UNIVERSITI PUTRA MALAYSIA

ASSESSING POTENTIAL USE OF GARBAGE ENZYME IN HEAVY METAL REMOVAL OF WATER TREATMENT SLUDGE AND ITS CONTRIBUTION TO HUMAN HEALTH

MUHAMMAD ASHRIL BIN MOHD ROSDI

ASSESSING POTENTIAL USE OF GARBAGE ENZYME IN HEAVY METAL REMOVAL OF WATER TREATMENT SLUDGE AND ITS CONTRIBUTION TO HUMAN HEALTH

BY

MUHAMMAD ASHRIL BIN MOHD ROSDI

Thesis submitted in fulfilment of the requirement for the degree of Bachelor Science (Environmental and Occupational Health) from the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia.

ACKNOWLEDGEMENTS

Foremost, I would like to express deepest gratitude to my supervisor, Dr Sharifah Norkhadijah Syed Ismail, for her encouragement, patience, financial support and valuable guidance given to me to complete this project throughout numerous consultations. Without her guidance and wisdom, I may not complete this project successfully.

A big thank to Department of Environmental and Occupational Health, Faculty of Medicine and Health Sciences for giving me the opportunity to do my final year project. Besides that, special gratitude to staffs from Faculty of Environmental Studies who involved in this study and gave some assistance for analysis of samples.

Special thanks to Bukit Nanas Water Treatment Plant especially PNSB Water Sdn Bhd, Kuala Lumpur for allowing sludge collection at one of their sludge tanks. Without the sludge samples, this study would be meaningless.

Special appreciation to my family especially my father, Mohd Rosdi bin Halim and my mother, Norazleena Binti Jusoh and other family members for giving a lot support and motivation to complete this project.

Last but not least, I would like to thank my friends, staffs from my department for their support, patience and care.

ABSTRACT

ASSESSING POTENTIAL USE OF GARBAGE ENZYME IN HEAVY METAL REMOVAL OF WATER TREATMENT SLUDGE AND ITS CONTRIBUTION TO HUMAN HEALTH

MUHAMMAD ASHRIL BIN MOHD ROSDI

Introduction: Heavy metals in Water Treatment Sludge (WTS) pose acute and chronic health risks to adult and children. Since there are limitations such as limited disposal facilities and expensive costs, most sludge is disposed at landfill and eventually degraded to form leachate that contaminate soil and water sources. A low cost and environmentally friendly technology like Garbage Enzyme (GE) is needed to tackle this issue. **Objectives:** To assess potential use of GE, derived from food wastes to remove heavy metals in water treatment sludge and its contribution to human health. **Methodology:** Six different types of food wastes (i.e. citrus peels, banana peels, carrot wastes, potato wastes, nut wastes and black bean wastes) were fermented for 4 weeks to produce GEs and the physico-chemical characteristics of GE (i.e. pH and temperature) were assessed using CyberScan pH 300 Portable pH meter (Eutech Instruments). Citric acid concentration of GE was also determined using acid-base titration technique. The sludge samples were collected at Bukit Nanas Water Treatment Plant using APHA standard methods for examination of water and wastewater, 1999. During treatment of sludge using GEs, the pH and temperature of sludge samples were assessed. The heavy metal concentrations (Lead, Zinc and Copper) of sludge samples were analysed using Inductively Coupled Plasma Mass Spectrometry (ICPMS). The obtained concentrations of heavy metals were then compared by types of sludge samples and also used for health risk calculation via ingestion. **Results:** There were significant mean difference of pH and temperature among GEs. For sludge samples, there was a significant mean difference of pH levels among sludge samples but no significant difference of temperature. There was a fair negative association (r = -0.358) between temperature and pH of GE. There was a good negative association (r = -0.73) between lead concentration in sludge samples and citric acid concentration of GE. All heavy metals in this study did not show any significant health risk (HQ < 1, LCR within acceptable risk). **Conclusion:** Garbage Enzyme is potential be used to remove heavy metals in water treatment sludge.

Keywords: Garbage Enzyme, Water Treatment Sludge, Heavy Metals, Health Risk

ABSTRAK

MENILAI POTENSI ENZIM SAMPAH DALAM PENYINGKIRAN LOGAM BERAT DARIPADA ENAPCEMAR RAWATAN AIR DAN SUMBANGANNYA KEPADA KESIHATAN MANUSIA

MUHAMMAD ASHRIL BIN MOHD ROSDI

Pengenalan: Logam berat dalam enapcemar loji rawatan air (WTS) menimbulkan risiko kesihatan akut dan kronik kepada orang dewasa dan kanak-kanak. Memandangkan terdapat batasan seperti kemudahan pelupusan yang terhad dan kos yang mahal, kebanyakan enap cemar dibuang di tapak pelupusan dan akhirnya mereput untuk membentuk leachate yang mencemari sumber tanah dan air. Teknologi kos rendah dan mesra alam seperti Garbage Enzyme (GE) diperlukan untuk menangani masalah ini. **Objektif:** Untuk menilai potensi penggunaan GE yang diperoleh daripada sisa makanan untuk menghilangkan logam berat dalam enapcemar rawatan air dan sumbangannya kepada kesihatan manusia. **Metodologi:** Enam jenis sisa makanan (contohnya kulit sitrus, kulit pisang, sisa lobak, Sisa Kentang, Sisa kacang tanah dan sisa kacang hitam) telah ditapai selama 4 minggu untuk menghasilkan GE dan ciri-ciri fiziko-kimia GE (iaitu pH dan suhu) telah dinilai menggunakan pH meter Mudah Alih pH 300 (Instrumen Eutech). Kepekatan asid sitrik GE juga ditentukan menggunakan teknik titrasi asid-bes. Sampel enap cemar dikumpulkan dari Loji Rawatan Air Bukit Nanas menggunakan Kaedah standard APHA untuk pemeriksaan air dan air sisa, 1999. Semasa rawatan enapcemar menggunakan GE, pH dan suhu sampel enapcemar dinilai. Kepekatan logam berat (plumbum, zink dan kuprum) daripada sampel enapcemar dianalisis menggunakan *Inductively Coupled Plasma Mass Spectrometry (ICPMS)*. Kepekatan logam berat yang diperoleh kemudiannya dibandingkan dengan jenis sampel enap cemar dan juga digunakan untuk pengiraan risiko kesihatan melalui ingesi. **Keputusan:** Terdapat perbezaan yang signifikan antara pH dan suhu dalam kalangan GE. Bagi sampel enapcemar, terdapat perbezaan tahap pH yang signifikan di antara sampel enapcemar tetapi tidak terdapat perbezaan suhu yang signifikan. Terdapat persamaan negatif yang sederhana ($r = -0.358$) antara suhu dan pH GE. Terdapat persamaan negatif yang baik ($r = -0.73$) antara kepekatan plumbum dalam sampel enapcemar dan kepekatan asid sitrik GE. Semua logam berat dalam kajian ini tidak menunjukkan risiko kesihatan yang signifikan (HQ <1, LCR dalam risiko yang boleh diterima). **Kesimpulan:** Enzim Sampah berpotensi digunakan untuk menghilangkan logam berat dalam enapcemar rawatan air.

Kata kunci: Enzim Sampah, Enapcemar Rawatan Air, Logam Berat, Risiko Kesihatan

TABLE OF CONTENTS

CHATER 2: LITERATURE REVIEW

CHAPTER 3: METHDOLOGY

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVATIONS

ADD	Average Daily Intake
AT	Averaging Time
BW	Body Weight
ICPMS	Inductively Coupled Plasma Mass Spectrometry
ED	Exposure Duration
GE	Garbage Enzyme
EF	Exposure Frequency
IR	Ingestion Rate
US EPA	United State Environmental Protection Agency
HQ	Hazard Quotient
CSF	Cancer Slope Factor
FAO	Food Administration Organization
LCR	Lifetime Cancer Risk
RfD	Reference Dose
IRIS	Integrated Risk Information System
LADD	Lifetime Average Daily Intake

Cu	Copper
Pb	Lead
Zn	Zinc
HNO_3	Nitric Acid
LOD	Limit of detection
C	Concentration
ppb	Part per billion

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Rising local population and industries will increase the demand for clean water supply and the river as one of water sources is involved to fulfil this demand. In Malaysia, raw water supplies from the rivers are no longer considered to be directly consumed without proper treatment or filtration. As a rule, waterways in Malaysia are considered as dirtied. Sg. Klang in Selangor, Sg. Juru in Penang and Sg. Segget in Johor are instances of contaminated streams in Malaysia (Zainudin, 2010). In this manner, a total of 488 water treatment plants (WTP) are worked all through this nation to treat the crude water from stream to create a protected and clean water before it very well may be provided for populace use (Wahid, 2009). However, the water treatment processes at each WTP produce amount of wastes in the form of sludge.

Because of the expansion of WTPs, the generation of sludge have expanded essentially. This sludge should be treated before being released as the effluent and needs to fulfil the required guideline level issued by Malaysian Department of Environment. Be that as it may, the issue that remaining parts unsolved is constraint in finding reasonable disposal sites (Puncak Niaga, 2019). Incineration and landfilling are the most widely recognized techniques used to discard sludge from water treatment plants (Arun and Sivashanmugam, 2015). The degradation of these sludge will create high level of leachates which consequently taint natural resources, for example, groundwater, surface water and soil (Naveen et al., 2016). Additionally, this sludge likewise high in natural issue that can influence the situations, for example, air, water and soil (Arun and Sivashanmugam, 2015).

Current enactment in Malaysia is controlling industries particularly WTPs to diminish the amount of sludge entering landfills for disposal by recycling the sludge. Anaerobic digestion is one of reasonable innovations to treat the solid waste and it has been considered as waste to wealth innovation (Arun and Sivashanmugam, 2015; Garg and Renuka, 2011; Sonakya, Raizada and Kalia, 2001). Also, the operating expense of treatment of modern wastewater utilizing anaerobic digestion is a lot less expensive than utilizing anaerobic composting (Clisso, 2010).

Various physical (Gayathri et al. 2015; Xu et al., 2014), chemical (Zhou et al., 2014; Kim et al., 2010; Heo et al., 2003) and biological methods (Kavita et al., 2015; Veera Lakshmi et al., 2014; Kavita et al., 2014; Merrylin et al., 2013) are available to solubilize the complex organic matter, but the biological methods such as microbial or enzyme are preferred due to being eco-friendly and having a low operating cost (Parawira, 2012; Imai et al., 2010). In addition, these methods are preferred to improve the solubility of sludge for further utilization or disposal.

Truth be told, a study showed that Malaysians have tossed 15 000 tons of food wastes every day from 38 000 tons of total solid waste in 2015 (Armi and Khairul, 2017; SW Corp, 2016). Another investigation revealed that 930 tons of unconsumed foods were discarded by Malaysians every day (Jereme et al., 2016). Food wastes are

60% of municipal solid waste in Malaysia and estimated to increase to 6.54 million tonnes in 2020 (Hamid et al., 2012).

Then again, food waste and sludge disposal via landfill techniques produce ozone depleting substances and leachate that influence the air and the water condition to a bigger degree (Pleissner and Sze Ki Lin, 2013). The food waste and sludge in landfill will be eventually degraded to create carbon dioxide and methane in this manner recycling carbon back to the environment and causing a global warming (Levlin, 2009). The discharge of ozone depleting substances (GHGs) into climate is relied upon to have noteworthy effect on nature, human wellbeing and the economy (Arun and Sivashanmugam, 2015). Consequently, environment-friendly and sustainable technology at a low cost is needed for the management of food waste and WTS (Luque & Clark, 2013).

The food wastes can be utilized to produce garbage enzyme (GE) through fermentation (Arun and Sivashanmugam, 2015) and the GE can be utilized as fertilizers, plant development hormone, pesticides, waste water treatment and antimicrobial solutions (Prakash, 2011). In the present work, an experiment was made to produce different types of garbage enzyme from different types of food wastes (citrus peels, banana peels, carrots wastes, potatoes wastes, black beans wastes and nuts wastes separately) and the characteristics of each garbage enzyme produced were investigated in term of pH, temperature and citric acid concentration. Also, the experiment was continued by conducting the treatment of WTS using different crude garbage enzymes. Parameters like pH, temperature and heavy metals concentration (lead, zinc, copper) of the treated sludge were studied to discover the impact of

Garbage Enzyme in removing heavy metal from WTS. Health risk was also calculated for each treated sludge sample prior to land application.

1.2 Problem Statement

Malaysia had produced almost 23,000 tonnes of waste each day and expected to be increased up to 30,000 tons by the year 2020 due to the increasing of human population and industrial development, and only 17% of the waste being recycled (Global Environment Centre, 2017). Sludge waste has become one of the largest scale solid wastes in Malaysia. Nowadays, the large amount of waste produced by water treatment plant has overloaded the landfill, not to mention other waste from different sources. Malaysia is currently facing the solid waste management problem. In 2017, Malaysia produced 226,748 metric tonnes of heavy metal sludge and 11.48 % of them are from drinking water treatment plant (DOE, 2017). The collection and disposal cost of municipal waste has become a burden to government and people. It is rather extravagant to spend on the handling, transportation, and collection of sludge. The sludge ends up in either sanitary landfills or secured landfills. Idrus (2008) stated that Malaysia has limited landfill site. Every day, each person produces about 1 kg of solid waste and the waste production rate is increasing at 15% per year due to the urbanization and population growth. The disposal rate of municipal waste is far higher than the decomposition rate at landfill. In a very short period, the current landfill in Malaysia will reach their design capacity. Although the volume of sludge is reduced after the incineration process, the sludge ash from the incineration process must be disposed.

While studies show that the volume of sludge is expected to rise, disposal options are limited due to stricter environmental regulations including a ban on burying sludge in soil, due to its high heavy metal content that could cause adverse impacts to the environment (Water World, 2015). The degradation of these sludge in landfills produce landfill leachate, a highly contaminated wastewater consisting toxic elements including organic and inorganic matter and high concentration of heavy metals where the direct release of heavy metals into the environment contaminates the soil and water bodies and becoming environmental and health hazards (Thirumalaisamy & Lakshumanan, 2012; Meenakshi, 2015). These pollutants will cause adverse effects to aquatic population, ecology, food chain as well as indirectly affect human health such carcinogenic effects, acute toxicity and genotoxicity after consuming those pollutants from affected sources.

On the other hand, a study conducted by Solid Waste Management and Public Cleansing Corporation (SWCorp) in 2017 showed that Malaysians throw 16,688 tons (16,688,000kg) of food on a daily basis (New Straits Time, 2018). Another research conducted by Malaysian Agricultural Research and Development Institute (MARDI) showed that 20 % to 50 % of fruits and vegetables are usually discarded (The Star, 2018). Most common disposal method in Malaysia is landfilling method and most of the landfills are open dumping areas which ultimately cause serious environmental and social threats. While the amount of waste generated is increasing day by day, Malaysia is facing solid waste management problem since the landfills are rapidly overloaded resulting serious environmental and human health problems (Mahmood, 2009). According to Kadir et al. (2016), Malaysia commonly uses landfill as a primary method of solid waste disposal and 50% of the waste at landfill consists of food waste.

The food wastes are being disposed in landfill and degradation process of food wastes produce high levels of leachate and methane gas (CH_4) which was proven in contributing to the environmental degradations such as global warming, water pollution, etc. (Tweib, Rahman, & Kalil, 2011). It is estimated that 7175 m^3 of leachate generated each year at landfills in Selangor (Tengku Nilam, Zalina & Faridah, 2016). Moreover, landfill generates greenhouse gases such as Methane (50-55%) and Carbon Dioxide (40-45%) from biodegradation process that contribute to global warming and climate change. Another study from Bo-Feng et al. (2014), they reported that methane emission from waste management shared 4% of global greenhouse gases (GHG) discharged in 2010 with half of them came from Municipal Solid Waste and Wastewater Treatment Plant.

Heavy metals have toxic, non-biodegradable, and persistent nature (Azimi et al., 2017). They can bioaccumulate through food chain (Bohli et al, 2013). Thus, ingestion is the most worrisome route of exposure to heavy metals (Muhammad, Shah & Khan, 2011). Furthermore, heavy metals pose health risk to both adult and children. Children have a low tolerance to toxins as well as the inadvertent behavior of coming into contact with significant quantities of sludge in their environment (Acosta et al, 2009). There is no safe blood lead level for children. (EPA; CDC; WHO, 2019).

Therefore, this study was conducted to assess the potential use of Garbage Enzyme (GE) to remove heavy metals from water treatment sludge by comparing physicochemical characteristics and citric acid concentration of the garbage enzyme and to determine the correlation of those components in GE with heavy metals concentration after the treatment with GE.

