CHAPTER 1

INTRODUCTION

Human interaction with the Earth's systems has been a very helpful one for ages. Recently, this interaction has become more intense than ever. This intensified interaction is caused by the accelerated socio economic development involving population growth (Johari et al., 2012). Increase in population brought about changes in technology, trade, production and consumption patterns, and governance among others. As a result, our natural resources such as minerals and crude oil are being increasingly consumed. Urbanization has also led to wide-ranging land use practices across the world food security. Desertification, soil erosion and degradation, looming consumption and exhaustion of our fossil fuel reserves are contemporary and increasing problems. For example, fossil fuels produce approximately 90 % of the energy we need. Therefore, there is so much dependence on fossil fuel (Johari et al., 2012). Globally, there are about three major interrelated problems that seriously threaten our world and civilization; Climate change (due to accumulation of greenhouse gases in the atmosphere), waste disposal and the need for renewable and environment friendly sources for energy (IPCC, 2011).

1.1 Climate Change

From observations on global average air and ocean temperatures, widespread melting of snow and ice and rising of sea levels, it is evident that global warming causes change in climate. During the last 50 years, the surface temperature of the earth has been on the increase 0.10 – 0.16 °C per decade. Sea levels have also risen at an average of 1.8 mm.yr^{-1}(1961- 2003) to 3.1 (1993 – 2003). Arctic sea ice extent has shrunk by 2.7 % per decade with larger decreases in summer of 7.4 % (IPCC, 2007). An increase in the intense tropical cyclone activity in the North Atlantic since 1970, Pakistan floods (2010), China floods (2011) can be attributed to the Oceans taking up over 80 % of the heat being added to the climate system (IPCC, 2011, 2007).

Several studies have been conducted and results have shown that accumulation of greenhouse gases (GHG) in the atmosphere are likely caused by global warming and climate change (IPCC, 2011; Stern, 2006). Carbon dioxide ($CO_{2)}$, methane (CH_4), nitrous oxide (N_2O) and halocarbons (gases containing chlorine, fluorine or bromine) are the main GHGs (IPCC, 2007). The global atmospheric concentration of these gases have increased from a preindustrial (1750) values of ~ 280 ppm to 379 ppm, 715 ppb to 1732 ppb and 270 to 319 ppb in 2005 for CO_2, $CH_4$and N_2O respectively (Stern, 2006; IPCC, 2007).

The main contributor to climate change is $CO_{2.}$ This is because it is the most abundant anthropogenic GHG in the atmosphere (Stern, 2006; IPCC, 2007). As of June 2011 CO_2produced from fossil fuel was 390 ppm at an average increase of 3.3 ppm/yr. CO_2 emission has been projected to reach 560 ppm if no action is taken to control it. This could double the concentration of GHG in the atmosphere by 2035. With a subsequent rise in temperature that could surpass 5°C which is above the maximum of 2^{o}C preindustrial levels target. This would mean serious major impacts on physical geography of the earth and on peoples' lives (Fig. 1.1). It would also mean that the world's major coastal cities would be lost (Stern, 2006). Economically, our current activities will have an effect in the next 40 to 50 years. If transformed into cost, there shall be a high global gross domestic product (GDP).yr^{-1} of 5 – 20 % if we fail to act now. Also floods, droughts, storms etc will be affecting the poorest countries and populations. But if we act now, the cost would imply an estimated 1 % of global GDP/yr and bring stabilization (Stern, 2006). The search for alternative energy is no more just an attractive option but is inescapable goal of human civilization.

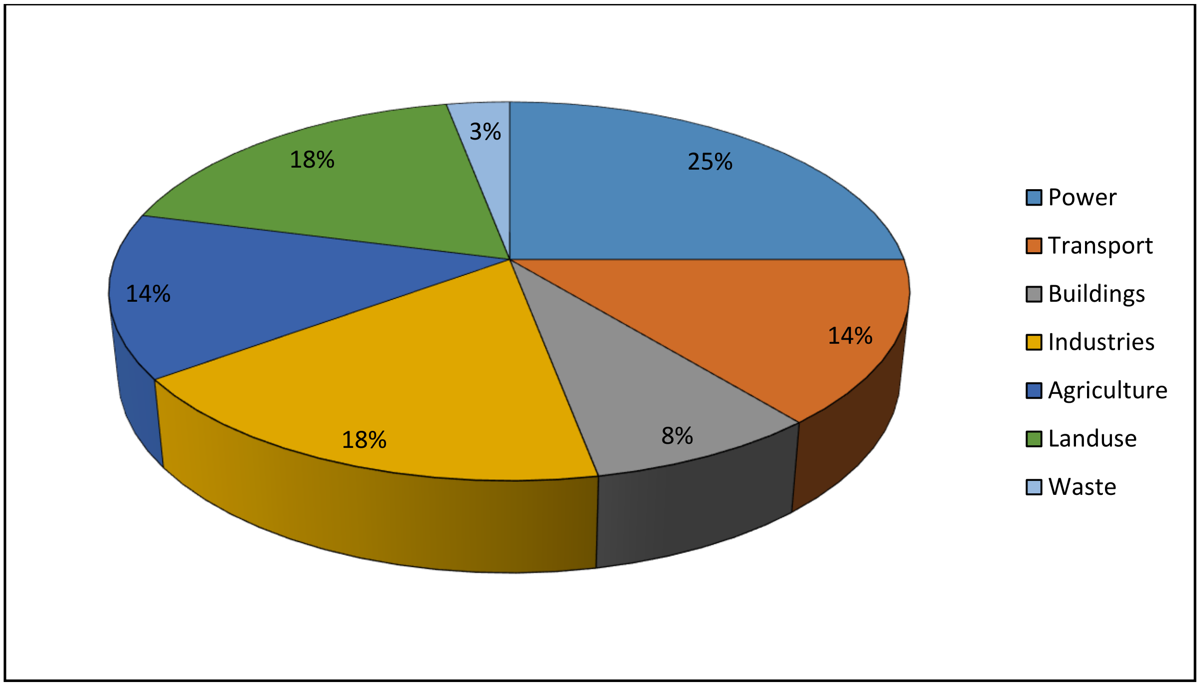
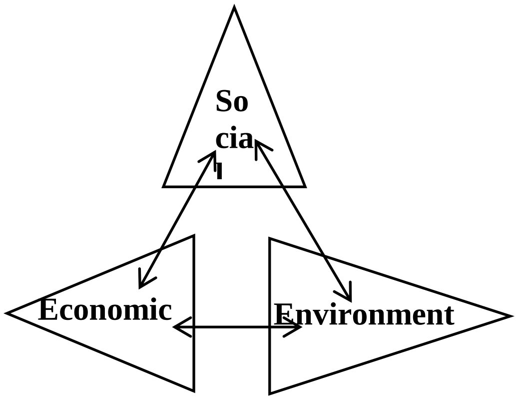


Figure1.1 GHG emissions by source of CO_2eq (IPCC, 2007).

1.2 Waste Disposal

All stages of the materials cycle (extraction, consumption and waste treatment) in urban and rural areas involves waste generation. The environment is impacted by the way this waste is managed and the amount of waste generated. For instance, human health is impacted by the way emissions in the landfills or refineries are managed. Important waste streams such as municipal solid wastes (MSW) and agricultural wastes have potentially high environmental impacts. When compared to other waste types, they are rich in organic biodegradable materials. They can decompose anaerobically or aerobically to generate methane (CH_4), $CO_2$and toxic leachate (Sathaye et al., 2011). The food consumption pattern of a city can change with an increase in the income of that city (Troschinetz and Mihelcic, 2009). This increase will cause some changes in waste types and quantities which will pose a greater challenge for the municipalities to handle. If waste was seen as a resource and managed rightly, the increase in the volumes of waste would not be a problem. This is because the more the cities generate waste, the more diversified products they will have. This increase in produce will mean increase in revenue and a subsequent increase in cost of living. This could lead to a sustainable development.

According to the Hoornweg and Perinaz, (2012) report sustainable development is one that meets the needs of the present without compromising the ability of future generations to meet their own needs (Hoornweg and Perinaz, 2012). According to the report, sustainable development suggests that meeting the needs of the future depends on how well we balance today's decision making as it concerns our social, economic, and environmental needs (Fig 1.2).



Services Household needs Industrial Growth Agricultural Growth Efficient Use of labor	Equity Participation Empowerment Social Mobility Cultural Preservation	Biodiversity Natural Resources Carrying Capacity Ecosystem Integrity Clean air and Water

Figure 1.2: Objectives of Sustainable development Source: (Hoornweg and Perinaz, 2012)

Sustainable development does not make the world ‘ready’ for the future generations, but it establishes a basis on which the future world can be built. A sustainable energy system may be regarded as a cost-efficient, reliable, and environmentally friendly energy system. This is because it can effectively utilize local resources and networks. It is not ‘slow and inert’ like a conventional energy system, but it is flexible in terms of new techno-economic and political solutions.

1.3 Renewable Energy (RE)

Globally, it is estimated that RE accounted for 12.9 % of primary energy supply in 2008 (Jagadish et al., 2011). The largest RE contributor was biomass (10.2 %). In as much as the modern use of biomass is on the increase, roughly 60 % of the biomass fuel are used in traditional cooking and heating applications in developing countries. In 2009, a rapid increase was recorded in use of RE despite global financial challenges. This includes; hydropower (3 %, 31 GW), geothermal power (4 %, 0.4 GW), solar hot water/heating (21 %, 31 GW), wind power (32 %, 38 GW) and grid-connected photo voltaic (53 %, 7.5 GW) (REN21, 2011). About 2% of global road transport fuel demand was met from Biofuels in 2008 and approximately 3 % in 2009 (Jagadish et al., 2011). From 2008 to 2009, 140 GW of the approximate 300 GW of new electricity generating capacity added globally came from RE additions. By the end of 2009 developing countries contributed 53 % of global RE power (IPCC, 2011). The use of RE (excluding traditional biomass) in meeting rural energy needs is also increasing, including small hydropower stations, various modern bioenergy options, and household or village PV, wind or hybrid systems that combine multiple technologies (REN21, 2011).

1.4 Problem Statement

Organic component is about 40% - 60% of the MSW stream of any developing countries. This translates to about 520 million tonnes of MSW stream out of 1.3 billion generated globally (Hoornweg and Perinaz, 2012). It is quite obvious that if this can be diverted to resources then we shall have less waste to handle. There has been several technological means developed to divert solid waste typically destined for a landfill. They include; incineration with energy production, composting of organic waste, and material recovery through recycling. These technologies have the potential to be more sustainable methods to manage MSW than through landfill.

These technologies so far have the potential to emit CH_4 and CO_2 gases which are greenhouse gases. In order to protect our planet and ourselves, action has to be taken to curb adverse climate change by reducing CO_2 and CH_4 emission which in turn will reduce global warming. However, it is also of paramount importance that the problem of waste management will also be resolved. A solution to this lies in finding a source of biofuel which is

1. Economically competitive,
2. non-toxic
3. Abundant on earth, and
4. environmentally friendly is needed

Bio-hydrogen seems to fulfill all these requirements, thus this research is based on the production of bio-hydrogen using food waste through anaerobic fermentation. At approximately 1ppm by volume H_2 is an invisible and a nontoxic light gas that is very rare in the atmosphere. H_2 reacts with other elements quickly because it is very reactive; it does not occur freely in the atmosphere but it is present in water, hydrocarbons, natural and artificial compounds and in living organisms. H_2 has an energy content of 285.9 $KJ.mol^{-1}$ which is 2.7 times higher than that of gasoline and the combustion of H_2 yields heat and water as by products (Armaroli and Balzani, 2011).

Table 1.1 shows that with the exception of hydrogen, other energy sources have direct emission of carbon dioxide. It also shows hydrogen to have a higher EE, LHV and higher fuel to energy conversion. It also shows the high conversion efficiency of hydrogen technology when compared to that of coal, natural gas, gasoline, diesel and ethanol.

Use of fossil fuels has caused more harm than good to the environment and the world at large. Their combustion emits greenhouse gases which depletes ozone layer and causes drastic climate change. The world therefore seeks for an alternative source of fuel which will be renewable and environmental friendly.

Table1.1 Advantages of hydrogen as a fuel for electricity production over other fuels

Fuel	**LHV [MJ/kg fuel]**	**Fuel–to-electricity Conversion**		**EE [MJ/kg fuel]**	**Direct CO_2 emission [kg C/kg fuel]**
		Technology	Typical efficiency[%]		
Hydrogen	120	FC	65	78	0.00
Coal	15-19	CCPP	58	8-11	0.50
Natural Gas	33-50	CCPP	58	19-29	0.46
Gasoline	42-45	ICE	33	13-15	0.84
Diesel	43	ICE	33	14	0.90
Ethanol	21	ICE	33	7	0.50

LHV-lower heating value; EE-electrical energy; FC-fuel cell; CCPP-combined cycle power plant; ICE-internal combustion engine (Marbán and Valdés-Solís, 2007).

1.5 Objectives of The Study

1. To enhance food waste degradation through dark fermentation.
2. To ascertain the optimum temperature and pH for bio-hydrogen production
3. To assess the effectiveness of acclimatization in bio-hydrogen production.
4. To determine hydrogen production potential using Gompertz kinetic model.

Chapter 2

LITERATURE REVIEW

2.1 Introduction

Most human activities have always generated waste. When human population was relatively small, this was not a major issue but with urbanization and population increase, it has become a serious issue. It is estimated that two-thirds of the world's population will be living in the cities by 2025 (Mars et al., 2010). This simply means that more resources will be used up and more waste will be generated. Urban population in developing countries grows by more than 150,000 people every day (Mars et al., 2010). When this urbanization is not planned it can be seen on the streets. For example, there will be problem of public space encroachment, riverbank encroachment, air and water pollution and solid waste generation (Mars et al., 2010).

2.2 Municipal Solid Waste

Since the formation of non-nomadic societies around 10,000 BC humans have been mass producing solid waste (Worrell and Vesilind, 2012). Small communities bury their solid waste just outside their settlements. Some of these communities dispose them into the nearby water bodies. These practices led to spread of disease and foul odor as population increased (Seadon, 2006). The accumulation of these waste led to people living in filth in these growing communities. The ancient city of Mahenjo–Daro in the Indus Valley by 2000 BC, implemented solid waste management processes (Worrell and Vesilind, 2012). Many other initiatives were implemented in cleaning up the streets. All these were possible when certain factors like public health and the environment were considered. Other factors include scarcity of resources and the value of waste, public awareness and participation, and climate change (Worrell and Vesilind, 2012).

2.2.1 MSW in Malaysia

The rate of municipal solid waste (MSW) generation in Malaysia varies from 0.5–0.8 to 1.7 kg/person/day (Manaf et al., 2009). The daily MSW generation has also been on the increase from 16,200 tonnes (t) in 2001 to 19,100 t in 2005, 17,000 t in 2007 to 30,000 t in 2008, 31,000 t in 2012 and 33,000 in 2013 (Manaf et al., 2009; Omran et al., 2009; Fauziah and Agamuthu 2008; Agamuthu, 2014; Abdul Rahman, 2013). The acceleration of waste generation in urban areas such as Kuala Lumpur as shown in Fig 2.1 was due to the increase in urban population from 6.05 million in 1988 to more than 16.5 million in 2007. In 2009, it was shown that Selangor and Kuala Lumpur was the highest generator of waste (Agamuthu, 2009). Kuala Lumpur, the, the capital city of Malaysia, showed increasing trends of waste generation since 1970. From Fig 2.1, it can also be seen that waste generation increased by approximately 300 % from 98.9 tonnes/day in 1970 to 311 tonnes/day in 1980 (Agamuthu, 2014). Up till now, the waste generation in Kuala Lumpur has increased from approximately 590 in 1990s to 3,000 tonnes/day. The total sold waste generation in Peninsular Malaysia was 5.6 million tonnes or 14,000 tonnes/day and of this 80% was domestic waste while the remaining 20 % was commercial waste (Agamuthu, 2014).

However, it is important to know the composition of waste because the best management option to adopt will depend on it (Johari et al., 2012). Waste characterization also allows for the estimate of biodegradable components. It also helps to monitor the effectiveness of programs designed to divert biodegradable and compostable materials from landfills (Zheng, 2005).

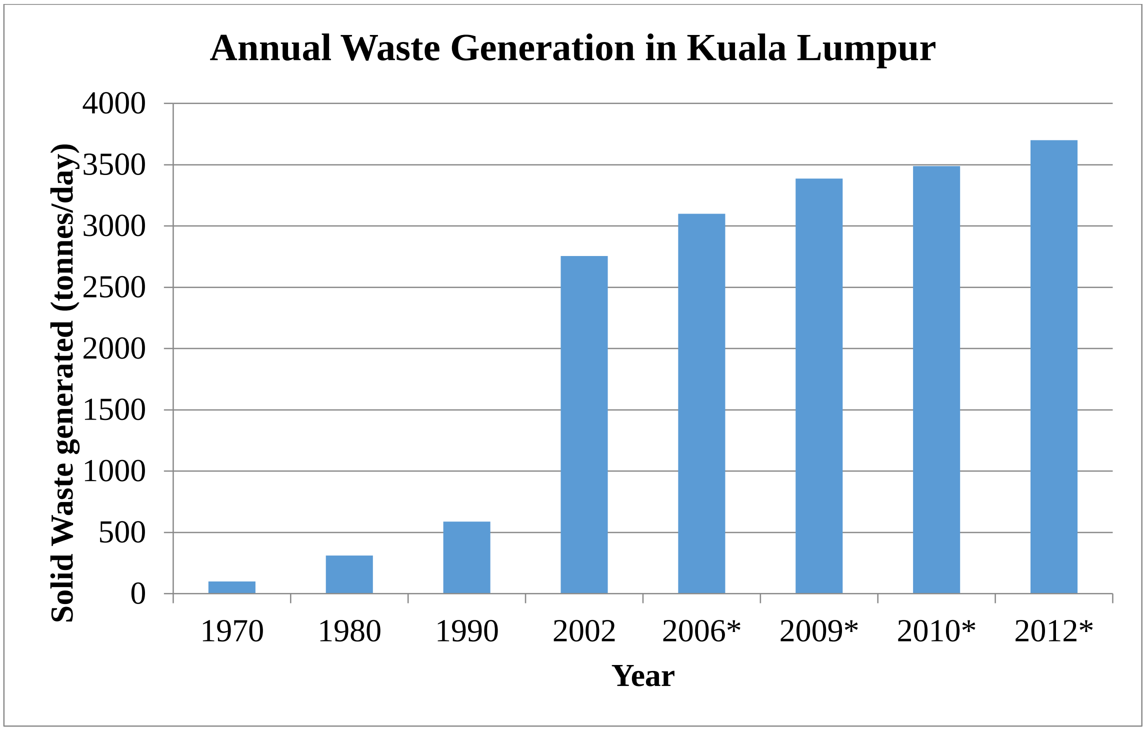


Figure 2.1 Annual Waste Generation in Kuala Lumpur. *- estimated figure

Source: (Agamuthu, 2014)

Table 2.1 shows the changing pattern of waste generation in Malaysia. The percentage of food waste has been on the increased from 37 % in 2004 to 59 % by 2009 (Noor et al., 2013), however, the amounts of paper and plastic has shown a substantial decrease. The large percentage of biodegradable organic matter (food waste and paper) creates a favorable environment for methane generation as well as hydrogen generation.

Table 2.1 MSW Composition in Malaysia

Material	Composition (%)					
	2004	**2005**	**2006**	**2007**	**2008**	**2009**
Food/organic	59.2	36.6	37.43	68.67	57	45
Plastic	12.6	30.7	18.92	11.45	15	24
Paper	8	8.9	16.78	6.43	17	7
Textile	1.4	1	8.48	1.5	1	NA
Wood	2.3	0.3	3.78	0.7	NA	NA
Yard waste	7.6	6.7	3.18	NA	5	NA
Rubber	0.7	NA	1.32	NA	1	NA
Glass	1.6	2.8	2.68	1.41	1	3
Organic fines	4	NA	4.37	NA	1	NA
Aluminium/metals	2.4	12.1	3.4	2.71	2	6
Others	NA	0.9	7.16	7.13	NA	15

Source: (Noor et al., 2013). NA → Not Available

2.3. Global MSW Generation

Globally, MSW is the most complex solid waste because it is not homogenous as opposed to industrial and agricultural activities (Noor et al., 2013). Global MSW generation levels are approximately 1.3 billion tonnes per year which equates to a daily generation rate of 3.6 million tonnes, and are expected to rise by 2025 approximately to 2.2 billion tonnes per year. This represents a significant increase in per capita waste generation rates, from 1.20 to 1.42 kg per person per day in the next fifteen years. Worldwide, the percentage of urban residents as a share of the global population is expected to increase to 70 % to 80 % in the coming decades leading to a growing amount of MSW to be managed (Hoornweg and Perinaz, 2012).

From Table 2.2, it is evident that with increase in population, waste generation in all regions will almost double by 2025. Increase in urbanization is highly correlated with increase in income level. As disposable incomes and living standards increase, the consumption of goods and services simultaneously increases, as does the amount of waste generated (Outlook, 2012; Shekdar, 2009). Globally, MSW costs are expected to increase from today's annual $ 205.4 billion to about $ 375.5 billion in 2025 (Hoornweg D. and Perinaz, 2012).

Table 2.2 Current Urban Waste Generation and Future Projections

Region	**Current Available Data**			**Projections for 2025**			
	Total Urban Population (millions)	Urban Waste Generation		Projected population		Projected Urban Waste	
		Per capital(kg/capital/day)	Total (t/day)	Total Population(millions)	Urban Population(millions)	Per Capita(kg/capital/day)	Total(t/day)
Africa	260	0.65	169.119	1,152	518	0.85	441,840
EAP	777	0.95	738,958	2,124	1,229	1.5	1,8565,379
ECA	227	1.1	254,389	339	239	1.5	354,810
LCA	399	1.1	437,545	681	466	1.6	728,392
MENA	162	1.1	173,545	379	257	1.43	369,320
SAR	426	0.45	1,938	1,938	734	0.77	567,545

Source: (Hoornweg and Perinaz 2012)

EAP – East Asia And Pacific region, ECA – Eastern Central Asia, LCA – Latin American and the Caribbean, MENA – Middle East and North Africa, SAR – South Asia Region

2.4 Global MSW Composition

MSW composition is influenced by the level of available income for goods and services, local culture, climatic conditions, geographical locations and energy sources (Chinellato et al., 2013). Geography influences waste composition by determining building materials for instance wood versus steel, amount of street sweepings and horticultural waste. The extent of reduction, reuse and recycling (3R's) programs and also the duration of year are also some factors that can influence MSW composition (Chinellato et al., 2013). MSW composition influences how often waste is collected and how waste is disposed (Hoornwegand Perinaz, 2012)

Waste composition in MSW varies widely in different regions and countries. It is evident from Figure 2.2 that MSW comprises mainly of organic waste, followed by paper, metal, other wastes, plastic, and glass. Generally the biodegradable portion is mainly due to food and yard waste, typical of the developing countries. The high paper and plastic content is typical of developed countries which could be as a result of purchasing prepared food, lots of office work and high recycling rate (Karak et al., 2012). When disposed in a landfill, it generates leachate which might seep into aquatic water bodies causing water pollution or into land causing land pollution. Furthermore, in benthic environment, leachate constituents can accumulate in poorly ventilated hypoxic and anoxic interstitial waters. Here, leachatemay be directly assimilated by benthic organisms; it could lead to death of the organisms. It could be said to cause nuisance to the society (Tomczak-Wandzel, 2013).

Although MSW composition is generally provided by weight, as a country increase in affluence, she tend to pay more attention toher waste volumes, particularly with regard to collection: organics and inerts generally decrease in comparative terms, while increasing paper and plastic increases overall waste volumes (Hoornwegand Perinaz, 2012)

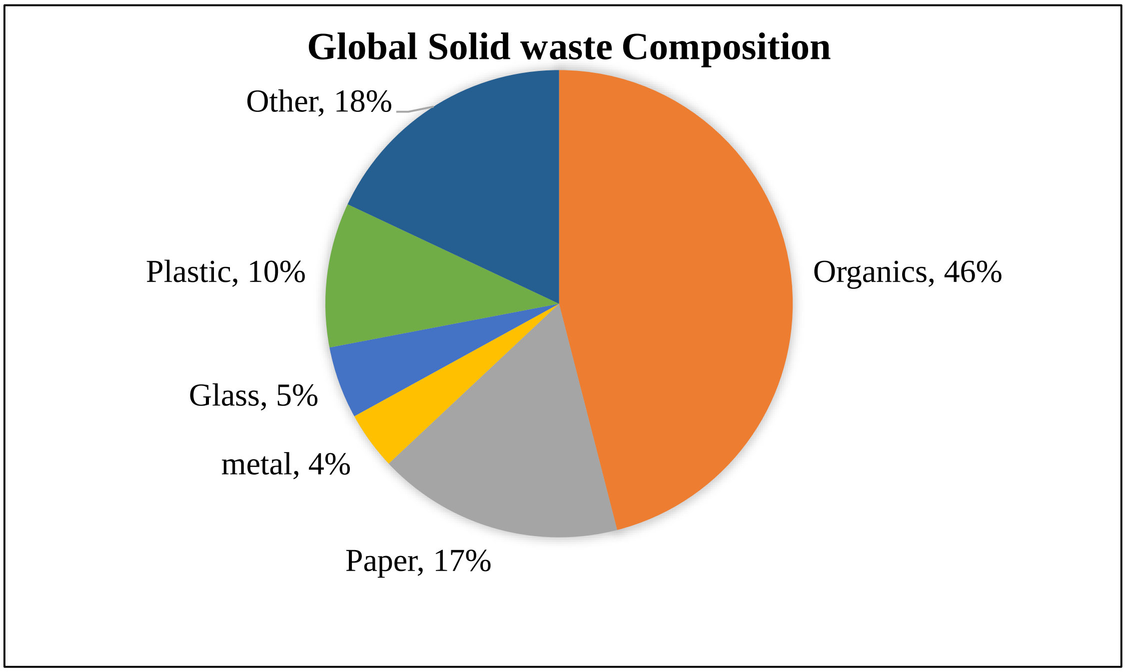


Figure 2.2 Global Solid Waste Composition(Hoornweg and Perinaz, 2012)

Source: eawag: Swiss Federal Institute of Aquatic Science and Technology

2.5 Solid Waste Management Practices

Increased generation and complexity of MSW has led to the development of many methods to help in its management. They are;

i) open dumping and landfilling,

ii) Biological treatment (composting and anaerobic digestion (AD))

iii) 3 R (Reduce, Reuse and Recycle)

iv) Thermal treatment (Incineration and Pyrolysis)

i) Open Dumping and Landfilling

This is a primitive type of waste disposal. It is the most cost effective method in many developing countries. Open dumping is basically, a situation where waste is dumped in a place and not covered with soil or other materials. This attracts flies and scavenging animals, thus, it is does not have aesthetic value. This method is commonly seen in developing countries such as India, Bangladesh, Most African countries and South East Asian countries (Agamuthu, 2001; Parrot et al., 2009).

A landfill is a carefully engineered depression in the ground (or built on top of the ground, having the resemblance of a football stadium) into which wastes are put by burial. Fundamentally, a landfill is a bathtub in the ground and a double-lined landfill is one bathtub inside another. Out the bottom is the leaking of leachate produced as a result of the decomposition of the organic matter. Leakage at the top is the release of gases such as CH_4, also produced due to the decomposition as well (Hoornweg and Perinaz, 2012).

ii) Composting and Bio-gasification

Composting is a process that involves the biological decomposition of organic matter, under controlled operation to produce a humus-like stable product (Worrell andVesilind 2012). The basic composting process is given in the following equation:

$$\text{[Organic complex materials]} + O_2 \xrightarrow[\text{(Oxidation)}]{\text{Aerobic}} CO_2, NO_2, NO_3 \tag{1}$$

The aerobic microorganisms' extract energy from the organic matters through a series of exothermic reactions that break the material down to simpler materials as shown in the equation above. For a proper function of a composting operation, non-compostibles such as metals, glassware and ceramic items must be removed(Worrell and Vesilind 2012).

Bio-gasification, on the other hand, is anaerobic and the breakdown process is reduction. The products are mainly CH_4 and CO_2 as shown below

$$\text{Organic compounds} \xrightarrow[\text{(Reduction)}]{\text{Anaerobic}} CH_4, CO_2\text{------------------------ (2)}$$

According to Agamuthu (2001), composting has four main objectives, which are; volume reduction, stabilization, sanitization and valorisation (includes compost and biogas). On dry weight basis, up to 75 % of the oraganic material could be decomposed while the weight loss of wet agrowaste is around 50 % (Agamuthu, 2001).

iii.) 3R (Recovery, Reuse and Recycling)

Reuse/Recycling refers to the collection and separation of waste and their subsequent transformation into usable or marketable materials (Nakahashi, 2008). For instance, plastic wastes can be used as feedstock in coke ovens or blast furnaces in iron and steel production. Plastic waste or a mixture of waste plastics and paper can substitute coal in boilers or kilns. Steel, cement and paper industries which are energy intensive industries are more effective in using recyclable wastes as feedstock or fuel in their production (Nakahashi, 2008). Recycling has major advantage of reducing the quantities of disposed waste and also returns materials to the economy (Daniel and Natalie, 2005). The use of recycled materials in inductries reduces energy use and emissions; lessens impacts when raw material is extracted and conserves raw materials (Agamuthu, 2001).

2.6 Thermal Treatment

As the name implies, it involves the use of heat in combusting waste. It could be solid, liquid or gaseous. There are two major processes here, which are incineration and pyrolysis (Agamuthu, 2009). In a broader sense, waste materials are treated at incinerator plants through the controlled application of heat that converts waste feed by high temperature oxidation to gaseous materials emitted as flue gas, viscous waste (slag) and solid residue (ash). During combustion, the moisture is vaporized while the combustible waste is also vaporized and oxidized resulting in the final products CO_2, water vapor, ash and non-combustibles or residue (Agamuthu, 2009).

2.7. Waste to Energy

While waste is generally perceived as a nuisance, it has hidden value as an energy fuel. One tonne of MSW can produce 535 kWh of electricity through incineration (Percy et al., 2012). This implies that, waste can become a resource. On the other hand, CH_4 gas is generated when organic waste dumped in the landfill decomposes. This CH_4 gas can be trapped and used to produce energy. CH_4 gas is also produced during the decomposition of livestock and human waste and can be trapped from these sources. Landfill gas collection systems can be installed at landfills to capture the CH_4 produced by trash as it decomposes (Dann et al., 2012).

2.7.1 Why Convert Waste to Energy?

Energy is the driving force that sustains our lifestyle. All our activities such as economic, physical and social welfare depends on it. The continuous supply of energy with an increasing worldwide demand institutes a significant challenge for our society. About 78% - 87 % of this energy demand has been met mainly through the exploitation of our natural reserves of fossil fuels (oil, coal, gas). As reported in 2012, global energy consumption was predicted to increase from 534 quadrillion joules in 2010 to 819.7 quadrillion joules in 2040 (Outlook, 2012) as shown in Fig 2.3. As of January 2006, it was reported that the total global natural gas reserves was 6112 trillion m^3, while 95 trillion m^3 has been consumed as of 2003 (EIA, 2011). Without considering the increase in demand, this would suggest that in approximately 60 years, natural gas will run out. Natural gas remains an important fuel for electricity production. This is because it is less capital intensive than those using coal, nuclear or most renewable energy sources. Global consumption of natural gas is projected to increase by 1.3 % per year from 108 trillion m^3 in 2007 to 156 trillion m^3 in 2035 (EIA, 2011).

Cars that run on petrol can be easily converted to run on natural gas. Natural gas and coal are used as raw materials to produce heat and electricity whereas oil serves dual purposes. The non-OECD countries are the highest energy consumers (Fig 2.3). This could be because most of these countries are developing and under-developed nations who do not have sufficient funds to use the recent energy reduction technologies (Outlook, 2012).

In the petroleum sector, the global demand for oil is on the increase, the petroleum industry has experienced about 30 % spike in oil use. Thus, on daily basis, it is becoming clearer that sustainability cannot be achieved by the current energy resources (Baxter, 2005).

