Intensive aerobic bioconversion of food waste into organic fertilizer

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Intensive Aerobic Bioconversion of Food Waste into Organic Fertilizer

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School of Civil & Environmental Engineering

A thesis submitted to the Nanyang Technological University
in fulfillment of the requirement for the degree of
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Summary

A popular way for recycling food waste is composting, a process to biologically stabilize and convert organic waste to useful material. However, composting of food waste in huge amount is always limited by the availability of land space. To overcome the drawbacks of composting process, an intensive aerobic bioconversion (IAB) technology was used to accelerate the stabilization and bioconversion of food waste into organic fertilizer in a reactor under controlled conditions of aeration, stirring and pH, at a temperature of 60°C.

Bench scale batch bioconversions were first conducted to understand the basic chemical and microbiological reactions in the IAB process of food waste. It was found that low pH in the beginning stage of the bioconversion inhibited the growth of thermophilic bacteria and thus slowed down the biodegradation. The addition of pH buffer such as CaCO_3 was essential as it provided the favourable pH condition for biodegradation activities of thermophilic bacteria. Meanwhile, the introduction of starter cultures, SWO9 and SW25, which were *Bacillus thermoamylovorans*, have successfully enhanced the IAB process of food waste by increasing cell contents of thermophilic bacteria to the optimum levels. The IAB process of food waste from different sources was also studied. Fruit waste was found not suitable for the IAB process because of its excessive acid production and low nitrogen content. Soybean residue was a good source of food waste as it produced high nitrogen organic fertilizer. However, co-bioconversion of soybean residue with other waste was required to increase the stability of the end product and reduce nitrogen loss. The bench scale IAB process successfully bioconverted food wastes into organic fertilizers in 5 days.

In the pilot scale batch bioconversion, it was proven that the big scale UB process was able to stabilize and bioconvert food waste into organic fertilizer in 5 to 6 days. The role of horticultural compost in the pilot scale batch bioconversion was

investigated. Horticultural compost, as a bulking agent, not only improved aeration by increasing pore volume inside the mixture, but also reduced nitrogen loss by providing carbon source to balance the carbon to nitrogen (C/N) ratio. On the other hand, because of the diversity of microorganisms it carried, horticultural compost was also used as a potential supplier of thermophilic bacteria to the bioconversion. Another possible function of horticultural composting during the bioconversion was pH buffering by physically increasing the volatilization of organic acids and releasing certain pH buffering chemicals. Through a first order kinetic study, it was found that, with horticultural compost, starter cultures (SW09 and SW25) accelerated the biodegradation of organic matter in the second phase of biodegradation. Thus the earlier stabilization of end product was achieved. The products from the IAB processes with horticultural compost, reached the required maturity in 5 to 6 days. The mature end product was used as organic fertilizer to improve plant yield at a rate up to 4% (of wet soil weight) without any adverse phytotoxicity.

Experimental results of the semi-continuous operation of IAB process, usually named as fed batch operation, revealed that the fed batch operation had practical advantage over the batch operation since the time needed for microorganisms to proliferate was reduced. However, the product from fed batch IAB process was required to undergo a batch bioconversion for further stabilization once the periodical feeding was ceased.

In general, the IAB process was able to stabilize and bioconvert food waste into organic fertilizer in less than a week. Horticultural compost included in the IAB process of food waste reduced nitrogen loss, provided the necessary microorganisms and improved the stability of end products.

Table of Contents

ACKNOWLEDGEMENT	i
SUMMARY	ii
TABLE OF CONTENTS	iv
LIST OF FIGURES	xii
LIST OF TABLES	xv

CHAPTER 1 INTRODUCTION

1.1 Background	1
1.2 Objectives and Scopes	3
1.3 References of Chapter 1	3

CHAPTER 2 LITERATURE REVIEW

2.1 Composting and Compost	5
2.1.1 An Overview of Composting Process	5
2.1.2 Impact of Compost at Different Status and Fresh Organic Materials on Soils and Plants	8
2.1.3 Functions and Utilizations of Compost	11
2.1.3.1 Compost as Amendment/Conditioner/Improver	11
2.1.3.2 Compost as a Fertilizer	13

2.1.3.3 Application Rate of Compost	13
2.2 Composting Science	14
2.2.1 Moisture Content	14
2.2.2 Carbon to Nitrogen Ratio (C/N)	14
2.2.3 pH Value	15
2.2.4 Microorganisms and Change of Temperature	18
2.2.5 Oxygen and Aeration	20
2.2.6 Particle Size and Bulking Agent	20
2.3 Traditional Composting Technologies	20
2.3.1 Windrow Composting	21
2.3.2 Aerated Static Pile Composting	23
2.3.3 In-Vessel Composting Systems	24
2.4 Operation Modes of Composting Process	26
2.4.1 Batch Mode Operation	26
2.4.2 Fed-batch Mode Operation	26
2.5 Enhancements of In-Vessel Composting Process	27
2.5.1 Co-composting: Way to Balance the Nutrient Ratio	28
2.5.2 Seeding Effect	30
2.5.2.1 No Effect of Seeding	31
2.5.2.2 Positive Effect of Seeding	31
2.5.3 Use of Thermophilic Degradation Stage	34
2.6 References of Chapter 2	37

CHAPTER 3 MATERIALS AND METHODS

3.1 Raw Materials	44
3.1.1 Food Waste	44
3.1.2 Horticultural Compost	44
3.1.3 Characteristics of Raw Materials	45
3.2 Starter Culture	46
3.2.1 Isolation Source and Procedure	47
3.2.2 Screening and Selection Procedure	47
3.2.3 Phylogenetic Identification of the Strains	48
3.3 Analytical Methods	50
3.3.1 Chemical Analysis	50
3.3.1.1 General Chemical Properties Analysis	50
3.3.1.2 Macro Element Analysis	50
3.3.1.3 Inorganic Nitrogen	50
3.3.2 Biological Analysis	51
3.3.2.1 Microbiological Enumeration	51
3.3.2.2 Phytotoxicity Test	51
3.3.2.3 Stability Test	51
3.3.3 Scanning Electron Microscopy Preparation	52
3.4 Bench Scale Reactor	53
3.5 Pilot Scale Reactor	55
3.6 References for Chapter 3	58

CHAPTER 4 RESULTS AND DISCUSSIONS:

BENCH SCALE BATCH BIOCONVERSION

4.1 Preliminary Study	60
4.1.1 Experimental Design	60
4.1.2 Results and Discussions	61
4.2 Bench Scale Batch Bioconversion (Phase 1)	62
4.2.1 Bench Scale Batch Bioconversion 1(BSBB1)	62
4.2.1.1 Experimental Design	62
4.2.1.2 Results and Discussions	63
4.2.2 Bench Scale Batch Bioconversion 2 (BSBB2)	65
4.2.2.1 Experimental Design	65
4.2.2.2 Results and Discussions	66
4.2.2.3 Scanning Electron Microscopy (SEM)	68
4.2.2.4 Plant Cultivation Test	71
4.3 Bench Scale Batch Bioconversion (Phase 2)	72
4.3.1 Bench Scale Batch Bioconversion 3 (BSBB3)	73
4.3.1.1 Raw Materials and Experimental Design	73
4.3.1.2 Results and Discussions	74
4.3.2 Bench Scale Batch Bioconversion 4 (BSBB4)	76
4.3.2.1 Raw Materials and Experimental Design	76
4.3.2.2 Results and Discussions	77
4.4 References of Chapter 4	79

CHAPTER 5 RESULTS AND DISCUSSIONS:

PILOT SCALE BATCH BIOCONVERSION

5.1 Pilot Scale Batch Bioconversion 1 (PSBB1)	80
5.1.1 Raw Material and Experimental Design	80
5.1.2 Results and Discussions	82
5.2 Pilot Scale Batch Bioconversion 2 (PSBB2)	84
5.2.1 Raw Material and Experimental Design	84
5.2.2 Results and Discussions	85
5.3 Pilot Scale Batch Bioconversion 3 (PSBB3)	88
5.3.1 Raw Materials and Experimental Design	88
5.3.2 Results and Discussions	89
5.4 Comparison of Pilot Scale Batch Bioconversions	92
5.4.1 pH	92
5.4.2 Thermophilic Bacteria	93
5.4.3 Stability Index	93
5.4.4 Nitrogen Loss	94
5.4.5 First Order Kinetic Constants and Maximum Degradation Rates	96
5.5 References of Chapter 5	104

CHAPTER 6 RESULTS AND DISCUSSIONS:

PILOT SCALE FED BATCH BIOCONVERSION

6.1 Experimental Design	105
6.2 Results and Discussions	107
6.2.1 Experimental Data	107
6.2.2 Calculated Data	110
6.2.2.1 VS Mass Reduction	110
6.2.2.2 Carbon Mass Reduction	112
6.2.2.3 Nitrogen Mass Loss	114
6.2.2.4 First Order Kinetic Constants and Maximum Degradation Rates of VS	116
6.3 References of Chapter 6	118

CHAPTER 7 RESULTS AND DISCUSSIONS:

EVALUATION OF END PRODUCTS

7.1 Maturity Index	120
7.2 Fertility	124
7.2.1 Plants Selected for Cultivation	124
7.2.2 Characteristics of End Products	124
7.2.3 Characteristics of Soil	125
7.2.4 Plant Cultivation Program and Procedure	125

7.2.5 Results and Discussions	128
7.2.5.1 Effect of End Products on Lengths of Plant Stems and Roots	128
7.2.5.2 Effect of End Products on Surface-Dry Weights of Plant Stems and Roots	131
7.2.5.3 Effect of Phosphorous Deficiency of End Products on Plant Growth	132
7.2.6 Summary	135

CHAPTER 8

CONCLUSIONS AND RECOMMENDATION

8.1 IAB, Starter Cultures and Horticultural Compost	136
8.2 Issues Related to IAB Process	136
8.2.1 Low pH in the Early Stage of IAB Process	136
8.2.2 Excessive Nitrogen Loss during IAB Process	137
8.2.3 Low Stability of End Products	138
8.3 Bench Scale Batch Bioconversion	138
8.4 Pilot Scale Batch Bioconversion	139
8.5 Pilot Scale Fed Batch Bioconversion	139
8.6 Maturity and Fertility of IAB Products	140
8.7 Overall Conclusion	140

8.8 References in Chapter 8	140
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List of Figures

Figure 2-1 Mass and Energy Balances: an Overview of Composting Process	6
Figure 2-2 A Simplified Working Flow Chart of a Typical Composting Process	7
Figure 2-3 A Representation of a Typical pH Curve in a Batch Composting Process	16
Figure 2-4 Temperature Change in Different Phases of Composting Process	19
Figure 2-5 Schematic Drawings of Three Traditional Composting Technologies	21
Figure 2-6 Typical Windrow Composting with an Elevating Face Windrow Turner	22
Figure 2-7 Typical Aerated Static Pile for Composting MSW	24
Figure 3-1 Changes of Cell Number with Time of the Different Strains in the Liquid Medium with Sewage Sludge	48
Figure 3-2 Schematic Drawing of Bench Scale Reactor	54
Figure 3-3 Photo of the Bench Scale Reactor	54
Figure 3-4 Photos of the Pilot Scale Reactor	56
Figure 3-5 Schematic Drawing of Pilot Scale Reactor	57
Figure 4-1 Variation of Experimental Data of Preliminary Flask Test	61
Figure 4-2 Variation of pH and Cell Content of Thermophilic Bacteria in BSBB1	64
Figure 4-3 Variation of VS Content and Stability in BSBB1	64
Figure 4-4 Variation of pH and Cell Contents of Thermophilic and Mesophilic Bacteria in BSBB2	67
Figure 4-5 Variation of VS Content and VS Loss in BSBB2	67
Figure 4-6 SEM Image of Fresh Food Waste in BSBB2 at 1000 Magnification	69
Figure 4-7 SEM Image of Day 2 Sample in BSBB2 at 1000 Magnification	69
Figure 4-8 SEM Image of Day 4 Sample in BSBB2 at 1000 (a), 2500(b) and 5000 (c) Magnifications	71
Figure 4-9 Images of Plants in B2F Plant Cultivation Test	72
Figure 4-10 Variation of VS Content and VS Mass Loss in BSBB3	75
Figure 4-11 Variation of VS Content and VS Mass Loss in BSBB4	77
Figure 4-12 Variation of pH, Stability and Cell Content of Thermophilic Bacteria in BSBB4	78
Figure 5-1 Variation of VS Content and VS,C and N Mass Loss in PSBB1	82
Figure 5-2 Variation of pH and Cell Content of Thermophilic Bacteria in PSBB1	83
Figure 5-3 Variation of Different Forms of Nitrogen in PSBB1	84

Figure 5-4 Variation of Stability Index in PSBB1	84
Figure 5-5 Variation of VS Content and VS, C and N Mass Loss in PSBB2	86
Figure 5-6 Variation of pH and Cell Content of Thermophilic Bacteria in PSBB2	87
Figure 5-7 Variation of Different Forms of Nitrogen in PSBB2	88
Figure 5-8 Variation of Stability Index in PSBB2	88
Figure 5-9 Variation of VS Content and VS, C and N Mass Loss in PSBB3	90
Figure 5-10 Variation of pH and Cell Content of Thermophilic Bacteria in PSBB3	91
Figure 5-11 Variation of Different Forms of Nitrogen in PSBB3	92
Figure 5-12 Variation of Stability Index in PSBB3	92
Figure 5-13 Comparison of pH Changes in Three Pilot Scale Batch Bioconversions	93
Figure 5-14 Comparison of Cell Content of Thermophilic Bacteria in Three Pilot Scale Batch Bioconversions	94
Figure 5-15 Comparison of Stability of Samples in Three Pilot Scale Batch Bioconversions	94
Figure 5-16 Comparison of N Loss in Three Pilot Scale Batch Bioconversions	95
Figure 5-17 Comparison of NH_3 Amount in Three Pilot Scale Batch Bioconversions	96
Figure 5-18 Comparison of VS Mass Loss in Three Pilot Scale Batch Bioconversions	98
Figure 5-19 Comparison of Food Waste VS Mass Loss in Three Pilot Scale Batch Bioconversions	98
Figure 5-20 Plotting of $\ln(S/S_0)$ vs. time for Each Phase of Three Pilot Scale Batch Bioconversions	102
Figure 6-1 Feeding Schedule of Compost and Food Waste In Pilot Scale Fed Batch Experiment (Wet Weight)	106
Figure 6-2 Feeding Schedule of Compost and Food Waste in Pilot Scale Fed Batch Experiment (Dry Weight)	107
Figure 6-3 Variation of pH, MC and Cell Content of Thermophilic Microorganisms in Pilot Scale Fed Batch Experiment	108
Figure 6-4 Variation of Stability in Pilot Scale Fed Batch Experiment	109
Figure 6-5 Degradation Pattern of VS in Pilot Scale Fed Batch Experiment	112
Figure 6-6 Bioconversion Pattern of C in Pilot Scale Fed Batch Experiment	114
Figure 6-7 Loss Pattern of N in Pilot Scale Fed Batch Experiment	116
Figure 6-8 First Order Kinetic Analysis: $\ln(S/S_0)$ vs. Time of Four Stages of Pilot Scale Fed Batch Experiment	118
Figure 7-1 G-1 Compost Maturity Assessment Process	121
Figure 7-2 G-2 Maturity Assessment Matrix	123
Figure 7-3 Effect of EP1 on Lengths of Plant Stems and Roots	129

Figure 7-4 Images of Plants Cultivated in Subsoil Amended with EP1	129
Figure 7-5 Effect of EP2 on Lengths of Plant Stems and Roots	130
Figure 7-6 Images of Plants Cultivated in Subsoil Amended with EP2	130
Figure 7-7 Effect of EP3 on Lengths of Plant Stems and Roots	130
Figure 7-8 Images of Plants Cultivated in Subsoil Amended with EP3	131
Figure 7-9 Effect of EP1 on Surface-Dry Weights of Plant Stems and Roots	131
Figure 7-10 Effect of EP2 on Surface-Dry Weights of Plant Stems and Roots	132
Figure 7-11 Effect of EP3 on Surface-Dry Weights of Plant Stems and Roots	132
Figure 7-12 Effect of Phosphorous Deficiency of EP1 on Stem and Root Length of Plants	133
Figure 7-13 Effect of Phosphorous Deficiency of EP1 on Stem and Root Weights of Plants	133
Figure 7-14 Effect of Phosphorous Deficiency of EP3 on Stem and Root Length of Plants	134
Figure 7-15 Effect of Phosphorous Deficiency of EP3 on Stem and Root Weights of Plants	134

List of Tables

Table 2-1 Temperature and Time Interval Required to Destroy Most Common Types of Pathogenic Microorganisms and Parasites Occasionally Present in Wastes	19
Table 3-1 Characteristics of Raw Materials	46
Table 3-2 Working Parameters of the Pilot Scale Reactor	58
Table 4-1 Parameters during BSBB1	63
Table 4-2 Parameters during BSBB2	66
Table 4-3 Planting Test Result of BSBB 2	72
Table 4-4 Composition of Initial Mixture in BSBB3	74
Table 4-5 Characteristics of Raw Materials in BSBB3	74
Table 4-6 Chemical and Biological Characteristics of Intermediate and End Products in BSBB3	75
Table 4-7 Composition of Initial Mixture in BSBB4	76
Table 4-8 Characteristics of Raw Materials in BSBB4	76
Table 4-9 Chemical and Biological Characteristics of Intermediate and End Products in BSBB4	77
Table 5-1 Composition of Initial Mixture in PSBB1	81
Table 5-2 Characteristics of Raw Material in PSBB1	81
Table 5-3 Chemical and Biological Characteristics of Intermediate and End Products in PSBB1	82
Table 5-4 Composition of Initial Mixture in PSBB2	85
Table 5-5 Characteristics of Raw Material in PSBB2	85
Table 5-6 Chemical and Biological Characteristics of Intermediate and End products in PSBB2	86
Table 5-7 Composition of Initial Mixture in PSBB3	89
Table 5-8 Characteristics of Raw Material in PSBB3	89
Table 5-9 Chemical and Biological Characteristics of Intermediate and End products in PSBB3	90
Table 5-10 First Order Kinetic Constants and Maximum Degradation Rates of Organic Matter	99
Table 6-1 Feeding Schedule of Compost and Food Waste in Pilot Scale Fed Batch Experiment	106
Table 6-2 Experimental Results Measured in Pilot Scale Fed Batch Experiment	107
Table 6-3 VS Reduction in Pilot Scale Fed Batch Experiment	111
Table 6-4 C Mass Reduction in Pilot Scale Fed Batch Experiment	113

Table 6-5 N Mass Loss in Pilot Scale Fed Batch Experiment	115
Table 6-6 First Order Kinetic Constants and Maximum Degradation Rates of VS in Pilot Scale Fed Batch Experiment	116
Table 7-1 G-1 Compost Maturity Index	120
Table 7-2 G-2 Compost Maturity Index Parameters	122
Table 7-3 G-3 Stability Indicator Thresholds Using Respirometry	122
Table 7-4 G-4 Maturity Indicator Thresholds	123
Table 7-5 Chemical and Biological Characteristics of End Products	124
Table 7-6 Chemical and Physical Characteristics of Soil	125
Table 7-7 Planting Program	126
Table 7-8 Physical Properties of Plants	128

Chapter 1

Introduction

1.1 Background

In Singapore, solid waste output had increased six folds from 1,260 tonnes per day in 1970 to 7,680 tonnes per day in 2001. If the waste output continues to increase at this rate, Singapore would need to build a new incineration plant every 5 to 7 years and a new landfill every 25 to 30 years. Due to restriction of land space availability in Singapore, it will be impossible to build new incineration plants years after years. The sustainable solution to this is to close the solid waste loop by minimizing waste and maximizing recycling.

Disposal of municipal solid waste (MSW) in Singapore amounted to approximately 2.8 million tonnes in 2001 (NEA Singapore Web Site, 2002). Of which, 18% was food waste collected from food companies, restaurants, food courts, markets and households. Food waste was only recycled at a low rate of 6% during the past 3 years. The recycled food waste was mainly used for feeding animal in livestock farms. The rest ended up at the incineration plants.

One of the popular ways for recycling food waste is composting. Composting is a natural aerobic biochemical process in which mesophilic and thermophilic microorganisms transform organic materials into a stable, soil-like product. The process is well established and has been comprehensively documented (Rynk, 1992; Haug, 1993; Eliot, 1997).

Composting process fulfils several waste management purposes: stabilization, volume reduction, and sanitation by thermal inactivation of pathogens. The aim of the stabilization is to produce a material that does not putrefy, self-heat, deplete oxygen, produce odours or attract vermins. Composting product can be beneficial by supplying nutrients for plant growth, organic matter for soil improvement and agents for plant disease suppression.

Composting technologies normally include three traditional types: windrow, aerated static pile, and in-vessel composting. Sorting, screening, and curing are

common supporting processes to the three composting methods. Additional odour control is always required to remove the offensive odours.

Operation duration of these traditional composting technologies is generally several months. Windrow and aerated static pile composting processes always take several months. In-vessel composting process requires the shortest time of up to one month, depending on the type of organic wastes and control factors such as intensity of aeration and moisture content. In terms of land space requirement, windrow and aerated static pile composting processes are suitable for countries or cities having plenty of spare land. In-vessel composting process is a feasible choice for the countries with scarce land resource, for instance, Singapore.

In-vessel composting systems enclose the feedstock into a chamber or vessel that provides adequate mixing, aeration, and moisture. The vessels are usually placed in a building. These systems, if properly operated, produce minimal odours and little or no leachate.

Based on the in-vessel composting technologies and the common microbiological and physico-chemical knowledge of the composting process, several improved composting technologies such as intensive aerobic bioconversion (IAB) technology (Wang, 2003 a, b) were developed. The IAB technology was initially developed and used successfully to convert sewage sludge and food waste into organic fertilizer. A bench scale reactor running under controlled conditions of aeration, stirring and pH, at a temperature of 60°C was employed to convert sewage sludge and food waste into organic fertilizer. The addition of a starter bacteria culture *Bacillus thermoamylovorans* improved the bioconversion process. The best organic fertilizer was obtained when sewage sludge was thermally pretreated and mixed with food waste, pH buffer CaCO_3 and an artificial bulking agent. In the IAB process, the content of volatile solids (VS) and carbon (C) decreased from 82.8% to 62.3% and from 37.7% to 32.5% of total solid (TS), during the 12 days of bioconversion. The stable organic fertilizer produced was a powder with moisture content less than 5% of TS, containing 3.4% of nitrogen (N), 0.4% of phosphorous (P) and 2.9% of potassium (K). The addition of 10 to 15 g of this fertilizer to 1 kg of poor fertility soil increased the growth of different plants by 113-164%.

However, the existence of high heavy metal content and pathogens in the sewage sludge may limit the use of sewage sludge as a raw source of organic fertilizer. At present, because of the lack of reliable technology to remove heavy metals from

sewage sludge, it is believed that the local government prefers using food waste only rather than involving sewage sludge.

1.2 Objectives and Scopes

This research project set out to investigate the IAB process of food waste into organic fertilizer and explored some effective approaches to improve the biodegradation of food waste. The IAB process must be performed as a thermophilic phase without additional curing and maturation as required by the traditional composting process.

The scopes of this research included:

- ✚ study of the IAB process of food waste in a bench scale reactor;
- ✚ study of the IAB process of food waste in a pilot scale reactor;
- ✚ investigation of the effect of horticultural compost as a bulking agent and a source of microorganisms on both bench and pilot scale IAB process of food waste;
- ✚ verification of the effect of bacterial starter culture on both bench and pilot scale IAB process of food waste;
- ✚ exploration of the feasibility of fed-batch operation of pilot scale IAB process of food waste;
- ✚ examination of the end product properties including chemical contents, maturity and fertility.

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Chapter 2

Literature Review

Organic waste is not only the result of human activities. Since the beginning of life on the earth, living organisms have produced waste, and other living organisms have used that waste by utilizing the energy and nutrients it contains. Recycling of organic waste is thus an integrated function of any ecosystem.

Composting is one of these processes in which the naturally occurring ability of organisms to recycle organic waste is used for the benefit of humans in the accelerated degradation of organic waste. With increased knowledge of how this process works, we can better control it and make it work more efficiently. Based on such understanding, a possible intensive bioconversion technology of organic waste, such as food waste, can be developed to accelerate the natural process.

This literature review firstly gives an overall description of the composting process and compost, secondly puts the emphasis on the science of the complex process interaction between the biology and physics. Then, it introduces the traditional composting technologies, describes the two operation modes of composting process and lastly reviews the most adopted enhancing measures of composting process.

2.1 Composting and Compost

2.1.1 An Overview of Composting Process

Composting is a natural, aerobic and biochemical process in which thermophilic microorganisms transform organic materials into a stable, soil-like product. The process is well established and has been comprehensively documented in several books (Rynk, 1992; Haug, 1993; Eliot, 1997).

Composting is environmentally preferable. It fulfils several waste management

purposes: stabilization, volume reduction and sanitation by thermal inactivation of pathogens. The aim of the stabilization is to produce a material that does not putrefy, self-heat, deplete O_2 , produce odours or attract vermin. The compost product can be beneficial, supplying nutrients for plant growth, organic matter for soil improvement and agents for plant disease suppression. In spite of these, there are several environmental issues to consider in composting. Ammonia emissions which can be considerable, contributes to atmospheric acidification and eutrophication of surface water body. The greenhouse gases methane and nitrous oxide are normally formed during composting in small amounts. Composting can also produce unpleasant odours.

Composting process is commonly described as aerobic degradation of organic matter where heat is released by the O_2 -consuming microbial metabolism, resulting in increased temperature (Figure 2-1). A composting system is dynamic, with very intense biological activity. This causes the system to change its own environmental conditions. Most notable is the increasing temperature. Equally important is the consumption of O_2 and production of CO_2 . In the active compost, the O_2 in the pore space is consumed within minutes, so a continuous supply of fresh air is crucial for the process to remain aerobic.

The carbon, chemical energy, protein, and water in the finished compost is less than that in the raw materials. The finished compost has more humus. The volume of the finished compost is 50% or less of the volume of raw material.

