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JUDUL	: CONTINUOUS HYDROGEN PRODUCTION WITH ANAEROBIC
	POME SLUDGE IMMOBILIZED BY SYNTHETIC POLYMER
SESI PEN	GAJIAN : <u>2011/2012</u>
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I declare that this project report entitled "Continuous Hydrogen Production with Anaerobic Palm Oil Mill Effluent (POME) Sludge Immobilized by Synthetic Polymer" is the result of my own research except as cited in the references. The project report has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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To my beloved mother and father also to all my family members. To my lovely friends Fairuz binti Rosni, Raja Nurshafarin binti Raja Osman, Nur'Ain binti Abdullah, Nik Shamim Uzaini binti Zulkiffli, Nor Aisahton binti Junet and all of BAE members.

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ABSTRACT

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. 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 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 temperature 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 temperatures, there is 30 °C, 35 °C, 40 °C and 45 °C at constant hydraulic retention times (HRT) in twelve hour, were tested to evaluate hydrogen productivity and operational stability of immobilized UASBR. The results showed higher system efficiency was achieved for temperature at 35°C, with maximum hydrogen production rate of 320 mL-H₂/h/L and hydrogen yield of 0.32 mol-H₂/mol-sucrose. COD removal reached in the range 45% - 50%. Butyrate was found to be the dominant metabolite in all temperature. Low concentration of volatile fatty acid (VFA) confirmed the state of stability and efficiency of the operation was achieved in immobilized UASBR.

ABSTRAK

Dengan pemprosesan minyak kelapa sawit yang meningkat telah menyebabkan pengeluaran Kumbahan Kilang Kelapa Sawit (POME) meningkat yang tidak dapat di rawat untuk dijajikan sebagai air minuman dan akan menyebabkan penambahan jumlah air sisa dikeluarkan di Malaysia. Hidrogen telah dikenal pasti sebagai sumber tenaga pada masa akan datang yang bersih, cekap dan boleh di kitar semula. Oleh itu, ia boleh mengantikan sumber tenaga yang tidak boleh di kitarsemula seperti arang batu, petrol, petroleum dan logam teras untuk tenaga. Kebolehlaksanaan penjanaan hidrogen daripada Kumbahan Kilang Kelapa Sawit (POME), merupakan air sisa yang mempunyai kepekatan yang tinggi dengan kandungan sisa pepejal yang tinggi, telah dinilai dalam UASBR. Kebanyakan kajian dalam penghasilan hidrogen mengaplikasikan penggunaan sistem sel terampai mengalami masalah dengan penyingkiran biojisim pada kadar kecairan yang tinggi dan memerlukan pengantian biojisim daripada biojisim untuk mengekalkan kepadatan sel untuk penggunaan dalam penghasilan hidrogen secara berterusan. Kajian ini perlu menentukan kesan suhu terhadap sel biojisim menggunakan UASBR untuk menentukan kualiti dan sifat-sifat POME dari segi COD, TSSdan VSS sebelum dan selepas sampel dirawat. Dalam empat perbezaan suhu iaitu 30 °C, 35 °C,40 °C dan 45 °C pada masa tahanan hidraulik dimalarkan pada dua belas jam telah diuji untuk menilai produktiviti hidrogen dan kestabilan operasi UASBR. Melalui keputusan yang diperolehi menunjukkan sistem yang lebih tinggi kecekapan ialah pada suhu 35 °C dengan kadar maksimum pengeluaran hidrogen 320 mL-H2/h/L dan hasil hidrogen 0.32 mol-H2/mol-sucrose. Kadar penyingkiran COD mencapai pada kadar yang telah ditetapkan iaitu dalam 45%-50%. Butyrate telah didapati menjadi metabolit dominan dalam semua suhu. Dengan kepekatan lemak yang rendah bagi lemak asid meruap (VFA) mengesahkan kestabilan keadaan dan kecekapan operasi yang telah dicapai oleh USBR.

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LIST OF ABBREVIATIONS

POME	- Palm Oil Mill Effluent
BOD	- Biochemical Oxygen Demand
TSS	- Total Suspended Solid
COD	- Chemical Oxygen Demand
TSS	- Total Suspended Solids
VSS	- Volatile Suspended Solid
UASBR	- Up-Flow Anaerobic Sludge Blanket Reactor
PEG	- Polyethylene Glycol Prepolymer

LIST OF SYMBOLS

H ₂	- Hydrogen
CO ₂	- Carbon Dioxide
%	- Percent
Kg	- Kilogram
m3	- Cubic meter
ppm	- Parts per million
mg/L	- Milligrams per liter
°C	- Degree Celsius
mL	- Mililiter

CHAPTER 1

INTRODUCTION

1.1.Background to the study

The demand of energy increasing day by days and lead to depletion of nonrenewable energy such as coal, gasoline, petroleum and metal cores. Since this problem has overwhelmed all over the world a lot of research have been carried out to utilize biomass as alternative renewable resource (Levin et al. 2004, Vijayaraghavan et al. 2006). Hydrogen generation from renewable biomass would reduce dependence on fossil fuel, decrease the carbon dioxide emission and produced usable bioenergy (Borkris 1973)

Hydrogen is widely produce and used in now days. Hydrogen (H_2) is an ideal energy carrier of the future and now days because H_2 is a clean, recyclable and has high conversion efficiency. It also has high energy content, and water is the sole end product after combustion. Hydrogen can be produced from many different sources, and different ways.

Hydrogen is best thought of as an energy carrier, more akin to electricity than the fossil fuels that we extract from the earth's crust. Hydrogen can be produced from any hydrocarbon fuel because by definition these fuels contain hydrogen. Hydrogen can also be produced from various biological materials and from water.

Hydrogen can be produced by various physical, chemical and biological methods that shown in Table 1.1.

