Composting microbiology ...................................................................................... 15
    2.12.1 Bacteria ........................................................................................................... 16
    2.12.2 Actinomycetes ................................................................................................. 17
    2.12.3 Fungi ............................................................................................................... 17
    2.12.4 Coliforms ......................................................................................................... 18
    2.12.5 Re-growth ......................................................................................................... 18
    2.12.6 Exploration on possible reasons for high-temperature survival ....................... 19
      2.12.6.1 Dissemination of thermal tolerance .......................................................... 19
      2.12.6.2 Mutation ................................................................................................... 19
      2.12.6.3 Genus Thermus ....................................................................................... 19
      2.12.6.4 Physical conditions ................................................................................... 20
  2.13 Inadequacy of previous data and findings ............................................................... 21
    2.13.1 Lack of information on the spatial and temporal temperature regime ............. 21
    2.13.2 Lack of information on linking the temperature regime to pathogen killing .... 21
  2.14 Bioaerosols .............................................................................................................. 22
    2.14.1 Endotoxin ......................................................................................................... 23
    2.14.2 β (1-3) glucan ................................................................................................. 23
    2.14.3 Aspergillus ....................................................................................................... 23
    2.14.4 Thermo-actinomycetes .................................................................................... 24
    2.14.5 Previous findings ............................................................................................. 24
    2.14.6 How turning may affect the bioaerosols from the perspective of kinetic ........ 24
    2.14.7 Downwind conclusion ...................................................................................... 25
    2.14.8 Andersen Sampler ............................................................................................ 25

CHAPTER 3 MATERIAL AND METHODS ................................................................................. 29
  3.1 Nutrients analysis ..................................................................................................... 29
3.2 Moisture content ......................................................................................................................................................... 29
3.3 Temperature Monitoring .................................................................................................................................................. 31
  3.3.1 The Smartbutton data logger ....................................................................................................................................... 31
  3.3.2 Pile construction ......................................................................................................................................................... 33
3.4 Bacteria Analysis ............................................................................................................................................................... 36
  3.4.1 Sample preparation ...................................................................................................................................................... 37
  3.4.2 Total Coliforms ............................................................................................................................................................ 38
  3.4.3 Thermo-tolerant Coliforms ......................................................................................................................................... 42
  3.4.4 E. Coli ........................................................................................................................................................................ 42
  3.4.5 Enterococcus ............................................................................................................................................................... 43
  3.4.6 Calculation .................................................................................................................................................................. 45
3.5 Bioaerosols Analysis ............................................................................................................................................................. 47
  3.5.1 Personal Sampling ....................................................................................................................................................... 47
  3.5.2 Andersen Sampling ...................................................................................................................................................... 49
3.6 Weather monitoring ............................................................................................................................................................. 51

CHAPTER 4 RESULTS AND DISCUSSION ............................................................................................................................. 52
4.1 Physical appearance of feedstock ....................................................................................................................................... 52
4.2 Physical Dimensions of Piles ............................................................................................................................................... 52
4.3 Moisture content ................................................................................................................................................................. 52
4.4 Nutrients (C:N ratio) ........................................................................................................................................................... 53
4.5 Temperature ......................................................................................................................................................................... 55
  4.5.1 Pile 1 .............................................................................................................................................................................. 56
   4.5.1.1 From start to the 1st turn .......................................................................................................................................... 56
   4.5.1.2 Demonstration of temperatures from data loggers in-situ at every layer for pile 1 .............................................. 62
   4.5.1.3 What can be seen from a 3D video demonstration ................................................................................................. 64
   4.5.1.4 From start to finish ................................................................................................................................................. 66
  4.5.2 Pile 2 .............................................................................................................................................................................. 68
   4.5.2.1 From start to the 1st turn .......................................................................................................................................... 68
   4.5.2.2 Demonstration of temperatures from data loggers in-situ at each layer for pile 2 .............................................. 75
   4.5.2.3 From start to finish ................................................................................................................................................. 76
  4.5.3 What can be found from the weekly average temperature profile ................................................................................... 78
   4.5.3.1 Pile 1 ........................................................................................................................................................................ 78
   4.5.3.2 Pile 2 ........................................................................................................................................................................ 79
   4.5.3.3 How the mean could be biased ................................................................................................................................. 80
  4.5.4 Highest temperature ..................................................................................................................................................... 82
  4.5.5 Comparison of average temperature for 5 positions between the two piles ................................................................. 83
   4.5.5.1 Comparison between two piles based on same positions ...................................................................................... 83
   4.5.5.2 Comparison among five positions within one individual pile .............................................................................. 84
  4.5.6 Each individual logger’s performance during a single day time ...................................................................................... 85
  4.5.7 Significance of the temperature monitoring study .................................................................................................... 86
4.6 Bacterial Analysis ................................................................................................................................................................. 88
  4.6.1 Performance of bacteria of interest at different stages in pile 1 ....................................................................................... 89
  4.6.2 Performance of bacteria of interest at different stages in pile 2 ....................................................................................... 91
  4.6.3 What can be found from the average temperature vs bacteria counts in pile 1 ............................................................... 93
  4.6.4 What can be found from total average temperature vs TC counts in pile 2 ................................................................. 95
  4.6.5 What can be found from the highest temperature vs bacteria counts ........................................................................... 96
  4.6.6 What can be found from the number of days staying above 55°C vs bacteria counts .............................................. 98
  4.6.7 What can be found from the log distributions of bacteria ............................................................................................ 100

CHAPTER 5 BIOAEROSOLS ANALYSIS ....................................................................................................................................... 102
**LIST OF TABLES**

Table 2-1 Major species of Actinomycetes, Fungi and Bacteria ........................................... 16
Table 3-1 SmartButton Specifications ....................................................................................... 31
Table 3-2 List of diameter ranges of particulate collected by stages ........................................ 50
Table 4-1 Number of samples analyzed for nutrients ............................................................... 54
Table 4-2 Number of loggers grouped for pile 1 from start to 1st turn ..................................... 56
Table 4-3 Number of loggers recovered and embedded for pile 1 from start to finish ............ 67
Table 4-4 Number of loggers grouped for pile 2 from start to 1st turn ..................................... 68
Table 4-5 Number of loggers embedded and recovered for pile 2 from start to finish ............ 77
Table 4-6 Number of samples analyzed for both piles ............................................................ 88
Table 5-1 Number of samples by tasks ..................................................................................... 107
Table 5-2 Number of samples by stages for pile 1 .................................................................. 109
Table 5-3 Number of samples at four stages for pile 2 .......................................................... 112
LIST OF FIGURES

Figure 1-1 Composting facility at VLF ................................................................. 2
Figure 2-1 Generalized representation of the composting process .......................... 4
Figure 2-2 Andersen six-stage sampler ................................................................. 26
Figure 2-3 Hazardous range of the six stages ....................................................... 27
Figure 3-1 The ceramic dish. ................................................................................. 30
Figure 3-2 Dishes ready to be used ...................................................................... 30
Figure 3-3 The oven used for getting moisture content ......................................... 30
Figure 3-4 The inside of the oven ......................................................................... 30
Figure 3-5 ACR Smartbutton .............................................................................. 31
Figure 3-6 The Smartbutton reader ..................................................................... 32
Figure 3-7 A bunch of loggers in painted and labelled PVC casing ......................... 33
Figure 3-8 New pile is built up by a front-end loader ............................................ 34
Figure 3-9 Schematic demonstration on placement of data loggers and construction of pile .... 35
Figure 3-10 Placement of loggers in pile 1 ............................................................ 35
Figure 3-11 Placement of loggers in pile 2 ............................................................ 35
Figure 3-12 A data logger in-situ ......................................................................... 36
Figure 3-13 Demonstration of how the serial dilution was done and working principle of MPN 38
Figure 3-14 Quantification and confirmation of Coliforms ..................................... 39
Figure 3-15 Comparison of a positive tube with a negative one ............................. 40
Figure 3-16 Laminar Flow Hood ....................................................................... 41
Figure 3-17 Positive colonies on MacConkey plate ............................................. 41
Figure 3-18 Tubes which fluoresce ..................................................................... 43
Figure 3-19 Quantification and confirmation of Enterococci ............................... 44
Figure 3-20 Positive colonies on mEnt plate ....................................................... 45
Figure 3-21 Personal sampling pump ................................................................. 47
Figure 3-22 Seven-hole sampling cassette with filter in ....................................... 47
Figure 3-23 Filters ready to be weighed and used ............................................... 48
Figure 3-24 Positive colonies on MEA plate ....................................................... 49
Figure 3-25 Positive colonies on TSA plate ....................................................... 49
Figure 3-26 Taking sample with Andersen sampler on site .................................. 50
Figure 4-1 Moisture content VS composting stages for Pile 1 and 2 ................. 53
Figure 4-2 C:N ratio vs stages for both piles ...................................................... 54
Figure 4-3 Cross Sectional View of the placement of loggers ............................. 55
Figure 4-4 Temperature VS time at 5 positions of pile 1 from start to 1st turn .... 57
Figure 4-5 Temperature profile with weekly standard deviation for position 1, pile 1 58
Figure 4-6 Temperature profile with weekly standard deviation for position 2, pile 1 ... 58
Figure 4-7 Temperature profile with weekly standard deviation for position 3, pile 1 ... 59
Figure 4-8 Temperature profile with weekly standard deviation for position 4, pile 1 ... 59
Figure 4-9 Temperature profile with weekly standard deviation for position 5, pile 1 ... 60
Figure 4-10 Rain profile for pile 1 with matching event .................................... 60
Figure 4-11 Temperatures from data loggers in-situ for pile 1 layer 1 one month after the start. 62
Figure 4-12 Temperatures from data loggers in-situ for pile 1 layer 2 one month after the start. 62
Figure 4-13 Temperatures from data loggers in-situ for pile 1 layer 3 one month after the start. 63
Figure 4-14 First five days temperature profile for logger #86 ............................ 64
Figure 4-15 Captured from the 3D video about 15 days after construction of the pile ... 65
Figure 4-16 Captured from the 3D video about 30 days after construction of the pile .... 66
Figure 4-17 Temperature VS time at 5 positions of pile 1 from start to finish ....... 67
Figure 4-18 Temperature VS time at 5 positions of pile 2 from start to 1st turn ....... 69
Figure 4-19 Temperature profile with weekly standard deviation for position 1, pile 2
Figure 4-20 Temperature profile with weekly standard deviation for position 2, pile 2
Figure 4-21 Temperature profile with weekly standard deviation for position 3, pile 2
Figure 4-22 Temperature profile with weekly standard deviation for position 4, pile 2
Figure 4-23 Temperature profile with weekly standard deviation for position 5, pile 2
Figure 4-24 Temperature VS time at position 2 of pile 2 from start to 1st turn
Figure 4-25 Rain profile for pile 1 with matching event
Figure 4-26 Temperatures from data loggers in-situ for pile 2 layer 1
Figure 4-27 Temperatures from data loggers in-situ for pile 2 layer 2
Figure 4-28 Temperatures from data loggers in-situ for pile 2 layer 3
Figure 4-29 Temperature VS time at 5 positions of pile 2 from start to finish
Figure 4-30 Weekly average temperature of pile 1
Figure 4-31 Weekly average temperature of pile 2
Figure 4-32 Two loggers' profiles to show how the mean could be biased
Figure 4-33 Temperature profile of one logger generated by the SmartButton software
Figure 4-34 Average temperatures of each position for both piles to first turning
Figure 4-35 Scatter plot of one day's temperature of each logger at five positions of pile 1
Figure 4-36 Scatter plot of one day's temperature of each logger at five positions of pile 2
Figure 4-37 Performance of bacteria of interest at different stages in pile 1
Figure 4-38 Performance of bacteria at different stages in pile 2
Figure 4-39 Average temperature vs TC counts for end samples of pile 1
Figure 4-40 Average temperature vs FC counts for end samples of pile 1
Figure 4-41 Average temperature vs TC counts for end samples of pile 2
Figure 4-42 Highest temperature vs TC for end samples from pile 1
Figure 4-43 Highest temperature vs FC for end samples from pile 1
Figure 4-44 Number of days staying with a temperature>55°C vs TC from end samples of pile 1
Figure 4-45 Number of days staying with a temperature>55°C vs FC from end samples of pile 1
Figure 4-46 LNT=Log(base e) distribution of CFU number of TC for all samples from pile 1
Figure 4-47 LNF=Log(base e) distribution of CFU number of FC for all samples from pile 1
Figure 4-48 LNE=Log(base e) distribution of CFU number of Ent for all samples from pile 1
Figure 5-1 Histogram of in-cab dust concentration
Figure 5-2 Histogram of out-cab dust concentration
Figure 5-3 Histogram of in-cab endotoxin concentration
Figure 5-4 Histogram of out-cab endotoxin concentration
Figure 5-5 Histogram of in-cab Glucan concentration
Figure 5-6 Histogram of out-cab glucan concentration
Figure 5-7 Comparison of AF CFU counts among different tasks
Figure 5-8 Comparison of thermo bacteria among different tasks
Figure 5-9 Comparison between log AF and bacteria counts
Figure 5-10 Comparison of thermo bacteria at the four phases for pile 1
Figure 5-11 Compost bacteria concentration of pile 1 at the four stages
Figure 5-12 Comparison of log AF countsat the four phases for pile 1
Figure 5-13 Comparison of thermo bacteria at the four phases for pile 2
Figure 5-14 Comparison of log Af counts at the four phases for pile 2
Figure 5-15 Compost bacteria concentration of pile 2 at the four stages
ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my supervisors, Prof. James Atwater and Prof. Karen Bartlett, for their help, support, stimulating suggestions, encouragement and patience during all the time of conducting the research and writing the thesis. I have learnt a lot from them, not only academically, but also spiritually.

I also want to thank Yat Chow, for his help with processing the data and the field work; Nicole Steglich and the composting crew, for their collaboration and support in setting up the pilot piles, sampling, field research and so forth; Susan Harper and Paula Parkinson, for their help in the environmental lab.

Funding for the research project was provided by Work Safety B.C. and the Natural Science & Engineering Research Council of Canada.

Also, thank the City of Vancouver for supporting this project.

Last but not least, I would like to thank my parents, my friends for their support, love and patience during the period of my study.
CHAPTER 1 INTRODUCTION

The on-site work of the study has been conducted at the composting facility of Vancouver Landfill (VLF) while the experimental work has been done in both the lab of environmental engineering and lab of occupational hygiene and environmental health at the University of British Columbia (UBC), Vancouver, Canada.

The Vancouver Landfill, located in Delta at 5400 72nd Street, is owned and operated by the City of Vancouver. The Landfill serves approximately 940,000 residents of Vancouver, Delta, Richmond, White Rock, University Endowment Lands, and some regions of Surrey. Each year, about 475,000 tonnes of municipal solid waste is deposited at the Landfill. Approximately 60% of that total is received via the Vancouver South Transfer Station located at 377 West Kent Avenue. Municipal solid waste is accepted at the Transfer Station or the Landfill for $65.00 per metric tonne. Loads less than 900 kg are charged lower rates to encourage residents to use City disposal facilities. Yard and garden trimmings are accepted at reduced rates, and loads containing only recyclable materials are accepted at no charge. (www.city.vancouver.bc.ca)

In 1989, the City of Vancouver began a leaf composting program at the Vancouver Landfill. In 1995, this facility was expanded to a 1.8-hectare paved composting facility to accommodate all types of yard and garden trimmings.

Over 37,000 tonnes of trimmings are composted each year. Composting diverts trimmings from the Landfill, conserving landfill space while helping to achieve the solid waste reduction goals set out in the regional Solid Waste Management Plan.
In February 2004, an outbreak of avian influenza A (H7N3) occurred in poultry in the Fraser Valley region of British Columbia (BC). The crisis led to the destruction of approximately 17 million birds, a large percentage of which were composted. Fecal coliform criteria were used to gauge the effectiveness of the composting process, and the treated compost derived from poultry waste that had tested negative for H7N3 was available for sale to the public. However, concern remains that not all composting processes are equally effective at killing pathogens in these and other situations (http://www.cidrap.umn.edu).

Agencies across Canada and in the United States use indicator pathogen measurements and time-temperature records to determine if pathogens of concern have been removed before the compost is distributed to the public. Growing concern about the safety of compost has highlighted the need to ensure that the required temperatures are consistently and uniformly achieved such that universal indicator organism die-off is realized (Hay & Kuchenrither, 1990).

Therefore, one objective of the study was to investigate the relationship between temperature and pathogen reduction in the composting method of windrow, which was come into practice from three parts: 1. to determine if the windrow uniformly achieves the required time-temperature regime during the thermophilic phase; 2. to determine if compost meeting the time-temperature
requirements also meets the pathogen criteria for indicator organisms; 3. to compare the consistency between the 4 indicators in predicting the degree of pathogen kill in the process.

All Canadian provinces, and most Canadian cities are investigating or have introduced composting as an adjunct to landfill for solid waste disposal. The composting industry grew three-fold between 1994 and 1999. In 1994 it was estimated there were 100 facilities across Canada composting 300,000 metric tons of organic materials annually. In 1999 there were over 300 public and private sector composting 1,650,000 metric tons and producing over 800,000 metric tons of compost for use. In 1998 there were 46 centralized composting facilities in British Columbia. The number of facilities is expected to increase (by comparison, in the same census, Alberta had 84 facilities, followed closely by Ontario with 71) (http://www.compost.org/compostinggrowsstronger.html).

There is every reason to expect the growth of this industry to continue, as Canadian cities have no alternative but to burn, bury, or convert solid waste. Although composting has wide public appeal, and produces marketable product from either green, biosolids or food wastes, the health risks resulting from exposing to airborne biohazards during composting operations for increasing numbers of workers were still poorly described, since exposures to microorganisms, organic dust and bacterial endotoxins in the waste composting industry can be substantial (Douwes et al., 2000).

Given that the industry is still relatively small, and it has not been expect to see many cases of chronic lung disease in this population yet. However, as the industry grows, and more workers are employed, the chances of recognizing disease increase. Rather than let the “natural experiment” proceed, which would result in waiting and counting cases after the fact, we have the opportunity to study different composting technologies now, which will allow us to make solid recommendations for controls to prevent illness.

