INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.
APPLICATION OF SPECTROSCOPIC ANALYSIS (FT-IR) TO A BENCH-SCALE SOLID SUBSTRATE FERMENTATION (COMPOSTING) SYSTEM

Dissertation

Presented in Partial Fulfillment of the Requirement for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

Yi Tseng, B. Eng., M.S.

The Ohio State University

1995

Dissertation Committee:

J. J. Chalmers
O. H. Tuovinen
S. T. Yang

Approved by

Advisor
Department of Chemical Engineering
To my parents
ACKNOWLEDGMENTS

I express my sincere appreciation to my advisor Prof. Jeffrey J. Chalmers, for his guidance, encouragement, understanding, and patience during the past three years. Thanks go to Prof. Olli H. Tuovinen for his advice, guidance and friendship during this study and Prof. S. T. Yang for serving my committee. Special thanks go to Prof. Harry A. J. Hoitink for his invaluable suggestions, insights and critical comments to my study. I owe special thanks to Prof. Sam J. Traina for his advice and kindness of using his instrument. Without his kindness, it is impossible to accomplish this study. Thanks also go to Profs. Warren A. Dick and Harold M. Keener for advice and motivational discussions.

To my fellow graduate students, special Mr. Mukul Agarwal, I am extremely grateful for the support, help and friendship along this study. Thanks go to all my family members. I hope my success in the future would be the best appreciation I can give you back.

Thanks go to everyone I met at OSU. Your encouragement will definitely inspire me to go on and accomplish the next level of excellence.
VITA

August 30, 1965 ....................................................... Born - Beijing, China

1989 .......................................................................... B. Eng., Tsinghua University
Beijing, China

1991 ........................................................................... M.S., The Ohio State University
Columbus, OH

1992-present ............................................................ Graduate Research Associate
The Ohio State University
Columbus, OH
FIELDS OF STUDY

Major Field: Chemical Engineering

Studies in: Biochemical Engineering, Fermentation Technology, and Environmental Biotechnology
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>TABLE OF CONTENTS</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEDICATION</td>
<td>ii</td>
</tr>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>iii</td>
</tr>
<tr>
<td>VITA</td>
<td>iv</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>viii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>x</td>
</tr>
<tr>
<td>CHAPTER</td>
<td>PAGE</td>
</tr>
<tr>
<td>I. GENERAL INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>1. Background</td>
<td>1</td>
</tr>
<tr>
<td>2. Operational Conditions of Composting</td>
<td>5</td>
</tr>
<tr>
<td>3. Lab-scale Study of Composting</td>
<td>9</td>
</tr>
<tr>
<td>4. Compost Maturity or Stability and Spectroscopic Analysis of Compost</td>
<td>10</td>
</tr>
<tr>
<td>5. Problems</td>
<td>13</td>
</tr>
<tr>
<td>6. Objectives</td>
<td>14</td>
</tr>
<tr>
<td>II. CHARACTERIZATION OF A BENCH-SCALE COMPOSTING SYSTEM FOR STUDYING THE BIODEGRADATION OF ORGANIC SOLID WASTES</td>
<td>17</td>
</tr>
<tr>
<td>1. Introduction</td>
<td>17</td>
</tr>
<tr>
<td>2. Materials and Methods</td>
<td>20</td>
</tr>
<tr>
<td>3. Results</td>
<td>25</td>
</tr>
<tr>
<td>4. Discussion</td>
<td>27</td>
</tr>
<tr>
<td>5. Summary</td>
<td>34</td>
</tr>
<tr>
<td>6. Notation</td>
<td>34</td>
</tr>
<tr>
<td>III. ATP MEASUREMENT IN COMPOST</td>
<td>53</td>
</tr>
<tr>
<td>1. Introduction</td>
<td>53</td>
</tr>
<tr>
<td>2. Materials and Methods</td>
<td>59</td>
</tr>
<tr>
<td>3. Results</td>
<td>62</td>
</tr>
</tbody>
</table>
### Table of Contents

I. **Discussion** ......................................................................................... 63
II. **Summary** .......................................................................................... 66

IV. **QUANTITATIVE FOURIER-TRANSFORM INFRARED SPECTROSCOPIC ANALYSIS OF ORGANIC MATTER DEGRADATION IN A BENCH-SCALE SOLID SUBSTRATE FERMENTATION (COMPOSTING) SYSTEM**

   - **Introduction** .................................................................................. 78
   - **Theory** ........................................................................................... 83
   - **Materials and Methods** ................................................................. 84
   - **Results** .......................................................................................... 87
   - **Discussion** .................................................................................... 89
   - **Summary** ....................................................................................... 96

V. **TEMPERATURE EFFECT ON ORGANIC MATTER DEGRADATION IN A BENCH-SCALE COMPOSTING SYSTEM** ....................................................................................................... 111

   - **Introduction** .................................................................................. 111
   - **Materials and Methods** ................................................................. 113
   - **Results** .......................................................................................... 117
   - **Discussion** .................................................................................... 119
   - **Summary** ....................................................................................... 124

VI. **RECOMMENDATIONS FOR FURTHER RESEARCH** ....................... 141

   - **References** ................................................................................... 148
   - **Appendices**
     - **A. Computer control program for composting system** ............. 160
LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1. Microbial diversity in composting process (Storm 1985b)</td>
<td>15</td>
</tr>
<tr>
<td>2.1. Compositions of simulated compost feed materials</td>
<td>36</td>
</tr>
<tr>
<td>2.2. Analysis of initial and final compost samples</td>
<td>37</td>
</tr>
<tr>
<td>2.3. Sieve numbers and pore sizes</td>
<td>38</td>
</tr>
<tr>
<td>2.4. O₂ and CO₂ concentrations at different locations of the reactor</td>
<td>39</td>
</tr>
<tr>
<td>2.5. O₂ and CO₂ concentrations inside and outside compost layer</td>
<td>40</td>
</tr>
<tr>
<td>2.6. Partical size distribution of dry and wet feed material</td>
<td>41</td>
</tr>
<tr>
<td>3.1. Summary of ATP measurement protocols for biomass measurement in compost systems</td>
<td>68</td>
</tr>
<tr>
<td>3.2. Compositions of simulated compost feed materials</td>
<td>69</td>
</tr>
<tr>
<td>4.1. Compositions of simulated compost feed materials</td>
<td>97</td>
</tr>
<tr>
<td>4.2. Some FT-IR bands and their proposed assignments</td>
<td>98</td>
</tr>
<tr>
<td>4.3. Peak area before and after normalization</td>
<td>99</td>
</tr>
<tr>
<td>4.4. Peak positions, widths and their proposed assignments from the second derivative and curve fitting</td>
<td>100</td>
</tr>
<tr>
<td>4.5. Spectra range and peak positions for peak area integration</td>
<td>101</td>
</tr>
<tr>
<td>4.6. Chemical analysis of initial and final samples</td>
<td>101</td>
</tr>
</tbody>
</table>
4.7. Results of wet chemistry measurements ..................................................... 101

5.1. Compositions in compost feed materials ..................................................... 125

5.2. Carbon, nitrogen, C/N ratio, ash, pH and loss of dry mass for the four temperature experiments ................................................................. 126

5.3. Wet chemistry measurements of polysaccharides and lignin concentrations for initial and final samples ......................................................... 127
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>FIGURES</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1. FT-IR spectra of CSM compost at different time stages</td>
<td>16</td>
</tr>
<tr>
<td>2.1. The bench-scale composting vessel and system</td>
<td>42</td>
</tr>
<tr>
<td>2.2. Experimental profiles under 40°C</td>
<td>45</td>
</tr>
<tr>
<td>2.3. Experimental profiles under 50°C</td>
<td>47</td>
</tr>
<tr>
<td>2.4. Cumulative CO$_2$ evolution and O$_2$ consumption for experiments under 40 and 50°C</td>
<td>49</td>
</tr>
<tr>
<td>2.5. Particle size distributions of the dry feed material before water addition and the wet feed material after water addition</td>
<td>49</td>
</tr>
<tr>
<td>2.6. Schematic presentation of the compost layer and the compost biofilm surrounding the particle</td>
<td>50</td>
</tr>
<tr>
<td>2.7. O$_2$ diffusion profiles into the compost layer</td>
<td>51</td>
</tr>
<tr>
<td>2.8. O$_2$ penetration profiles into compost film</td>
<td>52</td>
</tr>
<tr>
<td>3.1 Experimental profiles under Temperature of 60°C</td>
<td>70</td>
</tr>
<tr>
<td>3.2. Compost extraction and ATP measurement procedure</td>
<td>71</td>
</tr>
<tr>
<td>3.3. ATP concentration profiles of experiments under 40, 50, 60 and 70°C</td>
<td>73</td>
</tr>
<tr>
<td>3.4. CO$_2$ evolution and O$_2$ consumption profiles of experiments under 40, 50, 60 and 70°C</td>
<td>74</td>
</tr>
</tbody>
</table>
3.5. 3-D plot of ATP concentration versus RQ and time course under four temperature experiments ................................................................. 75
3.6. 3-D plot of ATP concentration versus CO₂ evolution and O₂ consumption rate during composting under 60°C .................................................. 77
4.1. Experimental profiles under 50°C for 350 hrs ................................................. 102
4.2. Typical DRIFT spectra of compost ................................................................. 104
4.3. Amplified peak region in the 2800-3000 cm⁻¹ .............................................. 106
4.4. Spectra area changing versus time course .................................................. 107
4.5. Spectra area changing in percentage ........................................................... 108
4.6. Correlation between FT-IR and wet chemistry results .................................. 109
4.7. NMR spectra for initial and final compost samples ...................................... 110
5.1. Experimental profiles under 40°C ................................................................. 129
5.2. Experimental profiles under 50°C ................................................................. 130
5.3. Experimental profiles under 60°C ................................................................. 131
5.4. Experimental profiles under 70°C ................................................................. 132
5.5. Total CO₂ evolved and O₂ consumed profiles ............................................. 133
5.6. Peak area of polysaccharide carbon contents peak region (1050 cm⁻¹) versus time course for those four experiments under 40, 50, 60 and 70°C ................................................................. 134
5.7. Peak area of aromatic carbon contents peak region (1651 cm⁻¹) versus time course for those four experiments under 40, 50, 60 and 70°C ................................................................. 135
5.8. Peak area of aliphatic carbon contents peak region (1050 cm⁻¹) versus time course for those four experiments under 40, 50, 60 and 70°C ................................................................. 136
5.9. CO₂ evolution and O₂ consumption rates versus decomposition of polysaccharides ................................................................. 137
5.10. RQ versus decomposition of polysaccharides ......................................... 138
5.11. SEM pictures of experiment under 50°C .................................................... 139
6.1. New water spraying system ......................................................................... 146
6.2. FT-IR spectra of compost with NaN₃ addition ........................................... 147
CHAPTER I

GENERAL INTRODUCTION

1.1 Background

In recent years, composting has been recognized as an effective method for organic solid waste treatment since it shows more advantages over landfill (He et al., 1992). Composting has been reviewed by many previous studies (Golueke 1972; Haug, 1980; Zucconi and de Bertoldi 1987; Inbar 1989; Fogarty and Tuovinen, 1991; Chen and Inbar 1993). Zucconi and de Bertoldi (1987) summarized and gave the definition of composting as:

"Composting is a controlled biooxidative process that: (i) involves a heterogeneous organic substrate in its solid state; (ii) evolves by passing through a thermophilic phase and temporary release of phytotoxins; and (iii) leads to production of CO$_2$, water, minerals and stabilized organic matter (OM)."

The raw materials of composting usually are agricultural crops, food wastes, urban refuse, manure, etc. Most of the microorganisms involved in the composting process are thermophilic bacteria, thermophilic fungi and
Compost can reduce erosion, improve water infiltration and retention, decrease soil temperature fluctuations, and improve plant health by controlling plant pathogens after it is applied to soil (Hoitink and Keener, 1993).

Compost are generally dark in color, humus like, have a crumbly texture, an earthy odor and resembles rich topsoil (Haug, 1980). Other by-products from composting process are CO\textsubscript{2}, heat, and small amount of ammonia. In general, compost is used as a soil conditioner, mulch, top dressing or as an organic base with fertilizer amendments because of its low nutrients. However, by adjusting the composition of the original raw materials, more nutrient rich compost can be produced. This type of rich compost is called as "mature compost", which can be added directly to agricultural field, and contributes toward plant growth. Compost can be generally divided into yard waste compost, sewage sludge compost (SSC) and municipal solid waste (MSW) compost.

The chemical properties of compost are very important to the composting process. Those factors include carbon/energy source, balanced amount of nutrients, moisture content, adequate oxygen, pH, the toxic material content, etc. The carbon source usually comes from food waste, leaf and paper. Nitrogen content is also important because of its nutrient characteristics as well as the fact that is quite often either in excess or limited amount. The carbon/nitrogen (C/N) ratio of compost is critical in determining the rate of decomposition. A C/N ratio lower than 30:1 is considered ideal. A higher ratio tend to retard the process of decomposition. Moisture and oxygen content are other two important chemical
factors. Physical characteristics include particle size, temperature and mixing conditions.

Composting has several advantages when compared to other kinds of waste treatment processes. First of all, composting can be particularly effective in converting wet materials to a more usable and disposable form. Secondly, composting can stabilize putrescible organic, destroy pathogenic organisms and provide significant drying of the wet substrate. Composting can also be used to destroy certain toxic industrial wastes, such as TNT, petroleum sludge and certain pesticide residues. Comparing to landfill or incineration, composting has several advantages, such as low operational cost, beneficial use of end products and less air and ground water pollution (He et al. 1992).

During the composting process, the presence of a large variety of thermophilic microorganisms have been reported to be present. One systematic attempt to identify the thermophilic microorganisms is that of Strom et al. (1985 b). Some of their results are summarized in Table 1.1. By examining samples both from laboratory units and large composting plants, 87% of the randomly picked colonies were identified as Bacillus spp. Other identified species include the actinomycetes, Streptomyces spp. and Thermoactinomyces spp., and the fungus Aspergillus fumigatus. Bacillus spp. includes B. circulans complex, B. stearothermophilus, B. coagulans types A and B, B. licheniformis, B. brevis, B. sphaericus, Bacillus spp. type i and ii, and B. subtilis.

The optimal temperatures of composting had been studied by many researchers, it is now generally agreed that the optimal temperature of composting is around
60°C and should not exceed 60°C (Nakasaki et al., 1985c; Kuter et al., 1985; Strom 1985a; McKinley and Vestal 1984, 1985; Sikora and Sowers 1985). Nakasaki et al. (1985c) found that the CO₂ evolution rate was attributed by thermophilic bacteria at the initial stage of 60°C and to thermophilic actinomycetes at the later stage of 60°C. However, at a temperature above 60°C, only a few bacterial species, mainly Bacillus spp. remained active (Storm, 1985a). Thermophilic fungi were found only at a narrow temperature interval from 55 to 61°C. The microbial diversity of microorganisms verse temperature is summarized in Table 1.1.

Due to the complex nature of the composting process, it is difficult to evaluate the relationship between microbial activity and temperature at given time points (Fogarty and Tuovinen, 1991). However, changes in number of mesophilic bacteria, thermophilic bacteria, and thermophilic actinomycetes during composting process can be observed (Nakasaki et al., 1985c). To isolate these different organisms, different medium were used to isolate different groups of microorganisms, trypticase soy agar for bacteria, malt-yeast extract agar medium for actinomycetes and potato-dextrose agar for fungi. Nakasaki et al. (1985) found that the changes in number of mesophilic bacteria was not remarkable compared with those of thermophilic bacteria and actinomycetes.

Haug (1980) classified composting system based on whether the composting material is contained in a reactor or not. Reactor systems have been divided into vertical flow reactors, horizontal and inclined flow reactors. For vertical flow reactor, it consists of a vertical tower with certain (e.g. 10) floors one above the other. Each floor contains a hydraulically operated valve which allows material to
be discharged to the next floor, while oxygen is introduced by forced aeration from the bottom. Other vertical flow reactor contains several separate vertical compartments, with residence times of 4-10 days. Air is pulled out of top of reactor, while screw extractors remove and agitate material from the bottom of the reactor. Other types of vertical reactor may have different internal structure but operate using the same principles. Horizontal and inclined flow reactors have two types. One is called tumbling solids bed reactor which consists of a complete-mix, rotating drum. Feed consists of dewatered sludge and recycled compost product. Another type is called agitated solids bed reactor. Feed consists of sludge, heat-dried recycled compost and shredded paper at certain dry weight ratio.

Although composting has served human being for many years, it remained as an ancient art until the early 1900's when researchers began to systematize the traditional composting procedures (Golueke, 1972). In the mid-1960's, research on composting received a considerable impetus as a result of the 1965 Solid Wastes Act. However, no sizable advances were made in compost technology until 1960's.

1.2 Operational Conditions of Composting

Temperature is considered one of the most important factors to microbial activity in composting and its effect on composting process has been studied both in lab-scale and pilot systems (Anderson 1990). During the composting process, each group of microorganism has its own optimal temperature range. If the microorganism grow under the temperature away from the optimal temperature, it
is manifested by a decline in growth and activity of the organism. In general, microorganisms can be grouped into three categories: psychrophile, mesophile, and thermophile. During composting, most microorganism are in mesophilic and thermophilic ranges.

For a natural heating composting process, such as windrow operation without turning, the temperature profile will have two phases. The first phase is the initial burst of microbial activity and heat generation will go to a point at which the temperature becomes inhibitory to the microbial activity. During this phase, there are two heat generation peaks, one is at 37°C and the other is at 55°C (Finstein and Hogan, 1993). The first heat generation peak at 37°C is caused by the mesophilic organisms. While the second heat generation peak at 55°C is contributed by the thermophilic microorganisms. However, if the heat is not removed, the temperature elevation continuous beyond the point of maximal temperature for thermophilic range, the microbial activity will begin to decline. This is the second phase, called the "curing" phase, of composting process.

There has been significant debate with respecting to the optimal temperature for the composting process. Golueke (1972) pointed out the range of optimal temperature for the composting process is from 35 to 55°C. Even though people found that mesophilic bacteria are more efficient than thermophilic bacteria and therefore composting proceeds more rapidly (Kuter et al., 1985), few composting plant run their process under mesophilic temperature range. One of the reasons is that pathogens can only be killed under thermophilic temperature range. In addition, approximately three-fold more air is needed to maintain a ceiling at 35°C than that at 55°C (Finstein and Hogan, 1993).
Aeration is another important factor in the composting process. In large scale composting process, aerobic respiration is required to produce the exothermic reactions necessary to achieve self-heating conditions. Additionally, sufficient aeration can prevent the formation of odor and produce a more stabilized and useful residue than anaerobic conditions (Anderson, 1990). The potential of aeration is directly related to free air space, particle size, composition of material, moisture content and agitation. Aeration can be achieved by diffusion, agitation, and forced air pressure. For windrow process, periodic turning is a common method to keep aeration.

O₂ is required for the aerobic respiration for microorganisms during the composting process. The aerobic condition of the composting process is commonly evaluated in terms of the O₂ concentration in the off-gas. The optimal O₂ for aerobic composting process is still unknown. De Bertolden et al. (1983) suggested that the O₂ concentration in the circulating air should not fall below 18% in windrows. While Finstein et al. (1986) suggested the minimum oxygen condition for the composting process should be around 5 to 10%. However, there were few experimental data to support these values and the oxygen concentration inside the compost pile may be lower than that in off-gas.

In large scale composting systems, aeration and temperature are two important factors which can interact with each other. Increasing air flow rate can not only provide sufficient oxygen to microbial activity, but also cool down the compost temperature. In addition, microbial activity may demand more oxygen at higher temperatures.
Moisture content is essential to microbial activity (Finstein and Hogan, 1993). Since it is believed that microorganisms survive in the liquid film on the surface of compost particles, low moisture content will seriously limit microbial activity. Usually, moisture is defined as the weight loss after the sample has been dried to constant weight at 100 to 110°C (Fogarty and Tuovinen, 1991). The moisture content is affected by many other factors, such as reactor feed, free air space, aeration, temperature, and other related physical factors.

The optimal moisture content is now generally agreed around to be 60%. Researchers found that bacterial metabolic activity is severely inhibited when the moisture content drops below 40% (Glueke 1972). Excess moisture content will inhibit oxygen diffusion through the water film and limit microbial activity. Under excess moisture conditions, composting process will switch from aerobic to anaerobic condition and extensive odor will be produced. In addition, frequently adding water to maintain moisture content is also used in some other composting operations.

During the biological decomposition of organic matter, all kinds of elements are required for the microbial activity. Those are required not only for microbial cellular matter, but also for metabolic activities as an energy source or enzyme constituent. One of the important balance is the C/N ratio. The optimal C/N ratio for the microbial degradation in composting process is within 20 or 25 to 1 (Golueke, 1989). This ratio is fairly close to the C/N ratio in the microbial biomass. Since the C/N ratio will decrease during the composting process as a result of CO₂ production, the C/N ratio used at the beginning of the composting
process may reach value as high as 30 to 1. If the C/N ratio is low, microbial degradation will lead to excess amount of ammonia formation. On the other hand, high C/N ratio will result in nitrogen limitation which will lead to organic acid production. Excess acid will lower the pH and finally limit the microbial activity.

1.3 Lab-scale Study of Composting

In large scale composting, operational parameters, such as oxygen supply, temperature, particle size and structure, moisture content, C/N ratio, affect the process efficiency. Understanding the interaction among those parameters will help to optimize the process. However, it is difficult to investigate the interaction in large scale system. Lab-scale reactor is generally used to provide control of the operational conditions in order to optimize microbial growth and activity (Anderson, 1990).

Lab-scale reactor can be classified into two types, cylinder type and rotating drum type, based on the reactor configuration (Ashbolt and Line, 1982). On the other hand, classification can be done with respect to temperature. Self-heating reactor uses heat generated by microorganisms in stead of external sources. Fix-temperature reactor impose a pre-set temperature regime. Adiabatic reactor employ an external heating source to compensate the heat loss from the reactor to control the temperature at set-point (Anderson, 1990). In previous studies, laboratory reactor study has made a lot of contribution to the fundamental understanding of the composting process (Schulze, 1962; Jeris and Regan, 1973; Suler and Finstein, 1977; Ashbolt and Line, 1982; Sikora et al., 1983; Bach et al. 1984; Nakasaki et al., 1985a, 1985b, 1985c; Hogan et al., 1989; Palmisano
et al., 1993). However, limitations are also exist in lab-scale reactors. Those limitations include the scale-up of those reactors to pilot and production scale reactors.

1.4 Compost Maturity or Stability and Spectroscopic Analysis of Compost

Compost maturity or stability is the degree of decomposition of the organic matter in solid waste materials. Maturity of compost critically affects its utilization. First of all, maturity of compost can interfere with plant growth. Immature composts with a high C/N ratio causes nitrogen immobilization. On the other hand, the excessive low C/N ratio causes ammonium toxicity (Inbar et al., 1991). Secondly, the maturity of compost is also an important factor for the design and operation of composting plant. For windrow type of composting operation, it takes a few months to produce desired compost. However, it only takes a few days in vessel. Finally, high concentrations of soluble organic nutrients present in immature compost support growth of certain types of plant pathogen, which need free nutrients.