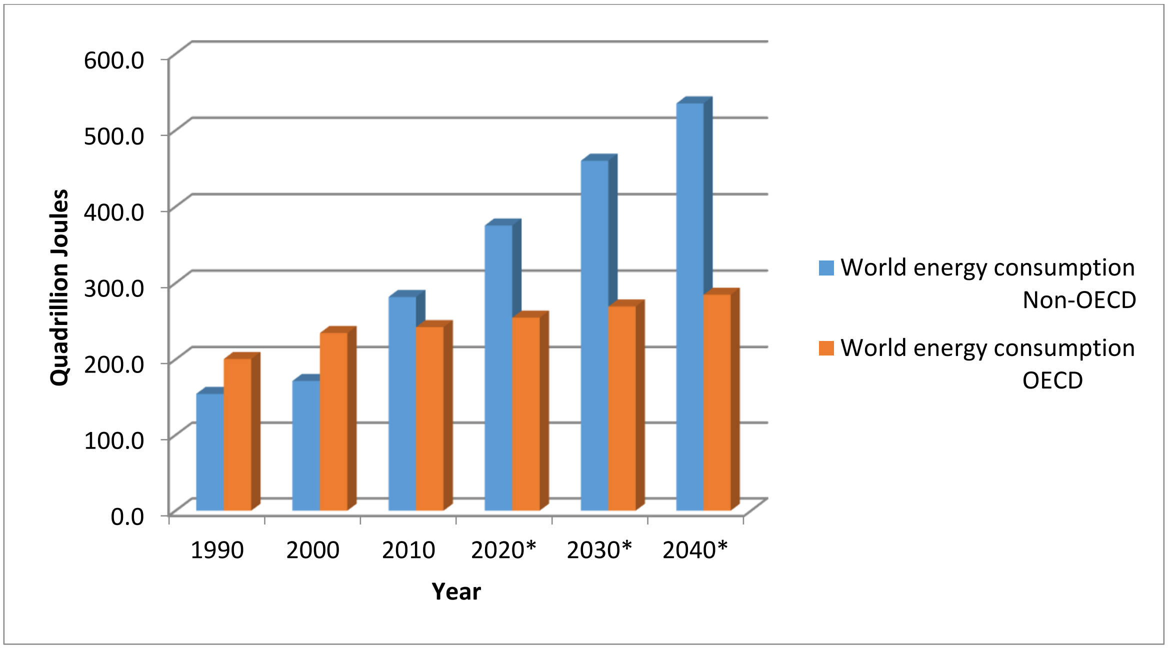


Figure 2.3 World energy consumption by OECD and non-OECD countries

Source (EIA, 2011) * - Prediction, OECD – Organization for Economic Cooperation and Development countries.

Coal contributes more than one-fourth of the world's total primary energy supply and more than one-third of the fuel used for electricity generation. Coal provides the largest share of world electricity generation which was 42 % in 2007 and remains unchanged through 2035 (EIA, 2011). The general fuel consumption is on the increase (Outlook, 2012).

Incomplete combustion processes, which result from the burning of fossil fuels produced a great amount of gases as carbon dioxide (CO_2) and nitrogen oxides (NO_x). CO_2 emissions are of particular concern, since CO_2 has been identified as a GHG. The atmospheric concentration of GHGs has been steadily rising. In 2005, the concentration of CO_2 rose to 378.9 ppm (Hou et al.,2013). This increase has been directly linked to human activity such as bush burning, transportation etc.

World nuclear power is expected to increase from 2.6 Trillion Kw/h in 2007 to an estimated 4.5 Trillion Kw/h in 2035 (Arvizu et al., 2011). Despite this fact, countries such as China, India and Russia accounts for the largest increase in world installed nuclear power with 114 Gigawatts of nuclear capacity of which 60 % belongs to China alone (Outlook, 2012).

From the Table 2.3, it is evident that the natural reserve will decrease with increasing consumption. In order to meet the demand of the increasing population, more of the natural will be used up and we shall face scarcity in the mere future. Therefore, there is the need to transform our current fossil fuel dependent energy systems to new clean renewable energy sources. These renewable energy sources include: Bioenergy, direct solar, geothermal, hydropower, wave and wind energy (IPCC, 2011).

Table 2.3. World fossil fuel reserve and consumption in 2009

	Oil	Natural Gas	Coal
World Reserves	(M barrels) 1.333 x 10^{12}	(T M^3) 187.5	(M tonnes) 826001
Consumption	(M barrels/day) 84.1	(B M^3/year) 2940.4	(M tonnes/year) 3278.3
R/P	Years		
	45.7	62.8	240

Source (BP 2010). R/P→Production Ratio

2.8 Technologies for Sustainable Energy Production

Hydropower is a renewable resource from the global water cycle, driven by the sun. It is basically the conversion of water's potential (or kinetic) energy into electricity using water turbines and electric generators. Globally, between 40,000 and 50,000 large dams have been built for different purposes such as irrigation, domestic water use, flood control, and power generation (Balmer and Spreng, 2008). Hydroelectric power is a major source of renewable energy growth in developing countries. For example, China, India and Brazil collectively accounts for 83 % of the total increase in hydroelectric production (Kumar and Schei, 2011).

On one hand, photovoltaic, wind and biomass, among others stand out in their technological innovation and prospects for future economic development. Alternatively, today in many parts of the world, civil nuclear power receives support from policy makers who are willing to expand its use (Cicia et al., 2012). The use of solar energy has rapidly increased in the past few years (30 – 40 % a year), yet, the current global nature of solar power output is equivalent to less than 1% of global demand for electricity (Arvizu et al., 2011). This suggests that the use of solar energy technology faces a big challenge globally. This is especially in developing and new industrialized countries, which are more oriented to rapid economic growth and tend to be less sensitive to environmental concerns (Dorian et al., 2006).

One of the first renewable technologies to be adopted on a large scale is wind energy. As of the end of 2006, the installed global capacity of wind energy technology was greater than 74,000 MW (Staudt, 2008). The economics of wind energy can be compared with fossil-fuel technology in the windier parts of the world. A significant percentage of the world's electricity can be supplied by the vast supply of wind energy resource. The differential heating of the earth's surface by the sun which causes wind results in low and high pressure systems as heated air rises and then falls (Staudt, 2008). Around the globe, wind turbines are already providing substantial amounts of sustainable, pollution-free electricity. There is also a high growth rates for wind powered electricity production in developing nations. For example, the total generation from wind power plants in China is projected to increase from 6 b KW/h in 2007 to 374 b KW/h in 2035 (Hoornweg and Perinaz, 2012).

2.8.1 Incineration

Waste incineration could be defined as controlled burning of solid, liquid or gaseous waste. Waste Incineration reduces the volume of waste by about 90% and the remaining ash goes to landfill (Masirin et al., 2008). These high volume reductions are only seen in waste streams with very high amounts of horticultural waste, packaging materials, plastics, paper and cardboards. It offers the solution of waste disposal to countries where land is scarce. It is also one way to prevent CH_4 release from landfills. For each tonne of MSW processed in a waste incineration plant, 1 tonne of CO_{2eq} is avoided (Dann et al., 2012). US Environmental Protection Agency has stated that waste incineration plants produce electricity with less environmental impact than almost any other source of electricity (Dann et al., 2012). With increasing regulatory focus on GHG emissions, waste incineration turns from an environmental problem to an environmental solution.

2.8.2 Pyrolysis

Pyrolysis is a thermochemical decomposition of organic materials at elevated temperatures in the absence of oxygen. It is also an irreversible process. The key products of biomass pyrolysis are water, permanent gases such as (H_2, CO, CO_2, and CH_4), C_2–C_3 hydrocarbon gases, tar and char (Consonni and Viganò, 2012). The formation of tar is the main issue in biomass pyrolysis. It causes blockage of equipment and fouling of down-stream application process which reduces the thermal efficiency. It is therefore necessary that tar is decomposed into gas products (H_2 and CO) during the pyrolysis of biomass. Generally, the main method for removing tar is by in situ tar cracking. Operating factors such as catalyst, reactor structure, heating rate, and temperature and residence time can be enhanced to maximize the effectiveness of pyrolysis and reduce tar formation (Qinglan et al., 2010). Air, steam or oxygen can be used as a gasification agent to increase energy value in the conventional gasification which is an old technology, in which biomass is heated at high temperatures and separated to combustible gas (Kalinci et al., 2009).

Gasification is simply the process that converts a solid or liquid combustible feedstock into an incompletely oxidized gas called "syngas" (mostly a mixture of CO, H_2, CO_2 and H_2O). The term "gasification plant" is commonly used to designate the entire system that converts the primary feedstock into useful energy carriers. In order to meet the requirements of high-efficiency, internally-fired cycles (gas turbines, internal combustion engines), proper syngas treatment is needed (Consonni and Viganò, 2012).

2.8.3 Landfill

A common final disposal site for waste is landfills and should be planned and operated to protect the health of the public and the environment. The CH_4 produced from the anaerobic decomposition of organic matter can be recovered and burned with or without energy recovery to reduce GHG emissions. Landfill CH_4 represents 12 % of total global CH_4 emissions (EPA, 2006). Furthermore, almost half of the CH_4 emission attributed to the municipal waste sector in 2012 comes from Landfill CH_4 emission (Johari et al., 2012). Different countries have different levels of CH_4 from landfills depending on waste compositions and waste disposal practices as shown in Table 2.4. Landfill gas, a by-product of anaerobic decomposition is composed of CH_4 (50%), CO_2 and other gases. In comparison with carbon dioxide, CH_4 has a global warming potential 21 times greater than carbon dioxide, and it is the second most common GHG than CO_2(Saeed et al., 2013).

Table 2.4 Landfill CH_4 Emissions and Total GHG emissions for selected Countries

Country	CH_4 emissions from post-comsumer Municipal Waste Disposal ($MtCO_2e$)	GHG emissions (CO_2, CH_4, N_2O) ($MtCO_2eq$)	% CH_4 from disposal Sites Relative to Total GHG Emissions
South Africa	16	380	4.3
Mexico	31	383	8.1
Brazil	16	659	2.4
India	14	1210	1.1
China	45	3650	1.2

Source (EPA, 2012)

According to the ministry of Housing and Local government website, there are generally 296 landfills/dumpsites in Malaysia out of which 165 are still in operation. This includes eight sanitary landfills (Manafet al., 2009). More sanitary landfills are been planned in the future either to replace or to upgrade the current dumpsites.

2.8.4 Hydrogen Production

The world has recognized hydrogen as an energy carrier that complies with all the environmental quality, energy security and economic competitiveness demands. Roadmaps such as “Hyways Roadmap Europe” by European Commission (EC), “the National

Hydrogen Energy Roadmap and the Hydrogen Posture Plan" by the US Department of Energy (DOE), "The Hydrogen Technology Roadmap" by the Australian Government Department of Resources, Energy and Tourism and the Future Fuels for the Asia Pacific Economic Cooperation (APEC), have already been developed as roadmaps to the transition to "Hydrogen economy (Hyways, 2007; Hurley, 2009; Armaroli, 2011). Hydrogen (H_2) is the third most abundant element on Earth and the most abundant element in the universe (Armaroli, 2011).

Many technologies have emerged in response to the environmental, economic security and energy needs. These include;

- Hydrogen from Biomass, (Kalinci et al., 2009)
- Hydrogen from steam reforming of fossil fuel, (Jean, 2010)
- Water Electrolysis, (thermochemical) (Richard, 2008)
- Biological methods (Nathao et al., 2013)
- Hydrogen Production from photosynthesis (Allakhverdiev, 2012)

2.8.4.1 Hydrogen from Biomass

Agricultural residues, forest resources, perennial grasses, energy crops, wastes (MSW, urban wood waste, and food waste), and algae are all biomass. Thus, biomass is an abundant renewable resource and it is said to be capable of supporting the future H_2 economy (EERE, 2011). For example, US department of energy reported that the total annual biomass consumption for both bioenergy and bio-products is about 190 million dry tonnes. This provides over 3 % of the total energy consumption in the United States (EERE, 2011). Among renewable energy resources, biomass has distinctive characteristics which is that in addition to power, it can be converted to carbon based fuels and chemicals (Kalinci et al., 2009). Thus, biomass stands as the only renewable resource with the potential to replace fossil based fuels. It is estimated that over a billion tonnes of sustainable biomass resources are produced in the United States (EERE, 2011). This can provide fuel for cars, trucks, and jets; make chemicals; and produce power to supply the grid. It also creates new opportunities and jobs throughout the country in agriculture, manufacturing, and service sectors.

The composition of biomass varies depending on its nature as shown in Fig 2.4. The most important components of biomass are starch, cellulose, hemicelluloses and lignin. These are also among the most abundant renewable resources on earth. Starch, cellulose and hemicelluloses are potential sources of fermentable hydrolysates into H_2, ethanol, butanol among others. Biomass such as agricultural food and food waste biomass is usually rich in starch (Sun and Cheng, 2002).

Starch, a main constituent of biomass, is present in many agricultural and staple food wastes such as potatoes, corn, rice, wheat, pasta and wastes from textile industries (Güllü and Demirbaş, 2001; Hanaoka et al., 2004). Starch molecules are more susceptible to enzyme and other hydrolysis systems, thus are easily broken down into glucose (Mars et al., 2010; Rosendahl et al., 2008). Cellulose, a major component of agro-food wastes is also one of the most abundant renewable organic compound on earth. Cellulose molecules under normal conditions are insoluble in water and are strongly resistant to enzymatic attack and chemicals such as acid compounds. Therefore, cellulose is more difficult to hydrolyze into glucose units than starch (Vijayaraghavanand Yun, 2008).

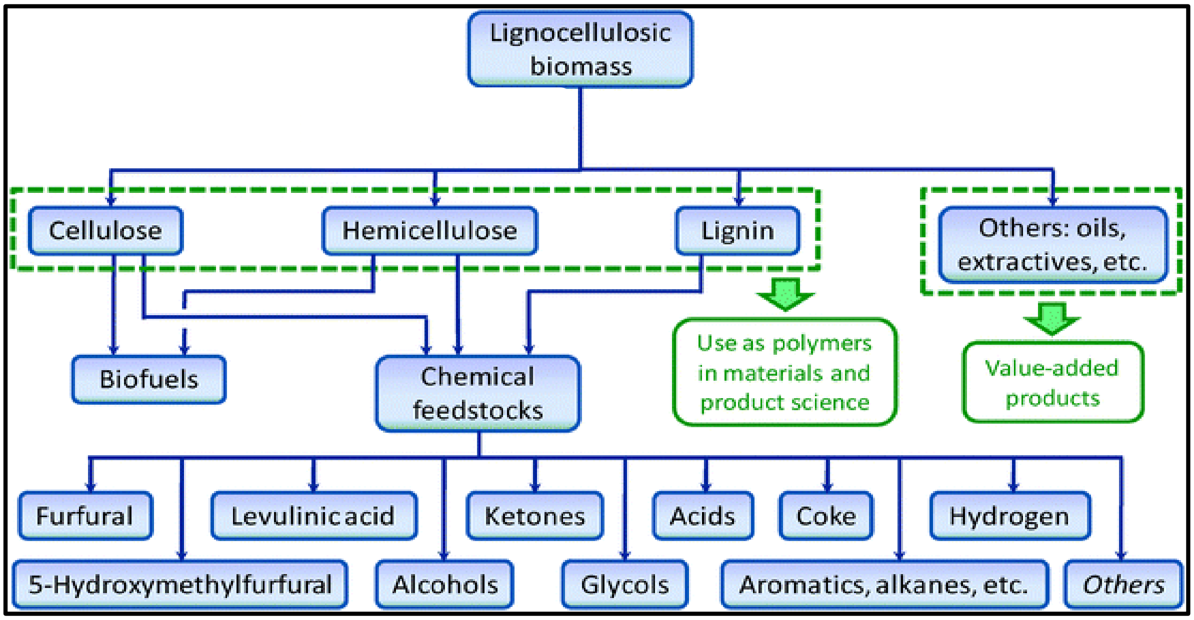


Figure 2.4:Components of Biomass.Source (Heidrich and Witkowski, 2010).

Waste such as agricultural and agro-industrial wastes not only provides an economical source of energy but also an effective low sulphur fuel (Capareda, 2011). In order to reduce environmental hazards, biomass could further be processed into other fuels e.g. biomass from sewage. Nevertheless, the conversion of light energy into biomass by plants is relatively of small percentage and there is somewhat low concentration of biomass per unit area of land and water (Capareda, 2011). Thus, the conversion methods into fuels are important.

There are different methods for converting biomass into fuel (Fig 2.5). The most efficient process of these is the conversion into heat energy process (Heidrich and Witkowski, 2010). In order to have diverse use of biomass resources, they need to be converted into chemical, electrical or mechanical energy. These take the form of solid fuel (charcoal), liquid fuel (ethanol) or gaseous fuel (methane). These fuels can be used in a wide range of energy conversion devices to satisfy the diverse energy needs.

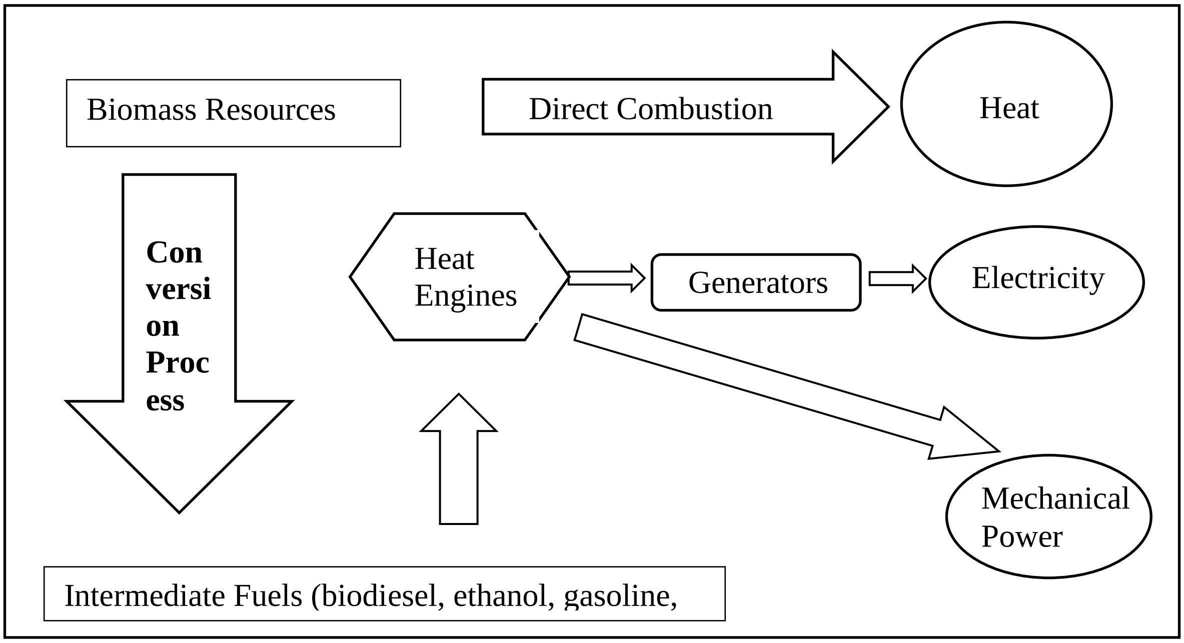


Figure 2.5 Methods of Using Biomass for Energy Source: (Heidrich andWitkowski, 2010)

2.8.4.2 Hydrogen from Steam Reforming of Fossil Fuels

Steam reforming is a process to reform hydrocarbons in the presence of H_20 to produce syngas using catalyst (supported Ni-based) at a prescribed reaction conditions. As shown in Fig 2.6, syngas is a mixture of H_2, CO and CO_2 in various proportions (Jean, 2010). Steam reformation of fossil fuel accounts for about 96 % of global H_2production, of which 49 % is natural gas, 29% is liquid hydrocarbon and 18% is coal (Matthew, 2009). Steam CH_4 reforming (SMR) is highly efficient having about (65 - 75 %) conversion of natural gas into $H_2$and syngas production (Matthew, 2009).

The integration of separation membranes to the SMR process help to overcome thermodynamic limitations. It also helps to achieve almost 100 % CH_4conversion to H_2 at lower temperatures. Carbon capture and storage (CCS) is seen as a way to reduce CO_2

emissions into the atmosphere, thus decreasing the threat of global warming. It involves the capture and transportation of CO_2 to a store location (Basu et al., 2010). Storing and maintaining the CO_2in CCS is currently limited and more research and demonstration projects to develop efficient and economic methods for carbon capture, transport and storage needs to be done. With ultimate CCS, SMR is projected to be the main source of H_2 to meet up with increasing demand. The problem with this approach is that fossil fuel power will still emit CO_2 through residual emissions from power plants due to limited capture efficiency.

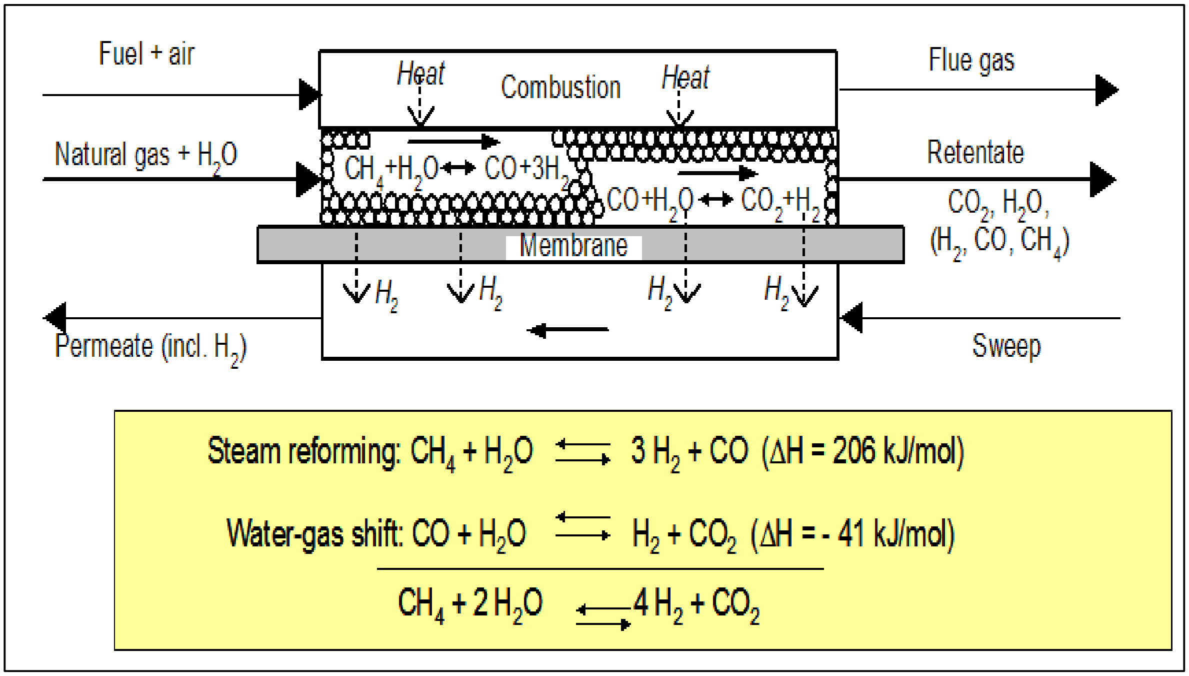


Figure 2.6 Steam reformation of fossil fuel (Jean, 2010)

2.8.4.3 Steam Reformation of Glycerol

This reforming process is the splitting of hydrocarbons in the presence of water and water–gas shift reaction as given below (Equation 1)

$$C_nH_{2n+2} + nH_2O \rightarrow nCO + (2n+1)\,H_2 \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

Stochiometrically, the moles of hydrogen obtained by steam reforming of natural gas is four, while that obtained from steam reforming of glycerol is seven as equation (2). Therefore, using stoichiometric study, glycerol will be preferred to fossil fuels because it provides a higher number of moles of hydrogen.

$C_3H_8O_3$ (g) + $3H_2O$ (g) $\leftrightarrow 7H_2$ (g) + $3CO_2$ (g) $\Delta H = 128$ kJ/mol ……………….(2)

However, this process also has some limitations such as control of high temperatures, the unavoidable CH_4 formation, and the formation of coke. The coke formed acts as a poison and clogs the pores of the catalyst, thus affecting the process, as well as, the yield and purity of hydrogen (Avasthi et al., 2013).

2.8.4.4 Electrolysis of water

Electrolysis of water is the splitting of water into oxygen and hydrogen gas by passing an electric current through it. This process requires large amounts of energy thus it is the most costly method of H_2 production (Matthew, 2009; Richard, 2008). The current breaks the chemical bond between the H_2 and O_2 thus, splitting them into atomic components.

At the cathode, water combines with electrons from the external circuit to form H_2^+ and O_2^-. The oxygen ions however, reacts at the anode to form oxygen gas and give up the electrons to the external circuit (Fig 2.7). The overall environmental friendliness depends on the fuel source. However, due to the high energy requirement involved in this process, people do not like to get involved in it(Millet and Grigoriev, 2013).

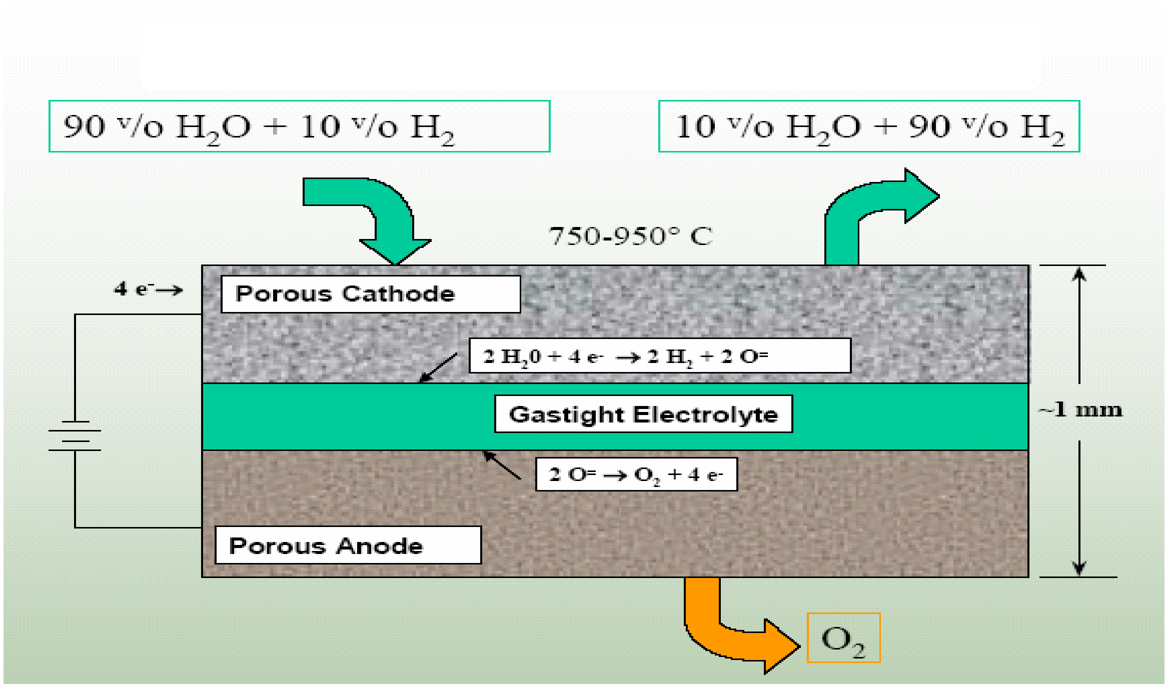


Figure 2.7 Schematic Diagram of Industrial water electrolysis to produce hydrogen. Source: (Richard, 2008).

2.8.4.5 Bio-catalyzed Electrolysis

This is a process whereby organic matter is converted into hydrogen by the use of electrochemically active enzymes inside an electrochemical cell via coupled anode-cathode reactions (Rozendal et al., 2008). This technology is mainly used to generate hydrogen from wastewater with high organic content.

At the anode, electrochemically active microorganisms oxidize the organic material from the wastewater. Consequently, the electrons resulting from this oxidation reaction are transferred by microorganisms to the anode by means of extracellular electron transfer (EET). The electrons are transported to the cathode, where they are consumed for oxygen reduction (in the case of MFCs) or product formation (in the case of MECs) via an electrical circuit. Electro neutrality is maintained in the system by the transport of ions in between the electrodes (optionally through a membrane). In an MFC, electrical energy can be extracted from the electrical circuit. In an MEC, however, electrical energy needs to be supplied to the electrical circuit by means of a power supply (Rozendal et al., 2008). See Figure 2.8

However, MFCs and bio-catalyzed electrolysis systems operate at low current densities (~1 to 10 A/m^2) and as a result, MFCs and bio-catalyzed electrolysis systems produce too little electricity or hydrogen per amount of platinum. Moreover, the platinum electrode is such an expensive material as the cathode catalyst. This has encouraged researchers to look for alternative hydrogen energy source (Renea et al., 2007).

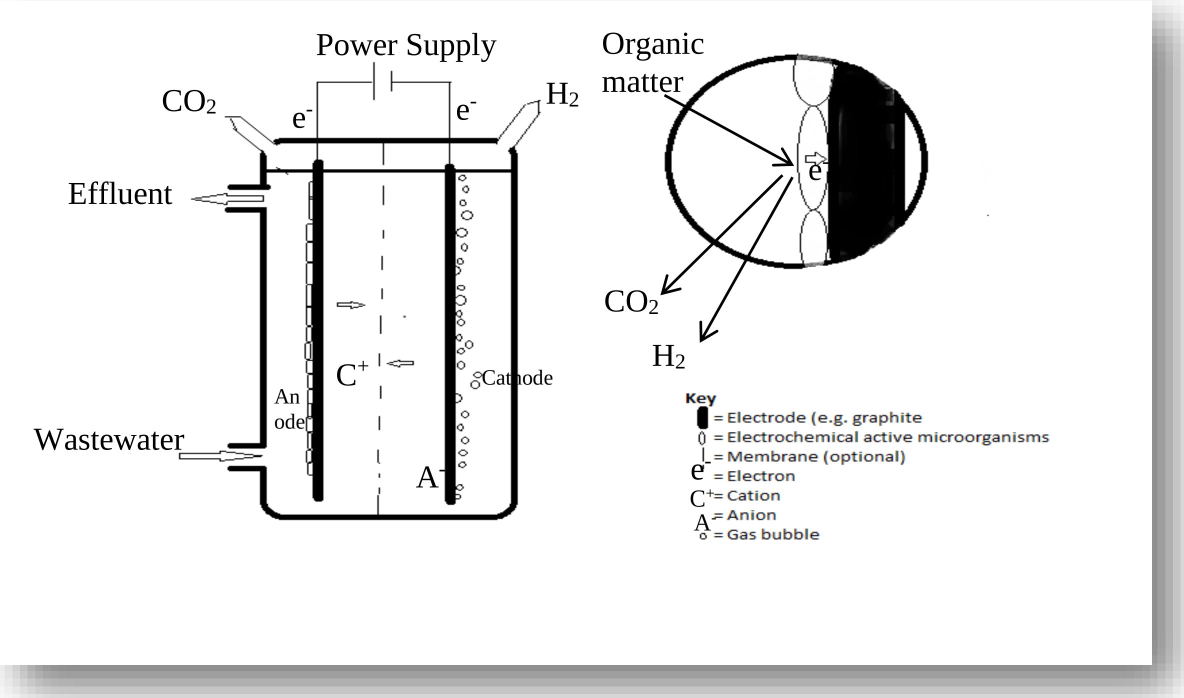


Figure 2.8 Schematic diagram of Bio-catalyzed electrolysis. Source (Rozendal et al., 2008).

2.8.4.6 Bio-photolysis-Green Algae/Cyanobacteria

Biological hydrogen can be generated from plants by bio-photolysis of water using microalgae (green algae and Cyanobacteria). Bio-photolysis is the decomposition of water by algae or cyanobacteria to hydrogen and oxygen with the aid of sunlight. Photosynthetic production of hydrogen from water is a biological process that can convert sunlight into useful, stored chemical energy as shown in equation 3.

$2H_2O \rightarrow 2H_2 + O_2$………………………… (3)

This process is attractive because it uses solar energy to convert a readily available substrate (water), to oxygen and hydrogen. Water splitting involves one enzyme (hydrogenase) in the case of unicellular algae to catalyze hydrogen generation, while two enzymes are involved in the case of Cyanobacteria; hydrogenase and nitrogenase to do the same work of catalyzing the hydrogen generation process. However, this process is not economically viable because it requires large bioreactor surface area, solar conversion efficiency of about 10 % and a large reactor foot-print (Matthew, 2009). Moreover, the oxygen which is generated during the process inhibits the algal hydrogenase(Renea et al., 2007).

2.8.4.7 Photosynthetic Hydrogen Production

Hydrogen is mainly generated here through the action of nitrogenase enzyme via photosynthetic bacteria such as *Rhodabactersphaeroides*. Nevertheless, the presence of oxygen and excess amounts of ammonia inhibits this activity (Harun, 2003). High nitrogen concentration has been linked to high biomass concentration instead of hydrogen production. The higher the biomass concentration, the less light that can diffuse into the bioreactor (Harun, 2003).