1.3 Study Justification

In order to solve problems regarding food wastes and sludge, this study must be conducted to assess the potential sludge treatment using garbage enzyme (GE) from food wastes. Garbage enzyme is obtained by fermenting fruit and vegetable wastes, which provides a solution to waste minimization and reduction since a large proportion of municipal solid waste consists of food waste (Ho, Ling & Abd Manaf, 2013). It is one of the green approaches that can help reuse the food waste to produce GE and reduce the volume of sludge by reusing them as fertilizer after treating them with GE to reduce the heavy metals concentration and this approach can contribute to environmental sustainability (Ahmad et al., 2016). The treated sludge with reduced heavy metal content has potential to be a very good soil fertilizer (Verlicchi & Masotti, 2000 & Pogrzeba et al., 2006). This approach can help saving costs in disposal of food wastes and sludge. Plus, the production of GE only requires an easy fermentation of food wastes in airtight container with molasses, sugars or jaggery.

GE contains biodegradable chelating agents such as organic acids which can bind to toxic metal ions to form more soluble structures which are easily excreted from the body (Flora & Pachauri, 2010). Organic acids, pectin and flavonoids are examples of chelating agents in GE. Organic acids such as citric acid can effectively remove heavy metals such as Zinc (Zn) and Copper (Cu) (Song et al, 2015). Kartel (1999) reported that pectin can bind with Pb, Cu, Co, Ni, Zn, Cd, Ba, Zn, Sr, Mn, and Mg to make them more soluble. On the other hand, a number of flavonoids have been shown to have chelation with heavy metal ions particularly with Copper (Cu) and iron (Fe) (Fernandez et al., 2002).

Therefore, this study tries to solve the problem of food waste by converting it into garbage enzyme (GE) that can be used to treat water treatment sludge. This study was designed to assess the potential of sludge treatment using GE to reduce the heavy metal levels. This approach is not only green, but also will reduce the volume of waste in the landfill. To date, very limited studies has been made to treat water treatment sludge (WTS) using garbage enzymes in Malaysia. The latest study regarding sludge treatment using garbage enzyme is a study of Garbage Enzyme's effects in domestic wastewater by Tang and Tong. Since sludge and food waste problems are becoming major public and environmental health issues in Malaysia, the need of more study on green approaches to cater these problems. The garbage enzyme is one of the approaches as it has potential to reduce the amount of food wastes by converting them to GE, at the same time, it has potential to reduce the contaminants in the sludge especially heavy metals so that the treated sludge will be safe for land application, reducing the amount of sludge in landfills. The garbage enzyme production cost is cheaper as it is produced from food waste. The active compounds in GE produced from food waste may help to remove certain heavy metals in the sludge.

1.4 Objectives

1.4.1 General Objective

To assess the potential use of Garbage Enzyme (GE) in heavy metal (lead, zinc and copper) removal of water treatment sludge and its contribution to human health.

1.4.2 Specific Objectives

1) To compare physico-chemical characteristics (i.e. pH and temperature) of Garbage Enzymes (GE) by weeks of fermentation (4 weeks).
2) To compare citric acid concentration of GE by types of GE after four weeks of fermentation.
3) To compare physico-chemical characteristics (i.e. pH and temperature) of sludge samples in 5 days of treatment (120 hours).
4) To compare heavy metals concentration (i.e. Lead, Zinc and Copper) of sludge samples after 5 days of treatment.
5) To determine correlation between pH level and temperature of GE.
6) To determine correlation between pH level and temperature of sludge samples.
7) To determine correlation between citric acid concentration of GE with heavy metals concentration of treated sludge samples.
8) To calculate human health risk for ingestion of heavy metals from sludge samples.

1.5 Hypothesis

1) There is significance mean differences of physico-chemical characteristics (i.e. pH and temperature) of Garbage Enzymes (GE) by weeks (4 weeks).
2) There is a significant mean difference of citric acid concentration of GE by types of GE.
3) There is a significant mean difference of physico-chemical characteristics (i.e. pH and temperature) of sludge samples in 5 days of treatment (120 hours).
4) There is a significant mean difference of heavy metals concentration (Lead, Zinc and Copper) of sludge samples after 5 days of treatment.
5) There is a significant correlation between pH and temperature of GE.
6) There is a significant correlation between pH and temperature of sludge samples.
7) There is a significant correlation between citric acid concentrations of GE with heavy metals concentrations of treated sludge samples.
8) There is no significant health risk for ingestion of heavy metals from sludge samples.

1.6 Definition of Terms

1.6.1 Conceptual Definition

i. Garbage Enzyme (GE)

Garbage Enzyme (GE) is a complex solution produced by the fermentation of food waste with sugars and water (Fazna & Meera, 2017).

ii. Water Treatment Sludge

According to Malaysian Environmental Quality Act 1974, sludge is defined as any deposit of particulate matter settled from any liquid, including deposit resulting from physical, chemical, biological or other treatment of water or industrial effluent or mixed effluent. The water treatment sludge (WTS) are large quantity residues or wastes generated from process of coagulation, flocculation, sedimentation, filtration and disinfection at water treatment plant (Ahmad et al., 2016).

iii. Heavy metals

Heavy metals are defined as metallic elements that have a relatively high density compared to water (Fergusson, 1990; Tchounwou et al., 2012). They are found naturally in earth's crust but technology-based and/or research-related activities have caused drastic changes in their geochemical cycles (Rehman et al., 2012). Moreover, heavy metals are also considered as trace elements because of their presence in trace concentrations (ppb range to less than 10ppm) in various environmental matrices (Kabata-Pendia, 2001, Tchounwou et al., 2012).

1.6.2 Operational Definition

i. Garbage Enzyme (GE)

Garbage enzyme is a product of fermentation of food waste for one month in an airtight container. 6 types of food wastes consist of orange peels, banana peels, carrots, potatoes, nuts and black beans were collected from cafeteria of Faculty of Medicine and Health Sciences and Seventeenth Residential College of Universiti Putra Malaysia, Serdang, Selangor. The food wastes were divided into each type and put in different airtight container for fermentation process in one month before filtration process can be conducted. After filtration, parameters such as pH, citric acid concentration, temperature, total dissolved solids and salinity were measured in lab.

ii. Water Treatment Sludge

The water treatment sludge was collected at Bukit Nanas Water Treatment Plant using APHA standard method 1999. The physicochemical parameters of the sludge such as pH and temperature were measured during treatment.

iii. Heavy metals

Heavy metals concentrations such as Lead (Pb), Zinc (Zn) and Copper (Cu) were detected using *Inductively Coupled Plasma Mass Spectrometry (ICPMS)*. Human health risk of heavy metals concentration via ingestion were also calculated using USEPA method (2009).

1.7 Conceptual framework

Figure 1.7 shows the conceptual framework of this study. The independent variable is type of food waste used to produce Garbage Enzyme (GE). In this study, six types of food waste (i.e. citrus peels, banana peels, carrot wastes, potato wastes, nut wastes and black bean wastes) to produce six types of GE. The dependent variables were psychochemical properties (i.e. pH and temperature) of GE (during fermentation) and treated sludge, the heavy metals (i.e. Lead, Zinc and Copper) concentration after treatment and the human health risk of ingestion of heavy metals from the sludge samples.

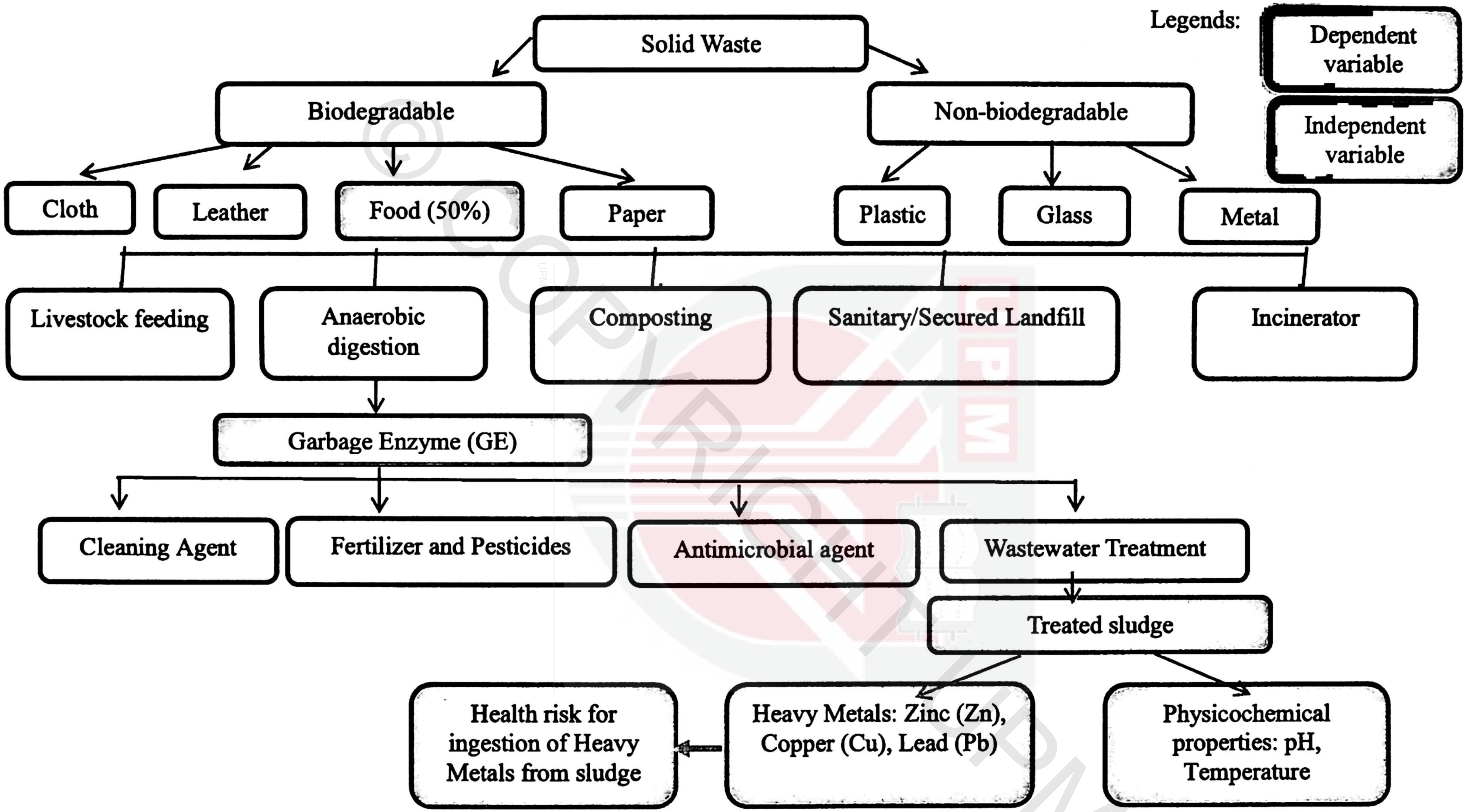


Figure 1.1: Conceptual framework

CHAPTER 2

LITERATURE REVIEW

2.1 Garbage Enzyme

GE is a multipurpose liquid and its applications covers household, agriculture (fertiliser, plant growth hormone, pesticides, and animal husbandry), waste water treatment and antimicrobial agent (Prakash, 2011; Fazna & Meera, 2017). A 9% solution of garbage enzyme in wastewater was found to be most economical in removing ammonia nitrogen and phosphorus, and in neutralizing the wastewater, within the digestion period of 5 days (Tang & Tong, 2011).

The experiments by Arun & Sivashanmugam (2015) confirmed the presence of hydrolytic enzyme activities (amylase, protease, and lipase) in all types of garbage enzyme solution at pH 7. When treatment time increased from 48-60 hours, 10% of pineapple and orange garbage enzymes showed slightly higher reduction of VSS and TSS of nearly 20%-25% and increased % solubility of Chemical Oxygen Demand, Total Kjeldahl method and Total Phosphorus of nearly 20-25%, 15-20% and 9-11% respectively in treated Waste Activated Sludge at pH 7 compared with other garbage. Another study conducted by Fazna & Meera (2013) regarding the treatment of grey water using 5% and 10% of garbage enzyme, the results showed that 10% garbage enzyme has the ability to reduce Biochemical Oxygen Demand, Chemical Oxygen Demand and Total Dissolved Solids by up to 70, 50 and 39% respectively. Furthermore, 100% removal of ammonia nitrogen and phosphates was possible using enzyme solution.

2.2. Relationship between organic acids and heavy metals

Garbage Enzyme contains various organic acids. Organic acids are alternative agents for the extraction of heavy metals from the soils. In contrast to strong acids, Organic acids cause less damage to the soil's crystalline structure over extended contact times and they are often suggested as alternatives to straight mineral acid use (Yu and Klarup, 1994; Wuana, Okieimen, & Imborvungu, 2010; Oustan, Neyshabouri, & Reyhanitabar, 2015). Natural organic acids include oxalic, citric, acetic, and lactic and malic acids which are natural products of root exudates, microbial secretions, and plant and animal residue decomposition in soils (Naidu and Harter, 1998; Wuana, Okieimen, & Imborvungu, 2010; Oustan, Neyshabouri, & Reyhanitabar, 2015). The chelating organic acids are able to dislodge the exchangeable, carbonate, and reducible fractions of heavy metals via washing procedures (Peters, 1999; Wuana, Okieimen, & Imborvungu, 2010; Oustan, Neyshabouri, & Reyhanitabar, 2015). Citrate has been reported to be one of the organic acids in soil solution. Among the organic acids used to simulate metal mobilisation, citric acid presents a high metal complexation strength (Labanowski et al., 2008; Huang et al., 2008; Oustan et al., 2015). Many researchers have studied various natural and synthetic chelating agents for their ability to remediate soils contaminated by heavy metals. Determining the effectiveness of a chelating agent for washing soils contaminated by heavy metals has commonly been accomplished in one-step batch extractions at the laboratory scale (Andrade et al., 2007;Oustan et al., 2015). A study by Heidari et al. (2015) reported that citric acid showed high efficiencies for removing Pb and Zn from a contaminated soil, while oxalic acid and acetic acid were mild extractants, but effective for removing Zn and Cd, respectively.

2.3 Heavy metals pollution in Malaysia

Rapid industrialization and urbanization in Malaysia has increased alarmingly due to the amount of toxic heavy metals entering the environment. Solid and/or liquid waste containing toxic heavy metals are generated in various industrial processes such as chemical manufacturing, coal and ore mining, smelting and metal refining, metal plating; and others (Nair et al., 2008; Yahya, 2008). The most common toxic heavy metals found in industrial wastes include copper (Cu), lead (Pb), zinc (Zn), cadmium (Cd), chromium (Cr), iron (Fe) and manganese (Mn). Sludge generated during various industrial processes has been found to contain undesirable levels of toxic metals. Disposal of sludge containing heavy metals is a complex and problematic situation for most waste generators because the metals are non-biodegradable and tend to bioaccumulate. Based on the Malaysia Environmental Quality Report (MEQR) 2011 (DOE, 2011), heavy metal sludge was one of the main wastes produced in Malaysia with the amount of 173,837.06 metric tonnes (MT) per year contributing 10.72% of total waste. There was a slight increase in the amount of waste produced when compared to 2010 where the total amount of heavy metal sludge generated was 157,381.38 MT per year or 8.37% of total waste. The waste generated was mostly from the electronic/electrical and metal/engineering industries. Realizing the potential danger of improper management of toxic and hazardous wastes, the Malaysian Government has taken initiatives to identify the possible options and necessary measures to ensure its proper management since 1979.

The regulations aimed to ensure hazardous waste produced in the country is safely managed and in an environmentally sound manner. The Environmental Quality (Scheduled Wastes) Regulation 1989 was further improved when the Environmental Quality (Scheduled Wastes) Regulation 2005 came into force on August 15, 2005. All these regulations are stipulated under the Environmental Quality Act (EQA), 1974 which are meant to prevent, abate and control pollution, as well as, further enhance the quality of the environment in this country. The Department of Environment (DOE) is responsible for the administration of this legislation to ensure that Malaysia will continuously have the benefit of both industrial growth and a healthy living environment. Scheduled wastes can be defined as any waste falling within the categories of waste listed in the First Schedule in Environmental Quality (Scheduled Wastes) Regulations 2005. According to the World Health Organization (WHO), hazardous waste possesses physical, chemical or biological characteristics, requires special handling and disposal procedures to avoid risk to health and adverse environmental effects. Table 2.1 present the categories of waste that are listed in the First Schedule under the Environmental Quality (Scheduled Wastes) Regulation 2005.

Table 2.1: The categories of waste listed in First Schedule under Environmental Quality (Scheduled Wastes) Regulation 2005, EQA 1974

Code	Waste Categories
SW 1	Metal and metal bearing wastes.
SW 2	Wastes containing principally inorganic constituent which may contain metals and organic material. As example SW 204 refer to sludge containing one or several metals including Cr, Cu, Zn, Pb, Cd, aluminium (Al), tin (Sn), vanadium (V) and beryllium (Be).