Technology	Feed stock
Steam reforming	Hydrocarbons
Autothermal reforming	Hydrocarbons
Plasma reforming	Hydrocarbons
Aquesous phase reforming	Carbohydrates
Ammonia reforming	Ammonia
Partial oxidation	Hydrocarbons
Biomass gasification	Biomass
Photolysis	Sunlight + water
Dark fermentation	Biomass
Photo fermentation	Biomass + sunlight
Microbial electrolysis cells	Biomass + electricity
Alkaline electrolyzer	H_2O + electricity
PEM electrolyzer	H_2O + electricity
Photo-electrochemical water	H_2O + heat
splitting	
Thermo-chemical water splitting	H_2O + sunlight

Table 1.1: Hydrogen production technologies used today

Hydrogen productions by biological method have been start on 1980's. The large amount of H_2 is produced as a by-product of colonic fermentation of dietary fibre and un-adsorbed carbohydrates. H_2 production by microorganism can be divided into two main categories:

- 1. Photosynthetic bacteria and algae under light condition and the other, (photo-fermentation)
- 2. Anaerobic fermentative bacteria under dark condition, (dark-fermentation)

Parameter	Concentration (mg/l)	
Biochemical oxygen demand (BOD)	22,100-54,200	
Chemical oxygen demand (COD)	75,100-96,300	
рН	4.0-5.0	
Total carbohydrate	16,200-20,000	
Total nitrogen	820-910	
Ammonium -nitrogen	25-30	
Total phosphorus	95-120	
Phosphorus	14-20	
oil	80100-10500	
Total solid	35,000-42,000	
Suspended solids (SS)	8400-12000	

Table 1.2: Chemical characteristics of palm oil mill effluent used in this study

In Malaysia, the palm oil industry annually generates about 15.2 million tons of waste water know as palm oil mill effluent (POME) and also considered as high strength complex wastewater with high COD and BOD value shown in Table 1.2.. POME also knows as high strength wastewater with high content of degradable organic matter. The raw POME is hard to degrade because it's containing significant amount of oil (tryacylglycerols) and degradative product such as di-and monoacylglycerols and fatty acids.

POME nutrient content is too low for aerobic treatment process, but sufficient for anaerobic treatment process. Currently, methane production is commonly used method to treat POME, but production of hydrogen could be an innovative alternative.

In this study, I will used of immobilized sludge using anaerobic fermentation for hydrogen production. In this study, Continuous Hydrogen Production with Anaerobic POME Sludge Immobilized by Synthetic Polymer using immobilized UASB (up-flow anaerobic sludge blanket) reactor.

Immobilized-cell has been common alternative to the suspended cell system, this method has been used more often as a result of being less expensive and more easily to handle compare to the other method. The immobilized-cell system was suitable for continuous hydrogen production. Immobilized-cell system is another option for the effective retention of biomass and also gifted with a restricted anaerobic environment, which is well suitable to oxygen sensitive fermentative for continuous hydrogen production and the effect of temperature.

1.2.Problem Statement

- 1. Hydrogen production from POME wastewater is low
- 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.

1.3.Research objective

- 1. To characterized the POME (palm oil mill effluent) before and after treatment
- 2. To determine the effect of temperature for continuous hydrogen production using immobilized UASB reactor

1.4.Scope of study

The scope of this study is to determine the effluent different of continuous hydrogen production from Palm Oil Mill Effluent (POME) using UASB (Up-flow anaerobic sludge blanket).

This study also studies about the characteristic of POME before and after anaerobic fermentation. Using POME as waste water, the effluent taken will be part of sample that will test according the Chemical Oxygen Demand (COD), Total Suspended Solid (TSS), Volatile Suspended Solid (VSS) and pH.

There are two vary that need to be consider during this study. The first thing is the temperature and the second is the COD of sample.

There are two stages of process in the laboratory's experimental which is the first, need to determine the characteristic of the POME without processing the POME using the UASB reactor. Then the second stage is to determine the amount of hydrogen production using continuous hydrogen production method with UASB reactor.

After the experiment done, the data collect use for characterized the characteristic of the POME and to determine the amount of hydrogen production using POME.

1.5.Expected outcomes

The expected outcomes of this study are:

- 1. To analyze what is the different of anaerobic fermentation process defect in term of COD and pH of POME (Palm Oil Mill Effluent).
- 2. To analyze factor of effect for continuous hydrogen production using UASB reactor according to the temperature when retention time is constant

1.6.Significant of study

The significant of study of this study are:

- 1. High cell concentrations and long retention time of biomass in the system.
- 2. Cell reuse without the costly processes of cell recovery and cell recycle.
- 3. Eliminate cell washout at high dilution rates.
- 4. 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 (H₂) 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. At present 95% of commercial hydrogen generated by steam reforming of natural gas and gasification of coal (Logan et al.2002), hence, these process 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 H₂ productions are still the major problems of biological process.

Large quantities of water are consumed during the extraction of crude palm oil from the fresh fruit bunch. About 50% of the water results in POME, the other 50% being lost as steam, mainly through sterilizer exhaust, piping leakages, as well as wash waters . Many research works on hydrogen 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.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.

In this chapter the main aims of this review, the things that will be find out is the concept of term of hydrogen production and to find out about the characteristic of POME. The continuous hydrogen production using UASB reactor is needed to find out.

2.2.Paper review

2.2.1. Hydrogen

 H_2 exists naturally in earth's crust as bound state and there is a need to produce H_2 . H_2 is an ideal energy due to its advantages including clean, efficient and non-polluting characteristics. Biological hydrogen production derived from renewable energy sources is a clean bio-energy replacement for fossil fuels.

2.2.1.1.Hydrogen production

First produced artificially in the 16th century and identified as a unique element in1766, H_2 helped power the first working fuel cell, which generates electricity from the reaction between H_2 and O2. 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.

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; Turner, 2004; McDowall and Eames, 2007; Kalia and Purohit, 2008; Kotay and Das, 2008; Holladay et al., 2009).

2.2.2. Waste water

Wastewater is liquid waste discharged by domestic residences, commercial properties, industry, agriculture, which often contains some contaminants that result from the mixing of wastewater from different sources.

Generally waste water is synonymously with sewage even though sewage is a more general term that refers to any polluted water including wastewater, which may contain organic and inorganic substance, industrial waste, groundwater that happens to infiltration and to mix with contaminated water, storm, runoff, and other similar liquids (Miretzky et al. 2004).

Wastewater is any water that has been adversely affected in quality by antrophogenic influence. It comprises liquid waste discharge by domestic residences, commercial properties, industries, or agriculture and can encompass a wide range of potential contaminant and concentration. In the most common usage, it refers to the municipal wastewater that contains a broad spectrum of contaminant resulting from the mixing of wastewater from different sources (Salt, 2001).

2.2.3. Palm Oil Mill Effluent (POME)

Today, oil palm is the leading agriculture crop in Malaysia, covering about three million hectares of the cultivated area and the area planted with oil palm increased 70 fold since 1960s. By the year of 2004, Malaysia remained the world's largest producer and exporter of palm oil with 13.9 million tons or 52% of world production and 12.2 million tons or 58% of total world exports, respectively. However, this is important economic activity generates an enormous amount of liquid effluent POME.