C/N ratio is often used as an indicator of compost maturity. Inbar et al. (1989) observed the C/N ratio decreased rapidly from 27 to 10 during the first 60 day of the composting process, while a small decrease of C/N ratio to 8.7 thereafter. The decrease of C/N was because the organic matter was degraded to CO₂ and H₂O during the composting process. The formation of humic substances has been characterized by many methods. The crude-fiber analysis can be used to measure the organic matter, such as cellulose, hemicellulose, soluble organic matter, and lignin in compost. In addition, ¹³C-nuclear magnetic resonance (¹³C-
NMR) spectroscopy and Infrared spectroscopy (IR) are promising methods to provide the information of humic substances in compost.

The application of infrared spectroscopy to evaluate the composition of organic matter is based on the difference in the chemical composition. Since the difference in chemical composition will change the ratios of infrared absorbing functional groups, different infrared spectra will be resulted. The infrared spectra can be used to determine the carbon content, botanical composition, degree of humification, cation exchange capacity (CEC), and nitrogen content (Lehtovaara et al., 1988).

Although Fourier Transform Infrared Spectroscopic analysis (FT-IR) does not provide quantitative data, it can determine the trends indicating transformation of organic matter during the composting process. Inbar et al. (1989, 1990, and 1991) successfully used FT-IR to study the CSM (separated cattle manure) composting. As shown in Figure 1.1, the FT-IR spectra representing several stages during composting of CSM, exhibit similar IR features regardless of time. According to Inbar et al. (1990), the main absorbance bands correspond to certain functional groups. These spectra show a unique feature that includes: a strong absorption band near 1650 cm\(^{-1}\); moderately strong absorption at 1540 cm\(^{-1}\); a strong absorption near 1050 cm\(^{-1}\); and a relatively pronounced near 2900 cm\(^{-1}\). Usually this unique feature indicates the presence of proteins and carbohydrates. Because of the degradation of carbohydrates, such as polysaccharides, the regions corresponding the polysaccharides (C-O, 1100-1040 cm\(^{-1}\)) reduce as the composting process proceeds. However, the aromatic
(C=C, 1655 cm⁻¹), alkyl C (CH₃, 1450 cm⁻¹) and carboxylate ion (C=O of COOH, 1720 cm⁻¹) regions rise as a result of accumulation of lignin in compost.

In order to quantify the relative changes in the FT-IR spectra intensities during the composting process, Inbar et al. (1989) calculated the ratios between the main peaks and the ratios were correlated to some chosen compost maturation parameters such as compost age, CEC, HS content and C/N ratio. As expected, the ratios of (COO⁻, CH₃/aliphatic C-H) and (COO⁻, CH₃/polysaccharides C-O) increased with time as a result of increase of carboxylate ion concentration and alkyl C and a decrease in aliphatic C and carbohydrates C -O during the composting process. This result were also observed for other correlations with CEC and substances contents. This method can be served as a semi-quantitative analysis for the levels of compost maturity and humification.

¹³C-NMR can provide carbon “fingerprint” of diverse solid samples such as compost (Wilson, 1990), particularly for the elements, C, N, and P. For example, ¹³C-NMR spectra provide us with an inventory of different types of C (paraffic, aliphatic and aromatic C, and C in CO₂H, ketonic, and quinonoid groups in HS (Schnitzer, 1990). The basic principle of ¹³C-NMR spectroscopy for chemical analysis is chemical shift since different structural groups in organic matter yield resonances at different chemical shifts. In addition, different structural groups may also have different relaxation times, which could be used for qualification analysis (Wilson, 1990).

As reported by Indar et al. (1989, 1990, and 1991), CPMAS ¹³C-NMR was used to analyze the compost. Their results confirmed the presence of appreciable
amounts of carbohydrates such as polysaccharides throughout the composting process. During the process, the level of polysaccharides decreased while levels of alkyl C, aromatic C and carboxyl groups increased. The data from CPMAS $^{13}$C-NMR experiments represented two stages of decomposition during the composting process. The CPMAS $^{13}$C-NMR spectra were divided into the following nine regions: (i) methyl, methylene and methane groups (0-50 ppm); (ii) methoxyl groups (50-60 ppm); (iii) C-OH groups (60-70 ppm); (iv) C to O or C to N groups (70-98 ppm); (v) anomeric C (98-112 ppm); (vi) aromatic C (112-145 ppm); (vii) phenolic carbons (145-163 ppm); (viii) carboxyl groups, esters and amides (163-190 ppm); and (ix) C=O (190-215 ppm) (Inbar et al., 1991).

1.5 Problems

Lack of the fundamental understanding of the composting process is the major reason to cause shutdown of many composting plants. This is highlighted by recent shutdown of two of the largest facilities ever built in the United States (Libby, 1991). The major reason for the shutdown is the odor formation, which is the result of poor process control. Composting can undoubtedly play a major role in the field of the solid waste management. However, its potential can not be realized through the empirical practice usual in this field (Finstein and Hogan, 1993). A more precise, scientific study of the fundamentals is very necessary for future decision making. Those fundamental understanding of the composting process include operational parameters, such as temperature, moisture, C/N ratio, reaction design and microbiology.
1.6 OBJECTIVES

The objectives of this study were as following:

(1). To design and build a bench-scale composting reactor and system which can provide a controlled environment for laboratory studies. Those controlled environmental conditions include temperature, aeration, moisture content, etc.

(2). To determine the biomass in compost by ATP measurement.

(3). To develop a non-destructive, quantitative, easy to use FT-IR (DRIFT) procedure to investigate the organic matter degradation during the composting process.

(4). To investigate the effect of temperature on organic matter degradation in composting process in the bench-scale composting system above.
Table 1.1. Microbial diversity in composting process (Storm 1985b).

<table>
<thead>
<tr>
<th>Name / percentage of random isolates samples</th>
<th>Temperature (°C)</th>
<th>49-57 (lab)</th>
<th>55-61 (lab)</th>
<th>60-65 (lab)</th>
<th>65-69 (lab)</th>
<th>55 (land)</th>
<th>59-65 (land)</th>
<th>66 (land)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fungus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspergillus fumigatus</td>
<td>-</td>
<td>17</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Actinomycete</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptomyces spp.</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Thermoactinomyces spp.</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bacillus spp. (total)</td>
<td>50</td>
<td>78</td>
<td>100</td>
<td>83</td>
<td>98</td>
<td>99</td>
<td>99</td>
<td>99</td>
</tr>
<tr>
<td>B. licheniformis</td>
<td>2.5+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>2</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B. coagulans type A</td>
<td>2.5-</td>
<td>42</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B. coagulans type B</td>
<td>21-</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>69</td>
<td>28-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B. circulans complex</td>
<td>22</td>
<td>8</td>
<td>-</td>
<td>-</td>
<td>91</td>
<td>18</td>
<td>27.5</td>
<td>-</td>
</tr>
<tr>
<td>B. stearothermophilus</td>
<td>2+</td>
<td>20</td>
<td>97</td>
<td>83</td>
<td>-</td>
<td>8</td>
<td>23.5</td>
<td>-</td>
</tr>
<tr>
<td>B. brevis</td>
<td>1.5+</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>6.5</td>
<td>-</td>
</tr>
<tr>
<td>B. sphaericus</td>
<td>-</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>2</td>
<td>13.5-</td>
<td>-</td>
</tr>
<tr>
<td>Other Bacillus spp.</td>
<td>9.5-</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Other nonsporeformer</td>
<td>42.5</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lost</td>
<td>2+</td>
<td>5</td>
<td>+</td>
<td>17</td>
<td>-</td>
<td>1</td>
<td>1-</td>
<td>-</td>
</tr>
</tbody>
</table>
Figure 1.1. FT-IR spectra of CSM compost at different time stages (Inbar et al., 1989).
2.1 INTRODUCTION

Composting has been recognized as an effective way to partially solve the growing concern of solid waste management. Many problems, however, such as odor formation and high operating costs, are associated with systems that are operating today (Miller, 1993). The comment of Finstein and Hogan (1993), "Its (composting) potential can not be realized through the empirical practices usual to this field" underscores the need for more precise, scientific studies of the fundamentals of the composting process.

The composting process is fundamentally based on process kinetics and microbial ecology. However, process kinetics and microbial ecology cannot be understood without characterizing and controlling the operational parameters during the process. Temperature is one of the most important operational parameters in composting (Golueke, 1972). In a typical composting process,
different zones of temperature typically exist which shift with time as a result of changes in microbial activity. These zones are colonized by different types of microbes. Consequently, different degradation rates exist (Strom, 1985a, 1985b). Systematic study of composting by isolating temperature from other operational parameters will greatly contribute to our understanding of these degradation rates.

The role of water in microbial activity during composting is often overlooked (Miller, 1993). Since a majority of the microorganisms typically grow in or below a liquid film on the surface of compost particles, low moisture content seriously limits microbial activity (Golueke, 1972). The optimal moisture content varies with the ability of the feed stock to hold water (Bach et al., 1984). For municipal sewage sludge, the optimal moisture content ranges from 50 to 60% (Suler and Finstein, 1977; Bach et al., 1984), while for yard wastes feed materials it is in the range of 65-70%. Microbial activity is severely inhibited if the moisture content is below 40% (Golueke, 1972). The role of water in microbial activity was extended further by Miller (1989) with the introduction of matric water potential as a measure of the degree to which water is held in small pores on the surface of particles.

$O_2$ concentration is another important parameter affecting the process (Golueke, 1972; Suler and Finstein, 1977; Haug, 1980; Miller et al. 1991; Nakasaki et al. 1992; Miller 1993). Predominantly aerobic conditions accelerate the degradation rate of most organic materials (Nakasaki et al., 1992). Suler and Finstein (1977) reported that the $O_2$ concentration in the exit gas needs to be at least within the range of 10% to 18% to prevent a decrease in metabolic activity based on $CO_2$
evolution. A recent model by Hamelers (1993) suggests that only the outer shell equivalent to 40% of the typical compost particle is aerobic even under "completely aerobic" conditions. Consequently, it is reasonable to assume that aerobic and anaerobic microbial activities exist in well-controlled composting systems.

Ashbolt and Line (1982) classified laboratory scale vessels into rotating drums and stationary cylinders. Several studies have addressed the performance of each type of vessels (Schulze, 1962; Jeris and Regan, 1973; Suler and Finstein, 1977; Ashbolt and Line, 1982; Sikora et al., 1983; Nakasaki et al., 1985; Hogan et al., 1989; Palmisano et al., 1993). Bach et al. (1984) combined the advantages of these two types of vessels and used a mixed isothermal reactor. In traditional composting reactors, evaporative cooling controls temperature (MacGregor et al. 1981; Sikora and Sowers, 1985; Nakasaki et al., 1992). Evaporative cooling and non uniform aeration (usually due to channeling) in composting systems can result in temperature gradients within the compost, as shown for full-scale systems by Kuter et al. (1985). In most systems it is difficult, if not impossible, to control temperature, moisture content, and O$_2$ concentration independently.

The objective of part of work was to design an experimental system in which temperature, moisture and aeration were controlled independently as opposed to a typical system representing a more semi-controlled process, in which all three are intimately linked. The goals for this system design were to maintain constant temperature in the reactor, control moisture content of the solid material in the range of 50 to 60%, and minimize temperature and O$_2$ gradients within the solid
material with uniform gas exchange. With this defined environment, microbial biomass and rates of O$_2$ consumption and CO$_2$ evolution as a function of temperature was determined. In addition, the rates and types of compounds degraded and volatile compounds produced can be determined. This paper describes the system and the results obtained at two constant temperature experiments carried out at 40 °C and 50 °C.

2.2 MATERIALS AND METHODS

**Laboratory-scale vessel.** The vessel used in this study was constructed of 1.27 cm acrylic plastic (Figure 2.1a). The empty, void space in the vessel was approximately 56 L. The vessel contained eight trays made of nylon mesh which supported thin layers (~1 cm) of feed material. Baffles were added at the end of each tray to direct air flow through as well as over the feed. A total of 10 thermocouples (Type K) were installed within the vessel to measure feed (one on each tray), air, and wet bulb temperatures. Two electrical fans (20 W, 106 CFM) installed in stack fashion circulated air inside the vessel.

O$_2$ and CO$_2$ concentrations in exit gas were determined by independent gas meters (Horiba). To provide a gas sample for each meter, an air pump with air flow rate at 2,500 ml/min was placed within the vessel and actuated by a computer at half hour increments. Prior to the experiment, the O$_2$ and CO$_2$ meters were calibrated with standard gas samples. To prevent air escaping from the vessel when the air pump was off, a computer controlled gas valve was installed. Each layer of compost was sampled periodically for gravimetric
determination of moisture content. A sealable opening (2 cm) adjacent to each tray facilitated removal of these samples.

A heat exchanger inside the reactor under some conditions created water condensate. This condensate was collected through a drain underneath the heat exchanger and its pH was determined. To control temperature in the reactor to a desired set-point, the vessel was placed in a modified incubator which contained a heater, a heat exchanger connected to a refrigerated water bath and four electric fans to increase the air circulation rate. Compressed, in-house air was used for aeration. Air flow rates for experiments reported here are shown in Figures 2.2e and 2.3e.

**Control Strategy.** Two major goals of the control strategy were: a sensitive, uniform compost temperature control and minimized condensation within the vessel. To maintain temperature inside the vessel at the set point and to prevent condensation on internal walls, the temperature within the incubator was maintained at the same set point as that of the vessel. Heating and cooling inside the vessel and incubator were controlled by a computer.

The relationship of all components is shown in Figure 2.1b. A PC installed with an A/D (DAS-8, Keithley-Metrabyte) and a D/A (DDA-6, Keithley-Metrabyte) conversion boards were used to interface with the system. Signals from O₂ and CO₂ meters were directly inputted into the computer by the D/A board. In order to input temperature data from thermocouples, a temperature compensation board (EXP-16, Keithley-Metrabyte) was used. The D/A board was connected to a relay box for analog output. The program (appendix A) was written in
QuickBasic (Microsoft) which was compatible with the standard driver for the A/D and D/A boards. Temperature and gas concentration data were stored on disk every 30 min.

An open loop moisture control strategy was used to maintain compost moisture content within a desired range by spraying a fine mist of water through perforated silicon tubing on top of each layer of compost as needed during the experiments. The rate of water addition to the compost was controlled based on historic data from prior experiments. The moisture content of the substrate was kept high enough so that added water moved freely through the compost, thus avoiding wet anaerobic zones.

**Oxygen Gradients.** To verify the absence of O$_2$ gradients in the system, O$_2$ concentrations were measured in different locations in the vessel. The locations of these measurements are indicated by numbers in Figure 2.1b. Gas samples were pumped to the O$_2$ meter through 6.4 mm I.D. tubing. Measurements within the compost bed were made after the meter had equilibrated in the gas above the bed. The tube was then placed within the bed and the analyzer was monitored for 20 seconds, the response time of the O$_2$ analyzer with the 6.4 mm tubing attached. The lowest concentration of the O$_2$ was then recorded.

**Feed Stock.** A simulated (semi-defined) feed was used to obtain reproducible data. The feed formula (Palmisano et al. 1993) was modified to decrease nitrogen availability (Table 2.1). The initial total carbon to nitrogen ratio (C/N ratio) of the feed was approximately 25:1. The initial moisture content of the feed was adjusted to approximately 55-60% through the addition of distilled water to
dry feed materials. To provide some buffering capability, 0.01 M each of Na$_2$HPO$_4$ and KH$_2$PO$_4$ was added to the water. A total of 550 g of feed was introduced as a 1 cm deep layer on each tray in the vessel (total feed was 4,400 g). Seed compost (1% of total feed) was added as starter inoculum.

**Feed Physical Properties.** The particle size of the feed before and after water addition was measured with ASTM sieves since it was observed that the addition of water significantly changed the particle size distribution. The material was thoroughly mixed before placed in the sieving apparatus. Ten sieves, ASTM 5/16" and No.'s 6, 10, 16, 20, 40, 50, 70, 120, and 200 were used for the dry material and six sieves 5/16" and No.'s 6, 10, 16, 20, and 40 were used for the wet material. The system was shaken for 15 min and the material on each sieve was then weighed.

The average density of the feed material was determined by adding a known mass to a graduated cylinder filled with water and recording the change in liquid volume. The average bulk density of the wet material was determined by measuring the volume that a known mass of wet feed occupied on a tray within the vessel.

**Compost Analysis.** Moisture content was determined every 12 hr by drying 5 g (approximately) of compost sample at 105°C for 12 hr. The pH of the feed material was determined by mixing 2 g compost in 100 ml of distilled water. This suspension was stirred 5 min and then allowed to settle 15 min before the pH was determined (Carnes and Lossin, 1970; Michel *et al*. 1993). The pH in condensed water was also measured. The C/N ratio and ash content of 50 g
compost samples were measured by the Research Extension Analytical Laboratory (OSU, Wooster, OH) using a carbon analyzer (ASTM D4129-82), a Macro-N analyzer (Dumas method), and for ash content the AOAC 967.04 method.

**Carbon Dioxide Dissolved in Water Condensate.** CO₂ dissolved in the water condensate was calculated based on the solubility of CO₂ using Henry’s law.

**ATP concentration.** Microbial biomass in the feed material was measured as ATP using a modified version of the bioluminescence method described by Suberkropp *et al.* (1983). Compost samples (0.5 g) were mixed with 5 ml cold 1.2 N H₂SO₄ containing oxalic acid (8 g/L) and 5 ml 0.05 M HEPES (Sigma Chemical Co.), pH 7.5. The samples were extracted for a total of 3 hr with hourly vortexing for 1-5 min. Solids were removed by centrifugation (10,000 x g, 10 min) and the supernatants were neutralized with NH₄OH to pH 7.8. The neutralized samples were centrifuged (10,000 x g, 5 min) again and stored at -4°C until analyzed.

To quantitatively measure the effectiveness of the ATP extraction technique, a known amount of ATP (10⁻⁸ M) was added to replicate samples. The ATP recovery was determined using Equation 1. The concentrations of ATP in the extracted samples were measured with an ATP Bioluminescent Assay Kit (Sigma Chemical Co.) and a portable luminescence photometer (Analytical Luminescence Laboratory, Inc.) by integrating the light emission during a 10 sec. period after the addition of the luciferin-luciferase reagent. The reagents were reconstituted with sterile double distilled water and set over night to reduce
background readings. All glassware used for measurements were cleaned with 1 N HCl and sterilized to reduce the background readings. Every sample (100 µg) was assayed in duplicate. A standard curve was generated for a concentration range of $10^{-6}$ to $10^{-9}$ M ATP. Standard solutions of ATP were prepared in the neutralized extraction reagent (pH 7.8).

2.3 RESULTS

The results of two experiments operated at a set point of 40 and 50°C are presented in Figures 2.2 and 2.3. To provide a measure of the temperature fluctuations, the average temperature for a 10 minute period is presented as data points in the Figure 2.2a and 2.3a. The error bars correspond to the standard deviations during those ten hours. Figure 2.2b and 2.3b present the average temperature of the compost as a function of time. Each data point corresponds to the average temperature over a 10 minute period. In addition to the temperature in the feed material and the surrounding gas, changes in the moisture content, O$_2$, CO$_2$ and ATP concentrations, rates of O$_2$ consumption and CO$_2$ evolution, and the pH of the condensed water during the time course are presented. The amount of water condensed in the vessel averaged between 1 and 5 ml/hr.

The rates of O$_2$ consumption and CO$_2$ evolution were calculated using Equations 2.1 and 2.2:

$$ R_{CO_2} = \left( V \cdot \frac{dC_{CO_2}}{dt} + F_n \cdot C_{CO_2} + S_{CO_2} \right) \frac{P}{D_w \cdot R \cdot T} $$

(2.1)
Data points in Figure 2.2f and 2.3f represent the average rates for 5-hr intervals. Fluctuations in the concentrations of O\(_2\) and CO\(_2\) and in the corresponding rates of O\(_2\) consumption and CO\(_2\) evolution are the result of variations in the air flow rate through the vessel. Figure 2.4 presents the cumulative consumption of O\(_2\) and evolution of CO\(_2\) for the two experiments.

The analytical recovery of ATP varied between 40 to 80%. The actual amounts of ATP were similar in duplicate samples when corrected for differences in the relative recovery. The recovery increased upon a longer intermittent vortexing time during the 3-hr extraction. Carbon and nitrogen content, C/N ratio, ash content and pH of the initial and final samples are listed in Table 2.2. There was relatively little change in the C/N ratio although the total lose of mass was 32-33% in both experiments. The ash content increased in agreement with the less of carbon content upon mineralization. The pH increased by 2 units because of formation of NH\(_3\).

The particle size distributions of dry and wet feed materials are presented in Figure 2.5. Table 2.3 presents the corresponding pore size for each sieve number. The average density of the feed material (wet) was 1.08 g/cm\(^3\). The average bulk density of the feed material was 0.6 g/cm\(^3\) which corresponds to a void fraction of 0.4 in the layers on each tray.
Visual observations revealed significant white mycelial growth after 150 hours in the 40°C experiment. On average, this mycelial growth reached 12 mm above the compost layers. In the 50°C experiment, white mycelial growth was practically absent.

2.4 DISCUSSION

Data in Figures 2.2 and 2.3 reveal that temperatures in both the 40 and 50°C runs were controlled reasonably well around the set points. This control includes both temperatures in the gas surrounding the material in the vessel and that within the material. The average and standard deviation of all of the air temperature readings were 40.0 + 0.6°C and 50.3 + 1.4°C for the 40 and 50°C runs, respectively. Temperature of the feed material itself were 40.4 + 0.6°C and 50.4 + 1.6°C for the 40 and 50°C runs, respectively. The wet bulb temperature readings were 39.8 + 0.7°C and 49.6 + 1.6°C, respectively, indicating that the humidity was maintained near saturation.

ATP was used as a measure of biomass in this work. As shown in Figure 2.2g and 2.3g, after an initial jump, the ATP concentration gradually increased, indicating microbial growth during composting. The final level of ATP was approximately 1 μg ATP/g dry wt. compost, equivalent to about 100-120 μg biomass carbon per gram dry weight, based on the conversion factor established in soil studies (Jenkinson and Oades, 1979; Oades and Jenkinson, 1979). Microbial respiration, measured as O₂ consumption and CO₂ evolution (Figures 2.2f and 2.3f), displayed a distinct lag phase in both the experiments. Both experiments were initiated with a seed from a mature compost. The lag phase
was more pronounced in the 50°C experiment, indicating that fewer organisms in
the seed were initially active in the 50°C environment as opposed to the 40°C
experiment. Accompanied with the lag phase was a gradual increase of about
one order of magnitude in the respective level ATP. Thus, the time course data
suggested that there was a relationship between the amount of biomass
(measured as ATP) and microbial respiration (O₂ consumption and CO₂
evolution) during the initial phase of composting. During the most intense
respiration, the concentration of ATP leveled off in the 40°C experiment. In the
50°C experiment, the trend of continuing formation of biomass after the peak
period of respiration was more discernible. Fluctuations in ATP levels were
evident during peak O₂ consumption and CO₂ evolution rates. It is concluded
that a major source of this variability was the moisture content which decreased
to levels almost prohibitive to microbial respiration.