Figure 2-1 Mass and Energy Balances: an Overview of Composting Process
(Rynk et al., 1992)

It is a microbial process, and the overall performance of the composting process is therefore the combined effect of the activities of various microorganisms. It is thus

important to understand and control the environmental factors that affect microbial life in composts.

In any composting process, the usual physical treatments of raw material such as sorting, shredding and mixing are normally required (Figure 2-2). Not all organic wastes can be biodegraded during composting. Plastic and textile are always considered non-biodegradable. Sometimes raw solid waste contains directly recyclable materials such as metal cans. In such cases sorting is a necessary step to remove plastic, textile and metal materials from raw materials. The particle size of raw material occurs always in a wide range varying from several meters in length to few millimetres in diameter. Homogeneity and smallness in size always make biodegradation of organic matters easier. On the other hand, proper compactness in densities will reduce the occupied place by the raw material. Hence, shredding is required. After shredding, mixing of raw material is followed because of the non-even distribution of nutrient composition in the raw material.

Figure 2-2 A Simplified Working Flow Chart of a Typical Composting Process

After going through the stage of active composting, the intermediate product or immature compost is further processed in the subsequent curing process. The curing period allows decomposition to proceed for further completion and stabilization. During this period, the compost should be tested periodically to ensure that it meets

applicable quality standards for final use (Canadian Council of Ministers of the Environment, 1996).

This is important because compost should provide nutrients to plant and humus to soil, without threatening the life or health of plants or animals (Mathur et al., 1993). The curing time required to produce good quality compost depends on the material involved and could be as short as a few months or as long as two years.

Screening is used to help separate the compost from the bulking agent, which is large in size and has a great surface to volume ratio. The bulking agent not only provides the microorganisms with a platform to actively degrade the organic matter but also increases the air space between organic matter particles to provide good ventilation. Non-easily biodegradable or lignin-cellulose rich materials such as wood chips, saw dust and wheat straw can be used as bulking agents.

Those bulking agents after separated from the compost can be recycled and participate in new round of composting. Such kinds of recycled bulking agents have an advantage over new bulking agents because they carry the seed for a new round of composting process. The high population and wide diversity of microorganisms in the recycled bulking agent can accelerate the composting process of new round.

The screened end product of the compost is either directly used or packaged for storage after dewatering to low moisture contents. Low moisture contents will prevent the reactivation of microorganisms in the compost. With enough moisture, some microorganisms will anaerobically convert organic matter into methane, CH_4 , which can cause fire if allowed to reach certain levels. The compost can also release NH_3 , a source of offensive odours. The proper control of moisture content before storing compost product is thus normally required.

2.1.2 Impact of Compost at Different Status and Fresh Organic Materials on Soils and Plants

Compost may be appropriately defined as "the stabilized and sanitized product of composting, which is still in the process of stabilization and is beneficial to plant growth". According to this definition, the organic matter in the product of composting must have been degraded to fine particles, have lost its original identity and been

extensively stabilized to a humus-like product, which can be applied on the soil without damage to plants (Zucconi and De Bertoldi, 1987).

Products used in agriculture taken from various stages of the composting process exhibit different properties and behaviours in soil.

"Fresh compost" may be defined as an intermediate product of the thermophilic stage, which has achieved sanitization, where necessary, and has undergone partial decomposition, but is not yet stabilized. This product may be used in agriculture with further decomposition and stabilization occurring in the soil with beneficial effects on soil structure improvement, increased microbial activity, and gradual release of nutrients through mineralization.

"Mature compost" is the end-product of the stabilization stage and can be considered as general purpose organic fertilizer which is fully suitable for application on the soil even in the presence of standing crops. Direct contact with roots should be avoided for products derived from substrates such as urban refuses, particularly in pot cultures, because it may cause a temporary delay in the growth of plants (Zucconi and De Bertoldi, 1987).

Finally, "cured compost" is defined as the highly stabilized product resulting from a prolonged stabilization and mineralization stage, beyond that of commonly accepted maturity. This material is often considered as a humus-like substitute possessing a high value for the preparation of artificial substrates used in direct contact with root systems in nursery-protected crops, potting, and flower cultivation.

Organic material without going through the thermophilic stage is generally unsuitable for agricultural use for several reasons (Senesi, 1989).

At any given time and condition, a dynamic equilibrium state characterizes the microbiological activity and the organic matter evolution level in any soil. This situation can be altered to various extents by the addition of fresh organic materials of different nature and origins, with beneficial or adverse effects on soil organic fertility. The introduction of organic substances into the soil causes an initial variation of both the content and quality of total organic matter present in the soil system. Such variation includes modification of all physical, chemical and microbiological equilibriums and reactions. Organic carbon may arrive into the soil in the form of either fresh, undecomposed wastes, or partially-decomposed/transformed materials, or "well" decomposed materials, in an advanced state of transformation and stabilization.

A vast amount of data available in the literature indicate that organic additives of

the fresh, undecomposed wastes, or partially-decomposed/transformed materials, generally exert more adverse than beneficial effects on soil organic fertility by a number of deleterious modifications on physical, chemical or biological soil properties, while the addition of stabilized, partly humified materials seems to affect soil properties beneficially (Stevenson, 1982).

An important effect of the addition of easily decomposable organic residues to soil is the modification of its normal biological activity at equilibrium and the broadening of the spectrum of soil microbial activity. This results in the immediate increase of the metabolism of the soil biomass with the production of enzymes which not only degrades the added residues, but also attacks the native soil organic matter at equilibrium. This effect results in a change of the decomposition rate of the native soil organic matter measured in terms of CO_2 evolution and is referred to as the "priming action" (Stevenson, 1982).

The availability of nutrients such as nitrogen (N), phosphorus (P) and sulphur (S) of organic waste is closely related to its maturity. A stabilized organic material, well balanced in nutrients, i.e. with low C/N, C/P and C/S ratios, will support slow rate of nutrient release. And fresh, labile materials, where nutrient release rates are high will cause a limitation of growth of microbial population, therefore, retard or even stop decomposition and transformation, unless nitrogen can be gardened from another source in the system (Simpson, 1983). The microorganisms will thus compete with higher plants for available nitrogen in the soil and nitrogen deficiency will occur in plants.

Anaerobic conditions may be induced in soils, with a lack of O_2 in the rhizosphere, by mineralization of large amounts of non-stabilized organic carbon with extensive O_2 consumption and CO_2 production. If O_2 supply is insufficient, soil microorganisms are forced to utilize progressively weaker electron acceptors (oxidants), such as NO_3^- , Fe^{3+} , SO_4^{2-} and H^+ . These conditions can result in increased denitrification, reduced nitrification, nitrogen-mineralization, and formation of phytotoxic compounds such as NO_2^- , H_2S , CH_4 and C_2H_2 (Clapp et al., 1986).

Application of stabilized organic additives generally has a favourable effect in increasing the pH-buffering capacity of soil, while application of not well-stabilized organic materials may result in alterations of soil pH, with beneficial or adverse effects in dependent on the natural soil pH (Clapp et al., 1986).

Seed germination can be inhibited and root elongation depressed in various crops

by addition of fresh organic material or immature compost to soil. It is probably due to the production of high amounts of ammonia and ethylene oxide, increased temperature, decreased available O₂, heavy metal additions and other effects (Gallardo Lara and Nogales, 1987). However, no negative effect appears, if the dosage of fresh organic waste or immature compost applied is low, or mature compost is used (Gallardo Lara and Nogales, 1987).

Other undesirable effects related to the use of non-stabilized, immature organic materials may occur, including the addition and/or production of low molecular weight acids toxic to plants and microorganisms, phytotoxins and other organic compounds at phytotoxic levels, allelopathic chemicals, plant and animal pathogens, soluble salts, toxic heavy metals and, finally, of certain beneficial micronutrients which may be raised to toxic levels for organisms (Senesi, 1989).

2.1.3 Functions and Utilizations of Compost

Compost has two functions: the main function as soil amendment, soil conditioner or soil improver; and the other function as soil fertilizer. It is why compost is called an organic fertilizer. Compost and organic fertilizer are often two interchangeable terms.

2.1.3.1 Compost as Soil Amendment/Conditioner/Improver

Compost is always used as soil amendment, conditioner or improver because compost as an organic matter resource improves the general properties of soils. The function of compost is mostly attributed to the characteristics of the organic matter in the compost. Soil organic matter content can be increased through frequent repeated applications of compost.

Occurrence and definition of organic matter

Organic matter is commonly the sum of substances containing organic carbon (Schnitzer, 1991), and in the soil science, defined as the total organic components including undecayed plant and animal tissues, their partial decomposition products, and the soil biomass exclusive of the macro fauna and macro flora (Vaughan et al., 1985).

The organic matter after composting is very high in humus substances. Humus

makes up a large fraction of organic matter and is important in soil ecology, soil fertility and soil structure. Humus is a material comprised of many organic substances and represents a state during the decomposition of organic materials. It is in a semi-finite state of decomposition (the finite state is ash) and is characterized by a very slow decomposition rate (Eliot, 1997).

Organic matter or humus consists of two broad categories known as non-humic and humic substances. The non-humic groups are simple compounds such as carbohydrates, aliphatic and aromatic hydrocarbons, amino acids, ethylene, and hydrogen sulphide that are easily degraded by soil organisms. In contrast, the humic fraction is made up of complex organic molecules, usually formed as by-products of decomposition and is resistant to further degradation. The two stable components of humic substances that play a dominant role in soil physical properties are humic and fulvic acids. These weak acids present in organic waste and compost are suggested to be chemically and structurally similar to humic substances in soil (Sposito et al., 1982).

The proportion of humus within compost increases with compost stability. In general, the relative proportion of humic carbon to total organic carbon content of organic matter increases as the compost stabilizes. This relationship varies with the nature of the raw materials used to form the compost. Raw materials high in lignin usually yield greater amounts of humus than materials poor in lignin.

Functions of Organic matter

Organic matter is an important reservoir of carbon and a dynamic component of soil and carbon cycle. It has impacts on the physical, chemical and biological properties of a soil.

Addition of organic matter to soil alters its physical characteristics by changing available soil water retention, infiltration, drainage and aeration. Structural parameters are optimized for plant growth by lowering soil bulk density, increasing water holding capacity and aeration.

Chemically, the soil nutrient status or nutrient carrying capacity is enhanced by additional organic matter such as organic matter of compost. Biologically, an enhanced soil organic matter fraction serves as a rich nutrient reservoir and energy source for beneficial microbes. The chemical and biological properties of soils are related to one of compost functions as fertilizer.

Physical effect of organic matter on soils

Physical effect of organic matter on soil include improved soil structure, increased aeration, and increased water holding capacity and decreased density. These physical modifications to soil structure modify conditions for root development. Enhanced root development improves water use efficiencies and nutrient uptake.

Most agricultural cropping systems return relatively low amount of organic matter to soil as crop residues. Soil structure is damaged under continuous cropping systems. Over time, this reduces root penetration and development, and soil aeration. Crop yields are negatively affected by the decreased soil aeration and drainage, due to the depletion of organic matter and increase in soil bulk density. Compost amended to such soil systems can reverse many negative factors associated with intensive crop production.

2.1.3.2 Compost as a Fertilizer

Compost's fertilizer function is related to the chemical property of soils. Organic matter acts as both a sink and source in the soil system. It is a large pool for storage of nitrogen (N), phosphorous (P) and sulphur (S), and can supply nutrients for plant growth. Mineralization of organic matter by microorganisms releases nitrogen (N), phosphorous (P) and sulphur (S) to plants. The mineralization of organic matter in grassland soils has contributed to much of the nitrogen and phosphorous nutrition of crops (Tiessen and Stewart, 1983).

Organic matter can be partitioned into fresh, slightly humified, and humified state of decomposition (Conti et al., 1993). The humified organic matter is chemically stable and mature (that is, free of organic phytotoxins). Humified organic matter releases nutrients slowly, similarly to a slow release fertilizer (Chen and Avnimelech, 1986). The rate of nutrient release varies with soil physical and chemical properties, climate, microbial population, and the degree of maturity. Composting is a humification process in which the fresh organic waste is stabilized and biodegraded and the compost contains high portion of humified organic matter.

2.1.3.3 Application Rate of Compost

The beneficial effects of compost on crop production and soil properties are directly related to the application rate of compost.

As soil physical, chemical and biological conditions vary across the landscape, so do the relative benefits of nutrient application (Malo and Worcester, 1975). Because compost is an expensive, relatively scarce and sometimes toxic soil amendment, it is difficult to justify high rate applications across entire fields and farms. The possible way to determine the optimum rate of compost application onto the soil is to use such compost for planting test and determine the optimum rate of application for local soil condition and type of plants.

2.2 Composting Science

No matter which method of composting is used, the requirements for the microorganisms to survive and grow are very specific. Factors that influence microbial activity include temperature, O₂ concentration, pH, moisture content, carbon to nitrogen ratio, particle sizes and substrate composition (Miller, 1993).

2.2.1 Moisture Content

All living organisms need water, so moisture is essential for the function of the composting process. Moisture is also important for heat storage in the compost bulk.

Moisture affects microbial activities in two ways. Like all organisms, microbes require water to function. Moisture content below 40% can inhibit biological activities. For the microorganisms there is no upper limit for moisture content, but excessive moisture reduces the airspace in the compost matrix and thus causes O₂ limitation (Miller, 1993). If air pore space is occupied by water, the availability of O₂ will be reduced and anaerobic decomposition will develop. Anaerobic decomposition produces several by-products with an offensive smell and is, therefore undesirable in uncontrolled composting processes.

2.2.2 Carbon to Nitrogen Ratio (C/N)

The activities of the heterotrophic microorganisms involved in the composting process are dependent upon the nitrogen (N) and carbon (C) contents. The above microorganisms use the carbon as the energy source, whereas the nitrogen is used for the synthesis of proteins. During the oxidation reactions that involve the release of CO₂, the major portion of the carbon (approximately 2/3) is used by the microorganisms as the energy source, while the remaining portion serves to form

protoplasm cells, along with nitrogen, phosphorus, potassium and other micro nutrients (Sharma et al., 1997). Nitrogen, in the form of ammonium ions (NH_4^+), is generally required as a major nutrient. Ammonia (NH_3) is produced from hydrolysis of protein and probably also from purines and pyrimidines.

The living organisms use average 30 carbon atoms for each nitrogen atom. Thus before decomposition can occur, the composting materials must have a C/N ratio that permits microbial activity and should be between 25 and 35 (Rynk, 1992; Haug, 1993; Eliot 1997). Therefore, mixtures of materials are often required to provide a suitable C/N ratio.

If the C/N ratio is too low, excess nitrogen is often lost as NH_3 , resulting in odour problems. During the composting process, the nitrogen loss is a commonly occurrence. Low C/N ratio is one of the causes. However, even in the case of proper C/N ratio of 25 to 35, if ventilation of air is strong, the nitrogen released from the degradation of organic matter will not be fully utilized by microorganisms. This is especially important for active aerated composting process such as aerated static pile and in-vessel composting in which strong aeration is a must to accelerate the biodegradation and stabilization.

If the carbon content is too high, the available nitrogen is used up before the carbonaceous material can be completely decomposed. The co-composting of lignocelluloses material with nitrogen rich material allows the re-equilibrium of the ratio and, thus, guarantees optimal conditions for the biological transformation process.

From experience so far, it can be concluded that a good quality compost product has a C/N ratio of the order of 15-20 (Sharma et al., 1997).

2.2.3 pH Value

The parameters, such as pH, alkalinity and volatile organic acids, are closely inter-related to the composting process (Sharma et al., 1997). The pH affects the growth response of microorganisms. Ideally, the pH should be in the range of 6-8, depending on the composting material, to allow for the highest rates of decomposition. If the pH is outside this range, microbial activity will be compromised and decomposition will slow down or even stop.

It is to be noted that, during the composting process, the pH undergoes considerable change. In the beginning, the formation of CO_2 and organic acids causes

pH values dropping below 6 even 5. As the process progresses, the pH value will reach even up to 8 to 8.5. This is mainly due to the decomposition of proteins, as well as the elimination of CO_2 (Sharma et al., 1997; Beck-Friis et al., 2003).

The pH in composting process is influenced by three acid-base systems.

- One is the carbonic system, where carbon dioxide (CO_2) is formed during decomposition, can escape as a gas or dissolve in the liquid, forming carbonic acid (H_2CO_3), bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}). This system has two dissociation constants (pK_a), 6.35 and 10.33 at 25°C , and thus it tends to neutralize the pH of the compost, increasing low pH and reducing high pH.
- The second system is ammonium (NH_4^+) – ammonia (NH_3), which is formed when protein is decomposed. During the initial phase of composting most of the metabolized nitrogen is retained by growing microorganisms, but during the high-rate phase, NH_3 is released. The NH_3 system has a pK_a of 9.24 at 25°C and thus increases the pH towards this value.
- The third system is composed of several organic acids, of which acetic and lactic acid dominate. This system can reduce pH down to 4.14, which is the pK_a of lactic acid at 25°C (Weast et al., 1989-1990), and it is important in the beginning of composting as described below.

These three systems combine to form the typical pH curve for batch composting, with initially falling pH and a sharp rise during high-rate degradation (Figure2-3).

Figure 2-3 A Representation of a typical pH Curve in a Batch Composting Process

(Rynk et al., 1992)

Note: A- Initial phase or mesophilic phase; B- High rate phase or thermophilic phase; C- Decay phase and sometimes including the curing phase

Organic acids are formed during fermentation of organic matter. There are several metabolic pathways for acid production. One is fermentation by anaerobic microorganisms. Acetic acid is the main product of such processes, but longer chained acids such as butyric or propionic acids are also formed. Another important organic acid found in composts is lactic acid, which is formed by lactic acid bacteria, a group of facultative anaerobes (Brock and T, 1988). Acetic acid is mainly produced anaerobically, but it can also be produced when O_2 is present, e.g. when *E. coli* is subject to high concentrations of glucose (Enfors and Häggström, 2000). The bacteria take up more glucose than they can oxidize aerobically and acetate is formed in what is called the overflow metabolic pathway.

Organic acids are not only formed in composts, they are also decomposed by microorganisms. Most microorganisms can utilize organic acids as a readily available substrate for aerobic oxidation. The acid concentration in composts is therefore influenced both by production and consumption of the acids. Organic acids are suppressive to microbial activity and growth at low pH (Cherrington et al., 1991). Different microorganisms have different sensitivity to organic acids. Generally, bacteria are more sensitive to acids than fungi (Atlas and Bartha, 1998). The acids interfere with cellular functions of the organisms. The CO_2 evolution acids can enter the cell when they are in their undissociated form, i.e. when the pH is low. The negative effect of organic acids on microbial activity is thus strongly dependent on the pH of the medium. The acids themselves reduce the pH, but in composts the pH is also influenced by the carbonic and ammonia systems. Acid concentration and pH are therefore connected and both influence the toxic effect of organic acids. In municipal solid waste, acetic and lactic acids are produced during storage, and in source-separated organic waste, these acids can reduce the pH to 4-5 (Eklind et al., 1997). During the initial phase of batch composting, reduced pH and high concentrations of organic acids can occur (Beck-Friis et al., 2003). During successful composting, the acids are decomposed and pH increases. Compared to well-aerated composting, composting at low O_2 levels results in a larger acid production and a slower break-down of acids (Beck-Friis et al., 2003). The initial period of low pH can be significantly reduced if the temperature in the compost stays below $40^\circ C$ until pH rises (Smårs et al., 2002).

2.2.4 Microorganisms and Change of Temperature

Microbiology is the heart of the composting process. The microbial populations involved are numerous, often reaching levels of 10^9 to 10^{10} /g compost. Composting involves mesophilic and thermophilic bacteria, fungi, and actinomycetes. Temperature is the single most important factor affecting microbial populations, their growth, and activities.

If other requirements (such as C/N, moisture content, pH and aeration) are satisfied, microbial activity will increase the temperatures within the composting mixture. As a result, a series of microbial populations, each with an optimal temperature range, flourish and decompose particular types of materials. Bacteria are better adapted to breaking down the easily decomposable material, whereas fungi are adapted to breaking down the more difficult materials such as cellulose and lignin.

Heat is produced as a result of aerobic decomposition of waste by microorganisms. Biodegradation is a highly exothermic process. The heat produced can either remain in the compost mass, resulting in an increased temperature, or leave it either by conduction or radiation from the surface, or with the air passing through it. The temperature elevation which is the result of the microorganism activities, reversely affects such activity. Each microbial species can only grow within a certain temperature range, and most microorganisms are killed by too high temperatures (Miller, 1993). Initially, mesophilic bacteria with an optimal temperature in the range of 20-50°C are responsible for decomposition. As a result of these microbial activities, temperatures rise to exceed the mesophilic range, and then thermophilic bacteria take over between 40°C and 70°C (the optimal range is 50-65°C). These thermophilic temperatures persist during the initial stage of high-rate of decomposition and are required to reduce pathogenic bacteria, weed seeds, and other undesirable organisms to acceptable level (Canadian Council of Ministers of the Environment, 1996). To ensure this occurs, compost must be maintained at temperatures above 55°C for 3-15 days depending on the composting methods. Temperatures will then fall and mesophilic microorganisms continue the process at a slower rate (Figure 2-4).

It has been observed that the thermophilic phase is advantageous because of increased organic removal efficiency, improved solid-liquid separation and destruction of pathogens. The negative points are attributed to the higher energy

requirement for maintaining high temperature, poor process stability, etc.

Figure 2-4 Temperature Change in Different Phases of Composting Process

It can be stated that, within the temperature range of 50 to 70°C, most of mesophilic microorganisms, as well as pathogenic species, are destroyed. As shown in Table 2-1, the higher temperature achieved during the course of composting assures the destruction of the pathogenic microorganisms contained within the solid wastes. Therefore, it can be stated that if the bulk organic material in the composting is subjected to a temperature of 55 to 60°C for a minimum period of 2 to 3 days, the end product obtained would be considered sufficiently hygienic (Sharma et al., 1997).

Table 2-1 Temperature and Time Interval Required to Destroy Most Common Types of Pathogenic Microorganisms and Parasites Occasionally Present in Wastes
(Sharma et al., 1997)

Salmonella typhosa	Further growth is stopped above 46°C; dies within 20-30 minutes at temperature of 55-60°C.
Salmonella sp.	Dies within 60 and 20 minutes at a temperature of 55 and 60°C, respectively.
Shigella sp.	Dies within 60 minutes at a temperature of 65°C.
Escherichia coli	A large portion is killed within 60 and 15-20 minutes at a temperature of 55 and 60°C, respectively.
Entamoeba histolytic	Dies within few minutes at 45°C and within few seconds at 55°C.
Taenia saginata	Dies within few minutes at a temperature of 55°C.
Trichinella spiralis	Dies rapidly at 55°C and instantaneously at 60°C.
Brucella abortus and Brucella suis	Dies in 3 minutes while at 62-63°C and within an hour at 55°C.
Micrococcus piogenes	Dies within 10 minutes at 50°C.
Streptococcus piogenes	Dies within 10 minutes at 54°C.
Mycobacterium tuberculosis var. hominis	Dies within 15-20 minutes at 66°C.
Corynebacterium diphtheria	Dies within 45 minutes at 55°C.
Nectar americanus	Dies within 30 minutes at 45°C.
Ascaris lumbricoides	Dies within 55-60 minutes at temperature > 50°C.

2.2.5 Oxygen and Aeration

Oxygen (O_2) is essential for the microbial activity in composting since it is an aerobic process. There are three principal aeration methods provide O_2 during composting: physical turning of the mass, convective air flow, and mechanical aeration. Windrow methods use the former two ways, whereas static systems provide O_2 by using blowers or through convective air flow. The latter, often called passive aeration is highly dependent on the porosity of the compost matrix. In-vessel composting is normally provided mechanical aeration by using air blowers. Sometimes, the compost matrix is physically turned in order to make full contact with fresh air.

The lack of O_2 results in anaerobic conditions. Consequently, putrescible compounds are formed. Odour control is required in most composting processes.

2.2.6 Particle Size and Bulking Agent

Porosity within the mixture basically depends on particle size if the moisture content is in the optimum range. Small particles result in a large surface area, which is desirable for microbial activity. Consequently, some materials may need to be ground or shredded before composting. However, small particles also result in lower porosity; thus, bulking agents such as wood chips and sawdust may need to be added to the mixture to ensure there is adequate air-filled porosity. Normally the wood chips are not necessarily decomposed and can be screened out at the end of the process for reuse. Presence of sawdust in the end product could be a problem, since its small particle sizes make it unsuitable for screening.

2.3 Traditional Composting Technologies

Many composting systems/technologies have been used for treatment of different wastes under different conditions. Common systems are (I) naturally aerated windrow systems in Figure 2-5(I): long rows with a triangular cross section; (II) forced aerated static pile systems in Figure 2-5(II); (III) in-vessel systems: closed rotating horizontal cylinders in Figure 2-5(III) or vertical silo-type cylinders in Figure 2-5(IV). Sorting,

screening, and curing are common supporting processes to the three composting methods. Additional odour control is always required to remove the offensive odours.

Figure 2-5 Schematic Drawings of Three Traditional Composting Technologies

The compost systems/technologies vary in the method of air supply, temperature control, mixing/turning of the material, and the time required for composting. Their capital and operating costs may vary as well.

2.3.1 Windrow Composting

A windrow is defined as regularly turned elongated pile and shaped triangular in cross section. Pile length exceeds its width and height and could be up to a hundred

meters. The width is usually about twice the height (USEPA, 1975 and 1995). The ideal pile height allows for a pile large enough to generate sufficient heat and maintain temperatures, yet small enough to allow O_2 to diffuse to the centre of the pile. For most materials the ideal height is between 1.5 and 3 m with a width from 3 to 6 m (Figure 2-6).

Figure 2-6 Typical Windrow Composting with an Elevating Face Windrow Turner
(Rynk *et al.*, 1992)

Turning the pile re-introduces air into the pile and increases porosity so that efficient passive aeration from atmospheric air continues at all times. As noted above, the windrow dimensions should allow conservation of the heat generated during the composting process and also allow air to diffuse to the deeper portions of the pile. The windrows must be placed on a firm surface so the piles can be easily turned. Piles may be turned as frequently as once per week. Turning the piles also moves material from the pile's surface to the core of the windrow, where it can undergo composting. Machines equipped with augers, paddles, or tines are used for turning the piles. Some windrow turners can supplement piles with water, if necessary. When piles are turned, heat is released as steam to the atmosphere. If inner portions of the pile have low levels of O_2 , odours may result when this portion of the pile is exposed to the atmosphere.

