UNIVERSITI MALAYSIA PAHANG

BORANG PENGESAHAN STATUS TESIS

JUDUL : CONTINUOUS HYDROGEN PRODUCTION WITH ANAEROBIC POME SLUDGE IMMOBILIZED BY SYNTHETIC POLYMER

SESI PENGAJIAN : 2011/2012

Saya FAIRUZ BINTI ROSNI (AE08070)
(HURUF BESAR)

mengaku membenarkan tesis (PSM/~~Sarjana/Doktor Falsafah~~)* ini disimpan di Perpustakaan Universiti Malaysia Pahang dengan syarat-syarat kegunaan seperti berikut :

1. Tesis adalah hakmilik Universiti Malaysia Pahang
2. Perpustakaan Universiti Malaysia Pahang dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.
4. **Sila tandakan (√)

☐ SULIT (Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHSIA RASMI 1972)

☐ TERHAD (Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan)

☑ TIDAK TERHAD

Disahkan oleh

(TANDATANGAN PENULIS)

(TANDATANGAN PENYELIA)

Alamat Tetap:
NO. 30 JLN EMAS 8, TMN BKT BARU,
BKT SIPUT, 85020 SEGAMAT, JOHOR.

Nama penyelia:
DR. ZULARISAM BIN AB WAHID
Timbalan Dekan (Penyelidikan & Pasca Siswazah)
FAKULTI KEJURUTERAAN AWAM & SUMBER AL

Tarikh: 6/6/12

Tarikh: 6/6/12

CATATAN:
* Potong yang tidak berkenaan.
** Jika tesis ini **SULIT** atau **TERHAD**, sila lampirkan surat daripada pihak berkuasa/organisasiberkenaan dengan menyatakan sekali sebab dan tempoh tesis ini perlu dikelaskan sebagai **SULIT** atau **TERHAD**.
*** Tesis dimaksudkan sebagai tesis bagi Ijazah Doktor Falsafah dan Sarjana secara penyelidikan, atau disertasi bagi pengajian secara kerja kursus dan penyelidikan, atau Lapuran Projek Sarjana Muda (PSM)

CONTINUOUS HYDROGEN PRODUCTION WITH ANAEROBIC PALM OIL MILL EFFLUENT (PO[illegible]IZED BY SYNTHETIC

FAIRUZ BINTI ROSNI
AE08070

PROF. MADYA DR ZULARISAM BIN ABD WAHID

A thesis submitted in fulfillment of the requirement for the award of the degree of
Bachelor on Civil Engineering (Environment)

Faculty of Civil Engineering & Earth Resources
University Malaysia Pahang

Semester II 2011/2012

"I hereby declare that I have read this thesis and in my opinion this thesis is sufficient in terms of scope and quality for the award of the Degree of Civil Engineering & Earth Resources. I also certify that the work described here is entirely my own except for excerpts and summaries whose sources are appropriately cited in the references."

Signature : ……………………………………………………..
Name : FAIRUZ BINTI ROSNI
Date : 21st MAY 2012

"I hereby declare that I have read this thesis and in my opinion this thesis is sufficient in terms of scope and quality for the award of the degree of Bachelor of Civil Engineering"

Signature : ....
Name of Supervisor : PROF. MADYA DR ZULARISAM BIN ABD WAHID
Date : 2/6/12

Dedicated to my beloved parents,
Rosni Mohd Sheh and Masirah Ahmad
Husband, Syahrul Nizam and son, Syahfarisz Nurziyad..

ACKNOWLEDGEMENT

Alhamdulillah, a great thank to The Great Almighty ALLAH S.W.T who grants me the knowledge, strength and determination to accomplish my degree research work. My deepest gratitude to my husband Mr. Syahrul Nizam bin Shamaudin for his love, understanding, encouragement, prayers and patience that supported me through the whole course of this study.

Prof. Madya Dr. Zularisam bin Abdul Wahid, my main supervisor, provided a motivating, enthusiastic, and critical atmosphere during the many discussions we had. It was a great pleasure to me to conduct this thesis under his supervision. I also acknowledge Mr. Lakhveer Singh Thakur who as my co-supervisors provided constructive comments during my study.

I would also like to acknowledge the cooperation of the staff of the Environmental Engineering Laboratory of University Malaysia Pahang who gave full cooperation.

Not forgetting, all friends in UMP especially Sakila Sabriani, Nor Aisahton, Nik Shamim Uzaini, Nur'ain, Raja Nurshafarin, and Rosmanira who have always provided an enjoyable and friendly working environment.

Last but definitely not least, my deepest and most heart-felt gratitude to my beloved mum, Mrs. Masirah Ahmad and my adored dad, Mr. Rosni Mohd Sheh for their endless love and support. They instilled in me a love for knowledge and a strong work ethic that has enabled me to accomplish anything I set my mind to.

Finally, it is my lovely sweet son, Syahfarisz Nurziyad bin Syahrul Nizam, who has been giving me so much happiness and joy that, gave me a duplex spirit to work.

ABSTRACT

Hydrogen has been recognized as a promising energy in the future for being clean, efficient and recyclable. Therefore, it can be replaced with other non-renewable energy such as coal, gasoline, petroleum and metal cores for energy. The increasing of the palm oil processing has resulted the increasing of the Palm Oil Mill Effluent (POME) that can't be treated for drinking water and can lead to an increase the total waste water in Malaysia. The feasibility of hydrogen generation from palm oil mill effluent (POME), a high strength wastewater with high solid content, was evaluated in an upflow anaerobic sequencing batch reactor (UASBR). Most studies for the hydrogen production in the applied cell suspension systems often encountered problem with biomass washout at high dilution rates and required recycling of biomass from the effluent to maintain sufficient cell density for continuous hydrogen production. This study is needed to determine the effect of hydraulic retention time (HRT) of the immobilized Upflow Anaerobic Sludge Blanket (UASB) reactor to the quality of the POME and to characterize the POME properties in terms of the COD, TSS and VSS before and after the sample is being treated. Four different hydraulic retention times (HRT), ranging from 4 h to 16 h at constant temperature of 35°C were tested to evaluate hydrogen productivity and operational stability of immobilized UASBR. The results showed higher system efficiency was achieved at HRT of 2 h with maximum hydrogen production rate of 330 mL-H_2/h/L and hydrogen yield of 0.33 mol-H_2/mol-sucrose. COD removal reached more 50%, respectively. Butyrate was found to be the dominant metabolite in all HRTs. Low concentration of volatile fatty acid (VFA) confirmed the state of stability and efficiency of the operation was achieved in immobilized UASBR.

ABSTRAK

Hidrogen telah dikenal pasti sebagai sumber tenaga yang bersih, cekap dan boleh di kitar semula pada masa akan datang. Oleh yang demikian, ia boleh menggantikan sumber tenaga yang tidak boleh di kitar semula seperti arang batu, petrol, petroleum dan logam teras untuk penjanaan tenaga. Peningkatan pemprosesan minyak kelapa sawit telah menyebabkan pengeluaran kumbahan kilang kelapa sawit (POME) meningkat yang tidak dapat di rawat untuk dijadikan air minuman dan akan menyebabkan penambahan jumlah air sisa yang dikeluarkan di Malaysia. Kebolehlaksanaan penjanaan hidrogen daripada kumbahan kilang kelapa sawit (POME) yang merupakan air sisa yang berkepekatan yang tinggi dengan kandungan sisa pepejal yang tinggi, telah dinilai dalam pencerna lapisan enap cemar anaerobik alir-naik (UASBR). Kebanyakan kajian dalam penghasilan hidrogen mengaplikasikan penggunaan system sel terampai telah mengalami masalah dengan penyingkiran biojisim pada kadar kecairan yang tinggi dan memerlukan penggantian biojisim untuk mengekalkan kepadatan sel untuk penggunaan dalam penghasilan hidrogen secara berterusan. Kajian ini diperlukan untuk menentukan kesan masa penahanan hidraulik (HRT) terhadap sel biojisim menggunakan pencerna lapisan enap cemar anaerobik alir-naik (UASBR) untuk menentukan kualiti dan sifat-sifat kumbahan kilang kelapa sawit POME dari segi COD, TSS dan VSS sebelum dan selepas sampel dirawat. Dalam empat perbezaan masa penahanan hidraulik (HRT) iaitu 4 jam, 8 jam, 12 jam dan 16 jam pada suhu yang dimalarkan sebanyak 35°C, sampel telah diuji untuk menilai produktiviti hidrogen dan kestabilan pencerna lapisan enap cemar anaerobik alir-naik (UASBR) beroperasi. Melalui keputusan yang diperolehi menunjukkan sistem yang lebih tinggi kecekapan ialah pada masa penahanan hidraulik (HRT) 2 jam dengan kadar maksimum pengeluaran hidrogen 330 mL-H2/h/L dan hasil hidrogen 0.33 mol-H2/mol-sucrose. Kadar penyingkiran COD mencapai pada kadar yang telah ditetapkan dengan sekata iaitu dalam 50%. Butyrate te;lah didapati menjadi metabolit dominan dalam semua masa penahanan hidraulik (HRT). Dengan kepekatan lemak yang rendah bagi lemak asid meruap (VFA) mengesahkan kestabilan keadaan dan kecekpan operasi yang telah dicapai oleh pencerna lapisan enap cemar anaerobik alir-naik (UASBR).

TABLE OF CONTENTS

1 INTRODUCTION

2 LITERATURE REVIEW

2.2 Paper Review

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

^{0}C = degree Celsius
BOD = Biochemical oxygen demand
COD = Chemical oxygen demand
HRT = Hydraulic retention
H_2 = Hydrogen
kJ/g = kilo Joule per gram
LCFAs = Long chain fatty acids
mg/L = Milligram per liter
mL = Mililiter
MLVSS = Mixed liquor volatile suspended solids
NaOH = sodium hydroxide
NO_3^- = Nitrate
NO_2^- = Nitrite
O_2 = Oxygen
PEG = Polyethylene Glycols
POME = Palm Oil Mill Effluent
SMP = Soluble Metabolite Products
TDS = Total Dissolved Solid
TSS = Total Suspended Solids
UASB = Upflow Anaerobic Sludge Blanket
VFA = Volatile Fatty Acids
VSS = Volatile Suspended Solids

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Hydrogen has been recognized as a promising energy in the future for being clean, efficient and recyclable. The demand of energy increasing day by days and lead to depletion of non- renewable energy such as coal, gasoline, petroleum and metal cores. “Direct and highly efficient conversion of H_2 into electricity by fuel cells makes the application of H_2 energy even more attractive” (Levin DB, Pitt L, Love M., 2004 and Moore RB, Raman V., 1998). Therefore, low-cost and sufficient supply of H_2 could soon become in urgent demand.

Today, the primary sources of H_2 are from fossil fuels are almost depleted. “Besides, energy-intensive thermal or chemical processes are often required for H_2 production using fossil fuels, making it expensive and polluting the environment” (Shu-Yii Wu, Chi-Neng Lin, Jian-Sheng Chang, Jo-Shu Chang, 2004). A lot of researches have been carried out to utilize biomass as alternative renewable resource since this problem has overwhelmed all over the world.

Oil palm is one of the most versatile crops in tropical countries. Palm oil industry is one of the most important contributors to Malaysia economy. “Today, Malaysia is the world’s largest producer and exporter of palm oil; contributing 49.5% of world production and 64.5% of world exports” (Malaysian Palm Oil Board, 2004).

The production of palm oil results in the generation of large quantities of polluted wastewater commonly referred to as palm oil mill effluent (POME). “Typically, 1 tonne of crude palm oil production requires 5-7.5 tonnes of water; over 50% of which ends up as POME.” (Ma, 1999a). “Based on palm oil production in 2005 (14.8 million tonnes), an average of about 53 million m^3 POME is being produced per year in Malaysia” (Malaysia Palm Oil Production Council, 2006).

The POME comprises a combination of wastewater from “three main sources viz. clarification (60%), sterilization (36%) and hydrocyclone (4%) units” (Ma, 2000). It contains various suspended components including “cell walls, organelles, short fibres, a spectrum of carbohydrates ranging from hemicellulose to simple sugars, a range of nitrogenous compounds from proteins to amino acids, free organic acids and an assembly of minor organic and mineral constituents” (Ugoji, 1997).

Hydrogen has been called the "most alternative" of the alternative fuels: if it is made by electrolysis of water using electricity from a nonpolluting source like wind or solar power, then no pollutants of any kind are generated by burning it in an internal combustion engine except for trace amounts of nitrogen oxides, and if it is used in a fuel cell then even these disappear. Furthermore, no greenhouse gases are generated because there's no carbon in the fuel. All that comes out the vehicle's exhaust is drinkable water. Using hydrogen as the "battery" to store energy from a nonpolluting, renewable source would result in a truly unlimited supply of clean fuel.

The advantage of using hydrogen to store energy rather than a battery pack is that a hydrogen tank can be refilled in minutes rather than recharged in hours, and it takes less space and weight to store enough hydrogen to drive a given distance on a single refueling than it does to carry enough battery capacity to go the same distance on a single recharging. The battery-electric drive train uses energy more efficiently, and can handle the vast majority of daily commute-and-errands driving that people do, but for long trips hydrogen could prove to be a lot more convenient.

1.2 Problem Statement

Most of the study for hydrogen production on applied suspended cell-systems often encounter problem with washout of biomass at high dilution rate and would require the recycling of biomass from the effluent to maintain sufficient cell density for continuous hydrogen production. "Biological hydrogen production using suspended-cell system is normally inefficient and / or difficult to control in continuous operation. The law hydraulic retention rate of the hydrogen producing bacteria in a freely suspended-cell system limits the productivity of a reactor because of the long hydraulic retention time" (Bo Hu, Shu Lin Chen, 2008).

Nowadays, the use of palm oil for biodiesel was prevalent in Malaysia. "Palm oil is now starting to be used as an ingredient in bio-diesel and as a fuel to be burnt in power stations to produce electricity. This is a new market for palm oil which has the potential to dramatically increase global demand for this commodity" (Friends of the Earth, 2006). The increasing of the palm oil processing has resulted the increasing of the Palm Oil Mill Effluent (POME). POME can't be treated for drinking water. Therefore, it can lead to an increase the total waste water in Malaysia.

