PERFORMANCE OF *Enterococcus faecalis* IN THE PRODUCTION OF METHANE USING SINGLE AND DOUBLE PHASE REACTORS

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UNIVERSITI SAINS MALAYSIA

2011

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By

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Thesis submitted in fulfillment of the requirements for the degree of Doctor of Philosophy (Environmental Engineering)

August 2011

ACKNOWLEDGEMENTS

In the name of ALLAH S.W.T all praise is due to Him, the only creator, who bestowed me the strength, knowledge and perseverance to bring this thesis to completion successfully.

I wish to express most sincere appreciation and deep gratitude to my kind supervisors, Associate Professor Dr Hj Ismail bin Abustan, for his motivation and enthusiasm during discussions; not forgeting to Dr Norli bt. Ismail and Dr Mohd. Suffian bin Yusoff, for their constant assistance, guidance and concern supervising towards my research and in writing up the thesis. Their willingness to share some sleepless night and valuable weekdays in reviewing my thesis will always be appreciated.

I would also like to thank all administrative and technical staffs of the School of Civil Engineering for their assistance during the period of research. The financial support provided by the Grant (1001/PAWAM/814021) Waste Management Cluster, Engineering Innovation and Technology Development Unit (EITD), Universiti Sains Malaysia is gratefully acknowledged. I am indebted to the Ministry of Higher Education and Universiti Malaysia Perlis (UniMAP) for offering me as their “Skim Latihan Akademik Bumiputera” (SLAB) fellowship to continue this postgraduate study.

I would also like to express my heartiest thanks and deepest gratitude to my beloved husband Mr. Abdul Rahim bin Abustan for his understanding, encouragement, prayers and patience that supported me through the whole course of this study. Not-forgetting to my father Zakarya bin Ahmad, my mother Haliza bt. Mohamad and to my father in law Abustan bin Othman, my mother in law Hamsiah bt Md.Isha, to my little heroes Ahmad Umar Naim and Ahmad Nizar Rayyan for their endless support and patient during my studies and also for both sides of my family. This thesis is earnestly dedicated to them.

Finally, sincere thanks to all my colleagues Encik Salleh, Cik Tengku Nuraiti, Cik Nurulilyana, Cik Nurikhwani Idayu, Cik Puganeshwary, Pn. Noor Ainee, Cik Siti Hidayah, Pn. Nurul Syakira, En. Ariff Nazry, Cik Sheena, Cik Haliza and Cik Azim at Postgraduate Student Room for their continuous support and their assistance in one way or another, towards the success of this undertaking.

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ASL	Anaerobic Solid-Liquid reactor
BOD	Biological Oxygen Demand
C/N	Carbon to Nitrogen Ratio
CH_4	Methane gas
CO_2	Carbon dioxide
COD	Chemical Oxygen Demand
FID	Flame Ionization Detector
GC	Gas Chromatography
H_2	Hydrogen
HMBs	Hydrogen scavenging bacteria
HRT	Hydraulic Retention Time
HS	High solids
LS	Low solids
MS	Medium solids
MSW	Municipal Solid Waste
OLR	Organic Loading Rate
POME	Palm Oil Mill Effluent
SEM	Scanning Electron Microscope
SLBR	Simulated landfill bioreactor
SP	Single Phase
SRT	Solid Retention Time
TKN	Total Kjeldhal Nitrogen

TOC	Total Organic Carbon
VFA	Volatile Fatty Acid
TAN	Total Ammonia Nitrogen

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KEUPAYAAN *Enterococcus faecalis* DALAM PENGHASILAN METANA MENGGUNAKAN REAKTOR SATU DAN DUA FASA

ABSTRAK

Penghasilan metana melalui proses pencernaan tanpa oksigen merupakan satu kaedah yang telah dikomersialkan dalam penglestarian tenaga yang boleh diperbaharui. Kajian terhadap keupayaan *Enterococcus faecalis* (*E. faecalis*) dalam penghasilan metana merupakan suatu kajian baru dalam mencernakan bersama sisa makanan dengan menggunakan reaktor satu dan dua fasa. *E. faecalis* dipencilkan daripada efluen kilang kelapa sawit (POME) yang diambil dari kolam takungan dari kilang kelapa sawit yang berdekatan. Sisa makanan diambil dari kafeteria universiti dan dibahagikan kepada tiga kumpulan iaitu kumpulan A (45% nasi, mi), kumpulan B (30% ikan, daging) dan kumpulan C (25% sayur-sayuran). Dua jenis reaktor pencernaan digunakan iaitu Simulated Landfill Bioreactor (SLBR) sebagai reaktor satu fasa dan reaktor Anaerobic Solid Liquid (ASL) sebagai reaktor dua fasa. Kajian yang dijalankan ini melibatkan beberapa objektif iaitu membuat perbandingan penghasilan metana di dalam reaktor satu dan dua fasa, membuat perbandingan terhadap parameter-parameter yang diuji di dalam SLBR dan reaktor ASL, memencilkan bakteria yang sesuai di dalam POME untuk meningkatkan penghasilan metana dan menilai keupayaan bakteria yang dipilih iaitu *E. faecalis* dalam menghasilkan metana menggunakan kedua-dua reaktor tersebut. Tiga kondisi suhu yang berbeza iaitu 35°C, 45°C dan 50°C ditetapkan pada kedua-dua reaktor dengan nisbah makanan kepada inokulum yang berbeza. Beberapa parameter digunakan untuk mengawasi penghasilan metana dan menganalisa sampel cecair di dalam reaktor melalui analisis kimia. Keputusan menunjukkan reaktor ASL adalah yang

terbaik berbanding dengan SLBR. Penghasilan metana yang tertinggi dicapai adalah 72% pada reaktor B-B1 ASL bagi suhu 50°C. Walaubagaimanapun, bagi SLBR, cuma 1.6% metana yang dapat dihasilkan. Sementara itu, keputusan bagi keupayaan *E.faecalis* dalam menghasilkan metana bagi suhu 50°C menunjukkan reaktor A-A1 ASL menghasilkan 21.7% metana berbanding B-B1 ASL cuma 11.8%. Walau bagaimanapun, bagi SLBR, *E. faecalis* hanya berupaya menghasilkan metana kurang dari 1%. Berdasarkan kepada keputusan yang ditunjukkan, *E.faecalis* berfungsi sebagai pemula di dalam fasa acetogenesis dalam proses fermentasi sebelum ia digunakan dalam proses seterusnya sebagai substrat dalam menghasilkan metana. Di samping itu, *E. faecalis* adalah bakteria tempatan yang dibantu oleh bakteria pembentuk-metana dalam meningkatkan metana.

