APPLICATION OF ENZYME-PRODUCING BACTERIA FOR MUNICIPAL SOLID WASTE BIODEGRADATION

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MASTER OF SCIENCE

(BIOTECHNOLOGY)

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APPLICATION OF ENZYME-PRODUCING BACTERIA FOR MUNICIPAL SOLID WASTE BIODEGRADATION

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the requirements for the award of the degree of
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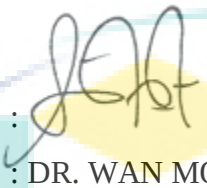
We hereby declare that we have checked this thesis and in my opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Master of Science in Biotechnology.

Signature : 
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I hereby declare that the work in this thesis is my own except for the quotations and summaries which have been duly acknowledged. The thesis has not been accepted for any degree and is not concurrently submitted for the award of any other degree.

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DEDICATION

Dedicated to my husband, my parents, my brothers, my sister and friends, who gave me never ending encouragements and priceless supports towards the success of this study.

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ABSTRACT

Population growth with increasing consumption levels leads to abundant waste in Kuantan. Jabor landfill, commonly known as Kuantan landfill, receives more than 500 tons of waste per day with a composition of 60% of domestic waste and 40% of commercial waste. The composition of domestic waste were organic waste, green waste, mixed paper, plastic, textile, ferrous, glass, rubber and leather, and others. Meanwhile, the compositions of commercial waste are food, plastic, yard waste, paper, cardboard, textile, glass, non-ferrous, rubber, wood, ferrous metals. Landfill system always produces leachate. This waste contains many types of bacteria with the potential to degrade the waste compound. Screening tests with selective media demonstrated the ability of bacteria to produce amylase, lipase, protease and cellulase enzymes. These enzymes are needed to accelerate the molecules breakdown of municipal solid waste in the biodegradation process. Samples for isolation of bacteria were taken from different places, namely landfill soil and leachate. Identification of bacteria was conducted using Gen III microplate BIOLOG microbial identification system. They were *Bacillus amyloliquefaciens*, *Bacillus ruris*, *Bacillus licheniformis*, *Bacillus subtilis* and *Kocuria varians*. There were 4 different treatments: composting pile without bacteria (T0), composting pile with inoculation of amylase and protease producing bacteria (T1), composting with inoculation of lipase and cellulase producing bacteria (T2), and composting with inoculation all enzyme-producing bacteria (T3). The result of biodegradation process of T3 treatment reached the highest temperature (53° C) with the longest thermophilic phase compared to other treatments. The lower value of C/N ratio, the more stable the level of maturity of compost. The lowest of C/N ratio value was T3 (10%). T3 treatment compared with other treatments can increase as much as 27% content of nitrogen, 67% of phosphorus and 33% of potassium. All the treatments with bacterial inoculation (T1, T2, and T3) are able to reduce the content of heavy metals (Fe, Zn, Cu) on municipal solid waste biodegradation. It can be concluded that the inoculation of potential enzyme-producing of bacteria on municipal solid waste biodegradation is effective to increase the nutrient content and decrease the heavy metals.

ABSTRAK

Pertumbuhan penduduk dengan tahap penggunaan yang semakin meningkat membawa kepada sisa pepejal yang banyak di Kuantan. Tapak pelupusan Jabor yang dikenali sebagai tapak pelupusan Kuantan menerima lebih daripada 500 tan sampah sehari dengan komposisi 60% daripada sisa domestik dan 40% daripada sisa komersial. Komposisi sisa domestik adalah sisa organik, sisa hijau, kertas campuran, plastik, tekstil, besi, kaca, getah dan kulit, dan lain-lain. Komposisi sisa komersial adalah makanan, plastik, sisa halaman, kertas, kadbod, tekstil, kaca, bukan ferus, getah, kayu, logam ferus. Sistem tapak pelupusan sentiasa menghasilkan larut resapan. Sisa ini mengandungi banyak jenis bakteria yang berpotensi untuk menguraikan kompaun sisa. Ujian saringan dengan media terpilih menunjukkan keupayaan bakteria untuk menghasilkan enzim amilase, lipase, protease dan selulase. Enzim ini diperlukan untuk mempercepatkan pecahan molekul sisa pepejal perbandaran dalam proses biodegradasi. Sampel untuk isolasi bakteri telah diambil dari tempat yang berbeza, iaitu tanah tapak pelupusan dan larut resapan. Pengenalan bakteria telah dijalankan oleh Gen III microplate BIOLOG sistem pengenalan mikrob. Mereka adalah *Bacillus amyloliquefaciens, Bacillus ruris, Bacillus licheniformis, Bacillus subtilis dan Kocuria varians*. Terdapat empat rawatan yang berbeza: kompos timbunan tanpa inokulasi bakteria (T0), kompos timbunan dengan inokulasi bakteria yang menghasilkan amilase dan protease (T1), pengkomposan dengan inokulasi bakteria yang menghasilkan lipase dan selulase (T2), dan kompos dengan inokulasi semua bakteria yang menghasilkan enzim (T3). Hasil daripada proses biodegradasi rawatan T3 telah mencapai suhu yang paling tinggi (53 ° C) dengan fasa thermophilic yang paling lama berbanding rawatan lain. Kompos yang nisbah C/N paling kecil adalah tahap yang lebih stabil dan matang, yang paling kecil daripada nilai nisbah C / N adalah T3 (10%). Rawatan T3 berbanding dengan rawatan lain boleh meningkatkan sebanyak 27% daripada kandungan nitrogen, 67% daripada kandungan fosforus dan 33% daripada kandungan potassium. Semua rawatan dengan suntikan bakteria (T1, T2, T3 dan) dapat mengurangkan kandungan logam berat (Fe, Zn, Cu) di biodegradasi sisa pepejal perbandaran. Dapat disimpulkan bahawa inokulasi potensi enzim-penghasilan bakteria di biodegradasi sisa pepejal perbandaran adalah berkesan untuk meningkatkan kandungan nutrien dan mengurangkan logam berat.

TABLE OF CONTENTS

CHAPTER 5 CONCLUSION AND RECOMMENDATIONS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

B	Boron
^{o}C	Celsius Degree
C	Carbon
Ca	Calcium
CH_4	Methane
CO_2	Carbon dioxide
Cu	Copper
Fe	Iron
g	Gram
H_2S	Hydrogen sulphate
K	Potassium
L	Litres
Mg	Magnesium
mL	Millilitres
Mo	Molybdenum
mS cm^{-1}	Millisiemens centimetre
N	Nitrogen
NH_3	Ammonia
O_2	Oxygen
P	Phosphorus
S	Sulfur
Si	Silicon
T0	Compost treatment without bacteria
T1	Compost treatment with amylase and protease enzymes producer
T2	Compost treatment with cellulase and lipase enzymes producer
T3	Compost treatment with all enzymes producer bacteria
V	Volume
W	Weight
Zn	Zinc

LIST OF ABBREVIATIONS

AAS	Atomic absorption spectroscopy
C/N Ratio	Carbon to Nitrogen ratio
CFU	Colony forming units
CMC	Carboxymethyl cellulose
DFT	Dry fermentation technology
DNA	Deoxyribonucleic acid
EC	Electrical conductivity
GHG	Green house gases
HC	Hydraulic compactor
HHE	Human health and the environment
HW	Horticultural waste
IPS	Initial particle size
JICA	Japan International Cooperation Agency
MC	Moisture content
MHLG	Ministry of housing and local government
MSW	Municipal solid waste
NA	Nutrient agar
NB	Nutrient broth
PGPR	Plant growth promoting rhizobacterium
PPSPPA	Solid Waste Management and Public Cleansing
PSA1	Pahang service area 1
SS	Sewage sludge
SWM	Southern waste management
TAD	Thermophilic aerobic digestion

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

Every year, the level of waste generation continues to rise because of uncontrolled consumption due to increasing population, attitudes towards spending and high living standards. Quantities of waste generated are growing in response to the rapid increase in population, accelerated urbanisation and industrialisation. However, with the increasing population, the volume of waste generated remains abundant. Solid waste is generated from various domestic (schools, hospitals, universities, offices) and commercial (from restaurants, hotels, markets and industry) sources and consists of biodegradable matter, as well as inert non-biodegradable matter. The most dominant variable in the municipal solid waste (MSW) flow is food waste. Relatively homogeneous residential waste, some differences in the waste depends on local factors and other demographics; most households dispose of the same type of waste (Yousuf and Rahman, 2007). Trends in the composition of MSW in Malaysia showed that food, paper and plastic are the main components of waste generated in most places (Agamuthu et al., 2007). The main household waste composition includes 71% organic waste, 12% plastic, 7.5% paper and paper products, 5% dirt and construction debris and 1% hazardous waste. The highest percentage is organic waste, although the composition of the waste varies depending on the source.

The handling and separation of wastes at the source is a critical step in waste management in Malaysia. Solid waste management can be defined as a discipline related to the control of waste generated. The storage of waste used various types of bins such as a small bin for household, medium and large bin for industry or

manufacture. The most used bins for residential areas are small bins. Also, the bins used are made of various materials, such as plastic, metal, rubber, mixed paper, and cardboard boxes. In the case of high-rise building, communal bins or central container is used. Waste collection activities are the most expensive activity in waste management systems. The introduction of intermediate treatment facilities such as transfer stations, composting and incinerator plants, may become the alternative treatment of waste in Malaysia. The government is also considering the various designs and modes of incineration process available in the market. One of the process is the thermal gasification process (Earth Observation Centre, 2011).

Jabor Landfill is the only landfill in Kuantan, which is located in Pahang Service Area 1 (PSA1) covering the district of Kuantan in the state of Pahang. It is approximately 300 km away from Kuala Lumpur and 25 km from the city of Kuantan. The total site area is 30 hectares. There is also a system of leachate containment, leachate collection system, leachate treatment plant and landfill gas management (Alam flora, 2006). Approximately 500 tons of waste are collected at Jabor landfills every day, using the concept of semi aerobic recirculatory system treatment or Fukuoka methods as a solid waste management.

Leachate production is one of the biggest problems associated with the operation of environmental sanitary landfill, because the liquid waste can cause harmful pollution problems by contaminating surfaces and ground water as well as surrounding soil surfaces. *Clostridium perfringens* and fungal filaments are usually contaminated leachate. In addition, there are bacteria, which includes aerobic, coliform and fecal coliform, psychrophilic and mesophilic bacteria, and spore-forming bacteria (Matejczyk et al., 2011). The two bacterial groups that showed a good adaptation and critical participation in the leachate treatment for almost the entire duration of treatment are *Actinomycetes* and *Bacillus* (Yahmed et al., 2009). Analysis of individual substrate utilisation patterns of bacteria isolated from the leachate collected at successive sampling dates showed a decrease in the percentage of Gram-negative bacteria, which are able to metabolize sugar selected by increasing the percentage of Gram-positive bacteria that are capable of metabolizing sugar (Hale Boothe et al., 2001). Therefore, one of the objective in this study is to identify and to compare the number of species of

bacteria that live in landfill soil and leachate, which have the potential to produce amylase, protease, cellulase and lipase enzymes. The bacteria could be the agent of biodegrader for municipal solid waste treatment. With that abundant amount, it needs various efforts to reduce the high production of waste. Sanitary landfills and incinerators cause further effects that are more harmful to living beings and environment. Biodegradation is one of the waste managements that can produce a product that are beneficial to plants. It can convert up to 50-60% of the waste into biofertilizer. The product will be richer in microbes, thus can improve plant nutrient uptake, which is also effective in increasing agricultural production.

Biodegradation process involves biological and chemical processes. The enzyme activity is affected by the type of substrate, temperature and microbiological activity to degrade waste. Monitoring enzyme activity during the composting process can provide valuable information related to the dynamics of essential nutrients such as C, N or P, and contribute to developing a better understanding of the transformation that occurs during composting (Vargas-García et al., 2010). Microorganism-induced degradation of organic materials depends on the activity of various hydrolytic enzymes (Raut et al., 2008). Each material source will demonstrate different enzyme activities. The rate of decomposition is highly dependent on the quality of the organic substrate, environmental conditions, the chemical nature of the substrate, and the activity of microorganisms (Jurado et al., 2014).

1.2 PROBLEM STATEMENT

To identify the trend of the various types of waste that goes into landfills each year, it is necessary to conduct waste classification update. This is useful to determine what management will be implemented to resolve the problem of municipal solid waste in Kuantan. Food is the majority biodegradable component of municipal solid waste, which consists of more than 50%. Municipal solid waste management costs are expensive and require other alternative management for example incineration, sanitary landfill and their technologies. Composting is one of solid waste management that is economical, convenient and can produce useful organic fertiliser for crops. However, Jabor landfill never applied this treatment as a combination for the waste treatment.

Biodegradable waste in landfill is the substrate for composting. In this study, the biodegradation method will be conducted by using bacterial consortium. The isolated bacteria in this research are indigenous bacteria that are expected to be utilised for the municipal solid waste degradation. The method uses a combination of microbes that have different abilities to produce enzymes to degrade organic waste. Therefore, it can accelerate the decomposition process to be more effective and efficient.

1.3 OBJECTIVES

The objectives of this study include:

1) To characterise the municipal solid waste in Jabor landfill, Kuantan, Pahang.
2) To isolate and identify the potential bacteria from Jabor landfill to those are able to produce amylase, protease, cellulase and lipase enzymes.
3) To determine the efficiency of municipal solid waste degradation by bacterial consortium.

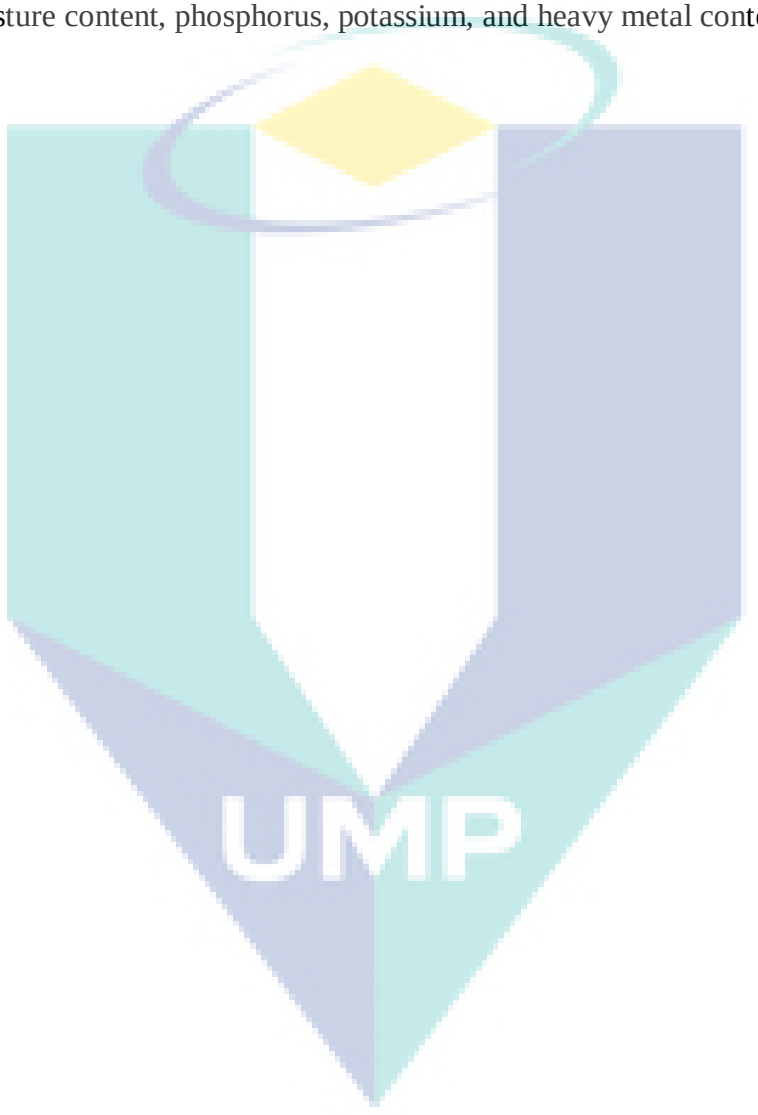
1.4 SCOPE OF STUDY

Based on the objectives, the major scope of this experiment is to find out the effectiveness of bacteria consortium from landfill soil and leachate that are capable to degrade municipal solid waste. To achieve the objective of this research, four scopes have been identified:

1. Characterisation of municipal solid waste using hand sorting methods. Generally MSW consists of different categories: food waste, green waste, paper (mixed), cardboard, plastic (rigid, film and foam), textile, wood waste, metals (ferrous or non-ferrous).
2. Isolation of bacteria using the standard serial dilution procedure and identification of bacteria using Gram staining and BIOLOG automated system.
3. To study the ability of bacterial consortium to produce lipase, protease, amylase and cellulase enzymes with selective media. The types of agar used are: skim

milk agar for protease, starch agar for amylase, rhodamine B agar for lipase and carboxymethyl cellulose agar (CMC) for cellulase activity.

4. Application of bacterial consortium for municipal solid waste degradation with small scale composting in a plastic container with duplicate sub sample were taken for the monitoring composting process. The monitored parameters were total carbon, total nitrogen, C/N ratio, temperature, pH, electrical conductivity, moisture content, phosphorus, potassium, and heavy metal content (Cu, Fe, Zn).



CHAPTER 2

LITERATURE REVIEW

2.1 MUNICIPAL SOLID WASTE

Municipal solid waste (MSW) is a term often used for the solid heterogeneous by-product of different human activities in the municipal area of the city. The waste generally contains discarded material like papers, plastic, glass, metal fine earth particles, ash, sewage sludge, dead animals, etc. (Thitame et al., 2010). Solid wastes are all the waste originating from human and animal activities that are normally solid and are discarded as useless or unwanted. It encompasses the heterogeneous mass of discarded residence and commercial activities as well as the more homogeneous accumulations of single industrial activities. The characteristics and composition of this waste depend on every activity and the amount varies by source, season, geography and time (Robert, 1999; Thitame et al., 2010). There are three primary purposes for solid waste characterisation. First, the data became the basis for planning economic analysis, design and subsequent management and operation of a disposal system or material – energy resource recovery facilities. Secondly, the solid waste characterisation for rehabilitation or retrofit of facilities redefines the quantity and type of waste for disposal. Third, plant optimisation and emission monitoring can be expedited by the characterisation of solid wastes being processed (Robert, 1999).

Three primary sources of waste are classified as municipal solid waste. These are residential, institutional and commercial waste, and municipal services wastes (street sweeping). Residential wastes are high in quantity and vary with time and season. Over time, municipal solid waste is not only increasing but the composition is also changing. The

organic wastes are decreasing and the paper and plastics are increasing in the waste stream, indicating the growing preference for consumption of packaged food in recent years (Yousuf and Rahman, 2007). According to Saeed (2009), the sources of municipal solid waste are street cleansing, landscape and garden, industrial & constructional, institutional, residential and commercial (Table 2.1). The generation of municipal solid waste by the public is a function of socio-economic background, i.e. the buying power, cultural background, locality i.e. urban or rural setting and the environment awareness (Johari et al., 2012). Analysis of the composition of the waste comes from restaurants, hotels, schools and roads (Dangi et al., 2011).

Table 2.1: Source Sector of MSW

Sector	**Percentage**
Street Cleansing	11%
Landscape & Garden	7%
Industrial & Constructional	4%
Institutional	6%
Residential	48%
Commercial	24%

Source : (Saeed et al., 2009)

2.2 CHARACTERISTIC OF MUNICIPAL SOLID WASTE

Generally, the solid waste composition in most Asian countries is highly biodegradable with high moisture contents such as food waste, paper, plastic/foam, agriculture waste, rubber/leather, wood, metal, glass and textiles. Basically, municipal solid waste is a heterogeneous mixture of waste; organic and inorganic, rapidly and slowly biodegradable and hazardous generated from various sources due to human and industrial activities. In major cities, it showed that food and vegetables wastes are the major components in the waste stream.

Other waste components are paper, paper product, polyethylene, plastics, textile, wood, rubber, leather, metal, tins, glass, ceramics, brick, concrete, dust, soil and etcetera (Yousuf and Rahman, 2007; Tsiko and Togarepi, 2012). Table 2.2 shows that the characteristic of municipal solid waste in Malaysia is organic waste as the major component, which includes paper, plastics, textiles, rubber & leather, wood, garden waste, dust and incombustibles (Muhd Yunus, 2012).

Table 2.2: Characteristic of MSW in Malaysia

Characteristic of MSW	Percentage
Organics	58.30%
Paper	8.20%
Plastics	13.10%
Textiles	1.30%
Rubber & leather	0.40%
Wood	1.80%
Garden Waste	6.90%
Dust	0.40%
Incombustibles	11.50%

Source : (Muhd Yunus, 2012)

The characteristics or the composition of Malaysian MSW is different from other countries. Due to its tropical climate with heavy rainfall, the Malaysian MSW contains high moisture content ranging from 52.6 % to 66.2 %, (Hassan et al 2001). Daniel and Laura summarize the sources and types of municipal solid waste in Table 2.3.

Table 2.3: Type and source of MSW

Source	Typical waste generators	Types of solid wastes
Residential	Single and multifamily dwellings	Food wastes, paper, cardboard, plastics, textiles, leather, yard wastes, wood, glass, metals, ashes, and household hazardous wastes
Industrial	Light and heavy manufacturing, fabrication, construction sites, power and chemical plants	Housekeeping wastes, packaging, food wastes, construction and demolition materials, hazardous wastes, ashes, special wastes
Commercial	Stores, hotels, restaurants, markets, office buildings, etc.	Paper, cardboard, plastics, wood, food wastes, glass, metals, special wastes, hazardous wastes
Institutional	Schools, hospitals, prisons, government centers	Same as commercial
Construction and demolition	New construction sites, road repair, renovation sites, demolition of buildings	Wood, steel, concrete, dirt, etc
Municipal services	Street cleaning, landscaping, parks, beaches, other recreational areas, water and wastewater treatment plants	Street sweepings, landscape and tree trimmings, general wastes from parks, beaches; and other recreational area, sludge
Process	Heavy/light manufacturing, refineries, chemical plants, power plants, mineral extraction and processing	Industrial process wastes, scrap materials, off specification products, slag, tailings
Agriculture	Crops, orchards, vineyards, dairies, feedlots, farms	Spoiled food wastes, agricultural wastes, hazardous wastes (e.g. pesticides)

Source: Daniel and Laura, 1999.

2.2.1 Biodegradable Municipal Solid Waste Content

Biodegradable wastes in the MSW also consist of vegetable kitchen waste. The various feedstocks could be classified into four groups according to nutrient composition (protein, fat, cellulosic materials and easily degradable carbohydrates). The various types of biodegradable municipal solid waste content are described in Table 2.4 according to Kobayashi et al., (2012).

Table 2.4: Different composition from various type of municipal solid waste

Volatile Solid (g/100 g wet)		Concentration (g/100 g VS)					
		Protein	Fat	Carbohydrates	Cellulose	Hemicellulose	Carbohydrates except cellulose and hemicellusose
AKW	33.3	54.4	35.7	9.9	3.9	n.d.	6.0
VKW	13.4	21.6	19.4	59.0	21.6	9.0	28.4
OKW	25.5	23.1	19.2	57.6	7.5	2.7	47.5
MP	31.7	7.3	20.8	71.9	59.9	4.1	7.9
CW	32.8	10.4	3.4	86.3	n.d.	n.d.	86.3
TCD	28.3	21.9	11.3	66.8	20.5	17.7	28.6
NP	33.6	5.7	7.1	87.2	66.4	5.1	15.8
WPC	63.4	2.1	2.1	95.9	91.0	5.0	0.0
UAKW	38.6	59.8	27.2	13.0	1.6	n.d	11.4
UVKW	10.8	19.4	11.1	69.4	19.4	n.d.	50.0
UP	59.2	2.0	7.4	90.5	75.5	6.4	8.6
VW	22.1	30.8	15.4	53.8	45.7	13.6	0.0
Mix	-	-	-	-	-	-	-
HQP	82.9	0.1	0.2	99.6	83.2	7.2	9.2
EGG	96.5	66.6	33.5	n.d.	n.d.	n.d.	0.0
WEO	28.5	0.2	94.5	5.3	n.d.	n.d.	5.3
ISC	66.9	5.4	1.0	93.6	n.d.	n.d.	93.6
FDN	98.2	-	-	-	-	-	-
BP	99.9	-	-	-	-	-	-
PGB	95.5	-	-	-	-	-	-

(1) Animal kitchen waste (AKW)
(2) Vegetable kitchen waste (VKW)
(3) Other kitchen waste (OKW)
(4) Miscellaneous paper (MP)
(5) Cereal waste (CW)
(6) Tea and coffee dregs (TCD)
(7) Newspaper used for wrapping (NP)
(8) Wrapping paper and container (WPC)
(9) Uneaten animal kitchen waste (UAKW)
(10) Uneaten vegetable kitchen waste (UVKW)
(11) Used paper (UP)
(12) Vegetation waste (VW)
(13) Mixture of 12 wastes (Mix)
(14) Waste high quality paper (HQP)
(15) Waste egg (EGG)
(16) Waste edible oil (WEO);
(17) Waste of Japanese style confection (JSC)
(18) Fiber drain net made of kenaf (FDN)
(19) Biodegradable plastics (BP)
(20) Paper garbage bag (PGB)

Source: Kobayashi et al., 2012.

2.3 MUNICIPAL SOLID WASTE MANAGEMENT

One of the characteristics of modern society is its ability to produce waste. Most human activities generate large amounts of waste that are apparently useless and can cause serious problems from both environmental and economic perspectives. Waste is generated in the human activity, such as municipal solid waste or sewage sludge, while the other, associated with certain economic activities, is recorded only in a few areas. Knowledge of quantity and composition of municipal solid waste is fundamental for the planning of waste management system. Most previous studies looked at the characteristics of municipal solid waste at the final disposal sites. For example, there is a case of horticultural waste in Southeastern Spain, a region with a strong agricultural sector that produces more than one million tons of organic waste per year. Many alternatives for the disposal of organic waste has been proposed, with composting being one of the most effective one due to low environmental impact and cost (Bustamante et al., 2008; Canet et al., 2008; Lu et al., 2008), capacity of composting to produce a valuable product used to improve soil fertility (Weber et al., 2007) or as a growing medium in horticulture. Common waste was used for composting are agricultural waste/plant residues, livestock/poultry waste, sewage sludge (SS) and municipal solid waste (MSW).

According to Atkinson and New (1993) regarding waste management strategies, shifting towards more recycling as well as determining the quantity and composition of waste at the sources of generation are getting more attention and concern. Therefore, in Malaysia, according to the report by the Ministry of Housing and Local Government, waste generation has been increasing gradually since 2000 (Table 2.5). These circumstances require the main focus to be placed on managing the solid waste and mitigating the negative environmental effects (Badgie et al., 2012). There are common municipal solid waste managements. Those are composting, landfill, and incineration.