"From environmental perspective, fresh POME is a hot and acidic brownish colloidal suspension, characterized by high amounts of total solids (40 500 mg/l), oil and grease (4 000 mg/l), COD (50 000 mg/l) and BOD (25 000 mg/l)." (Singh et al., 1999; Ma, 2000). POME has been identified as one of the major sources of aquatic pollution in Malaysia. The characteristic of a typical POME is shown in Table 1.1.

Table 1.1 Typical characteristics of POME (Ma, 2000)

Parameter	**Average**	**Metal**	**Average**
pH	4.7	Phosphorous	180
Oil and Grease	4 000	Potassium	2 270
Biochemical Oxygen Demand (BOD_5)	25 000	Magnesium	615
Chemical Oxygen Demand (COD)	50 000	Calcium	439
Total Solids	40 500	Boron	7.6
Suspended Solids	18 000	Iron	46.5
Total Volatile Solids	34 000	Manganese	2.0
Ammoniacal Nitrogen	35	Copper	0.89
Total Nitrogen	750	Zinc	2.3

***All in mg/L except pH**

1.3 Research Objectives

Basically this project is base on these objectives:

i. Determine the effect of hydraulic retention time (HRT) of the immobilized Upflow Anaerobic Sludge Blanket (UASB) reactor to the quality of the POME.
ii. Characterize the POME properties in terms of the COD, TSS and VSS before and after the sample is being treated.

1.4 Scope of the Research

The scope of this study is to determine the different of effluent quality from the Palm Oil Mill Effluent (POME) taken at Lepar Hilir before and after using the immobilized UASB reactor method.

The sample will be test according the Chemical Oxygen Demand (COD), Total Suspended Solid (TSS) and Volatile Suspended Solid (VSS).

There are three stages of process in the laboratory experimental. Firstly is to determine the quality of the samples without processing the POME by using immobilized UASB reactor. Then, the second process is to determine the quality of the samples after processing the POME by using immobilized UASB reactor. After that, the process is to determine the hydrogen production based on the different value of hydraulic retention time.

The factor that need to be considering during test by using immobilized UASB reactor is the hydraulic retention times (HRT) which are 4, 8, 12 and 16 hours based on the temperature 35°C.

After the experiment done, the data collected is use for a comparison between the quality of the POME for before and after the process by immobilized UASB reactor and the total of the hydrogen production that can be produce during the experiment.

1.5 Expected Outcomes

The expected outcomes from this project are:

i. To analyze the quality of the POME before and after the experiment according to COD, TSS and VSS by using immobilized UASB reactor.
ii. To analyze factor of effect for continuous hydrogen production using immobilized UASB reactor according to the hydraulic retention time when temperature is constant.

1.6 Significance of the Research

The significances of the study are:

i. High cell concentrations and long retention time of biomass in the system.
ii. Cell reuse without the costly processes of cell recovery and cell recycle.
iii. Eliminates cell washout at high dilution rates.
iv. May provide favorable micro-environmental conditions for cells (i.e. cell-cell contact, nutrient-product gradients, pH gradients), resulting in better performance of the biocatalysts (higher yields, growth and production rates) of hydrogen.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Hydrogen (H_2) is considered as a clean source of energy and an ideal substituent for fossil fuel due to its high energy content (122 kJ/g), recyclability and non-polluting character. Therefore, it will be the best alternative energy that will use for conversion of hydrogen to electricity.

"At present 95% of commercial hydrogen generated by steam reforming of natural gas and gasification of coal" (Logan et al.2002), hence, these process is expensive and not environmental friendly.

"Biological process for hydrogen production is less energy intensive and more eco-friendly compared to conventional chemical method" (Wang and Wan 2009). But low hydrogen yield, low production rate and instability for continue high H_2 productions are still the major problems of biological process.

"Many research works on biohydrogen production from complex wastewater such as POME focused on suspended cell culture system either under batch or continuous mode of operation" (Chong et al.2009 and Ismail et al. 2010). However, continuous operation of suspended-cell system often encounters problems with washout of biomass at high dilution rates and would require the recycling of biomass from the effluent to maintain the sufficient cell density for high H_2 production.

Immobilized cell system will be another option with a feature of creating a local anaerobic environment, which is well suitable to oxygen-sensitive fermentative H_2 production. The immobilization system enhances the separation of solid–liquid in the settling tank and therefore allows for further recycling of the biomass.

Entrapment of cells in synthetic and natural polymers for H_2 production from simple sugars has been successful which is shown in Table 2.1 below. In this study we are going to use first time poly (ethylene glycol) (PEG) for immobilization of activated POME sludge for bio-hydrogen production from POME waste water.

Table 2.1 Cell immobilized technique for enhancing bio-hydrogen production.

Material used as solid support matrices for immobilization	Reactor	Inoculums	Substrate	Hydrogen production / yield With free cell	Hydrogen production / yield With immobilized cell	References
Agar gel	Batch	Rhodopseudomonas faecalis strain RLD-53,	Acetate	—	3.15 mol H2/mol acetate	[Lin et al.2011]
PMMA collagen, and activated carbon	Continuous, conditions	Anaerobic sludge	Sucrose based synthetic water	1.9 mol H2/mol sucrose	2.0 mol H2/mol sucrose	[Wu et al 2007]
Ligno-cellulosic wastes-banana leaves and coconut coir	Batch	Mixed microbial cultures (MMCs)	Glucose	—	1.54-1.65 mol/mol glucose	[Patel et al.2010]
Calcium alginate matrix (CA+CH+TiO2)	Batch	Anaerobic sludge	Sucrose	6.8 mmol/L h	21.3 mmol/L h	[Park and Chang 2000]
Polyvinyl alcohol (PVA)-boric acid gel	Photobioreactor.	Rhodopseudomonas palustris CQK 01	Glucose	—	3.6 mmol/g cell dry weight/h	[Tian et al. 2009]
Ager gel or on porous glass beads	Column reactor	Enterobacter aerogenes strain HO-39	Glucose	0.54 mol Hz/mol glucose	0.73 mol Hz/mol glucose	[Yoki et al 1997]
Lignocellulosic agroresidues	Bioreactor	Enterobacte cloacae IIT-BT 08	Lignocellulosic	37 mmol/L h	62 mmol/L h	[Kumar and Das2001]
Polyurethane form	IBR	Clostridium tyrobutyricum JM1	Glucose	—	223 mL/g hexose	[Jo et al.2008]
Granular activated carbon (GAC)	AFBR	Activated sludge and digested sludge	Glucose synthetic wastewater	—	1.16 mol H2/mol glucose	[Zhang et al.2007]
EVA (ethylene vinyl actate copolymer)	Batch	Sewage sludge	Sucrose	0.89 mol H2/ mol sucrose	1.74 mol H2/mol sucrose	[Wu et al.2005]
Ager gel	Batch	Rhodobacter sphaeroides	Tofu wastewater	1.9 L/h m gel glucose medium(control)	2.1 L/h m gel glucose	[Zhu et al.1999]

2.2 Paper Review

2.2.1 Hydrogen Production

H_2 which helped power the first working fuel cell, which generates electricity from the reaction between H_2 and O_2 is the first produced artificially in the 16th century and identified as a unique element in 1766. H_2 production by microorganisms has been known for over 100 years but it was not until in the 1970′s that real development and research started. H_2 exists naturally in earth's crust as bound state and there is a need to produce H_2.

"Regarding the limited fossil energy resources, energy security and the global environment, a great attention is being paid to the usage of hydrogen as fuel to reduce the emission of CO2 to the atmosphere. Hydrogen as the future energy source has recyclability and non-polluting nature" (Argun H, 2008).

Hydrogen production using biological process is one of the attractive processes because it results less energy intensive compare to other method. "Biological hydrogen production is of great interest because it can be operated at ambient temperature and pressure and consequently is less energy intensive compared to traditional thermo-chemical and electro-chemical process. Production of hydrogen through biological process can be achieved by using anaerobic process" (Marzieh Badiei, 2011).

"Besides electrolytic or thermochemical process, both of which are energy intensive" (Rajeshwar et al, 1994), "hydrogen can also be produced from organic waste and wastewater by fermentation bacteria" (Fang and Liu, 2002; Li and Fang, 2006). "This process reduces pollution and produces a clean energy at the same time" (Fang et al, 2004).

At first the main focus was on photosynthetic bacteria and fermentation was mostly left unattended. It can be produced from fossil fuels and biomass via different methods. Although the processes involve renewable sources and involve expensive techniques, these are still practiced due to the abundant availability of low cost coal from water (thermal and thermo-chemical processes) or electrolysis and photolysis. "The H_2 economy has the potential to provide a sustainable and secure system and there is a wide growing literature promoting and exploring different possible H_2 future." (Nandi and Sengupta, 1998; McDowall and Eames, 2007; Kotay and Das, 2008).

2.2.2 Palm Oil Industry

"Over the last 30 years, Malaysian palm oil industry has grown rapidly and at present it is one of the largest agro-based industries in the world" (Wong et al. 2002; Wu et al. 2010).

"Malaysia currently accounts for 51% of world palm oil production and 62% of world exports. In 2004, it was reported that Malaysia produces 14 million tons of palm oil planted on 38 000 square kilometers of land" (Nur Azeera Binti Badroldin, 2010).

"Today, Malaysia is the world's largest producer and exporter of palm oil; contributing 49.5 % of world production and 64.5 % of world exports" (Malaysian Palm Oil Board, 2004). "The total oil palm planted area increased by 4.5 % or 174,000 hectares to 4.0 million hectares in 2005 compared to that in 2004. The production of crude palm oil continued to increase for seven consecutive years reaching 15.0 million tonnes in 2005 from 14.0 million tones in the previous year" (Malaysian Palm Oil Board, 2005).

2.2.3 Palm Oil Mill Effluent (POME)

A typical palm oil mill releases liquid effluent, known as palm oil mill effluent (POME), gaseous emissions from boilers and incinerators, solid waste materials and by-products that include empty fruity bunches (EFB), potash ash, palm kernel, fiber and shells (Wong et al., 2002). These wastes, if not disposed properly, will cause negative and deleterious effect to the environment as well as human beings.

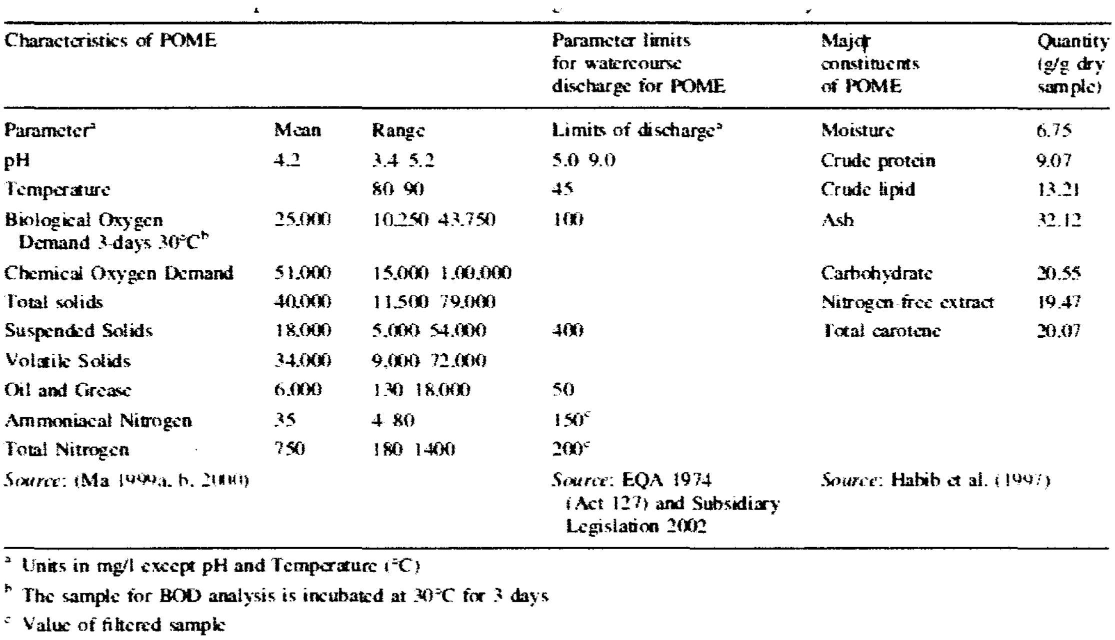
POME is a colloidal suspension of 95-96% water, 0.6-0.7% oil, 4-5% total solids including 2-4% suspended solids originating from the mixture of a sterilizer condensate, separator sludge and hydrocyclone wastewater (Abdul Latif et al., 2003).

Palm oil mill effluent is considered as one of the most polluting agro-industrial effluent due to its high values of COD and BOD. “Today, the percolation of palm oil mill effluents into the waterways and ecosystems, remain a fastidious concern towards the public health and food chain interference” (Foo and Hameed 2010). “This can cause considerable environmental problems if discharged without effective treatment by polluting land, water and destroying aquatic biota” (Cheng et al. 2010; Singh et al. 2011). “This generates an enormous amount of liquid effluent known as palm oil mill effluent (POME) and consequently creates significant amount of pollution when released into rivers and lakes without proper treatment” (Nur Azeera Binti Badroldin, 2010).

Therefore, we have to treat the POME before release it to the pond, lake and etc. “Palm oil mills are required to treat their POME prior to discharging it into streams and rivers. In the process of palm oil milling, POME is mainly generated from sterilization and clarification of palm oil in which a large amount of steam and hot water are used” (Zinatizadeh et al. 2006; Rupani et al. 2010).

"POME is acidic (pH 4–5), has discharge temperature of 80–90°C or 50–60°C and is non toxic (since no chemicals are added during extraction)" (Ahmad et al. 2003). The characteristics and the parameter limits for POME discharge into watercourses in Malaysia are summarized in Table 2.2. "It is rich in organic matter such as proteins, carbohydrates and lipids along with nitrogenous compounds and minerals" (Wu et al. 2007).