The production of palm oil, however, results in the generation of large quantities of polluted wastewater commonly referred as palm oil mill effluent (POME). Typically, 1.0 ton of crude palm oil production requires 5.0-7.5 ton of water; over50.0% of which ends up as POME. Moreover, POME was high in organic content (COD 50.0 g/l, BOD 25.0 g/l) and contains appreciable amounts of plant nutrient (Borja et al., 1996; Singh et al., 1999; Ahmad et al., 2005)

2.2.3.1.Characteristic of POME

The POME comprises a combination of the wastewaters which are principally generated and discharged from the following major processing operations (Department of Environment, 1999):

- 1. Sterilization of FFB-sterilizer condensate is about 36 % of total POME
- Clarification of the extracted crude palm oil-clarification wastewater is about 60% of total POME
- Hydrocyclone separation of cracked mixture of kernel and shell hydrocyclone wastewater is about 4% of total POME.

The typical quality characteristics of the individual wastewater streams from the 3 principal sources of generation are presented in Table 2.1. In most mills, all three

wastewater streams amounting to about 3 tonnes per tonne of palm oil produced, are combined together resulting in a viscous brown liquid containing fine suspended solids (Borja and Banks, 1994).

Palm oil mill effluent is basically a mixture of sterilizer condensate, separator sludge, and hydrocyclone wastewater. Freshly produced POME is a colloidal suspension made up of 95%-96% water, 0.6%-0.7% oil, and 4%-5% total solids including 2%-4% suspended solids which are mainly debris from the palm fruit mesocarp (Whiting, 1978)

Parameter	Average	Metal	Average
рН	4.7	Phosphorous	180
Oil and Grease	4000	Potasium	2270
Biochemical Oxygen Demand (BOD ₅)	25000	Magnesium	615
Chemical Oxygen Demand (COD)	50000	Calcium	439
Total Solids	40500	Boron	7.6
Suspended Solids	18000	Iron	46.5
Total Volatile Solids	34000	Manganese	2.0
Ammonical Nitrogen	35	Copper	0.89
Total Nitrogen	750	Zink	2.3

 Table: 2.1. Typical characteristics of POME (Ma, 2000)

all in mg/l except pH BOD₅= after incubation for 3 days at 30 °C

2.2.3.2. Chemical characteristic

Chemical of wastewater are typically classified as organic and inorganic. Organic constitutes in wastewater can be classified as aggrega and individual. Meanwhile, inorganic constitutes in water can be divided into individual elements such as Zinc (Zn), Iron (Fe), Chloride (CI) and a wide variety of compound, for example nitrate (NO₃) and Sulphate (SO₄), (Salt, 2001).

2.2.3.2.1. Organic compound

Normally, organic compound are composed of carbon, hydrogen and oxygen, together with nitrogen in some cases. Other important element, such as sulphur phosphorus and iron may also be present (Rock, 1997).

2.2.3.2.2. Chemical oxygen demand (COD)

The COD test is commonly used to indirectly measure the amount of organic compounds in water. Most applications of COD determine the amount of organic pollutants found in surface water (e.g. lakes and rivers) or wastewater, making COD a useful measure of water quality. It is expressed in milligrams per liter (mg/L), which indicates the mass of oxygen consumed per liter of solution. Older references may express the units as parts per million (ppm), (Wikipedia, 2011)

2.2.3.2.3. Inorganic chemical

Several organic components of wastewater are important in establishing and controlling wastewater quality. Industrial wastewater has to be treated for removal of the organic constituents that are added in the life cycle. Concentrations of inorganic constituents also are increased by the natural process, which removes some of surface water and leaves the inorganic substance in wastewater (Llorans, 2000).

2.2.3.2.4. pH

The hydrogen ion concentration is an important quality parameter of wastewater. The concentration range suitable for the existence of most biological life is quit narrow and critical. Wastewater effluent may alter the concentration in the natural water, (Barber, 2004).

2.2.3.2.5. Heavy metal

Trace quantity of many metals such as Nikel (Ni), Manganese (Mn), Led(Pb), Choromium (Cd), Zinc (Zn), Cooper (Cu), Iron (Fe) and mercury (Hg) are important constitutes of some industrial wastewater. The presence of any of these metal in excessive quantities will interfere with many beneficial use of the water because their toxicity. Therefore, it is frequently desirable to measure and control the concentration of these substances (Satyakala and Jamil, 2001)

2.2.3.3.Physical characteristic

2.2.3.3.1. Total solids

The total solids content of waste water (POME) is meaning as all the matter that remains as residue upon evaporation at 103 °C to 105 °C, matter that has a significant vapour pressure at this temperature is lost during evaporation it is not define as a solid. Total solids, or residue upon evaporation can be further classified as non filterable (suspended) or filterable by passing a know volume of liquid through a filter (Rovers, 1997).

The dissolved solid consist of both organic and inorganic molecules and irons that are present in true solution in water. The colloidal fraction cannot be removed by settling. The suspended solid are found in considerable quantity in many industrial wastewater, such as cannery and paper mill effluents. They are screened and settled out at the treatment plant. Solids are removed by settling and separated from wash water are called sludge, which may then be pumped to filter for extraction of additional water (Baber, 2004).

2.2.4. Immobilized cell system

Immobilized whole cell technique leads to high reaction rates and thus represents an efficient approach to bio-catalysis for carrying out several biochemical reactions including H₂ production and CH4 production. Most of the solid matrices used for the immobilization of the whole cells are synthetic polymers or inorganic materials.

Use of immobilized whole cells compared to free floating cells increases the mean cell residence time in the reactor. Studies to improve rate of hydrogen production through immobilization have resulted in up to 1.7 fold increase. A four-fold increase in H_2 production by immobilizing B. *licheniformis* on brick dust has resulted in a H_2 yield of 1.5 mol/mol glucose in batch culture. Ligno-cellulosic agricultural waste materials such as rice hull, sugarcane bagasse, wheat straw, peashells, banana leaves and coconut coir are regarded as abundant, inexpensive and readily available natural sources. These are potential support materials for retaining large populations of H_2 producers within the reactor.