Table 2.5: Generation on MSW in Malaysia according to states (1996-2009)

States	Solid waste generated (tons per day)							
	1996	**1998**	**2000**	**2002**	**2004***	**2006***	**2008***	**2009***
Johor	1613	1786	1915	2093	2255	2430	2578	2655
Kedah	1114	1215	1324	1447	1559	1680	1782	1835
Kelantan	871	950	1034	1131	1213	1302	1382	1423
Melaka	433	480	515	563	605	650	690	711
N. Sembilan	637	695	757	828	890	957	1015	1046
Pahang	806	879	957	1046	1125	1210	1284	1322
Perak	1286	1402	1527	1669	1375	1930	2048	2109
Perlis	165	180	196	214	3573	247	262	270
P. Pinang	916	999	1088	1189	1116	1375	1458	1502
Selangor	2380	2595	2827	3090	3322	3573	3790	3904
Terengganu	743	811	883	965	1038	1116	1184	1219
K. Lumpur	2105	2305	2520	2755	3025	3323	3525	3631
WP Labuan	NA	NA	46	70	74.3	81.2	86.1	88.7
Sabah	NA	NA	NA	2490	2642	2887	3062	3154.3
Sarawak	NA	NA	NA	1905	2021	2208	2343	2413
Total	13070	14589	15587	21452	23073	24969	26489	27284

Source: (MHLG, 2003; Agamuthu et al., 2009)

2.3.1 Composting

The presence of mixed organic substrates is a prerogative of composting. More specifically, according to its etymological meaning, composting (from the Latin compositum, meaning mixture) refers to a biodegradation process of a mixture of substrates carried out by the microbial community composed of various populations in aerobic condition and in the solid states. Microbial transformation of pure substrates goes under the name of fermentation or bio-oxidation, but not composting. The main product is called compost, which may be defined as the stabilized and sanitized product of composting that is compatible and beneficial to plant growth. Compost has undergone: (1) an initial, rapid stage of decomposition; (2) a stage of stabilization; and (3) an incomplete process of humification, (Bertoldi and Insam, 2007).

The biodegradable solid waste, however, is increasingly being viewed to be unsuitable for disposal through non-biological treatment technologies. Eventually, composting is one of the promising methods of Dry-Fermentation Technology (DFT) to handle a large amount of biodegradable waste (Baig et al., 2010). This process of biological treatment of wastes is also known as composting. It is a self-heating, aerobic solid phase biodegradative process of organic materials under controlled conditions, which distinguishes it from natural rotting (Sarkar et al., 2011).

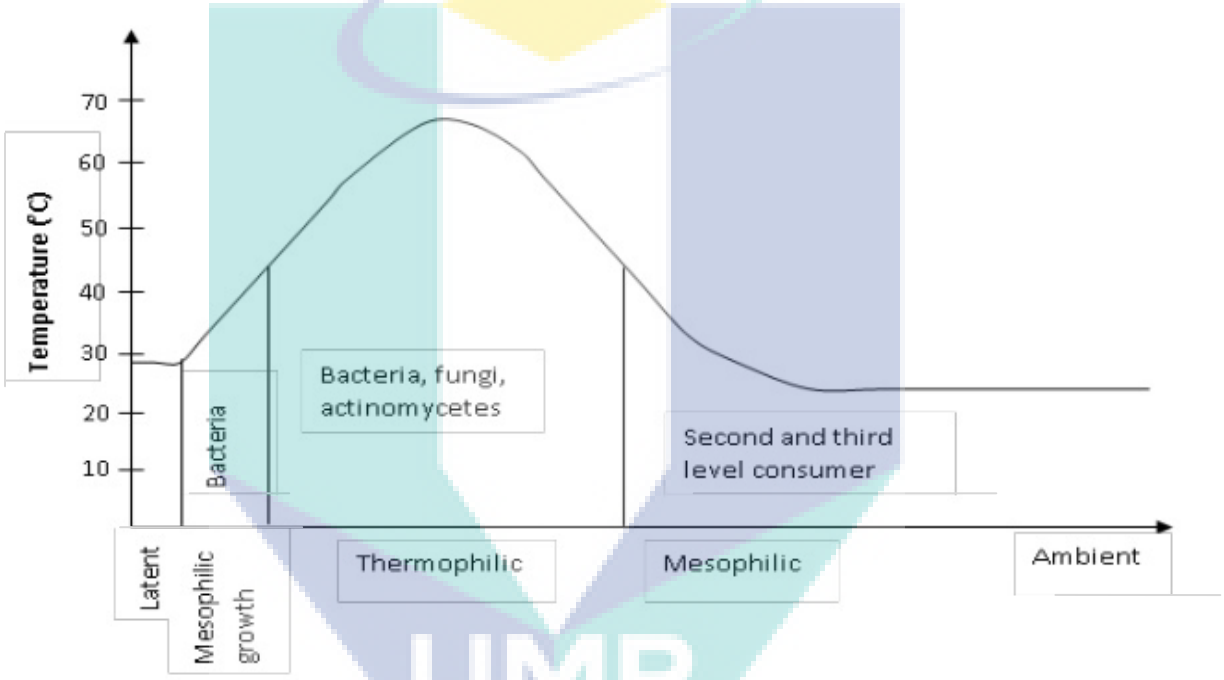


Figure 2.1: Pattern of temperature and microbial growth during composting process.

Source: Nangalia, 2013.

However, it is generally accepted that composting as a discontinuous process is essentially a four-phase process that is summarized in Figure 2.1. The first phase is the mesophilic phase (25-40°C) that is rich in energy, where easily degradable compounds like sugar and proteins are degraded by bacteria. The second phase is the thermophilic phase (35-65°C), the decomposition continues to be fast by fungi, actinomycetes, and bacteria, generally referred to as primary decomposers, and accelerates until a temperature of about

62°C. The third phase is cooling phase (second mesophilic phase), while in the starting phase, organisms with the ability to degrade sugars, oligosaccharides and proteins dominate, the second mesophilic phase is characterized by an increasing number of organisms that degrade starch or cellulose. Among them are both bacteria and fungi. During the maturation phase, the quality of the substrates declines, and in several successive steps, the composition of the microbial community is entirely altered. Usually, the proportion of fungi increases while bacterial numbers decline. Compounds that are not further degradable, such as lignin-humus complexes are formed and become predominant, (Bertoldi and Insam, 2007). Composting is a preferred and environmentally sound method whereby organic waste is reduced to organic fertiliser and soil conditioners through biological processes (Gautam et al. 2010; Alexander 1999).

2.3.2 Sanitary Landfill

Municipal solid waste (MSW) landfills represent the dominant option for waste disposal in many parts of the world. In general, the comparatively high costs of treatment and disposal alternatives are a major reason for the reliance on MSW landfills, particularly in developing economies (Brunner and Fellner, 2007). It can be divided into two categories, namely open dumpsite and engineered sanitary landfill. A sanitary landfill has features consisting of liners, leachate collection and treatment, gas harvesting as well as daily and final covers. A dumpsite is a MSW site without facilities such as liner or leachate collection/treatment. Landfilling solid waste is an anaerobic process. It produces landfill gases that consist of CO_2, CH_4, H_2S, NH_3 and other traces of gas. It can be harvested, treated and applied for electricity generation or direct heating if not being flared. Methane is known to be one of the contributors to global warming. The generation is a function of the amount of waste being deposited. Besides producing biogas, landfill requires huge land space and it also releases an unpleasant odor and leachate, which require further treatment (Chua et al., 2011).

The practice overview on MSW landfills highlights the significant variation among individual countries in both solid waste management practices and the extent of pre-treatment prior to waste disposal. However, there are at least two areas of commonality. First, the basic design elements of modern engineered landfills are similar (in this context, a modern landfill are one in which operation and maintenance are regulated at the national or sub-national level). Such landfills include a waste containment liner system to separate waste from the subsurface environment, systems for the collection and management of leachate and gas, and placement of a final cover after waste deposition is complete. Secondly, regardless of current approaches, the legacy of closed MSW landfills in almost all industrialized countries will continue to require aftercare (or post-closure care) until the protection of human health and the environment (HHE) is not compromised in the absence of such care (Laner et al., 2012).

An evaluation of final storage quality was also proposed by Knox et al., (2005) and criteria were derived from investigations at 14-year old MSW landfill test cells. The authors state that the biodegradation of MSW is the key process with respect to achieving final storage quality, and suggest target values for landfill gas and leachate emissions as well as tests to assess the residual biodegradability of the deposited waste. As long as a landfill represents a hazard and there is a receptor that can be damaged (e.g. human health or environmental media such as groundwater), some level of risk is associated with the landfill. Risk assessment has been applied to landfills, often with a focus on leachate emissions and groundwater pollution (Butt and Oduyemi, 2003).

Focus on the impact of leachate releases on groundwater quality, the long-term impact of leachate emissions is evaluated based on geochemical modeling and the subsequent comparison of maximum concentration levels to water quality criteria. The procedure is comparable to the source-pathway-receptor approach, which was applied to determine waste acceptance criteria within the Landfill Directive (Hjelmar et al., 2001, 2005; EC, 2003). Another impact assessment approach primarily based on modeling of the landfill leachate release and forward modeling of contaminant transport processes in the subsurface has been presented by Hall et al., (2006, 2007).

MSW is disposed on land without taking any specific precaution, which poses a serious threat to the environment (Sengupta et al., 1998). The effect is reported that the chemicals from solid waste disposal site polluted underground water, rendering it unfit for consumption (Samsudin et al., 2006; Singh et al., 1999). Similarly, dumping of solid waste result is an explosion of gases (LaMar et al., 1978). The solid waste contains a high proportion of organic matter, and this attracts the flies and rodents. The high temperature and humidity favor rapid bacterial growth and decomposition of waste that causes bad smell and odour which invite different diseases as well as disturb the aesthetic beauty of the area (Sharma, 2005).

2.3.3 Incineration

Another option for MSW treatment is incineration. The ignition of MSW allows huge volume reduction for both MSW and hazardous wastes. Even though incineration does not produce GHG, it produces harmful gases, particles and ashes. Incinerators are equipped with scrubbers and other prevention technology to remove those potential pollutants (Chua et al., 2011). Incineration is one of the major methods for the disposal of sewage sludge (Lin and Ma, 2012) that has been widely applied in many developed countries and some newly industrialized areas in recent years (Roy et al., 2011).

Incineration can achieve stabilization, volume reduction and resource recovery of sludge. It is an effective way of sewage sludge disposal (Khiari et al., 2006; Oral et al., 2005). The main shortcoming is a large quantity of ash formed when the furnaces operate at temperatures below the melting point of the sludge. The ash is contaminated with a high concentration of heavy metals, and is thus expensive to dispose and requires special and expensive filling sites (Werther and Ogada, 1999).

2.4 MUNICIPAL SOLID WASTE MANAGEMENT IN MALAYSIA

For many decades in the past, there was no clear or specific definition of solid waste and solid waste management in Malaysia. The specific legislation on Solid Waste and Public Cleaning Management were amended in 1971 and revised in 2007. According to the Solid Waste and Public Cleaning Management Act (Bill 2007), the definition of solid waste includes:

i. Any scrap material or other unwanted surplus substance or rejected products arising from the application of any process,
ii. Any substance required to be disposed of as being broken, worn out contaminated or otherwise spoiled, or
iii. Any other material that according to this act or any other written law, is required by the authority to be disposed of.

Approximately in Malaysia, between 70 to 80 percent of municipal solid waste is placed in landfills (Sumiani et al., 2009). For many years, landfill was inexpensive and the most common technique for solid waste disposal. Any type of waste was simply dumped in an open area of ground without any attempt for recovering or recycling. Solid waste generated from landed houses, apartments, flats, commercial shops, private and royal offices are levied and collected by the workers and put into baskets or trolleys, before being transported into a suitable solid waste lorry (Budhiarta et al., 2012). Waste in Malaysia is completely disposed to landfills. In 1988, there were 230 official dumping sites in Malaysia and about 49 sites are landfills. By the year 2002, there are 161 disposal sites that actively operate in Peninsular Malaysia. Most landfills in Malaysia were small-scale operations with varying levels of design sophistication, and the majority of the sites were poorly managed and approximately 50 percent of the landfills are open dumps.

According to data from the Ministry of Housing and Local Government, there are 296 landfills/dumpsites in Malaysia and 166 are still in operation, which include 9 sanitary landfills. More sanitary landfills are being planned in the future either to replace or to

upgrade the current dumpsites. There are four incinerators owned by the government and one operated by a private entity named Recycle Energy Sdn Bhd at Semenyih with a capacity of 1000 Metric tons per day. The other four incinerators are located in Pulau Pangkor, Pulau Langkawi, Pulau Tioman and Cameron Highlands.

Composting of municipal solid waste is another approach used by some people at the community or individual level. Some utilizes earthworm to decompose the solid waste, especially food waste in a method referred to as vermin-composting. Some private companies utilize anaerobic digesters to treat their organic waste in a small scale. Parallel with the government policies, which are to increase the recyclable percentage in Malaysia, recently a lot of government agencies and private companies are showing interest to involve composting in a bigger scale and settle the biofertiliser standard (Table 2.6). Based on the Kyoto protocol, Malaysia is trying to reduce their carbon emission, and one of the alternatives is through composting. One of the successful composting projects is at Takon Palm Oil Mill, Sabah (Amran et al., 2011).

Privatization and enforcement will make waste management solids more effective and efficient to ensure the states around the Peninsula have a clean, safe and healthy environment, besides protecting public health. Signing Ceremony Concession Act 672 is among the concessionaires for the privatization of solid waste management and public cleansing of Alam Flora Sdn. Bhd. (Kuala Lumpur, Putrajaya, Pahang, Terengganu and Kelantan), SWM Environment Sdn. Bhd. (Johor, Malacca and Negeri Sembilan) and Environment Idaman Sdn. Bhd. (Kedah & Perlis) with the Federal Government, represented by MHLG and Solid Waste Management and Public Cleansing (PPSPPA).

Table 2.6: Range of physical and chemical properties of organic fertilizer in Malaysia

	pH (1:5)	EC (dS m-1)	C/N %	O.M	TOC	TN	Lignin	Cellulose	P	K	Ca	Mg	Zn	Cu	Mn	Fe
													mg kg -1			
Maximum	9.8	12.28	43	79	45	4	67	29.1	8.9	6.9	12	3.3	353	88	827	24740
Minimum	4.5	0.08	3.8	25	8.4	1	5.3	9.3	0	1.3	0.1	0.3	45	17	89	912
Average	6.9	2.1	13	46	19.1	2	16.8	19.1	1.7	2.8	1.7	1	134	47	315	8602
SDa	1.3	2.76	7.2	16	7.9	1	10.8	6	2	1.7	3	0.8	70	17	200	5691

Source : (Kala et al., 2011)

2.5 JABOR LANDFILL MANAGEMENT

Jabor landfill (known as Kuantan landfill) was first opened in 1993 as the designated landfill for municipal solid waste for Kuantan town, Pahang, Malaysia. The landfill has two separate cells. Cell 1 was located northwest of the landfill and was closed in March 2006. Cell 2 was located south of the landfill was closed at the end of 2007. Cell 1 is a smaller landfill and its size is one third of cell 2 (Figure 2.2). The amount of waste received at Jabor landfill was from domestic waste weighing as much as 300-500 tons/day (residential area, market, business area) and commercial waste from 100-150 tons/day (small medium industry & entrepreneur area). The landfill was not designed as a sanitary landfill when it was first used by the Kuantan Municipal Council in 1993. As such, there was no proper landfill management carried out on site. After the disposal, the municipal waste was covered with a thin layer of soil for basic sanitary purpose only. There is a leachate treatment plant installed at the landfill for treatment of leachate prior to their discharge in the nearby drain.

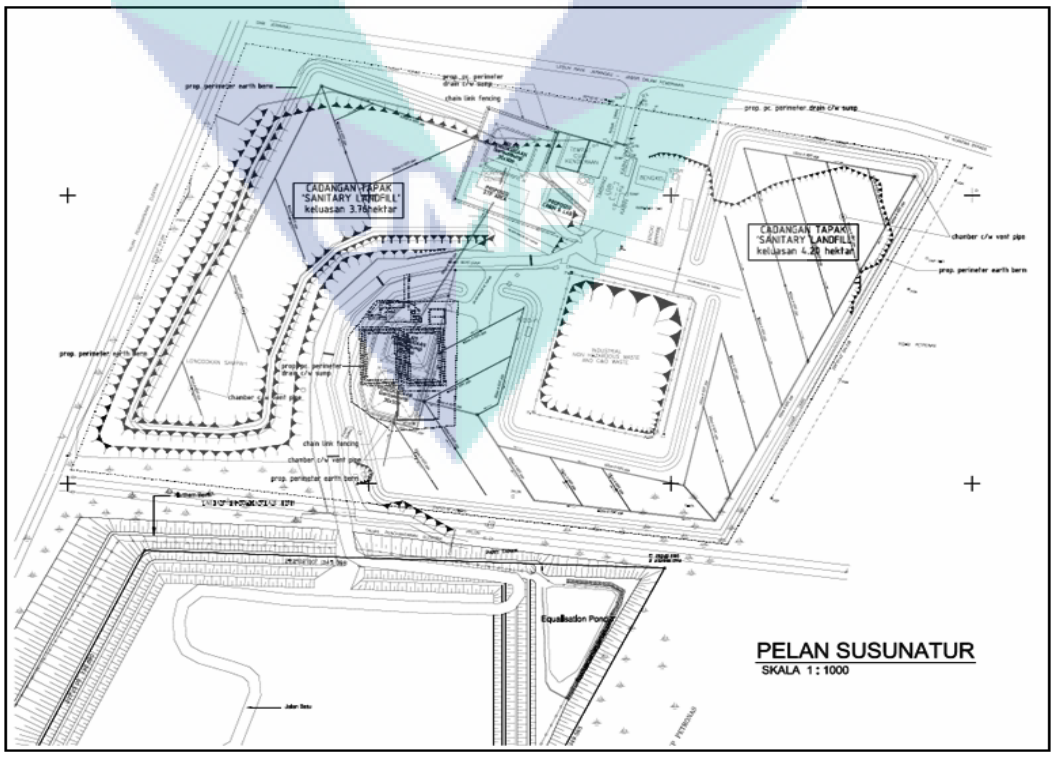


Figure 2.2: Jabor Landfill Site Map.

Jabor landfill covers an area of approximately 30 hectares. Terang Bersih Sdn Bhd is entrusted to manage and develop these into waste disposal landfill phase by phase using the concept of "semi-aerobic Recirculatory System" or "Fukuoka Method" (Figure 2.3). These methods have the capacity to take around 500 tonnes of solid waste per day, and can be used until it reaches maximum capacity within about 5 years. Among the advantages of using a semi-aerobic method are:

a. The quality of leachate is better and cost-effective to perform secondary treatment.
b. Generation/release of methane (CH_4) and hydrogen sulfide (H_2S) is a controlled and easily managed.
c. Accelerate the process of decay.
d. Cost-effective and easy-to-build Technology.
e. Shortening the time for other uses of landfill.

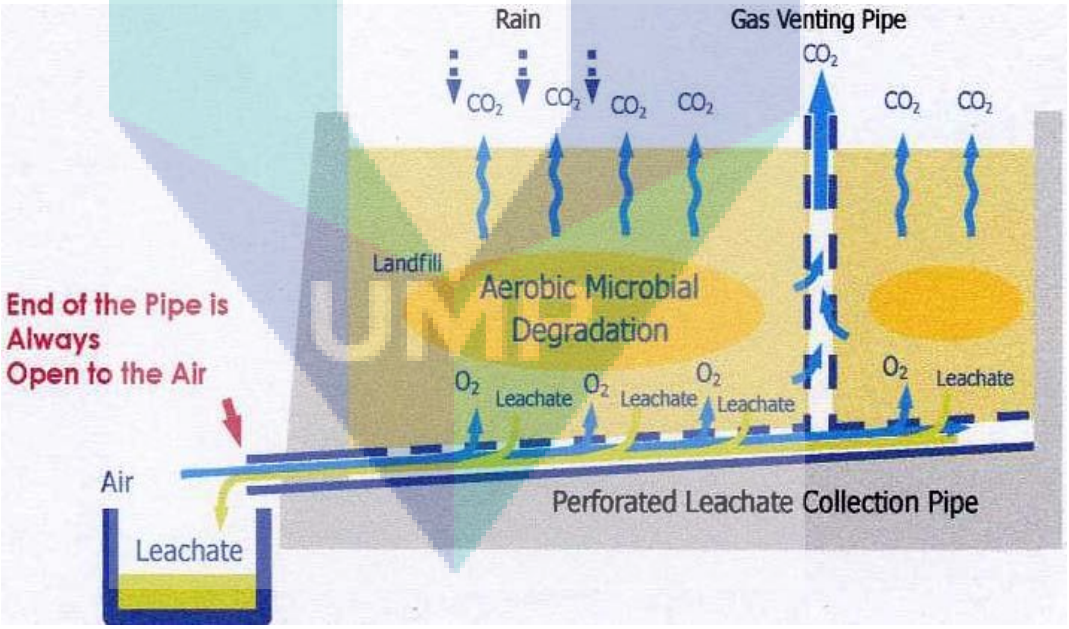


Figure 2.3: Semi-Aerobic Landfill structure (Fukuoka Method).

Source: JICA, 2010.

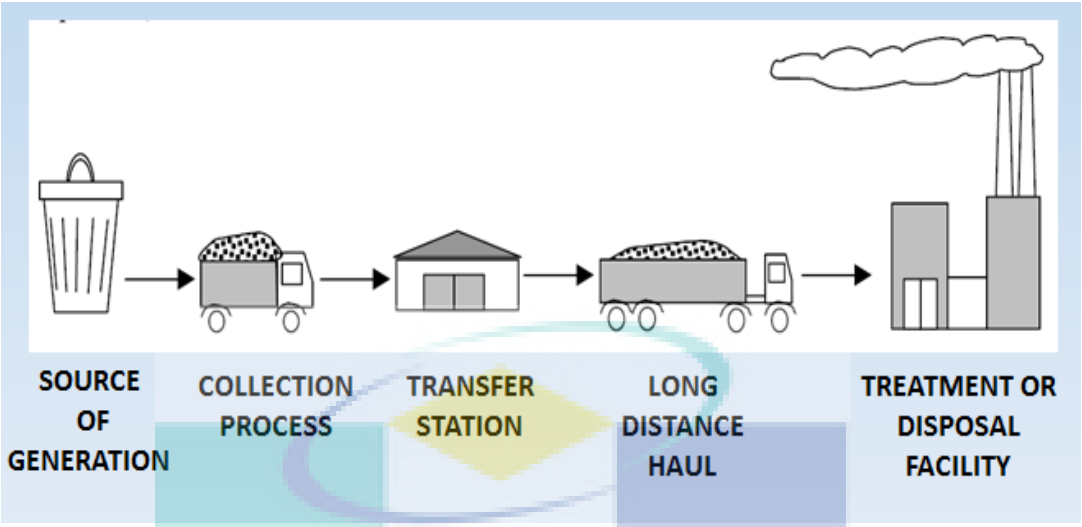


Figure 2.4: Waste collection flow in Kuantan to Jabor landfill.

Source: Terang Bersih Report, 2012.

Waste collected in the area is sent to landfill directly or indirectly depend on the source of waste generation (Figure 2.4). There are several different types of vehicles used to collect and send waste to landfill. There are Hydraulic Compactor (HC), Ro-Ro Bin, Prime Mover, Open Cart, etc. The operations carried out in a landfill are the inspection, weighing, arrangement and compaction, daily cover and environmental control. After inspection through a letter to the Jabor supervision, the waste is weighed to monitor the rate of incoming waste, and it is then compressed by the excavator, thus forming the tipping face flattened and covered by soil.

In addition, the Environmental Quality Control Process in Jabor landfill covers the following aspects:

a. Installation of a leachate collection system (waste water).
b. Installation of gas flow system.
c. Monitoring the quality of the river water, sea water, ground water and gas.
d. Waste water treatment process.
e. Preparation of monthly reports to all authorities involved.

2.6 BIODEGRADATION OF MUNICIPAL SOLID WASTE

Biological waste treatment process is also known as composting. It is a self-heating, aerobic solid phase biodegradation process organic materials under controlled conditions, which distinguish it from the natural degradation (Sarkar et al., 2011). Composting is another sustainable disposal option that increasingly plays a role in reducing the flow of easily degradable material to landfill. The effective result has been achieved by the engineering bioreactor system, which has brought significant improvements in traditional piles and windrow. Composting performance can be improved with process management, particularly as applied to the preparation of raw materials, moisture, aeration, and temperature control (Lloyd-Jones et al., 2010). According to Rynk (1992), the output of composting process is compost product and during the degradation process, water, heat and CO_2 with aerobic condition O_2 are produced (figure 2.5)

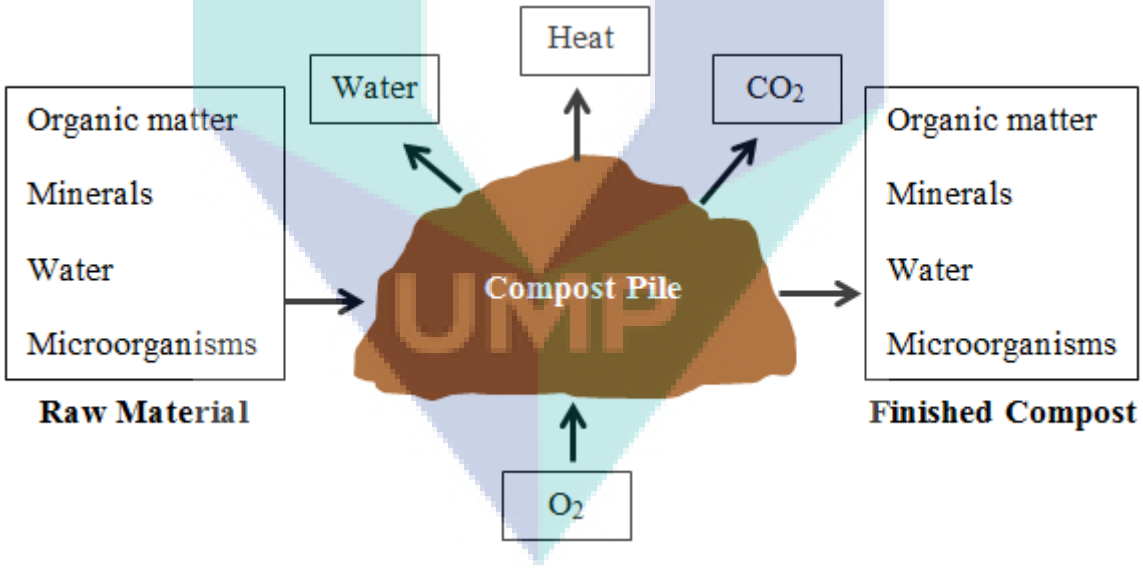


Figure 2.5: Input and output analysis of composting process.

Source: Rynk, 1992.