Table 2.2 Characteristics and parameter limits for POME discharge into water courses in Malaysia



Characteristics of POME			Parameter limits for watercourse discharge for POME	Major constituents of POME	Quantity (g/g dry sample)
Parameter[a]	Mean	Range	Limits of discharge[a]	Moisture	6.75
pH	4.2	3.4 5.2	5.0 9.0	Crude protein	9.07
Temperature		80 90	45	Crude lipid	13.21
Biological Oxygen Demand 3-days 30°C[b]	25.000	10.250 43.750	100	Ash	32.12
Chemical Oxygen Demand	51.000	15.000 1.00.000		Carbohydrate	20.55
Total solids	40.000	11.500 79.000		Nitrogen free extract	19.47
Suspended Solids	18.000	5.000 54.000	400	Total carotene	20.07
Volatile Solids	34.000	9.000 72.000			
Oil and Grease	6.000	130 18.000	50		
Ammoniacal Nitrogen	35	4 80	150[c]		
Total Nitrogen	750	180 1400	200[c]		
Source: (Ma 1999a, b, 2000)			*Source*: EQA 1974 (Act 127) and Subsidiary Legislation 2002	*Source*: Habib et al. (1997)	

[a] Units in mg/l except pH and Temperature (°C)

[b] The sample for BOD analysis is incubated at 30°C for 3 days

[c] Value of filtered sample

Therefore, it can be reused for biotechnological means. The various effluent treatment schemes, which are currently used by the Malaysian palm oil industry, are listed in descending order:

a) Anaerobic / facultative ponds tank digestion and mechanical aeration,
b) Tank digestion and facultative ponds,
c) Decanter and facultative ponds,
d) Physic-chemical and biological treatment,

e) Evaporation and a clarification coupled with filtration and aeration.

"Palm oil mill effluent (POME) and its derivatives have been exploited as fermentation media to produce various products and have shown high potential as a substrate for generation of hydrogen. The possibility of reusing POME as fermentation media is largely due to the fact that POME contains high concentration of carbohydrates, protein, nitrogenous compounds, lipids and minerals" (Marzieh Badiei, 2011).

"Many anaerobic fermentation studies used mixed culture of POME sludge to convert POME to hydrogen and their operating conditions and reactors configuration always affected on efficiency of microbial degradation of wastewaters by anaerobic fermentation." (Marzieh Badiei, 2011).

2.2.4 Dark Fermentative Hydrogen Production

Fermentative H_2 production is a novel aspect and ubiquitous phenomenon under anoxic or anaerobic conditions (i.e., no O_2 present as an electron acceptor). When bacteria grow on organic substrates (heterotrophic growth), these substrates are degraded by oxidation to provide building blocks and metabolic energy for growth.

Biological H_2 production processes particularly fermentation process is environment friendly and less energy intensive as compared to other methods. Biological H_2 production can be more attractive by using organic wastewater or other wastes. "Microorganisms can reduce protons on fermentation to get rid of excess reducer in cases where the medium contains no terminal electron acceptor [for example, O_2, nitrate (NO_3^-), nitrite (NO_2^-) or sulfate]" (Bartacek et al., 2008).

"Of the variety of possible substrates, practical H_2 fermentations are restricted to carbohydrate-rich materials. Proteins are hydrolysed to amino acids which are principally fermented in pairs by so-called Strickland reactions where one amino acid serves as the electron acceptor for the oxidation of the second amino acid. These reactions thus yield no H_2. Lipids are hydrolysed to glycerol and long chain fatty acids (LCFAs). LCFAs are degraded to acetate and H_2 in natural systems by syntrophic bacteria, but this reaction is only possible at the extremely low *p*H2 (partial pressure of H_2) maintained by the associated methanogenic or sulphate-reducing bacteria" (Bartacek et al., 2008).

2.2.5 Upflow Anaerobic Sludge Blanket (UASB) reactor

The Upflow Anaerobic Sludge Blanket (UASB) reactor is a form of anaerobic (oxygen free) digester that is used in the treatment of wastewater. "Upflow anaerobic sludge blanket (UASB) reactor is a popular anaerobic reactor for both high and low temperature" (Dinsdale, et al., 1997).

"The UASB reactor is by far the most widely used high rate anaerobic system for anaerobic sewage treatment. In the case of a relatively low strength wastewater such as sewage, the hydraulic retention time rather than organic loading rate is the most important parameter determining the shape and the size of the UASB reactor" (A. A. Azimi, 2004).

"The UASB reactor exhibits positive features, such as high organic loading rates (OLRs), short hydraulic retention time (HRT) and a low energy demand" (Borja and Banks, 1994; Metcalf and Eddy, 2003).

"Anaerobic sludge in UASB reactors spontaneously immobilizes into well settling granular sludge. It has been widely adopted for treatment of medium to high strength industrial wastewaters" (Lettinga and Hulshoff Pol, 1991; Fang et al., 1995).

Lettinga et al. (1980) has developed UASB reactor and this system has been successful in treating a wide range of industrial effluents including those with inhibitory compounds.

"The underlying principle of the UASB operation is to have an anaerobic sludge which exhibits good settling properties and efficiently retains complex microbial consortium without the need for immobilization on a carrier material (for example, as a biofilm) by formation of biological granules with good settling characteristics. Performance depends on the mean cell residence time and reactor volume depends on the hydraulic residence time, therefore, UASB reactor can efficiently convert organic compounds of wastewater into methane in small 'high-rate' reactors. " (Muhammad Asif Latif, 2011).

"Approximately 60% of the thousands of anaerobic full-scale treatment facilities worldwide are now based on the UASB design concept, treating a various range of industrial wastewaters" (Jantsch et al., 2002; Karim and Gupta, 2003).

According to previous research studies also suggest the possibility of this process to treat the domestic effluent. "The key feature of this system is the microbial aggregation into a symbiotic multilayer structure called a granule and retention of highly active biomass with good settling abilities in the reactor" (Schmidt and Ahring, 1996).

There are several advantages and limitation of the UASB reactor. Table 2.3 below shows the advantages and limitation of the UASB reactor (Muhammad Asif Latif, 2011).

Table 2.3 Advantages and limitation of the UASB reactor. (Muhammad Asif Latif, 2011).

Advantages	1.Good removal efficiency can be achieved in the system, even at high loading rates and low temperatures. 2.The construction and operation of these reactors is relatively simple and low demand for foreign exchange due to possible local production of construction material, plant components, spare parts and low maintenance. 3.Much less bio-solids waste generated compared with aerobic process because much of the energy in the wastewater is converted to a gaseous form and resulting in very little energy left for new cell growth. 4.The sludge production is low, when compared to aerobic methods, due to the slow growth rates of anaerobic bacteria. 5.The sludge is well stabilized for final disposal and has good dewatering characteristics. It can be preserved for long periods of time without a significant reduction of activity, allowing its use as inoculum for the startup of new reactors.
Limitation	1.Pathogens are only partially removed, except helminthes eggs, which are effectively captured in the sludge bed. Nutrients removal is not complete and therefore a post-treatment is required. 2.Due to the low growth rate of methanogenic organisms, longer startup takes before steady state operation, if activated sludge is not sufficiently available. 3.Hydrogen sulphide is produced during the anaerobic process, especially when there are high concentrations of sulfate in the influent. A proper handling of the biogas is required to avoid bad smell and corrosion. 4.Post-treatment of the anaerobic effluent is generally required to reach the surface water discharge standards for organic matter, nutrients and pathogens.

According to Singapore International Water Week, the benefits of using the Upflow Anaerobic Sludge Blanket (UASB) reactor are:

1. It is able to treat highly polluted water
2. Low energy requirement because aeration is not needed
3. Less operation and maintenance cost
4. Lower skill requirement for operation / supervision
5. Compact design
6. Less sludge production
7. Biogas produced by the process can be used for other purposes
8. Sludge can be used as fertilizers

The present work evaluates an important design parameter for a UASB reactor, that is, Hydraulic Retention Time (HRT). "The performance of a UASB reactor was assessed by applying various hydraulic retention times" (A. A. Azimi, 2004).

2.2.6 Chemical Oxygen Demand (COD)

Chemical oxygen demand is a vital test for assessing the quality of effluents and waste waters prior to discharge. The Chemical Oxygen Demand (COD) test predicts the oxygen requirement of the effluent and is used for monitoring and control of discharges, and for assessing treatment plant performance.

The impact of an effluent or waste water discharge on the receiving water is predicted by its oxygen demand. This is because the removal of oxygen from the natural water reduces its ability to sustain aquatic life. The COD test is therefore performed as routine in laboratories of water utilities and industrial companies.

In the COD method, the water sample is oxidised by digesting in a sealed reaction tube with sulphuric acid and potassium dichromate in the presence of a silver sulphate catalyst. The amount of dichromate reduced is proportional to the COD. A reagent blank is prepared for each batch of tubes in order to compensate for the oxygen demand of the reagent itself.

Over the range of the test a series of colours from yellow through green to blue are produced. The colour is indicative of the chemical oxygen demand and is measured using a Photometer. The results are expressed as milligrams of oxygen consumed per litre of sample. (http://www.palintest.com/media/uploads/137.pdf)

2.2.7 Total Suspended Solids (TSS)

Total suspended solids (TSS) include all particles suspended in water which will not pass through a filter. Suspended solids are present in sanitary wastewater and many types of industrial wastewater. There are also nonpoint sources of suspended solids, such as soil erosion from agricultural and construction sites.

As levels of TSS increase, a water body begins to lose its ability to support a diversity of aquatic life. Suspended solids absorb heat from sunlight, which increases water temperature and subsequently decreases levels of dissolved oxygen (warmer water holds less oxygen than cooler water). Some cold water species, such as trout and stoneflies, are especially sensitive to changes in dissolved oxygen. Photosynthesis also decreases, since less light penetrates the water. As less oxygen is produced by plants and algae, there is a further drop in dissolved oxygen levels.

TSS can also destroy fish habitat because suspended solids settle to the bottom and can eventually blanket the river bed. Suspended solids can smother the eggs of fish and aquatic insects, and can suffocate newly-hatched insect larvae.

Suspended solids can also harm fish directly by clogging gills, reducing growth rates, and lowering resistance to disease. Changes to the aquatic environment may result in a diminished food sources, and increased difficulties in finding food. Natural movements and migrations of aquatic populations may be disrupted.

For point sources, adequate treatment is necessary to insure that suspended solids are not present at levels of concern in waters of the state. Treatment typically consists of settling prior to discharge of the wastewater. Settling allows solids to sink to the bottom, where they can be removed. Some types of wastewaters, such as noncontact cooling water, are naturally low in suspended solids and do not require treatment.

For nonpoint sources, control measures should be implemented to reduce loadings of suspended solids to streams, rivers and lakes. Farming practices such as no-till minimize soil erosion and help protect water quality. For construction sites, controls such as silt fences and sedimentation basins are designed to prevent eroding soils from reaching surface waters. In urban areas, storm water retention ponds or a regular schedule of street sweeping may be effective in reducing the quantity of suspended solids in storm water run-off.
(http://www.michigan.gov/documents/deq/wb-npdes-TotalSuspendedSolids_247238_7.pdf)

2.2.8 Volatile Suspended Solids

"Volatile suspended solid is a water quality measure obtained from the loss on ignition of total suspended solids" (Wikipedia). The residue of Total Suspended Solid (TSS), Total Dissolved Solid (TDS) after heating to dryness for a defined amount of time and at a defined temperature is defined as fixed solids. Volatile solids are those solids lost on ignition (heating to 550^0C) after one hour. They are useful to the treatment plant operator because they give a rough approximation of the amount of organic matter present in the solid fraction of wastewater, activated sludge and industrial wastes.

CHAPTER 3

METHODOLOGY

3.1 Introduction

Methodology can be defined as the analysis of the principles of methods, rules, and postulates employed by a discipline. Other than that, it is also the systematic study of methods that are, can be, or have been applied within a discipline or a particular procedure or set of procedures. There are various methods in which you can collect the data such as interview, questionnaire, survey, experimental, case study and observation.

In this chapter, we are going to explain in details our methodology which is including sampling processes and laboratory assessment.

3.2 Project Flow Diagram

Refer to the Figure 3.1 below, the projects starts with literature review and research about the title. The tasks have been done through research on the interview, internet, books, journals and other sources.

After all relevant information is found, the project undergoes collecting the sample at Lepar Hilir. In this step, the sample that we use is Palm Oil Mill Effluent (POME).

Next, experimental in laboratory will be conducted by COD, TSS and VSS test to get the initial value for COD, TSS and VSS for raw sample.

Immobilized UASB reactor will be set up. The immobilized-cell matrix, polyethylene glycol prepolymer (PEG) will be used to entrap the activated POME sludge for continuous hydrogen production from POME waste water by immobilized UASB reactor.

The POME will be tested again to vary the COD, TSS and VSS value before and after the immobilizing process using immobilized UASB reactor.

After all the process mentioned is done, the result will be analyzed. All the materials for report writing are gathered. The report writing process will be guided by the UMP final year project report writing. This process also includes the presentation slide making for the final presentation of the project.

The project ended after the submission of the report and the slide presentation has been present.