2.9 Dark Fermentation

In biological hydrogen production, organic materials are metabolized by bacteria or microalgae actions to produce hydrogen. Biological hydrogen production generates less GHG. Furthermore, it reduced the negative environmental impact of biomass residue, domestic and food industrial waste waters (Hallenbeck, 2009). Dark fermentation is a biological process performed in anaerobic conditions. The bacteria are grown in the absence of light sources under appropriate conditions to produce H_2 from carbohydrate rich substrates. Dark fermentation simply put is the fermentative conversion of organic substrate to bio-hydrogen (Equation 4). The anaerobic degradation of carbohydrates by heterotrophic microorganisms has several important advantages. The advantages include high rates of H_2 production and constant H_2 production (during day and night). Fermentative bacteria have a good growth rate to supply the H_2 into the system. The utilization of agricultural and food industry wastes as resources provides a valuable way to divert these wastes from landfill (Johari et al., 2012).

$C_6H_{12}O_6 + 4H_2O \rightarrow 2\ CH_3COO^- + 2\ HCO_3^- + 4\ H^+ + 4\ H_2$ ---------------Equation (4)

As shown in Fig 2.9, anaerobic breakdown of organic matter are in four stages; hydrolysis, fermentation (or acidogenesis), acetogenesis and eventual methanogenesis(Gerardi, 2003).

Hydrolysis involves the conversion of complex molecules and compounds such as carbohydrates, Proteins and lipids – found in organic matter into simple sugars, amino acids and long chain fatty acids, respectively (Kalinci et al., 2009). Hydrolysis is a relatively slow process and generally it limits the rate of the overall anaerobic digestion process. The second step of the anaerobic digestion process is acidogenesis or acidification, this process results in the conversion of the hydrolyzed products into simple molecules like volatile fatty acids (e.g. acetic-, propionic- and butyric acid) with a low molecular weight, alcohols, aldehydes and gases like CO_2, H_2 and NH_3. The acidogenic bacteria are able to metabolize organic material down to a very low pH of 4 (Valdezand Poggi, 2009).

The third step is acetogenesis. Here, acetogenic bacteria convert the products of the acidification into acetic acids, hydrogen, and carbon dioxide. This process is affected by diverse group of bacteria, majority of which are strictly anaerobes. Luckily for these strict anaerobes, there are always bacteria present that will use oxygen whenever it is available. The presence of these bacteria is important to remove all oxygen that might be introduced into the system, for instance together with the excess sludge.

These three stages are called acid fermentation. It is important to note that in the acid fermentation, no organic material is removed from the liquid phase.

The final step of anaerobic digestion process is methanogenesis. The products of the acid fermentation (mainly acetic acid) are converted into CO_2 and CH_4. After this conversion, the organic material will be removed, as the produced CH_4 gas will largely dissolve from the liquid phase. Methanogens have the ability to produce CH_4 by using the carbon dioxide and hydrogen gas or the acetic acid produced from both the acetogenic or acidogenic phases. Dark Fermentation is meant to either inhibit or slows down this methanogens and harvest the hydrogen gas.

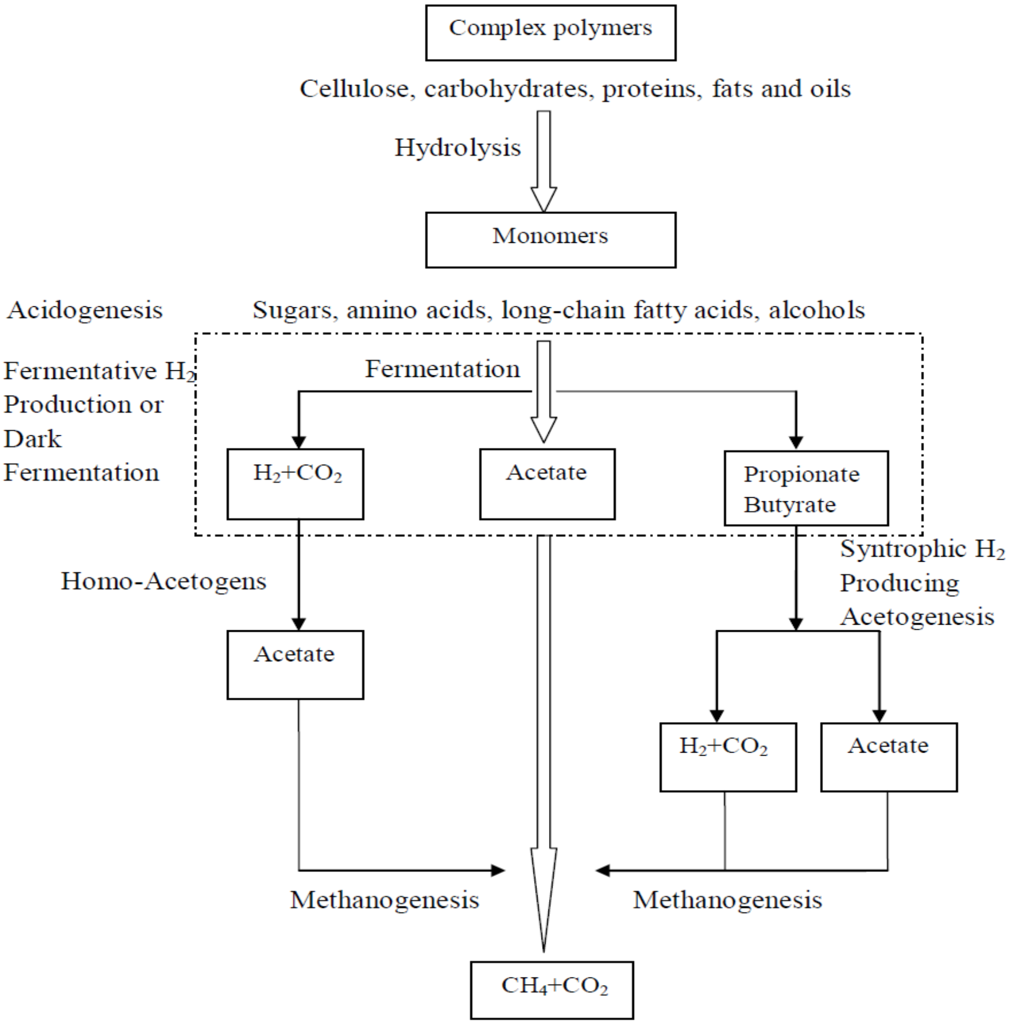


Figure 2.9 Diagram of anaerobic digestion and dark fermentation.

Source: (Basu et al., 2010)

Anaerobic or dark fermentation is one of the most widely studied techniques of producing bio-hydrogen. Anaerobic fermentation is known for its rapid hydrogen evolution rate and does not require large surface area or any external energy. Dark fermentation also yields other metabolites such as H_2 and electricity production, which can be further processed through microbial fuel cells (Logan and Regan 2006).

There are many factors that affect hydrogen production such as microbes, temperature, pH, type of substrate, reactor type among others.

2.9.1 Microbes

Inoculum sources for fermentative hydrogen production can be classified into two groups: mixed bacteria cultures from natural sources and pure cultures from bacterial culture collections. In the natural environment such as soil, wastewater sludge, compost among others, hydrogen producing bacteria widely exist (Kalogo and Bagley, 2008). Nevertheless, *Clostridium* and *Enterobacter* are most widely used as inoculum for fermentative hydrogen production. Bacteria of the genus *Clostridium* form endospores and are gram-positive, rod-shaped and strict anaerobes, while those of the *Enterobacter* species are gram-negative, rod-shaped, and facultative anaerobes (Kalogo and Bagley, 2008; Kraemer et al., 2007). Some other natural sources that has been used to provide inocula for H_2 production by mixed micro flora, includes; biosolid and biosolid pellets (Fang et al., 2006; Kalogo and Bagley, 2008; Keigo and Shigeharu, 2006). In order to eliminate methanogens and select spore formers, inocula are commonly pretreated. There are various pretreatment methods such as heat treatment, acid and alkali pretreatment (Jayalakshmi, 2007; Kimet al., 2013; Kraemer et al., 2007).

Heat treatment selects endospore forming bacteria, such as *Clostridium*, *Bacillus* and Thermo-anaerobacterium. However, it inactivates H_2-consuming methanogens and prevent consumption of the produced H_2. Nevertheless, a low pH of 5.5 has been reported to control methanogens (Fang et al., 2006; Kraemer et al., 2007; Mizuno et al., 2000).

Alkali-treated sewage sludge (SS) was used as a microbial source for H_2 fermentation of food waste leachate (FWL) and the highest H_2 yield of 2.1 mol H_2/mol hexose was achieved at pretreatment pH 10 and a mixing ratio of FWL to SS = 3:5 (Kim et al., 2013). It was also found that pretreatment pH 9 was not strong enough to suppress the activity of lactic acid bacteria (LAB) which are the non-H_2-producers in SS. Moreover, microbial analysis showed that LAB such as *Lactobacillus* sp. and *Enterococcus* sp. was the dominant species at pretreatment pH 9 while *Clostridium* sp., the main anaerobic H_2-producers, were dominant at pretreatment of pH 10 (Kim et al., 2013).

Aged refuse (AR) excavated from a typical refuse landfill with over 10 years of placement was also used for the augmentation of bio-hydrogen production from food wastes. It was found that below 0.4% of hydrogen concentration could be detected in the biogas produced due to its severe acidification properties. However, the hydrogen concentration in the biogas increased to over 26.6% with pH ascending from 4.36 to 5.81 when AR (50% in weight) was added. Meanwhile, it was also found that sterilizing the AR by heating at 160 °C for 2 h before being used as additive for bio-hydrogen production from food wastes decreased the hydrogen content in the biogas drastically to 3.3%. This signifies that the AR may chiefly function as a microbial inoculum (Li et al., 2008).

Food and microbe (F/M) ratios were analyzed in a two stage process and it was found to influence biogas yield, production rate, and potential. The highest H_2 and CH_4 yields of 55 and 94 mL g^{-1} VS in two stage process and the highest CH_4 yield of 82 mL g^{-1} VS in one stage process was observed at F/M of 7.5 (Nathao et al., 2013).

2.9.2 Temperature

Temperature is one of the most important factors that influenced the activities of hydrogen-producing bacteria and the fermentative hydrogen production. Akutsu et al, (2009) has shown that different organisms require different temperature range. Some organisms are better at very cold environment, while others are better at very hot environment and the rest are better in between, popularly called moderate organisms (Akutsuet al., 2009). Organisms can be classified as psychrophilic, mesophilic or thermophilic depending on the environment they can survive in. It is also important to note that these organisms have some adaptive features which they possess that make it possible for them to survive in any of these temperatures. Therefore, if they are removed from their original environment to another, they may die.

It has been validated that in an appropriate range, the ability of hydrogen-producing bacteria to produce hydrogen could increase with increase in temperature. Nonetheless, temperature at much higher levels could decrease hydrogen production with increasing levels of temperature (Linet al., 2008). However, the optimal temperature reported for fermentative hydrogen production has not always been the same, but it fell into the mesophilic range (around 37^0C) and thermophilic range (around 55^0C), respectively (Akutsu et al., 2009; Harun, 2003; Jingwei, 2008; Kim et al., 2013; Lin et al., 2008).

In studying the hydrogen production from food waste by the mesophilic and thermophilicacidogenic culture acclimated with sewage at 5 days HRT, it was shown that the biogas produced from the thermophilicacidogenic culture was free of CH_4 at all tested pH and VS concentrations (Shin et al., 2004). It was also shown that from the mesophilicacidogenicculture, CH_4 was detected at all tested pH. However, the amount of hydrogen produced from the thermophilicacidogenic culture was found to be much higher than that from the mesophilic culture at all tested pH. This could be due to CH_4 free condition and negligible propionate production. Increase of VS concentrations from 3 to 10 g VS^{-1} caused an increase in quantity and quality of hydrogen produced. The maximum hydrogen content was 69% (v/v) at 10 g VS^{-1}. The hydrogen yield was in the range of 0.9–1:8 mol-H_2 mol-hexose with the peak at 6 g VS^{-1} (Shin et al., 2004).

2.9.3 pH

Another important factor that influenced bio-hydrogen production is the pH because it affects both the metabolic pathway and also the activities of the hydrogenase. Most studies done on this were done using a batch reactor therefore only the initial pH were of major concern. It has been shown that in an appropriate range, the ability of hydrogen-producing bacteria to produce hydrogen during fermentative hydrogen production increased with increase in pH, nevertheless at much higher levels of pH, the hydrogen producing ability of the bacteria decreased (Fang et al., 2006; Herbert, 2002; Kim et al., 2009; Masset et al., 2010). Setting the initial pH dictates a delicate balance between obtaining optimum conversion efficiency, and acquiring the fastest rate of hydrogen production. A pH value outside of the acceptable range can inhibit hydrogen production by altering bacteria’s metabolism or cause a microbial population shift (mixed inoculum culture) bringing about a termination in hydrogen production and as such, reliable pH control is crucial.

A study with an initial pH of 5.5 has the highest hydrogen production rate of 2.90 mmolH_2/d, at 90 gTS/L, using food waste from cafeteria as substrate (Carlos, 2012). Using sugar cane bagasse hydrolysate, the optimum pH for hydrogen production was found to be

5.5 at a rate of 1611 mL H_2/L/day (Sakchai et al., 2008). Generally, it has been shown that initial pH has a significant effect on both the yield and rate of hydrogen production.

2.9.4 Substrate

In the quest to know which organic substrate will yield more hydrogen, many research have been carried out. Mostly used substrates are glucose, sucrose and starch. It has been demonstrated that in an appropriate range, increasing the amount of substrate could enhance the hydrogen-producing bacteria's ability to produce hydrogen during fermentative hydrogen production. Just like the temperature and pH, substrate concentrations at much higher levels could decrease hydrogen production with increasing substrate levels (Baron, 1996; Yasin et al., 2013; Kimia, 2013; Hori et al., 2005). For example, Akutsu et al, (2009) showed that there was 18 % decrease in hydrogen yield when substrate concentration was increased from 15 g/l starch to 20 g/l-starch. Furthermore, the study also revealed no change in hydrogen yield when substrate concentration was increased from 50 g/l to 70 g/l.

2.9.5 Reactor

There are three major reactors used globally for anaerobic fermentation. Such as serum batch reactors, continuous stirred tank type bioreactor (CSTR) and an up-flow anaerobic sludge blanket bioreactor (UASB). See plate 2.1 and 2.2

Hariklia et al., (2006) did a study to examine and compare the biological fermentative production of hydrogen from glucose in a CSTR and an (UASB) at hydraulic retention times varying from (2–12 h HRT) under mesophilic conditions (350C). It showed the UASB reactor configuration to be more stable than the CSTR as regards to hydrogen production, pH, glucose consumption and microbial by-products, such as, volatile fatty acids, alcohols etc at the tested HRTs.

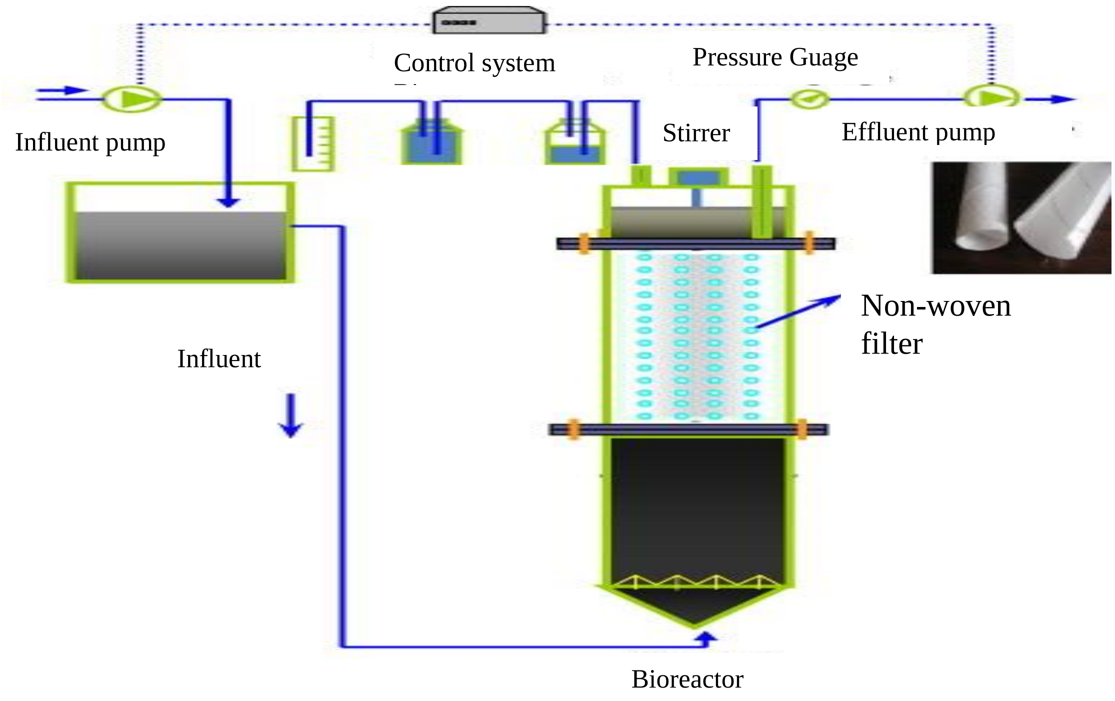


Plate 2.1 Continuous Stirred tank Bioreactor. Source: (Heidrich and Witkowski, 2010)

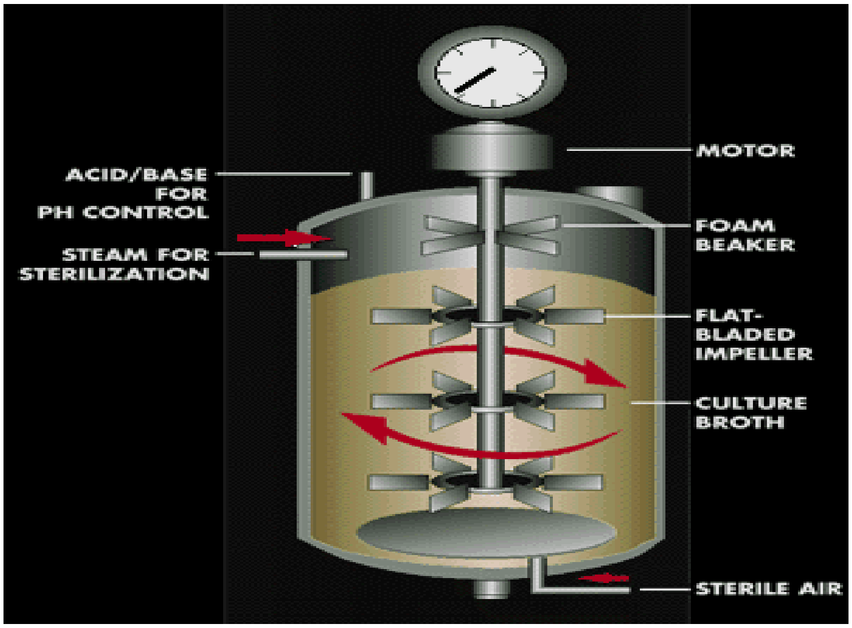


Plate 2.2 Up-flow sludge blanket bioreactor. Source : (Heidrich and Witkowski, 2010)

Tawfik and El-Qelish (2012) showed that the rate of hydrogen production in the UASB reactor was significantly higher compared to that of the CSTR at low retention times (19.05 and 8.42 mmole H_2/h/l, respectively at 2 h HRT). It also revealed that a higher hydrogen yield was found at the CSTR reactor at all tested HRT (Hariklia et al., 2006; Tawfik and El-Qelish, 2012).

Furthermore, it has been demonstrate that cassava and food waste could be ideal substrates for bio-hydrogen production using a two-step process combining dark fermentation and photo-fermentation. The average yield of hydrogen was approximately 199 ml H_2 g^{-1} cassava and 220 ml H_2 g^{-1} food waste in dark fermentation (Zong et al., 2009). The average yield of hydrogen from the effluent of dark fermentation was approximately 611 ml H_2 g^{-1} cassava and 451 ml H_2 g^{-1} food waste in subsequent photo-fermentation. A combination of the two has a total hydrogen yield of 810 ml H_2 g^{-1} cassava and 671 ml H_2 g^{-1} food waste (Zong et al. 2009).

2.9.6 Acclimatization

Acclimatization in its simple form can be defined as the process in which an organism adjusts to a gradual change in its environment so as to maintain performance across a range of environmental conditions (Eroğlu., 2006). This could be a change in temperature, humidity, or pH. In response to these changes, organisms can change the biochemistry of cell membranes. Specific proteins called heat shock proteins which help the cell maintain function under extreme stress may also be expressed by organisms. This adjustment ranges from days to couple of months depending on the environmental condition.

It has been observed that acclimatization plays an important role in enhancing bio-hydrogen production. For example, a comparative evaluation of anaerobic digester sludge (ADS) and acclimatized anaerobic digester sludge (AADS) for bio-hydrogen production was done by Nasr et al., (2011), it was found that a maximum hydrogen yield of 19.5 L H_2/L and 7.5 L H_2/L thin stillage was achieved for the AADS and ADS respectively (Nasr et al., 2011).

Another study conducted in a Sequential Batch Reactor with a pH of 5, a temperature of 35 °C was done to show the effect of acclimatization with sewage sludge. It was shown that acidogenic microorganisms which plays a major role in initiating hydrogen production increased from 0.160 h^{-1} to 0.125 h^{-1} during the acclimatization process. However, facultative microorganisms remained constant during the acclimatization process (Fernández et al., 2010).

Hydrogen production by anaerobic fermentation bacteria was investigated in a three-compartment anaerobic baffled reactor (ABR) by Li et al., (2007). The study showed that H_2 yields in the 1st compartment was lowest with the longest acclimatization period. The 2nd and 3rd compartments were found to have higher hydrogen yields but shorter acclimatization durations (Li et al., 2007).

2.9.7 Metal Ion

It has also been observed that the metal ion present in substrates may inhibit the activity hydrogen-producing bacteria especially if not in a trace level. It is well known that low concentrations of heavy metals such as magnesium (Mg), molybdenum (Mo), manganese (Mn), iron (Fe), and others are necessary for the growth of purple bacteria

(*Rhodobactersphaeroides). R. sphaeroides* produce H_2 under reducing conditions upon the drop in redox potential, which could determine electron transfer within bacterial membrane and generation of proton motive force (Hakobyanet al., 2012).

Fe, Mo and nickel (Ni) have been shown to be component of several enzymes involved in H_2 metabolism in photosynthetic bacteria such as nitrogenase and membrane-bound uptake hydrogenase.

It has been shown that Mg^{2+} aids more in hydrogen production than Fe^{2+}, Cu^{2+}, Zn^{2+} and Ca^{2+}(Wang and Wan 2009). It does so by activating almost 10 enzymes including hexokinase, phosphofructokinase and phosphoglycerate kinase during glycolysis process (Voet et al., 1999). The key enzyme for hydrogen production is hydrogenase which requires ferrodoxin formed from Iron. Thus, iron is an essential element in hydrogen production process (Nicolet et al., 2002).

However, Zhao et al., (2012) reported that the effects of metal ions on H_2 production by *C. beijerinckii* RZF-1108 was complicated. They also reported a maximum H_2 yield of 1.96 mol H_2/mol glucose and production rate of 106.0 ml H_2/l medium·h^{-1} using optimized culture medium supplemented with 0.2 g/l $FeSO_4 \cdot 7H_2O$ and 0.1 g/l $MgCl_2 \cdot 6H_2O$.(Zhao et al., 2012).

Bao et al, (2013) reported that the addition of Fe^{2+} and Mg^{2+} and L-cysteine has a higher H_2 yield than the control. Furthermore, the study showed an enhancement of the H_2 production by the sole addition of Fe^{2+} and L-cysteine was significant (by 105% and 60%, respectively). The sole addition of Fe^{2+} to the system had the highest effect with a maximum cumulative H_2 production of 1928 mL and H_2 yield of 1.94 mol H_2/mol glucose (Bao et al., 2013).

2.10 Gompertz Kinetic Model

Gompertz Kinetic model has been used to describe the progress of hydrogen production. For example Mu et al., (2007) used it to describe the growth of hydrogen-producing microorganisms, consumption of substrate and formation of product in this work. According to Das and Debabrata (2012), it was found that the modified Gompertz kinetic model was the most suitable to describe the progress of biohydrogen formation process. The hydrogen production yield and rate is dependent on the experimental conditions such as temperatures, pH, substrate etc (Mu et al., 2007).

Previous researches have been conducted using this model. Kim et al (2011) showed both the highest H_2 yield of 1.79 mol H_2/mol hexose and a production rate of 369.1 ml H_2/L/h were observed at 50^0C (Kim et al., 2011). Under standard temperature and pressure, Nazlina et al (2011) used the gompertz model to show that the bio-hydrogen production potential obtained from fermentation broth at controlled pH values of 5.0, 5.5 and 6.0 were 129 NmL, 444 NmL and 426 NmL, respectively.

Nathao et al., (2013) showed that the highest rate of hydrogen production to be at food 17.9 ± 2.7 (mL/h) at food to microorganism ratio of 7.5 (Nathao et al., 2013). Tawfik and El-Qelish (2012) found that H_2 production remained at the same level of 5.3 ± 1.04 L H_2/d at increasing the organic loading rate from 36 to 47 g CODtotal/L d. Under a controlled fermentation pH of 5.5, Hydrogen production rate and yield were about 108 mL/L·h and 128 mL/g $COD_{degraded}$ respectively (Jong et al., 2008)

2.11 Food Waste

In order to assess how feasible it is to use food waste as a means of feeding anaerobes for bio-hydrogen production, it is necessary to note the unique potentials.

2.11.1 Potential Environmental Benefits

Using Malaysian scenario, where about 50 % of 33,000 tonnes of MSW produced per day is food waste (Agamuthu, 2014). Food waste therefore is the largest component of the Malaysian MSW stream. It will imply that about 16,500 tonnes of food waste goes to the landfill daily. Thus food waste has become a problem in Malaysia and as such, diverting this organic component of MSW can serve to greatly reduce landfill loading rates. Furthermore, this diversion has a high potential of greatly expanding the service life of landfill.

As food waste decomposes in landfills, it is typically degraded into both CO_2 and CH_4 gas and emitted into the atmosphere. Sanitary landfills have gas collection systems to control these emissions while non-sanitary landfills do not. The ability to capture and collect CH_4 gas produced from food waste decomposition provides a significant means of reducing our overall GHG emissions. However, any technology that will reduce this CO_2 and CH_4 emission will be saving the environment a great deal of harm (IPCC, 2007).

Furthermore, from a purely space conservation standpoint, it makes sense to prevent the transportation of food waste to landfills since a significant portion of the MSW stream in Malaysia is food waste. Siting and operating landfills is a large and complex undertaking, therefore diverting any portion of it the refuse being sent to them will not only increase their lifespan but will also reduce those problems associated with the decay of the organic components disposed of, including odors and unwanted pest attraction, it will also reduce the cost of leachate treatment (Abdul Rahman, 2013).

2.11.2 Financial Considerations

There are other opportunities which will compete with food waste anaerobic digestion (AD) as recycling options. These include using food wastes as soil amendment through composting or using food waste as an animal feed. Economically speaking, the AD of food waste may not be the most preferred option financially for its ultimate food waste disposal. For example, in the United States, Disney World converts a great portion of its food waste into animal feed, and the product has even been approved for human consumption by the United States Department of Agriculture (Jaworski, 2007; Smith, 2010). Nevertheless, in constructing any AD plant, some factors are taken into consideration. These factors include space requirements of the facility, water demand, quality and quantity of wastewater discharged, the quality and quantity of the digestate residual, electricity use and electricity production and the local biogas markets (Rajendran et al., 2012).

Johari et al.,(2012) estimated that based on 8.2 million tonnes MSW generated in Peninsular Malaysia in 2010, about 310,220 tonnes per year of CH_4 will be emitted (Johari et al. 2012). This was further estimated to generate about 1.9 billion kWh of electricity per year worth over RM 570 million (US$ 190 million). Furthermore, about 6,514,620 tonnes year^{-1} of CO_2 will be reduced which is equivalent to carbon credit of over RM 257 million (US$ 85 million). Converting waste to energy could be economically viable, depending on factors such as cost of production etc.Factors such as acceptable rates of revenue from carbon credits, sales of renewable energy back to local power utilities or private purchasers, and tax incentives will determine the financial success of any food waste digestion enterprise (Lee and Chung, 2010). Organizations like the California Climate Action Registry (CCAR) serve as an exchange for carbon credits. A protocol was listed in 2009 for the digestion of organic waste which would also apply to an AD facility for food waste which attempts to earn revenue through carbon credits (Chum et al., 2011).

Companies who wish to offset their GHG (GHG) emissions will have to be certified by this organization before buying carbon credits. There are often set rates (usually on a kilowatt-hour (kWh) basis) by different utility companies at which they are willing to purchase renewably produced electricity. There is also need for different restaurant and food service industries to process and separate their waste before disposal. Without this source separation, additional operational costs will be incurred in AD of food waste in trying to separate this waste. Based on economic evaluation, two-phase hydrogen and CH_4 fermentation was found to have a greater potential for recovering energy than CH_4-only fermentation (Lee and Chung, 2010).

Hydrogen production technology will reduce the cost of maintaining a landfill because the waste will have little organic in it after diversion. It will also reduce CH_4 emission into the atmosphere. This will in turn reduce global warming and its adverse effect. It is therefore necessary that studies are conducted on how to commercialize hydrogen production.

The current study was conducted to increase the knowledge base on the conditions needed for hydrogen production from food waste.

CHAPTER 3

MATERIALS AND METHODS

3.1 Food Waste Preparation

The food waste substrates were collected from the University of Malaya cafes. It was further separated manually into the different components to remove bones, papers, tissues, plastics etc (Plate 3.1). A blender (super blender, model PB – 326) was used to grind the separated food waste in the laboratory.

The grinding was to increase the surface area which speeds up the rate of reaction and enhance microbial activities. It was then sealed in sterile plastic bags and stored in the freezer at 4^oC prior to use. The food waste and the anaerobic sewage sludge were both thawed before they were used for the experiment.

3.2 Sludge Preparation

Wet anaerobic sewage sludge used as seed sludge in this study was obtained from an anaerobic digester in Pantai Dalam sewage treatment plant, Kuala Lumpur, Malaysia. After collection, the sludge was transported to the laboratory and stored in a refrigerator prior to use for experiments at 4^oC. Before the experimental set up, the anaerobic sewage sludge was thawed to room temperature. It was sieved with 2 mm sieve to remove stones (Chyi-How et al., 2010). It was then pre-heated at 80^oC for 15 minutes. This was to inhibit the bioactivity of methane forming bacteria and other pathogenic microbes and also to promote the growth of hydrogen producing bacteria.

Plate 3.1: Food waste Collection and Separation

3.3 Alkalinity Determination of Sewage Sludge

Dry Na_2CO_3 (2.5 ± 0.2 g) was weighed out using a weighing balance. This was transferred to a 1-L volumetric flask. The flask was filled to the mark with distilled water. The dry Na_2CO_3 dissolved in the water forming a standard solution (0.05N).

Standard sulfuric acid 0.1N was prepared and standardize against 40.00 mL 0.05 N Na_2CO_3 solution, with about 60 mL water, in a beaker by titrating potentiometrically to pH ofabout 5. After titration, the electrodes were lifted out and rinsed into the same beaker, then the titrate was boiled gently for 3 to 5 min under awatch glass cover. It was further cooled to room temperature. The normality was calculated.

3.4 Volatile fatty acids in Sewage Sludge

Volatile fatty acids (acetic acid, propionic acid, n-butyric acid, isobutyric, valeric acid, iso-valeric acid) were quantified using a Clarus 500 Gas Chromatography (PerkinElmer®). The measurement follows the method of Kraemer and Bagley (2008). The oven temperature of the headspace sampler was set to 85°C. The sample vials went through six steps: equilibration (about 1 minute), thermostatting (30 min), pressurization (1.5 min), injection (less than 1 min), withdrawal (less than 1 min) and venting (less than 1 min). The gas sample was transferred to the Gas Chromatography (GC) by transfer line (105°C), and then analyzed by GC with a Zebron free fatty acid phase (FFAP) column (30 mm×0.32 mm×0.25 μm) and flame ionization detector (FID). The oven temperature program of GC started at 45°C for 1.8 minutes, and then ramped to 140°C at 45°C/min, was kept at 140°C for 2 minutes and finally ramped at 10°C/min to 166°C. The temperature of the FID was 250°C. The gas flow rate of air (Zero Grade) was 450 ml/min, the gas flow rate of H_2 was 45mL/min and the gas flow rate of N_2 was 2 ml/min. For volatile fatty acids, the retention times were: 4.619 minutes for acetic acid, 4.98 minutes for propionic acid, 5.462 minutes for n-butyric acid, 5.06 minutes for isobutyric acid, 6.2 minutes for valeric acid, and 5.67 minutes for isovaleric acid.