Piles may be placed under a roof or out-of-doors. Placing the piles out-of-doors, however, exposes them to precipitation, which can result in runoff or leachate. Piles with initial moisture content within the optimum range have a reduced potential for producing leachate. The addition of moisture from precipitation, however, increases

this potential. Any leachate or runoff created must be collected and treated or added to a batch of incoming feedstock to increase its moisture content. To avoid problems with leachate or runoff, piles can be placed under a roof, but doing so adds to the initial costs of the operation.

2.3.2 Aerated Static Pile Composting

Aerated static pile composting can be shaped much like windrows or in an elongated pile or bed. The essential difference is in the name: static piles are not mechanically agitated (Figure 2-7).

The piles are placed over a network of pipes connected to a blower, which supplies the air for composting. Air can be supplied under positive or negative pressure. That is to say, air supply blower either forces air into the pile or draws air out of it. Forcing air into the pile generates a positive pressure system, while drawing air out of the pile creates negative pressure. The blowers are controlled by a timer or a temperature feedback system. Air circulation in the compost piles provides the needed O_2 for the composting microbes and also prevents excessive heat build-up in the pile. Removing excess heat and water vapour cools the pile to maintain optimum temperatures for microbial activity. A controlled air supply enables construction of large piles, which decreases the need for land. Odours from the exhaust air could be substantial, but traps or filters can be used to control them. The temperatures in the inner portions of a pile are usually adequate to destroy a significant number of the pathogens and weed seeds present. The surface of piles, however, may not reach the desired temperatures for destruction of pathogens because piles are not turned in the aerated static pile technology. This problem can be overcome by placing a layer of finished compost 150 to 300 mm thick over the compost pile. The outer layer of finished compost acts as an insulating blanket and helps maintain the desired temperatures for destruction of pathogens and weed seeds throughout the entire pile.

Aerated static pile composting systems have been used successfully for MSW, yard trimmings, biosolids, and industrial composting. It requires less land than windrow composting. Aerated static pile composting can also be done under a roof or in the open, but composting in the open has the same disadvantages as windrows placed in the open (see previous section on windrows). Producing compost using this technology usually takes from 6 to 12 weeks. The land requirements for this method

are lower than that of windrow composting.

Figure 2-7 Typical Aerated Static Pile for Composting MSW
(Rynk et al., 1992)

2.3.3 In-Vessel Composting Systems

In-vessel composting systems enclose the feedstock in a chamber or vessel that provides adequate mixing, aeration, and moisture. There are several types of in-vessel systems available; most are proprietary. Drums, silos, digester bins, and tunnels are some of the common in-vessel type systems. These vessels can be single- or multi-compartment units.

Vertical composting reactors are generally over 4 m high, and can be housed in silos or other large structures. Organic material is typically fed into the reactor at the top through a distribution mechanism, and flows by gravity to an unloading mechanism at the bottom. Process control is usually by pressure-induced aeration, where the airflow is countercurrent to the downward materials flow. The height of these reactors makes process control difficult due to the high rates of airflow required per unit of distribution surface area (Bertoldi et al., 1988b). Neither temperature nor O_2 can be maintained at optimal levels throughout the reactors, leading to zones of non-optimal activity (Bertoldi et al., 1988b). Some manufacturers have minimized these difficulties by enhanced air distribution and collection systems, including changing the airflow direction from vertical to horizontal between alternating sets of inflow and exhaust pipes. As with static pile composting, a stable porous structure is

important in vertical reactors which usually lack internal mixing. Tall vertical reactors have been successfully used in the sludge composting industry where uniform feedstock and porous amendments can minimize these difficulties in process control (USEPA, 1989), but are rarely used for heterogeneous materials like MSW. A variation on the vertical reactor which is used for MSW is a discontinuous vertical design, where a series of shallow reactors are stacked vertically, each with a separate aeration system (Ambrose, 1983; Martegani et al., 1985).

Horizontal reactors avoid the high temperature, O_2 , and moisture gradients of vertical reactors by maintaining a short airflow pathway. They come in a wide range of configurations, including static and agitated, pressure and/or vacuum-induced aeration, and can be operated in series or as parallel process trains (Vastola et al., 1987; USEPA, 1989). Agitated systems usually use the turning process to move material through the system in a continuous mode, while static systems require a loading and unloading mechanism. Aeration systems are usually set in the floor of the reactor, and may use temperature and/or O_2 as control variables. Systems with agitation and bed depths less than 2-3 m appear effective in dealing with the heterogeneity of an MSW feedstock (Mousty et al., 1987; Bertoldi et al., 1988b).

Horizontal and vertical reactors are commonly referred to as in-vessel systems as differentiated from open systems such as windrows and static piles. Because of the higher capital and operation costs associated with these contained systems, residence time in the reactors is much less than is required for production of mature compost. As a result, in-vessel composting facilities typically also have a windrow or static pile system for the later stages of decomposition and curing.

A major advantage of in-vessel systems is that all environmental conditions can be carefully controlled to allow rapid composting. The material to be composted is frequently turned and mixed to homogenize the compost and promote rapid O_2 transfer. Retention times range from less than one week to as long as four weeks. The vessels are usually placed in a building. These systems, if properly operated, produce minimal odours and little or no leachate. In addition the air supply can be precisely controlled. Some units are equipped with O_2 sensors, and air is preferentially supplied to the O_2 -deficient portion of the vessel. In-vessel systems enable exhaust gases from the vessel to be captured and subjected to odour control and treatment.

2.4 Operation Modes of Composting Process

There are in principle two ways of composting, i.e., as a batch mode or a fed mode. In batch operation, a batch of waste is prepared and composted separately from other batches to a final product. In fed operation, fresh substrate is intermittently added and mixed with the active compost without discharging the product for a long period. Fed operation are often called fed-batch or continuous, but there is no consistent terminology within this field. Fed-batch operation is suitable for in-vessel composting where the physical, chemical and biological conditions are controlled constantly throughout the process. This operation allows treating organic wastes at the generating sites and a large number of such reactors have been developed in Japan in the recent years.

2.4.1 Batch Mode Operation

In this text, the batch process is classified into three phases which are divided based on process dynamics. The phases are (A) the initial phase or mesophilic phase, (B) the high-rate phase or thermophilic phase and (C) the curing phase and once again back to mesophilic phase (Figure 2-3). Apart from the particle size, temperature, and moisture, which affect the degradation rate in all phases, there are certain parameters that are more important in each phase. Microbial biomass growth is limited in the initial phase, often by low pH. The initial phase is characterized by temperature rising and CO₂ production increasing. The time of the initial phase can range from less than a day to a few weeks. The next phase is the high-rate phase, when neither microbial biomass, substrate availability nor low pH is rate-limiting. This phase is normally thermophilic, and one rate-limiting factor is O₂ diffusion into the solid-liquid compost particles. The time-span of the high-rate phase ranges from a few days to several months. In the later phase, the readily available substrate is depleted while the microbial biomass is still large, so the substrate availability is rate-limiting. The CO₂ production declines and the temperature may also decline.

2.4.2 Fed-batch Mode Operation

Fed-batch composting has not been as extensively investigated as batch

composting, although some work was documented more than 30 years ago (Schulze, 1962; Jeris and Regan, 1973). In recent years, interest in continuous composting has increased, especially in Asia (Nakasaki et al., 1998; Hwang et al., 2002). This is connected to an increased use of decentralized composting machines, used mainly for food waste in households and restaurants. The reported experiments have therefore been carried out on food waste, either real (Hwang et al., 2002) or artificial (dog food) (Nakasaki et al., 1998). Feeding of the compost was in both cases made on a daily basis. A peak in CO₂ evolution rate was noted daily shortly after waste addition, indicating a rapid degradation of the easily degradable matter in the waste. Condition of low pH during heavily loaded fed-batch composting was noticed (Schulze, 1962). Low pH during a period of low microbial activity and declining temperature was also observed (Hwang et al., 2002). The daily feed rates in fed-batch composting research have been about 10% of the starting culture (Nakasaki et al., 1998; Hwang et al., 2002) or 8.4-18.3% (Schulze, 1962). Because the time required for the microorganisms to proliferate is shortened, the time lag between the addition of the organic material and the time at which active degradation begins is shorter in the fed-batch operation than in the batch operation (Nakasaki et al., 2000).

2.5 Enhancements of In-Vessel Composting Process

The traditional composting technologies such as windrow and aerated static pile, normally not only need long durations to biologically degrade the waste to stabilized compost but also require plenty of land space to pile the wastes. It may be common to come across windrow and aerated static pile composting sites in USA, China, India, Australia and South Africa. In these countries, there are sufficient rural lands to compost urban solid wastes. Although the compost produced from the windrow and aerated static pile composting processes are poor and inconsistent in qualities, it can be cheaply sold or even given away for free to the farmers.

On the other hand, in-vessel composting technology is suitable for either high-density-population countries like Japan and Korea, or city-like countries, especially, Singapore. However, because of the higher capital and operation costs associated with in-vessel composting technology, a shorter residence time of organic waste in the reactor is required.

Many solutions have been sought and proposed to shorten the residence time of

organic waste in the reactor, and produce good quality of compost. It is a matter of how to enhance the biodegradation and stabilization of organic wastes. By controlling or improving one or several parameters of the composting processes, e.g., balancing the nutrients, increasing the population of microorganisms, or keeping good aeration, the bioconversion of organic wastes to good end products could be enhanced and subsequently the residence duration in the reactors would be shortened.

2.5.1 Co-composting: A Way to Balance the Nutrient Ratio

It was emphasized that mixtures of raw materials were required to provide a balanced initial C/N ratio. The composting process is a substrate-limited process. If the C/N ratio is too low, excess nitrogen is often lost as ammonia, resulting in odour problems. If the carbon content is too high, the available nitrogen is used up before the carbonaceous material is completely decomposed. The composting process will slow down and eventually stop. The living microorganisms use average 30 carbon atoms to fix each nitrogen atom. Thus the mixed raw materials must have a C/N ratio that permits microbial activity and should be between 25 and 35 (Rynk, 1992; Haug, 1993; Eliot, 1997).

The co-composting of lignocelluloses material with nitrogen rich material can allow re-equilibrium of the C/N ratio and, thus, guarantee optimal conditions for the biological transformation process.

This co-composting strategy has been widely introduced into the windrow and aerated static pile composting processes. A general rule of thumb is that “wet, green” materials (e.g. vegetable waste, leaf materials, sewage sludge and poultry manure) contain more nitrogen, and “dry, brown” materials (e.g. sawdust, paper, coffee pulp, wood chips and wheat straw) contain more carbon.

Once nitrogen rich waste materials are composted, other carbon rich material wastes will be amended to reduce the nitrogen loss and control the odours. The carbon rich materials, such as wood chips not only provide carbon source but also act as bulking agents because of their high volume to mass ratios.

- ✦ For example, soybean residues and leaves were amended with sawdust to achieve a C/N ratio of 30 before composting (Wong et al., 2001).
- ✦ In Spain, concentrated sugar beet vinasse was a high-density waste from the sugar industry. Co-composting of a concentrated sugar beet vinasse was made with

addition of two agricultural solid residues of different organic natures, namely grape marc (a lignin waste) and cotton gin trash (a cellulosic waste). Both materials were evaluated to be adequate for co-composting with concentrated sugar beet vinasse. The composts obtained had a high fertilizer value, high level of stability and absence of phytotoxicity (Madejon et al., 2001).

In most cases, the nitrogen rich materials are actively supplied to the carbon rich materials.

- ✦ The by-products generated in the manufacture of paper pulp were co-composted with other nitrogen rich material. Two nitrogen amendments, ammonium nitrate and chicken litter were added and compared to determine which provided a more rapid mass reduction and stabilization. It was recognized that the addition of nitrogen amendments the decreased C/N ratio and facilitated the composting process. Increasing the amount of ammonium nitrate did not increase the total dry matter reduction. The addition of chicken litter provided other benefits such as a wide variety of micronutrients compared to the inorganic amendment ammonium nitrate (Das et al., 2001).
- ✦ In Spain, huge amounts of barley wastes were produced by the brewery industry during malt preparation. Barley waste was low in nitrogen and high in carbon. Poultry manure supplemented the barley wastes very well by adding the required nitrogen and moisture when two co-composting of these two wastes was studied to compare the control composting of solid poultry manure alone. The co-composting of barley waste and solid poultry manure showed a better behaviour than the control (Guerra-Rodriguez et al., 2000).
- ✦ In another paper, co-composting of chestnut burr and leaf litter with solid poultry manure was reported. Chestnut burr and leaf litter were major agricultural wastes generated from North-western Spain. They were generally deficient in nitrogen and high in carbon. Co-composting of Chestnut burr and leaf litter amended with solid poultry manure balanced the C/N ratio into the optimum range. In conclusion, the co-compost of chestnut burr and leaf litter with solid poultry manure showed a better behaviour than the manure control compost (Guerra-Rodriguez et al., 2001).

Many papers have been published to show the enhancing effect of co-composting

of a carbon rich waste with a nitrogen rich waste on the biodegradation and stabilization of organic matter in the waste.

In a finished study related to this research, the co-conversion of sewage sludge with food waste was found to be effective. It was reported that the C/N ratio was balanced to near the optimum range and the O₂ transfer within the bulk mixture was improved. From the view of nutrients available to plants, sewage sludge provided the essential amounts of nitrogen and phosphorus, and food waste supplied the sufficient potassium (Wang et al., 2003a, b).

In this research, mixed food waste was the organic waste to be biodegraded and stabilized. Green leaves and roots from food preparation, was obtained from a university canteen, and soybean residues were obtained from Singapore Food Industrial Park. Soybean residues consisting of nutrient balanced contents (especially nitrogen), were produced from Soybean drinks and tofu factories. Both wastes were high in nitrogen and had low C/N ratios ranging from 10 to 15. It seemed necessary to amend them with carbon rich waste to obtain efficient biodegradation and stabilization of organic matter.

2.5.2 Seeding Effect

It may be practical to install small composting plants in densely populated cities, for example, one for each high rise apartment, so as to exclusively handle household organic waste. This will also reduce transportation costs to a central plant. Efficient processing of the waste is essential for the operation of small plants in urban areas. Thus, attention was focused on the effects of seeding (Nakasaki and Akiyama, 1988).

Since composting is a biological process, its course should be identified by the quantity and nature of the microbial population present. Composting may be accelerated if the number of microorganisms is artificially increased at the onset of composting.

There are mainly two ways to increase the number of microorganisms at the beginning of composting process: one is pure culture seeding in which the selected strain or strains are cultivated aside and added into the composting material (Goluecke, 1977; Nakasaki and Akiyama, 1988; Nakasaki et al., 1992, 1994 and 1996; Fang et al., 2001; Wang et al., 2003a, b). Another way is to add microorganisms-rich materials into the composting materials. The microorganisms-rich materials can be the active

compost taken from a previous composting process (Bolta et al., 2003), some garden soils (Goluecke, 1977), animal manure (Goluecke, 1977), or sewage sludge.

However, the use of microbial seeding has been under debate. Seeding has sometimes had either no effect on the degradation rate of compost, or a large positive effect.

2.5.2.1 No Effect of Seeding

No effects have been frequently observed (Finstein and Morris, 1975; Goluecke, 1977; de Bertoldi et al., 1983; Solbraa, 1984; Nakasaki et al., 1985; Faure and Deschamps, 1991; Nakasaki et al., 1992) in some research where the microorganisms added as inocula were not more effective than those indigenous to the raw material.

- ✦ The effects of an inoculum on refuse composting was examined by inoculating animal manure, garden soil, and a variety of propriety bacterial cultures, and it was stated that the microorganisms added as an inoculum did not appear to be any more effective than those indigenous to the refuse (Goluecke, 1977).
- ✦ The effect of seeding on the thermophilic composting of sewage sludge was examined by measuring the changes in CO₂ evolution rates and microbial numbers. Although the succession of thermophilic bacteria and thermophilic actinomycetes clearly reflected the effect of seeding, no clear difference was observed in the overall rate of composting or quality of the composted product (Nakasaki et al., 1985).
- ✦ The effect of bacterial inoculation on the process of composting of grape pulps was studied. The addition of an inoculum rich in cellulolytic and ligninolytic bacteria had no effect on the degradation of organic matter. The principal reason for this failure was possibility that microorganisms existing in grape pulps were already in sufficient quantity for adequate composting (Faure and Deschamps, 1991).

2.5.2.2 Positive Effect of Seeding

Sometimes, seeding or inoculation was effective only in the early stages of the process for raw materials containing indigenous microorganisms at low population density.

- ✦ The effect of seeding on the composting reaction rate of tofu refuse was

examined by measuring changes in CO₂ evolution, temperature, conversion of carbon, and microbial succession. The timing of CO₂ evolution and microbial succession differed substantially when sterilized tofu refuse was seeded with two different inoculum sources. This difference was restricted only to early composting stages and the total amounts of CO₂ evolved from both composts were similar in the later stage of refuse degradation. The analysis of microbial succession indicated that the types of microorganisms primarily responsible for composting of the refuse were the same in the later stages of both experiments. In contrast to the composting of sterilized tofu refuse, no measurable effect of seeding was observed when air-dried tofu refuse was composted. Large numbers of microorganisms responsible for composting inhabited the air-dried tofu refuse. Although the succession of thermophiles and patterns of CO₂ evolution were different in composting sterilized and dried tofu refuses, no clear difference was observed in the final conversion of carbon of the raw refuse in all treatments (Nakasaki et al., 1992).

- ✦ The seeding effect on fly ash-amended biosolids composting was investigated by inoculating a mixture of ash and biosolids with seeding inocula before composting. These inocula included thermophilic bacteria (*Bacillus brevis*, *Bacillus coagulans*, and *Bacillus licheniformis*) isolated from the ash-biosolids compost, a commercial decomposer, and recycled biosolids compost. Although the addition of these microbial additives to the ash-biosolids compost improved the population of thermophilic bacteria at the early stage of composting, the improvement was negligible after 4 days of composting (Fang and Wong, 2001).

The microbes added were not abundant in the substrate, and they have been specialized to the compost environment in question. One of examples is seeding with thermophilic bacteria and actinomycetes in thermophilic composting of household waste. The effects of seeding on the thermophilic composting of household organic waste were examined by measuring the changes in CO₂ evolution rate, temperature, conversion of volatile matter, pH, and microbial succession in the composting reaction. The courses of the above values differed substantially between the seeded and unseeded experiments. The rises in temperature and pH, as well as the appearance of large peaks in the rate of CO₂ evolution occurred at earlier times in the seeded composting than in the unseeded one. Furthermore, the final conversion of volatile

matter was greater in the seeded composting than in the unseeded one. The analysis of microbial succession indicated that the seeded composting was accelerated both in mesophilic and thermophilic stages (Nakasaka and Akiyama, 1988).

Seeding effect was obvious when thermophilic acid-tolerant microorganisms were added into low-pH compost.

- ✦ The efficacy of various compost starter cultures was examined in a bench-scale composting system at 60°C. Initially, when an initial compost pH of 7.0 was used, a laboratory produced starter culture (Culture A) was much less effective than a commercial culture (Culture B). Low activity in the experiment with Culture A was due to a low pH (<5.5) that developed within 30 hours after inoculation. However, when Culture A was inoculated with a thermophilic strain (HA1) of *Bacillus licheniformis* isolated from Culture B, the new Culture A prevented the decrease of pH and significantly increased the rate of decomposition. It also enhanced population growth of other thermophilic bacterial groups during the process. This bench scale composting demonstrated that inoculated with proper microbial strains, the composting process could be enhanced (Nakasaka et al., 1994).
- ✦ The effect of *Bacillus licheniformis* cell density on the acceleration of organic waste composting was examined using the same bench scale composting system at 60°C. It was found that the minimum cell density to accelerate organic decomposition was around 10^4 - 10^5 c.f.u./g dry matter of raw material. The cell density of microbial inoculants was one factor of determining whether such microbial inoculants would enhance the bioconversion of composting process (Nakasaka et al., 1996).

However, the seeding of recycled compost from a previous composting process could exhibit a comparable degradation to that of specially selected strain seeding. For instance, the seeding effect on fly ash-amended biosolids composting was investigated by inoculating a mixture of ash and biosolids with seeding inocula before composting. These inocula included thermophilic bacteria (*Bacillus brevis*, *Bacillus coagulans*, and *Bacillus licheniformis*) isolated from the ash-biosolids compost, a commercial decomposer, and recycled biosolids compost, respectively. The isolated *Bacillus* species were as efficient as the commercial decomposer in accelerating the decomposition rate during ash-amended biosolids composting as indicated by the high

amounts of CO₂ evolved and cumulative weight loss. Ash-biosolids compost inoculated with 15% (dry weight basis) of recycled compost showed a comparable decomposition activity to those inoculated with bacterial culture and the commercial decomposer with milk powder. Taking into consideration the lower operating cost and acceptable decomposition efficiency, recycled biosolids compost seemed to be a promising additive to ash-amended biosolids compost to improve composting efficiency (Fang and Wong, 2001).

In a recent study, a positive seeding effect was observed when the selected strain *Bacillus thermoamylovorans* was supplied to the aerobic thermophilic degradation of sewage sludge and food waste (Wang et al., 2003 a, b). The application of the selected strain of *Bacillus thermoamylovorans* SW25 improved the bioconversion of the mixture of sewage sludge and food waste.

A starter culture may be useful for start-up of the biodegradation of organic waste (Fang et al., 2001). After this initiation, the product, containing essential bacteria, can be recycled to improve the process of microbial degradation (Beffa et al., 1996b). However, the inoculation of composting organic waste by pure starter cultures is not practically used at present. Usually, finished compost, or the compost from the thermophilic phase, is used as an inoculum to speed up the process (Fujio and Kume, 1991; Furhacker and Habel, 1995; Beffa et al., 1996 a, b). Notwithstanding this practice, the use of a pure starter culture has theoretical advantages in giving greater control over desirable processes and lowering the risk of accumulation of harmful microorganisms in the final product of the biodegradation. It may also prevent the growth of actinomycetes and fungi releasing allergenic spores in the air.

There were also some successful applications of special cellulolytic fungi in composting straw and wood which were high in lignocellulose (Goluecke et al., 1954; Finstein et al., 1975; Yadav, 1982; Matthur, 1986).

2.5.3 Use of Thermophilic Degradation Stage

It has been observed that the thermophilic phase (temperature above 55°C and below 65°C) is advantageous over other phases because of increased organic removal efficiency, improved solid-liquid separation and destruction of pathogens.

There has been some debate regarding the optimum temperature for decomposition of organic matter. One reason for this controversy is that different feed

stocks or materials decompose more rapidly at different temperatures. However, most data in the literature indicated that the optimum temperature lied between 50 and 60°C.

- ✚ It was indicated that the maximum CO₂ production occurred at temperatures between 60 and 65°C for municipal solid wastes (MSW) which were mainly garbage and refuse (Wiley and Pierce, 1955).
- ✚ It was reported that maximum decomposition of MSW occurred at temperatures between 65 and 70°C (Schultz, 1961).
- ✚ It was reported that the maximum O₂ uptake rates occurred between 45°C and 66°C (Schultz, 1960; Regan and Jeris, 1970). Since O₂ uptake is a function of microbiological activities, the highest O₂ uptake rate should indicate the optimum decomposition temperature.
- ✚ It was reported that optimum temperature for the decomposition of MSW was near 60°C (Jeris and Regan, 1973).
- ✚ It was also reported that for biosolids composting, 60°C was the optimum temperature (Bach et al., 1984).
- ✚ It was found that two-thirds of the entire metabolic activity in sewage sludge was due to degradation of insoluble matter by the indigenous thermophilic bacteria (*B. stearothersophilus*) at 65°C (Bomio et al., 1989).

Nevertheless, the optimum temperature only occurs in the thermophilic phase of composting processes. In this phase, the biodegradation of organic matter was at the highest rate. Thermophilic bacteria, fungi and actinomycetes played a very important role during the biodegradation.

In windrow and aerated static pile composting processes, it is difficult to achieve good time control because of their large stock piles. The temperature changes in these two composting processes always passively follow a typical pattern: mesophilic phase in the beginning, then due to self-heating, thermophilic phase and lastly decay phase. In contrast, thermophilic composting is normally made in closed reactors. In-vessel composting reactors always have a relevant facility or device to control the temperature of compost matrix.

Many studies have been made by maintaining the composting process at the thermophilic stage to achieve a fast biodegradation of organic matter. In order to sustain or obtain the optimum temperature of around 60°C, temperature control seems

very crucial.

- ✦ In order to study the seeding effect on the thermophilic composting of household organic wastes, a composting reactor with a ribbon heater was used. A Styrofoam insulator was also provided to prevent heat exchange between the reactor and the atmosphere. Two kinds of experiments, autothermal and isothermal composting were studied. In the former no heater was used, but throughout the latter experiments the external heater was used to maintain the reactor temperature at 60°C for as long as possible. It was found that the effects of seeding in the isothermal composting were not as pronounced as in the autothermal composting. The experiments of autothermal and isothermal composting have also demonstrated that the composting can be accelerated both in mesophilic and thermophilic stages by seeding (Nakasaki and Akiyama, 1988).
- ✦ Dewatered sewage sludge was composted in a laboratory-scale reactor in which a constant temperature of 60°C was kept as long as possible by regulating the air feed rate. The succession of mesophilic bacteria, thermophilic bacteria, and thermophilic actinomycetes was also observed during the composting. Specific CO₂ evolution rates of thermophilic bacteria and actinomycetes in the constant-temperature region of 60°C were assessed quantitatively. It was found that the CO₂ evolution rate was attributed to thermophilic bacteria at the initial stage of 60°C and to thermophilic actinomycetes at the later stage of 60°C (Nakasaki et al., 1985 a). In another paper, the seeding effect of thermophilic composting was studied using the same reactor with a constant temperature of 60°C. It was concluded that in contrast to the composting of the organic wastes, the decomposition of sewage sludge was not affected by seeding (Nakasaki et al., 1985 a, b).