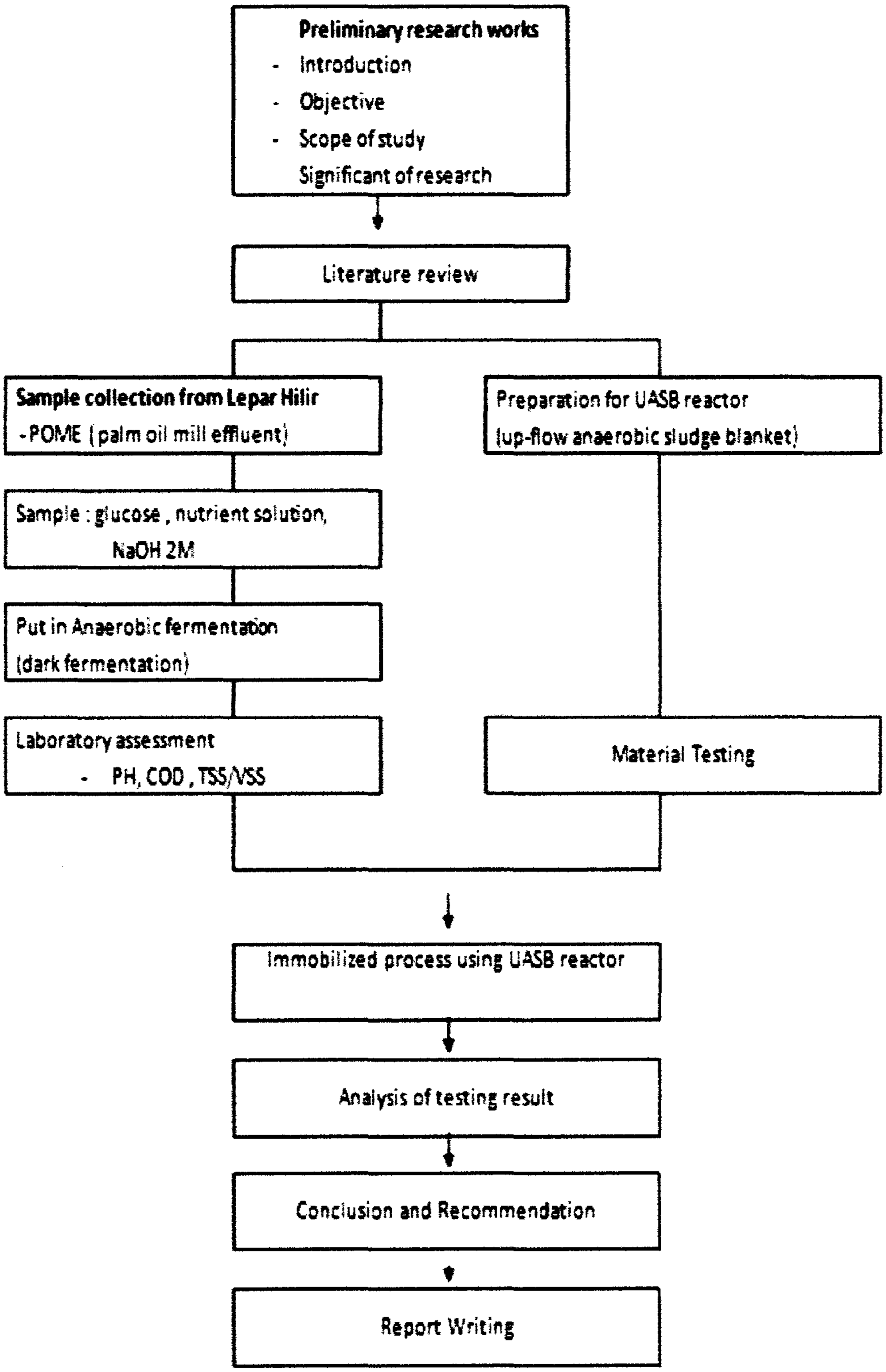


Figure 3.1 Project Flow Diagrams

3.3 Sampling Processes

The sample taken at Lepar Hilir must be prepared before the laboratory assessment start. In this step, the sample that we use is Palm Oil Mill Effluent (POME). The sample was added with nutrient, glucose and nutrient solution as the food to the bacteria inside the POME. The sample was covered by parafilm to put it in dark fermentation (anaerobic fermentation).

After the entire sample is prepared, 2 mol sodium hydroxide (2 M NaOH) was put inside the sample every 24 to 36 hours to maintain the pH value thus to activate the bacteria. To activate the sludge, the pH value of the sample must be in range 6.3 to 7.8.

3.4 Laboratory Assessment

Before do the laboratory assessment, the pH value was determined. Chemical Oxygen Demand (COD), Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS) have been tested to determine the characteristics of the POME. Polyethylene glycol prepolymer (PEG) has been used to entrap the activated POME sludge for continuous hydrogen production from POME waste water by immobilized UASB reactor. Finally, the POME was characterized again.

3.4.1 Chemical Oxygen Demand (COD) Test

3.4.1.1 Description

Chemical Oxygen Demand (COD) is the quantity needed to chemically oxidize the organic compound in sample, converted to carbon dioxide and water.

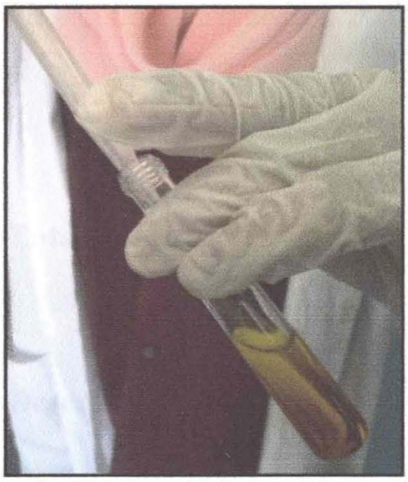
3.4.1.2 Apparatus

1. COD reactor
2. High Range COD Digestion Reagent Vials
3. Spectrophotometer

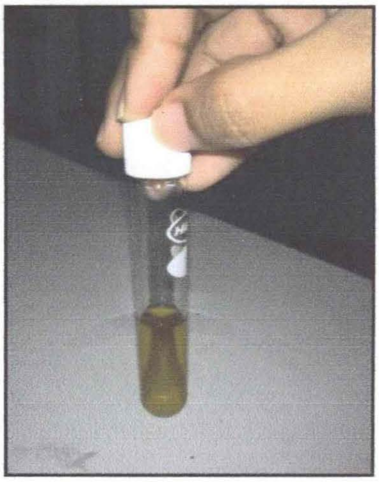
3.4.1.3 Procedure

1. Turn on the COD reactor and preheat to 150°C. Place the shield in front of the reactor.
2. Dilute 1.00 ml of sample with 200 ml of distilled water.
3. Set proportion of samples needed in volumetric pipet and for test is 2.00 ml
4. Hold one vial at 45 degree angle. Pour the sample into High Range COD digestion Reagent Vials using the volumetric pipet (be sure to use vials for appropriate range). See Figure 3.2 (a) and (b).
5. Hold a second vial at 45 degree angle. Use a clean volumetric pipet to add 2.00 mL of deionized water to the vial. This is for blank sample. See Figure 3.2 (c).
6. Cap the vials tightly. Rinse them with deionized water and wipe with clean paper towel.

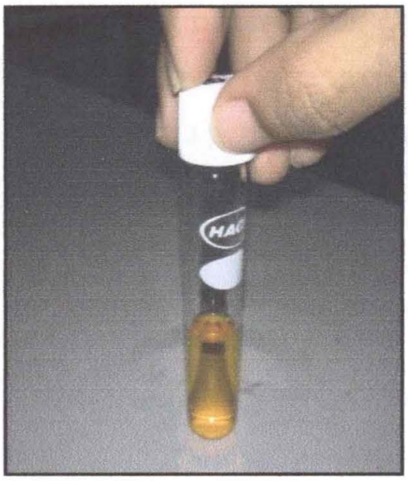
7. Hold the vials by cap over the sink. Invert gently several times to mix. Place the vials in the preheated COD Reactor. The samples vials will become hot during mixing. See Figure 3.2 (d).
8. Heat the vials for two hours in COD reactor.
9. After two hours, turn off the reactor and wait for 20 minutes for the vials to cool off. See Figure 3.2 (e)
10. By using spectrophotometer, set blank to zero. Then only, place the vial of sample into the cell holder. Wait for the timer to beep and the result will shown. See Figure 3.2 (f).



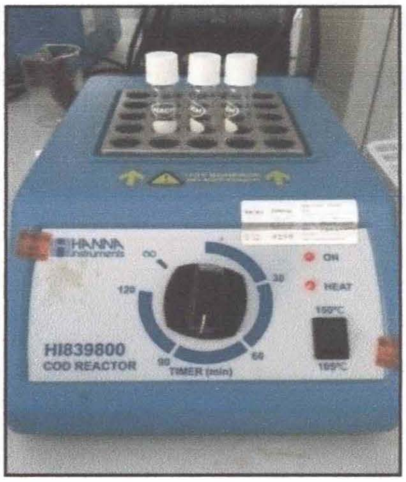
(a)



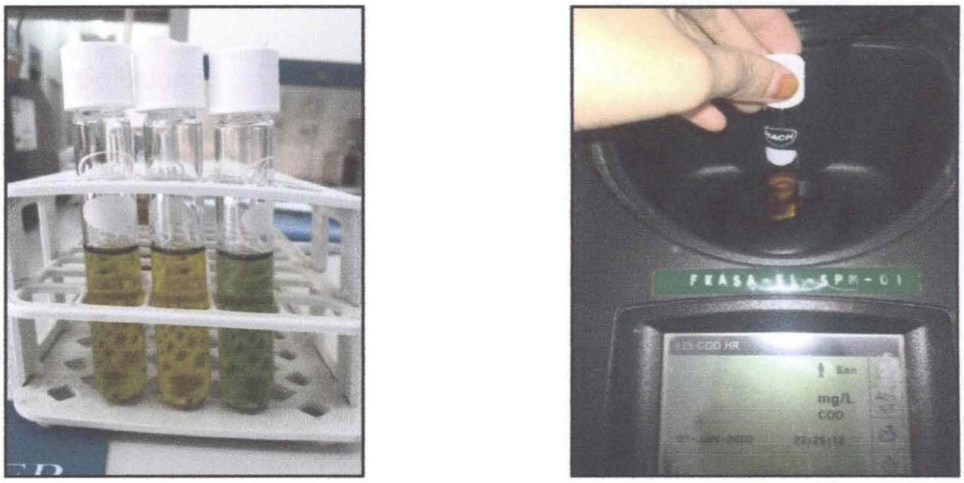
(b)



(c)



(d)



(e) (f)

Figure 3.2 Method to do the COD test

3.4.2 Total Suspended Solid

3.4.2.1 Description

TSS testing measures the total concentration of suspended (non-soluble) solid in the aeration stabilization basin (ASB) or in effluents. The total suspended solids (TSS) data is critical in determining the operational behavior of waste treatment system. It is usually a permitted test and solid must kept at minimum.

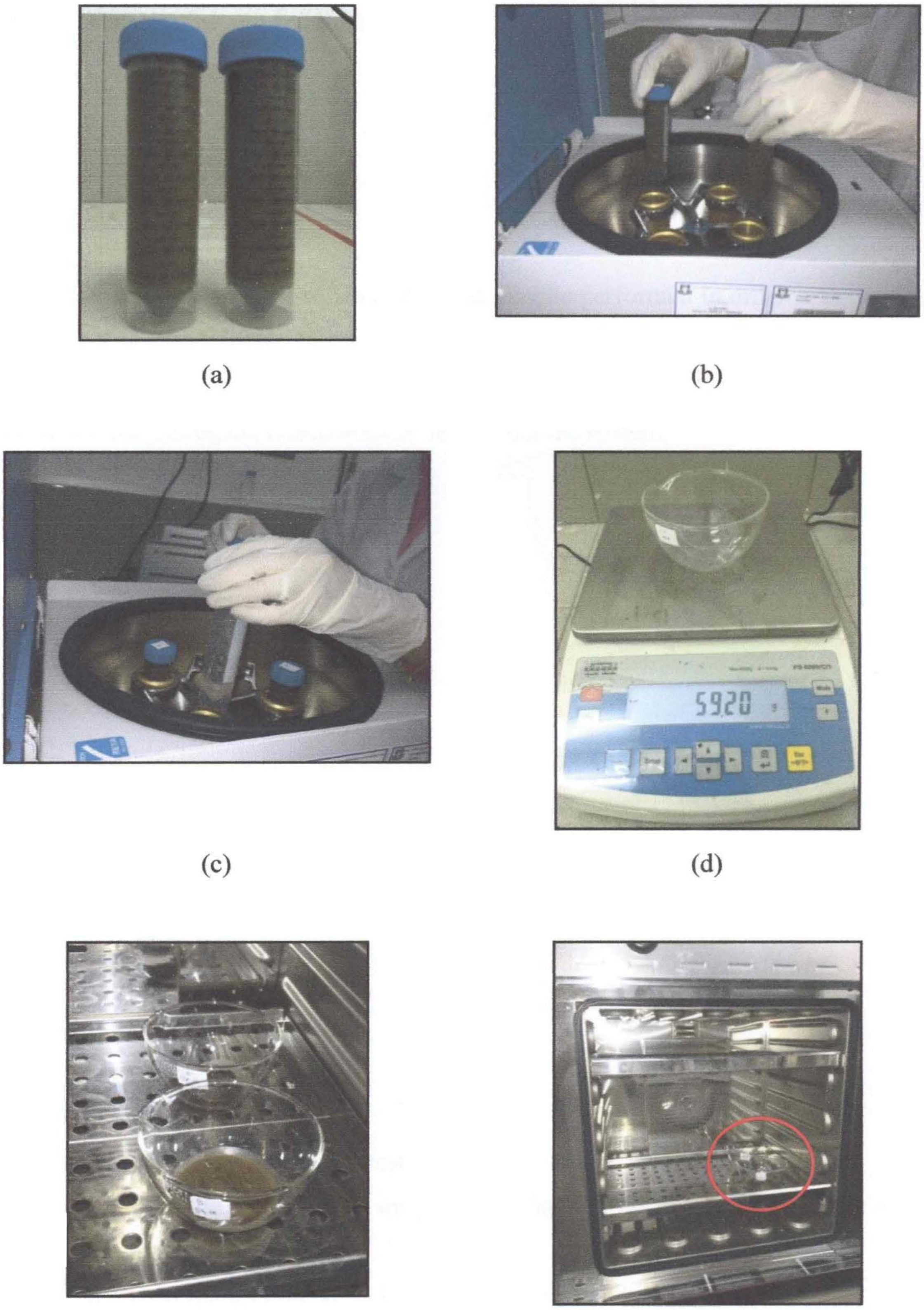
3.4.2.2 Apparatus

1. Polypropylene tube (PP tube 50ml)
2. Evaporation dish
3. Pipette 5ml

4. Spatula
5. Analytic balance
6. Desiccators
7. Oven

3.4.2.3 Procedure

1. Preparation of the apparatus and material.
2. Take 50ml sample and put it in polypropylene (PP tube 50ml). See Figure 3.3 (a).
3. To get the solid content from the sample, put the PP tube into centrifuge with a speed of 40 rpm for 10 minutes. See Figure 3.3 (b).
4. Put the PP tube contains with water into the centrifuge to balance the PP tube in the centrifuge. See Figure 3.3 (c).
5. Take the weight of empty evaporation dish. See Figure 3.3 (d).
6. Take the sample from the centrifuge and pour/take the solid contain in the PP tube into the evaporation dish. See Figure 3.3 (e)
7. Put the evaporation this that contain a solid from the sample into the oven with temperature 103.50° C for an hour. See Figure 3.3 (f)
8. After one hour carefully take the evaporation dish out and then cool in desiccators and weighed the evaporation dish with the sample.