PERFORMANCE OF *Enterococcus faecalis* IN THE PRODUCTION OF METHANE USING SINGLE AND DOUBLE PHASE REACTORS

ABSTRACT

Production of methane via anaerobic digestion process is the method that already commercialized in the sustainable of renewable energy. The study on performance of *Enterococcus faecalis* (*E. faecalis*) in the production of methane is new research in co-digestion with food waste using single and double phase reactors. *E. faecalis* isolated from palm oil mill effluent (POME) that taken from holding pond at the nearest palm oil mill. Food waste collected from the university cafeterias were sorted out in three different groups which are group A (45% of rice, noodle), group B (30% of meat, fish) and group C (25% of vegetables). Two type of anaerobic reactors is used which are Simulated Landfill Bioreactor (SLBR) as a single reactor and Anaerobic Solid Liquid (ASL) reactor as a double phase reactor. Studies conducted involves several objectives, namely to compare the methane production in single phase and double phase of anaerobic digester, to compare the parameters tested in two different reactors which are Simulated Landfill Bioreactor (SLBR) and Anaerobic Solid-Liquid reactor (ASL), to isolate the suitable bacteria from POME for methane production enhancement and to evaluate the performance of selected microbe which is *E. faecalis* in terms of methane production in both reactors. Three different temperature conditions which are 35°C, 45°C and 50°C were set up to the reactors with different ratio of food waste to inoculums. Several parameters were used to monitor the methane production and to analyze the liquid samples in the reactors by chemical analysis. Results show ASL reactor was the best reactor compare to SLBR. The highest production of methane achieved is 72% in reactor B-

B1 ASL for temperature 50°C. However, for SLBR, the methane production only achieved at 1.6%. Meanwhile, results for performance of *E. faecalis* in production of methane at temperature 50°C show that A-A1 ASL reactor achieved 21.7% of methane compare to B-B1 ASL reactor only at 11.8%. However, for SLBR, *E. faecalis* can only performs for methane production less than 1%. Based on the results show, *E. faecalis* play as the starter in the acetogenesis phase in the fermentation before further use as a substrate to produce methane. Moreover, *E. faecalis*, as the local bacteria could be supported by methane-forming bacteria to enhance the methane.

CHAPTER 1

INTRODUCTION

1.1 Introduction

Solid waste can be defined as wastes arising from human activities which are normally solid and unwanted. It can be classified into a variety of states such as physical (solid, liquid, gaseous), original use (packing waste), material (glass, paper, plastics), physical properties (combustible, compostable), origin (domestic, commercial, industrial, agricultural) and safety parameters (hazardous, radioactive) (Agamuthu, 2001; Franchetti, 2009; Tchobanoglous et al., 1993 and Ngoc and Schnitzer, 2009).

The generation of solid waste and its implications for people and the environment are global issues. The complexity of waste composition and the increase per capita of waste generation in every year is a challenge for waste management especially in developing country. The Malaysian government recorded that the total amount of solid waste generated in Peninsular Malaysia increased from 16,200 tonnes/day for year 2001 to 19,100 tonnes/day for year 2005. In an average of about 0.8 kg/capita/day and this amount is expected to reach 30,000 tonnes/day in 2020. Starting by 2006, solid waste generation in Malaysia has increased to 1.3 kg/capita/day and expected to reach 1.5kg/capita/day in most cities (Agamuthu et al., 2009).

Municipal Solid Waste (MSW) is basically household waste that includes commercial waste and institutional waste. It contains significant composition of organic material that can produce a variety of gaseous when dumped in landfills. MSW in Malaysia mostly involves the disposal of waste to landfills. However, this method of disposal causes pollution of groundwater and soil. Malaysian food waste are putrefies because of its high water content. This makes its transport and storage difficult and can cause a serious problem with the leachate produced when it is being dumped in the landfill (Idris et al., 2004). Approximately 46% of organic waste content are consisted of kitchen waste and food waste, followed by paper waste (14%) and plastic based waste (15%) (Fauziah et al., 2004 and Agamuthu et al., 2009).

Food waste can be categorized under Organic Fraction Municipal Solid Waste (OFMSW), by means of a specific waste and complexity. It can also be described as a complex substrate and requires more complex metabolic pathway to be degraded into a series of metabolic reactions before final conversion to methane gas (Mata-Alvarez, 2003). Normally food waste is treated by composting and most are dumped at landfill sites. The alternative way to reduce the usage of landfill sites and to control the groundwater and soil pollution is via anaerobic digestion process.

The anaerobic degradation of food waste needs the concerted action of varied microbial population, consisting of several groups of strict and facultative bacteria strains. The groups of bacteria that are involved in the process help to degrade the long-chain organic compounds (carbohydrates, protein and lipids) to the final products, methane gas and carbon dioxide (Sponza and Ağdağ, 2004 and Mata-Alvarez, 2003). The methane gas produced from the anaerobic process can be utilized as a renewable energy with useful application such as cooking gas, electricity and fuel.