The concentration of O₂ in the vessel for the two runs only briefly dropped below
5%. The concentration of O₂ in the system is a function of the rate of
consumption by the microorganisms and the rate at which fresh air is added to
the system (Miller and Macauley, 1988). This is particularly noticeable from the
continued decrease in the O₂ concentration at specific periods of operation
despite a large increase in air flow during both runs. With an electronic mass
flow meter and a solenoid air valve, it will be possible to achieve a much more
constant O₂ level in the system through feedback control, despite the change in
demand with time.

Two important questions need to be addressed with respect to O₂ concentration
and consumption: 1) What is the concentration of O₂ within the compost?, and 2)
What is the O₂ concentration at which significant metabolic changes in microbes take place?

To address the first question, experimental data and theoretical calculations were made. As reported previously, O₂ and CO₂ concentration measurements were made at 16 locations within the vessel as well as within the compost layer itself. These locations are shown in Figure 2.1b and the values of the O₂ and CO₂ concentrations at these locations are given in Tables 2.4 and 2.5. As can be observed, no significant bulk gradients exist within the vessel.

To calculate the O₂ concentration surrounding the particles within the compost bed (interstitial concentration) the particle size, structure, and bed void space are needed. As indicated in Figure 2.5, the average particle diameter increased with the addition of water. Visual observations indicated that smaller particles adhered to larger particles after water addition and remained as aggregates throughout the degradation period. A majority of these wet particles are 5.6 mm in diameter.

The absence of smaller particles has important implications with respect to interstitial O₂ concentration, i.e., the larger the average particle size the greater the void space within the bed. This is supported by the relatively large void fraction, 0.4, in the bed. Since the average distance within these void spaces is much larger than the mean free path of a gas molecule, ordinary fluid molecular diffusion can be assumed to apply.
A typical compost bed in the vessel is represented in Figure 2.6. Since the feed material is supported on a nylon mesh, gas molecules diffuse in and out of the compost layer from both the top and bottom. Taking into consideration the geometry of a layer, the calculated void space, a steady state concentration, and using the maximum rate of O\textsubscript{2} consumption for the experiments, the O\textsubscript{2} concentration gradient within each layer of compost can be calculated with the following relationship:

\[ D_{\text{O}_2} \frac{d^2 C_{\text{O}_2}}{dx^2} + r \cdot \rho = 0 \]  

(2.3)

Figure 2.7a presents the normalized concentration of O\textsubscript{2} as function of bed thickness when the diffusion of O\textsubscript{2} is assumed equal to the ordinary diffusion of O\textsubscript{2} in air (10\textsuperscript{-5} m\textsuperscript{2}/s). To determine the sensitivity of the calculation to the diffusivity of O\textsubscript{2}, a second calculation was conducted when the value was equal to 10\textsuperscript{-7} m\textsuperscript{2}/s; two orders of magnitude smaller. This calculation is presented in Figure 2.7b. As can be observed, using the ordinary diffusivity of O\textsubscript{2} in air, the bed could be an order of magnitude thicker before significant O\textsubscript{2} concentration gradients would be observed. Even for the extreme case of an O\textsubscript{2} diffusivity 1% of that in air, the center of the bed would still have a non-zero concentration of O\textsubscript{2}. These theoretical calculations were confirmed with the experimental results presented in Table 2.5, which indicated the absence of a detectable O\textsubscript{2} gradient within the bed.

While these theoretical and experimental calculations and measurements predict the concentration of O\textsubscript{2} around the particles within the bed, they do not describe the actual environment microbes experience. Very little work has been published
on this concept. It has been proposed that actual microbial activity in composting takes place in a thin aqueous layer surrounding each particle, which is called a "biofilm" (Harremoes, 1978; Hamelers, 1993). Hamelers (1993) suggested that the maximum depth that $O_2$ can penetrate into this biofilm can be predicted from:

$$L_p = \sqrt{\frac{2 \cdot D_{O_2} \cdot O_{2,p}}{K_0}}$$  \hspace{1cm} (2.4)

where $L_p$ represents the penetration depth of oxygen within the biofilm. This relationship assumes that the rate of consumption of $O_2$ is zero order and that the system is at steady-state. Using the maximum $O_2$ consumption rate at 40 and 50°C, and an $O_2$ diffusivity in the biofilm of $1 \times 10^{-9}$ m$^2$/s, the penetration depth of $O_2$ for various bulk $O_2$ concentrations is given in Figure 2.8a. As can be observed, the higher the interstitial $O_2$ concentration, the deeper the $O_2$ penetration. However, even at the highest $O_2$ concentrations (21%), $O_2$ only partially penetrates a theoretical biofilm in agreement with Hamelers' (1993) results. Within the region in which $O_2$ penetrates, the $O_2$ concentration rapidly decreases as shown in Figure 2.8b.

The above analysis applies only to small microorganisms in the size range of bacteria contained within a biofilm. As was mentioned above, after 150 hours the compost materials in the 40 °C experiment had noticeable white mycelial mold present. Much of this cell mass was far removed from the biofilm or the feed material itself; and consequently the gas transport between the bulk gas above the feed material and the organism is much different. This further increases the complexity of $O_2$ concentration and demand with respect to composting systems.
A final question which needs to be addressed with respect to O₂ concentration is the concentration that induces significant metabolic changes within a microbe, such as the shift from aerobic to anaerobic metabolism. An absolute answer to this question cannot be arrived at because the relative contribution of various microorganisms to microbial activity remains unknown. However, a reasonable estimate can be made if one considers the types of microbes isolated so far. The critical O₂ concentration inducing a shift from aerobic to anaerobic metabolism for several strains of bacteria ranges from 0.003 to 0.05 mol/m³ (Finn, 1967; Bailey and Ollis, 1986). As can be observed, when these numbers are compared to Figure 1-8b, even within the theoretical O₂ penetration depth, insufficient O₂ exists to support aerobic metabolism within much of the particle. This conclusion agrees with Hamelers (1993).

The experimental and theoretical analysis presented above underscores the complexity of the microbial environment during composting. The sensitivity of the O₂ concentration to biofilm thickness and microbial concentration reveals that it is difficult to accurately predict the degree to which a particular process is aerobic or anaerobic, as well as a critical interstitial concentration of O₂ required to maintain a bulk aerobic environment. However, it is important that the metabolism of microorganisms near air channels in large composting systems is aerobic. By-products of anaerobic metabolism produced deep inside particles must be consumed by aerobes on the surface to reduce odor generation.

Moisture content set-point was 50-55% during the experiments. Moisture content is greatly affected by air flow rate, rate of water addition to compost, and the growth rate of microbes in the compost. In the 40°C experiment, the moisture
content was maintained during the first 100 hr (Figure 2.2c). Thereafter, it
decreased to 45% at 130 hr because of the high microbial activity and high air
flow rate. Doubling of the water addition rate at 130 hr increased the moisture
content to 50-55% until the end of the run. In the 50°C experiment (Figure 2.3c)
the amount of water added was increased during the period of highest microbial
activity. Because on-line moisture measurement was not possible in this work
and the rate of water addition was based on historical data, moisture content
was sensitive to non-historical process changes.

One reason for a decrease in moisture content was the formation of condensate
(average 1 to 5 ml/hr) under the heat exchanger, which operated approximately
20% of the total operating time. This is undesirable since gases (CO₂ and NH₃)
dissolve in the condensate, creating difficulty in determining dynamic mass and
energy balances. The condensate was allowed to drain into a container which
significantly decreasing the absorption of these gases. With an ideal control of
the temperature of the incubator, heat generated by composting process could
be conducted through reactor wall while forming minimum water condensates.
Work is ongoing to address this issue.

An important question in the analysis of data obtained in these experiments is
whether a significant amount of CO₂ dissolved in the condensed water and was
thereby not included in the CO₂ evolution rate. The solubility of CO₂ in water is
9.73 x 10⁻⁴ g/ml at 40°C and 7.61 x 10⁻⁴ g/ml under 50°C. Assuming the average
CO₂ concentration to be 5%, the total CO₂ dissolved in the total water
condensed by Henry's law would be 1.74 x 10⁻³ mol. at 40°C and 6.30 x 10⁻³
mol. at 50°C. Total amounts of CO₂ evolved were 1.71 and 3.37 moles for the 40
and 50°C runs, respectively. Even if the condensed water was to be completely saturated with CO₂, only approximately 0.2% of the total evolved CO₂ would be absorbed by condensed water.

2.5 Summary

A novel bench-scale system was developed that through its use contributes toward a more fundamental understanding of the composting process. The computer controlled system maintained temperature and moisture levels within narrow ranges in thin layers of compost positioned on trays within the vessel. This system minimized complications caused by gradients observed in earlier systems. Specific O₂ consumption and CO₂ evolution rates were determined based on dynamic mass balances. Water was introduced intermittently and microbial biomass (ATP) was monitored at specific time intervals throughout the process. The system allows close observation of decomposition of feed materials at constant temperature and moisture levels throughout the process.

2.6 Notation

\begin{align*}
\text{C}_{\text{CO}_2} & \quad \text{CO}_2 \text{ concentration (v/v %)} \\
\text{C}_{\text{O}_2} & \quad \text{O}_2 \text{ concentration (v/v %)} \\
\text{D}_{\text{O}_2} & \quad \text{O}_2 \text{ diffusion coefficient (m}^2/\text{s}) \\
\text{D}_{\text{w}} & \quad \text{dry weight (g)} \\
\text{F}_{\text{ad}} & \quad \text{luminescence of sample with known amount of ATP added} \\
\text{F}_i & \quad \text{luminescence of sample without ATP added} \\
\text{F}_i & \quad \text{inlet flow rate (l/hr)}
\end{align*}
Fo  outlet flow rate (l/hr)
Fref  luminescence from reference sample at same concentration as that added to Fad
L  thickness of feed material layer
LP  depth of oxygen penetration into compost film surrounding particle (m)
K0  zero order reaction constant
O2, b  bulk oxygen concentration at gas-liquid interface based on solubility (mol/m3)
r  density of feed materials (g/cm3)
R  gas constant
Rr  percent recovery (%)
RCO2  CO2 evolution rate (mol/hr g)
RO2  O2 consumption rate (mol/hr g)
SCO2  CO2 dissolved in condensed water (mol)
t  time (hour)
T  temperature (K)
Vr  reactor empty volume (l)
Table 2.1. Compositions of simulated compost feed materials.

<table>
<thead>
<tr>
<th>Composition</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled Water</td>
<td>60</td>
</tr>
<tr>
<td>Rabbit chows (ground grass and leaf)</td>
<td>16.3</td>
</tr>
<tr>
<td>Corn cobs (chopped)</td>
<td>14</td>
</tr>
<tr>
<td>Sand</td>
<td>8</td>
</tr>
<tr>
<td>Seed inoculum (matured compost from commercial plants)</td>
<td>1</td>
</tr>
<tr>
<td>Manure</td>
<td>0.4</td>
</tr>
<tr>
<td>Newspaper</td>
<td>0.3</td>
</tr>
</tbody>
</table>
Table 2.2. Analysis of initial and final compost samples.

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>Sample</th>
<th>Carbon (%)</th>
<th>Nitrogen (%)</th>
<th>C/N ratio</th>
<th>pH</th>
<th>Ash Content (%)</th>
<th>Total Dry Mass (g)</th>
<th>Loss of Dry Mass (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>Start</td>
<td>33.0</td>
<td>1.47</td>
<td>22.5:1</td>
<td>6.38</td>
<td>25.5</td>
<td>1923</td>
<td></td>
</tr>
<tr>
<td>Finish</td>
<td>31.7</td>
<td>1.58</td>
<td>20.1:1</td>
<td>8.54</td>
<td>29.9</td>
<td>1312</td>
<td></td>
<td>31.8</td>
</tr>
<tr>
<td>50</td>
<td>Start</td>
<td>32.7</td>
<td>1.45</td>
<td>22.5:1</td>
<td>6.36</td>
<td>31.48</td>
<td>1795</td>
<td></td>
</tr>
<tr>
<td>Finish</td>
<td>32.9</td>
<td>1.42</td>
<td>23.2:1</td>
<td>8.27</td>
<td>40.37</td>
<td>1197</td>
<td></td>
<td>33.3</td>
</tr>
</tbody>
</table>
Table 2.3. Sieve numbers and pore sizes.

<table>
<thead>
<tr>
<th>Sieve Number</th>
<th>Pore Size (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5/16&quot;</td>
<td>8.00</td>
</tr>
<tr>
<td>6</td>
<td>3.36</td>
</tr>
<tr>
<td>10</td>
<td>2.00</td>
</tr>
<tr>
<td>16</td>
<td>1.19</td>
</tr>
<tr>
<td>20</td>
<td>0.841</td>
</tr>
<tr>
<td>40</td>
<td>0.420</td>
</tr>
<tr>
<td>50</td>
<td>0.297</td>
</tr>
<tr>
<td>70</td>
<td>0.210</td>
</tr>
<tr>
<td>100</td>
<td>0.125</td>
</tr>
<tr>
<td>120</td>
<td>0.074</td>
</tr>
</tbody>
</table>
Table 2.4. $O_2$ and $CO_2$ concentrations at different locations of the reactor.

<table>
<thead>
<tr>
<th>Location&lt;sup&gt;a&lt;/sup&gt;</th>
<th>$CO_2$ (%)</th>
<th>$CO_2$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15.92</td>
<td>4.54</td>
</tr>
<tr>
<td>2</td>
<td>15.88</td>
<td>4.60</td>
</tr>
<tr>
<td>3</td>
<td>15.92</td>
<td>4.52</td>
</tr>
<tr>
<td>4</td>
<td>15.88</td>
<td>4.59</td>
</tr>
<tr>
<td>5</td>
<td>15.83</td>
<td>4.59</td>
</tr>
<tr>
<td>6</td>
<td>15.83</td>
<td>4.64</td>
</tr>
<tr>
<td>7</td>
<td>15.86</td>
<td>4.54</td>
</tr>
<tr>
<td>8</td>
<td>15.84</td>
<td>4.54</td>
</tr>
<tr>
<td>9</td>
<td>15.83</td>
<td>4.53</td>
</tr>
<tr>
<td>10</td>
<td>15.83</td>
<td>4.55</td>
</tr>
<tr>
<td>11</td>
<td>15.83</td>
<td>4.52</td>
</tr>
<tr>
<td>12</td>
<td>15.83</td>
<td>4.54</td>
</tr>
<tr>
<td>13</td>
<td>15.81</td>
<td>4.54</td>
</tr>
<tr>
<td>14</td>
<td>15.81</td>
<td>4.62</td>
</tr>
<tr>
<td>15</td>
<td>15.84</td>
<td>4.60</td>
</tr>
<tr>
<td>16</td>
<td>15.88</td>
<td>4.55</td>
</tr>
</tbody>
</table>

<sup>a</sup>Locations refer to Figure 1.1c
Table 2.5. $O_2$ and $CO_2$ concentrations inside and outside compost layer.

<table>
<thead>
<tr>
<th>Measurements</th>
<th>$O_2$ (%)</th>
<th>$CO_2$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>In</td>
<td>10.65</td>
</tr>
<tr>
<td></td>
<td>Out</td>
<td>10.71</td>
</tr>
<tr>
<td>2</td>
<td>In</td>
<td>10.25</td>
</tr>
<tr>
<td></td>
<td>Out</td>
<td>10.28</td>
</tr>
</tbody>
</table>
Table 2.6. Particle size distribution of dry and wet feed material.

### a. Dry feed material.

<table>
<thead>
<tr>
<th>Particle Size Range (mm)</th>
<th>Interval (mm)</th>
<th>Average Size (mm)</th>
<th>Weight in Range (g)</th>
<th>Percentage in Range (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.07 to 0.012</td>
<td>0.05</td>
<td>0.095</td>
<td>13.76</td>
<td>7.1</td>
</tr>
<tr>
<td>0.12 to 0.21</td>
<td>0.09</td>
<td>0.165</td>
<td>21.0</td>
<td>10.8</td>
</tr>
<tr>
<td>0.21 to 0.29</td>
<td>0.08</td>
<td>0.25</td>
<td>38.25</td>
<td>19.72</td>
</tr>
<tr>
<td>0.29 to 0.42</td>
<td>0.13</td>
<td>0.355</td>
<td>15.75</td>
<td>8.12</td>
</tr>
<tr>
<td>0.42 to 0.84</td>
<td>0.42</td>
<td>0.63</td>
<td>19.83</td>
<td>10.22</td>
</tr>
<tr>
<td>0.84 to 1.2</td>
<td>0.36</td>
<td>1.02</td>
<td>4.46</td>
<td>2.30</td>
</tr>
<tr>
<td>1.2 to 2.0</td>
<td>0.8</td>
<td>1.6</td>
<td>4.82</td>
<td>2.48</td>
</tr>
<tr>
<td>2.0 to 3.3</td>
<td>1.3</td>
<td>2.65</td>
<td>29.36</td>
<td>15.1</td>
</tr>
<tr>
<td>3.3 to 7.9</td>
<td>4.6</td>
<td>5.6</td>
<td>46.51</td>
<td>24.0</td>
</tr>
</tbody>
</table>

### b. Wet feed material.

<table>
<thead>
<tr>
<th>Particle Size Range (mm)</th>
<th>Interval (mm)</th>
<th>Average Size (mm)</th>
<th>Weight in Range (g)</th>
<th>Percentage in Range (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.42 to 0.84</td>
<td>0.42</td>
<td>0.63</td>
<td>0.68</td>
<td>0.01</td>
</tr>
<tr>
<td>0.84 to 1.2</td>
<td>0.36</td>
<td>1.02</td>
<td>57.2</td>
<td>15.9</td>
</tr>
<tr>
<td>1.2 to 2.0</td>
<td>0.8</td>
<td>1.6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2.0 to 3.3</td>
<td>1.3</td>
<td>2.65</td>
<td>50.4</td>
<td>14</td>
</tr>
<tr>
<td>3.3 to 7.9</td>
<td>4.6</td>
<td>5.6</td>
<td>252</td>
<td>70</td>
</tr>
</tbody>
</table>
Figure 2.1. (1a) The bench-scale vessel. The numbers correspond to the following components: (1) inlet air, (2) inlet air humidifier, (3) heat exchanger, (4) condensation trap, (5) dry air temperature thermocouple, (6) inlet water, (7) recirculating fan, (8) air pump to gas meters, (9) air line to meters, (10) exit air line, (11) compost temperature thermocouple, (12) wet bulb temperature thermocouple, (13) compost layer. (1b) The bench-scale composting system.
Figure 2.1.
Figure 2.1. Continued.

1c
Figure 2.2. Experiment at 40°C. (a) The average dry (■) and wet (●) bulb temperature profiles in the vessel; (b) the average compost temperature profile inside the vessel; (c) moisture content of the compost samples; (d) CO₂ (thick line) and O₂ (thin line) concentration profiles; (e) air flow rate profile; (f) CO₂ (x) evolution and O₂ (■) consumption rate profiles; (g) ATP concentration; (h) pH of condensed water. The bars represent the standard deviation.
Figure 2.3. Experiment at 50°C. (a) The average, dry (■) and wet (●) bulb temperature profiles in the vessel; (b) the average compost temperature profile inside the vessel; (c) moisture content of the compost samples; (d) CO₂ (thick line) and O₂ (thin line) concentration profiles; (e) air flow rate profile; (f) CO₂ (x) evolution and O₂ (■) consumption rate profiles; (g) ATP concentration; (h) pH of condensed water. The bars represent the standard deviation.
Figure 2.4. Cumulative CO$_2$ evolution (■) and O$_2$ consumption (□) in the 40°C (a) and 50°C (b) experiment.

Figure 2.5. Particle size distributions of the dry feed material before water addition (■), and the wet feed material after water addition (□).
Figure 2.6. Schematic presentation of the compost layer (a) and the compost biofilm surrounding the particle (b).
Figure 2.7. $O_2$ diffusion profile into the compost layer: (a) 21% $O_2$ concentration, max. $O_2$ consumption rate, $10^{-5} \, m^2/s$ $O_2$ diffusion coefficient; (b) 5% $O_2$ concentration, max. $O_2$ consumption rate, $10^{-7} \, m^2/s$ $O_2$ diffusion coefficient; (■) 40°C experiment; (●) 50°C experiment.
Figure 2.8. $O_2$ penetration profile into compost film for 40°C (■) and 50°C (●) experiments.
CHAPTER III

ATP MEASUREMENT IN COMPOST

3.1 INTRODUCTION

Biomass along with cell numbers and microbial activity is the key ecological parameter (Atlas and Bartha, 1993) and can elucidate the fundamental kinetics and microbial ecology of the composting process. Microbial biomass only accounts for about 1-3% of the soil organic-C, but this small amount controls the turnover of most of organic matter present (Martens, 1995). During the composting process, microorganisms convert organic matter into heat, CO₂, partial degradation products, and new cell material.

The relationship between microbial activity, cell numbers, and biomass is relatively constant only under steady-state growth conditions. Changes in environmental determinants such as temperature or nutrient availability may directly influence microbial activity but not necessarily the amount of biomass. The fraction of metabolically active cells in the population is mostly related to environmental conditions. Different operational or nutrient conditions in
composting processes may result in variations in biomass levels even though they may display similar microbial activity (e.g., CO$_2$ evolution or O$_2$ consumption rates). Monitoring the biomass changes during the composting process by measuring ATP concentrations may be used to optimize the composting conditions (Lehtokari et al., 1983).

Microbial decomposition of compost material and cell growth are closely coupled processes. The amount of biomass is related to the net turnover of organic carbon and is a quantitative measure of the microbial community in a compost matrix. The kinetic parameters of the decomposition of organic material in compost are dependent on the presence of metabolically active cells. Therefore, degradation rates of organic compounds, including indicators such as O$_2$ consumption and CO$_2$ evolution, should be normalized to microbial biomass in composting systems. This is particularly important for comparisons of different operational conditions because the activity of microorganisms greatly depends on the microenvironment the microbes are contained within, such as the presence of anaerobic zones inside compost materials (Hamelers, 1993; Tseng et al., 1995). Many experimental approaches have been explored for biomass measurements in composting studies. Plate counts are often used for microbial enumeration in composting processes (Nakasaki et al., 1992). In general, non-selective, general-purpose media gives the best plate counts results because unknown recoveries plate counts represent unknown fractions of the total microbial counts.

An alternative to actual cell counts or measurements of metabolic activity, a parameter can be used to estimate microbial biomass. Such a parameter must be based on cellular constituents present only in living organisms
and proportional to the cellular entity or cell carbon. The measurement of the phospholipid content is one such parameter (White et al., 1979; Mckinley and Vestal, 1984, 1985). Although sensitive, this analytical approach may yield erroneous data because some compost feed materials such as alfalfa have a high P-lipid content which cannot be readily differentiated from microbial P-lipids without additional analytical separation. Nucleic acid (DNA or RNA) content can also be employed for biomass measurement. However, this method is subject to low recovery in soils with high clay content because of sorption of DNA and RNA by clay minerals. Internal standards of DNA and RNA are not available for routine use to correct for analytical recovery. Nucleic acids may also occur as free DNA in soils and sediments and thus its relative cellular ratio in environmental samples is difficult to assess. Another parameter is ATP which is present universally in all living organisms and meets all the basic requirements of biomass measure.