2.6.1 Biodegradation Process

Composting, being a biological decomposition process, has many advantages as well as disadvantages like any biological systems. Composting systems have the advantages of using lower technology equipment, simple operation outlay, and less undesirable impacts on the quality of environment (Yeoh et al., 2011).

a. Chemistry aspect

Chemical changes occur during the biodegradation process. Chemical breakdown is triggered by the action of enzymes produced by microorganisms. An enzyme secreted from bacteria and fungi will break down these complex organic compounds, and the simpler compound will then be absorbed by their cells. The enzyme catalyse reaction in which sugar, starches, proteins, and other organic compound are oxidized, in the end produces carbon dioxide, water, energy, and compound resistant to further decomposition. Specific enzymes are for a particular compound, such as cellulase to break down cellulose, amylase for starches and protease for proteins. The more complex the original molecule is, the more extensive the enzyme system is required to break it down. Lignin is the large polymers that cement cellulose fibers together in the wood, which is among the slowest compounds to decompose since their complex structure is highly resistant to enzyme attack. Nutrients such as nitrogen, phosphorus and potassium are released in various chemical forms during the decomposition of organic matter by the microorganisms and invertebrates that make up the compost food web (CWMI, 2007).

b. Biology aspect

Compost is a self-heating method due to biological activity. Although the microbial community naturally present in organic wastes usually carries out the process satisfactorily, inoculation with external microorganisms is a strategy that could potentially improve the way the process takes place and the properties of the final product (Liu et. al., 2011 and Rashad et. al., 2010). Certainly, some contradictory results have been reported on the

benefits of bio-inoculation, since composting closely depends on the original raw materials and the biological events that take place along the entire process. Taking into account all the biological, nutritional, physical and chemical aspects involved, it stands to reason the conditions are different in every situation, so it is really intricate to accurately estimate the efficacy of bio-inoculation in composting processes (Vargas Garcia et. al., 2006). Besides that, successful composting depends on a number of factors that have both direct and indirect influences on microbial activities.

The importance of non-mycelial bacteria during the composting process has been long neglected, probably because of the better visibility of fungi and actinobacteria. If temperatures are kept under 60 ^{o}C in sewage sludge composting, more than 40% of solids are degraded within the first 7 days, almost entirely through bacterial activity. Actinobacteria prefer neutral or slightly alkaline pH and are able to degrade relatively complex substrate. Several are thermotolerant, or even thermophilic, with a temperature range of 50 to 60 ^{o}C. Many bacteria in archaea group are known to be thermophilic or even hyperthermophilic. They have primarily been isolated from hypotermal vents. The reason for the relatively low abundance of archaea probably is that they are usually oligotrophic and their generation times are much higher than those of bacteria, which make them unsuitable in rapidly changing conditions. During the starting phase, fungi compete with bacteria for the easily available substrates, since the maximum specific growth rate of bacteria exceeds that of fungi by one order of magnitude, thus fungi are very soon out-competed (Insam and Bertoldi, 2007).

2.6.2 Biodegradation Factor

According to Diaz et al., 2002, the main factors in the control of a composting process include environmental parameters (temperature, moisture content, pH and aeration) and substrate nature parameters (C/N ratio and particle size).

a. Temperature

Composting is a complex process of biological activity. There are many different microbial communities that lay on environmental conditions and proper nutrition, but some of them can thrive during the whole process of it. Temperature, oxygen and organic substrate properties are the main factors which affect the dynamic growth of microbial (Steger et al., 2005; Tiquia, 2002). Temperature is the main factor controlling the reaction of composting as its effect on microbial metabolism (Tang et al., 2007). According to the study done by Lopez et al., (2006), inoculating composting of agricultural wastes can produce a greater amount of heat output. Increased microbial activity was induced by temperature increases. In this study, the temperature in the two mixed up immediately after composting (Liang et al., 2003).

According to Stentiford (1996), the maximum temperature that necessary to destroy pathogens is between 55-65 ^{o}C for at least three consecutive days. Even so, it must not be too high as this condition will kill all microorganisms and can cause the process to cease (Golueke and Diaz, 1996). Flynn and Wood (1996) found that each compost pile has a different temperature pattern depending on the carbon (C) source with small differences between nitrogen (N) source over time, and they also found that peanut hull and pine bark compost has the highest temperature.

b. pH

Bohacz, J., Korniłłowicz-Kowalska, (2007) reported that in the course of the experiments, a significant decrease in the pH value was observed in the composts, which coincided with the intensification period of mineral transformations in the forms of nitrogen and sulphur as discussed in the paper. There are many factors that affect the composting process, such as microbial diversity, moisture content, pH and C/N, proportions of the mixture, aeration rate, oxygen consumption, recycled compost, etc (Lu et al., 2009).

c. Moisture Content (MC)

Moisture content is another important factor in the preparation of compost. The optimal moisture content is different according to the material source, particle size and also the composting system used. Moisture content should be moderate, because if it is too low, it will disturb the biological process and if it is too high, it will clog the pores between particles (Yang, 1997). The moisture content of the compost indicates the capacity of the composts to hold water. Moisture content of the composting blend is an important environmental variable as it provides a medium for the transportation of dissolved nutrients required for the metabolic and physiological activities of microorganisms (McCartney and Tingley, 1998; Liang et al., 2003).

d. Aeration

In composting, aeration rate is the main factor influencing compost stability (Guo et al., 2012). One of the main factors that can be most influenced by technology and around which system design is developed is the provision of oxygen to the composting mass. The air contained in the interspaces of the composting mass during the microbial oxidative activity varies in composting. The carbon dioxide content gradually increases and the oxygen level falls. The average CO_2 and O_2 content inside the mass is about 20%. Oxygen concentration varies from 15 to 20% and carbon dioxide from 0.5 to 5% (MacGregor et al., 1981). When the oxygen level falls below this range, anaerobic microorganisms begin to exceed aerobic ones, after which fermentation and anaerobic respiration processes take over. It is very important that microorganisms have a constant supply of oxygen to maintain their metabolic activities unaltered. After a few hours of composting, the oxygen level drops to very low levels and oxygen has to be supplied through ventilation. Periodic pile turning, every one, or two days, without any ventilation of the mass, cannot guarantee a constant level of oxygen inside the mass.

e. Carbon to Nitrogen Ratio (C/N ratio)

One of the most important aspects of the total nutrient balance is the ration of organic carbon to total nitrogen (C/N). In the starting material, C/N value of about 25-30 is optimum for most types of waste. Living organisms in their metabolism utilise about 30 parts of carbon for each part of nitrogen. If the amount of carbon over that of nitrogen is too great, biological activity diminishes. In a composting operation, the manifestation could require an excessively long time to reduce the C/N to a more suitable level (Golueke, 1977).

Kayhanian and Tchobanoglous (1992) said that the C/N ratio is a dynamic factor for proper microbial growth and metabolism. Amongst the elements required by the microorganisms for decomposition, carbon and nitrogen are the most important and the most common limited. Carbon acts as both energy source and the basic building block, making up 50% of the mass microbial cells while nitrogen is necessary for cell growth and function, which is a crucial component of the proteins, amino acid, enzymes and DNA (Trautmann and Kransy, 1996). The C/N ratio mainly contributed to compost maturity (Guo et al., 2012).

f. Particle size

Numerous studies have demonstrated that the biodegradability of organic waste can be increased through chemical or biochemical measures, but few have investigated the effects of initial compost particle size (IPS). Adjusting the IPS could enhance microbial activity throughout the composting period and increase the rate at which macromolecules are degraded (Manpreet et al., 2005). Particle size will effect on moisture retention, free air space and porosity of the compost mixture (Naylor, 1996).

Smaller size organic material particle will have greater surface area available for attack by the microorganisms. However, it will be packed tightly together, which means that the space between particles will be small and narrow if the conditions of the particles

are very small. This will lead to the movement of air into the compost pile and the movement of carbon dioxide out of the pile, thus preventing the movement of carbon dioxide out of the pile. However, the surface area for microbial activity decreases if the particle size is too large, and the reaction may proceed slowly or stop all together (Dalzell et al., 1987).

2.7 APPLICATION OF BACTERIA IN COMPOSTING

Decomposer microorganism organic material is a biological activator that grows naturally or deliberately given to speed up and improve the quality of compost. The number and types of microorganisms determine the success of the composting process. The process of composting organic matter is not made by a single microorganism monoculture, but carried out by consortia of microorganisms. Several types of bacteria, including some types of Actinomycetes are also able to degrade the polymer cellulose, hemicellulose and lignin, but lower than the degradation ability of fungi. Bacteria play a role in the degradation of simple polysaccharides. Organic material decomposes bacteria can be found in places containing organic compounds derived from the remains of dead plants. Bacteria are able to decide carbon chains compiler lignin compounds (woody material), cellulose (fibrous material) and hemicellulose, which is a constituent component of organic matter in the rest of the plant, but the degradation process is slower when compared to simple polysaccharide compounds (starch, disaccharides and monosaccharides). Fungi have a better ability than bacteria in breaking down the remains of plants (cellulose, hemicellulose and lignin). Generally, microbes capable of degrading cellulose are also able to degrade hemicellulose. Microorganisms can accelerate the composting process depending on the chemical composition of organic matter. Cellulose is a polymer of glucose that is linked to each other by β-1-4 glycosidic bond is found in plants. Cellulolytic microorganisms are capable of hauling over the cellulose in plants because it can produce cellulase enzymes. Cellulase enzymes are a group of enzymes that are able to decide β-1-4 glycosidic bonds in cellulose molecules, selodekstrin, cellobiose and other cellulose derivatives contained in plants (Insam and de Bertoldi, 2007).

2.7.1 Bacterial Consortium

Mixed populations can perform functions that are difficult or even impossible for individual strains or species. Balancing two or more tasks efficiently so that they are completed in one organism may pose an insurmountable challenge in some situations. For example, it is difficult to work efficiently as independent metabolic pathways in the cell to allow it to consume five and six-carbon sugars produced by the degradation of lignocellulose; a synchrony degradation caused by glucose preferences could reduce productivity. However, if it is done by a mixed population, the process of lignocellulose degradation will be faster (Zaldivar et al., 2001).

Microbial consortia can perform even more complicated tasks and endure more changeable environments than monocultures can; they represent an important new frontier for synthetic biology (Brenner et al., 2008). Another important feature of microbial consortia is their ability to perform functions requiring multiple steps. Such tasks are possible when different steps are completed by dedicated cell-types. For example, cellulolytic microbes make and excrete several different protein components (e.g. Scaffolding proteins and enzymes) that assemble into an extracellular cellulosome that is capable of cellulose degradation. Various organisms in nature can secrete all of the necessary cellulase components, but these organisms are often difficult to manipulate genetically (Arai et al., 2007).

The composting process involves three phases, and uses diverse microflora such as bacteria, fungi and mesophilic (*Streptomyces rectus*) and thermophilic *Actinomycetes* (*Actinobifida chromogena* and *Thermomonospora fusca*), Microbispora (*Thermopolyspora*) bispora, *Therinomnonospora curvata*, *Thermoactinomyces* sp. eventually converting organic waste to humus. In composting, a large number of microorganisms such as fungi, actinomycetes and bacteria, which at the same time attack the organic residues, use a portion of them to form their cellular material and evolve carbon dioxide (Deporter et al. 1998).

Composting is an extraordinarily complex process which involves microbiological degradation, mass and energy transfer phenomena and coexistence of non-steady state conditions. Among the many parameters developed to measure the efficiency of composting processes are based on the survival of key degradation agents, the microorganisms. Wei et al., 2007 reported that compost or biofertiliser could be produced with the inoculation of appropriate functional microbes, which increase the decomposition rate, shorten the maturity period and improve the compost (biofertiliser) quality.

2.8 ENZYME ACTIVITIES IN COMPOSTING

Enzyme activity can be used in the evaluation of organic matter transformation in compost and to predict their maturity. Changes in the activity level of a particular enzyme class show the transformation of C, N, S, and P, and as a result, the available elements to plants will appear (Bohacz and Korniłłowicz-Kowalska, 2009). A sign of organic substrates is the high complexity of composition, therefore their complete biotransformation during composting requires various enzymes. Although the analysis of most of the enzymes is not feasible, investigation of some of the most significant ones (in this case, dehydrogenases, β-glycosidases, phosphatases, proteases and ureases) provide data to estimate the true events that took place throughout the process (Delgado et al., 2004).

Species that dominate the release of various hydrolytic enzymes (specifically, proteases, hemicellulases, phosphatases, cellulases) in composting process include *Bacillus* sp. (Ben-David et al., 2011; Portillo et al., 2011; Shi et al., 2011), which depolymerase various types of organic waste (Marx et al., 2001). Hence, in addition to species identification, the key of microbial enzymes activities involved in composting is characterized to obtain further information relevant to control the process (Maeda et al., 2010; Yamamoto et al., 2011; Xing et al., 2005).

2.8.1 Ligninolytic

Lignocellulose is a complex macromolecule composed of lignin, cellulose and hemicellulose (Huang et al., 2008); (Hofrichter, 2002). Lignin is an extremely irregular polymer which is hard to dissolve, chemically bound by covalent connection of hemicellulose. The high structural complexity of these polymers, together with the diversity of enzymes involved in the degradation process, make it difficult to estimate the activity. Therefore, polymer dyes are traditionally used for the screening of ligninolytic microorganisms because they are easy to use and responsible for ligninolytic system in their decolorization (Takano et al., 2001) because they indicate a structure that is similar to lignin and its derivatives. However, most of these studies have been conducted with axenic cultures (Chander et al., 2004), (Hernandez-Luna et al., 2008); (Levin et al., 2004); (Pointing et al., 2000), without disturbing other microorganisms. This decomposition trend is similar to decompose cellulose in two mixtures. It is gradually degraded during composting. Cellulose was slightly degraded during the initial phase of composting (0-6 days), whereas the rapid decomposition was identified during the thermophilic phase. Significant differences among the control and treatment were detected in the hemicellulose and lignin degradation during composting. Hemicellulose and lignin degradation rates in treatment increased by 5.24% and 11.74% ($P < 0.05$), respectively, compared to the control results (Feng et al. 2011).

In the study on the ability of ligninolytic enzymes to degrade natural lignin by Thompson et al., (1998), it was found that ligninolytic enzymes decreased the lignin content by 5%, decreased the solid mass by 11%, and released several typical low molecular weight lignin-derived products. To increase the degradation of lignin, the addition of ligninolytic enzyme into lignocellulosic waste composting and carbon utilization ability were also improved by microbial communities. All results indicate that it is feasible to apply the ligninolytic enzyme to increase lignocellulosic waste composting. This is probably from the ligninolytic enzyme that catalyzes lignin degradation. Further research is needed to investigate the mechanisms of ligninolytic enzymes to speed up the composting process, and develop an efficient composting technology with ligninolytic

enzyme for effective treatment of lignocellulosic waste (Feng et al., 2011). The bacteria are known for their ability to degrade complex molecules, especially the components of lignocellulose, which make them important agents in the decomposition process (Pathak et al., 2006); (Halet et al., 2006); (Ruberto et al., 2003). They survive under extreme environmental conditions (Ugwuanyi, 2008) by releasing soluble sugars from rice straw (Lei & VanderGheynst, 2000) to accelerate the bioconversion of lignocellulose components by producing lignocellulolytic enzymes (Lee et al., 2008).

2.8.2 Protease

Proteolytic activity seems to be very influenced by microbial populations. It is proven by the high correlation coefficients among the enzyme activity and aerobic mesophilic microbiota, fungi and actinomycetes. This correlation was also observed in the case of the MSW, although protease activity evolutionary pattern was different from that described in the case of the other two materials. During the maturation phase, the hydrolysis of proteins and polypeptides increased to the same level as that listed at the start of the process, indicating complete decomposition of protein during bio-oxidative phase (Raut et al., 2008). Proteases act on proteins and polypeptides and their degradation can be considered as a good indicator of the decomposition of organic materials due to their extreme dependence on substrate availability (Lazcano et al., 2008). Therefore, greater protease activity should be expected for the next phase of compost with a high concentration of macromolecule polymer and a low degree of stabilization. Sewage sludge (SS) and horticultural waste (HW) piles fit this profile, due to higher levels observed at the beginning of the process and during the bio-oxidative, while the next stage (maturation and final products) are characterized by values that were significantly lower on the other side, and as previously described (Aira et al., 2007).

Bohacz and Korniłłowicz-Kowalska (2009) reported that the results of the study concluded that the protease is active in the initial 3 weeks of composting, synthesized primarily by bacteria. Polysaccharides such as cellulose, as well as protein, perhaps with a less complex structure of keratin, have started to be hydrolyzed after 7-14 days of

composting of chicken feathers by mixing lignocellulosic waste. This was evidenced by increasing in caseinian protease, hydrolysis of peptide bonds of "standard proteins".

2.8.3 Dehydrogenase

Dehydrogenase activity is considered as a general index of biological activity because of its role in oxidative phosphorylation process, and also in the respiratory metabolism of microorganisms (Delgado et al., 2004). Tiquia (2002) reported in the study of poultry waste compost with plant material (Benito et al., 2003), during composting of plant waste materials and by Castaldi et al., (2008) the composting of municipal solid waste with sewage plants (leaves, grass clippings and shredded bark), that dehydrogenase activity increased rapidly in the early period of compost due to simple carbon substrate oxidation catalyzed by the enzyme. Dehydrogenase activity is an intensity index of basic oxidation process performed by microorganisms and positively correlated with their respiratory activity as measured by the amount of CO_2 emitted (Aguilera et al., 1998); (Camina et al., 1998). A decrease in respiratory activity and dehydrogenase activity were observed mainly in the treatment at the 4th week of composting, which indicated the diminishing of available carbon and energy source for the microorganisms. Hydrolysis activity in protease, urease, cellulase, β-glycosidase, and dehydrogenases, increased significantly over the past two weeks at the beginning of compost from municipal solid waste (MSW) with plant waste, and suggested evolution of enzymatic activity during composting as an indicator of the state and evolution of organic matter, but only for this particular type of waste (Castaldi et al., 2008).

2.8.4 Cellulase

The presence of a simple carbon sources can lead to the inhibition of cellulase activity (of sorts endoglucanase) by 50% (Emtiazi et al., 2001). Different results obtained by Castaldi et al., (2008) who observed decreased cellulase activity throughout the composting process of municipal solid waste and the plant residue. Polysaccharides such as cellulose will start to be hydrolyzed after 7-14 days of composting of chicken feathers by

mixing lignocellulosic waste. This was evidenced by an increase of cellulase activity in this type of endo-glucanase, breaking bonds of the cellulose molecules (Bohacz & Korniłłowicz-Kowalska, 2009). β-glucosidase and dehydrogenase activity were higher in the early stages of the process, with the maximum rate for a pile of SS, and at least at the end of the bio-oxidative phase. In the case of piled MSW, there was a big difference between phase 1 and 2 and between the two final phases, although the difference was with the opposite sign. In the initial stage, β-glucosidase activity was decreased, whereas an increase was observed among the maturation and the final product (Vargas-García et al., 2010).

The composting phase is characterized by higher availability of these compounds to be related to a greater β-glucosidase activity. β-glucosidase is responsible for the range of β-glucosidase hydrolysis, especially cellobiose, with glucose as the end product. The level of activity is determined by the presence of metabolic substrates easily. Therefore, the maximum rate was observed at the early stage and during the maturation stage. The last increase might be due to carbon compounds derived from cellulolytic and hemicellulolytic activities, which is mainly achieved during the final stage of thermophilic and cooling stage that precede maturation (Yu et al., 2007). Since the action of the two communities have preceded microbial enzymatic activity, the lack of a clear correlation among β-glucosidase activity and cellulolytic populations and hemicellulolytic remains insignificant. On the other hand, the complex formed by humic substances and enzymes during composting (Mondini et al., 2004), provides a different source of enzyme (Cayuela et al., 2008). This fact contributed higher levels when the population of microorganism producers is lower. Furthermore, β-glucosidase and cellulase are known to be important in the degradation of cellulose, and the level of their activity seems to correlate with each other (Liu et al., 2011).

2.8.5 Phosphatase

The activity of this enzyme plays an important role in the P cycle and is considered as an indicator of general microbes, even though some phosphatases are synthesized not only by microorganisms but also by plants. Phosphatase catalyzes the hydrolysis of organic

phosphorus to inorganic forms that plants can metabolizes differently (Makoi & Ndakidemi, 2008). In this study, higher levels of phosphatase were observed in the sewage sludge, which may be caused by a number of organic phosphorus compounds that characterize the biosolids (Benitez et al., 2005), coming from detergents, dish-washing powder and even urine and feces (Balmer, 2004). On the other hand, there is a positive correlation among the enzyme activity and bacterial populations N_2 it, especially in the case of the SS. Phosphatase activity seems to be influenced by microorganisms, because their activities modify the availability of P (Allison et al., 2006), (Wang et al., 2007) and even some of diazotrophic bacteria recognized as a phosphatase producer (Hoppe, 2003). Conversely, the lowest phosphatase activity was detected in the MSW. Similar results have been reported by Pramanik et al., (2007), which describe the least phosphatase activity in the vermicomposting MSW in relation to end product obtained from the three types of waste.

A decrease in urease activity with the progress of composting time is associated with a decrease in microbial biomass due to the diminishing availability of nitrogen compounds (Castaldi et al., 2008). The results of this study indicated that other causes that are important for reducing urease activity may be the accumulation of N-NH_4, an inhibitor of the biosynthesis of the enzyme, as has been reported in previous studies (Bohacz, J., Korniłłowicz-Kowalska, 2007). In contrast, phosphatase, protease, β-glucosidase and ureases are associated with a particular cycle. Protease and ureases take part in nitrogen mineralization. Both are hydrolyzed into ammonia nitrogen compounds using low molecular weight protein and urea as substrates. β-glucosidase is involved in the carbon cycle through the hydrolysis of glucoside, whereas phosphatase releases phosphate groups from organic compounds (Vargas-García et al., 2010)

2.8.6 Urease

Ureases are involved in the nitrogen cycle. Accumulation of substrates produced by another enzyme activity associated with nitrogen and ammonium loss during the previous phase of thermophilic that may be responsible for this activity are higher urease. In

contrast, their maximum activity was observed at the final stage of the process, during maturation (Cunha-Queda et al., 2007; Ros et al., 2006). The inhibitory effect was caused by ammonium, which was confirmed by a significant negative correlation among the urease and ammonifying population. A distinct pattern was observed in piled MSW, with the maximum urease activity at the initial of the process in accordance with the results described by (García et. al., 1993).

The authors reported a significantly higher activity in fresh municipal solid waste than in the compost in each category (bacteria degrading cellulose, hemicellulose degrading bacteria, lignocellulose degrading bacteria, with temperatures ranging from 42 ^{o}C to 62 °C). Strains that indicate the highest growth rates were evaluated (Table 2.7). All strains that were isolated were Gram-positive, rod shaped, spore production, motility positive, be positive aerobic and catalase as well as oxidase. The results show that the use of inoculation in composting depends on the conditions in which this process is carried out, especially the characteristics of raw materials and inoculant. Thus, it becomes possible to increase the decomposition of compost each waste characteristic.

2.8.7 Lipase

Fats and oils are among the main components of organic matter in solid wastes, especially those produced by the food industry (Galli et al., 1997; Mari et al., 2003). Fats and oils are essentially triglycerides consisting of linear fatty acids attached as esters to glycerol. The biodegradation of fats begins with the enzymatic hydrolysis of the ester bond carried out by lipases, followed by the consumption of glycerol and beta-oxidation of fatty acids (Lalman and Bagley, 2000). According to Ruggiere et al., lipolytic activity was detected during the second week of process and increased progressively during the thermophilic phase to reach and maintain maximum levels in the mesophilic cooling and maturation stages in the biodegradation of animal fats in a co-composting process with wastewater sludge. The presence of lipases is very low in plant residues, so that their role in the carbon cycle is limited in comparison to the role observed in other processes that use high-fat content substrates (Jurado, 2014).

Co-composting of sewage sludge and animal fat mixtures was studied in order to determine the possibility of using this technology to recycle fat-enriched wastes. Under these conditions a fat content reduction of 85% was achieved. Biological activity was highly dependent on the moisture content as shown by the respiratory quotient values. Lipases responsible for fat hydrolysis were monitored during the composting process and a sample from the thermophilic period was characterized in terms of stability in front of pH and temperature. Optimal conditions for lipase stability were found at 38.3 ^{o}C and pH 7.97, however, the maximum lipolytic activity was observed at thermophilic temperatures. Lipase from thermophilic composting environments showed a higher stability for mesophilic values of temperature and slightly alkaline values of pH, however, the maximum lipolytic activity was observed at thermophilic temperatures. Major lipases involved in the composting of sludge: fat mixture could not be identified by N-terminal sequence analysis (Gea et al., 2007).

2.8.8 Amylase

Amylase profile closely reflected the development of microbial activity in thermophilic aerobic digestion (TAD) of model agricultural waste slurry that was carried out in a continuously stirred tank reactor for a total of 156 h at 55 ◦C. The highest starch degradation obtained at pH 7.0 was related to the highest amylase activity obtained at this pH value. Regarding the effect of pH, amylase activity fluctuated rapidly during digestion at different aeration rates. Amylase activity developed rapidly in the digesting slurry. This is understandable since starch was the predominant polymer, and its breakdown product was probably the preferred carbon source (Ugwuanyi, 2003).

Thermophilic *Bacillus* spp. tend to secrete only limited amylase (Grueninger, 1984). *Bacillus* sp. IMD 435 that was isolated from mushroom compost produced a raw starch-digesting but non-raw starch-adsorbing a-amylase (Hamilton et al., 1999). Enzymatic activities implied in the carbohydrates metabolism were strongly correlated among them. This effect was especially observed in the case of xylanase, which showed a

positive correlation both with b-glucosidase, cellulase and amylase activities. Table 2.7 shows the correlation of material and enzyme activity in the composting process.