More experiments concerning the seeding effect on thermophilic composting with different inoculum has been studied by the same group of people in Japan (Nakasaki et al., 1992, 1994 and 1996). Seeded thermophilic composting has been proven to be effective in the degradation of organic matter.

The seeded thermophilic conversion of organic wastes in controlled reactors has also been studied in Singapore. In order to intensify conventional composting of a mixture of sewage sludge and solid food wastes, a one-stage thermophilic bioconversion of these wastes into an organic fertilizer was investigated. An intensive

process was carried out in a closed system, with or without addition of a starter culture of *Bacillus thermoamylovorans*. The temperature was controlled at 60°C. It was proven that the effective thermophilic bioconversion of the mixture of food waste and sewage sludge could be achieved with the addition of a starter culture with the pH buffered (Wang et al., 2003 a, b).

It can be concluded that use of thermophilic composting or thermophilic bioconversion is one way to accelerate the biodegradation of organic wastes from the above literature review. On the other hand it should be kept in mind that temperature exceeding 55°C must be maintained for several days if the waste contains pathogens. It is easier to achieve the high temperature required for disinfection earlier in the composting process when easily digestible carbon for maximum microbial activities is available.

2.6 References of Chapter 2

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Chapter 3

Materials and Methods

3.1 Raw Materials

3.1.1 Food Waste

Food wastes have been normally composted with green waste from gardens and parks, which gives a good structure and a well-balanced concentration of rapidly degrading organic waste.

Compared to other organic wastes, such as yard waste and paper waste, food waste has a higher content of easily degradable matter, but a poorer structure. It also has a lower pH. All these factors contribute to start-up problems during composting.

In this study, two sources of food waste were identified and used. One portion of the food waste was collected from the university's canteen consisting of mainly vegetable waste and some fruit waste. Another portion of the food waste, which was especially important in the pilot scale experiments, was soybean residue generated in massive amount by a local food industry park. Soybean is the raw material to manufacture Tofu, which is one of the most popular foods in Asia. In Singapore, there are more than 40 tonnes of soybean residue being generated daily. These two kinds of food wastes are either disposed of at the incineration plants or exported to other countries as feed material to livestock. No matter which disposal methods are adopted, the local food waste generators have to pay at a specific amount of disposal fee.

3.1.2 Horticultural Compost

Horticultural compost was chosen to not only function as a bulking agent but also to provide the necessary microorganisms for the bioconversion in this study. The

horticultural compost was provided by a local composting company at the age of 9 months. The company received feedstock for its compost product from horticultural waste such as tree pruning, yard trimmings and grass clippings. The feedstock was first shredded using a mechanical grinder and subsequently heaped onto the existing compost pile by excavators. The horticultural waste was left to self disintegrate and decompose in the compost pile. Excavators were employed to constantly agitate the compost pile to provide aeration. After a period of 9 to 12 months, the horticultural waste was considered composted. The unscreened horticultural compost or “Compost Biochips” was given away free of charge to landscaping contractors. Another form of horticultural compost produced by the company was the screened horticultural compost or “Processed Biochips”. The horticultural compost was mechanically screened to filter out particles larger than 6.35 cm in size and other impurities such as plastic bags. The end product consisted of 70% of fine particles (about 1 mm in size) and 30% of coarser materials (about 6.35 cm in size). The unscreened 9 months horticultural compost was used in this study, after removing some visible impurities.

Recycled compost is available at any compost facility. There are in principle two different reasons for using recycled compost as an amendment in batch composting. The first is to use it as a structural amendment, in order to dilute the fresh waste and increase the free airspace by moisture absorption and pore space increase. The second reason for using recycled compost is to add microbial biomass to improve the degradation process.

3.1.3 Characteristics of Raw Materials

The characteristics of raw materials are shown in Table 3-1. The vegetable waste has very high moisture content (around 94%) and a high VS content. Nitrogen content of vegetable waste is at a level of 4%. Soybean waste has a very high VS content and it is rich in nitrogen. Both two food waste components are biologically unstable and possess high stability index. The higher the stability index, the more unstable the raw material is. It implies that if vegetable waste and soybean waste is left untreated, the activities of microorganisms will take place and give rise to unpleasant odours.

The horticultural compost is low in moisture content and high in organic content. However, it is biologically stable compared to the two food wastes because organic matter in the horticultural compost is mainly rich in cellulose and lignin. The nitrogen in the compost is quite low.

Table 3-1 Characteristics of Raw Materials

Raw Material	TS, %	VS, % of TS	pH	C, % of TS	N, % of TS	C/N	Stability index, mg CO ₂ -C/g-VS d	
							Maximum	Average
Veg. Waste	5.6±0.2	75.2±1.1	5.9	32.2±0.4	4.3±0.3	6.3	9.08	6.07
Soy waste	16.0±0.1	96.2±0.1	6.6	45.54±0.2	5.4±0.1	8.5	23.92	21.33
Compost	43.5±1.3	76.3±1.1	6.6	40.19±2.7	1.4±0.1	29.3	1.96	1.23

3.2 Starter Culture

A starter culture may be useful for start-up of the biodegradation of organic waste (Fang et al., 2001). After the initiation, the product, containing essential bacteria, can be recycled to improve the process of microbial degradation (Beffa et al., 1996b). However, the inoculation of composting organic waste by starter cultures is not practically used. Usually, finished compost, or the compost from the thermophilic phase, was used as an inoculum to speed up the process (Fujio and Kume, 1991; Furhacker and Habel, 1995; Beffa et al., 1996 a, b). Notwithstanding this practice, the use of a pure starter culture has theoretical advantages by giving greater control over desirable processes and lower risk of accumulation of harmful microorganisms in the final product of the biodegradation. It may also prevent the growth of actinomycetes and fungi releasing allergenic spores in air (Wang et al., 2003 a, b).

In this study, two selected strains SW09 and SW25 from previous study (Wang et al., 2003 a, b) were adopted to enhance the biodegradation of food waste. In the previous study, these two strains were proven to effectively enhance the biodegradation of the mixture of food waste and anaerobically digested sludge.

3.2.1 Isolation Source and Procedure

Enrichment cultures were produced in a mixture of either sewage sludge and soil (1:0.1 by total solids), or in a mixture of sewage sludge, solid food waste, and soil (1:1:0.1 by total solids). Both mixtures were treated aerobically at 60°C in 250 ml flasks. Sewage sludge was the stored dewatered, anaerobic-digested sludge from a municipal wastewater treatment plant. Vegetable food waste was collected from a university canteen. The soil used was fertile topsoil from a plot where dewatered anaerobic sludge is collected for further disposal. Enrichment cultures were used for the isolation of pure cultures of thermophilic microorganisms. Microbiological isolation was carried out by a spread-plate method from serial 10-fold dilutions of the enrichment culture, or the suspension produced by the vortexing of 1 g of compost matter in 9 ml of phosphate-buffered saline solution (PBS), pH 7.2. The plates were prepared from nutrient agar or tryptic soy agar (Difco Laboratories, USA) and were incubated at 60°C for 1 day under aerobic conditions. The cells from individual colonies were transferred to a further set of nutrient agar or tryptic soy agar plates. In total, 38 strains of thermophilic bacteria were isolated from the original enrichment cultures.

3.2.2 Screening and Selection Procedure

The ability of the 38 isolated strains to digest sewage sludge was tested by their growth on a mixed agar sludge medium (SAM) at 60°C. The medium was prepared by mixing 10 g of dry sewage sludge and 18 g of Bactoagar (Difco Laboratories, USA) in 1 l of distilled water. The pH was adjusted to 7.0, and the medium was autoclaved for 15 min at 121°C. The five strains producing the biggest colonies on SAM were selected for further screening by cultivation in 100 ml of 1% (w/v) suspensions of dry sewage sludge in 250 ml flasks shaken at 150 rev /min and 60°C.

Five strains, SW06, SW09, SW11, SW19, and SW25, which produced the biggest colonies on SAM, were selected from the 38 isolated strains. All thermophilic isolates

were Gram-positive bacteria. The changes during the cultivation of these strains in the suspension of sewage sludge are shown in Figure 3-1. The initial pH was 7.1 and the final pH was in the range of 5.9–6.1 in all cultures. The population of the SW09 and SW25 strains rose after an initial decline. The highest cell numbers were obtained with the strains SW09 and SW25 after 72 h of cultivation. These two strains were selected for further testing as the starter cultures in the bioconversion of sewage sludge.

Figure 3-1 Changes of Cell Number with Time of the Different Strains in the Liquid Medium with Sewage Sludge

Strain SW09 (curve 1); strain SW25 (curve 2); strain SW19 (curve 3); strain SW06 (curve 4); strain SW11 (curve 5), control without inoculation (curve 6).

3.2.3 Phylogenetic Identification of the Strains

DNA of the strains was extracted from the cells of pure cultures grown on Tryptic Soy Agar using a protocol described by Kowalchuk et al. (1997). This method involved the blending of 200-300 mg of wet biomass by glass beads followed by extraction with phenol, phenol-chloroform (1:1) and chloroform: isoamylalcohol (24:1). The extracted DNA was precipitated overnight with a sodium acetate-ethanol mixture and dissolved in sterile deionised water. Universal eubacterial primers 27f (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492r1 (5'-TACGGYTACCTTGTTAC GACTT-3') were used to amplify the 16S rRNA gene from the genomic DNA as

described by Tay et al. (1998). The polymerase chain reaction (PCR) was performed in a 100 µl (total volume) reaction mixture containing 100 ng purified template DNA, 50 mM KCl, 10 mM Tris HCl (pH 8.3), 2.5 mM MgCl₂, 5% (w/v) acetamide, 0.05% (v/v) NP40, 200 µM dNTP, 0.2 µM each of forward and reverse primers and 2.5 U Taq polymerase (Promega, USA). After 30 cycles of thermal cycling (denaturation at 94°C for 1.5 min; primer annealing at 60°C for 1.5 min; and extension at 72°C for 1.5 min), PCR amplicons were purified with a Qiagen PCR purification kit (Qiagen, Germany). The nucleotide sequences of representative clones were determined using the dideoxy chain termination chemistry and the ABI model 310A sequencer (Applied Biosystems, Perkin-Elmer). The ABI PRISM[®] BigDye[™] Terminator Cycle Sequencing ready-reaction kit (version 2.0) (Applied Biosystems, Perkin-Elmer) was used as specified by the manufacturer. Both strands were sequenced by using forward primer 530F (5'- GTGCCAGCMGCCGCGG-3') and reverse primer 1100R (5'-TTGCGCTCGTTGCGGGACT-3') (Lane, 1991). Partial 550 bp sequences were produced from experimentally determined sequences using BioEdit program (Hall, 1999). For each partial sequence, the Basic Local Alignment Search Tool (BLAST) search program (Altschul et al., 1997) was used for the determination of the nearest phylogenetic neighbor sequences in the database of National Centre for Biotechnology Information (NCBI).

GenBank accession numbers for identified partial 16S rRNA gene sequences are AY197332 and AY197333 for the strains SW09 and SW25, respectively. The determination of the nearest phylogenetic neighbour sequences for 16S rDNA sequences of the strains SW09 and SW25 by the BLAST search program (Altschul et al., 1997), showed that these strains are the representatives of one species close to both the uncultured bacterium pPD10 (identities= 99%) isolated from synthetic food waste hot compost (Dees and Ghiorse, 2001), and also to the facultative anaerobic *Bacillus thermoamylovorans*, isolated by Combet-Blanc et al. (1995, 1999) (identities = 98%).

3.3 Analytical Methods

3.3.1 Chemical Analysis

3.3.1.1 General Chemical Properties Analysis

Total solid contents (TS) in the samples were determined by oven drying at 103°C-105°C for 24 hours and volatile solid content (VS) or organic matter content (OM), which are mainly organic substances, were obtained by weight loss after the ignition of the samples at 550°C (APHA, 1998).

The pH of samples was measured in a de-ionized water soluble extract of 1:10 (weight/volume) using pH meter (CORNING 145, England).

3.3.1.2 Macro Element Analysis

The contents of Carbon (C) and Nitrogen (N) in the samples were determined by Elemental Analyzer CHNS/O 2400 (Perkin Elmer, USA). Samples were oven-dried at 103°C for 24 hours, then ground to particles less than 0.2 mm in size, and each 2 mg of sample powders was put into the sample collector of the Elemental Analyzer.

The total contents of Potassium (K) and Phosphorus (P) were analyzed using an Inductively Coupled Plasma Atomic Emission Spectrometer (Perkin-Elmer ICP-AES).

3.3.1.3 Inorganic Nitrogen

Nitrate (NO_3^- -N) and Nitrite (NO_2^- -N) were determined by means of flow injection analysis (FIA) based on Lachat QuickChem Method 10-107-04-1-F (Wendt, 1997). Ammonia (NH_4^+ -N) was measured by the nesslerisation method (APHA, 1998). Prior to the analysis, extraction of water-soluble NO_3^- -N, NO_2^- -N and NH_4^+ -N was performed using 2M KCl solution (Keeney and Nelson, 1982).

3.3.2 Biological Analysis

3.3.2.1 Microbiological Enumeration

The enumeration of bacteria was carried out using spread-plate from a serial ten-fold dilution of the suspension produced by the vortexing of 1 ml of microbial suspension or 1 g of matter in 9 ml of phosphate-buffered saline solution. Tryptic Soy Agar (DIFCO Laboratories, USA) was used for the bacterial growth. The enumeration of colony forming units (c.f.u.) was provided after incubation of Petri dishes at 30°C for one or two days for mesophilic bacteria, respectively, and at 60°C for one day for thermophilic bacteria.

3.3.2.2 Phytotoxicity Test

Phytotoxicity level of the organic fertilizers was determined by the modified Germination and Root Elongation Test (Test methods for the examination of composting and compost materials, 2002). The extracts from samples were prepared by the following procedure: samples were suspended in water in the ratio of 1:9 (w/v), and incubated under 50 rpm shaking for 3 hours. The extract was separated from the solids by centrifugation at 4000 rpm for 5 minutes. The cucumber seeds were selected for the germination and root elongation test. Ten cucumber seeds were placed on a 9 cm diameter paper Whatman No.3 disk inside Petri dish. An aliquot of 10 ml of the extract in different dilutions was added to each Petri dish. The germination of the seeds in distilled water was used as control. Petri dishes were placed in lighted area, but not in direct sunlight to avoid rapid evaporation. Percentages of germinated seeds, the lengths of roots were determined after 5 days.

3.3.2.3 Stability Test

The stabilities of the raw material, intermediate products and organic fertilizers were measured by the carbon dioxide evolution rate (Q , mg CO₂-C/g-VS d) during 4

days of incubation (Test methods for the examination of composting and compost materials, 2002). The moisture contents of the samples were adjusted to 75% - 85% and the samples were pre-incubated at room temperature for 24 h. After that, each 5g pre-incubated samples was transferred into an incubation vessel, in which 50ml beaker with 20 ml of 1M NaOH was installed. The vessel was sealed and placed at $25\pm 1^{\circ}\text{C}$ for 4 days (Wu and Ma, 2002). The amount of CO_2 adsorbed by NaOH was determined daily over a four-day period by back titration of the residual NaOH with normalised HCl. Sample stability index was obtained as the average CO_2 evolution rate (Q , $\text{mg CO}_2\text{-C/g-VS d}$) over four days. The stability was then evaluated according the following range: very stable ($Q < 2$); stable ($Q = 2 - 4$); unstable ($Q > 4$).

3.3.3 Scanning Electron Microscopy Preparation

Critical point drying of samples were used in stead of air drying before the visual examination under scanning electron microscope.

- ✦ Washing: Specimens must be washed free of mucus, blood, serum or any other contaminant likely to be fixed on the surface of specimens.
- ✦ Fixation: Filter samples with $0.2\ \mu\text{m}$ filter, soak filter in beaker of 2% glutaraldehyde for 1 night.
- ✦ Dehydration: Dehydrate specimens in series of 10 minutes washes in 50, 70, 85 and 95% Ethanol and follow with storage of specimens in 100% ethanol.
- ✦ Substitution with CO_2 Miscible Liquid: After dehydration with ethanol, further substitution to amyl acetate is necessary before putting tissues in the pressure chamber.
- ✦ Substitution with liquid CO_2 : Fill the pressure chamber with liquid CO_2 and purge and soak repeatedly until all solvent removed.
- ✦ Heating to super critical temperature of CO_2 (above 31.5°C)
- ✦ Pressure release: Once the CO_2 has passed through its Critical Point (31.5°C , 1100 psi), release the pressure within the chamber.

✦ Vacuum Coating: Mount the specimens on microscope stub and coat them with Au film. (Coating machine: Fisons-Polaron LT 7480).

3.4 Bench Scale Reactor

The bench scale reactor was designed to meet the requirement of the intensive thermophilic aerobic bioconversion of food waste (Figures 3-2 and 3-3).

The cylindrical main body of the bench reactor was made of stainless steel. The thickness of steel cylinder was approximately 1 cm. The length and internal diameter of the reactor were 40 cm and 30 cm, respectively. The reactor had a total volume of 28 litres but a maximum working volume of 20 litres. Adhering to the stainless steel cylinder, there was a layer of thermo heater plate and another layer of heat insulating material. The thermo heater plate provided the external energy source to maintain the reactor and bioconversion matrix at 60°C. The heat insulating material minimized the heat loss from the reactor and the thermo heater layer to the atmosphere. Another function of this insulating material was in preventing water contact with the heater layer. A separated motor steered the rotating shaft to rotate the paddles inside the reactor. The rotating speed was set at 20 rpm. The paddles rotated and agitated the bioconversion matrix to gain good contact with O₂. Fresh air was pumped by an air blower to the reactor through the rotating shaft. Holes with a diameter of 1mm were drilled on the hollow shaft at a distance interval of 5 cm. From these tiny holes, fresh air was evenly dispersed into the reactor space. Air flow rate was 10 l/min. Waste gas was discharged through the sampling opening located on the top of the reactor. The sampling opening was covered during the bioconversion and only the waste air exhaust on the opening cover was opened. A portion of vapour in the waste air at a high temperature was precipitated into water which was cycled back to the reactor. A photograph of the bench scale reactor is shown in Figure 3-3.

Figure 3-2 Schematic Drawing of Bench Scale Reactor
(Part a: overview of bench scale reactor; Part b: details of bench scale reactor)

Figure 3-3 Photo of the Bench Scale Reactor

3.5 Pilot Scale Reactor

Figure 3-4 shows the photos of the pilot scale reactor from the front, left and right view points.

(a) Front View of Pilot Reactor

(b) Left Hand Side View

(c) Right Hand Side View

Figure 3-4 Photos of the Pilot Scale Reactor

The individual parts in the drawing (Figure 3-5) are explained in the following.

1. Control Box: Inside the control panel, there were the power switch, temperature controller, agitation speed adjuster, air compressor switch and timer. Mounted on the cover of control panel, there were operation light indicator, start and stop button, reset button and auto and manual operation switch.

2. Air blower and Motor Chamber: Air blower and agitation motor were located in this compartment. The air blower supplied fresh air by suction from air inlet (See air inlet).

3. Sampling and Input Gate: At the start of the process, raw material was fed into the reactor through this opening. Interval samples were taken from this opening. This opening was equipped with electrical sensors. Once the gate was open, the agitation in programmed mode would stop. This gate may be tightly closed to prevent evaporation of water from the material.

4. Cooling Tower: During the process, due to heating effect, liquid water was transformed into steam. Upon reaching this cooling tower, the up-rising steam was condensed into water and was recycled back to the reactor.

5. Heating Plate: The heating plate was comprised of one heating convection layer and one insulation layer.

6. Output gate: This gate was only opened at the end of the process. End product was cleaned out through this output gate from the reactor.

7. Air inlet: Fresh air was drawn into the reactor through this air inlet located on the right hand side wall. This air inlet was adjustable to control the real air flow to the reactor.

8. Air outlet: Waste air was discharged through this air outlet located on the left hand side wall.

Figure 3-5 Schematic Drawing of Pilot Scale Reactor

At the top of the drawing: Left section view, Front view and Right section view of reactor

At the bottom of the drawing: Top View of reactor

The pilot scale reactor had a total volume of 500 l. The actual maximum working volume was 300 l. The working parameters are listed in Table 3-2.

Table 3-2 Working Parameters of Pilot Scale Reactor

Parts	Process Parameters	Bioconversion Process	Heating Process
Heater	Temperature, °C	60±1	80±1
Agitator	Agitation, rpm	10	5
Air Blow	Working frequency, recycle/h	6	3
	Blowing Rate, l/min	50	50
	Blow Cycle Duration, min/recycle	5	5

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Chapter 4

Results and Discussions:

Bench Scale Batch Bioconversion

4.1 Preliminary Study

A preliminary study using flask was firstly carried out to understand the original evolution of some basic parameters related to the aerobic biodegradation of food waste under thermophilic condition. These parameters included pH, cell content of indigenous mesophilic and thermophilic bacteria, total solid content (TS) and volatile solid content (VS). Carbon and nitrogen contents were not considered in this preliminary study.

4.1.1 Experimental Design

A 2 l conical flask with 200 g of vegetable food waste and 100 ml of water was shaken at 150 rpm. The mixture of food waste and water had a volume of less than 500 ml. The ratio of flask volume to the mixture volume (ratio=4) ensured the biodegradation of food waste was aerobic. The conical flask was placed in an incubation shaker with temperature control. The temperature was set at 60°C throughout the whole experiment.

pH was not adjusted throughout the experiment. Indigenous thermophilic and mesophilic bacteria were purposely designed to grow freely and change accordingly with the natural change of pH during the aerobic treatment of food waste at 60°C. The water content was maintained at the level of 100 ml. pH, TS, VS, cell contents of thermophilic and mesophilic bacteria were monitored daily. The experiment lasted for 17 days.

4.1.2 Results and Discussions

The following Figure 4-1 presents the variations of pH, VS content and cell content of thermophilic bacteria in the 17-day preliminary study in the flask. The evolution pattern of the aerobic biodegradation of food waste under thermophilic condition was obtained by understanding the variation of some basic parameters.

Figure 4-1 Variation of Experimental Data of Preliminary Flask Test

pH decreased from 6.5 initially to 4.4 at the end of day 1 and maintained at this level till day 4. It gradually increased to 8.6 till the end of the experiment. The decrease in pH in the early stage of IAB process was inevitable without pH adjustment. The combined effect of the accumulation of CO_2 and the formation of organic acids caused pH to drop below 6. As the process progressed, it went up and reached 8 to 8.5. This was mainly due to the degradation of organic acids, the decomposition of proteins, as well as elimination of CO_2 (Sharma et al., 1997; Beck-Friis et al., 2003).

The cell content of mesophilic bacteria decreased significantly and finally disappeared after 6 days (not shown in the Figure 4-1). The cell content of thermophilic bacteria increased from 6.0×10^2 c.f.u./g TS in the beginning to 5.5×10^7 c.f.u./g TS after 7 days and then stayed unchanged for the next 7 days. The cell

content of mesophilic bacteria under the thermophilic condition inevitably diminished to an undetectable level as most of the mesophilic bacteria can not survive when temperature is higher than 50°C. On the other hand, thermophilic bacteria became the dominating species under the thermophilic condition. The cell content of thermophilic bacteria was also affected by other environmental factors. The initial low pH condition limited the rapid growth of thermophilic bacteria. Aeration condition might also influence thermophilic bacteria since they relied on O₂ to degrade the organic matters.

The unmodified biodegradation of food waste took more than 2 weeks to achieve total 60% loss of initial VS mass. The residue still had a high VS content of 75.5% after 17 days.

In order to enhance the biodegradation of food waste, pH adjustment may be necessary and the cell content of indigenous thermophilic bacteria may be increased by seeding similar or other special thermophilic bacteria.

4.2 Bench Scale Batch Bioconversion (Phase 1)

Phase 1 was aimed at examining the effect of pH adjustment and addition of selected strains SW09 and SW25, which have been proven to be high active in degrading organic matter.

4.2.1 Bench Scale Batch Bioconversion 1 (BSBB1)

4.2.1.1 Experimental Design

2 kg of fresh vegetable food waste was placed in the bench scale reactor. A suspension of starter cultures SW09 and SW25 was mixed with the fresh vegetable food waste to achieve an ideal cell content of 1.0×10^8 c.f.u./g TS. Another portion of starter cultures was added at the end of day 4 to bring the cell level back to 1.0×10^8

c.f.u./g TS. pH was adjusted daily by CaCO₃ during the first three days. Total dosage of CaCO₃ was normally 5% of initial food waste TS. 1/3 of the total CaCO₃ dosage was applied into the reactor after first day of operation for the next three consecutive days. The bioconversion was performed at 60°C under an aeration of 10 l/min and an agitation of 20 rpm. Water was added periodically to keep moisture content of food waste from falling below 80%. The experiment lasted for 11 days.

4.2.1.2 Results and Discussions

Daily variation of pH, TS, VS, cell content of thermophilic bacteria and stability index during BSBB1 is listed in Table 4-1. pH dropped from 6.5 to 5.7 in the first 2 days and was adjusted to 6.2 after addition of the first 1/3 dosage of pH buffer. However, such level did not sustain until the second 1/3 dosage brought it up to 6.5 again. Another 1/3 dosage increased the pH (less than 5 on day 3) to 6.5. In the subsequent 6 days, the pH remained above 6.5.

Table 4-1 Parameters during BSBB1

Duration, days											
	0	1	2	3	4**	5	6	7	8	9	10
pH	6.5	5.7/6.2*	4.7/6.5	5.1/6.5	6.5	6.5	6.6	6.7	6.7	6.4	6.2
TS,%	5.6±0.2	10.0±0.3	10.9±0.1	12.0±0.5	17.7±2.2	15.5±0.5	13.2±0.1	11.1±0.3	11.6±0.4	12.0±0.2	12.5±0.2
VS,% of TS	92.5±0.5	85.4±0.6	82.4±0.2	77.1±0.4	73.3±0.6	73.0±0.5	72.7±0.7	72.4±0.5	71.5±0.6	70.8±1.2	69.7±1.9
Cell content of thermophilic bacteria, c.f.u./g TS											
	1.0x10 ⁸	3.0x10 ⁶	5.0x10 ⁵	6.7x10 ⁴	1.1x10 ⁶	1.2x10 ⁷	6.5x10 ⁷	1.5x10 ⁸	1.3x10 ⁸	1.2x10 ⁸	1.1x 10 ⁸
Stability index (mg-CO ₂ -C/g-VS d)											
	12.5	9.1	8.1	7.9	7.5	7.4	7.4	7.3	7.3	7.2	7.1
Stability grade											
	Unstable										

Note: *pH/adjusted pH; **second portion of the starter bacterial biomass was added

The cell content of thermophilic bacteria decreased during the first three days, due to the decrease of pH and increased to an optimum level of 10⁸ c.f.u./g TS after pH stabilized above 6.5. This increment in cell content might be also due to another portion of starter culture added on day 4 (Figure 4-2).