In fact, it has been possible to increase H_2 yield up to 2.36 mol/mol glucose by immobilizing Bacillus strains on these ligno-cellulosic wastes (Kalia and Joshi, 1995; Kumar et al., 1995; Kalia et al., 1997; Kalia and Lal, 2006; Pandey and Pandey, 2007; Tortoriello and de Lancey, 2007; Patel et al., 2010).

However, the choice of proper support material for immobilization appears to be very important. The solid matrices used for the immobilization of whole cells were mostly synthetic polymer or inorganic materials. These materials possess disposal problems. On the other hand, natural polymers such as ligno-cellulosic materials present abundantly in agro residues are environment friendly and do not have these problems and are cheap as well (Patel et al., 2010). For this study, the synthetic polymer that I used is polyethylene glycol (*PEG*).

2.2.5. Anaerobic fermentation treatment

Anaerobic fermentation treatment has been considered have a number of advantages over the conventional aerobic fermentation process. The principal advantages and disadvantages of anaerobic process have been shown in Table 2.2.

Anaerobic digestion is a process which breaks down organic matter in simpler chemicals components without oxygen. This process can be very useful to treat arising organic waste such as:

- Sewage sludge
- Organic farm wastes
- Municipal solid wastes
- Green/botanical wastes
- Organic industrial and commercial wastes

The science underlying AD can be complex and the process is best understood if split into the three main stages: hydrolysis, acidogenesis and methanogenesis. During hydrolysis, the fermentative bacteria convert the insoluble complex organic matter, such as cellulose, into soluble molecules such as fatty acids, amino acids and sugars. The complex polymeric matter is hydrolyzed to monomers, e.g. cellulose to sugars or alcohols. The hydrolytic activity is of significant importance in wastes with high organic content and may become rate limiting. Chemicals can be added during this step in order to decrease the digestion time and provide a higher methane yield. In the second stage, acetogenic bacteria, also known as acid formers, convert the products from the first stage into simple organic acids, carbon dioxide and hydrogen. The principal acids produced are acetic acid, butyric acid, propionic acid and ethanol.

Finally, methane is produced during methanogenesis by bacteria called methane formers in two ways: by means of cleavage of two acetic acid molecules to generate carbon dioxide and methane, or by reduction of carbon dioxide with hydrogen. The acetate reaction is the primary producer of methane because of the limited amount of hydrogen available (Fabien Monnet, 2003)

Table 2.2: Advantages and disadvantages of anaerobic process compared to aerobic process (Tchobanoglous et al., 2003).

Advantages	Disadvantages		
- Less energy required	- Longer startup time to develop		
- Less biological sludge	necessary biomass inventory		
production	- May require alkalinity addition		
- Fewer nutrients required	- May require further treatment		
- Methane production, a potential	with an aerobic treatment		
energy source	process to meet discharge		
- Hydrogen production, a	requirements		
potential energy source	- Biological nitrogen and		
- Smaller reactor volume required	phosphorus removal is not		
- Elimination of off gas air	possible		
pollution	- Much more sensitive to the		
	adverse effect of lower		
	temperatures on reaction rates		

2.2.6. Technology

Recently, the considerable attention of research activity on fermentative H_2 production has been focused on the conversion of biomass (Table 1) and carbohydrates (Table 2) reproducible resources to H_2 by mixed cultures.

S. No.	Organisms	Substrate	Yield	References
Batc	h culture condition			
1	Anacrobic sludge	Dairy waste	0.0317 mmol/g COD	(Mohan et al., 2008)
2	Anaerobic sludge	Food waste	101 mL/g COD	(Chen et al., 2006)
3	Anaerobic sludge	POME effluent	6.33 L/L substrate	(O-Thong et al., 2008)
4	Anaerobic sludge	Wheat powder	281 mL/g starch	(Argun et al., 2009)
5	Anaerobic sludge	Non-fat dry milk	119 mL/g COD	(Chen et al., 2006)
6	Cow dung compost	Cornstalk waste	149.69 mL/TVS	(Zhang et al., 2007)
7	Cow dung sludge	Cellulose	2.8 mmol/g cellulose	(Lin and Hung, 2008)
8	Cow waste slurry	Cow waste slurry	392 mL/L slurry	(Yokoyama et al., 2007)
9	Mixed consortia	Vegetable waste	13.96 mol/kg COD	(Mohan, et al., 2009)
Cont	inuous culture cond	ition		
10	Anaerobic sludge	TPW	2.3 mol/mol glucose	(Kim and Lee, 2010).
11	Anaerobic sludge	Organic waste	360 ml/g VS	(Valdez-Vazquez et al., 2005)
12	Mixed culture	Food waste	2.5 mol/mol hexose	(Lee et al., 2009)
13	Mixed culture	Wheat straw	178 mL/g sugar	(Kaparaju et al., 2009)

Table 2.3: Mixed culture hydrogen production from bio-waste

S. No.	Organisms	Substrate	Yield	References			
Batcl	Batch culture condition						
1	Anacrobic shalge	Gingose	1.46 mol-mol-glacose	(Davila-Vozquez et al., 2008)			
7	Cattle manure	Glucose	1.0 mol-mol-glacose	(Cheong and Hansen, 2008)			
3	Cracked screals	Sucruse	2.73 mol mol sucrose	(Zhang et al., 2005)			
4	Digested studge	Glucose	3 fmot mot gaucose	(Wang and Wan, 2008)			
5	Digested studge	Sucruse	6.12 mol mal sucrose	(Zhu and Beland, 2006)			
6	Methanogerne granides	Gucose	1.2 mol/mol/glucose	(Hu and Chen, 3007)			
7	Mumeipal studge	Sucrose	2.46 mill mail sucrose	(Wang et al., 3006)			
1 	Murneipal studge	Nylose	2.25 mil mal xylose	(Lan and Cheng, 2006)			
ų	Murne pal sludge	Staryh	11.25 mmol g starch	(Lee et al., 2008b)			
Conti	Continuous culture condition						
2. . 2.	Anaerobic studge	Glucose	1.63 mal mal glacase	(Wulet al., 2008)			
1	Anaerobic studge	Sucrise	1.22 mol mathexase	(Kum et al., 2006)			
12	Anaerobic Sludge	Sucroise	1811,16	(Wa and Chang, 2007)			
13	Anaerobic sludge	Starch	1.5 mil mil bexuse	(Euryet al., 2008)			
14	Anaerobic shulge	Starch	0.97 mill mal glaçase	(Arooj et al., 2008)			
15	Mixed cultures	Sucrose	1.61 mul mul glacose	{Zhao and Yu, 2008}			
l'n	Mumerpal stadge	Fractione	1.68 mol/mol/lexuse	(Lee et al., 2007)			
17	Mumerpal studge	Sucrose	1.60 mol mul hexase	(Lee et al., 2007)			
18	Mumeripal studge	Sucrose	3-88 millional successe	(Lee et al., 2006)			
14	Momeinal studge	Xyinse	1.63 mil mal vylose	(Wulet al., 2008)			
30	Sludge waste water	Glucose	0.73 HH2 E.K	(Chenng et al., 2007)			

Table 2.4: Mixed culture hydrogen production from pure substrates

2.2.7. Up-flow anaerobic sludge blanket (UASB) reactor

There are several technologies for wastewater treatment system presently available for the high rate treatment. This system obviously will prefer for the most economical system in terms of operation and maintenance.