3.5 Ammonical Nitrogen in Sewage Sludge

Ammonia concentration was measured by using Method 10031 (Salicylate method) from the HACH company. Each sample was analyzed in triplicate. Liquid samples were filtered through 0.45 μm membrane filter (Millipore Express® PLS 0.45μm, 25mm in diameter), and the filtrate was used in the ammonia measurement. Appropriate dilution with distilled water was conducted to get the measured values within the detection range (0.4-50.0 mg/L NH_3-N).

3.6 Total Suspended Solids (TSS) of Sewage Sludge

A well-mixed sewage sludge was filtered through a weighed standard glass-fiber filter and the residue retained on the filter was dried to a constant weight at 103 to 105°C. The increase in weight of the filter represents the total suspended solids. The measurement of TSS followed Method 2540 D in Standard Methods (APHA, 2005).

3.7 Batch Fermentation

3.7.1 Acclimatization of food waste with Sewage Sludge

Acclimatization was done by mixing 100g of food wastes substrates with 100 mL thawed anaerobic sewage sludge using a sterile 250 mL conical flask. The conical flask was covered with aluminum foil (Plate 3.2) and then transferred into an incubator (Memmert 854 Schwabach W- Germany) for 31 days at 37^0C. 250 mL serum bottle used as the fermenter was washed clean, sealed with an aluminum foil, and then autoclaved to sterilize the medium. Using a measuring cylinder (150 mL), 30 mL of wet acclimatized food waste were then inoculated into the sterile serum bottle (sterilized using Hirayama HVE – 50 autoclave). 8g of each blended food waste substrate was weighed out using an electronic weighing balance (mark Bel 500, capacity 500,000g) with the weight of a crucible and added to the batch reactor. The crucible was weighed dry and the weight was noted. The electronic weighing balance was calibrated before the individual weighing out of 8g was made. 50 mL of anaerobic sewage sludge heated (pre-treatment) at 80^0C for 15 minutes was added to the mixture. The working volume was brought to 150 mL by adding distilled water. The initial pH was corrected to 4.0, 5.5 or 6.0 using 1N sodium hydroxide (NaOH) and 1N sulphuric acid (H_2SO_4). The pH meter (model: EL 20, mettle-toledo AG 8603 Schwerzenbach Switzerland) was used to measure this pH. The correction was done by first knowing the pH of the samples, and then NaOH or H_2SO_4 were added in drops simultaneously. The mixture was then stirred until the desired pH was obtained. The batch reactors were then corked with a septa and an aluminum seal using a crimper in order to make the batch reactors air tight. To maintain an anaerobic condition the headspace of the batch reactors were filled with pure N_2 gas. Mixing was done manually twice a day. Each experimental condition was carried out in triplicates. The batch reactors were then placed in a water bath (model: baird and Tatlock, made in England) and monitored at temperature range of 27^0C, 35^0C and 55^0C.

Plate 3.2: Acclimatized Food Waste Substrates

3.7.2 Non Acclimatization

Here, 8g of the blended food waste substrate was mixed with 50 mL of thawed anaerobic sewage sludge without adding any incubated mixture. The mixture was added into a 250 mL serum bottle (Plate 3.3). The working volume was adjusted to 150 mL using distilled water and the initial pH was corrected to pH values of 4.0, 5.5 and 6.0. The sludge was pre-heated in a 500 mL volumetric flask at 80°C for 15mins before mixing it with the food waste substrates. The head space of the bottle was also filled with N_2 gas. This is to make the medium completely anaerobic.

Plate 3.3 Food Waste in a 250 mL serum Bottle

After conditioning, the batch bottles were then placed in a water bath at 27, 35, and 55 ± 2°C till the end of the experiment. Attached to a transfusion tube was a transfusion needle at one end. The other end was bored into rubber cork at the mouth of a conical flask full of water. This same open end was made to touch the bottom of the conical flask. This was to ensure proper water displacement. The rubber cork was further made air tight using a sealant. The conical flask also has an outlet transfusion tube which also touches its bottom. This outlet transfusion tube conveys displaced water out of the conical flask. The displaced water was collected in another conical flask and measured using a measuring cylinder as shown in Fig 3.1. The amount of biogas produced was measured by the amount of water displaced as shown in plates3.4 and 3.5. Thus, the amount of water displaced was used to calculate biogas production and the amount of hydrogen gas produced was detected by the gas chromatography (GC, shimadzu 8A).

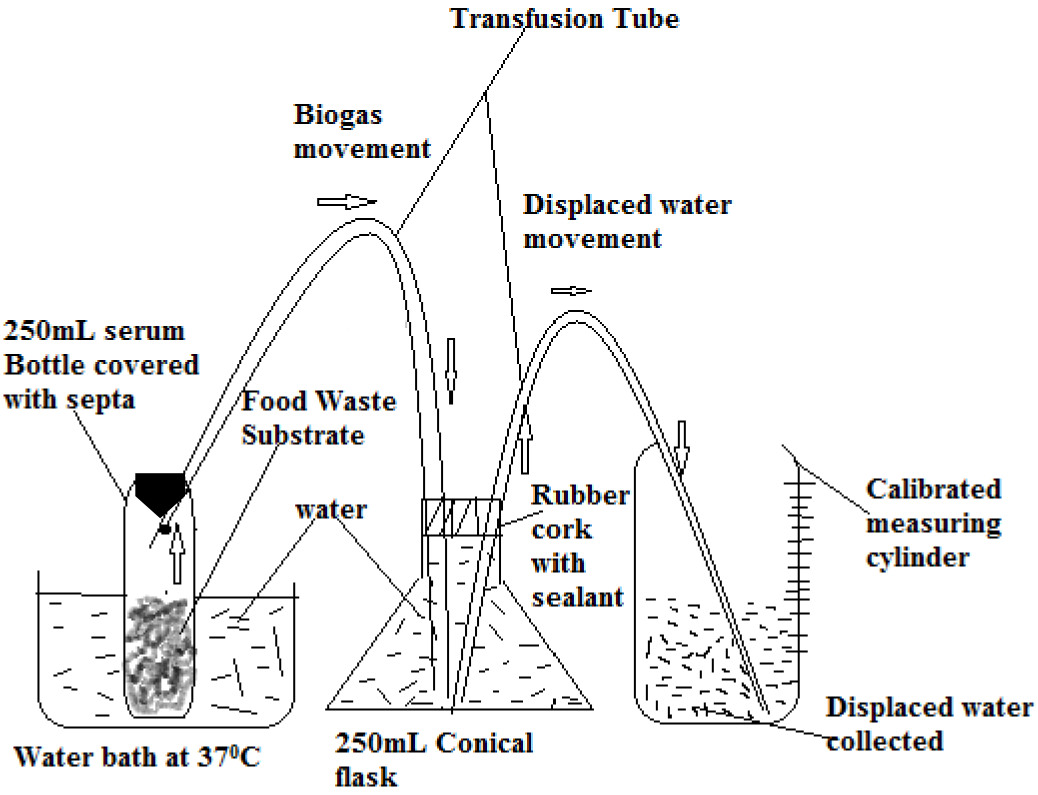


Figure 3.1 Schematic Diagram of Biogas Production

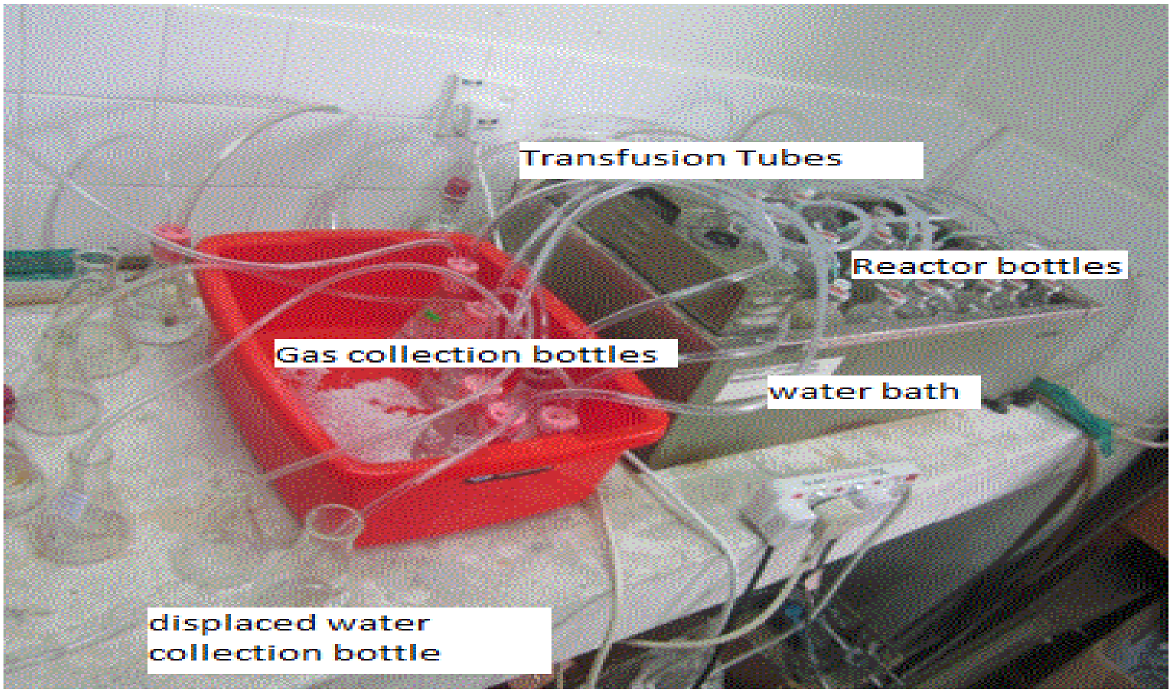


Plate 3.4 Biogas Production and water displacement

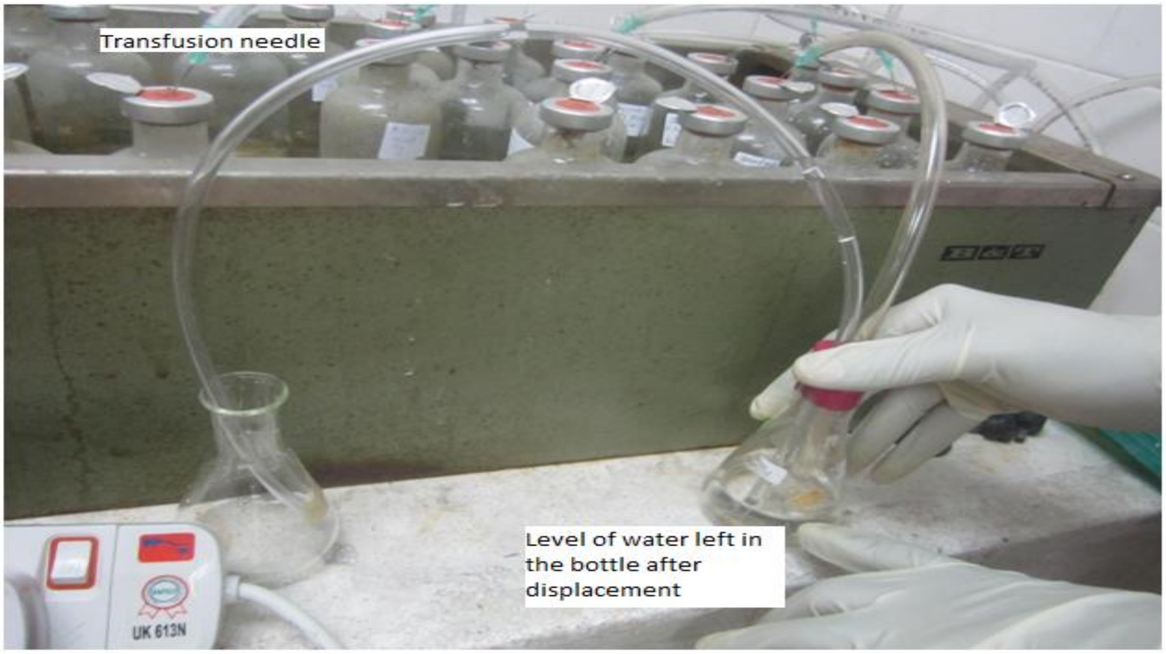


Plate 3.5 Level of Water displaced for Biogas Measurement

3.8 Effect of Metal Ions on Bio-hydrogen production

Furthermore, a study to know the effect of heavy metal ions on bio-hydrogen production from food waste substrates was conducted. This was because it is possible that batteries (containing lead (Pb)) could be found in the MSW stream.

8g of mixed food waste was weighed with an electronic weighing balance (mark Bel 500, capacity 500,000g) and added into a 250 mL serum bottle (batch reactor). Using the weighing balance, 5mg, 10mg and 15mg lead (Pb) were weighed out in a crucible and diluted with 1000 mL of deionized water. This mixture was carefully transferred into the serum bottles. 50 mL of acclimatized anaerobic sewage sludge was then added using a measuring cylinder to the reactor. Then distilled water was used to get a working volume of 150 mL. The pH was then corrected to 5.5 using 1N NaOH or 1N H_2SO_4. As usual, the reactor was purged with N_2 gas and put in a water bath at 35^0C. A control was set up alongside having no metallic ions in it. These experiments were conducted in triplicates to minimize experimental error.

3.9 Column Experiment

A column of 30 cm having only one outlet when sealed with a sealant was used to investigate the effect of a slightly bigger reactor on bio-hydrogen production. The column has two ends, the bottom was permanently sealed while the upper end was open for food waste substrate addition before sealing was done. A tap was tightly fitted just before the open end for biogas sampling and analysis (Fig 3.2). 24g of the food waste was added into the column, 5 mg, 10 mg and 15 mg of lead was weighed out with an electronic weighing balance. It was further diluted with 1000 mLs of deionized water and added into the column from the open end. The column size was 3 times the bottle size, therefore for easy comparison, the amount of substrates used were trice that used in the bottle experiments. 150 mL of heat treated anaerobic sewage sludge was then added to the mixture in the column, 150 mL Acclimatized anaerobic sewage sludge was also added, then distilled water was further added to get the working volume of 750 mL. The reactor was shaked vigorously and the pH corrected to 5.5 using 1N NaOH or 1N H_2SO_4. The air in the reactor was sucked out using a vacuum machine. N_2 was used to purge the system to make it anaerobic. The set up was then put in an incubator at 35°C ± 2°C. Daily measurement was conducted to measure the amount of biogas produced through water displacement. This was done by carefully placing a transfusion tube over the tap on the column. Then the free end of this transfusion tube was connected through a rubber cork into a conical flask full of water. The conical flask also has an outlet transfusion tube. This tube conveys the displaced water into an empty conical flask where the water was collected and measured using a measuring cylinder as shown in Fig 3.2. The amount of biogas produced equals the amount of water displaced.

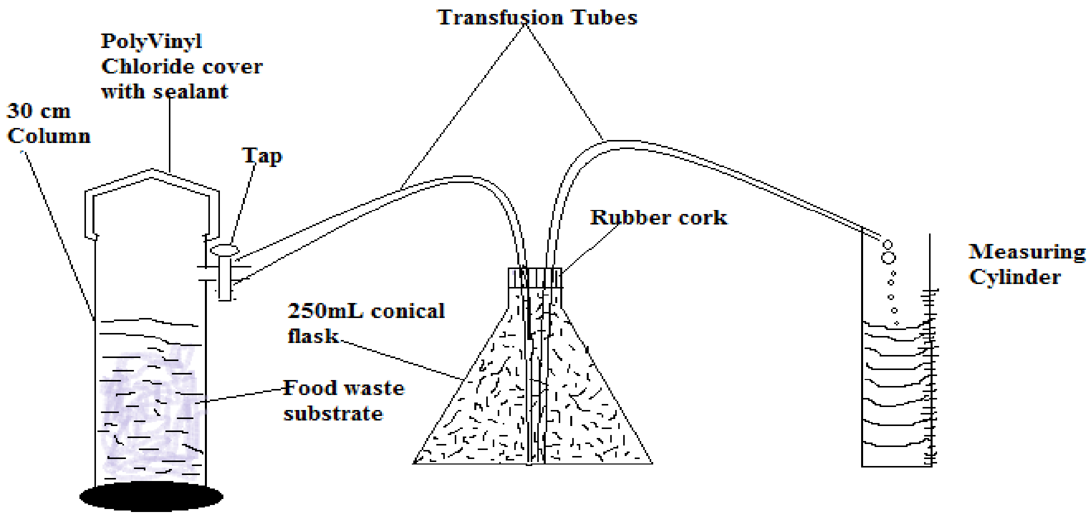


Figure 3.2 Schematic Diagram of Column Experiment on Biogas production

3.10 Moisture Content of Food Waste

The mass of the substrates were determined using an electronic weighing balance. In order to determine the characteristics of the food waste, a porcelain dish was weighed dry and the weight noted, then about 20 g of each substrate was added to it and the total weight was also noted. The dish containing the wet sample was then put in a desiccator for a day to avoid absorbing extra moisture from the environment. It was then put into an oven at a temperature of 105^0C for 24 hours. The dry samples were then brought out from the oven and weighed; the difference in weight was recorded as the moisture content of each sample.

3.11 Biogas Analysis

The volume of biogas production in each batch reactor was measured and recorded through the water displacement method. Biogas sampling began as soon as biogas was produced in the reactor. One mL of the biogas was taken with the 1mL gas tight syringe (model 1MR – GT, M04 – C3985) from the batch reactors. It was then injected into a gas chromatography (GC Shimadzu 8A) with thermal conductivity detector to analyze the H_2 content. Helium gas was used as the carrier gas at a flow rate of 60 mL/min. The injector, detector and column operated at 160^{o}C, 130^{o}C and 130^{o}C respectively (Plate 3.6). Pure hydrogen gas was used for calibration.

The hydrogen production potential and the rate of production were analyzed using the modified Gompertz kinetic model (Zwietering et al., 1990).

$$H(t) = P\,.\,\exp[-exp\{\frac{Rm\,.e(\lambda - t)}{P} + 1\}]\text{………………….(6)}$$

Where H (t) is cumulative hydrogen production (mL), P is hydrogen production potential (mL), R_m is maximum hydrogen production rate (mL/d), e = 2.71828, ƛ is the lag phase (d) and t is the time (d).

Plate 3.6: Gas chromatography (GC) used for biogas analysis.

3.12 Statistical Analysis

Statistical analysis were carried out using Microsoft excel software because the data to be analyzed was not very large. To compare the amount of gas produced from each substrate, a one way single factor ANOVA was used. One way single factor ANOVA was also used to compare the mean values in all four substrates (rice versus fish, rice versus vegetable, rice versus mixed, mixed versus fish, mixed versus vegetable, fish versus vegetable). This was to determine their significance. The level of statistical significance was set at 5% Post-hoc analysis and 95% confidence level.

The Kuskal Wallis non parametric test was used to compare the amount of cumulative biogas production produced by all four substrates between the acclimatized and non-acclimatized food waste substrate. The level of statistical significance was set at 5% Post-hoc analysis and 95% confidence level.

One Way factor ANOVA was also used to compare the amount of biogas and hydrogen produced by the food waste containing metallic ion (Pb) at different concentrations.

Mean values were used in graphical representations.

CHAPTER 4

RESULTS AND DISCUSSION

This chapter discusses the effect of temperature and pH on bio-hydrogen production using food waste substrates. Gompertz kinetic model was used to determine the rates and potentials of hydrogen production. The model also identifies the substrate that has the highest H_2 production rate.

4.1 Composition of Food Waste

Composition of kitchen waste used in this study is given in Table 4.1. The notable characteristics of the food waste were their high moisture content (70% - 80%). This definitely forms leachate when dumped in a landfill. The food waste substrates used in this study were obtained from different batches collected at different times. The composition of the different batches showed that the standard deviation was low. TS and TSS represented the solid content in food waste substrates. The large amount of TS and TSS might be due to the varieties of leftover raw and cooked food, as well as, the peels of fruits and vegetables. The pH became acidic probably because upon dark fermentative transformation, hydration of glucose molecule elucidates a simultaneous formation of acetic acid and hydrogen (Yasin et al., 2013).

This result is similar to previous studies by Han and Shin (2004), Zhang and Wang (2005), where the moisture content of food waste used for biogas production was 70 % and 79 % respectively. However, the current study has higher TS and lower pH when compared with that of Han and Shin (2004). This might be due to the different sources of food waste collection(Ruihong, 2007).

Table 4.1 Characteristics of Food Waste used

Food Waste Substrates	**pH (initial)**	**pH (final)**	**Total Solid(TS) (g/l)**	**Total Suspended Solid(TSS) (g/l)**	**Moisture (%)**
Rice	5.9±0.22	4.3±1.22	100.50±0.52	70.42 ± 1.22	80 ± 2.25
Vegetable	5.2±0.24	4.5±0.34	102.04±0.32	86.32 ± 2.34	72 ± 1.76
Fish	5.0±0.21	4.0±0.42	98.32 ± 0.72	87.42 ± 2.50	70 ± 1.64
Mixed	5.2±0.22	4.2±1.36	110.21±0.68	88.51 ± 1.25	70 ± 2.34

ASS means Anaerobic Sewage Sludge

4.1.1 Characteristics of Anaerobic Sewage

The sewage sludge characteristics are subject to change depending on its nature and treatment of sewage, which affects the properties of these wastes. The characteristics of the sewage used in this study are given in Table 4.2. The pH of 7.57 can be said to be neutral. Thus, the sludge remains in the methane digestion stage (Kijo-Klecckzkowska et al., 2012). The low alkalinity of 286 mg/L shows that the amount of dissolved alkaline compounds in the liquid sludge is low. The Low Volatile Fatty Acids and Ammonical Nitrogen all points to the fact that the sludge was well digested. This might be because it was collected from anaerobic sludge digester tank (Kijo-Klecckzkowska et al., 2012).

The characteristics of the sludge in the current studies agree with the characteristics of the well digested sludge as classified in previous studies (Kijo-Klecckzkowska et al., 2012; Heidrich, 2004; Heidrich, and Witkowski, 2010). Nevertheless, the alkalinity of the sludge used in the current study falls under the secondary sludge. This might be because the moisture content (50 %) of the sludge used in the current study falls into secondary sludge characteristics.

Table 4.2 Characteristics of the Anaerobic Sewage Sludge used in this study

Test Parameter	**Result**
Ammonical Nitrogen	5.39 mg/L
Alkalinity (as CaCO3)	286 mg/L
Volatile fatty acids	120 mg/L
Volatile Suspended Solids	5782 mg/L
Organic Carbon	1.75%
pH	7.57
Moisture	50 %

4.2 EFFECT OF TEMPERATURE

4.2.1 Effect of Temperature on Cumulative Biogas and H_2 Production Using Rice Waste as Substrate

When the substrate was subjected to a temperature of 35^0C, biogas production was recorded on day zero as opposed to H_2 gas which commenced on day one (Figure 4.1). This might be because the temperature was favorable for H_2 producing bacteria to proliferate. There was a rapid increase in the production of biogas and H_2 reaching its peak on the 9th day before stabilizing from the 10th day onward. This could also be because the temperature made sugar conversion easier for the Hydrogenase which in turn increases H_2 production (Jingwei et al., 2008).

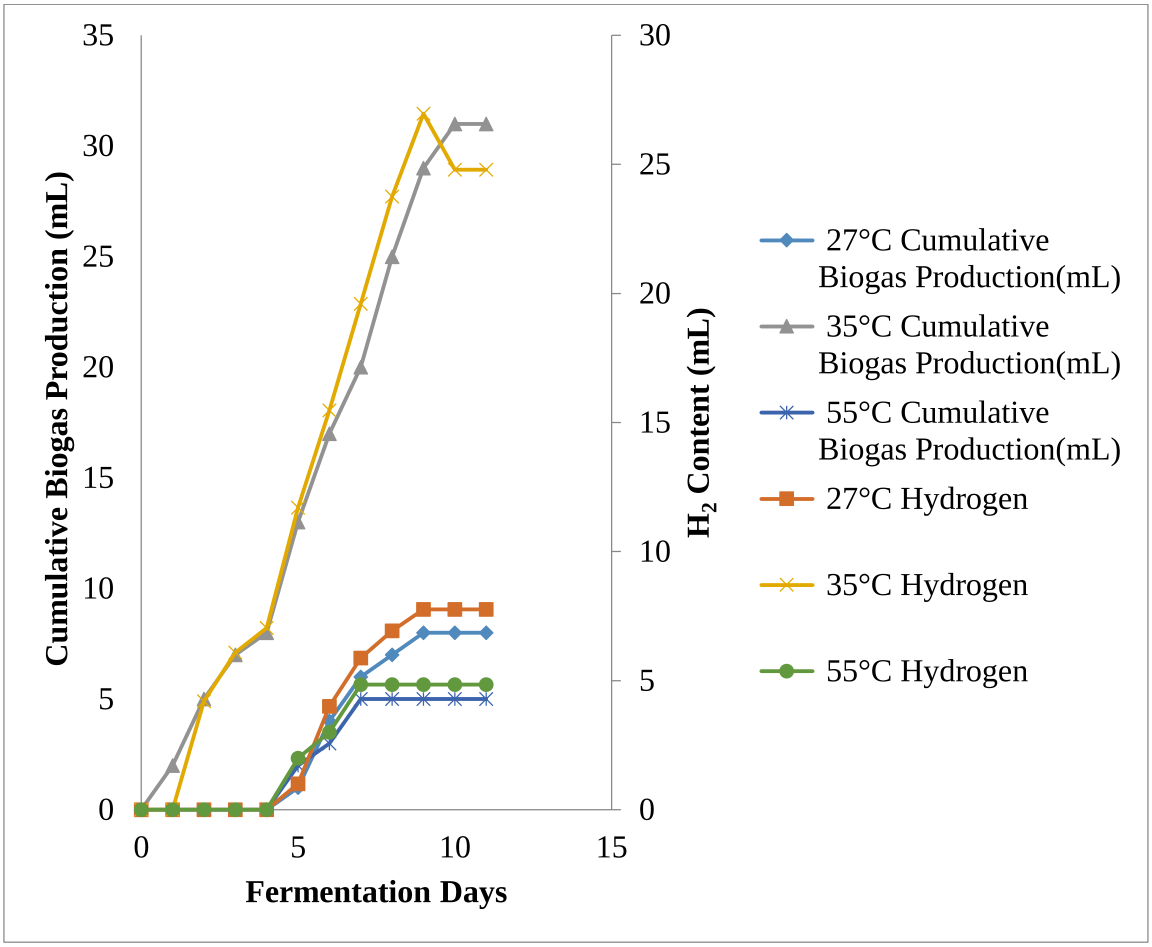


Figure 4.1 Effect of temperature on Cumulative Biogas and H_2 Production Using Rice waste as Substrate

Also, considering biogas and H_2 production at other temperatures, it was observed that at 27^0C and 55^0C, H_2 and biogas production commenced on the 4th day. There was a slow increase in the H_2 production observed for substrates at 27^0C before reaching its peak on the 7th day with a H_2 yield of 7.76 mL. The long lag period was probably because the temperature was unfavorable for H_2 producing bacteria (Jianlong and Wan, 2009).

Furthermore, it was observed that the maximum H_2 production recorded at 55^0C was 4.85 mL. This was probably because the H_2 producing bacteria survived for a while but were inhibited by the high temperature. Generally, the optimum temperature for bio-H_2 production using rice waste was found to be 35^0C in this study.

This agrees with previous report by Fang et al., (2006) showing the optimum temperature for H_2 production from rice waste to be 5.5 even though Fang et al., (2006) recorded a higher maximum H_2 yield of 346 mL. This higher yield might be because the substrate used by Fang et al (2006) was rice slurry which provides an enabling environment for the microbes. Results in this research was not in agreement with those of Lee et al., (2008) and Elijah et al., (2009) where the optimum temperature was 55^0C, which might be because they used rice husk and as such higher temperature was needed to get the nutrients out (Elijah et al., 2009).

Moreover it was statistically shown that H_2 production from rice waste as substrate was statistically significant at 37^0C ($P < 0.001$) than at 27^0C and 55^0C. The same was the case when statistical analysis was conducted for cumulative biogas production.

4.2.2 Effect of Temperature on Cumulative H_2 production and H_2 Content Using Fish waste as Substrate

H_2 and biogas production commenced on the 6th day when the fish waste were subjected to a temperature of 27^0C (Fig 4.2). This was probably because of unfavorable temperature coupled with the acidic content of fish waste. A slight increase in H_2 production was observed from the 7th day before reaching its peak on the 9th day after which it decreased to zero on the 10th day.

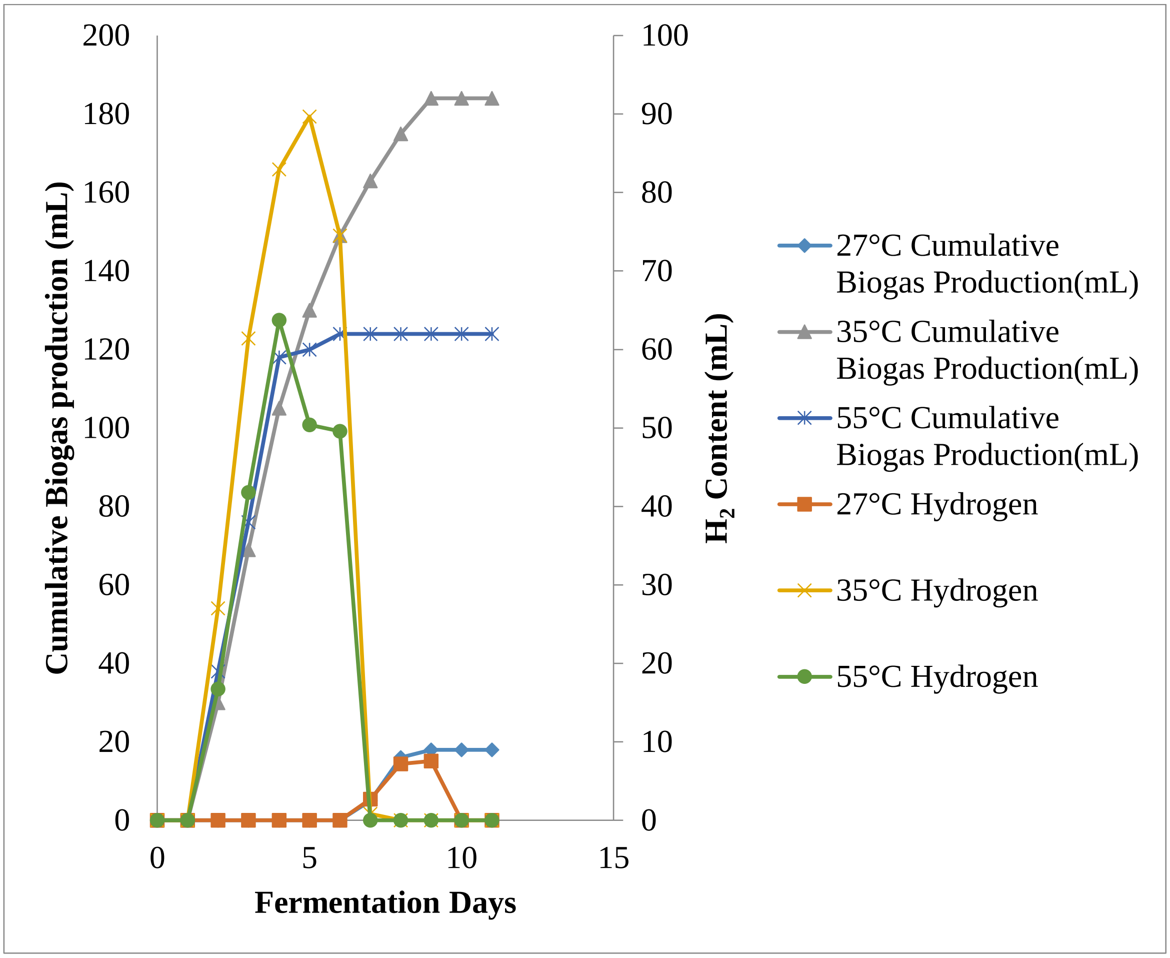


Figure 4.2 Effect of Cumulative Biogas production and H_2 content Using Fish waste as Substrate

At 35^0C, biogas and H_2 production commenced at day one. A rapid increase was observed until it reached its peak on day 5, then a sudden fall in H_2 production was observed after the 5th day. The rapid increase and fall could be because temperature 35^0C was favorable at the start but the production of carbon-dioxide made the system more acidic that the medium became harsh for the survival of hydrogenase enzyme (Nazlina et al., 2009). For 55^0C, $H_2$and biogas production commenced on the 1st day. We also observed maximum H_2

production of 63.72 mL which also decreased gradually until no H_2 gas was produced. This might also be due to the high carbon dioxide content observed in the fish which helps to increase the acidic content of the mixture (0-thong et al., 2007). H_2 and biogas production commenced after 6 days when fish waste substrate was subjected to 27^0C which lasted for just 3 days. This might be because the temperature was low and as such hydrogen producing bacteria had tough time adjusting with the temperature (Nazlina et al., 2009). The 3 days production period was probably due to the carbon-dioxide produced by fish which makes the system acidic, thus, inhibiting hydrogenase(O-Thong et al., 2007; Okamoto, 2000).