SW 3	Wastes containing principally organic constituents which may contain metals and inorganic materials.
SW 4	Wastes which may contain either inorganic or organic constituents.
SW 5	Other wastes.

2.4 Health effects of heavy metals

With the assumption that heaviness and toxicity are inter-related, heavy metals also include metalloids, such as arsenic, that are able to induce toxicity at low level of exposure (Duffus, 2002, Tchounwou et al., 2012). Some metals such as zinc, copper, iron, manganese, and cobalt are required by human body for normal physiological processes (Lane & Morel, 2000; Ouyang et al., 2002; Rehman et al., 2017), however; they may be harmful if ingested at higher or excessive concentrations (Chronopoulos et al., 1997; Ouyang et al., 2002; Rehman et al., 2017). The heavy metals such as lead and mercury which are not known to have any beneficial effects on human health but in fact are deleterious to human health if accumulated in body over time (Chronopoulos et al., 1997; Rehman et al., 2017). Heavy metals such as lead, Zinc and Copper can cause serious impact on health (Table 2.2). Ingestion of water containing certain amount of heavy metals at high level may cause health problems including shortness of breath (Kavcar, 2009).

Table 2.2: Health impacts of Lead, Zinc and Copper at toxic level

Heavy Metals	Health Impact
Lead(Pb)	Neurotoxic effects on intelligence (Khalil et al.2009), decreased memory (Schwartz et al., 2000), hemolytic anaemia (Vij, 2009), cardiovascular diseases (Navas-Acien et al., 2007), reproductive toxicity (Levin & Goldberg 2000), lung cancer, bladder cancer (IARC, 2004).
Zinc (Zn)	Excessive intake can cause fever, coughing, stomach pain, fatigue and many other health problems (Plum et al., 2008).
Copper (Cu)	Toxic effects including liver damage and gastrointestinal disturbances (Fewtrell et al., 1996).

Therefore, any heavy metal can be considered as a contaminant if it can cause harmful effect to either human or environment or both (Singh et al., 2011). The human activities such as agricultural activities also contribute to contamination of heavy metals in river (Shazili et al., 2006).

2.4.1 Zinc

Zinc (Zn) is one of the most abundant components in the Earth's crust. It is found naturally in the environment in the form of zinc sulphide. Moreover, zinc compounds are generally utilized in manufacturing industry as they are used to make white paints, ceramic and other products. As a consequence of the industrial activities such as mining and purifying of zinc, steel production i.e. alloys and other human activities such as coal burning and burning of wastes, zinc can enter natural resources such as air, water and soil, increasing the zinc levels in the environment. In addition, zinc also being discharged into waterways through zinc and other metal manufacturing, zinc chemical industries, domestic waste water and run-off from soil containing zinc (ATSDR, 2006). The recommended dietary allowance (RDA) of zinc consumption for men is 11 mg/day and 8 mg/day is RDA of zinc consumption for women (FNB IOM, 2001). In fact, excessive intake of zinc can cause fever, coughing, stomach pain, fatigue and many other health problems (Plum et al., 2010).

2.4.2 Copper

Copper (Cu) is a type of metal that found naturally in rock, soil, water and other natural resources. Although the level of copper in surface and ground water is generally very low in nature, the high levels of copper may enter environment through anthropogenic activities such as mining, farming, manufacturing operations as well as municipal or industrial waste water released into rivers and lakes. A study tested on hair cells on zebrafish larvae has shown acute exposure to an overload of this metal can easily lead to Fenton-type redox reactions with hydrogen peroxide (H_2O_2), resulting in oxidative cell damage and cell death (A Olivari et al., 2008) while some studies have suggested that copper overloading increases the formation of an undefined oxidant in mammalian cells (Archielo et al., 2005; Krumschnabel et al., 2005).

2.4.3 Lead

Lead is a naturally occurring toxic metal found in the Earth's crust. Its widespread use has resulted in extensive environmental contamination, human exposure and significant public health problems in many parts of the world. People can become exposed to lead through occupational and environmental sources. This mainly results from inhalation of lead particles generated by burning materials containing lead, for example, during smelting, recycling, stripping leaded paint, and using leaded gasoline or leaded aviation fuel; and ingestion of lead-contaminated dust, water (from leaded pipes), and food (from lead-glazed or lead-soldered containers). Large numbers of heavy metals from urban areas, agricultural areas and industrial sites are discharged into aquatic environments where they are transported in the water column, accumulated in sediment, and biomagnified through the food chain (Yi et al., 2011). Lead in the body is distributed to the brain, liver, kidney and bones. However, human exposure is usually assessed through the measurement of lead in blood. (WHO, 2018). Generally, it is stored in the teeth and bones, where it accumulates over time. Therefore, in pregnant women, lead in bone is released into blood during pregnancy and becomes a source of exposure to the developing foetus. High lead exposure probably results in foetal death. Since lead is a cumulative toxicant that affects multiple body systems, it is particularly harmful to young children. The digestive system of children absorbs 50% of lead they ingest (NRCLPI, 2019). The World Health Organization estimates that 15-18 million children in developing countries are suffering from permanent brain damage due to lead poisoning. Hundreds of millions of children and pregnant women in practically all the developing countries are exposed to elevated levels of lead.

2.5 Solid waste

According to Malaysian Solid Waste Management Act 2007 (Act 672), solid waste is defined as any unwanted substances or materials that are broken, contaminated or worn out which are required to be disposed by authority. It is also stated as useless, unwanted or discarded materials generated from domestic, commercial, industrial or agricultural sectors (Shyamala & Belagali, 2012). Solid wastes in Malaysia can be categorized into three main categories namely Municipal Solid Waste (MSW), scheduled or hazardous waste and clinical waste. They are managed through numerous methods including recycling, composting, incineration, inert landfill, sanitary landfill and other disposal sites (Ghafar, 2012).

United States Environmental Protection Agency (USEPA) defined MSW as wastes generated from daily items such as food waste, garden waste, newspapers, electronic items and other items produced from homes, institutions and commercial sectors. However, sludge, industrial wastes, automobile wastes, combustion products as well as construction and demolition debris are not included as parts of MSW by USEPA.

2.5.1 Food waste

Food waste is described as a discarded food starting from primary production until the end of consumer level and they are decreasing in subsequent stages of food supply chain intended for human consumption (FAO, 2017). Moreover, food waste also can be defined as all food materials that left uneaten or thrown away produced from human consumption and they are disposed from various sources including agriculture, household, commercial, institutional and industries (Ghafar, 2017). Jereme et al. (2016) reported that Malaysians has thrown 930 tonnes of food waste for each day whereas Hamid et al. (2012) stated that 60% of Municipal Solid Waste in Malaysia are dominated by food waste and it is estimated to increase 6.54 million tonnes in 2020. Lim et al. (2016) categorize food waste into three main groups namely food losses, unavoidable food waste and avoidable food waste. Ghafar (2017) states that almost 17% of agriculture products will be thrown as they do not fulfil marketing standards or criteria. According to National Solid Waste Management Department (2013), 50 % of solid waste in Malaysia consist of food waste at source and 70% of them are disposed through landfilling.

2.5.2 Sludge

Sludge is a waste, residue or by-product that generated during process of treatment of domestic and industrial waste water (Edwards et al., 2017). As the population increases drastically every year, the generation of sewage sludge waste in Malaysia has been rapidly increasing. Malaysia produces 3.2 million cubic metres of domestic sludge every year and increases to 4.3 million cubic metres by the year of 2005. Indah Water Konsortium Sdn Bhd (2010) recorded that an estimation of 7 million cubic metres of sewage sludge will be produced annually in the year of 2020.

Although there are methods to consolidate, stabilize and dewater the sludge, but most of the sludge is ended up to be disposed by landfill even after treated. Landfill has become dominant manner of sludge waste. However, landfill is only a temporary solution for the disposal of sludge waste because there is limited space for the sludge waste to be disposed. The waste sludge generated from the water treatment plant consists of organic and inorganic matters. Rizzardini & Goi (2014) stated that the disposal of waste sludge by landfill has become a serious threat to the environment due to the toxic content of sewage waste in Italy. The untreated sewage sludge has caused serious pollution to the soil condition.

In Malaysia, the pollution caused by the disposal of sludge cannot be ignored and needs immediate remedial. As the problem triggered by landfill become worst, awareness from the public and government has been raised upon this problem. To encounter the problem, there are researchers studied the properties of sludge so that it can be reused. The wasted sludge can be utilized as resources after treatment process. For example, treated and stabilised sludge can be utilised for soil conditioning. Treated sludge is inert and stable where it might be suitable for agriculture use. The sludge consists of chemical composition of phosphorus, nitrogen and organic matters which has fertilizer properties. These components in sludge are proved for the ability to improve the condition of agriculture soil.

However, Lin et al. (2012) concluded that sludge consists more than 5% content of heavy metal which shows that sludge has high amount of heavy metal. The content of heavy metal might be harmful for human consumption. Donatello & Cheeseman (2013) stated that there are also limitations to the application because sludge contains heavy metal that may contaminate agriculture soil. Public also consider the risk of pathogen from sludge transferred to the crops. Application of sewage sludge for agriculture has become even difficult as fertilizer quality is standardized.

Conventional water treatment plants (WTPs) transform crude water into potable water utilizing a series of processes: coagulation, flocculation, decantation and filtration. The process of coagulation involves the use of Fe or Al salts that form floccules with impurities in water, which sediment (or float) and are later filtered out. This treatment produces a solid residue (alum or ferric sludge) with a high water content, whose composition depends on the origin of the crude water collected (surface water or groundwater through wells), the type of soil of the region, the material discharged into the river, chemical products present, the process of treatment employed, etc. The main components of the sludge from WTPs are (sometimes known as water treatment residues): clay minerals, very fine-grained minerals (mainly oxides and hydroxides of aluminium and iron), organic matter and contaminants from the discharge of urban and industrial effluents and other human activities (Teixeira et al., 2011).

In general, this sludge is dumped directly into rivers and streams or into the drain system, causing a significant environmental impact, which compromises the quality of drinking water and the health of the public and animals that utilize it (Pamukoglu, F. Kargi, 2006; Sawalha et al., 2007; Teixeira et al., 2011). The growing concern of environmental organizations, due to the risks to health and to the environment, has led to the restriction or prohibition of discharging this residue into the environment (streams, landfills, soil, etc.) (Teixeira et al., 2011).

CHAPTER 3

METHODOLOGY

3.1 Study design

This is an experimental study to assess the effectiveness of Garbage Enzyme (GE) on sludge treatment prior to heavy metal removal and its contribution to human health risk reduction.

3.2 Sampling location

3.2.1 Location of food waste sampling

Food wastes were collected from cafeteria of Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia. Six different types were collected and separated in different containers namely as citrus peels, banana peels, carrot wastes, potato wastes, nut wastes and black bean wastes. The brown sugar for fermentation mixture was bought from local supermarket in Serdang.

3.2.2 Location of sludge sampling

600 grams of sludge were sampled from sludge tank at Bukit Nanas Water Treatment Plant, Federal Territory of Kuala Lumpur, Malaysia on 18th February 2019. This phase involved sampling of food wastes, preparation of materials for GE production,

sampling of Water Treatment Sludge (WTS) and fermentation of food waste for one month.

3.3 Food wastes sampling and preservation

Six types of food wastes (i.e. citrus peels, banana peels, carrot wastes, potato wastes, nut wastes, black bean waste) were collected from cafeteria of Faculty of Medicine and Health Sciences and kept separately in different airtight containers. At laboratory, the containers were kept in refrigerator at 4 °C until further use.

3.4 Production of Garbage Enzyme (GE)

The production of Garbage Enzyme (GE) using 1: 3: 10 ratio referring to 1 ratio of brown sugar, 3 ratio of food wastes and 10 ratio of ultrapure Milli-Q water. In this study, about 1000 ml of GE was produced, it means that 100g of brown sugar, 300g of food wastes and 1000 ml of filtered water were used as a mixture. The food wastes were rinsed with tap water and air-dried. The samples were then pre-treated (crushed and grinded using pestle and mortar). The brown sugar was then dissolved into an airtight plastic container filled with filtered water. Six different food wastes (i.e. citrus peels, banana peels, carrot wastes, potato wastes, nut wastes and black bean wastes) were added separately into six labelled containers respectively and mixed evenly. Each container was labelled; CR for citrus peels, BN for banana peels, CT for carrot wastes, PT for potato wastes as well as NT for nut wastes and BB for black bean wastes. The mixture was fermented for one month in a cool, dry and well ventilated place (Arun & Sivashanmugam, 2017; Fazna & Meera, 2017; Tang & Tong, 2013). During fermentation process, gases were released. Pressure built up in the containers was released daily to avoid rupturing of the container. Fruits/vegetables/legumes wastes were pushed downward every once in a while. After one month of fermentation, the light brownish solution yielded, separated from solids. The solution from each container was sieved to remove large solids and then filtered with Whatman qualitative filter paper No. 1. Grade 1 to remove the remaining residues. [redacted] Each solution was then stored in a volumetric flask and labelled.

Figure 3.1: Mixture ratios for GE preparation

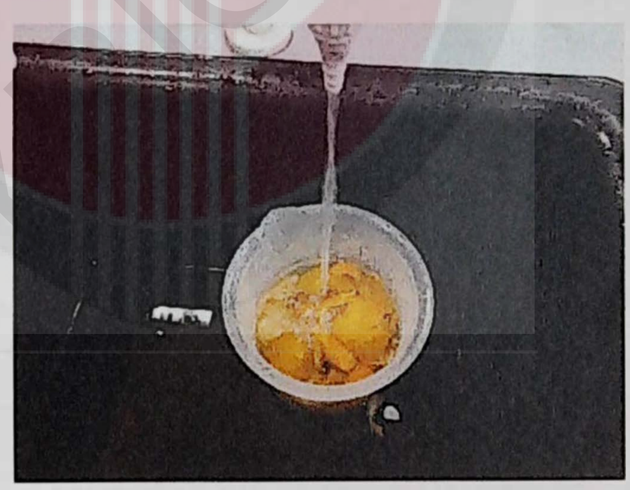


Figure 3.2: The citrus peels sample was rinsed with tap water before air-dried.

Figure 3.3: The carrot and black bean wastes samples were crushed and grinded using pestle and mortar.

Figure 3.4: Brown Sugar (Gula Prai) was used as one of the ingredients

Figure 3.5: Each GE mixture was sieved to remove large solids

Figure 3.6: Each GE was filtered with Whatman No.1 filter paper to remove residues

Figure 3.7: The samples after filtration

Figure 3.8: The samples were stored in volumetric flasks until further analysis

3.5 Sludge sampling

600 grams of sludge was sampled from Bukit Nanas Treatment Plant, Kuala Lumpur on 18th February 2019. The sampling followed American Public Health Association (APHA) standard method 1999. The wide-mouthed Polyethylene containers were rinsed with 50% Nitric Acid fortnight. For Quality Control, the

samples were duplicated. For preservation of the sludge during transportation from sampling field to the laboratory, the containers filled with sludge samples were stored in the cooler filled with cooling agents. The samples were transported immediately to laboratory for proper cooling using refrigerator at 4°C ± 2°C in the dark (covered with aluminum foil).

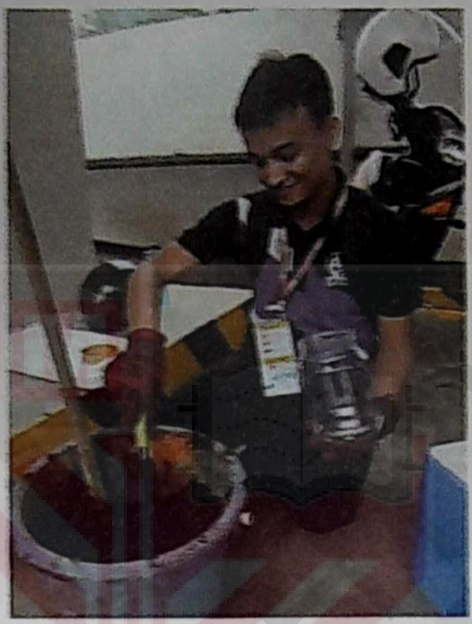


Figure 3.9: Process of collecting sludge

3.6 Determination of pH and temperature of GE

The test was conducted by one-week interval for a month (4 intervals). About 50 milliliter (ml) of each type of GE was transferred to six different beakers prior to measurement. The pH and temperature of each GE was determined using CyberScan pH 300 Portable pH meter (Eutech Instruments).