Malaysia is gifted with suitable climatic and geographical factors for the cultivation of oil palm scientifically known as *Elaeis guineensis Jacq*. The palm oil industry is very important to Malaysia and it has contributed significantly to the country's Gross Domestic Product (GDP). The export earnings from palm oil, palm kernel oil, and its by-products in 1998 amounted to almost US$5.6 billion, equivalent to 5.6% of the country's GDP (Yusoff, 2006). Today, Malaysia is the world's largest producer and exporter of palm oil. In year 2008, crude palm oil production rose strongly by 12.1% to 17.7 million tonnes driven by favorable weather conditions and, in part, by strong increase in crude palm oil prices of 16.3% to an average of RM2,875 per tonne. Palm oil yields in Peninsular Malaysia recorded a total output of 10.1 million tonnes and increase of 17.4% (MPOA, 2010). However, despite the high economic returns, the generation of liquid waste or Palm Oil Mill Effluent (POME) is also huge. It was estimated that for every tonne of fresh fruit bunch processed, between 0.5 and 0.75 tonne of POME is produced (Yacob et al., 2006).

POME is generated from the combination of sterilization, clarification and hydrocyclone washing processes during palm oil processing (Hassan et al., 2004). More than 85% of the palm oil mills in Malaysia use the conventional pond systems for the treatment of POME due to its lower operating costs (Najafpour et al., 2006). In the future, anaerobic treatment of POME coupled with methane gas recovery will be the preferred choice for sustainable development of the palm oil industry.

Anaerobic treatment of POME by a closed anaerobic digestion system offers several advantages in comparison with other treatment technologies such as lower energy requirements with no aeration, producing methane gas production as a valuable end product and sludge generation from the process which can be used as fertilizer or for land application (Poh and Chong, 2009).

Many types of reactors have been developed to treat wastes in an efficient, economical and environmentally acceptable way. The technologies vary from wet process to dry one, single-phase to multi-phase, from batch to continuous and variety of feedstock. Single phase reactor is a one stage reactor where all the anaerobic process (hydrolysis, acidogenesis, acetogenesis and methanogenesis) occurs in the system. In double phase reactor, two reactor was used to separate the reaction process. The first reactor which is called acidogenic reactor occur the hydrolysis and acidification reactions. In the second reactor is called as methanogenic reactor occur the acetogenesis and methanogenesis reactions. Both reactors had their own advantages and disadvantages.

In this study, a single phase reactor namely as Simulated Landfill Bioreactor (SLBR) and double phase reactor, Anaerobic Solid-Liquid reactor (ASL) is used to compare the production of methane gas from food waste co-digestion with *Enterococcus faecalis* from POME.

1.2 Problem Statement

The solid waste management in Malaysia displays a problem such as, low collection coverage and irregular collection services, crude open dumping, burning without air and water pollution control, breeding of flies and vermin and the handling and control of informal waste picking and scavenging activities. These problems can be caused by various factors which have an impact on development of waste management system in Malaysia (Abd Manaf et al., 2009 and Lau, 2004).

Malaysian solid waste contains a very high concentration of organic waste (46%) and consequently high moisture content and bulk density above 200kg/m^3. A study done by Kathirvale et al. (2003), in waste characterizations found that the main components of Malaysian waste were food, paper and plastic which comprises 80% of the overall weight. These characteristics shows the rapid development and changing in lifestyle of Malaysian population, which also affect the nature, where food that are dumped in landfill site pollutes the groundwater, while plastic, paper and packaging materials that are complex to nature (Idris et al., 2004).

Food waste which is putrefies can causes the leachate problem when it dumps to the landfill site. By segregate food waste in the municipal solid waste stream for being use as the source energy should be less depending of dumping in the landfill site. Food waste also known as a source of high in carbon and hydrogen which has the potential to produce methane gas when co-digest with other material and can be a new source of fuel and electricity.

The common method to treat POME is by using open digestion tank systems, which have particular disadvantages such as a long hydraulic retention time of 45–60 days, bad odour, difficulty in maintaining the liquor distribution to ensure smooth performance over huge areas and difficulty in collecting biogas, with a mixture of about 65% methane, 35% carbon dioxide and true amount of hydrogen sulfide which can have harmful effects on the environment (Ma, 1999; Yacob et al., 2005; Borja and Banks, 1994).

POME which is contains high in bacteria consortium give the opportunity to study in further the potential bacteria involve in the production of methane gas and the ability of POME when co-digestion with food waste to produce the better quality of methane gas. Therefore, it is very important to conduct the research on its characteristic of the wastes (food waste and POME) and experimental analysis to help in minimizing the pollution and landfill usage.

In this study, it is required to investigate the bacteria involved in POME that enhancing the methane gas production while co-digesting with food waste. The characteristics of bacteria used also are lack of information in the literature on the use in anaerobic digestion process in the ability to enhance the production methane gas.

Even the single phase reactor appeared as the attractive system because of it similarity to the demonstrated technology in use for decades in anaerobic stabilization of biosolids produced in wastewater treatment plants, there are several disadvantages of the reactor where the high Organic Loading Rate (OLR) is inhibition of acetogenesis and methanogenesis in the system. Beside, it is particularly sensitive to shock loads as inhibitors spread immediately in the reactor (Vandevivere et al., 2002).

Single phase reactor and double phase reactor was study separately by other researcher which are focusing on their subject interest. In my research this was the opportunity to compare these two types of reactor in term of methane gas production by different temperature condition and other characteristics.

1.3 Hypothesis

The increasing of solid waste in every year leads to the increasing of landfill site either sanitary landfill or illegally dumps. Therefore, the usage of anaerobic digestion process should be the alternative treatment in solid waste management, besides the methane gas produced from the treatment, can be use as the source of renewable energy. From that matter, the two types of reactor which are single and double phase will be study for their performance. The double phase rector may give the better performance compare to single phase reactor.

The analysis of the liquid sample from the two reactors will be analyze using the parameter such as Volatile Fatty Acid (VFA), pH, Total Organic Carbon (TOC), Alkalinity, Total Kjeldhal Nitrogen (TKN) and calorific value. So that, the parameter tested results (VFA, pH, TOC, alkalinity, TKN and calorific value) are similar in both reactors.

Due to the variable of bacteria involve in POME, the isolation process was done and used as the selected microorganism in both rectors. Therefore, the selected microorganism that had been isolated from the POME produce more methane gas in both reactors.