**ATP in Soil and Compost.** Oades and Jenkinson (1979) established a linear relationship between the concentration of biomass determined by chloroform fumigation technique and that measured as ATP in soil samples. Garcia et al. (1992) showed that ATP concentration can be used as an index of the microbial biomass and activity in the composting process. Marambe et al. (1993) used ATP for measurement of microbial biomass in animal-waste compost, and Suberkropp et al. (1992) measured ATP in leaf litter approximating yard waste compost systems. Bach et al. (1984) found ATP concentration increased along with the pattern of CO$_2$ evolution rate in the composting of dewatered sewage sludge. Although ATP turnover times are relatively fast, its concentration has a direct and constant relation with living biomass in various environmental
materials. On a pure culture level, a coefficient can be used to convert ATP content to cellular C.

In previous studies, ATP levels have ranged from 0.6 to 7.0 μg/g in soil and from 0.4 to 2 μg/g for different compost materials (Patterson et al., 1970; Jenkinson and Oades, 1979; Lehtokari et al., 1983; Suberkropp et al., 1983; Garcia et al., 1992; Garcia et al., 1993; Marambe et al., 1993). The conversion factor between ATP and biomass carbon depends on the type of the sample; for aquatic samples it is in the range of 250-286 and for soil samples in the range of 100-120 (Atlas and Bartha, 1993). Conversion factors have not been estimated for composting processes but they are likely to be in the same range as for surface soils rich in organic-C.

ATP concentration in soil and compost is usually measured with the luciferin-luciferase bioluminescence system of the firefly (Photinus pyralis). This bioluminescence system is extremely specific to ATP and involves the following sequence of reactions:

\[
\begin{align*}
\text{ATP} + \text{Luciferin} & \rightarrow \text{Adenyl-luciferin} + \text{PPi} \quad (3.1) \\
\text{Adenyl-luciferin} + \text{O}_2 & \rightarrow \text{Oxyluciferin} + \text{AMP} + \text{CO}_2 + \text{light} \quad (3.2)
\end{align*}
\]

The firefly bioluminescence system is extremely specific for ATP and has a detection level of approximately $10^{-14}$ M ATP with commercially available reagents (Karl and Holm-Hansen, 1976). The level of detection and sensitivity are greatly affected by the luciferase preparation and assay conditions.
Sampling and Storage of Samples. It is most important to ensure sample homogeneity in order to obtain data which are representative of the distribution of microbial biomass in environmental samples. In the composting processes, heterogeneity is inevitable because of the physical, chemical, and biological differences in the interior and exterior zones (Tseng et al., 1995). Therefore, the sampling protocol for representative microbial population should include lateral and vertical profiles with relatively large sample sizes to minimize heterogeneity. For some bench-scale systems, compost matrix can be considered to be homogeneous because of the lack of thermal or gaseous gradients. For example, Lehtokari et al. (1983) used 1 g samples for ATP measurement to characterize a bench-scale compost reactor. Tseng et al. (1995) used 0.5 g compost samples in replicates from a bench-scale composting system which was maintained as thin layers at constant incubation temperatures.

Sample storage conditions greatly influence the ATP concentration. Low, subzero temperatures (-20°C) are highly recommended because they stop significant microbial activity. Microbial activity is not completely prevented in the temperature range of -4 to 4°C because of ATPase activities which would influence the ATP concentration of the sample upon prolonged holding time. This problem can be circumvented by storage at temperature below -20°C.

ATP Extraction from Compost. Many different ATP extraction reagents have been tested for soil samples (Karl, 1980; Webster et al., 1984). An extraction reagent should release ATP quantitatively from biomass, inactivate the ATP-hydrolyzing enzymes present in the sample matrix, and prevent the sorption of the released ATP on soil colloids (Jenkinson and Ladd, 1981). With some
environmental matrices, poor ATP recovery may seriously underestimate the amount of microbial biomass. Sometimes, microbial cells may colonize inter-aggregate spaces in soil and compost particles and may not be easily accessed with a solvent treatment for cell lysis and release of ATP. During sample extraction, ATP may be sorbed by soil or compost particles instead of being released to the solution. Metals may form complexes with ATP, thereby preventing the reaction of ATP in the bioluminescence system. Under some circumstances, the intracellular concentration of ATP may change under nutritional or other environmentally induced stress conditions.

The relative recovery of ATP can range from <1% to >80% for different extraction reagents (Sparrow et al., 1988), and it also varies with the sample matrix. Many of the commonly used extraction reagents contain 0.6-1.5 N H₂SO₄ for cell lysis. Lehtokari et al. (1983) reported that 18 w/v-% trichloroacetic acid (TCA) was the most effective extractant for ATP measurement in compost. Any increase did not improve the yield of ATP. The extraction temperature will also affect the yield of ATP from biomass depending on the extraction reagent. The H₂SO₄ extraction is usually used at 0-4°C (Suberkropp et al., 1983; Tseng et al., 1995); on the other hand, Patterson et al. (1970), using Tris buffer, claimed that sample extraction at 100°C increased ATP yields. Several extraction methods for ATP measurement suitable for compost systems have been summarized in (Table 3.1).

**Recovery Efficiency.** The efficiency of ATP extraction in environmental samples is dependent on the combination of the sample matrix, solvent, and extraction conditions. Because the efficiency of extraction varies for different samples and from one treatment condition to another, the use of internal ATP
standards is necessary for estimating quantitative recovery. Internal standards are normally added in the form of a known amount of an ATP stock solution (Lehtokari et al., 1983; Suberkropp et al., 1983). Werster et al. (1984) used Escherichia coli cells as an internal standard in an effort to determine the efficiency of extraction and assay of ATP. The use of bacterial cells as internal standards may, however, give misleading results because the cellular ATP content cannot be standardized to a constant amount. According to Ciardi and Nannipieri (1990) the recovery efficiency estimated with standard solutions of ATP varied in the range of 55-85% depending on the extraction method. In different soils, the analytical recovery ranges between 3 and 76% (Anderson and Davies, 1973).

The purpose of this work is to evaluate the luciferin-luciferase assay for ATP as a measure of biomass in experimental compost samples. Data, from a bench-scale composting system, were used to investigate the relationship between ATP (biomass) and microbial activity as determined by CO₂ evolution and O₂ consumption rates.

3.2 Materials and Methods

**Bench-scale System and Feed Material.** The bench-scale reactor was made of Acrylic plastic. It held eight trays of thin layer feed material (Table 2). Two circulating fans were installed inside the reactor to insure uniform aeration. One thermocouple was buried in each tray to measure the temperature of feed material. A computer system was interfaced with this reactor to control the temperature in the reactor and monitor the CO₂ and O₂ concentrations. Air flow
rate into the reactor was manually controlled. A detailed description of this system and data acquisition has been presented by Tseng et al. (1995). The seed inoculum originated from a mature municipal sewage sludge compost and it was added at 1% of total weight to feed material before the experiment. The experiment was conducted under 60°C for 250 h. Every 12 h, samples (approx. 2-3 g) were taken from the reactor and kept under -4°C until assayed. CO₂ evolution and O₂ consumption rates were calculated based on a mass balance surrounding the reactor (Tseng et al., 1995). Respiratory quotient (RQ) is calculated as:

$$RQ_{t_1,t_2} = \frac{\int_{t_1}^{t_2} R_{CO_2} dt}{\int_{t_1}^{t_2} R_{O_2} dt}$$ (3.3)

where RCO₂ and RO₂ are CO₂ evolution and O₂ consumption rates respectively.

**ATP Extraction.** The ATP extraction method was modified from that described by Suberkropp et al. (1983). Compost samples (0.5 g) were mixed in 10 ml of cold (on ice) extractant which contained 1.2 N H₂SO₄ (5 ml) and 0.05 M HEPES (N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid] (5 ml with 0.089 M oxalic acid, pH 7.5). The HEPES solution was sterilized before extraction. To determine the efficiency of ATP recovery, all compost samples were extracted in duplicates. The samples were extracted for 3 h on ice while vortexing once an hour before the solids were removed by centrifugation at 10,000 x g for 5 min. Aliquots of supernatants (~8 ml) were neutralized with NH₄OH to pH 7.8, centrifuged again, and stored at -4°C until assayed. A known amount of ATP (10⁻
8 mol/l) was added to one sample of each duplicate pair as an internal standard. A standard ATP curve was prepared prior to ATP assay by diluting known amounts of ATP in the extraction solution (HEPES and H₂SO₄ at 1:1 with 5 mM MgCl₂, pH 7.8) without a sample matrix present. The reagents for the ATP bioluminescence assay (Adenosine 5'-Triphosphate Bioluminescent Assay kit FL-AA, Sigma Chemical Co.) were reconstituted with sterile double distilled water and set over night to reduce background readings. All glassware were cleaned with 1 N HCl and sterilized before use to reduce background readings.

**ATP Bioluminescence Measurement.** ATP concentrations in the samples were measured with a portable luminescence photometer (Monolight 350, Analytical Luminescence Laboratory) by integrating the light emission during the first 10-sec interval after the addition of the reagent. Each sample was assayed twice. In order to determine the actual concentration of ATP in a sample, the relative recovery of ATP was calculated for each sample combination. This value was determined using the following relationship:

$$ R = \frac{F_{ad} - F_i}{F_{ref}} \cdot 100\% $$

(3.4)

where $R$ is percent recovery, $F_{ad}$ is luminescence of sample with known amount of ATP added, $F_i$ is luminescence of sample without ATP added, $F_{ref}$ is luminescence from reference sample at same concentration as that added to $F_{ad}$. 
3.3 Results

The experimental results from 60°C experiment are presented in Figure 3.2. Both dry and wet bulb temperatures (Figure 3.2a) inside the reactor were well controlled during the experiment. The wet bulb temperature which was slightly lower than dry bulb temperature indicated the air inside the reactor was unsaturated. The temperature inside the compost material (Figure 3.2b) was maintained constant slightly lower than 60°C. The moisture content (Figure 3.2c) was fluctuated around 55%. CO$_2$ and O$_2$ concentrations (Figure 2d) were monitored and used to calculated CO$_2$ evolution and O$_2$ consumption rates (Figure 3.2f). pH in condensed water (Figure 3.2h) increased from around 5 to 8.5 during the experiment. No water was condensed between 70 to 130 hr. The experimental results for 40 and 50°C experiments has been published (Tseng et al., 1995).

The ATP concentration profiles for the experiments under 40, 50, 60 and 70°C are presented in Figure 3.3. The ATP concentration profile of 50°C experiment was only showed for the first 250 hr in Figure 3.3b. The CO$_2$ evolution and O$_2$ consumption rates for those four experiments are shown in Figure 3.4. Again, rates of 50°C experiment was only shown for the first 250 hr.

In order to elucidate the relation between ATP concentration and RQ value with respecting to time course, 3-D plot of RQ, time course and ATP concentrations for those four experiments are presented in Figure 3.5. CO$_2$ evolution and O$_2$ consumption rates of 60°C experiment are also plotted verse time course and ATP concentrations in Figure 3.6a and 3.6b respectively.
3.4 Discussion

For compost materials, the highest ATP concentration was found in fresh sewage sludge while municipal solid waste showed the lowest ATP levels (Garcia et al., 1993). The time course of ATP concentration characteristically showed an interim decrease in the early stage of composting process for sewage sludge and city refuse which may reflect the depletion of readily biodegradable organic substrates (Garcia et al., 1992). The ATP concentrations in compost materials reported in previous and current studies are summarized in Table 3.1. The average ATP concentrations in compost materials are around 0.1-11 μg/g compost. ATP concentrations appear particularly high for leaf litter and may be a result of a high background level of endogenous ATP in leaf litter. The recovery efficiency associated with the extraction methods is usually in the range of 40-90%.

The extraction of ATP from compost matrix is a critical step in the procedure (Powlson, 1994). The main difficulties in the standardization of the ATP methodology stem from incomplete extraction and enzymatic and chemical hydrolysis of ATP. In addition, ATP is readily sorbed on soil constituents (Martens, 1995). During ATP extraction, samples and extracts should not be contaminated by external materials, and this problem can be minimized by using aseptic techniques. In general, strong acids, such as sulfuric acid or trichloroacetic acid, can be used to inactivate ATPase activities and to prevent the sorption of ATP on particulate materials. In this study, H₂SO₄ reagent was used to extract the ATP, as indicated in Figure 3.1. In the present work it was
found that the increase in the vortexing time enhanced the release of ATP from the compost matrix. The recovery efficiency of 40-80% achieved in this study was deemed acceptable in view of the low recoveries reported in the literature for soil-ATP studies. Other, more complex reagent formulations have been proposed (Webster et al., 1984; Vaden et al., 1987; Pangburn et al., 1994) but the improved relative recoveries may not be universal for all environmental materials.

In this study, the composting experiments were carried out under 40, 50, 60 and 70°C. Typical experimental profiles of the temperature, moisture content, CO₂ evolution, O₂ consumption, and ATP concentration in the 60°C experiment are shown in Figure 3.2. The temperature in both the reactor and the compost materials (Figure 3.2a and 3.2b) was controlled around the set-point. The moisture content varied around 50% and decreased to around 45% after 150 h of operation (Figure 3.2c). The CO₂ evolution and O₂ consumption rates (Figure 3.2f) were calculated from the corresponding CO₂ and O₂ concentrations (Figure 3.2d), based on the mass balance surrounding the composting reactor.

As shown in Figure 3.2g, the ATP concentration in 60°C experiment is gradually increased from 0.1 µg/g to over 5 µg/g compost dry weight and then remained relatively constant at approximately 3 µg/g until the end of the experiment. Using the soil conversion factor, this range would be equivalent to about 12-600 µg biomass-C/g compost. For 40°C experiment, the ATP concentration (Figure 3.5a) increased from around 0.01 µg/g to 0.5 µg/g in the first 20 hr and then gradually increased to around 8µg/g at the end of the run. The ATP concentration of 50°C experiment had a long lag phase and began to increase
from 130 hr. The ATP concentrations around 220 to 250 hr for 40, 50 and 60°C experiment were very similar. However, the ATP concentrations of 70°C experiment were fairly constant around 1 µg/g during the experiment.

The time courses of changes in the ATP concentration and the corresponding RQ values are shown in Figure 3.5. The temperature inside the reactor had little direct impact on the ATP concentration. The ATP levels for the 40, 50 and 60°C experiments were in the range of 0.1-10 µg ATP/g compost. As shown in Figures 3.2f and 3.2g, the microbial activity decreased toward the end of the experiment although the level ATP was constant. The ATP concentration of 40°C experiment is somewhat elevated because of the presence of a filamentous fungus which was the predominant form of biomass under these experimental conditions. The time courses of changes in the ATP concentration and the corresponding CO$_2$ evolution and O$_2$ consumption rates for 60°C experiment are shown in Figure 3.6. They show the similar trends as such in RQ plots.

CO$_2$ evolution and O$_2$ consumption activities as well as ATP levels were adversely affected by incubation at 70°C. The RQ values were lower at the beginning and at the end of each experiments, in keeping with initial lag periods and eventual, gradual depletion of the substrate. The RQ value was high during 100-150 h because the degradation at this temperature favored catabolism. Nakasaki et al. (1985) suggested that elevated temperatures enhance catabolism over anabolic reactions, thereby increasing the respective RQ values. With raw sewage sludge compost maintained at 50, 60 and 70°C, the RQ values increased from 0.6 to 0.8 as the temperature increased (Nakasaki et al., 1985). Nakasaki et al. (1992) also suggested that high RQ values may indicate the presence of
anaerobic zones inside compost materials, because anaerobiosis would support catabolism through fermentative decomposition and anaerobic respiration. The RQ ratios in the present study suggested catabolic enhancement as the temperature was maintained at 70°C, but these results also reflect the relative change in the microbial community. Strom (1985) demonstrated that the temperature of the composting process had a major impact on microbial diversity in the laboratory-scale and in field samples. In the moderately thermophilic range of temperatures, bacilli, actinomycetes and fungi become dominant while non-spore forming microorganisms are virtually absent (Miller, 1993). At temperatures approaching 70°C, fungi are virtually absent and actinomycetes are rare in compost materials (Miller, 1991, 1993). This trend is in agreement with the finding in the present study that the experiment at 70°C supported the lowest levels of biomass measured as ATP.

In conclusion, ATP measurement was an effective method for biomass estimation in composting process. The sample extraction was a critical step for the ATP measurement. With an internal standard addition, the recovery efficiency can be obtained and used for the calculation of ATP concentrations. It was found that temperature has little effect on ATP concentration in the temperature range from 40 to 60°C. However, an increase to 70°C became inhibitory to biomass formation.

3.5 SUMMARY

Changes in microbial biomass were monitored by measuring ATP levels in a bench-scale composting bioreactor maintained at 40, 50, 60, and 70°C. The ATP
levels were comparable and in the range of 0.1 to 10 mg/g dw except for the 70°C experiment where the level of biomass was reduced although the elevated RQ values suggested enhanced respiration. Internal standard was used during the extraction to obtain the recovery efficiency.

<table>
<thead>
<tr>
<th>Compost Feed</th>
<th>Extraction Reagent</th>
<th>Sample Storage</th>
<th>Recovery Efficiency (%)</th>
<th>ATP Concentration</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activated sludge Leaf litter</td>
<td>Tris buffer</td>
<td>N/A&lt;sup&gt;a&lt;/sup&gt;</td>
<td>100</td>
<td>0.4-1.9 µg/g VSS 180-300 µg/g dry wt.</td>
<td>Patterson et al., 1970 Suberkropp et al., 1983 Lehtokari et al., 1983</td>
</tr>
<tr>
<td>Sewage sludge</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>10% TCA</td>
<td>N/A</td>
<td>42-87</td>
<td>6.2-11.2 µg/g dry wt. 4.7-6.8 µg/g dry wt.</td>
</tr>
<tr>
<td>Sewage sludge</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>N/A</td>
<td>100 (reference) 60 (related to TCA)</td>
<td>0.3-1.2 µg/g dry wt.</td>
<td>Garcia et al., 1992</td>
</tr>
<tr>
<td>City refuse mixture</td>
<td>Phosphoric acid mixture</td>
<td>4°C and -8°C</td>
<td>N/A</td>
<td>0.3-1.2 µg/g dry wt.</td>
<td>Garcia et al., 1992</td>
</tr>
<tr>
<td>Animal wastes</td>
<td>Boiling water</td>
<td>N/A</td>
<td>N/A</td>
<td>2-26 mg/imbibed sorghum seed dry wt.</td>
<td>Marambe et al., 1993</td>
</tr>
<tr>
<td>Municipal wastes Simulated wastes</td>
<td>TCA with Na&lt;sub&gt;2&lt;/sub&gt;HPO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>N/A</td>
<td>N/A</td>
<td>0.5-3 µg/g dry wt. 0.1-1.0 µg/g dry wt.</td>
<td>Garcia et al., 1993 This study</td>
</tr>
<tr>
<td></td>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>-4°C</td>
<td>40-80</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>N/A, information not available.
Table 3.2. Compositions of Simulated Compost Feed Materials.

<table>
<thead>
<tr>
<th>Composition</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled Water</td>
<td>60</td>
</tr>
<tr>
<td>Rabbit chows (ground grass and leaf)</td>
<td>16.3</td>
</tr>
<tr>
<td>Corn cobs (chopped)</td>
<td>14</td>
</tr>
<tr>
<td>Sand</td>
<td>8</td>
</tr>
<tr>
<td>Seed inoculum (matured compost from commercial plants)</td>
<td>1</td>
</tr>
<tr>
<td>Manure</td>
<td>0.4</td>
</tr>
<tr>
<td>Newspaper</td>
<td>0.3</td>
</tr>
</tbody>
</table>
Figure 3.1. Compost extraction and ATP measurement procedure.
Figure 3.2. Experiment under Temperature of 60°C. (a) The average
temperatures in the vessel; (b) the average compost temperature
inside the vessel; (c) moisture content of the compost samples; (d)
CO₂ and O₂ concentrations; (e) air flow rate; (f) CO₂ evolution (x)
and O₂ consumption (■) rates; (g) ATP concentration; (h) pH of
condensed water. The bars represent the standard deviation.
Figure 3.2.
Figure 3.3. ATP concentration profiles of experiments under (a) 40°C, (b) 50°C, (c) 60°C, (d) 70°C.
Figure 3.4. CO$_2$ evolution and O$_2$ consumption profiles of experiments under (a) 40°C, (b) 50°C, (c) 60°C, (d) 70°C.
Figure 3.5. 3-D plot of ATP concentration versus RQ and time course. (a) 40°C; (b) 50°C; (c) 60°C; (d) 70°C.
Figure 3.6. 3-D plot of ATP concentration versus CO₂ evolution rate (a) and O₂ consumption rate (b) during composting at 60°C.
CHAPTER IV

QUANTITATIVE FOURIER-TRANSFORM INFRARED SPECTROSCOPIC ANALYSIS OF ORGANIC MATTER DEGRADATION IN A BENCH-SCALE SOLID SUBSTRATE FERMENTATION (COMPOSTING) SYSTEM

4.1 INTRODUCTION

Composting, especially lab-scale study with simulated feed media, is one kind of a solid substrate fermentation (SSF), which involves aerobic or anaerobic microbial activity and "non-liquid" substrate (Hesseltine, 1972; Rathbun and Shuler, 1983). In recent years, composting has been recognized as a more effective method for solid waste treatment than landfill (He et al., 1993). However, finding a good method to predict compost stability or maturity, the degree to which composts have been decomposed, is critical for successful compost production, marketing and utilization. It has been shown that energy bioavailability, determined by carbohydrate content, in the organic matter controls the microbial community and its activity once compost is introduced into the soil (Boehm et al., 1993). Methods, such as respirometry (Iannotti et al., 1993, 1994); heat output (Jourdan 1988); NMR and FT-IR spectroscopy (Chen and
Inbar 1993) have been used in previous studies to provide useful information of compost stability or maturity.

Composting. Composting has been used to treat all kinds of solid wastes, such as agriculture waste, sewage sludge, food waste, manure sludge waste, etc. and has been reviewed extensively by previous studies (Golueke, 1972; Finstein and Morris, 1975; Haug, 1980; Zucconi and de Bertoldi, 1987; Chen and Inbar, 1993). It is a complex microbial process that involves self-heating, multi-stage temperature conditions, a diversity of microorganisms and leads to production of CO$_2$, water, minerals and stabilized organic matter (Finstein and Morris, 1975; Haug, 1980; Strom, 1985a, 1985b; Bach et al., 1987). This microbial diversity is largely a function of the process temperature. Some microorganisms identified are mesophilic bacteria, fungi and actinomyces in mesophilic range. Thermophilic fungus, actinomyces and bacteria, such as Bacillus spp., are often found in thermophilic range (Strom 1985a, 1985b).