One of the most notable developments in anaerobic treatment process technology was the UASB reactor in the late 1970s in the Netherlands by Lettinga and his coworkers (Tchobanoglous et al., 2003). Considering the present application of anaerobic treatment, apparently reactors based on the UASB principle look most favourable. In the meantime the feasibility of the UASB concept for treating mainly soluble wastewaters has been sufficiently demonstrated at full scale, at demonstration scale, and at pilot plant scale (Malina and Pohland, 1992).

The anaerobic treatment is practical and useful process to treat various industrial and domestic wastewaters. Although this process had numberless advantages, a lot of designers and operators have preferred to use aerobic processes than to anaerobic processes. It was because there were some misunderstandings for anaerobic processes as well as the lack of knowledge, experience, and skills. , there have been continuously studying so many research on high-rate anaerobic treatment processes and accumulated technologies and know-how obtained in the fields do not make the anaerobic process useless any more (Seung J. Lim,2001)

Hydrogen-producing granules with an excellent settling ability have been cultivated in an up-flow anaerobic sludge blanket (UASB) reactor treating a synthetic sucrose-rich wastewater. The physicochemical characteristics of the H₂-producing granules have been well elucidated .In another work, the feasibility of employing a granule-based UASB reactor for long-term continuous H₂ production has been explored. In order to further understand the H₂-producing UASB reactor, it is essential to develop a model to quantitatively describe the reactor performance. (Yang and Han, 2007) An up-flow anaerobic sludge blanket (UASB) process is an extensively applied anaerobic treatment system with high treatment efficiency and a short hydraulic retention time (HRT). Recently, UASB hydrogen production systems have been used in granulation enhancement and granule microstructure .However, the performance of hydrogen-producing UASB systems has not been discussed in detail. (Feng and Chiu, 2003)

Advantage	Disadvantage		
- Good removal efficiency can be	- Proper temperature control (15-		
achieved in the system, even at	35 °C) required for colder		
high loading rates and low	climates.		
temperatures.	- Post-treatment of the anaerobic		
- The construction and operation of	effluent is generally required to		
these reactors is relatively simple	reach the surface water		
and low demand for foreign	discharge standards for organic		
exchange due to possible local	matter, nutrients and pathogens.		
production of construction	- Hydrogen sulphide is produced		
material, plant components, spare	during the anaerobic process,		
parts and low maintenance.	especially when there are high		
- Anaerobic treatment can easily be	concentrations of sulphate in the		
applied on either a very large or a	influent. A proper handling of		
very small scale.	the biogas is required to avoid		
- When high loading rates are	bad smell and corrosion.		
accommodated, the area needed for	- Due to the low growth rate of		
the reactor is small thus reducing	methanogenic organisms, longer		
the capital cost.	start-up takes before steady		
- As far as no heating of the influent	state operation, if activated		
is needed to reach the working	sludge is not sufficiently		
temperature and all plant	available.		
operations can be done by gravity,	- Pathogens are only partially		
the energy consumption of the	removed, except helminthes		
reactor is less. Moreover, energy is	eggs, which are effectively		
produced during the process in the	captured in the sludge bed.		
form of methane.	Nutrients removal is not		
	complete and therefore a post-		
	treatment is required.		

Table 2.5 Advantages and disadvantages of UASB (Muhammad Asif Latif, RumanaGhufran, Zularisam Abdul Wahid, Anwar Ahmad, 2011)

CHAPTER 3

METHODOLOGY

3.1.Introduction

In this study, the continuous hydrogen production with anaerobic POME sludge immobilized by synthetic polymer will be analyzed by a laboratory assessment. The POME (Palm Oil Mill Effluent) that will use in this test is from Lepar Hilir because only POME from Lepar Hilir is hard to treat. The laboratory tests that will be doing are COD, TSS, VSS, pH, Immobilized cell system, H₂ production using UASB reactor.

3.2. Flow chart of study

Refer to the Figure 3.1 below; this study 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 before the sample treated.

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 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 in different temperatures.

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.

This study ended after the submission of the report and the slide presentation has been present to the panels.

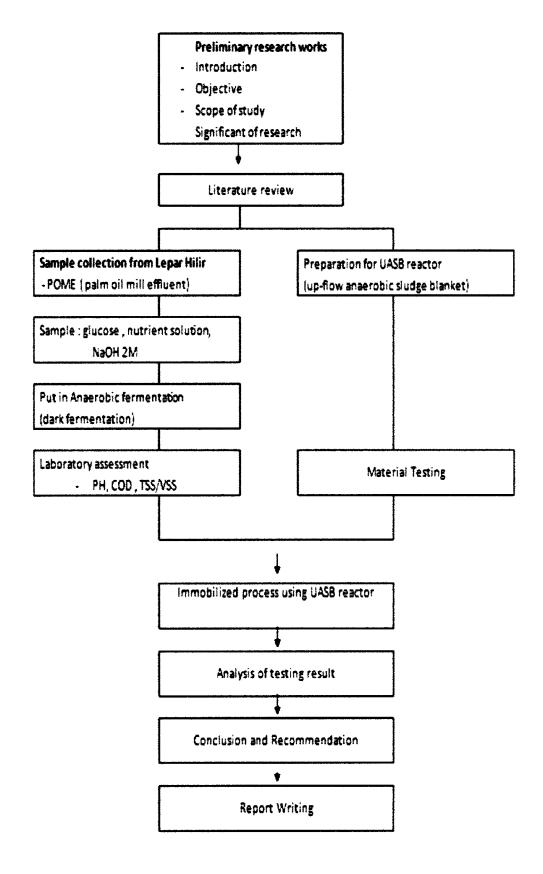


Figure 3.1 Flow chart of study

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 will be added with nutrient, glucose and nutrient solution as the food to the bacteria inside the POME. The sample will cover by parafilm to put it in dark fermentation (anaerobic fermentation).