Figure 3.10: CyberScan pH 300 Portable pH meter (Eutech Instruments)

3.7 Determination of citric acid concentration

3.7.1 Preparation of 0.1 N Sodium Hydroxide (NaOH)

4 g of NaOH pellets (R&M Chemicals) were dissolved in 1 Liter (L) of ultra-pure water from Milli-Q® Integral Water Purification System, to form 1 L of 0.1 N NaOH solution.

3.7.2 Titration

The test was conducted at the end of pre-treatment process. The required apparatus including 25 ml-burette, filter funnel retort stands and 250 ml-conical flasks were set up as in Figure 3.13. Five millilitre of each Garbage Enzyme (GE) solution was diluted using 25 ml of ultra-pure water. (The solution is called an analyte) 25 ml of 0.1 N NaOH solution was added into burette through filter funnel (The solution is called as a titrant). About 3-4 drops of phenolphthalein was used as indicator. The titration process began for each GE starting from CR (citrus peels) ending with BB (black bean wastes). The titration process for each GE was stopped when the pale pink colour appeared indicated the end-point of titration has reached.

Figure 3.11: Titration Setup

3.7.3 Calculation of citric acid concentration

Calculations were then performed to find the unknown concentration of the analyte (citric acid):

$$M_{acid} \times V_{acid} = M_{base} \times V_{base}$$

Where:

M_{acid} = Molarity of the acid

V_{acid} = Volume of the acid

M_{base} = Molarity of the base

V_{base} = Volume of the base

If the titrant and analyte have a 1:1 mole ratio, the formula equation above can be used to determine the unknown concentration, if the ratio is not 1:1 then a modified version should be used. In this study, the balanced equations for chemical reactions used for calculation were as following. The reaction between citric acid with sodium hydroxide:

$$C_3H_5O(COOH)_3\,(aq) + 3\,NaOH\,(aq) \rightarrow Na_3C_3H_5O(COO)_3\,(aq) + 3\,H_2O\,(l)$$

In this reaction equation, the mole ratio of acid ($C_3H_5O(COOH)_3$) and base (NaOH) is 1: 3. In this case, a modified version of $M_{acid} \times V_{acid} = M_{base} \times V_{base}$ is required, therefore $M_{acid} \times V_{acid} = 3 \times M_{base} \times V_{base}$ equation is used. Since, 5 ml of each GE sample was diluted with 25 ml of ultrapure water to form 30 ml of diluted GE solutions. Thus, the dilution factor of 1:6 was applied for the concentration of acid obtained from the earlier formula and equation in order to obtain the undiluted sample's concentration of acid. In short, the concentration of acid obtained from the earlier equation should be multiplied by 6.

3.8 Treatment of Water Treatment Sludge (WTS) using GE

Ninety grams of well mixed WTS was inserted into a beaker and 50 ml of garbage enzyme solution was added into the same beaker (Arun & Sivashanmugam, 2015). The mouth of the beaker was then covered with aluminium foil to retain the mixture from evaporating and avoided from direct sunlight (Fazna & Meera, 2017). After addition, the beaker was shaken in an orbital shaker PSU-10i (BOECO Germany) for 5 days at 250 rpm (Figure 3.14). Simultaneously in another beaker 90 g of sludge and 50 ml of distilled water were added and kept in orbital shaker as a blank (untreated sludge) (Arun & Sivashanmugam, 2015). At regular time intervals (12 hours) parameters like pH and temperature of sludge samples were measured and the measurements were repeated twice to determine the consistency in the result determined (Arun & Sivashanmugam, 2015).

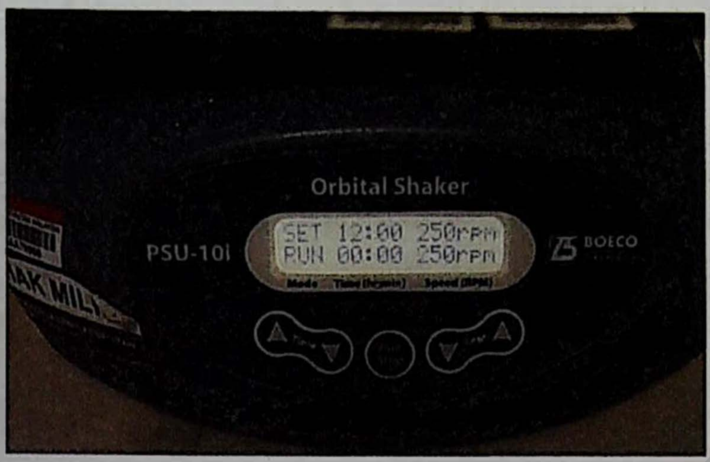


Figure 3.12: The agitation for orbital shaker was set as 250 rpm in duration of 12 hours

Table 3.1: Treatment time

Day of treatment	Treatment hour	Time frame
1	12	8 a.m.-8 p.m.
	24	8 p.m.-8 a.m.
2	36	8 a.m.-8 p.m.
	48	8 p.m.-8 a.m.
3	60	8 a.m.-8 p.m.
	72	8 p.m.-8 a.m.
4	84	8 a.m.-8 p.m.
	96	8 p.m.-8 a.m.
5	108	8 a.m.-8 p.m.
	120	8 p.m.-8 a.m.

3.9 Determination of pH and temperature of Water Treatment Sludge (WTS)

The test was conducted by 12 hours- interval for 5 days (10 intervals). The pH and temperature of each GE was determined using CyberScan pH 300 Portable pH meter (Eutech Instruments). The probe of the pH meter was held perpendicularly and immersed to the approximate midpoint of sludge-GE mixture.

3.10 Determination of heavy metal concentration

The process began with the preparation of samples using nitric acid digestion and ended with injecting the samples into *Inductively Coupled Plasma Mass Spectrometry (ICPMS)*.

3.10.1 Preparation of samples for heavy metal analysis

Firstly, the treated sludge samples were dried under sun. Then they were dried in an oven for 105 °C in an hour (Chen, Afzal & Salema, 2014). Samples were then subjected to nitric acid digestion according to the EPA guidelines (EPA, 1998; Hseu, 2004; Shamuyarira & Gumbo, 2014). Firstly, 2 g (or 0.002 kg) of the sludge sample was weighed and placed in a conical flask. Next, 20 ml of HNO_3 (55% concentration) were added. For each sample, 16 ml of 69% HNO_3 (R&M Chemicals) was diluted using ultrapure water in a 20 ml-volumetric flask to form 20 ml of 55% HNO_3. The samples were then heated at 90 °C on hot plate for 45 minutes. Then temperature of the hot plate was then the increased to 150 °C and heated for 10 min. During heating and boiling, 10 mL of HNO_3 (55% concentration) was added periodically three times to make sure that the liquid remains. Next, the mixture was allowed to cool at room conditions. Following cooling, the samples were filtered into 100 ml volumetric flasks and filled to the mark with distilled water.

3.10.2 Heavy metal analysis by *ICP-MS*

The digested samples were filtered through 0.45 µm membrane filter (GVS Filter, Indianapolis, IN, USA) into 15 mL centrifuge tubes, labelled ("1" for Citrus Peels GE treated sample, "2: for Banana Peels GE treated sample, "3" for Carrot wastes GE treated sample, "4" for potato wastes GE treated sample, "5" for nut wastes GE treated sample as well as "6" for Black Bean wastes GE treated sample and "7" for control sample) and sent for heavy metal analysis at Faculty of Environmental Studies, Universiti Putra Malaysia, Serdang, Selangor, Malaysia in triplicate to be injected to *ICP-MS* (Agilent 7700 instrument, Agilent Technologies Inc., Tokyo, Japan) for measurement of three heavy metals namely lead (PB), zinc (Zn) and copper (Cu). The Agilent 7700 instrument reported the trace element concentrations in parts per billion (ppb). This instrument is able to measure trace and ultra-trace element concentrations even down to parts per trillion (ppt). The range of detection of ICPMS for each standard was 15% of lower percentile and 15% of upper percentile. For example, if the standard of 100 ppb was used, the range of detection will be between 85 ppb to 115 ppb. The *ICP-MS* analysed samples using one standard at a time. The results were converted to mg per kg by multiplying them with 1000 as they would be used in health risk calculation later on.

Table 3.2: Limit of detection (LOD) of heavy metals measured by ICP-MS

Heavy Metal	LOD (µg/L)
Cu	0.005
Zn	0.01
Pb	0.01

3.11 Calculation of human health risk for ingestion of heavy metals.

Heavy metals have toxic, non-biodegradable, and persistent nature (Azimi et al., 2017). They can bioaccumulate through food chain (Bohli et al, 2013). Thus, ingestion is the most worrisome route of exposure to heavy metals (Muhammad, Shah & Khan, 2011). Heavy metals pose health risk to both adult and children. Children have a low tolerance to toxins as well as the inadvertent behavior of coming into contact with significant quantities of sludge in their environment (Acosta et al, 2009). There is no safe blood lead level for children. (EPA; CDC; WHO, 2019).

The equation for calculation of human risk for ingestion of heavy metals via ingestion was adapted from United States Environmental Protection Agency (USEPA) (1989; 1994; 1995; 2011). The equation for non-carcinogenic health risk was applied to ingestion of Zinc and Copper while the equation for carcinogenic health risk applied for ingestion of Lead. The calculation of health risk involved both adult and children.

3.11.1 Non-carcinogenic Health Risk Calculation

According to United States Environmental Protection Policy (2011), the intake of heavy metals such as Zinc and Copper can cause non-carcinogenic health effects to humans. Therefore, Average Daily Dose (ADD) was used in the calculation of non-carcinogenic health risk (Table 3.2). ADD was calculated (USEPA 1991, 2011; Duan et al., 2017) as following:

Average Daily Dose, ADD for Ingestion of Heavy Metals =

$$\frac{C \times IR \times EF \times ED}{BW \times AT}$$

Table 3.3: The variables involved in non-carcinogenic health risk calculation

Variable	Value	Source
C = Average concentration of heavy metal (Zinc, copper) [mg/kg]	Depend on the average results from this study	USEPA (2011)
IR = Soil ingestion rate (kg/day)	Adult = 0.0001 Children = 0.0002	
EF = Exposure frequency (days/year)	Adult = 350 Children = 350	
ED = Exposure duration (years)	Adult = 30 Children = 6	
BW = Body weight of the exposed Individual (kg)	Adult = 70 Children = 15	
AT = Average time (days)	Adult = 365xED Children =365xED	

Note: USEPA refers to United States Environmental Protection Agency

For non-carcinogenic health risk, Hazard Quotient (HQ) >1 indicates significant non-carcinogenic health risk otherwise HQ<1 indicates non-carcinogenic health risk is not significant. HQ was calculated as following:

$$\text{Hazard Quotient, HQ} = \frac{ADD}{RfD}$$

Oral reference dose for given heavy metals were used in this study as shown in Table 3.4.

Table 3.4: Oral reference dose, RfD of Zinc and Copper

Heavy Metal	**Oral Reference dose, RfD (mg/kg/day)**	**Sources**
Zinc	3.0×10^{-1}	USEPA-IRIS (2005)
Copper	4.0×10^{-2}	CalEPA (2005)

Note:

1) USEPA-IRIS refers to Integrated Risk Information System developed by United States Environmental Protection Agency.
2) CalEPA refers to California Environmental Protection Agency

3.11.2 Carcinogenic Health Risk

According to United States Environmental Protection Policy (2011), the intake of heavy metals such as Lead can cause carcinogenic health effects to humans. Therefore, Lifetime Average Daily Dose (LADD) was used in the calculation of carcinogenic health risk. (Table 3.3). LADD was calculated (USEPA 1991, 2011) as following:

Lifetime Average Daily Dose, LADD for ingestion of Heavy Metals=

$$\frac{C \times IR \times EF \times ED}{BW \times AT}$$

Table 3.5: The variables involved in carcinogenic health risk calculation

Variable	Value	Source
C = Average concentration of heavy metal (Lead)[mg/kg]	Depend on the average results from this study	USEPA (2011)
IR = Soil ingestion rate (kg/day)	Adult = 0.0001 Child= 0.0002	
EF = Exposure frequency (days/year)	Adult = 350 Child= 350	
BW = Body weight of the exposed Individual (kg)	Adult = 70 Child=15	
ED = Exposure duration (years)	Adult = 30 Child= 15	
AT = Average time (days)	Adult and Child= 365daysx70 years	

Note: USEPA refers to United States Environmental Protection Agency

For carcinogenic health risk, Lifetime Cancer Risk (LCR) between LCR between $1x10^{-6}$ and $1x10^{-4}$ is considered as acceptable risk. LCR $<10^{-6}$ is considered as clearly acceptable whereas LCR $> 10^{-4}$ is considered as clearly unacceptable indicating that the health risk is significant. LCR was calculated as following:

$$LCR = LADD \times Cancer\ Slope\ Factor,\ CSF$$

Cancer Slope Risk (CSF) for given heavy metal was used in this study as shown in Table 3.6.

Table 3.6: Cancer Slope Factor (CSF) For Lead

Heavy Metal	Cancer Slope Factor, CSF (Mg/Kg/Day)	Source
Lead	8.5×10^{-3}	CalEPA (2011)

Note: CalEPA refers to California Environmental Protection Agency

3.12 Quality Control and Quality Assurance

Before sludge collection, the wide-mouthed Polyethylene containers were rinsed with 1+1 Nitric Acid fortnight. Before sludge collection, samples were duplicated and preserved in the cooler filled with cooling agents, and transported immediately to laboratory for proper cooling using refrigerator at 4°C ± 2°C in the dark (covered with aluminum foil).

Figure 3.13: Container filled with sludge samples were kept temporarily in cooler filled with cooling agents

During measurement of pH & temperature, the measurements were repeated twice and the average readings were taken. The pH meter was calibrated weekly using standard buffer solutions (i.e. pH 4.01, pH 7.0 & pH 10.01) throughout study process.

During 5 days of treatment, the mouth of the beaker was covered with aluminium foil to retain the mixture from evaporating and avoided from direct sunlight. A control sample with blank ultrapure water was available throughout the treatment.

Before titration, 25 ml-burette and filter funnel were rinsed with 0.1 N NaOH. During titration, the samples were triplicated and the average values were taken.

During heavy metal analysis using *Inductively Coupled Plasma Mass Spectrometry (ICPMS)*, the samples were triplicated and the average values were taken. It was calibrated using the US EPA method 6020A. The standard used for *ICP-*

MS was Multi-Element Calibration Standard 3, Matrix per Volume: 5% HNO3 per 125 ml (PerkinElmer Inc., 710 Bridgeport Ave Shelton, CT). The standard originally has 10,000 ppb. The standard was then diluted to ultra-pure water from Milli-Q® Integral Water Purification System to form five standards with different concentration; 10 ppb, 30 ppb, 50 ppb, 100 ppb and 500 ppb. The ultra-pure water was used as the blank standard for the *ICP-MS*. The acceptable correlation coefficient of calibration curve for *ICPMS* was 0.995 and higher.

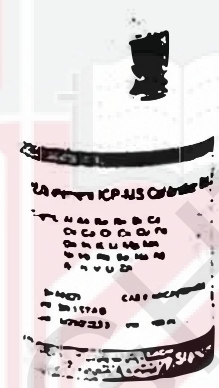


Figure 3.14: Multi-element Calibration Standard 3, Matrix per Volume: 5% HNO3 per 125 ml was used to produce standards

3.12 Data Analysis

MS Excel was used calculation of average, standard deviation and drawing of graphs. IBM SPSS Statistics Version 22 was used to analyse comparison and correlation of samples.

CHAPTER 4

RESULTS

4.1 Comparison of pH level of Garbage Enzyme (GE) in four weeks of fermentation.

Table 4.1 highlights the comparison of pH level of 6 types Garbage Enzymes (i.e. citrus peels GE, banana peels GE, carrot wastes GE, potato wastes GE, nut wastes GE and black bean GE) in four weeks of fermentation. There was a significant mean difference of pH level for all Garbage Enzyme from first week to fourth week of fermentation (p-value<0.001). Across first week to fourth week, most GEs showed a decreasing trend of pH level.

The pH level of citrus peels was ranged from 3.47±0.01 to 2.99±0.01. The pH range of citrus peels GE was the lowest among the others since it was the only GE that reached pH below than 3 in four weeks of fermentation. For banana peels GE, the pH level was ranged from 4.01±0.01 to 3.40±0.01. Banana peels GE showed a slight increase of pH level from second week to third week (from 3.48±0.01 to 3.50±0.01).

In the meantime, the pH level of carrot wastes GE was ranged from 3.85±0.01 to 3.38 ±0.04. Carrot wastes GE also portrayed a slight increase of pH level from third week to fourth week (from 3.18±0.01 to 3.38±0.04). The pH level of potato wastes GE was ranged from 3.98±0.01 to 3.13±0.02, showing a decrease trend of pH level throughout four week of fermentation.

