Effects of temperature and waste age on stabilization of aged waste during landfill in situ aeration

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**NANYANG
TECHNOLOGICAL
UNIVERSITY**

**EFFECTS OF TEMPERATURE AND WASTE AGE
ON STABILIZATION OF AGED WASTE DURING
LANDFILL IN SITU AERATION**

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List of Publications

Journals

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- Huanhuan Tong, Ke Yin, Apostolos Giannis, Jing-Yuan Wang, Influence of temperature on stabilization of MSW during in situ aeration of simulated landfill bioreactors, 2015, *Bioresource Technology*, 2015, 192: 149-156
- Ke Yin, Huanhuan Tong, Omar Noh, Jing-Yuan Wang, Apostolos Giannis, Mapping Refuse Profile in Singapore Old Dumping Ground through Electrical Resistivity, S-Wave Velocity and Geotechnical Monitoring, *Bulletin of Environmental Contamination and Toxicology*, 2014, 94 (3): 275-281

Conferences

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Abstract

The increasing demands for land space and pollution hazards incurred from old dumping grounds and landfills drive the need to employ in situ aeration technique to solve landfill aftercare problems rapidly. By injecting air into the landfill body, in situ aeration establishes aerobic condition in later stage of landfill, accelerates carbon transformation, reduces organic load, decreases landfill post-closure period and achieves earlier beneficial reuse of land. Several successful in situ aeration projects have been reported worldwide in the past decades with a variety of objectives.

The efficiency of landfill aeration depends on the proper system design and operation. That is why lots of efforts have been paid on selecting the appropriate well design and spacing, the effective air distribution method, the suitable air pressure and aeration rate, and sufficient duration of aeration. However, the inception time for aeration is yet to be answered. As the waste age defines the initial landfill status before aeration, the inception time for aeration plays an essential role in the success of the aeration project. Moreover, the effects of temperature on the process of aerobic bioconversion need for clarification. This information is important to indicate if there is a need for temperature control during in situ aeration and foresee the characteristics of waste from different temperature zone in the landfill. Furthermore, no studies have been particularly cared for the microbial profiles in the course of in situ aeration. Since in situ aeration mainly relies on the local microbes to degrade the organic substances, the comprehension of microbial community and its function, especially in the incipient stage, is vital for the rapid startup and the high performance in long-term running of aeration. To address raised all above concerns, the following studies were conducted to examine the impacts of waste age and temperature on stabilization of aged waste as well as the population dynamics of microorganism at different temperature during the startup phase.

In the current study, synthetically aged waste was utilized to simulate the waste composition of closed landfill or dumping ground. One reason was because the Lorong Halus Dumping Grounds (LHDG) accepted high amount of inorganic waste (> 50%) during dumping, while current waste characterization further showed that the waste ingredients were biologically inert and stabilized. Therefore waste materials from LHDG were considered unsuitable for the present study, which focused on the bioconversion process of organic matter. Another reason was ascribed to better control of the experiments, in which artificial aged wastes would minimize the influences induced by heterogeneity on the components and thus allow for more scientific comparisons.

The effects of waste age on the emissions and stability of waste materials during in situ aeration were first investigated. In bioreactors with young aged waste materials, dissolved organic carbon (DOC) rapidly accumulated in the bioreactor leachate after aerobic conditions were established. Fractional analysis showed that the elevated concentration of humic acids (HAs) was primarily responsible for the increment of leachate strength. Interestingly, elevation of HAs concentration was not observed in the aeration reactor with a prolonged waste age, as the mobility of HAs was inhibited by the high molecular weight (MW) derived from extended waste age. Without significant release of organic substrate, later aeration promoted carbon biodegradation more profoundly. In sum, the beneficial properties of higher molecular weight and more stable humic substances related with later aeration are weighed up against the benefits of aerating earlier, which facilitates earlier stabilization and larger avoidance of greenhouse gas emission but also generates higher concentrations of leachable lower molecular weight compounds. The results suggest that waste age shall always be taken into account during design of aeration system, in order to maximize the aeration efficiency.

The effects of temperature on carbon and nitrogen compounds during in situ aeration of aged waste were investigated by setting the operation temperature of three landfill bioreactors at 35, 45 and 55 °C, respectively. The results demonstrated that the bioreactor operated at 55 °C presented the higher carbon mineralization rate

throughout the experimental period, suggesting accelerated biodegradation rates under thermophilic conditions. The leachate nitrogen speciation study indicated that organic nitrogen transcended among other species, because ammonia was significantly stripped out due to aeration. In order to elucidate the fate of individual nitrogen containing constituent, fractionation of leachate organic nitrogen was conducted. Detailed investigation revealed that the most intensive bioconversion of humic nitrogen occurred in thermophilic conditions. At the end of aeration, the waste from 55 °C bioreactor featured the highest degree of biostability and richest level of humic content. All the benefits observed in 55 °C bioreactor suggested that remediation through aeration would be more efficient if thermophilic temperature could be maintained. Moreover, thermophilic zone usually exists in the middle depth of the landfill if temperature control is not implemented. Thus, after aeration the waste from this layer could provide product with a better quality if biofertilizer and soil conditioner is the expected end use for the excavated fine particles, because thermophilic temperature could increase the yield and maturity of HAs, which is a significant factor in soil fertility.

The variation of microbial community under different temperature during startup phase were assessed by comparing the 16S rRNA clone libraries derived from leachate sample of three landfill aeration bioreactors at 35, 45 and 55 °C, respectively. Compared to anaerobic control bioreactor, the microbial population under aerobic condition was more diverse as more operational taxonomic units (OTUs) were identified. In addition, the gradually established aerobic condition caused the descending of proteolytic *Bacteroidetes* in aerated bioreactors, while the rising in the percentage of metabolically diverse *Proteobacteria* with the increased temperature could partially explain why the biodegradation efficiency was increased by high temperature. Moreover, phylogenetic analyses revealed the predominant role of microaerophilic *Symbiobacterium* at 35 °C and obligate aerobic *Vulgatibacter incomptus* at 55 °C in the microbial community, verifying that a more profound aerobic condition was established at thermophilic condition most probably due to the enhanced oxygen diffusion by high temperature.

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List of Acronyms

Acronym	Definition of Acronym
ACN	Acetonitrile
ANOVA	Analysis Of Variance
AR35	Aerated Landfill Bioreactor Under 35 °C
AR45	Aerated Landfill Bioreactor Under 45 °C
AR55	Aerated Landfill Bioreactor Under 55 °C
ATP	Adenosine-5'-Triphosphate
BH	Borehole
BOC	Biodegradable Organic Carbon
BOD	Biochemical Oxygen Demand
C&D	Construction and Demolition
COD	Chemical Oxygen Demand
CPG	CPG Consultances Pte Ltd
DI	Deionised
DM	Dry Material
DNA	Deoxyribonucleic Acid
DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
EU	European Union
FAs	Fulvic Acids
GC	Gas Chromatography
GS ₂₁	Gas Generation Potential Detected in 21 Days
HAs	Humic Acid
HS	Humic Substances
Hyl	Hydrophilic Compound
IC	Ion Chromatography
JTC	Jurong Town Corporation
LFG	Landfill Gas
LHDG	Lurong Halus Dumping Grounds
LR35	Aerated Landfill Bioreactor With Longer Waste Age Under 35 °C
L/S	Liquid/Solid
LSD	Least Significant Difference
LSRs	Landfill Simulation Reactors
MALDI	Matrix-assisted Laser Desorption/ Ionization
MH	Monitoring well converted from borehole
MS	Mass Spectrometry
MSW	Municipal Solid Waste
MW	Molecular Weight
MWC	Montgomery Watson Consultances Pte Ltd
NMR	Nuclear Magnetic Resonance
NMOCs	Nonmethane Organic Compounds
NR35	Nonaerated Landfill Bioreactor Under 35 °C
ORP	Oxidation-Reduction Potential

Acronym	Definition of Acronym
OTU	Operational Taxonomy Unit
PAH	Polycyclic Aromatic Hydrocarbons
PCR	Polymerase Chain Reaction
R3C	Residues and Resource Reclamation Centre
RFLP	Restriction Fragment Length Polymorphism
RL ₄	Respiration Index Detected in 4 days
TC	Thermal Conductivity Detector
TFA	Trifluoroacetic Acid
TKN	Total Kjehldahl Nitrogen
TN	Total Nitrogen
TOF	Time of Flight
TOC	Total Organic Carbon
TPH	Total Petroleum Hydrocarbons
VFAs	Volatile Fatty Acids
VOCs	Volatile Organic Compounds
XOC	Xenobiotic Organic Compounds
ZR35	Aerated Landfill Bioreactor With Longest Waste Age Under 35 °C

Chapter 1 Introduction

1.1 Background

The natural environment is suffering the negative consequences of municipal solid waste (MSW) landfilling, as many old dumping grounds did not incorporate the engineering design to better isolate the waste from the surrounding environment. Even in landfills designed with protection layers, uncontrolled leachate and landfill gas (LFG) may be released due to defects of the aged liner system, eventually causing threats to atmosphere and water bodies and groundwater resources. On top of that, the long-term adverse environmental impact caused by MSW landfills may last for decades, or even centuries (Bilgili et al., 2007b).

The most concern about landfill redevelopment is the health and safety issue of the future users on the reclaimed land. A comprehensive remedial action at a closed landfill could be listed as follows: capping of the site, managing landfill leachate and gas, monitoring the impact on the environment, and mitigating the potential of site differential settlement (Ayalon et al., 2006). As a matter of fact, the key issue is to stabilize the organic fraction in waste materials, as it is the active part of landfill that generates the organic emission and thereby the environmental risks.

Provided that adequate precaution has been undertaken to minimize the potential hazardous problems prior to redevelopment, building on landfill sites can be carried out in an acceptable fashion. For example, the Hiriya landfill in Israel, which received waste from 1952 to 1999, has been successfully rehabilitated and a green park was constructed directly over the encapsulated waste (Sandler, 2011). Intensive development of reclaimed landfill is also documented. Take Seaboard Point Resort in North Wildwood of New Jersey for instance, residential condominiums were built on pile structures over the capped waste (Wiley and Assadi, 2002).

Among all the rehabilitation techniques, in situ aeration is a promising one. By supplying a controlled amount of air to landfills, in situ aeration promotes

aerobic biodegradation via interruption of existent anaerobic process (Ritzkowski et al., 2006). In contrast to the slow rate presented by anaerobic pathway, in an aerobic environment biodegradable organic matter together with those semi-degradable and hardly degradable organics such as cellulose and lignin could be oxidized into carbon dioxide in a significantly shorter timeframe (Jayasinghe et al., 2014). This provides more immediate benefits, such as the rapid reduction of biological oxygen demand (BOD), ammonia, methane and odor compounds (El Fadel et al., 2013; Hrad et al., 2013; Raga and Cossu, 2014; Read et al., 2001a). In the long term, landfill stabilization is achieved more rapidly by an factor of 2-4, thereby lowering the cost for post closure care, and eventually earlier beneficial reuse of the land may be possible (Heyer et al., 2005b; Rich et al., 2008; Ritzkowski and Stegmann, 2013).

Over the last several decades, full scale aeration has been successfully demonstrated worldwide with various aims (Chong et al., 2005; Hrad et al., 2013; Read et al., 2001b). In Germany, in situ aeration has been used as a landfill remediation technique to seek regulatory relief of closure and postclosure liability (Ritzkowski and Stegmann, 2013). In Italy and Netherland, aeration has been practiced as pretreatment method before landfill mining (Jacobs et al., 2003; Raga and Cossu, 2014). To optimize the aeration process, a series of operation parameters has been studied such as air pressure, aeration rate, air injection mode (active or passive, continuous or intermittent), aeration efficiency, moisture and temperature control, and off-gas treatment (Chong et al., 2005; Heyer et al., 2005b; Hrad et al., 2012; Öncü et al., 2012; Prantl et al., 2006; Ritzkowski and Stegmann, 2012; Yazdani et al., 2010).

Laboratory simulation and full-scale in situ aeration systems have provided useful information on the performance and procedures of aeration technique (Rich et al., 2008). However, there are a number of areas that remain little touched, such as the influences of waste age, the effects of temperature, and the transition of microbial profile. The exploration on the above mentioned aspects is necessary, as the gained insights could provide useful references for the system design and

operation in an aeration project, such as the proper time point of starting aeration, the target value of temperature control, and the effective strategy for enhancing the performances of indigenous microbes.

1.2 Objective

The objective of this research is to study the effects of waste age and temperature on waste stabilization during in situ aeration as well as the variation of microbial community during the start up phase mediated by temperature. Eventually the key parameters associated with in situ aeration were identified to serve for the better design of landfill stabilization and redevelopment.

1.3 Scopes

To obtain the above objective, the following scopes were conducted (Figure 1-1):

- To select proper waste materials for laboratory experiments
- To investigate the effects of waste age on the performance of in situ aeration to determine the best inception period for aeration in traditional anaerobic landfills and dumping grounds.
- To understand the influences of temperature on waste stabilization processes during in situ aeration and provide references for temperature controlling
- To analyze the population variation of microorganism at different temperature in the beginning stage of aeration so as to develop the strategy for rapid startup

Figure 1-1 Schematic diagram of the proposed research scopes

1.4 Organization of the thesis

This thesis consists of 8 chapters. It starts with a brief introduction to the research background, objective, scopes of the work, and the organization of the report. Chapter 2 covers the literature review about the demands for landfill reclamation, the development of in situ aeration remediation technology, and the research gaps. Chapter 3 presents the possibility study of using the waste materials from a local dumping ground as the experimental substrate. Based on the conclusion drawn from Chapter 3, synthetic aged waste was selected as the studying substrate instead of waste excavated from local dumping site. Chapter 4 includes the preparation procedure of synthetic aged waste and the methodologies applied to realize the research scope. The experiment results regarding the effects of waste age are presented, analyzed and discussed in Chapter 5, while Chapter 6 focuses on the influences of temperature on the carbon and nitrogen conversion during aeration. Chapter 7 contains the findings related with the microbial population and their contribution to the biodegradation process during startup phase under different operation temperature. At last, the important findings of this study and recommendations for further research are summarized in Chapter 8.

Chapter 2 Literature Review

2.1 MSW generation

Solid waste is a consequence of life, as it comprises all the solid-state materials thrown away as useless or unwanted during living activities. MSW is made up of household and commercial waste, and does not contain industrial, hazardous, or construction waste. MSW generation rate is closely correlated to population, urbanization and prosperity. According to 2007 statistical data from the Intergovernmental Panel on Climate Change (Bogner et al., 2007), solid waste generation rates fluctuated in the range from <0.1 t/cap/yr in low-income countries to >0.8 t/cap/yr in high-income industrialized countries. Globally, approximately 1.3 billion tons of MSW are generated per year currently, and the number is expected to increase to approximately 2.2 billion tons per year by 2025 (WorldBank, 2013). In particular, MSW generation rates will more than double over the next twenty years in lower income countries. As shown in Figure 2-1, only about 18% of MSW generated is recycled, 18 % is converted to energy through incineration, 9% is composted, 9% is treated by other methods, and the rest (46%) is deposited in open dumpsites or landfills (WorldBank, 2013).

Figure 2-1 Contribution of different MSW disposal methods worldwide

2.2 Traditional anaerobic landfill process

As demonstrated by Figure 2-1, landfilling is still most common practice of waste disposal nowadays, due to its cheapest initial investment and easy operation (Masi et al., 2014). A landfill is an engineered site for waste disposal or waste burial. Although temporarily the waste is hid away from human living area, potentially this could generate numerous environmental problems and eventually threatens human society. The main culprit is the organic fraction in MSW, as their biodegradation processes produce gaseous and liquid emissions and bring the environmental risks.

In general, the biodegradation of organic component after placement in landfill is considered to occur in five sequential phases, as illustrated in Figure 2-2 (Tchobanoglous et al., 2003; Vesilind et al., 2002).

- Phase I (Initial adjustment): The biological decomposition starts with an aerobic stage, as a certain amount of air is contained in the waste layer during landfilling. With the presence of oxygen, the organic compound is bioconverted into carbon dioxide, water and partially degraded organic materials. The temperature is increased due to the exothermal microbial metabolism and low amount of carbon dioxide is detected. This stage will continue until the oxygen trapped in the voids of the refuse is consumed.
- Phase II (Transition phase): The landfill transfers from aerobic condition to anaerobic condition gradually as oxygen is depleted. Facultative anaerobic microorganisms predominate and nitrate and sulfate replace oxygen becoming the electron acceptors. Organic polymers and lipids are hydrolyzed into complex dissolved organic compounds and organic acids. The pH and alkalinity of the leachate drops due to the dissolution of elevated concentrations of carbon dioxide and organic acids. High level of chemical oxygen demand (COD) and BOD are often found in the leachate.
- Phase III (Acid phase): The acidogens are responsible for converting the dissolved organic compounds and organic acid into volatile fatty acids

(VFAs), CO₂ and small amount of H₂. The accumulation of VFAs in acid phase is characterized by a decrease in pH (a value of 5 or lower) and an increase in COD and BOD. In some cases, the low pH solubilizes a number of inorganic heavy metals into leachate.

- Phase IV (Methane fermentation): VFAs and H₂ are utilized in this phase by methanogens to generate end products, principally methane and carbon dioxide. The removal of VFAs boosts the pH to more neutral values of 6.8 to 8, and the concentration of COD and BOD drops accordingly.
- Phase V (Maturation phase): It occurs after significant reduction of readily biodegradable organic matter. Methane generation and other landfill gases production declines, and oxygen may begin to diffuse back into the landfill via the atmosphere. This phase presents stable low concentrations of leachate constituents and slowly biodegradation.

The general anaerobic transformation of solid waste can be described by the following equation:

As discussed before, anaerobic degradation is realized by mutualistic consortia of organisms. Methane and carbon dioxide are the two main carbon products. Apart from utilization in cell growth, the left carbon is retained in the resistant organic matter, e.g. humus. Nitrogen and sulfur are released in the forms of ammonia and hydrogen sulfide.

Figure 2-2 The five phases of waste degradation in anaerobic landfill (Reinhart and Townsend, 1998)

2.3 Problems associated with redeveloping anaerobic landfill

During landfill reclamation, the developer faces with a number of problems, such as potential hazards caused by natural occurring biological and physicochemical processes. Concerns include LFG release, leachate pollution and uneven settlement.

2.3.1 Landfill gas

LFG consists mainly of methane (60%) and carbon dioxide (40%), from a typical domestic waste landfill undergoing anaerobic decomposition. When methane emits into air and reaches concentration in the range of 5~15%, an explosion might occur if a source of ignition is present. Moreover, methane is a strong greenhouse gas, which has a notorious global warming effect 20-25 times higher than CO₂ due to its stronger ability to absorb infrared radiation (Singh and Strong, 2016). According to Ritzkowski and Stegmann (2010), landfills emitted 40-60 M tons of CH₄ annually and was the third largest source (11-12%) of anthropogenic methane.

The other potential problems related to LFG production are odor and some nonmethane organic carbons (NMOCs). NMOCs, including aromatics, chloro compounds, carbonyls and nitro compounds, usually take up 0.01–0.6% of landfill gas (Majumdar et al., 2014). These gaseous nuisances could expose redevelopment workers and future land users to a toxic circumstance, as many of them are carcinogens or pose serious non-cancer hazard (Majumdar et al., 2015; Zhang et al., 2014).

2.3.2 Landfill leachate

Leachate forms when precipitation, surface run-off, or water originating from waste biodegradation flows through landfill cells, picks up dissolved and suspended materials and moves to the bottom or sides of a landfill. Leachate contains a high pollutant load and its strength varies widely depending on many factors like weather, waste composition, landfill age, depth of the fill, compaction and permeability (Mukherjee et al., 2015). The major groups of pollutants likely to be found in

landfill leachate are organic matter, inorganic ions and nutrient, heavy metals and xenobiotic organic compounds (XOCs) (Mouser et al., 2010).

Lack of reliable information and budget, many landfills/dumping sites do not incorporate engineering barriers to isolate the waste from surrounding environment. Interaction between landfill and shallow unconfined aquifer or nearby water body can negatively affect groundwater and surface water quality. The negative consequences include depletion of dissolved oxygen by organic matter, eutrophication by inorganic nutrient, carcinogenicity and ecotoxicity by heavy metals, and genotoxicity and cytotoxicity by XOCs (Clarke et al., 2015; Le Mer and Roger, 2001; Masoner and Cozzarelli, 2015).

2.3.3 Settlement

The major geotechnical problems arising from construction on landfills are caused by settlement. Over an extended period of time, the landfill may lose about 20-30% of its initial height (Liu et al., 2011b). Moreover, landfill settlement generally shows a non-uniform pattern due to the great spatial unevenness in waste composition and biodegradation (Viswanadham et al., 2012). This creates differential settlement which significantly compromises the structure integrity constructed in the landfill. Problems associated with differential settlement include structural failures of buildings, breach of the surface cover, and damage to buried services such as the surface water drainage system, leachate and LFG gas collection systems, and underground utilities pipelines and connections for water, wastewater, fuel, electricity, gas, and telecommunications systems (Simões and Catapreta, 2013). Therefore, landfill settlement potential should be sufficiently reduced before infrastructure construction.

The settlement behavior in landfills is complex and may potentially be divided into three main stages. They are immediate settlement (resulting from the void reduction due to the compression of landfill loading), primary settlement (caused by the slow dissipation of pore pressures of liquids and gases), and secondary settlement (attributed to biodegradation and mechanical creep) (Siddiqui et al.,

2013). The first two stages occur during and shortly after waste placement, while the last stage can last decades (Fei and Zekkos, 2013). During the long-term settlement, biodegradation is identified as the major contributor. Reducing the amount of biodegradable component and increasing the biostability of waste materials are effective methods to decrease the magnitude and rate of long-term landfill settlement.

2.4 Fundamental processes during in situ aeration

Many approaches have been developed to counteract the negative effects induced by landfill, such as capping, reactive barrier, and phytoremediation. However, the objections of these technologies for reasons of high cost and/or limited capacity have hindered their wide adoption. Differently, in situ aeration targets to reduce the degradation potential of the organic waste before problems occur (Read et al., 2001b).

Major technical components of a typical in situ aeration system include (1) gas inject wells, (2) air compressors, and (3) off-gas collection piping and treatment equipment. The atmospheric oxygen is compressed into the waste layer through gas inject wells. After the oxygen is utilized by the aerobes, the exhausted gas is collected and further treated in a controlled manner. Figure 2-3 shows the conceptual arrangement of the in situ aeration system.

The anaerobic degradation in landfill is interrupted by the added air. Instead, these biologically available matters are converted into their fully oxidized forms of carbon dioxide and water. The generalized chemical reaction for the aerobic decomposition of solid waste can be expressed as:

As illustrated in Eq. (2.2), the end products of an aerobic process are primarily carbon dioxide and water which are the stable, oxidized forms of carbon and

hydrogen. Nitrogen and sulfur in the starting material are also transformed into their oxidized forms-nitrate and sulfate.