Table 2.7: Correlation of compost material and enzyme activity in composting

No	Material	Microorganism	Enzyme Activity	pH	Temperature	Reference
1	Chicken manure, rice husk, rice bran, mushroom residue	*Bacillus* sp., *Arcobacter* sp., *Marinospirillum minotulum*, *Cohnella fontinalis.*	Dehidrogenase, celullase, β glukosidase, protease, urease.	7	55 °C	(He et al., 2013a)
2	Municipal solid waste	*Bacillus* sp.	Celullase, hemicelullase, lignocelullase, protease, lipase, amylase	-	42 °C	(Mirdamadian and Ghanavati, 2011)
3	Rice straw	*Bacillus pumilis*	Lignocelullase, amylase.			(Ismail et al., 2012)
4	Horticultural waste	Fungi, yeast, actinomycetes, ammonifying bacteria,	Dehidrogenase, celullase, β glukosidase, protease,	8.85	-	(Vargas-García et al., 2010)
	Sewage sludge	N_2 fixing bacteria, cellulolytic xylanolytic,	Phospatase, Urease	7.89	-	
	Municipal solid waste	Ligninolytic microorganism.		8.47	-	
5	Pig manure, wheat straw, spent mushroom substrate	-	Urease, cellulase, protease polyphenol oxidase activity	-	50-55 °C	(Guo et al., 2012)
6	Pruning waste	*Phanarochaete sordida*	Polygalacturonase, amylase, endoxylanase, endogluconase, lacase.	5.6	-	(Diorio et al., 2009)
7	Poultry waste,	*Pseudomonas*, *Achromobacter*, *Stenotrophomonas* sp.	Protease, keratinase	-	-	(Lloyd-Jones et al., 2010)
	Feather waste	*Chryseobacterium* sp., *Bacillus* sp. *Rhodococcus erythropolis*	Protease, keratinase	-	-	
8	Chicken feather, pine bark, rice straw	-	Phospatase, urease protease, cellulose dehidrogenase.	4.2	41 °C	(Bohacz and Korniłłowicz-Kowalska, 2009)

CHAPTER 3

MATERIALS AND METHODS

3.1 MATERIALS

In this study, municipal solid waste from Jabor landfill was used as the main sources for batch scale composting. Bacterial consortium was inoculated to the raw material to enhance the biodegradation process. This bacterium was isolated from soil and leachate obtains from Jabor landfill, Kuantan. All the materials were shredded and composted together in a plastic container. Figure 3.1 shows the flow chart of the methodology from the preparation of materials and data analysis. Materials that were used for identification were nutrient agar (NA), nutrient broth (NB), skim milk powder, carboxy methyl cellulose (CMC), congo red, gelatin, casein enzymic hydrolysate, $CaCO_3$, NH_4NO_3, $Na_2HPO_4.7H_2O$, $KH_2PO_4.MNCl_2.7H_2O$, $MgSO_4.7H_2O$, yeast extract, Rhodamine B (olive oil, NaCl, agar, nutrient broth), peptone, phenol red and Gram-staining reagents, which were supplied by Merck, Malaysia Division. Meanwhile, automated BIOLOG identification system kit was used to identify bacteria. Sulphuric acid, potassium sulphate, copper sulphate, glycerol and selenium powder were supplied by Merck.

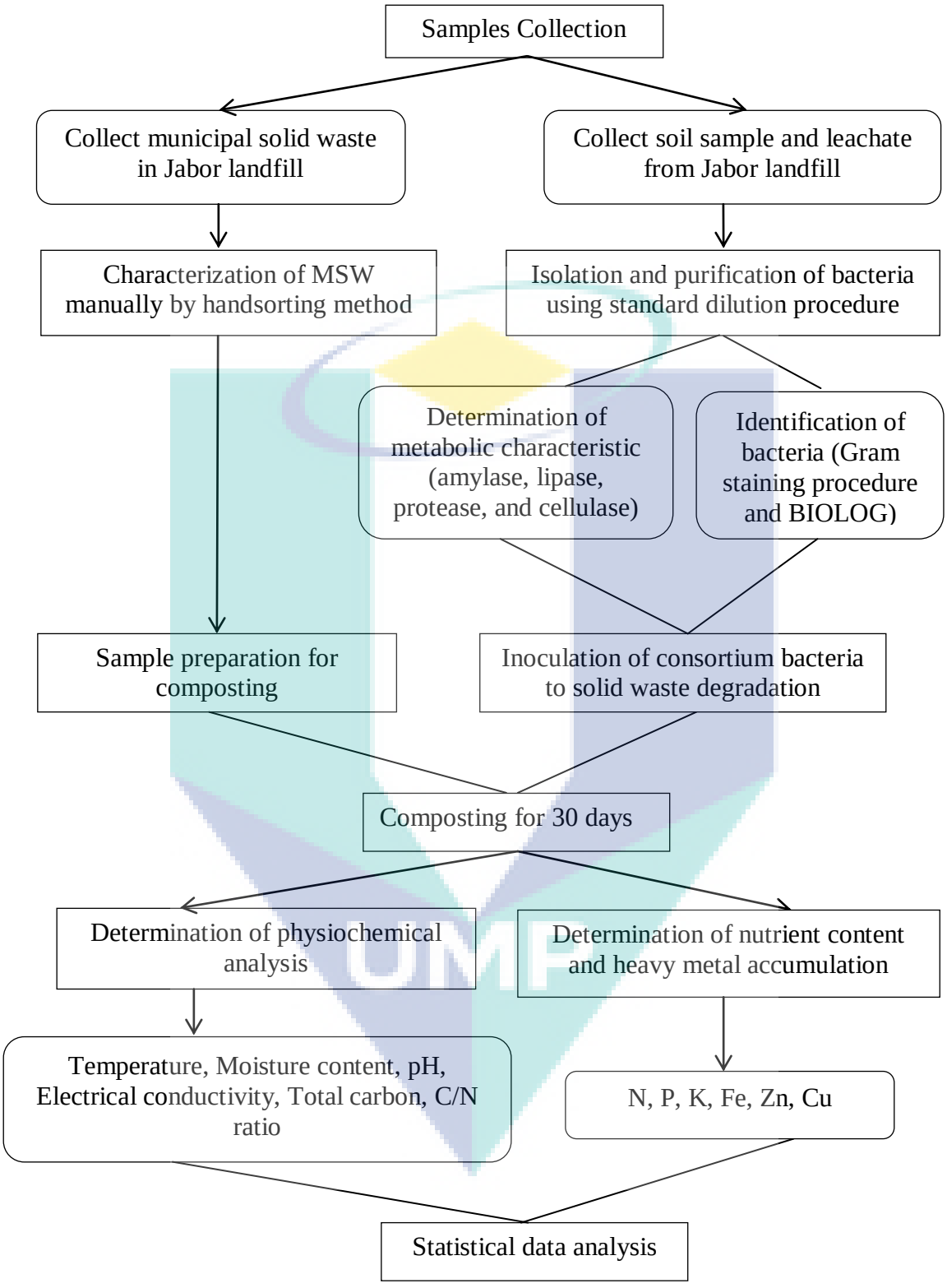


Figure 3.1: Flow chart of the methodology.

3.2 CHARACTERIZATION OF MUNICIPAL SOLID WASTE IN JABOR LANDFILL

Solid wastes were categorized into four main sections, namely municipal solid waste, hazardous waste, agricultural waste and commercial waste. This classification is important in order to identify several criteria that include all the sources, types, classification and composition. It is also to ensure that the landfill operations can be carried out more regularly and systematically (Agamuthu, 2001).

The municipal solid waste was generated from residential areas, housing estates, villages, office, institutional and commercial areas. Most municipal solid waste generated in a residential area consists of the remnants of the house, such as food waste, paper, plastic, metal, glass and yard waste (Razman, Othman and Marzuky, 1993). Industrial waste is a source of waste generation and it includes waste generated as a result of industrial and manufacturing activities. Examples of the component are generated debris, dust, dirt, construction waste and hazardous waste (Agamuthu, 2001).

The characterization methodology, which is site-specific, sampling, sorting, and weighing the individual components of the waste stream could be used. This methodology is useful in defining a local waste stream, especially if large numbers of samples are taken over several seasons. In addition, quantities of MSW components such as food scraps and yard trimmings can only be estimated through sampling and weighing studies. Source of waste in Jabor landfill were from commercial waste and domestic waste, which are separated in different disposal wastes. The solid wastes from domestic waste (100 kg) and commercial waste (100 kg) from Jabor landfill were separated manually using a hand-sorting method according to food waste, green waste, plastic, paper, aluminum, glass, solid waste and others. The solid waste used for composting was biodegradable matter and was then analyzed for chemical and biochemical properties during the biodegradation process.

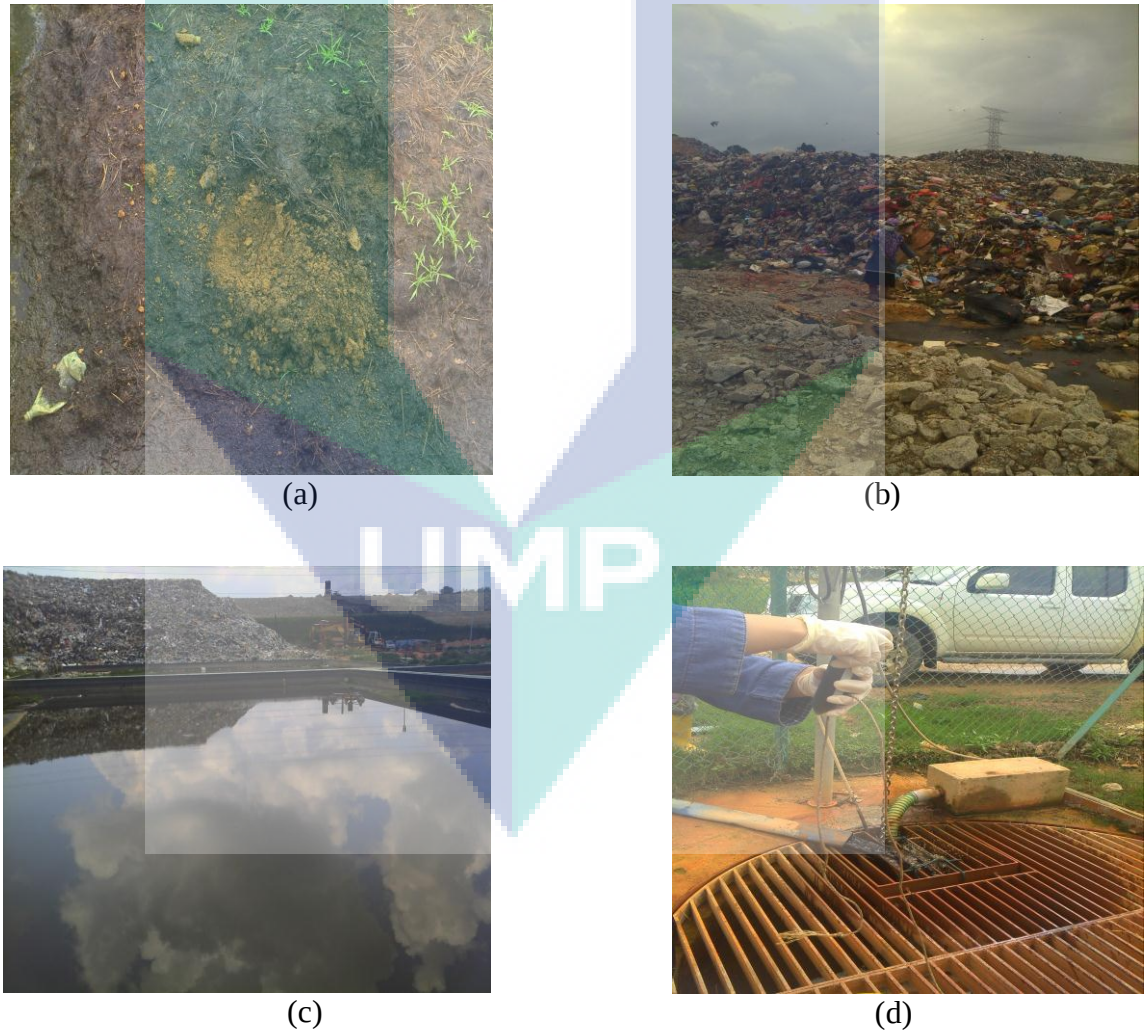
The classification used the following formula:

Percent type of waste = (weight of the type of waste/total weight of waste) x 100

3.3 ISOLATION AND IDENTIFICATION OF BACTERIA

3.3.1 Sampling and Isolation

Soil samples and leachate were collected from Jabor landfill. The location of the landfill is located at 30 56’ 53” N, 1030 21’ 03” E, along the Jabor-Jerangau Road, District of Kuantan, State of Pahang, Malaysia. Soil samples were taken as a composite at depths of 0-20cm as much as five points in every composite and mixed. The leachate samples were taken from different leachate tanks (Figure 3.2). There were four sampling points and every point made five repetitions of samples and mixed together. All samples were kept in a temperature of 4 ^{o}C.



(a) (b)

(c) (d)

Figure 3.2: Soil sampling point at different places (a) & (b); leachate sampling points (c) leachate treatment pond, (d) leachate storage wells.

One gram of soil sample was added in 9 mL of distilled water and shaken. 1 mL of leachate was then added into 9 mL of distilled water. After that, 1 mL from the sample was taken out and added into 9 mL of distilled water. This step was continuously repeated from soil and leachate sample until nine fold dilution, 0.1 mL from each dilution tube was spread on the nutrient agar plate. For each plate, L stick was used to spread the dilution on the medium. The plates was labeled and kept in a temperature of 37 °C in an incubator for 24 hours.

3.3.2 Isolation, Purification and Morphological Characteristics of Bacterial Isolates

These microorganisms were isolated until a single colony was obtained using the serial dilution method and streaking method. The preparation media from commercial products was according to the manual. To prepare agar plates, 24 g of nutrient agar (NA) was mixed in 1L distilled water. The suspension was mixed in an Erlenmeyer flask, then dispensed and sterilized for 20 minutes at 121 °C. The agar was poured into a petri plate to make a nutrient agar plate. Agar pours containing 15 mL of media were often used to prepare agar plates (Chowdhury et al., 2011). After nutrient agar plate was ready, the fast growing bacteria plate was counted. Plates were incubated to count colony forming units (CFU) to determine the average number of CFU per milliliter of leachate or wet weight of bulk sample. The different shapes of bacteria on the plate were chosen. By using aseptic technique, the selective bacteria on the agar plate were streaked. The streak agar plate is sealed and kept in an incubator at a temperature of 37 °C for growing. After 24 hours, the purification of bacteria was conducted by another streak technique and incubation. The next step was Gram staining observation under microscope. The cocci and the rods were counted and identified following Gram staining and microscopic observation of the CFU isolated in Nutrient Agar. The morphology observation and Gram staining results were analyzed together. The general characteristics of bacterial colonies were then described in terms of shape, margin, elevation and color, after which an accurate description of the colonies was given. All the microorganism cultures were maintained at 4 ^{o}C in nutrient agar as stock and were subcultured at a 15-day interval.

3.3.3 Qualitative Screening of Bacterial Enzymes Production

Various techniques were devised to analyze the structure and function of microorganisms. Some techniques are qualitative while others are quantitative. These techniques provide numerical information about a sample. Qualitative technique to screen the enzyme production was conducted with selective media. Starch agar was a differential medium that tests the ability of an organism to produce extracellular enzymes (exoenzymes) α -amylase and oligo-1, 6-glucosidase that were secreted out of the bacteria and diffused into the starch agar. These enzymes hydrolyzed starch by breaking the glycosidic linkages between glucose subunits and allowing the products of starch hydrolysis to enter the cell. Skim milk agar was a differential test medium for determining lactose fermentation and for detecting proteolytic enzymes that hydrolyze casein. Rhodamine B agar demonstrated the ability of some bacteria to produce lipase and degrade food oils, such as olive oil, in a clear and easily interpreted manner. To indicate the cellulose activity of the organisms, the diameter of clear zone around the colony and hydrolytic value on cellulose Congo Red agar media were measured. The use of Congo-Red as an indicator for cellulose degradation in an agar medium provides the basis for a rapid and sensitive screening test for cellulolytic bacteria. Colonies showing discoloration of Congo-Red were taken as positive cellulose-degrading bacterial colonies, and only these were taken for further study.

a) Qualitative test of amylase (Starch agar)

Meat Extract 3 (g/L), peptic digest of animal tissue 5 (g/L), starch soluble 2 (g/L), agar 15 (g/L). Final pH is (at 25℃) 7.2±0.1. Heat the agar solution to boil and to dissolve the medium completely. Sterilized the solution by autoclaving at 15 lbs pressure (121℃) for 10 minutes. Mix well and pour in sterile petri plates. The positive result of amylase production shows a clear zone around the colonies after being flooded by iodine.

b) Qualitative test of protease (Skim milk agar)

Skim milk powder 28 (g/L), casein enzymic hydrolysate 5 (g/L), yeast extract 2.5 (g/L), dextrose 1 (g/L), agar 15 (g/L). Final pH is (at 25 ℃) 7.0±0.2. Heat to boil and to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121 ℃) for 15 minutes. Mix well and pour into

sterile Petri plates. Proteolytic bacteria hydrolyze casein to form soluble nitrogenous compounds, which indicate a clear zone surrounding the colonies.

c) Qualitative test of lipase (Rhodamine B agar)

Agar plate containing rhodamine B 0.001 % (w/v), nutrient broth 0.8 %, NaCl 0.4 % (w/v), agar 1 % (w/v) and olive oil 2 % was prepared in distilled water, adjusted pH of 6.5. The assay was incubated at 55 °C for 18 h and lypolitic activity was identified as a pink halo around colonies under UV light at 350 nm.

d) Qualitative test of cellulase (CMC agar)

Cellulose congo red agar media contain KH2PO4 0.5 (g/L), MgSO4 0.25 (g/L), cellulose 2 (g/L), agar 15 (g/L), congo red 0.2 (g/L), gelatin 2 (g/L) and distilled water. Adjusted pH for 6.8-7.2. Mix well completely and sterilize by autoclaving. The positive result shows a clear zone around the colonies.

3.3.4 Identification of Bacteria

Identification of bacteria was conducted using Biolog Microplate analyses. It contained 94 phenotypic tests: 71 carbon source utilization assays and 23 chemical sensitivity assays. The test panel provides a phenotypic fingerprint of the microorganism that can be used to identify it at the species level. All necessary nutrients and biochemicals were prefilled and dried into 96 wells of the microplate (Figure 3.3). The tetrazolium redox dyes were used to colorimetrically indicate utilization of the carbon source or resistance to inhibitory chemicals. The isolate should be a pure culture and freshly grown since many strains lose viability and metabolic vigor in stationary phase. The incubation period for most organisms was 4-24 hours. The calibration of the turbidimeter was checked before testing. The cell suspension was poured into the multichannel pipet reservoir to microplate well and incubated for 24 hours at 330 C. Negative wells remained colourless, as did the negative control and the positive reaction was purple because of the increased respiration, which caused a reduction of the tetrazolium redox dye. There was also a positive control well used as a reference for the chemical sensitivity assay in columns.

GEN III MicroPlate ™

A1 Negative Control	A2 Dextrin	A3 D-Maltose	A4 D-Trehalose	A5 D-Cellobiose	A6 Gentiobiose	A7 Sucrose	A8 D-Turanose	A9 Stachyose	A10 Positive Control	A11 pH 6	A12 pH 5
B1 D-Raffinose	B2 α-D-Lactose	B3 D-Melibiose	B4 β-Methyl-D-Glucoside	B5 D-Salicin	B6 N-Acetyl-D-Glucosamine	B7 N-Acetyl-β-D-Mannosamine	B8 N-Acetyl-D-Galactosamine	B9 N-Acetyl Neuraminic Acid	B10 1% NaCl	B11 4% NaCl	B12 8% NaCl
C1 α-D-Glucose	C2 D-Mannose	C3 D-Fructose	C4 D-Galactose	C5 3-Methyl Glucose	C6 D-Fucose	C7 L-Fucose	C8 L-Rhamnose	C9 Inosine	C10 1% Sodium Lactate	C11 Fusidic Acid	C12 D-Serine
D1 D-Sorbitol	D2 D-Mannitol	D3 D-Arabitol	D4 myo-Inositol	D5 Glycerol	D6 D-Glucose-6-PO4	D7 D-Fructose-6-PO4	D8 D-Aspartic Acid	D9 D-Serine	D10 Troleandomycin	D11 Rifamycin SV	D12 Minocycline
E1 Gelatin	E2 Glycyl-L-Proline	E3 L-Alanine	E4 L-Arginine	E5 L-Aspartic Acid	E6 L-Glutamic Acid	E7 L-Histidine	E8 L-Pyroglutamic Acid	E9 L-Serine	E10 Lincomycin	E11 Guanidine HCl	E12 Niaproof 4
F1 Pectin	F2 D-Galacturonic Acid	F3 L-Galactonic Acid Lactone	F4 D-Gluconic Acid	F5 D-Glucuronic Acid	F6 Glucuronamide	F7 Mucic Acid	F8 Quinic Acid	F9 D-Saccharic Acid	F10 Vancomycin	F11 Tetrazolium Violet	F12 Tetrazolium Blue
G1 p-Hydroxy-Phenylacetic Acid	G2 Methyl Pyruvate	G3 D-Lactic Acid Methyl Ester	G4 L-Lactic Acid	G5 Citric Acid	G6 α-Keto-Glutaric Acid	G7 D-Malic Acid	G8 L-Malic Acid	G9 Bromo-Succinic Acid	G10 Nalidixic Acid	G11 Lithium Chloride	G12 Potassium Tellurite
H1 Tween 40	H2 γ-Amino-Butyric Acid	H3 α-Hydroxy-Butyric Acid	H4 β-Hydroxy-D,L-Butyric Acid	H5 α-Keto-Butyric Acid	H6 Acetoacetic Acid	H7 Propionic Acid	H8 Acetic Acid	H9 Formic Acid	H10 Aztreonam	H11 Sodium Butyrate	H12 Sodium Bromate

Figure 3.3: Layout of assay in the Biolog gen III microplate.

After overnight incubation, the phenotypic fingerprint of purple well was compared to Biolog's database. If a match was found, a species level identification of the isolate was made. The interpretation of result was read using Biolog's microbial identification systems software (e.g. Omnilog Data Collection). The GEN III Microplate performance characteristics were determined by establishing a large collection database of microorganisms. The database was designed to identify all species in the database, in accordance with current standards of classical identification methods and current taxonomic nomenclature.

3.4 APPLICATION OF BACTERIAL CONSORTIUM IN COMPOSTING

3.4.1 Preparation of Municipal Solid Waste

Interest has recently emerged in engineering microbial consortia–multiple interacting microbial populations – because consortia can perform complicated functions that individual population cannot and also because consortia can be more

robust to environmental fluctuations. These attractive traits rely on two organizing features. First, members of the consortium communicate with one another. Whether by trading metabolites or by exchanging dedicated molecular signals, each population or individual detects and responds to the presence of others in the consortium (Keller and Surette, 2006). The bacterial consortiums were able to produce enzyme prepared for substrate inoculation. Twenty (20) kg small heaps of biodegradable solid wastes were collected from Jabor landfill in a plastic container. Solid wastes were shredded prior to increasing the surface area and reducing the size for easy handling by using a shredding machine. The organic municipal solid waste were food waste, green waste, yard waste and paper. Raw materials were mixed and analyzed for physiochemical, nutrients and heavy metal.

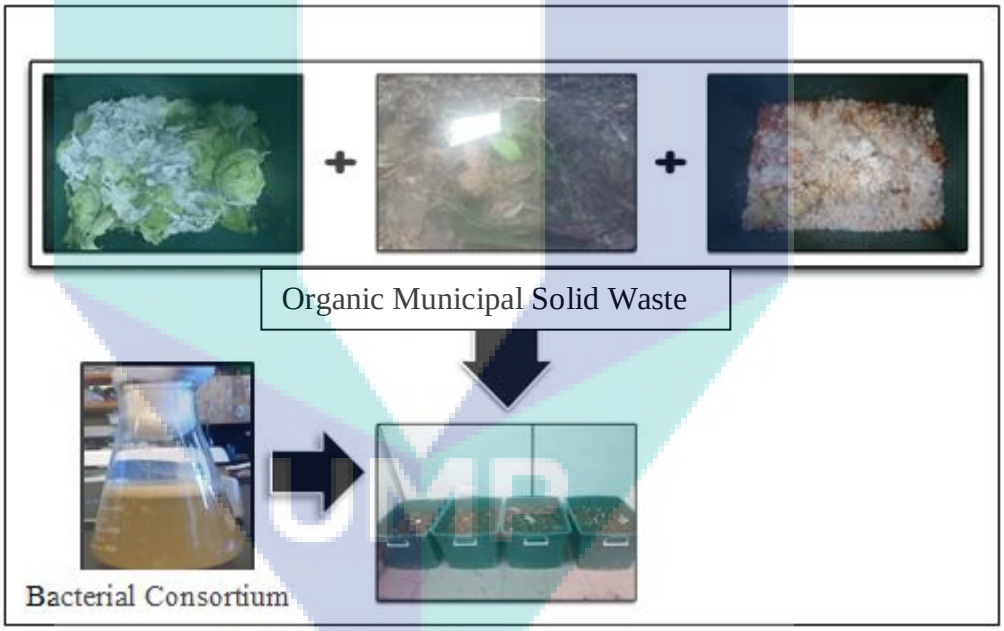


Figure 3.4: Preparation of Municipal Solid Waste degradation

Each heaps were inoculated with 5% (w/v) of consortium by evenly mixing the inoculum with the solid waste and kept under aerobic condition for 30 days until mature enough to observe the visual rate of degradation (Figure 3.4). The heaps were periodically altered and water was sprinkled for proper aeration and moisture

There were different treatments in municipal solid waste degradation:

1. T0 : Control (without bacteria)
2. T1 : Consortium of protease and amylase producer bacteria (TD+TMO+LD2)
3. T2 : Consortium of lipase and celullase producer bacteria (TK+TE+TS22)
4. T3 : Bacterial consortium of all enzyme-producing bacteria (TD, TMO,LD2,TK,TE, TS22)

Sampling and monitoring of the biodegradation process was conducted by collecting samples from at least 5 spots in pile and duplicates.

3.5 SAMPLING AND MONITORING OF COMPOST

3.5.1 Determination of Temperature

The temperature was measured daily throughout the composting period. The temperature was taken daily at 10 a.m using a digital thermometer. The temperature was measured at four different points in the compost pile. The thermometer was dipped into the compost pile approximately 15 and 30 cm depth for about five minutes before taking the reading (Figure 3.5).

Figure 3.5: Determine of temperature status.

3.5.2 Determination of Moisture Content

The moisture content was determined by gravimetric method. Five grams of sample were dried in an oven for 24 hours at 105 ^{o}C (weighed and re-weighed until a constant weight was reached). The sample was then allowed to cool at room temperature and the final weight was taken at the end of this process.

3.5.3 Determination of pH and Electrical Conductivity

Solid waste was mixed with distilled water at 1: 2.5 ratio and its pH was determined by glass electrode using a digital pH meter ("Systronics" model 335). The EC of solid waste was determined using a conductivity meter and expressed as dS/m, by mixing with distilled water at 1:2.5 ratios. Salinity was determined using an Electrical Conductivity Meter.

3.5.4 Determination of Total Carbon

The organic carbon in the compost samples was estimated by taking known quantities of dried samples (15 g) in a pre-weighed silica crucible. The samples were kept in a muffle furnace at a temperature of 6000 ^{o}C for 2 hours. The crucibles were later transferred to desiccators, cooled and immediately weighed to a constant weight (ash weight). The total presence of organic matter was calculated by taking the difference of dry weight of samples and ash weight of the sample. Then organic carbon was calculated by dividing the percentage of organic matter by the factors 1.724 (Jackson, 1973).

3.5.5 Determination of Total Nitrogen

Nitrogen in the sample was estimated by following the micro Kjeldhal method as outlined by Jackson (1973). Dried sample (0.5g) was digested using 10 mL of concentrated sulphuric acid in the presence of 0.3g of catalytic mixture containing potassium sulfute, copper sulphate and selenium powder in the ratio 50:10:1 in the micro Kjeldhal digestion unit. The digested samples were diluted with distilled water

and distilled after the addition of sufficient quantities of 40 percent NaOH to make the sample alkaline in the micro Kjeldhal distillation unit. The ammonia evolved was trapped in two percent boric acid mixed indicator solution and titrated against 0.05 N sulphuric acids. The nitrogen content was calculated from the volume of acid consumed.