Consequently, another part of the study was designed to measure airborne exposures to selected biohazards: endotoxin, (1-3) glucan, Aspergillus fumigatus, Thermophilic spore forming bacteria as well as record potential determinants related to airborne concentrations of selected biohazards, such as different phases of piles, work tasks or various environmental conditions. Therefore, not only for the public regarding the safety of the compost, the research also has great relevance to British Columbia compost workers.
CHAPTER 2 LITERATURE REVIEW

2.1 Composting Principle

Composting is a thermophilic biological process involving the decomposition of organic material into a relatively stable end product (Hay & Kuchenrither, 1990). When properly managed, the process can reduce the volume, weight and water content and kill pathogenic organisms. Factors affecting the process include nutrients, oxygen, moisture, pH, and the presence of toxic chemicals (Hay & Kuchenrither, 1990). Furthermore, quality of raw material also plays a role in the end product.

Figure 2-1 Generalized representation of the composting process (Adapted from Young et al., 2005)
2.2 Compost Feedstocks

"Feedstock" is the raw material which is used to make compost. Feedstock is usually high in human pathogens including biosolids, food, yard waste, animal manure and animal carcasses (Stoffella & Kahn, 2001).

Yard waste is vegetative matter resulting from gardening, horticulture, landscaping, or land clearing operations and includes materials such as tree and shrub trimmings, plant remains, grass clippings, and chipped trees. Yard waste generally constitutes approximately 15 percent of municipal solid waste flow. The C:N ratio of leaves and other woody yard wastes is relatively high compared with other feedstocks (CCME), 2005).

Biosolids are organic product obtained from the physico-chemical and/or biological treatment of wastewater. Biosolids result from primary wastewater treatment (primary biosolids), or from secondary wastewater treatment (secondary biosolids), and these two types of biosolids are often combined (mixed biosolids). These biosolids can be derived from the treatment of either municipal wastewater or industrial wastewater (CCME), 2005).

Food waste includes fruits, vegetables, dairy products, grains, bread, unbleached paper napkins, coffee filters, eggshells, meats. Basically, if it can be eaten or grown in a field or garden, it can be composted. Food waste has unique properties as a raw compost agent. Because it has a high moisture content and low physical structure, it is important to mix fresh food waste with a bulking agent that will absorb some of the excess moisture as well as add structure to the mix. Bulking agents with a high C:N ratio, such as sawdust and yard waste, are good choices (Risse & Faucette, 2000). Regardless of feedstock, BC regulations apply the same temperature and pathogen criteria to assess compost safety.

2.3 Compost Methods

There are two commonly used composting methods in Canada: windrow and static aerated piles. A third technology, in-vessel composting, is also mandated for use in BC; however, this technology is more expensive to operate than the other methods, and thus is less commonly used (Compost Council of Canada, 2002; Hay & Kuchenrither, 1990).
Windrow is a method used for yard waste that involves a much lower level of technology. In windrow composting, leaves are laid down in elongated piles, agitated and aerated by turning with either a loader or a specialized piece of equipment (windrow turner). The leaf windrows are exposed to the weather, unlike an in-vessel system where they are contained (Hay & Kuchenrither, 1990).

Currently, regulations do not specify how piles should be turned, thus there is no guarantee as to the degree of compost uniformity within the pile. Two common options for windrow turning are front-end loaders, which is a relatively crude method, also very dependant on the operators to be effective, and the straddle-type windrow turners, which straddle and thoroughly mix the pile from above as it travels along the pile length (Biernbaum & Fogiel, 2004; Hay & Kuchenrither, 1990).

Static aerated piles are built over perforated plastic pipes, aeration cones or a perforated floor to allow for airflow. Aeration can be forced in either the positive (blowing) or negative (sucking) direction (Sesay et al., 1998). The timing, duration and movement of air need to be controlled to ensure uniform oxygen transfer, moisture content and temperature distribution. However, forced aeration may cause crusts, dry zones and air channels that short-circuit airflow throughout the pile. These deficiencies foster conditions that may prevent the uniform sanitization of the pile.

2.4 Compost Regulations

The issue of pathogens in compost is important in BC for two reasons: First, compared to other Canadian provinces, BC has one of the highest percentages of composting facilities that compost biosolids. Second, BC has a very large agricultural sector, and any livestock crisis in the province (such as a repeat of the 2004 Avian Flu crisis) could generate a massive feedstock influx for compost facilities to treat (OMRR, 2002).

In British Columbia, composting falls under the Organic Matter Recycling Regulations (OMRR, 2002), under the Waste Management Act and Health Act. OMRR is the legislation that governs the production, distribution, storage, sale and land application of compost.

Under OMRR, finished compost is deemed as either Class A, which can be distributed to the public, or Class B, which can be applied to land under limited conditions (OMRR 2002). Compost meeting 'Class A' maturity and pathogen reduction criteria at the end of the composting
process may be sold and distributed without restriction to the public. Effective pathogen reduction during the compost process is therefore essential to protect consumer health from compost-borne disease. This study will only address Class A compost, to which the public is most likely to be exposed.

Three compost methods are mandated under the OMRR: Aerated Static Piles, Windrows, and In-vessel composting. Acceptable compost feedstock (inputs) include materials high in human pathogens such as digested sewage sludge, manure and chicken carcasses.

The generalized compost process, and the timing of OMRR mandated maturity and pathogen tests, are shown as follows (OMRR, 2002):

Temperature Criteria
Under OMRR, compost piles must meet the following temperature ‘treatment’ criteria:
- 3 consecutive days at a temperature greater than 55°C in Static Aerated Piles
- 15 days (non-consecutive) at a temperature greater than 55°C in a turned Windrow pile

Pathogen Criteria
Under OMRR, the only indicator organism measured is fecal coliform. However, *Salmonella* and *E.coli* are being considered for use in BC. All three indicator organisms will therefore be examined in this study.

The OMRR pathogen indicator criterion is:
- A fecal coliform value of less than 1000 MPN per gram of dry weight

Other indicator criteria are (OMRR, 2002):
- A *Salmonella* value less than 3 MPN per 4g of total solids, dry weight
- An *E.coli* value less than 1000 MPN per gram of dry weight

There are some researchers putting forward that Salmonellae monitoring may sometimes be substituted for total coliforms monitoring.

The required time-temperature ratios for hygienic composting varies between countries and reflects different assessments of the hygienic efficiency and risk (Risse & Faucette, 2000), as well as different product uses and safety standards.
2.4.1 Trends and Comparison in Regulation All Over the World

EU Biological Treatment of Biowaste
Requirements:
- Leachate must be collected and treated
- Odors must be controlled
- Measures taken to minimize:
  - Exposure to birds, vermin and insects
  - Noise, dust and the formation of aerosols

UK Risk Assessment
- Risk Assessment focuses on potential human and animal pathogens
- Any waste that potentially contains uncooked meat will:
  - Be composted for at least 2 days inside a vessel at temperatures > 60°C
  - Will be composted further for at least 14 days and turned at least three times
  - Raw materials are delivered to an enclosed reception area

California Rule 1133.2 Air Quality
- The objective is to reduce VOC and ammonia emissions to improve air quality
- Active composting
  - The first 22 days of active composting will occur in the confines of an enclosure
  - All process air to be biofiltered
  - The curing process is performed with negative aeration 90% of the time (40 days or until Solvitas Maturity Index of 7)
- There are provisions for alternates - have to show 80% reduction of VOC and ammonia emissions

BC Organic Matter Recycling Regulation
Composting operations need to have:
- A leachate management plan
- All receiving, storage, processing and curing must be on an impervious surface that will prevent Release of leachate to environment
- Or be covered to prevent leachate formation
- An odor management plan
• Need to have and report pathogen reduction
• Need to have and report vector attraction reduction
• Unrestricted use of Class A compost

(Paul, 2001)

2.5 Composting Process

Composting is a degradation process in which aerobic bacteria and fungi break down organic matter into humus-like material called compost. The process can be described in equations as follows (Stoffella & Kahn, 2001):

“Organic matter + O2 + Aerobic Bacteria = CO2 + NH3 + Products + Energy”

For some anaerobic systems, O2 is absent, thus the overall equation takes a different form:

“Organic matter + Anaerobic Bacteria = CO2 + NH3 + Products + Energy + H2S + CH4”

Based on the equations shown above, it is not hard to see that the process control objective of composting is to maximize microbial activity at the expense of the organic matter, in other words, to maximize metabolic heat output, i.e. energy as shown in the equation. As temperature is a function of the accumulation of heat generated metabolically, and simultaneously it is also a determinant of metabolic activity, thus it could be concluded that temperature is both effect and cause in this self-heating ecosystem. The interaction between heat output and temperature is the centerpiece of rational control of the composting process (Macgregor et al., 1981).

Composting is a very complex and heterogeneous process. Many factors, including moisture content, substrate availability, nutrients, oxygen supply rate, bulk density, porosity, wind speed, solar radiation, ambient temperature and humidity all could affect the process (Turner et al., 2005). Therefore, there is no doubt that such a large number of variables (many of which cannot be measured) make it very difficult to predict and effectively control the process.

According to previous study, it has been found that during composting, pile temperatures can reach up to ~75°C (Hassen et al., 2001). And also it has been recorded by Fischer et al. (1998) that in their study, 80°C has been observed to be the highest temperature achieved during the process.
2.6 Temperature of compost

Monitoring the temperature of composting materials is important for two reasons. Firstly it is an indirect way of measuring the activity of micro-organisms within a pile, thus it can indicate if there is a problem with the process. Secondly, it enables an operator to assess whether the composting materials have been sanitized. Elevated temperatures are important during the composting process, as theoretically, they could help destroy many pathogenic micro-organisms, weed seeds and weed propagules, thereby reducing the risk to human, animal and plant health (Brinton & Droffner, 1994).

The organisms in composting systems can be divided into three classes: cryophiles or psychrophiles (0°C -25°C); mesophiles (25°C -45°C); and thermophiles (>45°C) (Young, 2005). Consequently, the process can be divided into four phases based on temperature. The first stage is the mesophilic stage, where mesophiles generate large quantities of metabolic heat and energy due to availability of abundant nutrients, but gradually this will pave the way for the dominance of thermophiles. Then with depletion of food sources, overall microbial activity decreases and temperature falls to ambient, leading to the second mesophilic stage, where microbial growth will be slower as readily available food is consumed. Finally, compost material enters the maturation stage, or curing stage, which might take some months (Young, 2005).

Normally, cryophiles are found only during winter composting. Mesophiles, in association with thermophiles, generally predominate the process. The temperature can range from near freezing to 70°C. Starting at ambient temperature, the compost can reach 40°C -60°C in less than two days depending on the composition and environmental conditions (Young, 2005).

2.6.1 Debate on optimal temperature for composting

Several researchers have tried to define the optimal temperature for composting. Leton & Stentiford (1990) proposed the optimum operating temperatures are between 45°C and 55°C with an initial period above 55°C to sanitize material; showed microbial activity is greatest at 52 to 60°C and a steep decline starts above this upper boundary; also, it was recorded in Nakasaki’s (1985) paper that the optimal temperature for composting process as a whole is from 35°C to 55°C while Strom (1985) concluded in his work that the maximum desirable composting temperature based on species diversity is 60°C.
Therefore, generally speaking, moderate is favored rather than extreme thermophilic temperatures during composting (Macgregor et al., 1981). The reason could be attributed to the two kinds of systems existing in the composting process: those that are and those that are not temperature self-limiting. When the self-limiting system reaches inhibitive temperatures (>60°C), it will debilitate the microbial community, suppressing decomposition, heat output, and water removal. In contrast, non-self-limiting temperatures (<60°C) support a robust community, promoting decomposition, heat output, and water removal (Macgregor et al., 1981). Hence, researchers reached different conclusions about the optimum temperature for composting possibly because of the speciality of each study, difference could exist in many factors such as original feedstock, weather conditions, experimental set up and so on. However, no matter how heated they debate on the optimum points, it can be seen that to some extent, they are all within 35°C to 60°C.

### 2.6.2 Temperature distribution

80°C has been achieved in the centre of composting piles observed by Fischer et al. (1998), and their measurements carried out within the pile showed that important temperature gradients existed in the pile, temperatures in the outer or lower zones were found up to 30°C below that of the core. Furthermore, the material in the upper part of a triangular pile is always the hottest (chimney effect), while the bottom and the outer layers are much colder, the former due to the inflow of fresh air, the latter through important heat losses to the environment (Fischer et al., 1998; Lynch et al., 1997).

Therefore, as far as the compost quality standards are concerned, as mentioned before, both the United States and Canada require temperatures above 55°C for 15 days for windrow composting; some of the regulations also include a minimum number of turnings (Fischer et al., 1998). However, considering the profile of temperature distribution in the pile, some researchers have showed great concern about the reliability of the regulatory requirements, because these requirements could be meaningless as none of the regulations state where in the compost pile these temperatures have to be measured (Fischer et al., 1998). Therefore, certain minimal number of turnings would be necessary to help thoroughly mix the inherently heterogeneous material and evenly redistribute the heat (Fischer et al., 1998).
2.7 Moisture

Moisture is a key environmental factor that affects many aspects of the composting process. Biodegradation kinetics is affected by moisture through changes in oxygen diffusion, water potential and water activity, and microbial growth rates. Meanwhile, these relationships are made more complex by the dynamic nature of the composting process, with changes in particle size and structure occurring over time (Richard et al., 2002).

When considering the metabolic process of the microbes, if the mixture is too dry, the microorganisms cannot survive, and composting stops; if there is too much water, the oxygen from the air is not able to penetrate to where the micro-organisms are, and the mixture becomes anaerobic or at least anoxic (Young et al., 2005). In conclusion, moisture management requires a balance between two functions: encouraging microbial activity and permitting adequate oxygen supply (Richard et al., 2002).

The optimum moisture content for biodegradation can vary widely for different compost mixtures and times in the composting process, ranging from near 50 to over 70% on a wet basis. There is a significant reduction in biodegradation rate when operating outside the optimum range (Richard et al., 2002). Moisture can be controlled either directly by adding water or indirectly by changing the operating temperature or the aeration regime. Feedstock with different moisture holding capacities can be blended to achieve an ideal moisture content (Young et al., 2005).

2.8 Nutrient balance-carbon/nitrogen ratio

Nutrient balance is very much dependent on the type of feed materials being processed. In the process, carbon provides the preliminary energy source and nitrogen quantity determines the microbial population growth. Hence, maintaining the correct C:N ratio is important to obtain good quality compost. Bacteria, actinomycetes, and fungi require carbon and nitrogen for growth. These microbes use 30 parts of carbon to 1 part of nitrogen. Composting is usually successful when the mixture of organic materials consists of 20-40 parts of carbon to 1 part of nitrogen. However, as the ratio exceeds 30, the rate of composting decreases. Furthermore, as the ratio decreases below 25, excess nitrogen is converted to ammonia. This is released into the atmosphere and can result in undesirable odour (Young et al., 2005).
During bioconversion of the material, concentration of carbon will be reduced while that of nitrogen will be increased, resulting in the reduction of C:N ratio at the end of composting process. The reduction can be attributed to the loss in total dry mass due to losses of C as CO2 (Young et al., 2005).

Maintaining C:N ratio after composting is also important to determine the value of finished compost as soil amendment for crops. The final C:N ratio of 15 to 20 will be expected and the value of more than 20 might have a negative impact and will damage the crop and seed germination. The value of 10 has been suggested as ideal (Young et al., 2005).

2.8 pH

The pH of the compost pile has been observed to vary with time, showing an initial drop and then increasing to between 8.0 and 9.0, finally levelling off between 7.0 and 8.9 (Young et al., 2005). It was found by McKinley & Vestal (1985) that the pH of the low-temperature piles tended to increase faster than that of the high temperature piles. These results confirm other work in which increased rates of CO2 production were detected in materials composted at lower temperatures (Stoffella & Kahn, 2001). If the compost pile becomes anaerobic, however, the pH will continue to drop due to the action of the anaerobic acid formers. As long as the pile stays aerobic, the buffering has been proved sufficient within the compost to allow the pH to stabilize at an alkaline level (Young et al., 2005).

2.9 Windrow size

Windrow size markedly influences the composting process. For the process to work at all, the heat liberated by the thermophilic decomposition process must exceed the heat lost through the exposed surface of the windrow. Increasing windrow size greatly decreases the rate of heat loss. As the cross section increases, the surface-to-volume ratio decreases, thereby decreasing heat losses and increasing the internal temperature (Hay & Kuchenrither, 1990).

One key to high temperature development is to build windrows as large as is practical within the constraints imposed by the mobile composting equipment. Building large windrows can also reduce the effects of rain on a composting operation (Hay & Kuchenrither, 1990).
2.10 Turning

Besides helping mix the non-homogeneous material and redistribute the heat thus promoting a better thermo-hygienization in the pile as mentioned earlier, turning also has further beneficial effects on the composting process, such as reducing particle size, increasing porosity to promote aerobic conditions. This was especially true when composting more woody material, which contained less easily degradable and larger particle material, and where temperatures were more difficult to maintain at a level sufficient for pathogen elimination (Fischer et al., 1998).

During the treatment of more woody wastes, the turning machine was mechanically breaking apart the wood pieces, reducing the size of the particles and providing new surfaces for microbial attack. It was also been found by Fischer et al. (1998) that the bulk density of the compost in frequently turned yard trimming compost increased faster compared to windrows turned only once per month, and that the rejection percentage during sieving was greatly reduced by the intensive turning regime.

Moreover, it also has been noticed that the control of compost humidity was possible only by frequent mixings: for the material with slightly higher moisture content, turning could also help promoting drying through release of water vapour (Fischer et al., 1998); and when it comes to very dry material, an effective addition of water can only be carried out during turning. Nevertheless, during very wet periods, the turning frequency should be lowered, otherwise the compost gets too humid through the incorporation of the very wet material from the base of the windrow (Fischer et al., 1998). And also, turnings should be carried out with moderation in very cold weather, especially for windrows in an advanced state of maturation, because of the risk of cooling out the compost too much (Fischer et al., 1998).

Also, as far as the safety issue related to the site workers' exposure to the bioaerosols is concerned, with the advantage of promoting a better thermo-hygienization in the pile as stated earlier, there is no doubt that intensive turnings of the pile can help diminish the health risks for the personnel working on the composting sites. Fischer et al. (1998) have pointed out that although more frequent turnings result in more frequent exposures, comparing to dealing with a pile much less turned, the cumulative risk of that more frequent exposures resulted from more frequent turning is still smaller, because of the better thermo-hygienization achieved.
To sum up, the advantages resulted from frequent turnings of compost windrows could be concluded as enhancing aeration conditions, fast temperature rise, good thermo-hygienization, better process control and less dispersion of bioaerosols during turning (Fischer et al., 1998).