1.4 Objectives of Research

The objectives of this research are:

1. To compare the methane gas production in single phase and two-phase of anaerobic digester.
2. To compare the parameters tested in two different reactors which are Simulated Landfill Bioreactor (SLBR) and Anaerobic Solid-Liquid reactor (ASL).
3. To isolate a suitable bacteria from POME for methane production enhancement.
4. To evaluate the performance of the selected microbe as the catalyst in the digestion process to enhance the methane production in both reactors.

1.5 Scope of Research

This research mainly focuses on the production of methane gas in the food waste which then should be compassed with other parameters such as VFA, pH, TOC, alkalinity, TKN and calorific value in the leachate sample. VFA and pH are the important compounds in metabolic pathway of methane fermentation and causes microbial stress if present in high concentration. Therefore, the monitoring of all the parameters mentioned is essential for the operation performance of an anaerobic digester. Furthermore, it is crucial to investigate the optimum conditions and efficiencies of digesters by examining those parameters.

1.6 Outline of Thesis

The arrangement of the thesis is as follow:

Chapter 1 introduces the general background of the research, the objectives, and the scope of this research and the outline of the thesis.

The literature review will be covered in Chapter 2. The chapter covers the definition, categories, composition and generation of municipal solid waste, the overview of anaerobic processes and the microorganisms involved in the process. This is followed by the parameters influence for the anaerobic digestion process and finally a general review of POME characteristics.

Chapter 3 will discuss the methodology of experiments that are being performed as well as the materials and apparatus used, the experimental setup of the SLBR and ASL reactor and the methodology flow chart.

Chapter 4 further outlines the results obtained as well as discussion of the results that are obtained from the experiments with regards to the objectives of the research. For both reactors SLBR and ASL reactor, the discussion will cover the co-digestion with and without inoculums.

Finally, the conclusions derived from the results of the experiments are described in Chapter 5. The achievements and findings of the research are concluded in this chapter. Recommendations for future research were also included.

CHAPTER 2

LITERATURE REVIEW

2.1. Introduction

For the purpose of this study, there are several scientific literatures that needed to be reviewed. These subjects include the definition, categories, composition and generation of municipal solid waste. Other important related subjects include concept and technology of anaerobic digestion, and phases involves in anaerobic digestion system. In addition, literatures related to parameter of anaerobic digestion, inhibition of the system and the microorganisms involved in the system are also reviewed. Besides, the general overview of POME also discussed.

2.2. Municipal Solid Waste

2.2.1 Definition of Municipal Solid Waste

Municipal Solid Waste (MSW) is generated by households, commercial activities and other sources which are similar to those of households and commercial enterprises for example, wastes from offices, hotels, supermarkets, shops, schools, institutional and from municipal services such as street cleaning and maintenance of recreational areas (Ngoc and Schnitzer, 2009; Chiemchaisri et al., 2010).

The major types of MSW are food waste, plastic, rags, metal and glass, with some hazardous household wastes such as electric light bulbs, batteries, discarded medicine and automotive parts. The composition of MSW typical of cities in Southeast Asian countries is presented in Table 2.1. It shows that the highly urbanized cities generated a high percentage of organic and mixed inorganic waste (55-70%), with about 15-28% from paper and cardboard, 10-16% made of plastic, 4-10% of glass and 4-12% of metal. Malaysia shows the highest organic waste (62%) compared to other countries, followed by 7% of paper and cardboard, 12% of plastic, 3% of glass and 6% of metal. According to Visvanathan et al. (2003) and Nguyen et al. (2007), the MSW stream in Asians cities is almost similar by composing of high fraction of biodegradable material of more than 50% with high moisture content and the increasing of generation rate with time.

Table 2.1 Composition of Municipal Solid Waste in Southeast Asian Countries

Country	***Waste composition (%)***					
	Organic waste	**Paper cardboard**	**Plastic**	**Glass**	**Metal**	**Others**
Brunei	44	22	12	4	5	13
Cambodia	55	3	10	8	7	17
Indonesia	62	6	10	9	8	4
Loas	46	6	10	8	12	21
Malaysia	**62**	**7**	**12**	**3**	**6**	**10**
Myanmar	54	8	16	7	8	7
Philippines	41	19	14	3	5	18
Singapore	44	28	12	4	5	7
Thailand	48	15	14	5	4	14
Vietnam	60	2	16	7	6	9

(Source: Ngoc and Schnitzer, 2009)

2.2.2 MSW Generation in Malaysia

The average amount of MSW generated in Malaysia is between 0.5-0.8 kg/person/day and has increased to 1.5 kg/person/day in the year 2007 in most cities. Figure 2.1 shows the trend of per capita generation of MSW in Malaysia from 1985 to 2007 (RMK 9, 2006; Fauziah et al., 2004; Agamuthu et al., 2009). The increasing trend shows the changes in consumption habits and also the increasing of the affordability of consumer goods.

The main components of the Malaysian MSW were found to be food, paper and plastic, which almost 80% of the waste by weight. Food/organic waste was highly contributed by the residential area (up to 60%) but with low contribution from the institutional area (only 25%).The average moisture content of the MSW was about 55% with calorific value between 1500 and 2600 kcal/g (Kathirvale et al., 2003). However, data reported by Department of National Solid Waste Management (Ministry Housing and Local Government, 2010), in 2005 reports that solid waste in Malaysia comprise of 45% of food waste, 24% of plastics, 7% of paper, 6% of metal, 3% of glass and 15% of others (Figure 2.2).