3.5.6 Determination of C/N Ratio

The C:N ratio was calculated by dividing the percentage of organic carbon by the percentage of total nitrogen.

3.5.7 Determination of Nutrient Content

Analysis for nutrients such as nitrogen was carried out using the Kjeldahl method. The Kjeldahl method was broken down into three main steps. The first was digestion, the decomposition of nitrogen in organic samples utilizing a concentrated acid solution. This was accomplished by boiling a homogeneous sample in concentrated sulfuric acid. The end result was an ammonium sulfate solution. The second step was distillation, adding excess base to the acid digestion mixture to convert NH_4 + to NH_3, followed by boiling and condensation of the NH_3 gas in a receiving solution. The third step was titration, to quantify the amount of ammonia in the receiving solution. The amount of nitrogen in a sample can be calculated from the quantified amount of ammonia ions in the receiving solution. A sample of 0.25 g (dry weight) of each sample was transferred to digestion flask and 0.5 mL of concentrate sulfuric acid (H_2SO_4) was added and digested at 450 ^oC for 7 minutes. Two mL of H_2O_2 was added, and the reactions were allowed to continue for about 4 minutes. Finally, the solution made up to 100 mL with distilled water and filter through Whatman No. 1 filter paper. The nitrogen content was determined using Auto Analyzer.

Phosphorus (P) was carried out by Spectrophotometry and Potassium (K) by Flame Photometer. The sample was dried at 105 ^oC for 24 hours, followed by combustion at 550 ^oC in the muffle furnace oven with the ash was dissolved in 20 mL of HCl (32%) and filled up to 100 mL after 12 hours in the dark with distilled water. Total

P was measured by spectrophotometer using vanadate-molybdate-method (Gericke and Kurmies, 1952) and K was measured using flame photometer.

3.5.8 Determination of Micronutrient Content

Determination of micronutrient content was conducted for zinc (Zn), while iron (Fe) and copper (Cu) were conducted using Atomic Absorption Spectroscopy (AAS). The samples were digested using the ISO 11466 (1995) standard method (the aqua regia digestion method). 3 g of samples was placed in a 100 mL round bottom flask with 21 mL of concentrated HCl (35%) and 7 mL of concentrated HNO_3 (65%). The solution was kept at room temperature overnight before a water condenser was attached, and the solution was boiled for 2 hours. 25 mL of water was added down the condenser before filtration of the mixture using Whatman No. 42 filters. The filtered residue was rinsed twice with 5 mL of water and the solution was made up to 100 mL. All solutions were prepared with mili-Q deionized water. The above procedure was also used to obtain a blank and control sample and all samples were blank correlated.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 CHARACTERIZATION OF MUNICIPAL SOLID WASTE IN JABOR LANDFILL

Jabor landfill, also commonly known as Kuantan landfill, receives more than 500 tons of waste per day with a composition of 60% of domestic waste and 40% of commercial waste as described in Table 4.1 (Zahari et al., 2010). This is a disposal place of waste from all villages in Kuantan city. Manual hand sorting method is used to separate and group the waste. The types of waste that could be composted are wood, paper, yard waste, food and organic/green waste (Figure 4.1 & 4.2).

Table 4.1: Waste flow in Jerangau-Jabor landfill in 2013

Month	Domestic	Industry (Tons)	Total
January	13,969.28	1,303.23	15,923.47
February	11,422.98	1,019.57	15,993.07
March	11,831.73	1,508.10	13,681.68
April	12,024.71	1,033.60	12,802.06
May	12,750.94	1,603.69	14,354.63
June	11,939.61	1,138.93	13,078.54
July	13,230.94	1,259.93	14,490.87
Augustus	13,328.86	1,066.00	14,394.86
September	12,943.79	1,303.09	14,246.88
October	12,927.93	1,548.02	14,475.95
November	12,219.23	1,512.57	13,731.80
December	18,289.45	973.79	19,263.24

Waste that goes into Jabor landfills were sourced from domestic waste and commercial waste. Domestic waste comes from housing, market, business areas and gardens while commercial waste comes from small-scaled and large industrial factories around Kuantan. Figure 4.1 shows that the majority of domestic waste comprises of organic waste (27%), green waste (23%), mixed paper (21%) and plastic (14%), which comes from residential areas, market and the central office. Biodegradable material that can be used for composting is food waste, green waste and mixed paper.

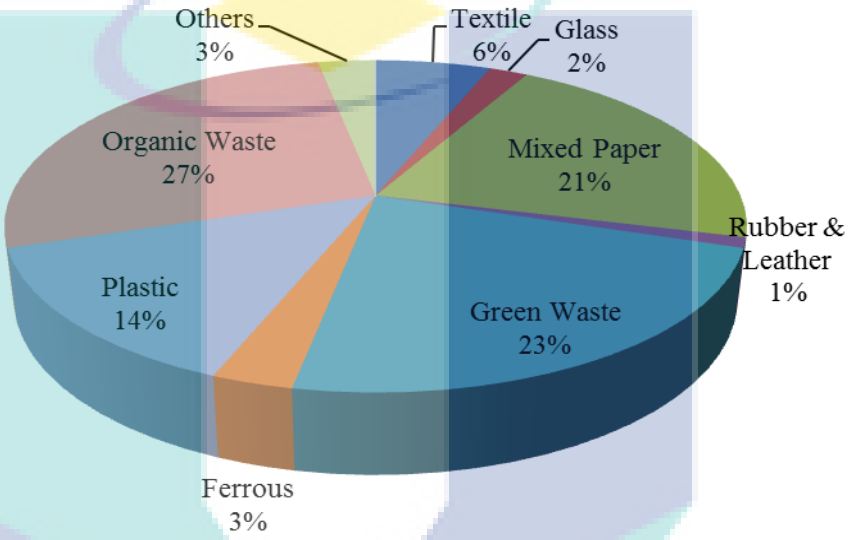


Figure 4.1: Domestic waste generation in Jabor landfill

The dominant types of commercial waste are food (35.7%), plastic (22.2%), yard waste (13.3%) and paper (10.6%). Most of the industries in Kuantan are food and manufacturing industries. Other types of waste are residues of the manufacturing industry such as plastic, cardboard, textile, paper and others, as can be seen in Figure 4.2.

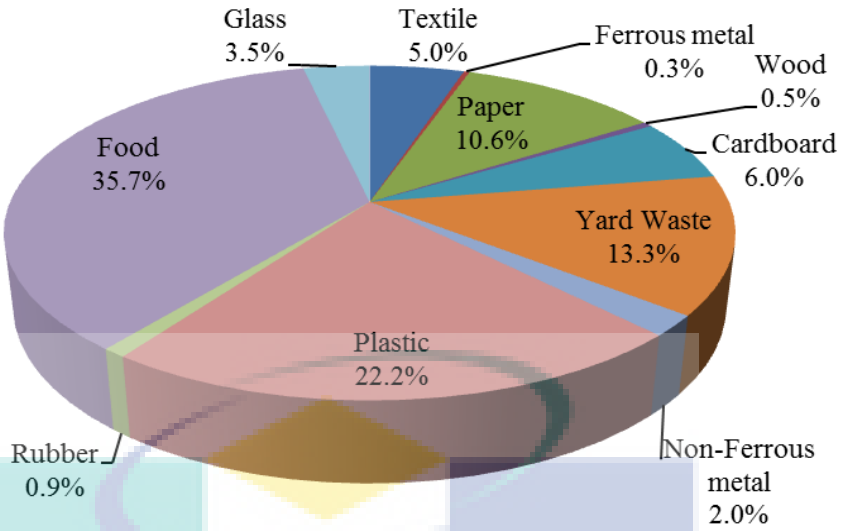


Figure 4.2: Commercial waste generation in Jabor landfill

4.2 ISOLATION AND IDENTIFICATION OF BACTERIA

4.2.1 Bacterial Population Morphology

Thirty seven isolates were found from different sites (soil and leachate). Bacteria with different morphological characteristics were transferred to a new nutrient agar plate with a streaking method to find a single colony and pure culture (Figure 4.3). The isolation showed estimates of colony forming units. The smaller dilution rate is the greater population of bacteria according to the concentration of each dilution. Bacteria found in the soil were more numerous than leachate.

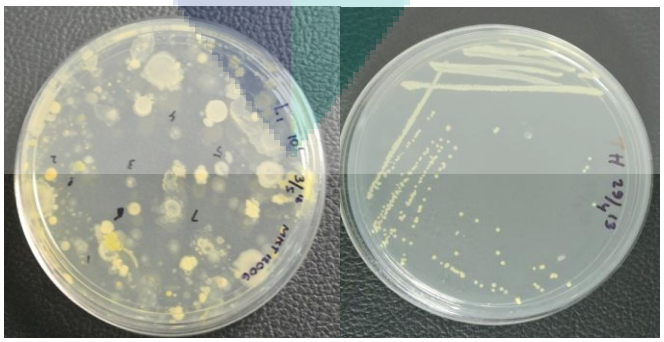


Figure 4.3: Morphological Characteristics and Pure Culture

Total colonies derived from landfill soil were approximately $50x10^4$ to $40x10^9$ CFU/mL, while total colonies derived from leachate were approximately $7x10^4$ to $1x10^9$ CFU/mL. From Table 4.2, it can be seen that the population of bacteria from landfill soil is more numerous than bacteria from leachate. This is due to the condition of leachate, which contains toxic material, heavy metals and limited oxygen availability, which makes only few specific bacteria could thrive. After determining the number of colonies that grow, the next step is to transfer different bacteria to a new plate to obtain a single colony that is separated with streaking method. From 3 times of streaking, the expected growing bacteria were pure culture and contained a single colony which would then be tested using the method of Gram staining.

Table 4.2: Population of bacteria from Jabor landfill (CFU/mL)

No	Dilution Factor	Soil	Leachate
1	10^3	$50x10^4$ CFU/ml	$7x10^4$ CFU/ml
2	10^7	$15x10^8$ CFU/ml	$1x10^8$ CFU/ml
3	10^9	$40x10^9$ CFU/ml	$1x10^9$ CFU/ml

Gram staining consists of four components; primary stain (crystal violet), mordant (lugol's iodin), decolourizer (ethanol) and counterstain (safranin). Many theories explain why some bacteria were able to retain the dye while others do not. Moreover, the thickness of the cell wall of Gram-positive and more lipid content in the cell wall of Gram-negative is a more acceptable reason for Gram stain reaction. Another factor is decolorized process. When over decolorized, even Gram-positive bacteria may appear pink and when under decolorized, gram-negative bacteria may appear Gram-positive. Gram reaction also depends on the age of the cell. Then, the glass slide was observed under a microscope and oil immersion. After decolorization, the Gram-positive cell remains purple and the Gram-negative cell loses its purple color. The counterstain is applied at the end to decolorize Gram-negative bacteria into a pink or red color (Figure 4.4).

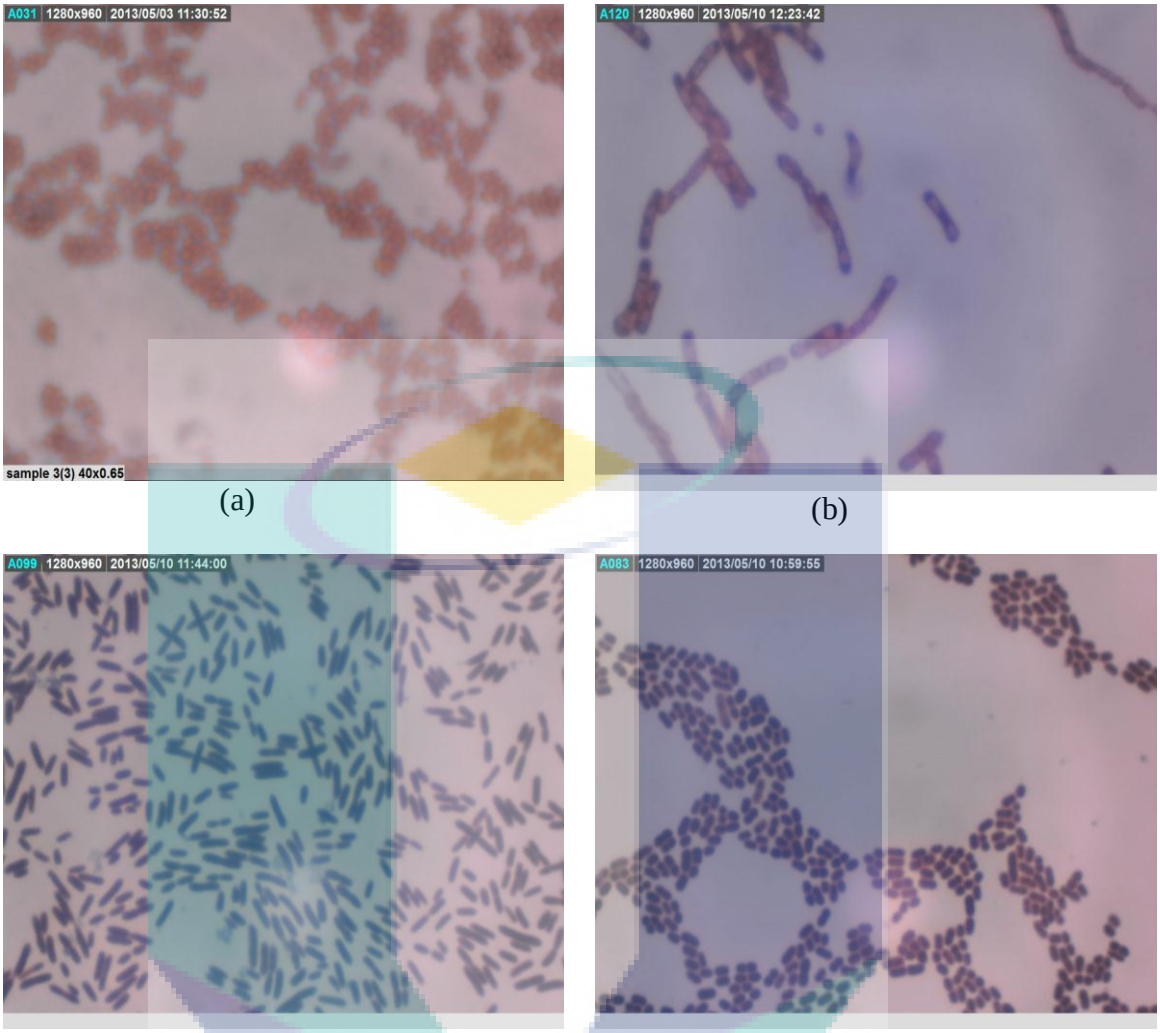


Figure 4.4: Gram reaction shows different bacterial shapes (a. staphylococci; b. streptobacilli; c. bacilli; d. diplococci).

4.2.2 Enzyme Production Screening

Nineteen different species of bacteria have been found in the soil of Jabor landfill (Table 4.3). They were eight Gram positive and 11 Gram negative bacteria. The positive result of enzyme production (Figure 4.5) in selective media shows a clear zone around the colony for skim milk agar, which indicates protease activity. A correlation between the sizes of the hydrolysis rings and the protease catalytic activity was demonstrated. Halo zone showed in starch agar after flooding by iodine indicated that the bacteria produced amylase. Lipolytic activity in rhodamine B agar showed the pink halo under a UV lamp (350nm). Almost all bacteria produced amylase and protease,

while just a few bacteria could produce lipase. Proteases as a good indicator of organic matter decomposition on account of their dependence on substrate, also act on proteins and polypeptides degradation. Bacteria convert starch molecules to glucose by producing amylases enzyme (Kausar et al., 2012). Therefore, from landfill soil, there were TD, TE, TK, TM, TS22 and TO as the best bacteria.

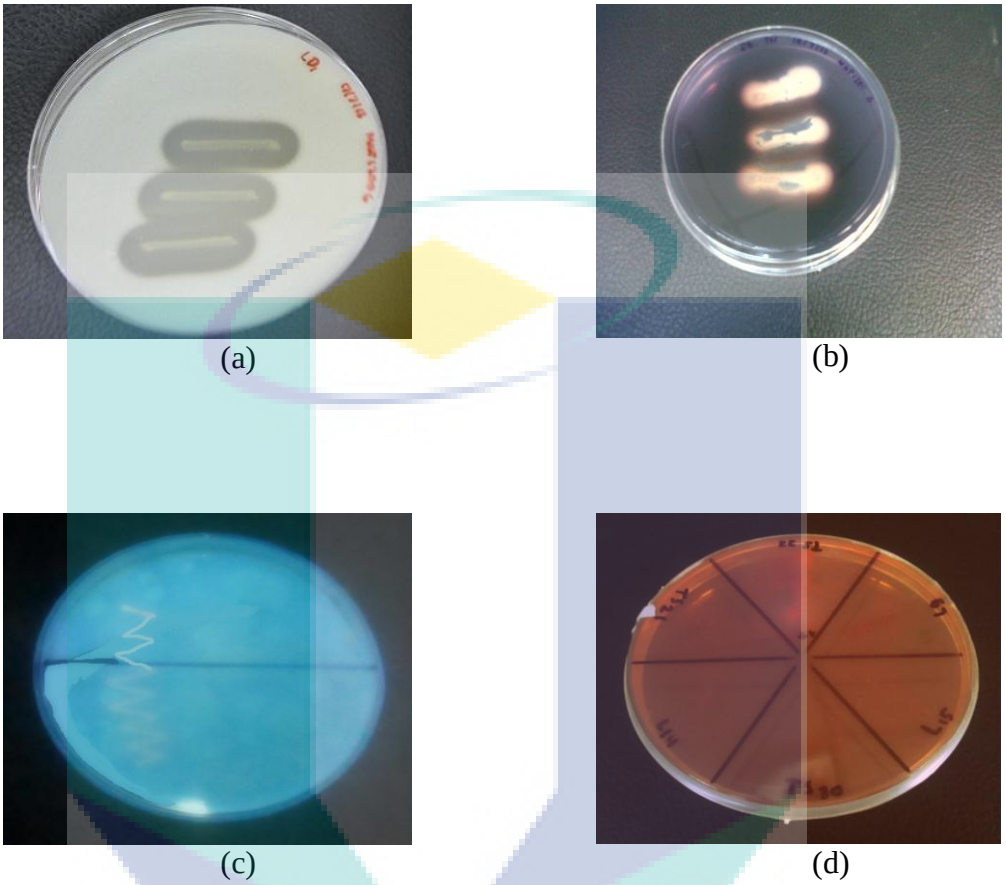
Table 4.3: Gram reactions and enzyme production screening from soil sample

No	Code	Morphology	Gram Character	Enzyme Production			
				Amylase	Protease	Lipase	Celullase
1	TB	Bacilli	Positive	+++	++	-	+
2	TA	Bacilli	Negative	+	+	-	-
3	TC	Bacilli	Negative	+++	+	-	-
4	TD	Bacilli	Positive	+++	+++	+	-
5	TE	Bacilli	Positive	-	+	+	+
6	TF	Bacilli	Negative	+++	++	-	-
7	TG	Streptobacilli	Positive	++	++	-	-
8	TH	Staphylococcus	Negative	-	+	-	-
9	TI	Tetrad	Negative	++	+	-	-
10	TJ	Streptobacilli	Negative	+++	+	-	-
11	TK	Bacilli	Positive	+++	+	+	+
12	TL	Bacilli	Negative	-	-	-	-
13	TM	Bacilli	Positive	+++	+++	-	-
14	TN	Streptobacilli	Negative	+++	++	-	+
15	TO	Bacilli	Positive	+++	+++	-	-
16	TP	Bacilli	Negative	+++	+	-	-
17	TQ	Bacilli	Negative	+++	++	-	-
18	TR	Bacilli	Negative	+++	-	-	-
19	TS22	Bacilli	Positive	++	+	-	++

(+): Low reaction, (++): Moderate reaction, (+++): Strong reaction, (-): No reaction

Productivity test using carboxymethyl cellulose (CMC) shows the indication of a clear zone as a positive reaction. Only a few bacteria can produce cellulase enzyme, one of the most clearly visible ones is bacteria with the code name TS22. High content of degradable organic compounds in the initial mixture may have stimulated microbial growth and enzyme synthesis, as available substrate decreased, the enzyme activity decreased as well (Castaldi et al., 2008). The production of enzyme depends on microbial biomass, which implies that when this biomass is degraded, enzymatic activity decreases (Ayuso et al., 1996). Cellulase, due to its massive applicability, has been used in various industrial processes, such as biofuels like bioethanol, triphasic

biomethanation as well as agricultural and plant waste management; chiral separation and ligand binding studies.



(a) (b)

(c) (d)

Figure 4.5: Screening of enzyme production in selective media. (a). Skim milk (protease activity); (b). Starch agar (amylase activity); (c). Rhodamine B agar (lipase activity); (d). CMC agar (cellulase activity).

Bacteria obtained in leachate tank of Jabor landfill were found to be from 18 different species. They were Seven Gram positive and 11 Gram negative bacteria (Table 4.4). Among the Gram positive and Gram negative bacteria isolated, rods were more frequently isolated than cocci of both samples. Species in the first two genera have been previously identified in landfill material. The microbiological analyses indicate that four bacterial groups were responsible for the biological treatment in leachate in Tunisia, which were *Actinomycetes, Bacillus, Pseudomonas* and *Burckholderia* (Yahmed et al., 2009). Activity of enzyme production of leachate samples was less than the landfill soil

because of the higher content of starch, protein and lipid in landfill soil than in leachate. The best enzyme producing bacteria from leachate were LD1 and LD2.

Table 4.4: Gram reactions and enzymes production screening from leachate

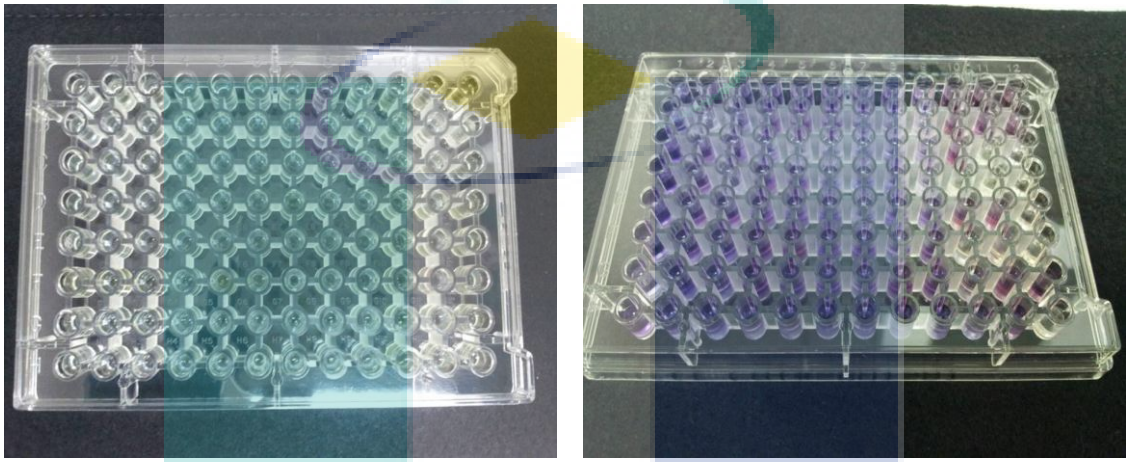
No	Code	Morphology	Gram Character	Enzyme Production			
				Amylase	Protease	Lipase	Celullase
1	LA1	Diplococcus	Negative	-	-	-	-
2	LA2	Diplococcus	Negative	++	-	-	-
3	LA3	Diplococcus	Negative	-	-	-	-
4	LB1	Bacilli	Negative	-	-	-	-
5	LB2	Bacilli	Negative	-	-	-	+
6	LCA	Bacilli	Positive	-	+	-	-
7	LD1	Streptobacilli	Positive	++	++	+	+
8	LD2	Streptobacilli	Positive	++	++	+	+
9	LE	Bacilli	Negative	-	+	-	-
10	LF	Diplococcus	Negative	-	-	-	-
11	La	Diplococcus	Negative	+	-	-	-
12	Lb	Bacilli	Negative	+	-	-	-
13	Lc	Staphylococcus	Positive	++	-	-	+
14	Ld	Bacilli	Positive	-	++	-	-
15	Le	Bacilli	Positive	+++	+	-	-
16	Lf	Bacilli	Negative	+	+	-	-
17	Lg	Bacilli	Positive	++	+	-	+
18	Lh	Streptobacilli	Negative	-	+	-	-

(+): Low reaction, (++): Moderate reaction, (+++): Strong reaction, (-): No reaction

4.2.3 Identification of Bacteria

Identification of bacteria was conducted by Gen III microplate Biolog microbial identification system (Figure 4.6). The GEN III MicroPlate™ test panel provides a standardized micromethod using 94 biochemical tests to profile and identify a broad range of Gram-negative and Gram-positive bacteria. Biolog's Microbial Identification Systems software (OmniLog® Data Collection) is used to identify the bacterium from its phenotypic pattern in the GEN III MicroPlate. From 37 bacteria that have been isolated, only 7 bacteria showed the best results through a screening test (Table 4.5).

They were *Bacillus amyloliquefaciens, Bacillus ruris, Bacillus licheniformis, Bacillus subtilis* and *Kocuria varians*. Similar to the study by He et al., (2013), the *Bacillus* species was found to almost appear as a majority in relation to the degradation of organic molecules. Bacteria that are the dominant in the process include *Bacillus* species. It was reported that 87% of bacteria in thermophilic composts is of genus *Bacillus* (Tongpim et al., 2014).



(a) (b)

Figure 4.6: Identification using BIOLOG automated system: (a). Microplate before incubation and (b). Microplate after incubation.

The strains show sequence similarity of more than 97% to known cellulolytic and hemicellulolytic bacteria. Those strains were closely related to *Bacillus licheniformis* and *B. subtilis*. They were found during degradation of lignocellulosic materials, with the majority belonging to *Firmicutes*, especially *Actinobacteria* and *Firmicutes* play a major role in lignocellulose degradation during thermophilic stage of composting (Li et al, 2013). *Bacillus subtilis* was stable at wide range of pH (5–10) and temperatures (20–60 ◦C) and its minor effect on collagen and fibrin fibers makes it a promising protease enzyme that may be used in tannery dehairing processes (Tork et al., 2013).

Table 4.5: Identification of bacteria using BIOLOG automated system

No	Sample Code	Result
1	LD2	*Kocuria varians*
2	TD	*Bacillus amyloliquefaciens*
3	TE	*Bacillus ruris*
4	TK	*Bacillus licheniformis*
5	TM	*Bacillus subtilis*
6	TS22	*Bacillus licheniformis*
7	TO	*Bacillus subtilis*

Bacillus amyloliquefaciens was able to effectively degrade and grow using small pieces of chrome as the protein source and produced in the spent medium high levels of a keratinolytic serine protease that can be proficiently applied for the pre-tanning processing step of hide dehairing, as this bacteria are also chromium-resistant (Pillai and Archana, 2012). *Bacillus amyloliquefaciens,* a well-known plant with growth-promoting rhizobacterium (PGPR), is used as a bio-control agent due to its ability to suppress soil-borne plant-pathogenic microorganism within the plant rhizosphere (Koumoutsi et al., 2007*). Bacillus amyloliquefaciens, Bacillus subtilis, Bacillus licheniformis*, *Bacillus pumilus* and some other *Bacillus* species strains are both phenotypically and genetically similar species and can easily be confused because they are all placed in 16S rRNA/DNA group 1 (Ruckert et al., 2011). Using *Bacillus licheniformis* increases the efficient degradation of melanised feathers by this keratinase, which may offer an environmentally friendly solution to the degradation of feather waste and other organic matter of similar molecular composition.