Figure 2-3 Schematic diagram of typical components of an in situ aeration system (Ritzkowski et al., 2006)

2.4.1 Quick decomposition through in situ aeration

Compared to anaerobic decomposition, aerobic composition has faster kinetics. It means that in a certain period, the carbon discharge may be increased by aeration or in other words for a certain carbon discharge the time span could be shortened. Taking the Kuhstedt landfill in Germany as an example, after 1400 days of aeration, 180% carbon discharge was achieved in comparison with the calculated carbon discharge under anaerobic conditions (Ritzkowski et al., 2006). Moreover, acceleration factor of 3-4 was reported for the Milmersdorf landfill, Germany (Heyer et al., 2005a). This phenomenon could be understood in terms of the principles of cell energy conservation in fermentation and respiration. Taking glucose degradation for example, different degradation pathway under fermentation and respiration could be summarized as the following (Madigan et al., 2006):

In fermentation, the redox reaction occurs in the absence of oxygen, which only releases a few ATPs. The mechanisms behind are due to that (1) the carbon atoms in the initial substrate are only partially oxidized, and (2) the difference in reduction potential between the primary electron donor and final fermented product is small. By contrast, in respiration, with the presence of oxygen, the glucose is oxidized completely to CO_2 and yields more ATP. As a consequence, respiration heterotrophs enjoy higher growth rates and cell yield than heterotrophic organisms which are strictly fermentative (Murphy et al., 1994). In situ aeration takes this primary advantage to achieve a rapidly stabilized landfill.

2.4.2 Changes in landfill gas quality through in situ aeration

Another driving force for in situ aeration is gas pollution control. The main components of LFG are CH_4 and CO_2 , with approximate concentrations of 45-60% CH_4 to 40-60% CO_2 in the stable methane fermentation phase. By shifting to aerobic conditions, the methane concentration in LFG drops to below 5%, while the carbon dioxide contents range between 6 and 12% (Heyer et al., 2001). The reduction of CH_4 concentration can be attributed to three possible factors: dilution from added air, suppression in methane production due to the aerobic conditions, and biological oxidation of methane. Although dilution takes a large part of responsibility, the significant decrease of CH_4/CO_2 ratio indicates the aerobic decomposition takes place in the large area (Powell et al., 2006). After aeration ceases, the anaerobic condition may gradually be regained. Since the waste has already been stabilized during aeration, the subsequent anaerobic condition will not be able to generate high amount of CH_4 . In laboratory, very low gas generation (with no methane) was observed after stopping aeration (Prantl et al., 2006). Data from the Modena landfill showed that although methane reappeared after stopping 3-month aeration, the values measured were much lower than the initial ones (35% before start, 15% after aeration ceased) (Cossu et al., 2003a). Methane has a much stronger greenhouse potential than carbon dioxide. By means of in situ aeration,

greenhouse gas releases can be significantly reduced. It was reported that 77% of theoretically emitted CO₂ equivalents caused by methane emission was reduced by aeration in Kuhstedt landfill, Germany (Ritzkowski et al., 2006).

Other gases emitted from landfill including hydrogen sulfide and NMOCs are also reduced by aeration. The NMOCs concentrations was found around 37.0 ppmv (as hexane) in the aerobic portion in a Georgia landfill, US, while concentration from anaerobic portions averages 146.99 ppmv as hexane (Berge et al., 2006b). However, CO concentration increases as the result of aeration (Powell et al., 2006). This could be caused by oxidation of methane and NMOCs. Since CO can have adverse effects on human health and tropospheric formation of ozone, more attention should be paid on it.

2.4.3 Changes in leachate quality and quantity through in situ aeration

Aeration achieves improvement in several measures of leachate quality, including BOD, COD, ammonium and volatile organic compounds (VOCs). The rapid reduction of the COD occurs in the first period of aeration. During further operation, only a slight decrease occurs and finally the COD value would reach a limited value, because the further COD reduction is rather caused by chemical-physical leaching processes. Prantl et al. (2006) reached a value of 180 mg/L after 273 days of aeration from initial value of 1000 mg/L in landfill simulation reactors (LSRs), while Cossu (2003b) decreased the COD value from 30000 mg/L to 1000 mg/L after 120 days aeration for lab columns.

Hudgins and Harper (1999) reported a 75%-99% reduction by aeration in leachate VOCs of Columbia County landfill, which had been accepted MSW, C&D waste, treated lumber and wood waste. After 5 months of air injection, acetone dropped from 750 ppm to less than 250 ppm; methyl-ethyl ketone decreased from 1,800 ppm to 300 ppm, and toluene was lowered down from 250 to 50 ppm.

Nitrogen is mainly present in the form of protein. As protein is hydrolyzed into amino acid and the latter substance is further broken down, ammonia is liberated

into leachate. This deamination can take place in aerobic and anaerobic conditions, with some differences in reactions (Sawers, 2001).

The oxidative deamination only occurs under aerobic condition and can be illustrated as follows:

The NAD-linked oxidative deamination and elimination deamination can be written as Eq. (2.6) and (2.7). These two reactions can take place both under anaerobic and aerobic conditions.

There are more deamination pathways under aerobic condition than anaerobic condition (Shao et al., 2013). Thus the ammonium release speed and the degradation rate of amino acid are faster under aeration condition.

Leachate with high concentration of ammonium could be emitted from anaerobic landfills and cause high environmental risk, because of no biodegradation pathway for ammonium under anaerobic condition (Chung et al., 2015). By contrast, aeration could remove leachate ammonium effectively, because ammonium could be biologically converted into nitrate and nitrite following below pathways (Berge et al., 2005).

However, the elevation of nitrate and nitrite concentrations observed was not expected as much as the stoichiometrical calculation if nitrification had occurred, suggesting de-nitrification processes occurred as well (Ritzkowski and Stegmann,

2005). Although air was continuously added to the system, anaerobic pocket still existed in the system due to the heterogeneous nature of solid waste (Giannis et al., 2008). Nitrate removal may occur via heterotrophic denitrification and autotrophic denitrification in those anaerobic pockets, as shown in the following reactions (Ghafari et al., 2008; Moraes et al., 2012):

Berge et al. (2006a) suggested that almost 70% of the nitrate was denitrified via heterotrophic denitrification, while 30% of nitrate removal was attributed to autotrophic denitrification.

Nitrous oxide was produced as the byproduct of either partial nitrification or denitrification. Considering the heterogeneous nature of solid waste and high temperature characteristics of aerobic landfill, it is difficult to maintain and control sufficient oxygen levels throughout the landfill, which may result in oxygen deficiency in certain local area. Under oxygen-limiting conditions, nitric and nitrous oxides may be produced by autotrophic ammonia-oxidizing bacteria (Mampaey et al., 2013). On the other hand, nitrous oxide can also be byproduct of denitrification when sufficient organic carbon source is not available (Cheng et al., 2004; Khalil et al., 2004). However, the nitrous oxide may be converted to nitrogen gas before emitting the landfill, given that long residence times are presented. Data from the field did not show an obvious change in the concentration of N_2O during aeration (Powell et al., 2006).

In-situ aeration also has the potential to reduce the volume of leachate which needs ex-situ treatment. Due to intensive aerobic conversion processes, the thermal energy being released could bring the temperature in the landfill body up to the thermophilic range (50-70 °C) (Berge et al., 2006b). This high temperature will lead to leachate volume reduction through enhanced evaporation process. It is reported that the leachate treatment need was reduced by 86% in the 14 months of aerobic treatment in Columbia County Landfill (Read et al., 2001a).

2.4.4 Changes in waste material though in situ aeration

In-situ aeration could reduce the emission potential of MSW remarkably, in terms of organic content and its stability. The characterization of waste stability by only one single parameter is rather difficult due to the distinctive heterogeneity of waste sample. Therefore a combination of several parameters is used to determine the effects of in situ aeration process. The respiration index (RI_4) and the gas generation potential (GS_{21}) were used to describe the biological activity of the waste. In details, RI_4 measured the accumulated oxygen consumption of waste materials under aerobic condition in consecutive 4 days, while GS_{21} detected the volume of gas generated by the waste materials seeded with digested sludge under anaerobic condition in consecutive 21 days. In addition, leaching test was applied to define the stabilization status. Total organic content (TOC) and Total Kjeldahl nitrogen (TKN) can shed light on the organic matter content, although both of them do not sufficiently describe the stabilization process of the waste material under the influence of aeration (Ritzkowski et al., 2006).

A number of previous studies have shown that emission potential could be significantly reduced in the course of landfill aeration. Cossu et al. (2001) has noticed low values ($< 0.5 \text{ mg O}_2/\text{g dry materials (DM)}$) of RI_4 (10% of the initial value) and zero biogas production in GS_{21} test after 10 weeks aeration in lab test. In line with these parameters, the BOD and COD concentration in leaching test also showed significant reduction in the experiment. It was found that more than the half reduction of TOC and TKN of the wastes from Kuhstedt landfill after six years of aeration (Ritzkowski and Stegmann, 2007). It is obvious that during in situ aeration

the nitrogen pool (potential for ammonium-nitrogen release) and carbon pool (potential for methane and leachate COD release) have been reduced considerably.

Aeration leads to accelerated accumulation of humic acids, which are recalcitrant organic compounds and have a low degree of reactivity (Tesar et al., 2007). Moreover, compared to anaerobic area, humic acid extracted from aerobic area are more stable, possessing the characteristics of higher contents of aromatic compounds and higher degree of polymerization and condensation (Lguirati et al., 2005). Rapid maturation of humic acid reflects quick stabilization of organic matter caused by aeration.

2.4.5 Changes in settlement speed through in situ aeration

The process of biological decomposition of the waste mass causes differential subsidence, which could compromise the stability of the structure (Chakma and Mathur, 2012). However, this risk would be greatly reduced if most of the organic matter in the wastes was decomposed before the redevelopment (Read et al., 2001b). Erses et al. (2008) showed a 37% settlement of the waste height at 374 days in aerobic bioreactor as compared 5% after 630 days in the anaerobic bioreactor. Since settlement in aerobic condition will be largely complete soon after landfill closure, the owner not only save annual landfill post-closure maintenance costs, but also obtain the potential economic benefits associated with the land value of sites reclaimed for other uses.

2.4.6 Changes in post-closure period through in situ aeration

Once a landfill has reached its design capacity and closed, the site owner is required to monitor and maintain the closed landfill to ensure that waste is not posing a polluting threat to the surrounding environment. Indian regulation specifies a 15-year post-closure (Agamuthu, 2006), while the post closure care should continue at least 30 years in US (Laner et al., 2012). EU council and Australian Environment Protection Authority stipulate that the landfill operator shall be responsible in the aftercare phase until the landfill could not present hazards to environment (European Commission, 1999; NSW EPA, 2015). Different regulations among the countries/regions require a general duration of decades in

waste post-closure. Actually, the polluting potential of landfill will not be eliminated even in decades if just letting the site be subjected to natural attenuation after closure. Through the modeling of the Kiefer Landfill in Sacramento County, California, Hudgins and Richgels (2007) pointed out that it would take more than a half century after landfill closure to let the emitting NMOCs decrease below the threshold value of 50 Mg/year.

In situ aeration could reduce these troublesome decades of natural attenuation to merely a couple of years, and at the same time assure the same stabilization of the landfill to be achieved. According to Hudgins and Peck (2005), through aeration the waste can be stabilized in generally less than 4 years. In this way, in situ aeration releases the landfill owner from long-term burden.

One important concern of applying in situ aeration is cost, as it requires a considerate expenditure of energy to overcome the natural act of anaerobic degradation. Since air must be supplied to the landfill, capital costs will increase due to the requirement of air blowers and piping. The subsequent operating cost of electricity is also high in aerobic system, as the power is required for air compression and injection (Reinhart and Townsend, 2001). Table 2-1 shows the aeration cost for the three German sites. Cost are calculated to 0.5-1 €/m³ under optimized condition. When the general conditions are unfavorable, the cost may rise up to 2-3 €/m³ (Heyer et al., 2005b). However, these additional costs could be balanced by future economic benefits, which bring earlier profitable after-use, lower leachate treatment cost, shorter post-closure period, less strict surface sealing and lesser monitoring frequency (Berge et al., 2009). Heyer et al. (2005b) pointed out that compared to anaerobic conditions at least 20-25% cost reduction could be achieved by aeration if long-term monitoring and aftercare of the landfill was considered.

Table 2-1 Investment and operating costs for three German landfills using low pressure aeration (Heyer et al., 2005b)

Component	Kuhsteht	Amberg	Milmersdorf
Investment and operating	650,000	650,000	640,000
Landfill volume (m³)	220,000	420,000	580,000
Unit cost (€/m³)	3.0	1.5	1.1

2.4.7 System monitoring during in situ aeration

Along with aeration, monitoring scheme should be carefully designed, which is necessary for evaluating system performance, adjusting aeration parameter, and determining the endpoint of aftercare. In the gaseous phase, gas composition (CO₂, CH₄ and O₂) and oxygen utilization rate should be measured to reflect the bioconversion rate (Giannis et al., 2008; Ritzkowski et al., 2006). Evolution of temperature and settlement in the waste layer could be used as auxiliary tools to assess the stabilization (Heyer et al., 2005b; Raga and Cossu, 2014). Leachate load needs to be analyzed in terms of dissolved organic carbon (DOC), COD, BOD, ammonium, nitrite/nitrate, heavy metal and toxicity (Bilgili et al., 2007a).

Characterization of leachate and gas emission can indicate maturity and stability of waste materials. However, these indirect measurements are insufficient to assess waste decomposition state. For example, observation of low gas production does not always lead to the conclusion that the waste in the landfill is well-decomposed. It could also be caused by degradation inhibition, like what happens in dry landfill. When infiltration water is minimized in those dry tombs, low emission rate is present, however with significant amount of active waste remaining (Read et al., 2001a). Barlaz et al. (2002) also argued that low BOD/COD ratio was necessary but not sufficient indicator to claim a mature landfill. As leachate sample is collected at the bottom of a landfill, its strength is more associated with the bottom layer refuse. When the upper layer is in the different stage with the bottom layer, the leachate investigation may misrepresent the real situation. Thus waste characterization is required to clearly understand the waste

stability. In this regards, the RI_4 , GS_{21} and TOC on solid sample were proposed (Prantl et al., 2006). A carbon balance could help to validate if the targeting reduction of waste emission potential has been achieved (Ritzkowski and Stegmann, 2013). Investigation on the stable isotope signatures of leachate was newly employed as a cost-effective method to characterize landfill stabilization state (Wimmer et al., 2013).

2.4.8 Termination criteria of in situ aeration

Termination criteria of in situ aeration were used to define an endpoint of aeration practice. Considering the heterogeneity of the waste, Ritzkowski et al. (2006) suggested criteria which encompass all important aspects of the aeration process. More details are listed in Table 2.2. The limit values for RI_4 and GS_{21} were set to 4 mg O_2 /g DM and 10 L/kg DM respectively. These demands are considered to be met if 4 out of 5 waste samples are below the limit values and none exceed 10 mg O_2 /g DM and 20 L/kg DM. The eluate criteria resembles that in Appendix II of the German Waste Disposal regulation (AbfAbIV, 2001), and modified to allow single parameter value to exceed the target value by up to 50%. By operating LSRs, the carbon load in the leachate phase and gas phase is obtained. The sum of the carbon discharge in these two pathways (referred to the unit dry mass) could be set at the maximum amount of biodegradable organic carbon (BOC) under optimized condition. In full scale application, a reduction of 90% of BOC could be set as a targeting value (Ritzkowski and Stegmann, 2013), due to the suboptimal conditions in the field. Additionally, clearly decreasing tendency of settlement and temperature inside the landfill body could be used as supplement parameters, because both of them indicate low bioactivity.

Table 2-2 Criteria of termination of in situ aeration measures (Ritzkowski et al., 2006)

Criteria	Definition	Suggested value resp. dimension
BOC-conversion rate	Determination of the actual converted TOC by means of off-gas monitoring, target value according to LSR-tests carried out with original waste material	$C_{\text{discharge, aerated landfill}} > 90\%$ BOC
GS₂₁	Residual amount of LFG to be produced after the completion of the active aeration (determined by means of gas formation potential tests)	$GS_{21} < 10 \text{ l/kg DM}$
RI₄	Residual biological activity measured indirectly via the respiration index	$RI_4 < 4 \text{ mgO}_2/\text{g DM}$
Eluate criteria	Extractable residues and water soluble compounds determined as pH, conductivity, organics (TOC, AOX), ammonia-nitrogen and heavy metals	As prescribed in Annex II of the German waste disposal regulation; where applicable 50% exceeding permitted
Settlement and subsidence	Measuring data extrapolated for the total landfill area, average value	Clearly decreasing tendency
Temperatures inside the landfill body	Waste temperature, measured in monitoring wells. Depths of the wells should represent the total landfill thickness	Declining, tendency towards initial waste temperature prior to aeration

2.4.9 Transferability of laboratory results to full-scale project

Laboratory studies of landfill aeration bioreactors have successfully demonstrated the positive effects of aerobic biodegradation on carbon conversion, gas composition and leachate quality. Ritzkowski and Stegmann (2013) observed that > 97.5% of the maximal biodegradable carbon discharge could be reached within 19-month aeration in the laboratory study. Almost no methane was released and leachate ammonium concentration immediately dropped below detection limit after aeration commencement in bioreactors (Hrad et al., 2013). However, similar results were not achieved in full-scale projects. In order to achieve the same amount of carbon discharge as that had been realized within 19-month in the lab-scale, a period between 5.4 and 8.2 years may be required in a full-scale aeration (Ritzkowski and Stegmann, 2013). Meanwhile, low level of methane (5%-10%) was persistent in the extraction gas and ammonium decreased slower than expectation (Hrad et al., 2013).

Several factors could contribute to the different performance between the lab-scale and full-scale aeration. First, the aeration was more homogenous in the bioreactor. In the real landfill, the undesired anaerobic activity was increased due to the heterogeneous nature of the waste, the existence of gas preferential pathways, areas with water pooling as well as the inevitable shutdown of aeration measures because of system failure and maintenance. Moreover, the operation conditions in the lab were optimized, such as the optimal temperature, sufficient aeration rate and enough water supplies. In contrast, these perfect situations are unrealistic or, at least very difficult to be realized. Besides, the homogenization and size reduction of the waste materials in lab study also provided larger surface area to maximize the bioactivity and shorten the whole landfill aging period (Hrad et al., 2013). The major aspect to be concerned should be the temperature. Aeration could increase the temperature of waste bodies up to 60 °C. However, most lab reactors in the literature were operated in temperature control room of 35 °C. The heat generated soon dissipated in the atmosphere, leading to a constant temperature of 35 °C inside the waste body. These experiment set-ups lessen the influence of temperature, which however is the key factor influencing the bioprocess during landfill aeration.

In order to better adopt the laboratory results, the following measures are suggested. When choosing the target value for the complete carbon bioconversion during full-scale projects, it was recommended to make a reduction of 10 % from the maximum value obtained from laboratory investigation (Ritzkowski and Stegmann, 2012). Moreover, laboratory experimental design should take the temperature effects into account. Last but not least, further research is needed to improve the air distribution, as one significant hurdle in the field application will be to overcome channeling effects which will occur even in homogenous materials. With the presence of the channels, or the preferential pathways, injected air tends to flow along these shortcuts, and bypass other areas in the landfill, which results in incomplete oxygen utilization and limited influencing zone of aeration.

2.4.10 System optimization during full-scale projects

In situ aeration is a relative new and popular research field and many aspects still need further improvement.

Aeration rate: In the literature, aeration rate have been carried out differently. falling in the range of 0.03 L/(min • kg waste) to 1.0 L/(min • kg waste)(Bilgili et al., 2007b). However according to Prantl et al. (2006), the high aeration rate can only achieve minor benefits in the beginning stage. Thus based on economical principal, aeration rate should be controlled at an expedient level. Similar to bioventing, suitable air supply rate could be estimated by waste oxygen utilization rate (Giannis et al., 2008), which could be measured by respiration tests in the field.

Air injection: To aerate landfill as efficiently as possible, air injection system should be properly designed to meet the oxygen demanding in different portion of the site. Ritzkowski and Stegmann (2012) directed the air to the target zone by flexibly changing the function of the gas wells between injection and extraction. In order to make sure air intrude into waste at various depths, a series of vertical gas injection wells with different length could be employed. The perforated gas well could even be inserted into the unsaturated soil zone underlying the wastes. In this case, the soil could serves as air distribution layer for even aeration.

Moisture control: Water, as a fundamental element, is needed by metabolic and physiological activities of microorganisms. Moreover, the liquid convection could facilitate microorganism transportation, which can improve organic matter degradation (Shimaoka et al., 2011). However, high water content reduces the air-filled porosity, and limits oxygen diffusion (He et al., 2011). Therefore, the water content during aeration should be carefully adjusted based on site condition. In one Italian aeration project (Cossu et al., 2003a), leachate extraction wells were installed to lower the high level of accumulated leachate. In other situation, leachate may be recirculated or extra water could be added to compensate the moisture loss due to evaporation. In some times, irrigation would even be used as a measure to

lower the refuse temperature in order to lower down the risks of self-ignition (Öncü et al., 2012).

2.5 Different operation mode of aeration

According to specific objective and local situation, aeration has been operated in different modes to best meet the requirements.

2.5.1 Continuous aeration

In this operation mode, air is continuously pressed into the landfill body through gas wells. Take the aeration project on Kuhstedt Landfill for example, the net operation time took up 81% during the 5 years operation. The stop of aeration was mainly due to testing and maintenance needed (Ritzkowski and Stegmann, 2007). The aeration volume was adjusted to the oxygen demand of the waste, in order to supply sufficient oxygen as well as prevent energy wasting. Wide range of aeration rates were used in pilot and full scale landfill aeration studies (Bilgili et al., 2007b). Prantl et al. (2006) pointed out that in the initial stage high aeration rate could achieve faster degradation rate than low aeration rate. However, the effect of aeration rate in the latter stage was less obvious.

2.5.2 Semi aeration

Continuous active aeration consumes a huge amount of energy to pump air into the landfill. In semi-aerobic landfill, however, air could flow into the waste layer spontaneously (Figure 2-4). The reason is that the ends of leachate collection pipes are open to the air instead of isolated. The temperature inside the landfill body is raised up by exothermic microorganism activity. The hot air buoys up and flows out through gas collection pipes, thus resulting in a negative pressure which could suck fresh air through the open leachate collection system. As a consequence, waste degradation was accelerated by air convection. A series of research results showed that semi-aerobic landfill can not only reduce the leachate strength and methane production, but also accelerate the degradation (humification) of the organic matters (Chong et al., 2005; Huang et al., 2008; Huo et al., 2008a). Compared to mechanically forced aeration, semi-aerobic takes usage of natural ventilation, thus requiring less energy input.

Figure 2-4 Schematic diagram of semi-aerobic landfill (Aziz et al., 2010)

Air ventilation in semi-aerobic landfill is strengthened by the perforated vertical gas pipes which are erected at each intersection of the main leachate collection pipes with branch pipes, as well as at the end of each branch pipes. The exit of the well is always open. While the fresh air is taken into the landfill body, the exhaust landfill gas is also vented to the atmosphere (Yoshida, 2007). Herein it increases the difficulty in landfill gas management and limits the usage of the surrounding area.