2.11 Seasonal variability

Seasonal variability was found in the composting operation. Temperatures peaked in the summer months and declined in the winter. The exposure time that indicated the days the internal temperature exceeded the critical temperatures also exhibited a seasonal trend, which helped give a rough approximation of time needed to achieve thermo-hygienization. Cool air temperatures and rainfall in winter slow the composting process by cooling the windrows and thus extended the composting time. To achieve adequate pathogen inactivation, windrows must be composted longer in the winter than in the summer. It was found by that longer exposure time couldn’t fully compensate for the lower temperature during winter time (Hay & Kuchenrither, 1990).

Compost dryness also varied seasonally, with the driest material produced in the summer and fall. Wet weather, cool air temperature and lower air drying caused by decreased solar radiation reduced the drying rate. It was observed that drying and not disinfection were the limiting factors during winter months (Hay & Kuchenrither, 1990).

2.12 Composting microbiology

Composting is a complex process involving a wide variety of micro-organisms attacking organic substrate. The micro-organisms involved in the process are primarily bacteria, actinomycetes, and fungi (Stoffella & Kahn, 2001). Major species forming these three microbial families are shown in Table 2-1. Under aerobic conditions, temperature is the major factor that determines the species diversity, types of micro-organisms, and the rate of metabolic activities (Hassen et al., 2001). Although considered bacteria, actinomycetes are actually intermediate between bacteria and fungi because they look similar to fungi and have similar nutritional preferences and growth habits. It was observed that they tend to be more common in the later stages of the composting process, and are generally thought to follow the thermophilic bacteria in succession. They, in turn, are followed predominantly by fungi during the last stages of composting (Young et al., 2005).
### Table 2-1 Major species of Actinomycetes, Fungi and Bacteria

<table>
<thead>
<tr>
<th>Actinomycetes</th>
<th>Fungi</th>
<th>Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinobifida ahromogenia</td>
<td>Aspergillus fumigatus</td>
<td>Alcaligenes faecalis</td>
</tr>
<tr>
<td>Microbispora bispora</td>
<td>Humicola grisea</td>
<td>Bacillus brevis</td>
</tr>
<tr>
<td>Micropolyspora faeni</td>
<td>H.insolens</td>
<td>B.circulans complex</td>
</tr>
<tr>
<td>Nocardia sp.</td>
<td>H.lanuginosa</td>
<td>B.coagulans typeA</td>
</tr>
<tr>
<td>Pseudocardiathermophilia</td>
<td>Malbranchea pulchella</td>
<td>B.coagulans typeB</td>
</tr>
<tr>
<td>Streptomyces rectus</td>
<td>Myriococcum thermophilum</td>
<td>B.licheniformis</td>
</tr>
<tr>
<td>S.thermofuscus</td>
<td>Paecilomyces variotti</td>
<td>B.megaterium</td>
</tr>
<tr>
<td>S.thermoviolaceus</td>
<td>Papulospora thermophilia</td>
<td>B.pumilus</td>
</tr>
<tr>
<td>S.thermovulgaris</td>
<td>Scytalidium thermophilum</td>
<td>B.sphaericus</td>
</tr>
<tr>
<td>S.valeceus-ruber</td>
<td>Sporotrichum thermophile</td>
<td>B.stearothermophilus</td>
</tr>
<tr>
<td>Thermoactinomyces sacchari</td>
<td>B.subtilis</td>
<td></td>
</tr>
<tr>
<td>T.vulgaris</td>
<td>Clostridium thermocellum</td>
<td></td>
</tr>
<tr>
<td>Thermomonospora curvata</td>
<td>Escherichia coli</td>
<td></td>
</tr>
<tr>
<td>T.viridis</td>
<td>Flavobacterium sp.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pseudomonas sp.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Serratia sp.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Thermus sp.</td>
<td></td>
</tr>
</tbody>
</table>

(Adapted from Young et al., 2005)

#### 2.12.1 Bacteria

Normally, bacteria are about 100 times more prevalent than fungi, pointed out by (Stoffella & Kahn, 2001). It had been estimated that at least 80 to 90% of the microbial activity in composting is due to bacteria (Stoffella & Kahn, 2001). Certainly, either the feedstock, the ongoing process itself, or environmental conditions as well as many other factors, could affect the bacteria populations. A large number species of bacteria had been found in composting, which also indicated micro-organisms initially present in wastes dominate the composting process (Brinton & Droffner, 1994). And when temperature increases, the species variety has been found to decrease (Stoffella & Kahn, 2001). During the high temperature stage, as stated earlier, the mesophilic bacteria are inhibited while the thermophilic bacteria are prevalent. However, as
observed to occur, which got inactivated during thermophilic stage (Stoffella & Kahn, 2001), which also brings about the concerns for regrowth.

2.12.2 Actinomycetes

Actinomycetes belong to the order Actinomycetales. Although they are similar to fungi, in that they form branched colonies, they are more closely related to bacteria. Not like either bacteria or fungi, which indigenously exist in the pile with significant amount and varieties, normally, they are not present in appreciable numbers until the composting process is well established. Visual growth of actinomycetes may be observed under favourable conditions, usually between 5 to 7 days into the composting process. It was stated by previous researchers that actinomycetes are responsible for the faint “earthy” smell that the compost emits under favourable conditions and which generally increases as the process proceeds. The species of actinomycetes can be spore formers and are able to withstand adverse conditions, such as inadequate moisture. Because the actinomycetes can utilize a relatively wide array of compounds as substrates, they play an important role in the degradation of the cellulotic component. To some extent, they can also decompose the lignin component of wood (Stoffella & Kahn, 2001).

2.12.3 Fungi

Fungi appear within the composting process about the same time as the actinomycetes. Two general growth forms in fungi exist—molds and yeasts. Although some fungi are very small, most are visible in the form of fruiting bodies throughout the composting pile (Stoffella & Kahn, 2001).

The upper limit for fungal activity seems to be around 60°C. However, at temperatures below 60°C, the thermophilic fungi can recolonize the compost pile. At temperature below 45°C, the mesophilic fungi reappear. One of the few thermophilic fungi that survive above 60°C is the thermotolerant species *Aspergillus fumigatus*. The spores of this species readily withstand temperatures above 60 °C and this species becomes the dominant fungus in the compost pile at those temperatures. *Aspergillus fumigatus* is a mold and has a special significance as a cellulose and hemicellulose degrader (Stoffella & Kahn, 2001). However, the airborne spores can be a health hazard at the composting facility, to site workers who have once experienced respiratory illnesses.
2.12.4 Coliforms

As it is known that one of the requirements of a commercial operation is to maximize the destruction of pathogens that may be present in the composting feedstock. Theoretically, if the feedstock does not contain manures or biosolids, there should be few enteric pathogens. However, where composting operations allow some contaminants like pet feces to be part of the waste collection, this may not be the case. Therefore, coliforms are always of great concern when it comes to the issue of composting hygiene, and also they are supposed to be good indicators of the safety of compost. Coliform is a lactose-fermenting member of the family Enterobacteriaceae. It is commonly associated with the intestinal tract of animals, including humans, fish, birds and insects. But many are also known and reported to be free-living in the environment and associated with plants and soil. As the temperature rises during the composting process, coliforms usually could be destroyed as they reach their thermal death points (Stoffella & Kahn, 2001). However, surviving temperatures even higher than the theoretical thermal death points of some pathogenic bacteria such as E. coli had been observed and the explanations for this will be discussed in more details in the coming section.

2.12.5 Re-growth

It was pointed out that the regrowth of bacteria may be possible under certain conditions. Regrowth depends upon a number of factors such as moisture content, bio-available nutrients, temperature and indigenous microorganisms (Sidhu et al., 2001). As a result of regrowth, pathogen levels may be 'unsafe' in finished compost even if pathogens are reduced to low levels during the thermophilic composting phase (Brinton & Droffner, 1994). Consequently, it needs to be taken into consideration about what "negative" results mean. When pathogens are not found, it does not necessarily mean they are absent. Because of the possible occurrence of re-growth, the likelihood that the organisms may have been present all along, but not detected must be evaluated (Brinton & Droffner, 1994). Therefore, long-term storage of compost is not recommended as this may increase the pathogen regrowth potential (Sidhu et al., 2001) and also an active population of non-pathogenic bacteria is important to be maintained in order to prevent explosive regrowth of the pathogenic bacteria (Stoffella & Kahn, 2001).
2.12.6 Exploration on possible reasons for high-temperature survival

2.12.6.1 Dissemination of thermal tolerance

Therefore, to get a better understanding of the real case, effort had been given to conducting research on finding the reasons or mechanisms resulting in the survival. It has been indicated in J. Ryckeboer et al. (2003)’s work that under certain conditions, thermo-tolerance spreads rapidly to organisms not believed to be heat resistant. The dissemination of thermal tolerance may take place through similar, complicated mechanisms as what are responsible for the spread among bacteria of antibiotic resistance (Ryckeboer et al., 2003).

2.12.6.2 Mutation

Conversely, it also could be the result of mutations enabling the bacteria to adapt to the high temperatures in the composting process (Brinton & Droffner, 1994). It had been demonstrated by Brinton et al. (1994) that the genera *Escherichia*, *Salmonella* and *Pseudomonas* all have the capacity to produce mutants able to grow at elevated temperature. Both *E. coli* and *Salmonella* had been recorded of surviving for long periods of time at 60°C. An *E. coli* mutant had been isolated which was even capable of growing at 65°C by Brinton & Droffner (1994). There is also good evidence that *Salmonella* will mutate to grow at 68°C (Brinton & Droffner, 1994). Isolation of thermophilic mutants of *Pseudomonas aeruginosa* also was undertaken and the isolation and demonstration of the origin of the mesothermophilic (54°C) mutant strains had also been described (Brinton & Droffner, 1994).

Question was brought up by Brinton et al. (1994) that whether surviving mutants actually are pathogenic, and whether it is possible to shift the populations to effective and safe organisms. Clearly, concerns were raised that if heat was not the mechanism which removed pathogens, then other routes like bacterial competition must be given more attention (Brinton & Droffner, 1994).

2.12.6.3 Genus Thermus

Furthermore, it was also reported a diversitie and numerous bacteria related to the genus Thermus in thermogenic (65 to 82°C) composts taken from 2- to 5-week-old organic-waste samples. The majority of the isolates are probably Thermus strains which have adapted to the conditions present in the hot-compost ecosystem (Beffa et al., 1996).
Thermophilic bacteria related to the genus Thermus had been isolated from many neutral and alkaline geothermal and manmade thermal environments throughout the world. It is assumed that the natural habitat of Thermus spp. is the hot-springs ecosystem. Since either the feedstock for composting or the windrow process itself has so large chance of being contaminated by the environmental organisms, so the genus Thermus theory may help partly explain the finding of survival of bacteria in hot compost (Beffa et al., 1996).

2.12.6.4 Physical conditions

In addition, survival of potential pathogens in composts may be partly dependent on physical conditions. For example, pathogens may reside in cooler zones of windrows or as clumps along edges of static piles (Brinton & Droffner, 1994).

Last but not least, in addition to the already discussed microbes, what also needs to be mentioned here is that it is not only the heat of the compost that destroys all these pathogens; it is a combination of factors including (Young et al., 2005):

- Competition for food from compost micro-organisms;
- Inhibition and antagonism by compost micro-organisms;
- Consumption by compost micro-organisms;
- Biological heat generated by compost micro-organisms; and
- Antibiotics produced by compost micro-organisms.

Generally speaking, from what has been discussed above, it can been concluded that the composting microbiology is indeed very complex, and the composting process can, if not properly managed, induce the proliferation and dispersion of potentially pathogenic and/or allergenic thermotolerant/thermophilic fungi and bacteria (Franke-Whittle T et al., 2005). Among the bacteria, Salmonella, E. coli, Streptococci can emerge and cause infections for compost handlers and agricultural users. Among the fungi, the mold Aspergillus fumigatus can be predominant because of its cellulolytic and thermotolerant properties (Hassen et al., 2001). Due to the difficulties of obtaining sound knowledge on micro-flora during the whole process, it may be hard for engineered compost technologies to achieve desired levels of control (Brinton & Droffner, 1994).
2.13 Inadequacy of previous data and findings

2.13.1 Lack of information on the spatial and temporal temperature regime

A major deficiency of previous compost studies is the poor spatial and temporal temperature data. For large-scale field studies, the intensive measurements of these data are challenging, expensive and time consuming to conduct. Some studies have obtained detailed, three-dimensional temperature data over time by using static thermocouples on rods or embedded wooden grids (Sartaj et al., 1995), however, the resulting data are rarely dense enough to describe areas of rapid temperature fluctuations (i.e. localized variations are unobserved), while others have produced more generalized two-dimensional representations (Turner, Williams, White, & Tillett, 2005). Single plane temperature evolution measurements are more common (Fernandes et al., 1994). In contrast, detailed three-dimensional spatial and temporal data are rare, especially for windrow composting.

2.13.2 Lack of information on linking the temperature regime to pathogen killing

Pathogen sampling is expensive and relatively complex, temperature is routinely used as a surrogate measure of compost quality and process completion. Pathogen testing is therefore only done as a final regulatory requirement before compost can be sold to the public (Compost Council of Canada, 2002). To ensure compliance with temperature criteria, compost operators usually take a small number of temperature measurements randomly throughout the pile. These sparse measurements, as stated earlier, may not detect cool zones resulting from forced aeration or improper mixing.

Few studies have directly correlated pathogen levels to temperatures in the same sample. Stentiford et al. (1990) have monitored E.coli, Streptococci and Salmonella in a windrow pile turned with a front-end loader, but the temperature was only monitored at six locations, and thus, few spatial data were generated. Other work examined a 2-stage windrow method, (which is not an OMRR mandated process), but again, spatial relationships were generalized and poorly illustrated (Hay and Kuchenrither 1990). Similarly, in static piles, limited research has attempted to correlate indicators (E.coli, fecal coliform, fecal streptococci) to time-temperature regimes. Because results were based on a small number of samples, the impact of the temperature regime on the level of sanitization can not be clearly discerned (Sesay, Lasaridi, & Stentiford, 1998).
To conclude, there are no reliable data describing the fraction of real compost systems that achieve the time temperature regime required to kill pathogens. Therefore, inadequate evidence exists to indicate that pathogens are uniformly killed in areas that reach the mandated temperature regime, so uncertainty remains about the suitability of using temperature to estimate pile sanitization in real compost piles and thus the safety of the finished compost product is unclear.

2.14 Bioaerosols

As described in the previous section, the presence of bacteria and fungi in high concentrations are fundamental to the composting process. Besides in terms of staying in the pile, whenever composting materials are moved around, for example during the shredding, turning and screening processes, these micro-organisms can be aerosolised, forming what is termed a bioaerosol (CAHSL, 2003).

There has been a popular misconception that what is “organic” (used in the sense meaning “natural”) is good as opposed to “non-organic” (used in the sense “produced through engineering”). While this may be true for the end product compost, there is evidence from countries that have already adopted composting as a primary solid waste management strategy that exposures to compost workers are not benign.

As an overall pattern emerges, workers on composting facilities are potentially exposed to considerably higher concentrations of bacteria, including Gram-negative bacteria, actinomycetes, fungi and their associated toxins than are likely to be present in background air away from bioaerosol sources, while the microbial components of compost bioaerosols have a known pathogenic and allergenic potential to cause respiratory ill health (Thorn et al., 1998).

In general, the potential harm in terms of public health triggered from composting process can be categorized into both acute and chronic health effects. Acute health effects include an inflammatory response of the upper airways with congested nose, sore throat, and dry cough frequently in connection with symptoms of the eyes like redness and lacrimation—the so-called mucous membrane irritation (MMI)—subsiding several hours after cessation of exposure and the organic dust toxic syndrome (ODTS) (Bünger et al., 2007; Olenchock et al., 2007), a limited, flu-like illness caused by the response of alveolar macrophages to high concentrations of antigen.
Chronic health effects include permanent lung scarring as a result of unchecked inflammation and development of hypersensitivity pneumonitis (Olenchock et al., 2007).

2.14.1 Endotoxin

The causative agents of these diseases are micro-organisms that are intrinsic to the composting process. Endotoxin is a structural component of the cell wall membrane of most Gram negative bacterial cells, regardless of the ability of the intact bacteria to cause disease. Endotoxin is stable, and can persist in the environment for long periods of time. Previous study on rural or agricultural occupations has identified endotoxin as the primary cause of both acute and chronic lung inflammation. Lipid A is now known to be the active pro-inflammatory molecule of endotoxin (CAHSL, 2003; Weber et al., 1993).

2.14.2 \( \beta (1-3) \) glucan

A structural component of fungal spores, \( \beta (1-3) \) glucan, also acts on the innate immune system. Dr. Rylander, an occupational health physician in Sweden, has established that the inflammatory properties of \( \beta (1-3) \) glucan are synergistic with endotoxin. In nature, fungal and bacterial contamination is common in any organic material. Analogous to endotoxin, the pro-inflammatory effects of \( \beta (1-3) \) glucan are independent of the fungal species being either “toxic” or pathogenic.” Like endotoxin, fungal spores and \( \beta (1-3) \) glucan are stable in the environment (CAHSL, 2003).

2.14.3 Aspergillus

Luckily, this organism rarely, if ever, infects normally healthy individuals. The source of the fungus is any organic material, and Aspergillus is unique amongst environmental fungi in that the optimal temperature range of the organism is above 20°C. The upper limit of growth is around 50°C. The ability of the organism to thrive in warm conditions allows Aspergillus to successfully compete for survival, and to grow at above ambient temperatures. Aspergillus fumigatus reproduces asexually by the production of many millions of spores, which are easily dislodged from the parent colony into air by any disturbance of the growth substrate. The process of making
Compost has many steps where organic dust is aerosolized by turning, raking, grinding, screening, etc (CAHSL, 2003).

2.14.4 Thermo-actinomycetes

Another group of micro-organisms that reproduce asexually and produce very small spores are the thermophilic bacteria, or thermo-actinomycetes. There are several bacterial genera that make up this group. Early studies of the lung disease called Farmer's Lung identified precipitating antibodies in serum to thermo-actinomycetes antigens. The very small diameters of the spores (<1.5 μm) allow deposition into the alveolar region of the lung (Rautiala et al., 2003). These organisms are normally found in organic material, and their ability to survive and grow at high temperatures creates an optimal ecologic niche in composting materials. Species of this group of bacteria remain primary antigens in cases of hypersensitivity pneumonitis (CAHSL, 2003).