Figure 4-2 Variation of pH and Cell Content of Thermophilic Bacteria in BSBB1

VS content decreased from 92.6% to 73.3% of TS and total VS mass reduction was 73.4% of initial VS mass in the first 3 days. The addition of new starter culture on day 4 only resulted in a slight decrease of VS to 69.7% of TS in the next 6 days (Figure 4-3).

Figure 4-3 Variation of VS Content and Stability in BSBB1

The stability index was noticed to have remarkable drop from 12.5 to 7.9 mg-C/g-VS d in the first 3 days. However, reviewed with the change of pH, the

microbiological stability may not have been really improved. Theoretically, the stability test utilizes the indigenous microorganism, to evaluate the Carbon Dioxide Evolution Rate. The microorganism activities are influenced by the surrounding pH. Most of the microorganisms can not survive under the condition with a pH below 5.5. In such cases, CO₂ produced by the respiration of the microorganisms definitely gave a low Carbon Dioxide Evolution Rate. The samples taken in the early stage of the experiment possessed an intolerably low pH for microorganisms. As a result, the stability index (or Carbon Dioxide Evolution Rate) was low and inaccurate. Therefore, the real biological stability of food waste residue was low.

The addition of starter cultures SW09 and SW25 and pH buffer effectively enhanced the biodegradation of food waste under thermophilic condition. In 10 days, BSBB1 achieved 73.3% reduction of initial VS mass in food waste. In the preliminary study, the biodegradation relying on the growth of indigenous thermophilic bacteria in fresh food waste only obtained 56.3% reduction of initial VS mass in fresh food waste in the same duration of 10 days.

pH buffer to the food waste during the bioconversion successfully reduced the time to obtain an ideal pH environment for thermophilic bacteria. In BSBB1, 3 days was only needed to reach a stabilized pH level above 6.5 with the aid of pH buffer. In the preliminary study, more than 8 days was needed to achieve such a pH level.

4.2.2 Bench Scale Batch Bioconversion 2 (BSBB2)

4.2.2.1 Experimental Design

2 kg of fresh vegetable food waste was placed in the bench scale reactor. Starter cultures, SW09 and SW25, were added only at the end of day 2. pH adjustment by CaCO₃ (5% dosage) was carried out 2 hours before addition of starter cultures. Another portion of food waste of 500 g was added on day 4 to investigate the performance of the fed-batch operation and the bioconversion continued for another 3 days.

4.2.2.2 Results and Discussions

Daily variations of the parameters of BSBB2 are listed in the Table 4-2. The results of day 4 are divided into two columns. One column contains the results before the addition of food waste, and the other is for those obtained after the addition.

Table 4-2 Parameters during BSBB2

Parameter	Duration, day								
	0	1	2*	3	4	4***	5	6	7
pH	6.5	5.3	4.6/6.9**	7.0	6.6	6.0	6.4	6.4	6.3
TS,%	9.1±0.9	12.3±0.5	12.5±1.9	11.5±0.9	28.1±0.5	12.2±0.3	11.5±0.3	11.0±0.3	10.5±0.3
VS,% of TS	87.5±0.8	75.4±0.6	61.7±3.0	58.5±2.3	56.2±3.9	66.3±1.7	63.5±0.3	62.5±0.6	61.3±3.9
Cell Contents of thermophilic and mesophilic bacteria, c.f.u./g TS									
thermophilic	1.0x10 ³	1.0x10 ⁵	3.6x10 ⁶ /4.0x10 ⁸	6.0x10 ⁸	1.0x10 ⁸	2.0x10 ⁶	6.0x10 ⁷	2.0x10 ⁸	4.0x10 ⁸
mesophilic	1.4·x10 ⁸	1.0x10 ⁷	9.0x10 ⁶	8.0x10 ⁵	4.0x10 ⁴	1.0x10 ⁷	1.0x10 ⁶	7.010 ⁵	2.5x10 ⁵

Note: *Starter culture was added to the reactor; **pH/adjusted pH; ***The second batch of food waste was added to the reactor.

pH dropped from 6.5 to 4.5 in the first 2 days and was raised to 6.9 in a short period of time after 5% dosage of pH buffer was added. Such level (around 6.5) of pH maintained for 2 days and dropped immediately to 6.0 when the second portion of the food waste was added. In the next 3 days, pH had not significant change (Figure 4-4).

The cell content of thermophilic bacteria increased in the first 2 days from 1.0x10³ to 3.6x10⁶ c.f.u./g TS, which, however, was not sufficient to stimulate a high rate biodegradation of food waste. It was brought to a level of 4x10⁸ c.f.u./g TS by the addition of starter cultures SW 09 and SW25 on day 2. Such cell content maintained for the next 2 days and then dropped to 2.0x10⁶ c.f.u./g TS because of the dilution of more fresh food waste added on day 4. In the last 3 days, the cell content rapidly recovered back to an ideal level of 4.0x10⁸ c.f.u./g TS. The stabilized pH at above 6.0 and the additional food waste on day 4 were probably the main reasons for such a rapid growth of thermophilic bacteria (Figure 4-4).

The cell content of mesophilic bacteria started to decrease on day 1 and continued to decrease after experiencing an increment on day 4 because the addition of fresh food waste brought more mesophilic bacteria into the reactor (Figure 4-4).

Figure 4-4 Variation of pH and Cell Contents of Thermophilic and Mesophilic Bacteria in BSBB2

The VS content decreased from 87.5% to 56.2% in the first 4 days and achieved more than 80% reduction of VS mass in the initial food waste. Then the VS content on day 4 was increased immediately from 56.2% to 66.3% by the addition of fresh food waste and decreased to 61.3% at the end of day 7. The actual VS reduction of the second portion of food waste in the last 3 days was only 20% (Figure 4-5).

Figure 4-5 Variation of VS Content and VS Loss in BSBB2

In this bioconversion, starter cultures, SW09 and SW25, were only supplemented to the reactor after the pH of food waste was adjusted to be stable at near neutral level. Both actions were implemented on day 2. The pH adjustment effectively raised the pH on day 2 and was not required in the subsequent days. In BSBB1, the daily pH adjustment in the first 2 days could not bring the pH back to near neutral level. Conversely, in this bioconversion, addition of SW09 and SW25 after pH adjustment on day 2, gave an increase in the cell content of thermophilic bacteria. In BSBB1, no significant effect of SW09 and SW25 added in the beginning was observed on the cell content of thermophilic bacteria in the subsequent bioconversion.

The reduction of VS mass in the first 4 days of this bioconversion was as high as 80% of the VS mass in the initial food waste. Such VS mass reduction in the 10 days of BSBB1 was only 73.3%. The performance of BSBB2 was obviously much better than BSBB1.

The performance of fed batch operation in food waste bioconversion was also examined. The addition of fresh food waste on day 4 did not significantly bring down the pH and the cell content of thermophilic bacteria. The common delay occurring in the early stage of batch operation by low pH was not observed.

4.2.2.3 Scanning Electron Microscopy (SEM)

The fresh food waste and the samples taken on day 2 and day 4 were virtually examined under Scanning Electron Microscope. The SEM images are displayed in Figure 4-6, 4-7 and 4-8.

1. *SEM image of fresh food waste at 1000 magnification (Figure 4-6)*

It was seen that the surface of fresh food waste was very smooth and there were no traces of microorganism attack.

2. *SEM image of day 2 sample at 1000 magnification (Figure 4-7)*

It was found that a portion of food waste was undergoing degradation. However, there was a big percentage of non degraded food waste in day 2 sample.

3. *SEM image of day 4 sample at 1000, 2500 and 5000 magnifications (Figure 4-8 (a, b, c))*

It was seen that most of food waste on day 4 had been biodegraded and only those portion built up with microorganisms-attack-resistant cellulose and lignin was left and undergoing a very slow degradation.

Figure 4-6 SEM Image of Fresh Food Waste in BSBB2 at 1000 Magnification

Figure 4-7 SEM Image of Day 2 Sample in BSBB2 at 1000 Magnification

(a) 1000 Magnification

(b) 2500 Magnification

(c) 5000 Magnification

Figure 4-8 SEM Image of Day 4 Sample in BSBB2 at 1000 (a), 2500(b) and 5000 (c) Magnifications

4.2.2.4 Plant Cultivation Test

Fertility of bioconversion 2 product (B2F) was examined by a plant cultivation test in comparison with that of a commercial organic fertilizer (COF). Infertile subsoil was taken as the planting soil and tomato was chosen as tested plant. B2F had two dosages of 1% and 2% (dry weight to the wet weight of soil) in the subsoil. The negative and positive controls were subsoil only and soil with COF, respectively. The dosage of COF had the same amount of nitrogen as 2 % of B2F. The duration was 6 weeks. The results are presented in Table 4-3. The images of plants are shown in Figure 4-9.

It was found that addition of B2F to subsoil increased the yield and growth of tomato plant by 4–7 times compared to that cultivated in subsoil only. It was also shown that B2F was compatible with COF in nutrient supply to plants.

Table 4-3 Planting Test Result of BSBB2

Parameters	B2F in subsoil, % of subsoil wet weight			COF with equivalent amount N to 2% of B2F**
	0*	1	2	
Length of stem, cm	65.0 ± 7.1	165.5 ± 5.1	143.3 ± 16.1	140.0 ± 8.7
Length of root, cm	11.0 ± 0.0	21.0 ± 0.30	46.7 ± 10.4	45.0 ± 5.0
Wet weight of stem, mg	218.3 ± 10.6	1531.9 ± 9.8	1550.57 ± 21.6	1403.7 ± 137.5
Wet weight of root, mg	25.3 ± 1.3	99.1 ± 1.0	99.47 ± 20.8	150.0 ± 19.3
Note: * means negative control; ** means positive control.

Figure 4-9 Images of Plants in B2F Plant Cultivation Test

From the left to the right: tomato plants cultivated on (1) subsoil only, (2) subsoil with COF, and (3) subsoil with 2 % of B2F.

4.3 Bench Scale Batch Bioconversion (Phase 2)

There are many sources of food waste found in Singapore. In BSBB1 and BSBB2, food waste used was mainly from vegetable food waste. Other than vegetable food waste, soybean residue is a good source to achieve a nutrient rich organic fertilizer after IAB process. It is rich in nitrogen and high in VS. Singapore food industry generates a huge amount of soybean residue each year. Due to its high VS content (>

95% of TS), degradation of soybean residue immediately occurs after it is generated and dumped on the waste collection ground. An unpleasant smell always develops and insects usually live in the soybean residue. Currently the soybean residue has been sent to neighbouring countries to be disposed of at their livestock farms. IAB process of soybean residue to organic fertilizer on site may be a way to save the transportation cost and generate revenue.

Fruit waste is another main source of food waste. Especially in the tropical countries like Singapore, the high consumption of fruits definitely generates an enormous amount of fruit waste. Fruit waste normally has high carbon and low nitrogen. Its pH is generally acidic and sometimes below 5 and may cause problems to IAB process. Anaerobic digestion method is generally suitable for bioconversion of fruit waste into biogas. In this study, the IAB process of fruit waste was investigated and reported below.

The bench scale batch bioconversion (phase 2) were designed to investigate the aerobic degradation pattern of fruit waste and soybean waste in IAB process.

4.3.1 Bench Scale Batch Bioconversion 3 (BSBB3)

4.3.1.1 Raw Materials and Experimental Design

A mixture composed of mainly soybean residue and small amount of vegetable waste was prepared and bioconverted in a bench scale reactor. Vegetable waste was used as a bulking material, which amounted to 40% of the final mixture volume. The composition of this mixture and general characteristics of the raw materials are listed in Tables 4-4 and 4-5.

CaCO_3 (5% of total TS of the mixture) was mixed with raw materials to prevent pH drop in the beginning. After 1 day, 600 ml of SW09 and SW25 suspension (10^8 c.f.u./ml) was added into the mixture as starter cultures. Samples were collected before addition of water. This mixture was studied in the batch experiment for 7 days.

Table 4-4 Composition of Initial Mixture in BSBB3

Raw materials	Wet weight, g	% of total wet weight	TS, g	% of total TS weight
Soy waste	900	75%	144	89.4%
Vegetable Waste	300	25%	17	10.6%

Table 4-5 Characteristics of Raw Materials in BSBB3

Raw Material	TS, % of Raw Weight	VS, % of TS	pH	C, % of TS	N, % of TS	C/N	Stability index, mg CO ₂ -C/g VS-day	
							Maximum	Average
Veg. Waste	5.57±0.23	75.20±1.12	5.93	32.19±0.43	4.26±0.13	6.3	9.08	6.07
Soy waste	15.98±0.10	96.22±0.11	6.63	45.54±0.23	5.37±1.27	8.5	23.92	21.33
Mixture	13.42	94.00	6.4	44.13	5.25	8.4	22.67	20.04

4.3.1.2 Results and Discussions

The chemical and biological characteristics of intermediate and end products are listed in Table 4-6. VS content of the mixture decreased from around 94% to 89% and the total VS mass reduction was 45 % of VS mass in the initial mixture during the 6-day bioconversion (Figure 4-10). Most mass reduction of VS occurred in the first 2 days. Carbon and nitrogen content were reduced from 44.1% to 43.5% of TS and from 5.3% to 3.1% of TS, respectively. The actual carbon and nitrogen reduction were equal to 45.7 % and 68% of carbon and nitrogen mass in the initial mixture, respectively. The initial C/N ratio was about 8.4, which was not recommended as starting C/N ratio for either IAB process or composting. Such low C/N ratio indicated that carbon available to microorganisms would not be sufficient to provide the carbon sources and energy sources to fix the available nitrogen. NH₃ hydrolyzed from proteins would be evaporated into the air, under any circumstances especially in the IAB process where active agitation and aeration would accelerate the loss of NH₃. C/N ratio was 14.3 after 6 days.

Table 4-6 Chemical and Biological Characteristics of Intermediate and End Products in BSBB3

	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
TS , %	13.42	8.83±0.88	14.42±0.29	15.42±0.19	15.02±0.43	15.42±0.23	19.96±0.40
VS, % of TS	94.00	93.29±0.77	90.93±0.47	90.71±0.51	90.51±0.54	89.51±0.14	89.17±0.26
pH	6.6	5.7	5.2	6.6	7.0	6.9	6.8
C, % of TS	44.13	N.A.					43.54±0.34
N, % of TS	5.25						3.05±0.23
C/N	8.4						14.3
Stability index	20.04	11.54	11.64	11.54	10.76	9.56	8.44
Stability grade	unstable						
cell content of thermophilic bacteria, c.f.u./ g TS							
	1.0×10 ⁸	9.0×10 ⁷	1.0×10 ⁸	4.5×10 ⁸	6.5×10 ⁸	8.2×10 ⁸	1.1×10 ⁹

Figure 4-10 Variation of VS Content and VS Mass Loss in BSBB3

The mixture was acidic in the first 4 days after 5 % of CaCO₃ had been added at start. It was mainly due to excessive amount of organic acids anaerobically produced in some dead zones of the bioreactor. Soybean residue has an extraordinarily high VS content and vegetable waste is also rich in VS. Most of VS in both wastes are easily degradable and unstable. When the anaerobic and other conditions were favoured, organic acids which were mainly volatile fatty acids were formed excessively.

The cell content of thermophilic bacteria was 10⁹ c.f.u./g TS at the end of the

bioconversion. The end product had an average stability index of 8.44 mg CO₂-C/g-VS d. The stability was significantly improved compared to 20.04 mg CO₂-C/g-VS d of the initial mixture. However, the end mixture was still microbiologically unstable and longer bioconversion duration was required to decompose the remaining organic matter.

4.3.2 Bench Scale Batch Bioconversion 4 (BSBB4)

4.3.2.1 Raw Materials and Experimental Design

A mixture composed of vegetable and fruit waste with the same wet weights was prepared and studied. The composition of this mixture and general characteristics of the raw materials are listed in Tables 4-7 and 4-8. Fruit waste in the initial mixture was 72.52% of total dry weight, and produced a low pH of 4.5 in the mixture. VS, carbon and nitrogen contents of the initial mixture were 88.10%, 41.57% and 2.96% of TS, respectively.

Table 4-7 Composition of Initial Mixture in BSBB4

Raw materials	Wet weight, g	% of total wet weight	TS, g	% of total TS weight
Fruit Waste	1250	50	184	72.52
Vegetable Waste	1250	50	70	27.48

Table 4-8 Characteristics of Raw Materials in BSBB4

Raw Material	TS % of Raw Weight	VS % of TS	pH	C % of TS	N % of TS	C/N	Stability index (mg CO ₂ -C/g VS-day)	
							Maximum	Average
Fruit waste	14.70±0.76	93.81±0.14	3.8	45.01±0.23	2.13±0.10	21.1	0.94	0.58
Veg. Waste	5.57±0.23	75.20±1.12	5.9	32.19±0.43	4.26±0.13	6.3	9.08	6.07
Mixture	10.1	88.10	4.5	41.57	2.96	14	2.84	1.86

Both CaCO₃ and 1 N NaOH solution were used to adjust the pH. 1000 ml of SW09 and SW25 suspension with 10⁸ c.f.u./ml was added as starter cultures two hours before the first pH adjustment by NaOH at the end of day 1. This mixture was

bioconverted for 8 days.

4.3.2.2 Results and Discussions

During the 8 days of bioconversion, the VS content decreased from 88% to 64% and total 75.7% of VS mass in the initial mixture was bioconverted (Table 4-9 and Figure 4-11). Most mass reduction of VS occurred in the first 4 days. Carbon and nitrogen contents decreased from 41.6% to 33.5% and from 3% to 2.6% of TS, respectively. The actual carbon and nitrogen reduction were equal to 73 % and 70% of carbon and nitrogen mass in the initial mixture, respectively.

Table 4-9 Chemical and Biological Characteristics of Intermediate and End Products in BSBB4

	Day 0	Day 1	Day 2	Day 3	Day 4	Day 6	Day 7	Day 8
TS, %	10.1	8.07±0.59	11.55±0.64	11.39±0.34	10.02±0.06	12.13±2.87	6.79±1.91	95.16±1.64
VS, % of TS	88.10	83.10±1.35	76.34±2.20	74.11±0.93	69.88±0.11	68.72±1.76	64.99±3.15	64.26±0.96
pH	4.5	5.4/6.5	5.3/6.8	5.2/6.8	6.4	6.9	7.4	7.3
C, % of TS	41.57	N.A.						33.5
N, % of TS	2.96							2.6
C/N	14							12.9
Stability index	1.86	2.23	3.12	4.53	7.2	6.80	5.67	4.76
Cell content of thermophilic bacteria, c.f.u./ g TS								
	1.0x10 ⁴	8.5x10 ⁵ / 2.0x10 ⁸	1.0x10 ⁵	4.3x10 ⁶	1.0x10 ⁸	1.2x10 ⁸	1.5x10 ⁸	1.5x10 ⁸

Figure 4-11 Variation of VS Content and VS Mass Loss in BSBB4

Although 5 % of CaCO_3 was added, the mixture remained acidic in the first 4 days (Figure 4-12). It was mainly due to the presence of fruit waste which produced excessive amount of organic acids. Additional CaCO_3 was not able to increase pH to the neutral in the short period of time. 1 N NaOH solution was then used for rapid neutralization. 50, 80 and 80ml of 1 N NaOH were needed to increase the pH to above 6.5 on days 1, 2 and 3, respectively.

The cell content of thermophilic bacteria increased from 10^6 to 10^8 c.f.u./g TS after the first NaOH pH adjustment. However, it dropped to 10^5 c.f.u./g TS in one day. After three NaOH pH adjustments in the first 4 days, it finally stabilized at a level of 10^8 c.f.u./g TS.

Figure 4-12 Variation of pH, Stability and Cell content of Thermophilic Bacteria in BSBB4

The end product had an average stability index of 4.76 mg-C/g-VS d. The initial stability index was 1.86 mg-C/g-VS d. This increase of stability index from the beginning to the end could be explained by the fact that the initial mixture contained a great amount of organic acids, which inhibited or deactivated the growth of microorganisms in the carbon dioxide evolution rate test and thus CO_2 from respiration of microorganisms was low. However, the end mixture was still

microbiologically unstable. Longer duration bioconversion could decompose the remaining organic matter in the fruit waste. Anaerobic digestion is more suitable for fruit waste which is a good source of volatile fatty acids.

4.4 References of Chapter 4

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Chapter 5

Results and Discussions:

Pilot Scale Batch Bioconversion

Several pilot scale batch bioconversions were designed to simulate the real situation of food waste bioconversion in a pilot scale bioreactor after the study of bench scale batch bioconversion of food waste.

Due to the restriction of small reactor volume in the bench scale bioconversions, the effect of bulking agent usually used in real bioconversion was not studied. In this research, horticultural compost was used as bulking agent. There were several reasons to select horticultural compost as bulking agent in pilot scale bioconversions. The horticultural compost was at the end of active degradation period and thus could provide the necessary microorganisms to the bioconversion if mixed with the fresh food waste. In a short time, the degradation of horticultural compost was considered negligible because of its high lignocellulose content. Hence the sole degradation of food waste could be calculated and studied. At the end of the bioconversion, the horticultural compost could be either screened out and recycled to the new process, or directly applied with organic fertilizer of food waste. The local horticultural compost is a cheap material. Large quantity of horticultural compost can be obtained with low cost.

5.1 Pilot Scale Batch Bioconversion 1 (PSBB1)

5.1.1 Raw Material and Experimental Design

A mixture, composed of vegetable waste and soybean residue, was prepared and bioconverted in the pilot scale reactor. This bioconversion was studied as control.

There was neither bulking agent nor starter culture provided.

The composition of the mixture and general characteristics of the raw materials are listed in Tables 5-1 and 5-2. The initial mixture had the same wet weight of vegetable food waste and soy waste. VS, carbon and nitrogen of the initial mixture were 90.79, 42.09 and 5.08% of TS, respectively.

Table 5-1 Composition of Initial Mixture in PSBB1

Raw materials	Wet weight, g	% of total wet weight	TS, g	% of total TS weight
Vegetable Waste	40000	50	2228	25.85
Soy waste	40000	50	6392	74.15

Table 5-2 Characteristics of Raw Materials in PSBB 1

Raw Material	TS, % of raw weight	VS, % of TS	pH	C, % of TS	N, % of TS	C/N	Stability index, mg CO ₂ -C/g VS-day	
							Maximum	Average
Vegetable waste	5.57±0.23	75.20±1.12	5.93	32.19±0.43	4.26±0.13	6.3	9.08	6.07
Soy waste	15.98±0.10	96.22±0.11	6.63	45.54±0.23	5.37±1.27	8.5	23.92	21.33
Mixture	10.78	90.79	6	42.09	5.08	7.7	20.74	18.06

5% of CaCO₃ was mixed with raw materials in the beginning to prevent pH from dropping. In all pilot scale bioconversions of this research, the moisture level of the mixture was monitored daily and compensated by adding the necessary amount of water so as to maintain the optimum value at between 75 and 80%. Samples were collected before addition of water. A representative sample of 30 g was taken daily by mixing 3 sub-samples from 3 locations of the mixture (in the whole profile: from the left to the centre and then to the right of the mixture). Each sample was divided into two parts, one of which was immediately sealed and stored at 4°C, while the other part was dried and ground to particles less than 0.2 mm in size. All measurements were done in triplicate. This mixture was bioconverted for 10 days. At the end of day 9, addition of tap water was stopped and the temperature of the reactor was maintained at 80°C for 12 hours before the whole process was ceased.

5.1.2 Results and Discussions

VS content decreased from around 83 to 71% and 50% of VS mass in the initial mixture was bioconverted during the 9-day bioconversion (Table 5-3 and Figure 5-1). Carbon and nitrogen contents decreased from 42.1% to 38.6% and from 4.7% to 2.8% of TS, respectively. The actual carbon and nitrogen reduction were equal to 46% and 65% of carbon and nitrogen mass in the initial mixture, respectively. C/N ratio was about 9 at start and finally stabilized at 13.6 after 6 days.

Table 5-3 Chemical and Biological Characteristics of Intermediate and End Products in PSBB1

	Day 0	Day 1	Day 2	Day 3	Day 5	Day 6	Day 7	Day 9
TS, %	9.67±0.26	12.65±0.61	13.74±0.49	14.29±0.43	22.41±1.40	31.42±0.62	34.63±1.82	95.06±0.08
VS, % of TS	82.79±0.77	76.27±0.47	75.6±0.57	75.06±0.54	75.8±0.26	74.03±0.69	71.96±0.98	70.75±0.44
pH	5.91	6.50	6.24	5.76	5.82	5.90	6.18	5.90
C, % of TS	42.14±0.39	38.72±0.19	39.51±0.30	39.64±0.25	39.47±0.34	39.34±0.02	38.32±0.27	38.57±0.32
N, % of TS	4.73±0.17	4.25±0.19	3.89±0.05	3.28±0.01	3.07±0.23	2.89±0.11	2.78±0.13	2.82±0.10
C/N	8.91	9.11	10.16	12.09	12.87	13.61	13.78	13.61
Stability index (mg-CO ₂ -C/g-VS d)								
	6.88	10.09	10.40	11.62	8.26	8.41	7.55	7.68
Stability Grade	Unstable							
Cell content of thermophilic bacteria, c.f.u./ g TS								
	1×10 ⁸	2.2×10 ⁸	3.3×10 ⁸	2.6×10 ⁸	2.3×10 ⁸	2.3×10 ⁸	1.6×10 ⁸	-

Figure 5-1 Variation of VS Content and VS, C and N Mass Loss in PSBB1

The mixture was acidic in the whole process mainly due to excessive amount of organic acids being produced anaerobically. The cell content of thermophilic bacteria was at a high level of 10^8 c.f.u/g TS regardless of the low pH condition (Figure 5-2).