Similarly, nut wastes GE showed a decrease pattern of pH level in four week of fermentation that was ranged from pH 4.00±0.01 to pH 3.33±0.02. The same pattern occurred to black bean wastes GE as the pH level of 4.05±001 after one week of fermentation was kept decreasing and reached pH level of 3.28±0.01 after four week of fermentation. Overall, all pH levels of Garbage Enzymes were in acidic conditions from first week to fourth week of fermentation.

Table 4.1: Comparison of pH level of GE in 4 week of fermentation.

Week	Mean pH level of Garbage Enzyme (SD)						F-statistics (df)	p-value
	Citrus Peels [N=2]	Banana Peels [N=2]	Carrot Wastes [N=2]	Potato Wastes [N=2]	Nut Wastes [N=2]	Black Bean Wastes [N=2]		
1	3.47 (±0.01)	4.01 (±0.01)	3.85 (±0.01)	3.98 (±0.01)	4.00 (±0.01)	4.05 (±0.01)	1247.58 (5)	<0.001
2	3.14 (±0.01)	3.48 (±0.01)	3.37 (±0.01)	3.35 (±0.01)	3.44 (±0.01)	3.41 (±0.01)	307.80 (5)	<0.001
3	3.02 (±0.01)	3.50 (±0.01)	3.18 (±0.01)	3.25 (±0.01)	3.35 (±0.01)	3.35 (±0.03)	301.93 (5)	<0.001
4	2.99 (±0.01)	3.40 (±0.01)	3.38 (±0.04)	3.13 (±0.02)	3.33 (±0.02)	3.28 (±0.01)	127.531 (5)	<0.001

One-Way ANOVA test

**p*-value is significant at 0.05 level

4.2 Comparison of temperature of Garbage Enzymes (GE) in four weeks of fermentation.

Table 4.2 highlights the temperature of 6 types Garbage Enzymes (i.e. citrus peels GE, banana peels GE, carrot wastes GE, potato wastes GE, nut wastes GE and black bean GE) in four weeks of fermentation. The temperature of most GEs was not stable since there were varied readings of temperature among GEs over four week of fermentation. However, there was a significant mean difference of Garbage Enzyme (p-value<0.001) except fourth week (p-value=0.817) which showed no significant difference of temperature among the GEs.

The temperature of was decreasing from first week (22.30±0.01 °C) to third week of fermentation (21.80±0.01 °C). In the fourth week of fermentation, the temperature of Citrus Peels GE increased to 23.30±0.28 °C. For Banana Peels GE, the temperature was slightly decreasing from first week (21.80 ±0.01 °C) to second week of fermentation (21.75±0.07 °C); however, it increased to 22.10±0.01 °C at third week and 23.30±0.28 °C at fourth week. Similarly, Carrot Wastes GE showed a slight decrease of temperature from first week (22.25±0.07 °C) to second week of fermentation (21.70±0.01 °C) and it suddenly increased at third week (22.30±0.01 °C) and fourth week (23.10±0.28 °C).

In the meantime, Potato Wastes GE showed an increasing trend of temperature as the temperature values kept increasing from 21.70±0.01 °C at first week until 23.10±0.28 °C at fourth week of fermentation. Next, Nut wastes GE also showed the pattern as the same as Banana Peels GE and Carrot Wastes GE.

At first, the temperature value was decreasing from first week (22.25 ±0.07 °C) to second week (21.60±0.01 °C) but it increased to 21.95±0.07 °C at third week and 23.15±0.07 °C at fourth week of fermentation. The same pattern also occurred to Black Bean Waste GE as the temperature value was slightly decreased from first week (21.90±0.01 °C) to second week (21.80±0.01 °C) of fermentation and increased at third week (22.15±0.07 °C) and fourth week (23.05±0.07 °C) of fermentation.

Table 4.2: Comparison of temperature of GE in 4 weeks of fermentation.

Week	Mean temperature of Garbage Enzyme, °C (SD)						F-statistics (df)	p-value
	Citrus Peels [N=2]	Banana Peels [N=2]	Carrot Waste [N=2]	Potato Waste [N=2]	Nut Waste [N=2]	Black Bean Waste [N=2]		
1	22.30 (±0.01)	21.80 (±0.01)	22.25 (±0.07)	21.70 (±0.01)	22.25 (±0.07)	21.90 (±0.01)	83.60 (5)	<0.001*
2	22.10 (±0.01)	21.75 (±0.07)	21.70 (±0.01)	21.95 (±0.07)	21.60 (±0.01)	21.80 (±0.01)	39.20 (5)	<0.001*
3	21.80 (±0.01)	22.10 (±0.01)	22.30 (±0.01)	22.00 (±0.01)	21.95 (±0.07)	22.15 (±0.07)	36.00 (5)	<0.001*
4	23.30 (±0.28)	23.30 (±0.28)	23.10 (±0.28)	23.10 (±0.28)	23.15 (±0.07)	23.05 (±0.07)	0.42 (5)	0.817

Note: One-Way ANOVA test

**p*-value is significant at 0.05 level

Room temperature: ≤22°C

4.3 Comparison of citric acid concentration of Garbage Enzymes (GE) after four weeks of fermentation.

Table 4.3 shows the comparison of citric acid concentration of 6 types Garbage Enzymes (i.e. citrus peels GE, banana peels GE, carrot wastes GE, potato wastes GE, nut wastes GE and black bean GE) after four weeks of fermentation. There was a significant mean difference of citric acid concentration of Garbage Enzymes [F (5) =150.73, p-value<0.001*].

Citrus peels GE had 1.33 ±0.05 mol/L of citric acid concentration which was the highest among the other GEs. The second highest citric acid concentration was carrot wastes GE with concentration of 1.16±0.10 mol/L. Next, the third highest was banana peels GE with 1.00±0.09 mol/L followed by potato wastes (0.44±0.03 mol/L), nut wastes (0.30±0.06 mol/L) and the lowest one which was black bean wastes GE with citric acid concentration of 0.19±0.06 mol/L.

Table 4.3: Comparison of citric acid concentration of Garbage Enzymes (GE) after four weeks of fermentation.

Mean Citric Acid Concentration of GE, mol/L (SD)						F-statistics (df)	p-value
Citrus Peels [N=3]	Banana Peels [N=3]	Carrot Wastes [N=3]	Potato Wastes [N=3]	Nut Wastes [N=3]	Black Bean Wastes [N=3]		
1.33 (±0.05)	1.00 (±0.09)	1.16 (±0.10)	0.44 (±0.03)	0.30 (±0.06)	0.19 (±0.06)	150.73 (5)	<0.001*

Note: One-Way ANOVA test

**p*-value is significant at 0.05 level

4.4 Comparison of pH level of sludge samples in 5 days (120 hours) of treatment.

Figure 4.4 portrays the trend of mean pH level of sludge samples in 5 days of treatment while Table 4.4 highlights the comparison of pH level of 7 types of sludge samples treated with different GEs (i.e. citrus peels GE, banana peels GE, carrot wastes GE, potato wastes GE, nut wastes GE, black bean wastes GE and control or sludge sample with blank ultrapure water) in 5 days (120 hours) of treatment. There was a significant mean difference of pH level among sludge samples in 5 days of treatment ($p<0.001^*$).

For sludge sample with citrus peels GE, the pH levels were decreasing from pH level of 4.49±0.01 after 12 hours of treatment to pH level of 4.34(±0.01) after 24 hours of treatment. However, the pH level of sludge sample with Citrus peels GE was then increasing after 36 hours (4.69±0.01) until 48 hours (4.73±0.03) of treatment and then decreasing again after 60 hours of treatment (4.52±0.01) until 72 hours of treatment (4.17±0.01). The pH level of the sample with citrus peels GE increased after 84 hours of treatment (4.82±0.01) and then decreased again after 96 hours of treatment (4.25±0.01). After 108 hours of treatment, the pH values of the sample increased (4.70±0.01) until the 5 days of treatment ended at pH level of 4.97±0.01. All the pH values for sludge sample with citrus peels GE were outside the acceptable range of pH level stipulated by Industrial Effluent Standards, EQA 1979 (Standard A: range of pH 6.0-9.0 and Standard B: range of pH 5.5-9.0)

The pH level of sludge sample with Banana Peels GE was decreasing from pH level of 4.68±0.01 after 12 hours of treatment to pH level of 4.59±0.03 after 24 hours of treatment. Then the pH value of the sample was then increasing after 36 hours of treatment until 48 hours of treatment. The pH value then dropped drastically after 60 hours of treatment until 72 hours of treatment. The pH value was then increasing after 84 hours of treatment and then decreased again in 96 hours of treatment. The pH value was then increasing until the treatment ended at pH level of 5.00±0.01. All the pH values for sludge sample with Banana Peels GE were outside the acceptable range of pH level stipulated by Industrial Effluent Standards, EQA 1979 (Standard A: range of pH 6.0-9.0 and Standard B: range of pH 5.5-9.0). Other than Citrus and Banana, other samples also had an increase and a decrease of pH level indicates that the pH levels were not stable.

For sludge sample with Carrot Wastes GE, the pH level was ranged from 4.36±0.01 to 4.75±0.01 from 12 hours of treatment until 120 hours of treatment respectively. The sample reached the lowest pH value in 24 hours of treatment at pH of 4.00±0.01 and highest pH level of 4.75±0.01 at the end of the treatment. All the pH values for sludge sample with Carrot Wastes GE were outside the acceptable range of pH level stipulated by Industrial Effluent Standards, EQA 1979 (Standard A: range of pH 6.0-9.0 and Standard B: range of pH 5.5-9.0).

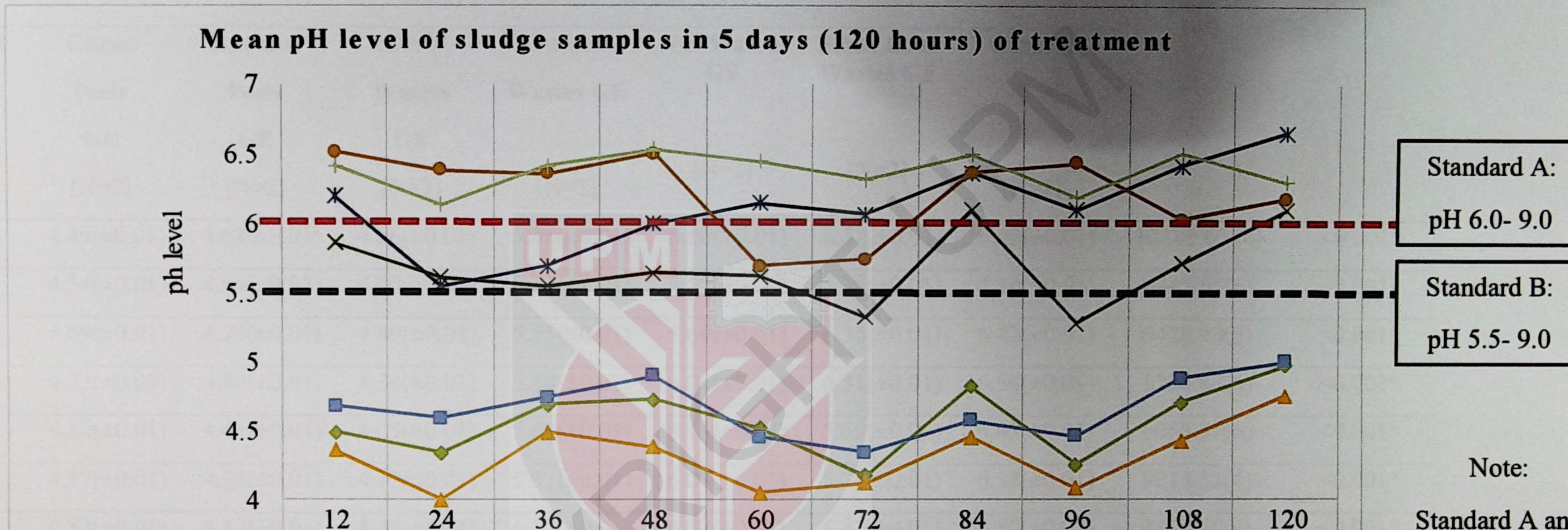
For sludge sample with Potato Wastes GE, the pH level was ranged from 5.86±0.01 to 6.10±0.02 from 12 hours of treatment until 120 hours of treatment respectively. The sample reached the lowest pH value in 96 hours of treatment with pH level of 5.28±0.01 and the highest pH level of 6.10 ±0.01 in 84 hours of treatment and 6.10±0.02 in 120 hours of treatment. All the pH values for sludge sample with Potato Wastes GE were within the acceptable range of pH level stipulated by Industrial Effluent Standards, EQA 1979 (Standard A: range of pH 6.0-9.0 and Standard B: range of pH 5.5-9.0) except in 72 hours and 96 hours of treatment.

In the meantime, pH level of sludge sample with Nut Wastes GE was ranged from 6.20±0.01 to 6.66±0.01 from 12 hours of treatment until 120 hours of treatment. The sample reached the lowest pH value of 5.55(±0.04) in 24 hours of treatment and the highest pH level of 6.66±0.01 after 120 hours of treatment. All the pH values for sludge sample with Nut Wastes GE were within the acceptable range of pH level stipulated by Industrial Effluent Standards, EQA 1979 (Standard A: range of pH 6.0-9.0 and Standard B: range of pH 5.5-9.0).

The pH level of sludge sample with Black Bean Wastes GE was ranged from 6.52±0.01 to 6.18±0.01 from 12 hours of treatment until 120 hours of treatment. The sample reached the lowest pH value of 5.74(±0.01) in 72 hours of treatment and the highest pH level of 6.52±0.01 after 12 hours of treatment. All the pH values for sludge sample with Black Bean Wastes GE were within the acceptable range of pH level stipulated by Industrial Effluent Standards, EQA 1979 (Standard A: range of pH 6.0-9.0 and Standard B: range of pH 5.5-9.0).

For control sludge sample with blank ultrapure water, the pH level was ranged from 6.42±0.01 to 6.3±0.01 after 12 hours of treatment until 120 hours of treatment. The sample reached the lowest pH value of 6.14±0.01 in 24 hours of treatment and the highest pH level of 6.54±0.01 after 48 hours of treatment. All the pH values for control sludge sample were within the acceptable range of pH level

stipulated by Industrial Effluent Standards, EQA 1979 (Standard A: range of pH 6.0-9.0 and Standard B: range of pH 5.5-9.0)



	12	24	36	48	60	72	84	96	108	120
CITRUS	4.49	4.34	4.69	4.73	4.52	4.17	4.82	4.25	4.7	4.97
BANANA	4.68	4.59	4.74	4.9	4.45	4.34	4.57	4.46	4.88	5
CARROT	4.36	4	4.49	4.38	4.05	4.12	4.45	4.08	4.43	4.75
POTATO	5.86	5.61	5.55	5.64	5.62	5.32	6.1	5.28	5.71	6.1
NUT	6.2	5.55	5.69	6	6.15	6.07	6.37	6.1	6.42	6.66
BLACK BEAN	6.52	6.39	6.36	6.51	5.69	5.74	6.37	6.44	6.03	6.18
CONTROL	6.42	6.14	6.42	6.54	6.45	6.32	6.5	6.19	6.51	6.3

Note:

Standard A and B adopted from Industrial Effluent Standards, EQA 1979

Figure 4.4: Comparison of pH level of sludge samples in 5 days (120 hours) of treatment

Table 4.4: Comparison of pH level of sludge samples in 5 days (120 hours) of treatment.

Treatment time (hour)	Mean pH of Sludge Sample (SD)							F-statistics (df)	p-value
	Citrus Peels GE [N=2]	Banana Peels GE [N=2]	Carrot Wastes GE [N=2]	Potato Wastes GE [N=2]	Nut Wastes GE [N=2]	Black Bean Wastes GE [N=2]	Control [N=2]		
12	4.49(±0.01)	4.68(±0.01)	4.36(±0.01)	5.86(±0.01)	6.20(±0.01)	6.52(±0.01)	6.42(±0.01)	10282.60 (6)	<0.001*
24	4.34(±0.01)	4.59(±0.03)	4.00(±0.01)	5.61(±0.01)	5.55(±0.04)	6.39(±0.01)	6.14(±0.01)	4445.65(6)	<0.001*
36	4.69(±0.01)	4.74(±0.01)	4.49(±0.01)	5.55(±0.01)	5.69(±0.01)	6.36(±0.01)	6.42(±0.01)	9428.98(6)	<0.001*
48	4.73(±0.03)	4.90(±0.01)	4.38(±0.01)	5.64(±0.01)	6.00(±0.01)	6.51(±0.01)	6.54(±0.01)	5754.87(6)	<0.001*
60	4.52(±0.01)	4.45(±0.01)	4.05(±0.01)	5.62(±0.01)	6.15(±0.03)	5.69(±0.01)	6.45(±0.01)	6053.57(6)	<0.001*
72	4.17(±0.01)	4.34(±0.01)	4.12(±0.01)	5.32(±0.01)	6.07(±0.01)	5.74(±0.01)	6.32(±0.01)	9814.81(6)	<0.001*
84	4.82(±0.01)	4.57(±0.01)	4.45(±0.04)	6.10(±0.01)	6.37(±0.03)	6.37(±0.01)	6.50(±0.01)	3394.70(6)	<0.001*
96	4.25(±0.01)	4.46(±0.01)	4.08(±0.01)	5.28(±0.01)	6.10(±0.01)	6.44(±0.01)	6.19(±0.01)	10026.24(6)	<0.001*
108	4.70(±0.01)	4.88(±0.01)	4.43(±0.01)	5.71(±0.01)	6.42(±0.01)	6.03(±0.01)	6.51(±0.01)	9676.33(6)	<0.001*
120	4.97(±0.01)	5.00(±0.01)	4.75(±0.01)	6.10(±0.02)	6.66(±0.01)	6.18(±0.01)	6.30(±0.01)	6218.57(6)	<0.001*

Note: One-Way ANOVA test; **p*-value is significant at 0.05 level

Standard A : PH 6.0-9.0 and Standard B: PH 5.5-9.0 in Industrial Effluent Standard (EQA,1979).