2.14.5 Previous findings

Exposures to biologic agents have been reported in a variety of composting settings. Marsh et al. (1979) reviewed literature reporting exposures to Aspergillus fumigatus in sludge composting, but included other natural substrates held at temperatures between 40-50°C, including grain silos, boiler rooms, sauna baths, and the cooling canals from nuclear power generators. Rautiala et al. (2003) reported high concentrations of thermotolerant fungi and thermophilic actinobacteria (up to 105 CFU/m3) in swine confinement buildings where the composting system was functioning properly. Douwes et al. (2000) found endotoxin exposures to be related to job tasks and the production of inflammatory markers.

However, no studies were found in the literature that compared exposures between different composting technologies. Nor were any studies found reporting the efficacy of engineering controls in reducing bioaerosol exposures to compost workers.

2.14.6 How turning may affect the bioaerosols from the perspective of kinetic

Dropping the composting material from the bucket of the front end loader, over the windrow, releases the bioaerosols. The position of these releases moves along the length of a windrow during the turning process and takes place over a number of hours. There is no initial momentum.
to the source though the initial temperature of the material could lead to buoyancy effects, as
described later. The release of bioaerosols, due to turning during composting, could be described
by a series of sources and emissions: representing the discrete releases from a bucket and their
movement along a windrow. With the possible exception of buoyancy effects the source and
emissions will not affect the atmospheric flow. The total effect of turning a windrow could then
be found by summing the results of a calculation of the time varying dispersion of the release
from a single bucket. The effect of this calculation could then be summed, at positions in space
and time representing the turning of a windrow, to give the total effect due to turning a windrow
(Thorn et al., 1998).

2.14.7 Downwind conclusion

Concerning the health risks for the population living near the composting site, measurements
showed that spore concentrations in 10m downwind of the machine yielded numbers already 2 to
3 orders of magnitude lower than directly behind the machine. Outside the perimeter of the site,
AF concentration were normally not higher than in locations far away from composting
installations(Fischer et al., 1998).

2.14.8 Andersen Sampler

It is well recognized that the particle size of an aerosol of pathogenic organisms determines the
degree of infectivity by the respiratory route(Andersen, 1958).

Nasal efficiency for screening out airborne particles entering the respiratory tract has been found
practically 100 percent for particles above 5 micrometers and decreases with particle size to zero
for 1 micrometer particles; that depth of penetration into the respiratory tract increases with
decreasing size; that alveolar retention is complete for particles larger than 1 micrometer which
escape being trapped in the upper respiratory tract; and that from 1 to ¼ micrometer, alveolar
retention decreases (Andersen, 1958).

Therefore, any instrument used to assess the health hazard or infection potential of particulate
aerosols should determine the number and the size of the airborne particles or, preferably, classify
them aerodynamically since penetration and deposition in the respiratory tract is a matter of
aerodynamics (Andersen, 1958).
In this study, a six-stage Andersen sampler is used to collect the airborne bacteria and fungi samples. It takes samples by drawing in air through 400 holes and impacts the particles onto a collection medium. At this sampling rate, the particulate that impacts on the first stage has aerodynamic diameters (AD) of about 7 – 10 micrometers. The second stage collects particulate matter of around AD 4.7 - 7.0 micrometers; the third stage, AD 3.3 - 4.7; the fourth stage, AD 2.1 - 3.3 micrometers; the fifth stage, AD 1.1 - 2.1 micrometers; and the sixth stage, AD 0.65 - 1.1 micrometers (Andersen, 1958).

The size of the holes is constant for each stage, but smaller in each succeeding stage; consequently, the jet velocity is uniform in each stage but increases in each succeeding stage. When the velocity imparted to a particle is sufficiently great, its inertia will overcome the aerodynamic drag and the particle will leave the turning stream of air and be impinged on the surface of the medium; otherwise the particle remains in the stream of air and proceeds to the next stage (Andersen, 1958).

![Andersen six-stage sampler](image)

**Figure 2-2 Andersen six-stage sampler**

Different parts in human-beings’ respiratory system mimicked by the Andersen six stage sampler is listed as follows:

- **Stage 1** = captured by the nose;
- **Stage 2** = impacts in the nasopharynx;
- **Stage 3** = impacts in the trachea & primary bronchi;
- **Stage 4** = impacts in the secondary bronchi;
- **Stage 5** = impacts in the terminal bronchioles;
Stage 6 = small enough to enter the alveoli (the gas exchange region of the lung).

**Figure 2-3 Hazardous range of the six stages** (Adapted from Andersen, 1958)

2.14.9 Difficulty of achieving universally applicable conclusion about bioaerosols

Levels of airborne micro-organisms generated during the handling of compost can vary greatly from site to site depending on the scale and type of operation. Different sampling methods, different site of samplers and other method variations e.g. whether sampling took place during compost pile activity or not, make it difficult to compare results directly from study to study. Even at one particular site concentrations can vary greatly from hour to hour by more than ten times (Neef et al., 1999). Different composting activities have a dramatic effect on the levels of microbial emissions, such as weather conditions, wind speed and direction. The moisture content of the compost also affects the bioaerosol levels (Harrison, 2007). As a result, different studies have reported widely differing levels. It is not possible in many instances to deduce from the data presented in the published studies how the different types of composting activity, containment and automation might have affected the levels of bioaerosol generated.

Reported concentrations of bioaerosols generated from composting activities were measured at fixed point locations may not reflect accurately the personal exposure levels of workers carrying
out the composting process. For much of the work done on site, workers may be using tractors or front end loaders and therefore are protected by vehicle cabs which may also be air conditioned. Assuming that this is the case, and doors and windows are kept closed in the close proximity of actively handled compost, the workers’ exposure will be considerably less than the bioaerosol levels outside the vehicle cab (Flannigan et al., 2002). Effort has been made on examining the protection afforded by vehicle cabs, it demonstrated that potential exposure could be reduced by several orders of magnitude (Flannigan et al., 2002). However, leakage of air around poorly fitted or badly maintained cab filters compromises the protection, and opening a door or window even for a short period in the vicinity of a bioaerosol source negates the protective effect within a very short period of time.

For work activities outside of vehicles and in the vicinity of composting material, suitable respiratory protection would significantly reduce potential exposure. Few studies provided any detail about the use of respiratory protective equipment.
CHAPTER 3 MATERIAL AND METHODS

To accomplish the objectives outlined above, the study has been conducted primarily in three parts: one, collect both temporal and spatial thermal data; two, quantify the number of organisms of interest in compost samples while the third part is to collect and analyse bioaerosol samples to test the workers' exposure to organisms of concern. In addition, moisture content and nutrients (C, N) concentration analysis were determined on the compost samples. The weather condition had been monitored continuously during the whole process.

The study was conducted at the composting facility located on the Vancouver landfill in Delta, BC. The composting method used is windrow. The equipment used for turning are Volvo 180 and Volvo 220 front-end loaders. Feedstock is plant and yard waste, both city and commercially hauled.

3.1 Nutrients analysis

Total Carbon (TC) and Total Nitrogen (TN) of the compost from feedstock, after turnings, and after screening were tested in this study. The samples were dried and then sieved into particles smaller than 2 mm. Tests were conducted by Carol Dyck with LECO CN-2000 analyzer in the Agroecology lab in Land and Food Systems, University of British Columbia.

3.2 Moisture content

Moisture content was tested for every compost sample in this study. Samples were put into the ceramic dishes which have already been weighed and labelled in advance.
The net weight of each sample was obtained by subtracting the total weight of dish and sample by the net weight of the dish itself. Then the dishes were placed into the muffle oven, the temperature of which had been set at 80°C.

![The ceramic dish](image1)

![Dishes ready to be used](image2)

The dishes were taken out of the oven after two days and weighed on the balance when they cooled down, then they were put back to the oven again and after one day, were weighed again to determine if the weight was consistent; if so, the sample was dry and the sample was ready; if not, the sample was returned to the oven again, until the weight was to be consistent with the previous one. Moisture content was calculated as \((\text{pre-weight} - \text{post-weight})/\text{net weight of sample} \times 100\%\).
3.3 Temperature Monitoring

3.3.1 The Smartbutton data logger

The ACR Smartbutton was chosen for recording temperature over time in the pile. The SmartButton is a miniature-sized temperature logger, the size of it is 17.35 mm diameter x 5.89 mm height while the weight is 4 grams, and it is made of stainless steel. Specifications of the SmartButton in more details are listed in Table 3-1 that follows:

![Figure 3-5 ACR Smartbutton](image)

**Table 3-1 SmartButton Specifications**

<table>
<thead>
<tr>
<th>Type</th>
<th>Temperature Sensor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size</td>
<td>17.35 mm diameter x 5.89 mm height (0.68” x 0.23”)</td>
</tr>
<tr>
<td>Weight</td>
<td>4 g</td>
</tr>
<tr>
<td>Material Type</td>
<td>Silicon</td>
</tr>
<tr>
<td>Accuracy</td>
<td>±1.0°C from -30°C to 45°C (± 1.8°F from -22°F to 113°F) ±1.5°C from 45.5°C to 85°C (± 2.7°F from 114°F to 185°F)</td>
</tr>
<tr>
<td>Capacity</td>
<td>2048 consecutive temperature measurements in internal memory</td>
</tr>
<tr>
<td>Operating Range</td>
<td>-40°C to 85°C (-40°F to 185°F)</td>
</tr>
<tr>
<td>Working principles</td>
<td>Measuring temperature in 0.5°C increments; Automatically waking up and measures temperature at user-programmable intervals from 1 to 255 minutes; Programmable temperature-high and temperature-low alarm trip points</td>
</tr>
<tr>
<td>Sampling Methods</td>
<td>Continuous (First-in, First-out) or Stop When Full (Fill-then-stop)</td>
</tr>
<tr>
<td>Software Requirements</td>
<td>SmartButton Reader™</td>
</tr>
<tr>
<td>Communication</td>
<td>RS232 Serial/ACR SmartButton® interface</td>
</tr>
</tbody>
</table>

(Source: www.acrsystems.com)
In this study, the data logger was initialized for data collection at 3 hours' time interval. Given the capacity of the memory, the logger could collect 8 data points per day for 256 days available to stay in the pile, which was more than enough to capture the overall temperature profile during the whole windrow process period. The temperature limits were set from 0°C to 85°C.

As mentioned in the table, both a Smartbutton reader (shown as in Fig. 3-6) and the corresponding software are included in the Smartbutton starter pack. When the software is installed on the computer, with the systematic serial port reader, the data collected and saved in the thermistors could be transferred and downloaded to the computer in both the excel database format and visualized Figure which can show the trend of the temperature changing over time.

![Smartbutton reader](image)

**Figure 3-6 The Smartbutton reader**

The data loggers were encased in watertight PVC casings, given an identification number and painted in a bright colour. They were cycled through the pile as it was turned and recorded temperatures experienced during the entire pile treatment.

Calibration tests were conducted to see if the data loggers could work correctly under high temperatures. A few loggers placed into casings as well as loggers without casing were put in an oven with the temperature set as 65°C, after 24 hours, the temperature then was set directly to 70°C, then after another 24 hours, temperature was increased to 75°C, similarly, this was repeatedly done till the temperature reaching 85°C, the upper testing limit of the Smartbutton. Based on the data collected by the loggers during this process, temperature profiles were plotted
to observe if the loggers behaved consistently with the temperature increment of the oven over this range of relatively high temperatures and also whether the casings interfered with the accuracy of the loggers’ performance. The test proved that the data loggers could work accurately even under high temperatures and the casings didn’t show any influence on the behaviour of loggers.

Figure 3-7 A bunch of loggers in painted and labelled PVC casing

3.3.2 Pile construction

Ninety-eight data loggers were located in a constructed pile (pile 1) on September 14, 2006, which was turned on November 9th, January 9th, then broken down and screened on February 1, 2007.

One hundred loggers were put in a second pile (pile 2) on November 9, 2006, first turned on January 9th, and then on February 1st, broken down and screened on March 27th. The test duration was similar to that of pile 1 at about 4 and half months.
The data loggers (in PVC casings) were embedded in the pile in three vertical layers as the pile was constructed and were expected to act as a surrogate compost particle. They were evenly distributed with each one representing about 0.5m$^3$ of compost material. The schematic demonstration of how the data loggers are laid out in the pile is shown as in Figure 3-9. Data loggers, seen as big dots in the figure, were placed at about 0.8 m interval respectively in both width and height from bottom to top from the cross sectional view; after finishing one layer, more feedstock was added in by front-end loader along the direction as the arrow shown in Figure 3-9, then data loggers were embedded into the new cross sectional interface the same way as layer 1; a third layer was built up also in the same manner. The study section of the pile was located within an elongated full-scale pile, so in length, the three layers were all in the middle of the pile and there was no substantial difference between them. The analysis and discussion will be only based on the cross sections with observing how the temperature varies from bottom to top of pile (in height) as well as from the outer area, i.e. close to ambient, to the core of pile (in width).
Figure 3-9 Schematic demonstration on placement of data loggers and construction of pile

Shown in Figure 3-10, 11 below are the placement the data loggers from a cross-sectional view.

Figure 3-10 Placement of loggers in pile 1
Figure 3-11 Placement of loggers in pile 2
During each turning, effort was given to locating loggers in-situ, in order to be taken out with the compost material surrounding it. This allowed for matching a specific temperature profile to the bacteria data. With each turning, 2-5 loggers were located in-situ while more (about 10 loggers in-situ) were found when the piles were broken down for screening.

![Figure 3-12 A data logger in-situ](image)

Sixty-eight data loggers out of 98 were recovered from pile 1 at the end of the process while 92 out of 100 were recovered from pile 2. Pile 2 was screened twice which helped to improve the recovery rate. 14 loggers (5 for pile 1, 9 for pile 2) were disabled and failed to transfer the data into the computer.

3.4 Bacteria Analysis

The bacteria test on the compost samples in the study were prepared and analyzed following the Test Methods for the Examination of Composting & Compost (TMECC, 2001).

TMECC provides detailed protocols for the composting industry to verify the physical, chemical, and biological condition of composting feedstocks, material in process and compost products at the point of sale. TMECC provides protocols to sample, monitor, and analyze materials at all stages of the composting process, i.e., prior to, during and after composting to help maintain process control, verify product attributes, assure worker safety, and to avoid degradation of the environment in and around the composting facility.
TMECC is released by U.S. Composting Council and it is a laboratory manual modeled after American Society for Testing and Materials (ASTM). It provides benchmark methods for compost analysis to enable comparison of analytical results and it is recognized as the “Gold Method” in the field (http://tmecc.org/tmecc/index.html).

According to the OMRR requirements and the suggested testing parameters in TMECC, Total coliforms, Thermo-tolerant coliforms, *E.coli* and Enterococci were confirmed and quantified for the study while only confirmation test was conducted to monitor for the presence of *Salmonella*.

### 3.4.1 Sample preparation

The compost samples were put into clean Ziploc bags, labelled, shipped to the lab in a cooler with icepacks. Then the following steps were taken.

1. Prepare 0.2% buffered peptone water (BPW) with Oxoid Special Peptone with distilled water. (formula per litre: 2g Peptone in 1000 ml distilled water)
2. Prepare $10^{-1}$ Homogenate: place 20 g of compost into blender, bring weight up to 200 g by adding 0.2% BPW for a 1:10 dilution ($10^{-1}$).
3. Homogenize for 2 mins in blender.
4. Prepare 5 additional dilutions by making 1:10 serial dilutions in 0.85% NaCl. Adding 1mL sample homogenate ($10^{-1}$) to 9 mL 0.85% NaCl, then serially dilute $10^{-2}$, $10^{-3}$, $10^{-4}$, $10^{-5}$, $10^{-6}$. 

3.4.2 Total Coliforms

Apparatus, reagents and materials:
Culture tubes, Fisher brand 95*15 mm Petri Dishes, inverted gas tubes, hockey sticks, Difco™
MacConkey's Agar, Oxoid Lauryl-Tryptose (LT) Broth

The flow chart shown below generally demonstrates how the confirmation and quantification on Coliforms were performed based on TMECC and the practicing procedures in detail will be stated afterward.
Figure 3-14 Quantification and confirmation of Coliforms
(Note: for treated compost, i.e. already being turned, only $10^4$, $10^3$ dilutions were tested with spread plating)
MPN Procedure:
1. Prepare nine culture tubes (three dilutions $10^{-1}$-$10^{-3}$ in triplicate), each containing 9 mL sterile LT broth and inverted gas tube.
2. Aseptically transfer 1 mL of the 1:10 ($10^{-1}$) sample homogenate into each of three culture tubes containing 9 mL sterile LT, which makes the 1:100 final concentration.
3. Aseptically transfer 1 mL of the 1:100 ($10^{2}$) sample homogenate into each of three culture tubes containing 9 mL sterile LT, which makes the 1:1000 final concentration.
4. Aseptically transfer 1 mL of the 1:1000 ($10^{3}$) sample homogenate into each of three culture tubes containing 9 mL sterile LT, which makes the 1:10,000 final concentration.
5. Incubate tubes for 24h to 48h in a 37±2°C incubator.
6. Observe the inverted gas tubes for the presence of small air bubbles. Gas formation indicates a positive result for lactose fermentation, and is therefore a positive result for a coliform. Record the number of tubes in each dilution set that are positive for gas formation. Convert dilutions to a dry weight basis by multiplying tube concentration by the total solids ratio as determined on a parallel aliquot. This number will be used to calculate the MPN $g^{-1}$ for total coliforms. Keep the record.

Spread Plates:
1. Prepare MacConkey agar in the NUAIR NU-201-430 Laminar Flow Hood. Air-dry the surface of the plates by maintaining them covered at room temperature for one day.
2. Place 100uL from $10^{-4}$-$10^{-6}$ dilutions onto the surfaces of different MacConkey plates.

3. Spread the liquid evenly onto the surface of each agar plate using a sterilized glass "hockey-stick." The hockey sticks are sterilized easily by dipping in 70% ethanol and briefly passing through a flame to eliminate trace residue of the alcohol.

4. Incubate at 37±2°C incubator for 18h-24h.

5. Observe the agar surface for colonies that are bright pink or red. These colonies are considered to be coliforms (gram negative, lactose-fermenting members of the family Enterobacteriaceae).

Figure 3-16 Laminar Flow Hood

Figure 3-17 Positive colonies on MacConkey plate
3.4.3 Thermo-tolerant Coliforms

Apparatus, reagents and materials:
Culture tubes, inverted gas tubes, Fisher brand 95*15 mm Petri Dishes, hockey sticks, Difco™ MacConkey's Agar, Oxoid EC Broth with 4-methylumbelliferyl-p-D-glucuronide (MUG) broth.