Figure 5-2 Variation of pH and Cell Content of Thermophilic Bacteria in PSBB1

The changes in different forms of nitrogen are shown in the Figure 5-3. Although excessive $\text{NH}_3\text{-N}$ was produced from the hydrolysis of proteins and simple peptides at the start, the intensive agitation and aeration of the reactor removed most of the hydrolysis products. The decrease of $\text{NH}_3\text{-N}$ concentration in the mixture continued until day 4. Another portion of $\text{NH}_3\text{-N}$ loss in the beginning was due to the conversion of $\text{NH}_3\text{-N}$ to $\text{NO}_3^-\text{-N}$. The concentration of $\text{NO}_3^-\text{-N}$ increased to a level of around 1000 mg/kg TS. This indicated that probably some favourable conditions such as a temperature below 40°C existed at some locations of the reactor, for nitrifying bacteria to transform $\text{NH}_3\text{-N}$ to $\text{NO}_3^-\text{-N}$. The total organic nitrogen was maintained at a level of above 97% of total nitrogen.

The stability index of intermediate and end products are shown in Figure 5-4. The end product after the 9-day bioconversion had an average stability index of 7.7 mg $\text{CO}_2\text{-C/g-VS}$ d which indicated that the bioconverted mixture was still microbiologically unstable and longer bioconversion duration was required to decompose the remaining easily biodegradable organic matter.

Figure 5-3 Variation of Different Forms of Nitrogen in PSBB1

Figure 5-4 Variation of Stability Index in PSBB1

5.2 Pilot Scale Batch Bioconversion 2 (PSBB2)

5.2.1 Raw Material and Experimental Design

A mixture consisting of vegetable waste, soybean residue and horticultural compost, was prepared and bioconverted in the pilot-scale reactor. Compared to the PSBB1, this bioconversion had the horticultural compost to function as mainly a

bulking agent and probably a microorganism provider. No extra starter culture was added.

The composition of this mixture and general characteristics of the raw materials are presented in Tables 5-4 and 5-5. Horticultural compost accounted for 20% of total wet weight but contributed 50% of total volume to the initial mixture. The wet weights of vegetable food waste and soy waste were mostly the same. VS, carbon and nitrogen contents of the initial mixture were 84.03, 41.16 and 3.37% of TS, respectively.

Table 5-4 Composition of Initial Mixture in PSBB2

Raw Material	Wet weight , g	% of total wet weight	TS , g	% of total TS weight
Vegetable Waste	27000	40.3	1620	14
Soy waste	28000	41.8	4474	40
Compost	12000	17.9	5222	46

Table 5-5 Characteristics of Raw Materials in PSBB2

Raw Material	TS % of raw Weight	VS % of TS	pH	C, % of TS	N, % of TS	C/N	Stability index, mg CO ₂ -C/g VS-day	
							Maximum	Average
Vegetable Waste	5.57±0.23	75.20±1.12	5.93	32.19±0.43	4.26±0.13	6.28	9.08	6.07
Soy waste	15.98±0.10	96.22±0.11	6.63	45.54±0.23	5.37±1.27	8.5	23.92	21.33
Compost	43.52±1.32	76.34±1.05	6.57	40.19±2.72	1.37±0.14	29.3	1.96	1.23
Mixture	16.89	84.03	6.2	41.16	3.37	11.8	12.81	10.95

2.5% of CaCO₃ was mixed with raw materials in the beginning and the same dosage was added on day 2. During the whole process, there was no starter culture added. Samples were collected before addition of water. This mixture was bioconverted for 10 days.

5.2.2 Results and Discussions

The chemical and biological characteristics of intermediate and end products are listed in Table 5-6. During the 10-day bioconversion, the VS content of the mixture

decreased from around 76 to 64% and 45% of VS mass in the initial mixture was bio-converted (Table 5-6 and Figure 5-5). Most mass reduction of VS occurred in the first 5 days. Carbon and nitrogen contents decreased from 39% to 33% and from 2.4% to 2.2% of TS, respectively. The actual carbon and nitrogen reduction were equal to 44% and 40% of carbon and nitrogen mass in the initial mixture, respectively. The initial C/N ratio was about 16 and stabilized at 15 after 7 days.

Table 5-6 Chemical and Biological Characteristics of Intermediate and End products in PSBB2

	Day 0	Day 1	Day 3	Day 4	Day 5	Day 6	Day 8	Day 10
TS, %	19.74±1.37	21.18±1.41	30.01±0.39	31.89±0.43	31.88±1.37	36.03±0.70	34.52±0.08	37.00±.054
VS, % of TS	76.23±1.07	74.38±1.87	69.92±0.95	67.65±1.22	65.88±0.66	65.34±0.39	65.25±0.67	63.77±0.44
pH	6.1	5.65/7.2	7.4	7.2	7.5	7.6	8.2	8
C, % of TS	39.03±0.02	37.71±0.44	35.07±0.84	34.57±0.78	34.31±0.21	34.15±0.20	33.35±0.32	33.25±0.77
N, % of TS	2.41±0.07	2.51±0.09	2.63±0.13	2.36±0.01	2.27±0.04	2.21±0.08	2.2±0.33	2.18±0.08
C/N	16.19	15.02	13.33	14.65	15.11	15.45	15.16	15.25
	Stability index (mg-CO ₂ -C/g-VS d)							
	6.78	7.07	8.32	7.80	5.83	4.01	3.14	2.91
Stability Grade	Unstable					stable		
	Thermophilic bacterial cell content, c.f.u./g TS							
	2×10 ⁵	3.5×10 ⁷	5×10 ⁷	4×10 ⁷	4×10 ⁷	4.2×10 ⁸	9.8×10 ⁷	2.1×10 ⁸

Figure 5-5 Variation of VS Content and VS, C and N Mass Loss in PSBB2

The mixture contained nearly 50% of horticultural compost on dry matter basis which had a neutral pH and was mainly composed of non-easily biodegradable organic matter. Thus, pH became neutral or slightly basic from day 2 onwards. The cell content of thermophilic bacteria started to increase from the beginning, and maintained at a high level of 10^8 c.f.u./g TS. (Figure 5-6).

Figure 5-6 Variation of pH and Cell Content of Thermophilic Bacteria in PSBB2

The change of different forms of nitrogen is shown in the Figure 5-7. The decrease of $\text{NH}_3\text{-N}$ concentration in the mixture was immediately observed on day 1. Another portion of $\text{NH}_3\text{-N}$ loss in the beginning may be due to the conversion of $\text{NH}_3\text{-N}$ to $\text{NO}_3^-\text{-N}$. The concentration of $\text{NO}_3^-\text{-N}$ increased to a level of 4000 mg /kg TS in the first 2 days and then decreased. Organic nitrogen decreased from 97% to 83% of total nitrogen in the first 2 days when $\text{NO}_3^-\text{-N}$ had the maximum level, and maintained at above 95% level in the following days.

The stability index of intermediate and end products are shown in Figure 5-8. The product after the 6-day bioconversion had an average stability index of 4 mg-C/g-VS d, which indicated that after 6-day bioconversion, the mixture residue became microbiologically stable and longer bioconversion duration may not be required.

Figure 5-7 Variation of Different Forms of Nitrogen in PSBB2

Figure 5-8 Variation of Stability Index in PSBB2

5.3 Pilot Scale Batch Bioconversion 3 (PSBB3)

5.3.1 Raw Materials and Experimental Design

A mixture consisting of vegetable waste, soybean residue and horticultural compost was prepared and bioconverted. In this bioconversion, extra starter culture

was prepared and added. The effect of such starter culture on the bioconversion was investigated in the presence of horticultural compost.

The composition of this mixture and general characteristics of the raw materials are listed in Table 5-7 and Table 5-8. Horticultural compost accounted for around 20% of total wet weight but contributed 50% of total volume to the initial mixture. The wet weights of vegetable food waste and soy waste were the same. VS, carbon and nitrogen contents of the initial mixture were 83.68%, 41.56% and 3.25% of TS, respectively.

Table 5-7 Composition of Initial Mixture in PSBB3

Raw material	Wet weight, g	% of total wet weight	TS, g	% of total TS weight
Veg. waste	25000	40.3	1400	13.1
Soy waste	25000	40.3	4000	37.6
Compost	12000	19.4	5250	49.3

Table 5-8 Characteristics of Raw Materials in PSBB3

Raw Material	TS, % of Raw Weight	VS, % of TS	pH	C, % of TS	N, % of TS	C/N	Stability index mg CO ₂ -C/g VS-day	
							Maximum	Average
Veg. Waste	5.57±0.23	75.20±1.12	5.93	32.19±0.43	4.26±0.13	6.28	9.08	6.07
Soy waste	15.98±0.10	96.22±0.11	6.63	45.54±0.23	5.37±1.27	8.5	23.92	21.33
Compost	43.52±1.32	76.34±1.05	6.57	40.19±2.72	1.37±0.14	29.3	1.96	1.23
Mixture	17.11	83.68	6.2	41.56	3.25	12.7	12.31	10.50

2.5% of CaCO₃ was mixed with raw materials in the beginning and the same dosage was added on day 2. 2000 ml of 10⁹ c.f.u/ml SW09 and SW25 suspensions were added 2 hours after the first pH adjustment. The mixture was bioconverted for 14 days.

5.3.2 Results and Discussions

The chemical and biological characteristics of intermediate and end products are listed in Table 3-3. During the 12-days bioconversion, VS content decreased from around 80% to 66% and 52% of VS mass in the initial mixture was bioconverted

(Figure 5-9). Most mass reduction of VS had a linear relationship with time during the first week and then such reduction slowed down towards the end of the process. Carbon and nitrogen contents decreased from 40% to 35.4% and from 2.56% to 2.38% of TS, respectively. The actual carbon and nitrogen reduction were equal to 48.3% and 45.7% of carbon and nitrogen mass in the initial mixture, respectively (Figure 5-9). The C/N ratio was initially about 15.7 and finally stabilized at 15 after 7 days.

Table 5-9 Chemical and Biological Characteristics of Intermediate and End products in PSBB3

	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12
TS (%)	19.91±1.07	23.26±0.90	22.97±2.37	30.16±0.45	39.47±3.03	34.16±0.08	98.87±.054
VS, % of TS	80.25±0.33	77.86±1.51	74.20±1.00	70.40±3.07	69.51±0.84	67.09±0.67	66.19±0.44
pH	5.65/7.2	6.0/6.8	7.0	7.8	7.9	8.1	8.1
C, % of TS	40.1±0.19	39.38±0.84	39.10±0.78	36.54±0.49	36.01±0.48	35.98±0.12	35.41±0.77
N, % of TS	2.56±1.46	2.58±0.13	2.62±0.01	2.51±0.04	2.45±0.12	2.42±0.33	2.38±0.08
C/N	15.67	15.26	14.92	14.56	14.89	14.87	14.88
	Stability index, mg-C/g-VS day						
	9.88	10.05	7.83	3.32	2.33	2.09	1.89
Stability Grade	Unstable			Stable		Very stable	
	Thermophilic bacterial cell content, c.f.u./ g TS						
	1×10 ⁸	8.6×10 ⁶	4.5×10 ⁷	5.8×10 ⁷	4.8×10 ⁷	6.8×10 ⁷	6.5×10 ⁷

Figure 5-9 Variation of VS Content and VS, C and N Mass Loss in PSBB3

pH became neutral or slight basic from day 2 onwards. The cell content of thermophilic bacteria was initially 10^8 c.f.u./g TS and slightly dropped to 10^7 c.f.u./g TS after the first 2 days due to the production of organic acids. After pH was adjusted to neutral level, it went back to a level of 10^8 c.f.u./g TS (Figure 5-10).

Figure 5-10 Variation of pH and Cell Content of Thermophilic Bacteria in PSBB3

The variation in different forms of nitrogen is shown in Figure 5-11. The decrease of $\text{NH}_3\text{-N}$ concentration in the mixture was immediately observed in the first 2 days. Another portion of $\text{NH}_3\text{-N}$ loss may be due to the conversion of $\text{NH}_3\text{-N}$ to $\text{NO}_3^-\text{-N}$. The concentration of $\text{NO}_3^-\text{-N}$ increased to a level of 1100 mg /kg TS in the first 2 days and then decreased. Organic nitrogen content decreased from 98% to 95% of total nitrogen in the first 2 days when $\text{NO}_3^-\text{-N}$ hit the maximum level, and maintained at above 98% level in the following days.

The stability index of intermediate and end products are shown in Figure 5-12. The product after 6 days, had an average stability index of 3.5 mg-C/g-VS d which indicated that after 6-day bioconversion, the residue became microbiologically stable and longer bioconversion duration may not be required.

Figure 5-11 Variation of Different Forms of Nitrogen in PSBB3

Figure 5-12 Variation of Stability Index in PSBB3

5.4 Comparison of Pilot Scale Batch Bioconversions

5.4.1 pH

In Figure 5-13, it was observed that in PSBB1, pH was below 6 during most of the first 9 days. On the other hand, in PSBB2 and PSBB3, pH increased above 7 after

going through a slight decrease in the first 1 or 2 days. Such difference in pH behaviour may be due to the presence of horticultural compost. Physically horticultural compost was used as a bulking agent to increase the bulk density of the mixture to ensure the aeration inside the mixture was sufficient to diminish the development of anaerobic zone. Chemically, it was reported that the compost may possess the capacity of pH buffering (Clapp et al, 1986).

Figure 5-13 Comparison of pH Changes in Three Pilot Scale Batch Bioconversions

5.4.2 Thermophilic Bacteria

The cell contents of thermophilic bacteria in three bioconversions were at levels of between 10^7 to 10^8 c.f.u./g TS independent of the addition of starter culture or horticultural compost (Figure 5-14).

5.4.3 Stability Index

In the PSBB1, all of the intermediate products were not microbiologically stable. Conversely, the products on day 6 of the PSBB2 and PSBB3 were tested and found to be microbiologically stable. Obtaining a microbiologically stable end product in a short period of time is one of the goals of IAB. Comparing these three bioconversions, it was concluded the horticultural compost have helped to improve the biological

stability of the bioconversion products (Figure 5-15).

Figure 5-14 Comparison of Cell Content of Thermophilic Bacteria in Three Pilot Scale Batch Bioconversions

Figure 5-15 Comparison of Stability of Samples in Three Pilot Scale Batch Bioconversions

5.4.4 Nitrogen Loss

Nitrogen loss in composting process is a big issue since high nitrogen content makes compost attractive to the end user. Nitrogen loss is undesirable but also inevitable. In IAB, nitrogen loss is also a very important issue. In the PSBB1, it was

found that nitrogen loss in the 9 days was as high as 60%. The nitrogen loss in PSBB2 and PSBB3 were 35% at the end of 6 days (Figure 5-16). The high nitrogen loss in such short period of time might be resulted from the intensive aeration and agitation. Addition of CaCO_3 to the mixture was another reason causing nitrogen loss. It was reported that CaCO_3 conditioned composting had a nitrogen loss of as high as 60% (Witter and López-Real, 1988).

The high-rate organic matter degradation was coupled with the high-rate nitrogen loss. NH_3 volatilization was the main causing mechanism of nitrogen loss. In PSBB1, the massive amount of NH_3 was produced on day 1 (Figure 5-5 and Figure 5-7 in PSBB1). The $\text{NH}_3\text{-N}$ was formed by the hydrolysis of organic nitrogen, like protein and peptides. The $\text{NH}_3\text{-N}$ produced in the beginning of PSBB2 and PSBB3 was quite high but less than that of PSBB1 (Figure 5-17). Hence, under the condition of intensive aeration and agitation, ammonia volatilization loss in PSBB1, was definitely higher than PSBB2 and PSBB3. Thus additional horticultural compost reduced the nitrogen loss. These findings agree with those of Witter and López-Real (1988), Morisaki et al., (1989), and Paredes et al. (1996), who emphasized the importance of adding lignocellulosic materials to reduce nitrogen losses.

Figure 5-16 Comparison of N Loss in Three Pilot Scale Batch Bioconversions

Figure 5-17 Comparison of NH_3 Amount in Three Pilot Scale Batch Bioconversions

5.4.5 First Order Kinetic Constants and Maximum Degradation Rates

A simple equation can be applied to describe the degradation of organic matter,

$$\frac{dS}{dt} = -k_s S \quad \text{Equation (5-1)}$$

where S is the weight of biodegradable substrate (g), k_s is a kinetic constant based on substrate weight (d^{-1}) and t is the time (d).

By integrating both sides of Equation (5-1), it becomes $\int_{S_0}^S \ln S = -\int_0^t k_s dt$, or

further $\ln \frac{S}{S_0} = -k_s t$, and finally reaches another expression format,

$$S = S_0 e^{-k_s t} \quad \text{Equation (5-2)}$$

where S_0 is the initial weight of biodegradable substrate (g).

Many researchers used Equation (5-1) and Equation (5-2) for simplicity (Fuzita, 1993; Haug, 1993; Marugg et al., 1993; Shin et al., 1999). These simplified equations have worked well in describing composting process as reviewed by Haug (1993). This study also used the simplified first order kinetic equation to define the organic matter degradation pattern in intensive aerobic bioconversion under isothermophilic condition.

The kinetic constants were obtained for each feeding stages by plots of $\ln(S/S_0)$ vs time. It was possible to describe the composting rate and microbial activity using kinetic constants. It was also a good measure for simulation as well as for the validation of experimental data (Haug, 1993).

However, it was pointed out that the kinetic constant itself could not be compared directly for the interpretation of biodegradation rate because the initial substrate amount in different experiments may be different (Shin et al., 1999). Therefore, maximum degradation rates of biodegradable substrate should be calculated and compared using the following equation,

$$\left(\frac{dS}{dt} \right)_{max} = -k_s S_0 \quad \text{Equation (5-3)}$$

The maximum degradation rate of biodegradable substrate is a comparable index of composting activity exerted by the microorganisms, as it has a kinetic constant term and substrate term.

In Figure 5-18 and Figure 5-19, it was noticed that there could be two exponential VS degradation patterns in each pilot scale bioconversion. For instance, in Figure 5-19, PSBB1 had a much faster food waste VS degradation rate in day 1 than the rest days. Thus, each bioconversion could have two VS degradation phases: the 1st phase had a high rate of VS degradation pattern, whilst the 2nd had a slow one. So the kinetic constants were estimated separately for two phases.

Figure 5-18 Comparison of VS Mass Loss in Three Pilot Scale Batch Bioconversions

Figure 5-19 Comparison of Food Waste VS Mass Loss in Three Pilot Scale Batch Bioconversions

The degraded food waste VS in each phase, in term of % of the initial food waste VS mass was listed in Table 5-10. The 1st phase and 2nd phase in the same bioconversion process had a different S_0 , the initial available biodegradable substrate, since the kinetic constants were estimated separately for two phases. For example, in the PSBB1, the 1st phase had a S_0 equal to 100% of the initial VS food waste mass, whereas the 2nd phase had a S_0 equal to 66.81% of the initial VS food waste mass, which was the portion of VS food waste mass left from the 1st phase. The VS reduction in the 1st phase was 33.19% of the initial food waste VS mass.

Table 5-10 First Order Kinetic Constants and Maximum Degradation Rates of Organic Matter

Parameters	PSBB1 with compost	PSBB2 with compost	PSBB3 with compost and starter culture
<i>Durations of high rate phase (1st phase) and slow rate phase (2nd phase), days</i>			
The 1 st phase	1	5	6
The 2 nd phase	8	5	6
<i>Biodegraded VS, % of initial food waste VS mass</i>			
The 1 st phase	33.19	68.19	75.26
The 2 nd phase	16.53	9.13	18.78
The whole process	49.72	77.32	94.04
<i>S₀, expressed as % of initial food waste VS mass</i>			
S ₀ for the 1 st phase	100	100	100
S ₀ for the 2 nd phase	66.81	31.81	24.74
<i>S, expressed as % of initial food waste VS mass</i>			
Day 0	100.00	100.00	100.00
Day 1	66.81	83.77	87.85
Day 2	64.41	68.31	75.59
Day 3	62.56	52.84	61.28
Day 4	62.13	40.38	46.97
Day 5	61.70	31.81	35.86
Day 6	59.26	29.36	24.74
Day 7	53.35	29.17	22.55
Day 8	51.82	28.97	20.35
Day 9	50.28	25.83	14.96
Day 10	-	22.68	9.57
Day 11	-	-	7.77
Day 12	-	-	5.96
<i>First order kinetic constants k, d⁻¹</i>			
The 1 st phase	0.4033 (r ² =1.0000)	0.2219 (r ² = 0.9948)	0.2136 (r ² = 0.9708)
The 2 nd phase	0.0329 (r ² =-0.9612)	0.0557 (r ² = 0.8612)	0.2274 (r ² = 0.9586)
<i>Maximum degradation rates= kS₀, % of initial food waste VS mass d⁻¹</i>			
The 1 st phase	40.33	22.19	21.36
The 2 nd phase	2.2	1.77	5.63
<i>Maximum degradation rates= kS₀, g food waste VS d⁻¹</i>			
The 1 st phase	2583	1243	1045
The 2 nd phase	141	99.14	275

Table 5-10 summarizes the calculated first order kinetic constants and the maximum degradation rates of organic matter in two phases of three pilot scale

bioconversions. PSBB1, PSBB2 and PSBB3 had 1, 5 and 6 days in the 1st phase, and 8, 5 and 6 days in the 2nd phase, respectively.

The $\ln(S/S_0)$ was plotted against the duration of each phase, t , in Figure 5-20 a, b and c. The calculation of the data points of PSBB1 was demonstrated in the following text. The same way of calculation was also applied on the data points of PSBB2 and PSBB3.

For instance, in the 1st phase of PSBB1, S_0 was equal to 100% of initial food waste VS mass, or $S_0=100\%$. The 1st phase of PSBB1 included two data points at day 0 and day 1, respectively.

On day 0, S was equal to S_0 , or $S=S_0=100\%$, thus $\ln(S/S_0)=0$ and $t=0$; on day 1, S was equal to 66.81% of initial food waste VS mass, or $S=66.81\%$, thus $\ln(S/S_0)=-0.4033$ and $t=1$. There were two data points of the 1st phase plotted in the upper part of Figure 5-20a.

And in the 2nd phase of PSBB1, S_0 was equal to 66.81% of initial food waste VS mass, or $S_0=66.81\%$. The 2nd phase of PSBB1 included 9 data points for each day from day 1 to day 9.

On day 1, S was equal to 66.81% of initial food waste VS mass, or $S=66.81\%$, thus $\ln(S/S_0)=0$ and $t=0$. Day 1 was the transition point from the 1st phase to the 2nd phase, i.e., the end point of the 1st phase and also the starting point of the 2nd phase. Thus t was equal to 0, or $t=0$ for day 1 in the 2nd phase.

On day 2, S was equal to 64.41% of initial food waste VS mass, or $S=64.41\%$, thus $\ln(S/S_0)=-0.0366$ and $t=1$. On day 3, S was equal to 62.56% of initial food waste VS mass, or $S=62.56\%$, thus $\ln(S/S_0)=-0.0657$ and $t=2$.

The same way of calculation was applied to the rest of the days. There were 9 data points for the 2nd phase plotted in the lower part of Figure 5-20a.

Each set of data points was fitted with a linear curve which had the most reasonable first order kinetic equation for such set of data points. The first order kinetic constant k was the slope of the individual fitting linear line. The k values and the corresponding relative variations r^2 for all phases of three bioconversions were listed in Table 5-10. The most relative variations r^2 were very close to 1. It was shown

that all the linear fittings were statistically satisfactory. The r^2 of the k value for the 2nd phase of PSBB2 was slightly low but still in an acceptable range.

(a) PSBB1

(b) PSBB2

(c) PSBB3

Figure 5-20 plotting of $\ln(S/S_0)$ vs. time for Each Phase of Three Pilot Scale Batch Bioconversions

It was difficult to compare the k values from this study directly to those of previous studies because the conditions of bioconversions such as substrate, pH and C/N ratio differed in each experiment (Shin et al., 1999). However, the k values obtained in this study were in the range of 0.03 to 0.4 d⁻¹ which was similar to those found in previous studies (Fuzita, 1993; Haug, 1993; Marugg et al., 1993).

The first order kinetic constant k for the 1st phase of PSBB1 was 0.4 d⁻¹ and twice of that for the 1st phase of another two bioconversions. There were probably several reasons to achieve this high k value. During that period, not only CO₂ as a result of microorganism consumption of easily degradable organic matter was produced and discharged into the ambient atmosphere, but also some easily degradable organic constituents, mostly volatile organic acids and somewhat ammonia were also subject to direct and massive discharge to the air through intensive aeration and agitation. During such period, strong acidic odour was detected. This odour did not occur in the PSBB2 and PSBB3. Such high rate biodegradation of VS lasted for 1 day only. After that, the k value of the 2nd phase of PSBB1 dramatically dropped to 0.033 d⁻¹ which

was only one tenth of the 1st phase k value. Such notable change was the consequence of the 1st day massive loss of easily biodegradable organic matter through both microorganism consumption and intensively direct evaporation into the air. In the beginning of the 2nd phase of PSBB1, the available food (easily degradable organic matter) to microorganisms became limited and at the same time, the destruction of the intermediate organic matter to easily degradable matter had a slow rate. So a decrease in microorganism cell content was observed in the beginning of the 2nd phase (Figure 5-2).

In PSBB2 and PSBB3, horticultural compost was added as bulking agent. When the degradation pattern of easily biodegradable garbage such as food waste was discussed, many papers always excluded the degradation of lignocellulose bulking agents such as sawdust and wood chips by presuming in a short period they had no degradation (Shin et al., 1999). In this study, the degradation of horticultural compost was not taken into account to predict the degradation of food waste.

The 1st phase k values for both PSBB2 and PSBB3 were almost the same. The only difference was that the 1st period of PSBB2 was slightly shorter than that of PSBB3. The 1st phase k values of both PSBB2 and PSBB3 were half lower than that of PSBB1. Such k values reflected the real biodegradation pattern of organic matter, and provided more precise scientific data to the study of intensive aerobic bioconversion. The 1st phase of PSBB2 and PSBB3 with relevant high k values was 4 to 5 days longer than that of PSBB1. It means the high rate degradation phases sustained longer in PSBB2 and PSBB3 than in PSBB1. In term of the cumulative VS mass loss, there was only 33% loss in the 1st phase of PSBB1 while there were 68% and 75% loss in the 1st phases of PSBB2 and PSBB3, respectively.