Humic Substance. During the composting process, microorganisms convert organic matter into biomass, CO$_2$, thermo-energy minerals and stabilized organic matter called humic substance (HS) (Inbar, 1989). HS includes humic acid, fulvic acid and humin and has been carefully defined by MacCarthy et al. (1990). The organic matter in nature can be grouped into six major types: lignin, polysaccharide, proteins, lipids, resins and pigments (Wilson, 1987). Soil organic matter contains high-molecular-weight organic material (e.g. polysaccharides and proteins), simple substances (e.g. sugars, amino acid, and other small molecules), and humic substances (MacCarthy et al., 1990). Among them, polysaccharides and lignin are the two most important organic precursors
to HS formation (Inbar, 1989). In soil, organic matter contains approximately 5-20% carbohydrates which are mostly in the form of polysaccharides. Polysaccharides can be found in all living organisms and make up approximately 75%, on a dry weight basis of plants (Lowe, 1978; Cheshire, 1979). Cellulose and starch are two structurally different polysaccharides consisting entirely of glucose. Lignin is another source of HS formation (Stevenson, 1982) with a basic structure of phenyl propane (Wilson, 1990). Lignin contains blocks of conifery alcohol, p-hydroxycinnamyl alcohol and sinapyl alcohol. The basic structure of lignin contains phenyl propane and phenolic, hydroxyl, and methoxyl groups (Wilson, 1990).

HS in soil or compost affects soil ecology, fertility, structure, plant growth and pathogen suppression (Vanghan and Malcolm, 1985; Chen and Aviad, 1990; Hoitink et al., 1993). In general, different organic matter precursors for HS formation have different biodegradation rates. A further complexity in the formation of HS is the diversity of microorganism present during the composting process. As stated above, this diversity is a function of the operational temperature as well as other operational parameters. For example, polysaccharides and proteins are decomposed more slowly than simple sugars and acids (Stott and Martin, 1990).

**FT-IR Analysis of Compost.** Organic matter present in compost usually is chemically complex and difficult to fractionate. Extraction procedures may only remove a fraction of the organic matter in the solid waste (Inbar, 1989; Inbar et al., 1989). Consequently, a non-destructive method is desired to investigate the transformation of carbon content during the composting process. Both $^{13}$C-
Nuclear Magnetic Resonance (NMR) and Fourier-transform Infrared Spectroscopy (FT-IR) are reliable methods for characterization of soil HS (Gerasimowicz and Byler, 1985; Lehtovaara, 1988; Lobartini and Tan, 1988; Inbar, 1989; Inbar et al., 1989; Inbar et al., 1990). Compared to the ability of creating a C "fingerprint" of solid samples by 13C-NMR technique, FT-IR usually serves as a qualitative tool for characterization of the chemical groups of organic matter (Inbar et al., 1989). However, FT-IR analysis is more practical and economical than NMR.

In FT-IR spectra, specific peak regions correspond to certain carbon bonds which can then be related to specific compounds. By measuring these specific peak regions of interest, it is possible to determine over a period of time (corresponding to samples taken at discreet time increment) the change in the organic matter which correspond to these peak regions. However, because the peak intensity of each FT-IR spectra only provides relative concentration of certain carbon content in each individual sample, a quantitative comparison cannot be achieved by just comparing the peak intensity from different FT-IR spectra. By Bouguer-Beer law (Equation 1), the absorbance of a sample, A(v), is affected by extinction coefficient α(v), optical pathlength (l), and substance concentration ([C]). Other deviations from Bouguer-Beer law could be caused by spectrometer and cell imperfections or systems effects.

Despite this limitation, quantitative FT-IR analysis has been practiced by employing standardization methods which is one of the methods commonly used for solids analysis. Examples of internal standards are calcium carbonate, and sodium azide. (Smith, 1979).
For compost analysis, previously only semi-quantitative analysis has been conducted by taking the ratio between specific peaks. For example, the ratios among peak regions, 1050 cm\(^{-1}\) (polysaccharide C-O), 1385 cm\(^{-1}\) (COO\(^{-}\), CH\(_3\)), 1425 cm\(^{-1}\) (COO\(^{-}\), CH\(_2\)) 1655 cm\(^{-1}\) (aromatic C=C, COO\(^{-}\)), and 2930 cm\(^{-1}\) (aliphatic C-H), Inbar et al. (1989) found that ratios of 1385/2930, 1385/1050, 1655/2930, and 1425/1050 increased with composting time. They concluded that the polysaccharide decreased while the aromatic C=C, alkyl C and carboxylate ions increased. However, no quantitative conclusions could be made using this approach.

The objective of this work was to develop a quantitative FT-IR technique to measure the degradation of particular organic matter during the composting process. FT-IR spectra was processed with second derivative, curve fitting, and peak area integration. The peak area of individual peak region was normalized against that of internal standard. Degradation of specific organic matter (e.g. polysaccharides and lignin) was characterized by quantitative comparison of the changing of the corresponding peak area. NMR and wet chemistry measurement for initial and final samples were used for calibration.

In order to obtain high resolution FT-IR spectra, Diffuse Reflectance Fourier-Transform Infrared Spectroscopy (DRIFT) was used. Advantages of using DRIFT for compost analysis have been discussed by previous studies (Fuller and Griffiths 1978; Griffiths 1975; Baes and Bloom, 1989; Niemeyer et al., 1992).
4.2 THEORY

FT-IR. A FT-IR measurement includes generation of interferogram and Fourier Transform (Griffiths, 1975; Smith 1979; Griffiths, 1983; Jones 1985; Colthup et al., 1990; Griffiths, 1992). A spectrum, $E(\nu)$, in the wavenumber domain, and its interferogram, $I(x)$, are a pair of Fourier Transforms. The n-th order derivatives of an original spectrum is in Equation 4.2. Derivative spectroscopy has been proved as an useful technique for identifying overlapped peaks (Gillette et al., 1985). Gerasimowicz et al. (1986) shown that the detail of information from second derivative of an original spectrum was also increased.

$$\frac{d^n E(\nu)}{d\nu^n} = \int \frac{1}{i2\pi n} I(x) \exp(i2\pi \nu x) dx \quad (4.2)$$

Curve fitting has often been used to resolve the overlapped spectra (Painter et al., 1985). This technique has been carefully reviewed by Maddams (1980). However, the accuracy of curve fitting depends on the knowledge of the number and positions of bands in the spectral region of interest. Derivative and self-deconvolution are usually used to serve this purpose (Painter et al., 1985).

Spectra Region. The infrared bands and their proposed assignment are summarized from previous studies (Stevenson 1982; Gerasimowicz and Byler, 1985; MacCarthy and Rice, 1985; Painter et al., 1985; Inbar 1989; Inbar et al., 1989, 1990; Colthup et al., 1990) in Table 4.1. There is little difference between the assignment of FT-IR bands and those of DRIFT (Baes and Bloom 1989; Niemeyer et al., 1992).
The typical DRIFT spectra is shown in Figure 4.2: an absorption around 3300-3400 cm\(^{-1}\) region (O-H hydrogen bonding); an absorption bands 3000-2700 cm\(^{-1}\) (aliphatic C-H stretching vibrations); an absorption band at 2930 cm\(^{-1}\) (aliphatic CH\(_2\) symmetric stretch); an absorption region of 1650-1500 cm\(^{-1}\) (aromatic stretch, e.g. C=C); a distinct band at 1655 cm\(^{-1}\) (aromatic C=C, COO\(^{-}\), or hydrogen-bonded C=O stretching); a broad peak around 1100 to 1020 cm\(^{-1}\) (C-O stretch of polysaccharides); and a band from 2650 to 2500 cm\(^{-1}\) (internal standard, CaCO\(_3\)).

4.3 MATERIALS AND METHODS

**Bench-scale System and Feed Material.** The bench-scale reactor was made of Acrylic plastic. It held eight trays of thin layer feed material (Table 1). Two circulating fans were installed inside the reactor to insure uniform aeration. One thermocouple was buried in each tray to measure the temperature of feed material. A computer system was interfaced with this reactor to control the temperature inside the reactor and monitor the CO\(_2\) and O\(_2\) concentrations during the experiment. The inlet air flow rate was manually controlled. A detailed description of this system has been described by Tseng *et al.*, (1995). Seed inoculum (1 % of total weight) was added to feed material before the experiment. The results presented and discussed in this paper were conducted under 50°C for 350 hr.

**Sample Preparation** Every 12 hrs, samples were taken from the bioreactor for moisture content, ATP concentrations and FT-IR analysis. After drying...
(approximately 3 g) at 105 °C in an oven for 12 hr., samples were sieved (495 μm), ground and diluted with KBr. 8 mg samples were mixed with 382 mg KBr and 4 mg internal standard (CaCO₃ or NaN₃). The mixture was then re-ground and stored in a P₂O₅ desiccator overnight prior to the measurement.

**FT-IR.** The FT-IR analysis were conducted in a Mattson (Polaris™) DRIFT spectrometer operating at a nominal resolution of 2 cm⁻¹, the mid-infrared spectrum from 4000 to 400 cm⁻¹; iris of 40%; transmittance type of spectra; signal gain factor of 1; blocks of 5; scans of 100. The spectrometer was equipped with a liquid nitrogen cooled Lg MCT (large mercury cadmium telluride) detector.

**Carbon-13 Nuclear Magnetic Resonance Spectroscopy.** ¹³C-NMR spectra for the initial and final samples were obtained on a ¹³C-NMR (Bruker, MSL 300) at the Chemical Intrumental Center, The Ohio State University, with the following parameters: ¹H frequency: 300.13; ¹³C frequency 75.476; spinning rate RO; contact time 1ms=D5; external standard, glycine; acquisition, D0=4s; sweep width, 29411; line broadening, LB; acquisition time, D0=4s; type of rotor, ??; spun frequency, RO. Peak area under 10-45 ppm, 45-95ppm, 95-120 ppm and 160-180 ppm were integrated.

**Spectra Processing.** The second derivative, curve fitting, and peak area integration were performed using the software GRAM386 (Galactic, Co.). Second derivative was obtained by Savitsky-Golay function with second degree polynomial and 50 convolution points. The identified peak positions and width from second derivation were used for the purpose of curve fitting and peak area
integration. The curve fitting of the spectra was performed with the interactive mode in the software. The initial guess of the peak type is Mix of Guassian and Lorentzian (1:1). The initial guess of peak position and width is shown in Table 4.4. Iterations were conducted until converged.

**Wet Chemistry Measurement.** The extraction procedure for wet chemistry was modified from that of liyama et al. (1994). Initial and final samples (30 g) were oven dried at 105 °C overnight and sieved (495 μm). Sample (3 g) was extracted with 50 ml boiling anhydrous ether for 4 hr., extracted with 50 ml 80% boiling ethanol for 4 hr., and extracted with 50 ml water at 40°C for 24 hr. All extracts from above three extractions were filtered with 202 grade filter paper (VWR Sci.), oven dried, and weighed. In order to verify the FT-IR results, samples were used for polysaccharides and lignin assays without any extraction.

Polysaccharides content was measured by total sugar reduction method with anthrone (Doutre et al., 1978; Chshire, 1979). 100 mg sample was digested with 12 M H$_2$SO$_4$ (4 ml) for 2 hr. with occasionally shaking. Then the solution was diluted with distilled water to 0.5 H$_2$SO$_4$ (192 ml). Anthrone reagent (1% w/v in concentrated H$_2$SO$_4$, 2 ml) was pipetted to the sample solution (1 ml). After setting 50 min at ambient temperature, the absorbance were determined at 625 nm. The glucose standard curve was made prior to the measurement.

For lignin assay (liyama and Wallis, 1988, 1990), 20 mg samples were placed in a glass bottle (20 ml) with a solution of 25 % (w/w) acetyl bromide in acetic acid (5 ml) containing 0.2 ml perchloric acid (70 %). The bottles were capped and placed in an oven at 70 °C for 2 hr. The bottles were shaken at 10-30 min
intervals. After digestion, the solution was mixed with 2 M NaOH (20 ml) and acetic acid (25 ml). The lignin content was measured by absorbence at 280 nm and used the SAC value for lignin, 20.0 g/L cm⁻¹.

Carbon content, nitrogen content, and ash content were measured by Research Extension Analytical Laboratory (REAL, The Ohio State University, Wooster, OH). pH of initial and final compost samples were also measured (Carnes and Lossin, 1970; Michel et al. 1993; Tseng et al., 1995).

4.4 RESULTS

The temperature profiles, both within and surrounding the compost material, are present in Figure 4.1a and 4.1b. Along with temperature profiles, moisture content (Figure 4.1c), CO₂ and O₂ concentrations (Figure 4.1d), air flow rate (Figure 4.1e), ATP concentrations (Figure 4.1g), and pH in condensed water (Figure 4.1h) are presented. CO₂ evolution rate and O₂ consumption rates on a dry mass basis are presented in Figure 4.1f. The experimental data for first 250 hr was previously published (Tseng et al., 1995).

The FT-IR (DRIFT) spectra of compost (Figure 4.2) gave a broad range of peaks in the mid-infrared region (400-4000 cm⁻¹). The peak around 3300-3400 cm⁻¹ belongs to H-bonded OH groups. A couple of peaks at 2930 cm⁻¹ and 2870 cm⁻¹ correspond to aliphatic carbon content. A slight shoulder at 2600 cm⁻¹ and a sharp peak at 2508 cm⁻¹ belong to CaCO₃ internal standard. Other peaks are: a peak at 1877 cm⁻¹, a sharp peak at 1800 cm⁻¹ (CaCO₃), a slight shoulder at 1720 cm⁻¹ (C=O of COOH), and a peak at 1655 cm⁻¹ (aromatic C=C, COO⁻, H-
bonded C=O), a broad peak at 1440 cm\(^{-1}\) (CaCO\(_3\) internal standard.), a slight shoulder at 1200 cm\(^{-1}\) (aromatic C, C-O), a peak at 1150 cm\(^{-1}\) (aliphatic CH\(_2\), OH or C-O stretch of various groups), and a couple of peaks at 1084 cm\(^{-1}\) and 1042 cm\(^{-1}\) (C-O of polysaccharides carbon contents).

The typical FT-IR spectra of compost before and after internal standard addition are shown in Figure 4.2a and 4.2b respectively. The peak around 2508 cm\(^{-1}\) results from the addition of CaCO\(_3\). Three peak area regions (1050 cm\(^{-1}\), 1655 cm\(^{-1}\) and 2930 cm\(^{-1}\)) were integrated and normalized against this internal standard. These integrated peak area before and after normalization for initial and final samples are shown in Table 4.3a and 4.3b. The changes of these peak area in percentages for initial and final samples are also shown in Table 4.3.

Before peak area integration and normalization, the peak regions were determined by the method of second derivative and curve fitting. A example of the processing of aliphatic carbon content region (2921 cm\(^{-1}\)) is shown in Figure 4.3. The aliphatic carbon contents region (2800-3000 cm\(^{-1}\)) is amplified from Figure 4.2a and shown in Figure 4.3a. The second derivative and curve fitting of the aliphatic spectra region are shown in Figure 4.3a and 4.3b. Same kind of analysis was also conducted for peak region corresponding to polysaccharides and aromatic carbon contents. The positions, widths and possible assignments for those identified peaks under 2921 cm\(^{-1}\), 1655 cm\(^{-1}\), 1050 cm\(^{-1}\) and 2508 cm\(^{-1}\) regions are listed in Table 4.4. The spectra regions chosen for peak integration are listed in Table 4.5. Those three peak area after normalization are plotted verse time course in Figure 4.4. Relative changes in percentages of those three peak regions are shown in Figure 4.5.
Carbon and nitrogen content, C/N ratio, pH and ash contents for the initial and final samples before sieving are shown in Table 4.6. NMR spectra of compost sample for initial and final samples are shown in Figure 4.7. Wet chemistry measurements for initial and final samples are presented in Table 4.7. Polysaccharides concentrations, based on wet chemistry data, at six different time intervals versus their corresponding FT-IR spectra area are shown in Figure 4.6.

4.5 DISCUSSION

During the composting process, organic matter is degraded by microorganisms under suitable conditions. As shown in the CO\textsubscript{2} evolution and O\textsubscript{2} consumption rates profiles (Figure 4.1f), the microbial activity was low for the first 100 hr and began to increase after 130 hr. The slight peak around 20 hr, also found in other temperature experiments (Tseng et al., 1995), may be caused by the early degradation of free sugar or easily degradable carbon sources. The following long lag phase may indicate that fewer organisms in the seed was initially active at 50°C temperature. The high microbial activity region (from 130 to 210 hr) was associated with biomass accumulation, as shown by an increased ATP concentration, and organic matter degradation, as shown by the changes in polysaccharides carbon content. The CO\textsubscript{2} evolution and O\textsubscript{2} consumption rates reached the maximum of 0.04 mmol/g hr and 0.08 mmol/g respectively around 170 hr. As shown in Figure 4.1g, the ATP concentration, which is an indicator of biomass (1 ug/g = 100-120 ug/g biomass), increased from 0.1 to 4 ug/g dry
weight. pH in condensed water which indirectly reflects the pH in compost material increased from around 5 to 8.5 during the run (Figure 4.1h).

Internal standardization method is required for quantitative FT-IR analysis of solid samples (Smith, 1979) because peak intensities of FT-IR analysis are affected by many external factors and comparison of results without normalization is not accurate. The internal standard used in this study is CaCO$_3$ because of its easy accessible and handling nature. Selection of internal standard for FT-IR analysis affects how accurate the results are. As shown in Figure 4.1b, the FT-IR spectra of compost sample with CaCO$_3$ addition does not show a peak around 2508 cm$^{-1}$. However, a peak at 2508 cm$^{-1}$ along with some other peaks appear in the FT-IR spectra after CaCO$_3$ addition (Figure 4.1a). Since the peak around 2508 cm$^{-1}$ of CaCO$_3$ does not interfere with any other peaks of compost after CaCO$_3$ addition, this peak was chosen as the internal standard peak. However, peak overlapping occurred in the aliphatic peak region (2930 cm$^{-1}$) after CaCO$_3$ addition. The peak at 2984 cm$^{-1}$ in the second derivative spectra could belong to CaCO$_3$. This overlapping may affect the analysis of aliphatic carbon content. Using other kind of internal standard without any peak interferers with peaks of compost, such as NaN$_3$, could give better results. But because of the easy accessible nature of CaCO$_3$, it is still preferred as the internal standard for FT-IR analysis in this study.

As shown in Table 4.4, the difference of the peak area before and after normalization for initial and final samples is pronounced. The changes of peak area which corresponding to polysaccharides carbon contents is -38.5% before peak normalization (Table 4.4a). However, the percentage changes of the peak
area is -50.9% after peak area normalization (Table 4.4b). This change of polysaccharides carbon contents for initial and final samples was close to the results of wet chemistry analysis (Table 4.7).

In general, FT-IR spectra of compost are highly overlapped because of the complex nature of compost material. Both visual inspection and derivative functions can be used for peak finding (Maddams, 1980). Using second derivative technique is a promising method for peak identification and is generally used as an enhancement method to identify the individual peaks under overlapping spectra region (O'Haver 1979; Maddams 1980; Whitbeck, 1981). The second derivative of $A(\lambda)$ (Equation 1) is given in Equation 3.

$$\frac{d^2A}{dv^2} = l[C] \frac{d^2\alpha}{dv^2}$$  \hspace{1cm} (4.3)

Where $d^2A/dv^2$ is the rate of change of the slope of the absorption line. The narrower the line the greater $d^2A/dv^2$. On the other hand, the broader the line the smaller $d^2A/dv^2$. This feature can be used to enhance the sharp spectral feature and decrease the effect of gradual curvature of the background (Whitbeck, 1980). The condition of presence of a valley in a spectra is clearly as $d^2A/dv^2 = 0$. As shown in Figure 4.3b, the second derivative of aliphatic carbon region showed six peaks under this region. The proposed assignments for these peaks are CaCO$_3$ (2984 cm$^{-1}$); -CH$_3$ (2958 cm$^{-1}$); -CH$_2$- (2921 cm$^{-1}$); long CH group (2894 cm$^{-1}$); -CH$_3$ sym. stretch (2873 cm$^{-1}$) and -CH$_2$ sym stretch (2855 cm$^{-1}$) respectively.
Peak width is another parameter required for peak integration or curve fitting. This has always been a problem for curve fitting studies (Maddams, 1980). Change in half-width of peaks in curve fitting will affect the peak area and the implications of quantitative work. Although people have assumed the same peak width for peaks under spectra region, it has been proved to be a wrong assumption (Maddams, 1980). Vandeginste and de Galan (1975) showed that second derivative offered the possibilities for half-width determination. The half-band width can be estimated from the distance between the two inflection points. This method was used to find the peak width in this study. The peak width for peaks under those three spectra regions are listed in Table 4.4 and used for peak area integration. The optimization of finding the peak width is difficult to access because all parameters, such as peak height, numbers, positions, baseline and half-band width, are interacted to certain degree (Maddams, 1980).

Selection of baseline is very important for peak area integration or curve fitting. As shown in Figure 4.2, extraneous background is present in the low wavenumber region. This kind of background should be taken into account for peak integration by choosing appropriate baseline. Despite the complex mathematical representation of the background, empirical baseline procedures are still commonly used. The simplest approach is to assume a linear baseline (Maddams, 1980). However, the most difficult part is to determine the end points. Smith (1979) suggested the criterion is to "draw the baseline as nearly as possible where the pen tracing would go if the band were not present." In this study, we found the end points selected by this criterion was close to the end points (e.g. points a and b in Figure 4.3b) determined by the second derivative. Therefore, second derivative was used to determine the end points of the
baseline. Even with diluting the sample concentration to 1% and 0.5% respectively, FT-IR spectra of the compost showed the same kind background (data not shown). This further proved the validity of the baseline selection in this study.

Polysaccharides contain many C-C and C-O bonds which show up in the 1100 to 1050 cm$^{-1}$ region (C-O) in the FT-IR spectra (Figure 4.2). Polysaccharides is usually degraded by hydrolysis (Aspinall 1982). During the hydrolysis of polysaccharides, the cleavage of C-O linkages formed oligosaccharide. As shown in Figure 4.4a, the polysaccharide carbon contents decreased from 130 to 210 hr. The decline of microbial activity from 170 hr may be caused by the partial depletion of easy degradable polysaccharides carbon sources. This is confirmed by the FT-IR results which showed a decrease from 130 hr and remained constant from 220 hr. However, the microbial activity still remained at low level which may indicated the degradation of other carbon sources. The reason and timing for the microorganisms switching nutrient source are still unknown.

The FT-IR results were compared to wet chemistry measurement. Although the accuracy of wet chemistry analysis is debatable (Mclellan et al., 1991), it is still an effective way to calibrate FT-IR results (Inbar 1989; Inbar et al., 1989, 1990). As shown in Table 4.7, the total reduced sugar (in terms of glucose) decreased from 21% in initial sample to 8.4% in final sample. This result close to the result of FT-IR measurement. As shown in Figure 4.6, samples at six different time intervals (~60 hr apart) were chosen to compare the change of polysaccharides contents from wet chemistry measurement with that of FT-IR analysis. Despite
the fluctuations which may be results from the wet chemistry measurements, it shows a linear relationship between these two measurements.