MPN Procedure:
1. Prepare nine culture tubes (three dilutions $10^{-1}-10^{-3}$ in triplicate), each containing 9 mL sterile EC-MUG broth and inverted gas tube.
2. For each positive LT tube, aseptically transfer 20uL-40uL into a culture tube containing 9mL EC-MUG.
3. Incubate all EC-MUG tubes at 44.5°C for 18h to 24h.
4. Observe EC-MUG gas tubes for presence of air bubbles. Gas formation indicates a positive result.

Spread Plates:
1. Prepare MacConkey agar using standard spread plating techniques in the NUAIR NU-201-430 Laminar Flow Hood. Air-dry the surface of the plates by maintaining them covered at room temperature for one day.
2. Place 100uL L from $10^{-4}$-$10^{-6}$ dilutions onto the surfaces of different MacConkey plates.
3. Spread the liquid evenly onto the surface of each agar plate using a sterilized glass "hockey-stick." The hockey sticks are sterilized by dipping in 70% ethanol and briefly passing through a flame to eliminate trace residue of the alcohol.
4. Incubate at 44.5+1°C incubator for 18h-24h.
5. Count colonies with bright pink or red color.

3.4.4 E.Coli

1. Observe the EC-MUG tubes under long wave ultraviolet light. Tubes which fluoresce and contain gas in the inverted gas tube were considered positive for E.coli.
3.4.5 Enterococcus

Apparatus, reagents and materials:
Culture tubes, inverted gas tubes, hockey sticks, Difco™ Modified Enterococcus (mEnt) Agar, Difco™ Azide Dextrose Broth (AD Broth), Difco™ Brain Heart Infusion (BHI)

The flow chart shown below generally demonstrates how the confirmation and quantification on Enterococci have been performed based on TMECC and the practicing procedures in detail will be stated afterward.
MPN:

1. Prepare nine culture tubes (three dilutions $10^{-1}$-$10^{-3}$ in triplicate), each containing 9 mL sterile AD broth and inverted gas tube.
2. Aseptically transfer 1 mL of sample into each tube.
3. Incubate tubes for 24h in a $37\pm2^{o}C$ incubator.
4. Observe the AD tubes for presence of growth turbidity. Vortex each tube and streak (one loopful) onto the surface of mEnt agar plate. Simultaneously pipet 20-40uL from each tube into 9mL BHI containing 6.5% NaCl.

**Figure 3-19** Quantification and confirmation of Enterococci
(Note: for treated compost, i.e. already being turned, only $10^4$, $10^3$ dilutions were tested with spread plating)

MPN:
5. Incubate mEnt plates for 24-48h in a 37°C incubator. Record the number of plates in each dilution set that are positive for growth. This number will be used to calculate the MPN g\(^{-1}\) for *fecal Streptococci*, which includes *Enterococci*.

6. Incubate the BHI+6.5%NaCl tubes in a 37°C for 24h. Record the number of tubes in each dilution set that are positive for growth. This number will be used to calculate the MPN g\(^{-1}\) dw for *Enterococci*.

**Spread plates:**

1. Place 100uL from \(10^4\)-\(10^6\) dilutions onto the surface of a different mEnt plates.
2. Incubate at 37°C for 24h to 48h.
3. Count colonies which may appear red, purple or absent of colour. All are considered to be *Enterococcus*.

![Figure 3-20 Positive colonies on mEnt plate](image)

### 3.4.6 Calculation

MPN- Record the number of positive tubes in each dilution set. Select the highest dilution that gives results in all tubes, plus the next two higher dilutions. For dilutions prepared with 'as received' samples, i.e., wet weight basis, convert the test aliquot size from we: weight basis to dry weight basis by multiplying each dilution times the total solids ratio and compute the MPN g\(^{-1}\) dw using the MPN calculator (http://www.i2workout.com/mcuriale/mpn/index.html).
Colony Forming Units Technique (CFU)-Quantify the total coliforms as colony-forming units (cfu g⁻¹ dw). Count the pink colonies and perform the calculation using the following formula:

\[ CFU = \frac{C}{V} \times D \times TS \]

Where:

- CFU: colony forming units per gram of sample; number of cells in original sample, cfu g⁻¹ dw,
- C: number of colonies of the target organism, (e.g., coliforms=pink or red colonies),
- V: volume plated, mL, i.e., 100uL=0.1mL; 50uL=0.05mL, etc.,
- D: dilution factor, mL g⁻¹;
- TS: total solids ratio = mass of oven dried aliquot/mass of aliquot at as received moisture.

3.4.7 Salmonella

Apparatus, reagents and materials:

Culture tubes, hockey sticks, Difco™ Tetraphionate Broth Base, Difco™ XLT4 Agar Base +Supplement, Difco™ Triple Sugar Iron Agar (TSI)

Procedure:

1. Incubate the 10⁻¹ homogenate in 37°C incubator for 18 to 24h.
2. Transfer two * 1 ml homogenate to two 9 ml Tetraphionate broth, respectively.
3. Place tubes into a 35°C incubator for 18 to 24h.
4. Vortex the tubes for 5-10 sec, and aseptically transfer two loops of broth mix onto XLT4 agar. Streak for isolation.
5. Incubate for 24h at 35°C, if no black colonies are seen, the plates are then incubated for an additional 24h.
6. All red colonies, red colonies with black centers, and black colonies are considered presumptively positive salmonellae.
7. Prepare TSI Agar into slants with a generous slant on the top of the tube.
8. Pick the presumptive colony from XLT4 plate using sterile loop and inoculate the medium in the following manner: Inoculate TSI agar by first streaking the slant, then stabbing into the solid non-slant end of agar, on the deep end of the tube.
9. Incubate for 18hr at 35°C.
10. Observe coloration of medium. Presumptive salmonellae on TSI will have an acid butt (yellow) and basic slant (red). Some will present a black colour in the butt.
Bioaerosols Analysis

Apparatus, and materials:
Andersen six-stage sampler, Aircon2 High Volume Air Sampler pump, personal sampling cassette, Airchek Sampler pump, fiber filters, PHOENIX Biomedical 100*15 mm Petri Dishes, PVC tubing, Difco™ Tryptic Soy Agar(TSA), Difco™ Malt Extract Agar(MEA), Ziploc bags, markers, alcohol wipes.

3.5.1 Personal Sampling

Samples were captured on 25mm glass fibre filters which are fitted into the cassettes. The glass fibre filters were baked (depyrogenated) in the oven at a temperature of 356 °F to render the filters free of contaminating endotoxin, then were put in the balance room, where both the temperature and humidity were kept constant and insulated from air flow for 24 hours. After field samples were taken, the filters were put in the drying cabinet which contained desiccant, for removing moisture on the filters, then left in the balance room again for at least for 24 hours before taking post-weight to get prepared for the same measurement condition as the pre-weight thus making the results matchable and comparable. The cassettes were rinsed in the Branson 2200 Ultra-sonic cleaner before each use.

Figure 3-21 Personal sampling pump  Figure 3-22 Seven-hole sampling cassette with filter in

To capture the workers’ exposure to endotoxin and β(1-3)glucan, seven-hole sampling cassettes were attached to personal sampling pumps calibrated to 2 liters per min flow rate, to collect particulate matter with aerodynamic diameters of 100 micrometers or smaller. Cassettes were taped both inside and outside the front-end loader cab for the study. It would be ideal if the
workers could carry the cassettes personally, considering the inconvenience of working around with wearing the pumps, the alternatives put into practice was to tape the cassettes right above the seat back inside the cab, close to the driver and almost at the same height as the upper respiratory tract, which still enabled the collection of representative samples. Outside, the cassettes were attached right above the door of the cab.

By deducting the pre-weight from post-weight, the net weight of the airborne particulate matter could be obtained. With knowing the sampling time and flow rate, the volume of the air collected by the personal pump would be available, and when dividing the net weight of airborne particles by the volume, the concentration (mg/m³) was then obtained.

Endotoxin Analysis: Samples were sent to the laboratory of Dr. Karen Bartlett, School of Occupational and Environmental Hygiene, University of British Columbia, for Endotoxin analysis.

β-(1-3) glucan Analysis: Glucan was extracted from the filters by denaturing the molecule by moist heat (autoclave). The samples were sent to the laboratory of Dr. Peter Thorne, Institute of Rural and Environmental Health, University of Iowa, for analysis by end-point, enzyme linked immunoabsorbant assay (ELISA) for fungal glucan.
3.5.2 Andersen Sampling

Airborne bacteria and fungi were collected with a six-stage Andersen sampler operating for 5 minutes and located about 1m above the ground. The Andersen sampler was equipped with plastic Petri dishes containing 45 ml of the appropriate media and was calibrated with plates in place as field blanks. TSA agar was prepared for collecting airborne thermo-tolerant bacteria. MEA agar was used for the fungus Aspergillus fumigatus. The plates were prepared in the NUAIR NU-201-430 Laminar Flow Hood. The surfaces of the plates were air dried by maintaining them covered at room temperature for one day.

Bacterial plates were incubated at 55 °C for 48h, and Aspergillus fumigatus plates were incubated at 37°C for 48h. The colonies were then counted. Only colonies in the hole pattern were counted, and the positive hole correction was applied (Andersen, 1960).

The flow rate on the pumps was set so that they capture particulate matter of a certain size range, when paired with the sampling head. In this case, the large pump ran at 28.3 lpm when it was used with the Andersen six-stage sampler.
Table 3-2 List of diameter ranges of particulate collected by stages

<table>
<thead>
<tr>
<th>Stages</th>
<th>Diameters of particulate collected (micrometers)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7-10</td>
</tr>
<tr>
<td>2</td>
<td>4.7-7.0</td>
</tr>
<tr>
<td>3</td>
<td>3.3-4.7</td>
</tr>
<tr>
<td>4</td>
<td>2.1-3.3</td>
</tr>
<tr>
<td>5</td>
<td>1.1-2.1</td>
</tr>
<tr>
<td>6</td>
<td>0.65-1.1</td>
</tr>
</tbody>
</table>

(Source: Andersen, 1958)

Sampling sites in the composting facility were selected to be representative of worker locations for both waste processing and compost manipulation. For material processing, the sampling site was at the grinder. Sampling sites in the compost area were beside the front-end loaders manipulating compost piles such as moving or turning and at other locations such as screening. Upwind and downwind samples were taken as well to help get an understanding of how the surrounding area could be affected by the composting practice. Sampling covered the various stages of the whole process, namely, new feedstock, thermophilic stages (two turnings), and screening.

Figure 3-26 Taking sample with Andersen sampler on site
3.6 Weather monitoring

- Precipitation was collected continuously using a RL-Loader V2.1.4 rain gauge.
- Ambient temperature was collected in duplicate using two Smartbutton temperature data loggers.
- During aerosol sampling, wind speed, temperature and relative humidity were measured using a Kestrel 4000 Pocket Weather Tracker.
CHAPTER 4 RESULTS AND DISCUSSION

4.1 Physical appearance of feedstock

Pile 1 was constructed on September 14, 2006, which was the end of summer in Vancouver. The material of feedstock in this pile was primarily woody stuff, such as branches and trimmings, but also grass, etc. Pile 2 was built up on November 9, 2006, which was right about the peak time for leaf drop, so the fresh feedstock was almost all leafy material. The whole pile appeared yellow and quite compacted compared with pile 1.

Although no measurements were made on the physical parameters of the material, e.g. particle size, porosity, bulk density etc. It was not hard to tell the difference in physical make-up of the material from pile 1 and pile 2 by visual observation. Particle size and porosity were visually larger in pile 1, but bulk density was smaller; while the particle was finer in pile 2 and more compacted, therefore, the porosity of pile 2 was consequently smaller while the bulk density was higher.

4.2 Physical Dimensions of Piles

Pile 1 was about 18 feet high, 26 feet wide as the cross section, and about 16 feet in length as the logger section; pile 2 was about 16 feet high, 26 feet wide and 13 feet long as the logger section, which were both within about 150 feet long piles.

4.3 Moisture content

Moisture content is a critical variable in composting. If the mixture is too dry, the microorganisms cannot survive, and composting stops. If there is too much water, the oxygen from the air is not able to penetrate to where the micro-organisms are, and the mixture becomes anaerobic. The optimum moisture content for biodegradation could vary widely for different compost mixtures and times in the composting process, ranging from near 50 to over 70% on a wet basis. Significant reduction in biodegradation rate appears when operating outside the optimum range (Richard et al., 2002). Figure 33 shown below is the average moisture content at each stage, feedstock, first turned, second turned, and screened, for both pile 1 and pile 2(Appendix F).
From Figure 4-1, it can be seen that for either pile 1 or pile 2, the moisture content is for the most part within the optimum range, near 50 to 70%. Moisture was not likely a limiting factor as far as the study is concerned.

As it may be noticed, the moisture content of pile 2 is generally, higher than that of pile 1, the reasons for the difference could be attributed to the seasonal variation in both the weather condition (rain season just got started when pile 2 was built up) and the raw material of feedstock (foliage peak for pile 2 while more woody stuff in pile 1). Given that pile 2 started with smaller particles and porosity, the higher moisture content of pile 2 might lead to greater compaction of the pile and therefore possibly lower porosity at the bottom. See the attached CD for all the appendixes of this thesis.

4.4 Nutrients (C:N ratio)

In Figure 4-2 are the C:N ratio changes over time for both pile 1 and pile 2 while number of samples included in this figure(See Appendix G for data) is listed in Table 4-1:
As it has been mentioned before, theoretically, 20-40 parts of carbon to 1 part of nitrogen is the acceptable range for successful composting and if the ratio exceeds 30, the rate of composting decreases. Also, C:N ratio should have decreased at the end of composting process. A final C:N ratio of 15 to 20 would be expected and a value of more than 20 might have a negative impact (Young et al., 2005). As can be seen from Figure 4-2 both pile 1 or pile 2, from the start till before being screened, had C:N ratio all within the optimum range with decreased ratios at the end of the process, within the expected and favourable range for finished product.

Although based on quite limiting number of samples, it would appear that nutrient ratios were well within desirable ranges.
4.5 Temperature

As has been described before, Smartbutton data loggers were placed in the pile with each one representing 0.5 m$^3$ material, which was realized by setting each logger about 0.8m*0.8m*0.8m in length, width, height, respectively, apart from the other logger. According to the physical similarity of data loggers’ placement in the pile, they were grouped into five positions as shown in Figure 4-3 for analysis and to help get a clearer picture of the temperature profile over time within the whole pile from nearly a hundred scattered loggers.

![Cross Sectional View of the placement of loggers](image)

Figure 4-3 Cross Sectional View of the placement of loggers

Temperature data from the recovered and usable data loggers were downloaded with the data each saved as an individual excel file.

Note that up to the first turn, the data loggers were all as originally placed, in other words, perfectly matching the original placements and the position layout. Once the pile was turned, all the loggers moved with the compost, thus the positions could not be predicted anymore. Therefore, only during the period from start to the first turn, was the profile clearly associated with position and could be used to describe how the temperature changed spatially and temporally in the pile.
4.5.1 Pile 1

4.5.1.1 From start to the 1st turn

The number of loggers from which data were grouped together based on the defined positions is listed in Table 4-2:

<table>
<thead>
<tr>
<th>Position</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Total loggers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of loggers grouped</td>
<td>16</td>
<td>9</td>
<td>15</td>
<td>12</td>
<td>4</td>
<td>56</td>
</tr>
</tbody>
</table>

Therefore, Figure 4-4 was made by taking means (Appendix B) of all the data obtained at each time point from the data loggers (Appendix A) starting with the same position, then plotting each mean versus time to get the temperature changing curve of each individual position, the “average” in the figure was obtained by taking means of the mean values of all the positions and plotting versus time.

As mentioned before, pile 1 was constructed on September 14, 2006, the material of feedstock in this pile was primarily woody stuff. Ninety-eight data loggers were embedded in the pile. The pile was turned on November 9th and January 9th, was screened on February 1st. Figure 4-4 describes how the average temperature of each group of loggers in positions 1-5 as shown in figure changes with time from the start of the run till the first turn (Nov 9th). As pointed out in Figure 4-4, most obvious temperature fluctuations occurred in section 2, the outside layer.

Given that the rain data was available only starting from October 22, 2006, which was after the construction of pile 1 and the temperature fluctuation no. “1” occurred before the availability of rain data, so rain data profile is also provided below as Figure 4-10 only with one rain event, “2” marked as the matching-up of the “2” pointed out in both Figure 4-4 and Figure 4-10 based on occurrence at the same time.
The five graphs listed below are simply temperature profiles separated from Figure 4-4 into five individual positions to show the standard deviations of the data grouped by weeks on each curve, i.e. each position. Considering the loggers recorded data by every three hours, which was so intense that made it not possible to plot the standard deviation at each time point, therefore, to make the demonstration more interpretable, weekly standard deviations for each position are shown individually in five figures as follows:
Figure 4-5 Temperature profile with weekly standard deviation for position 1, pile 1

Figure 4-6 Temperature profile with weekly standard deviation for position 2, pile 1
Figure 4-7 Temperature profile with weekly standard deviation for position 3, pile 1

Figure 4-8 Temperature profile with weekly standard deviation for position 4, pile 1
Figure 4-9 Temperature profile with weekly standard deviation for position 5, pile 1

Figure 4-10 Rain profile for pile 1 with matching-up event as the temperature profile
After the temperature fluctuations only at position 2 have been observed from Figure 4-4, while the temperatures of other positions just keep on rising without getting any influence from the ambient and showing any fluctuations. When looking back to the rain profile, there seems to be some correlation between the rain events and the temperature fluctuation at position 2. As pointed out by "2" in Figure 4-4 and 4-10, the big rain event in Figure 4-10 and the fluctuation in Figure 4-4 occurred on the same day, therefore, it is likely that position 2, the outer layer, may get some influence from the ambient while other positions not. In addition, although lack of rain data before October 22, 2006, according to the historical weather records found on-line, a rain event occurred around September 21, 2006 too, which was the same time when the fluctuation appeared and is shown as number "1" in Figure 4-4.
4.3.1.2 Demonstration of temperatures from data loggers in-situ at every layer for pile 1

Figure 4-11 Temperatures from data loggers in-situ for pile 1 layer 1 one month after the start

Figure 4-12 Temperatures from data loggers in-situ for pile 1 layer 2 one month after the start
To illustrate the range of temperatures within two piles, Figure 4-11, 12, 13 plot for each data logger in situ, the mean of one day's temperature data. The positions of the loggers were mapped at the time the pile was constructed. Temporarily the pile was one month post-construction and before the first turn. Each of the three cross section positions is shown. These data were examined to determine if solar radiation or dominant wind direction may have influenced pile temperatures.