The 2nd phase k values for PSBB2 and PSBB3 had a big difference. It seemed that the 2nd phase degradation pattern of PSBB3 followed that of the 1st phase. The 2nd phase degradation rate of PSBB2 decelerated to only one quarter of that of the 1st phase. From Figure 5-14, it was found that, the cell content of thermophilic bacteria in PSBB2 started to decrease from day 5 (i.e. from the beginning of the 2nd phase) while the cell content in PSBB3 was maintained at a constant level. The indigenous

thermophilic bacteria in either food waste or horticultural compost might not be as active as SW09 and SW25 in degrading non-easily degradable organic matter. In PSBB3, the addition of starter culture SW09 and SW25 effectively improved the 2nd phase of bioconversion. It may prove again the SW09 and SW25 have strong degradation abilities.

5.5 References of Chapter 5

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Chapter 6

Results and Discussions:

Pilot Scale Fed Batch Bioconversion

6.1 Experimental Design

The fed batch bioconversion was designed based on the results of PSBB2. The same horticultural compost was used as a bulking agent and a microorganism source supplier as in the PSBB2. No previously selected inoculums were added in this fed batch bioconversion.

In fed batch bioconversion, the mass of bulking agent should be the same amount as or several times of that of initial added food waste (Nakasaki et al., 1998; Hwang et al., 2002). 23 kg of the wet horticultural compost and 18.6 kg of the wet food waste (10 kg and 2 kg in dry weight, respectively) were mixed and prepared in the beginning of the process. The mixture was buffered by 5% of CaCO_3 . In order to simulate the real fed batch operation, the food waste in every periodical feeding was always small. Each periodical feeding had 18.6 kg (wet weight) or 2 kg (dry weight) of food waste. Three periodical feedings were made at the end of day 4, 6 and 8, respectively (Table 6-1, Figure 6-1 and Figure 6-2). After the 3rd feeding, the bioconversion went back to batch mode to further stabilize the residue.

The food waste had a composition of vegetable food waste and soy waste with equal wet weights (refer to PSBB2 in Chapter 5). The food waste was air-dried and stored in a refrigerator at 4°C. A portion of the dried food waste was adjusted to the optimum moisture content (75%) before being added into the reactor (Hwang et al., 2002).

If plotted against time, food waste only and total mixture with horticultural compost show staircase-like curves (Figure 6-1 for wet weight and Figure 6-2 for dry matter). These curves stand as reference baselines for the coming curves of VS and carbon during the fed batch bioconversion.

Table 6-1 Feeding Schedule of Compost and Food Waste in Pilot Scale Fed Batch Experiment

Time (day)	Horticultural compost		Food waste		Total mixture	
	Weight added (kg)	Total weight added (kg)	Weight added (kg)	Total weight added (kg)	Weight added (kg)	Total weight added (kg)
0	23	23	18.6	18.6	41.6	41.6
1		23		18.6		41.6
2		23		18.6		41.6
3		23		18.6		41.6
4 *		23		18.6		41.6
4 **		23	18.6	37.2	18.6	60.2
5		23		37.2		60.2
6 *		23		37.2		60.2
6 **		23	18.6	55.8	18.6	78.8
7		23		55.8		78.8
8 *		23		55.8		78.8
8 **		23	18.6	74.4	18.6	97.4
9		23		74.4		97.4
10		23		74.4		97.4
11		23		74.4		97.4
12		23		74.4		97.4
13		23		74.4		97.4
14		23		74.4		97.4
15		23		74.4		97.4

* means the weight was calculated before the periodical feeding of food waste;
** means the weight was calculated after the periodical feeding of food waste.

Figure 6-1 Feeding Schedule of Compost and Food Waste in Pilot Scale Fed Batch Experiment (Wet Weight)

Figure 6-2 Feeding Schedule of Compost and Food Waste in Pilot Scale Fed Batch Experiment (Dry Weight)

6.2 Results and Discussions

6.2.1 Experimental Data

Table 6-2 lists pH, moisture content (MC), VS, carbon (C) content, nitrogen (N) content, cell content and stability index in daily samples.

Table 6-2 Experimental Results Measured in Pilot Scale Fed Batch Experiment

Time	pH	MC	VS	C	N	Thermophilic microbial cell content c.f.u./ g TS	Stability Index	
							Maximum	Average
		%	%	%	%		mg-C/g-VS d	
0	6.50	60.12	70.00	40.51	1.95	1.98x10 ⁶	15.6	14.2
1	7.20	61.23	68.72	40.20	1.52	6.60x10 ⁷	12.4	10.29
2	7.30	59.86	65.69	40.25	1.49	1.80x10 ⁸	8.71	7.26
3	7.40	60.23	62.52	40.29	1.45	4.10x10 ⁸	5.01	4.22
4*	7.60	59.86	61.60	40.02	1.46	6.00x10 ⁸	1.34	1.08
4**	7.10	68.56	67.00	40.36	1.72	6.80x10 ⁷	11.74	11.74
5	7.34	60.02	65.00	40.73	1.66	2.30x10 ⁸	13.64	11.11
6*	7.84	54.01	64.00	40.20	1.52	1.86x10 ⁹	6.48	6.38
6**	7.01	68.52	68.50	40.40	1.63	2.15x10 ⁸	13.79	11.94
7	7.25	66.25	67.36	40.18	1.63	5.70 x10 ⁸	19.91	17.21
8*	7.95	56.23	66.20	39.79	1.44	7.78 x10 ⁸	14.56	12.52
8**	7.29	72.12	69.97	41.20	1.62	7.98 x10 ⁸	17.32	15.19
9	6.50	64.23	68.80	40.80	1.63	2.78 x10 ⁸	22.8	19.88
10	5.90	60.45	67.86	40.71	1.65	4.78 x10 ⁸	28.27	24.56
11	5.91	58.58	67.40	40.44	1.61	5.08 x10 ⁸	27.56	23.93
12	5.80	50.14	67.00	39.66	1.52	5.08 x10 ⁸	26.28	22.44
13	5.70	45.25	66.82	39.59	1.52	3.88 x10 ⁸	26.14	21.35
15	6.09	16.27	66.50	39.48	1.51	1.66 x10 ⁷	19.09	18.6
14	5.78	38.59	66.64	39.52	1.51	1.58 x10 ⁸	25.71	20.27
*means the sample was taken before the periodical feeding of food waste;								
** means the sample was taken after the periodic feeding of food waste.								

pH experienced a gentle increase in the first 4 days. Because the amount of food waste in the initial mixture was relatively small and pH buffer was also added, the decrease in pH, which was commonly observed in the previous bioconversions, did not occur in the first 4 days. The 1st and 2nd periodical feeding caused pH to drop immediately and however the pH bounced back to the neutral level in a short period of 2 days. From the 3rd periodical feeding, pH decreased and did not go back to the neutral level (Figure 6-3).

The cell content of thermophilic bacteria sustained at a level of 108 c.f.u./g TS throughout the whole process. The periodical feedings diluted the cell content of thermophilic bacteria instantly. However, the food waste provided the additional easily degradable food to thermophilic bacteria, so the cell content rapidly bounded back to its normal level in one day. A balance of a high cell content of thermophilic bacteria and a high amount of easily biodegradable food was reached in the fed batch bioconversion. At the balance status, the bacteria achieved the possible maximum growth rate. The easily degradable organic matter was sufficient to sustain such high bacterial growth rate. When the ‘food’ available to bacteria started to be depleting, the additional food (e.g. periodical feeding of food waste) had to be brought into the reaction to maintain the bacterial growth at the maximum rate.

Figure 6-3 Variation of pH, MC and Cell Content of Thermophilic Microorganisms in Pilot Scale Fed Batch Experiment

This phenomenon is commonly seen in fed batch operation and is one of the main advantages over the batch operation. The batch operation normally needs a start-up

period before the active growth of bacteria occurs. In fed batch operation, once the active growth of bacteria is achieved, periodical feeding of organic matter will sustain such high cell content of bacteria. The balance has its preconditions. This balance has to be under a favourable environment with near neutral level pH, sufficient O₂, optimum moisture content and stable temperature.

The variation pattern of biological stability of intermediate products from fed batch operation is shown in Figure 6-4. In the first 4 days, the stability status of the mixture changed from unstable to very stable. As long as the periodical feedings were made, the mixture had more unstable organic matter which resulted in the decrease of the stability. The stability of the whole mixture has improved in the 2 days between two consecutive feedings. The overall stability of mixture decreased from day 4 onwards. For instance, the sample taken before the 3rd periodical feeding, had a lower stability than that before the 2nd periodical feeding. It could be explained by the accumulation of unstable organic matter from the consecutive periodical feeding. Such accumulation also had a negative impact on the pH. From Figure 6-3, the pH could not go back to its normal level of 6.5 to 7.5 after the 3rd periodical feeding.

Figure 6-4 Variation of Stability in Pilot Scale Fed Batch Experiment

6.2.2 Calculated Data

6.2.2.1 VS Mass Reduction

VS reduction of this fed batch bioconversion is listed in Table 6-3. Three kinds of VS reduction were calculated according to the following definitions.

P1(t) is the total or cumulative VS reduction up to time t, as % of the total VS (including VS of initial compost, initially and periodically added food waste, up to the time t). This percentage gave overall performance of bioconversion process. However, as mentioned earlier in Chapter 5, horticultural compost is mostly of lignocellulose material which is considered non-biodegradable in a short period of time. Including VS mass of horticultural compost in the total initial VS mass may not be able to reflect the real biodegradation pattern of food waste.

P2(t) is the total or cumulative VS reduction up to time t, as % of the total food waste VS added up to time t. The total VS of food waste up to time t included the VS portion of food waste both initially and periodically added, up to time t. This percentage reflected the real VS reduction of food waste. This percentage was based on the assumption that the VS of horticultural compost was not degraded in this bioconversion. This percentage could possibly exceed 100% when food waste was completely degraded, the microorganisms also started to attack horticultural compost.

P3(t) is the VS reduction of each dosage of food waste feeding before the next feeding, as % of VS mass in the food waste of each periodical feeding. P3(t) predicted the VS degradation of food waste added in previous periodical feeding. This percentage had a presumption. It is well known that when microorganisms are incubated in the presence of two or more substrates, the substrates will be degraded in order of their ease of degradation, e.g., catabolite repression (Stanier, 1976). In this bioconversion, thermophilic bacteria were presumed to stop to attack the previous added portion and start to degrade the freshly added food waste portion until both portions were biodegraded to the same extent. This method had been successfully used in predicting the degradation pattern of organic materials in fed batch operation in the recent researches (Nakasaki et al., 1998 and 2000).

Table 6-3 VS Reduction in Pilot Scale Fed Batch Experiment

Time (day)	VS (%)	Total VS added (g)	VS at time t (g)	Total FW VS added (g)	FW VS reduction of each dosage of FW (g)					VS reduction, (%)		
					Day 0	Day 4	Day 6	Day 8	Sum	P1	P2	P3
t		$a(t)$	$b(t)$	$c(t)$	$d(t)$	$e(t)$	$f(t)$	$g(t)$	$h(t)$	$P1(t)$	$P2(t)$	$P3(t)$
0	70.00	8509	8509	1957	0				0	0.00	0.00	0.00
1	68.72	8509	8010	1957	498				498	5.86	25.47	25.47
2	65.69	8509	7420	1957	1088				1088	12.79	55.60	55.60
3	62.52	8509	6837	1957	1671				1671	19.64	85.40	85.40
4*	61.60	8509	5848	1957	2660				2660	31.26	135.9	135.9
4**	67.00	10466	7805	3914		0			2660	25.42	67.97	0.00
5	65.00	10466	7139	3914		666			3326	31.78	84.98	34.03
6*	64.00	10466	6834	3914		971			3631	34.70	92.77	49.61
6**	68.50	12423	8791	5871			0		3631	29.23	61.85	0.00
7	67.36	12423	8342	5871			449		4080	32.84	69.49	22.93
8*	66.20	12423	7917	5871			874		4505	36.26	76.73	44.65
8**	69.97	14380	9876	7828				0	4505	31.33	57.55	0.00
9	68.80	14380	9357	7828				519	5024	34.94	64.17	26.51
10	67.86	14380	8949	7828				927	5432	37.77	69.39	47.36
11	67.40	14380	8762	7828				1114	5618	39.07	71.77	56.90
12	67.00	14380	8604	7828				1271	5776	40.17	73.79	64.97
13	66.82	14380	8535	7828				1341	5846	40.65	74.67	68.51
14	66.64	14380	8466	7828				1410	5914	41.13	75.56	72.03
15	66.50	14380	8413	7828				1463	5968	41.50	76.23	74.74

1. * means the sample was taken before the periodical feeding of food waste;

2. ** means the sample was taken after the periodical feeding of food waste;

3. $P1(t)$ refers to the total or cumulative VS reduction as % of the total VS (including VS of compost and food waste up to time t), $P1(t) = \frac{h(t)}{b(t)} \times 100\%$;

4. $P2(t)$ refers to the total VS reduction as % of the total food waste VS added up to time t , $P2(t) = \frac{h(t)}{c(t)} \times 100\%$;

5. $P3(t)$ refers to the VS reduction of each dosage of food waste feeding before the next dosage, as % of each dosage food waste VS (around 1957 g):

From day 0 to day 4, $P3(t) = \frac{d(t)}{1957} \times 100\%$; from day 4 to day 6, $P3(t) = \frac{e(t)}{1957} \times 100\%$;

From day 6 to day 8, $P3(t) = \frac{f(t)}{1957} \times 100\%$; From day 8 to the end, $P3(t) = \frac{h(t)}{1957} \times 100\%$.

The data in Table 6-3 is plotted and visualized in Figure 6-5.

In the curve of $P1(t)$, the total VS reduction in term of % of total VS was found to be only up to 40% at the end of the bioconversion. The real degradation pattern of food waste is not obvious in this curve.

The curve of $P2(t)$ provides detailed information about the degradation of food waste VS. In the first 4 days, the initial food waste in the initial mixture was mostly degraded and horticultural compost started to be degraded. This resulted in a more

than 100% of degradation of the VS mass in the initial food waste. Overall, the degradation of food waste in the fed batch operation was between 75-90%. For instance, P2(t) was brought down to 70% of TS by the 1st periodical feeding, and then increased to 90% of TS in the next 2 days.

The curve of P3(t) has 4 segments. The segment for the first 4 days was coincided with the first 4-day portion of the curve of P2(t). The next 3 segments showed that the food waste from each periodical feeding had almost the same degradation percentage of 50% in 2 days. It proved that the fed batch operation had a stable performance in food waste degradation. However, it was also noted that after the periodical feeding stopped, a long time was required to further polish and stabilize the products (Figure 6-4 and Figure 6-5).

Figure 6-5 Degradation Pattern of VS in Pilot Scale Fed Batch Experiment

6.2.2.2 Carbon Mass Reduction

The carbon reduction of the fed batch bioconversion was listed in Table 6-4 and plotted in Figure 6-6. The reduction pattern of carbon was theoretically and experimentally similar to the degradation pattern of VS. The discussions on carbon reduction were neglected.

Table 6-4 Carbon Mass Reduction in Pilot Scale Fed Batch Experiment

Time (day)	C (%)	Total C added (g)	C at time t (g)	Total FW C added (g)	FW C reduction of each dosage of FW, (g)					C mass reduction, (%)		
					Day 0	Day 4	Day 6	Day 8	Sum	P1	P2	P3
<i>t</i>		<i>a(t)</i>	<i>b(t)</i>	<i>c(t)</i>	<i>d(t)</i>	<i>e(t)</i>	<i>f(t)</i>	<i>g(t)</i>	<i>h(t)</i>	P1(t)	P2(t)	P3(t)
0	40.51	4924	4924	841	0				0	0.00	0.00	0.00
1	40.20	4924	4686	841	238				238	4.84	28.33	28.33
2	40.25	4924	4547	841	377				377	7.66	44.87	44.87
3	40.29	4924	4406	841	518				518	10.53	61.64	61.64
4*	40.02	4924	3799	841	1125				1125	22.85	133.7	133.7
4**	40.36	5765	4701	1682		0			1125	19.51	66.88	19.51
5	40.73	5765	4473	1682		228			1353	23.46	80.41	27.06
6*	40.20	5765	4293	1682		408			1533	26.60	91.16	48.56
6**	40.40	6606	5185	2523			0		1533	23.21	60.78	0.00
7	40.18	6606	4975	2523			210		1743	26.39	69.09	24.94
8*	39.79	6606	4758	2523			427		1960	29.68	77.70	50.79
8**	41.20	7447	5815	3364				0	1960	26.33	58.28	0.00
9	40.80	7447	5549	3364				266	2227	29.90	66.19	31.65
10	40.71	7447	5368	3364				447	2408	32.33	71.57	53.17
11	40.44	7447	5257	3364				558	2518	33.82	74.86	66.32
12	39.66	7447	5093	3364				722	2682	36.02	79.74	85.83
13	39.59	7447	5057	3364				758	2719	36.51	80.81	90.15
14	39.52	7447	5021	3364				794	2755	36.99	81.89	94.45
15	39.48	7447	4995	3364				820	2781	37.34	82.66	97.54

Note: 1. * means the sample was taken before the periodical feeding of food waste;

2. ** means the sample was taken after the periodical feeding of food waste;

3. **P1(t)** refers to the total carbon reduction as % of the total carbon added (including carbon of

compost and food waste up to time t), $P1(t) = \frac{h(t)}{b(t)} \times 100\%$; 4. **P2(t)** refers to the total carbon

reduction as % of the total food waste carbon added up to time t, $P2(t) = \frac{h(t)}{c(t)} \times 100\%$;

5. **P3(t)** refers to the carbon reduction of each dosage of food waste feeding before the next dosage, as % of each dosage food waste carbon Mass (around 841 g):

From day 0 to day 4, $P3(t) = \frac{d(t)}{841} \times 100\%$; from day 4 to day 6, $P3(t) = \frac{e(t)}{841} \times 100\%$;

From day 6 to day 8, $P3(t) = \frac{f(t)}{841} \times 100\%$; from day 8 to the end, $P3(t) = \frac{h(t)}{841} \times 100\%$.

Figure 6-6 Bioconversion Pattern of C in Pilot Scale Fed Batch Experiment

6.2.2.3 Nitrogen Mass Loss

The nitrogen loss of the fed batch bioconversion is listed in Table 6-5 and plotted in Figure 6-7. Three kinds of nitrogen loss were also calculated according to the following definitions.

P1(t) is the total or cumulative nitrogen loss up to time t, as % of the total nitrogen (including nitrogen of initial compost, food waste initially and periodically added, up to the time t). This percentage gave overall nitrogen loss in term of total nitrogen added up to time t. From the Figure 6-7, the overall nitrogen loss, as percentage of total nitrogen, was less than 40%.

P2(t) is the total or cumulative nitrogen loss, up to time t, as % of the total food waste nitrogen added up to time t. The total nitrogen of food waste, up to time t included the nitrogen mass in food waste initially and periodically added, up to time t. Based on the previous assumption that the organic matter of horticultural compost was not involved in degradation, this percentage could reflect the real nitrogen loss of food waste. From Figure 6-7, it was observed that the more the food waste periodically fed, the less the total or cumulative nitrogen loss was.

P3(t) is the nitrogen loss of each dosage of food waste feeding before the next feeding, as % of nitrogen mass in the food waste of each periodical feeding. P3(t)

predicted, the nitrogen loss of food waste previously fed. Figure 6-7 indicated that the food waste from each periodical feeding had a constant nitrogen loss of 40%.

Table 6-5 Nitrogen Mass Loss in Pilot Scale Fed Batch Experiment

Time (day)	N (%)	Total N added (g)	N at time t (g)	Total FW N added (g)	FW N loss for each dosage of periodical feeding, (g)					N loss, (%)		
					Day 0	Day 4	Day 6	Day 8	Sum	P1	P2	P3
<i>t</i>		<i>a(t)</i>	<i>b(t)</i>	<i>c(t)</i>	<i>d(t)</i>	<i>e(t)</i>	<i>f(t)</i>	<i>g(t)</i>	<i>h(t)</i>	<i>P1(t)</i>	<i>P2(t)</i>	<i>P3(t)</i>
0	1.95	237	237	106	0				0	0.00	0.00	0.00
1	1.52	237	177	106	60				60	25.25	56.46	56.46
2	1.49	237	168	106	69				69	28.99	64.82	64.82
3	1.45	237	159	106	78				78	33.10	74.01	74.01
4*	1.46	237	139	106	98				98	41.52	92.84	92.84
4**	1.72	343	200	212		0			98	28.69	46.42	0
5	1.66	343	182	212		18			116	33.95	54.93	17.02
6*	1.52	343	162	212		38			136	39.79	64.37	35.90
6**	1.63	449	209	318			0		136	30.39	42.91	0
7	1.63	449	202	318			7		144	32.02	45.22	6.90
8*	1.44	449	172	318			37		173	38.63	54.54	34.88
8**	1.62	555	229	424				0	173	31.25	40.90	0
9	1.63	555	222	424				7	180	32.50	42.55	6.57
10	1.65	555	218	424				11	184	33.24	43.51	10.44
11	1.61	555	209	424				19	193	34.74	45.47	18.25
12	1.52	555	195	424				33	207	37.28	48.79	31.56
13	1.52	555	194	424				34	208	37.47	49.04	32.54
14	1.51	555	192	424				37	210	37.88	49.59	34.73
15	1.51	555	191	424				38	211	38.03	49.78	35.49

Note:

- * means the sample was taken before the periodical feeding of food waste;
- ** means the sample was taken after the periodical feeding of food waste;
- P1(t)*** refers to the total nitrogen loss as % of the total nitrogen added (including nitrogen of compost and food waste up to time *t*), $P1(t) = \frac{h(t)}{b(t)} \times 100\%$;
- P2(t)*** refers to the total nitrogen loss as % of the total food waste nitrogen added up to time *t*, $P2(t) = \frac{h(t)}{c(t)} \times 100\%$;
- P3(t)*** refers to the nitrogen loss of each dosage of food waste feeding before the next dosage, as % of each dosage food waste nitrogen Mass (around 106 g):
 From day 0 to day 4, $P3(t) = \frac{d(t)}{106} \times 100\%$; from day 4 to day 6, $P3(t) = \frac{e(t)}{106} \times 100\%$;
 From day 6 to day 8, $P3(t) = \frac{f(t)}{106} \times 100\%$; from day 8 to the end, $P3(t) = \frac{h(t)}{106} \times 100\%$.

Figure 6-7 Loss Pattern of N in Pilot Scale Fed Batch Experiment

6.2.2.4 First Order Kinetic Constants and Maximum Degradation Rates of VS

Table 6-6 summarizes the first order kinetic constants and maximum degradation rate of VS in the pilot scale fed batch bioconversion. Figure 6-8 (a, b, c and d) demonstrates the reasonable data fitness of the first-order kinetic equation.

Table 6-6 First Order Kinetic Constants and Maximum Degradation Rates of VS in Pilot Scale Fed Batch Experiment

	Initial Stage (day 0 to 4)	First feeding (day 4 to 6)	Second feeding (day 6 to 8)	Third Feeding (day 8 to 10)
Kinetic Constant, k (d^{-1})	0.6298	0.3427	0.2957	0.3209
Relative variation, r^2	0.9188	0.9850	0.9953	0.9995
S_0 , (g)	1957	1957	1957	1957
Maximum degradation rate, $(dS/dt)_{max}$, (g/d)	1233	671	579	628

The periodical feedings at day 4, day 6 and day 8 had a consistent first order kinetic constant k values with an average of $0.32\ d^{-1}$. The standard deviation for the

three k values was 0.023 d^{-1} . The k values in the periodical feedings were higher than k ($k=0.22\text{ d}^{-1}$) obtained in the 1st phase of PSBB2. An average maximum degradation VS rate of the periodical feeding was about 616 g/d which was much higher than 249 g/d in the 1st phase of PSBB2. This meant that the fed batch bioconversion of food waste was quite stable and also faster than the batch operation.

(a) Initial Stage

(b) First Feeding

(c) Second Feeding

(d) Third Feeding

Figure 6-8 First Order Kinetic Analysis: $\ln(S/S_0)$ vs. Time of Four Stages of Pilot Scale Fed Batch Experiment

6.3 References of Chapter 6

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Chapter 7

Results and Discussions:

Evaluation of End Products

Maturity index and fertility of end products from three pilot scale bioconversions were evaluated and discussed in this chapter.

7.1 Maturity Index

The stability and maturity of end products were examined according to the standard method ‘Test Methods for the Examination of Composting and Compost’ (TMECC) by the U.S. Composting Council. TMECC is adapted after ‘American Society for Testing and Materials’ (ASTM). TMECC provides benchmark methods for compost analysis to enable comparison of analytical results.

In maturity rating system, compost is tested and classified as "very mature, mature, or immature" according to the Compost Maturity Index (Table 7-1 G-1).

Table 7-1 G-1 Compost Maturity Index

VERY MATURE	MATURE	IMMATURE
Well cured compost	Cured compost	Uncured or raw compost
No continued decomposition	Odor production not likely	Odor production likely
No toxicity potential	Limited toxicity potential	High toxicity potential
No impact on plant-available soil nitrogen	Minimal impact on plant-available soil nitrogen	Significant impact on plant-available soil nitrogen

The compost maturity index is implemented using a three-tier decision process as illustrated in Figure 7-1 G-1. The maturity index considers three characteristics of a product: C/N ratio, stability (microbial activity by respirometry), and potential phytotoxicity (bioassay tests and chemical analyses).

The first step excludes materials with a high tendency to immobilize nitrogen (high C/N ratio). Compost-like material with a C/N ratio equal to or greater than 25 is

categorized as immature, whereas compost with a C/N ratio less than 25 is further evaluated in the second step and third step.

The second step excludes material that is still undergoing active microbial decomposition, e.g., samples that contain adequate levels of carbon to sustain aerobic microbial activity. A stability rating assigns the material: very stable, stable or unstable. Compost rated as unstable is classified as immature, and compost with the stability rating of very stable or stable is further evaluated in the third step.