The spectra area (1655 cm\(^{-1}\), C=C of aromatic carbon content) corresponding to aromatic carbon content decreased from 23.1%. However, the wet chemistry measurement of lignin showed a constant concentrations from initial sample (12.4%) to final sample (12.7%). The relative constant of lignin content from wet chemistry result may indicate the non-easy degradable nature of lignin. The opposite results from FT-IR analysis may indicate that 1655 cm\(^{-1}\) spectra area not only corresponds to lignin content but also represents mainly aromatic carbon content which could be degraded during the process. If we calculated the relative changes of those three carbon contents as shown in Figure 4.5, the increasing of polysaccharides carbon contents (Figure 4.5a) was accompanied with the decreasing of aromatic carbon content (Figure 4.5b) or visa versa. The aliphatic carbon contents were relatively constant during the experiment.

Wet chemistry extractions also provided the information of the degradation of different carbon contents during the composting process. Liyama et al. (1994) stated that "the ether extract contains low molecular-weight hydrophobic organic compounds such as fatty acids and other lipids. The ethanol-water and water extracts consists of lower-molecular-weight carbohydrates, soluble salts and some UV absorbing materials (e.g. proteins, low-molecular-weight aromatic compounds, and hydrophilic polyphenols)". In this study, all of those extracts decreased from initial to final samples as shown in Table 4.7. In NMR spectra for initial and final samples (Figure 4.7), there are two pronounced peaks, one at 72 ppm (carbohydrate or aliphatic alcohols) and another at 107 ppm
(polysaccharides). The total spectra area of these two peaks decreased 20% from initial to final samples.

Self-deconvolution has been approved as a good method to treat overlapping peaks. Kauppinen et al. (1981a, b) has shown that the area of the peak after deconvolution is equal to the original peak area. However, this technique was not successfully conducted for the spectra analysis in this study. The problems involved that shifting of peak positions as well as selecting the correct baseline and width for the peak area integration in curve fitting. But more work could be done in the future to explore this technique for compost analysis.

In conclusion, quantitative FT-IR analysis of organic matter degradation in composting process can be achieved by internal standardization method with CaCO$_3$. The change of normalized spectra area corresponding to specific compound (e.g. polysaccharides) decreased during the composting process and the results were verified by wet chemistry measurements. Further work should be done to pick the more precise peak area which corresponds to compounds of interest (e.g. lignin). The improvements could be brought by using a different internal standard or using curve fitting analysis to deconvolute peak of interest from the overlapping spectra region. The ultimate goal of this research in our lab is to provide an on site method to measure the compost stability for composting plants.
4.5 Summary

Degradation of organic matter in composting was evaluated by a quantitative FT-IR analysis using an internal standardization method with CaCO$_3$. Composting was conducted in a bench-scale reactor at controlled operational conditions under 50°C temperature, 50-60 % moisture content, and uniform aeration for 325 hr. The normalized FT-IR spectra area (1050 cm$^{-1}$) corresponding to polysaccharides carbon content decreased at 130 hr until 210 hr. The normalized FT-IR spectra area corresponding to aromatic carbon content (1651 cm$^{-1}$) and aliphatic carbon content (2921 cm$^{-1}$) were fairly constant for the first 210 hr and decreased only as the polysaccharides carbon content became constant. Wet chemistry measurement of polysaccharides and other carbon contents was conducted to verify the FT-IR results.
Table 4.1. Compositions of simulated compost feed materials.

<table>
<thead>
<tr>
<th>Composition</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled Water</td>
<td>60</td>
</tr>
<tr>
<td>Rabbit chows (ground grass and leaf)</td>
<td>16.3</td>
</tr>
<tr>
<td>Corn cobs (chopped)</td>
<td>14</td>
</tr>
<tr>
<td>Sand</td>
<td>8</td>
</tr>
<tr>
<td>Seed inoculum (matured compost from commercial plants)</td>
<td>1</td>
</tr>
<tr>
<td>Manure</td>
<td>0.4</td>
</tr>
<tr>
<td>Newspaper</td>
<td>0.3</td>
</tr>
</tbody>
</table>
Table 4.2 Some FT-IR bands and their proposed assignments.

<table>
<thead>
<tr>
<th>Band (cm⁻¹)</th>
<th>Proposed Assignment</th>
<th>Band (cm⁻¹)</th>
<th>Proposed Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>3400-3300</td>
<td>H-bonded O-H strech</td>
<td>1620-1600</td>
<td>aromatic C=C</td>
</tr>
<tr>
<td>3090-3070</td>
<td>aromatic C-H strech</td>
<td>1510</td>
<td>C=C of aromatic ring</td>
</tr>
<tr>
<td>2958-2960</td>
<td>-CH₃ asym strech</td>
<td>1460-1450</td>
<td>aliphatic C-H</td>
</tr>
<tr>
<td>2926-2930</td>
<td>aliphatic -CH₂ sym strech</td>
<td>1420</td>
<td>aromatic ring</td>
</tr>
<tr>
<td>2873</td>
<td>-CH₃ sym strech</td>
<td>1420-1300</td>
<td>strech COO⁻</td>
</tr>
<tr>
<td>2840-2855</td>
<td>aliphatic -CH₂ sym strech</td>
<td>1280-1200</td>
<td>aliphatic C-H strech</td>
</tr>
<tr>
<td>2850</td>
<td>-CH sym strech</td>
<td>1125</td>
<td>-C-O- strech, OH</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>deformation of COOH</td>
</tr>
<tr>
<td>1730-1715</td>
<td>C=O strech of CO₂H</td>
<td>1100-1020</td>
<td>C-O strech of polysaccharides</td>
</tr>
<tr>
<td>1650-1655</td>
<td>aromatic C=C, COO⁻, H-bonded C=O</td>
<td>1000-700</td>
<td>aromatic C-H out of plane bend</td>
</tr>
</tbody>
</table>
Table 4.3 Peak area before and after normalization.

a. Before normalization

<table>
<thead>
<tr>
<th>Spectra (cm$^{-1}$)</th>
<th>Initial sample</th>
<th>Final sample</th>
<th>Change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2508 (internal standard)</td>
<td>1.13</td>
<td>1.41</td>
<td>-38.5</td>
</tr>
<tr>
<td>1050</td>
<td>3.17</td>
<td>1.95</td>
<td>-50.9</td>
</tr>
<tr>
<td>1655</td>
<td>1.95</td>
<td>1.87</td>
<td>-4.1</td>
</tr>
<tr>
<td>2930</td>
<td>1.86</td>
<td>1.34</td>
<td>-28</td>
</tr>
</tbody>
</table>

b. After normalization.

<table>
<thead>
<tr>
<th>Spectra (cm$^{-1}$)</th>
<th>Initial sample</th>
<th>Final sample</th>
<th>Change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2508 (internal standard)</td>
<td>1.13</td>
<td>1.41</td>
<td>-50.9</td>
</tr>
<tr>
<td>1050</td>
<td>2.81</td>
<td>1.38</td>
<td>-23.1</td>
</tr>
<tr>
<td>1655</td>
<td>1.73</td>
<td>1.33</td>
<td>-42.4</td>
</tr>
<tr>
<td>2930</td>
<td>1.65</td>
<td>0.95</td>
<td>-28</td>
</tr>
</tbody>
</table>
Table 4.4. Peak positions, widths and their proposed assignments from the second derivative and curve fitting.

<table>
<thead>
<tr>
<th>Band and Peak Position (cm⁻¹)</th>
<th>Peak Width (cm⁻¹)</th>
<th>Proposed assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aliphatic Carbon Contents</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2984</td>
<td>24</td>
<td>CaCO₃</td>
</tr>
<tr>
<td>2958</td>
<td>24</td>
<td>-CH₃ asym strech</td>
</tr>
<tr>
<td>2921</td>
<td>41</td>
<td>-CH₂- asym strech</td>
</tr>
<tr>
<td>2898</td>
<td>19</td>
<td>long CH group</td>
</tr>
<tr>
<td>2874</td>
<td>23</td>
<td>-CH₃ sym strech</td>
</tr>
<tr>
<td>2852</td>
<td>28</td>
<td>-CH₂- sym strech</td>
</tr>
<tr>
<td><strong>Internal Standard</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2600</td>
<td>95</td>
<td>CaCO₃</td>
</tr>
<tr>
<td>2508</td>
<td>48</td>
<td>CaCO₃</td>
</tr>
<tr>
<td><strong>Aromatic Carbon Contents</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1651</td>
<td>69</td>
<td>-C=C-</td>
</tr>
<tr>
<td>1625</td>
<td>23</td>
<td>-C=C- strech</td>
</tr>
<tr>
<td>1603</td>
<td>44</td>
<td>C=C aromatic ring strech</td>
</tr>
<tr>
<td><strong>Polysaccharides Carbon</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1200</td>
<td>46</td>
<td>-C=C- strech of O-H</td>
</tr>
<tr>
<td></td>
<td></td>
<td>deformation of CO₂H</td>
</tr>
<tr>
<td><strong>Contents</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1160</td>
<td>38</td>
<td>primary alcohols ?</td>
</tr>
<tr>
<td>1106</td>
<td>67</td>
<td>C-O of polysaccharides</td>
</tr>
<tr>
<td>1056</td>
<td>24</td>
<td>C-O of polysaccharides</td>
</tr>
<tr>
<td>1036</td>
<td>35</td>
<td>C-O of polysaccharides</td>
</tr>
<tr>
<td>994</td>
<td>38</td>
<td>hydrocarbons</td>
</tr>
</tbody>
</table>
Table 4.5. Spectra range and peak positions for peak area integration.

<table>
<thead>
<tr>
<th>Corresponding Carbon content</th>
<th>Spectra Range (cm⁻¹)</th>
<th>Peak Position (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Internal Standard</td>
<td>2538-2490</td>
<td>2508</td>
</tr>
<tr>
<td>CaCO₃</td>
<td>1070-974</td>
<td>1050</td>
</tr>
<tr>
<td>Polysaccharides</td>
<td>1705-1614</td>
<td>1651</td>
</tr>
<tr>
<td>Aromatic Carbon Content</td>
<td>2995-2887</td>
<td>2921</td>
</tr>
</tbody>
</table>

Table 4.6. Chemical analysis of initial and final samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Carbon (%)</th>
<th>Nitrogen (%)</th>
<th>C/N ratio</th>
<th>pH</th>
<th>Ash (%)</th>
<th>Dry weight (g)</th>
<th>Loss mass (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>32.7</td>
<td>1.45</td>
<td>22.5:1</td>
<td>6.36</td>
<td>31.48</td>
<td>1795</td>
<td></td>
</tr>
<tr>
<td>Final</td>
<td>32.9</td>
<td>1.42</td>
<td>23.2:1</td>
<td>8.27</td>
<td>40.37</td>
<td>1197</td>
<td>33.3</td>
</tr>
</tbody>
</table>

Table 4.7. Results of wet chemistry measurements.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ether Extract (%)</th>
<th>Ethanol Extract (%)</th>
<th>Water Extract (%)</th>
<th>Polysaccharides (%)</th>
<th>Lignin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>7</td>
<td>4.2</td>
<td>5.5</td>
<td>21</td>
<td>12.4</td>
</tr>
<tr>
<td>Final</td>
<td>2.2</td>
<td>2.9</td>
<td>4.4</td>
<td>8.4</td>
<td>12.7</td>
</tr>
</tbody>
</table>
Figure 4.1. Experiment was under 50°C for 350 hr. (a) The average, dry (■) and wet (●) bulb temperature profiles in the vessel; (b) the average compost temperature profile inside the vessel; (c) moisture content of the compost samples; (d) CO₂ (thick line) and O₂ (thin line) concentration profiles; (e) air flow rate profile; (f) CO₂ (x) evolution and O₂ (■) consumption rate profiles; (g) ATP concentration; (h) pH of condensed water. The bars represent the standard deviation.
Figure 4.2. Typical DRIFT spectra of compost. (a). Spectra without internal standard, (b). spectra with internal standard. The peak regions and their corresponding bonds are: 2930 cm$^{-1}$ (aliphatic -CH$_2$ stretch); 2850 cm$^{-1}$ (-CH stretch); 1655 cm$^{-1}$ (aromatic C=C, COO', H-bonded C=O); 1050 cm$^{-1}$ (C-O stretch of polysaccharides).
Figure 4.2.
Figure 4.3. Amplified peak region in the 3000-2800 cm\(^{-1}\). (a) original spectra; (b) second derivative; and (c) curve fitting.
Figure 4.4. Spectra area changing versus time: (a) polysaccharide carbon contents; (b) aromatic carbon contents; and (c) aliphatic carbon contents.
Figure 4.5. Spectra area changing in percentage: (a) polysaccharide carbon contents; (b) aromatic carbon contents; and (c) aliphatic carbon contents.
Figure 4.6. Correlation between FT-IR and wet chemistry results.
Figure 4.7. NMR spectra for initial and final compost samples.
CHAPTER V

EFFECT OF TEMPERATURE ON ORGANIC MATTER DEGRADATION IN A BENCH-SCALE COMPOSTING SYSTEM

5.1 INTRODUCTION

Understanding the effect of operational conditions on compost stability, the degree of decomposition of organic matter, is very important for exploring compostings' potential for solid waste treatment. Among the operational conditions, temperature is one of the predominant parameters during the composting process. The role of temperature during the composting process has been extensively reviewed in previous studies (Glueke 1972; Finstein and Morris 1975; Haug 1980; Anderson 1990; Fogarty and Tuovinen, 1991; Finstein and Hogan, 1993). Because composting is usually a self-heating process, microbial activity is not only responsible for the heat generation but also affected by its consequence. During the composting process, temperature affects microbial activity, degradation rate of organic matter and microbial diversity.
The optimal temperature for a composting process has been investigated by many previous studies (Schulze, 1962; Jeris and Regan 1973; Suler and Finstein 1977; Macgregor et al., 1981; Bach et al., 1984; Strom 1985a; Nakasaki et al., 1985; Kuter et al., 1985; McKinley and Vestal 1984, 1985; Sikora and Sowers 1985; Viel et al., 1987; Inbar et al., 1988). It is generally agreed that the optimal temperature should not exceed 60°C. Schulze (1962) showed that the O$_2$ consumption rates increased with temperature logarithmically in the range of 20-70°C. Jeris and Regan (1973) worked with high paper content compost and reported optimum CO$_2$ production and O$_2$ consumption rates under 60°C. The optimum temperature for newsprint, stabilized municipal refuse, and mix refuse are 48, 40, and 59°C respectively. Suler and Finstein (1977) reported the cumulative amount of CO$_2$ evolved was maximal at 56 to 60°C. By measuring the microbial activity, McKinley and Vestal (1984, 1985a, 1985b) found the maximal activity in the low temperature range (25-45°C) and relatively low activity in the high temperature range (55-74°C). For full scale reactor, Kuter et al. (1985) found that the highest composting rates were achieved when temperatures were kept below 60°C. However, other researchers have concluded that high temperature is preferred for high degradation rates and reduction of pathogens (Finstein and Morris, 1975). This conflict is partially caused by the different types of feed materials and environments with which the work has been done, and the indirect and incomplete nature of the measurement of microbial activity and biomass.

During the composting process, microorganisms convert organic matter to CO$_2$, heat and stabilized organic residue according to the following equation (Finstein et al., 1986):
Fresh organic waste + O₂ → \textit{Microbial Metabolism} → Stabilized organic residue + CO₂ + H₂O + Heat \quad (5.1)

Because the CO₂ evolution and O₂ consumption rates directly link to the decomposition rates of organic matter in compost, they can be used to evaluate process performance and determine operational conditions’ effect on the composting process.

The purpose of this work is to study the effect of temperature on the degradation of organic matter in a bench-scale composting system which can provide constant temperature control and minimum temperature gradients in the compost material. The degradation rates of this organic matter was characterized by the CO₂ evolution and O₂ consumption rates as well as FT-IR analysis. In FT-IR analysis, degradation of organic matter, such as polysaccharides, was evaluated by the change of the corresponding peak area (1050 cm⁻¹) in FT-IR spectra. The FT-IR analysis was verified with wet chemistry measurement for the absolute organic matter (e.g. polysaccharides and lignin) content.

5.2 MATERIALS AND METHODS

\textbf{Bench-scale Reactor.} The bench-scale reactor was made of Acrylic plastic. It held eight trays of thin layer feed material (Tseng \textit{et al.}, 1995). Two circulating fans were installed inside the reactor to insure uniform aeration. One thermocouple was buried in each tray to measure the temperature of feed material. A computer system was interfaced with this reactor to control the
temperature in the reactor and monitor the CO$_2$ and O$_2$ concentrations in the reactor. Inlet air flow rate into the reactor was manually controlled. Silicon tubing with small holes was installed on top of each tray for water spraying based on an open loop control strategy in order to maintain the moisture content around the set-point. A detailed description of this system and data acquisition has been presented by Tseng et al. (1995). The seed inoculum originated from a mature municipal sewage sludge compost and was added at 1% of total weight to feed material before the experiment.

**Experimental Conditions.** Experiments were run under 40, 50, 60 and 70°C conditions. A total 4,400 g simulated feed material (550 g as a 1 cm depth layer on each tray) was loaded into the reactor for each run. The simulated feed material was adjusted to 55-60% moisture content and C/N ratio of 25:1. 0.01 M each of NaHPO$_4$ and KH$_2$PO$_4$ was added to the feed material to provide some buffer capability. The compositions of the simulated feed material is listed in Table 5.1. During the experiments, dry and wet bulb temperature of air, temperature of each compost layer, and CO$_2$ and O$_2$ concentrations were monitored. The temperature inside the reactor was maintained using a feed-back control scheme. The moisture content for 40 and 50°C experiments was open-loop controlled by spraying water to the compost material based on historical data. Because of rapid water vaporization, pumping rate for water spraying to the compost material was manually increased during the period of high microbial activity for 60 and 70°C experiments. Air flow rates were manually adjusted during the experiments. The air flow rates were 104-426, 154-426, 154-426 and 104-0 ml/min. for experiments under 40, 50, 60 and 70°C temperatures.
respectively. Because of the low microbial activity at 70°C temperature experiment, the air flow rate was shut down after 56 hr.

Compost Analysis. Every 12 hrs, a sample (2-3 g) was taken from the reactor for moisture content, FT-IR analysis and ATP assay. Moisture content was determined by weight difference in drying of approximately 5 g compost sample at 105°C for 12 hrs. The pH of the feed material was determined by mixing 2 g compost in 100 ml of distilled water. This suspension was stirred 5 min. and then allowed to settle 15 min. before the pH was determined (Carnes and Lossin, 1970; Michel et al. 1993). C/N ratio and ash content of compost samples (approximately 50g) were measured by the Research Extension Analytical Laboratory (OSU, Wooster, OH) using a carbon analyzer (ASTM D4129-82), a Macro-N analyzer (Dumas method), and for ash content the AOAC 967.04 method. ATP concentration was measured by the bioluminence assay (Tseng et al., 1995).

FT-IR Analysis. Compost samples for FT-IR analysis were dried in an oven at 105°C for 12 hrs. Samples were sieved (495 μm) and ground into fine powder. 8 mg sample powder was mixed thoroughly with 4 mg internal standard (CaCO₃) and 388 mg KBr (background). The mixture was desiccated on P₂O₅ overnight before FT-IR analysis.

FT-IR spectra was obtained on a Mattson (Polaris™, WI) DRIFT (Diffuse Reflectance Infrared Fourier Transform) spectrometer operating at a nominal resolution of 2 cm⁻¹, the mid-infrared spectrum from 4000 to 400 cm⁻¹; iris of 40%; transmittance type of spectra; signal gain factor of 1; blocks of 5; scans of
100. The spectrometer was equipped with a liquid nitrogen cooled Lg MCT (large mercury cadmium telluride) detector.

**Spectra Processing.** FT-IR spectra was processed in a software GRAM/386 (Galactic Co.). In the typical FT-IR spectra of compost material, four spectra regions (1050 cm\(^{-1}\), 1651 cm\(^{-1}\), 2508 cm\(^{-1}\) and 2921 cm\(^{-1}\)) were chosen for analysis. The spectra regions of interest were first amplified and the second derivative (Savitsky-Golay function with second degree polynomial and 50 convolution points) was conducted to determine the peak positions and width. Curve fitting was used to verify the presence of individual peak which corresponds to specific carbon bond under the highly overlapping peak region. The detail description of the selection of the four spectra ranges (974-1070, 1705-1614, 2538-2490, and 2995-2887 cm\(^{-1}\)) for peak area integration was discussed by Tseng et al. (1995b).

**SEM Analysis.** SEM pictures for initial and final compost samples were viewed and photographed using an ISI 40 Scanning Electron Microscope at The Ohio Agricultural Research and Development Center (OARDC, Wooster, OH). Prior to the analysis, samples were first freeze dried at -40°C for 3 days and coated with platinum using a Polaron E5100 coater.

**Wet Chemistry Assay.** The extraction procedure for wet chemistry was modified from that of liyama et al. (1994). Initial and final samples (30 g) were oven dried at 105°C overnight and sieved (495 μm). A 3 g sample was extracted with 50 ml boiling anhydrous ether for 4 hr., extracted with 50 ml 80% boiling ethanol for 4 hr., and extracted with 50 ml 40°C water for 24 hr. All extracts from
the above three extractions were filtered with 202 grade filter paper (VWR Sci.), oven dried, and weighted. In order to verify the FT-IR results, samples were used for polysaccharides and lignin assays without any extraction.

Polysaccharide content was measured by total sugar reduction method with anthrone (Doutre et al., 1978; Chshire, 1979). 100 mg samples were digested with 12 M H$_2$SO$_4$ (4 ml) for 2 hr. with occasionally shaking. Next the solution was diluted with distilled water to 0.5 H$_2$SO$_4$ (192 ml). Anthrone reagent (1% w/v in concentrated H$_2$SO$_4$, 2 ml) was pipetted to the sample solution (1 ml). After setting 50 min at ambient temperature, the absorbencies were determined at 625 nm. The glucose standard curved was made prior to the measurement.

For lignin assay (Iiyama and Wallis, 1988, 1990), 20 mg samples were placed in a glass bottle (20 ml) with solution of 25% (w/w) acetyl bromide in acetic acid (5 ml) containing 0.2 ml perchloric acid (70%). The bottles were capped and placed in an oven at 70°C for 2 hr. The bottles were shaken at 10-30 min intervals. After digestion, the solution was mixed with 2 M NaOH (20 ml) and acetic acid (25 ml). The lignin content was measured by absorbencies at 280 nm and used the value for lignin, 20.0 g$^{-1}$ L cm$^{-1}$.

5.3 RESULTS

The temperature profiles in the compost material are presented in Figure 5.1a, 5.2a, 5.3a, and 5.4a as the average temperature of each compost tray for a 5 hr period. The CO$_2$ and O$_2$ concentrations were monitored during the experiments (Figure 5.1b, 5.2b, 5.3b and 5.4b). The CO$_2$ evolution and O$_2$ consumption rates
(Figure 5.1c, 5.2c, 5.3c and 5.4c) were calculated based on a mass balance surrounding the reactor (Tseng et al., 1995). ATP concentrations in these four temperature experiments measured by bioluminescence assay are shown in Figure 5.1d, 5.2d, 5.3d and 5.4d.