Proper operation is critical in the success of semi-aerobic landfill. For example, sufficient leachate drainage is necessary to keep a portion of drainage pipe empty, so that air could be allowed to get into the waste layer. Otherwise, if the drainage pipe is full of leachate, the fresh air inlet pathway would be blocked. If this is the case, forced aeration is needed.

2.5.3 Intermittent aeration

Ammonia is one of the most significant long-term pollutants in the anaerobic landfill, since there is no mechanism for its biodegradation (Chung et al., 2015). Intermittent aeration could attenuate the organic carbon and nitrogen pollutants simultaneously, with less power requirement. By supplying the air into the landfill system from the bottom intermittently, the whole landfill body could be divided into three separate zones from bottom to top: aerobic zone, anaerobic zone and anoxic zone. Ammonia is oxidized to nitrate at the bottom aerobic area, and then the recycled leachate is fed into the top anoxic area for denitrification and organic

compound hydrolyzation. The middle layer serves as a methanogenic zone. The methanogenesis could provide alkalinity and reduce the oxygen competition from organic carbon biodegradation with denitrification in the aerobic zone (Jun et al., 2007; Long et al., 2008; Ruo and Shen, 2006).

Intermittent aeration enjoys a name of cultivating a cocktail of aerobic, anaerobic, and anoxic microbes. These microbial populations are capable of a variety reaction and facilitate the substrate removal. Shao et al. (2008) reported that intermittent aeration could enhance phase separation and avoid inhibition of denitrification by persistent existence of oxygen. However, it is also claimed that simultaneous nitrification and denitrification can greatly reduce nitrogen load in the continuous aeration landfill (Berge et al., 2006a). Due to the diversities in waste materials and various conditions among these studies, it is hard to conclude with a best operation mode. Further research is therefore required before any consolidation can be made.

2.5.4 Sequencing air-injection

In a sequencing air-injection system, initial intensive aerobic condition is followed by anaerobic condition. Waste is compacted in lifts. Air is directed into the first layer for months. After the second layer construction is finished, air addition to the first lift stops and aeration of the second lift begins (Hater et al., 2001). The aerobic activity has positive effects on the anaerobic population. The high temperature arisen by aerobic degradation is favorable for anaerobes growth, especially in cold winter. Moreover, the initial aeration could cut down the initiating unproductive period and speed the onset of methanogenesis process. Thus, the LFG could be used for energy as soon as possible (Lefebvre et al., 2000).

2.6 Different usage of aeration

Aeration techniques have been successfully used in many countries for a variety of purposes. Aerobic landfill has been adopted by US as a sustainable solid waste management approach to reduce the demand on declining landfill voids (Berge et al., 2006b). In Germany, in situ aeration has been employed as a landfill

remediation technique to seek regulatory relief of closure and post-closure liability (Ritzkowski et al., 2006). In addition, several projects picked aeration as a pretreatment of landfill mining (Cossu et al., 2003a; Jacobs et al., 2003).

2.6.1 Aerobic/Semi-aerobic landfill as a waste treatment option

As aerobic process could degrade organic matter in a shorter time period than the anaerobic ones, the aerobic/semi-aerobic landfill could replace traditional anaerobic landfill as a waste treatment method. In aerobic/semi-aerobic landfill, the aeration pipes and the other related infrastructure are constructed before waste disposal rather than after landfill closure. Moreover, aeration is commenced immediately after new waste emplacement and continued throughout the life of the site. In contrast, aeration is recommended to start in the later stage of methanogenesis of the existing anaerobic landfill for the in situ aeration projects. Aerobic landfill techniques have achieved considerable success in US (Read et al., 2001b). Semi-aerobic landfill in Japan has demonstrated its superiority and this technique was transferred to Malaysia in 1990 (Chong et al., 2005).

2.6.2 In situ aeration remediation

In the past, landfills were often allocated on the boundaries of towns and rural area isolated from the city. However, due to the growth of population and urbanization, many of these closed landfills have been bordered or even centered with the new developments. These areas represent a significant portion of reclaimable urban land, carrying high value for further development. Applying in situ aeration in the later stage of anaerobic landfill could mitigate the adverse effects and achieve earlier beneficial reuse of the site. Rich et al. (2008) commented that the best scenario appears to be to have anaerobic condition fulfill its work in the first 20 years and then introduce aeration to shorten the aftercare periods. The early anaerobic condition could provide more opportunities for removal of certain pesticide, heavy metal fixation and landfill gas recovery. The subsequent in situ aeration could reduce organic load, decrease landfill post-closure period and achieve earlier beneficial reuse of land.

2.6.3 In situ aeration as a pretreatment of landfill mining

During landfill mining, several problems may arise, like odor nuisance and gas explosion relevant to air-methane mixture. In order to facilitate excavation works and protect the worker's health, suitable pretreatment measures should be defined. Aeration is considered to be a promising alternative, which has been applied in many projects (Cossu et al., 2003a; Jacobs et al., 2003). Establishing aerobic conditions in the landfill body, aeration could reduce methane concentration below lower limit of explosive range, bring waste stabilization forward, and confine odor emission to an acceptable level. According to Stessel and Bernreuter (2001), landfill mining would only be productive if enhanced waste degradation occurs. This could be another added value for applying aeration before landfill mining.

2.7 Temperature evolution during in situ aeration

Intensive microbial metabolism during aeration could raise the waste temperature via exothermic processes. The temperature may reach to 40-60 °C over a long period of time up to years (Heyer et al., 2005b; Oncu et al., 2013). In certain extreme cases, the temperature exceeded 70 °C inside the aerobic landfill (Yazdani et al., 2010). Generally, the temperature decreases as the most of the organic matter has been degraded. Thus, the temperature variation may be wide and change dramatically over time (Heyer et al., 2005b). In addition, the spatial distribution of temperature could highly vary as well (Hanson et al., 2010): the maximum temperature is often noticed at central locations within the middle third depth of the waste layer (temperature curve at 4 m in Figure 2-5). Near the surface, waste temperature resembles the ambient temperature with seasonal fluctuation (temperature curve at 0.5 m in Figure 2-5), while elevated values above mean annual ambient temperature would be encountered close to the landfill base (temperature curve at 6 m/8 m in Figure 2-5).

It is well known that all biological and physicochemical processes are highly influenced by temperature (Hanson et al., 2010). First, the high temperature accelerates water evaporation, which could cause moisture scarcity for microbial activity. Second, moderately thermophilic temperatures (40-50 °C) could delay the

acclimation of microorganisms in aerobic conditions (Raga and Cossu, 2013), as it falls out of the optimum growth temperature range for thermophiles and mesophiles. In addition, thermophilic temperature (50-60 °C) potentially inhibit nitrification (Raga and Cossu, 2013), likely due to the thermal death of *Nitrosomonas* cultures between 54-58 °C (Berge et al., 2005). Some researchers have also pointed out a poor system stability and reliability in the thermophilic temperature range, mainly associated with the less diverse microbial community and transient accumulation of potentially inhibitory intermediates (Labatut et al., 2014; Levén et al., 2007). However, several important advantages have been recognized in thermophilic conditions, such as higher growth rates of microorganisms, larger hydrolytic activities of extracellular enzymes, faster biochemical conversion rate, shorter retention time, smaller system footprint and essentially safer end products with significant low concentration of pathogens (Fernández-Rodríguez et al., 2013; Kim et al., 2012; Ugwuanyi et al., 2005).

Figure 2-5 Temperature profile during in situ aeration in Landfill Konstanz Dorfweiher (Oncu et al., 2013)

To our knowledge, there are many uncertainties in the responses of in situ aeration system to thermophilic temperatures, and information is lacked in this area as references are only up to 45 °C (Raga and Cossu, 2013). Moreover, in order to provide a more complete understanding associated with complex situations in the

real scenario, the aeration performances at different temperature should be examined.

2.8 Effects of waste age during in situ aeration

Landfill stabilization is often a matter of time. Landfill waste carries varied characters and reacts differently to the external stimuli at its different periods of time. In this regard, the incept time of aeration is of great significance for success in landfill treatment.

First, when prioritizing uncontrolled landfills for remedial actions, young landfill should be ranked higher due to its greater potential for future pollution (Singh et al., 2011). Second, waste age is usually considered as the main parameter affecting the composition of biodegradable organic matter, the quality of leachate, the generation rate of landfill gas, and the level of site biostability. Therefore, the best operating procedure of aeration is age-specific. For example, there is always a need of further reduction of the remaining methane in the exhaust gas during aeration, as in full scale application anaerobic pocket cannot be eliminated because of the heterogeneous nature of waste and methane is generated as a consequence of anaerobic degradation. In a relative young landfill, apart from air injection, gas-extraction well and off-gas treatments are usually included to further treat the methane residue. At an old landfill, the methane flux (i.e., the amount of methane extracted during a defined period of time) has already been low. A well designed landfill biocover, using compost materials as well as mineral soil, may be sufficient to oxidize the residue landfill methane through methanotrophic activity of indigenous microorganisms. Compared to the high cost and large energy input in the operation and maintenance of the gas extraction and off-gas treatment, the biocover is more cost-effective (Hrad et al., 2012). Third, the efficiency of individual bioprocess significantly varies along with the landfill age. For instance, denitrification is more favored in young waste because of a sufficient carbon source for heterotrophic denitrifiers, while autotrophic nitrifiers may oxidize ammonia-nitrogen more effectively in young waste due to less competition with heterotrophs (Berge et al., 2005).

Ritzkowski et al. (2006) recommended that aeration should start when methane concentration is too low to be profitable for energy use. However, this statement is ambiguous and more clear guidance is required. Thus, more sound evidences should be collected about the effects of waste age, and thus the right time to start aeration.

2.9 Landfill Humic Substance (HS)

During waste decomposition detritus become rich in reactive phenolic and carbohydrate groups, which may polymerize together with amino acids to form HS (Barje et al., 2012). The reactive phenolic compounds are mainly from the abundant lignin sources in the initial waste materials, such as yard waste and paper. Other humic precursors may be provided by food waste, which could provide protein, polysaccharides, lipids and nucleic acids (Zhao et al., 2013). The HS are generally subdivided into three fractions based on their alkali and acid solubility. Humins are non-soluble at all pH values, humic acids (HAs) are insoluble at acidic conditions ($\text{pH} < 1$), while fulvic acids (FAs) are soluble at the full pH range. Regarding their structure, HS are supermolecular associations by relatively small and heterogeneous molecules of various origins, which self-assemble by hydrophobic interactions and hydrogen bonds (Piccolo et al., 2000; Sutton and Sposito, 2005). The ideal building block of humic and fulvic acids are presented in Figure 2-6 and Figure 2-7 (Alvarez-Puebla et al., 2006; Colombo et al., 2015). Humic acid features the highly condensed aromatic rings, while fulvic acid is relative small and abundant in COOH groups.

As shown in Figure 2-6, HAs and FAs are composed of a set of active chemical groups, which gives the HAs and FAs surface charge and reactivity. For example, the phenolic-OH groups together with COOH groups are providing the acidic characteristics as well as the capacity for chelation, ion and metal binding (Wang and Mulligan, 2009). The aromatic functional groups contribute to the hydrophobic association between HS and other hydrophobic compounds (Chai et al., 2010). The above-mentioned chemical reactions may finally affect the fate of persistent organic pollutants and heavy metals in the environment (Chai et al., 2010; Wang and Mulligan, 2009). Moreover they may lead to the formation of disinfection

by-products in the following wastewater treatment system (Kim and Yu, 2005). Accordingly, HS have received increasing attention in the past several decades.

Figure 2-6 Typical structures of humic acids (Colombo et al., 2015)

Figure 2-7 Typical structures of fulvic acids (Alvarez-Puebla et al., 2006)

In a landfill, percolated water washes out HS in the appearance of yellowish or brownish color. HS are abundant in the leachate representing up to 90% of DOC, which the HAs account for 0.4%-44% while FAs occupy a fraction about 23%-56 % (Huo et al., 2008b; Nanny and Ratasuk, 2002; Zhao et al., 2013). Along with landfill aging and humidification processing, HS percentage in leachate generally increases (Han et al., 2009; Huo et al., 2008b). However, Nanny and Ratasuk (2002) observed an opposite change over time.

The properties of HS are affected by the landfill age and landfill operation mode (Chai et al., 2013; Kang et al., 2002; Lguirati et al., 2005). Kang suggested that the aromaticity and molecular weight of HS increase as landfill age increases (Kang et al., 2002). In particular, the aromatic C=C content in HS structure rose over time, while the percentage of aliphatic C in the long-fatty acid chain decreased significantly with the landfill age (Chai et al., 2008). Compared to HAs from anaerobic landfill, HAs from semi-aerobic landfill was richer in oxygen-containing group, which may be caused by the intensive oxidation under semi-aerobic condition (Chai et al., 2013). Moreover, HS with the higher level of polymerization and condensation was found in landfill site particularly with aeration and irrigation (Lguirati et al., 2005).

The interaction of HS with other refractory organic compounds, heavy metals and microbes will be significantly influenced by the properties of HS structure. Thus, the information on the characteristics of leachate HS in the target landfill is important for the optimization of leachate treatment and selection on suitable remediation procedures for the leachate-contaminated groundwater (Kang et al., 2002). However, to the author's best knowledge, there is no comprehensive studies that is pertinent on the characterization of leachate HS during in situ aeration.

2.10 Summary

Literature show that in situ aeration is an effective landfill rehabilitation technique, which could enhance biological stabilization, accelerate settlement and offer good potential for redevelopment. Although successful full-scale applications have been reported worldwide, there are a number of areas being rarely touched, which are identified and summarized as following:

(1) Limited information of temperature effects on bioconversion process and waste stability during and after aeration. The spatial distribution of temperature in landfill site highly varies, which in turn may result in different rate and pattern in biological and physicochemical processes. However, comparison of the aeration

performance between mesophilic (30-40 °C) and thermophilic (50-60 °C) conditions is limited due to the shortage of reference data particularly from thermophilic studies. Apart from system controlling, the study on these processes is also important for waste recycling given an option for excavation from different temperature zones.

(2) Limited information of waste age effects on aeration. As the different waste ages indicate bunch of information with regards to not only the organic materials but the distribution of other ingredients in previous anaerobic stage, it defines the current status of the targeted landfill and how well the aeration can contribute to the site stabilization in the future. Moreover, knowledge on these time-dependent processes could help to optimize configuration of the aeration system to best suit the different landfills.

(3) Limited information on the formation, transfiguration and humification of HS in aerobic landfill conditions. Since the HS is formed during landfill biodegradation process, its properties are significant indicator for the bioconversion progress of in situ aeration.

(4) Limited information on the variation of microbial community in the course of in situ aeration. The success of in situ aeration largely depends on interaction among microbial communities. Therefore, analysis of microbial population and its function can provide a better understanding of the degradation mechanism and tremendously improve the process design.

The research gaps listed above motivated the present research works aiming to verify the influences of temperature, to explore the impacts of waste age, to clarify the humification process and at last to confirm the function of indigenous microbes during aeration.

Chapter 3 Selection of Waste Materials

In situ aeration aims to enhance waste stabilization in the later life of traditional anaerobic landfill, as in the earlier life methane gas could generate revenue as an energy resource. The targeting materials during in situ aeration therefore should be aged waste, which is almost depleted of the methane generation potential. The following aeration could lead to a much higher level of stabilization, by accelerating the biodegradation of the remaining organics, especially those hardly or slowly biodegradable compounds during anaerobic degradation. This chapter discussed the feasibility of using the buried waste from a local dumping ground for lab scale aeration studies.

Figure 3-1 Location of LHDG

3.1 Background for Lorong Halus Dumping Ground

The LHDG, previously termed the Tampines Dumping Ground, were formerly mangrove swamps. It was in operation from the early 1970s to the late 1990s in Singapore. Situated in the northern part of Singapore, it is bordered on its northern and western perimeter by a river: the Sungei Serangoon River. Across the river on its northern orientation lie the newer residential developments of Sengkang and Punggol. Despite its main designation for municipal solid waste, it has been

receiving a wide range of material including construction debris, incineration ash and inert stabilized hazardous waste.

The LHDG is comprised of 5 different segments with different operational periods with a total site area of approximately 234 hectares (Figure 3-1). The earliest portion in operation termed the “Old Dumping Grounds” is located in the southwestern corner of LHDG. This is followed by Phase I in 1974, Phases IIA and IIB in 1976, Phase III in 1983, and the latest Phase IV in 1990. The waste materials were excavated from Phase III for characterization.

Phase III lies at the northeast of the Old Dumping Grounds, and is landmarked by Buangkok East Drive on its western edge. It covers an area of 44 hectares, with the operational lifetime from 1983 to 1989. It was an uncontrolled dump site without any liners or leachate collection system in place. Initially, the buried waste in Phase III mainly consisted of municipal waste. Due to the starting of incinerators in 1979 and city development in the late 1970s, the main component of receiving waste gradually shifted from domestic wastes to more diverse forms, including construction and demolition (C&D) waste and incineration bottom ash.

In its current state, phase III has a network of landfill gas collection system that methane was captured, and guided to a connected flare station for burning since 2002. It is also heavily vegetated with trees and shrubbery, with dirt tracks in place to allow for access to each gas collection well/trench point.

3.2 Review of previous studies

A few studies have been carried out on LHDG during 1996-2005 with different purposes. Therefore the crossed application among the studies may be limited. Nonetheless, they are helpful to recognize the transitional profiles of the site particularly associated with the current research of interests, beneficial in speeding up the preparation of the site characterization/field work, thus to delineate the overall picture of the aboveground and subsurface conditions – both quantitative and qualitative of the buried wastes.

In details, three studies have been gone through as follows:

1. Consultancy Services for the Rehabilitation of Tampines Dumping Ground – Site Investigation and Alternatives Analyses (Montgomery Watson, May 1997)
2. Feasibility Study on the Rehabilitation of Lorong Halus Dumping Grounds for Industrial Development (Meinhardt, October 2004)
3. Tampines and Lorong Halus Landfill Sites – Environmental Site Characterization Study (CPG Consultants Pte Ltd, May 2005)

The Montgomery Watson Consultancy (MWC) study was commissioned in 1997 by the Ministry of Environment at the moment. The aim of the study was to rehabilitate the Tampines Dumping Ground and reclaim it in compliance with the overall city state development. The MWC report comprised a site investigation, rehabilitation alternatives study as well as design and construction associated with it. The rehabilitation measures meant to stabilize the buried wastes by soil capping and static surcharging, collect leachate through extraction wells/collection trenches, and flare landfill gas with a controlled system.

Meinhardt (Singapore) Pte Ltd was appointed by JTC (Jurong Town Corporation) in early May 2004 to undertake the feasibility study on the rehabilitation of Lorong Halus Dumping Grounds for possible industrial redevelopment. After site characterization relevant to landfill hazards and development constraints, suitable remediation/restoration measures and designs were proposed for further assessment. Apart from common strategies for pollutant control and mitigation, the raised engineering scheme involved soil redistribution and reconsolidation as well as construction of retaining wall in large perimeters before the LHDG can be put into use. Moreover, long bored pile was chosen as the building foundation to alleviate the differential settlement effect.

The CPG Consultants Pte Ltd (CPG) study was commissioned in 2005 by the Public Utilities Board (PUB) to assess the feasibility of turning Sungei Serangoon into a fresh water reservoir. In general, a variety of contaminants were found to be above standard levels in groundwater assessment. In this regards, vertical barrier

walls along the Sungei Serangoon foreshore was proposed to avoid leachate migration.

Table 3-1 The landfill gas and leachate data extracted from 3 reports

	Testing Results	MWC Study (1997)	Meinhardt Study (2004)	CPG Study (2005)
Landfill Gas	CH₄%	60	45.2	45.8
	CO₂%	20	28.5	N.A.
	O₂%	<1	2.1	N.A.
Leachate	pH	6.8	7.00	N.A.
	COD(mg/L)	374	416	547
	BOD(mg/L)	53	18	N.A.
	BOD/COD	0.14	0.04	N.A.
	Ammonia(mg/L)	N.A.	356	346
	Barium(µg/L)	N.A.	1201	762
	TPH(µg/L)	N.A.	5358	N.A.
	PAH(µg/L)	N.A.	N.D.	N.D.
	Aromatic Compounds(mg/L)	N.A.	N.D.	N.A.
	Pesticides(µg/L)	N.A.	N.D.	N.A.

Notes:

N.A. indicates the sample was not analyzed for the parameter.

N.D. indicates the compound concentration was below the detection limit.

TPH: Total Petroleum Hydrocarbons; PAH: polycyclic Aromatic Hydrocarbons

Table 3-1 shows a breakdown of the common parameters found in groundwater and landfill gas from the 3 studies. All studies covered the LHDG as a whole without any phase specific. Therefore, the information only related to phase III was extracted to gain an insight. In the MWC study, a total number of 40 boreholes were drilled, designated MWC01 through MWC40 over LHDG from October to November 1996. Whereas five of them were particularly drilled in Phase III: MWC23, MWC24, MWC25, MWC26 and MWC27. As a result, the values of the 5 boreholes were presented on average in Table 3.1. In the Meinhardt study, although 5 boreholes were located in Phase III, only MS16 was converted into a monitoring well. Thus its value is shown in Table 3.1. However, most monitoring wells in the CPG study were along the foreshore and presented less waste materials

compared to others, therefore the upper value from the report is taken as a reference.

The neutral pH (7.0) and the low BOD/COD ratio (<0.1) in leachate samples in the three studies indicated an aged leachate and advanced waste decomposition. Furthermore, the gas composition was also consistent with expectation considering the age of refuse in Phase III, as 40~60% of CH_4 and 20~30% CO_2 are the typical LHG composition in methanogenesis. Comparing these data chronologically, slight abatement in the methane concentration and leachate strength was observed. Overall, the pollutant concentration kept stable while at a low level. It was concluded that the organic fraction in the buried wastes were highly decomposed.

To sum up, the data from the three reports delivered a message of aging-derived stability at Phase III, offering a potential baseline for subsequent investigation. Although the historical data could not reflect the latest updates on the site, but it provided indicatively a progress and trend that can be potentially followed and projected.

3.3 Waste sample collection from LHDG

A typical site investigation at the phase III of LHDG was carried out by the author's research group from the Residues and Resource Reclamation Centre (R3C). Seven boreholes (BH1 to BH7) with a termination depth of 30 m had been drilled during the period from 23rd July 2011 to 28th August. The location of the seven boreholes in phase III is shown in Figure 3-2. The drilling was undertaken using an YBM-3SJ rotary hydraulic drilling machine (Figure 3-3).

Figure 3-2 Location of seven borehole drilled in phase III of LHDG

The diameter of the borehole was around 100 mm. The sampling of waste materials started when the drill rod reached the waste layer. Waste from every 1 m vertical section were placed in one bag, labeled as one subsample of this borehole, and immediately transferred to a cold room for storage in the laboratory.