Generally, the optimum temperature for H_2 production using fish waste as substrate was 35^0C. The low yield in fish waste could also be attributed to the amino acid which is the catalytic end product of protein (Michael, 2006).

The amount of H_2 produced by fish waste at 35^0C was statistically significant than the amount produced at 27^0C and 55^0C ($P < 0.05$). Likewise, the amount of biogas produced at 35^0C was statistically significant than the amount produced at 27^0C and 55^0C ($P < 0.001$).

This agrees with previous reports where the optimum temperature for H_2 production using protein substrate was 35^0C (Zhu, 2011; Keigo and Shigeharu, 2006; Shin et al., 2004).

4.2.3 Effect of Temperature on Cumulative Biogas production and H_2 Content Using Vegetable Waste as Substrate

It was observed that no gas production was recorded when vegetable waste substrate was subjected to 27^0C and 55^0C (Figure 4.3). This might be because at 27^0C, the lactic acid fermentation bacteria which operate better at this temperature range liberated ascorbic acid which is richly present in vegetables. This liberation therefore makes the medium more acidic and thus unsuitable for H_2 producing bacteria as shown in Table 4.1 (Leon, 2011). According to Okamoto (2000), H_2 producing bacteria are more active at temperatures 35 - 40; therefore 55^0C might be too high for the bacteria to act on vegetable waste substrate (Okamoto, 2000).

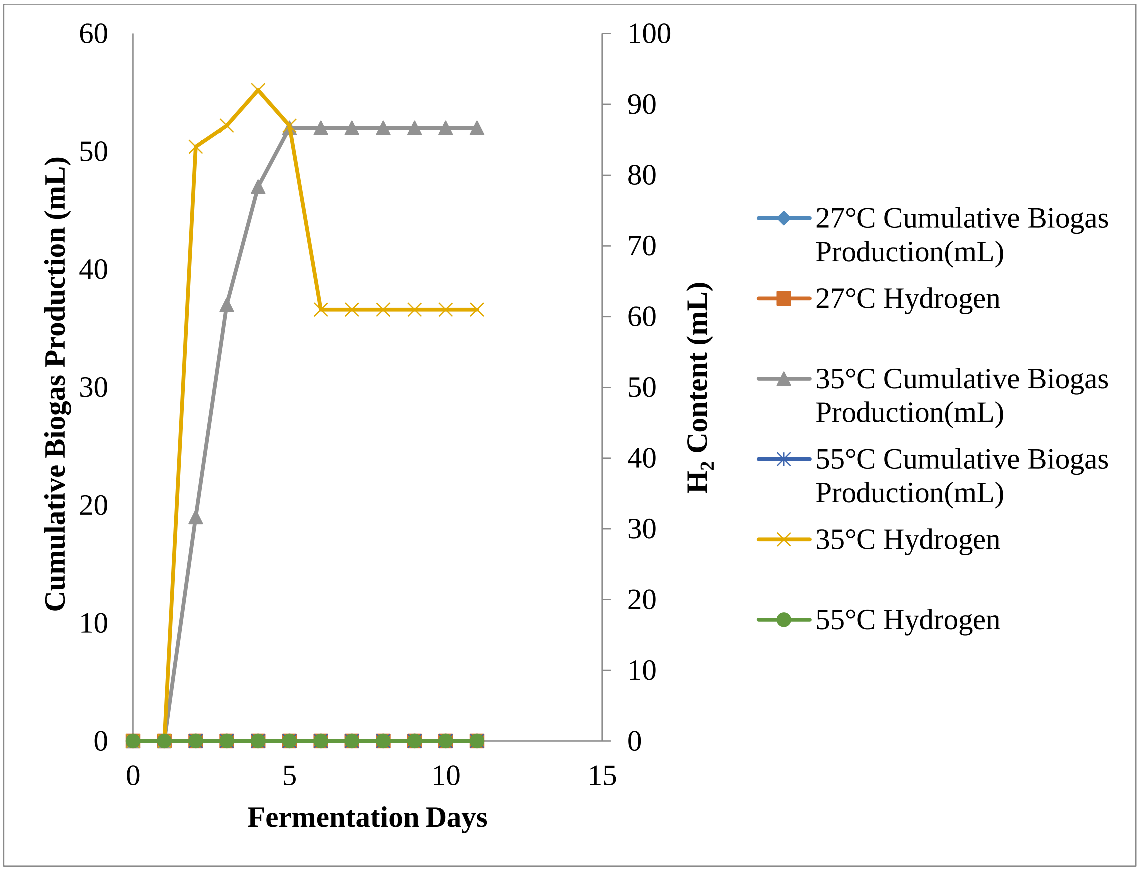


Figure 4.3 Effect of Temperature on Cumulative and H_2 Production Using Vegetable Waste as Substrates

Nevertheless, biogas and H_2 production commenced when the vegetable waste as substrate was subjected to a temperature of 35^0C. On day one, gas production was recorded. A rapid increase in H_2 production was observed from the second day until a maximum of 92 mL was reached on the 4th day. The subsequent days showed a gradual decrease in H_2 production until it stabilized from the sixth day onward. This was probably because the temperature favored H_2 producing bacteria. It could also be because most plants that grows in temperate regions like Malaysia contains indigenous bacteria which do well at 35^oC (Merrill, 2010).

This result is different with previous reports by Krishnan et al. (2007) and Chu et al. (2008). They recorded gas production at 55^0C. This was probably because only one vegetable waste type was used in these studies compared to more than one that was used in the present study. Nevertheless, this result agrees with Okamoto (2000) who reported 35^0C as the optimum temperature for bio-H_2 production from vegetable waste substrate.

The amount of H_2 produced by vegetable at 35^0C was statistically significant than the amount produced at 27^0C ($P < 0.001$) and 55^0C ($P < 0.001$). This is because no gas production was observed when vegetable substrate was used for bio-H_2 production at 27^0C and 55^0C.

4.2.4 Effect of temperature on Cumulative Biogas Production and H_2 Content Using Mixed Food Waste as Substrate

Cumulative biogas and H_2 production of mixed food waste substrate, at 27^0C and 35^0C, commenced on the 5th day (Figure 4.4). There was a slow but steady increase in the production of biogas and H_2 at 27^0C until it reached its peak on the 8th day. However, on the 9th day, no H_2 production was observed. Furthermore, a rapid increase in H_2 production was recorded at 35^0C on the 5th day until a maximum cumulative H_2 production of 108.9 mL was recorded on the 7th day before dropping sharply to zero on the 8th day. No biogas or H_2 production was recorded for mixed waste substrates at 55^0C.

The five days lag period observed at 35^0C could be because this waste has rice, a source of carbohydrate, fish, a source of protein and vegetable, a source of vitamins, hence it will take a longer time for these three to be acclimated. The higher cumulative biogas and H_2 yield recorded at 35^0C could be because H_2 producing bacteria were able to maintain the pH of 5.5 at 35^0C and as such more biogas and hydrogen were produced (Nazlina et al., 2011).

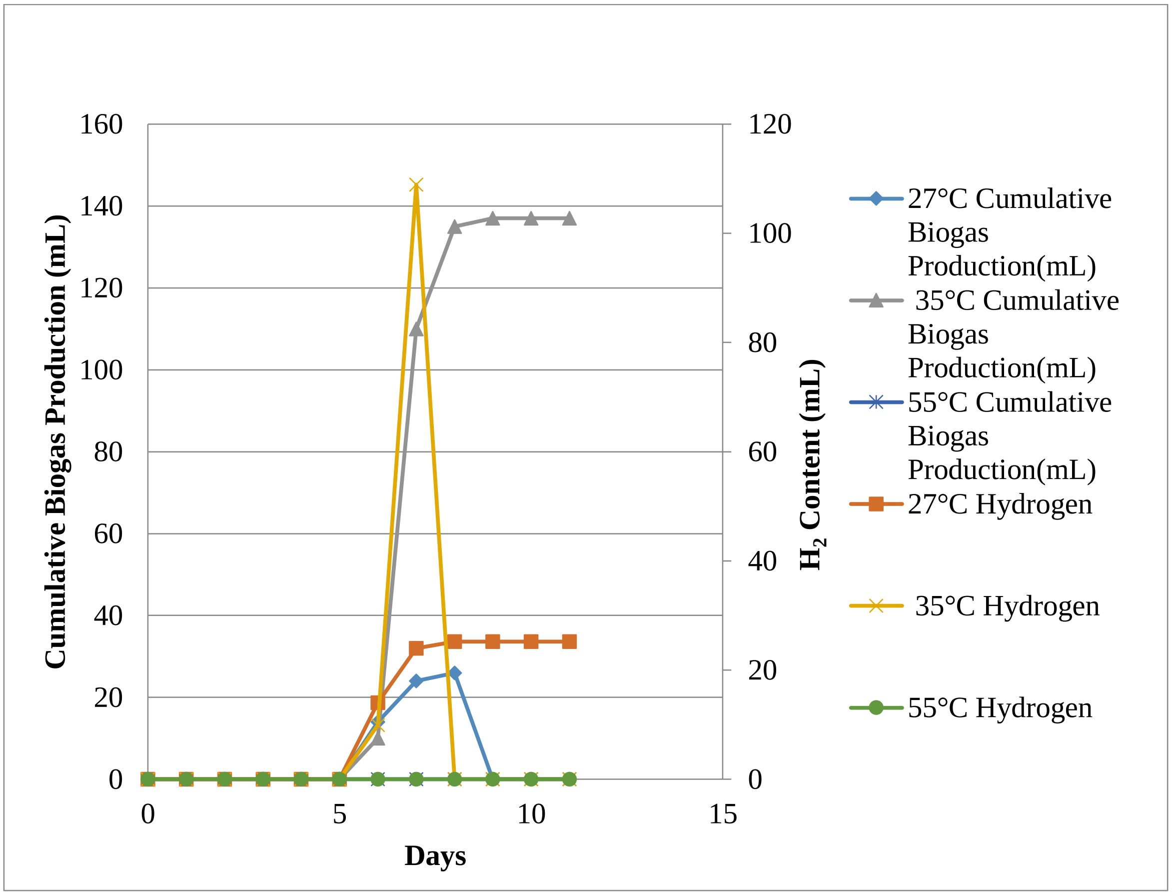


Figure 4.4 Effect of temperature on cumulative biogas production and H_2 Content Using Mixed Food waste as Substrate

This result agrees with previous reports by Xiao et al., (2013) having optimum temperature of 37^0C. It also agrees with that of Chen et al., (2006) having the maximum H_2 yield of 101 mL. This might be because anaerobic sewage sludge from anaerobic digester was used in both studies. Similarly, previous report by Pan et al. (2008) recorded H_2 production at 50^0C. This might be because of the temperature difference of 5^0C which could be harmful to H_2 producing bacteria (Lin et al., 2008). Nevertheless, a report by Shimizu (2008) agrees with this study, recording no gas production at 55ºC.

Statistically, there was no significant difference in the amount of H_2 gas produced at the two temperature of 27ºC and 35ºC, where gas production was recorded using mixed food waste as substrate ($P = 0.25$). Nevertheless, the amount of biogas produced by mixed food waste at 35^0C was statistically significant than the amount produced at 27^0C ($P < 0.05$) and 55^0C ($P < 0.01$). Thus, we could say that for an enhanced bio-H_2 production using mixed food waste substrate, room temperature ranges are favorable.

Vegetable waste produced no gas when subjected to experimental condition of 27ºCand 55ºC (Table 4.3). However, rice and fish waste produced gases at all the experimental conditions of 27ºC, 35ºC and 55ºC. Furthermore, it was observed that all the waste substrates used were able to produce biogas and hydrogen gas at 35ºC. It can be observed that mixed food waste substrate also recorded the highest cumulative hydrogen production while fish waste recorded the highest cumulative biogas production at 35ºC. It can be observed also that throughout the food waste substrates, the biogas and hydrogen produced was highest when they were subjected to a temperature of 35ºC. Thus, we can say that the optimum temperature for bio-hydrogen production from food waste was found to be 35ºC in this study.

This is in agreement with number of studies carried out previously (Kim et al., 2004, Jo et al., 2007, Zhu et al., 2011, Munoz -Páezet al., 2012). The biogas and hydrogen yield tends to vary across these studies. This is probably because of the differences in the types of substrates used for bio-hydrogen production.

Table 4.3 The maximum cumulative biogas and hydrogen production from different temperatures

FWS	27ºC		35ºC		55ºC	
	MCB (mL)	**MCH (mL)**	**MCB (mL)**	**MCH (mL)**	**MCB (mL)**	**MCH (mL)**
Rice	8.00± 1.25	7.76±0.35	31.00±1.22	26.97±0.76	5.00±2.23	4.85±1.87
Fish	18.00± 2.27	7.56 ± 1.18	184.00±3.46	89.70 ± 1.25	124.00±1.23	63.74 ±2.23
Vegetable	Nil	Nil	52.00±2.25	42.00±1.76	Nil	Nil
Mixed Waste	26.00±2.45	25.22±0.76	137.00±3.20	108.90±1.42	Nil	Nil

Number of replicates = 3. FWS = Food Waste Substrates, MCB = maximum cumulative biogas, MCH = maximum cumulative hydrogen, Nil = no gas production was recorded.

4.3 Effect of pH at 35^{0}C on Bio-hydrogen Production Using Food Waste as Substrates

It is very important that proper pH is provided when fermenting food waste for bio-H_2 production purposes (Wang and Wan, 2009). Optimum pH can enhance bio-H_2 production. This is because pH has effects on the H_2 production metabolic pathways (Kimet al., 2011). pH can affect the H_2 production yield, biogas content, organic acids produced and

H_2production rate. Four different food waste substrates were used; Rice, Fish, vegetable and mixed for this study.

4.3.1 Effect of pH on Cumulative biogas Production and H_2 Content of Biogas from Rice Waste

Figure 4.5 shows the effect of pH on Bio-H_2 production using rice waste substrate. It can be observed that when the substrate was subjected to pH of 5.5, cumulative biogas production was recorded on day zero while H_2 gas production commenced on day one. There was a rapid increase in the production of H_2 and biogas reaching its peak on the seventh day, before it gradually decreased.

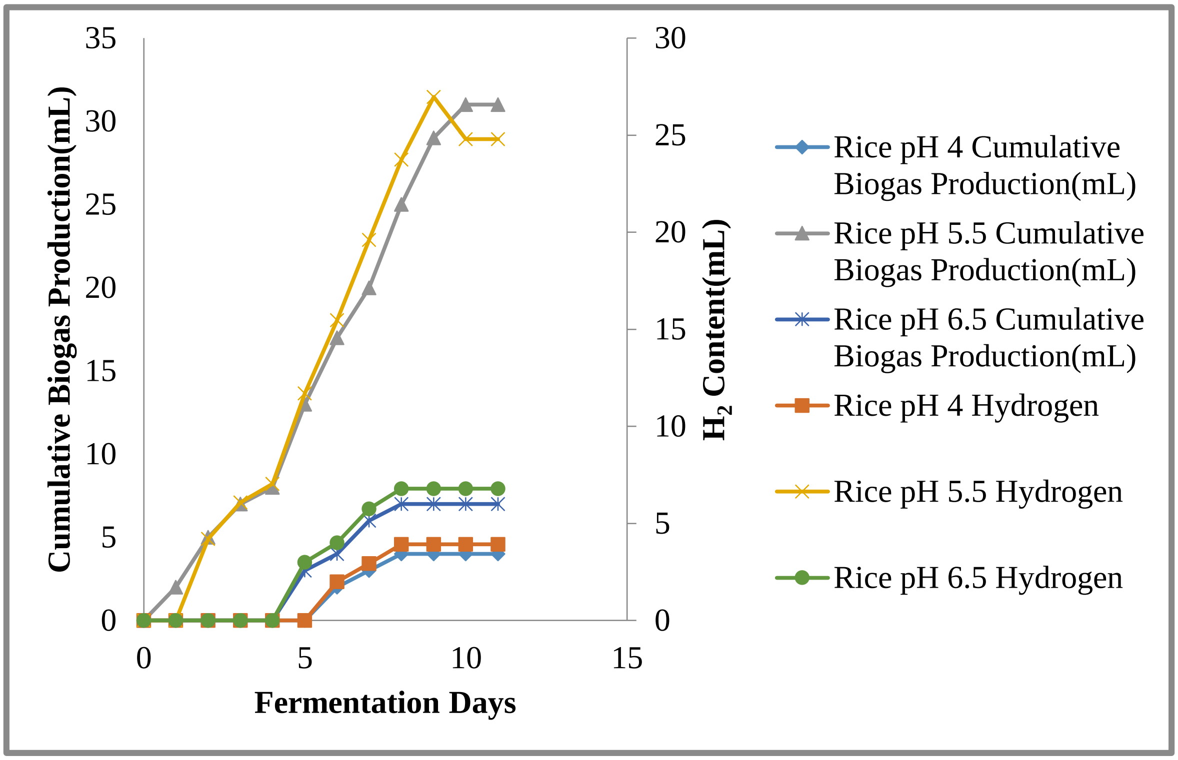


Figure 4.5 Effect of pH on Cumulative Biogas and H_2 Production using Rice Waste as Substrate

Also, looking at the amount of gas produced at different pH values, it was observed that, at pH 5.5, rice waste produced the highest amount of H_2 gas (26.97 mL). This was probably because the H_2 producing bacteria are more active at an initial pH of 5.5 as was previously reported (Keigo and Shigeharu, 2006). On the other hand, Fang et al., (2006) in a similar study using a different substrate as waste (rice slurry) observed that the production of H_2 was highest at pH 4.5. The current study did not incorporate rice slurry and as such was unable to draw comparison between their studies (Fang et al., 2006) and the present study. At pH 6.5, there was a delay of about 4 days in the production of H_2 and cumulative biogas at pH 5.5. This delay could be attributed to the time taken for volatile fatty acids to be converted to H_2 at pH 6.5 as was observed by Jayalakshmi et al., (2007). However, despite the lag in the production of H_2 and cumulative gas, a rapid increase in H_2 production was observed reaching a stable value on the 8th day, yielding maximum H_2 gas of 6.76 mL. This quantity of H_2 gas was much lower than 32.9 mL produced by rice waste in Nazlina et al study (2011) in which unlike the present study, the pH was controlled throughout the study period. There was a delay of about 5 days before production of H_2 gas commenced when the substrate was treated at pH 4. However, the gas production also increased rapidly afterwards up till the eighth day when a maximum yield of 3.9 mL was observed. In a previous study (Masset etal., 2010), it was observed that rice waste produced maximum H_2 cumulative yield of 45 mL when the experiment was conducted at pH 4.4. This amount of H_2 gas produced was considerably higher than that reported in the present study mostly because of the different pretreatment methods used in their study and the different carbohydrate sources employed. It is therefore very important that proper pH be selected so that H_2 production can be maximized. The results presented here reveals that, a pH of 5.5 is the optimum pH required to enhance H_2 production when rice waste is used as a substrate.

4.3.2 Effect of pH on Cumulative Biogas and H_2 Production of Using Fish Waste as Substrate

Production of H_2 commenced on day one for both pH 5.5 and 6.5, when fish substrate was used (Fig 4.6). At pH 5.5 there was a rapid increase in production of H_2 reaching a maximum of 88.4 mL on the fifth day, before the production dropped sharply to zero on day six. In contrast, the increase in cumulative gas production followed a curvilinear pattern while stabilizing at a maximum production of 184 mL from the ninth day to 12th day. The sudden drop in H_2 gas production observed at this pH state of the fish waste could be because of the drop in pH as observed at the end of the experiment (Table 4.1). For this reason, after the 5th day, the H_2 producing bacteria were inhibited as such H_2 production was also inhibited (Shimizu, 2008).

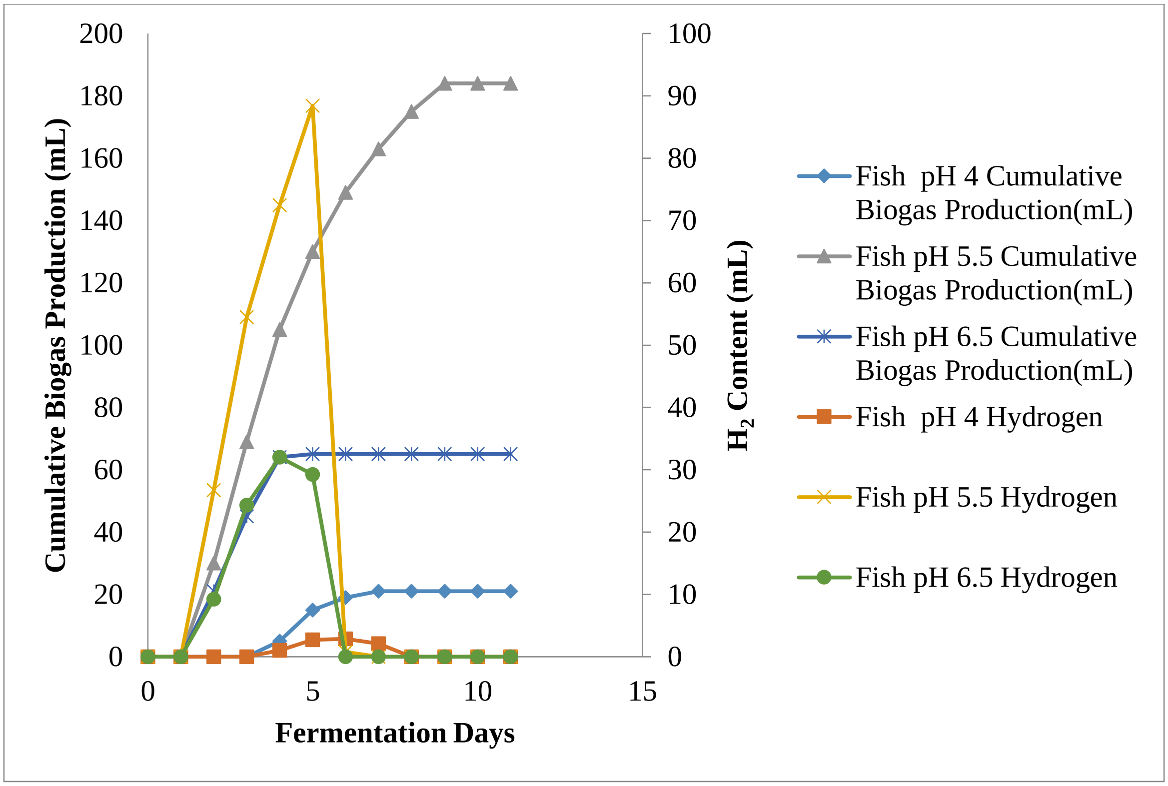


Figure 4.6 Effect of pH on Cumulative Biogas and H_2 Production of Using Fish waste as substrate.

On the other hand, at pH 6.5, the production of H_2 gas and cumulative biogas increased gradually reaching a maximum of 32 mL and 65 mL respectively on the 4th day. However, the subsequent days showed a decrease in the amount of H_2 gas produced. This decrease meant that at day six no H_2 was observed in the system. It was observed that cumulative gas production continued and appeared to have stabilized from the 4th day onward. We also observed a maximum production of H_2 gas (15 mL) on the 5th day, at pH 4, which also decreased till no H_2, was produced on the 8th day. We had also observed that after the experiment, that across the studied pH ranges, the pH reduced until it became acidic at pH 3.0. Again, this was because of the increased production of carbon dioxide at these low pH ranges which meant that the system was so acidic that the H_2 producing bacteria were unable to as noted also by Li (2007).

4.3.3 Effect of pH on Cumulative Biogas and H_2 Production of Vegetable Waste As Substrate

Just like rice and fish waste substrates, H_2 production was observed first at pH 5.5 on day 1, which then increased rapidly and reached a peak 45.24 mL at the 4th day and then decreased to zero (Figure 4.7). The drop in H_2 production could be because of the acidic nature of vegetable and of the individual components of vegetable (Krishnan et al., 2007; Yap, 2013). At pH 4.0, H_2 production began on day 4 but the biogas was more of carbon dioxide than H_2 and also dropped after 4 days. pH 4 is acidic therefore when combined with continuous production of carbon-dioxide, the H_2 producing bacteria could not survive the high acidic medium (Lee et al., 2008; Yap, 2013). The H_2 content of the cumulative biogas was quite small at pH 4. At pH 6.0, no gas production was observed. This could be as a result of the nearness of the pH to neutrality and possibly the H_2 producing bacteria may not do well above pH 5.5. Nevertheless, research by Lee et al (2008) also revealed a contrast optimum pH of 7.0. This might be because of kitchen waste compost that was added to the reactor which will have similar microbes as the ones present in the system. Another previous study by Chyi-How et al. (2010) revealed the optimum pH for bio-H_2 production was 6.0. This might be due to the use of preserved fruit soaking solution as substrate without any additional microbes from sewage sludge or compost.

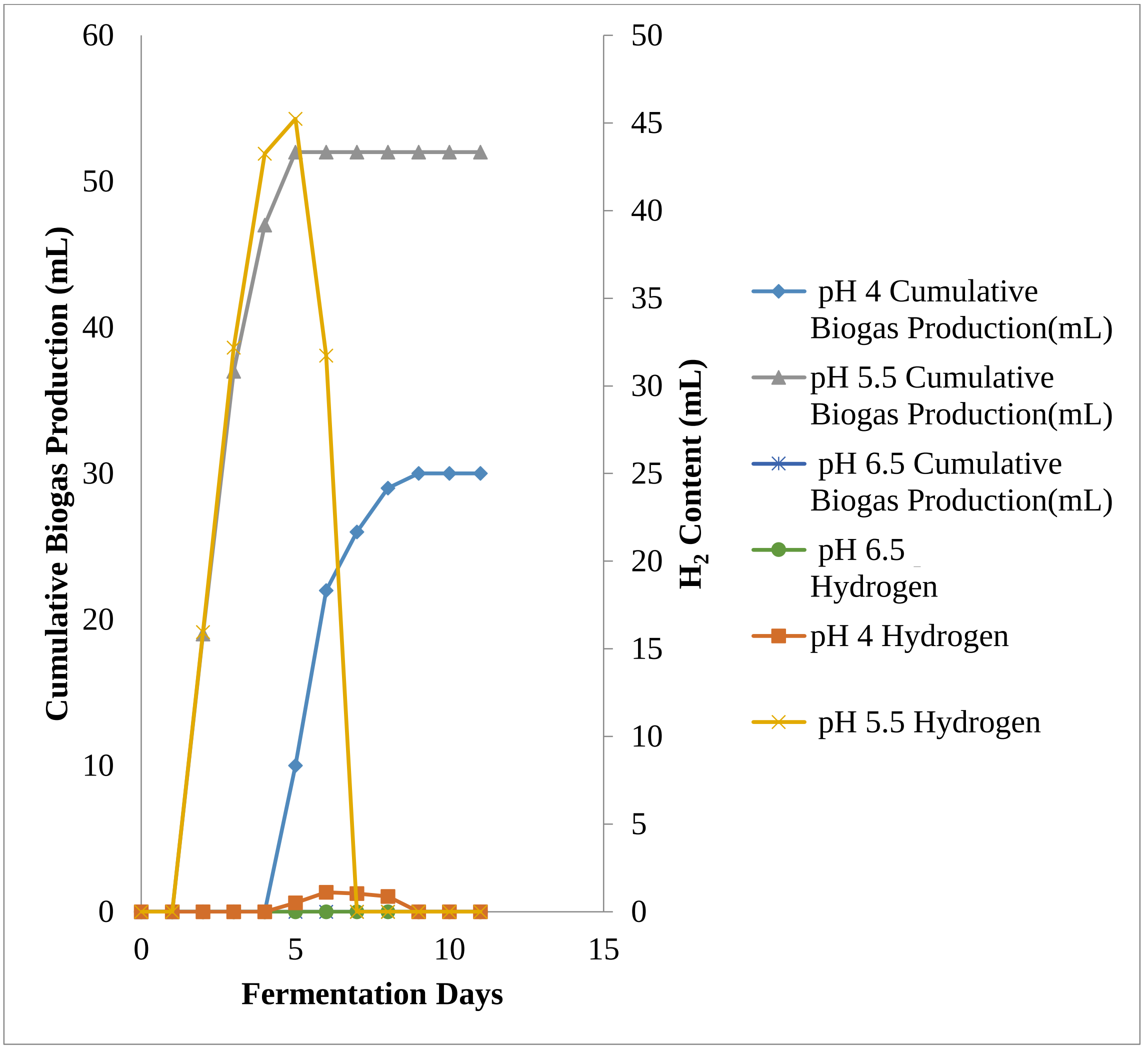


Figure 4.7 Effects of pH on Cumulative Biogas and H_2 Production Using Vegetable Waste as Substrate

4.3.4 Effect of pH on Cumulative Biogas Production and H_2 Content Using Mixed Food Waste as Substrate

Substrates subjected to pH of 4.0, caused cumulative biogas production as well as H_2 gas production on the 4th day (Figure 4.8). This was probably because of the acidic nature of the medium which is not too favorable for the H_2 producing bacteria (Jayalakshmi et al., 2007). There was a slow increase in production of H_2 and biogas reaching its peak on the 9th day, before sharply dropping to zero on the 10th day. This could be because the H_2 producing bacteria were trying to adjust to the environment but eventually they could not survive so the H_2 production stopped (Masset et al., 2010).

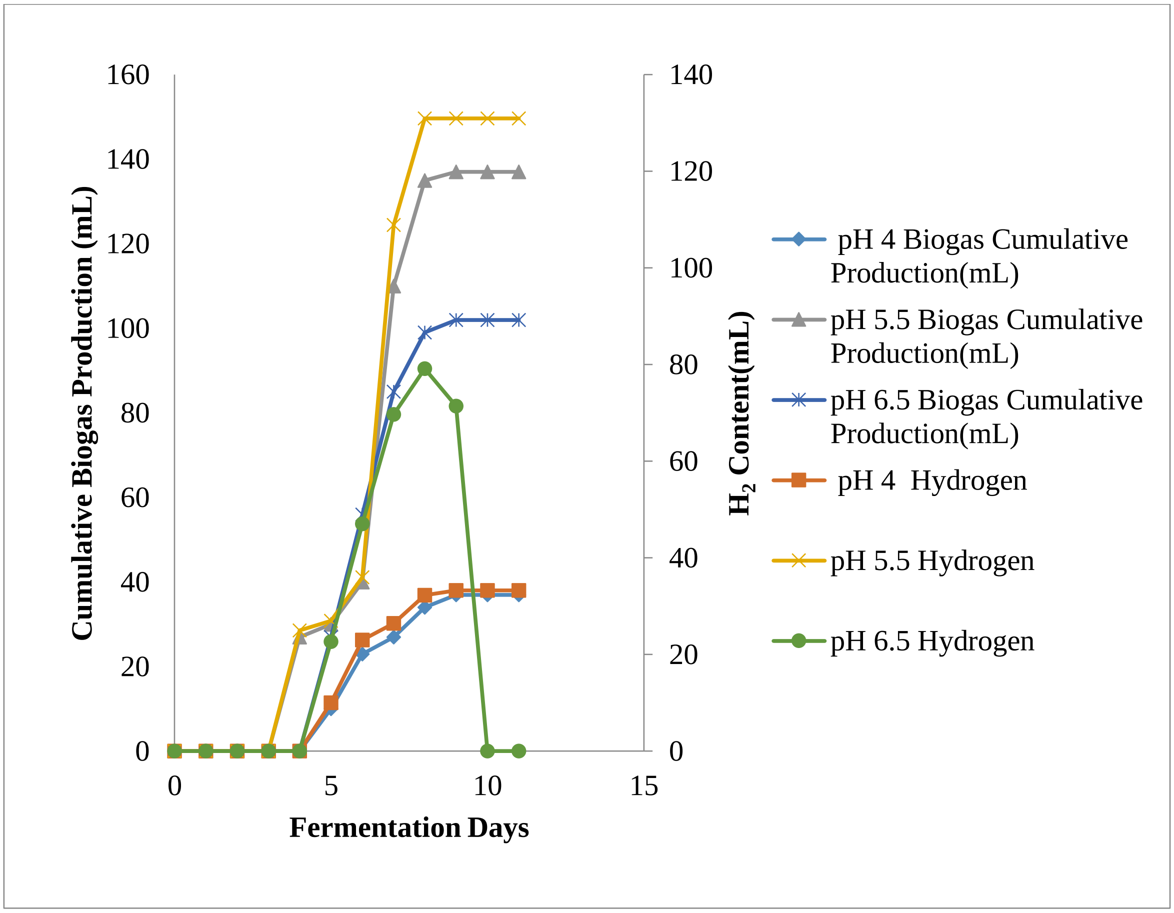


Figure 4.8 Effects of pH on Cumulative Biogas and H_2 Production Using Mixed food waste as Substrate

Also, looking at the amount of gas produced at different pH values, it was observed that, at pH 6.5, biogas and H_2 production commenced together on the 4th day. This was probably because the pH was close to being neutral. A rapid increase was recorded from the 5th day before reaching its peak on the 8th day, before it gradually decreased to zero on the 10th day. This was probably because the pH being slightly acidic still allows H_2 producing bacteria to grow but was also favorable for H_2 consuming bacteria which probably inhibited the H_2 production (Fang et al., 2006). It was also observed that at pH 5.5 biogas and H_2 production commenced on the 3rd day before increasing slowly on the 4th and 5th day. However, the subsequent days showed a rapid increase before stabilizing from 8th day onward. The shorter lag period observed in pH 5.5 might be because the H_2 producing bacteria were favored by pH 5.5 (Shimizu, 2008).