Total CO$_2$ evolved and O$_2$ consumed were calculated based on the average CO$_2$ evolution and O$_2$ consumption rates between two adjunct data points (0.5 hr). Figure 5.5 shows the total CO$_2$ evolved and O$_2$ consumed for these four experiments. Carbon and nitrogen content, C/N ratio, pH of initial and final samples, total loss of dry organic matter for these four experiments are listed in Table 5.2.

Four peak regions were processed with second derivative and curve fitting to identify the peak positions and width during the FT-IR analysis. Those peak area under 1050 cm$^{-1}$, 1651 cm$^{-1}$ and 2921 cm$^{-1}$ were normalized against the peak area of internal standard (2508 cm$^{-1}$). The change of the peak area corresponding to polysaccharide, aromatic and aliphatic carbon contents versus time course are shown in Figure 5.6, 5.7 and 5.8 respectively. The y-axis is arbitrary units. Total CO$_2$ evolved versus the conversion of polysaccharides from FT-IR analysis for these four experiments are presented in Figure 5.9. Total RQ from those four experiments versus 1/T are shown in Figure 5.10.

The organic matter in compost was verified with wet chemistry measurements. The percentages of polysaccharides and lignin content in the initial and final samples are listed in Table 5.3. The decrease in concentration of polysaccharides from initial to final samples are 50.2, 60.0, 51.7 and 21.3%
respectively. Since we assume that the CO\textsubscript{2} evolution during the experiments were mainly contributed by the degradation of polysaccharides, the plot of total CO\textsubscript{2} evolved versus the conversion of polysaccharides from FT-IR analysis provides the information of the degradation rate (Figure 5.9). The change of the percentage of other extracts which reflect the change of other organic matter such as lipid or free sugars, from ether, ethanol and water extractions are also listed in Table 5.3. SEM pictures were also taken for initial and final samples from the experiments under those four different temperatures. Figure 5.10 showed the SEM pictures of the initial and final samples from 50°C temperature experiment.

5.4 DISCUSSION

Controlling temperature and isolating it from other parameters was one of the goals for the design of this computer controlled bench-scale composting system. Although previous studies (Bach \textit{et al.} 1984; Nakasaki \textit{et al.} 1985) had controlled temperature and studied its effect on degradation rate, it has been demonstrated that this system can minimize both temperature and O\textsubscript{2} gradient in the compost materials by using a thin layer configuration (Tseng \textit{et al.}, 1995). As shown in Figure 5.1a, 5.2a, 5.3a and 5.4a, the temperatures inside the compost material was well controlled around the set-point. The temperature profiles for 60 and 70°C experiments were slightly low because the set-point during the experiments were slightly lower than 60 and 70°C respectively. Different from the temperature profiles of previous studies, the temperature profiles in this study were fairly constant throughout the experiments. The CO\textsubscript{2} and O\textsubscript{2} concentration profiles were also monitored during the experiments. Since air flow rates were manually
adjusted during the experiments in order to avoid $O_2$ limiting conditions, the $CO_2$ evolution and $O_2$ consumption rates based on mass balance reflected the microbial activity more precisely than the concentration profiles.

Temperature affects microbial diversity during the composting process (Kane and Mullins, 1973; Savage et al., 1973; Godden et al. 1983; Strom 1985; Nakasaki et al., 1985). In previous studies, mesophilic bacteria, thermophilic bacteria, thermophilic actinomycetes, and thermophilic fungus have been studied in lab-scale, pilot-scale and field-scale composting processes. Mesophilic bacteria increased in number until 50°C but declined as temperature increased over 50°C. The mesophilic bacteria and actinomycetes are usually present in the thermophilic stage, which is generally considered essential for the compost production and minimization of pathogens. Microorganisms present in different temperature range are not only diverse but also have different degradation rates toward organic matter. Nakasaki et al. (1985a) concluded that mesophilic bacteria contributed mainly the $CO_2$ evolution in the early stage of composting. In the thermophilic stage of 60°C, the initial stage of the $CO_2$ evolution at the initial stage was attributed by the thermophilic bacteria, while the later stage of 60°C was attributed by thermophilic actinomycetes. Although mesophilic bacteria may be present under thermophilic temperature range, its contribution to $CO_2$ evolution was negligible.

In order to study the microbial activity in composting processes, $CO_2$ evolution rate (Bach et al., 1984; Nakasaki et al., 1985a, 1985b; Nakasaki et al., 1992; Michel 1993), $O_2$ consumption rate (Viel et al., 1987; Inbar et al., 1988) enzyme activities (Godden et al., 1983), and incorporation of $^{14}C$ acetate into microbial
lipids (McKinley and Vestal, 1984, 1985) had been used by previous researchers. Bach et al. (1984) showed that the CO\textsubscript{2} evolution rate was mostly associated with degradation of volatile matter in raw sludge in the composting process. In this study, both CO\textsubscript{2} evolution and O\textsubscript{2} consumption rates, calculated from a mass balance surrounding the reactor, were used to represent the microbial activities during the composting process. As shown in Figure 5.1c, 5.2c, 5.3c and 5.4c, CO\textsubscript{2} evolution and O\textsubscript{2} consumption rates showed the change of microbial activities during the composting process under four different operational temperatures. Except for 70°C, which has low microbial activity because of the inhibitory temperature, the other three profiles (at 40, 50, and 60°C) have similar patterns, a slight peak of CO\textsubscript{2} evolution rate around 20 hr (or valley of O\textsubscript{2} consumption rate) followed by a broad peak of CO\textsubscript{2} evolution rate. This may be caused by the microbial activities of different types of microorganism. Nakasaki et al. (1985a) observed two peaks in the CO\textsubscript{2} evolution profiles and interpreted that the peaks were associated with the activity of themophilic bacteria and thermophilic actinomycetes.

The maximum CO\textsubscript{2} evolution and O\textsubscript{2} consumption rates in this study were reached at 50°C temperature. In addition, the total O\textsubscript{2} consumed and CO\textsubscript{2} produced also reached the highest at 50°C experiment (Figure 5.5). Both of these observations indicated that the microbial activity was the highest under 50°C temperature. This result agrees with some previous researchers' conclusions. Microbial activity under 40°C was high because of the present of large amount of mycelia organisms, observed during the later stage of the run. The microbial activity was inhibited under 70°C and showed low CO\textsubscript{2} evolution and O\textsubscript{2} consumption rates (Figure 5.4c). However, microbes will start to grow
when temperature went back to proper temperature range. As shown in Figure 5.4c, the microbial activity increased around 150 hr when the temperature declined to around 50°C as a result of power failure. The increasing in microbial activity also caused the increase of biomass as reflected by ATP concentration (Figure 5.4d).

It unlikely that temperature affected the formation of biomass during the favourable microbial temperature range. In general, ATP concentration is an indicator of biomass in composting process. Conversion factor of 100-120 is usually used to convert ATP concentration to biomass in soil or compost. The ATP concentrations were ranged from 0.1 µg to 10 µg/g dw for experiments under 40, 50, and 60°C. No difference could be observed from the ATP concentrations of these three experiments. The ATP concentrations were slightly higher in experiment at 40°C because fungus dominated the microbial population during the later stage of the experiment. ATP concentrations were extremely low at 70°C experiment because of the inhibitory effect of the high temperature. However, the ATP concentration increased to 1 µg/g dw around 150 hr when the temperature decreased accidentally.

During the composting process, CO₂ evolution results from the decomposition of organic matter. It has been shown that carbohydrates, such as polysaccharides, will be degraded first during the composting process. Using FT-IR technique, degradation of polysaccharides and other organic matter can be monitored during the composting process. The change of spectra area (after normalization) which correspond to polysaccharide carbon contents versus time are shown in
Figure 5.6. The polysaccharides carbon contents decreased in all four experiments.

As shown in Figure 5.9, the CO$_2$ evolution rate versus decomposition of polysaccharides show a linear relation. It indicates that the CO$_2$ evolved was resulted from the degradation of polysaccharides carbon contents during the composting process. In addition, the slope of this correlation tells how the organic matter was converted into CO$_2$. As shown in Figure 5.9, the slope of the correlation is the highest of 50°C experiment. On the other hand, the slope from 70°C experiment is the lowest. This indicates the enhanced respiration under 70°C experiment. The aromatic and aliphatic carbon contents from FT-IR results are fairly constant for 40, 50 and 70°C experiments. However, both aromatic and aliphatic carbon contents decreased for 60°C experiment. Unknown microbes under 60°C may contribute to the degradation of aromatic and aliphatic carbon contents.

The effect of temperature on organic matter degradation was also reflected on the loss of dry mass, pH, and C/N ratio for initial and final compost samples (Table 5.2). The loss of dry mass under 50°C temperature experiment, 33.3%, is the highest, while the loss of dry mass under 70°C temperature experiment, 16.9%, is the lowest among the four experiments.

Because of the effect of temperature on microbial diversity, different microbes are supposed to be present in the compost sample under different temperature experiments. The SEM is served for the purpose to observe the microbes present on the surface of the compost sample. As shown from the SEM pictures
of 50°C temperature experiment, the surface of the compost particles was fairly flat and few microorganisms were present before the composting process. However, the surface of the compost particles became rough and a lot of microorganisms colonized on the surface of the compost materials of the sample which was taken at the end of the experiment (350 hr).

The wet chemistry results as shown in Table 5.3 indicated the degradation of different types of organic matter during the composting process under different temperature experiments. The ether extract contained mainly lipids, whereas ethanol and water extracts contain low molecule aromatic and free sugars. The polysaccharide carbon contents decreased around 50% for 40, 50 and 60°C experiments and only decreased around 20% for 70°C experiment. The lignin contents in both initial and final samples were fairly constant for all four temperature experiments.

In conclusion, the effect of temperature on organic matter degradation was conducted in a well controlled bench-scale composting system. The microbial activity and total CO$_2$ evolved and O$_2$ consumed were at the maximum under 50°C temperature. From the FT-IR analysis of the compost samples, polysaccharides decreased in all four experiments.

5.6 SUMMARY

Composting processes at 40, 50, 60 and 70°C temperatures were conducted in a bench-scale composting system which maintained constant temperatures during the experiments. The maximum CO$_2$ evolution and O$_2$ consumption rates were
observed at 50°C temperature experiment. The total CO$_2$ evolved and O$_2$ consumed were also the highest at 50°C temperature experiment. Temperature above 70°C became inhibitory to the microbial activity. However, temperature has little impact on biomass formation in the temperature range of 40-60°C.
Table 5.1. Compositions in compost feed materials.

<table>
<thead>
<tr>
<th>Composition</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled Water</td>
<td>60</td>
</tr>
<tr>
<td>Rabbit chows (ground grass and leaf)</td>
<td>16.3</td>
</tr>
<tr>
<td>Corn cobs (chopped)</td>
<td>14</td>
</tr>
<tr>
<td>Sand</td>
<td>8</td>
</tr>
<tr>
<td>Seed inoculum (matured compost from commercial plants)</td>
<td>1</td>
</tr>
<tr>
<td>Manure</td>
<td>0.4</td>
</tr>
<tr>
<td>Newspaper</td>
<td>0.3</td>
</tr>
</tbody>
</table>
Table 5.2. Carbon, nitrogen, C/N ratio, ash, pH and loss of dry mass for the experiments.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Carbon (%)</th>
<th>Nitrogen (%)</th>
<th>C/N</th>
<th>ash (%)</th>
<th>pH</th>
<th>Weight (g)</th>
<th>Loss of dry mass (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40 - I&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.0</td>
<td>1.47</td>
<td>22.5:1</td>
<td>25.5</td>
<td>6.38</td>
<td>1923</td>
<td></td>
</tr>
<tr>
<td>40 - F&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31.7</td>
<td>1.58</td>
<td>20.1:1</td>
<td>29.9</td>
<td>8.54</td>
<td>1312</td>
<td>31.8</td>
</tr>
<tr>
<td>50 - I</td>
<td>32.7</td>
<td>1.45</td>
<td>22.5:1</td>
<td>31.5</td>
<td>6.36</td>
<td>1795</td>
<td></td>
</tr>
<tr>
<td>50 - F</td>
<td>32.9</td>
<td>1.42</td>
<td>23.2:1</td>
<td>40.4</td>
<td>8.27</td>
<td>1197</td>
<td>33.3</td>
</tr>
<tr>
<td>60 - I</td>
<td>32.85</td>
<td>1.44</td>
<td>23:1</td>
<td>29.13</td>
<td>6.12</td>
<td>1755.6</td>
<td></td>
</tr>
<tr>
<td>60 - F</td>
<td>26.55</td>
<td>1.46</td>
<td>18:1</td>
<td>33.96</td>
<td>7.83</td>
<td>1212.98</td>
<td>30.9</td>
</tr>
<tr>
<td>70 - I</td>
<td>29.48</td>
<td>1.4</td>
<td>21:1</td>
<td>27.27</td>
<td>6.3</td>
<td>1767.48</td>
<td></td>
</tr>
<tr>
<td>70 - F</td>
<td>33.17</td>
<td>1.34</td>
<td>25:1</td>
<td>27.78</td>
<td>6.35</td>
<td>1469.04</td>
<td>16.9</td>
</tr>
</tbody>
</table>

<sup>a</sup>Initial sample  
<sup>b</sup>Final sample
Table 5.3. Wet chemistry measurements of polysaccharides and lignin concentrations for initial and final samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ether Extract (%)</th>
<th>Ethanol Extract (%)</th>
<th>Water Extract (%)</th>
<th>Polysaccharides (%)</th>
<th>Lignin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40 - I&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.2</td>
<td>6.1</td>
<td>3.1</td>
<td>23.3</td>
<td>11.9</td>
</tr>
<tr>
<td>40 - F&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.0</td>
<td>4.4</td>
<td>3.8</td>
<td>11.6</td>
<td>12.5</td>
</tr>
<tr>
<td>50 - I</td>
<td>7.0</td>
<td>4.2</td>
<td>5.5</td>
<td>21.0</td>
<td>12.4</td>
</tr>
<tr>
<td>50 - F</td>
<td>2.2</td>
<td>2.9</td>
<td>4.4</td>
<td>8.4</td>
<td>12.7</td>
</tr>
<tr>
<td>60 - I</td>
<td>7.6</td>
<td>3.9</td>
<td>2.4</td>
<td>26.3</td>
<td>11.9</td>
</tr>
<tr>
<td>60 - F</td>
<td>7.5</td>
<td>3.9</td>
<td>6.7</td>
<td>12.7</td>
<td>13.8</td>
</tr>
<tr>
<td>70 - I</td>
<td>7.3</td>
<td>4.0</td>
<td>3.7</td>
<td>26.7</td>
<td>11.5</td>
</tr>
<tr>
<td>70 - F</td>
<td>5.8</td>
<td>4.3</td>
<td>3.8</td>
<td>21.0</td>
<td>11.5</td>
</tr>
</tbody>
</table>

<sup>a</sup>Initial sample  
<sup>b</sup>Final sample
Figure 5.1. Experiment under 40°C. (a). Temperature in compost. (b). CO\textsubscript{2} (thicker line) and O\textsubscript{2} concentration (thinner line) profiles. (c). CO\textsubscript{2} evolution (x) and O\textsubscript{2} consumption (■) rates. (d). ATP concentration profile.
Figure 5.2. Experiment under 50°C. (a). Temperature in compost. (b). CO₂ (thicker line) and O₂ concentration (thinner line) profiles. (c). CO₂ evolution (x) and O₂ consumption (■) rates. (d). ATP concentration profile.
Figure 5.3. Experiment under 60°C. (a). Temperature in compost. (b). CO₂ (thicker line) and O₂ concentration (thinner line) profiles. (c). CO₂ evolution (x) and O₂ consumption (■) rates. (d). ATP concentration profile.
Figure 5.4. Experiment under 70°C. (a). Temperature in compost. (b). CO$_2$ (thicker line) and O$_2$ concentration (thinner line) profiles. (c). CO$_2$ evolution (x) and O$_2$ consumption (■) rates. (d). ATP concentration profile.
Figure 5.5. Total CO$_2$ produced and O$_2$ consumed: 40°C; 50°C; 60°C; and 70°C.
Figure 5.6. Peak area of polysaccharide carbon contents peak region (1050 cm\(^{-1}\)) versus time course for those four experiments. (a) 40°C. (b) 50°C. (c) 60°C. (d) 70°C.
Figure 5.7. Peak area of aromatic carbon contents peak region (1651 cm\(^{-1}\)) versus time course for those four experiments. (a). 40°C. (b). 50°C. (c). 60°C. (d). 70°C.
Figure 5.8. Peak area of aliphatic carbon contents peak region (2921 cm\(^{-1}\)) versus time course for those four experiments. (a) 40°C. (b) 50°C. (c) 60°C. (d) 70°C.
Figure 5.9. Total CO$_2$ evolved verse conversion of polysaccharides from FT-IR results. (a). 40°C, (b). 50°C, (c). 60°C, (d). 70°C.
Figure 5.10. RQ versus 1/T for these four temperature experiments.
Figure 5.11. SEM of experiment under 50°C. (a) fresh compost under magnitude of 770. (b) final compost under magnitude of 770. (c) fresh compost under magnitude of 1.5 k. (d) final compost under magnitude of 1.5k.
CHAPTER VI

RECOMMENDATIONS FOR FUTHER RESEARCH

6.1 Improvement of this system

The results showed that this bench-scale system can provide fairly well controlled environment, such as temperature and moisture content, for laboratory studying of the composting process. The advantages of this system are: (1). constant temperature control and minimum temperature gradient inside compost material. (2). uniform aeration, non-odor formation and minimum \( O_2 \) gradient inside compost. (3). controlled-moisture content. (4). ability to calculate \( CO_2 \) evolution and \( O_2 \) consumption rates by mass balance surrounding the reactor. However, certain modifications of this system will improve its performance in the future.

As shown in the pervious experiments, moisture control is the major issue in this system. Because of the absence of an on-line moisture probe, moisture control was based on historical data (previous experiment without moisture control). In general, this kind of open-loop control worked fairly well during most of the
experiments. However, fluctuation of the moisture content occurred during the time period of high microbial activity.

Two improvements can be applied to overcome the fluctuations in moisture content: (1) on-line manually adjust the moisture control strategy; (2) rebuild the water spraying system. The water pumping rate could be adjusted on-line based on the moisture content of the last measurement (e.g. .10 hr past). This kind of adjustment would be very useful during the time period of high microbial activity. The current water spraying system was built by silicon tubing which was on top on each compost tray. Water was sprayed from certain amount of small holes on the tubing. Because of the low pumping rate and time lag to build the pressure, water was occasionally dropped onto the compost in certain region of the compost. This kind of water dropping might cause anaerobic zone inside the compost. A new water spraying system has been built for the new compost reactor (Figure 6.1). Tap water with 30-40 psi instead of water from pump would be used. A small nozzle was installed on the glass tubing. Fine water spray could be obtained with this kind of spraying system. In addition, water could be sprayed to the corner of the compost tray.

6.2 Research Needed on Process Kinetics

O₂ effect on composting has been studied in previous studies (Nakasaki et al., 1992; Suler and Finstein, 1977), however no precise conclusion was made. One of the impossibility for them to study the effect of O₂ on the composting process is lacking the ability to control O₂ concentration during composting. By using this system, the O₂ concentration can be on-line measured and the air flow rate can
be controlled by the computer to maintain a constant $O_2$ level inside the reactor. However, a mass flow meter is required to implement this goal. With the installation of the mass flow meter, we can not only study the $O_2$ effect on biodegradation rates, but also maintain the $O_2$ concentration always above a certain set-point (e.g. 18 %) during the experiment to study the effect of other parameters on the composting process.

Composting is a natural heating process. Microorganisms will generate heat during the process. Adiabatic experiment by allowing the temperature to follow the natural temperature profile can be investigated by using this composting system in the future study. Although this kind of experiment has been investigated in a so called "Rutgers System", certain gradients, such as temperature and $O_2$ gradients, presented in the compost materials in their studies (Hogan et al., 1989). Those kinds of gradients could be eliminated and a simulated nature heating process could be investigated by using this system.

6.3 Research Needed on FT-IR Analysis of Composting

Using CaCO$_3$ as an internal standard in this study is because of its non-toxic and easy accessible nature. Although no peak overlapped around aromatic and polysaccharides region, peak overlapping is occurs around aliphatic peak region. Better internal standard without peak interference, such as NaN$_3$, (Figure 6-2), can be used to verify the results from the analysis using CaCO$_3$ as the internal standard. In addition, careful selection of peak region used for peak normalization is also very important for the accuracy of the results.
Further research may be required on the FT-IR spectra analysis. In the above study, the spectra analysis was processed by second derivative, peak position and width identification, and curve fitting. The peak position and width were manually picked from second derivation of the spectra region. The curving fitting was performed by manually selecting the initial guesses of the peak position and width. The whole processing was time consuming and not accurate. By modifying the program, the second derivation, peak position and width identification, and curving fitting could be conducted at once. In addition, the position and width can be used for each individual peak region instead of using the fix position and width for all of the samples. Peak area integration from curve fitting has been conducted in this study but not successful. Future work may focus on developing an effective curve fitting protocol and related the change of peak area with process kinetics.

The FT-IR analysis shown in this study was conducted only for the outer-layer (or biofilm) of the compost material because we assumed that most of organic matter degradation was happened in the out-layer. However, certain anaerobic activity may occur below the biofilm even in the corn cob particle during the composting. FT-IR analysis of the whole compost sample may be conducted to investigate the degradation process during the experiment.

Near Infrared (NIR) (700-2000 nm) spectroscopic analysis has shown great advantages comparing to MIR (2-8 μm). Those advantages includes: greater penetration depth, easy sample preparation, less spectra peaks, etc. Future studies may focus on the application of NIR analysis to this bench-scale composting system.
6.4 Applications of this Bench-scale System and recommendations for future studies:

(1). Improve the system performance by installing new water spraying system and adjusting the water pumping rate control program.

(2). Study the O$_2$ effect on composting in this system by controlling the O$_2$ concentration during the experiment.

(3). Simulate the temperature to follow the natural temperature profile during the experiment to study the effect of individual parameter on the composting process.

(4). Use different internal standard to verify the quantitative analysis.

(5). Modify the spectra analysis program for better curve fitting results.

(6). The applications of this bench-scale system include:
     (a). Study the degradation of PLA in this system.
     (b). Study the degradation of Atrazine in this system.