After the entire sample is prepared, sodium hydroxide will be put inside the sample everyday to maintain the pH value thus to activate the bacteria. To activate the bacteria, the pH value of the sample must be in range 5.5 to 6.0.

After the sludge were in activated sludge the inoculate process have been done. Inoculate process is the process to seed the bacteria in the POME. The raw POME need to mix with the activated sludge before put in the reactor.

Next, the sample have been heated at various temperatures there is 30°C,35°C,40°C and 45°C in constant HRT 12hours. In every temperature the laboratory tests need to be done.

3.4.Laboratory Tests

There are four (4) laboratory tests that will use to determine the characteristics of the POME which are Chemical Oxygen Demand (COD), Total Suspended Solids (TSS), Volatile Suspended Solids (VSS), pH test.

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)

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

Apparatus

- i. COD reactor
- ii. High Range COD Digestion Reagent Vials
- iii. Spectrophotometer

Procedure

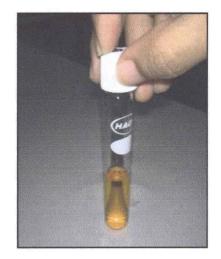
- i. Turn on the COD reactor and preheat to 150°C. Placed the shield in front of the reactor.
- ii. Dilute 1.00 ml of sample with 200 ml of distilled water.
- iii. Set proportion of samples needed in volumetric pipette and for test is 2.00 ml
- iv. Hold one vial at 45 degree angle. Pour the sample into High Range COD digestion Reagent Vials using the volumetric pipette (be sure to use vials for appropriate range). Refer Figure 3.2 (a) and (b).
- v. 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. Refer Figure 3.2 (c).
- vi. Cap the vials tightly. Rinse them with deionized water and wipe with clean paper towel.
- vii. 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. Refer Figure 3.2 (d).
- viii. Heat the vials for two hours in COD reactor.

- ix. After two hours, turn off the reactor and wait for 20 minutes for the vials to cool off. Refer Figure 3.2 (e)
- x. 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. Refer Figure 3.2 (f).



(a)





(c)



(d)



(e)



(f)



Method of COD test

3.4.2. Total Suspended Solid (TSS)

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.

Apparatus

- i. Polypropylene (PP tube 50ml)
- ii. Evaporation dish
- iii. Pipette 5ml
- iv. Spatula
- v. Analytic balance
- vi. Desiccators
- vii. Oven

Procedure

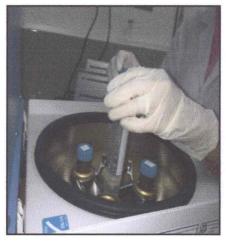
- i. Preparation of the apparatus and material.
- ii. Take 50ml sample and put it in polypropylene (PP tube 50ml). Refer Figure 3.3 (a).
- iii. To get the solid content from the sample, put the PP tube into centrifuge with a speed of 40 rpm for 10 minutes. Refer Figure 3.3 (b)
- Put the PP tube contains with water into the centrifuge to balance the PP tube in the centrifuge. Refer Figure 3.3 (c)
- v. Take the weight of empty evaporation dish. Refer Figure 3.3 (d)
- vi. Take the sample from the centrifuge and pour/take the solid contain in the PP tube into the evaporation dish. Refer Figure 3.3 (e)
- vii. Put the evaporation this that contain a solid from the sample into the oven with temperature $103.50 \square C$ for an hour. Refer Figure 3.3 (f)
- viii. After one hour carefully take the evaporation dish out and then cool in desiccators and weighed the evaporation dish with the sample.



(a)



(b)







(e)



(d)



(f)

Figure 3.3 Method

Method of TSS test

3.4.3. Volatile Suspended Solid (VSS)

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 behaviour and biological concentration throughout the system.

Apparatus

- i. Aluminium dish
- ii. Spatula
- iii. Chamber furnace
- iv. Analytic balance
- v. Glove

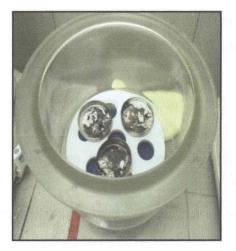
Procedure

- i. Use the sample from TSS test.
- Take out the sample from evaporation dish and put it in the aluminium dish.Refer Figure 3.4 (a).
- iii. Weighed the aluminium dish with residue before ignition process. ReferFigure 3.4 (b)
- iv. Put the aluminium dish with residue into the oven with temperature 500°C for an hour and after one hour switch off the oven and let the aluminium dish for ½ hour in the oven.
- V. Carefully take the aluminium dish out from oven and cool in desiccators. Refer Figure 3.4 (c)
- vi. After 15 minutes weighed the aluminium dish with residue.





(b)



(c)

Figure 3.4 Method of VSS test

3.4.4. pH

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.

Apparatus

- i. pH meter
- ii. Beaker
- iii. Distilled water

Procedure

i. The electrode(s) of the pH meter was rinsed by distilled water before immersing into the water sample.

- 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.
- iii. 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.
- iv. The pH value was recorded.
- v. 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

- i. The strength of the influents were depends on the COD of the diluted POME.
- The pHs of influents were adjusted to (pH 5.5). The working volume (V_w) for the UASB reactor was 5 L and the hydraulic retention time was constant (12 hours) the flow rate of Pump could be determined.
- The influents were continuously flowed into the reactor with the different temperatures which is 30°C, 35°C, 40°C, and 45°C. Parameters such as pH, COD, TSS and VSS of the both influent and effluent were determined. The

analysis methods for the desired parameters were referred to standard methods.

iv. The startup of the reactor was proceeding until the steady state condition. The controlled operating parameters were illustrated in Table 3.1.