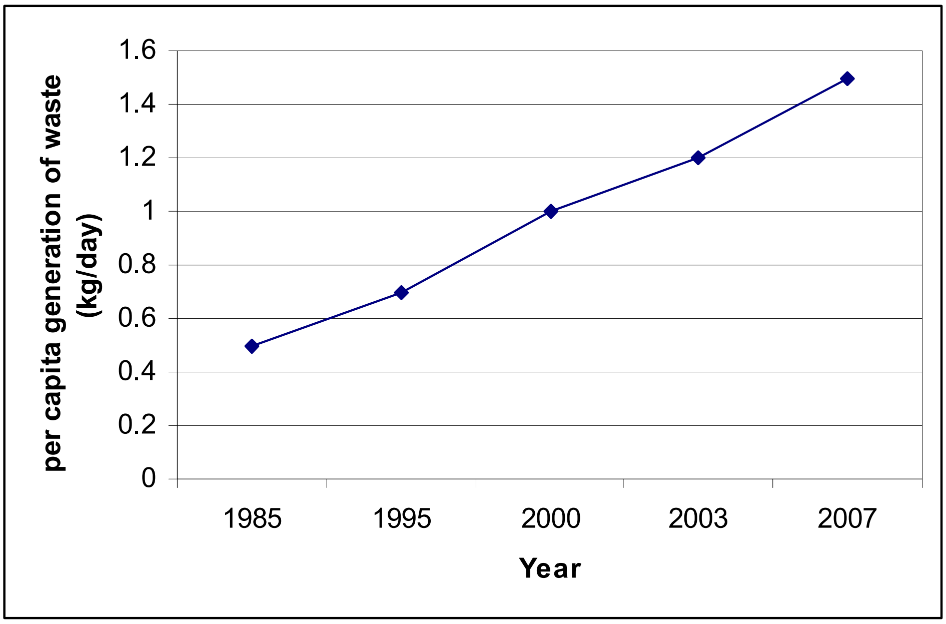

Figure 2.1 Increasing Trend in Per Capita Generation of Municipal Solid Waste in Malaysia from 1985 to 2007 (Source: Agamuthu et al., 2009)

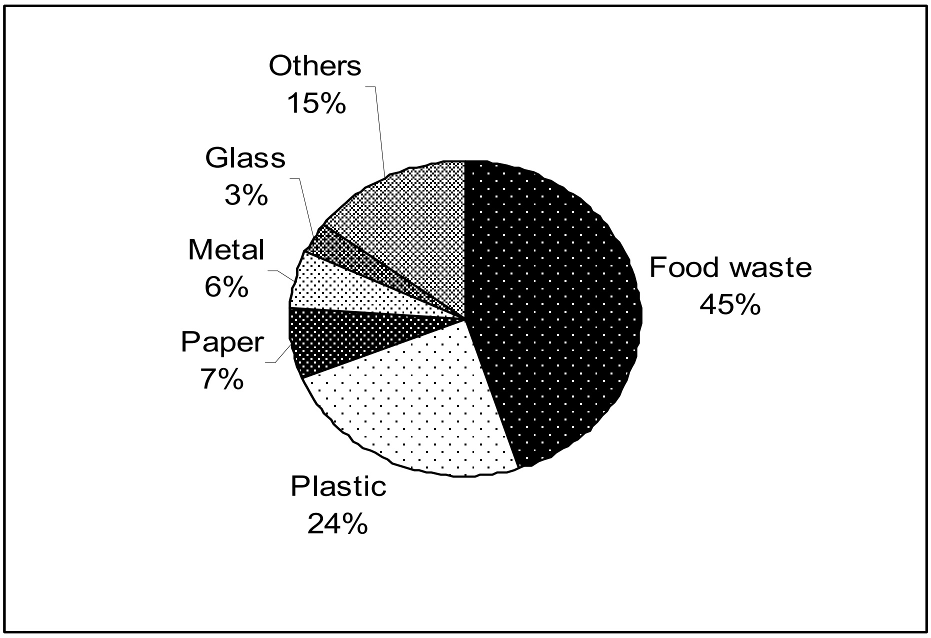

Figure 2.2 Solid Waste Generation in Malaysia 2005 (Source: Ministry Housing and Local Government, 2010)

2.2.3 MSW Generation in Pulau Pinang

Research done by Majlis Perbandaran Pulau Pinang (MPPP) that has been focusing on the state of Pulau Pinang on solid waste generation for the year 1992 was reported about 184,812 tonnes and increasing at 282,707 tonnes by 2006. However, a sudden decrease to 217193 tonnes has occurred in year 2007. It could be due to recycle rate of 4.53% per year (13475 tonnes of recycled waste) (MPPP, 2010). From the data available, we can calculate that the waste generated per capita daily is 0.9kg/capita/day (MPPP, 2010). Table 2.2 shows the daily average total weight waste disposed in Pulau Pinang.

Table 2.2 Daily Average Total Weight Waste Disposed in Pulau Pinang from 1992 to 2007

Year	Total Waste Disposed (Tonnes)	Average Daily (Tonnes)
1992	184,812	505
1993	205,973	564
1994	232,625	637
1995	192,016	526
1996	187,921	515
1997	184,192	505
1998	174,686	479
1999	177,691	486
2000	199,185	545
2001	199,878	547
2002	237,983	652
2003	252,271	691
2004	240,039	656
2005	272,844	749
2006	282,707	785
2007	217,193	603

(Source: MPPP, 2010)

While focusing on the area of Nibong Tebal town in Pulau Pinang, the report by Majlis Perbandaran Seberang Perai (MPSP) (2008) shows that waste disposed at landfill site Pulau Burung is 310000 tonnes for the year 2008 and an average of 93.21 tonnes/day was being disposed. This means, the amount of solid waste generated in the residential areas was found to be 0.6kg/capita per day. As the Pulau Pinang averaged of 0.9kg/capita/day, this figure is reasonable as Nibong Tebal is the small developing town and therefore the waste generation rate is expected to be lower. Figure 2.3 shows the percentage distribution of different waste components. Most of the waste consisted of food waste (52%), paper (16.5%), and plastics (15%). The remaining 16.5% comprised yard waste, textile, wood, glass and aluminium/tin cans (Isa et al., 2005).