4.5 Comparison of temperature of sludge samples in 5 days (120 hours) of treatment.

Figure 4.5 indicates the trend of mean temperature of 7 types of sludge samples treated with different GEs (i.e. citrus peels GE, banana peels GE, carrot wastes GE, potato wastes GE, nut wastes GE, black bean wastes GE and control or sludge sample with blank ultrapure water) in 5 days of treatment while Table 4.5 highlights the comparison of temperature of sludge samples in 5 days (120 hours) of treatment. There were significant mean differences of temperature among sludge samples after 12 hours of treatment [$F (6) = 42.83$, $p\text{-value}<0.001$] and after 60 hours of treatment [$F (6) = 7.66$, $p\text{-value}=0.01$] but there was no significant difference of temperature of sludge samples in other intervals.

For sludge sample with citrus peels GE, the temperature values were ranged from 23.55 ±0.07 °C to 23.60 ±0.01 °C from 12 hours of treatment until 120 hours of treatment. The lowest temperature was identified after 48 hours of fermentation at 22.60±0.42 °C and the highest temperature was exhibited after 96 hours of treatment at 30.05±1.34 °C. Overall, all temperature values of the sample were not exceeding acceptable limit of 40 °C stated in Industrial Effluent Standard (EQA,1979).

For sludge sample with banana peels GE, the temperature values were ranged from 23.35±0.07 °C to 23.65±0.07 °C from 12 hours of treatment until 120 hours of treatment. The lowest temperature was shown after 36 hours of treatment at 22.50±0.28 °C and 84 hours of treatment at 22.50±0.28 °C while the highest temperature was shown after 96 hours of treatment at 29.65±1.63. Overall, all temperature values of the sample were not exceeding acceptable limit of 40 °C stated in Industrial Effluent Standard (EQA,1979).

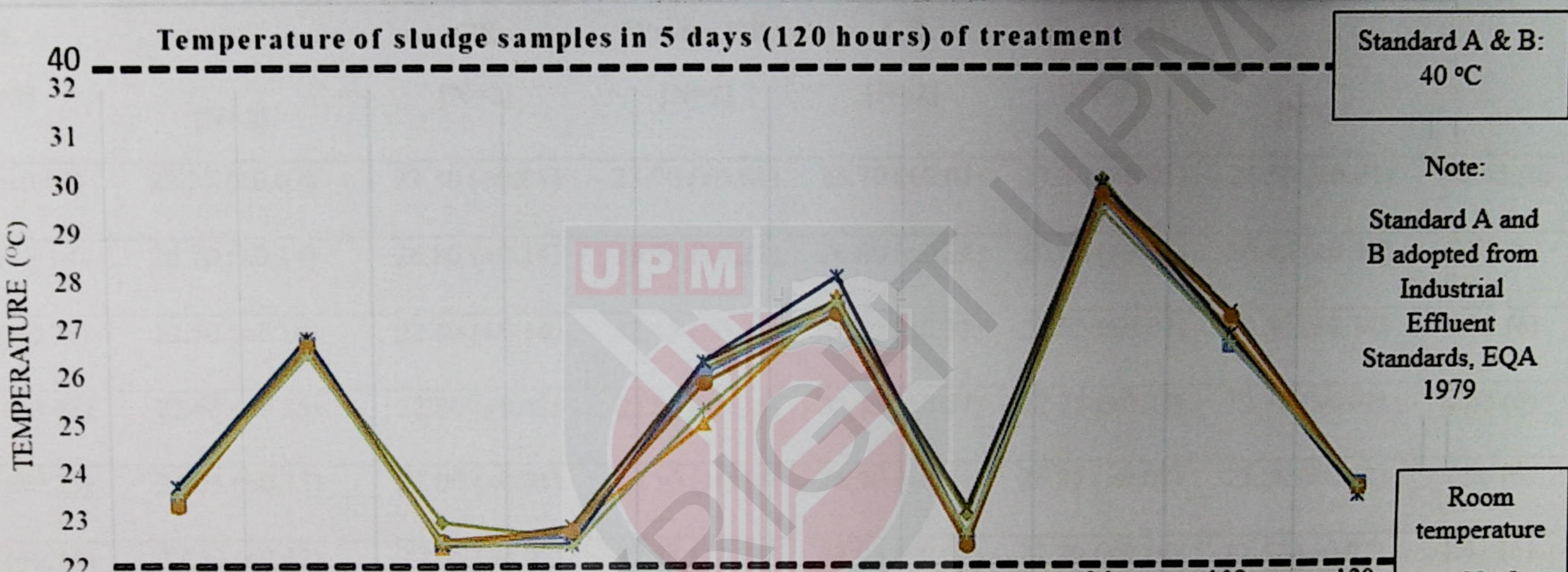
For sludge sample with carrot wastes GE, the temperature values were ranged from 23.30±0.01°C to 23.60±0.01 °C from 12 hours of treatment until 120 hours of treatment. The lowest temperature was shown after 36 hours of treatment at 22.40±0.14°C and the highest temperature was shown after 96 hours of treatment at 29.70±1.56 °C. Overall, all temperature values of the sample were not exceeding acceptable limit of 40 °C stated in Industrial Effluent Standard (EQA,1979).

For potato wastes GE, the temperature values were ranged from 23.70±0.01 °C to 23.55±0.07 °C from 12 hours of treatment until 120 hours of treatment. The lowest temperature was shown after 36 hours of treatment at 22.45±0.35 and the highest temperature was shown after 96 hours of treatment at 29.90±1.56°C. Overall, all temperature values of the sample were not exceeding acceptable limit of 40 °C stated in Industrial Effluent Standard (EQA,1979).

For Nut Wastes GE, the temperature values were ranged from 23.70±0.01 °C to 23.40±0.14°C from 12 hours of treatment until 120 hours of treatment. The lowest temperature was shown after 36 hours of treatment and 48 hours of treatment at 22.45±0.35 °C and 22.45±0.07°C respectively. The highest temperature was shown after 96 hours of treatment at 29.95±1.48 °C. Overall, all temperature values of the sample were not exceeding acceptable limit of 40 °C stated in Malaysian standard for effluent wastewater (EQA, 1974).

For sludge sample Black Bean Wastes GE, the temperature values were ranged from 23.30±0.01°C to 23.60±0.01°C from 12 hours of treatment until 120 hours of treatment. The lowest temperature was shown after 84 hours of treatment at 22.40±0.14 °C while the highest temperature was shown after 96 hours of treatment at 29.75±1.48 °C. Overall, all temperature values of the sample were not exceeding acceptable limit of 40 °C stated in Industrial Effluent Standard (EQA,1979).

For control sludge sample with blank ultrapure water, the temperature values were ranged from 23.50±0.01 °C to 23.60±0.01 °C from 12 hours of treatment until 120 hours of treatment. The lowest temperature was shown after 48 hours of treatment at 22.40±0.01°C while the highest temperature was shown after 96 hours of treatment at 29.40±1.56°C. Overall, all temperature values of the sample were not exceeding acceptable limit of 40 °C stated in Industrial Effluent Standard (EQA,1979).



	12	24	36	48	60	72	84	96	108	120
CITRUS	23.55	26.6	22.95	22.6	26.15	27.45	23.05	30.05	26.65	23.6
BANANA	23.35	26.7	22.5	22.65	26.05	27.4	22.5	29.65	26.6	23.65
CARROT	23.3	26.8	22.4	22.8	25	27.65	22.5	29.7	26.9	23.6
POTATO	23.7	26.65	22.45	22.85	26.3	27.55	23.2	29.9	27.3	23.55
NUT	23.7	26.8	22.45	22.45	26.3	28.05	22.6	29.95	26.8	23.4
BLACK BEAN	23.3	26.65	22.55	22.75	25.85	27.3	22.4	29.75	27.2	23.6
CONTROL	23.5	26.45	22.5	22.4	25.3	27.55	22.65	29.4	26.65	23.6

Figure 4.5: Comparison of temperature of sludge samples in 5 days (120 hours) of treatment.

Table 4.5: Comparison of temperature of sludge samples in 5 days (120 hours) of treatment.

Treatment time (hour)	Mean temperature of Sludge Sample, °C (SD)							F-statistics (df)	p-value
	Citrus Peels GE [N=2]	Banana Peels GE [N=2]	Carrot Wastes GE [N=2]	Potato Wastes GE [N=2]	Nut wastes GE [N=2]	Black Bean Wastes GE [N=2]	Control [N=2]		
12	23.55 (±0.07)	23.35 (±0.07)	23.30 (±0.01)	23.70 (±0.01)	23.70 (±0.01)	23.30 (±0.01)	23.50 (±0.01)	42.83 (6)	<0.001*
24	26.60 (±0.14)	26.70 (±0.14)	26.80 (±0.14)	26.65 (±0.21)	26.80 (±0.28)	26.65 (±0.21)	26.45 (±0.35)	0.58 (6)	0.74
36	22.95 (±0.21)	22.50 (±0.28)	22.40 (±0.14)	22.45 (±0.35)	22.45 (±0.35)	22.55 (±0.07)	22.50 (±0.14)	1.15 (6)	0.42
48	22.60 (±0.42)	22.65 (±0.35)	22.80 (±0.42)	22.85 (±0.35)	22.45 (±0.07)	22.75 (±0.21)	22.40 (±0.01)	0.63 (6)	0.71
60	26.15 (±0.35)	26.05 (±0.35)	25.00 (±0.01)	26.30 (±0.14)	26.30 (±0.14)	25.85 (±0.07)	25.30 (±0.42)	7.66 (6)	0.01*
72	27.45 (±0.35)	27.40 (±0.42)	27.65 (±0.07)	27.55 (±0.49)	28.05 (±0.07)	27.30 (±0.14)	27.55 (±0.07)	1.41 (6)	0.33
84	23.05 (±0.07)	22.50 (±0.01)	22.50 (±0.28)	23.20 (±0.28)	22.60 (±0.42)	22.40 (±0.14)	22.65 (±0.35)	2.64 (6)	0.12
96	30.05 (±1.34)	29.65 (±1.63)	29.70 (±1.56)	29.90 (±1.56)	29.95 (±1.48)	29.75 (±1.48)	29.40 (±1.56)	0.04 (6)	1.00
108	26.65 (±0.49)	26.60 (±0.57)	26.90 (±0.57)	27.30 (±0.14)	26.80 (±0.28)	27.20 (±0.28)	26.65 (±0.21)	0.99 (6)	0.50
120	23.60 (±0.01)	23.65 (±0.07)	23.60 (±0.01)	23.55 (±0.07)	23.40 (±0.14)	23.60 (±0.01)	23.60 (±0.01)	3.06 (6)	0.09

Note: One-Way ANOVA test; *p-value is significant at 0.05 level

Acceptable temperature limit in Industrial Effluent Standard = 40 °C (EQA, 1979). Room temperature: ≤22°C

4.6 Comparison of heavy metals (Lead, Zinc and Copper) of sludge samples after 5 days (120 hours) of treatment.

Table 4.6 highlights the comparison of heavy metals (lead, zinc and copper) of sludge samples after 5 days (120 hours) of treatment. There were significant mean differences of lead concentration among sludge samples [F (6) =2080.62, p-value<0.001*].

Sludge sample with banana peels GE had 35.03±0.16 ppb of lead concentration which was the lowest among the other GEs. The second lowest lead concentration was sludge sample that contained citrus peels GE with lead concentration of 36.30±0.53 ppb. Next, the third lowest lead concentration was shown by control sludge sample (43.85±0.22 ppb) followed by sludge with carrot wastes GE (48.74±0.05 ppb), potato wastes GE (51.00±0.20 ppb) and Black Bean GE (51.59±0.26 ppb).

For zinc concentration, there was a significant mean difference of zinc concentration among sludge samples [F (6) =31516.62, p-value<0.001*]. The sludge sample with citrus peels GE had the lowest concentration of 212.60±2.68 while the highest zinc concentration was originated from sludge sample with banana peels GE (1856.33±14.30 ppb).

For copper concentration, there was a significant mean difference of copper concentration among Garbage Enzymes [F (6) =10667.30, p-value<0.001*]. The sludge sample with banana peels GE had the lowest copper concentration (52.67±0.30 ppb) while the sludge sample with potato wastes GE has the highest copper concentration (114.40±0.24 ppb).

Table 4.6: Comparison of heavy metals (Lead, Zinc and Copper) of sludge samples after 5 days (120 hours) of treatment.

Heavy Metals (ppb)	Mean, ppb (SD)							Standards for industrial effluent discharge (DOE, 1979) [unit: ppb]		F-statistics (df)	p-value
	Citrus Peels GE	Banana Peels GE	Carrot Wastes GE	Potato Wastes GE	Nut Wastes GE	Black Bean Wastes GE	Control	A	B		
Lead	36.30 (±0.53)	35.03 (±0.16)	48.74 (±0.05)	51.00 (±0.20)	49.53 (±0.53)	51.59 (±0.26)	43.85 (±0.22)	100	500	2080.62 (6)	<0.001
Zinc	212.60 (±2.68)	1856.33 (±14.30)	247.22 (±1.49)	426.19 (±1.51)	523.65 (±2.68)	449.25 (±1.68)	290.66 (±0.41)	1000	1000	31516.62 (6)	<0.001
Copper	76.77 (±0.16)	52.67 (±0.30)	78.84 (±0.35)	114.40 (±0.24)	60.95 (±0.16)	75.24 (±0.42)	87.38 (±0.22)	200	1000	10667.30 (6)	<0.001

Note: One-Way ANOVA test; *p-value is significant at 0.05 level

4.7 Correlation between pH level and temperature of GE.

Table 4.7 shows the correlation between two physicochemical characteristics (i.e. pH and temperature) of GE. There was a significant correlation between temperature and pH level. There was a fair negative correlation ($r = -0.358$) between temperature and pH of GE. This means pH of GE was inversely proportional to temperature of GE. When the temperature of GE is increased, the pH level of GE is decreased or vice versa.

Note: <0.25 (Poor); 0.26-0.50 (Fair); 0.51-0.75 (Good); 0.76-1.00 (Excellent)

Table 4.7: Correlation between pH level and temperature of GE

Physico-chemical characteristics of GE	**Temperature**	
	Correlation coefficient, r	p-value
pH	-0.358	0.012*

Note: Pearson's correlation

**p*-value is significant at 0.05 level

4.8 Correlation between pH level and temperature of sludge samples.

Table 4.8 shows the correlation between two physicochemical characteristics (i.e. pH and temperature) of sludge samples. There was no significant correlation between temperature and pH level of sludge samples.

Note: <0.25 (Poor); 0.26-0.50 (Fair); 0.51-0.75 (Good); 0.76-1.00 (Excellent)

Table 4.8: Correlation between pH level and temperature of sludge samples

Physico-chemical characteristics of sludge	**Temperature**	
	Correlation coefficient, r	p value
pH	-0.138	0.103

Note: Pearson's correlation

**p*-value is significant at 0.05 level

4.9 Correlation between heavy metals and citric acid concentration of GE.

Table 4.9 shows the correlation between heavy metals (i.e. lead, zinc and copper) of treated sludge samples with citric acid concentration of GE. There was a significant correlation between lead concentration in sludge samples and citric acid concentration in GEs but there was no significant correlation between zinc and copper with citric acid concentration. There was a good negative correlation ($r = -0.73$) between lead concentration in sludge samples and citric acid concentration of GE. This means citric acid concentration of GE was inversely proportional to the lead concentration of the sludge samples. When the citric acid concentration of GE is increased, the lead concentration of sludge samples is decreased or vice versa.