(7). Use NIR to investigate the compost sample from the experiments in this study and make comparison of the NIR results with that by MIR analysis.
Figure 6.1. Design of the new water spraying system.
Figure 6.2. FT-IR spectra of compost with NaN$_3$ addition (a). spectra with NaN$_3$ addition, (b). spectra without internal standard.
REFERENCES


APPENDIX A

Control Program for the Bench-scale Composting System

DECLARE SUB EXP16 (CH%, D())
DECLARE SUB TEMP (CJC!, V!, TC, TF, NK%, SIK!, SVK!, TK())
DECLARE SUB OUTSCREEN (CJC!, T(), HUMI, O2, CO2, MOIS)
DECLARE SUB HUMIDITY (T(), HUMI)
DECLARE SUB CONTROL (T(), ETIME, TIMEON, TON%, PUMPON, PON%, PMON%, TCOM)
DECLARE SUB CALTEMP (T())
DECLARE SUB OUTPRINT (CT(), CJC!, T(), HUMI, ETIME, T1$, D1$, T$, D$, O2, CO2, MOIS)
DECLARE SUB GASMETER (O2, CO2, ETIME, GASON, gon%, GMON%)
DECLARE SUB PRUNT (ETIME, TCOM, PRUN)
DECLARE SUB DAS8 (MODE%, BYVAL DUMMY%, FLAG%)
DIM D(0 TO 15) '16 ELEMENTS, ONE FOR EACH EXP-16 CHANNEL
DIM TK(0 TO 308)
DIM ET(0 TO 2)
DIM CT(0 TO 2)
DIM LT%(0 TO 1)
DIM T(0 TO 15) 'CORRESPONDING REAL ARRAY TO RECEIVE TEMPERATURE DATE
DIM TC(0 TO 15)
DIM DIO%(0 TO 4)
COMMON SHARED D%(,), LT%(.)

'----------------------INITIALIZATION OF SOME VALUES-----------------------------
SCREEN 0, 0, 0: KEY OFF: CLS : WIDTH 80
CT(0) = CT(1) = CT(2) = 0!
TON% = 0
TIMEON = 0!
PON% = 0
PUMPON = 0!
PMON% = 0
GASON = 0!
gon% = 0
GMON% = 0

'----------------------INITIALIZATION OF DASH 8 AND DASH16-----------------------
CLEAR, 49152!
DATA 309, 0.2,-6.6
READ NK%, SIK!, SVK!
LOCATE 25, 1: COLOR 0, 7: PRINT "-PLEASE WAITE-"; : COLOR 7, 0:
'----------------------LOADING DASH 8 I/O ADDRESS-----------------------------
LOCATE 1, 1
OPEN "DASH8.ADR" FOR INPUT AS #1
INPUT #1, BASADR%    'INITIALIZE & DECLARE PARAMETERS
CLOSE #1
FLAG% = 0
MD% = 0           'MODE 0 = INITIALIZATION
CALL DASH8(MD%, VARPTR(BASADR%), FLAG%)
IF FLAG% <> 0 THEN PRINT "INSTALLATION ERROR"
GOTO DATABASE    'LOAD THERMOCOUPLE LINEARING LOOKUP DATA
CONTINUE:
AV = 50     'GET GAIN SETTING OF EXP-16

CLS
TI$ = TIME$
HRI$ = LEFT$(TI$, 2)
MMI$ = MID$(TI$, 4, 2)
SSI$ = RIGHT$(TI$, 2)
HRI = VAL(TI$)
MMI = VAL(MMI$)
SSI = VAL(SSI$)
DI$ = DATE$
DDI$ = MID$(DI$, 4, 2)
DDI = VAL(DDI$)
IF HRI < 12 THEN Ampml$ = " AM" ELSE Ampml$ = " PM"
HRIP = HRI
IF HRI > 12 THEN HRIP = HRI - 12
ET(0) = 0!

OPEN "LPT1:" FOR OUTPUT AS #2
PRINT #2, "TIME"; SPC(2); "DRY TEMP"; SPC(2); "T1"; SPC(5); "T2"; SPC(5); "T3"
CLOSE #2

REPEAT:

D$ = DATES
DD$ = MID$(D$, 4, 2)
DD = VAL(DD$)
T$ = TIMES
HR$ = LEFT$(T$, 2)
MM$ = MID$(T$, 4, 2)
SS$ = RIGHT$(T$, 2)
HR = VAL(HR$)
MM = VAL(MM$)
SS = VAL(SS$)

IF HR < 12 THEN AMPMS = " AM" ELSE AMPMS = " PM"
HRP = HR
IF HR > 12 THEN HRP = HR - 12

DH = (DD - DDI) * 24!
ETIME = (HR - HRI + DH) * 3600! + (MM - MMI) * 60! + (SS - SSI) * 1!
ET(1) = ETIME - ET(0)
LOCATE 1, 1
PRINT "THE START DATE IS "; D$; " THE CURRENT DATE IS "; D$
PRINT "THE START TIME IS "; STR$(HRIP); RIGHT$(T$, 6); Ampml$; " "
PRINT "THE CURRENT TIME IS"; STR$(HRP); RIGHT$(T$, 6); AMPMS; " 
PRINT "THE ELASP TIME IS "; ETIME / 3600; "HOURS "
PRINT "THE TIME FROM LAST MEASUREMENT IS"; ET(1); "SECONDS 
IF ET(1) < 3 THEN
GOTO REPEAT
ELSE
ET(0) = ET(1) + ET(0)
ET(1) = 0!
END IF

'----------------GET COLD JUNCTION COMPENSATION TEMPERATURE-----------------
'OUTPUT OF CJC CHANNEL IS SCALED AT 24.4MV/DEG. C. THIS CORRESPONDS TO 0.1DEG.
'C./BIT. DIVIDING OUTPUT IN BITS BY 10 YIELDS DEGREES C.

'LOCK DASH-8 TO CHANNEL #7 (CJC CHANNEL SELECTED) USING MODE 1
MD% = 1
LT%(0) = 7
LT%(1) = 7
CALL DAS8(MD%, VARPTR(LT%(0)), FLAG%)
IF FLAG% <> 0 THEN PRINT "ERROR IN SETTING CJC CHANNEL": END

'GET CJC DATE FROM THIS CHANNEL USING MODE 4
MD% = 4: CJ% = 0
CALL DAS8(MD%, VARPTR(CJ%), FLAG%)
IF FLAG% <> 0 THEN PRINT "ERROR IN CJC": END

'CHANGE OUTPUT IN BITS TO REAL TEMPERATURE
CJC = CJ% / 10
CH% = 0
CALL EXP16(CH%, D())
FOR I = 0 TO 15
  V! = (D(I) * 5) / (AV * 2048)
  CALL TEMP(CJC!, V!, TC, TF, NK%, SIK!, SVK!, TK())'PERFORM LOOK-UP LINEARIZATION
  T(I) = TC
NEXT I

'PRINT "d(5)=", d(10): END

'MOISTURE CONTENT
VMOIS = D(3)
MOIS = VMOIS
CALL CALTEMP(T())
CALL HUMIDITY(T(), HUMI)
CALL CONTROL(T(), ETIME, TIMEON, TON%, PUMPON, PON%, PMON%, TCOM)
CALL GASMEATER(O2, CO2, ETIME, GASON, gon%, GMON%)

'---------------------THERMOCOUPLE CALIBRATION-----------------------------
'IF II = 200 THEN
'LOCATE 10, 1
'PRINT "T4=", TC(4) / 200!: "T5=", TC(5) / 200!: "T6=", TC(6) / 200!
'PRINT "T7=", TC(7) / 200!: "T8=", TC(8) / 200!
'PRINT "T9=", TC(9) / 200!; "T10=", TC(10) / 200!
'PRINT "T11=", TC(11) / 200!; "T12=", TC(12) / 200!
'PRINT "T13=", TC(13) / 200!; "T14=", TC(14) / 200!
'PRINT "T15=", TC(15) / 200!
'END
'ELSE
'II = II + 1
'J = 0
'DO WHILE J < 15
'J = J + 1
'TC(J) = TC(J) + T(J)
'LOOP
'END IF

'-------------------------DISPLAY TEMPERATURE DATA---------------------------------
LOCATE 8, 1
CALL OUTSCREEN(CJC!, T(), HUMI, O2, CO2, MOIS)
CALL OUTPRINT(CT(), CJC!, T(), HUMI, ETIME, T1$, D1$, T$, D$, O2, CO2, MOIS)
GOTO REPEAT 'REPEAT SCAN OF CHANNELS

DATABASE:
'------- Table lookup data for K type thermocouple ---------------------
'Run this subroutine only in the initialization section of your program
'Number of points, voltage step interval (mV), starting voltage (mV)
'Temperature at -6.6mV, -6.4mV, -6.2mV etc.
DATA -353.5,-249.3,-224.0,-207.6,-194.3,-182.8,-172.3,-162.8,-153.8,-145.4
DATA -137.3,-129.6,-122.3,-115.2,-108.3,-101.6,-95.1,-88.7,-82.5,-76.4
DATA -70.4,-64.6,-58.8,-53.1,-47.5,-42.0,-36.6,-31.2,-25.9,-20.6
DATA -15.4,-10.2,-5.1,-0.0, 5.0, 10.1, 15.1, 20.0, 25.0, 29.9
DATA 34.8, 39.7, 44.6, 49.5, 54.3, 59.1, 64.0, 68.8, 73.6, 78.4
DATA 83.2, 88.0, 92.9, 97.7, 102.5, 107.4, 112.2, 117.1, 122.0, 126.9
DATA 131.8, 136.7, 141.7, 146.6, 151.6, 156.5, 161.5, 166.5, 171.5, 176.5
DATA 181.6, 186.6, 191.6, 196.6, 201.6, 206.6, 211.6, 216.6, 221.5, 226.5
DATA 231.5, 236.4, 241.4, 246.3, 251.2, 256.1, 261.0, 265.9, 270.8, 275.6
DATA 280.5, 285.3, 290.2, 295.0, 299.8, 304.6, 309.4, 314.3, 319.1, 323.9
DATA 328.7, 333.4, 338.2, 343.0, 347.8, 352.6, 357.3, 362.1, 366.9, 371.6
DATA 376.4, 381.1, 385.9, 390.6, 395.4, 400.1, 404.8, 409.6, 414.3, 419.0
DATA 423.8, 428.5, 433.2, 437.9, 442.6, 447.3, 452.0, 456.8, 461.5, 466.2
DATA 470.9, 475.6, 480.3, 485.0, 489.7, 494.4, 499.1, 503.8, 508.5, 513.1
DATA 517.8, 522.5, 527.2, 531.9, 536.6, 541.3, 546.0, 550.7, 555.4, 560.0
DATA 564.7, 569.4, 574.1, 578.8, 583.5, 588.2, 592.9, 597.6, 602.3, 607.0
DATA 611.7, 616.4, 621.2, 625.9, 630.6, 635.3, 640.0, 644.8, 649.5, 654.2
DATA 658.9, 663.7, 668.4, 673.2, 677.9, 682.7, 687.4, 692.2, 696.9, 701.7
DATA 706.5, 711.3, 716.1, 720.8, 725.6, 730.4, 735.2, 740.0, 744.8, 749.7
DATA 754.5, 759.3, 764.1, 769.0, 773.8, 778.7, 783.5, 788.4, 793.3, 798.1
DATA 803.0, 807.9, 812.8, 817.7, 822.6, 827.5, 832.4, 837.3, 842.2, 847.2
DATA 852.1, 857.1, 862.0, 867.0, 872.0, 876.9, 881.9, 886.9, 891.9, 896.9
DATA 901.9, 906.9, 911.9, 916.9, 922.0, 927.0, 932.0, 937.1, 942.2, 947.2
DATA 952.3, 957.4, 962.5, 967.6, 972.7, 977.8, 982.9, 988.0, 993.1, 998.2
DATA 1003.4, 1008.5, 1013.7, 1018.8, 1024.0, 1029.2, 1034.4, 1039.6, 1044.8, 1050.0
DATA 1055.2, 1060.4, 1065.6, 1070.8, 1076.1, 1081.3, 1086.6, 1091.9, 1097.2, 1102.4
DATA 1107.7, 1113.0, 1118.3, 1123.7, 1129.0, 1134.3, 1139.7, 1145.0, 1150.4, 1155.8
DATA 1161.2, 1166.6, 1172.0, 1177.4, 1182.9, 1188.3, 1193.8, 1199.2, 1204.7, 1210.2
DATA 1215.7, 1221.2, 1226.8, 1232.3, 1237.9, 1243.5, 1249.1, 1254.7, 1260.3, 1265.9
DATA 1271.6, 1277.3, 1282.9, 1288.6, 1294.3, 1300.1, 1305.8, 1311.5, 1317.3, 1323.1
DATA 1328.9, 1334.7, 1340.5, 1346.4, 1352.2, 1358.1, 1363.9, 1369.8, 1375.7

FOR I = 0 TO NK% - 1: READ TK(I): NEXT I
GOTO CONTINUE

SUB CALTEMP (T())

T(4) = .496709451# + 1.008751932# * T(4)
T(5) = .478177172# + 1.009451494# * T(5)
T(6) = .284698517# + 1.011721809# * T(6)
T(7) = .447190171# + 1.009458627# * T(7)
T(8) = -1.371321087# + 1.015642932# * T(8)
T(9) = -1.251650511# + 1.014139128# * T(9)
T(10) = -1.266388724# + 1.016118691# * T(10)
T(11) = -1.186403979# + 1.016540124# * T(11)
T(12) = -1.186162823# + 1.016333496# * T(12)
T(13) = -1.164081227# + 1.013566589# * T(13)
T(14) = -1.457476257# + 1.019570659# * T(14)
T(15) = -1.07963164# + 1.015365527# * T(15)

END SUB

SUB CONTROL (T(), ETIME, TIMEON, TON%, PUMPON, PON%, PMON%, TCOM)

' D = data (range 0-4095), NC = channel (0-5), BASE = I/O address
TG1! = T(14) - 39!
IF TG1! >= 0! THEN
D1 = 0
ELSE
D1 = 3000

EX.BAS: Subroutine to output data to D/A on DDA-06

165
END IF

BASADR = &H300
CN = 0
XH% = INT(D1 / 256) 'work out high byte
XL% = D1 - 256 * XH% 'remainder = low byte
OUT BASADR + 2 * CN, XL% 'write low byte to D/A
OUT BASADR + 1 + 2 * CN, XH% 'write high byte & load D/A

TG2! = T(4) - T(14)
IF TG2! >= 1! THEN
TIMEON = ETIME
TON% = 1
D2 = 3000
ELSE
D2 = 0
END IF

IF TON% = 1 THEN
D2 = 3000
ELSE
D2 = 0
END IF

TCOOL = ETIME - TIMEON

IF TCOOL >= 30! THEN
TIMEON = ETIME
TON% = 0
ELSE
END IF

BASADR = &H300
CN = 1
XH% = INT(D2 / 256) 'work out high byte
XL% = D2 - 256 * XH% 'remainder = low byte
OUT BASADR + 2 * CN, XL% 'write low byte to D/A
OUT BASADR + 1 + 2 * CN, XH% 'write high byte & load D/A

'---------WATER PUMP CONTROLLING PROGRAM---------

PCHECK = ETIME - PUMPON
PRINT "THE TIME FOR NEXT WATER PUMPING IS": 6 - PCHECK / 3600!;
"HOURS"
IF PCHECK >= 6 * 60 * 60 THEN
'CALL PRUNT(ETIME, TCOM, PRUN)
PON% = 1
PMON% = 1
PUMPON = ETIME
ELSE
PON% = 0
END IF

PCHECK = ETIME - PUMPON
IF PMON% = 1 AND PCHECK < 48 THEN
PON% = 1
ELSE
PON% = 0
PMON% = 0
END IF

IF PON% = 1 THEN
D3 = 3000
ELSE
D3 = 0
END IF

BASADR = &H300
CN = 2
XH% = INT(D3 / 256)   'work out high byte
XL% = D3 - 256 * XH%   'remainder = low byte
OUT BASADR + 2 * CN, XL%   'write low byte to D/A
OUT BASADR + 1 + 2 * CN, XH%   'write high byte & load D/A

END SUB

SUB EXP16 (CH%, D())

'SUBROUTION TO GET THE THERMOCOUPLE DATE
'FIRST LOCK DASH-8 ON THE ONE CHANNEL THAT EXP-16 IS CONNECTED TO

LT%(0) = CH%: LT%(1) = CH%: MD% = 1
CALL DAS8(MD%, VARPTR(LT%(0)), FLAG%)
IF FLAG% <> 0 THEN PRINT "ERROR IN SETTING CHANNEL": END

'NEXT SELECT EACH EXP-16 CHANNEL IN TURN AND CONVERT IT.
'DIGITAL OUTPUTS OP1-4 DRIVE THE EXP-16 SUB-MULTIPLEXER ADDRESS, SO USE
'MODE 14 TO SET UP THE SUB-MULTIPLEXER CHANNEL.
FOR CHAN% = 0 TO 15 'NOTE USE OF INTEGER INDEX SUB%
MD% = 14
CALL DAS8(MD%, VARPTR(CHAN%), FLAG%) 'ADDRESS SET
IF FLAG% <> 0 THEN PRINT "ERROR IN EXP-16 CHANNEL NUMBER"; END
'NOW THAT CHANNEL IS SELECTED, PERFORM A/D CONVERSION USING MODE 4.
'TRANSFER DATA TO CORRESPONDING ARRAY ELEMENT D%(SUB%)
MD% = 4 'DO 1 A/D CONVERSION
CALL DAS8(MD%, VARPTR(TEST%), FLAG%)
D(CHAN%) = TEST%
IF FLAG% <> 0 THEN PRINT "ERROR IN PERFORMING A/D CONVERSION"
'NOW REPEAT SEQUENCE FOR ALL OTHER EXP-16 CHANNELS

NEXT CHAN%

'ALL DONE - RETURN FROM SUBROUTINE
END SUB

SUB GASMETER (O2, CO2, ETIME, GASON, gon%, GMON%)

DIM DIO%(0 TO 1)

GCHECK = ETIME - GASON
PRINT "THE TIME FOR NEXT O2 & CO2 MEASUREMENT IS"; 1800 -
GCHECK; "SECONDS"
IF GCHECK >= .5 * 60! * 60! THEN
gon% = 1
GMON% = 1
GASON = ETIME
ELSE
gon% = 0
END IF

GASCHECK = ETIME - GASON
IF GMON% = 1 AND GASCHECK < 40! THEN
gon% = 1
ELSE
gon% = 0
GMON% = 0
END IF
IF gon% = 1 THEN
D3 = 4000
ELSE
D3 = 0
END IF

BASADR = &H300
'02 AND CO2 METER
CN = 3
XH% = INT(D3 / 256) 'work out high byte
XL% = D3 - 256 * XH% 'remainder = low byte
OUT BASADR + 2 * CN, XL% 'write low byte to D/A
OUT BASADR + 1 + 2 * CN, XH% 'write high byte & load D/A

'IF GMON% = 1 AND GASCHECK = 24! THEN
MD% = 1
LT% (0) = 1
LT% (1) = 1
CALL DAS8(MD%, VARPTR(LT%(0)), FLAG%)
IF FLAG% <> 0 THEN PRINT "DASH8 SETTING ERROR"

'MEASURE O2 CONCENTRATION
MD% = 4
CALL DAS8(MD%, VARPTR(DIO%), FLAG%)
IF FLAG% <> 0 THEN PRINT "DASH8 D/A ERROR": END
O2 = .0179 * DIO% + 1.2912

MD% = 1
LT% (0) = 2
LT% (1) = 2
CALL DAS8(MD%, VARPTR(LT%(0)), FLAG%)
IF FLAG% <> 0 THEN PRINT "DASH8 SETTING ERROR"

'MEASURE CO2 CONCENTRATION
MD% = 4
CALL DAS8(MD%, VARPTR(DICO%), FLAG%)
IF FLAG% <> 0 THEN PRINT "DASH8 D/A ERROR": END
CO2 = -2.4558 + .00683 * DICO%

'ELSE
'END IF
END SUB

SUB HUMIDITY (T(), HUMI)

END SUB

SUB OUTPRINT (CT(), CJC!, T(), HUMI, ETIME, T1$, DI$, T$, D$, O2, CO2, MOIS)
CT(0) = ETIME
OUTTIME! = ETIME / 3600!
IF CT(0) - CT(1) >= 1800! THEN
CT(1) = CT(0)
'OPEN "LPT1:" FOR OUTPUT AS #2
'PRINT #2, USING "###.###"; OUTTIME!; T(4); T(6); T(7); T(8); T(9); T(10);
T(11); T(12); T(14); T(15); O2; CO2
'CLOSE #2
ELSE
END IF

IF CT(0) - CT(2) >= 1840! THEN
CT(2) = CT(0)
OPEN "A:\COMPOST1.DAT" FOR APPEND AS #3
WRITE #3, OUTTIME!, T(4), T(5), T(6), T(7), T(8), T(9), T(10), T(11), T(12),
T(13), T(14), T(15), O2, CO2, MOIS
CLOSE #3
ELSE
END IF

END SUB

SUB OUTSCREEN (CJC!, T(), HUMI, O2, CO2, MOIS)
FOR I = 4 TO 5
IF I = 4 THEN
PRINT USING "DRY TEMPERATURE = ######.## DEG. C"; T(I)
ELSE
PRINT USING "WET TEMPERATURE = ######.## DEG. C"; T(I)
END IF
NEXT I
FOR I = 6 TO 13
PRINT USING "TRAY ## TEMPERATURE = ######.## DEG. C"; I - 5; T(I)
NEXT I
PRINT USING "COLD JUNCTION TEMPERATURE = ######.## DEG. C"; CJC!
FOR I = 14 TO 15
IF I = 14 THEN
PRINT USING "OUTSIDE INCUBATOR TEMPERATURE = #######.## DEG. C"; T(I)
ELSE
PRINT USING "WATER BATH TEMPERATURE = #######.## DEG. C"; T(I)
END IF
NEXT I

PRINT USING "O2 CONCENTRATION = #######.## % VOL/VOL"; O2
PRINT USING "CO2 CONCENTRATION = #######.## % VOL/VOL"; CO2
PRINT USING "MOISTURE CONTENT = #######.## COUNTS"; MOIS
END SUB

SUB PRUNT (ETIME, TCOM, PRUN)

WATERP! = -106.23 + 20.8 * 12!
PRUN = ((WATERP! + 7.9943) / 3.1134)

END SUB

SUB TEMP (CJC!, V!, TC!, TF, NK%, SIK!, SVK!, TK())

'----- Interpolation routine to find K thermocouple temperature -----
' Entry variables: -
'  CJC = cold junction compensator temperature in deg. C.
'  V = thermocouple voltage in volts
' Exit variables: -
'  TC = temperature in degrees Centigrade
'  TF = temperature in degrees Fahrenheit
' Execution time on std. IBM P.C. = 46 milliseconds
' Perform CJC compensation for K type
' VK! = 1000! * V! + 1! + (CJC - 25) * .0405*VK in mV

' Find look up element
EK = INT((VK! - SVK!) / SIK!)

IF EK < 0 THEN
TC = TK(0)
GOTO BOUND  'Out of bounds, round to lower limit
ELSE
END IF
IF EK > 306 THEN
TC = TK(308)
GOTO BOUND  'Out of bounds, round to upper limit
ELSE
END IF
'Do interpolation
'Centigrade

TC! = TK(EK) + (TK(EK + 1) - TK(EK)) * (VK! - EK * SIK! - SVK!) / SIK!
BOUND:
TF = TC * 9 / 5 + 32 'Fahrenheit

END SUB