This result was similar with previous reports showing that the optimum pH for H_2 production from mixed food waste was ranging from 6.5 (Han and Shin, 2004), 5.6 (Keigoand Shigeharu, 2006), 7.0 (Renet al., 2006) and 6.0. Many of these studies used a continuous batch reactor rather than the batch reactor as used in the present study and as such the difference in the reactor sizes may account for the differences in the reported results. Nevertheless our results agree with those of Atif et al. (2005) and O-Thonget al.*(*2007) where the optimum pH for H_2 gas production was 5.5 even though palm oil mill effluent (POME) was used. Another study (Shimizu, 2008) recorded little or no H_2 production at pH 5 and 6, using only one bacteria species, while the current study has more than one bacteria species. It was furthermore shown in a recent study that bio-hydrogen production stopped as pH dropped below 5 (Xiao et al., 2013).

After a period of 12 days, it was observed that pH reduced across the entire food waste substrates fermentation. This decrease in pH might be due to the organic acids formed during the fermentation process, which in turn reduced the buffering capacity of the medium. Such drop in pH is capable of changing the metabolic pathway from a hydrogenic to a non-Hydrogenic process and can inhibit substrate utilization (Kim et al., 2011). In addition, a drop in pH was also shown (Keigo and Shigeharu, 2006) to be capable of directly limiting the production of H_2 gas due to the decline in the activity of Hydrogenase which occurs at excessively low pH.

It can be observed that the maximum cumulative biogas hydrogen production across the food waste as substrate was highest when the food waste was subjected to pH of 5.5 (Table 4.4). Fish waste substrate recorded the highest maximum cumulative biogas production (184.00 ± 3.46) mL. Mixed food waste substrate recorded the highest maximum cumulative hydrogen production (102.00 ± 1.42) mL. As shown in Table 4.4, gas production was not recorded for vegetable waste substrate at pH of 6.5. Furthermore, the maximum hydrogen production from vegetable waste at pH 4.0 was 1.1±077 mL.

Fish waste produced more carbon dioxide than hydrogen gas. This may account for its high amount of cumulative biogas production. Fish waste however, is also a rich source of protein and ammonia nitrogen, this may also account for its high biogas yield (Michael et al., 2006; Tomczak-Wandzel and Levilin, 2013). The highest maximum cumulative hydrogen gas production from mixed food waste substrate could be due to the combined effect of hydrogenase enzyme found in all the substrates. This effect led to a longer lag phase but higher maximum cumulative hydrogen yield (Valdez and Varaldo, 2009).

Table 4.4 Maximum Cumulative Biogas and Hydrogen Production at Various pH

FWS	4.0		5.5		6.5	
	MCB (mL)	**MCH (mL)**	**MCB (mL)**	**MCH (mL)**	**MCB (mL)**	**MCH (mL)**
Rice	4.00± 1.25	2.92±0.25	31.00±1.22	26.97±0.76	7.00±2.23	4.75±1.87
Fish	65.00± 2.27	32.00± 1.18	184.00± 3.46	88.40± 1.25	21.00± 1.23	2.70± 2.23
Vegetable	30.00±1.53	1.1±.077	52.00±2.25	45.24±1.76	Nil	Nil
Mixed	37.00±2.45	33.30±0.76	137.00±3.20	102.00±1.42	102±2.54	79.20±2.24

Number of replicates = 3. FWS = Food Waste Substrates, MCB = maximum cumulative biogas, MCH = maximum cumulative hydrogen, Nil = no gas production was recorded.

4.4 EFFECTS OF ACCLIMATIZATION

Acclimatization refers to a mixture of the substrate with anaerobic sewage sludge which was allowed to acclimate for a certain period at mesophilic temperature (Dong et al., 2009). Heat pre-treatment was used to enhance the growth of H_2 producing bacteria and inhibit the methanogens from anaerobic sewage sludge before they were added to the various food wastes substrates (Chen et al., 2012). This process would encourage additional waste minimization because two different waste are used (food waste and anaerobic sewage sludge), thus reducing waste disposal and treatment needs. In order to know if the use of anaerobic sewage sludge was helpful in the production of H_2 by the H_2 producing bacteria, effect of acclimatization was studied and below are the findings on the various food waste substrates used in this study.

4.4.1 Effects of Acclimatization on Cumulative Biogas and H_2 Production Using Rice Waste as Substrate

It was observed that H_2 production commenced on the second day as for the acclimatized rice waste as opposed to the 4th day for non-acclimatized rice waste (Figure 4.9). The maximum H_2 production was recorded on the 10th day (13.6 mL) for non-acclimatized rice waste and on the 9th (26.97 mL) for the acclimatized rice waste respectively. H_2 production stabilizes from the 11th day for the acclimatized and on the 9th day for the non-acclimatized. Nevertheless, we observed that for the acclimatized rice waste, H_2 production decreased after the 10th day with increase in biogas production. This was not the case for the non-acclimatized, H_2 and biogas production stabilized the same day.

The shorter lag period observed for acclimatized rice waste could be because acclimatization hastened the activities of the hydrogen producing bacteria. The increasing biogas in the acclimatized rice waste could be as a result of the presence of methanogenic bacteria which were also enhanced by acclimatization even though they were affected by pre-heating (Ueno, 2001; Ahn, 2005; Kim et al., 2006).

Statistical analysis nevertheless showed no significant difference ($P = 0.05$) when the amount of hydrogen produced by acclimatized rice waste was compared with that of non-acclimatized rice waste. However, Duncan multiple comparison test showed the amount of biogas produced by acclimatized rice waste to be significantly higher when compared to that of non-acclimatized rice waste ($P = 0.0455$).

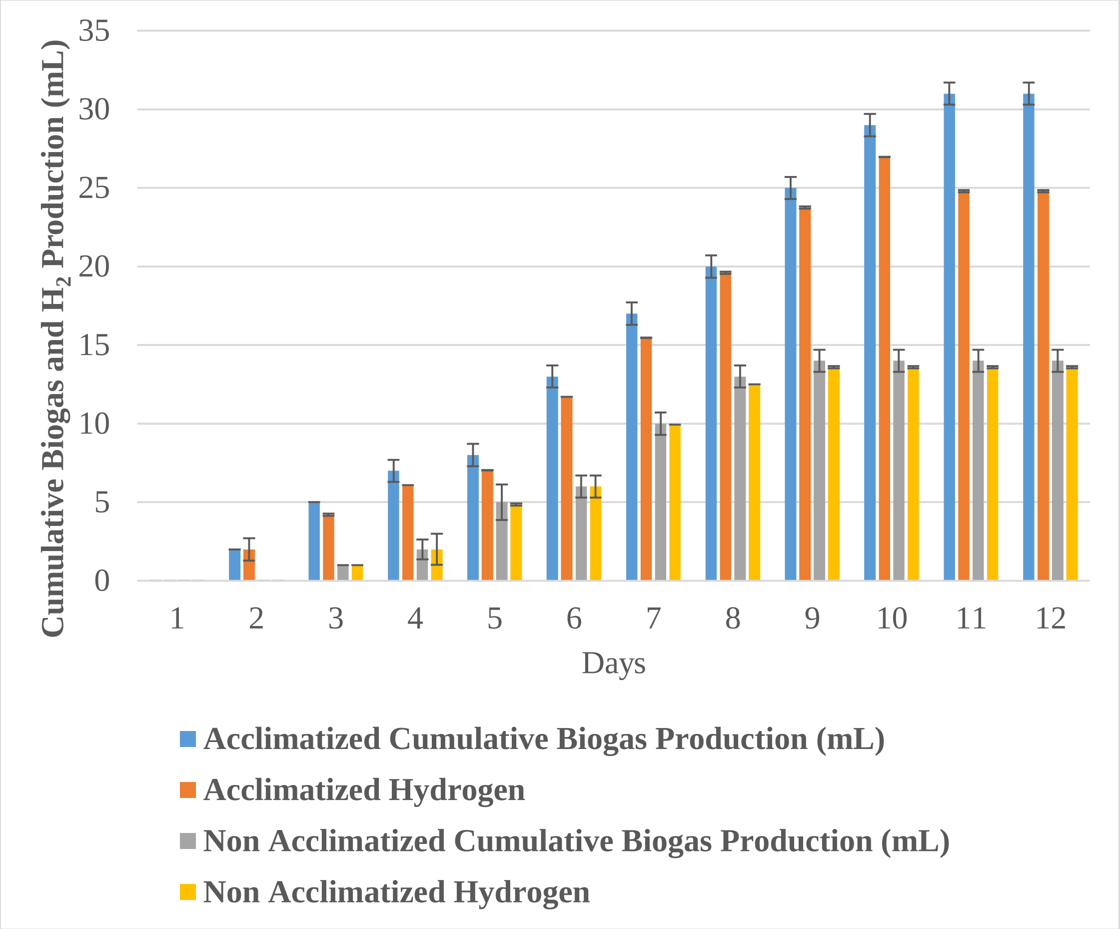


Figure 4.9 Effects of Acclimatization on Cumulative Biogas and H_2 Production Using Rice Waste as Substrate

4.4.2 Effects of Acclimatization on Cumulative Biogas and H_2 Production When Fish Waste was Used as Substrate

Cumulative biogas production and H_2 production commenced on day 3 for both acclimatized and non-acclimatized fish waste substrate (Figure 4.10). A rapid increase in biogas and H_2 production was observed in the acclimatized fish waste as opposed to the slow increase observed for the non-acclimatized substrate. The maximum H_2 production was 89.7 mL for acclimatized and 20 mL for the non-acclimatized fish waste substrate. We also observed that H_2 production stopped on the 8th day and on the 6th day for the acclimatized and the non-acclimatized fish waste respectively. We observed that CO_2

production was increasing with a decrease in H_2 content of the biogas both in both experimental conditions.

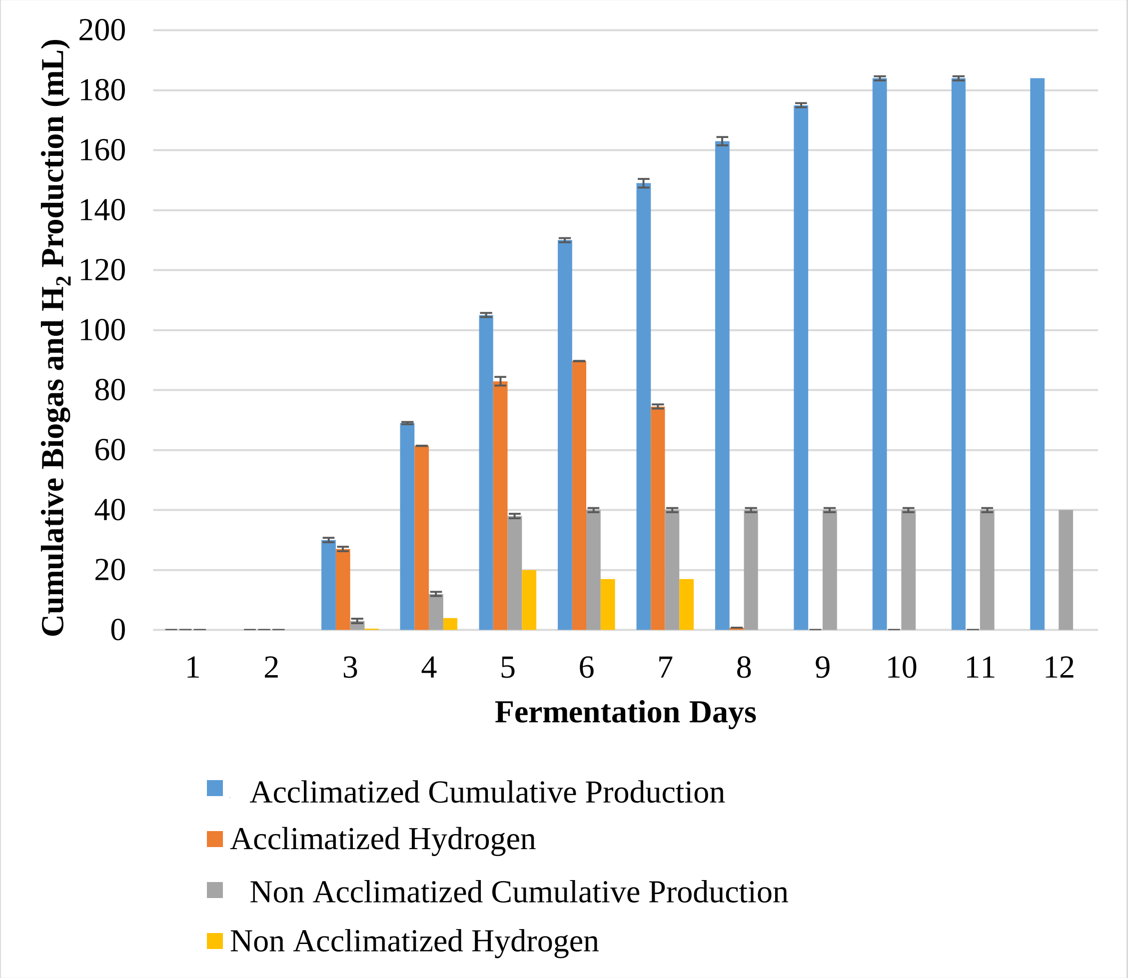


Figure 4.10 Effect of Acclimatization on Cumulative Biogas and H_2 Production when Fish Waste was used as Substrate

The 3 day lag period in both conditions could be because acclimatization could not enhance hydrogen production. Fish waste as observed in this study produced more carbon dioxide than rice, vegetable and mixed waste substrate. Thus, reducing the effect of acclimatization. This implies that in both conditions, H_2 production commenced on the same day but the difference is in the amount produced. It was also observed that the H_2 production lasted longer when acclimated fish waste was used as substrate than when non-acclimatized fish waste. This is probably because; acclimatized fish waste could withstand the carbon dioxide effect longer than the non-acclimatized (Ren et al., 2006).

This result is in agreement with previous studies that acclimatization has effect on bio-hydrogen production (Nasr et al., 2011; Voet et al., 1999). However, Dunn's multiple comparison test showed that the differences in cumulative biogas production was statistically significant in the comparison between acclimatized fish waste and non-acclimatized fish waste (P = 0.0006).

4.4.3 Effect of Acclimatization on Cumulative Biogas and H_2 Production Using Vegetable Waste as Substrate for Fermentation

Hydrogen production commenced on the 3rd day in the acclimatized vegetable waste and on the 6th day for the non-acclimatized vegetable waste (Figure 4.11). It was observed that for the acclimatized vegetable waste, H_2 content increased with increasing biogas production. It continued until a maximum of 45.24 mL on the 6th day was reached beyond which H_2 production stabilized. When compared with non-acclimatized vegetable waste, H_2 content of biogas in the acclimatized vegetable was almost 80 % while that of the non-acclimatized was less than 10 %. This shows that acclimatization enhanced H_2 production by 70 %. It might not be wrong to say that acclimatization reduced the formation of CO_2 when vegetable waste is used as substrate for bio-hydrogen production.

However, Dunn's Multiple Comparisons Test showed a higher significant difference (P = 0.0029) when the Cumulative biogas production of acclimatized vegetable waste was compared with that of non-acclimatized. It further showed a higher significant difference (P

< 0.05) when the hydrogen produced by acclimatized vegetable waste was compared with that of non-acclimatized vegetable waste.

The reason for the lower yield in non-acclimatized vegetable waste might be because the H_2 production was sustained by acclimatization in the acclimatized vegetable. Accumulated acidic medium will lower the pH of the reactor since the pH was not controlled. Thus, H_2 producing bacteria involved were unable to sustain its metabolic activity (Nazlina et al., 2009; Yap, 2013). It might as well be because only the indigenous microbe was in the fermenter. Vegetables are also known to contain high amount of vitamins and minerals (Leon, 2011) which can affect the pH of the medium, thus, affecting hydrogen production.

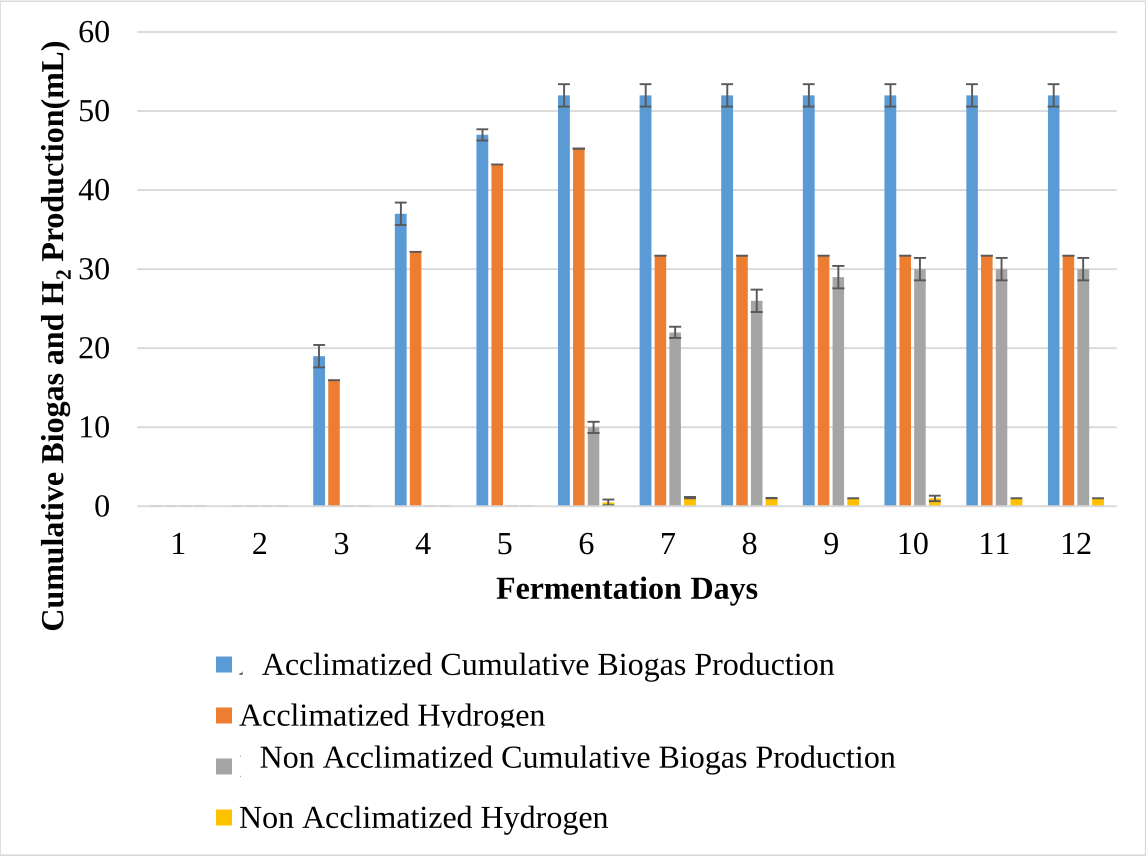


Figure 4.11 Effects of Acclimatization on Cumulative Biogas and H_2 Production Using Vegetable Waste as Substrate

4.4.4 Effect of Acclimatization on Cumulative Biogas and H_2 Production Using Mixed Food Waste as Substrate.

It was observed that Biogas production commenced on the 5th day in the acclimatized as opposed to the non-acclimatized which commenced on the 6th day (Figure 4.12). A rapid increase in H_2 production was observed in the acclimatized non-acclimatized experimental conditions from the 6th and 7th day respectively. Furthermore we observed that biogas and H_2 gas production seems to stabilize on the 9th day for acclimatized and on the 10th day for the non-acclimatized mixed food waste. The maximum H_2 production was 130.95 mL for acclimatized and 33.3 mL for non-acclimatized mixed food waste substrate.

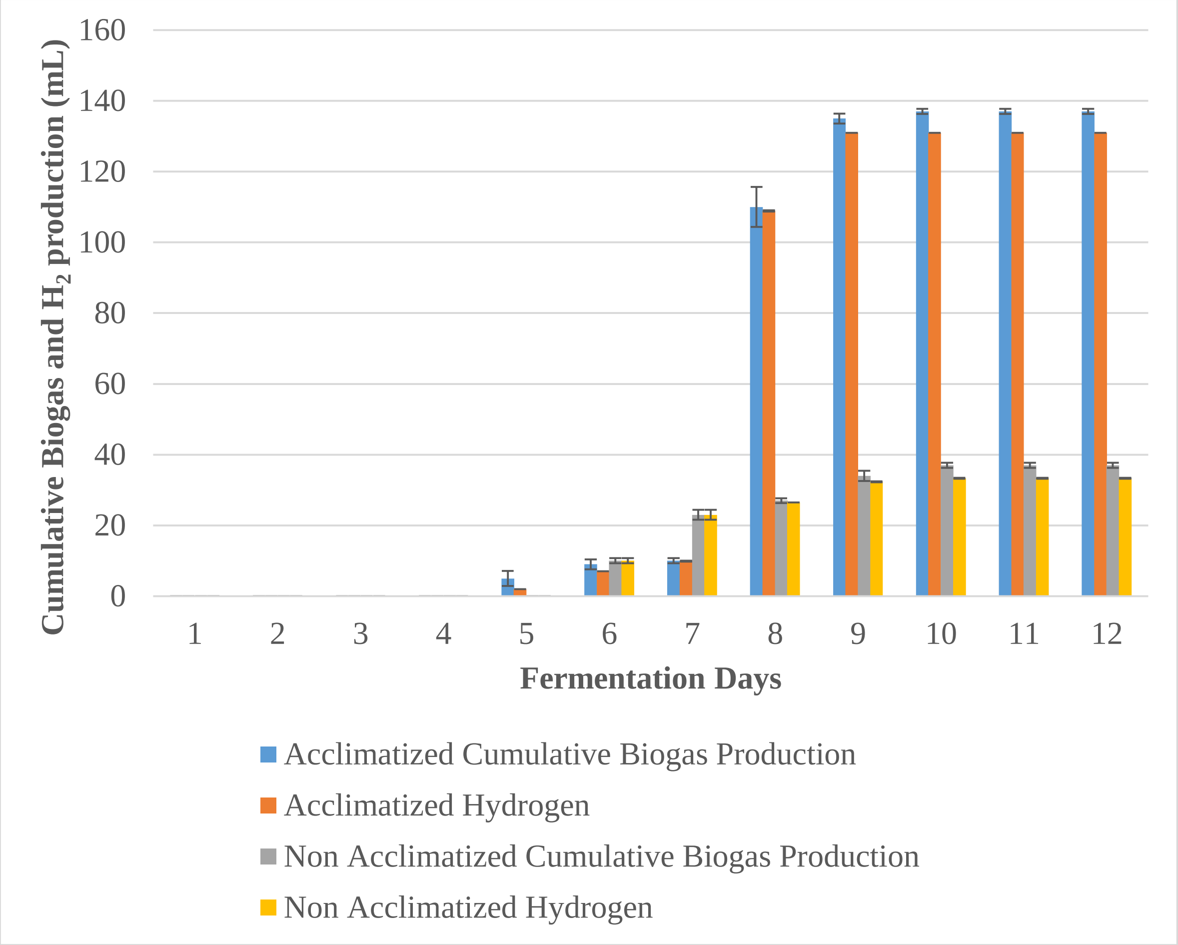


Figure 4.12 Effects of Acclimatization on Cumulative Biogas and H_2 Production Using Mixed Food Waste as Substrate

The long lag period observed in both acclimatized and non-acclimatized food waste substrate could be because of the heterogeneous nature of the substrate. Being mixed, it has various components which also have various rates of decomposition. The 97.65 mL difference in the maximum H_2 yield could be because the hydrogen producing bacteria in the non-acclimatized were not much and so cannot sustain the gas production (Nasr et al., 2011).

Statistical analysis showed that H_2 produced by acclimatized vegetable was significantly more than that produced by the non-acclimatized vegetable waste ($P < 0.0001$).

From this study, it is obvious that acclimatization improved the H_2 yield from the different food waste substrates used for bio-H_2 production. This study agrees with previous studies (Fang et al 2006, Massanet et al 2008, Nazlina et al. 2011) where acclimatization was used to enhance bio-H_2. Nevertheless, some studies also showed enhanced bio-hydrogen production without acclimatization (Kim et al., 2004 and Pan et al., 2008).

4.5 Gompertz Kinetic Model

This is a kinetic model used to determine the hydrogen production potential (P) and hydrogen production rate (Rm) of the different food waste used as substrates.

Mixed food waste was observed to have higher rate of H_2 production than other food waste substrates followed by rice, fish and lastly vegetable (Table 4.5). Vegetable waste had the lowest H_2 production rate while mixed food waste has the highest H_2 production rate. It is interesting to note that rice recorded the highest H_2 production potential, followed by mixed food waste, fish and vegetable. It was also observed that the cumulative H_2 production of mixed food waste substrate is higher 130.95 mL while fish, rice and vegetable were 26.97 mL, 30 mL, and 31 mL respectively.

Table 4.5 H_2 Production rate and Production Potential for Acclimatized Food Waste Substrate

FWS	**Rm (mL/d)**	**ƛ**	**H (mL)**	**ƛ-t**	**Rm.e**	**P (mL)**
Rice	41.215	1	26.97	-10	112.02	83.04
Fish	30.7	2	30	-9	83.44	55.8
Vegetable	16.1	1	31.72	-10	43.76	32.7
Mixed	60.5	5	130.95	-6	164.44	74.2

FWS = food waste substrate, Rm = maximum hydrogen production rate (mL/d), H = cumulative hydrogen production (mL), T = time (11 days), ƛ = lag period, P = hydrogen production potential (mL), e = exponential (2.718)

The higher H_2 production rate by mixed food waste might be because mixed waste has higher organic load. Higher organic loads has been reported to increase hydrogen production yield (Chen et al., 2006; Kraemer, 2007). It could also be because mixed food waste also contains some rice and other carbohydrate food sources which could enhance bio-hydrogen production.

The highest H_2 production potential by rice waste is probably because of rice is a rich source of carbohydrate while fish is a rich source of protein, vegetable, a rich source of vitamins and minerals (Leon, 2011; Steele, 2011; Yap, 2013). Mixed food waste having more iron tends to have lower H_2 production potential (Liu and Shen 2004). Higher cumulative H_2 by mixed food waste substrate can be attributed to the fact that much (60 % - 90 %) of the biogas it produced was H_2 (Guo et al., 2010; Keigo and Shigeharu, 2006).

From this Gompertz kinetic model, it can be concluded that even though rice has the highest H_2 production potential, mixed food waste produced H_2 faster than rice.

Table 4.6 shows the Gompertz kinetic parameters for non-acclimatized food waste substrates. Fish waste and mixed waste seems to have the same H_2 production potential with mixed waste having the high H_2 production rate which suggest that acclimatization was a great boost for the microbes in the mixed waste but not that great in the fish waste. It was also observed that even though vegetable waste and mixed waste had the same lag period of 4 days, the H_2 production potential of mixed waste is 15.5 mL more than that of vegetable. This might be because, the mixed waste is a combination of rice waste, fish waste and vegetable waste substrates. It is also evident that even though gas production started after 2 days for rice waste and fish waste, rice waste produced H_2 faster and also has a higher H_2 production potential then the fish waste. This might also be attributed to rice being a rich source of carbohydrate. It could also be as because 80 % - 90 % of the biogas content measured from rice waste was hydrogen (Pan et al., 2013)

This study agrees with previous studies with mixed food waste having a relatively high H_2 production rates (Jayalakshmi et al., 2007, Karlsson et al., 2008). The difference in H_2 yield in comparison with the current study could be attributed to the difference in reactor types used. A previous study by Dong et al. (2009) revealed that rice has a greater H_2 production potential than the other food waste substrates studied in this research. This is in agreement with the current study.

Gompertz kinetic model was also used to determine if acclimatized food waste has higher H_2 production potential than the non-acclimatized food waste. It was shown that the difference in the rate of H_2 production was 19.7 mL/d, 20.95 mL/d, 15.55 mL/d and 49 mL/d for rice waste, fish waste, vegetable waste and mixed food waste. This means that when compared to the non-acclimatized substrates, the rate at which H_2 is produced in the four acclimatized substrates was twice the rate at which H_2 was produced in non-acclimatized condition. Furthermore, we also observed that the hydrogen production potential of the acclimatized food waste substrates were greater than those of the non-acclimatized food waste substrates by 44.04 mL for rice waste, 38.1 mL for fish waste, 31.9 mL, 57.9 mL. These values were more than twice that of non-acclimatized food waste substrates. It will be necessary to analyze the individual differences within each condition.

Table 4.6 H_2 Production rate and production potential for non-acclimatized Food waste Substrate

FWS	Rm (mL/d	ƛ	H (mL)	ƛ-t	Rm.e	P (mL)
Rice	21.5	2	13.6	-9	58.44	39
Fish	9.75	2	13.6	-9	26.5	17.7
Vegetable	0.55	4	1.04	-7	1.49	0.8
Mixed	11.5	4	10	-7	31.257	16.3

FWS = food waste substrate, Rm = maximum hydrogen production rate (mL/d), H = cumulative hydrogen production (mL), T = time (11 days), ƛ = lag period, P = hydrogen production potential (mL), e = exponential (2.718)

Acclimatization of food waste with anaerobic sewage sludge will not only remove food waste from the MSW that goes to landfill, it will also remove the sludge from water and waste water treatment plants. The product after the experiment can also be used as compost. Therefore, it will not only be a useful resource for H_2 production, but can also be composted to provide nutrient supplement for plants (Okamoto, 2000).

4.6 Effect of Metal Ions Concentration on Bio-Hydrogen Production

One common metallic ion which can be found in MSW stream is lead (Pb). This is contained in batteries which were not separated before disposal. Thus a study to see if food waste containing this metallic ion can be used as a substrate for bio-H_2 production was conducted. The effect of different concentration of Pb in bio-H_2 production from food waste substrate is discussed in this section.

4.6.1 Effect of Metal ion Concentration of 5 mg/l of Pb on Bio-Hydrogen Production

Figure 4.13 shows the effect of 5 mg/L of Pb concentration on the bio-hydrogen production from food waste. It was observed that biogas and H_2 production commenced after day one. The reaction time for the control experiment was just 2 days after a lag period of 1 day as opposed to 6 days reaction time observed in the food waste with 5 mg/l of Pb. The biogas and hydrogen production increased until a maximum of 16.6 mL of H_2 was produced on the 5th day as opposed to 10.3 mL of H_2 on the 3rd day for the control. Beyond the 5th day, H_2 content of the biogas dropped to 3.7 mL for the food waste with 5 mg/L of Pb while no gas production was observed after the 3rd day for the control.