The third step screens for significant levels of phytotoxic compounds and considers chemical maturity parameters. Compost is assigned a maturity indicator rating of immature, mature or very mature.

The outcome from steps two and three are contrasted in a two-way decision matrix and assigned a final maturity rating.

Figure 7-1 G-1 Compost Maturity Assessment Process

Table 7-2 G-2 lists some selectable compost maturity index parameters. There are two groups of maturity index parameters: parameters in group A is related to biological stability of compost; those in group B is used to examine the compost

maturity. TEMCC suggests at least one parameter from each group should be tested in order to determine the maturity index of compost. Carbon Dioxide Evolution Rate Test from Group A and In-Vitro Germination and Root Elongation from Group B were chosen to first determine the stability rating and maturity rating so as to evaluate the maturity index of end products.

Table 7-2 G-2 Compost Maturity Index Parameters

Carbon Nitrogen Ratio (C:N, TMECC 05.02-A)	
Group A (Stability)	Group B (Maturity)
Respirometry Tests (TMECC 05.08): <ul style="list-style-type: none">▪ Specific Oxygen Uptake Rate (TMECC 05.08-A);▪ Carbon Dioxide Evolution Rate (TMECC 05.08-B);▪ Dewar Self-Heating Test (TMECC 05.08-D);▪ Solvita CO₂ (TMECC 05.08-E); and/or▪ Biologically Available Carbon (TMECC 05.08-F)	Ammonium (TMECC 04.02-C); NH ₄ -N:NO ₃ -N Ratio ² (TMECC 05.02-C); Biological Assays (TMECC 05.05): <ul style="list-style-type: none">▪ Emergence and Seedling Vigor▪ In-Vitro Germination and Root Elongation, or▪ Earthworm Bioassay: The Minnerota "Z"-Test; Solvita NH ₃ (TMECC 05.08-E); and/or Volatile Fatty Acids (TMECC 05.10-A)

In Table 7-3 G-3, compost stability rating can be matched with the results of stability test. The Carbon Dioxide Evolution Rate for pilot scale batch bioconversion 1, 2 and 3 were 7.68, 2.91 and 1.89 mg CO₂-C/g VS-day, respectively. Table 7-3 G-3 gave the corresponding stability rating: unstable, stable and very stable for end products of bioconversions 1, 2 and 3, respectively.

Table 7-3 G-3 Stability Indicator Thresholds Using Respirometry

Group A (Stability)	Rating		
	Very Stable	Stable	Unstable
Specific Oxygen Uptake Rate (mg O ₂ per g OM per d)	< 3	3 – 10	> 10
Carbon Dioxide Evolution Rate (mg CO ₂ -C per g OM per d)	< 2	2 – 4	> 4
Dewar Self-Heating Test (Dewar Index)	V		< V
Headspace Carbon Dioxide (color-code for Solvita CO ₂)	7 – 8	5 – 6	1 – 4
Biologically Available Carbon (mg CO ₂ -C per g OC per d)	< 2	2 – 4	> 4

In Table 7-4 G-4, compost maturity rating can be matched with the outcome of maturity test. Though the Germination and Root Elongation test, it was found that the end products of bioconversions 1, 2 and 3 were immature, mature and very mature, respectively.

Table 7-4 G-4 Maturity Indicator Thresholds

Group B (Maturity Indicator)	Rating		
	Very Mature	Mature	Immature
Ammonium, (mg kg ⁻¹ dw)	< 75	75 - 500	> 500
Ammonium:Nitrate Ratio ³ , (unitless ratio)	< 0.5	0.5 - 3.0	> 3.0
Seedling Emergence, (% of control), AND Seedling Vigor, (% of control)	> 90 and > 95	80 - 90 and 85 - 95	< 80 and < 85
In-Vitro Germination and Root Elongation, (% of control)	> 90	80 - 90	< 80
Earthworm Bioassay: The Minnesota "Z"-Test (% weight gain)	< 20	20 - 40	> 40
Ammonia, (color-code for Solvita NH ₃)	5	4	3 - 1
Volatile Fatty Acids, (mmoles g ⁻¹ dw)	< 200	200 - 1,000	> 1,000

After both the stability and maturity ratings of end products were determined, Figure 7-2 G-2 was used to perform the maturity assessment of end products. For instance, the end product from PSBB1 was unstable in stability rating and immature in maturity rating. Therefore its final maturity assessment result was immature.

		Group B Outcome		
		Very Mature	Mature	Immature
Group A	Very Stable	Very Mature		
	Stable		Mature	
	Less Stable			Immature

Figure 7-2 G-2* Maturity Assessment Matrix
(*Applied when the C/N ratio is equal to or less than 25:1)

The stability rating, maturity rating and final maturity assessment of all pilot scale end products are shown in Table 7-5.

Table 7-5 Chemical and Biological Characteristics of End Products

	EP1	EP2	EP3	COF
Bioconversion Duration (day)	10	10	11	-
TS (%)	95.06±0.08	97.00±.054	98.87±.054	74.9±0.5
VS (%)	70.75±0.44	63.77±0.44	66.19±0.44	41.2±0.4
EC (dS/m)	9.00	3.92	5.45	20.65
pH	5.90	8.00	8.10	7.6
C (% of TS)	38.57±0.32	33.25±0.77	35.41±0.77	18.2±0.6
N (% of TS)	2.82±0.10	2.18±0.08	2.38±0.08	7.0±0.4
C/N	13.61	15.25	14.88	2.60
P (% of TS)	0.34	0.25	0.23	2.9±0.4
K(% of TS)	2.99	2.59	3.15	2.4±0.2
Stability index (mg C/ g VS-day)	7.68	2.91	1.89	14.56
Stability Grade	Unstable	Stable	Very Stable	Unstable
Phytotoxicity	High	Low	Low	Very High
Maturity	Immature	Mature	Very Mature	Immature

7.2 Fertility

The fertilities of three end products were examined based on their use as organic fertilizers in plant cultivation test.

7.2.1 Plants Selected for Cultivation

Kang Kong (*Ipomoea aquatica*), a tropical vegetable, was used in plant cultivation experiment. Kang Kong, also known as water glorybind, water spinach, water convolvulus, or swamp cabbage, is an important green leafy vegetable in Southeast Asia. It is a common local vegetable and it processes characteristics of grass. Kang Kong can be easily cultivated as long as wet soil is provided. Harvesting may start in 4-week time after planting under wet soil condition.

7.2.2 Characteristics of End Products

End product 1 (EP1) was produced from PSBB1 with vegetable waste and soy waste. End products 2 (EP2) and 3 (EP3) were from PSBB2 and PSBB3 with not only vegetable waste and soy waste, but also the horticultural compost. In PSBB3, starter cultures were added. Commercial organic fertilizer (COF), named “Horti Bloom”

(Horti Flora Pte. Ltd., Singapore) was used as the positive control. It was added after seed germination because this commercial organic fertilizer was tested toxic to the seeds. The chemical and biological characteristics of three end products are shown in Table 7-5.

7.2.3 Characteristics of Soil

A poor quality subsoil was chosen as cultivating media. Sole use of subsoil was considered as the negative control. The characteristics of such subsoil and a commercial topsoil are listed in the Table 7-6.

pH of the topsoil was near neutral while pH of the subsoil was 4.4. It was reported that the optimal pH for growth of the majority of plants was between 6.5 and 7.0 (Smith, 1994). Before the plant cultivation test, pH of the subsoil was adjusted to neutral level using 0.1% CaCO₃ suspension. Low organic matter (OM) content was observed in the subsoil (2.6%), compared to a fertile soil with OM content of 4 to 8%. The subsoil was found generally deficient in macronutrients.

Table 7-6 Chemical Characteristics of Soil

Parameter	Commercial Topsoil	Local Subsoil
pH	6.4	4.4
TS, %	96.2 ±0.3	80.0±0.6
VS, % of TS	4.8 ±0.2	2.6±0.2
C, % of TS	0.63±0.3	Under detect limit
N, % of TS	0.29±0.02	0.25±0.01
P, % of TS	0.0±0.0	0.0±0.0
K, % of TS	0.16±0.1	0.80±0.4

7.2.4 Plant Cultivation Program and Procedure

In the plant cultivation test, each pot carried 3 kg (wet weight) of the subsoil. 4 dosages of three end products were applied into the subsoil. In terms of percentages, the 4 dosages were 1%, 2%, 3% and 4% (dry weight to wet weight of the subsoil), respectively. 3 dosages of COF, which contained the same amount of nitrogen as 2% of three end products, were applied into the subsoil to act as the positive controls. The pot with subsoil only was used as the negative control. Mixtures of subsoil and end products were homogenously mixed before being transferred into the pots.

EP1, EP2 and EP3 were deficient in phosphorous. The lack of phosphorous could limit the growth of Kang Kong. A phosphorous fertilizer was added in another three pots to examine the effect of phosphorous deficiency on the nitrogen availability to Kang Kong. The International Fertilizer Industry Association (IFIA) recommended that normal application of 20-40 kg P₂O₅ per ha is required for outdoor tomato yielding of 40-50 tonnes per ha. The common plant density is 3 to 4 plants/m². Hence the estimated application of phosphorous fertilizer is around 1g P₂O₅ per plant. The same rate is applicable for Kang Kong since it can grow under poor nutrient condition. Na₂HPO₄ was chosen as the phosphorous fertilizer and it also acted as a good pH buffer in the acidic subsoil. Each plant needed about 2 g of Na₂HPO₄ as phosphorous source. The phosphorous fertilizer was applied after germination and pre-selection of young plants.

The planting test is designed as follows.

Table 7-7 Planting Program

(A) Pot Content of Negative Control

Pot Name	Subsoil, kg in wet weight
K1	3

(B) Pot Contents of Positive Controls

Pot Name	Subsoil, kg in wet weigh	COF with equivalent N content to 2% of EP1, EP2 or EP3, g in dry weight
R1	3	24.1
R2	3	20.2
R3	3	19.9

(C) Pot Contents of EP1 Group

Pot Name	EP1 dosage, % of Subsoil	Subsoil, kg in wet weight	EP1, g in dry weight	Additional P source, g Na ₂ HPO ₄ /plant
K2	1	3	30	-
K3	2	3	60	-
K4	2	3	60	2
K5	3	3	90	-
K6	4	3	120	-

(D) Pot Contents of EP2 Group

Pot Name	EP2 dosage, % of Subsoil	Subsoil, kg in wet weight	EP2, g in dry weight	Additional P source, g Na ₂ HPO ₄ /plant
K7	1	3	30	-
K8	2	3	60	-
K10	3	3	90	-
K11	4	3	120	-

(E) Pot Contents of EP3 Group

Pot Name	EP3 dosage, % of Subsoil	Subsoil, kg in wet weight	EP3, g in dry weight	Additional P source, g Na ₂ HPO ₄ /plant
K12	1	3	30	-
K13	2	3	60	-
K14	2	3	60	2
K15	3	3	90	-
K16	4	3	120	-

The mixtures were placed in pots of 18 cm in diameter and 40 cm in depth. All pots were placed away from direct sunlight and rainfall. Before sowing, the potting mixtures were first incubated for one week for releasing nutrients. During the incubation period, the potting mixtures were kept moist by applying water once a day.

Pre-selection of good Kang Kong seeds was performed to minimize errors. Seeds with the same size and colour were picked and stored for sowing. After one week, each pot was sowed with 10 Kang Kong seeds. Even distribution was made for each seed to occupy equal space. All pots were irrigated twice a day. After all seeds germinated (around 10 days), only the four best young plants were kept in each pot. The whole planting experiment included an incubation period of 1 week, a germination and pre-selection period of 2 weeks and normal growth period of 5 weeks. At harvest, the plants were removed from the pots. The plants were air-dried for 2 hours to obtain surface dry status before the length and weight of stems and roots were measured.

7.2.5 Results and Discussions

Physical properties of the plants including the average lengths and surface-dry weights of stems and roots are shown in Table 7-8. The effect of three end products on Kang Kong growth is discussed.

Table 7-8 Physical Properties of Plants

Pot Content	End product dosage, % of subsoil	Remark	Average lengths of plant stems and roots, mm		Average surface dry weights of plant stems and roots, g	
			Stems	Roots	Stems	Roots
Subsoil	0		127±32	70±0	0.84±0.10	0.19±0.2
Subsoil with EP1	1		193±21	150±51	2.14±0.09	1.19±0.12
	2		161±34	130±39	2.44±0.21	1.23±0.11
	2	With P	213±16	133±11	5.81±0.21	1.63±0.22
	3		124±17	116±13	1.61±0.05	0.98±0.02
	4		100±0.00	100±0.00	0.65±0.00	0.1±0.00
Subsoil with EP2	1		167±11	122±23	1.88±0.21	0.48±0.05
	2		170±13	130±46	2.30±0.10	0.54±0.04
	3		188±13	156±21	2.52±0.15	1.00±0.11
	4		190±29	170±43	2.70±0.03	1.23±0.01
Subsoil with EP3	1		140±0	75±23	1.10±0.01	0.22±0.01
	2		150±19	80±15	1.42±0.12	0.25±0.03
	2	With P	205±15	115±32	2.08±0.20	0.47±0.05
	3		185±5	130±34	2.64±0.14	0.91±0.04
	4		189±10	140±35	3.50±0.21	0.78±0.03
Subsoil with COF	-	R1	307±34	240±49	9.02±0.54	3.81±0.58
		R2	300±20	245±5	8.83±0.41	1.27±0.14
		R3	230±7	235±42	5.98±0.38	2.27±0.21

7.2.5.1 Effect of End Products on Lengths of Plant Stems and Roots

Generally, 1% to 3% of EP1 applied to the subsoil enhanced the growth of Kang Kong stem and root lengths (Figure 7-3). However, 4% amendment of EP1 had negative effect on the stem and root lengths. The plants cultivated in the subsoil amended with 1% of EP1, had the longest stems and roots compared to those from the subsoil with 2%, 3% and 4% of EP1.

EP1 was produced from food waste only. It had been found to be phytotoxic on germination of plant seeds. In the plant cultivation experiment, it exhibited obviously high phytotoxic effect on the plant growth at high application dosage (Figure 7-4). When applied onto land, low application dosage of EP1 was recommended.

Figure 7-3 Effect of EP1 on Lengths of Plant Stems and Roots

Figure 7-4 Images of Plants Cultivated in Subsoil Amended with EP1

(Plants from the left to right were cultivated in subsoil amended with 0%, 1%, 2%, 3% and 4% of EP1, respectively)

All of the 1% to 4% application dosages of EP2 enhanced the growth of stem and root lengths (Figure 7-5 and Figure 7-6). Plants cultivated in the subsoil amended with 4% of EP2 had the longest stems and roots. It seemed that a higher dosage of EP2 than 4% would still enhance the growth of plants without adverse effect. EP2 and EP3 had the same raw materials. The similar results were observed in the EP3 plant cultivation experiment (Figure 7-7 and Figure 7-8). Additional horticultural compost reduced the phytotoxicity of EP2 and EP3 compared to EP1.

Figure 7-5 Effect of EP2 on Lengths of Plant Stems and Roots

Figure 7-6 Images of Plants Cultivated in Subsoil Amended with EP2
(Plants from the left to right were cultivated in subsoil amended with 0%, 1%, 2%, 3% and 4% of EP2, respectively)

Figure 7-7 Effect of EP3 on Lengths of Plant Stems and Roots

Figure 7-8 Images of Plants Cultivated in Subsoil Amended with EP3
(Plants from the left to right were cultivated in subsoil amended with 0%, 1%, 2%, 3% and 4% of EP3, respectively)

7.2.5.2 Effect of End Products on Surface-Dry Weights of Plant Stems and Roots

Generally, 1% to 3% of EP1 amended to the subsoil increased Kang Kong stem and root surface-dry (SD) weights compared to negative control (Figure 7-9). Among these three dosages of EP1, the dosage of 2% cultivated the stem and root with the maximum SD weight. However, 4% of EP1 showed negative effect on plant growth.

Figure 7-9 Effect of EP1 on Surface-Dry Weights of Plant Stems and Roots

All of 1% to 4% dosages of EP2 and EP3 enhanced stem and root weights (Figure 7-10 and Figure 7-11). Plants cultivated in the subsoil amended with 4% of both EP2

and EP3 had the maximum weights of stems and roots among their own cultivation group.

Figure 7-10 Effect of EP2 on Surface-Dry Weights of Plant Stems and Roots

Figure 7-11 Effect of EP3 on Surface-Dry Weights of Plant Stems and Roots

7.2.5.3 Effect of Phosphorous Deficiency of End Products on Plant Growth

Products from the bioconversions of food waste were commonly deficient in phosphorous. The planting results were extracted and divided into two groups so as to study the effect of phosphorous deficiency on the plant growth. In each group, each

pot of subsoil was fertilised with the same amount of nitrogen, but different amount of phosphorous.

Group 1 results were obtained from the plants cultivated in the subsoil amended with 2% of EP1, with 2% of EP1 and additional mineral phosphorous fertilizer, and with COF with the same amount of nitrogen as 2% of EP1. Group 2 results were of the plants cultivated in the subsoil amended with 2% of EP3, with 2% of EP3 and additional mineral phosphorous, and with COF with the same amount of nitrogen as 2% of EP3.

COF was made from chicken mature. It was rich in nitrogen (7%), phosphorous (3%) and potassium (2.4%). EP1 had 2.8 % of nitrogen, 0.34% of phosphorous and 3% of potassium. EP3 had 2.38 % of nitrogen, 0.25% of phosphorous and 3.15% of potassium.

Figure 7-12 Effect of Phosphorous Deficiency of EP1 on Stem and Root Length of Plants

Figure 7-13 Effect of Phosphorous Deficiency of EP1 on Stem and Root Weights of Plants

It was found that subsoil amended with COF produced Kang Kong with longer and heavier stems and roots than those amended with N-equivalent 2% of EP1 only (Figure 7-12 and Figure 7-13). Subsoil amended with both 2% of EP1 and additional phosphorous fertilizer, also produced longer and heavier stems and roots. It seemed that phosphorous deficiency restricted the use of EP1 as organic fertilizer. From Figure 7-14 and Figure 7-15, it was found that the phosphorous deficiency also similarly restricted the use of EP3.

Figure 7-14 Effect of Phosphorous Deficiency of EP 3 on Stem and Root Length of Plants

Figure 7-15 Effect of Phosphorous Deficiency of EP3 on Stem and Root Weights of Plants

7.2.6 Summary

The fertilities of three end products from pilot scale bioconversions were examined based on their use as organic fertilizer to plants. Kang Kong was selected as the experimental plant. The results are summarized in the following:

- The three end products were rich in nitrogen and potassium and generally enhanced the growth of the plants tested;
- The deficiency of phosphorous in the three end products limited their nutrient supply to the plants;
- The EP1 at high application dosage rate (more than 1%) was phytotoxic;
- The EP2 and EP3 at high application dosage (e.g., 4%) did not show any phytotoxic effect on the tested plants and it could be concluded that the horticultural compost reduced the phytotoxicity of EP2 and EP3.

Chapter 8

Conclusions and Recommendation

8.1 IAB, Starter Cultures and Horticultural Compost

In this study, IAB processes in both bench and pilot scale were proven to be able to effectively bioconvert food waste into biologically stable organic fertilizers. This process shortened the normal processing time of several months required by composting, to less than one week.

Special strains, SW09 and SW25, were found to be able to enhance IAB process and to shorten the retention time of organic waste in reactors. Theoretically, addition of starter cultures had its advantages, but the complicated preparation to obtain useful starter cultures confined the seeded bioconversion currently only in scientific research stage. In fact, in nature, many organic wastes carry a diversity of microorganisms, which can enhance biodegradation of other organic wastes. The horticultural compost was one of them. In the pilot scale IAB processes, horticultural compost was used as both a bulking agent and a source of microorganisms.

8.2 Issues Related to IAB Process

8.2.1 Low pH in the Early Stage of IAB Process

The combined influence of accumulation of organic acids and excessive production of CO₂ in the early stage of IAB process caused rapid decrease of pH and thus inhibited the growth of thermophilic bacteria. The acidic period could last for 2-5 days depending on the aeration condition (see Chapter 4, Sections 4.1, 4.2 and 4.3). pH buffer was used to maintain pH at the acceptable range for thermophilic bacteria. Sometimes, even the addition of pH buffer could not prevent the pH from dropping if

organic wastes, such as fruit waste in BSBB4, produced excessive amount of organic acids.

It was found that if starter culture had to be added to enhance IAB with pH buffering, the best time was after the first 1-2 days. By doing so, the wastage of expensive starter culture was avoided and the biodegradation of food waste was enhanced. In BSBB2, starter culture with 5% of CaCO_3 added on day 2 brought the thermophilic bacteria concentration from 3.6×10^6 c.f.u./g TS to 4.0×10^8 c.f.u./g TS immediately and such concentration sustained at a high level of 10^8 c.f.u./g TS throughout the rest of the IAB process. When the starter culture was added with pH buffer at the start of BSBB1, the thermophilic bacteria concentration did not increase due to the excessive production of organic acid in the next few days.

Horticultural compost physically increased the bulk density of the mixture to improve the aeration and diminish the development of anaerobic zone where excessive organic acids were easily produced. Chemically, it was reported that the compost may possess the capacity of pH buffering (Clapp et al, 1986). With horticultural compost mixed with food waste in PSBB2 and PSBB3, the extent of pH drop was less in the beginning stage and the lowest pH was just around 6.

8.2.2 Excessive Nitrogen Loss during IAB Process

In the conventional composting processes, loss of valuable nitrogen nutrient has always been a big issue of concern. There is no exception for IAB process of food waste. Aeration and agitation enhanced the biodegradation of organic matter in the food waste, however, they also caused the loss of nitrogen. Especially when food waste alone was put under IAB process, the rate of excessive nitrogen loss was greater than that of reduction in organic matter. In BSBB3, loss of nitrogen was as high as 68%, which was much more than 45% of VS reduction in 6 days. The similar difference was observed in PSBB1, in which nitrogen loss was 65% and VS reduction was only 50% in 9 days.

The presence of horticultural compost in IAB process significantly reduced the

nitrogen loss. In PSBB2 and PSBB3, where horticultural compost was added, nitrogen loss in 9 days was only 40%, which was much lower than 68% of no-horticulture-compost PSBB1. This finding agreed with those of Witter and López-Real (1988), Morisaki et al. (1989), and Paredes et al. (1996), who emphasized the importance of adding lignocellulosic materials to reduce nitrogen losses.

8.2.3 Low Stability of End Products

Stabilization of organic waste is always one of the purposes of either composting or IAB. In this study, it was found that, even after 70% in BSBB1 in 10 days and 50% in PSBB1 in 9 days, of reduction in organic matter of food waste had been achieved, the end product still behaved microbiologically unstable.

In PSBB2 and PSBB3, The end products after screened through 4 mm sieve to remove most of horticultural compost, reached the requirement of stabilization in 6 days. Horticultural compost played an important role to improve the product stability in PSBB2 and PSBB3.

8.3 Bench Scale Batch Bioconversion

Bench scale batch bioconversion showed that it was possible to achieve organic matter degradation of up to 80% in 4 to 5 days (in BSBB 2) if the pH was adjusted and selected strains, i.e., SW09 and SW25 were added on day 2. It was confirmed that pH adjustment was necessary and selected strains enhanced the IAB process. However, the biological stabilities of the products were still low.

Soybean waste is a good resource to produce high value organic fertilizer. IAB process of soybean waste required a long time, which was economically unfeasible. Due to its initial low C/N ratio, excessive nitrogen loss occurred. IAB process of fruit waste was limited by its low pH during the whole process. Strong alkaline solution was needed initially to bring the pH to neutral level in 3 to 4 days.

The results from a small scale planting test revealed that the successful organic

product obtained was compatible in fertility with one of the commercial organic fertilizers.

8.4 Pilot Scale Batch Bioconversion

The pilot scale batch experiments further proved that degradation of organic matter in food waste could reach 70% to 80% in 5 to 6 days in PSBB2 and PSBB3. The sustainable high rate degradation of organic matter in PSBB2 and PSBB3 had advantages over the abrupt reduction of organic matter in PSBB1 without horticultural compost because nitrogen loss was controlled and reduced, the period of low pH was shortened and biological stability of end products was improved.

In the first order kinetic study of the pilot scale batch bioconversions, it was also found that, selected strains SW09 and SW25 with horticultural compost enhanced the biodegradation of organic matter in the second phase of IAB process. It resulted in an earlier stabilization of the end product. However, it was recommended that practically the addition of selected strains into the intensive aerobic bioconversion was not necessary since the end products had been already biologically stable after the first phase.

8.5 Pilot Scale Fed Batch Bioconversion

The study of pilot scale fed batch bioconversion was based on the results obtained in PSBB2. It was found that in the fed batch operation periodically feeding of food waste did not cause a long-period low pH in the subsequent process, which usually occurred in the batch operation, and the time required for the microorganisms to proliferate was shortened. Thus, the time lag between the addition of fresh food waste and the time at which active degradation began was shorter in the fed-batch operation than in the batch operation (Nakasaki et al., 2000). Such finding has its practical benefit since IAB is an energy consuming process.

In spite of this advantage, the fed batch process had drawbacks because of low microbiological stability of products caused by periodical feeding of fresh food waste.

Hence it was recommended that after the stop of periodical feeding, a reasonably time is needed to the stabilization of the product.

8.6 Maturity and Fertility of IAB Products

The maturity and fertility of organic products applied onto soil have the most important influence on soil properties and plant growth. The organic fertilizer has to be mature before being considered as fertilizer. In this study, the products from PSBB2 and PSBB3 with horticultural compost as bulking agent and a source of microorganisms, reached the required maturity. The mature end product can be used as fertilizer by applying a rate up to 4% without any adverse phytotoxicity.

The fertility of organic fertilizer from food waste bioconversion was proven in the planting test. However, the lack of phosphorus in food waste, downgraded the fertilizer value of organic products.

8.7 Overall Conclusion

In general, IAB process can rapidly biodegrade and effectively stabilize the organic matter in food waste in less than one week. The products obtained from IAB process were rich in nitrogen and considered as organic fertilizers. The functions of horticultural compost in the bioconversions were very important. Fed batch operation of such bioconversion was practically advantageous over batch operation.

8.8 References in Chapter 8

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