Bacillus licheniformis is mildly thermo-tolerant with its optimum temperature of 50 oC, it may be adapted for microbial composting of organic wastes (Okoroma et al., 2012). *Kocuria varians* is a Gram positive coccus that is found in tetrads, irregular clusters, and cubical packets of eights. It is catalase positive, oxidase positive, and exhibits strictly aerobic metabolism as well as able to use glucose aerobically. Optimum growth temperature is 25-37 C. It is primarily isolated from mammalian skin, but can also be infrequently found in soil and water. Very little evidence of its involvement in the disease process has been found, but has been recovered infrequently from tissue

samples of immunocompromised patients. It was once considered a species of the genus *Micrococcus*.

4.3 BIODEGRADATION MONITORING EVALUATION

4.3.1 Temperature

All the trials showed similar changes in temperature and pH throughout the biodegradation process (Figure 4.7). The results of temperature after inoculation bacterial consortium showed significant results on the second day. Thermophilic phase lasts for 7 days after each treatment. T3 showed the highest increase in temperature compared to other treatments. The highest temperature obtained is 53 ^{o}C, which also was experienced by T0. However, T4 treatment in 7 days increased temperatures higher than T0 treatment, followed by T1 and T2. The temperature was peaked over 50 ^{o}C in the first 5 days and remained between 49–35 ^{o}C for 1 week. By the 23rd day, it dropped to 30 ^{o}C, which is considered to have entered the maturation phase.

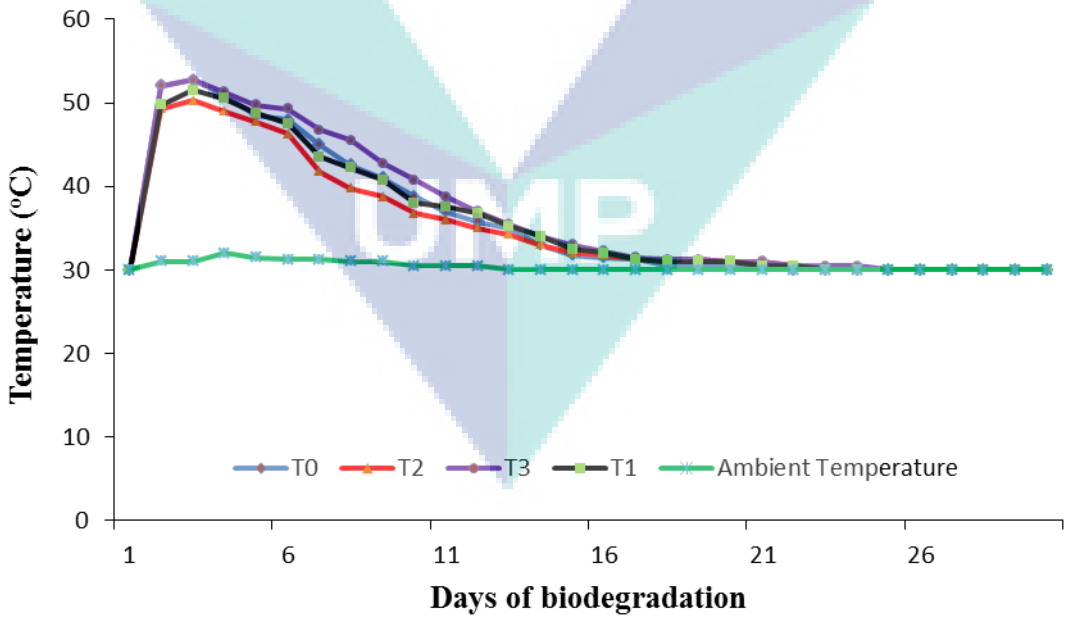


Figure 4.7: Changes of temperature in biodegradation process

Temperature variation plays a significant role during the biodegradation process, both in the evolution and succession of the communities of microbial in maximizing the biodegradation rate, sanitization and the microbial diversity (Hassan et al., 2001). The increases in temperature in biodegradation process are caused by the heat generated from respiration and decomposition of sugar, starch and protein by microorganism population, where the microorganisms convert organic matter into CO_2 and humic substances release heat. The increments in temperature are a good indicator that there is a microbial activity in the compost pile where the higher the microbial activity is, the higher the temperature will be increased.

In this study, the thermophilic phase lasted for only a week starting from the first day, with the maximum temperature of 53 ^{o}C retained only for 2 days. This is common in systems that use a smaller volume as in this experiment where it is susceptible to heat loss due to the high surface–volume ratio (Nair et al., 2006). Temperature, oxygen and nature of organic substrates are the main driving forces in relation to microbial dynamics. Thus, differences in microbial populations must be expected as decomposition progresses and the temperature rises. At the early stage of the process the presence of readily available carbon substrates, as well as the prevalence of mesophilic temperatures, there are high levels of microbial populations.

4.3.2 pH

The pH decreased at the early stage in a tight acidic state. Towards the 10th day, it gradually increased to a neutral pH and at the end process the pH was almost neutral in all treatments. This is in accordance with the pH changes that occur in a composting system, with initial drop followed by pH stabilization. The mineralization process is the conversion of organic nitrogen to inorganic nitrogen such as nitrates. Also, the degradation of soluble and easily degradable carbon sources, such as starch, monosaccharide and lipids increases organic acids of the material. In the next stage, proteins are degraded to ammonium and would result in an increase in pH (Caceres et al., 2006). The initial pH value of raw material is 7.83, after being inoculated with consortium bacteria, the pH value of all treatments decreased in the first week and gradually increased in the second week. The highest peak is 8.5 for T1 in 15th, then T3

and T0 with almost similar values at 8.4, while the pH value of T2 is 8.05 (Figure 4.8). After that, all treatments graduallydecrease around pH 7. According to Rynk et al. (1992), the initial pH tends to decrease in the early stage of the biodegradation process due to the release of organic acid and then gradually increases when the ammonia was released during protein degradation. Increase in pH could also occur due to protein degradation of the compost material so that the ammonia is liberated and causes the pH value to increase while the pH decreases due to the nitrification process or increased production of organic acids.

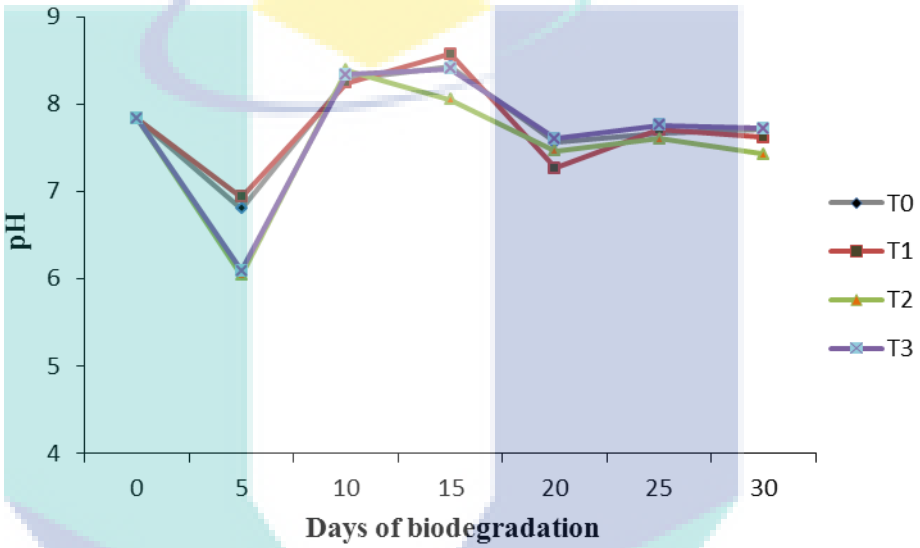


Figure 4.8: Changes of pH in biodegradation process

During the thermophilic stage process, the ammonia produced increased the pH values and then declined. This may be attributed to the nitrification process, which is constantly accompanied with the release of hydrogen ions. The optimal pH range for biodegradation with microorganisms under a condition of constant temperature and moisture concludes that the degradation with more microorganisms and higher microbial activity with a levelling off of activity at the higher pH level (pH 7.8) over a range of pH 5.6 to 8.4 and a constant moisture content of 59.6 and 65%.

4.3.3 Electrical Conductivity (EC)

The initial EC of raw material is 3.907 ms/cm^{-1}, for 10 days all the treatments

decreased the EC values. The highest peak is T3, which is 3.7 ms/cm^{-1}, then T2, T0 and T1 (Figure 4.9). Starting the 6th day, all the treatments decreased. This is similar to Lee et al. (2002), who elaborated that the decrease of EC values in the early stage of the composting period may be due to the adsorption of extractable salts by well-structured organic materials. Besides, the decrease also can be attributed to the transformation of complex organic compounds into simpler forms, thus the situation may consume some of ionic molecules.

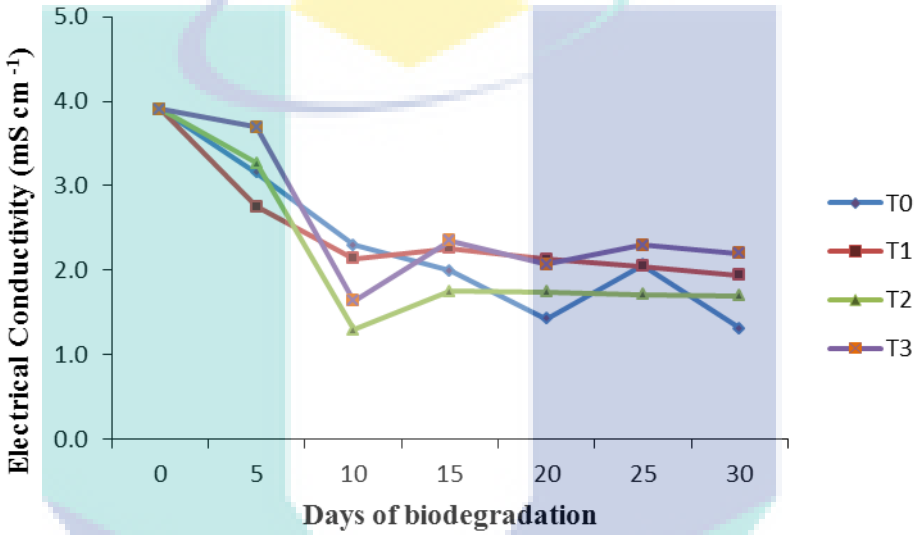


Figure 4.9: Changes of EC in biodegradation process

The increase in EC on the 10th day has been attributed to the release of bases and other nutrients from the mineralization of organic matter during composting (Betran et al., 2004). Furthermore, according to Lee et al. (2002), the increase is also attributed to the solubilization of the immobilized salts by the second decomposition of the amendment and microorganisms.

4.3.4 Total Organic Carbon

The content of organic carbon in the compost produced decreased from initial content (Canet et al., 2008). This show that the microorganisms require carbon as food for biodegradation activity of organic matter.

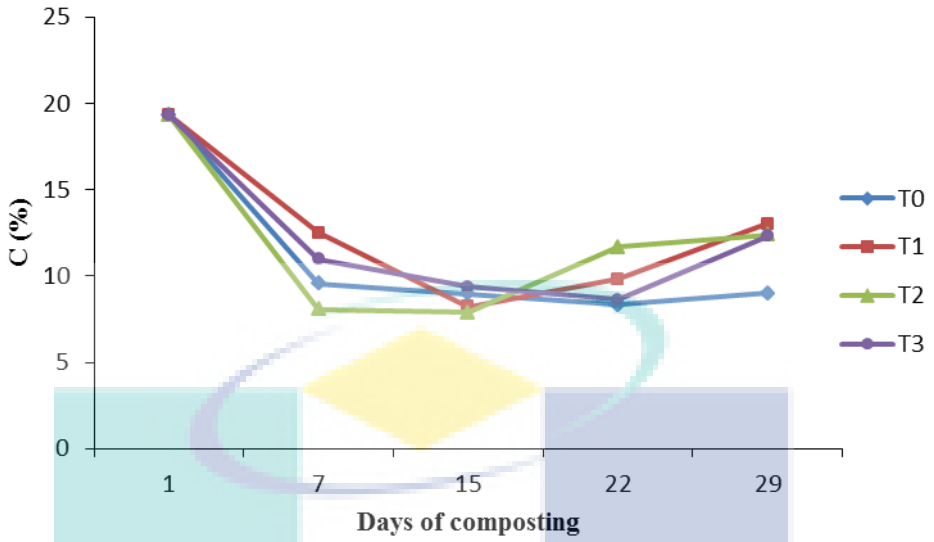


Figure 4.10: Changes of total carbon in biodegradation process

The initial process of total carbon value before treatment is 19.35%, which then tightly decreased until the 15th day. It shows that the microbial activity uses carbon as their food to degrade organic molecules. Slowly in the 22nd day, total carbon value was increased in all treatment except T0, and the final result of total carbon for T0 is 9%, T1 is 13.2%, T2 is 12.38% and T3 is 12.3% (Figure 4.10).

4.3.5 C/N Ratio

C/N ratio is an important parameter in determining the level of maturity of the compost. Mature compost has a C/N ratio value of 10-20% because in these conditions there is no nitrogen immobilization process by microorganisms that causes reduced nitrogen availability to plants. C/N ratio should not be too high because it leads to immobilization of N and if it is too low, it causes N volatilization (Stoffella and Kahn, 2001). Loss of nitrogen is influenced by elements of carbon during the composting process (Barrington et al., 2002).

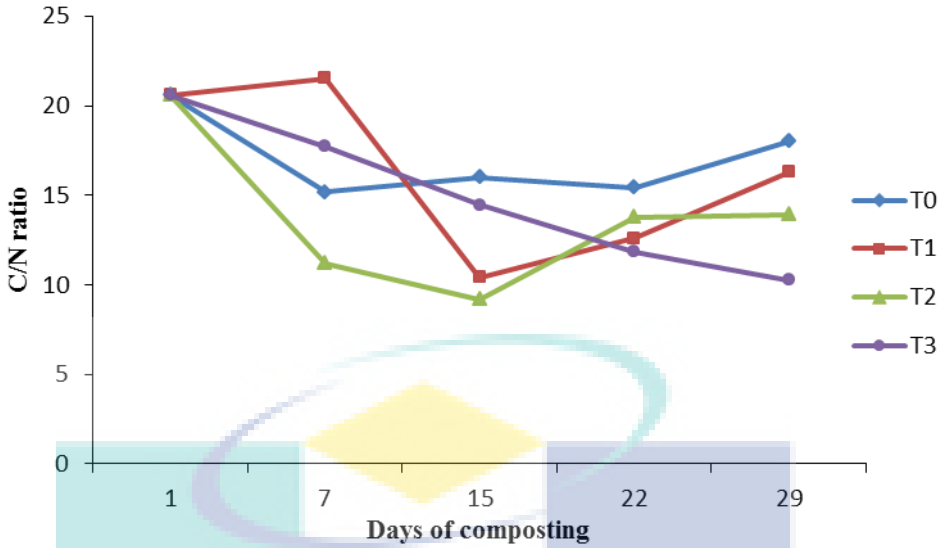


Figure 4.11: Changes of C/N ratio in biodegradation process

The initial process of total carbon value before treatment is 21 then decreases until the last day for T3, the final result of C/N ratio T3 is 10. The other treatments were unstable, but they were mature on the 15th day. The final result of the C/N ratio for T0 is 18%, T1 is 16%, and T2 is 14% (Figure 4.11). Morais and Queda (2003) suggested that a ratio under 20 is optimal for maturity and the best ratio is lower than 15. The best treatment is T3 because it had the C/N smallest value.

4.4 MACRONUTRIENT CONTENT

4.4.1 Nitrogen

According to Goyal et al. (2005), decreased levels of total nitrogen in the early stages depend on the type of material and the initial C/N ratio material that will be composted. Sanchez-Monedero et al. (2001) state that composting with a substrate which has C/N value lower than N, which is an easily removable organic material with a C/N ratio is high. In addition, a decrease in N content can be caused by the immobilization of N while volatilization is affected by moisture or aeration during composting.

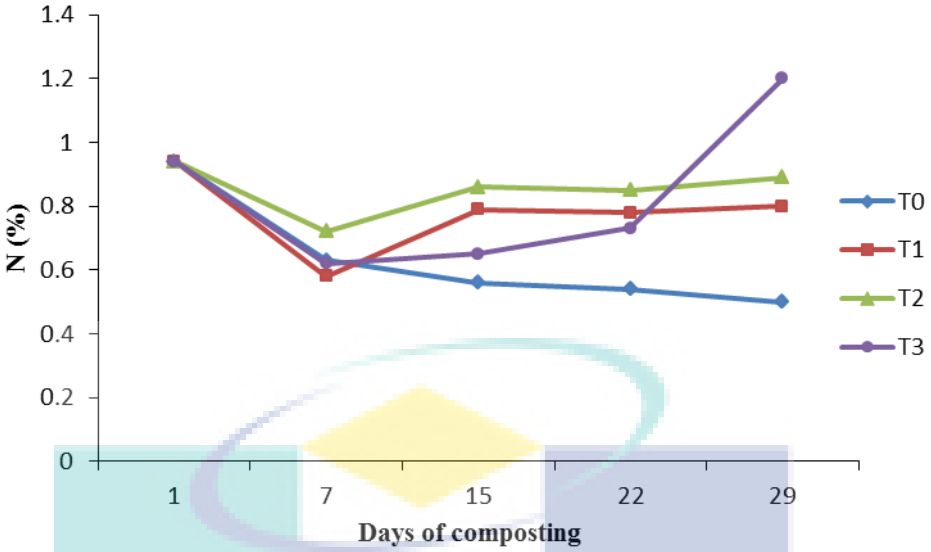


Figure 4.12: Changes of Nitrogen (N) in biodegradation process

Nitrogen is an essential nutrient. The higher the content of nitrogen in the compost, the higher the nutritional value of the compost. To improve the nutritional value, especially for materials with a low nutrient content, the addition of nutrients is needed and is usually carried out simultaneously with the addition of microbial inoculum. Proteases and ureases take part in nitrogen mineralization. Both of them hydrolyze nitrogen compounds into ammonia using low molecular weight proteins and urea as a substrate, respectively. T3 is the only treatment that has increased in value from 0.9%-1.2%, while the value of other treatments under 1% is as described in Figure 4.12.

4.4.2 Phosphorus

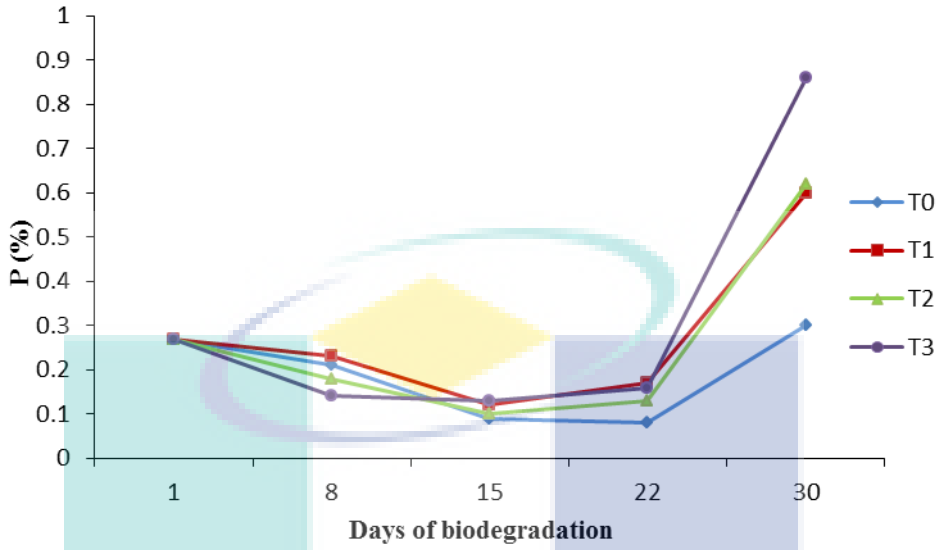


Figure 4.13: Changes of Phosphorus (P) in biodegradation process

In soils with low content of available P, organic phosphate form has an important role in supplying plant nutrients because most of the pottasium contained in the P-organic compounds. Most P-organic plant organs are such as fitin, phospholipids, and nucleic acids. There is only a limited quantity available in the soil organic matter because the compound is used by soil microorganisms. Derivatives of these compounds are very important in the soil because of its ability to form compounds with polyvalent cations. There is a relatively high number, but the decomposition is slow. In alkaline soil, inositol phosphate with Ca or Mg is formed, while on acid soils, inositol phosphate with Al or Fe is formed. Inorganic P in the form of Al-Fe; Ca-P are not available to the plant, and will be overhauled by organisms P into P-solvent-soluble inorganic or available to plants (Zhang et al., 2006). In the final result of all treatments, P has increased significantly from 0.27% to 0.60% (T1&T2) and 0.83% (T3), only the control treatment shows a tiny progress (0.30%) as described in Figure 4.13.

Most of the modifications that organic matter undergoes during composting are mediated by enzymes. Thus, the monitoring of enzyme activities throughout the process gives valuable information related to the dynamics of important nutritional elements like

C, N or P and contributes to a better understanding of the transformations that take place during composting.

4.4.3 Potassium

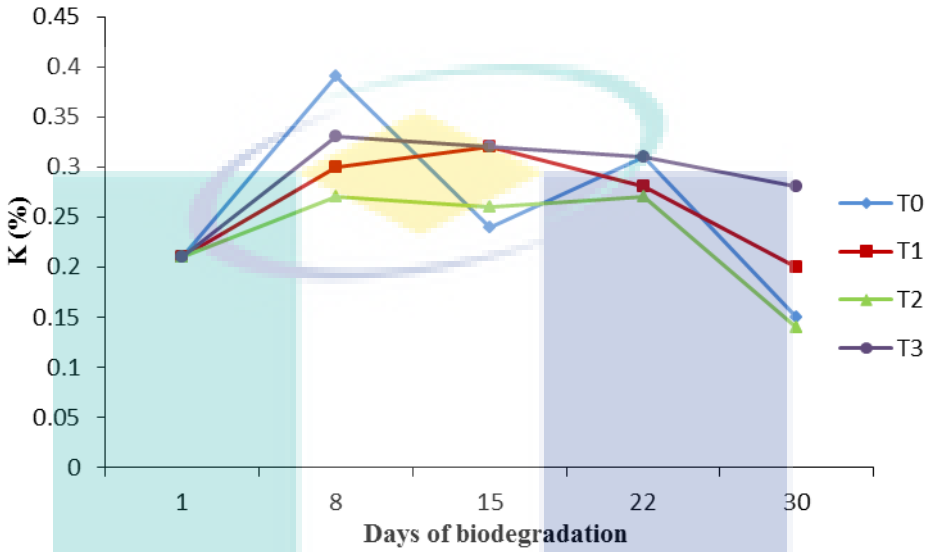


Figure 4.14: Changes of Pottasium (K) in biodegradation process

Changes in the macro-micro-nutrient content of each treatment showed an increase that indicates that mineralization process nutrients are present during decomposition, so the macro and micro nutrients become detached and available. Increased nutrient substrate increases with time and is associated with loss of composting organic material. Elements of macro-micro increased due to the release of elements previously bound in the cell components were, for example, nitrogen in protein and magnesium in chlorophyll.

An increases in nutrient substrate composted are also very dependent on the type of composting and the base substrate material. Nutrients can also be decreased, due to the process of evaporation or dissolved in water produced during composting. In addition, climatic conditions during composting can also affect changes in the nutrient content of compost. In this study, some elements, especially potassium, experience unstable conditions for all treatments (Figure 4.14). The initial value is 0.21% and the

final result for T0 is 0.15%, T1 is 0.20% and T2 is 0.14% and the best result is T3, which is increased to 0.28%.

4.5 MICRONUTRIENT CONTENT

4.5.1 Iron

Figure 4.15 shows the changes of Fe content in compost pile during the composting period. From the first to the second week, all treatments decreased the value of Fe. However, in the second week, T3 treatment rose from 2317 mg kg^{-1} to 2589 mg kg^{-1}. During the third week, Fe increased at T0 and T2 treatment. Overall, at the end of the composting period, the value of Fe is smaller than the initial condition. T3 treatment experienced the greatest a decline of Fe in the value from 2878 mg kg^{-1} down to 1669 mg kg^{-1} compared to all treatments (Figure 4.15).

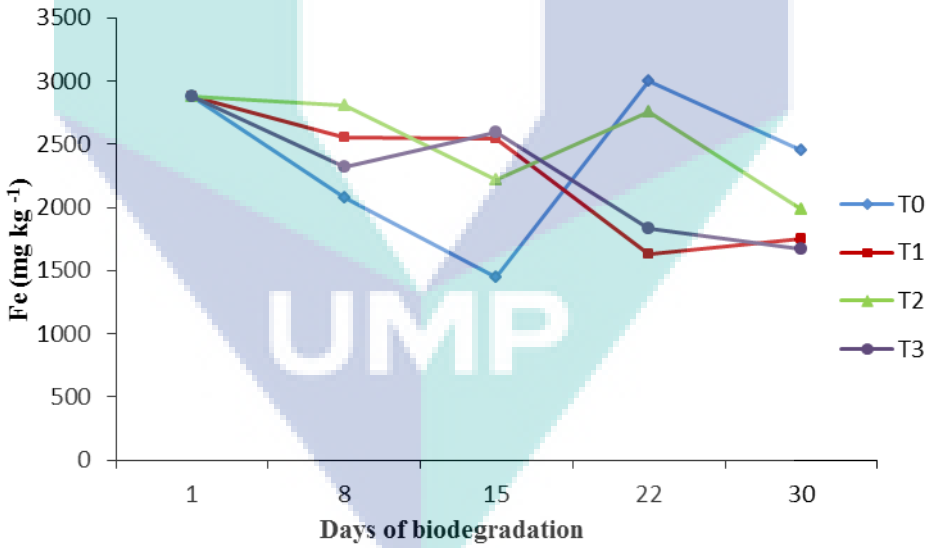


Figure 4.15: Changes of Fe in biodegradation process

Fe is an essential element for the survival of all microorganisms that us used to produce siderophores compounds capable to bind Fe in Fe-deficient soil conditions, resulting in inhibition of growth of pathogens due to the unavailability of Fe for pathogens (Naik, 2007). The change of nutrient content in the compost indicates that the

composting process has resulted in the mineralization of element, so that it becomes detached and nutrients become available to plants. Nutrients can also be decreased due to the process of evaporation or dissolved in water during composting. In addition, climatic conditions can also affect changes in the nutrient content of compost.