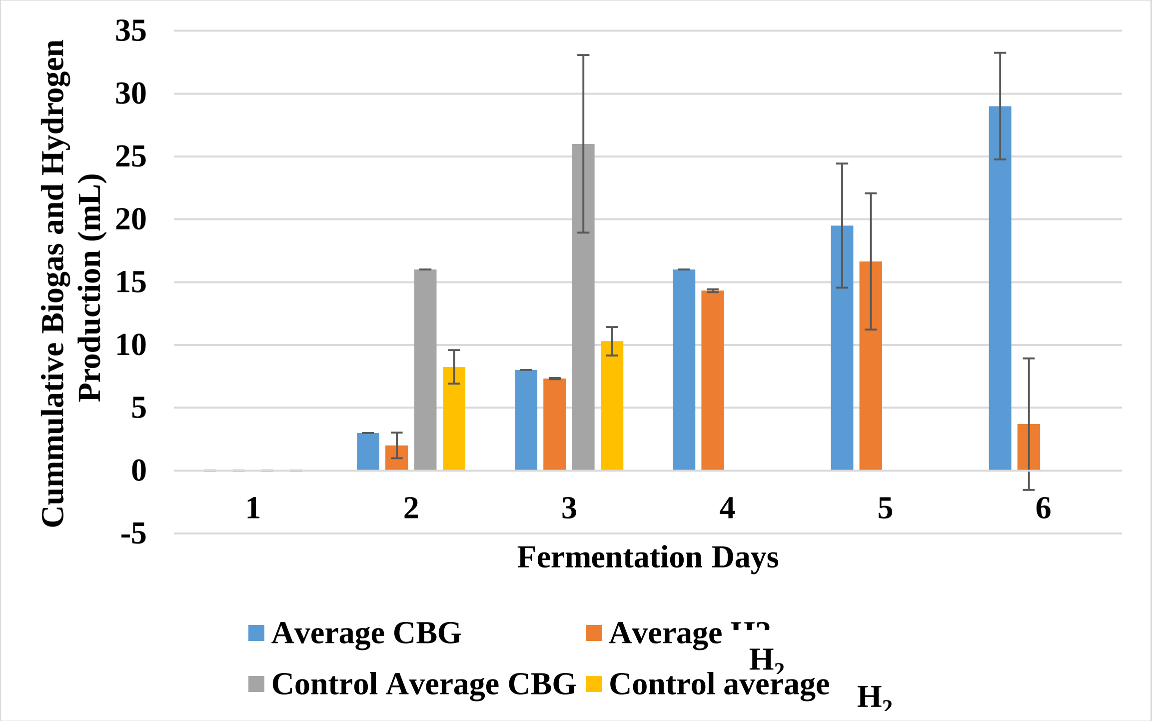


Figure 4.13 Effect of metallic ion (5 mg/L of Pb) on Bio-Hydrogen Production from Food Waste. CGB => cumulative Biogas Production

The difference observed in reaction days could be because lead ion at this trace concentration was a co-factor to the transport of hydrogenase thus making the reaction days longer (Wang and Wan, 2009). The decrease observed after day 5 could be when the lead ion became toxic to the system. It is important to note that no methane was observed throughout the experiment. We could say that the presence of lead at 5 mg/L was also an inhibitor to methane forming bacteria (Wang and Wan, 2009).

4.6.2 Effect of Metal ion Concentration at 10 mg/L on Bio-H_2 Production

It can be observed from Fig 4.14, that gas production started after the 1st day, nevertheless, the biogas and H_2 produced by the mixture containing 10 mg/L of lead was lower than the control. After the 3rd day, gas production in the control reduced to zero while that in food wastes containing 10 mg/L of Pb was on the increase. The maximum H_2 yield of 41.6 mL was recorded on the 5th day for the food waste with 10 mg/L of Pb while that of the control was 10.3 mg/L on the 3rd day. There was a drop in H_2 production on the 6th day to 1.8 mL. It was observed that the maximum H_2 from food waste with 10 mg/L was considerably more than that from 5 mg/L.

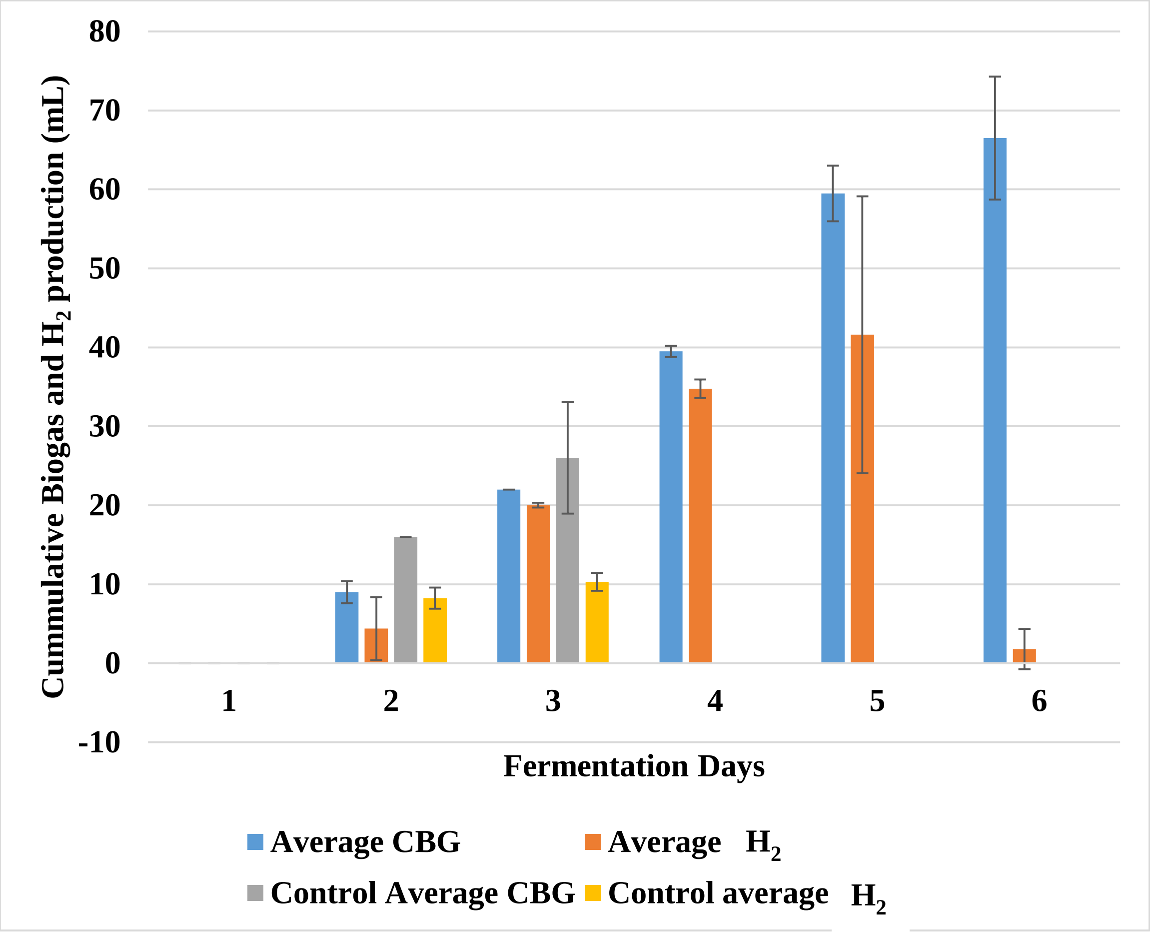


Figure 4.14 Effect of metallic ion (10 mg/L of Pb) on bio-hydrogen production from food waste.

The lower H_2 yield by the food waste with 10 mg/L could be due to the time it took for the Pb to be evenly distributed into the cells of the indigenous bacteria (Stohs and Bagchi, 1995). This result is in agreement with previous studies that increase in the concentration of metal ions increases the hydrogen yield (Sinha and Pandey, 2011; Wang and Wan, 2009). However, the H_2 yield varies in these studies which could be due to the substrates and types of reactors used.

4.6.3 Effect of Metal ion Concentration at 15 mg/L on Bio-Hydrogen Production

From Fig 4.15, it was observed that biogas and H_2 production increased simultaneously after a 1 day lag period until after the 5^{th} day when the H_2 content of the biogas dropped to almost zero. Maximum H_2 production of 52.6 mL was recorded on the 5^{th} day which was higher than 10.3 mL recorded as maximum H_2 production for the control. With a difference of 42.3 mL, one can say that addition of 15 mg/L of lead increased the production of H_2.

The low yield observed through the experiment after the 5^{th} day could be because the readily useable carbohydrate had been used up by the H_2 producing bacteria which in turn led to the stop in H_2 (Fadhil and Maleek, 2010). The high yield experienced throughout the 3 levels used in this study could be because the presence of metal ion in fermentation medium facilitate the bacterial growth by increasing the percentage of glucose consumed thereby increasing the H_2 productivity (Fadhil and Maleek, 2010).

Despite the changes in H_2 production observed at different level of Pb addition, the H_2 produced at these levels showed no statistical significance (P = 0.2) but it was only significant when compared with the control (P = 0.001).

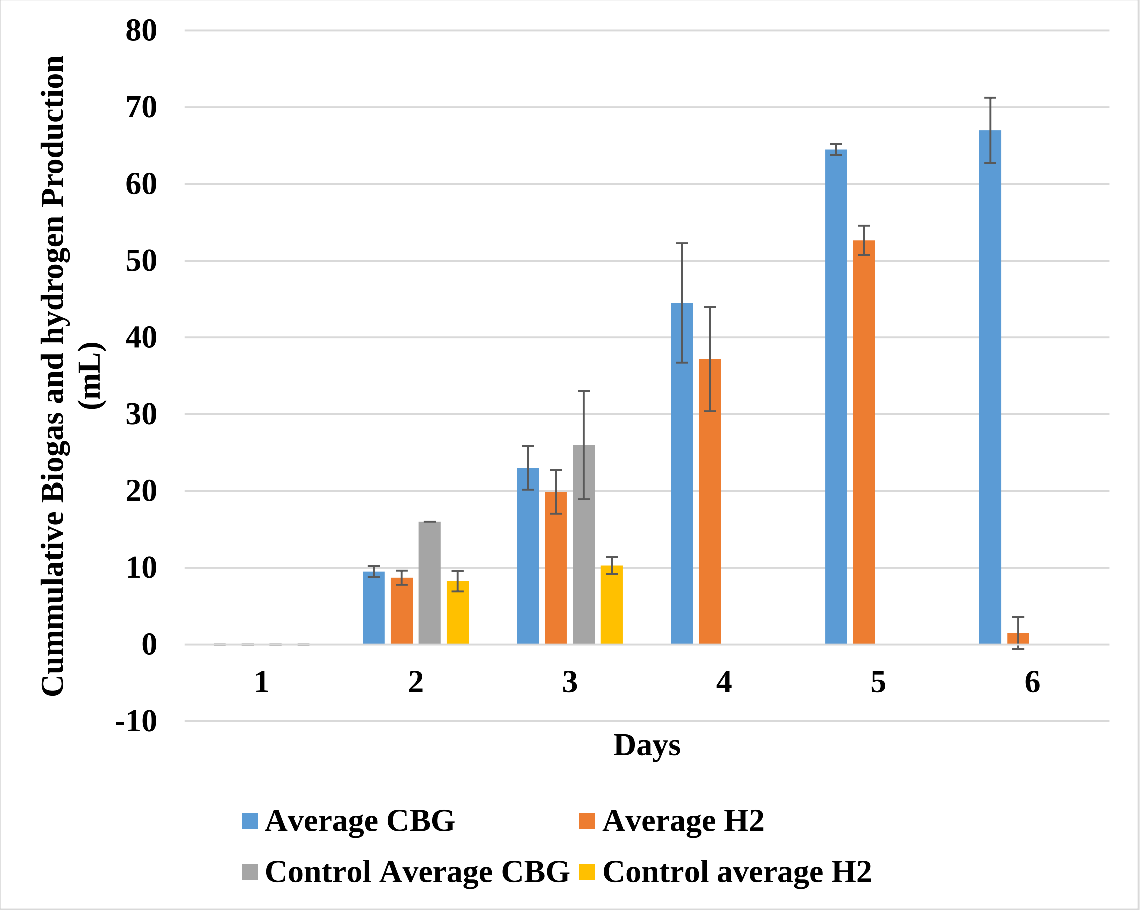


Figure 4.15 Effect of metal ion (15 mg/L of Pb) on Bio-Hydrogen production from food waste

It has been reported that metal ion in trace levels enhance H_2 production and this is supported by this result, for example, the photosynthetic bacteria, *Rodospirillumrubrum*, produced H_2 when grown in cheese whey in presence of light, then the addition of Fe ions (5 mg/L) enhanced H_2 production of treated whey to about 6000 ml in 10 days (Fadhil and Maleek, 2010). In another study, trace metal addition showed enhanced H_2 yield from 391 mLg^{-1} to 408 mLg^{-1} (Hisham et al., 2008). It has also been observed that metals such as Magnesium and calcium additions were better for growth of bacteria and not for H_2 production (Fadhil and Maleek, 2010).

4.7 Gompertz Kinetic Parameters for Metallic ion content

The control and the medium with metal additions had the same lag period of 1 day while the highest cumulative H_2 production was at 15 mg/L (Table 4.7). One could then say that the cumulative H_2 production increased as the concentration of the metal ions increases, with 5 mg/L for 44 mL/d, 10 mg/L for 102 mL/d and 15 mg/L for approximately 120 mL/d. It was also observed that the mixture with the metal ions have higher H_2 production rates and higher H_2 production potential than the control. All these parameters were also increasing with increase in metal ion concentration.

Table 4.7 H_2 Production Rate and Potential

Conc.	Rm (mL/d)	ƛ	H(mL)	ƛ-t	Rm.e	P (mL)
Control	5.15	1	5.15	-2	14.0	2.12
5mg/L	6.2	1	44	-4	16.85	5.42
10mg/L	15.2	1	102.3	-4	41.31	13.23
15mg/L	16.4	1	119.9	-4	44.58	14.37

FWS = food waste substrate, Rm = maximum hydrogen production rate (mL/d), H = cumulative hydrogen production (mL), T = time (11 days), ƛ = lag period, P = hydrogen production potential (mL), e = exponential (2.718).

Considering the H_2 production potential, 5 mg/L was 3.3 mL greater than the control, 10 mg/L was 11.11 mL greater than the control and 15 mg/L was 12.25 mL greater than the control. Considering the H_2 production rates, 5 mg/L was 0.05 mL/d greater than the control while 10 mg/L and 15 mg/L were 10.05 mL/d and 11.25 mL respectively greater than the control. The result got from Gompertz kinetic model revealed that food waste with trace amounts of lead ion has the potential to produce H_2 even more than the food waste without lead (Nasr et al., 2011).

Both the control and food waste with metal ions having the same lag period could be because lead ions had not been absorbed properly and as such could not catalyze the reaction faster than it had started (Wang and Wan, 2009). The increase in hydrogen yield as the concentration lead ion increased may be because lead ion served as a nutrient supplement for hydrogenase, thus increasing hydrogen production (Heidrich and Witkowski, 2010).

The problem with this method would be where to dispose this waste after it has been used for H_2 production. This is because it now contains metallic ions which have the ability to bio-accumulate and cause various problems in any environment. For example, Lead can cause: disruption of the biosynthesis of haemoglobin and anaemia, rise in blood pressure, kidney damage, miscarriages and subtle abortions (Lenntech, 2013).

4.8 Column Experiments

This section used batch reactor to know its effect on bio-hydrogen production. However, this experiment was conducted using mixed food waste substrate.

Figure 4.16, 4.17 and 4.18 shows the effect of metal ion concentration of 5 mg/L, 10 mg/L and 15 mg/l on bio-hydrogen production in a column respectively. The biogas contained in the column only consists of H_2 and CO_2.

4.8.1 Effect of 5 mg/L of Pb on Bio-hydrogen Production using Food Waste as a Substrate

Biogas production commenced on the second day for both the control and the food waste with Pb ion at 5 mg/L (Fig 4.16). The control was observed to produce its maximum biogas of 48 mL on the second day. However, the maximum hydrogen yield by the control was 10.3 mL on the 3rd day. The food waste with 5 mg/L of Pb produced a maximum biogas of 68 mL on the 6th day. Furthermore, it showed a maximum hydrogen yield of 42.96 mL on the 4th day beyond which hydrogen production was stabilized.

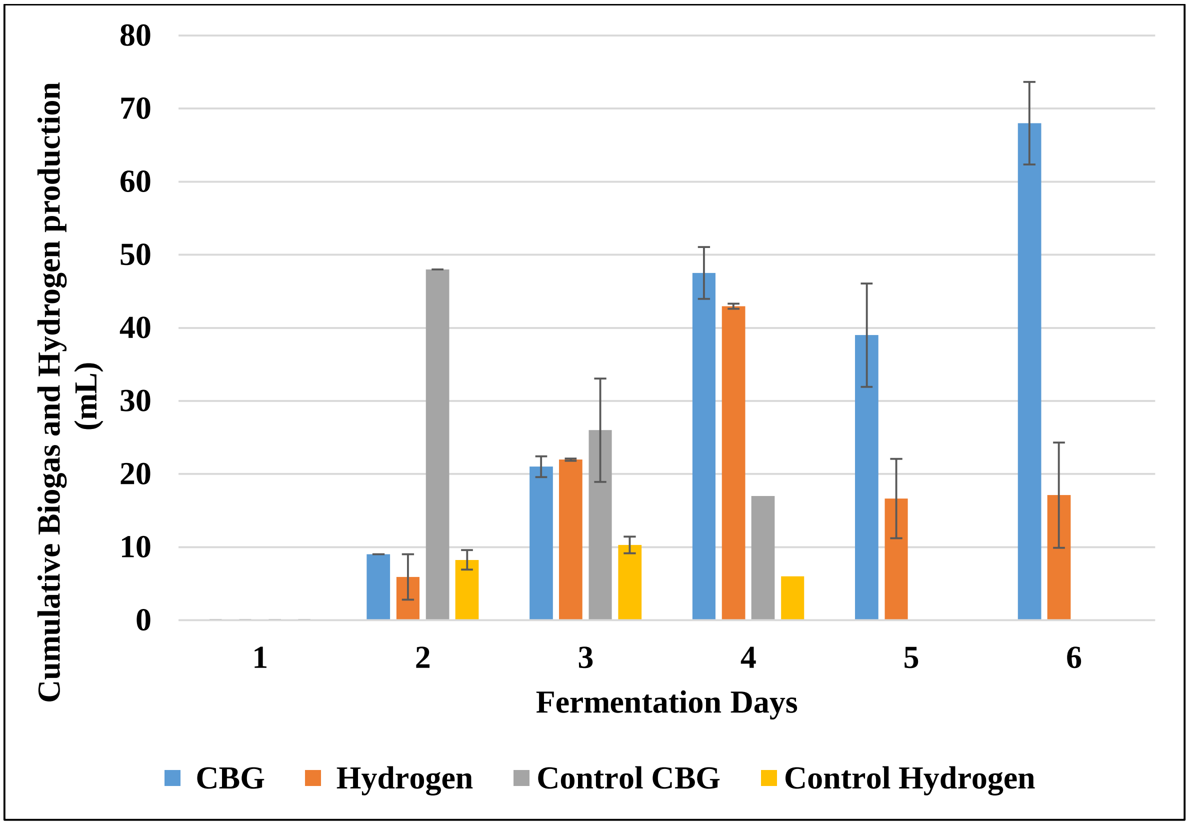


Figure 4.16 Effect of Metal ions (5 mg/L of Pb) on bio-hydrogen production. CBG → Cumulative Biogas

The difference observed in the lag period could be because concentration of the ion did not affect the lag period. This agrees with a previous study Liu et al, 2009. The higher amount of biogas and hydrogen observed in the food waste with 5 mg/L of lead could be due to the lead it contains. This was possible because a trace level of the ions is required for activation of function of many enzymes including the hydrogenase (Zheng et al., 2005). The inhibition after 6 days could be mostly due to the disruption of the hydrogenase structure because of its chemical bonding to Pb (Zheng et al., 2005).

4.8.2 Effect of 10 mg/L of Pb on Bio-hydrogen Production using Food Waste as a Substrate

Biogas production commenced on the second day for both the control and the food waste with Pb ion at 10 mg/L (Fig 4.17). A decreasing order in biogas and hydrogen production was observed in the control while the reverse was the case in the food waste with 10 mg/L of Pb. The control produced maximum biogas of 48 mL on the second day as opposed to maximum biogas production 185 mL by food waste with 10 mg/L.

However, the control has a maximum hydrogen yield of 10.3 mL while food waste with 10 mg/L has 124.8 mL. Gas production stopped after the 3rd day in the control while it stopped after the 6th day in the food waste with 10 mg/L of Pb. It is important to note that at the control had 40 % of its biogas as hydrogen at the last day while the food waste with 10 mg/L of Pb had less than 10 % of its biogas content as hydrogen.

The higher biogas and hydrogen produced by the food waste with 10 mg/L of lead could be because lead acts as a co-enzyme factor hydrogenase, thus increases increasing its transportation and action across the system (Heidrich and Witkowski, 2010). It could as well be the reason why the fermentation days were longer than the controls'.

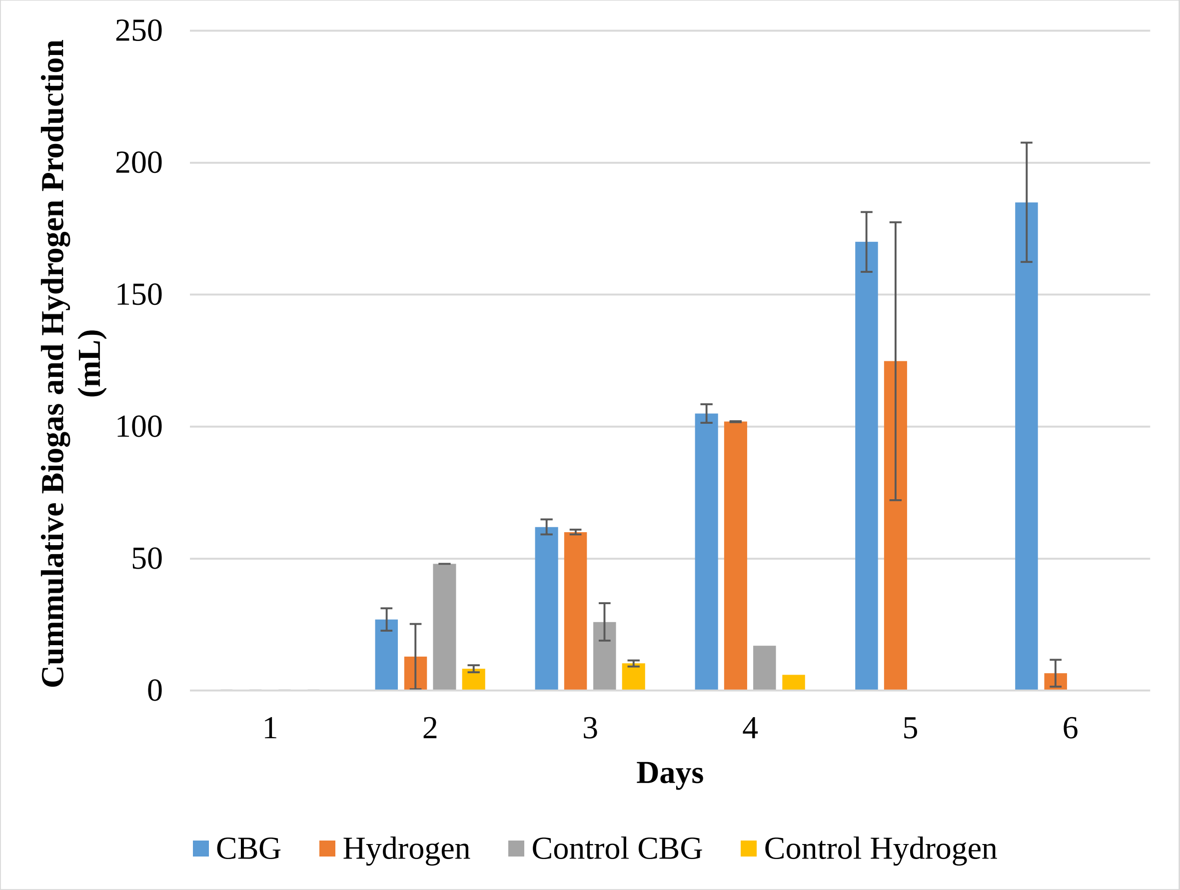


Figure 4.17 Effect of Metal ions (10 mg/L of Pb) on Bio-hydrogen production using food waste as substrate.

CBG → Cumulative Biogas

4.8.3 Effect of 15 mg/L of Pb on Bio-hydrogen Production using Food Waste as a Substrate

The control and the food waste with Pb ion at 10 mg/L commenced biogas production on the second day (Fig 4.18). A decreasing order in biogas (48 mL, 26 mL and 17 mL) was observed in the control while the reverse (28.5 mL, 69 mL, 118.5 mL, 193.5 mL and 201 mL) was the case in the food waste with 15 mg/L of Pb. Maximum hydrogen yield of 157.95 mL was observed for food waste containing Pb ions as opposed to 10.3 mL observed in the control.Just like the previous lead ion concentrations of 5 mg/L and 10 mg/L, the fermentation period was also 6 days for 15 mg/l and 4 days for the control. On the 6th day, only 5 % of the biogas produced by food waste with 15 mg/L was hydrogen while the rest 95 % was CO_2. This could be because at this stage Pb has effectively bonded with hydrogenase thus reducing its potency to produce hydrogen (Stohs and Bagchi, 1995). It could be said be said that the more the fermentation days, the less the hydrogen produced and the more the CO_2 produced. This will in turn acidify the system, which lowers the pH, thus reducing hydrogen production until no hydrogen will be produced (Yu et al., 2010).

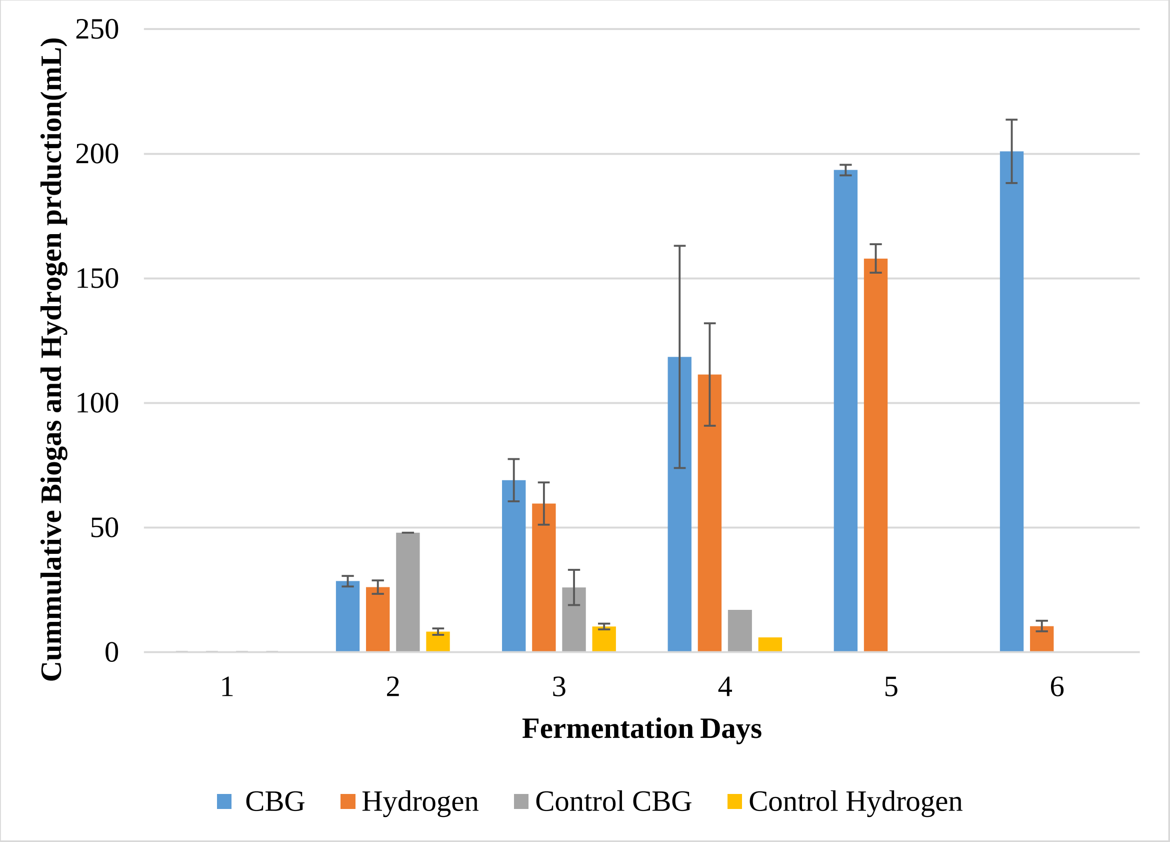


Figure 4.18 Effect of Metal ions (15 mg/L of Pb) on Bio-hydrogen production using food waste as substrate.CBG → Cumulative Biogas

This study is in agreement with previous studies showing that trace amount of metal ions improve hydrogen production but also inhibits hydrogen production on a longer fermentation days (Hakobyan et al., 2012; Heidrich and Witkowski, 2010; Hisham et al., 2008; Sinha and Pandey, 2011; Wang and Wan, 2009).

In comparison with the hydrogen produced when Pb ions were added to the bottle experiment, the column hydrogen yield tends to be 3 times more. This could be because of the higher amount of substrate used for the column study. However, there was no significant difference when hydrogen produced from bottle experiment with Pb ions was compared to that produced from column experiment.

CHAPTER 5

CONCLUSION

In this current study, hydrogen was produced from different food waste substrates with rice having the highest hydrogen production potential. However a combination of the food waste was also a good substrate for bio-hydrogen production. Temperature of 35^0C and pH 5.5 were found to be optimal when considering the optimum parameters for bio-hydrogen production. Acclimatization with anaerobic sewage sludge was found to enhance bio-hydrogen production through dark fermentation process. Hydrogen production potential and hydrogen production rate of the food waste substrates were determined using the Gompertz kinetic model in which rice waste and mixed waste showed highest hydrogen production potential and the highest hydrogen production rate respectively. Addition of metal ion such as Pb ions in trace levels also enhanced bio-hydrogen production.

REFERENCES

Abdul Rahman D. (2013). *Solid Waste Management in Malaysia.* International Conference On Waste Management and Environment, University of Malaya, Malaysia, Borneo Post online. doi: http://dx.doi.org/10.1016/j.enpol.2005.04.003

Agamuthu P. (2001). *Solid Waste: Principles and Management*. Malaysia, University of Malaya Press.

Agamuthu P. (2009*). Challenges and opportunities in agro-waste management: An Asian perspective*. Paper presented at the Inaugural meeting of first regional 3R forum in Asia.

Agamuthu P. (2014). MSW Management in Malaysia-Changes for Sustainability. *Municipal Solid Waste Management in Asia and the Pacific Islands*. Agamuthu, P and Tanaka, M. Springer Singapore**:** 195-232.

Ahn Y. P. E., Oh YK, Park S., Webster G., Weightman A.J. (2005). Biofilm microbial community of a thermophilic trickling biofilter used for continuous biohydrogen production. *FEMS Microbiol Lett.* ***249***(1), 31-38.

Akutsu Y., Li Y.Y., Harada H., and Yuc H.Q. (2009). Effects of temperature and substrate concentration on biological hydrogen production from starch. *International Journal of Hydrogen Energy* ***34***(6): 2558-2566.

Allakhverdiev S. I. (2012). Photosynthetic and biomimetic hydrogen production. *Interernational Journal of Hydrogen Energy* ***37***(10): 8744-8752.

APHA A. (2005). *Standard Methods for the Examination of Water and Wastewater*. 21st ed. American Public Health Association. Washington, DC. Part, 8000, 94-100.

Armaroli N, and Balzani, V. (2011). The hydrogen issue. *Chem Sus Chem* ***4***(1): 21-36.

Arvizu D, Palani B., Luisa F. C., Terry H. K. G., Arnulf J.-W., Michio K., Charles K., et al. (2011). Technical Summary. *IPCC Special Report on Renewable Energy Sources and Climate Change Mitigation*. O. Edenhofer, R. Pichs-Madruga, Y. Sokonaet al. Cambridge, United Kingdom and New York, NY, USA, Cambridge University Press.

Atif A. A. Y., Fakhru'l-Razi A., Ngan M. A., Morimoto M., Iyuke S. E., and Veziroglu N. T. (2005). Fed batch production of hydrogen from palm oil mill effluent using anaerobic microflora. *International Journal of Hydrogen Energy* ***30***(13–14): 1393-1397. doi: http://dx.doi.org/10.1016/j.ijhydene.2004.10.002

Avasthi K. S., Reddy R. N. and Patel S. (2013). Challenges in the Production of Hydrogen from Glycerol – A Biodiesel Byproduct Via Steam Reforming Process. *Procedia Engineering* ***51***(0), 423-429. doi: http://dx.doi.org/10.1016/j.proeng.2013.01.059

Balmer M. and Spreng D. (2008). Chapter 11 - Hydroelectric Power. In M. L. Trevor (Ed.), Future Energy. Oxford: Elsevier. Pp. 193-209.

Baron, S. (1996). *Medical microbiology*. Galveston. Texas, University of Texas Medical Branch

Bao M. D., Su H. J., and Tan T. W. (2013). Dark fermentative bio-hydrogen production: Effects of substrate pre-treatment and addition of metal ions or L-cysteine. *Fuel* ***112***(0): 38-44. doi: http://dx.doi.org/10.1016/j.fuel.2013.04.063

Basu S., Asim L K., Angels C.-O., Chunqing L., and Ivo FJ V. (2010). Membrane-based technologies for biogas separations. *Chemical Society Reviews* ***39***(2): 750-768.