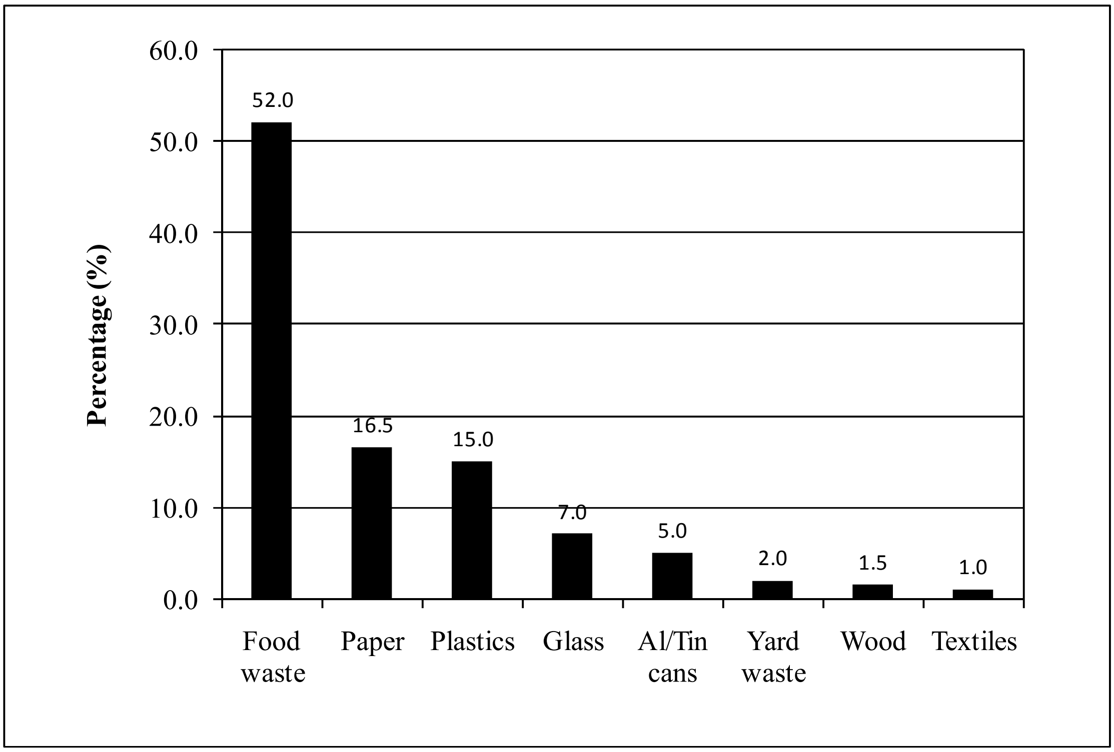

Figure 2.3 Solid Waste Composition of Nibong Tebal, Penang (Isa et al., 2005)

2.3 Anaerobic Digestion

2.3.1 Overview of Anaerobic Digestion

Scientific interest in the gasses produced by the natural decomposition of organic matter was first reported in the sixteenth century by Robert Boyle and Stephen Hale, who noted that flammable gas was released by disturbing the sediment of streams and lakes (Meynell, 1982). In 1808, Sir Humphry Davy determined that methane was present in the gasses produced by cattle manure. The first anaerobic digester was built by a leper colony in Bombay, India in 1859 (Meynell, 1982). In 1895 the technology was developed in Exeter, England, where a septic tank was used to generate gas for street lighting. Also in England, in 1904, the first dual purpose tank for both sedimentation and sludge treatment was installed in Hampton. In 1907, in Germany, a patent was issued for the Imhoff tank, an early form of digester. Through scientific research anaerobic digestion gained academic recognition in the 1930s. This research led to the discovery of anaerobic bacteria, the microorganisms that facilitate the process. Further research was carried out to investigate the conditions under which methanogenic bacteria were able to grow and reproduce. This work was developed in both Germany and Denmark where there was an increase in the application of anaerobic digestion for the treatment of manure (Neves et al., 2008).

During the last decades the anaerobic digestion has been considered as an alternative biotechnological process for degrading a variety of polluting organic wastes. It is one type of the biological treatment processes in the solid waste management. Anaerobic digestion is the natural process where bacteria convert the organic matter into the biogas. The process occurs in anaerobic conditions (absence of oxygen) through the acid- and methane-forming (methanogenic) bacteria that break down the organic material and produce methane (CH_4) and carbon dioxide (CO_2) and also a trace of other gaseous that form a biogas.

The organic material can be processed using this system. This includes biodegradable waste materials such as waste paper, leftover food, sewage, grass clipping and animal waste. Anaerobic digester can also be fed with the specially grown energy crops to boost the biodegradable content and increase the production of biogas. Anaerobic digesters have been used for a long time and are commonly used at municipal wastewater facilities, sewage treatment, to process industrial and agricultural waste and also for managing animal waste (Biomethane Report, 2003).

According to Zhang et al. (2007), many factors affect the design and performance of anaerobic digestion processes. These include feedstock characteristics, reactor design and operation conditions. The physical and chemical characteristics of the organic waste were also important for designing and ensuring a good performance of anaerobic digestion process towards the production of biogas. It includes moisture content, volatile solids content, nutrient content, particle size and biodegradability.

2.3.2 Phases of Anaerobic Digestion

There are two conventional operational temperature levels which are mesophilic and thermophilic. Mesophilic takes place optimally around 37-41°C and at ambient temperature of 20-45°C. While thermophilic takes place at the optimal temperature around 50-52°C and could get elevated to 70°C with thermophile bacteria.

Mata-Alvarez (2003) discussed that there are four phases of anaerobic digestion. The first stage is hydrolysis, where complex organic molecules are broken down into simple sugars, amino acids, and fatty acids with the addition of hydroxyl groups. The second phase is acidogenesis where a further breakdown into simpler molecules occurs, producing ammonia, carbon dioxide and hydrogen sulphide as byproducts. The third phase is acetogenesis where the simple molecules from acidogenesis are further digested to produce carbon dioxide, hydrogen and mainly acetic acid. In the second and third phases, decomposition is performed by fast-growing acid forming (acidogenic) bacteria. Protein, carbohydrate, cellulose and hemicellulose in the organic waste are hydrolyzed and metabolized into mainly short fatty acids- acetic, propionic and butyric along with CO_2 and hydrogen (H_2) gases.