Figure 3-3 (a) Rotary hydraulic drilling machine and (b) Drilled borehole from which waste sample was collected

Table 3-2 LHDG waste composition (by wet weight)

Constituents	% by weight	Constituents	% by weight
Wood	25.1%	C&D	4.4%
Plastics	7.6%	Metal	2.3%
Textiles	1.4%	Batteries	0.1%
Rubber	1.1%	Rock/Gravel	4.4%
Leather	0.1%	Glass	0.8%
Paper	2.2%	Fine Fraction (<2cm)	50.8%

3.4 Characterization of waste sample from LHDG

In phase III, the waste layer was covered by the marine clayey sand with thickness in a range of 5 to 12 m. In other words, the waste layer starts from 5-12 meter below the ground level and terminates variously with a thickness less than 15 meters. The bagged waste sample from one borehole was divided into two categories: sample collected from the upper part and sample collected from the lower part of the borehole. From each category, one bag was randomly selected and subjected to the following characterization.

According to Binner and Zach's study (1999), the waste fraction < 20 mm was representative for biological parameters (RI_4 and GS_{21}) and leaching test. After waste separation, the unclassified fine fraction (shown in Table 3-2) was used for the leaching and RI_4 test.

The cumulative oxygen consumption in 4 consecutive days (RI_4 , mg O_2 /g DM) was measured in an Oxitop apparatus (WTW GmbH, Germany). 50 g DM was loaded into the reaction bottle and adjusted a water content of 50%. The sample was agitated at a constant room temperature (20 °C) for the whole reaction period. The CO_2 produced by micrological activity was eliminated by sodium hydroxide tablets. The negative pressure resulted from oxygen utilization was recorded by the Oxitop controller (Cossu and Raga, 2008).

For leaching test, sample with a dry weight of $0.900 \text{ kg} \pm 0.005 \text{ kg}$, was firstly put into the reaction bottle. Deionized water was then added until a liquid to solid ratio (L/S) of 10 was attained. The reaction bottle was capped and agitated with a tumbler rotator at 10 rpm for 24 h. The eluate was filtered through the $0.4 \text{ }\mu\text{m}$ membrane filter and stored at 4°C for further analysis (British Standard, 2002).

3.5 Results of Waste Characterization for LHDG

The biological stability of solid waste was one of the main indicators to evaluate the long-term emission potential and environmental impacts of a landfill. The waste biostability can be quantitatively determined by RI_4 and leaching test. The results are tabulated in Table 3-3. European Communities Council (2003) has set out the correspondent criteria for the acceptable levels of wastes being admitted into landfill for inert waste and landfill for non-hazardous waste, and the criteria are also included in Table 3-3. Results of the leaching tests showed that the contaminants of concerns in the refuse from the LHDG met the inert waste criterion. European Communities Council defined the “inert waste” as follows:

Inert waste means waste that does not undergo any significant physical, chemical or biological transformations. Inert waste will not dissolve, burn or otherwise physically or chemically react, biodegrade or adversely affect other matter with which it comes into contact in a way likely to give rise to environmental pollution or harm human health. The total leachability and pollutant content of the waste and the ecotoxicity of the leachate must be insignificant, and in particular not endanger the quality of surface water and/or groundwater.

In addition, the detected RI_4 for all waste samples was less than $2 \text{ mg O}_2/\text{g DM}$, and much lower than the termination criteria ($5 \text{ mg O}_2/\text{g DM}$) proposed by Ritzkowski et al. (2006). All the result demonstrated a low reactive potential in the solid wastes, suggesting no need for aggressive measures such as in situ aeration.

Table 3-3 LHDG waste characterization (results of RI₄ and leaching test) and waste stabilization criteria in the literature

Sources	RI ₄		Leaching Test													
	mgO ₂ /g DM		mg/L							µg/L						
			F ⁻	Cl ⁻	NO ₂ ⁻	NO ₃ ⁻	SO ₄ ²⁻	Br ⁻	NH ₄ ⁺	COD	Cd	Cr	Cu	Pb	Zn	Ni
BH1-T^a	8-9 m	0.82	0.27	19.18	0.24	0.15	61.50	0.00	0.10	72.00	2.02	4.13	21.35	19.33	43.09	7.49
BH1-B^a	12-13 m	0.03	0.17	11.41	0.00	0.00	509.49	0.20	1.21	44.00	1.54	3.44	11.87	20.93	17.93	4.37
BH2-T	12-13 m	0.93	0.28	39.96	0.00	0.00	884.27	0.81	10.50	108.00	2.53	4.44	45.45	20.26	110.26	5.58
BH2-B	15-16 m	0.95	0.48	150.95	0.00	0.00	403.98	0.54	5.80	117.00	1.48	1.78	26.74	3.85	59.52	3.38
BH3-T	9-10 m	0.64	0.32	43.89	0.44	3.54	100.58	0.00	4.41	157.00	NA	3.12	106.13	18.86	23.54	4.71
BH3-B	14-15 m	0.22	0.59	204.60	0.00	2.09	508.00	0.00	74.40	449.00	0.65	5.64	267.73	41.69	458.06	31.07
BH4-T	6-7 m	1.76	0.19	21.04	0.17	0.71	230.03	0.51	14.80	46.00	2.47	5.77	26.39	13.47	43.95	18.02
BH4-B	11-12 m	1.13	0.28	39.31	0.00	0.53	368.46	0.34	15.40	65.00	1.58	3.90	18.44	15.85	36.52	4.49
BH5-T	11-11 m	N.A.	0.26	32.31	0.23	1.48	223.89	0.74	5.76	55.00	0.73	1.10	22.42	3.68	13.48	4.61
BH5-B	15-16 m	0.34	0.13	22.53	0.40	2.19	96.29	0.00	7.02	51.00	0.19	0.77	42.75	9.40	14.45	4.57
BH6-T	8-9 m	N.A.	0.24	10.13	2.35	25.46	62.39	0.00	5.18	43.00	0.13	0.51	14.45	2.78	9.32	6.35
BH6-B	11-12 m	0.65	0.35	7.93	0.00	0.40	160.93	0.00	0.09	15.00	0.40	0.38	8.84	0.31	14.77	5.27
BH7-T	9-10 m	0.64	0.25	119.90	17.92	17.78	129.83	0.66	1.12	50.00	1.42	0.91	12.42	N.D.	31.53	4.14
BH7-B	11-12 m	0.45	0.25	290.42	0.00	0.80	213.68	1.40	11.80	124.00	3.89	17.40	39.21	0.27	29.19	26.89
Aeration Termination^b		5.00							200.00		100.0	100.0	5000.0	1000.0	5000.0	1000
Inert^c			0.90	72.00			90.00				4.00	50.00	200.00	50.00	4000.0	40.00
Non-hazardous^c			13.50	1350.0			1800.0				1000	10000	50000	10000	50000	10000

^aT and B indicated that the waste sample was collected from the upper or lower part of the borehole, respectively.

^b Criteria of termination of in situ aeration measures, which were proposed by Ritzkowski et al. (2006)

^c Limit values for waste acceptable at this class of landfills, which were proposed by European Council (2003)

3.6 Field investigation on leachate and gas quality

After collection of waste samples, all boreholes were converted into monitoring wells for further monitoring (MH1 to MH7). Leachate samples were collected from the seven monitoring wells subsequently (14th-15th September 2011). They were then transferred to the lab for further analysis. Landfill gas quality was monitored concurrently at the end of leachate sampling by a portable gas analyzer (Sewerin Multitech 520, Germany) (Figure 3-4). All the sampling and testing were conducted by a commercial lab, the SETSCO Services Pte Ltd. The measured values on parameters in leachate and gas are list in Table 3-4.

Figure 3-4 (a) Leachate sample collection and (b) LFG measurement

The CO₂ and CH₄ concentration in LFG from MH1-MH4 falls in the range of a typical value from a matured landfill. The gas generation rate around MH5-MH7 was found to be very low. It indicated the oxygen intrusion via atmosphere. As a consequence high level of oxygen was detected in the three boreholes. Moreover, the pH value maintained neutral around 7 while BOD/COD ratio was found about 0.10 in all the monitoring wells. In general it indicated that the landfill was possibly at the late stage of phase V (Figure 2-1).

Table 3-4 Field testing data from seven monitoring wells

Well No	MH1	MH2	MH3	MH4	MH5	MH6	MH7
CH₄ %	50.3	61.2	68.8	61.0	0.6	0.3	18.1
CO₂ %	27.3	40.3	16.1	39.8	0.1	0.4	0.2
O₂ %	4.0	0.1	0.9	0.1	15.2	20.0	16.5
pH value	6.8	7.0	6.7	6.9	8.8	7.0	6.8
BOD (mg/L)	32.2	20.4	5.5	21.6	7.4	7.8	11.6
COD (mg/L)	310.1	380.1	N.D.	300.1	60.0	N.D.	210.0
BOD/COD	0.10	0.05	N.A	0.07	0.10	N.A	0.05

3.7 Conclusion

Waste samples were collected from LHDG phase III and subjected to characterization particularly relevant to its stabilization. The results from RI₄ and leaching test implied that the material was inert and stabilized. These observations were in accordance with the field measurements of landfill gas and leachate quality. All indicated that the maturation phase of the LHDG has been achieved. Given the phase III being 20 years after closure and an approximate 50% of C&D waste, there were little chance that significant amount of biodegradable organic matter were present. The necessity in performing in situ aeration was quite low for the LHDG and the buried wastes were not suggested for in situ aeration study.

In situ aeration mainly works on the putrescible organic substances. If the waste materials contain a high amount of active organic fraction, aeration can quickly demonstrated its advantages, as it can significantly reduce the content and biodegradability of these organic components. This kind of waste materials is also the suitable substrate for laboratory experiment, because it has the potential to reflect the variances between different treatments notably.

LHDG is the largest closed landfill in Singapore, and the only accessible landfill to the authors' research group. After the waste from LHDG was considered as unsuitable, the artificial aged waste was chosen to be used in the subsequent experiments. Moreover, the synthetic aged waste had its own benefit, as it can make the composition of waste materials easier to control and minimize the influences induced by heterogeneity on waste component, allowing the results more comparable.

Chapter 4 Materials and Methodologies

4.1 Aged waste preparation

Synthetically aged waste was prepared in the lab simulating the waste composition of closed landfill or dumping ground. By this way, the variance of aged waste composition among the bioreactors was minimized, allowing for more comparable results. Fresh MSW was prepared by mixing food waste, yard waste, paper, plastics and textile at a ratio of 60/15/10/10/5 (w/w). The composition of waste materials was determined according to other studies (Alkaabi et al., 2009; Francois et al., 2007; Ledakowicz and Kaczorek, 2004). In particular, the food waste composed of 35% fruit, 12% cabbage, 12% potato, 12% carrot, 10% rice, 5% bread, 5% noodle, 10% meat and 4% egg (Tang et al., 2004). The initial water content of the fresh waste mixture was measured as 52%. The element composition was determined as 63% C, 0.6% N, 10% H and 25% O (by weight). The synthetic MSW mixture (55 kg) was mingled with 10% compost (w/w) (Corona Florist & Nursery Pte. Ltd., Singapore) and loaded into an acrylic bioreactor, with dimensions 1100mm × 500mm (L × D). The compost, which was less reactive, served as a dilution agent, and at the same time buffered the pH to prevent undesirable acidification (Heerenklage and Stegmann, 2006). Figure 4-1 shows the schematic diagram of the reactor for aged waste preparation.

After waste loading, air was injected bottom-up into the drainage layer with a flow rate of 5 L/min in the first two weeks. The porous drainage layer served as an air distribution layer to achieve a homogeneous aeration. After two weeks, aeration was ceased. At that time 5 L of digested sludge from local wastewater treatment plant was inoculated to shorten the acid phase and bring forward the methanogenesis phase. Moreover, N₂ was pumped into the reactor to ensure anaerobic condition.

Figure 4-1 Schematic diagram of the aged waste preparation reactor (L×D: 1100 mm×500 mm)

Since the beginning, three times a week 2 L of DI water were added from the top and excess leachate was collected in a reservoir tank, reaching a volume of approximately 20 L at the end of week 4. From then on, 5 L of leachate (approximately 5% (v/v) of the MSW in LSRs) were recirculated three times per week until the end of the aged waste preparation. No leachate neutralization was conducted as the pH value remained at around 7. Moreover, under anaerobic condition oxidation-reduction potential (ORP) of the leachate was recorded and kept between -220 ~ -280 mV, falling within the typical range of -100 to -300 mV for methanogenesis. The generated landfill gas was ventilated from a gas collection

port on the top cover. The gas volume was recorded with a gas counter (Ritter Apparatebau GmbH, Germany) while the gas composition was determined by a portable gas analyzer (Sewerin Multitech 520, Germany).

Methane was detected in the outlet gas on next day of the sludge inoculation. The duration of aged waste preparation was determined by the methane production rate and methane concentration curves. The low methane generation rate and methane percentage suggested a well-decomposed aged waste at day 150 in Figure 4-2. The laboratory preparation of aged waste within 6 months was also successfully conducted by other researchers (Price et al., 2003).

Figure 4-2 Methane production rate and methane concentration during aged waste preparation

4.2 Operation of landfill aeration reactors

The aged waste (66% in water content (w/w)) was transferred into six bioreactors (L×D: 500mm×190mm) containing each approximately 6.5 kg of waste (Figure 4-3).

Figure 4-3 Schematic diagram of aeration bioreactor (1) measuring cylinder for leachate; (2) bioreactor column covered by heating belt; (3) temperature controller with thermoprobe; (4) air injection pipe; (5) gravel layer; (6) water distributor; (7) rubber septum for gas sampling during carbon mineralization rate measurement; (8) syringe for gas sampling; (9) gas flow meter; (10) air pump; (11) open port for outlet gas; (12) gas counter and (13) gas analyzer.

The landfill aeration bioreactors were preloaded with 5 cm high glass drainage marbles and the Nylon geonet above to prevent large particles from leaching out. After loading, the waste materials were carefully compacted to a depth of 35 cm, with a final bulk density of 655 kg/m³. In the end, the waste materials were stabilized with a thin layer of bulk gravels on its surface. Each reactor was equipped with three ports on top: one for air injection, one for gas discharge and one for water supply. Water distributors were applied to ensure even distribution. The aeration pipes were 2 cm in diameter placed in the middle of the reactor, and the end of the pipe was rested slightly above the geonet. The bottom section of the pipe with a length of 5 cm was slotted and the spacing of the slots was 5 mm.

Table 4-1 Operation data of the six landfill bioreactors

	Column	Operation Mode		T °C
Control	NR35	Anaerobic (396 d)		35 °C
	AR35	Aerated (396 d)		35 °C
Temperature effects	AR45	Aerated (396 d)		45 °C
	AR55	Aerated (396 d)		55 °C
Waste age effects	LR35	Anaerobic (155 d)	————→ Aerated (241 d)	35 °C
	ZR35	Anaerobic (274 d)	————→ Aerated (122 d)	35 °C

Upon loading, the bioreactors were purged with N₂ and operated under anaerobic condition for additional two weeks for adaptation, at room temperature (35 °C). Reactors were designed at variety of operations. Three reactors were immediately aerated at 35°C, 45°C and 55°C respectively, correspondent to AR35, AR45 and AR55. Two has been subjected to anaerobic aging followed by aeration, termed as LR35 and ZR35. The last one was maintained under anaerobic cinditions throughout the whole experimental period as a control (NR35). The differences of the bioreactor operation were summarized in Table 4-1. In brief, all three aeration bioreactors (AR35, AR45 and AR55) were operated with continuous air injection with the flow rate of approximately 0.016 L/min·kg dry material. It was considered as time “zero” once the air injection began. The flow rate was controlled by a ball valve and monitored by a digital flow meter (TSI 4000 series, USA). The air supply

was considered as sufficient, as the O₂ fraction in the outlet gas was measured above 15%. Meanwhile the ORP in both AR35 and AR55 was between 50-150 mV. The aerobic condition was double checked with dissolved oxygen in the leachate sample, achieving higher than 5 mg/L after aeration. For AR45 and AR55, the reactors were heated up with a heating belt, with a ramp of 2 °C/day until 45 and 55 °C respectively. The temperature was recorded by a thermoprobe in the middle of the waste layer. The two reactors LR35 and ZR35 were maintained in anaerobic conditions similar to NR35 until day 155 and day 274, respectively, and afterwards they were converted to aerobic conditions as AR35, separately.

On day 1, 3 and 5 per experimental week, 200 mL of deionized (DI) water was trickled from the top of each reactor. The produced leachate was temporarily withheld in the bottom marble layer before being fully discharged every other water addition. This means that discharge took place after two water additions. Generally, an average of 370 mL leachate was collected in the anaerobic reactor during each discharge process, whereas an average of 320 mL leachate was obtained in the aerobic reactor, suggesting more water losses. Losses were likely attributed to evaporation, while aeration accelerated such resulting in a lower volume collection in aerobic reactors. Leachate sample was passed through 0.45 µm syringe filter (Cronus, UK) and stored at 4 °C for chemical analysis. Chemical parameters like ammonium-nitrogen and COD were determined using Hach test kit and spectrophotometer (Hach, US). Nitrate and nitrite analysis was conducted by ICS-1100 ion chromatography system (Dionex, US). DOC and total nitrogen (TN) were measured in the Multi N/C 2100 system (Analytik Jena, UK). All testing results were normalized based on 400 mL of the initial water addition. The duration of the experiments lasted for 396 days until a monitored stable state, in which limited concentration of pollutants were identified from the leachate.

4.3 Extraction and purification of leachate HS

HS analysis needed a larger quantity of sample than that from current sample collection each time. To obtain enough volume for HS extraction, collective sampling approach was adopted at two different ranges of days. In this regards, the

sample in period I represented an average property from the 4 consecutive days of leachate collection, i.e. days 73, 77, 82 and 87, when 200mL leachate from each day were combined together to reach a total volume of 800mL. Period II was at days 215, 220, 224 and 229. Each 800 mL leachate sample was analyzed in duplicates.

Amberlite XAD-8 resin (Sigma-Aldrich, US) and AG-MP-50 resin (Bio-Rad, US) were employed to retain FAs by adsorption and purify FAs by ion exchange. Proper preparation for these two resins was required before usage. The XAD-8 resin was first washed in 0.1N NaOH for 24 hours and decanted for removal of organic impurities. The soaking/decanting process was repeated five times. The NaOH-treated XAD-8 resin was then Soxhlet-extracted for 24 hours sequentially with methanol, diethyl ether, acetonitrile and methanol, after which the clean resin was stored in methanol for use. 40 ml of the methanol-resin slurry was rinsed with DI water, and packed in a chromatography column (Supelco Co., US). Fifty bed volumes of DI water (2 L) were pumped through the column to remove the residual methanol. Finally, the packed resin was rinsed three times, alternating from 0.1N NaOH to 0.1NHCl and remained saturated in a solution of 0.1N HCl in the column until used (Aiken et al., 1979).

After packing into the column, AG-MP-50 resin (30 ml) was washed with DI water until 50 bed volumes to get rid of the impurities. Hydrogen-saturation and final purification was achieved by running through 3 bed volume of 1 M HCl and 50 bed volumes of DI water, respectively (Leenheer, 1981).

Figure 4-4 Isolation and purification procedure for leachate HS

Leachate HS was isolated and purified using the method described by Christensen et al. (1998), which is shown in Figure 4-4. In detail, 400 ml of leachate was filtered through a 0.45 μm nylon membrane to exclude the interference from suspended particles. The filtered leachate was further acidified to pH lower than 1

with concentrated HCl, and preserved at a temperature of 4°C for at least 12 hours to ensure complete precipitation of HAs. Following precipitation, the leachate sample was centrifuged at 5000 rpm for 30 min and filtered with a 0.45µm nylon membrane. The supernatant contains FAs and HyI fraction, while the precipitate was HAs. Purification and protonation of HAs were conducted by dialyzing HAs solution (HAs redissolved in 0.05 M NaOH) against DI water, 0.1 M HCl and DI water sequentially using a dialysis membrane with 1000-Dalton cut-offs (Spectrum Lab, US). The purified HA fraction was then freeze-dried. FAs were absorbed by Amberlite XAD-8 resin from supernatant. The dissolved organic matter after FAs adsorption not absorbed onto the XAD-8 resin was considered as HyI. FAs retained by XAD-8 resin was further eluted out by a 0.1 M NaOH solution, which was subjected to flow through a H-saturated cation exchange resin (AM-PG-50) for purification later. FAs powder was yielded after freeze-drying.

The concentration of HyI fraction was represented by the DOC content after the XAD-8 resin adsorption. The amount of HAs was calculated by the difference between the initial DOC content in the sample and the one after acidification and filtration in the sample. The theoretical content of FAs was determined by the difference between the DOC after acidification and filtration and the one after passing the XAD-8 resin column. The recovery rates of the FAs from the resin adsorbents were around 90% while the recovery rates of the FAs after AG-MP-50 resin were about 80%. The obtained HAs powder from the duplicate was ground together and subjected to structural analysis as one effective sample. The same procedure was adopted for FAs sample preparation.

4.4 CO₂ generation rate and carbon mineralization rate

In detailed, the air supply was stopped right before the in situ respiration tests. Afterwards, CO₂ emission in the headspace of the bioreactors was monitored over time. Gas samples were collected by syringe through a septum on the top cover of the reactor. Gas analysis were conducted using gas chromatograph equipped with a thermal conductivity detector (GC-TCD) (Agilent Technologies, US) over 4-10 hours interval. The slope of the CO₂ percentage versus time determined the carbon

dioxide generation rate in terms of percentage per hour. The CO₂ mass generation rate (mg/d) and carbon gasification rate (mg/d) was calculated by the Eq. (4.1) and Eq. (4.2), respectively:

$$k_m = (24)k_o(V_r - V_g - V_w + V_w\theta_a)\rho_{CO_2} \quad (4.1)$$

$$C_g = k_m\left(\frac{12}{44}\right) \quad (4.2)$$

where k_m is CO₂ mass consumption rate (mg/d); k_o is CO₂ consumption rate (%/hr); V_r is the working volume of the bioreactor (13.312 L); V_g is the volume of the gadgets inside the bioreactor, including aeration pipe, water distributor, thermoprobe, and bottom glass marbles (1.288 L); V_w is waste bulk volume (9.924 L); θ_a is gas-filled pore space (0.19 cm³ gas/cm³ waste measured via water-filled void volume of the waste); ρ_{CO_2} is the density of carbon dioxide (1.726 g/L at 35 °C, 1.671 g/L at 45 °C and 1.619 g/L at 55 °C); C_g is the carbon gasification rate (mg/d).

4.5 Nitrogen distribution in leachate

As mentioned earlier, four leachate samples were obtained at two different periods for mixing first to extract dissolved HS. The concentration of dissolved HAs, FAs, and HyI were calculated in terms of DOC (mg organic carbon/L). In the next step, organic nitrogen distribution among HAs and FAs was obtained by multiplying the DOC with N/C mass ratio of them, which was measured by the Vario EL cube (Elementar Analysensysteme GmbH, Germany). The nitrogen content in HyI was calculated by the difference between TN and the sum of all types of nitrogen in the other parts (i.e. ammonium, nitrite, nitrate, organic nitrogen in HAs and FAs) (Westgate and Park, 2010).