4.5.2 Copper

Figure 4.16 shows the Cu content in compost treatments with days. Based on the picture, the content of Cu decreased sharply at the end of the process of composting in all treatments (Figure 4.16). The final result of each treatment is almost the same at 0.6 mg kg^{-1} and 0.7 mg kg^{-1}.

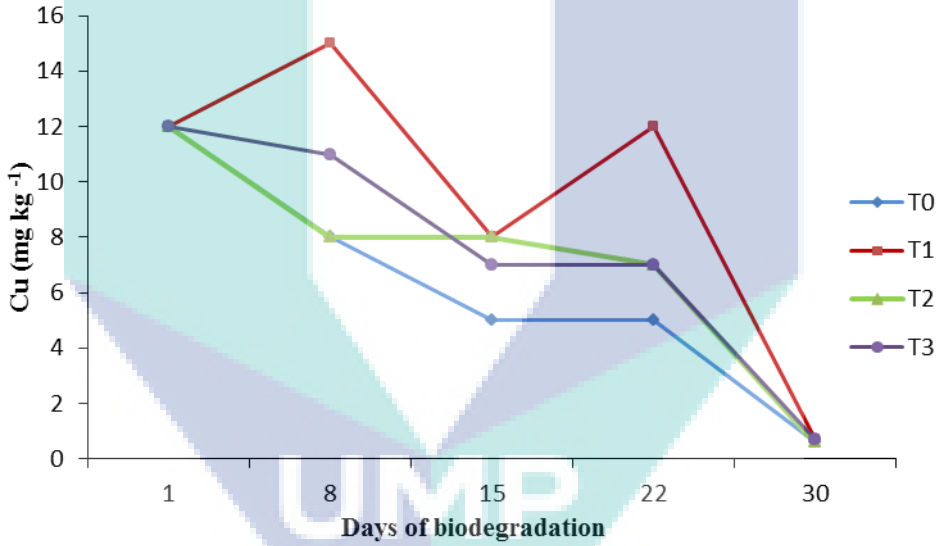


Figure 4.16: Changes of Cu in biodegradation process

Compost is a nutrient source and micro minerals are complete in a relatively small amount (N, P, K, Ca, Mg, Zn, Cu, B, Zn, Mo, and Si). In the long term, giving compost can improve the pH and increase the yield of agricultural crops on the land sour.

4.5.3 Zinc

Figure 4.17 shows the changes of Zn in the composting process. Changes in The Cu declining pattern are similar to Zn and Fe values. The value of Zn also decreased until the last day, but there is an unstable pattern. T0 treatment had increased at day 15 to 91 mg kg^{-1}, T2 treatment increased at day 8 to 102 mg kg^{-1} but the trend until the end of the process is decreased. T2 treatment on day 22 increased to 60 mg kg^{-1} from 55 mg kg^{-1}. While the T3 treatment is not stable during the day 22 to 71 mg kg^{-1}, it drastically decreased to 25 mg kg^{-1}.

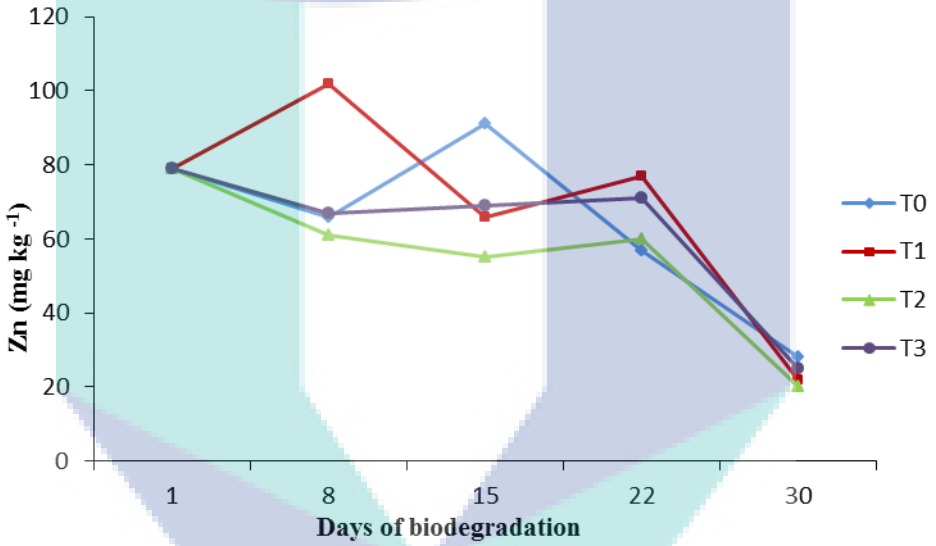
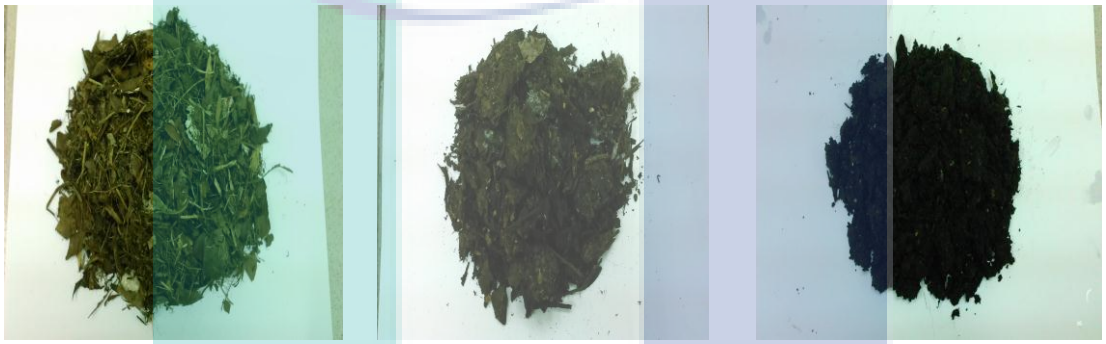


Figure 4.17: Changes of Zn in biodegradation process

In general, all micronutrient/heavy metal contents (Fe, Cu, Zn) in all treatments decreased through the composting process. The reduced content of heavy metals in the compost will have a positive impact on crops and can reduce the toxicity of metals in the process of absorption of nutrients. Micronutrients are needed only in a small amount by the plant and it would be poisoned if that amount is exceeded.

4.6 MATURITY STATUS

Discoloration on compost formulas without inoculant at the beginning of composting shows light brown to dark brown color at the end of composting while the compost material given inoculum formula showed more rapid color change, which is brown. According to Murbandono (2000), the substrate that is added with microbes in the composting process will mature faster so that it will reach maturity color. Compost maturity is reached when the color has become dark brown.



(a) (b) (c)

Figure 4.18: Changes of texture and color in biodegradation process: (a). Raw material at the first week; (b). at the second week; (c). at the end of process.

Compost becomes darkened due to the formation of humic acid substances in the composting process. Stevenson (1994) states that the polyphenol compounds produced in the composting process lignocellulosic compounds into quinones and then reacts with amino compounds to form dark fulvic acid (black). Color changes on all blackish brown compost formulas are associated with changes in the material form, with more crumbs crushed into a smoother texture (Figure 4.18). Colored dark brown compost contains life humus compounds such as humic acid, hematomelanic fulvic acid and others.

Table 4.6: Physiochemical characteristic of final compost

Parameter	Compost treatment			
	T0	**T1**	**T2**	**T3**
pH	7.7	7.6	7.4	7.7
EC (mS cm^{-1})	1.3	1.9	1.7	2.2
TOC (%)	9	13.02	12.38	12.3
C/N ratio	18	16	14	10
Moisture content (%)	51	47	49	48
N (%)	0.5	0.8	0.89	1.2
P (%)	0.3	0.6	0.62	0.82
K (%)	0.15	0.2	0.14	0.28
Zn (mg kg^{-1})	28	22	20	25
Cu (mg kg^{-1})	0.6	0.7	0.6	0.7
Fe (mg kg^{-1})	2450	1748	1986	1669

EC: Electrical conductivity, TOC: Total organic carbon, C/N ratio: Carbon/Nitrogen ratio, N: Nitrogen, P: Phosphorus, K: Pottasium, Zn: Zinc, Cu: Copper, and Fe: Iron.

Generally, maturity and quality compost can be determined simply by examining the temperature, smell, color, physical form decreasing the volume/weight, C/N ratio, foreign material and content of trace elements or heavy metals. Table 4.6 shows physiochemical characteristics of the final resultant compost between the treatments.

Figure 4.19 shows the correlation between temperature and C/N ratio of all treatments. Generally, the maturity phase started at 15^{th} day for all treatments, but T3 is the only treatment that has a very stable condition at the initial process until a maturity phase with the smallest C/N ratio is reached.

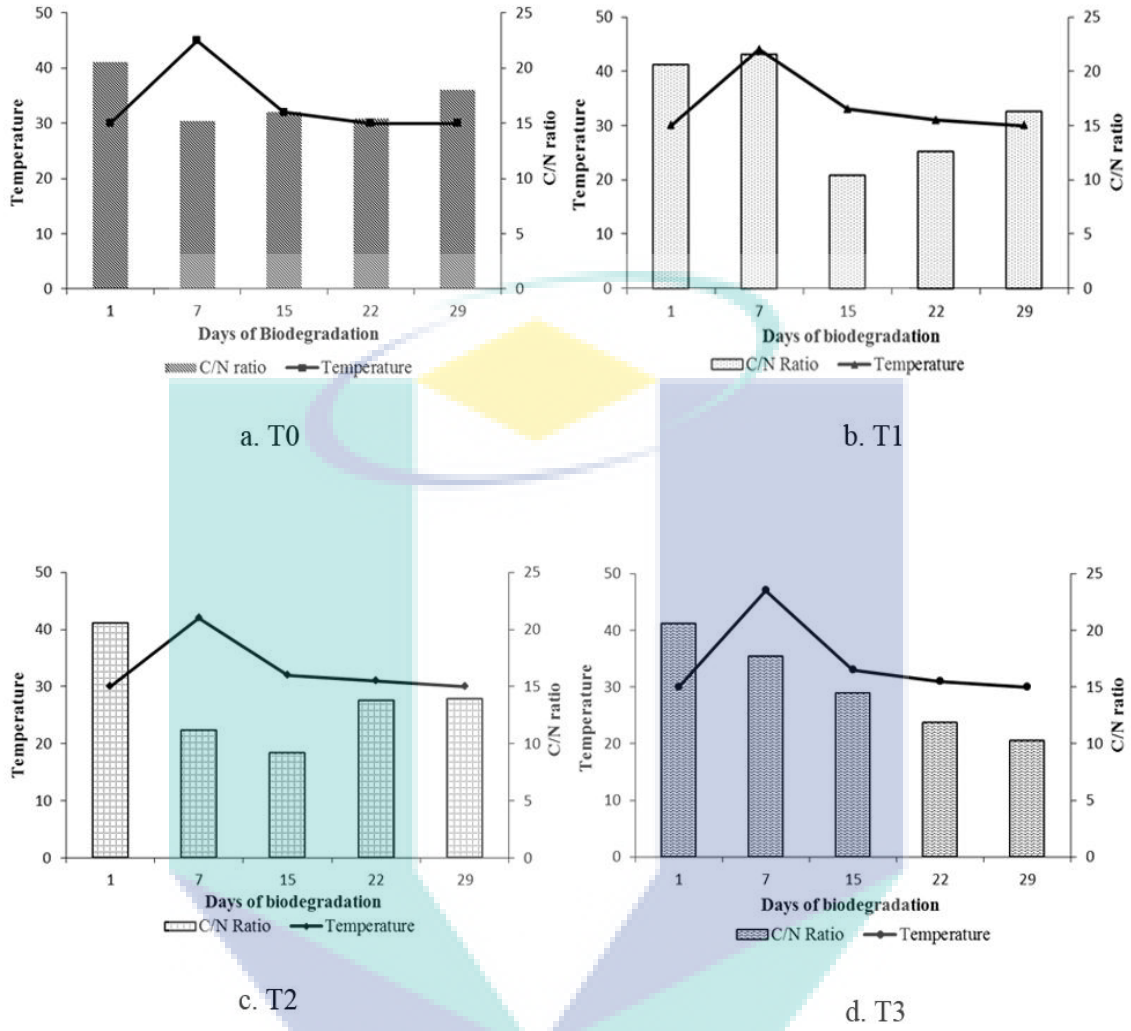


Figure 4.19: Correlation of temperature and C/N ratio as indicator of maturity status.

Compost as a soil amendment function is similar to that of chemical fertilizers to enrich the soil N, P, K, but the effect is to stimulate the stabilization principle of physical, biological, soil chemistry, and balance of mineral elements. Humus is the final product humification where compounds derived from lignin, polysaccharides, nitrogen compounds are converted into stable materials. Many alternatives for the disposal of these organic wastes have been proposed, with composting being one of the most attractive on account of its low environmental impact and cost (Bustamante et al., 2008; Canet et al., 2008; Lu et al., 2008), as well as its capacity for generating a valuable

product used for increasing soil fertility (Weber et al., 2007) or as a growing medium in horticulture

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

The experiments conducted have produced several outcomes that can be concluded as listed below. The conclusions are based on the experiments.

1. The waste in Kuantan was sourced from domestic waste and commercial waste. The main types of commercial waste are food (35%) and plastic (22.20%), while the domestic wastes consist of mixed paper (21%), green waste (23%), and organic waste (27%).

2. There are 37 isolated bacteria and identification of bacteria that was done by Gen III microplate BIOLOG microbial identification system. There are 7 bacteria that have the best result of enzyme production. Their code names are TE, TD, TK, TM, TO, TS22, and LD2. They are *Bacillus amyloliquefaciens, Bacillus ruris, Bacillus licheniformis, Bacillus subtilis* and *Kocuria varians.*

3. Treatment with the inoculation of all bacteria that is capable of producing the enzyme amylase, lipase, protease and cellulose (T3) produces the best result among the other treatments. T3 treatment effectively increases the value of N, P, K and lowers the heavy metal content (Fe, Zn, Cu). T3 treatment also has the lowest C/N ratio value, indicating a stable level of maturity.

5.2 RECOMMENDATION

This research has successfully investigated a series of experiments on biodegradation. A number of recommendations are proposed to enhance the whole research as listed below:

1) For further research, more studies are needed to measure the enzymatic activity assay and the mechanism of molecules breakdown during the biodegradation process.

2) In this study, no microbial test was done on the sample because of time constraint; hence, future studies should focus on identifying the bacteria after the biodegradation process.

3) The actual effect on plant growth using the final compost product should be tested. This will testify the product effectiveness.

REFERENCES

Agamuthu, P. 2001. *Solid Waste: Principle and Management*. Kuala Lumpur. University of Malaya Press.

Agamuthu, P., Fauziah, S.H., Khidzir, K.M. and Aiza, A.N. 2007. Sustainable Waste Management - Asian Perspectives. In *Proceedings of the International Conference on Sustainable Solid Waste Management*. 15–26.

Agamuthu P, Fauziah SH and Khidzir K. 2009. Evolution of solid waste management in Malaysia: impacts and implications of the Solid Waste Bill 2007. *Journal of Material Cycles and Waste Management*. **11**: 96–103.

Aguilera, M., Borie, G., Rokov, P., and Peirano, P. 1998. Bioquimica de derivados de cenizas volcanicas VII Determinacion de deshidrogenasas, *Agricultura Tecnica (Chile)*, **48**: 147–151.

Aira, M., Monroy, and Dominguez, J. 2007. Earthworms strongly modify microbial biomass and activity triggering enzymatic activities during vermicomposting independently of the application rates of pig slurry, *Science of the total environment*, **385**: 252–261.

Alam flora. 2006. *Briefing on kuantan waste disposal site*.

Alexander, R. 1999. Compost markets grow with environmental applications. *Bio Cycle Mag*, **40**:43–44.

Allison, S.D., Nielsen, C., and Flint Hughes, R. 2006. Elevated enzyme activities in soil under the invasive nitrogen-fixing tree Falcaria mollucana, *Soil Biology and Biochemistry*, **38**: 1537–1544.

Amran, A., Zainuddin, Z. and Mohamad Zailani,S. H. 2011. *Carbon Trading in Malaysia: Review of Policies and Practices*.

Arai, T. et al. 2007. Synthesis of Clostridium cellulovorans minicellulosomes by intercellular complementation. *Proc. Natl. Acad*. Sci. U. S. A. **104**: 1456–1460.

Atkinson W. and New R. 1993. *Kerbside Collection of Recyclables from Household Waste in the UK – A Position Study*. Warren Spring Laboratory, Stevenage, UK.

Ayuso, M., Hernandez a, T., Garcı C., and Pascual, J.A., 1996. Biochemical and chemical-structural characterization of different organic materials used as manures. *Bioresource Technology*. **57**: 201–207.

Badgie D., Mohd Armi A.S., Latifah A. M., and Azizi M. 2012. Assessment of municipal solid waste composition in Malaysia: Management, practice and challenges. Polish *Journal Environmental Studies*. **21**, (3): 539-547.

Baig, S. Nadeem, M., Syed, Q. Nelofer, R and Ahmed, B. 2010. Studies on the microbial consortium in open composting of urban bio-waste. *Pak. J. Food Sci*., **20**(1-4):19-23.

Balmer, P. 2004. Phosphorus recovery – an overview of potentials and possibilities, *Water Science and Technology*, **49**: 185–190.

Barrington, S., Choiniere, D., Trigui, M., and Knight, W. 2002. Effect of carbon source on compost nitrogen and carbon losses. *Bioresource Technology*, ***83***: 189-194.

Ben-David, E.A., Zaady, E., Sher, Y., and Nejidat, A. 2011. Assessment of the spatial distribution of soil microbial communities in patchy arid and semiarid landscapes of the Negev Desert using combined PLFA and DGGE analyses, *FEMS Microbiol. Ecol*, **76**: 492–503.

Benitez, E., Sainz, H. and Nogales, R. 2005. Hydrolytic enzyme activities of extracted humic substances during the vermicomposting of a lignocellulosic olive waste., *Bioresource technology*, **96**(7): 785–90.

Benito, Marta. Masaguer, Alberto., Moliner, Ana., Arrigo, Nilda., and Palma, R.M. 2003. Chemical and microbiological parameters for the characterization of the stability and maturity of pruning waste compost, *Biol Fertil Soils*, **37**: 184–189.

Betran, E., Sort, X., Soliva, M and Trillas, I. 2004. Composting winery waste sludge's and grape stalk, *Bioresource technology*. **95**: 203-208.

Bohacz, J., and Korniłłowicz-Kowalska, T. 2007. Choice of maturity indexes for featherplant material composts on a base of selected microbiological and chemical parameters-preliminary research, *J. Environ. Stud*, **16**: 719–725.

Bohacz, J. and Korniłłowicz-Kowalska, T. 2009. Changes in enzymatic activity in composts containing chicken feathers., *Bioresource technology*, **100**(14): 3604–3612.

Brenner, K., L. You, F. and Arnold. 2008. Engineering microbial consortia: a new frontier in synthetic biology. *Trends Biotechnol*. **26** (9): 483-9.

Brunner, P.H., and Fellner, J. 2007. Setting priorities for waste management strategies in developing countries. *Waste Management and Research* **25** (3): 234–240.

Budhiarta, I., Siwar, C. and Basri, H. 2012. Advanced Science Information Technology Current Status of Municipal Solid Waste Generation in Malaysia, *International Journal on Advanced Science Engineering Information Technology*, **2**(2): 16–21.

Bustamante MA, Moral R, Paredes C, Vargas-García MC, and Suárez-Estrella F, M.J. 2008. Evolution of the pathogen content during co-composting of winery and distillery wastes, *Bioresour Technol*, **100**: 3604–3612.

Butt, T.E., Oduyemi, K.O.K. 2003. A holistic approach to concentration assessment of hazards in the risk assessment of landfill leachate. *Environ. Int.* **28**: 597–608.

Caceres, R., Flotats, X., and Marfa, O. 2006. Changes in the chemical and physicochemical properties of the solid fraction of cattle slurry during composting using different aeration strategies. *Waste Manage*. **26** (10): 1081– 1091.

Camina, F., Trasar-Cepeda, C., Gil-Sotres, F., and Leirros, C. 1998. Measurement of dehydrogenase activity in acid soils rich in organic matter, *Soil Biol. Biochem*, **30**: 1005–1011.

Canet, R., Pomares, F., Cabot, B., Chaves, C., Ferrer, E., Ribó, M. and Albiach, M. a R. 2008. Composting olive mill pomace and other residues from rural southeastern Spain., *Waste management (New York, N.Y.)*, **28**(12): 2585–92.

Castaldi, P., Garau, G. and Melis, P. 2008. Maturity assessment of compost from municipal solid waste through the study of enzyme activities and water-soluble fractions., *Waste management (New York, N.Y.)*, **28**(3): 534–40.

Cayuela, M.L., Mondini, C, Sánchez-Monedero, M. and Roig, A. 2008. Chemical properties and hydrolytic enzyme activities for the characterisation of two-phase olive mill wastes composting., *Bioresource technology*, **99**(10): 4255–62.

Chander, M., Arora, D. S., and Bath, H.K. 2004. Biodecolourisation of some industrial dyes by white-rot fungi, *Journal of Industrial Microbiology and Biotechnology*, **31**: 94–97.

Chowdhury, a., Iqbal, a., Uddin, M.G. and Uddin, M. 2011. Study on Isolation and Identification of *Salmonella* and *Escherichia coli* from Different Poultry Feeds of Savar Region of Dhaka, Bangladesh, *Journal of Scientific Research*, **3**(2).

Chua, K.H., Jati, E., Sahid, M. and Leong, Y.P. 2011. Sustainable Municipal Solid Waste Management and GHG Abatement in Malaysia, *15th International Conference on ISO & TQM*, ,1–8.

Cunha-Queda, a C., Ribeiro, H.M., Ramos, a and Cabral, F. 2007. Study of biochemical and microbiological parameters during composting of pine and eucalyptus bark., *Bioresource technology*, **98**(17): 3213–20.

CWMI, The Science of Composting, 2007. http://cwmi.css.cornell.edu/chapter1.pdf (Accessed on 5 April 2015)

Dalzell, H. W., Biddlestone, A., J., Gray, K. R. and Thurairajan, K. 1987. Soil management; Compost production and use in tropical and subtropical environments, *FOA Soil Bulletin*. **56**: 177.

Dangi, M.B., Pretz, C.R., Urynowicz, M. a, Gerow, K.G. and Reddy, J.M. 2011. Municipal solid waste generation in Kathmandu, Nepal., *Journal of environmental management*, **92**(1): 240–9.

Daniel and Laura, T. 1999. *What A Waste: Solid Waste Management in Asia*. Working Paper Series Nr. 1. Urban Development Sector Unit. East Asia and Pacific Region. Hoornweg, Page 5.

Delgado, A., Río, R.S. and Sales, D. 2004. Study of the composting process of municipal solid waste and sewage sludge : stability and maturity. In *Proceedings of the 11th Conference of the FAO on Recycling of Agricultural, Municipal and Industrial Residues in Agriculture (RAMIRAN)*. 257–260.

Deporter I, Benoit-Guyod JL, Zmirou D and Bouvier M. 1998. Microbial Disinfection capacity of municipal solid waste water (MSW) composting. *J Appl Microbiol* **85**: 238-246.

Diaz, M.J., Madejon, E., Lopez, F., Lopez, R., and Cabrera, F. 2002. Optimization of the rate vinasse/grape marc for co-composting process. *Process Biochemistry* **37**, 1143–1150.

Diorio, L., Galati, B., García, M.A. and Papinutti, Leandro. 2009. Degradation of pruning wastes by Phanerochaete sordida growing in SSF: Ultrastructural, chemical, and enzymatic studies, *International Biodeterioration & Biodegradation*, **63**(1): 19–23.

Earth Observation Centre. 2011. Municipal solid waste management in Malaysia: Solution for sustainable waste management, **6**(1): 29–38.

EC (European Commission). 2003. Establishing criteria and procedures for the acceptance of waste at landfills pursuant to Article 16 of and Annex II to Directive 1999/31/EC. Official Journal of the European Communities, 11/ 27.

Emtiazi, G., Naghavi, N. and Bordbar. 2001. Biodegradation of lignocellulosic waste by Aspergillus terreus., *Biodegradation*, **12**(4): 259–63.

Feng, C., Zeng, GuangMing, Huang, DanLian, Hu, S., Zhao, M., Lai, C., Huang, C., Wei, Zhen and Li, N. 2011. Effect of ligninolytic enzymes on lignin degradation and carbon utilization during lignocellulosic waste composting, *Process Biochemistry*, **46**(7): 1515–1520.

Flynn, R. P. and Wood, C. W. 1996. Temperature and chemical changes during composting of broiler litter. *Compost Science and Utilization*. **4**(3): 62-70.

Galli, E., Pasetti, L., Fiorelli, F., and Tomati, U., 1997. Olive-mill wastewater composting:microbiological aspects. *Waste Management and Research* **15**: 323–330.

García, C., Hernández, T., Costa, C., Ceccanti, B., Masciandaro, G., and Ciardi, C. 1993. A study of biochemical parameters of composted and fresh municipal wastes, *Bioresource technology*, **44**: 17–23.

Gautam, S.P., Bundela, P.S., Pandey, A.K., Awasthi, M.K., and Sarsaiya, S. 2010. Microbial consortium or effective composting of municipal solid waste by enzymatic activities. *Journal of Applied Sciences in Environmental Sanitation* **5**: 301-308.

Gericke, S and Kurmies, B. 1952. The colorimetric determination of phosphoric acid with ammonium vanadate molybdate and its application in plant analysis. **59**: 235-247.

Golueke, C.G. 1977. *Biological reclamation of solid waste*, Pennsylvania, USA. Rodale Press Incorporation.

Golueke, C. G. and Diaz, L. F. 1996. *Historical review: Composting and its role in municipal solid wastemanagement in The science of composting* (Ed.) M. de Bertoldi, P. Sequi, B. Lemmes, T. Papi, Blackie, Glasgow: Blackie Academic and Professional.

Goyal, S., Dhull, S.K., and Kapoor, K.K. 2005. Chemical and biological changes during composting of different organic wastes and assessment of compost maturity. *Bioresour. Technol*. **96**: 1584-1591.

Grueninger H, Sonnleitner B, and Fiechter A. 1984. Bacterial diversity in thermophilic aerobic sewage sludge. III. A source of organisms producing heat stable industrially useful enzymes, e.g. amylase. *Appl Microbiol Biotechnol*. **19**: 414–21.

Guo, R., Li, G., Jiang, T., Schuchardt, F., Chen, T., Zhao, Yuanqiu and Shen, Y. 2012. Bioresource Technology Effect of aeration rate , C / N ratio and moisture content on the stability and maturity of compost, *Bioresource Technology*, **112**: 171–178.

Guo, X. et al. 2012. Effects of Cu on metabolisms and enzyme activities of microbial communities in the process of composting., *Bioresource technology*, **108**: 140–8.

Hale Boothe, D.D., Smith, M.C., Gattie, D.K. and Das, K. 2001. Characterization of microbial populations in landfill leachate and bulk samples during aerobic bioreduction, *Advances in Environmental Research*, **5**(3): 285–294.