(a) (b)

(c) (d)

(e) (f)

Figure 3.3 Method to do the TSS test

3.4.3 Volatile Suspended Solid

3.4.3.1 Description

Volatile suspended solids (VSS) test may be performed in order to determine the concentration of volatile suspended solids in an aeration stabilization basin system. Volatile suspended solids data is critical in determining the operational behavior and biological concentration throughout the system.

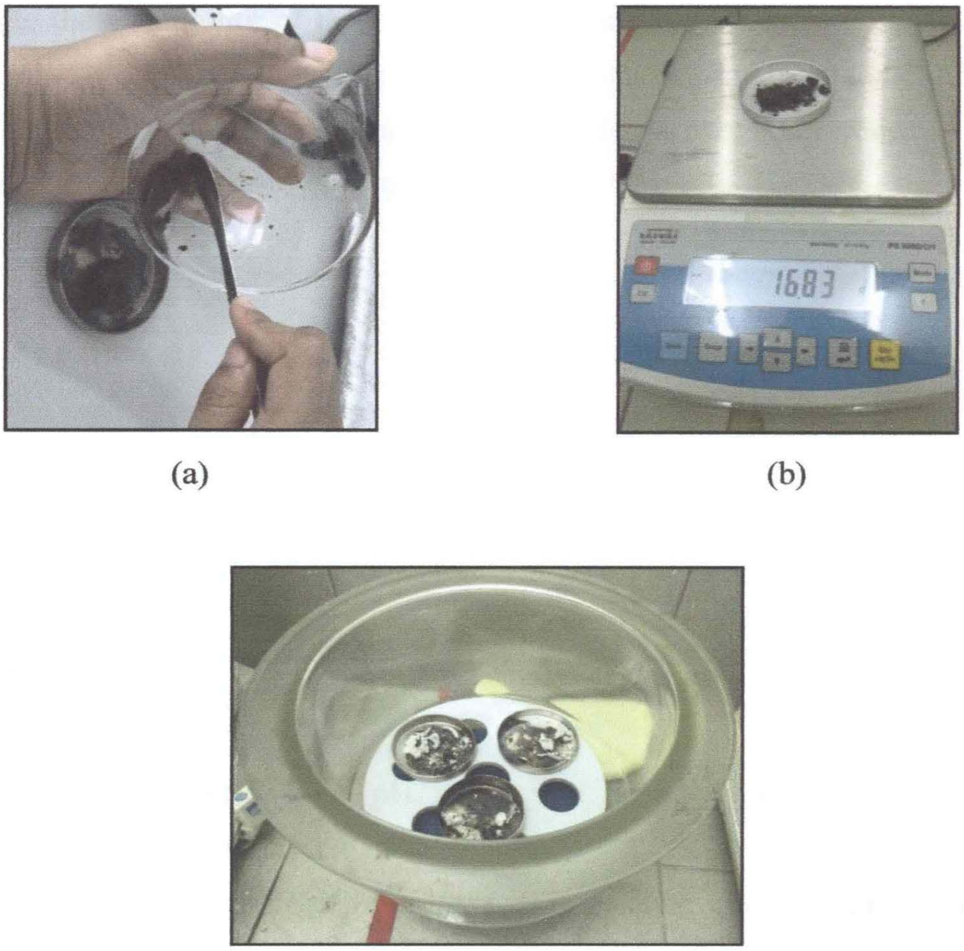
3.4.3.2 Apparatus

1. Aluminum dish
2. Spatula
3. Chamber furnace
4. Analytic balance
5. Glove

3.4.3.3 Procedure

1. Use the sample from TSS test.
2. Take out the sample from evaporation dish and put it in the aluminum dish. See Figure 3.4 (a).
3. Weighed the aluminum dish with residue before ignition process. See Figure 3.4 (b).
4. Put the aluminum dish with residue into the chamber furnace with temperature 550°C for an hour.

5. After that, switch off the oven and let the aluminum dish in the chamber furnace for about 30 minutes.
6. Carefully take the aluminum dish out from oven and cool in desiccators. See Figure 3.4 (c).
7. After 15 minutes weighed the aluminum dish with residue.



(a) (b)

(c)

Figure 3.4 Method to do the VSS test

3.4.4 pH

3.4.4.1 Description

The pH of water affects the solubility of many toxic and nutritive chemicals and therefore, the availability of these substances to aquatic organisms is affected. As acidity increases, most metals become more water soluble and more toxic. Alkalinity is the capacity to neutralize acids, and the alkalinity of natural water is derived principally from the salts of weak acids.

3.4.4.2 Apparatus

i. pH meter

3.4.4.3 Procedure

1. The electrode(s) of the pH meter was rinsed by distilled water before immersing into the water sample.
2. The electrode(s) of the pH meter was immersing into the water sample and turn the beaker slightly to obtain good contact between the water and the electrode(s). See Figure 3.5.
3. The electrode(s) required 30 seconds or longer in the sample to immersion before reading allowing the meter to stabilize. If the meter has an auto read system, it will automatically signal when stabilized.
4. The pH value was recorded.
5. The electrode(s) was rinsed well with distilled water, then dab lightly with tissues to remove any film formed on the electrode(s).

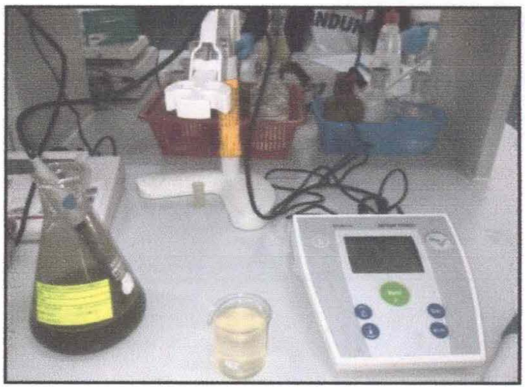


Figure 3.5 The electrode(s) of the pH meter was immersing into the water sample

3.4.5 Reactor Setup and Operation

1. The experimental set up is shown in Figure 3.6.
2. The strength of the influents were depends on the COD of the diluted POME. The preparation of diluted POME samples as influent for the reactor startup were controlled at 5000 mg/L COD. To produce such strength of influents, about 2 L of raw POME was diluted into 30 L.
3. The pHs of influents were adjusted to neutral (pH 7). The working volume (V_w) for the UASB reactor was 12.5 L and the hydraulic retention time (HRT) are 4, 8, 12 and 16 hrs, the flow rate of feed pump could be determined. Temperature is set to be constant at 35 °C. Table 3.1 shows the operating parameters at various hydraulic retention time (HRT).
4. The influents were continuously flowed into the reactor every 4 hours. Parameters such as COD, TSS and VSS of the both influent and effluent were determined. The analysis methods for the desired parameters were referred to standard methods.

5. The startup of the reactor was proceeding until the steady state condition. Steady state condition was defined when the COD removal was constant (not more than 5% difference).

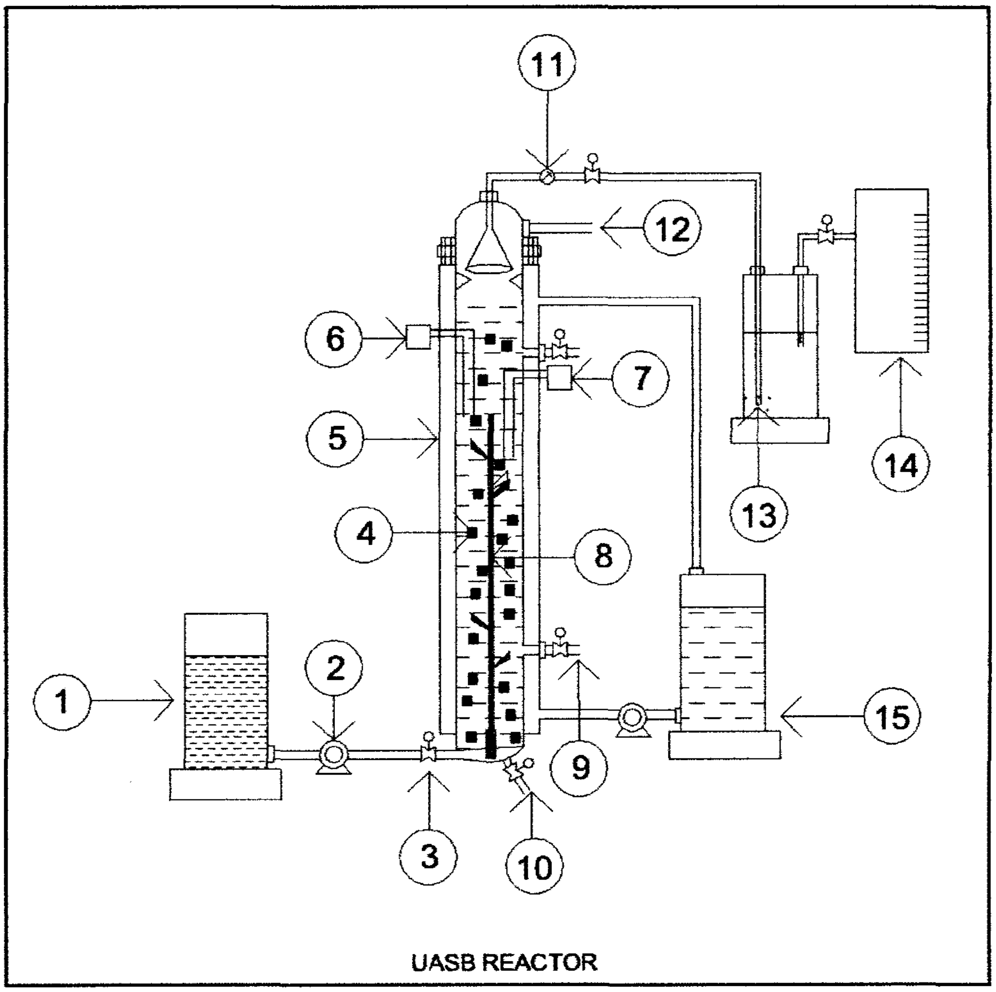


Figure 3.6 Schematic description of UASB reactor filled with immobilized beads for continuous hydrogen production. (1. POME feed tank; 2. Feed pump; 3. Manual valve; 4. Immobilzed bead; 5. Water jacket; 6. Temperature indicator; 7. pH indicator; 8. Stirred rod; 9. Sampling point; 10. Drain; 11. Gas flow meter; 12. Effluent outlet line; 13. Biogas collection system; 14. Hydrogen gas holder; 15. Hot water tank.)

Table 3.1 Operating parameters at various hydraulic retention time (HRT)

Operating parameters	**Desired value at HRT**			
Hydraulic retention time, HRT	**4 hrs**	**8 hrs**	**12 hrs**	**16 hrs**
Immobilized bead dosage (%)	6	6	6	6
Influent pH	5.5	5.5	5.5	5.5
Working volume (L)	5	5	5	5
VSS (g/L)	3.99	3.99	3.99	3.99
Influent COD (g/L)	5	15	25	35
Organic loading (g/L.h)	1.25	1.88	2.08	2.19
Flow rate at pump (L/hr)	1.25	0.63	0.42	0.31
F/M	0.31	0.47	0.52	0.55

3.4.6 Analytical methods

1. Hydrogen gas was determined with a Shimadzu GC-14A gas chromatography equipped with a thermal conductivity detector.
2. The carrier gas used was argon gas and the column was packed with Porapak Q (80/100 mesh, Waters Corp., USA).
3. Detection of VFA and ethanol were also done with gas chromatography using a flame ionization detector.
4. Volatile suspended solid (VSS; to represent the biomass concentration) was measured according to the procedures described in Standard Methods (APHA, 1995).

CHAPTER 4

RESULT AND DATA ANALYSIS

4.1 Introduction

This chapter will be focus on data collection and analysis result. This data was getting from the laboratory tests and the experiments have been done starting on early February until the end of May 2012.

The purpose of this analysis is to represent the result of the effect of hydraulic retention time to the hydrogen rate and characterizing the POME before and after using the continuous hydrogen production with anaerobic POME sludge immobilized by synthetic polymer. This purpose also can determine the effect of hydraulic retention time to the performance of the immobilized UASB reactor.

The analysis of sample was fully operated at laboratory. Firstly, I have characterized the raw sample of POME. After that, I have activated the sludge for a week to inoculate with raw sample inside the immobilized UASB reactor. Activated sludge can be determined by pH range of 7. PEG also has been added inside the sample.

The sample has been monitored for every 4h, 8h, 12h and 16h for COD, TSS, VSS and hydrogen production. Syringe was used to collect the gas at gas collector and analyzed by gas chromatography.

After the data was collected, it must be computed in excel to make a table and line graph to give an easy explanation of the result before making the conclusion.

4.2 Result Consideration

This result shown weather the objective has been achieve or not in this study. There are two objectives have to be achieve for this study there is to characterize the POME before and after the experiment and the effect of hydraulic retention time (HRT) in continuous hydrogen production using immobilized synthetic polymer. All tests are done within three month period to classified, tested and reported of the performance of the sample with the parameters during the study. For this study I only used three parameters to monitor the condition of the sample which are COD, TSS and VSS.

4.2.1 The Characteristic of raw POME

In order to study the performance of immobilized UASB system, selected parameters were chosen to characterize raw POME samples. These parameters are of great importance for effluent characterization and the values need to meet the level of statutory discharge limits in the Environmental Quality Act (Prevailing Effluent Discharge Standards for Crude Palm Oil Mills, 1984) prior being released into watercourses. The parameters are Chemical Oxygen Demand (COD), Total Suspended Solid (TSS), Volatile Suspended Solid (VSS) and pH.

Tables 4.1 shows the characteristics of the raw sample of POME. The sample extracted from cold room with temperature -20°C should be placed at room temperature before tested.