Note: <0.25 (Poor); 0.26-0.50 (Fair); 0.51-0.75 (Good); 0.76-1.00 (Excellent)

Table 4.9 Correlation between heavy metals and citric acid concentration of GE.

Heavy Metals (ppb)	**Citric Acid Concentration (M)**	
	Correlation coefficient, r	p-value*
Lead (Pb)	-0.73	0.001*
Zinc (Zn)	0.08	0.75
Copper (Cu)	-0.17	0.49

Pearson's correlation *p-value significant at 0.05 level

4.10 Non-carcinogenic and carcinogenic health risk on ingestion of heavy metals from sludge (lead, zinc and copper).

Table 4.10.1 shows non-carcinogenic and carcinogenic health risk on ingestion of heavy metal for adults. For ingestion of zinc and copper, the Hazard Quotient (HQ) for all samples were less than 1, indicating the non-carcinogenic health risk is not significant to human via ingestion of Zinc and Copper from sludge samples. For ingestion of lead, the Lifetime Cancer Risk (LCR) for all samples were less than 1×10^{-6} and considered as clearly acceptable.

Table 4.10.1: Non-carcinogenic and carcinogenic health risk on ingestion of heavy metal for adults

Sludge Sample	Heavy Metal, mg/kg [n=3](±SD)		HQ	LCR
With Citrus Peels GE	Pb	0.04(±0.16)	-	1.81×10^{-10}
	Zn	0.21(±1.74)	$9.71\times10^{-}$[illegible]	-
	Cu	0.08(±0.50)	2.63×10^{-6}	-
With Banana Peels GE	Pb	0.05(±0.26)	-	2.57×10^{-10}
	Zn	0.45(±1.68)	2.05×10^{-6}	-
	Cu	0.08(±0.42)	2.58×10^{-6}	-
With Carrot Waste GE	Pb	0.05(±0.05)	-	2.43×10^{-10}
	Zn	0.25(±1.49)	1.13×10^{-6}	-
	Cu	0.08(±0.35)	2.70×10^{-6}	-
With Potato Waste GE	Pb	0.05(±0.20)	-	2.54×10^{-10}
	Zn	0.43(±1.51)	1.95×10^{-6}	-
	Cu	0.11(±0.24)	3.92×10^{-6}	-
With Nut Waste GE	Pb	0.05(±0.53)	-	2.47×10^{-10}
	Zn	0.52(±2.68)	2.39×10^{-6}	-
	Cu	0.06(±0.16)	2.09×10^{-6}	-
With Black Bean Waste GE	Pb	0.04(±0.16)	-	1.75×10^{-10}
	Zn	1.86(±14.30)	8.48×10^{-6}	-
	Cu	0.05(±0.30)	1.80×10^{-6}	-
Control	Pb	0.04(±0.22)	-	2.19×10^{-10}
	Zn	0.29(±0.41)	1.33×10^{-6}	-
	Cu	0.09(±0.22)	2.99×10^{-6}	-

Table 4.10.2 shows non carcinogenic and carcinogenic health risk on ingestion of heavy metals for children. For ingestion of zinc and copper, the Hazard Quotient (HQ) for all samples were less than 1, indicating the non-carcinogenic health risk is not significant to human via ingestion of Zinc and Copper from the sludge samples. For ingestion of lead, the Lifetime Cancer Risk (LCR) for all samples were less than 1×10^{-6} and considered as clearly acceptable.

Table 4.10.2: Non carcinogenic and carcinogenic health risk on ingestion of heavy metals for children

Sludge Sample	Heavy Metal (mg/kg)		HQ	LCR
With Citrus Peels GE	Pb	0.04(±0.16)	-	3.47×10^{-10}
	Zn	0.21(±1.74)	9.06×10^{-6}	-
	Cu	0.08(±0.50)	2.46×10^{-5}	-
With Banana Peels GE	Pb	0.05(±0.26)	-	4.93×10^{-10}
	Zn	0.45(±1.68)	1.92×10^{-5}	-
	Cu	0.08(±0.42)	2.40×10^{-5}	-
With Carrot Waste GE	Pb	0.05(±0.05)	-	4.66×10^{-10}
	Zn	0.25(±1.49)	1.05×10^{-5}	-
	Cu	0.08(±0.35)	2.52×10^{-5}	-
With Potato Waste GE	Pb	0.05(±0.20)	-	4.87×10^{-10}
	Zn	0.43(±1.51)	1.82×10^{-5}	-
	Cu	0.11(±0.24)	3.66×10^{-5}	-
With Nut Waste GE	Pb	0.05(±0.53)	-	4.73×10^{-10}
	Zn	0.52(±2.68)	2.23×10^{-5}	-
	Cu	0.06(±0.16)	1.95×10^{-5}	-
With Black Bean Waste GE	Pb	0.04(±0.16)	-	3.35×10^{-10}
	Zn	1.86(±14.30)	7.91×10^{-5}	-
	Cu	0.05(±0.30)	1.68×10^{-5}	-
Control	Pb	0.04(±0.22)	-	4.19×10^{-10}
	Zn	0.29(±0.41)	1.24×10^{-5}	-
	Cu	0.09(±0.22)	2.79×10^{-5}	-

CHAPTER 5

DISCUSSION

5.1 Comparison of pH level and temperature of Garbage Enzyme (GE) in four weeks of fermentation.

During four week of fermentation, most GEs showed a decrease of pH level and all of them were in acidic condition (pH less than 7). Organic acids generated from decomposition of organic matters by microorganism caused the decrease of pH (He et al., 2012). Food waste contains high levels of organic compounds, nutrients, carbohydrates, fat and protein. The carbohydrate will be degraded into smaller compounds like sucrose, fructose, lactose and these simpler sugars can be easily taken up by indigenous acid-producing bacteria (Zhang et al., 2008). Anaerobic digestion consists of a few stages including hydrolysis, acidogenesis, acetogenesis and methagonesis. Acidogenesis is involved in the breakdown of the simple sugars, fatty acids and amino acids by acidogenic bacteria into volatile fatty acids and produces ammonia, carbon dioxide and hydrogen sulfide as by-products (Stabnikova et al., 2006). The acidic conditions may be occurred due to formation of organic acids in anaerobic condition or accumulation of organic acids produced from abundance of carbonaceous substrates (U.S. Department of Agriculture, 2000). The citrus peels GE showed the lowest range of pH. This may be due to the predominant presence of citric acid and amino acid in the citrus peels which lower the pH level (Fazna & Meera, 2017). Overall, there were significant mean differences of pH level for all GEs from first week to fourth week of fermentation (p-value<0.001). [ANOVA test]

During four week of fermentation, most GEs showed both increase and decrease of temperature. Due to the exothermic nature of fermentation, temperature increases as sugars are metabolized (Centinari, 2015) resulting heat generation which is corresponding to sugar consumption rate and ethanol production rate during fermentation process (Kumar et al, 2013). However, the external temperature which is the room temperature of the laboratory where the fermentation took place might influence the increase and decrease of the temperature since the containers that were being used for fermentation were made up from plastics that are not heat resistant or equipped with good heat insulator. Since the air conditioning of the laboratory only operated between 8.00 a.m. to 5.00 p.m., the results of sludge temperature for treatment hours after 5 p.m. might be influenced by the room temperature without air conditioning which was more than 22 °C and this higher room temperature might cause the increase of temperature of the sludge. Thus, the room temperature was the confounding variable that must be controlled at all time during the treatment. Overall, there were significant mean differences of temperature values among GEs during 4 weeks of fermentation (p<0.001) except the fourth week. [ANOVA test]

5.2 Comparison of citric acid concentration of Garbage Enzymes (GE) after four weeks of fermentation.

From acid-base titration, this study found out that Citrus Peels GE had the highest concentration of citric acid. This can be explained by the presence of citrate which is the most important organic acid in citrus fruit (Lin et al., 2016) however the citric acid is also produced during fermentation process (Arun & Sivashanmugam, 2015). Organic matter in food waste was converted to hydrogen, soluble by-product (organic acids and alcohols) and biomass through biohydrogen fermentation. The conversion of food waste to organic acids and alcohols would enhance the reduction of waste volume (Kim et al., 2004). Overall, there was a significant mean difference of citric acid concentration of Garbage Enzymes [F (5) =150.73, p-value<0.001*]. (ANOVA test)

5.3 Comparison of pH level and temperature of sludge samples in 5 days (120 hours) of treatment.

From 12 hours to 24 hours, most sludge sample showed decreasing pH level. All of them were in acidic condition (pH<7). This condition was likely occurred due to mineralization of organic acid by acid forming bacteria (Fei-Baffoe et al, 2015) which lowered the pH level. Biological fermentation of food waste and sludge produce hydrogen accompanied by the formation of acidic metabolites (Nazlina et al., 2009).

However, the pH values started increasing after 24 hours of fermentation. This condition was likely occurred due to the process of breaking down of protein (Saad et al, 2013) and the transformation of organic acids to carbon dioxide caused by microbial activity (Fan et al. 2017). Intense proteolysis (protein degradation) and rapid metabolic degradation of organic acid must have released alkaline ammonia compound (Fei-Baffoe et al, 2015) which increased the pH level. In addition, Huang et al., 2004 also state that this condition was probably occurred due to high production of ammonia during ammonification and mineralization of organic nitrogen as a result of microbial activities. Mat Saad et al. (2013) also stated that the increasing level of pH is due to break down of protein. Adhikari, Barrington, Martinez and King (2009) also stated that the depletion of organic acids and the process of denitrification occurs lead to higher pH level while Fazna and Meera (2013) also claimed that the denitrification process by the microbes is the cause of increased pH level. In addition, this condition also indicates that the sludge samples were reaching stabilization of organic matter (Fan et al., 2017).

There was a significant mean difference of pH level among sludge samples in 120 hours of treatment ($p<0.001$). Comparing to permissible limits for industrial effluent discharge (DOE Malaysia, 1979), all samples did not comply with Standard A (6.0-9.0) and Standard B (5.5-9.0) but sludge samples with Nut and Black Bean GE comply with Standard B.

During 5 days (120 hours) of treatment, most sludge samples showed both increase and decrease of temperature. A study concluded that heat generation during anaerobic digestion of sludge was correlated with the Carbon: Nitrogen ratio of sludge under identical aeration conditions (Solowiej, 2017). Temperature signifies the metabolism of microorganisms during decomposition process and it affects the activity of microorganism and the process of decomposition of the sludge (Zhu, 2006). Besides, Fan et al. (2017) also stated that the heat also produced from the decomposition of the food wastes.

However, the external temperature which is the room temperature of the laboratory where the fermentation took place might influence the increase and decrease of the temperature since the containers that were being used for fermentation were made up from plastics that are not heat resistant or equipped with good heat insulator. Since the air conditioning of the laboratory only operated between 8.00 a.m. to 5.00 p.m., the results of sludge temperature for treatment hours after 5 p.m. might be influenced by the room temperature without air conditioning which was more than 22 ^{o}C and this higher room temperature might cause the increase of temperature of the sludge. Thus, the room temperature was the confounding variable that must be controlled at all time during the treatment.

Overall, there were significant mean differences of temperature among sludge samples after 12 hours of treatment [F (6) = 42.83, p-value<0.001] and after 60 hours of treatment [F (6) = 7.66, p-value=0.01] but there was no significant difference of temperature of sludge samples in other intervals.

Comparing to permissible limits for industrial effluent discharge (DOE Malaysia, 1979), all samples did not exceed acceptable limit of 40 °C for temperature in Standard A and B.

5.4 Comparison of heavy metals (Lead, Zinc and Copper) of sludge samples after 5 days (120 hours) of treatment

There were significant mean differences of lead concentration among sludge samples [F (6) =2080.62, p-value<0.001]. [ANOVA test]. Banana peels GE showed lowest lead concentrations (35.03 ppb). Banana peel is a cheap and effective adsorbent for the adsorption of Pb (II) ions from aqueous solution (Kumari, 2017). Comparing to permissible limits for industrial effluent discharge (DOE Malaysia, 1979), all samples did not exceed acceptable limit for lead concentration in Standard A (100 ppb) and Standard B (500 ppb).

There were significant mean differences of zinc concentrations among sludge samples [F (6) =31516.62, p-value<0.001]. [ANOVA test]. Citrus peels GE showed lowest zinc concentration (212.60 ppb). Citrus Peels GE had highest concentration of citric acid by which it can effectively remove zinc from sludge (Song et al., 2015). Comparing to permissible limits for industrial effluent discharge (DOE Malaysia, 1979), all samples did not exceed acceptable limit of 1000 ppb for zinc concentration in Standard A and B.

There were significant mean differences of copper concentration among sludge samples [F (6) =10667.30, p-value<0.001]. [ANOVA test]. Banana peels GE showed lowest copper concentrations. Banana peel is a high capacitate, economically viable and low cost adsorbent for copper removal, and the favorable alternative of copper removal from water as 1g of banana peel can adsorb 28 mg of copper in a favorable condition (Hossain et al., 2012). Comparing to permissible limits for industrial effluent discharge (DOE Malaysia, 1979), all samples did not exceed acceptable limit in Standard A (200 ppb) and Standard B (1000 ppb).

5.5 Correlation between pH level and temperature of GE.

There was a fair negative correlation (r = -0.358) between temperature and pH of GE indicating that pH of GE was inversely proportional to temperature of GE.

Temperature has great impact on hydrolysis and acidification of food waste; as the temperature increased, the hydrolysis rate increased which produce organic acids that lower the pH value (He et al., 2012).

Reasonable temperature and pH are essential for a successful and productive anaerobic digestion process of food waste (Leung & Wang, 2016).

5.6 Correlation between pH level and temperature of sludge samples.

There was no significant correlation between temperature and pH level of sludge samples. Both physicochemical properties might not influence each other. The pH level is influenced by the acidic by-products from fermentation by microorganisms which decrease the pH values and also influenced by the depletion of the organic acids.

During metabolism of those microbes during fermentation, the heat is generated. However, the temperature measured during treatment might be influenced by external temperature which is the room temperature instead of the temperature influenced by the heat production of the microbes. This might be the reason why there is no correlation between pH level and temperature of the sludge samples.

5.7 Correlation between heavy metals and citric acid concentration of GE.

There was a good negative correlation (r = -0.73) between lead concentration in sludge samples and citric acid concentration of GE, indicating that citric acid concentration of GE was inversely proportional to the lead concentration of the sludge samples.

However, there was no significant correlation between concentration of zinc and copper of sludge with citric acid concentration of GE. A study also stated that citric acid was more effective in removing heavy metals from sludge, with the removal rate of 52.0% for Pb (Huang, Zhou & Zhang, 2008). Another study reported that citric acid seemed to be highly effective (100%) in extracting Pb at pH 3 at 1-day leaching time (Dacera & Babel, 2006).

5.8 Non-carcinogenic and carcinogenic health risk on ingestion of heavy metals (Lead, Zinc and Copper) from sludge.

Lead, zinc and copper in treated sludge samples showed no significant health risk to both adults and children as HQ is lower than 1 for non-carcinogenic health risk and the lifetime cancer risk was within acceptable risk. A study by Kendir, Kentel and Sanin (2014) in Turkey reported that the health hazards originating from only the "child ingesting sludge" pathway due to heavy metals were low and not likely to result in adverse non-cancer health effects. In contrast, a study in Taiyuan, China showed comparison of the non-carcinogenic risk and carcinogenic risk for adults and children and indicated that children were more sensitive and vulnerable than adults when exposed to the same pollutant in the environment (Duan et al., 2017).

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Garbage Enzyme has potential in removing heavy metal from water treatment sludge as the treated sludge sample showed low concentration of heavy metals. Banana Peels GE showed lowest concentration of Lead and Copper but the highest concentration of Zinc. Otherwise, Citrus Peels GE showed lowest concentration of Zinc. Despite that, all of them still showed significant health risk to adults and children for ingestion of lead, zinc and copper from the treated sludge. Comparing to permissible limits for industrial effluent discharge (DOE Malaysia, 1979), all samples did not comply with Standard A (6.0-9.0) and Standard B (5.5-9.0) but sludge samples with Nut and Black Bean GE comply with Standard B. Meanwhile, all samples did not exceed acceptable limit of 40 °C for temperature in Standard A and B.

6.2 Limitation

Time constraint was main limitation of this study. Most literatures suggested that 3 months of fermentation to produce better yield of GE but this study only involved 4 weeks (one month) of fermentation to produce GE. The high cost for sample analysis and unavailability of instruments to measure certain parameters were also some of the limitations in this study. Besides, field blank and recovery data (spikes) could not be analysed to enhance the quality of the findings due to those constraints.