4.6 Waste characterization

At the end of the experiment, waste materials were excavated for characterization. The measurement of TOC was performed on dried and milled sample through Multi N/C 2100 system (Analytik Jena, UK). Waste biostability was characterized through GS₂₁ and HS content. GS₂₁ was defined as the total volume of gas generated for 21 days under the anaerobic condition, determined by

the Automatic Methane Potential Test System (Bioprocess Control, Sweden). The sample incubation vials was first loaded with 50g waste sample. Afterwards, digested sludge (50 ml) from local wastewater treatment plant was inoculated and water was added to reach a final volume of 300 ml. The mixture was stirred by a slow rotating agitator and incubated in the water bath with constant temperature (35 °C). Two reference vials (only with digested sludge and water) were also set up simultaneously. During the investigation period, the produced biogas volume was recorded using a wet gas flow measuring device and calculated at normal conditions (20 °C, 1atm). The net gas volume for each sample was obtained by deducting the amount of from reference vials. The data evaluation period lasted for 21 days after the initial lag phase. The end of the lag phase was defined as 25% of the maximum gas-generation rate (3-day mean values).

Isolation and purification procedures of HS from waste sample were carried out according to the method by the International Humic Substance Society (IHSS). In brief, fifty gram of solid waste sample was equilibrated with 1 M HCl to a pH value of 1 to 2, and continuing a supply of 0.1M HCl until the final liquid/solid ratio (L/S) = 10. The suspension was shaken for 1 h, and then the supernatant was separated from the waste residue through centrifugation and filtration (0.45 µm nylon membrane). The supernatant was collected as FAs Extract 1 for later isolation. The waste residue was neutralized with 1 M NaOH and followed by additional 0.1 M NaOH until a final L/S ratio of 10:1. The suspension was shaken for 4 hours and preserved in a 4 °C freezer, allowing the suspension to settle overnight. The NaOH-extracted solution was filtered through 0.2 µm polyethersulfone filter membrane (Supelco Co., US) to minimize the influences of suspended particles and mineral ash. HAs precipitation was collected through adjusting pH of the NaOH-extracted solution to 1 by concentrated HCl. The HAs was obtained by the DOC difference between NaOH-extracted solution and the one in the supernatant (FAs Extract 2) after acidification and filtration. The HAs content in the end waste was denoted as DOC per gram of dry waste sample. The FAs Extract 1 and FAs Extract 2 were combined together for FAs isolation by using XAD-8 resin. The extraction of HS was conducted in duplicate for end waste sample from each reactor.

The yielded HS powder from duplicate was ground together and subjected to characterization as one effective sample.

4.7 HS characterization

Elemental analysis was performed on freeze-dried samples of HS (5 mg) by using the Vario EL cube (Elementar Analysensysteme GmbH, Germany). Carbon, hydrogen, nitrogen and sulfur content were given directly by the apparatus.

^{13}C Solid-state nuclear magnetic resonance (NMR) spectra were performed on a Bruker AVANCE III 400WB spectrometer (Bruker, US) operation at 9.4 T, with frequency of 100.62 MHz and 400.13 MHz respectively. Samples were packed into 4-mm zirconia rotors. For ^{13}C , the contact time was 0.5 ms, the recycle delay was 0.5 s -2 s, a ramp-contact and spinal 164 decoupling pulse programs were used. The numbers of scans of ^{13}C CP-MAS was 5000-20000 for all samples with rotor spinning rates of 14 kHz. The spectra was calibrated using the methine carbon atoms of adamantane as an external standard ($\delta = 29.47$ ppm).

Matrix-assisted laser desorption/ionization (MALDI) had the advantage of forming singly charged ions. Combined with time-of-flight mass spectrometry (TOFMS), it could detect the absolute MW of HS rather than the apparent MW detected in the high performance size exclusion chromatography (Mugo and Bottaro, 2004). In details, the HS samples were dissolved in 50% acetonitrile (ACN) and 0.1% trifluoroacetic acid (TFA) and then mixed with same amount of matrix solution containing 10 mg/mL of α -cyano-4-hydroxycinnamic acid in 0.1%TFA-50% ACN, and latterly spotted onto a 384-well stainless-steel MALDI target plate (Applied Biosystems, US). An ABI 4800 MALDI TOF/TOF mass spectrometer (Applied Biosystems, US) was used to analyze the samples on the MALDI target plates. For MS analyses, typically 1600 shots were accumulated for each sample.

In conclusion, the Table 4-2 summarized all the parameters measured during the experiment. The percentage of CH_4 , CO_2 and O_2 was determined in the off-gas. Leachate was characterized in terms of dissolved oxygen (DO), ORP, DOC, COD,

TN, NH₄-N, NO₃-N, NO₂-N, HAs, FAs, and HyIs, while the waste materials were subject to tests for TOC, HS content, and GS₂₁.

Table 4-2 Parameters analyzed for landfill bioreactors

Phase	Parameters
Off-Gas	CH ₄ , CO ₂ , and O ₂
Leachate	DO, ORP, DOC, COD, TN, NH ₄ ⁻ -N, NO ₃ ⁻ -N, NO ₂ ⁻ -N, HAs, FAs, and HyIs
Waste	TOC, HS content, GS ₂₁

4.8 DNA extraction and amplication

At the start-up phase of aeration, the in situ condition was changed from anaerobic to aerobic. Accordingly, the dominant microbial community shifted from anaerobes to aerobes. Comprehension of the evolution of microbial community and its function at this stage is necessary, as the investigation could help to select the best operation parameters for rapid startup and even long-term stability. The microbial diversity at start-up phase was characterized through 16S rRNA gene analysis on DNA extracted from leachate sample. For NR35, AR35, AR45 and AR55, the leachate sample represented an average property from the 2 consecutive days of leachate collection (day 28 and day 32), when 100 mL leachate from each day were combined together to reach a total volume of 200 mL. The total 200 ml leachate sample was centrifuged at 13,000 rpm for 15 min to concentrate microbial cell. The supernatant was decanted and the genomic DNA was extracted using ZR Soil Microbe DNA Kit (Zymo Research, US) according to the manufacturer's introduction.

First, the microbial cell was lysed in the lysis buffer by bead beating FastPrep-24 instrument (MP, US). Then the lysate was applied to the adsorption column, which the DNA was adsorbed to. The undesired contaminants (such as humic acid and phenols) were removed in the washing step by using washing buffer. The purified target DNA was eluted out by introducing TE buffer to the column.

In order to amplify bacterial 16S rRNA gene from the total-community DNA, Primer sets 8F (5'- AGA GTT TGA TYM TGG CTC -3') and 1490R (5'- GGY TAC CTT GTT ACG ACT T -3') were used. The thermal profile used for amplification of 16 rRNA gene was as follows: incubation at 94 °C for 3 min, then 30 cycles of denaturation at 95 °C for 30 S, annealing at 54 °C for 30 S and extension at 72 °C for 45 S, and a final extension at 72 °C for 5 min (Lim et al., 2013).

4.9 TA cloning

TOPO TA cloning kit (Invitrogen, CA) was used for clone library construction in accordance with the manufacturer's instruction. The detailed protocol is as follows:

1. Prepare selective LB agar plate with antibiotics ampicillin (50 mg/ml) and kanamycin (50 mg/ml).
2. Set up the 50 µL PCR reaction as mentioned in section 3.8. The PCR product was checked by agarose gel electrophoresis.
3. Use 5 µL PCR products as template to run another round PCR. The number of thermal cycles could be reduced to 10 times, and a 30 minute extension at 72 °C was added after the last thermal cycle. The recondition PCR product was checked by agarose gel electrophoresis.
4. Purify the recondition PCR product using QIAquick PCR Purification Kit (Qiagen, Netherlands)
5. Ligate the PCR inserts with the vector by gently mixing the following reagents in a clean PCR tube and incubating for 15 min at 30 °C incubator: 4 µL of purified recondition PCR product, 1 µL of Salt Solution (1.2 M NaCl, 0.06 M MgCl₂), 2 µL of RNAase-free Water, 1 µL of Topo Vector.
6. Thaw on ice 1 vial of One Shot competent *E.coli* cells for transformation.
7. Transform the recombinant vector into the Chemically Competent *E. coli*.
 - Add 3-6 µL of the recombinant vector solution into a vial of one shot chemically competent *E.coli* and mix gently.
 - Incubate on ice for 30 minutes.
 - Heat shock the cells for 35 seconds at 42°C without shocking.
 - Immediately transfer the tube to ice for 2mins.

- Add 250 μ L S.O.C medium into the tube at room temperature.
 - Cap the (wrap with parafilm) tube tightly and shake the tube horizontally at 37°C for 1.5-3 hrs.
 - Spread 50 μ L, 50 μ L, 80 μ L, 80 μ L and 20 μ L mixture from each transformation on a prewarmed selective agar plate and incubate overnight at 37°C.
8. Detect positive clone from overnight cultures by the appearance of white colonies. Using a toothpick to pick 150 clones from the agar plates and transfer them to fresh agar medium. Draw a few strips gently on the fresh agar medium in the specific zone for each clone.
9. Incubate the transferred clones overnight at 37°C.
10. Extract each clones from the agar medium using toothpicks and transfer the cloned cultures into individual PCR tubes filled with 100uL filtered H₂O (biotechnology grade).
11. Subject the clone cells collected in all the PCR tubes to a Freeze (-80°C) /Thaw (60°C) treatment process for 3 cycles.
12. Store the cell lysate containing the plasmids at -20°C for future verification.

4.10 Screening of recombinant clones

Preliminary screening was done by amplifying the plasmid DNA using primers M13F (5'-GTA AAA CGA CGG CCA G -3') and M13R (5'- CAG GAA ACA GCT ATG AC -3') and analyzed for plasmid containing inserts by 1% agarose gel electrophoresis. Secondary screening was conducted through restriction fragment length polymorphism (RFLP) to remove the possible redundant clones. The M13-PCR products of positive clones were digested with restriction enzymes MspI and RsaI (New England BioLabs, UK) respectively, and were electrophoresed on 1.5% agarose gel. Clones were grouped according to their RFLP pattern (Liu et al., 2011a).

4.11 Sequencing and phylogenetic anlaysis

Representative clones from each unique RFLP pattern were sequenced, by 1st BASE (Singapore). Nearly full-length 16s rRNA gene sequences were aligned to

rRNA gene sequences in SILVA-compatible alignment database by Mothur 1.33.3. Chimeric artifacts were checked by using the sequence collection as its own database through Mothur program. Operational taxonomy unit (OTU) assignments were based on a similarity threshold of 97%. Taxonomic classification at the phylum level were done with naïve Bayesian classifier method using SILVA full length sequences database and taxonomy, bootstrapped 1000 times with 70% cutoff.

The closest relatives in GenBank for the representative sequence for each OTU were identified by using the NCBI BLAST program. Phylogenetic trees were constructed with MEGA program version 5.2 on the basis of evolutionary distances that were calculated by the Neighbor-Joining method with Jukes-Cantor model. Bootstrap resampling analysis was performed for 1000 replicates to estimate degrees of confidence in tree topologies. Identical sequence was recognized by analysis of phylogenetic trees and manual comparisons, in which sequences that were more than 99% similar were defined as identical (Maspolim et al., 2015).

4.12 Statistical analysis

One-way analysis of variance (ANOVA) and least significant difference (LSD) were used to test the differences in the properties of HS compound (element composition and atomic ratio) and waste characteristics (i.e., HS content, TOC, etc.) among bioreactors. Type I error α was specified at a level of 0.05.

Chapter 5 Effects of Waste Age on Stabilization of Aged Waste during Aeration in Simulated Landfill Bioreactors

It brings a lot of benefits to apply aeration in the later stage of landfills. However, the question remains on when the best time would be to introduce aerobic condition to achieve these benefits. In bioreactor AR35, LR35 and ZR35, aeration was initiated at different time after bioreactor loading, representing the waste age of 5 months, 10 months and 14 months when aeration commenced. In this chapter, the emission behaviors of AR35, LR35 and ZR35 were compared to quantify the effects of the waste age with NR35 as anaerobic control. The number “35” in the name of the bioreactor indicated that all the bioreactors were operated in the warm room, with a constant room temperature of 35 °C.

5.1 Leachate DOC

The monitored DOC concentrations in leachate sample from NR35, AR35, LR35 and ZR35 are plotted in Figure 5-1. The DOC in NR35 gradually decreased from 811 mg/L to less than 30 mg/L at the end. Differently, DOC in AR35 showed a rise immediately after aeration and reached the maximum of 1440 mg/L at day 9, which was 1.8 times of the value in NR35 at the moment. Afterwards, DOC in AR35 was characterized by a rapid reduction to 215 mg/L before day 200, and then a slow decrease to 128 mg/L towards the end. The DOC in LR35 and ZR35 bioreactors also presented an increase by 30% and 90%, respectively, when converted into aerobic conditions, confirming that aeration enhanced dissolution of organic matter in the present study. Thereafter, the DOC in LR35 and ZR35 fluctuated around 145 mg/L and 113mg/L, respectively.

Figure 5-1 Variation of TOC in leachate sample from NR35, AR35, LR35 and ZR35. “↔” indicates the sampling periods for humic matter analysis: period I (day 73, 77, 82 and 87) and period II (day 215, 220, 224 and 229). The first “↑” indicates the start of aeration in LR35, while the second “↑” indicates the start of aeration in ZR35

Similar results were reported by Oncu et al. (2013) observing increased organic loads in the leachate during aeration. Over a 32-month aeration period in Dorfweier landfill, concentrations of COD and BOD were dramatically increased from 2000 mg/l up to 8100 mg/l and 70 mg/l up to 570 mg/l, respectively. Moreover, both concentrations showed a rolling change during aeration and no reduction trend was observed (Oncu et al., 2013). The persistent enhancement in leachate strength during the whole aeration period was different with the temporal increment found by Prantl et. al. (2005) and Raga and Cossu (2013), where COD only rose up in the first few days or months, while followed by a rapid reduction. The initial increase period was explained by the mobilization effects of the mechanical disturbance by air injection (Prantl et al., 2005). However, the persisting increased leachate strength in current and Oncu et al.’s (2013) studies suggested other mechanisms behind this. In addition, the darker color of leachate after aeration in the present study indicated the presence of high concentration of HS. Further investigations were conducted as a result.

5.2 Analysis by MALDI-TOF-MS of HS molecules

The leachate samples were collected from NR35 and AR35 in period I and NR35, AR35 and LR35 in period II for HS isolation. MALDI-TOF-MS determined the MW distribution of HS and two MALDI-TOF mass spectra are presented in Figure 5-2 as examples. The spectra of HS in current study were consistently found to contain a number of common peaks. It was difficult to assign the origins to peaks in the low mass to charge ratio (m/z) range, as they could be attributed to matrices, contaminants or HS fragments during ionization. Thus only the largest m/z peak with signal-to-noise ratio over 3 in each spectrum, which was more likely associated with the ionized HS molecule, was used to calculate the weight-average molecular weight.

Tables 5-1 and 5-2 summarize the largest m/z peak observed in MALDI spectra for HAs and FAs leachate samples, respectively. The highest mass detectable for HAs in NR35-I, AR35-I were at m/z 1914, and 2480, respectively, whereas peaks as high as m/z 2212, and 2483 were observed for HAs from NR35-II, and AR35-II, respectively. Compared to period I, the HAs in period II contained a higher MW, indicating humification progress along with time. This observation is in consistent with Kang (2002), who also reported that the MW of HAs tended to be higher as landfill aged. Moreover, the HAs in aerobic reactors displayed higher MW than anaerobic ones from the same sampling period, implying more intensive polymerization process in aerobic conditions. According to Piccolo (2002), the MW of HAs could be increased by incorporation of new components through -C-O- or -C-C- bonds. These new covalent bonds could be achieved via an oxidative-coupling reaction catalyzed by oxidative enzymes such as peroxidases and phenoloxidases (Dec and Bollag, 2000; Piccolo et al., 2000). Since these enzymes are more abundant and more active under aerobic condition (Freeman et al., 2001), the HS elongation is expected to be more efficient in aeration reactors.

Figure 5-2 MALDI mass spectra for AR35-I FAs and HAs

It was further noted that HAs from LR35-II exhibited the largest MW (3548), 43% higher than that of HAs from AR35-I. Both reactors had been subjected to the same duration of aeration (2 months), but starting at different points. The commencement of aeration in younger waste age produced HAs with a smaller size (such as AR35-I). The early aeration interrupted the size evolution of HAs, as it leached out a substantial amount of organic matter. It resulted in deficiency of

precursor substances from forming larger HAs molecules via intermolecular association (Sutton and Sposito, 2005). The bigger size of HAs in LR35-II was likely ascribed to the already attained higher molecular weight due to waste aging under previous prolonged anaerobic condition.

Interestingly, the changes in MW among different FAs were minor whereas the composition and structure were significantly varied (Figure 5-1 and Table 5-4). It could be ascribed to the alteration of side-chains in lignin-like constituent (Chai et al., 2008). Due to the preservation of the main aromatic carbon network, changes of the MW were minor.

5.3 Element analysis of leachate HS

According to elemental analysis, the element content for HS was in the typical range as reported in the literature (Tables 5-1 and 5-2). The low H/C and high N/C values were associated with high degree of humification, due to mineralization of alkyl carbon branch and preservation of nitrogen (Tan, 2003). Thus, the decreasing H/C ratio and slight increasing N/C ratio along with time suggested that the degree of aromaticity and maturity of FAs increased with operation time in NR35, AR35 and AR55, respectively.

It is difficult to presume the same trend of the compositional change for HAs along with time, attributed to the complexity in the formation of HAs. On one hand, alkyl carbon was frequently incorporated in the HAs molecule, such as lipid, peptide and polysaccharide. On the other hand, the existent alkyl carbon was lost as CO₂ during humification, as aliphatic structures also present a carbon source for microorganism (Barje et al., 2012). The ambiguity in the temporal variation of H/C and N/C ratios in HAs was also observed in other relative studies (Han et al., 2009; Kang et al., 2002).

Table 5-1 Elemental composition^a, atomic ratio and MW for HAs samples in present study and for landfill leachate HAs in the literatures

HAs	NR35-I	AR35-I	AR55-I	NR35-II	AR35-II	AR55-II	LR35-II	Literature^b
N% (mass%)	4.23±0.01	5.93±0.01	4.68±0.02	3.85±0.03	4.06±0.01	3.61±0.01	7.04±0.01	1.79-8.30
C% (mass%)	51.71±0.22	51.02±0.21	51.67±0.06	52.56±0.71	53.46±0.04	53.51±0.21	49.86±0.02	40.06-64.94
H% (mass%)	5.68±0.04	5.47±0.01	5.53±0.01	6.09±0.07	6.02±0.01	5.72±0.03	6.08±0.01	5.54-8.80
H/C (atomic ratio)^c	1.31±0.00	1.28±0.00	1.28±0.00	1.38±0.00	1.34±0.00	1.27±0.00	1.45±0.00	1.25-1.88
N/C (atomic ratio)^d	0.07±0.00	0.10±0.00	0.08±0.00	0.06±0.00	0.07±0.00	0.06±0.00	0.12±0.00	0.02-0.11
MW^e	1914	2480	2480	2212	2483	2870	3548	1490-3940 ^f

^aMean value (n=2)

^bRef.(Chai et al., 2013; Chai et al., 2010; Kang et al., 2002; Nanny and Ratasuk, 2002)

^cThe differences of H/C atomic ratio are significant. In period I, AR55-I = AR35-I < NR35-I at $p \leq 0.05$ level, while in period II, AR55-I < AR35-I < NR35-I at $p \leq 0.05$ level

^dThe differences of N/C atomic ratio are significant at $p \leq 0.05$ level.

^eThe largest m/z peak detected by MALDI-TOF-MS.

^fRef.(Christensen et al., 1998; Lou and Xie, 2006; Mugo and Bottaro, 2004)

Table 5-2 Elemental composition^a, atomic ratio and MW for FAs samples in the present study and for landfill leachate FAs in the literatures

FAs	NR35-I	AR35-I	AR55-I	NR35-II	AR35-II	AR55-II	LR35-II	Literature ^b
N% (mass%)	2.36±0.02	3.27±0.01	3.53±0.01	2.18±0.03	3.59±0.01	3.73±0.02	2.07±0.01	1.68-12
C% (mass%)	48.31±0.30	47.33±0.16	47.47±0.02	52.06±0.83	49.19±0.04	46.82±0.19	51.42±0.27	36.8-55.66
H% (mass%)	5.30±0.08	4.82±0.02	4.75±0.05	5.65±0.03	4.92±0.01	4.66±0.01	5.33±0.02	4.3-9.6
H/C (atomic ratio)^c	1.31±0.01	1.21±0.00	1.19±0.01	1.29±0.03	1.19±0.00	1.19±0.00	1.23±0.01	1.34-2.53
N/C (atomic ratio)^d	0.04±0.00	0.06±0.00	0.06±0.00	0.04±0.00	0.06±0.00	0.07±0.00	0.03±0.00	0.03-0.23
MW^e	2072	2071	2071	2072	2071	2072	2097	1000-2310 ^f

^aMean Value (n=2)

^bRef.(Kang et al., 2002; Nanny and Ratasuk, 2002; Pelaez et al., 2009)

^cThe differences of H/C atomic ratio are significant. In period I, AR55-I < AR35-I < NR35-I at $p \leq 0.05$ level, while in period II, AR55-I = AR35-I < NR35-I at $p \leq 0.05$ level

^dThe differences of N/C atomic ratio are significant at $p \leq 0.05$ level.

^eThe largest m/z peak detected by MALDI-TOF-MS.

^fRef.(Chin et al., 1994; Christensen et al., 1998; Mugo and Bottaro, 2004)

The observed differences in the H/C ratio of HS in the same sampling period are statistically significant and had the following order: AR55 $\leq$ AR35 < NR35 at $p \leq 0.05$ level. This suggests that aeration could enhance the humification degree of HS. This conclusion is consistent with the findings of previous research, which claimed that aeration could help to achieve rapid maturation of the humic acid in waste materials (Lguirati et al., 2005). Moreover, it was noticed that the maturation of the HS was further accelerated by high temperature.

5.4 Analysis of leachate HS structure by ^{13}C -NMR

The ^{13}C -NMR spectra of dissolved HAs and FAs are shown in Figure 5-3 and Figure 5-4, respectively. Based on previous NMR studies (Bartoszek et al., 2008; Duarte et al., 2013; Keeler et al., 2006), the spectra could be split into eight main spectral regions: 0-44 ppm (alkyl chain carbon); 44-60 ppm (OCH_3 group or α carbon of polypeptides), 60-93 ppm (O-alkyl in carbohydrate or alcohol); 93-110 ppm (anomeric carbon of a glucose fragment, O-C-O anomeric); 110-140 ppm (unsubstituted aromatic C and alkyl-substituted aromatic C); 140-162 ppm (oxygen-substituted aromatic ring C); 162-190 ppm (carboxyl C or carbonyl C from amides and polypeptides); and 190-220 ppm (carbonyl C of aldehydes or ketones).