Table 4.1 Characteristic of the raw POME

Parameters	Rate
Initial pH	5.29
Total Suspended Solid, g/L	39.6
Volatile Suspended Solid, g/L	36.6
COD, g/L	70.6

Raw effluent collected from the palm oil mill was viscous, oily, dark brown in colour with an obnoxious odour. Comparatively, POME sample comprised much more solids than that of the typical one. The COD values were as well around ten thousand units higher than usual, indicating that the sample contained higher concentration of organic and inorganic compounds ready to be utilized or oxidized.

The activated sludge of the POME that has been prepared will be tested for VSS 2 days for a week. Result of VSS for activated sludge shown in graph line at Figure 4.1 below.

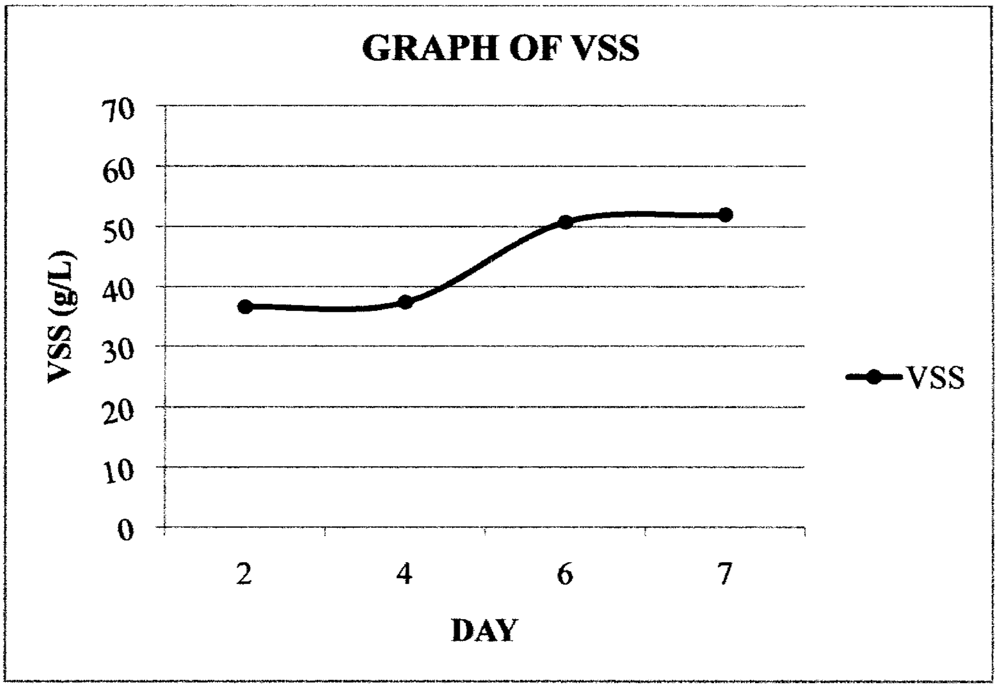


Figure 4.1 Graph of VSS versus day for activated sludge

4.2.2 Hydrogen production at various HRT

After the start-up period, the value of COD removal, hydrogen content, hydrogen production rate, and hydrogen yield was determined.

Figure 4.2 shows the effect of HRT on COD removal. The graph line shows that the COD removal in biogas increased from 50.24% at 16h to 55.14% at 4h. In other words, the value of COD concentration is decrease from hydraulic retention time at 16h to 4h. High COD removal can only achieve at short time period.

The variation of hydrogen content of biogas was plotted in Figure 4.3. It shows that hydrogen content in biogas decreased from 59.8% at 16 h to 55% at 4 h.

Figure 4.4 shows that hydrogen yield increased from 0.27 mol-H_2/mol-sucrose at 16 h to 0.33 mol-H_2/mol-sucrose at 8 h, but drastically decreased to 0.29 mol-H_2/mol-sucrose at 4 h. Based on previous study, it shows that the longer the time, the bacteria inside the reactor was inhibited by low substrate supplied. "One explanation for low hydrogen yield in HRT 96 h is that bacteria are inhibited by low substrate supplied which facilitate microbial population shift and growth of non-hydrogen producing bacteria in the reactor." (Marzieh Badiei, 2011)

Figure 4.5 shown that hydrogen production rate increased significantly from 260 mL-H_2/h/L at 16 h to 330 mL-H_2/ h/L at 8 h. The biogas produced consisted of H_2 and CO_2 and was free of methane, CH_4 which support the effectiveness of pretreatment process on seed sludge.

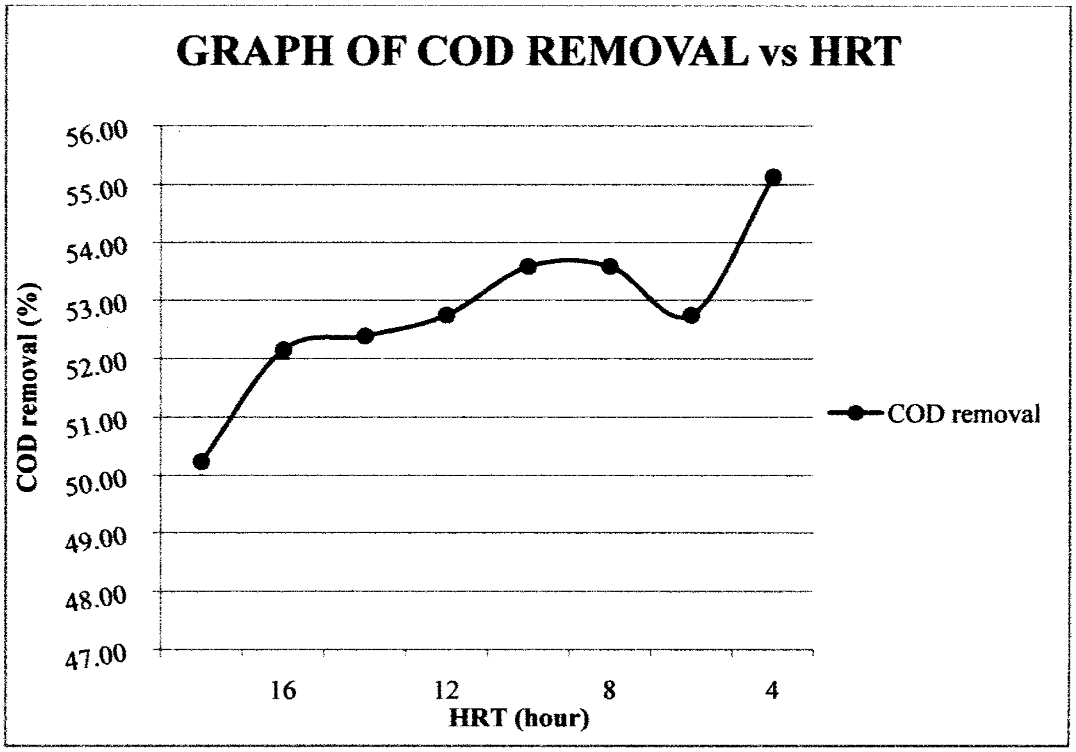


Figure 4.2 Graph of COD removal versus HRT

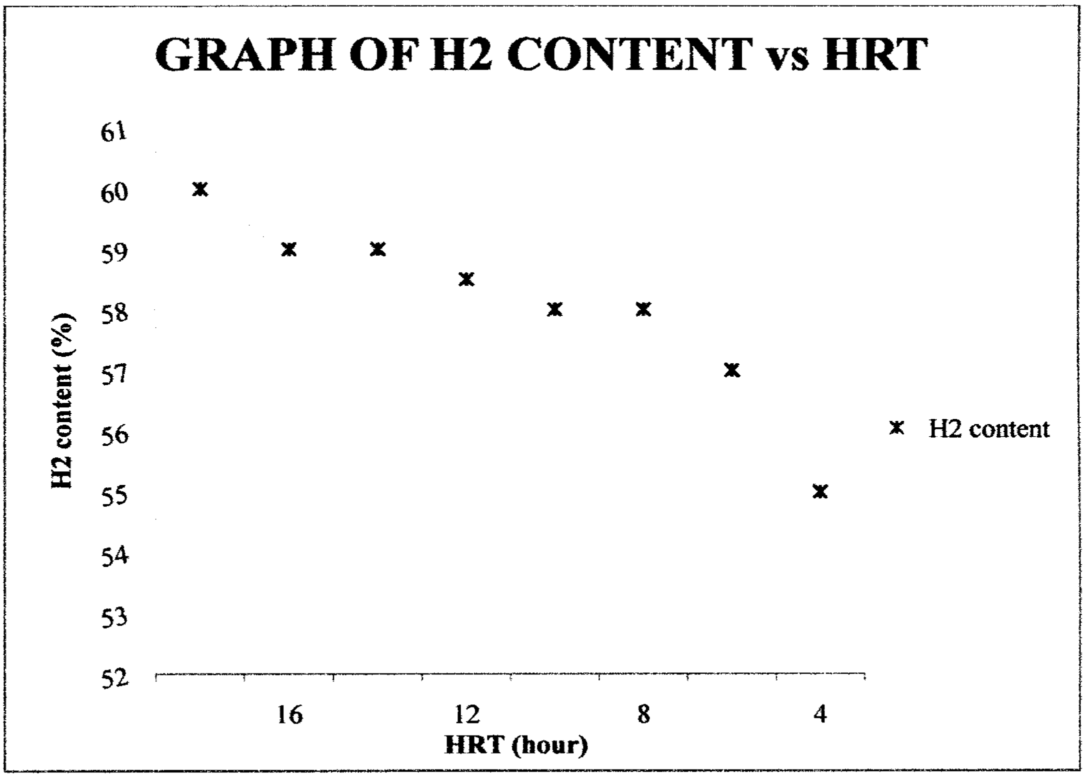


Figure 4.3 Graph of H_2 content versus HRT

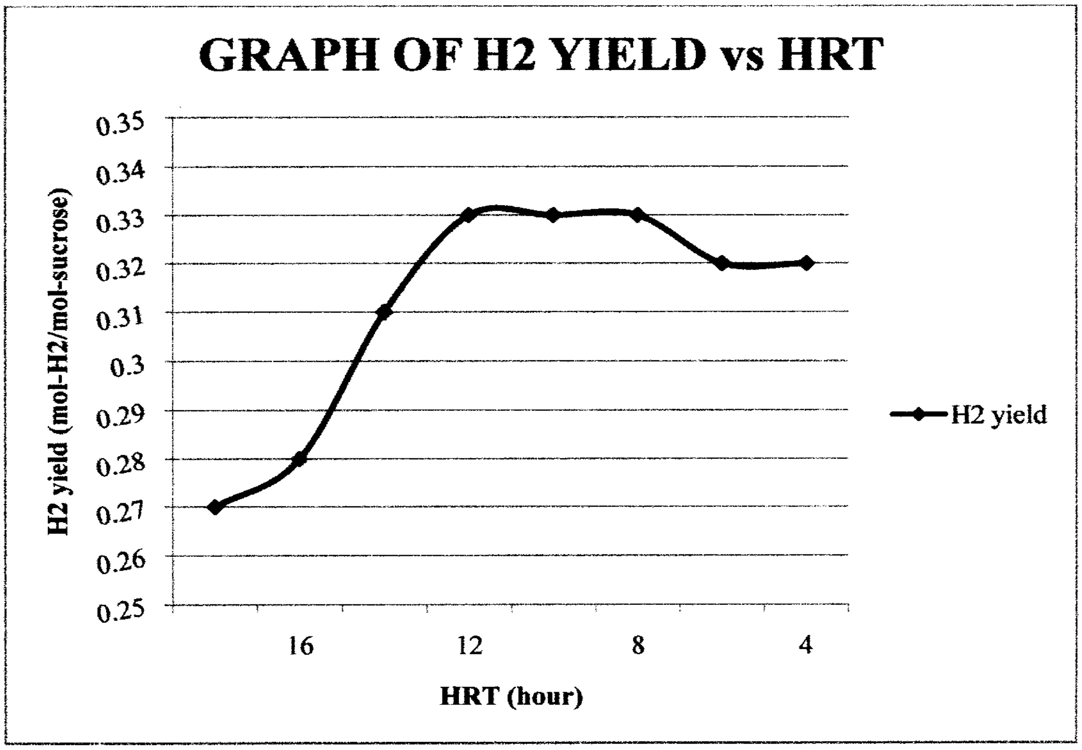


Figure 4.4 Graph of H_2 yield versus HRT

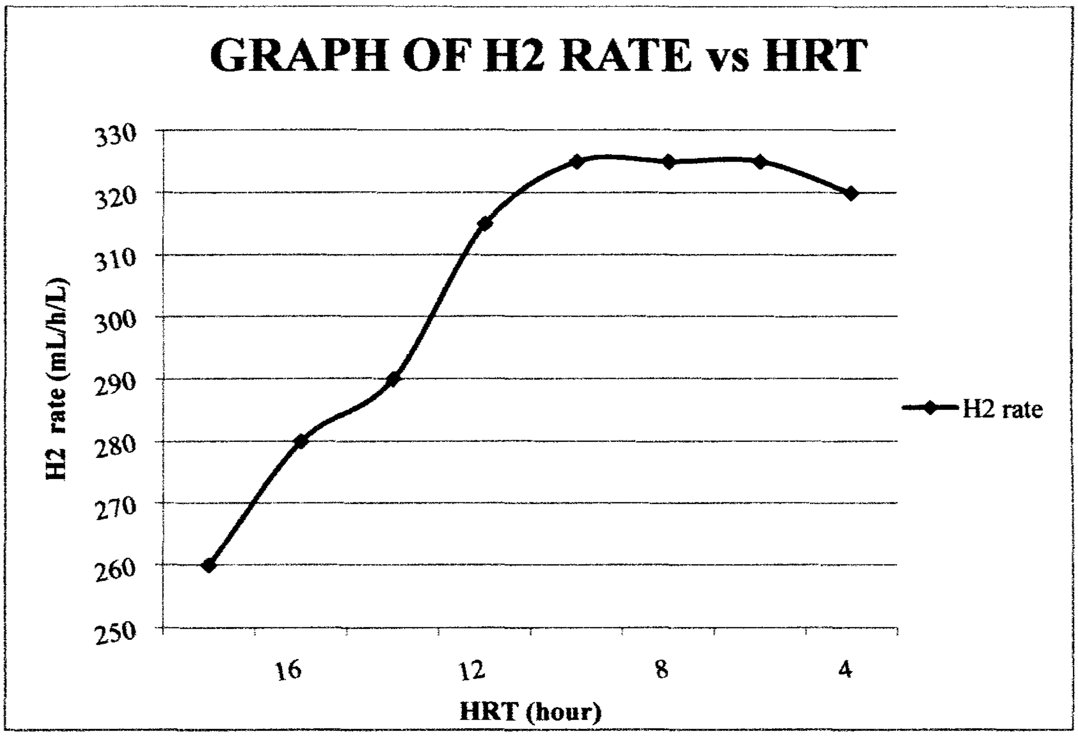


Figure 4.5 Graph of H_2 production rate versus HRT

4.2.3 Variation of VFA and solvent concentration

Results of soluble metabolite composition and concentration at various HRTs, presented in Table 4.2 can describe the performance of reactor.