Table 3.1Operating Parameters for the Reactor Start up for temperatures 30°C,
35°C, 40°C, 45°C

Operating Parameters	ating Parameters Desired Value	
influent pH	5.5	
HRT	12.00	hrs
organic loading	35.00	g/L.d COD
working volume	5.0	L
flow rate at pump 1 (influent)	2.9	L/hr
Immobilized bead dosages (w/v)	6	%

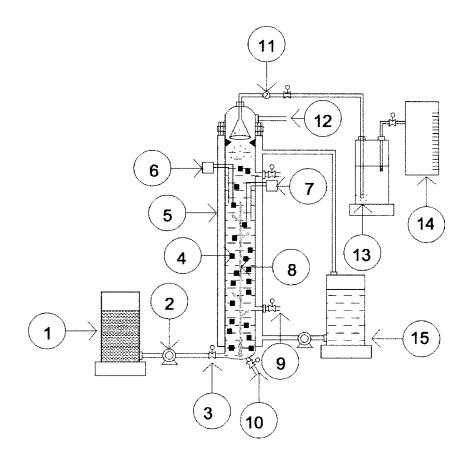


Figure 3.6: Schematic description of UASB reactor filled with immobilized beads for continuous hydrogen production.

- 1. . POME feed tank
- 2. Feed pump
- 3. Manual valve
- 4. Immobilized 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

3.4.6 Analytical methods

- 1. Hydrogen gas was determined with a Shimadzu GC-14A gas chromatography equipped with a thermal conductivity detector.
- 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.
- 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 temperature to the hydrogen rate using the continuous hydrogen production with anaerobic POME sludge immobilized by synthetic polymer.

The analysis of sample was fully operated at laboratory. Firstly, I have to characterize the sample of POME and after the finish the characterized the sample I have to further my experiment or test for second step that is I have to set-up the reactor for the gas collecting.

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

Laboratory studies have been conducted to analyze and determine the use of POME in hydrogen production. For the laboratory studies I have chosen four parameters to conduct this study which is Total Suspended Solid (TSS), Volatile Suspended Solid (VSS), Chemical Oxygen Demand (COD) and pH.

4.2. Result Consideration

The result of this report was to shown that is the objective was archive or not in this study. There is two objective have to be archive for this study there is to characterize the POME and the effect of temperature in continuous hydrogen production using immobilized synthetic polymer. All tests are done within three month period to classifying, testing and reporting of the performance of the sample with the all parameters during the study. For this study I only used two of parameter to monitor the condition of the sample which is COD test and VSS test.

4.2.1: The characteristic of Palm Oil Mill Effluent (POME)

The results have been shown in the table below. Tables 4.1 show the characteristics the raw sample of POME. After take the sample from the cold room put sample in room temperature before proceeds to the test.

Parameters	Sample
Initial pH	5.29
Total Suspended Solid, g/L	35.6
Volatile Suspended Solid, g/L	26.6
COD ,g/L	70.6

Table 4.1: The characteristic of the raw POME

		TSS	VSS
Temperature (°C)	COD (g/L)	(g/L)	(g/L)
30°C	80.6	55.2	50.4
35°C	90.6	58.4	54.8
40°C	77.6	52.8	50.1
45°C	73.6	50.6	46.6

Table 4.2: The result of COD, TSS and VSS for treated sample

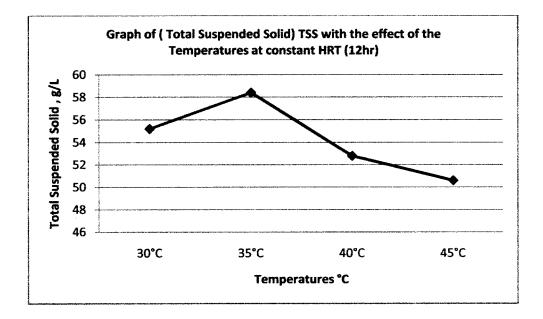


Figure 4.1: The value of TSS of the treated POME

Figure 4.1 show the value of total suspended solid of the treated POME at various temperatures. The results show that the TSS of the sample was increase at 35°C and decreased after 35°C.

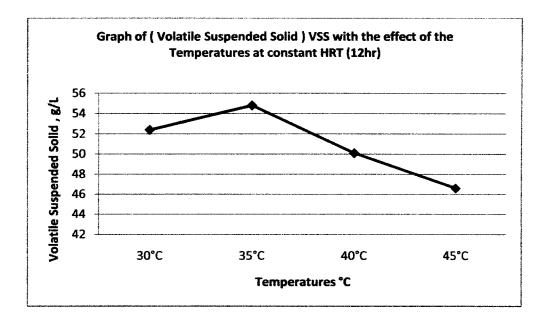


Figure 4.2: The value volatile suspended solid of the treated POME

Figure 4.2 show the result for the VSS for the sample of POME at various temperatures in the UASB reactor. The result shows that the VSS increase at 35°C and decrease when the sample was heated at high temperature.

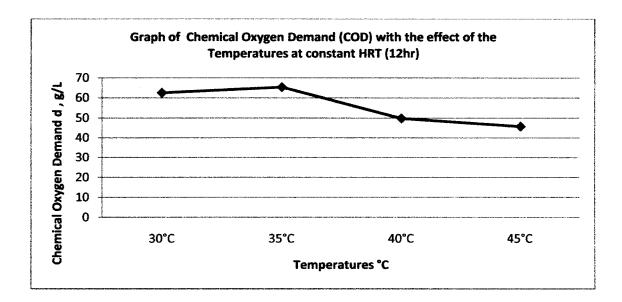


Figure 4.3: The chemical Oxygen Demand of the treated POME

Figure 4.3 show the value of COD for the sample of POME at various temperatures in the UASB reactor. The result shows that the COD for the sample was high at 35°C and decreased at high temperature.

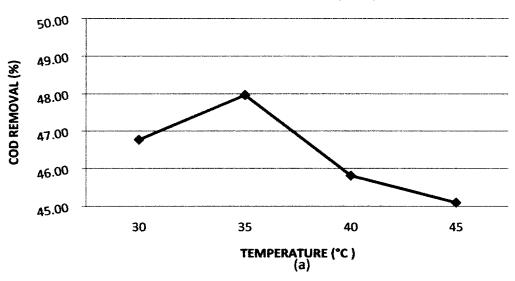
From the data that obtain from the experiment the raw POME sample was slightly same compared to the reported typical quality of POME.

From the data that obtain from treated POME it show the improvement of the value in terms of TSS, VSS and COD in the treated POME. Its mean this technology was the right way to treat POME.