The relative distribution (%) of each C-type was calculated by dividing the peak area in this specific region by the total spectral peak area and is summarized in Table 5-1. The signals in 43-60 ppm and 141-162 ppm are typical in spectral of lignin structural units, which could be attributed to methoxyl group (O-CH_3) and oxygen-substituent aromatic carbon (aromatic C-O) (Duarte et al., 2013). These two lignin fingerprints were most abundant in AR35-II HAs, indicating the significant contribution of lignin structure which may be derived from paper and yard waste (Zhao et al., 2013). Moreover, a clear decrease of alkyl carbon ($\delta = 0-43$ ppm) was noticed in AR35-II HAs. The loss of alkyl carbon were most probably caused by the biomineralization of methine and methylene groups in aliphatic chains (Keeler et al., 2006), as their typical peaks at 24 ppm, and 30 ppm in the spectra of AR35-II HAs were correspondingly depressed. As a consequence, HAs with higher aromaticity was achieved under aerobic condition. It was also noticed the distinct drop in of COOH

content in HAs of NR35 and AR35 along with time, owing to C loss in the form of CO₂ during biological mineralization (Barje et al., 2012).

Figure 5-3 Solid-state ¹³C-NMR spectra of leachate HAs

Figure 5-4 Solid-state ¹³C-NMR spectra of leachate FAs

Table 5-3 Molecular weight and relative distribution (%) of signal area over chemical shift regions (ppm) in ^{13}C -NMR spectra of dissolved HAs

HAs	Assignment	NR35-I	AR35-I	NR35-II	AR35-II	LR35-II
0-43ppm	alkyl	20.74	22.53	20.38	16.58	29.40
43-60ppm	O-CH ₃ /NCH	12.57	12.96	13.36	15.54	16.30
60-92ppm	O-alkyl	17.64	15.73	20.91	20.29	18.27
92-110ppm	O-C-O anomers	5.00	4.52	4.87	5.10	3.65
110-141ppm	aromatic C-H & C-C	20.89	19.24	21.52	22.75	13.21
141-162ppm	aromatic C-O	9.92	8.94	9.15	9.74	4.02
162-190ppm	COO/N-C=O	10.06	12.70	8.05	7.73	12.84
190-220ppm	C=O	3.17	3.39	1.77	2.28	2.31
aromaticity^a		0.36	0.34	0.34	0.36	0.20

^a aromaticity (%) = [aromatic C peak area (110-162 ppm)/total peak area (0-162 ppm)]

Table 5-4 Molecular weight and relative distribution (%) of signal area over chemical shift regions (ppm) in ^{13}C -NMR spectra of dissolved FAs

FAs	Assignment	NR35-I	AR35-I	NR35-II	AR35-II	LR35-II
0-43ppm	alkyl	43.30	36.23	43.22	36.99	41.07
43-60ppm	O-CH ₃ /NCH	8.22	8.93	7.48	8.91	8.93
60-92ppm	O-alkyl	13.68	12.70	13.88	12.10	13.09
92-110ppm	O-C-O anomers	1.27	2.10	2.10	2.29	2.23
110-141ppm	aromatic C-H & C-C	12.83	14.11	13.96	13.64	11.96
141-162ppm	aromatic C-O	4.69	5.82	5.18	6.02	4.78
162-190ppm	COO/N-C=O	13.48	16.03	11.72	16.56	14.67
190-220ppm	C=O	2.53	4.07	2.48	3.50	3.27
aromaticity^a		0.21	0.25	0.22	0.25	0.20

^a aromaticity (%) = [aromatic C peak area (110-162 ppm)/total peak area (0-162 ppm)]

By contrast, spectra study of HAs from LR35-II indicated the abatement of aromatic carbon and an increase for alkyl carbon. Considering the meanwhile increase in MW, the reduction in aromaticity was relatively a result of the introduced aliphatic components by coupling interactions during polymerization, such as lipid and peptides. The augmentation of N content (Table 5-1) in its element composition indicated the involvement of peptides during the evolution of HAs molecules. Such incorporation could also be evidenced by the increase of the relative intensity in 43-60 ppm and 162-290 ppm, as resonances in these two regions may also derive from α carbon and carbonyl carbon of polypeptides, respectively.

Similar to HAs, the degree of aromaticity in FAs was increased by aeration (Table 5-2). Moreover, compared with the anaerobic bioreactors, FAs from aeration bioreactors revealed 20%-40% higher content in carboxylic carbon. The increase in carboxyl carbon was accompanied by the decrease in alkyl carbon and O-alkyl carbon, indicating that the main structural variation in FAs originated from the oxidation of alcohol side chain of lignin structure to yield by carboxylation a COOH group (Plaza et al., 2007).

5.5 DOC fractionation

The measured distribution of leachate DOC into HAs, FAs and HyI fractions is shown in Figure 5-5. It was observed that the percentage of HAs was increased by aeration. In NR35-I, HAs contributed 46% of DOC, while it was for 76% in AR35-I.

Two potential mechanisms played significant roles for the enhancement of HAs dissolution from waste matrix. First, aeration could increase the pH value of leachate from 7 to 8-9 (Figure 5-6). At higher pH, more acidic groups of humic acids were deprotonated, giving the humic particles more negative charges. The resulted higher electrostatic repulsion dispersed large aggregates into smaller humic associates and consequently increased the dissolution of HAs (Brigante et al., 2009; Klučáková and Pekař, 2005). Second, aerobic condition enriched carboxylic group

in the structure of HAs. For instance, the COOH abundance was 26% higher in HAs from AR35-I (12.7%) than HAs from NR35-I (10.1%). The larger hydrophilicity (due to higher content of carboxylic group) of HAs could prevent strong molecular association by hydrophobic forces, resulting in HAs molecular aggregate with smaller size (Sutton and Sposito, 2005) and higher leachability.

Figure 5-6 Distribution of DOC into HyI, FAs, and HAs in the leachate from period I and period II

Figure 5-5 pH variation in leachate from each reactor. The first “↑” indicates the start of aeration in LR35, while the second “↑” indicates the start of aeration in ZR35

In period II, the HAs percentage in all fractions considerably decreased, which accounted for about 41% and 59% in NR35-II and AR35-II, respectively. Most likely, the key factor for the decrease of HAs in period II was the reduced HAs pool in the waste matrix, as lots of HAs had already been leached out. Furthermore, the decrease of COOH content in HAs structure in period II meant reduction of the mobility (Table 5-3), as the decrement in hydrophilicity (due to lose of COOH group) tightened the hydrophobic forces between humic assembling. When humic molecules remained confined in strong hydrophobic association of apparently large molecular size (Shin et al., 1999), it could hardly be liberated to dissolve in water. Moreover, the increase in size of the single HAs molecules was another possible reason for the reduction of HAs abundance. However, as the pH kept higher in aeration reactors, the HAs abundance in AR35-II was still larger than that in NR35-II.

Different from AR35, there was no increase of HAs concentration in LR35 when aeration was commenced later. It might be explained that larger MW (3548) in HAs from LR35-II had a lower solubility, resulting in limited dissolution. Although after aeration pH was increased and carboxylic groups were accumulated in molecular structure, the larger MW of HAs from LR35-II could cushion the dissolution effects of the abovementioned alterations and prevent itself from being washed out. As MW of HAs was positively correlated with the landfill age, it is assumed that aeration could lead to less leaching of larger molecular weight HAs in old landfills. Subsequently it could reduce the risks of elevated leachate loading.

The abovementioned assumptions are supported by the literature. Increased organic loads in the leachate have been observed Oncu et al (2013). COD decrease after aeration was reported by Ritzkowski et al. (2006), Prantl et al. (2006) and Raga and Cossu (2013). Many factors including waste age, waste types as well as aeration procedures, might play different roles in these studies leading to different results. Nevertheless, it is noticeable that waste age in the current study and Oncu's pilot project (4~11 years) was much younger compared with the waste materials used by Ritzkowski et al. (13~35 years) (2006), Prantl et al. (8~17 years) (2006) and Raga and Cossu (5~15 years) (2013).

5.6 CO₂ generation rate and Carbon mineralization rate

CO₂ generation rate could be used to estimate the biodegradation rates of hydrocarbon. These rates are important (1) for determining the potential application of in-situ aeration at a given landfill site; and (2) for estimating the time required for aeration at a given landfill site (Ritzkowski and Stegmann, 2013). Although many parameters are known to interfere with microbial activity, CO₂ generation rate integrates all factors to simply reveal to which extent organic substances are decomposed by the aerobes (Leeson and Hinchee, 1997).

Figure 5-7 (a)-(c) show the CO₂ generation data for AR35, LR35 and ZR55, respectively. In each test, the percentage of CO₂ displayed a linear relationship with time and the slope of the fitting line determined the CO₂ generation rate. The higher the slope, the higher the microbial activity and organic substrate degradation rate are. In total, 14, 9 and 5 tests were carried out for AR35, LR35 and ZR55, respectively. The calculated carbon mineralization rates versus time are presented in Figure 5-7(d). Overall, the variation of carbon mineralization rates can be divided into adjustment phase, initialization phase, active degradation phase and maturation phase. In AR35, the carbon mineralization rate in the adjustment phase started from 27 mg/d at day 0 and slightly dropped to 11 mg/d around day 56. During the adjustment phase, the indigenous anaerobic condition was interrupted when air was introduced artificially. As a consequence, aerobes overwhelmed anaerobes and gradually became the dominant microbial species. Being acclimated to the newly established situation, the microbial population began the fast growth in the initialization phase, which resulted in the carbon mineralization rate rising to 63 mg/d around day 87. When aerobes reached the highest growth rate, the carbon source in waste was exploited at maximum in the active degradation phase. In AR35, the rate maintained around 60 mg/d until day 183. During the maturation phase, the microbial activity diminished since the biodegradable components were eventually depleted. In AR35, the carbon gasification rate gradually reduced to approximately 30 mg/d around day 246 and no significant change was observed afterwards.

Figure 5-7 CO₂ generation during each test in AR35 (a), LR35 (b), ZR35 (c) and carbon mineralization rate versus time (d)

Distinct from the performance in AR35, the carbon mineralization rate in LR35 was marked by an immediate increase once aeration began. The adjustment phase was bypassed, probably due to the later aeration compared to AR35. As in the later stage of previous anaerobic degradation the landfill gas generation rate was much lower. Therefore the diffuse of oxygen through either water addition port or leachate drainage port became more significant. Before aeration, aerobes may already coexist at spots, which was able to start the initialization phase without extra adaptation. Moreover, the extra five-month anaerobic degradation in LR35 could further break the structurally complex and aromatic macromolecules into simpler forms, although in a slow rate. This reaction could help to provide viable substrate to quickly start the aerobic bioconversion. The carbon mineralization rate reached the maximum of 166 mg/d around day 211 (56 days after aeration) and leveled off until day 275 (120 days after aeration). Afterwards, it gradually decreased to 120 mg/d at the end of the experiment (241 days after aeration). During the whole monitoring period, the carbon mineralization rate in LR35 was considerably larger than that in AR35. In AR35, because of the young waste age, aeration enhanced the dissolution of organic matter, which later was washed out from the bioreactor and avoided the biomineralization. The carbon removal in each leachate drainage process was calculated by DOC and the discharged leachate volume. The total organic carbon load in terms of accumulated DOC discharge was determined at 14.4 g and 6.0 g in AR35 and LR35, respectively. The lower leachate loading and higher carbon mineralization rate in LR35 suggested a larger contribution of biomineralization than that in AR35. This provides one additional benefit of later aeration, since the emission of CO₂ caused lower environmental concerns compared with discharge of dissolved organic carbon.

Unlike the considerable differences with AR35, the change of carbon mineralization rate in ZR35 over time followed a very similar trend with that in LR35 but varied in intensity. For example, ZR35 began with a carbon mineralization rate of 89 mg/d, which was slightly higher than the beginning value of LR35 (74 mg/d). This finding further supported the previous statement drawn from the comparison between AR35 and LR35. When the waste age of a matured

anaerobic landfill increased, the waste matrix would be exposed to air intrusion for a longer period. This may trigger the proliferation of facultative or even obligate aerobes, leading to a rapid and efficient startup. Longer period of aging before aeration in ZR35 also resulted in a deeper depletion of the carbon pool in the waste materials. This could explain why the mineralization rate of ZR35 only peaked at 159 mg/d, which was lower than that in LR35 (168 mg/L). Until the end of the experiment, ZR35 was only aerated for four months and merely at the early stage of maturation phase. In total, the carbon removal through biomineralization in ZR35 was around 5.3 g, which was only one third as compared to LR35 (18.2 g). However, the carbon discharge ratio of leachate/biomineralization in aerobic stage was almost the same in LR35 and ZR35 (around 10), which was significantly higher than that of AR35 (around 3). This again suggested that later aeration may promote biomineralization mechanism over leaching pathway.

5.7 Characterization of end waste

Table 5-5 provides the data of solid waste analysis as well as carbon discharge through different pathways. Beginning at 11.5 L kg⁻¹ DM, GS₂₁ value in AR35 decreased to 4.8 L kg⁻¹ DM, which was about 76% of the value in NR35. The enhanced biostability by aeration corresponded to the finding of Hrad et al. (2013), who observed a reduction of 83% of the initial GS₂₁ value in landfill aerobic bioreactors in comparison to 71% under anaerobic bioreactors.

Aeration enhances the biodegradation of organic compound through several steps (Kristensen et al., 1995). First, oxygen serves as a reactant in the oxygenase catalyzed primary attack on the substrate to hydrolyze the organic matter into low-molecular compounds. Of the long list of primary reaction, only a few can be realized in the absence of oxygen. Then, all the products from the primary reactions are rapidly metabolized by microorganism with the presence of oxygen.

Table 5-5 Waste Characterization before and after bioreactor running and carbon discharge through different pathways

	NR35	AR35	LR35	ZR35
HAs content^{a,b} (mg C/g DM)	5.8±0.3	7.8±0.0	16.6±2.0	10.3±0.3
FAs content^{a,c} (mg C/g DM)	0.9±0.0	1.1±0.1	2.1±0.1	1.9±0.0
Initial TOC (g C/kg DM)	583.6	583.6	583.6	583.6
End TOC (g C/kg DM)	574.8	547.2	558.0	559.3
TOC difference (g C/kg DM)	8.8	36.5	25.6	24.3
Anaerobic leachate C discharge^d (g C/kg DM)	2.7	0.0	1.8	2.5
Aerobic leachate C discharge^e (g C/kg DM)	0.0	7.9	1.5	0.5
Anaerobic C mineralization^f (g C/kg DM)	5.0 (4.1 ^e)	0.0 (0.0 ^e)	4.0 (3.4 ^e)	4.6 (3.8 ^e)
Aerobic C mineralization^g (g C/kg DM)	0.0	20.2	18.2	5.3
Initial GS₂₁^h (L kg⁻¹ DM)	11.5±2.7	11.5±2.7	11.5±2.7	11.5±2.7
End GS₂₁^{h,i} (L kg⁻¹ DM)	6.3±1.0	4.8±0.1	7.7±0.0	8.3±0.3

^amean value (n=2)

^bThe differences of HAs content are significant and LR35 > ZR35 > AR35 > NR35 at p ≤ 0.05 level.

^cThe differences of FAs content are significant and LR35 = ZR35 > AR35 = NR35 at p ≤ 0.05 level.

^dcumulative carbon discharge through leaching under anaerobic condition

^ecumulative carbon discharge through leaching under aerobic condition

^fcarbon removal through anaerobic metabolization, including CH₄ and CO₂

^fThe value in the brackets indicates the carbon discharge in the form of CH₄ during anaerobic operation period, which was 396 days, 0 day, 155 days and 275 days in NR35, AR35, LR35 and ZR35, respectively.

^gcarbon removal through aerobic metabolization, emitting as CO₂. The amount of mineralized carbon was estimated by integrating the corresponding carbon mineralization curve in Figure 5-7 (d).

^hmean value (n=3)

ⁱThe differences of GS₂₁ are significant and ZR35 > LR35 > AR35 = NR35 at p ≤ 0.05 level.

In the first step, as the supply of labile substrate was increased, the GS_{21} value was expected to rise temporarily. Along with time, however, aeration eventually leads to a state where only the hardly- or non-degradable compounds have been remained. As a consequence much lower GS_{21} value would be expected than that under anaerobic condition. The GS_{21} values in LR35 and ZR35 were significantly higher than that in NR35 ($p \leq 0.05$), suggesting that the level of biodegradability of waste was augmented by aeration, whereas the reaction at the first step was dominant in LR35 and ZR35 by the end of experiments. Assuming aeration continues, a lower GS_{21} value than that in NR35, may be quickly obtained in LR35 and ZR35. After 396 days operation, the lowest GS_{21} was detected in AR35 in comparison with LR35 and ZR35. The results indicated that the earlier the aeration commenced, the earlier the waste getting stabilized.

When aerobic condition is established by aeration in traditional landfills, the carbon, which would under formerly anaerobic conditions have been emitted as CH_4 , is emitted as CO_2 instead. Since the CO_2 from landfill is considered to have no greenhouse impact, the greenhouse gas emission of the landfill is significantly reduced by aeration (Ritzkowski and Stegmann, 2010). The carbon discharge via gas release under anaerobic conditions was calculated by utilizing the analyzed gas composition (CO_2 and CH_4) and the measured gas volume. The results, referred to the unit dry mass, are presented in Table 5-5 as anaerobic C mineralization. Among the 5.0 g C/kg DM gas emission in NR35, 4.1 g C was released as CH_4 . This part of CH_4 emission was totally avoided in AR35, as no more methane detection after aeration. In agreement of our study, Ritzkowski et al. (2006) only measured the CH_4 concentration in a range from 0.5% to 2.5% v/v in the off-gas of the aerated landfill. Unlike AR35, methane was still generated in the first 155 days and 274 days in LR35 and ZR35 respectively, which could have been prevented if aeration started earlier.

Comparing the carbon discharge through leachate pathway in AR35, LR35 and ZR35, it demonstrated that later aeration could lower the increment in leachate loading. The cumulative carbon discharge by leaching in ZR35 (3.0 g C/kg DM)

was 94% and 38% of that in LR35 (3.3 g C/kg DM) and AR35 (7.9 g C/kg DM), respectively. The organic carbon, which was not washed out by leachate, may be involved in biological respiration and removed as CO₂ emission. It is why the ratio between the two carbon removal pathway (mineralization/leaching) after aeration was higher in older waste materials. The ratio in LR35 and ZR35 was 10.6 and 12.1, respectively, which was more than four times of that in AR35 (2.6).

During biodegradation, part of the organic matter is mineralized to carbon dioxide, ammonia and water, while the other is converted into stabilized HS. The quantity and properties of HS determine the degree of maturity of the treated waste, as the biostability of organic substrate refers to the inherent recalcitrance related to aromatic structures in complex macromolecules, such as HS (Serramiá et al., 2013). Compared to anaerobic reactor, the rise of HAs content (34%) and FAs content (22%) were noted in end waste materials from all aeration bioreactors. The abundance of HS in AR35 could be related to the enhanced humification process under aerobic condition. Given that the discharged leachate from AR35 contained high amount of HAs, the difference between the HAs level in AR35 and NR35 should be more than those shown in Table 5-5.

As later aeration in LR35 and NR35 did not wash out the organic substrate as much as that in AR35, the HS precursors and formed HS molecules could be largely retained. This was why higher amount of HS was observed in the end waste materials from LR35 and ZR35, although they were subjected to a shorter period of aeration than AR35. If HS was not lost through washing enhanced by aeration, the HS content should be positively correlated with the duration of aeration because of longer reaction time. In this event, LR35 was found to be richer in HS content than ZR35.

5.8 Conclusion

Accumulation of the organic carbon in the leachate after aeration in the current study was observed. Further studies on HAs, FAs and HyI fractionation revealed that aeration dramatically raised the percentage of HAs, which were contributable to

the DOC elevation. Enriched functional groups -COOH in HAs structure under aerobic condition was responsible for the enhanced dissolution of HAs. The higher pH after aeration further facilitated the HAs release. However, aeration did not enhance HAs release from waste materials with prolonged aging, most likely due to the limitation in mobilizing the aged HAs, which had higher MW.

Earlier aeration could eliminate methane generation to a large extent and achieve earlier stabilization of landfill site for profitable reuse. However, earlier aeration could create new problems in leachate treatment, as it may increase the leachate strength especially due to HAs accumulation. Because HAs are not readily biodegradable and they can pass through the biological treatment processes compromising the quality of the effluent. Herein more emphases should be put on physical-chemical processes, such as coagulation, membrane filtration and high advanced oxidation. By contrast, later aeration could avoid the problems associated with increased HAs concentration in the leachate, and allow more carbon to be engaged in the biodegradation pathway. As a result, more HAs could be generated and retained in the waste materials, which could be feasible for usage as biofertilizer or soil conditioner, although many other factors should be considered, such as heavy metal and pathogen content. Since earlier and later aeration both have their own pros and cons, a compromise should be made when designing the starting time of aeration. Moreover, the site condition, surrounding environment and government regulations need also to be taken into account during a successful landfill redevelopment.

Chapter 6 Effects of Temperature on Stabilization of Aged Waste during Aeration in Simulated Landfill Bioreactors

This chapter presents the observation on the temperature-mediated biochemical processes during in situ aeration of aged MSW. In particular, the effects of different temperatures (35, 45, and 55 °C) on carbon degradation rate, leachate quality and waste stability were investigated in lab-scale simulated landfill bioreactors. Particularly, the leachate organic nitrogen distribution in different fractions, i.e. the humic acid (HAs), fulvic acid (FAs) and hydrophilic compound (HyI), was measured the first time during landfill aeration to the best of the authors' knowledge. The findings from this study may provide as useful references to predict the emissions and stability of landfill refuse at different temperatures during aeration process.

6.1 CO₂ generation and carbon mineralization

Figure 6-1(a)-(c) show the CO₂ generation data for AR35, AR45 and AR55, respectively. To reveal the temporal evolution of microbial activity and organic substrate degradation rate, fourteen tests were conducted for each bioreactor at a frequency of approximately once per month. The estimated carbon mineralization rates versus time are presented in Figure 6-1(d). Overall, the variation of carbon mineralization rates can be divided into initialization phase, active degradation phase and maturation phase. In AR55, the carbon mineralization rate in the initialization phase started from 26 mg/d and reached rapidly to 111 mg/d on day 56. The initial increase was attributed to the rapid metabolic activity of aerobic microbial community. During initialization phase, aerobes gradually replaced anaerobes to become the dominant microbial species. Carbon aerobic mineralization was triggered and further promoted as aeration proceeded. When aerobic conditions were fully established and aerobes reached the highest growth rate, the carbon source in waste was exploited at the maximal rate in the active degradation phase. In AR55, the rate maintained around 80 mg/d until day 183. During the maturation phase, the microbial activity diminished since the biodegradable components were eventually depleted. In AR55, the carbon gasification rate gradually reduced to

approximately 40 mg/d around day 250 and no further change was observed afterwards.

Figure 6-1 CO₂ generation during each test in AR35(a), AR45 (b), AR55 (c) and carbon mineralization rate versus time (d)

Different with the immediate rising in AR55, carbon mineralization rate in AR35 first declined to 11 mg/d around day 56 and then began the initialization phase. The extra adjustment phase at the beginning indicated that the acclimation of the microbial community was relatively slow at 35 °C. Furthermore, compared to AR55, the carbon mineralization rate was lower in AR35 during the whole experimental period, suggesting that biodegradation process was more efficient at thermophilic temperature than mesophilic temperature. This observation agrees well with the findings from other bioprocesses (Fernández-Rodríguez et al., 2013; Ryu et al., 2009; Ugwuanyi et al., 2005).