VFA is known as intermediate and indicator in the anaerobic process. In all ranges of HRT, butyrate followed by acetate were the main acids throughout the fermentation in ASBR along with much lower amounts of propionic acid and iso-butyric acid with small amounts of heptanoic acid.

In this study, the VFA/SMP ratio was essentially higher than 0.59. "The abundance of VFAs in total SMP (VFA/SMP) suggests that H2 production was metabolically favorable since acidogenic pathway was predominant over solventogenesis." (Sreethawong T, 2010)

Moreover, HBu/VFA ratio was higher than 0.68 in this study, and fraction of butyric acid in soluble metabolites increased by decreasing HRT. Simultaneous decrease in the acetate concentration and an increase in H_2 production observed which also reported in previous studies in which mixed culture were used.

Table 4.2 Soluble metabolites obtained at steady-state conditions at different HRTs.

HRT, h	Soluble metabolites										
	Et, %	Bu, %	HAc, %	HPr, %	Iso-Bu, %	HBu , %	HHp , %	SMP, mg/L	VFA, mg/L	VFA/SMP	HBu/VFA
4	0.67	19.5	28.3	0.85	0.86	88.3	0.25	1545	1820	1.18	0.68
8	0.45	16.8	9.39	0.14	1.03	69	0.19	3880	3170	0.82	0.94
12	0.1	13.5	12.35	0.24	0.6	70.5	0.12	2652	2270	0.86	0.93
16	2.85	37.8	13.1	0.14	1.13	43.1	0.55	2867	1690	0.59	0.71

4.2.4 Characteristic of POME at various HRT

The sample from the reactor has been monitored for COD, TSS and VSS at hydraulic retention time 4h, 8h, 12h and 16h. Table 4.3 shows the comparison value of COD, TSS and VSS at various HRT.

Table 4.3 Value of COD, TSS and VSS at various HRT

HRT (h)	Initial pH	COD (g/L)	TSS (g/L)	VSS (g/L)
0	7	83.6	56	52.8
4		37.5	49.5	48.3
8		38.5	38.2	35
12		39.5	29.3	28.9
16		40	27.1	25.7

Figure 4.6 have shown the value of COD at various hydraulic retention time (HRT). The COD value was decreased rapidly from 83.6 g/L to 37.5 g/L at hydraulic retention time 4h but increased uniformly to 38.5 g/L at 8h, 39.5 g/L at 12h and 40 g/L at 16h. This was due to the soluble microbial products (SMP) production which in turn contributed to the COD value in the effluent. Various articles have proven that the formation of SMP in anaerobic systems indeed gave rise to a certain percentage of COD in the effluent of wastewater treatment system (Duncan et al., 2000; Aquino and Stuckey, 2003; Goorany and Ozturk, 2000; Lu et al., 2002).

Figure 4.7 shows the value of TSS at various hydraulic retention time (HRT). The result shows that the value of TSS was decrease smoothly from 56 g/L to 27.1 g/L at hydraulic retention time 16h.

Figure 4.8 shows the value of VSS at various hydraulic retention time (HRT). The result shows that the value of TSS was decrease smoothly from 52.8 g/L to 25.7 g/L at hydraulic retention time 16h.

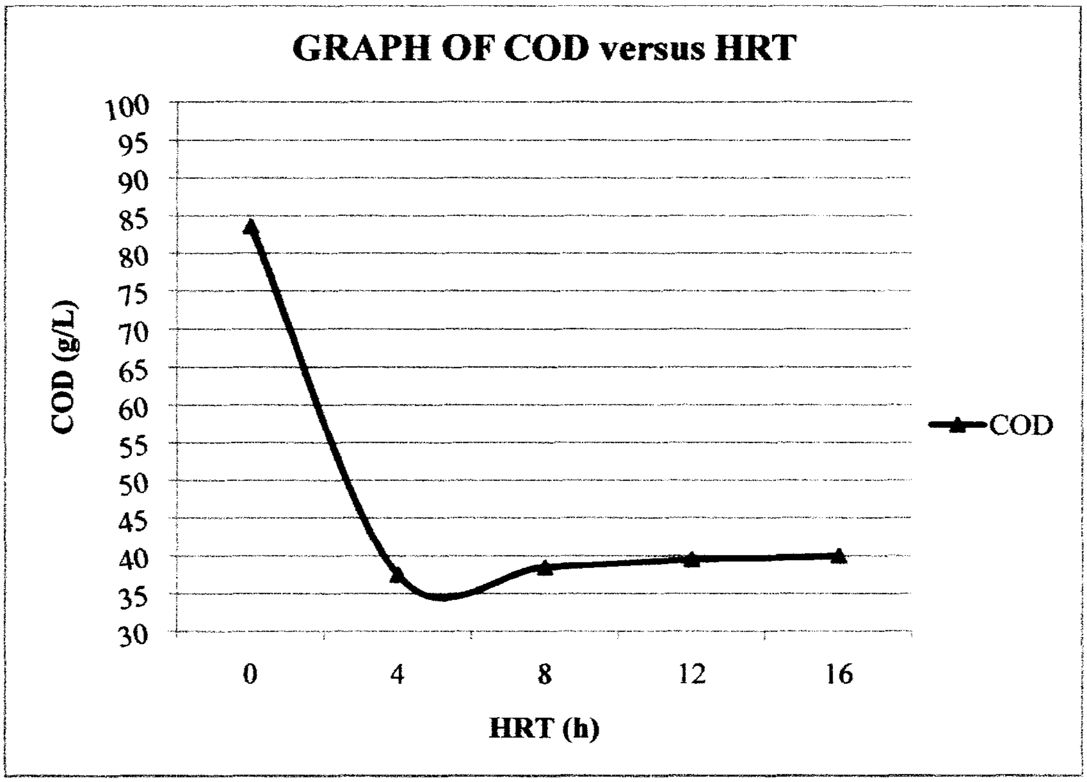


Figure 4.6 Value of COD at various HRT

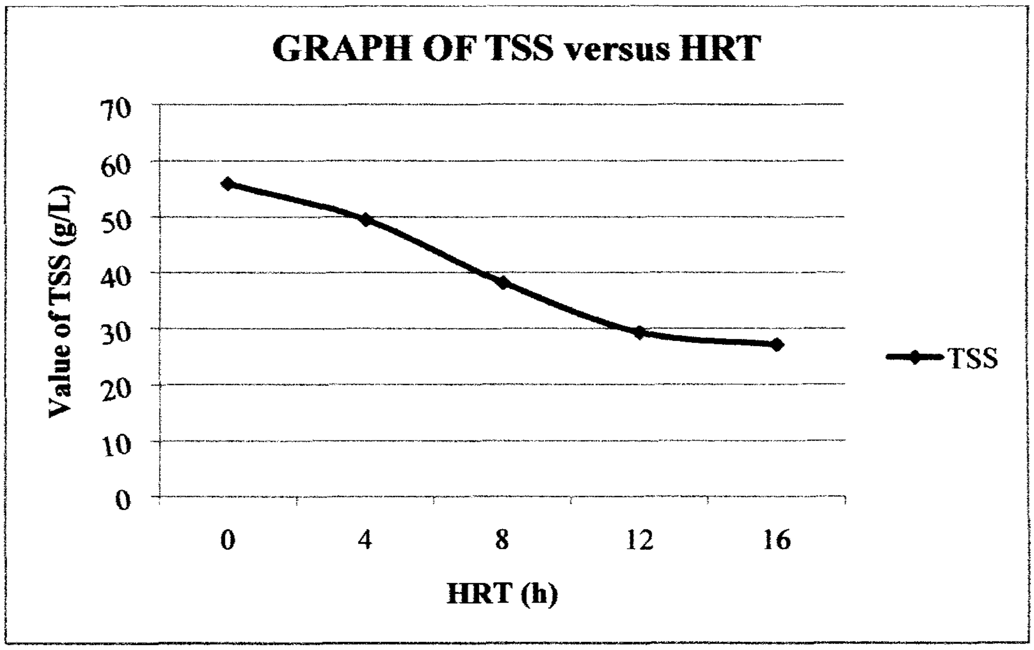


Figure 4.7 Value of TSS at various HRT

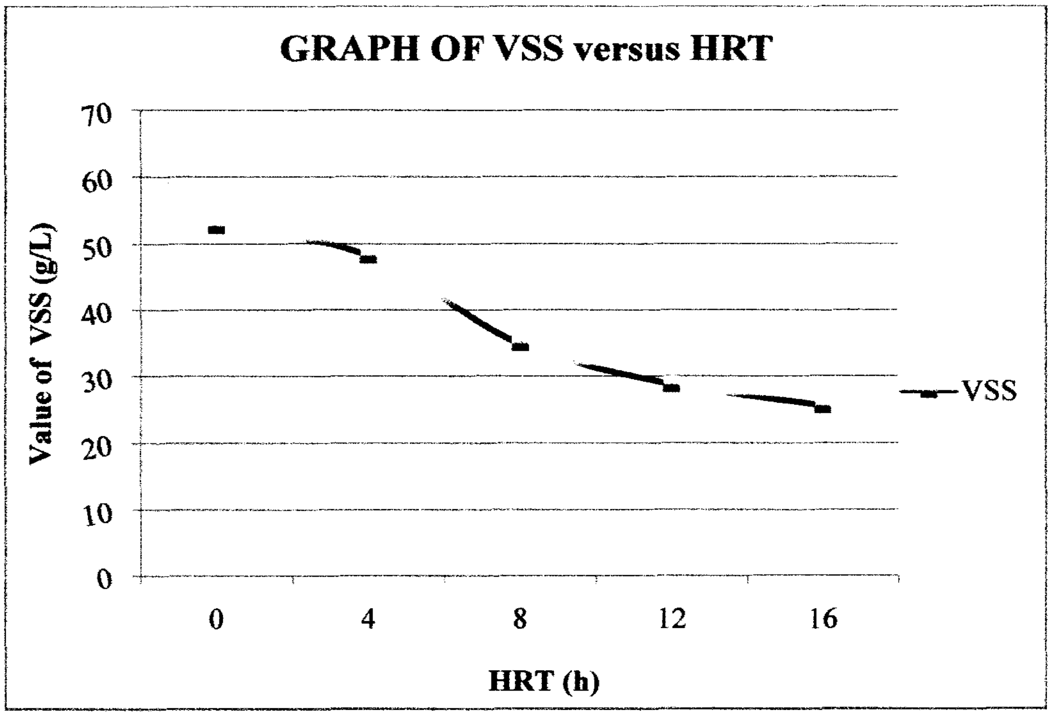


Figure 4.8 Value of VSS at various HRT

4.3 Summary

According on these results for first objective, optimal HRT for hydrogen production rate was 8 h, which concurred with the report (2.2 h) by Lin et al (2006). Optimal HRT for hydrogen yield was 8 h, because longer time was required for sucrose digestion. The maximum hydrogen production rate (300 mL-H_2/h/L) and yield (0.33 mol-H_2/mol-sucrose).

According to the result for second objective shows that the use of immobilized UASB reactor was a good strategy to accelerate anaerobic granulation and to achieve high COD removal efficiency in a short period of time. TSS and VSS concentrations values improved by the increasing of hydraulic retention time. Therefore, the reactor was very efficient in the treatment of diluted and high strength POME at high OLR and short HRT.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Introduction

This chapter comprises two part namely as conclusion and recommendation. For the conclusion, it was described the finding and summary of the experiment result that obtain from all aspect that based on the objective and scope of the study. The conclusion will explain that the objectives of the study were achieved or not. For recommendation, it has the purpose to give for the future study in this research and solution for the problem that faced in the research.

5.2 Conclusion

All of the objectives were done for this study which is to characterize the Palm Oil Mill Effluent (POME) properties in term of pH, COD, TSS and VSS before and after the sample was in startup period and to determine the effect of hydraulic retention time in continuous hydrogen production using immobilized synthetic polymer. Both objectives were achieved.

To achieve the goals, the samples have been analyzed in Environmental Laboratory. The sample was tested based on four parameters that have been discuss in previous chapter.

After all the experiment has been done, it shows that the hydrogen production rate was decreased at longer hydraulic retention time (HRT).

In this study, there was clearly showed that the optimal HRT for hydrogen production rate was 2 h which concurred with the report (2.2 h) by Lin et al (2006). Based on these results, optimal HRT for hydrogen yield was 8 h, because longer time was required for sucrose digestion. The maximum hydrogen production rate is 300 mL-H_2/h/L at 2 h and the maximum H_2 yield is 0.33 mol-H_2/mol-sucrose at optimal HRT 8 h. Shifted bacterial community was likely the reason for superior hydrogen production in immobilized biomass to suspended biomass under harsh environment.

The value of COD removal can be obtained up to 60% means that the COD concentration is decreased due to hydraulic retention time. Therefore, it is one of the process that can be used to treated the POME due to its high value of COD.

The value of TSS and VSS concentration also decreased smoothly by longer HRT. Hence, the POME also has been treated in terms of TSS and VSS.

As a conclusion, this study has potential to produce hydrogen by using immobilized cell system for continuous H_2 production because this method was not expensive and easy to operate, it may give an economical method to do in practicing the hydrogen production.

5.3 Recommendation

In order to save the environmental aspect and saving the cost for treat the POME, this method was a good way to use to the POME as sources in addition of hydrogen production. From the analysis, we can reduce the cost to treat the POME from being polluted and produce hydrogen which is one of the sources of energy.

Although this system can be implemented in the production of hydrogen fermentation of wastewater containing sucrose, but the technology is still in infancy. It is clear that research on several key areas needed for further development of this technology.