6.3 Recommendation

For future study, the researcher should add more physiochemical parameter for sludge treatment such as other heavy metals (e.g. cadmium, arsenic, mercury, etc.), total suspended solids (TSS), biochemical oxygen demand (BOD) and chemical oxygen demand (COD). In addition, nitrogen, phosphorus and potassium (NPK) content of treated sludge can be measured to assess its potential as fertilizer. Heavy metal concentration should be measured before treatment to compare with the concentration after treatment to provide percentage of removal of heavy metals by GE and to select the best GE for heavy metal removal. In addition, the dry weight before and after treatment must be measured to provide data on moisture content that may influence the sludge properties. Future study is also suggested to add more characterization of components in GE that involved in heavy metal removal (e.g. pectin, flavonoid, enzymes and other organic acids). The risk calculation can be calculated by using primary data (e.g. through survey) instead of secondary data, in order to have more accurate information on exposure of certain population.

7.0 REFERENCES

Acosta, J.A.; Cano, A.F.; Arocena, J.M.; Debela, F.; Martínez-Martínez, S. Distribution of metals in soil particle size fractions and its implication to risk assessment of playground in Murcia City (Spain). Geoderma **2009**, 149, 101–109.

Adhikari, B. K., Barrington, S., Martinez, J., & King, S. (2009). Effectiveness of three bulking agents for food waste composting. *Waste Management*, 29(1), 197-203. https://doi.org/10.1016/J.WASMAN.2008.04.001

U.S. Department of Agriculture. (2000). Composting. *Environmental Engineering: National Engineering Handbook*. https://doi.org/210-VI-NEH

Ahmad, Tarique & Ahmad, Kafeel & Alam, Mehtab. (2016). Sustainable management of water treatment sludge through 3'R' concept. *Journal of Cleaner Production*. 124. 10.1016/j.jclepro.2016.02.073.

American Public Health Association. (1999). *Standard methods for the examination of water and waste water*. American Public Health Association: Washington, DC.

Armi, M., Samah, A., Manaf, L. A., Ahsan, A., Nor, W., Sulaiman, A., Lawrence D'silva, J. (2013). Household Solid Waste

Composition in Balakong City, Malaysia: Trend and Management. *Pol. J. Environ. Stud.*, 22(6), 1807-1816.

Arun, C. & Sivashanmugam, P. (2015). Solubilization of waste activated sludge using a garbage enzyme produced from different pre-consumer organic waste. *RSC Advance*, 5 (3). DOI: 10.1039/C5RA07959D.

Arun, C & Sivashanmugam, Palani. (2014). Investigation of biocatalytic potential of garbage enzyme and its influence on stabilization of industrial waste activated sludge. *Process Safety and Environmental Protection*. 94. 10.1016/j.psep.2014.10.008.

Azimi, A., Azari, A., Rezakazemi, M. and Ansarpour, M. (2017), Removal of Heavy Metals from Industrial Wastewaters: A Review. ChemBioEng Reviews, 4: 37-59. doi:10.1002/cben.201600010w22

Babel, S. & Del Mundo Dacera, D. (2006). Heavy Metal Removal from Contaminated Sludge for Land Application: A Review. *Waste management* (New York, N.Y.). 26. 988-1004. 10.1016/j.wasman.2005.09.017.

Bo-Feng, C., Jian-Guo, L., Qing-Xian, G., Xiao-Qin, N., Dong, C., Lan-Chui, L., Zhang-Sheng, Z. (2014). *Estimation of Methane Emissions from Municipal Solid Waste Landfills in China Based on Point Emission Sources*, 5(2), pp. 81- 91.

Bohli T., Ouederni A., Fiol N., and Villaescusa I., "Single and binary adsorption of some heavy metal ions from aqueous solutions by activated carbon derived from olive stones," *Desalination and Water Treatment*, 2013.

California Environmental Protection Agency. (2005). *Human-Exposure-Based Screening Numbers Developed to Aid Estimation of Cleanup Costs for Contaminated Soil.* Retrieved from: https://oehha.ca.gov/media/downloads/crnr/screenreport010405.pdf

California Environmental Protection Agency. (2011). *Appendix A: Hot Spots Unit Risk and Cancer Potency Values*. Retrieved from: https://oehha.ca.gov/media/downloads/crnr/appendixa.pdf

Chalvatzaki, E & Lazaridis, Mihalis. (2010). Estimation of greenhouse gas emissions from landfills: Application to the Akrotiri landfill site (Chania, Greece). *Global NEST Journal*. 12. 108- 116.

Chatterjee, Sandipan & Chatterjee, Sudipta & Chatterjee, Bishnu & K. Guha, Arun. (2004). Clarification of fruit juice with chitosan. *Process Biochemistry*. 39. 2229-2232. 10.1016/j.procbio.2003.11.024.

Chen, Z., Afzal, M., T., & Salema, A. (2014). Microwave Drying of Wastewater Sewage Sludge. Journal of Clean Energy Technologies. 2. 282-286. 10.7763/JOCET. 2014.V2.140.

DOE Malaysia. (1979). *Environmental Quality Act 1974, Environmental Quality (Sewage and Industrial Effluents): Selected parameter limits of effluent of Standards A and B.*

Department of Environment. (2017). Chapter 5: Pollution Sources Inventory in Publications Section Strategic Communications Division Department of Environment Malaysia (Ed.), *Environmental Quality Report 2017* (pp. 134, 135, 144). Putrajaya, Federal Territory of Putrajaya: DOE.

Department of Environmental Affairs. (2010). *The Framework for the Management of Contaminated Land, South Africa.* Retrieved from: http://sawic.environment.gov.za/documents/562.pdf (accessed on 24 December 2018).

Duan, Baoling & Zhang, Wuping & Zheng, Haixia & Wu, Chunyan & Zhang, Qiang & Bu, Yushan. (2017). Comparison of Health Risk Assessments of Heavy Metals and as in Sewage Sludge from Wastewater Treatment Plants (WWTPs) for Adults and Children in the Urban District of Taiyuan, China. *International Journal of Environmental Research and Public Health*. 14. 1194. 10.3390/ijerph14101194.

Edwards, J., Othman, M., Crossin, E., Burn, S. (2017). Anaerobic co-digestion of municipal food waste and sewage sludge: a comparative life cycle assessment in the context of a waste service provision. *Bioresour. Technol.*, 223, pp. 237-249.

EPA (1998). Inductively Coupled Plasma-Mass Spectrometry Method 6020A. Retrieved from: http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/6020a.pdf (accessed on 30 December 2018).

Fan, Y., Lee, C.T., Klemes, J.J., Chua, L.S., Sarmidi, M. R., & Leow, C.W. (2017). Evaluation of Effective Microorganisms on Home Scale Organic Waste Composting. *Journal of Environmental Management.* https://doi.org/10.1016/j.jenvman.2017.04019

Fauziah, Shahul Hamid & Periathamby, Agamuthu. (2012). Trends in sustainable landfilling in Malaysia, a developing country. Waste management & research: *The Journal of the International Solid Wastes and Public Cleansing Association*, ISWA. 30. 656-63. 10.1177/0734242X12437564.

Fazna, N., Meera, V. (2013). Treatment of Synthetic Greywater Using 5% and 10% Garbage Enzyme Solution. *Bonfring International Journal of Industrial Engineering and Management Science*, 3(4), 111-117. DOI: 10.9756/BIJIEMS.4733.

Fernandez MT, Mira ML, Florêncio MH, Jennings KR (2002) Iron and copper chelation by flavonoids: An electrospray mass spectrometry study. J Inorg Biochem 92: 105-111.

Fewtrell, L., Kay, L., Jones, F., Baker, A. & Mowat, A. (1996). Copper in drinking water — an investigation into possible health effects. *Public Health,* 110, 175- 177.

Flora, S. J., & Pachauri, V. (2010). Chelation in metal intoxication. *International journal of environmental research and public health*, *7*(7): 2745-88. Ghafar., S. (2017). Food waste in Malaysia: Trends, current practices and key challenges, 1-10. Retrieved from http://ap.ffc.agnet.org/ap_db.php?id=774&print=1

Global Environment Centre (2017). *Solid Waste in Malaysia*. Retrieved from http://www.gecnet.info/index.cfm?&menuid=83

Graham-Jones. (2014). Evaluating Reuse of Sludge from Water Treatment for Wastewater Treatment. Retrieved from: https://va-tekniksodra.se/wp-content/uploads/2013/12/Evaluating-reuse-of- sludge-from-water-treatment-for-wastewater-treatment-%E2%80%93-an- assessment-of-effects-of-spent-alum-sludge-on-wastewater-treatment-in- Gothenburg-Sweden.pdf

Ho, Y., M., & Ling, L., K., and Abd Manaf, L. (2013) *Garbage enzyme as a solution to waste minimization.* In: International Conference on Environmental Forensics 2013, 11-14 Nov. 2013, Marriot Hotel, Putrajaya, Malaysia. (pp. 347-350).

Hseu, Z.Y. (2004). Evaluating heavy metal contents in nine composts using four digestion methods. *Bioresour. Technol.*, *95*, 53–59.

Idrus, M. (2008). An overview of landfill management and technologies: A Malaysian Case Study at Ampar Tenang 1. Journal of Environment, Development and Sustainability, pp. 157-165.

International Agency for Research on Cancer (2004). IARC monographs on the evaluation of carcinogenic risks to humans. *Inorganic and Organic Lead Compounds*.

Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B. B., & Beeregowda, K. N. (2014). Toxicity, mechanism and health effects of some heavy metals. *Interdisciplinary toxicology*, *7*(2), 60-72.

Jereme, I. A., Siwar, C., Begum, R. A., & Talib, B.A. (2016). Addressing the Problems of Food Waste Generation in Malaysia. *International Journal of Advanced and Applied Sciences,* 3(8), pp. 68-77. https://doi.org/10.21833/ijaas.2016.08.012

Kadir, A.A., & Abidin, S.Z. (2016). Solid Waste Composition and Quantification at Taman Melewar, Parit Raja, Batu Pahat. *IOP Conference Series: Materials Science and Engineering*, 136 (1), pp. 12-47: IOP Publishing.

Kartel MT, Kupchik LA, Veisov BK (1999) Evaluation of pectin binding of heavy metal ions in aqueous solutions. *Chemosphere*, 38: 2591-2596.

Kendir, E., Kentel, E., & F. Sanin, D. (2014): Evaluation of Heavy Metals and Associated Health Hazards in a Metropolitan Wastewater Treatment Plant's Sludge for Its Land Application, Human and Ecological Risk Assessment: An International Journal, DOI: 10.1080/10807039.2014.966590. Retrieved from: http://dx.doi.org/10.1080/10807039.2014.966590

Khalil, N., Morrow, L. A., Needleman, H., Talbott, E. O., Wilson, J. W., & Cauley, J. A. (2009). Association of cumulative lead and neurocognitive function in an occupational cohort. *Neuropsychology, 23*(1), 10-19.

Levin SM, Goldberg M. (2000). Clinical evaluation and management of lead-exposed construction workers. *Am J Ind Med.*, 37(1): 23– 43.

Mahmood, Z. B. (2009). *Solid Waste Management by Considering Composting Potential in Malaysia Towards a Green Country*, 4(1), pp. 48-55.

Muhammad, S.; Shah, M.T.; Khan, S. Health risk assessment of heavy metals and their source apportionment in drinking water of Kohistan region, Northern Pakistan. *Microchem*. J. 2011, 98, 334–343

Meenakshi A. K. (2015). Removal of Heavy Metal from Landfill Leachate Using Vertical Flow Construction Wetland. *IOSR Journal of Mechanical and Civil Engineering*, pp. 46-51.

Navas-Acien, A., Guallar, E., Silbergeld, E. K., & Rothenberg, S. J. (2006). Lead exposure and cardiovascular disease--a systematic review. *Environmental health perspectives*, *115*(3), 472-82.

National Solid Waste Management Department (2013). *Development of National Strategic Plan for Food Waste Management in Malaysia.*

Naveen, B.P. & Madhab Mahapatra, Durga & Thallak, Sitharam & Puvvadi, Sivapullaiah & Ramachandra, T V. (2016). Physico- chemical and biological characterization of urban municipal landfill leachate. *Environmental Pollution*. 220.10.1016/j.envpol.2016.09.002.

New Straits Time (2018). *'Amount of food wasted by Malaysians enough to feed 12 million people a day.* Retrieved from https://www.nst.com.my/news/nation/2018/12/441882/amount-food-wasted- malaysians-enough-feed-12-million-people-day

Pamukoglu, M., Y., & Kargi, F. (2006). Batch Kinetics and Isotherms for Biosorption of Copper(II) Ions onto Pre-treated Powdered Waste Sludge (PWS), J. Hazard. Mater. 138, 479 – 484.

Plum, L. M., Rink, L., & Haase, H. (2010). The essential toxin: impact of zinc on human health. *International journal of environmental research and public health*, 7(4), 1342- 65.

Rehman, Kanwal & Fatima, Fiza & Waheed, Iqra & Akash, Muhammad Sajid Hamid. (2017). Prevalence of Exposure of Heavy Metals and Their Impact on Health Consequences. *Journal of cellular biochemistry*. 119. 10.1002/jcb.26234.

Sawalha et al., Thermodynamic and Isotherm Studies of the Biosorption of Cu(II), Pb(II), and Zn(II) by Leaves of Saltbush (Atriplex canescens), J. Chem. Thermodyn. 2007, 39, 488 – 492.

Schwartz BS, Stewart WF, Bolla KI, Simon PD, Bandeen-Roche K, Gordon PB, et al. (2000). Past adult lead exposure is associated with longitudinal decline in cognitive function. *Neurology*, 55(8):1144–1150.

Shamuyarira, K. & Gumbo, J. (2014). Assessment of Heavy Metals in Municipal Sewage Sludge: A Case Study of Limpopo Province, South Africa. International journal of environmental research and public health. 11. 2569-79. 10.3390/ijerph110302569.

Song, S.W. & Wang, L & Wang, X.X. & Qi, G.C. & Yu, L & Liu, J. (2015). Speciation Analysis and Removal of Heavy Metals Zn, Cu, Cd from Sludge by Organic Acid. 10.2991/icadme-15.2015.158.

Tang, F. E. and Tong C. W. (2011). A Study of the Garbage Enzyme's Effects in Domestic Wastewater World Academy of Science, *Engineering and Technology International Journal of Environmental and Ecological Engineering*, 5 (12), 887-892.

Teixeira, S., R., Santos, G., T., A., Souza, A., E., Alessio, P., Souza, S., A., & Souza, N., R., (2011). The effect of incorporation of a Brazilian water treatment plant sludge on the properties of ceramic materials. *Applied Clay Science.* 53. 561-565. 10.1016/j.clay.2011.05.004.

The Star Online. (2018). *Malaysians throwing away food at alarming rate.* Retrieved from: https://www.thestar.com.my/news/nation/2018/10/15/malaysians-throwing-away-food-at-alarming-rate/

Tweib, S. A., Rahman, R. A., & Khalil, M. S. (2011). Composting of solid waste from wet market of Bandar Baru Bangi Malaysia. *Australian Journal of Basic and Applied Sciences*, 5(5), 975-983. Retrieved from www.scopus.com

United States Environmental Protection Agency. (2011). *Exposure Factors Handbook: 2011 Edition*. Retrieved from:

http://ofmpub.epa.gov/eims/eimscomm.getfile?p_download_id=5229 96

USEPA (1989) Risk Assessment Guidance for Superfund. Human Health Evaluation Manual, (Part A); Office of Emergency and Remedial Response: Washington, DC, USA

USEPA (1994.) A Plain English Guide to the EPA Part 503 Biosolids Rule; U.S. Environmental Protection Agency Office of Wastewater Management: Washington, DC, USA.

USEPA. (1995). A Guide to the Biosolids Risk Assessments for the EPA Part 503 Rule; U.S. Environmental Protection Agency Office of Wastewater Management: Washington, DC, USA.

Verlicchi P., Masotti L. (2000). Reuse of Drinking Water Treatment Plants Sludges in Agriculture: Problems, Perspectives and Limitations (pp.19-25) Retrieved from: https://pdfs.semanticscholar.org/2295/ebca70c845b1d944dc7eed76bb79c009c4f8.pdf

Vij, A.G. (2009). Hemopoietic, Hemostatic and Mutagenic Effects of Lead and Possible Prevention by Zinc and Vitamin C. *Al Ameen Journal of Medical Sciences*.; 2:27–36.

Zainu, Z., & Songip, A. (2017). Policies, Challenges and Strategies for Municipal Waste Management in Malaysia. *Journal of Science,* *Technology and Innovation Policy, 3*(1). Retrieved from http://www.jostip.org/index.php/jostip/article/view/47/23

Zhu, N. (2006). Composting of high moisture content swine manure with corncob in a pilot-scale aerated static bin system. *Bioresource Technology*, 97(15), 1870-1875. https://doi.org/10.1016/j.biortech.2005.08.011