Carbon mineralization rate in AR45 displayed the similar four phases as that in AR35. In particular, the delay phase in AR45 extended to day 87, which was one month longer than that in AR35. The longer adaption time at 45 °C was also observed in the landfill aeration study of Raga and Cossu (2013). After the lag phase, the gasification rate increased rapidly in AR45, reached up to 90 mg/d between day 211 and day 308, and then slightly declined at the maturation phase. At the end of the experiment, AR45 was just at the beginning of the maturation phase, as the biomineralization activity was still high. In order to achieve the same steady state as that in AR35 and AR55, a prolonged aeration was foreseen for AR45, which inevitably would increase the operation cost.

6.2 Leachate strength

The influences of temperature on organics load in the leachate characterized by COD are demonstrated in Figure 6-2. COD in NR35 showed a gradual descending from 2240 mg/L in the beginning to 100 mg/L at the end, due to leaching processes and anaerobic degradation. By contrast, COD concentrations were rapidly increased to their highest values up to day 18 in all aerobic bioreactors at 4750 mg/L, 6460 mg/L and 6020 mg/L in AR35, AR45 and AR55, respectively. The initial increment after air injection may be induced by suddenly enhanced hydrolysis process (Kristensen et al., 1995), which may not be as favorable in the previously anaerobic condition. As the percolating water was not sufficient enough to remove COD in pace with its production, the accumulation of COD in leachate were observed

(Giannis et al., 2008). Before day 32, the COD in AR45 and AR55 were up to 38% higher than that in AR35, indicating exaggerated solubilization of organic matter at higher temperature.

Figure 6-2 Evolution of COD concentrations in bioreactor leachate

After reaching the maximum value, all COD values in aerobic reactors declined. The fastest COD attenuation was noticed in AR55, as from day 32, COD in AR55 kept at the lowest value among all the aeration bioreactors, which could be caused by the accelerated biodegradation at 55 °C. Before day 161, COD in AR45 was slightly lower than that in AR35, most probably because of the significant reduction of the soluble carbon during the intensive leaching at the beginning. However, after day 161, the COD concentration in AR45 surpassed that in AR35, becoming the highest one until the end. It could be assigned to the elongated lag phase in the first 87 days and the inefficient bioremoval in 45 °C in the following 74 days, since microbes prefer two temperature range dominant at around 35 °C and 55 °C, while not in between (Raga and Cossu, 2013). Eventually, the COD stabilized at 400 mg/L, 700 mg/L, and 250 mg/L in AR35, AR45 and AR55, respectively.

6.3 Inorganic nitrogen in the leachate

The variations of NH_4^+ -N (ammonium ion as N) concentration in the leachate sample, over 396 days, were plotted in Figure 6-3. The NH_4^+ -N concentration in the leachate from all the four bioreactors started with 1625 ± 54 mg/L, indicating the homogeneous load of the synthetic aged waste among the bioreactors. In NR35, NH_4^+ -N concentration decreased slowly. Because of the low removal rate in anaerobic condition, ammonia is considered as the most significant long-term problem at traditional landfills (Barlaz et al., 2002).

Figure 6-3 Ammonium-Nitrogen concentration in leachate sample. “→” indicates the two sampling periods for nitrogen speciation analysis.

Regarding the aerobic bioreactors, ammonium concentration was reduced drastically below 500 mg/L directly after in-situ aeration. The main reason for ammonia attenuation was related to air stripping. Since pH in the bioreactors was alkaline, ammonia volatilization was significant (Berge et al., 2005b). The free ammonia fraction is estimated in Eq. (6.1).

$$[\text{NH}_3 - \text{N}] = \frac{[\text{NH}_3 - \text{TN}] \times 10^{\text{pH}}}{\frac{K_a}{K_w} + 10^{\text{pH}}} \quad (6.1)$$

where $\text{NH}_3\text{-N}$ is the free ammonia as N (mg/L); $\text{NH}_3\text{-TN}$ is the total dissolved ammonia as N (mg/L) and K_a and K_w are the dissociation constants of ammonia and water. The ratio of K_a/K_w is related to temperature in Eq. (6.2).

$$\frac{K_a}{K_w} = e^{\left(\frac{6344}{273+^{\circ}\text{C}}\right)} \quad (6.2)$$

At temperature 35 °C and pH around 8.5 (average pH value in aerobic bioreactors), 26% of ammonia present was in the form of free ammonia. In comparison, the percentage of free ammonia present at neutral pH (average pH value in anaerobic bioreactors) was only 2%. At higher temperatures, ammonia volatilization could be further promoted. When the temperature was at 45 and 55°C, the fraction of free ammonia at pH 8.5 increased to 41% and 56%, respectively. Since the ammonia volatilization was governed by the temperature, especially in the first place, the $\text{NH}_4^+\text{-N}$ concentration in bioreactors leachate followed the order $\text{AR55} < \text{AR45} < \text{AR35}$ before day 115.

In aerobic conditions, $\text{NH}_4^+\text{-N}$ can be also converted into other forms by the combination of nitrification and denitrification. No matter the efficiency of aeration system, anaerobic or anoxic pockets always exist. In these microenvironments, simultaneous nitrification (in aerobic zone) and then denitrification (in anoxic/anaerobic zone) could occur, similar to a designed wastewater treatment facility but in micro scale (Ritzkowski et al., 2006). However, these pathways took place after partial ammonia volatilization because of the low growth rate of autotrophic nitrifiers and potential competition with heterotrophs when organic substrate was abundant (Berge et al., 2005a). As the nitrifiers have optimal temperature range between 30-35 °C and they are inhibited at thermophilic temperature range (Willers et al., 1998), the highest ammonium removal efficiency through nitrification-denitrification was expected in AR35. It could potentially explain the low $\text{NH}_4^+\text{-N}$ concentration (below detection limit) found in AR35 at the end of the experiments, which was much lower than that in AR45 (20 mg/L) and AR55 (3 mg/L).

Figure 6-4 depicts the evolution of nitrite and nitrate in the leachate samples of aerobic bioreactors. Nitrite first increased at day 168 in AR35 and declined at day 200, with a peak around 22 mg/L at day 173. Nitrate appeared in AR35 at around day 190, leveled at 7 mg/L before day 359 and showed a slight increase in the end. In AR55, nitrite and nitrate increased simultaneously at day 196, maintained around 7 mg/L before day 261 and raised to 14 mg/L towards the end. The appearances of nitrite and nitrate in AR35 and AR55 were consorted with a considerable drop of carbon mineralization rate, which was a sign of a substantial declined carbon source. Similarly, Valencia et al. (2011) reported an elevated level of nitrate after day 200 due to depleted organic substrate for denitrifier in the bioreactor landfill study. Different from AR35 and AR55, nitrate and nitrite concentrations were found below the detection limit (0.1 mg/l) in AR45, owing to the relative high organic substrate supply for denitrifiers all the time, especially evidenced by the highest COD value in the leachate in the last half of the period. This in turn supports the previous finding that COD removal was not favored under 45 °C.

Figure 6-4 Variation of leachate NO₂⁻ and NO₃⁻ concentration. Since no nitrite was observed for NR35 and AR45, they are excluded from the figure.

6.4 Organic nitrogen in the leachate

The organic fraction in the leachate collected from period I and period II was fractioned into HAs, FAs and HyI. The organic nitrogen in HAs (HAs-N) was calculated based on the DOC concentration of HAs (HAs-C) and the N/C mass ratio of HAs. The same calculation was also conducted for nitrogen in FAs (FAs-N). All the data for HAs and FAs were presented in Table 6-1 and 6-2, respectively.

The nitrogen content in each organic fraction (HAs, FAs, and HyI) is displayed together with inorganic nitrogen species in Figure 6-5. The TN value for each sample is indicated on top of the bar chart. It was found that the TN was decreased by aeration up to 87%, with NH_4^+ -N fraction undergoing the greatest percentage of reduction (up to 99.9%). Abatement of HyI-N was also noticed in aerobic bioreactors. In period I, compared to NR35, 23%, 37%, and 58% less of HyI-N were detected in AR35, AR45 and AR55, respectively. In period II, except AR45 with slightly higher HyI-N, AR35 and AR55 were both measured with 41% lower of HyI-N than NR35. The relatively low concentrations of HyI-N in aerobic reactors were most likely related to the enhanced degradation of hydrophilic protein and amino acid under aerobic condition (Shao et al., 2013).

The HS-N, which was composed of HAs-N and FAs-N, played a minor role in NR35, with concentrations of 12 mg/L (1.9% of TN) in period I and 3 mg/L (1.6% of TN) in period II. By contrast, HS-N showed significant increment in aerobic bioreactors, accounting for 100 mg/L (43% of TN), 67 mg/L (43% of TN), and 36 mg/L (43% of TN) in AR35-I, AR45-I, and AR55-I respectively and 11mg/L (43% of TN), 35 mg/L (36% of TN) and 9 mg/L (32% of TN) in AR35-II, AR45-II and AR55-II, respectively. The lowest contribution of HS-N in AR55 could be relevant to the most intensive humification process in the waste matrix under thermophilic temperature, which raised the molecular weight and polycondensation of HS and thus effectively prevented themselves from leaching out (Tong et al., 2015). Among HS group, HAs-N exceeded FAs-N, as the major source of humic nitrogen. In details, HAs-N/FAs-N ratio was 9, 6, 3 in AR35-I, AR45-I, and AR55-I, and 2, 3, 2 in AR35-II, AR45-II and AR55-II, respectively. The HAs-N/FAs-N ratio decreased

remarkably in period II, probably because more HAs was washed out than FAs in the previous 215 days. Moreover, as the humification processes mainly occurred in the HAs fraction rather than the FAs fraction (Huang et al., 2006), the level of polycondensation was increased greater in HAs than FAs. Thus, leaching of HAs was diminished in the aged landfill waste compared to FAs.

Figure 6-5 Nitrogen distribution among different nitrogen containing compounds in collective leachate sample from two periods. The TN value for each sample was indicated on top of the bar.

The main nitrogen fraction was ammonium in anaerobic bioreactor with a dominant percentage of 89% in period I and 86% in period II, while under aerobic conditions organic nitrogen was the major constituent, in the range of 62-94 %. Therefore, special focuses should be paid during aeration with regards to the organic nitrogen management. According to literature, HS-N could be removed by adsorption onto activated sludge, while electrolysis was proved to be efficient in HyI-N elimination (He et al., 2006).

Table 6-1 Carbon and nitrogen weight percentage in purified leachate HAs powder, leachate HAs-C concentration and leachate HAs-N concentration

HAs	NR35-I	AR35-I	AR45-I	AR55-I	NR35-II	AR35-II	AR45-II	AR55-II
N%^a	4.23	5.93	6.07	4.68	3.85	4.06	5.58	3.61
C%^a	51.71	51.02	50.93	51.67	52.56	53.46	50.84	53.51
N/C^b	0.07	0.10	0.10	0.08	0.06	0.07	0.09	0.06
HAs-C (mg/L)^c	116.83	964.05	627.67	362.26	30.12	132.75	280.56	111.29
HAs-N (mg/L)^d	9.55	112.05	37.46	32.78	2.21	10.07	30.80	7.50

^a Value measured in purified leachate HAs powder

^b $N/C = (N\%)/(C\%)$

^c $HAs-C (mg/L) = HAs-N (mg/L) \cdot N/C$

Table 6-2 Carbon and nitrogen weight percentage in purified leachate FAs powder, leachate FAs-C concentration and leachate FAs-N concentration

FAs	NR35-I	AR35-I	AR45-I	AR55-I	NR35-II	AR35-II	AR45-II	AR55-II
N%^a	2.36	3.27	3.88	3.53	2.18	3.59	4.59	3.73
C%^a	48.31	47.33	48.51	47.47	52.06	49.19	47.81	46.82
N/C^b	0.04	0.06	0.07	0.06	0.04	0.06	0.08	0.07
FAs-C (mg/L)^c	87.90	170.96	149.41	127.29	23.58	56.44	119.68	59.97
FAs-N (mg/L)^d	4.29	11.81	11.95	9.47	0.99	4.12	11.49	4.78

^a Value measured in purified leachate HAs powder

^b $N/C = (N\%)/(C\%)$

^c $FAs-C (mg/L) = FAs-N (mg/L) \cdot N/C$

6.5 HS in the end waste

During waste decomposition detritus became rich in reactive phenolic and carbohydrate groups, which may polymerize together with amino acid to form HS. Compared to NR35, HAs content in AR35 and AR55 showed a statistically significant increase (Table 1), suggesting the positive effects of aeration on waste biostability under mesophilic and thermophilic conditions. The significant decrease of HAs in AR45 indicated that the humification and stabilization was not favored at 45 °C as in mesophilic or thermophilic temperatures.

Figure 6-6 Solid-state ^{13}C -NMR spectra of HAs extracted from end waste materials

Figure 6-7 Solid-state ^{13}C -NMR spectra of FAs extracted from end waste materials

Table 6-3 HS content in the end waste and relative distribution (%) of different C-types in ^{13}C -NMR

	HAs				FAs			
	NR35	AR35	AR45	AR55	NR35	AR35	AR45	AR55
Content^{a,b} (mg C/g DM)	5.83±0.29	7.78±0.00	3.68±1.38	7.93±0.50	0.93±0.02	1.11±0.09	1.68±0.34	1.43±0.23
0-43ppm alkyl-C	28.00	19.70	21.02	19.72	31.80	28.57	28.37	30.48
43-60ppm O-CH₃	15.60	15.66	14.89	15.58	13.45	12.74	12.65	12.29
60-110ppm O-C-O anomers & O-alkyl	18.80	24.75	22.89	21.16	19.25	19.14	18.31	18.07
110-141ppm aromatic C-H & C-C	16.86	19.15	19.23	22.66	14.90	15.00	14.87	14.97
141-162ppm aromatic C-O	6.69	8.18	7.88	9.13	4.89	5.63	6.48	6.05
162-190ppm COOH	11.32	9.71	10.83	9.66	14.06	15.41	16.49	15.59
190-220ppm C=O	2.74	2.85	3.26	2.09	1.65	3.51	2.84	2.55
aromaticity^c (%)	0.27	0.31	0.32	0.36	0.23	0.25	0.26	0.26

^amean value of duplicates

^bThe differences of HA content are significant and AR55= AR35 > NR35 > AR45 at $p \leq 0.05$ level. FA content among bioreactors displays no significant difference at $p \leq 0.05$ level.

^caromaticity (%) = [aromatic C peak area (110-162 ppm)/total peak area (0-162 ppm)]

The solid-state ^{13}C -NMR of HAs and FAs extracted from end waste materials are presented in Figure 6-6 and 6-7. The spectra can be divided into seven resonance regions to reflect different carbon structures (Barje et al., 2012; Spaccini and Piccolo, 2009). The relative distribution (%) of each C-type was calculated by dividing the peak area in this specific region by the total spectral peak area (Table 6-3). Compared to anaerobic HS, a clear decrease of alkyl carbon ($\delta = 0\text{-}43$ ppm) was noticed in aerobic HS. The loss of alkyl carbon were most probably caused by the biomineralization of terminal methyl, methine and methylene groups in aliphatic chains (Keeler et al., 2006), as the abatement of their typical peak at 20 ppm, 24 ppm, and 30 ppm in the spectra of aerobic HS was observed. Unlike easily biodegradable alkyl structure, the recalcitrant aromatic portion would be preserved during humification. The higher content of aromatic carbon ($\delta = 110\text{-}162$ ppm) in aerobic HS thus indicated a more decomposition of alkyl structure. This corresponds to the finding by Lguirati et al. (2005), where a higher level of aromaticity and condensation of humic molecules in the aerated landfill waste was observed. Interpretation of NMR data further revealed that high temperature benefited the maturation of HAs, as the degree of aromaticity increased with a higher temperature.

NMR spectra of aerobic HAs exhibited higher intensity in the chemical shift region of 60-110 ppm, which was assigned to O-alkyl carbon in the monomeric units in oligo- and polysaccharidic chains of yard waste. The rise of O-alkyl carbon in aerobic HAs could be relevant to the polymerization progress, in which new polysaccharide component was incorporated into HAs structure by covalent bonds (Serramiá et al., 2013). As a matter of fact, this was important in improving the long term stability of the waste materials. Since easy biodegradable organic compounds were converted into more stable polymeric HAs, the pollution potential was therefore reduced. In contrast to the observation for HAs spectra, no significant changes were found in FAs spectra apart from the variation of alkyl-C and aromatic C. This was supported by the previous finding that more structural alteration occurred in HAs than FAs (Huang et al., 2006).

The thermophilic temperature and aeration both increased the yield and maturity of HS. The implication is that the organic fraction, excavated from middle depth of landfill (where the thermophilic temperature most probably happens), are more promising as biofertilizers or organic soil amendments than those from other layers with lower temperature. It has been shown that HS contributes substantially to soil fertility by exerting a number of important functions such as improving soil structure, behaving as a pH buffer, serving as a micronutrient carrier and exerting hormonal effects on plant development (Canellas et al., 2012; Jindo et al., 2011).

6.6 Waste characterization and total carbon discharge

The results of waste characterization before and after bioreactor operation are shown in Table 6-4. The observed differences in end TOC and GS₂₁ were not statistically significant. Due to the nature of severe heterogeneity of waste sample, the measurement of TOC commonly has a large variance. Ritzkowski (2006) also commented that testing on TOC alone was not sufficient to describe the stabilization process of the waste material under aeration. Therefore, a calculation of carbon discharge on each pathway should be conducted.

Table 6-4 Waste Characterization^a before and after bioreactor running and carbon discharge through different pathways

	NR35	AR35	AR45	AR55
Initial TOC^b (g C/kg DM)	583.6±13.2	583.6±13.2	583.6±13.2	583.6±13.2
End TOC^b (g C/kg DM)	574.8±19.5	547.2±14.7	539.2±13.6	537.3±1.3
TOC difference (g C/kg DM)	8.8	36.4	44.4	46.3
Leachate C discharge(g C/kg DM)	2.7	7.9	10.1	7.0
Mineralization C discharge (g C/kg DM)	5.0	20.2	24.6	23.3
Initial GS₂₁^c (L kg⁻¹ DM)	11.5±2.7	11.5±2.7	11.5±2.7	11.5±2.7
End GS₂₁^c (L kg⁻¹ DM)	6.3±1.0	4.8±0.1	5.2±0.9	4.0±0.9

^a There was no significant difference in values of end TOC and end GS₂₁ at $p \leq 0.05$ level.

^b Mean value of triplicates

^c Mean value of duplicates

Table 6-4 lists the carbon discharge through leaching and mineralization pathway, respectively. The carbon load in each leachate drainage process was calculated by multiplying TOC and the volume of discharged leachate. In this regards the total carbon removal through leaching was obtained with sum of each TOC discharge. The amount of mineralized carbon through bioconversion was estimated by integrating the corresponding carbon mineralization curve in Figure 6-1(c). The carbon discharge via gas release in NR35 was calculated by utilizing the analyzed gas composition (CO_2 and CH_4) and the measured gas volume.

Carbon removal through leaching, referred to the unit dry mass, were 7.9, 10.1 and 7.0 g C/kg DM in AR35, AR45 and AR55, respectively, while a relative lower value of 2.7 g C/kg DM was obtained in NR35. The enhanced leaching of the organic carbon in aerobic bioreactors in current study was significantly correlated with the elevated concentration of HAs after aeration. Our previous studies found that the enhanced dissolution of HAs in present work was due to the inherent young waste age of the synthetic aged waste used (Tong et al., 2015). However, it was worthy to highlight the positive effects of thermophilic temperature on humification process, which reduced the dissolution of HAs and decreased the carbon load in the leachate.

Due to accelerated biological process under aerobic condition (Ritzkowski and Stegmann, 2013), the carbon discharge through biodegradation (mineralization) was largely promoted with the value of 20.2, 24.6 and 23.3 g C/kg DM in AR35, AR45 and AR55, respectively, which was more than fourfold of that under anaerobic condition (NR35). Interestingly, although the bioprocess got delayed in AR45, it caught up at the later stage and eventually achieved the largest amount of carbon conversion through mineralization. A similar phenomenon was also observed by Raga and Cossu (2013). Additionally, aeration could also change the carbon removal pathways. In NR35, the ratio of carbon discharge between mineralization and leaching was 1.8, while it increased to 2.6, 2.4 and 3.3 in AR35, AR45 and AR55, respectively. Similarly, Matsufuji (2005) and Ritzkowski and Stegmann (2013) investigated the carbon discharge and also notified the boosted carbon

discharge through mineralization under aerobic and semi-aerobic conditions. However, the relatively lower ratio in AR45 among all aerobic bioreactors indicated more carbon load in the leachate, adding extra burden to the downstream leachate treatment system.

Regarding total carbon discharge, it was estimated by the difference in TOC of waste material before and after column experiments. The value was larger than the sum of carbon removal through mineralization and leaching, because several carbon reduction pathways were not taken into account during calculation, such as CO emission and direct volatilization of organic compounds (Powell et al., 2006). CO may be formed during aerobic degradation of green waste and livestock waste, or from abiotic oxidation of methane and non-methane hydrocarbons, while volatilization of organic compounds relies on the mechanism of air stripping. Most of these pathways are not microbe related processes. As shown in Table 6-4, the TOC differences increased as temperature increased, suggesting that the rate of those chemical reactions were positively correlated with temperature. If only looking at the TOC difference, the greatest carbon removal was from AR55, further echoing the previous finding that the thermophilic conditions may have attained the fastest kinetics of carbon reduction.

6.7 Conclusions

The study examined the influence of temperature (35, 45 and 55 °C) during in situ aeration of simulated landfill bioreactors. A higher biodegradation rate was occurred at 55 °C, which corresponded to a larger carbon mineralization rate than that at 35 °C. Consequently, the enhanced bioactivity under thermophilic temperature eventually led to the lowest COD in leachate among all the aeration reactors. Extended acclimation period in biodegradation process was observed at 45 °C, in turn the COD reduction was not as efficient as the others. Volatilization played a main role in ammonium removal under aerobic condition. As a result organic fraction of nitrogen (>60%) present in aerobic leachate was shifted to be the majority, while ammonium remained dominant in anaerobic conditions (80%). Considering carbon balance, it was found that carbon transformation rate was

significantly enhanced by aeration and high temperature. An acceleration factor of 3 - 4 was observed for TOC reduction in waste materials during aeration. The highest biostability, in terms of the lowest GS₂₁ value and the largest richness of highly condensed HAs, was achieved at 55 °C. Interestingly, the formation of HAs was not favored at 45 °C, resulting in the dearth of HAs in the end waste materials.

All the benefits observed in 55 °C bioreactor suggested that remediation through aeration would be more efficient if thermophilic temperature could be maintained. Moreover, thermophilic zone usually exists in the middle depth of the landfill if temperature control is not implemented. Thus, after aeration the waste from this layer could provide product with a better quality if biofertilizer and soil conditioner is the expected end use for the excavated fine particles, because thermophilic temperature could increase the yield and maturity of HAs, which is a significant factor in soil fertility.