The final phase is methanogenesis where methane, carbon dioxide and water are produced. At this phase, most of the organic acids and H_2 are metabolized by methane-forming bacteria. The methane-forming bacteria are slower growing and more sensitive to pH, air and temperature than the acidogenesis bacteria. Typically, the methanogenic bacteria require pH range between 6–7, adequate time (typically more than 15 days) and temperatures at 37–70 °C (depend on the temperature level) (Biomethane Report, 2003).

Digestion is not complete until the substrate has undergone all the phases describe above. Each of the phases has a physiologically unique bacteria population responsible that requires disparate environmental conditions. The full process can be described as the illustrated in Figure 2.4 with hydrolysis, where complex molecules are broken down to constituent monomers; acidogenesis which acids are formed; acetogenesis, where the production of acetate occurs and methanogenesis, the stage which methane is produced from either acetate or hydrogen (Ostrem, 2004).

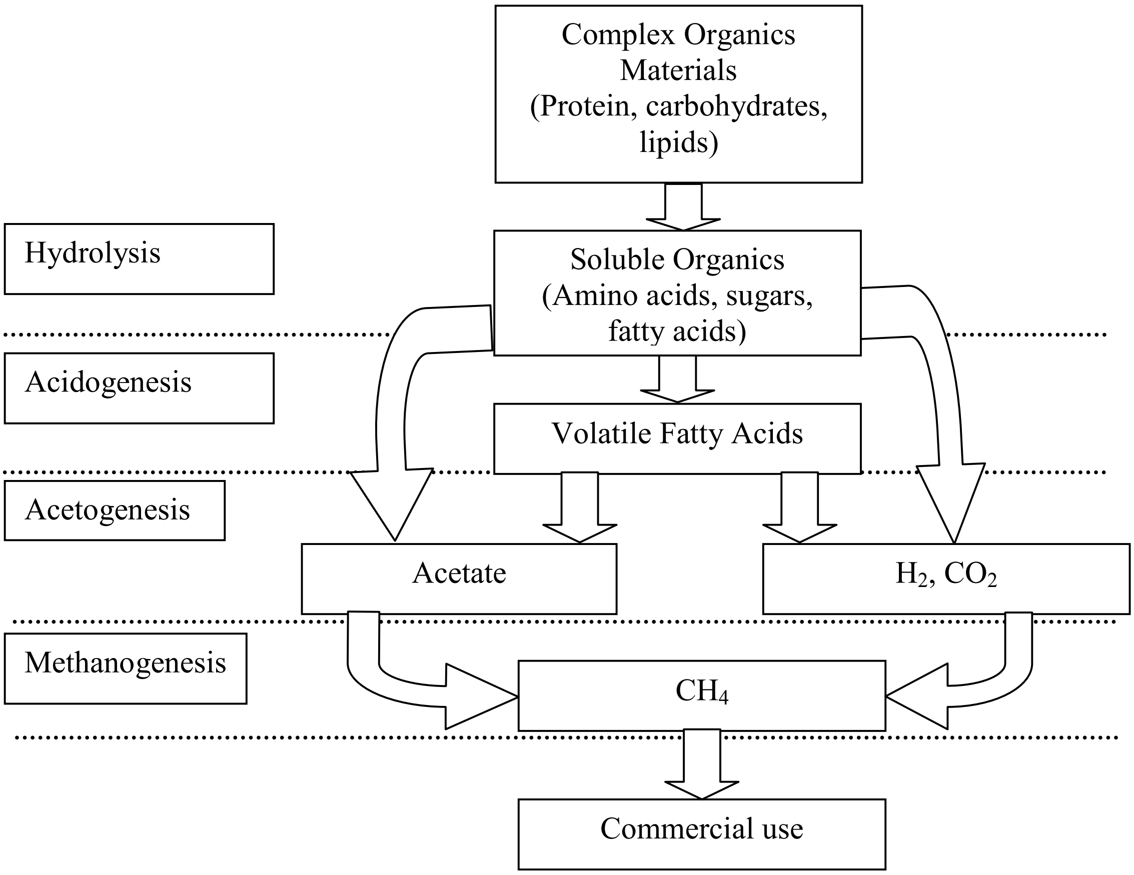

Figure 2.4 The Phases of Anaerobic Digestion (Ostrem, 2004).

2.3.2.1 Hydrolysis

In the hydrolysis stage, complex organic materials are broken down into constituents parts. The result is soluble monomers where proteins are converted to amino acids; fats to fatty acids, glycerol and triglycerides; complex carbohydrates such as polysaccharides, cellulose, lignin, starch and fiber converted to simple sugars likes glucose.

Hydrolytic or fermentative bacteria are responsible for the creation of monomers, which are then available to the next group of bacteria. Hydrolysis is catalyzed by enzymes excreted from bacteria such as cellulose, protease and lipase. If the feedstock is complex, hydrolytic phase is relatively slow. This is especially true for raw cellulolytic waste, which contain lignin (United Tech, 2003). Wood is therefore not an ideal feedstock for the anaerobic digestion process. On the other hand, carbohydrates are to be rapidly converted via hydrolysis to simple sugars and subsequently fermented to volatile fatty acids (Mata-Alvarez, 2003). A hydrolysis reaction where organic waste is broken down into a simple sugar which is glucose can be presented as:

$$C_6H_{10}O_4 + 2H_2O \rightarrow C_6H_{12}O_6 + 2H_2 \qquad (2.1)$$

In an anaerobic environment, lipids are the first hydrolyzed to glycerol and free long-chain fatty acids (LCFAs). The process is catalyzed by extracelullar lipases that are excreted by the acidogenic bacteria. Glycerol is converted to acetate by acidogenesis, while LCFAs are converted to acetate (or propionate in the case of odd-number carbon LCFAs) and hydrogen through the β-oxidation pathway (Cirne et al., 2007). From this study, it indicates that the addition of lipase enhances the hydrolysis of lipids and affects to a certain degree the concentration of the individual intermediate compounds which is the methane production rate.