Halet, D., Boon, N. and Verstraete, W. 2006. Community dynamics of methanotrophic bacteria during composting of organic matter., *Journal of bioscience and bioengineering*, **101**(4): 297–302.

Hall, D.H., Drury, D., Gronow, J.R., Rosevear, A., Pollard, S.J.T., and Smith, R. 2006. Estimating pollutant removal requirements for landfills in the UK: II. Model development. *Environmental Technology*. **27**(12): 1323–1333.

Hall, D.H., Drury, D., Gronow, J.R., Pollard, S.J.T., and Smith, R., 2007. Estimating pollutant removal requirements for landfills in the UK: III. Policy analysis and operational implications. *Environmental Technology* **28** (1): 25–32.

Hassan, M. N., Yusoff, M. K., Sulaiman, W. N., and Abdul Rahman, R. 2001. Solid waste management in Southeast Asian contries with special attention to Malaysia. In: *Proceedings 8th International Waste Management and Landfill Symposium*, Italy.

He, Y., Xie, K., Xu, P., Huang, X., Gu, W., Zhang, F. and Tang, S. 2013. Evolution of microbial community diversity and enzymatic activity during composting., *Research in microbiology*, **164**(2): 189–98.

Hjelmar, O., van der Sloot, H.A., Guyonnet, D., Rietra, R.P.J.J., Brun, A., and Hall, D. 2001. Development of acceptance criteria for landfilling of waste: an approach based on impact modelling and scenario calculations. *In: Sardinia Waste Management and Landfill Symposium*, S. margarita di Pula, Cagliari, Italy, CISA.

Hjelmar, O., Holm, J., Gudbjerg, J., Bendz, D., Suèr, P., Rosquvist, H., Wahlström, M., and Laine-Ylijoki, J. 2005. *Development of Criteria for Acceptance of Monolithic Waste at Landfills Nordic Council of Ministers*, NMR Landfill Group.

Hernandez-Luna, C.E., Gutierrez-Soto, G. and Salcedo-Martinez, S.M. 2008. Screening for decolorizing basidiomycetes in Mexico : Screening and selection of ligninolytic basidiomycetes with decolorizing ability in Northeast Mexico, *World journal of microbiology & biotechnology*, **24**(4): 465–473.

Hofrichter, M. 2002. Review: lignin conversion by manganese peroxidase (MnP), *Enzyme and Microbial Technology*, **30**(4): 454–466.

Hoppe, H. 2003. Phosphatase activity in the sea, *Hydrobiologia* 493: 187–200.

Huang, D.-L. et al. 2008. Degradation of lead-contaminated lignocellulosic waste by Phanerochaete chrysosporium and the reduction of lead toxicity., *Environmental science & technology*, **42**(13): 4946–51.

Insam, H and de Bertoldi, M. 2007. Microbiology of the composting process. In E. Diaz, Luis F., de Bertoldi, M., Bidlingmaier, W., and Stentiford, ed. *Compost science and technology*. Elsevier Ltd, page 27.

Ismail, Mohd Razi, Saud, H.M., Habib, S.H., Siddiquee, S. and Kausar, H. 2012. Physical, chemical and biological changes during the composting of oil palm frond, *African Journal of Microbiology Research*, **6**(19): 4084–4089.

Jackson, M. L., 1973. *Soil chemical analysis*. Prentice hall (India) Pvt. Ltd, New-Delhi.

Johari, A., Ahmed, S.I., Hashim, H., Alkali, H. and Ramli, M. 2012. Economic and environmental benefits of landfill gas from municipal solid waste in Malaysia, *Renewable and Sustainable Energy Reviews*, **16**(5): 2907–2912.

Jurado, M.M., Suárez-Estrella, F, Vargas-García, M.C., López, M.J., López-González, J. a and Moreno, J. 2014. Evolution of enzymatic activities and carbon fractions throughout composting of plant waste., *Journal of environmental management*, **133**: 355–64.

Kala, D.R., Rosenani, A.B., Fauziah, C.I., Ahmad, S.H., Radziah, O. and Rosazlin, A. 2011. Commercial Organic Fertilizers and their Labeling in Malaysia, *Malaysian Journal of Soil Science*, **15**: 147–157.

Kausar, H., Sariah, M., Ismail, M Razi, Saud, H.M. and Habib, S.H. 2012. Development of a potential lignocellulolytic resource for rapid bioconversion of rice straw, *African Journal of Biotechnology*, **11**(38): 9235–9242.

Kayhanian, N. A. and Tchobanoglous, G. 1992. Computation of C/N ratio for various organic fractions, *Biocycle*. **5**: 58-60.

Keller, L. and Surette, M.G. 2006 Communication in bacteria: an ecological and evolutionary perspective. *Nat. Rev.Microbiol*. **4**: 249–258.

Khiari, B., Marias, F., Zagrouba, F., and Vaxelaire, J. 2006. Use of a transient model to simulate fluidized bed incineration of sewage sludge. *J. Hazard. Mater*. **135**: 200–209.

Knox, K., Braithwaite, P., Caine, M., and Croft, B. 2005. Brogborough landfill test cells: the final chapter. A study of landfill completion in relation to final storage quality (FSQ) criteria. *In: Sardinia 2005 – 10th International Waste Management and Landfill Symposium*, S. Margherita di Pula, Cagliari, Italy, CISA.

Kobayashi, T., Xu, K.-Q., Li, Y.-Y. and Inamori, Y. 2012. Evaluation of hydrogen and methane production from municipal solid wastes with different compositions of fat, protein, cellulosic materials and the other carbohydrates, *International Journal of Hydrogen Energy*, **37**(20): 15711–15718.

Koumoutsi, A., Chen, X.H., Vater, J., and Borriss, R. 2007. Deg U and Ycz E positively regulate the synthesis of bacillomycin D by Bacillus amyloliquefaciens strainFZB42. *Appl. Environ. Microbiol*. **73** (21): 6953–6964.

Lalman, J.A., and Bagley, D.M. 2000. Anaerobic degradation and inhibitory effects of linoleic acid. *Water Research* **34**: 4220–4228.

LaMar, J. J., Daniel, D. E., Abeele, W. V., Ledbetter, J. O., and Hansen, W. R. 1978. Effects from past solid waste disposal practices. *Journal of Environmental Health and Perspective*, **27**: 215–221.

Laner, D., Crest, M., Scharff, H., Morris, J.W.F. and Barlaz, M. 2012. A review of approaches for the long-term management of municipal solid waste landfills., *Waste management (New York, N.Y.)*, **32**(3): 498–512.

Law of Malaysia. 2007. *Act 672, Solid Waste and Public Cleansing Management Act*. Malaysia: Government of Malaysia.

Lazcano, C., Gómez-Brandón, M. and Domínguez, J. 2008. Comparison of the effectiveness of composting and vermicomposting for the biological stabilization of cattle manure., *Chemosphere*, **72**(7): 1013–9.

Lee, I. B., Kim, P. J., and Chang, K. W. 2002. Evaluation of stability of compost prepared with korean food waste, *Soil science plant nutrition*. **1**: 1-8.

Lee, Y.-J., Kim, B.-K., Lee, B.-H., Jo, K.-I., Lee, N.-K., Chung, C.-H., Lee, Y.-C. and Lee, J.-W. 2008. Purification and characterization of cellulase produced by Bacillus amyoliquefaciens DL-3 utilizing rice hull., *Bioresource technology*, **99**(2): 378–86.

Lei, F. and VanderGheynst, J. 2000. The effect of microbial inoculation and pH on microbial community structure changes during composting, *Process Biochemistry*, **35**(9): 923–929.

Levin, L., Papinutti, L and Forchiassin, F. 2004. Evaluation of Argentinean white rot fungi for their ability to produce lignin-modifying enzymes and decolorize industrial dyes., *Bioresource technology*, **94**(2): 169–76.

Liang, C., Das, K.C. and McClendon, R.W. 2003. The influence of temperature and moisture contents regimes on the aerobic microbial activity of a biosolids composting blend., *Bioresource technology*, **86**(2): 131–7.

Lin, H. and Ma, X. 2012. Simulation of co-incineration of sewage sludge with municipal solid waste in a grate furnace incinerator., *Waste management (New York, N.Y.)*, **32**(3): 561–7.

Liu J, Xu X, Li H, and Xu Y. 2011. Effect of microbiological inocula on chemical and phys- ical properties and microbial community of cow manure compost. *Biomass Bioenergy*. **35**: 3433–9.

Liu, D., Zhang, R., Wu, H., Xu, D., Tang, Z., Yu, G., Xu, Z. and Shen, Q. 2011. Changes in biochemical and microbiological parameters during the period of rapid composting of dairy manure with rice chaff., *Bioresource technology*, **102**(19): 9040–9.

Lloyd-Jones, G., Prŏlß, J., Trapski, C. and Brijs, E. 2010. Protease- and keratinase-producing microbial strains for compost bioaugmentation, *International Biodeterioration & Biodegradation*, **64**(7): 574–580.

Lopez, M.J., Del Carmen Vargas-García, M., Suárez-Estrella, Francisca and Moreno, Joaquín. 2006. Biodelignification and humification of horticultural plant residues by fungi, *International Biodeterioration & Biodegradation*, **57**(1): 24–30.

Lu, L.-A., Kumar, M., Tsai, J.-C. and Lin, J.-G. 2008. High-rate composting of barley dregs with sewage sludge in a pilot scale bioreactor., *Bioresource technology*, **99**(7): 2210–7.

Lu, Y., Wu, X. and Guo, J. 2009. Characteristics of municipal solid waste and sewage sludge co-composting., *Waste management (New York, N.Y.)*, **29**(3): 1152–7.

MacGregor S. T., F. C. Miller, K.M.P. and M.S.F. 1981. Compostingg process control based on interaction between microbial heat output and temperature, *Applied and environmental microbiology*, **39**: 1000–1009.

Maeda, K., Hanajima, D., Morioka, R. and Osada, T. 2010. Characterization and spatial distribution of bacterial communities within passively aerated cattle manure composting piles., *Bioresource technology*, **101**(24): 9631–7.

Makoi, J.H.J.R. and Ndakidemi, P.A. 2008. Selected soil enzymes : Examples of their potential roles in the ecosystem, **7**(3): 181–191.

Manpreet, S., Sawraj, S., Sachin, D., Pankaj, S., and Banerjee, U.C. 2005. Influence of process parameters on the production of metabolites in solid-state fermentation. *Malays. J. Microbiol*. **1**: 1–9.

Mari, I., Ehaliotis, C., Kotsou, M., Balis, C., and Georgakakis, D. 2003. Respiration profiles in monitoring the composting of by-products from the olive oil agro-industry. *Bioresource Technology* **87**: 331–336.

Marx, M., Wood, M. and Jarvis, S.C. 2001. A microplate fluorimetric assay for the study of enzyme diversity in soils, *Soil Biol. Biochem*, **33**: 1633–1640.

Matejczyk, M., Płaza, G. a, Nałęcz-Jawecki, G., Ulfig, K. and Markowska-Szczupak, A. 2011. Estimation of the environmental risk posed by landfills using chemical, microbiological and ecotoxicological testing of leachates., *Chemosphere*, **82**(7): 1017–23.

McCartney, D., and Tingley, J. 1998. Development of a rapid moisture content method for compost materials. *Compost Science and Utilization* **6**: 14–25.

Ministry of Housing and Local Government (MHLG). 2000. *Ministry of Housing and Local Government Reports*. Kuala Lumpur: Government Printers.

Ministry of Housing and Local Government Malaysia (MHLG). 2003. Waste Generation Statistics. http://www.kpkt.gov.my/statistik/perangkaan2002. Accessed 15 April 2014.

Mirdamadian, S.H. and Ghanavati, H. 2011. Reduce of Fermentation Time in Composting Process by Using a Special Microbial Consortium, *Engineering and Technology*, **52**: 533–537.

Mondini, Claudio, Fornasier, F. and Sinicco, T. 2004. Enzymatic activity as a parameter for the characterization of the composting process, *Soil Biology and Biochemistry*, **36**(10): 1587–1594.

Morais, F.M.C., and Queda, C.A.C. 2003. Study of storage in Xuence on evolution of stability and maturity properties of MSW composts. In: *Proceedings of the fourth International Conference of ORBIT association on Biological Processing of Organics: Advances for a sustainable Society* Part II, Perth, Australia.

Muhd Yunus, M.N. 2012. *From municipal solid waaste to radioactive waste: positions taken by nuclear malaysia*,

Murbandono, L. 2000. *How to make compost (TNH)*. Penebar Swadaya, Jakarta.

Naik, P.S. 2007. Studies on microbial consortia for production and enrichment of bio-compost from grapevine residues. *Master thesis*. University of Agricultural sciences, Dharwad.

Nair, J., Sekiozoic, V., and Anda, M. 2006. Effect of pre-composting on vermicomposting of kitchen waste. *Bioresour. Technol.* **97**: 2091– 2095.

Nangalia, S. 2013. Spectroscopic and thermal analysis of composting during agitated pile and rotary drum composting. *Module*.

Naylor, L. M. 1996. Composting, Environmental and science and pollution series. **19**(69): 193-269.

Okoroma, E. a., Garelick, H., Abiola, O.O. and Purchase, D. 2012. Identification and characterisation of a Bacillus licheniformis strain with profound keratinase activity for degradation of melanised feather, *International Biodeterioration & Biodegradation*, **74**: 54–60.

Oral, J., Stehlík, P., Sikula, J., Puchy´ , R., Hajny´ , Z., and Martiná, P. 2005. Energy utilization from industrial sludge processing. *Energy* **30**: 1343–1352.

Pathak, H., Singh, Ramandeep, Bhatia, A. and Jain, N. 2006. Recycling of rice straw to improve wheat yield and soil fertility and reduce atmospheric pollution, *Paddy and Water Environment*, **4**(2): 111–117.

Pillai, P. and Archana, G. 2012. A novel process for biodegradation and effective utilization of chrome shavings, a solid waste generated in tanneries, using chromium resistant Bacillus subtilis P13, *Process Biochemistry*, **47**(12): 2116–2122.

Pointing, S.B., Road, P. and Avenue, T.C. 2000. Dye decolorization by sub-tropical basidiomycetous fungi and the effect of metals on decolorizing ability.

Portillo, M.C., Villahermosa, D., Corzo, A and Gonzalez, J.M. 2011. Microbial community fingerprinting by differential display-denaturing gradient gel electrophoresis., *Applied and environmental microbiology*, **77**(1): 351–4.

Pramanik, P., Ghosh, G.K., Ghosal, P.K. and Banik, P. 2007. Changes in organic - C, N, P and K and enzyme activities in vermicompost of biodegradable organic wastes under liming and microbial inoculants., *Bioresource technology*, **98**(13): 2485–94.

Priest, F.G., 1985. Synthesis and secretion of extracellular enzymes by bacilli. *Microbiol. Sci.* **2**: 278–282.

Rashad F. M, Saleh W. D, and Moselhy A. A. 2010. Bioconversion of rice straw and cer- tain agro-industrial wastes to amendments for organic farming systems: 1. Composting, quality, stability and maturity indices. *Bioresour Technol*; **101**: 5952–60.

Raut, M.P., Prince William, S.P.M., Bhattacharyya, J.K., Chakrabarti, T. and Devotta, S. 2008. Microbial dynamics and enzyme activities during rapid composting of municipal solid waste - a compost maturity analysis perspective., *Bioresource technology*, **99**(14): 6512–9.

Razman M, S., Othman, F. and Marzuky, S. 1994. Problems and Challenges of Solid Waste Management: A Case Study in South Johor, Malaysia. *Jurnal Fakulti Kejuruteraan*. Universiti Teknologi Malaysia. **7**(1): 1-8.

Robert, A.C. 1999. *Standard Handbook of Environmental Engineering*, McGraw-Hill Companies, U.S.A.

Ros, M., García, C. and Hernández, T. 2006. A full-scale study of treatment of pig slurry by composting: kinetic changes in chemical and microbial properties., *Waste management (New York, N.Y.)*, **26**(10): 1108–18.

Roy, M.M., Dutta, A., Corscadden, K., Havard, P., and Dickie, L. 2011. Review of biosolids management options and co-incineration of a biosolid-derived fuel. *Waste Manage*. 06-08.

Ruberto, L., Vazquez, S.C. and Mac Cormack, W.P. 2003. Effectiveness of the natural bacterial flora, biostimulation and bioaugmentation on the bioremediation of a hydrocarbon contaminated Antarctic soil, *International Biodeterioration & Biodegradation*, **52**(2): 115–125.

Ruckert, C., Blom, J., Chen, X., Reva, O., and Borriss, R. 2011. Genome sequence of B. amyloliquefaciens type strain DSM7T reveals differences to plant-associated B. amyloliquefaciens FZB42. *J. Biotechnol.* **155** (1): 78–85.

Rynk, R., van de Kamp, M., Wilson, G. G., Singley, M. E., Richard, T. L., Kolega, J.J., Gouin, F. R., Laliberty Jr., L., Kay, D., Murphy, D., Hoithink, H. A. J., and Brinton, W. F. 1992. *On farm composting handbook. Ithaca. NY*: Natural resource, Agriculture and engineering service.

Saeed, M.O., Hassan, M.N. and Mujeebu, M.A. 2009. Assessment of municipal solid waste generation and recyclable materials potential in Kuala Lumpur, Malaysia., *Waste management (New York, N.Y.)*, **29**(7): 2209–13.

Samsudin, A. R., Elwali, B. C., Rahim, A., Wan Zahairi, W. Y., and Hamazah, U. 2006. Mapping of contami- nation plumes at municipal solid waste disposal sites using geoelectric imaging technique: Case studies of Malaysia. *Journal of Spatial Hydrology*, **6**(2): 13–22.

Sanchez-Monedero, M. A., Roig, A., Paredes, C., and Bernal, M. P. 2001. Nitrogen transformation during organic wastes composting by the Rutgers system and its effect on pH, EC and maturity of the composting mixtures, *Bioresource Technology*. **78**: 301-308.

Sarkar, P., Meghvanshi, M. and Singh, R. 2011. Microbial Consortium : A New Approach in Effective Degradation of Organic Kitchen Wastes, **2**(3): 170–174.

Sengupta S, J., Trehan, C., and Kaul, S. 1998. Methane emission from MSW by gas chromatography. *Journal of Pollution Research*, **17**(4): 367–369.

Sharma, B. K. 2005. Environmental chemistry (Ed.). 9th. Meerut: Goel.

Shi, S., Richardson, A.E., O'Callaghan, M., DeAngelis, K.M., Jones, E.E., Stewart, A., Firestone, M.K. and Condron, L.M. 2011. Effects of selected root exudate components on soil bacterial communities, *FEMS Microbiology Ecology*, **77**(3): 600–610.

Singh, C. P., Kumar, P., and Gupta, R. K. 1999. Generation of solid waste and its management in Govt. opium and alkoloids works, Ghazipur, India. *Journal of Industrial Pollution Control*, Enviromedia Publication, **15**(1): 51–55.

Steger, K., Eklind, Y., Olsson, J. and Sundh, I. 2005. Microbial community growth and utilization of carbon constituents during thermophilic composting at different oxygen levels., *Microbial ecology*, **50**(2): 163–71.

Stentiford, E.I. 1996. Composting control: principals and practice. In: de Bertoldi, M., Sequi, P., Lemmes, B., Papi, T. (Eds.), The Science of Composting. Blackie, London.

Stevenson, F.J., 1994. *Humus Chemistry. Genesis, Composition, Reactions*. Wiley, New York.

Sumiani, Y., Onn, C. C., Mohd, D., and Wan, W. Z. 2009. Strategies for planning of optimum landfill sitting decision making. *Journal of Sains Malaysiana*, 457-462.

Takano, M., Nishida, A., and Nakamura, M. 2001. Screening of wood-rotting fungi for kraft pulp bleaching by the Poly R decolorization test and biobleaching of hardwood kraft pulp by Phanerochaete crassa WD 1694, *Journal of Wood Science*, **47**: 63–68.

Tang, J.-C., Shibata, A., Zhou, Q. and Katayama, A. 2007. Effect of temperature on reaction rate and microbial community in composting of cattle manure with rice straw., *Journal of bioscience and bioengineering*, **104**(4): 321–8.

Thitame, S.N., Pondhe, G.M. and Meshram, D.C. 2010. Characterisation and composition of municipal solid waste (MSW) generated in Sangamner City, District Ahmednagar, Maharashtra, India., *Environmental monitoring and assessment*, **170**(1-4): 1–5.

Thompson D. N, Hames B. R, and Reddy C. A,. 1998. In vitro degradation of natural insoluble lignin in aqueous media by the extracellular peroxidases of Phanerochaete chrysosporium, *Biotechnology and Bioengineering*, **57**: 704–717.

Tiquia, S.M. 2002. Evolution of extracellular enzyme activities during manure composting., *Journal of applied microbiology*, **92**(4): 764–770.

Tongpim, S., Meidong, R., Poudel, P., Yoshino, S., Okugawa, Y., Tashiro, Y., Taniguchi, M. and Sakai, K. 2014. Isolation of thermophilic L-lactic acid producing bacteria showing homo-fermentative manner under high aeration condition., *Journal of bioscience and bioengineering*, **117**(3): 318–24.

Tork, S.E., Shahein, Y.E., El-Hakim, A.E., Abdel-Aty, A.M. and Aly, M.M. 2013. Production and characterization of thermostable metallo-keratinase from newly isolated Bacillus subtilis NRC 3., *International journal of biological macromolecules*, **55**: 169–75.

Trautmann, N. and Kransy, M. 1996. Composting in the classroom: scientific inquiry for highschool students.

Tsiko, R.G. and Togarepi, S. 2012. A Situational Analysis of Waste Management in Harare, Zimbabwe, **8**(4): 692–706.

Ugwuanyi, J.O. 2008. Yield and protein quality of thermophilic Bacillus spp. biomass related to thermophilic aerobic digestion of agricultural wastes for animal feed supplementation., *Bioresource technology*, **99**(8): 3279–90.

Vargas-García MC, Suárez-Estrella F, López MJ, and Moreno J. 2006. Influence of micro- bial inoculation and co-composting material on the evolution of humic-like substances during composting of horticultural wastes. *Process Biochem.* **41**: 1438–43.

Vargas-García, M.C., Suárez-Estrella, F, López, M.J. and Moreno, J. 2010. Microbial population dynamics and enzyme activities in composting processes with different starting materials., *Waste management (New York, N.Y.)*, **30**(5): 771–8.

Wang, Y.-P., Houlton, B.Z. and Field, C.B. 2007. A model of biogeochemical cycles of carbon, nitrogen, and phosphorus including symbiotic nitrogen fixation and phosphatase production, *Global Biogeochemical Cycles*, **21**(1).

Weber, J., Karczewska, a., Drozd, J., Licznar, M., Licznar, S., Jamroz, E. and Kocowicz. 2007. Agricultural and ecological aspects of a sandy soil as affected by the application of municipal solid waste composts, *Soil Biology and Biochemistry*, **39**(6): 1294–1302.

Wei, Zimin, Xi, B., Zhao, Yue, Wang, S., Liu, H. and Jiang, Y. 2007. Effect of inoculating microbes in municipal solid waste composting on characteristics of humic acid., *Chemosphere*, **68**(2): 368–74.

Werther, J. and Ogada, T. 1999. Sewage sludge combustion. Progress in Energy and Combustion Science **25**: 55-116.

Xing, D., Ren, N., Gong, M., Li, J., and Li, Q., 2005. Monitoring of microbial community structure and succession in the biohydrogen production reactor by denaturing gradient gel electrophoresis (DGGE). *Sci. China Ser. C* **48**: 155-162.

Yahmed, A. Ben, Saidi, N., Trabelsi, I., Murano, F., Dhaifallah, T., Bousselmi, L. and Ghrabi, A. 2009. Microbial characterization during aerobic biological treatment of landfill leachate (Tunisia), *Desalination*, **246**(1-3): 378–388.

Yamamoto, N., Asano, R., Yoshii, H., Otawa, K. and Nakai, Y. 2011. Archaeal community dynamics and detection of ammonia-oxidizing archaea during composting of cattle manure using culture-independent DNA analysis, *Applied Microbiology and Biotechnology*, **90**(4): 1501–1510.

Yang, S. S. 1997. Preparation of compost and evaluating its maturity. (http://www.agnet.org./library/bc/53007/ (accessed 6 April 2015)

Yeoh, C.Y., Chin, N.L. and Tan, C.S. 2011. Co-composting of palm oil mill wastes, **9**.

Yousuf, T. Bin and Rahman, M. 2007. Monitoring quantity and characteristics of municipal solid waste in Dhaka City., *Environmental monitoring and assessment*, **135**(1-3): 3–11.

Yu, H., Zeng, Guangming, Huang, H., Xi, X., Wang, R., Huang, Danlian, Huang, G. and Li, J. 2007. Microbial community succession and lignocellulose degradation during agricultural waste composting., *Biodegradation*, **18**(6): 793–802.

Zahari, M.S., Mohd, W., Wan, F., Armi, M. and Samah, A. 2010. Study on solid waste generation in Kuantan, Malaysia. **2**(5): 1338–1344.

Zaldivar, J., J. Nielsen, and L. Olsson. 2001. Fuel ethanol production from lignocellulose: a challenge for metabolic engineering and process integration. *Appl. Microbiol. Biotechnol*. **56**:17–34.

Zhang, Y., and He, Y. 2006. Co-composting solid swine manure with pine sawdust as organic substrate. *Bioresource Technology*, **97**: 2024-2031.

APPENDIX

LIST OF PUBLICATIONS

1. Oral Presentation

- **Nailah Sa'adah**, W.M.F. Wan Ishak, Essam A. Makky. *Comparison of Enzymes Production of Bacteria from Landfill Soil and Leachate: A Case Study- Jabor Landfill Kuantan. Pahang, Malaysia*. Presented at the 4th International Conference on Environment and Industrial Innovation (ICEII 2014), 12nd – 14th March 2014, The Gurney Resort Hotel & Residences, Penang, Malaysia.
- **Nailah Sa'adah**, W.M.F. Wan Ishak, Essam A. Makky. *Comparison of Bacteria from Landfill Soil and Leachate Based On Gram Characteristic and Enzyme Production in Jabor Landfill, Pahang, Malaysia.* Presented at national Conference on Industry-Academia Joint Initiatives in Biotechnology (CIA: Biotech 13), 5th – 7th December 2013, Equatorial Hotel, Cameron Highland, Pahang, Malaysia.

2. Journal

- **Nailah Sa'adah**, W.M.F. Wan Ishak, Essam A. Makky. *Comparison of Enzymes Production of Bacteria from Landfill Soil and Leachate: A Case Study- Jabor Landfill Kuantan. Pahang, Malaysia*. International Journal of Innovation, Management and Technology (IJIMT, ISSN: 2010-0248). http://dx.doi.org/10.7763/IJIMT.2014.V5.487
- **Nailah Sa'adah**, W.M.F. Wan Ishak, Essam A. Makky. *Correlation of Compost Materials and Enzyme Activity in Composting – A Review,* (**Under review)**. Submitted to *Waste Management*.