It seems that the temperatures of the right side of the pile as shown in the figures appear a little bit higher than those of the left side. In general, no big difference in temperature between the two sides of the pile has been observed in these figures, more research may need to be conducted to look into this issue.

It can be seen from Figure 4-4 that the temperatures at other positions just kept increasing without any apparent influence from the ambient environment. The temperature at every position rose quickly, within 4 days. The Figure 4-14 is the temperature profile over five days for one data logger (No. 86) as an example, which located at the lower part of position 3:
It can be seen from our findings that at the very beginning of the process, when all required factors, e.g. nutrient, oxygen, etc. are sufficiently satisfied, plus no heat self-limiting mechanism has occurred yet, the microbial activities are very active and thus temperature inside the pile rapidly increased. The ambient temperature during this period was also relatively high considering it was between the end of summer and start of fall.

Figure 4-4 also shows that in position 4, which basically has the least portion of material either close to the ambient or the ground, the temperature rises to the highest compared to the same day data recorded at the other positions. Position 1, not affected by the ambient, and the temperature overall stayed relatively cooler than other positions probably because the large surface area of material on the ground greatly increases the chance of heat exchange with the ground. The chimney effect also drives the heat from position 1 to go up. At the same time, the temperatures of both position 3 and 5 tend to be quite consistent with their positions in the whole pile, being medium in general.

4.5.1.3 What can be seen from a 3D video demonstration

3D videos were made to show how the temperature was changing dynamically in the pile from the day of construction to the first turn. A series of videos for both piles were made. Two frames
of starting phase of pile 1 were captured as representative and are presented in Figure 4-15,16 (Appendix A).

The Figures illustrate what is happening inside the pile both temporally and spatially in detail. The colour of green in the video represents colder temperature, while red is hotter, so the change of colour, from green to red, indicates the rising of temperature. The picture presents the profile from the cross sectional view. A, B, C, D are four individual profiles chosen to help illustrate the findings.

![Figure 4-15 Captured from the 3D video about 15 days after construction of the pile](image)

It can be seen from this picture that the temperature shown by C,D, goes to above 55°C much faster comparing with that from bottom or inside lower part, shown by A,B.
Figure 4-16 Captured from the 3D video about 30 days after construction of the pile

From this picture it can be seen that the temperature of the bottom and lower layers start to increase, comparing the A, B here with the same positions in the previous graph, while that of outer layer drops, similarly, comparing to the previous performance of C, D. The reasons could possibly be because the oxygen and substrate have been used up very quickly due to the intensive microbial activities in the outer part, while the heat keeps on accumulating inside the pile which partly contributes to the increase of temperature among the inner parts, on the other hand, the accumulative heat may result in the occurrence of chimney effect inside the pile, which drives heat up and draws the oxygen in from the ambient environment thus providing oxygen to the inner part for their microbial activities, leading to the continuous but slow increase in temperature in those parts. And the reason for the slow increase as well as not being able to reach as high as the outer parts is probably because either the chimney effect or the oxygen diffusion can not provide as abundant an amount of oxygen as the outer parts got at the very beginning.

4.5.1.4 From start to finish

The number of data loggers originally embedded in the pile and recovered during screening as well as the recovery rate of loggers from each position are listed in Table 4-3 shown below. There were 5 loggers from which the data could not be read by the Smart button reader thus not included here. The recovery from each position is similar.
### Table 4-3 Number of loggers recovered and embedded for pile 1 from start to finish

<table>
<thead>
<tr>
<th>Initial Position</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of loggers recovered</td>
<td>16</td>
<td>9</td>
<td>15</td>
<td>12</td>
<td>4</td>
<td>56</td>
</tr>
<tr>
<td>Number of loggers embedded in the pile</td>
<td>26</td>
<td>18</td>
<td>22</td>
<td>22</td>
<td>9</td>
<td>97</td>
</tr>
<tr>
<td>Recovery rate</td>
<td>62%</td>
<td>50%</td>
<td>68%</td>
<td>55%</td>
<td>44%</td>
<td>58%</td>
</tr>
</tbody>
</table>

**Figure 4-17 Temperature VS time at 5 positions of pile 1 from start to finish**

Figure 4-17 was made by plotting the mean value (Appendix B) of the data loggers at each position at every single time point (Appendix A) versus time of the whole process, from start to finish. As it has been mentioned above, only the data loggers were identified by their starting position and were in different positions after the first turn. Even so, the data has still been grouped in this way to provide a better view on what happened to the compost material represented by these data loggers from different positions after being turned. Observations found from the figure with data sorted in this way were still quite meaningful and constructive.
It can be seen from Figure 4-17 that after the first turn (09/11/2006), the data loggers from high-temperature levels fall while the low temperature data logger increased, which indicates that the material was thoroughly mixed after being turned.

Furthermore, the profiles show that the highest temperatures appeared after the first turn. Considering the ambient temperature actually started to decline during this period of time (September-November), hence, the finding helps illustrate that turning plays a role in improving the microbiological condition, thus strengthening the microbial activities and generating more heat while other factors such as nutrients are not limiting. Highest average temperatures were also between the first and second turn. Temperatures above 75°C were seen after the second turn with a maximum recorded temperature of 80°C.

However, after the pile remained in the thermophilic phase for some days, it was turned and aerated but at colder air (winter time) and temperature. The pile reheated but did not on average return to the temperature prior to the turn. However, individual growing did once again demonstrate the effect of turning.

4.5.2 Pile 2

4.5.2.1 From start to the 1st turn

Pile 2 was built up on November 9, 2006, which corresponded to peak leaf drop, the fresh feedstock was predominantly leaf material. The whole pile appeared yellow and quite compacted comparing with pile 1. Ninety eight data loggers were originally embedded into the pile and the pile was turned on January 9th and February 1st, and was screened on March 27th. The number of data loggers plotted for pile 2 from start to first turn is shown as below in Table 4-4. Presented in Figure 4-18 is the average temperature (Appendix C, D) of each position in pile 2.

| Table 4-4 Number of loggers grouped for pile 2 from start to 1st turn |
|-------------------------|---|---|---|---|---|---|
| Position | 1 | 2 | 3 | 4 | 5 | Total |
| Number of loggers grouped | 21 | 18 | 18 | 16 | 7 | 80 |
Figure 4-18 Temperature VS time at 5 positions of pile 2 from start to 1st turn
Weekly standard deviations for each position of pile 2 are shown individually in five graphs as follows:

Figure 4-19 Temperature profile with weekly standard deviation for position 1, pile 2

Figure 4-20 Temperature profile with weekly standard deviation for position 2, pile 2
Figure 4-21 Temperature profile with weekly standard deviation for position 3, pile 2

Figure 4-22 Temperature profile with weekly standard deviation for position 4, pile 2
In Figure 4-18, the labelled arrows 1 to 5 point out the occurrence of downside fluctuations at position 2 during the period of time from start to first turn, which are all matched up by the rain events labelled correspondingly as number 1 to 5 in the rain profile based on the same period of time. Again, since the position 2 is the only affected one, to help provide a clearer view on these fluctuations, a figure plotting the temperature versus time purely of position 2 is provided as Figure 4-24 shown below, with all the five matching up curves marked as well.
There are some similarities of the trends shown here with that shown in pile 1. As could be expected, position 2 (outside layer) seems to be the only one which was influenced by the changes of ambient weather while the temperatures at other positions simply and smoothly increase. It can be observed that the occurrence of every low curve, to some extent, comes following some rain events, which again, shows the ambient environment may play a role in affecting the temperature of position 2, the outer layer.

The difference between pile 2 and pile 1 can also be seen by comparing Figure 4-4 and 4-18: although the temperatures rise in pile 2, they don't increase as fast as those in pile 1, and the performance of positions is also different comparing to that of each matching position in pile 1.

Physical parameters such as particle size, porosity, bulk density etc., based on the visual observation are quite different with the porosity as well as smaller bulk density comparing with pile 2, while smaller particle size results in reduced air space and less porosity. Particle size also affects moisture retention and therefore reduces gas permeability.
Considering aeration is one of the key elements in composting, particle size plays a role in the process, affects the capacity for oxygen penetration. Moreover, although smaller particle size reduces the permeation of the oxygen supply, aerobic decomposition increases with smaller particle size. In pile 1, the mean temperature recorded in position 4 was the highest while in pile 2, the mean temperature recorded in position 2 (outer layer) was the highest while inside positions stayed much lower. Because the particle size was bigger and porosity was much greater in pile 1, the oxygen is able to get into the center of the pile, and the temperature at position 4 was the highest. Conversely, as the particle size in pile 2 was much smaller as well as the porosity was correspondingly lower. The rain season in Vancouver just started before pile 2 was constructed, and as is known the smaller particles have more moisture retention capacity, which results in even more reduced air space and more compaction in the inner zone and bottom. Oxygen may have been limited in the core zone of pile 2, thus the temperature of inner positions was not able to increase to the same extent as in pile 1 and all mean temperatures were lower than the mean of position 2.

In theory, by turning regularly, the particle size within the pile is decreased. By employing grinding and sieving equipment, problems like big particle can also be avoided. At the end of the process, the bulk density of the compost would be expected to increase due to breakdown in the particle size of the material, resulting in more compact compost.
4.5.2.2 Demonstration of temperatures from data loggers in-situ at each layer for pile 2

Figure 4-26 Temperatures from data loggers in-situ for pile 2 layer 1

Figure 4-27 Temperatures from data loggers in-situ for pile 2 layer 2
Figure 4-28 Temperatures from data loggers in-situ for pile 2 layer 3

Figure 4-26, 27, 28 are the demonstration of one day's temperatures from data loggers as placed originally in-situ by layers, 35 days after the pile was constructed.

Similar to pile 1, the temperatures on the right side of the pile as shown in the three figures above appear to be a little bit higher than those of the left side. Again, generally speaking, no big difference in temperature between the two sides of the pile was observed in these figures.

4.5.2.3 From start to finish

Number of data loggers originally embedded in the pile and recovered during screening as well as the recovery rate of loggers from each position are listed in Table 4-5 shown below. There were 9 loggers which turned out not to be downloaded by the Smart button reader thus not included here. The recovery of data logger was similar for all positions.
Table 4-5 Number of loggers embedded and recovered for pile 2 from start to finish

<table>
<thead>
<tr>
<th>Position</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of loggers recovered</td>
<td>21</td>
<td>18</td>
<td>18</td>
<td>16</td>
<td>7</td>
<td>80</td>
</tr>
<tr>
<td>Number of loggers embedded</td>
<td>29</td>
<td>22</td>
<td>21</td>
<td>17</td>
<td>9</td>
<td>98</td>
</tr>
<tr>
<td>Recovery rate</td>
<td>72%</td>
<td>82%</td>
<td>86%</td>
<td>94%</td>
<td>78%</td>
<td>82%</td>
</tr>
</tbody>
</table>

Figure 4-29 Temperature VS time at 5 positions of pile 2 from start to finish

From the overall trend of pile 2 shown in this figure (Appendix C, D), some similar conclusions could be drawn as for pile 1, such as turning does help mixing and aerating. However, there are also some differences presented in pile 2 comparing with pile 1. For example, after the first turn, the average temperature in general was almost the same as that before the turn and also the highest temperature occurred at the first phase (before first turn), which was different from what was observed in pile 1. The major reason is probably because of the ambient temperature, the ambient temperature was colder after the first turn was made, which may have affected the temperature of the pile.
An unresolved question is why there are so many temperature fluctuations after the second turn in pile 2, at the last phase of the process, which was not observed in pile 1.

52-60°C is recognized as the optimum temperature for microbial decomposition in green waste composting. Therefore, although pile 2 didn’t reach temperature as high as that in pile 1, it can be seen from the Figure 4-29 that its average temperature did stay within the optimum range for most of the whole period of time, which is more favourable to the microbial activities rather than the high temperature.

*What can be found from the weekly average temperature profile*

4.5.3.1 Pile 1

![Vancouver Pile 1: Weekly Average Temperature](image)

The weekly temperature is plotted to illustrate the weekly maximum, minimum and average values (Appendix A, E). The max is taken from the maximum temperature achieved of all the data loggers available during every week, correspondingly, the min means the minimum temperature reached weekly while the average is from taking the means of all the data points captured within the week.
Even knowing that the temperature of pile 1 was able to reach very high levels within quite a short period of time. It is still surprising to see the weekly average of the maximum temperatures of pile 1 stayed above 75°C, or most time above 80°C (85°C is the maximum temperature the logger could record) for almost the whole period of time except the first two weeks, which also indicated that the thermophilic phase was not completed. As afar as the mean temperature was above 55°C for 14 weeks, which is obviously far more than 15 days, the regulatory requirement. Hence, when it comes to the thermal killing, it seems no doubt for pile 1 to be able to do a good job. Will that be the case? Statistical data about pathogen will be shown in next section.

In addition, it can also be seen from the Figure 4-30 that the minimum temperatures are quite close to the ambient temperature, showing that the lowest temperatures were at the bottom of the pile, while the inner part of the pile was not affected by the ambient.

4.5.3.2 Pile 2

![Figure 4-31 Weekly average temperature of pile 2](image)

It can be seen that the temperature in general (Appendix C, E) was lower in pile 2 than in pile 1. Considering pile 1 was constructed on Sep 14, 2006, while pile 2 on Nov 9, 2006, part of the reason could be attributed to seasonal variation, both in temperature and precipitation. Therefore, a conclusion is not hard to be made that for the outdoor windrow composting, the weather
condition (temperature, precipitation, wind, etc) plays an important role in the thermal regime inside the pile.

The difference in feedstock would contribute to the difference in temperature between the two piles. Because according to what has been discussed above, the porosity, bulk density and particle size can make great difference in the thermal profile. Additionally, the difference of nutrients concentration in two piles is also a factor which can not be neglected.

4.5.3.3 How the mean could be biased

Last but not least, attention must be paid to the mean temperature shown in Figure 4-31. It appears that the whole pile failed to meet the regulation (15 days over 55°C). However, when looking into the data profile for each retrieved individual logger, it was found that only 20% of the data loggers were in compost that truly failed. By examining the individual loggers, the reason for such big bias could be attributed to the range above and below 55°C. The measured temperatures below 55°C went much lower than 55°C and stayed much longer compared with those going above 55°C, which rose a little bit higher than 55°C and also didn’t remain long. All those “low points” played a significant role in dragging the mean down to below 55°C. Temperature profiles of two loggers (ID No. 81 and 196) from pile 2 are shown in Figure 4-32 as examples to better illustrate the description made above. The black line represents the 55°C baseline and the other two red lines show both the highest and the lowest data points reached. From the graphs, it can be seen that both loggers record more than 15 days above 55°C, 42 days for logger 81 while 19.6 days for logger 196, however, it can also be seen how much the difference is, comparing the distance from the upper red line to the black line with that from the lower red line to the black line, which leads to the conclusion of “process failure” as far as the mean is concerned.
Furthermore, as far as this issue is concerned, the traditional way of testing temperature with probes in the composting piles becomes problematic. The answer is certain as comparing with the data loggers which could be evenly laid out in the whole pile and covering the whole process, according to the time of staying at low temperatures as shown in Figure 4-32, there is much larger chance for probes to capture the low temperature points, thus conclusion could possibly be made as “process failure” which, as a matter of fact, is “passing” if tested with more temporally and spatially intensive measurements by using these Smartbutton data loggers. In conclusion, probe testing may not be reliable and may lead to false result.

Besides finding out actually 80% of pile 2 met the time and temperature criteria, although this is not an exciting number itself, still much better than the 0% passing ratio at the first glance of the mean profile. Lessons could also be learnt from the large data range that examining the mean sometimes can help get a clue from large amount of data and provide a general and clear idea about it, but sometimes, it may block the sight on the real picture and mislead to the wrong conclusion.
4.5.4 Highest temperature

From the data collected from the retrieved data loggers in pile 1, all of them recorded data while reached the standard set by OMRR, over 55°C for at least 15 days. Moreover, it is found that the temperature of some parts of the pile reached 85°C, which was the upper range of the loggers. The extremely high temperature was achieved within 15 days of pile 1 being built.

![Figure 4-33 Temperature profile of one logger generated by the SmartButton software](image)

Figure 4-33 Temperature profile of one logger generated by the SmartButton software

Shown in Figure 4-33 is the temperature profile of logger No. 112 as an example showing how high the temperature could rise in the pile. This logger was located at top right part of position 4 in pile 1. Since the upper detection limit of the data logger was 85°C, shown as the broken line in Figure 4-33 this was the highest temperature recorded during the process. It appears from the temperature profile opened with the Smartbutton software, the temperature actually went beyond the upper limit, but could not be recorded. Comparing with 80°C recorded in previous studies, the result found in the present study is surely of great interest and significance. It not only challenges the highest temperature recorded in the literature, but also makes the issue of composting microbiology which will be talked about in the coming section, more intriguing with being through so high temperatures.
Interestingly, many PVC casings for the data loggers were deformed in the pile presumably, because of the high temperature. However, although the PVC was broken down during the composting process, micro-organism survived, even proliferated.

4.5.5 Comparison of average temperature for 5 positions between the two piles

![Cross Sectional View](image)

Figure 4-34 Average temperatures of each position for both piles to first turning

Presented in Figure 4-34 is a comparison between the average temperatures at each position of both piles up to the first turn. The temperatures for pile 1 are all written at their corresponding positions, in green, on the left side of the figure, labelled “Pile 1”. Temperatures for piles 2 are written in red and are plotted on the right side.

4.5.5.1 Comparison between two piles based on same positions

It is clear to see that at position 1, the temperature of pile 2 is much lower than that of pile 1, explanations could be that firstly, the material of pile 2 was much more compacted and more moisture saturated, so it was much harder for oxygen to get into the bottom of the pile; in addition, pile 2 has experienced a snow storm at the end of November in Vancouver, the snow was about 30 inches above the ground and lasted for about 5 days, which possibly influenced the average temperature of pile 2, especially for position 1.

At position 2, although it was colder when pile 2 was built than pile 1, especially considering locations like position 2, which has the largest chance of being influenced by the ambient
temperature, yet the average temperature of both piles were the same. Oxygen is believed to be sufficient for the microbial activities at this position of both piles, plus the finer particle of the feedstock in pile 2, was easier to be degraded, thus resulting in more heat generated balancing the colder ambient temperature. Similarly, particle size can also help explain why the average temperature of pile 2 at position 3 is a little bit higher than that of pile 1.

However, for position 4 and position 5, temperatures in pile 2 are lower than those in pile 1, the reasons for which can also be concluded as the difference of oxygen penetration resulting from the difference of porosity in between piles and the difference in weather condition during the residency of the two piles.