2.3.2.2 Acidogenesis

Hydrolysis is immediately followed by the acid-forming phase of acidogenesis. In this process, acidogenic bacteria turn the products of hydrolysis into simple organic compounds, mostly short chain (volatile) acids such as propionic, formic, lactic, butyric or succinic acids; ketones such as ethanol, methanol, glycerol, acetone; and alcohols. The specific concentrations of products formed in this stage vary with the type of bacteria as well as in the culture conditions, such as temperature and pH (United Tech, 2003). In equation (2.2), glucose is converted to ethanol and equation (2.3) shows glucose is transformed to propionate.

$$C_6H_{12}O_6 \leftrightarrow 2CH_3CH_2OH + 2CO_2 \quad (2.2)$$

$$C_6H_{12}O_6 + 2H_2 \leftrightarrow 2CH_3CH_2COOH + 2H_2O \quad (2.3)$$

2.3.2.3 Acetogenesis

The next stage of acetogenesis is often considered with acidogenesis to be part of a single acid-forming stage. Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) are reduced through this stage. Acetogenesis occurs through carbohydrate fermentation, which acetate is the main product, and other metabolic processes. The result is the combination of acetate, CO_2 and H_2. The role of hydrogen as an intermediary is of critical importance to anaerobic digestion reactions. Long chain fatty acids, formed from the hydrolysis of lipids, are oxidized to acetate or propionate and hydrogen gas is formed. Under standard conditions, the presence of hydrogen in the solution inhibits the oxidation. The reaction only proceeds if the hydrogen partial pressure is low enough for thermodynamic to allow the conversion. The presence of hydrogen scavenging bacteria (HMBs) that consume hydrogen, thus lowering the partial pressure, is necessary to ensure thermodynamic feasibility and thus the conversion of all the acids. As a result the concentration of hydrogen, measured by partial pressure, is an indicator of the health of the digester (Mata-Alvarez, 2003).

When acetate and hydrogen are consumed by bacteria, the free energy becomes negative. In general, for reactions producing H_2, it is necessary for hydrogen to have a low partial pressure for the reaction to proceed.

$$CH_3CH_2COO^- + 3H_2O \leftrightarrow CH_3COO^- + H^+ + HCO_3^- + 3H_2 \quad (2.4)$$

Other important reactions in the acetogenesis stage involve the conversion of glucose (2.5), ethanol (2.6) and bicarbonate (2.7) to acetate.

$$C_6H_{12}O_6 + 2H_2O \leftrightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (2.5)$$

$$CH_3CH_2OH + 2H_2O \leftrightarrow CH_3COO^- + 2H_2 + H^+ \quad (2.6)$$

$$2HCO_3^- + 4H_2^+ + H^+ \leftrightarrow CH_3COO^- + 4H_2O \quad (2.7)$$

The transition of the substrate from organic material to organic acids in the acids forming stages causes the pH of the system to drop. This is beneficial for the acidogenic and acetogenic bacteria that prefer a slightly acidic environment, with a pH of 4.5 to 5.5, and are less sensitive to changes in the incoming feed stream, but is problematic for the bacteria involved in the next stage of methanogenesis (Veeken et al., 2000; Gas Technology, 2003)

2.3.2.4 Methanogenesis

The methanogenic anaerobic bacteria involved in the forth stage, known as methanogenesis or methane fermentation, is the same fastidious bacteria that occur in deep sediments or in the rumen of herbivores. This bacteria population converts the soluble matter into methane in about two thirds of which is derived from acetate conversion (equation (2.8) followed by (2.9)) , or the fermentation of an alcohol, such as methyl alcohol, equation (2.10) and one third is the result of carbon dioxide reduction by hydrogen, equation (2.11) (United Tech, 2003).

$$2CH_3CH_3OH + CO_2 \leftrightarrow 2CH_3COOH + CH_4 \qquad (2.8)$$

$$CH_3COOH \leftrightarrow CH_4 + CO_2 \qquad (2.9)$$

$$CH_3OH + H_2 \leftrightarrow CH_4 + H_2O \qquad (2.10)$$

$$CO_2 + 4H_2 \leftrightarrow CH_4 + 2H_2O \qquad (2.11)$$

Methanogens are very sensitive to changes and prefer a neutral to slightly alkaline environment. If the pH is allowed to fall below 6, methanogenic bacteria cannot survive. Methanogenesis is the rate-controlling portion of the process because methanogens have a much slower growth rate than acidogens. Therefore, the kinetics of the entire process can be describes by the kinetics of methanogenesis (United Tech, 2003 and Gas Technology, 2003).

2.3.3 By-products of Anaerobic Digestion

The end products of anaerobic digestion are biogas and digestate, a moist solid which is normally dewatered to produce a liquid stream and a drier solid. The components of biogas depend on the process of digestion, but predominately methane and carbon dioxide. The solids is a humus-like, stable, organic material, the quality and subsequent use of which determined by the characteristics of the feedstock to the anaerobic digestion process. The liquid contains soluble material, including dissolved organic compounds. In a typical anaerobic digestion facility processing organic fraction municipal solid waste, the gas mass comprises about 15% of the output stream and the liquid and solid compose approximately equal parts, or 42.5% each (Strategic Policy Unit, 2005 and Mahony et al., 2003).

There are three by-products from anaerobic digestion. First is biogas, a gaseous mixture comprising methane and carbon dioxide. It also contains a small amount of hydrogen and trace level of hydrogen sulfide. Biogas can be burned to produce electricity, usually to reciprocating engine or microturbine. It also used to generate the electricity and use waste heat to warm the digester or the buildings. Excess electricity can be sold to the electricity suppliers.

The second by-product is acidogenic digestate, which is a stable organic material comprising largely of lignin and chitin. A variety of mineral components in a matrix of dead bacterial cells and some plastic may also be present. This compost can be used as low grade building products such as fibreboard. The third by-product is a liquid which is methanogenic digestate, that is rich in nutrients and can be an excellent fertilizer