Chapter 7 Investigation on the Variations of Microbial Profile under Different Temperature during Aeration Start-up Phase

The change of condition, from anaerobic to aerobic, at the start-up phase of aeration resulted in the shift of the dominant microbial community from anaerobes to aerobes. Since successful application of in situ aeration depends on a good understanding of the microbiological process involved, it is important to understand the mechanism and variation in the microbial community and its associated functions during aeration process. This information could help establishing the most efficient and optimum operational parameters needed for rapid startup and promotion of long-term stability. In this study, at the first month DNA was extracted from the leachate samples collected from the bottom of the four aeration landfill bioreactors operated under different temperature (35 °C, 45 °C, and 55 °C) as well as anaerobic landfill bioreactors as a control. Phylogenetic analysis based on 16S rRNA gene clone library was conducted to characterize microbial profile and investigate their roles during biodegradation process mediated by temperature.

7.1 Bioreactor Performance

Table 1 shows the leachate characteristics before aeration commencement and the average properties of leachate of day 28 and 32, during which leachate sample was collected for DNA extraction. In NR35, the leachate pH was neutral and ORP was around -230 mV, which fell within the typical data range of methanogenesis in literature. In addition with significantly decreased methane generation rate, the results indicated that NR35 was moving towards maturation phase under anaerobic condition. The COD declining from 2240 mg/L to 1235 mg/L and ammonium-N dropping from 1625 mg/L to 944 mg/L could be ascribed to anaerobic biodegradation and washing effects from the added water.

Unlike the declining trend in NR35, COD, DOC and Org-N in aerobic bioreactors increased after air injection, which was possibly caused by the release of leachate stored in pore spaces in the newly formed leachate pathways due to the agitation of aeration (Öncü et al., 2012). Similarly, Prantl et al. (2005) also observed

a rise of COD concentration after 6 month of aeration during a field trial, and linked this phenomenon to the mobilization effect of aeration. Moreover, the enhanced solubilization and hydrolysis of solid organic matter, which otherwise may be considered as recalcitrant under anaerobic condition, also contributed to the elevated leachate organic load. In addition, according to our previous investigation on the profile of leachate organic matter, the dissolution of humic substances played a role too.

Table 7-1 Average leachate characteristics of day 28 and day 32, when the DNA was extracted

	pH	ORP	Carbon Mineralization rate	NH ₄ ⁺ -N	COD	DOC	TN	Org-N
		mV	mg C/day	mg/L	mg/L	mg/L	mg/L	mg/L
Initial^a	7.7	-237	26 ^b	1625	2240	811	1867	242
NR35	7.7	-229	NA	944	1235	430	1147	203
AR35	8.7	51	12 ^c	147	3415	1211	337	190
AR45	8.5	76	31 ^c	115	4351	1554	356	241
AR55	8.5	74	87 ^c	110	3925	1402	331	221

^a Leachate characteristics before aeration

^b Carbon mineralization test was conducted on day 35-36.

^c Carbon mineralization test was conducted after first day of aeration.

The initial detachment increased the availability of organic substrate, which in turn facilitated the proliferation of microorganisms. This could be supported by the fact that from the same volume of leachate sample the amount of extracted DNA in aerobic bioreactors was 1.5-2 times of that from anaerobic bioreactor (data not shown here). This finding partially explained why the biodegradation rate was accelerated in aerobic condition.

Furthermore, the positive effects of high temperature on the bioactivity and ammonium volatilization were evidenced. The increased carbon mineralization rate reflected an accelerated biodegradation rate under thermophilic condition, which could be attributed to higher growth rates of microbes, larger hydrolytic activities of

extracellular enzymes, and faster metabolism activity associated with the thermophilic temperature.

Regarding ammonium, its main attenuation mechanism was related to air stripping during this period. As nitrifying bacteria requires more than 30 days for full development under optimal condition (Valencia et al., 2011), bioremoval of ammonium through nitrification may not be significant in the incipient stage of aeration. Therefore, when temperature increases, ammonium concentration was further reduced because of the temperature enhancement of volatilization.

7.2 Microbial community characterization

In summary, 28 OTUs (114 clones), 36 OTUs (116 clones), 46 OTUs (109 clones) and 34 OTUs (89 clones) were obtained in NR35, AR35, AR45, and AR55 libraries, respectively. The number of OTUs for these three aerobic bioreactors exceeded that for NR35. The higher species richness in the aerobic bioreactors can be attributed to the increase in availability of labile substrate by aeration.

Figure 7-1 Taxonomic classification of bacterial phylum in leachate sample from four bioreactors

Fig. 7-1 presents the relative abundance of bacterial phyla in the four samples tested, while the Neighbor-joining trees showing the phylogenetic relationship among OTUs of the four clone libraries are presented in Fig. 7-2, Fig. 7-3, Fig. 7-4, and Fig. 7-5, respectively. Fig. 7-1 clearly illustrates the variation of microbial profile influenced by temperature. With the additional information from phylogenetic trees, it could be easy to identify in each bioreactor the major microbial groups that actually played a significant role in affecting the performance of each bioreactor.

7.3 Bacterial community in NR35

The bacterial community structure of NR35 was exclusively consisted of the phyla *Bacteroidetes*, *Firmicutes*, *WWEI* and *Spirochaetes*. Within the total 28 OTUs, 8 was classified as *Bacteroidetes*, 17 as *Firmicutes*, 2 as *Spirochaetes* and 1 as *WWEI*, in proportion of 50%, 45%, 3% and 2% of the clones, respectively. The most detected OTU (OTU2), representing 31% of the bacterial clones, was highly related to the uncultured *Bacteroidetes* clone (AB175366), obtained from mesophilic anaerobic protein degradation process, with 99% similarity (Fig. 7-2).

Six *Firmicutes* OTUs, comprising 14% of the clones, were closely affiliated with known species. OTU60 and OUT64 clustered with *Syntrophomonas wolfei* (NR_122058) and *Syntrophomonas sapovorans* (NR_028684) within the family *Syntrophomonadaceae*, which are strict anaerobes and are involved in β -oxidation of long-chain fatty acid. OTU19 was related to *Gracilibacter thermotolerans* (NR_115692), a heterotrophic fermentative bacteria with 96% similarity. The closet cultured relative of OTU35 was *Mobilitalea sibirica* (KF931641), which is able to ferment a variety of mono-, di- and polysaccharide. OTU36 was 97% identical with *Clostridium straminisolvens* (NR_024829), an anaerobic cellulolytic bacterium. Although apart from the above-mentioned five OTUs, more than half of the *Firmicutes* clones in NR35 were affiliated to as yet uncultured lineages, they seemed all to be involved in anaerobic degradation process based on their closet relatives, which were all retrieved from landfill leachate or methanogenic digester of MSW or organic waste.

Figure 7-2 Neighbour-joining tree illustrating the phylogenetic relationship of OTUs found in the leachate sample from NR35. Only OTU that includes at least two clones are used to construct the phylogenetic tree. *Methanococcus vannielii* served as outgroup.

7.4 Bacterial community in AR35

In AR35, the bacterial community was dominated by *Firmicutes*, accounting for 81% of the clones (20 OTUs). Except 3 OTUs unclassified, the other 13 OTUs, occurring less frequently, were affiliated with the phyla *Bacteroidetes* (4 OTUs), *Proteobacteria* (4 OTUs), *Actinobacteria* (1 OTU), *Synergistetes* (1 OTU), *Gemmatimonadetes* (1 OTU), *WWEI* (1 OTU) and *Spirochaetes* (1 OTU), representing 5%, 5%, 2%, 2%, 1%, 1% and 1% of the clones, respectively.

Among the 36 OTUs of AR35, OTU1 is the most frequently detected OTU (Fig. 7-3). It grouped with OTU18 and OTU31 together corresponding to 48% of the total clones. This predominant bacterial group clustered with *Symbiobacterium* related to the strain *Symbiobacterium toebii* (AF190460). *Symbiobacterium* is widespread in compost, soil, animal intestine, and sea water (Ueda et al., 2004).

Figure 7-3 Neighbour-joining tree illustrating the phylogenetic relationship of OTUs found in the leachate sample from AR35. Only OTU that includes at least two clones are used to construct the phylogenetic tree. *Methanococcus vannielii* served as outgroup.

The second predominant OTU group in proportions of 9 % of the bacterial clones, consisted of OTU65, OTU69 and OTU12. Their closet cultured relatives are

related to genus *Paenibacillus*. The ecologically important ability of this genus is to aid composting of plant materials through the secretion of extracellular carbohydrases and other enzymes.

7.5 Bacterial community in AR45

A total of 46 OTUs were identified in AR45. The dominant OTU included members related to *Firmicutes* (28 OTUs) and *Proteobacteria* (13 OTUs), taking up 61% and 30% of the clones, respectively. The minority OTUs were classified as *Bacteroidetes* (2 OTUs), *Cyanobacteria* (1 OTU) and unclassified (2 OTUs).

OTU3, the most detected one in AR45, accounted for 11% of the total bacterial count and was affiliated with *Vulgatibacter incomptus* strains B00001 (NR_126181) (Fig. 7-4). This myxobacterial strain, isolated from soil, is strictly aerobic and chemoheterotrophic (Yamamoto et al., 2014). The second abundant bacterial group (10% of the clones), including OTU4, OTU15 and OTU49, was related to uncultured *Firmicutes* bacterium clone (DQ346500) obtained from the early thermophilic stage (57±5)°C of compost.

7.6 Bacterial community in AR55

In AR55, the detected 34 OTUs represented the *Bacteroidetes* (2 OTUs, 6% of the clones), *Deinococcus-Thermus* (1 OTU, 1%), *Gemmatimonadetes* (2 OTUs, 2%), *Synergistetes* (2 OTUs, 8%), unclassified (2 OTUs, 2%), and the most abundant phyla *Proteobacteria* (11 OTUs, 42%) and *Firmicutes* (14 OTUs, 39%).

Similar with AR45, the predominant OTU in AR55 (Fig.7-5) was also OTU3 (affiliated with *Vulgatibacter incomptus*), but with higher percentage (20% in AR55 compared to 11% in AR45). The second predominant OTU group in AR55 was composed by OTU4 (a shared OTU between AR45 and AR55) and OTU 59, which were related with thermophilic *Firmicutes* bacterium clone (DQ346500) and together represented 19% of the total bacterial count.

Figure 7-4 Neighbour-joining tree illustrating the phylogenetic relationship of OTUs found in the leachate sample from AR45. Only OTU that includes at least two clones are used to construct the phylogenetic tree. *Methanococcus vannielii* served as outgroup.

Figure 7-5 Neighbour-joining tree illustrating the phylogenetic relationship of OTUs found in the leachate sample from AR45. Only OTU that includes at least two clones are used to construct the phylogenetic tree. *Methanococcus vannielii* served as outgroup.

7.7 Variation of *Firmicutes* in all bioreactors

By examining the bacterial communities in all the bioreactors, it seemed that despite the different operation conditions *Firmicutes* represented a ubiquitous albeit highly fluctuating group (39-82% of all the clones) being most abundant in AR35 (82%). Their indispensable role in all the bioreactors may be due to the fact that most members of this phylum can produce a variety of extracellular enzyme and exhibit diverse metabolic capabilities (Lim et al., 2013). They are known to be actively involved in the bioconversion of a wide range of macromolecules, including lipid, protein, cellulose and other carbohydrates (Kratat et al., 2011).

In AR35, 58% of *Firmicutes* was related to microaerophilic *Symbiobacterium toebii*, which was originally isolated from compost sample. *Symbiobacterium toebii* is a commensal symbiotic bacterium whose growth depends on coculture with *Geobacillus toebii*. It is reported that the anaerobic or microaerobic conditions are essential for a commensal interaction between these two bacteria, as *S. toebii* cannot grow under aerobic conditions (Sung et al., 2003). Based on the experiment observation, Kim et al. (2011) further suggested that the enzymatic defense system of *G. toebii* reduced the oxidative stresses from reactive oxygen, which may favor the syntrophic growth of *S. toebii*. The high abundance of *Symbiobacterium* may indicate the presence of large portion of anaerobic or at least anoxic pockets in the waste matrix. Due to the heterogeneous nature of waste, anaerobic zone is considered persistent during in situ aeration process, even when excess air is supplied (Ritzkowski et al., 2006). Yazdani et al. (2010) argued that advective-dispersive gas transportation was hindered by pore water in these anaerobic domain and only promising gas transfer mechanism was diffusion.

Unlike AR35, only less than 2% of the total clones were identified as *Symbiobacterium* in AR45 and AR55, suggesting a more profound aerobic condition when temperature was increased. This could be explained by enhanced diffusion coefficient under high temperature, which could speed up the oxygen intrusion into the immobile zone.

7.8 Variation of *Bacteroidetes* and *Proteobacteria* in all bioreactors

A notable reduction was observed for *Bacteroidetes* after aeration from 50% in NR35 to $\leq 6\%$ in aerobic bioreactors, while the population of *Proteobacteria* was promoted by aeration and high temperature from non-detection in NR35 to more than 40% in AR55.

Bacteroidetes is a major microbial component of anaerobic reactors and known to be proteolytic bacteria (Lim et al., 2013). They first intervene in the degradation of protein and are able to ferment amino acids into acetate. In contrast to NR35, the significant drop of their abundance in aeration bioreactors may be ascribed to the interruption on anaerobic condition by air injection.

Generally, *Proteobacteria* is recognized as an important contributor to biogeochemical processes or organic matter degradation (Zhang et al., 2011). The higher percentage of this phylum after aeration revealed that *Proteobacteria* may play an important role in aerobic biodegradation. The most detected *Proteobacteria*, representing 11% and 20% of the clones in AR45 and AR55, was affiliated to *Vulgatibacter incomptus*. Yamamoto et. al (2014) reported that pure culture of this obligate aerobe grew between 20–37°C and no growth occurred above 42 °C. Thus, the only niche for this microbe should be pockets of lower temperature, which most probably existed in the fractures and inter-particle pores of solid organic matter (“side pockets” or stagnant zone). As the biodegradation proceeded and solid matter was disintegrated, the inside microbes might be carried out by the percolating water. Considering that *Vulgatibacter incomptus* could not survive without oxygen, their appearance in AR45 (11% of total clones) and their prosperity in AR55 (20% of total clones) suggested the high efficiency of oxygen intrusion into the “side pockets”. Since molecular diffusion might be the dominant transport mechanism in these stagnant zones, the high aerobic condition in AR45 and AR55 could be attributed to the increased diffusion coefficient of oxygen by high temperature. This is cross-identified by the previous conclusion drawn from the variation on the percentage of *Symbiobacterium*. All the facts directed that aerobic condition may be

largely promoted by elevated temperature. This could partially contribute to a fast organic removal due to higher degradation rate of aerobes than anaerobes.

7.9 Conclusions

The study investigated the influence of temperature (35, 45 and 55 °C) on the microbial community structure during the start up stage of in situ aeration. Compared to anaerobic control bioreactor, the microbial population under aerobic condition was more diverse as more OTUs were identified.

The highest carbon mineralization rate was observed at 55 °C, followed by 45 °C. The enhanced bioconversion rate under thermophilic condition could be attributed to the elevated abundance of *Proteobacteria*, as *Proteobacteria* is recognized as an important contributor to biogeochemical processes or organic matter degradation. The high percentage of *Symbiobacterium* in the leachate sample from 35 °C suggested that the large area of anaerobic or anoxic zone may be the reason of lowest biodegradation rate in this aeration bioreactor. The increasing percentage of *Vulgatibacter incomptus* at 45 °C and 55 °C verified the profound aerobic condition in these two bioreactors, which could be ascribed to the increased diffusion coefficient of oxygen by high temperature.

Chapter 8 Conclusions and Recommendations

Nowadays, landfill is still the most popular disposal route for the majority of solid waste, due to its cheapest initial investment cost and easy operation. However, landfilling, as it is currently practiced, is fundamentally unsustainable. First, the traditional landfills require long-term aftercare, which is a heavy financial and environmental burden. Second, landfills create eyesores and result in considerable wastage of land. Third, lots of recyclables are buried, wasting huge quantity of resources. For these reasons, landfill reclamation attracts increasing attention.

In situ aeration has been proven to be a successful landfill remediation technique in lab and full scale research. By supplying a controlled amount of air to landfills, in situ aeration promotes aerobic biodegradation via interruption of existent anaerobic processes. Under aerobic conditions, increased waste decomposition, accelerated settlement, reduced leachate strength and enhanced carbon discharge via gas phase could be achieved. The aeration cost could be offset by earlier reuse, less strict requirement for subsequent capping and sealing system, and lower expenditure with decontamination.

Although over the last decade full scale aeration has been successfully demonstrated worldwide, there is still a lack of understanding regarding the specific biochemical processes influenced by temperature and waste age. Moreover, no studies have specifically investigated microorganisms in the course of in situ aeration in the literature. The information in these areas is important for the designer and operator of aeration system, as they would have direct references to rely on to select the best starting time of aeration, the proper operating temperature and the strategy for quick start-up. The current study through laboratory investigation provided insights on the above-mentioned topics, and the major conclusions of the work are outlined in this chapter followed by a list of recommended areas for future work.

8.1 Conclusions

8.1.1 Effects of waste age on waste stabilization during aeration

The study on the effects of waste age focused on variation of leachate strength, carbon removal pathways, and quality of the end waste materials. Besides the general parameters, leachate HS were characterized to indicate the biological and chemical processes involved.

- The accumulation of HAs in the leachate after aeration was observed in the waste with younger waste age. However, aeration did not enhance HAs release from waste materials with prolonged aging. The structural analysis of leachate HAs molecules revealed that HAs from older waste had higher MW, which may prevent them being washed out by leachate.
- Leaching made a significant contribution on carbon removal during aeration in the waste of younger age. By contrast, a higher percentage of carbon removal was ascribed to biomineralization in the bioreactors with older waste age. As less carbon was leached out by percolating water from the waste with elongated aging, they may have a higher chance to be trapped by indigenous microbes and enter into the biodegradation pathway.
- The earlier aeration started, the earlier landfill stabilization could be realized. However, later aeration could enrich HS content in the waste materials and minimize the extra troubles for leachate treatment. A compromise should be made between earlier aeration and later aeration when deciding the starting time of aeration.

8.1.2 Effects of temperature on waste stabilization during aeration

The effects of temperature were mainly investigated on carbon discharge rate, nitrogen speciation and HS transformation. The major findings are summarized below.

- A higher carbon mineralization rate was occurred at 55 °C than that at 35 °C, because of higher biodegradation rate under thermophilic condition. It was also the reason of the lowest leachate COD in the bioreactors at 55 °C.
- Microbes suffered a longer lag phase at 45 °C before the population effectively metabolized the organic substrate. However, after reaching this stage, the microbial community at 45 °C kept pace and even exceeded with the aerobes at 35 °C and 55 °C in terms of carbon mineralization rate.
- Ammonium in leachate was significantly reduced by aeration through volatilization and nitrification-denitrification. In contrast to the decrease in the inorganic nitrogen, the contribution of organic nitrogen in the leachate was increased after aeration. In particular, the percentage of HS to the total organic nitrogen was prominent, especially at 45 °C. Detailed investigation also revealed the higher bioconversion rates of N-humic and N-fulvic compounds compared to hydrophilic compounds in thermophilic conditions.
- In terms of waste biostability, GS₂₁ showed that waste material in 55 °C bioreactor was more biostable. The formation and transfiguration of HS were also accelerated by high temperature, resulting more richer and more condensed HS in the waste materials at 55 °C.

8.1.3 Variation of microbial profile during start up phase of aeration

After one month operation of bioreactor at different temperature, microbial profiles in leachate sample were characterized through constructing 16S rRNA clone libraries to develop a deeper understanding of the microbiological process involved in the startup stage of aeration treatment.

- Leachate COD, DOC and Org-N in aerobic bioreactors increased after air injection. Several mechanisms may contribute to this phenomenon, such as the release of previously stored leachate, the enhancement of hydrolysis of solid organic matter, and the dissolution of humic substances.
- The increased concentration of organic substrate in leachate could facilitate the proliferation of microorganisms. Compared to anaerobic control bioreactor, the microbial population under aerobic condition was more diverse as more OTUs were identified.
- A notable reduction was observed for *Bacteroidetes* after aeration, while the population of *Proteobacteria* was promoted by aeration and high temperature. As *Proteobacteria* is recognized as an important contributor to biogeochemical processes or organic matter degradation, its abundance probably resulted in the enhanced bioconversion rate under thermophilic condition.
- Microaerophilic *Symbiobacterium* was predominant in the leachate microbial community at 35 °C, while the dominance of obligate aerobic *Vulgatibacter incomptus* was observed at 45 °C and 55 °C indicating the more profound aerobic condition in these two bioreactors, which could be ascribed to the increased diffusion coefficient of oxygen by high temperature.

8.2 Recommendations

Based on the experience gained through the present study, a few topics are recommended here for future research.

- The current study investigated the individual roles of temperature and waste age in aeration process separately. Based on the knowledge obtained from current study, further steps could be taken to assess the combination effects imposed by these two factors together.
- Apart from the above mentioned two factors (waste age and temperature), waste stabilization is also affected by other variables, such as the composition of the waste, the pretreatment of waste, water content and the operation procedure of waste landfilling. Further endeavors are needed to answer these questions in order to adjust the setting of aeration system to maximize the benefits in various types of landfill.
- In real landfill site, the features would be considerably complicated by the high heterogeneity in the waste material and large variation in the boundary conditions. The current study was based on laboratory scale experiments, and it has not yet attained a certainty to match with a scaled-up setup and/or the real scenario. Therefore, in the next step, more data collected from field aeration projects should be collected to further verify the conclusion obtained in current work.
- In field text, because of the notably heterogeneity of the buried waste, injected air tends to rapidly escape from the landfill body along preferential pathways, while the other areas remain un-aerated. To overcome these channeling effects, impulsive injection of air with variant pressure is suggested based on the author's knowledge. The impulsive aeration could be realized by shock release of pressured air from nozzles mounted on the injection wells, and air pressure could also be changed from time to time. The intermittent discharge of air could avoid the

dominance of certain air channels, which may form under the continuous and steady air supply. The air flow with different pressure could penetrate weakly as well as highly compacted waste materials, and consequently maximize the influential zone of aeration. Furthermore, a series of vertical gas injection wells with different length could be employed to meet the air demanding of waste at various depths.

- Positive effects of aeration on methane reduction, COD mitigation and ammonium elimination were intensively documented in the literature. However, it is still unclear about the fate of other harmful pollutants, such as BTEX (benzene, toluene, ethylbenzene, and xylenes), chlorinated solvents (e.g. trichloroethene), and phthalic acid esters (PAEs). Clearly further research is required to provide information on the transportation and transformation of those compounds during aeration.
- The stabilization of landfill depends on the activities of microorganisms in the layers of waste matrixes. The current study revealed the variation of microbial profile during the start-up phase of aeration. The dynamics of microbial community along the whole process of aeration remained unknown. It is important to gain knowledge about the temporal changes in microbial population, which are relevant to biological and chemical processes at different stage of aeration and are essential to improve the efficiency of aeration.

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