4.5.5.2 Comparison among five positions within one individual pile

Within an individual pile, the highest temperature in pile 1 occurred at position 4, which is probably because this position is isolated from the outside, and as a result, both the accumulative heat and the heat drawn up from bottom by the chimney effect act together at this position. The low bulk density, high porosity of the material make it possible for oxygen to penetrate into this position because of the chimney effect.

Positions 2 and 3 are affected by the ambient temperature to a large extent, consequently, position 4 takes the leading place in pile 1.

However, in pile 2, the low porosity, small particle size and high compaction make it hard for oxygen to get into the inner part, especially hard for the chimney effect to either draw heat upward or get the oxygen diffused into the pile, so insufficient oxygen results in the much lower temperature in position 4 than in position 2 and 3, also can help explain the even lower temperature occurred at position 1 and 5. While with relatively more oxygen available, smaller particle did contribute to the higher temperature achieved at position 2.
4.5.6 Each individual logger's performance during a single day time

Figure 4-35 Scatter plot of one day's temperature of each logger at five positions of pile 1

Figure 4-36 Scatter plot of one day's temperature of each logger at five positions of pile 2

Shown in Figure 4-35,36 are pile temperature as recorded by individual logger on a particular day (one month since the start of the process) still sorted by positions. Each dot represents one logger
and the line just links loggers of the same position together but not to suggest a trend. From the
two figures, it can be seen that the temperature of each logger from the same position varies
greatly, the difference in temperature could even reach about 30°C within one position, which
proves the existence of “cool zones” and also indicates either the composting process or compost
pile is so heterogeneous and unpredictable that hard to reach any uniformity. The explanations in
detail for the difference could be attributed as follows: 1. The compost around each particular
logger could vary a lot, such as the porosity, particle size, moisture content, oxygen and bacterial
concentration, etc.; 2. The wind direction of that day and the long-term solar radiation also may
play a role. It also suggests very strongly that pile temperature is based on microbiology actively
at each location as opposed to general energy dissipation.

The temperatures of position 2 present the relatively small variation in general for either pile 1 or
pile 2, which may also have something to do with the oxygen supply. Because there is much
larger chance for position 2 to get sufficient oxygen supply, while it is quite likely for other
positions to have “dead zones” where hard to have oxygen get in. As oxygen plays a significant
role in the microbial activities and temperature regime, oxygen supply is very likely to be the
explanation to the relatively small variation in temperature at position 2.

Pile 2 has smaller variation in general, which may be because of the finer particles, better
uniformity of the whole pile in many ways, such as material of feedstock, particle size, bulk
density, bacteria concentration etc., and also smaller porosity which could make less sensitive to
ambient weather condition changes.

To sum up, existence of cool zones is, to some extent, closely related to oxygen supply. The
material with sufficient and uniform oxygen supply is less prone to fluctuation in temperature,
thus finer particles can reduce the chance of occurrence of cool zones.

4.5.7 Significance of the temperature monitoring study

This study provide more detailed and extensive data about both spatial and temporal temperature
distributions in real, operational compost piles than any previous studies, by using data loggers to
allocate at every part of the pile(approximately surrogate of each 0.5 m3 material) and
continuously record fluctuating temperature data from building-up the new pile through to
screening.
Furthermore, few, if any previous studies have correlated the time-temperature regime with level of microbial destruction for the full-scale operational composting practice. The discussion on microbial destruction will be in the sections following.
4.6 Bacterial Analysis

According to the OMRR requirements and the suggested testing parameters in TMECC, Total coliforms, Thermo-tolerant coliforms, *E. coli* and *Enterococci* were confirmed and quantified for the study while only confirmation tests were done for *Salmonella*.

Compost samples were taken adjacent to the data loggers in-situ so that the results of analysis could be related to the monitored temperature. Besides those, some compost samples from the outside of the pile were also taken at every stage for bacteria analysis. The number of compost samples analyzed and grouped at each stage is listed in Table 4-6 as follows:

<table>
<thead>
<tr>
<th>Table 4-6 Number of samples analyzed for both piles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of compost samples taken</td>
</tr>
<tr>
<td>Pile 1</td>
</tr>
<tr>
<td>Pile 2</td>
</tr>
</tbody>
</table>

All the data (Appendix F) presented are on a dry weight basis. Data has been grouped and graphed in various ways to show a better and representative view on the picture of pathogen killing over time in the pile.

What also needs to be pointed out here is attention should be paid to both the false “positive” and false “negative” results, which means the results obtained are not completely reliable because even when the result is positive, it may be due to the interference of some other “mimic” organisms while when it appears negative, it could because the organisms of interest actually do exist, but are below the detection limits and as soon as the condition for growth is met, they will grow and proliferate.

In order to analyze the data using data distributions, those “0” counts, or below detection limits, observed by spread plating in the study, with referring to the matching-up batch of MPN tests, which is known for higher sensitivity of detection, are given real numbers by taking the half of the possible limit baseline number for minimizing the bias and bettering the statistical analysis (eg. Log distribution). For example, $10^{-1}$ to $10^{-3}$ diluted samples were tested with spread plating while $10^{-4}$ to $10^{-5}$ with MPN in the study, so if certain organism was all positive in $10^{4}$ diluted
sample in MPN test, but showed up "0" counts in the three serial spread plating tests, then the detection limit was regarded as 1000 and then result as "500" was entitled to this sample.

4.6.1 Performance of bacteria of interest at different stages in pile 1

Figure 4-37 shows obvious decrease in bacterial concentration from the time the pile was newly built until after the first and second turning, especially for the first turning. High temperature does play an effective role in killing bacteria at the thermophilic stage during composting. However, a dramatic increase in total coliform and thermo-tolerant coliform is observed from after the second turn to screening of the pile. There are various possibilities working together to help explain this phenomena, such as drop of temperature, re-growth of bacteria, and accidental contamination. At the end of the thermophilic stage, the temperature of the pile decreases, and is related to a decline in microbial heat due to shortage of substrate to maintain the microbial metabolism plus limited oxygen supply resulting from porosity's getting smaller due to breaking down of feedstock and higher moisture content; moreover, because the temperature has gone up quite high, some were
even reaching up to 85°C during the thermophilic stage, so the self-limiting mechanism may also play a role in hindering the generation of microbial heat, all of which could also result in the observed re-growth here.

Furthermore, there is also the possibility that the pattern shown in the figure could be attributed to the test methodology. Because the medias for growing both total and thermo-tolerant coliforms are not very selective nor exclusive, hence, it is likely that the bacteria of interest have been killed during the process, however, the occurrence of re-growth plus significant amount and variety of environmental bacteria manage to survive, proliferate and even successfully "mimic" the bacteria of interest, growing on the medias, with similar appearing colonies, which leads to the result seen in the figure. What can help support the conclusion that the killing indeed has been going on in the pile is the performance of Enterococci. It is seen that the number of Enterococci continuously decreased to below the detection limit. The media used for growing Enterococci is Difco modified Enterococci, which is designed specially for Enterococci. To conclude, it is a highly selective media thus can inhibit the growth of any other background bacteria. Therefore, without the interference of other environmental organisms, the performance of Enterococci provides a robust proof that significant destruction of microbial organisms is accomplished during the composting process. Testing protocols for other organisms are not as specific and may account for the results that follow.
4.6.2 Performance of bacteria of interest at different stages in pile 2

In pile 2, both the total and thermo-tolerant coliforms behaved quite differently from those in pile 1. As a matter of fact, the original bacteria concentration was much lower in pile 2 compared to pile 1, which may be because the feedstock of pile 2 was mostly yellow leafy material and very uniform, more like pure collection of foliage from streets, in contrast, the feedstock of pile 1 was highly variable, containing trees, branches, trimmings, grass, and all kinds of yard waste, which undoubtedly bears much higher chance of contamination as it is well-recognized that backyard is heaven not only for kids but also for pets, squirrels, raccoons and birds.

For total coliforms, the figure shows the concentration just keeps going all the way up, not as expected at all, the reasons might be as follows: 1. the average temperature of pile 2 ranged from 35-55°C, plus with optimum moisture content, which possibly was providing a favourable environment for the microbial organisms to grow and proliferate. Due to the limitation of the
media, it is very likely that the result shown here may not represent the actual growth of enteric origin bacteria but the large family of environmental organisms, as a result, these all could lead to the increasing concentration from the low starting point.

Consequently, the reasons for the increase of total coliforms could also apply to the thermo-tolerant coliforms, and as the incubating temperature, 35 –37°C for growing total coliforms on the media also allows growth of environmental organisms. Thermo-tolerant coliform concentration does not go up as radically as total coliforms.

It was also observed that after the second turning to screening, the number of total coliforms drops but was still higher than the starting point. Based on this trend, more curing time could be of help to reduce the amount of total coliforms. As the substrate becomes limited, porosity gets smaller thus oxygen is less, which may lead to the significant reduction in the number of total coliforms.

However, the same as in pile 1, the performance of Enterococci indicated the successful pathogen killing of composting after seeing the unexpected behavior of total coliforms and thermo-tolerant coliforms. The concentration of Enterococci are reduced to detection limit. Also, based on the findings discussed above, selection of indicator organisms from the regulatory perspective may need to be re-examined.

In addition, the presence of both E.coli and Salmonella has been observed over the whole process and also showed a bit trend of re-growth.
4.6.3 What can be found from the average temperature vs bacteria counts in pile 1

Figure 4-39 Average temperature vs TC counts for end samples of pile 1
Interestingly, from the final compost samples of pile 1, it is observed that when plotting the average temperature versus the bacteria counts, the highest counts occurred right at 55°C, which is exactly the regulatory criteria set for defining the failure or success of Class A compost. Based on this single graph, it seems the standard just picks an optimum condition for incubating the bacteria to grow and proliferate. Still, not much evident tendency of pathogen reduction over time is observed by the TMECC protocol, which again, proved that with so many factors involved, composting is such an unpredictable and heterogeneous process.
4.6.4 What can be found from total average temperature vs TC counts in pile 2

Figure 4-41 Average temperature vs TC counts for end samples of pile 2

In this figure, the average temperature in total represents the mean temperature that the sample has been through the whole period of time the sample’s staying in the pile. Because of the insufficiency in samples being analyzed, it wouldn’t be fair to draw any sound conclusions related to the relationship between pathogen killing and temperature from this graph. However, it could still be observed that, to some extent, from 40°C to 60°C, the counts generally show a lower trend comparing with the count numbers out of this range, which matches the optimum range proposed by previous researchers.
4.6.5 What can be found from the highest temperature vs bacteria counts

Figure 4-42 Highest temperature vs TC for end samples from pile 1
Figure 4-43 Highest temperature vs FC for end samples from pile 1

These figures plot the bacteria CFU number versus the highest temperature the final samples have gone though during their residency in the pile. The two graphs show almost all highest temperatures happened to these samples ranging from about 65°C to 75°C, which is supposed to be high enough to kill bacteria, however, it can be seen that there is still a significant amount of bacteria in these samples, or even higher in general than those experiencing relatively lower highest temperatures. As mentioned earlier, when operating out of the optimum range of temperature, the extreme high temperatures may conversely, play a hindering role the bacteria growth as well as pathogen killing.

In addition, because there used to be some concerns that maybe the highest temperature plays a role in the performance of pathogen killing, from what is seen here, the highest temperature, to a large extent, seems not to be able to affect the pathogen killing performance during the composting process.
Surprisingly, both the figures show either total or thermo-tolerant coliforms manage to survive at some extremely high temperature like 83°C, even there is the possibility that some environmental organisms, such as *Pseudomonas aeruginosa*, which had been found to be able to produce thermo-tolerant mutants thus can survive at very high temperatures in the pile and also can successfully mimic the coliforms on the medias, undoubtedly, there must be some mechanisms behind this, enabling the survival, even proliferation.

4.6.6 *What can be found from the number of days staying above 55°C vs bacteria counts*

![Figure 4-44 Number of days staying with a temperature >55°C vs TC from end samples of pile 1](image)

Figure 4-44 Number of days staying with a temperature >55°C vs TC from end samples of pile 1
These two figures shown above, which plot the number of days during which the temperature has been above 55°C versus the bacteria number, present more or less some tendency of re-growth. When the material has been staying above 55°C in the pile for about 70 days, it has relatively high bacteria counts, when staying for 100 days, has the lowest counts, and while for about 110 and 120 days, shows some occurrence of bacteria re-growth. However, even 70 days is already supposed to be long enough to kill pathogens and enable the compost to meet the criteria. Consistent with the bacteria results shown before, there are significant number of organisms existing; Due to the limitation of number of samples, conclusion can not be made about if any particular number of days' staying over 55°C can obtain an optimum pathogen killing. What may be concluded that the longer in the pile till the end stage of the process, the more chance for the re-growth to happen.
4.6.7 What can be found from the log distributions of bacteria

- **Figure 4-46 LNT=Log(base e) distribution of CFU number of TC for all samples from pile 1**

- **Figure 4-47 LNF=Log(base e) distribution of CFU number of FC for all samples from pile 1**

- **Figure 4-48 LNE=Log(base e) distribution of CFU number of Ent for all samples from pile 1**
The Log distributions are not normally distributed as expected, which can help prove that composting is such a heterogeneous process, various factors and possibilities, such as killing failure from incomplete mixing, weather condition, accidental contamination, deficiency and limits of the detection methodology and so forth, are involved in it thus no obvious tendency of normal distribution is observed.

To sum up, from the overall performance of Enterococci and the performance of both total coliform and thermo-tolerant coliforms during the first stage (from start to 1st turn), it is fair to make the conclusions that the thermo-hygienization did take effect during the process and just when the thermophilic stage had been reached for a period of time, the microbial performance became complex. Given regular microbial activities along with changes in temperature, occurrence of re-growth, species’ tendency toward thermo-tolerant mutation, plus with the interference of the significant amount of environmental organisms, some physical factors as well as the limitation of test methods and lack of knowledge on micro-flora during the whole process, all what has made the composting microbiology such a complex issue and hard for engineered compost technologies to achieve desired levels of control. What also needs to be mentioned is that from the regulatory perspective, some further study may be needed to look into selection of the indicator organisms.
CHAPTER 5 BIOAEROSOLS ANALYSIS

Both bioaerosols and workers’ exposure samples were collected at various locations to monitor how the different compost handling tasks could potentially affect the health of personnel working on sites or have the potential for community exposure in the neighbourhood. Refer to Appendix H for all the data involved below.

5.1 Personal sampling

There are 12 groups of data (dust, endotoxin and β-glucan) from in cab while 11 from out-cab (one pump fell down from the cab and was lost). The results show that the enclosed front-end loader, to a large extent, does play an effective role in protecting the workers from being exposed to the bioaerosols contamination. The exposure results taken from inside were generally significantly lower than the ones from outside, no matter in terms of the dust or endotoxin. And all the inside data were negligibly small, which can to some extent, assure workers that the enclosed cab provides protection from the organic dust.

For the concentration of dust collected, the average obtained from all the outside cab data (14mg/m³) was about 100 times higher than it was on the inside of the cab (0.14mg/m³); meanwhile, the concentration of endotoxin presents almost exactly the same trend as the dust, with outside cab (856EU/m³) while inside (8.6EU/m³). When it comes to the concentration of β-glucan, the outside (107ug/m³) to inside (27ug/m³) ratio is about 4, much smaller than the other two.

The histograms shown below will provide a better view on the difference between the in-cab and the out-cab results.
Inside cab: Histogram of dust concentration (mg/m³)

Figure 5-1 Histogram of in-cab dust concentration

Outside cab: Histogram of Dust concentration (mg/m³)

Figure 5-2 Histogram of out-cab dust concentration
Inside cab: Histogram of Endotoxin concentration (EU/m³)

Figure 5-3 Histogram of in-cab endotoxin concentration

Outside cab: Histogram of Endotoxin concentration (EU/m³)

Figure 5-4 Histogram of out-cab endotoxin concentration
Inside cab: Histogram of Glucan concentration (ug/m³)

![Bar chart showing Glucan concentration distribution inside the cab.]

Figure 5-5 Histogram of in-cab Glucan concentration

Outside cab: Histogram of Glucan concentration (ug/m³)

![Bar chart showing Glucan concentration distribution outside the cab.]

Figure 5-6 Histogram of out-cab glucan concentration
The endotoxin concentration was highly correlated to dust concentration, in other word, they enjoy the consistent level of being high or low in their own system, which tells that the more dust collected, it is very likely the more endotoxin existed.

Health based occupational exposure limit for airborne endotoxin of 50 EU/m³ was initially recommended by the Netherlands exposure standards setting committee DECOS, and DECOS has agreed upon a limit of 200 EU/m³ which is progressing toward a statutory value (CAHSL, 2003). Therefore, when looking into each individual data from inside the cab of the study, they are all even far smaller than 50 EU/m³, ranging from 2-20 EU/m³, which assures that the cab could greatly protect the workers from being exposed to endotoxin thus it is fair to conclude that it is safe for the workers to work inside the cab, at least from the view of endotoxin.

Also, the individual data pair (inside and outside from the same cab), reveals that the outside to inside ratio could vary from 6000 to 2, for which the reasons could be 1. Tasks performed play a significant role, i.e. when one particular front-end loader took very heavy duty on that whole day, such as moving quickly with the load full of soil on the site, considering the optimistic effect of the enclosed cab, it is possible to have so big difference between inside and outside; 2. There is no doubt the samples collected under different weather conditions may vary greatly, as stated earlier, the precipitation could significantly reduce the airborne transport while the wind could possibly worsen the situation; 3. Different stages of the compost soil the front-end loader has been mainly working with on the sampling day can possibly exert some influence as well; 4. The bias may exist, such as from improperly handling the samplers which possibly results in inaccuracy in weighing the filters or failing to nicely remove the whole fibre filter from its holder.

5.2 Andersen Sampling

There were 27 sets of samples in total taken for bioaerosol monitoring, one set of samples includes one for *Aspergillus fumigatus* (Af) while one for thermo-tolerant bacteria. The sampling locations covered both the compost handling sites and the place where the workers rest and eat.