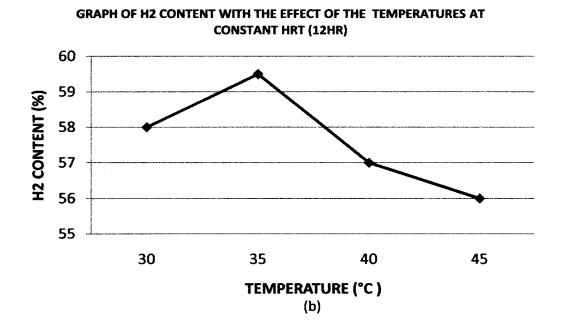
The characteristics of the raw POME would guide to the UASB reactor designs. By knowing the high strength of the raw POME was not possible directly apply to the UASB reactor, dilution of this raw POME was necessary in order to reduce the strength of the original raw POME. The strength of the wastewater was depending on the COD concentration.

4.2.2: Hydrogen production using Up flow Anaerobic Sludge Blanket Reactor at various temperatures

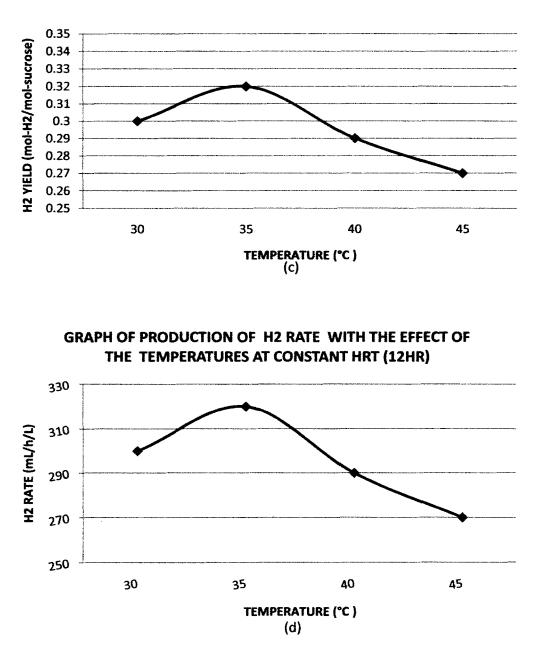
Figure 4.4 illustrated the performances of third series experiments for the POME sample .There was a period of days with low rate of hydrogen production when temperature was raised in high temperature.



GRAPH OF COD REMOVAL WITH THE EFFECT OF THE TEMPERATURES AT CONSTANT HRT (12HR)



42



GRAPH OF H2 YIELD WITH THE EFFECT OF THE TEMPERATURES AT CONSTANT HRT (12HR)

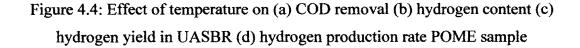


Figure 4.4 (a) shows the effect of the temperature on COD removal (%). The result shows that the COD removal was increased from 46.77% at 30° C to 47.97% at 35° C, decreased at 40°C and 45°C.

Figure 4.4 (b) shows the effect of the temperature on H2 content (%). The result shows that the H2 content in POME was increased linearly with the temperature from 58% at 30°C to 59.5% at 35°C and decreased after that.

Figure 4.4 (c) shows the effect of the temperature on hydrogen yield (mol-H2/mol-sucrose). The result shows that the hydrogen yield was increased significantly with temperature from 0.3mol-H2/mol-sucrose at 30°C to 0.32mol-H2/mol-sucrose at 35°C and decreased at high temperature 0.29mol-H2/mol-sucrose at 40°C to 0.27mol-H2/mol-sucrose at 45°C.

Figure 4.4 (d) shows the effect of the temperature on hydrogen production rate (mL/h/L). The graph shows the hydrogen production rate, which increased from 300 mL/h/L at 30°C to 320 mL/h/L at 35°C and decreased at high temperature 290 mL/h/L at 40°C to 270 mL/h/L at 45°C.

Based on these results, the optimal temperature was found as 35°C. It possibly due the ambient temperature cultivated seed sludge for POME sample. I can conclude that the 35°C was an optimal temperature to activate the sludge for high rate of hydrogen production and it has been proven that POME is good substrate for hydrogen production.

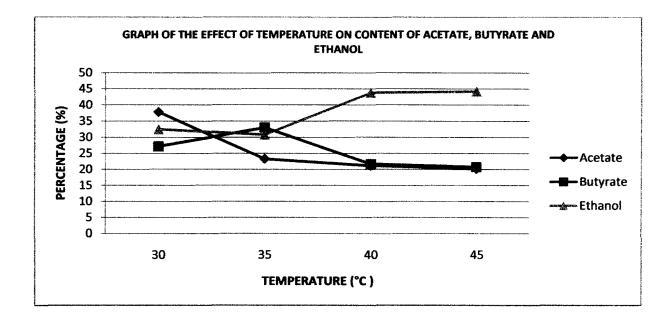


Figure 4.5: Effect of temperature on content of acetate, butyrate and ethanol in effluent (COD basic)

Generally, increasing temperature would result in decreasing concentration of acetate, but increasing concentration of ethanol, as shown in Figure 4.6. The result was concurred with the finding in suspended biomass reported by Valdez-Vazquez et al (2005).

CHAPTER 5

CONCLUSION AND DISCUSSION

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 objective of the study were archive or not. For recommendation, it has the purpose to give for the future study in this topic and solution for the problem that faced in the research.

5.2. Conclusion

When 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 the VSS before and after the sample was fermented for the both of sample. And for the second objective is to determine the effect of temperature in continuous hydrogen production using immobilized synthetic polymer. The both of objective were achieve. To achieve the goals, the sample have been analyze in laboratory which is Environmental Laboratory, the sample was tested based on four parameters that have been discuss in previous chapter.

After the experiment has been done the sample that show the hydrogen production rate were decreased at high temperature and the ambient temperature for the high rate of hydrogen production were 35 °C for the sample of POME in continuous hydrogen production by UASBR..

In this study, there was clearly show that the best temperature was 35° C respectively giving a H2 production rate of 320 mL/h/L and a yield of 0.32mol-H2/mol-sucrose for the POME sample.

As a conclusion, based on the result and the outcome of this study this works has potential of using immobilized cell system for continuous H2 production because this method was not expensive and easy to operate, it may be give an economical method to do in practice in 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 the POME as sources in hydrogen production.

From the analysis, we can reduce the cost to treat the POME and the pollutant from POME because we have another method to use the POME there is as a sources of energy.

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 the sample. Further treatment of the effluent is deserved either in anaerobic digestion or photosynthetic process.

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