Baxter L. (2005). Biomass-coal co-combustion: opportunity for affordable renewable energy. *Fuel,* ***84***(10), 1295-1302.

BP (2010) Statistical Review of World Energy

Capareda S. P. (2011). Biomass Energy Conversion, Sustainable Growth and Applications in Renewable Energy Sources, Dr. Majid Nayeripour (Ed.), ISBN: 978-953-307-408-5, InTech, Available from: http://www.intechopen.com/books/sustainable-growth-and-applications-in-renewable-energy-sources/biomassenergy-conversion.

Carlos Ramos G., Iva´n Moreno-Andrade B., Chamy R. (2012). Effect of the initial total solids concentration and initial pH on the bio-hydrogen production from cafeteria food waste. *International Journal of Hydrogen Energy*: 1 - 8.

Chen W.-H., Chen S.-Y., Kumar Khanal S., and Sung S. (2006). Kinetic study of biological hydrogen production by anaerobic fermentation. *International Journal of Hydrogen Energy* ***31***(15): 2170-2178. doi: http://dx.doi.org/10.1016/j.ijhydene.2006.02.020

Chen Y., Naidong X., Yuxiao Z., and Hui M. (2012). Enhancement of hydrogen production during waste activated sludge anaerobic fermentation by carbohydrate substrate addition and pH control. *Bioresource Technology* ***114***(0): 349-356.

Chinellato G., Cavinato C., Bolzonella D., Heaven S. and Banks C. J. (2013). Biohydrogen production from food waste in batch and semi-continuous conditions: Evaluation of a two-phase approach with digestate recirculation for pH control. *International Journal of Hydrogen Energy* ***38***(11): 4351-4360.

Chu C.-F., Li Y.-Y., Xu K.-Q., Ebie Y., Inamori Y., and Kong H.-N. (2008). A pH- and temperature-phased two-stage process for hydrogen and methane production from food waste. *International Journal of Hydrogen Energy* ***33***(18): 4739-4746. doi: http://dx.doi.org/10.1016/j.ijhydene.2008.06.060

Chum H., Faaij A., Moreira J., Berndes G., Dhamija P., Dong H., Pingoud K. (2011). *Bioenergy*. In O. Edenhofer, R. Pichs-Madruga, Y. Sokona, K. Seyboth, P. Matschoss, S. Kadner, T. Zwickel, P. Eickemeier, G. Hansen, S. Schlömer and C. von Stechow (Eds.), *IPCC Special Report on Renewable Energy Sources and Climate Change Mitigation.* Cambridge, United Kingdom and New York, NY, USA: Cambridge University Press.

Chyi-How L., Chin-Chao C., How-Chih L., Chian-Yu L., Chia-Wen L., and Chiu-Yue L. (2010). Optimal Ph And Substrate Concentration For Fermentative Hydrogen Production From Preserved Fruits Soaking Solution *J. Environ. Eng. Management* ***20***(1): 35-41.

Cicia G., Cembalo L., Del Giudice T., and Palladino A. (2012). Fossil energy versus nuclear, wind, solar and agricultural biomass: Insights from an Italian national survey. *Energy Policy* ***42***: 59-66.

Consonni S. and Viganò F. (2012). Waste gasification vs. conventional Waste-To-Energy: A comparative evaluation of two commercial technologies. *Waste Management* ***32***(4): 653-666.

Daniel H. and Natalie G. (2005). *Managing municipal solid waste in Latin America and the Caribbean Public Private Infrastructure Advisory Facility*. Washington, DC 20433, USA,

Dann C., Vandenberg J. et al. (2012). Reconsidering Waste-to-Energy. *Public Utilities Fortnightly* ***150***(3): 44-51.

Das K. M. and Debabrata (2012). State of the Art and Progress in Production of Biohydrogen. A. Nuri, David, B, Levin. India, Bentham Science Publishers: 127-136.

Doman L . (2006). International Energy Outlook 2006. *Energy Information Administration No. DOE/EIA-0484*.

Dong L., Zhenhong Y., Yongming S., Xiaoying K., and Yu Z. (2009). Hydrogen production characteristics of the organic fraction of municipal solid wastes by anaerobic mixed culture fermentation. *International Journal of Hydrogen Energy* ***34***(2): 812-820. doi: http://dx.doi.org/10.1016/j.ijhydene.2008.11.031

Dorian J. P., Franssen H. T. and Simbeck D. R. (2006). Global challenges in energy. *Energy Policy* ***34***(15): 1984-1991.

EERE (2011). U.S. Billion-Ton Update: B*iomass Supply for a Bioenergy and Bioproducts Industry*. U. S. D. o. Energy. Oak Ridge National Laboratory, Oak Ridge**:** 227.

Elijah T. I., Ibifuro A. M. and Yahaya S. M. (2009). The study of cow dung as co-substrate with rice husk in biogas production. *Scientific Research and Essay* **4**: 861-866.

Energy Information Administration (EIA 2011). U. S. *Annual Energy Outlook with projection to 2035.*

EPA (2012). A life cycle assessment of emission and sinks. *Solid Waste management and Green house gases.* Washinton DC, U.S. Environmental Protection Agency.

Eroğlu E., İnci E., Ufuk G., Lemi T., and Meral Y. (2006). Biological hydrogen production from olive mill wastewater with two-stage processes. *International Journal of Hydrogen Energy* ***31***(11): 1527-1535.

Fang H. H. P., Li C. and Zhang T. (2006). Acidophilic biohydrogen production from rice slurry. *International Journal of Hydrogen Energy* ***31***(6): 683-692. doi: http://dx.doi.org/10.1016/j.ijhydene.2005.07.005

Fadhil M. S. and Maleek M. I. (2010). Influence of Metal Ions on Hydrogen Production by Photosynthetic Bacteria Grown in Escherichia coli Pre-Fermented Cheese Whey. *Journal of Environmental Protection* ***1***: 426-430.

Fauziah S. H. and Agamuthu P. (2008). Challenges and issues in moving towards sustainable landfilling in a transitory country - Malaysia. *Waste Manag Res.,* ***29***(1): 13-19.

Fernández-Morales F. J., Villaseñor J. and Infantes D. (2010). "Modeling and monitoring of the acclimatization of conventional activated sludge to a biohydrogen producing culture by biokinetic control." *international journal of hydrogen energy* **35**(20): 10927-10933.

Gerardi M. H. (2003). *The microbiology of anaerobic digesters*. John Wiley and Sons.

Güllü D. and Demirbaş A. (2001). Biomass to methanol via pyrolysis process. *Energy Conversion and Management* ***42***(11): 1349-1356. doi: http://dx.doi.org/10.1016/S0196-8904(00)00126-6

Guo X. M., Trably E., et al. (2010). Hydrogen production from agricultural waste by dark fermentation: A review. *International Journal of Hydrogen Energy* ***35***(19): 10660-10673.

Hakobyan L., Gabrielyan L., Armen T. (2012). Ni (II) and Mg (II) ions as factors enhancing biohydrogen production by *Rhodobacter sphaeroides* from mineral springs. *International Journal of Hydrogen Energy* ***37***(9): 7482-7486.

Hallenbeck P., C. (2009). "Fermentative hydrogen production: Principles, progress, and prognosis." *International Journal of Hydrogen Energy* **34**(17): 7379-7389.

Han S.-K. and Shin H.-S. (2004). Biohydrogen production by anaerobic fermentation of food waste. *International Journal Of Hydrogen Energy* ***29***(6): 569-577.

Hanaoka T., Fujimoto S., Yoshida T., Kamei K., Harada M., Suzuki Y., . . . Minowa T. (2004). Hydrogen production from woody biomass by novel gasification using CO2

Sorbent. In J.-S. C., Sang-Eon P. and L. Kyu-Wan (Eds.), *Studies in Surface Science and Catalysis* ***153***: 103-108.

Hariklia N., Gavala I. V.,Birgitte S., and Ahring K. (2006). Biological hydrogen production in suspended and attached growth anaerobic reactor systems. *International Journal Of Hydrogen Energy* ***31***: 1164 – 1175.

Harun K, İnci E, Ufuk G, Meral Y, Lemi T. (2003). Kinetics of biological hydrogen production by the photosynthetic bacterium *Rhodobacter sphaeroides* O.U. 001 : Koku, H. et al. *International Journal of Hydrogen Energy* ***28***(4): 381–388.

Heidrich Z. (2004). Sewage system. *Wydawnictwo WSiP*.

Heidrich Z. and Witkowski A. (2010). Equipment for sewage treatment. Design examples of calculations. *Wydanie II, Wydawnictwo „Seidel – Przywecki, Sp. z o.o*.

Herbert P.F. and Hong L. (2002). Effect of pH on Hydrogen Production from glucose by mixed culture. *Bioresource Technology* ***82***: 87-93.

Hisham A, Mohd S. K, Aidil A. H, and Wan M, Wan Y. (2008). Trace Metal Effect on Hydrogen Production Using *C.acetobutylicum*. *Online Journal of Biological Sciences* ***1***(8): 1-9.

Hoornweg D. and Perinaz T. B (2012). What a waste : a global review of solid waste management. *Urban development series. U. D. S. k. papers*. Washinton DC. **15**.

Hori M., Matsui K., Tashimo M., and Yasuda I. (2005). Synergistic hydrogen production by nuclear-heated steam reforming of fossil fuels. Progress in Nuclear Energy, **47**(1–4), 519-526. doi: http://dx.doi.org/10.1016/j.pnucene.2005.05.053

Hou Y., Wang S., Zhou Y., Yan F., and Zhu J. (2013). Analysis of the carbon dioxide concentration in the lowest atmospheric layers and the factors affecting China based on satellite observations. *International Journal of Remote Sensing*, **34**(6), 1981-1994.

Hurley E. E. (2009). *Securing Australia's Energy for the Future*: Kokoda Foundation.

Hyways (2007). *The European hydrogen roadmap*. www.Hyways.de.

IPCC, Ed. (2007). *Climate Change 2007: Mitigation. Contribution of working Group III to the fourth assessment Report of the Intergovernmental panel on climate Change.* Waste Management, Cambridge University Press.

IPCC (2011). IPCC Special Report on Renewable Energy Sources and Climate Change Mitigation. *Summary for Policymakers.* Y. S. R.P.-M. O. Edenhofer, K. Seyboth, P. Matschoss, S. Kadner, T. and P. E. Zwickel, G. Hansen, S. Schlömer, C. von Stechow. Cambridge, United Kingdom and New York, NY, USA, Cambridge University.

Jagadish H P, Ma L, Raj A, Gavima C. C., and Thonart P. (2011). Study on Effect Of Pretreatment Methods on Biomethanation Of Water Hyacinth. *International Journal of Advanced Biotechnology and Research* ***2***(1): 143-147

Jaworski, C. (2007). UF Helps Animals Get Leftover Treats From Theme Parks and Restaurants. Retrieved 19th June, 2013, from http://news.ufl.edu/1997/11/25/food.

Jayalakshmi S., Sukumarak K., and Kurian J. (2007). Hydrogen production from kitchen waste using heat treated anaerobic biogas plant slurry. I*nternational Sustainable Solid Waste Management*. **34**(21), 8854-8858. doi: http://dx.doi.org/10.1016/j.ijhydene.2009.08.048

Jean-Pierre P. (2010). Membrane reactors for power stations. *Dutch newsletter*. Netherlands.

Jianlong W. and Wan W. (2009). Factors Influencing Fermentative Hydrogen Production: A Review. *International Journal of Hydrogen Energy* ***34***: 799–811

Jingwei Ma, Shuizhou K., Yinguang C. (2008). *Mesophilic Biohydrogen Production from Food Waste*. Bioinformatics and Biomedical Engineering, 2008. ICBBE 2008.

Johari A., Saeed I. A., Haslenda H., Habib A., and Mat R. (2012). Economic and environmental benefits of landfill gas from municipal solid waste in Malaysia. *Renewable and Sustainable Energy Reviews* ***16***(5): 2907-2912.

Jong k., Kim., Le Nhat., Young N, Chun, S, Wouk, K. (2008). Hydrogen Production Conditions from Food Waste by Dark Fermentation with *Clostridium beijerinckii* KCTC 1785. *Biotechnology and Bioprocess Engineering* ***13***: 499-504.

Kalinci Y., Arif H., and Ibrahim D. (2009). Biomass-based hydrogen production: A review and analysis. *International Journal Of Hydrogen Energy* ***34***(21): 8799-8817.

Kalogo Y. and Bagley D. M. (2008). Fermentative hydrogen gas production using biosolids pellets as the inoculum source. *Bioresource Technology* ***99***(3): 540-546.

Karak T., Bhagat R., and Bhattacharyya P. (2012). Municipal solid waste generation, composition, and management: the world scenario. *Critical Reviews in Environmental Science and Technology* ***42***(15): 1509-1630.

Karlsson A., Vallin L., and Ejlertsson J. (2008). Effects of temperature, hydraulic retention time and hydrogen extraction rate on hydrogen production from the fermentation of food industry residues and manure. *International Journal of Hydrogen Energy* ***33***(3): 953-962. doi: http://dx.doi.org/10.1016/j.ijhydene.2007.10.055

Keigo Y. and Shigeharu T. (2006). Fermentative Hydrogen Production from Artificial Food Wastes. *WHEC 16*. Lyon France.

Kijo-Klecckzkowska A., Otwinowski H. and Sroda K. (2012). Properties and production of sewage sludge in Poland with reference to the methods of neutralizing. *Archives of Waste Management and Environmental Protection* ***14***(4): 59-78.

Kim, Sang-Hyoun H, Sun-Kee S, Hang-Sik. (2004). Feasibility of Biohydrogen Production by Anaerobic Co-Digestion of Food Waste and Sewage Sludge. *International Journal of Hydrogen Energy* ***29***(15): 1607-16.

Kim S.H., Han S.K. and Shin H.S. (2006). Effect of substrate concentration on hydrogen production and 16S rDNA-based analysis of the microbial community in a continuous fermenter. *Process Biochemistry* ***41***: 199-207.

Kim D. H., Kim S.-H., and Shin H. S. (2009). Hydrogen Fermentation of Food Waste Without Inoculum addition. *Enzyme and Microbial Technology* ***45***: 181-187.

Kim D.-H., Jieying W., Kyung-Won J., Mi-Sun K., and Hang-Sik S. (2011). Natural inducement of hydrogen from food waste by temperature control. *International Journal Of Hydrogen Energy* ***36***(17): 10666-10673.

Kim M.-S.,Jaehwan C., and Dong-Hoon K.. (2013). Chapter 11 - Fermentative Biohydrogen Production from Solid Wastes. *Biohydrogen.* P. Ashok, C. Jo-Shu, C. H. Patrick, J.-S. C. P. C. H. Christian LarrocheA2 - Ashok Pandey and L. Christian. Amsterdam, Elsevier**:** 259-283.

Kimia S. (2013). *Hydrogen Synthesis*. http://kimia.um.edu.my/staff_new/sharifah_bee/2324/SCES2340_P3_Hydrogen_Synthesis_041218.pdf.

Kraemer J. T. and Bagley D.M. (2007). Effects of Gas Sparging in Biohydrogen Production. *Biotechnology Lett 29*: 685-695.

Krishnan V., Desa A., and Christianto S. (2007). Bio-hydrogen generation from mixed fruit peelwaste using anaerobic contact filter. *International Journal of Hydrogen Energy* ***32*:** 4754 – 4760.

Kumar A., Tormod S., Alfred A., Rodolfo C. R., Jean-Michel D., Marcos F., Douglas H., Ånund K., and Zhiyu L. (2011). Hydropower. *IPCC Special Report on Renewable Energy Sources and Climate Change Mitigation*. O. Edenhofer, R. Pichs-Madruga, Y. Sokonaet al. Cambridge, United Kingdom and New York, NY, USA, Cambridge University Press.

Lee K., Hsu Y., Lo Y., Lin P., Lin C., and Chang J. (2008). Exploring optimal environmental factors for fermentative hydrogen production from starch using mixed anaerobic microflora. *International Journal of Hydrogen Energy* ***33***: 1565 - 1572.

Lee Y-W. and Chung J. (2010). Bioproduction of hydrogen from food waste by pilot-scale combined hydrogen/methane fermentation. *International Journal of Hydrogen Energy* ***35***(21): 11746-11755.

Lenntech B. V. (2013). *Water Treatment Solutions*. from http://www.lenntech.com/periodic/elements/pb.htm#ixzz2ljMY2F5I.

Leon A. T. (2011). Health-promoting Properties of Fruit and Vegetables. Cranfield University, UK, Cranfield University, UK.

Li J., Baikun L., Gefu Z., Nanqi R., Lixin B., and Junguo H. (2007). Hydrogen production from diluted molasses by anaerobic hydrogen producing bacteria in an anaerobic baffled reactor (ABR). *International Journal of Hydrogen Energy* ***32***(15): 3274-3283.

Li Z., Wanga H., Tanga Z., Wanga X., and Bai J. (2008). Effects of pH value and substrate concentration on hydrogen production from the anaerobic fermentation of glucose. *International Journal of Hydrogen Energy* ***33***: 7413-7418.

Lin C.-Y., Chein-Chung W., and Chun-Hsiung H. (2008). Temperature effects on fermentative hydrogen production from xylose using mixed anaerobic cultures. *International Journal of Hydrogen Energy* ***33***(1): 43-50.

Liu, G., and Shen, J. (2004). Effects of culture and medium conditions on hydrogen production from starch using anaerobic bacteria. *Journal of Bioscience and Bioengineering 98*(4): 251-256. doi: http://dx.doi.org/10.1016/S1389-1723(04)00277-4

Logan B. E. and Regan J. M. (2006). Microbial fuel cells-challenges and applications. *Environmental Science and Technology 40*(17): 5172-5180.

Manaf L. A., Mohd A. A. S., and Nur I. M. Z. (2009). Municipal solid waste management in Malaysia: Practices and challenges. *Waste Management* ***29***(11): 2902-2906.

Marbán G., and Valdés-Solís T. (2007). Towards the hydrogen economy? *International Journal of Hydrogen Energy* ***32***(12): 1625-1637.

Mars A. E., Veuskens T., Budde M. A. W., van Doeveren P. F. N. M., Lips S. J., Bakker R. R., Claassen P. A. M. (2010). Biohydrogen production from untreated and hydrolyzed potato steam peels by the extreme thermophiles Caldicellulosiruptor saccharolyticus and Thermotoga neapolitana. *International Journal of Hydrogen Energy* ***35***(15): 7730-7737. doi: http://dx.doi.org/10.1016/j.ijhydene.2010.05.063

Masirin M., Idrus M., Ridzuan M. B., and Mustapha S. (2008). An overview of landfill management and technologies: a Malaysian case study at Ampar Tenang.

Massanet-Nicolau J., Dinsdale R., and Guwy A. (2008). Hydrogen production from sewage sludge using mixed microflora inoculum: Effect of pH and enzymatic pretreatment. *Bioresource Technology* *99*(14): 6325-6331. doi: http://dx.doi.org/10.1016/j.biortech.2007.12.012

Masset J., Serge H., Christopher H., Laurent B., Fabrice F., and Philippe T. (2010). Effect of pH on glucose and starch fermentation in batch and sequenced-batch mode with a recently isolated strain of hydrogen-producing *Clostridium butyricum* CWBI1009. *International Journal of Hydrogen Energy* ***35***(8): 3371-3378.

Matthew M., Yung W., Jablonski S., Kimberly, A., Magrini-Bair. (2009). Review of Catalytic Conditioning of Biomass-Derived Syngas. *Energy and Fuels* ***23***: 1874-1887.

Merrill G. m. L. (2010). The Effects of Temperature Stress on the Quality and Yield of Soya Bean. *Journal of Agricultural Science* ***2***(1): 172-179.

Michael B. S., Wooshin. P., Zuoc, Y., Bruce E. L, (2006). Inhibition of biohydrogen production by ammonia. *Water Research* ***40***: 1167 – 1172.

Millet P. and Grigoriev, S. (2013). Chapter 2 - Water Electrolysis Technologies. *Renewable Hydrogen Technologies*. L. M. Gandía, G. Arzamendi and P. M. Diéguez. Amsterdam, Elsevier**:** 19-41.

Mizuno O., Dinsdale R., Freda H., Dennis L. and Tatsuya N. (2000). Enhancement of hydrogen production from glucose by nitrogen gas sparging. *Bioresource Technology* ***73***(1): 59-65.

Mu Y., Yu H. Q. and Wang G. (2007). A kinetic approach to anaerobic hydrogen-producing process. *Water Res* ***41***(5): 1152-1160.

Muñoz-Páez K.M., Elvira R.L., Idania V.V., Noemí R.S., and Héctor M., Poggi-Varaldo. (2012). Re-Fermentation of Washed Spent Solids from Batch Hydrogenogenic Fermentation for Additional Production of Biohydrogen from the Organic Fraction of Municipal Solid Waste. *Journal of Environmental Management 95,* Supplement 3: S355-S59.

Nakahashi J. (2008). Recent life cycle assessment (LCA) activities by the Plastic Waste Management Institute, *JLCA News Letter,* pp. 3–8.

Nasr N., Elsayed E., Hisham H., George N., and Hesham M.., El Naggar (2011). Bio-hydrogen production from thin stillage using conventional and acclimatized anaerobic digester sludge. *International Journal of Hydrogen Energy* ***36***(20): 12761-12769.

Nathao, C., Ubonrat S., and Nipon P. (2013). Production of hydrogen and methane by one and two stage fermentation of food waste. *International Journal of Hydrogen Energy* 1-6pp.

Nazlina, H. M. Y., Abd Rahman. N.'A., Che M.. Hasfalina, M. Z., Mohd Y., and Mohd A.H.(2011). Microbial characterization of hydrogen-producing bacteria in fermented food waste at different pH values. *International Journal of Hydrogen Energy* ***36***: 9571 - 9580.

Nazlina, H. M. Y.,Abd Rahman. N.’A., Che M.. Hasfalina, M. Z., Mohd Y., and Mohd A.H (2009). Effect of Different Temperature, Initial pH and Substrate Composition on Biohydrogen Production from Food waste in Batch Fermentation. *Asian Journal of Biotechnology1* (2): 42-50.

Nicolet Y., Cavazza C., Fontecilla-Camps J. C. (2002)Fe-Only Hydrogenases: Structure, Future and Evolution. *J Inorganic Biochemistry **91***: 1-8.

Noor Z. Z., Yusuf R. O., et al. (2013). "An overview for energy recovery from municipal solid wastes (MSW) in Malaysia scenario." *Renewable and Sustainable Energy Reviews* **20**(0): 378-384.

O-Thong S., Prasertsan P. et al. (2007). Improvement of biohydrogen production and treatment efficiency on palm oil mill effluent with nutrient supplementation at thermophilic condition using an anaerobic sequencing batch reactor. *Enzyme and Microbial Technology **41***(5): 583-590.

Okamoto M, N. T., Miyahara T., Mizuno O. (2000). Biological hydrogen potential of materials characteristic of the organic fraction of municipal solid wastes. *Water Sci Technol **41***(3): 25-32.

Omran A., Mahmood A., Abdul Aziz, H. and Robinson G .M. (2009). Investigating Households Attitude Toward Recycling of Solid Waste in Malaysia: A Case Study. *International Journal of Environmental Research, **3***(2): 275-288.

Outlook, I. E. (2012). *International Energy Outlook*. USA: U.S. Energy Information Administration.

Pan J., Chen X., Sheng K., Yu Y., Zhang C., and Ying Y. (2013) Effect of ammonia on biohydrogen production from food waste via anaerobic fermentation. *International Journal of Hydrogen Energy*. doi: http://dx.doi.org/10.1016/j.ijhydene.2013.06.093

Pan J., Ruihong Z., Hamed M. El-M., Huawei S., and Yibin Y. (2008). Effect of food to microorganism ratio on biohydrogen production from food waste via anaerobic fermentation. *International Journal of Hydrogen Energy* ***33***(23): 6968-6975.

Parrot L., Sotamenou J. et al. (2009). Municipal solid waste management in Africa: Strategies and livelihoods in Yaoundé, Cameroon. *Waste Management* ***29***(2): 986-995

Percy K. E., Hansen M. C., and Dann T. (2012). Chapter 4 - Air Quality in the Athabasca Oil Sands Region 2011. In E. P. Kevin (Ed.). *Developments in Environmental Science*. **11**: 47-91pp Elsevier.

Qinglan H., Chang W., Dingqiang L., Yao W., Dan L., and Guiju L. (2010). Production of hydrogen-rich gas from plant biomass by catalytic pyrolysis at low temperature. *International Journal of Hydrogen Energy* ***35***(17): 8884-8890. doi: http://dx.doi.org/10.1016/j.ijhydene.2010.06.039

Rajendran K., Aslanzadeh S., and Taherzadeh M. J. (2012). Household biogas digesters—a review. *Energies* ***5***(8): 2911-2942.

Ren N., Jianzheng L., Baikun L., Yong W., and Shirui L. (2006). Biohydrogen production from molasses by anaerobic fermentation with a pilot-scale bioreactor system. *International Journal of Hydrogen Energy* ***31***(15): 2147-2157. doi: http://dx.doi.org/10.1016/j.ijhydene.2006.02.011

REN21. (2011). Renewables. *Global Status Report*, 116.

Renea R., Adriaan W., Jeremaisse M., Hubertusv H., and Ceesj B. (2007). Hydrogen Production with a Microbial Biocathode. *Environ. Sci. Technol.* ***42***: 629–634.

Richard A. (2008). Milestone for H2 Production by High-Temperature Electrolysis Retrieved 14/11/2013, 2013, from http://www.greencarcongress.com/2004/11/milestone_for_h.html

Rozendal R. A., Hamelers H. V. M., Rabaey K., Keller J., and Buisman C. J. N. (2008). Towards practical implementation of bioelectrochemical wastewater treatment. *Trends in Biotechnology* ***26***(8): 450-459. doi: http://dx.doi.org/10.1016/j.tibtech.2008.04.008

Ruihong Z., Hamed M., E., Karl H., Fengyu W.,Guangqing L., Chris C., Paul G. (2007). Characterization of food waste as feedstock for anaerobic digestion. *Bioresource Technology* ***98***: 929–935.

Saeed Isa, A., Johari A., Hashim H., Mat R., and Alkali H. (2013). Landfill gas and its renewable energy potentials in Johor,Malaysia. *International Journal of Emerging Trends in Engineering and Development* ***1***(3): 506-520.

Sathaye J., Lucon O., Rahman A., Christensen J., Denton F., Fujino J., . . . Shmakin A. (2011). Renewable Energy in the Context of Sustainable Development. In O. Edenhofer, R. Pichs-Madruga, Y. Sokona, K. Seyboth, P. Matschoss, S. Kadner, T. Zwickel, P. Eickemeier, G. Hansen, S. Schlömer and C. von Stechow (Eds.), *IPCC Special Report on Renewable Energy Sources and Climate change Mitigation*. Cambridge, United Kingdom and New York, NY, USA: Cambridge University Press.

Seadon, J. K. (2006). Integrated waste management – Looking beyond the solid waste horizon. *Waste Management,* ***26***(12): 1327-1336. doi: http://dx.doi.org/10.1016/j.wasman.2006.04.009

Shekdar, A. V. (2009). Sustainable solid waste management: an integrated approach for Asian countries. *Waste Management* ***29***(4): 1438-1448.

Shin H.-S., Youn J.H., and Kim S.H. (2004). Hydrogen production from food waste in anaerobic mesophilic and thermophilic acidogenesis. *International Journal of Hydrogen Energy,* ***29***(13): 1355-1363. doi: http://dx.doi.org/10.1016/j.ijhydene.2003.09.011

Shimizu S., Mizuno A. F., Kameda T., Yoshioka T. (2008). *Fermentative Hydrogen Production From Food Waste*. Water Dynamics, American Institute of Physics

Sinha P., and Pandey A. (2011). An evaluative report and challenges for fermentative biohydrogen production. *International Journal of Hydrogen Energy* ***36***(13): 7460-7478. doi: http://dx.doi.org/10.1016/j.ijhydene.2011.03.077

Smith J. L. (2010). The Suitability of Using Food Waste as an Anaerobic Digester Feedstock. Doctorate, University of Florida, Gainesville.

Staudt L. (2008). Chapter 6 - Wind Energy. In M. L. Trevor (Ed.), Future Energy Oxford: Elsevier. Pp. 93-110.

Stern, N. (2006). The economics of climate change (pp. 700).

Stohs S. J., and Bagchi D. (1995). Oxidative mechanisms in the toxicity of metal ions. *Free Radical Biology and Medicine* ***18***(2), 321-336. doi: http://dx.doi.org/10.1016/0891-5849(94)00159-H

Sun Y. and Cheng J. (2002). Hydrolysis of lignocellulosic materials for ethanol production: a review. *Bioresource Technology* ***83***(1): 1-11. doi: http://dx.doi.org/10.1016/S0960-8524(01)00212-7

Tawfik A. and El-Qelish M. (2012). Continuous hydrogen production from co-digestion of municipal food waste and kitchen wastewater in mesophilic anaerobic baffled reactor. *Bioresource Technology* ***114***(0): 270-274.

Tomczak-Wandzel R., Levilin E. (2013). *Biogas Production From Fish Waste in Co-digestionwith Sewage Sludge*. Sweden Environmental Institute. Swedish Environmental Institute.

Troschinetz A. M. and Mihelcic J. R. (2009). Sustainable recycling of municipal solid waste in developing countries. *Waste Management* ***29***(2): 915-923.

Ueno Y, H. S., Ishii M., Igarashi Y. (2001). Changes in product formation and bacterial community by dilution rate on carbohydrate fermentation by methanogenic microflora in continuous flow stirred tank reactor. *Appl Microbiol Biotechnol* ***57***(1-2): 65-73.

Valdez-Vazquez, I. and Poggi-Varaldo, H. M. (2009). Hydrogen production by fermentative consortia. *Renewable and Sustainable Energy Reviews* ***13***(5): 1000-1013.

Vijayaraghavan K. and Yun Y.S. (2008). Bacterial biosorbents and biosorption. *Biotechnology Advances* ***26***(3): 266-291. doi: http://dx.doi.org/10.1016/j.biotechadv.2008.02.002

Voet D, Voet JG, Pratt CW. (1999). *Fundamentals of Biochemistry*. US: John Wiley,

Wang J. and Wan W. (2009). Factors influencing fermentative hydrogen production: A review. *International Journal of Hydrogen Energy* ***34***(2): 799-811.

Worrell W.A., Vesilind P. A. (2012) *Solid Waste Engineering*. **S**econd ed. Cengage Learning, Stamford, CT.

Xiao L, Zhiyi D, Ka Y. Fung, and Ka M. Ng. (2013) Biohydrogen Generation from Anaerobic Digestion of Food Waste. *International Journal of Hydrogen Energy* ***38***(32): 13907-13.

Yap S. (2013). Alkalinity For Better Health. *The Sun.* Malaysia**:** 21.

Yasin N. H.M., Tabassum M., Mohd Ali H., and Nor'Aini A R (2013). Food waste and food processing waste for biohydrogen production: A review. *Journal of Environmental Management* ***130***(0): 375-385.

Yu W, Wang H, Xiaoqiong F, Xiaofang W, Jianxin H. (2010). Biohydrogen production from cornstalk wastes by anaerobic fermentation with activated sludge. *International Journal of Hydrogen Energy* ***35***: 3092 – 3099.

Zhang J., and Wang Q. (2005). Buffering and nutrient effects of white mud from ammonia–soda process on thermophilic hydrogen fermentation from food waste. *International Journal of Hydrogen Energy*. doi: http://dx.doi.org/10.1016/j.ijhydene.2013.08.047

Zhao X., Defeng X., Bingfeng L., Lu L., Jun Z., and Nanqi R. (2012). The effects of metal ions and l-cysteine on hydA gene expression and hydrogen production by Clostridium beijerinckii RZF-1108. *International Journal of Hydrogen Energy* ***37***(18): 13711-13717.

Zheng X-J. and Han-Qing, Y. (2005). Inhibitory effects of butyrate on biological hydrogen production with mixed anaerobic cultures. *Journal of Environmental Management* ***74***(1): 65-70.

Zhu G. F., Jiang Z. L., Liu C-X. (2011). Fermentative Hydrogen Production from Soybean Protein Processing Wastewater in an Anaerobic Baffled Reactor (ABR) Using Anaerobic Mixed Consortia. *Applied biochemistry and biotechnology* ***1***(168): 91-105.

Zong W., Ruisong Y., Peng Z., Meizhen F., and Zhihua Z. (2009). Efficient hydrogen gas production from cassava and food waste by a two-step process of dark fermentation and photo-fermentation. *Biomass and Bioenergy* ***33***(10): 1458-1463.