One of the advantages of biomass immobilization technology is the improvement of inhibitor tolerance, such as oxygen, heavy metal, low pH, etc. Further study on immobilized biomass' tolerance to the above inhibitors is warranted.

Microbial analysis had revealed that certain species of bacteria can produce superior hydrogen production immobilized biomass to suspended biomass. The genetic isolation of this potential species needs further studies.

Lastly, fermentative hydrogen production process can only removes about 55% of COD from sample. Further treatment of the effluent is deserved either in anaerobic digestion or photosynthetic process.

REFERENCES

A. A. Azimi and M. Zamanzadeh (2004). Determination of design criteria for UASB reactors as a wastewater pretreatment system in tropical small communities. Int. J. Environ. Sci. Tech. Vol. 1, No. 1, pp. 51-57, Spring 2004.

Abass O. Alade, Ahmad T. Jameel, Suleyman A. Muyibi, Mohamed I. Abdul Karim and Md. Zahangir Alam (2011). Application of semifluidized bed bioreactor as novel bioreactor system for the treatment of palm oil mill effluent (POME). African Journal of Biotechnology Vol. 10(81), pp. 18642-18648, 16 December, 2011.

Abdul Latif Ahmad, Suzylawati Ismail, and Subhash Bhatia (2003). Water recycling from palm oil mill effluent (POME) using membrane technology. Desalination 157: 87-95.

Ahmad Zuhairi Abdullah, Mohamad Hakimi Ibrahim & Mohd. Omar Ab. Kadir (2004). Treatment Of Palm Oil Mill Effluent (POME) Supernatants Using Aerobic Attached-Growth System: Trickling Filter As A Case Study. Universiti Teknologi Malaysia.

Ali Akbar Zinatizadeh Lorestani (2006). Biological Treatment Of Palm Oil Mill Effluent (Pome) Using An Up-Flow Anaerobic Sludge Fixed Film (Uasff) Bioreactor. University Sains Malaysia.

Anwar Ahmad, Rumana Ghufran, Zularisam Abd. Wahid (2011). Bioenergy from anaerobic degradation of lipids in palm oil mill effluent. Rev Environ Sci Biotechnol (2011) 10:353–376. DOI 10.1007/s11157-011-9253-8.

APHA, AWWA, WEF (1998) 'Standard Methods, American Public Health Association, American Water Works Association and Water Environment Federation', Washington D.C, USA.

Argun H, Kargi F, Kapdan IK, Oztekin R. Biohydrogen production by dark fermentation of wheat powder solution: effects of C/N and C/P ratio on hydrogen yield and formation rate. Int J Hydrogen Energy 2008;33:1813-9.

Bo Hu, Shu Lin Chen (2008). Biological Hydrogen Production using Chloroform-treated Methanogenic Granules. Appl Biochem Biotechnol 148:83-95.

Chaisri, R., Boonsawang, P., Prasertsan, P. and Chaiprapat, S. (2007). Effect of organic loading rate on methane and volatile fatty acids productions from anaerobic treatment of palm oil mill effluent in UASB and UFAF reactors. Songklanakarin J. Sci. Technol., May 2007, Suppl 2 : 311-323.

Cheng J, Zhu X, Borthwick A (2010). Palm oil mill effluent treatment using a two-stage microbial fuel cells system integrated with immobilized biological aerated filters. Bioresour Technol 101:2729–2734.

Chong ML, Rahin RA, Shirai Y, Hassan MA. (2009). Biohydrogen production by *Clostridium butyricum* EB6 from palm oil mill effluent. Int. J. Hydr. Energy, 34: 764-771.

Dinsdale, R. M., F. R. Hawkes, D. L. Hawkes, (1997). Comparison of mesophilic and themophilic upflow anaerobic sludge blanket reactors treating instant coffee production wastewater. Wat. Res., 31: 163-169.

D. Das and T.N. Veziroglu, (2008). Advances in biological hydrogen production processes. Int J Hydrogen Energy, 33 pp. 6046–6057.

Fang HHP, Liu H. (2002). Effect of pH on hydrogen production from glucose by a mixed culture. Bioresource Technol 82(2): 87-93.

Fang HHP, Liu H, Zhang T. (2004). Bio-hydrogen production from wastewater. Water Sci. Technol 4(1): 77-85.

Feng-Yung Chang, Chiu-Yue Lin (2004). Biohydrogen production using an up-&ow anaerobic sludge blanket reactor. International Journal of Hydrogen Energy 29 (2004) 33 – 39.

Friends of the Earth's (2006). The use of palm oil for biofuel and as biomass for energy. Briefing August 2006.

Foo KY, Hameed BH (2010). Insight into the applications of palm oil mill effluent: a renewable utilization of the industrial agricultural waste. Renew Sustain Energy Rev 14:1445–1452.

F.G. Fermoso, G. Collins, J. Bartacek, V. O'Flaherty, and P. Lens, (2008). Acidification of methanol-fed anaerobic granular sludge bioreactors by cobalt deprivation: Induction and microbial community dynamics. Biotechnology and Bioengineering 99 (2008) 49-58.

Hannes-Christian Moeller, Matthew K. Mian, Shamit Shrivastavaa, Bong Geun Chunga, Ali Khademhosseinia, (2008). A microwell array system for stem cell culture. Biomaterials 29 (2008) 752–763.

http://www.engr.subr.edu/ce/CE%20Labs/CE%20421%20Water-Wastewater%20Analysis%20Lab/Lab7,8_Solids.pdf

http://www.palintest.com/media/uploads/137.pdf

Ismail I, Hassan MA, Abdul Rahman N, Chen SS (2010). Thermophilic biohydrogen production from palm oil mill effluent (POME) using suspended mixed culture. Biomass and Bioenergy, 34: 42-47.

Isnazunita Ismail, Mohd Ali Hassan, Nor'Aini Abdul Rahman and Chen Sau Soon (24 January, 2011). Effect of retention time on biohydrogen production by microbial consortia immobilised in polydimethylsiloxane. African Journal of Biotechnology Vol. 10 (4), pp. 601-609.

Jantsch, T.G., Angelidaki, I., Schmidt, J.E., de Hvidsten, B.E.B., Ahring, B.K., (2002). Anaerobic biodegradation of spent sulphite liquor in a UASB reactor. Bioresour. Technol. 84 (1), 15-20.

Karim, K., Gupta, S.K., 2003. Continuous biotransformation and removal of nitrophenols under denitrifying conditions. Water Res. 37, 2953 - 2959.

Lemi Turker, Selcuk Gumus and Alper Tapan (2008). Biohydrogen production: molecular aspects. Journal of Scientific & Industrial Research. Vol. 67, November 2008, pp. 994 – 1016.

Lettinga, G. and Hulshoff Pol, L. W. (1991). "UASB Process Design for Various Types of Wastewater." Water Sci. Technol. 24 (8), 87-107.

Lettinga, G. van Velsen, A.F.M. Hobma, S.W. de Zeeuuw, W. and Klapwijik, A. (1980). Use of upflow sludge blanket (USB) reactor concept for biological wastewater treatment, especially for anaerobic treatment. Biotechnol. Bioeng. 22: 699-734.

Levin DB, Pitt L, Love M., (2004). Biohydrogen production: prospects and limitations to practical application. Int J Hydrogen Energy 2004; 29: 173–85.

Logan BE, Oh SE, Kim IS, van Ginkel S. Biological hydrogen production measured in batch anaerobic respirometers. Environ Sci Technol 2002;36:2530–5.

Malaysian Palm Oil Board (2004).

Malaysian Palm Oil Production Council (2006).

Marzieh Badiei, Jamaliah Md Jahim, Nurina Anuar, Siti Rozaimah Sheikh Abdullah (2011). Effect of hydraulic retention time on biohydrogen production from palm oil mill effluent in anaerobic sequencing batch reactor. International journal of hydrogen energy 36: 5912 – 5919.

McDowall & Eames (2007). Towards a sustainable hydrogen economy: a multi-criteria sustainability appraisal of competing hydrogen futures. International Journal of Hydrogen Energy 32: 4611-4626.

Muhammad Asif Latif, Rumana Ghufran, Zularisam Abdul Wahid, Anwar Ahmad (2011). Integrated application of upflow anaerobic sludge blanket reactor for the treatment of wastewaters. Faculty of Civil Engineering & Earth Resources, University Malaysia Pahang (UMP).

Nadeem Khalila, Rajiv Sinhab, A K Raghava, A K Mittal (2008). UASB Technology for Sewage Treatment in India: Experience, Economic Evaluation and Its Potential in Other Developing Countries. Twelfth International Water Technology Conference, IWTC12 2008, Alexandria, Egypt.

Nandi et al., R. Nandi, S. Ray and S. Sengupta, (2001). Thiosulphate improves yield of hydrogen production from glucose by the immobilized formate hydrogenlyase system of Escherichia coli. Biotechnology and Bioengineering, 75 4 (2001), pp. 492–494.

Nur Azeera Binti Badroldin (2010). Treatment of Palm Oil Mill Effluent (Pome) Using Hybrid Up Flow Anaerobic Sludge Blanket (Huasb) Reactor. Faculty of Civil and Environmental Engineering, Universiti Tun Hussein Onn Malaysia.

Prepared at the 31st JECFA (1987). Polyethylene Glycols. published in FNP 38 (1988) and in FNP 52 (1992). Metals and arsenic specifications revised at the 61st JECFA (2003). An ADI of 0-10 mg/kg bw was established at the 23rd JECFA (1979).

Rajeshwar K, Ibanez JG, Swain GM. (1994). Electrochemistry and the environment. J Appl Electrochem 24(11): 1077-1091.

Rupani PF, Singh RP, Ibrahim MH, Esa N (2010). Review of current palm oil mill effluent (POME) treatment methods vermicomposting as a sustainable practice. World Appl Sci J 11(1):70–81.

Schmidt, J.E., Ahring, B.K., 1996. Granular sludge formation in upflow anaerobic sludge blanket (UASB) reactors. Biotechnol. Bioeng. 49 (3), 229-246.

Shu-YiiWu, Chi-Neng Lin, Jian-Sheng Chang, Jo-Shu Chang (2005). Biohydrogen production with anaerobic sludge immobilized by ethylene-vinyl acetate copolymer. International Journal of Hydrogen Energy 30 : 1375 – 1381.

Sim Lin Lin (2005). Microbial community analysis in upflow anaerobic sludge blanket for the treatment of palm oil mill effluent. Faculty of Civil Engineering Universiti Teknologi Malaysia.

Singh, G., Huan, L.K., Leng, T. and Kow, D.W. (1999). Oil palm and the environment. SDN. Bhd, Kuala Lumpur.

Singh RP, Embrandiri A, Ibrahim MH, Esa N (2011). Management of biomass residues generated from palm oil mill vermicomposting a sustainable option, resources. Conserve Recycling 55:423–434.

Sompong O-Thong, Chonticha Mamimin, Poonsuk Prasertsan. Effect of temperature and initial pH on biohydrogen production from palm oil mill effluent: long-term evaluation and microbial community analysis. Electronic Journal of Biotechnology ISSN: 0717-3458 . DOI: 10.2225 / vol14-issue5-fulltext-9.

Sreethawong T, Niyamapa T, Neramitsuk H. Hydrogen production from glucose-containing wastewater using an anaerobic sequencing batch reactor: effects of COD loading rate, nitrogen content and organic acid composition. Chem. Eng J 2010;160:322 - 32.

Ugoji EO (1997). Anaerobic digestion of palm oil mill effluent and its utilization as fertilizer for environmental protection. Renewable Energy, 10: 291-294.

Wong PW, Nik MS, Nachiappa M, Balaraman V (2002). Pretreatment and membrane ultra filtration using treated palm oil mill effluent (POME). Songklanakarin J Sci Tech 24 (suppl) : 891–898.

Wu TY, Mohammad AW, Jahim JMd, Anuar N (2007). Palm oil mill effluent (POME) treatment and bioresources recovery using ultrafiltration membrane: effect of pressure on membrane fouling. Biochem Eng J 35:309–317.

Wu TY, Mohammad AW, Jahim JM, Anuar N (2010) Pollution control technologies for the treatment of palm oil mill effluent (POME) through end-of-pipe processes. J Environ Management 91:1467–1490.

Yang Mua, Han-QingYua, (2007). Simulation of biological hydrogen production in a UASB reactor using neural network and genetic algorithm. International Association for Hydrogen Energy.

Zinatizadeh AAL, Mohamed AR, Abdullah AZ, Mashitah MD, Husnain Isa M, Najafpour GD (2006). Process modeling and analysis of palm oil mill effluent treatment in an upflow anaerobic sludge fixed film bioreactor using response surface methodology (RSM). Water Res 40:3193–3208.

Z. Ahmad, Z. Ujang, G. Olsson and A.A. Abdul Latiff. Evaluation of Hybrid Membrane Bioreactor (MBR) For Palm Oil Mill Effluent (POME) Treatment. International Journal of Integrated Engineering (Issue on Civil and Environmental Engineering).

Design Parameters

$$Q\ (L/h) = \frac{V}{HRT}$$

Where;

Q = Flow rate (L/h)

V = Volume of sample inside the feed tank (L)

HRT = Hydraulic retention time (h)

$$OLR\ (g/L.d) = \frac{COD}{HRT}$$

Where;

OLR = Organic loading rate (g/L.d)

COD = Chemical oxidation demand (g/L)

HRT = Hydraulic retention time (d)

Formula for Total Suspended Solids

$$TSS\ (g/L) = \frac{(A - B)\ \times\ 10^3}{C}$$

Where;

A = Weight of filter and dish + residue, g

B = Weight of filter and dish, g

C = Volume of sample filtered, mL

Formula for Volatile Suspended Solids

$$VSS\ (g/L) = \frac{(A - B)\ \times\ 10^3}{C}$$

Where;

A = Weight of aluminium dish + residue, g

B = Weight of aluminium dish + residue (after ignition), g

C = Volume of sample filtered, mL

COD Removal

$$COD\ Removal = \frac{COD_{in} - COD_{effluent}}{COD_{in}} \times 100$$

Where;

COD_{in} = Initial value of COD

$COD_{effluent}$ = Effluent value of COD