5.2.1 Comparison among different tasks

Comparison of both *Aspergillus fumigatus* and thermo-tolerant bacteria among different tasks is graphed to demonstrate how dispersion of bioaerosols is affected by different tasks performed on
site and also how much potential influence the neighbourhood may get from the dispersion through the downwind and upwind samples. The number of samples involved is listed as follows:

Table 5-1 Number of samples by tasks

<table>
<thead>
<tr>
<th>Tasks performed</th>
<th>Screening</th>
<th>Turning</th>
<th>Upwind</th>
<th>Downwind</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of samples</td>
<td>5</td>
<td>7</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

Figure 5-7 Comparison of AF CFU counts among different tasks

Figure 5-8 Comparison of thermo bacteria among different tasks
From the Andersen sampling results, conclusion can be made that both turning and screening could result in relatively high concentration of airborne thermo-tolerant bacteria and fungi, comparing with samples taken at other sites. Samples have also been collected in both the downwind (150m away) and upwind (50m away) direction of the composting facility. Results from downwind are very small, generally even smaller than that from the upwind, which shows influence of the composting facility to the downwind neighbourhood area is minor.

Samples also have been collected in the workers' on-site lounge room, and the results were found to be negligibly small, thus, to some extent, it can be assured that the lounge area is safe and clean for workers to eat and rest in.

### 5.2.2 Comparison between AF and thermal bacteria

A figure of comparing the log Af and thermo bacteria counts at the six stages is made by plotting the overall 27 sets of samples. It clearly shows that among the six stages of the Andersen sampler, the 4th stage had highest counts for *Aspergillus fumigatus*.
In theory, the 4th stage represents the particulate which would enter into the lung and be deposited in the secondary bronchi. A large portion of Af captured in the air were of a size to enter the human-being’s secondary bronchi.

The concentration captured on the 6th stage was the lowest and also overall very small, mostly below detection limit. The average concentration on stage 6 shown here is resulted from the only two samples with concentration at the maximum possible for the sampler (too numerous to count), which brought the average up, therefore, generally speaking, certain tasks like grinding and screening produced the highest concentration of the smallest particles.

Moreover, according to the Figure 5-9, Af has been found to be more prevalent than thermo-tolerant bacteria under the airborne conditions, the ratio of Af to gram-negative bacteria varied between 58 and 138 in areas where material was handled, which is relatively smaller than what has been recorded in previous study (Olenchock et al. 2007).

As far as thermo-tolerant bacteria is concerned, the Figure 5-9 reveals that the numbers of counts are very low, thus it can tell that airborne thermo-tolerant bacteria contamination is not a pressing issue to solve for the composting industry, however, certain measures to control Af may need to be taken into consideration and put into practice.

5.2.3 Comparison among the four phases for the both piles

Figure 5-10,12 show how the concentrations of airborne thermo-tolerant bacteria and Aspergillus fumigatus, respectively, change with the four phases of the whole process, i.e. feedstock, first turn, second turn, and screened. And number of samples plotted is listed in table as follows:

<table>
<thead>
<tr>
<th>Process Stages</th>
<th>Feedstock</th>
<th>1st Turning</th>
<th>2nd Turning</th>
<th>Screened</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of samples</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 5-2 Number of samples by stages for pile 1
The performance of thermo-tolerant bacteria and Af presents different trends. In Figure 5-10, the thermal bacteria generally presents the trend of decreasing over time except the first turn as shown here. Because there are various factors involved in the process, such as tasks performed during this phase, weather conditions etc. and it is not possible to adjust for other factors but only plot a single factor, so it is very likely to be the task performed at the moment the sample was collected, but not the material itself results in the extremely high concentration of the first turn. Certainly, at this point, there is also the possibility that the weather condition, i.e. occurrence of rain events leads to the relatively low concentrations at other stages. And undoubtedly, the number of samples was too small to make any fair conclusions.

Figure 5-11 shown below is from the compost bacteria data, which has been discussed. Due to the insufficiency of samples and all the possible factors involved, it is hard to see any obvious consistency between bacteria concentration of the compost and the bioaerosol samples. It would be of great interest to compare the trend shown by samples collected from these two types of source by taking more samples to increase the sample size.
Figure 5-11 Compost bacteria concentration of pile 1 at the four stages

Pile 1: A. Fumigatus of 4 phases of the whole process

Figure 5-12 Comparison of log AF counts at the four phases for pile 1
From Figure 5-10 and 12, it is interesting to see that the pattern of Af concentration in air is a totally different trend from the matching samples taken for thermal bacteria shown above. The second turn shows high concentration while the other phases appear trivial. And except stage 5, all the other five stages for the second turning present very high concentrations, which is not usual to be observed on the media plates, especially for stage 6. Furthermore, for samples collected during screening, it is unusual to see the concentration on stage 6 increased while that from other stages are lower. Besides the various possibilities and randomness involved, the reasons for what is observed here are still not clear and larger number of samples is needed for drawing any fair conclusion.

Number of samples grouped for pile 2 is listed below:

Table 5-3 Number of samples at four stages for pile 2

<table>
<thead>
<tr>
<th>Process Stages</th>
<th>Feedstock</th>
<th>1st Turning</th>
<th>2nd Turning</th>
<th>Screened</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of samples</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

Pile 2: Thermo-tolerant bacteria at 4 phases of the whole process

Figure 5-13 Comparison of thermo bacteria at the four phases for pile 2
Pile 2: A. Fumigatus of 4 phases of the whole process

These two figures for pile 2 reveal some consistency with the compost bacteria results, both the *Aspergillus fumigatus* and airborne thermo-tolerant bacteria of pile 2 present much lower count in general compared to that of pile 1, which matches what has been observed from the compost bacteria analysis, i.e. the bacteria concentrations of pile 2 are generally much lower than that of pile 1 over the whole process. Besides the reasons mentioned in the bacterial discussion, which probably can be applied here, as the moisture content of pile 2 is relatively higher than that of pile 1, which has been discussed earlier in previous section, so that may help explain what’s seen here considering high humidity and water vapour rate are assumed to more or less, benefit the reduction of airborne contamination. Especially for AF as shown in Figure 5-14, almost all the results appear negligible, besides the explanations as pointed out earlier, the bacteria concentration of pile 2 is generally lower than that of pile 1, also pile 2 received more precipitation than pile 1, all these may also play an role in the generally lower concentration of bioaerosols in pile 2.

Moreover, Figure 5-13,14 also indicate that the weather condition, especially precipitation must contribute to what is seen here, for example, according to the weather record, it was rainy on the day the second turning of pile 2 occurred while no rain during both first turning and screening, which probably is the major reason for much fewer bacteria captured during the second turning.
Also, being consistent with the compost bacteria results, it seems there is still no significantly reduced number of bacteria when the process is finished. Besides the possibility mentioned before, in the pathogen discussion could be applied here as well. To conclude, as far as the same handling task is concerned, the precipitation and humidity may contribute the most to the reduction of airborne transport.

![Figure 5-15 Compost bacteria concentration of pile 2 at the four stages](image)

When referring to the compost bacteria data shown above in Figure 5-15, it seems the trend of bioaerosols changing with four stages is to some extent, consistent with that shown from the compost bacteria results, especially for the performance of airborne thermo-tolerant bacteria and thermo-tolerant coliforms. The airborne thermo-tolerant bacteria concentration was very low in feedstock, rises up significantly in the 1st turn, decreases during the 2nd turn and increases when being screened, which exactly matches what the thermo-tolerant coliforms presents in compost according to Figure 93. Therefore, although so many variables and possibilities exist either in the samples themselves or the ways of taking them, a general idea still can be obtained that there is some correlation between both feedstock and airborne contamination and bacteria which has the similar optimum temperature regime for growth shows the similar performance no matter in which form of existence.
5.2.4 Ratio of respirable particles

For an assessment of the respirable fraction of the airborne colony-forming particles, the number of CFU on plates 3 through 6 was expressed as a percentage of the total CFU. For *Aspergillus fumigatus*, about 70% were in the respirable size range and when it comes to thermo-tolerant gram negative bacteria, the percentage was about 64%.

To sum up: 1. Generally speaking, sites where material was handled, such as sites near compost piles being moved, turned or screened, showed equally high values for both *Aspergillus fumigatus* and thermo-tolerant bacteria. Sites where material was not handled generally had low values; 2. The weather condition, especially precipitation plays an indispensable role in the level of airborne contamination; 3. Moisture content may contribute to the reduction of airborne contamination; 4. About 70% of the bioaerosols were within the respirable range; 5. If based on the temporal scale, i.e. over the four phases of composting process, there seems to be correlation between the trend of concentration fluctuation from bacteria collected in the air and collected from pile 2, but not applicable for pile 1; while if based on the material itself, the results shown for the airborne samples are to some extent, consistent with that for the soil samples. 6. Af has been found to be generally more prevalent than thermo-tolerant bacteria under the airborne conditions.

Additionally, although the data, in terms of taking samples at both downwind and upwind direction of each sampling site, is sparse in the present study, thus it is hard to conclude how much significance the prevailing wind can make to the airborne process, yet, based on the limited data obtained, it is believed to have some influence on bioaerosols.

At last, for both Andersen sampling and personal sampling, to take more samples under various certain conditions is highly recommended and it will help find out more representative situations thus enabling to provide more prudent and feasible suggestion on improving the composting workers' working conditions to protect them from potential harm to health.
CHAPTER 6 CONCLUSION

Moisture Content
- Either for pile 1 or pile 2, from the start to being screened, the moisture content is to some extent, all within the optimum range, near 50 to 70%, which means moisture is not a markedly limiting factor as far as the study is concerned.

C:N Ratio
- The ratio has all been within the optimum range (20-40:1) and it does show the decrease at the end of the process, and is still within the expected and favourable range (15-20:1) for finished product.

Temperature
This study managed to provide more detailed and extensive data about both spatial and temporal temperature distributions in real, operational compost piles ever than any previous studies, by using data loggers to allocate at every part of the pile(approximately surrogate of each 0.5 m³ material) and continuously record fluctuating temperature data from building-up the new pile till screening.

Furthermore, few, if any previous studies have correlated the time-temperature regime with level of pathogen-killing for the full-scale operational composting practice, like what has been done in this study.

- Among the five positions, position 2 was the only one affected by the ambient weather conditions, other positions just kept increasing without any influence from the ambient.
- The temperature of position 4, 2 in general for pile 1, 2, respectively, goes to the highest comparing with other positions; while position 1, overall stays relatively lower than other positions; the temperatures of both position 3 and 5 tend to be quite consistent with their positions in the whole pile, being medium in general.
- It is found out that the temperature of some data loggers could reach up to 85°C, or even higher than that (beyond detection limit) which is out of the available records so far, and the extremely high temperature has been achieved within a very short period of time (less than 15 days) since the pile was newly built. PVC casings for the data loggers even got deformed in the pile because of the high temperature.
• “Cool zones” have been observed to exist in the pile. It is thought to be closely related to the oxygen supply. The material with sufficient and uniform oxygen supply is prone to less fluctuation in temperature, finer particles can reduce the chance of occurrence of cool zones.

• Using the means sometimes can help get a relatively clear picture from large amount of data; however, for some occasions, it may obscure the real picture, leading to the wrong conclusion.

• Probe testing may not be reliable and may lead to false results according to what has been found from the study.

• The influence of solar radiation on the process was observed and it is recommended to conduct further study on that.

• Turning does better mixing of the material, help reduce the “cool zones” as well as aeration; and also plays a role in breaking down the material into finer particles for better degradation.

• Difference in the material of even only yard waste, e.g. sources, types of the yard waste, moisture content as well as those physical parameters mentioned above, all could greatly influence the performance of the process.

• Seasonal variation, as expected, does make some difference in the performance of two piles constructed at different time of the year.

Bacteria

• The number of *Enterococci* has been continuously decreasing to below detection limit, the performance of *Enterococci* provides a robust proof that significant destruction and inactivation of microbial organisms do happen during the composting process; while concentration of Coliforms also showed reduction till the first turning but did presented some funny and unexpected trends afterwards, which brings up the issue of whether Coliforms are good indicator organisms for composting or rather to be replaced by *Enterococci*.

• Both *E.coli* and *Salmonella* have been observed over the whole process and also showed a bit of a trend of re-growth.

• With average temperatures within 40°C to 60°C, the counts generally show a lower trend comparing with numbers out of this range, which just matches the optimum range proposed by previous researchers.

• When operating out of the optimum range of temperature, the extreme high temperatures may conversely, play a hindering role the bacteria growth as well as pathogen killing.
• There used to be some concerns that maybe the highest temperature plays a role in the destruction of bacteria, from what has been observed in the study, the highest temperature, to a large extent, seems not to be able to affect the bacteria killing performance during composting process.

• Some environmental organisms, such as Pseudomonas aeruginosa, which had been found to be able to produce thermo-tolerant mutants thus can survive at very high temperatures in the pile and also can successfully mimic the coliforms on the medias, which all could result in the occurrence of survival, even proliferation of coliforms seen in the study.

• Tendency of re-growth has been observed and also it was found that the longer staying in the pile till the end stage of the process, the more chance for the re-growth to happen.

• Exclusive media like modified Enterococci agar can work in screening or blocking the growth of any other background bacteria on it and it is highly recommended to use more selective mediums for Coliforms too.

• Most Probable Number (MPN) may not be a good test method for monitoring different stages during the whole composting process. It is too sensitive to differentiate any different performance of bacteria during the process, i.e. it is hard to see any die-off happen from one stage to another from the MPN results.

Generally speaking, composting microbiology is truly complex and it is still well recognized as a “black box”. Given regular microbial activities along with changes in temperature, occurrence of re-growth, species’ tendency toward thermo-tolerant mutation, plus with the interference of the significant amount of environmental organisms, some physical factors as well as the limitation of test methods and lack of knowledge on micro-flora during the whole process, all has made the composting microbiology a complex issue and hard for engineered compost technologies to achieve desired levels of control.

Bioaerosols

• The enclosed front-end loader, to a large extent, does play an effective role in protecting the workers from being exposed to the bioaerosols contamination. And from the data collected from inside the cab, in theory, it is safe for workers to work inside the cab from the perspective of hygienic safety.

• The influence on the adjacent communities from composting facility is and trivial and negligible.
Sites where material was handled, such as sites near compost piles being moved, turned or screened, showed equally high values for both *Aspergillus fumigatus* and thermo-tolerant bacteria. Sites where material was not handled generally had low values.

There are some correlation between the pathogen concentration in the pile and in the air, but with the interference from so many other factors, e.g. weather conditions, tasks performed, incidental occasions etc., it is hard to draw any sound conclusions without a large number of data.

The weather condition, especially precipitation plays an indispensable role in the level of airborne contamination.

Moisture content may contribute to the reduction of airborne contamination.

About 70% of the bioaerosols from composting is within the respirable range.
CHAPTER 7 RECOMMENDATIONS

- To conduct measurements on the particle size, porosity, bulk density etc. of the feedstock. These physical parameters are found out to play an indispensable role in the composting process, significantly affecting the oxygen penetration, heat distribution, temperature regime, occurrence of cool zones, biological decomposition and so on. Only visual observation has been made in the study thus it is of great need to quantify these parameters for more convincing analysis in future study.

- To collect soil samples for bacteria analysis not only following the data loggers in-situ but also taking a few samples from every positions, i.e. outer layer, bottom, inner core zone etc., which could help provide a more systematic and clear view on the pathogen killing performance of different positions in the pile. It was somewhat random and not able to see the picture of sanitation in the pile as a whole when only doing bacteria tests to the compost material surrounding the data loggers found in-situ.

- To monitor oxygen saturation rate at all positions in the pile, what has been found in the study shows oxygen supply is very likely to play a role in the composting process, such as temperature regime, microbial activities and so on; meanwhile, it could be greatly affected by the physical parameters of the feedstock, like porosity, bulk density, also moisture content and weather conditions. Therefore, monitoring the oxygen saturation rate might be able to help find out the quantitative correlation between oxygen and temperature as well as oxygen and microbial activities, also how it could be quantitatively affected by the physical parameters.

- To explore a way not to lose track of data loggers after turnings, which definitely will provide more interesting and unprecedentedly valuable data.

- To take pH tests to all the phases of the process. It is one of the parameters that does not require much effort to be done but could provide a clue for the analysis on the microbial activities, biological decomposition and C:N ratio fluctuation.

- To pay a little more attention to the issue of solar radiation, which appears to possibly play a role during the process in this study. And also information about the wind direction may be of help to explain the variable performance of data loggers, i.e. material, from different side of the pile.

- To conduct pathogen tests at the end of curing stage, if possible. Compost right after being screened was tested in the study for pathogen concentration as the last stage of the whole process of pathogen monitoring, which is not, the curing phase, but the stage after being
cooled down for a while. It will be ideal to have data about this phase to help provide a better understanding on the behaviour of pathogen during the whole theoretical cycle.

- To choose more selective media for growing total coliforms and thermo-tolerant coliforms to minimize the interference of the environmental bacteria on the results. From what has been seen in the study, many environmental organisms managed to mimic coliforms on the MacConkey agar such as *Pseudomonas aeruginosa* thus leading to the somehow unexpected findings; however, with the strictly selective medium Modified Enterococcus (mEnt) Agar, number of enterococcus showed significant reduction all the way along with the rise of temperature, which, without any confusion, proved the thermal killing did occur during the composting process.

- Although being recommended in the “Test Methods for the Examination of Composting & Compost” by U.S. Composting Council, performing the Most Probable Number (MPN) technique to windrow composting & compost is found out to be still questionable. It should be OK to be used for only monitoring the final product. However, to monitor the whole composting process, for windrow composting, MPN, is too sensitive to differentiate the results from different stages (i.e. feedstock, after being turned, after being screened) of the process which is supposed to present different results because of the occurrence of thermal killing during the process. In other words, since it is not easy for windrow composting to achieve high level of sanitation, even having met the regulation of “staying for 15 days above 55°C, according to all the possible influences as discussed before in chapter, therefore, it is very likely that majority of MPN tests, no matter from which stage of the process, would present “positive” results, i.e. the same results, which made it not possible to obtain any findings or draw any conclusions from the same results, considering the high consumption of time and money of MPN tests, it may not be a wise solution to monitor the bacteria concentration of the whole windrow composting process. In addition, if MPN is chosen to monitor the bacteria concentration, it is suggested to do dilutions at least to 10⁴ sample homogenate for the MPN tests. The MPN results are normally given as “greater than” or “smaller than” a number, especially for the “greater than” results, as one of the regulatory requirements is to be “< 1000 MPN g⁻¹ TS on a dry weight basis”, if only 10⁻¹ to 10⁻³ is done, it is very likely that the results may come out as “> 600 MPN g⁻¹ TS”, which will make it tricky to tell whether it meets the standard or not. Therefore, it is highly suggested to conduct tests on high dilutions to avoid confusion.
- To collect large amount of data under specific conditions such as different tasks, different weather conditions etc. for the bioaerosols study in order to determine the influence of different factors possibly through multiple regression analysis.
REFERENCES


