Biohydrogen Production from Palm Oil Mill Effluent (POME) Using Immobilized Mixed Culture (Sludge)

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Thesis submitted in fulfillment of the requirements

for the award of the degree of

Bachelor of Civil Engineering

Faculty of Civil Engineering and Earth Resources

UNIVERSITI MALAYSIA PAHANG

JUNE 2013

ABSTRACT

The cell immobilization techniques by using immobilized anaerobic sludge as the seed culture were used to generate hydrogen. The substrate carbon source was taken from Palm Oil Mill Effluent (POME). It was revealed that by using the same concentration of POME, the suspended cell reactor was able to produce hydrogen at an optimal rate of 0.3491I H2/I POME at HRT 6 h. However, the immobilized cell reactor was able to exhibit better hydrogen production rate at 0.5871 I H2/I POME h at HRT 2 h. The present of beads enhanced the production of hydrogen during lower HRT without encountering wash out of cell. This could be shown by higher production of hydrogen at HRT 1 h with 0.535 I H2/I POME by using immobilized cell reactor. Compared to suspended cell reactor, the production of hydrogen at HRT 1 h was very low with 0.092 I H2/I POME due to wash out of cell during this time. The side products during hydrogen production were soluble metabolites including butyric acid and acetic acid, followed by propionic acid and ethanol.

ABSTRAK

Teknik sel tetap dengan menggunakan bahan buangan campuran anaerobik sebagai benih bahan buangan telah digunakan untuk menghasilkan hidrogen. Sumber substrat telah diambil daripada Air Buangan Kilang Kelapa Sawit. Telah didedahkan bahawa dengan menggunakan kepekatan Air Buangan Kilang Kelapa Sawit yang sama, sel reaktor terampai berupaya menghasilkan hidrogen pada kadar optimum iaitu 0.3491I H2/I POME pada HRT 6 h. Walapun begitu, sel reaktor tetap berupaya utuk meghasilkan hidrogen pada kadar yang lebih baik iaitu 0.5871 I H2/I POME h pada HRT 2 h. Penggunaan manik telah membantu mempertingkat penghasilan hidrogen sekitar HRT yang lebih rendah tanpa menghadapi pengeluaran sel. Hal ini dapat dilihat dari sudut penghasilan hidrogen yang lebih tinggi pada HRT 1 h iaitu 0.535 I H2/I POME dengan menggunakan sel reaktor tetap. Berbanding sel reaktor terampai, penghasilan hidrogen adalah pada kadar yang sangat rendah iaitu 0.092 I H2/I POME yang disebabkan pengeluaran sel pada waktu ini. Produk sampingan dalam penghasilan hidrogen ialah metabolit larut termasuk asid butirik, asid asetik, diikuti dengan asid propionik dan etanol.

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POME	Palm Oil Mill Effluent
NH_3-N	Ammonium Nitrate
COD	Chemical Oxygen Demand
BOD	Biological Oxygen Demand
TSS	Total Suspended Solid
PEG	Polyethylene glycol
HRT	Hydraulic Retentation Time
VFA	Volatile Fatty Acid
HBu	Butyric acid
HAc	Acetic acid
HPr	Propionic acid
EtOH	Ethanol

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

The demand of energy has been increased and causes to depletion of non- renewable energy such as coal, gasoline, petroleum and metal cores. Nowadays, this problem arises at every country and a lot of researches have been carried out to utilize biomass as alternative renewable resource (Levin *et al.,* 2004, Vijayaraghavan *et al.* 2006). Carbon dioxide emission and production usable bioenergy can be decreased as well as reduced dependence on fossil fuel by using hydrogen generation from renewable biomass (Borkris 1973).

Hydrogen is proved as a viable alternative source to replace conventional fossil fuel due to its characteristics as a clean, renewable and high energy yield nature. Hydrogen is new energy resource which is very productive because after hydrogen is used as a fuel, water is the sole end combustion product which can be recycled again to produce more hydrogen.

Hydrogen is an ideal energy resource nowadays and for the future. Hydrogen production is totally different compared to other fossil fuels because it is not readily available in nature. It needs some production methods that are quite expensive to extract it.

However, these processes are energy exhaustive, expensive and less environmental friendly because they involved with electricity and come from fossil fuel combustion.

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

Table 1.1: Hydrogen production technologies used today

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 splitting	H_2O + heat
Thermo-chemical water splitting	H_2O + sunlight

Biological process is one of the alternatives methods to generate hydrogen because this process can be operated at ambient temperatures, pressures, less energy intensive and more eco-friendly compared to conventional chemical method. This biological process allows opening new path for the exploitation of renewable energy resources which are unlimited. Biologically production of hydrogen can be achieved by two methods.

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

The palm oil mill industry in Malaysia has been recognized as the one who discharges the largest pollution load into the river compared to other country who also implemented palm oil industry (Hwang *et al.,* 1978). The palm oil industry in Malaysia annually generates about 15.2 million tons of waste water known as palm oil mill effluent (POME). POME is considered as an acidic brownish colloidal suspension wastewater with high total oxygen demand (COD) and biological oxygen demand (BOD) value. It is discharged at temperature of 80°C-90°C. The COD and BOD value of POME shown in **Table 1.2.**

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

Parameter	Concentration (mg/l)
Biochemical oxygen demand (BOD)	22,100-54,200
Chemical oxygen demand (COD)	75,100-96,300
pH	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

Immobilized-cell system has been common alternative to the suspended cell system in continuous operations. It can be operated at high dilution rate or low retention times without encounter washout of cells. It is also gifted with a local anaerobic environment which is well suitable to oxygen sensitive dark hydrogen fermentation. Immobilized cells also more flexible to environment distract, process stability, reusable and higher biological activity since higher cell density can be implemented.

In this study, I will used of immobilized sludge using anaerobic fermentation for hydrogen production. In this study, dark fermentation consists of high rate of cell growth, no light energy required and no oxygen limitation problems to perform and it has ability to run on low capital cost. The immobilized bead that has been cut into 3mm beads are prepared under anaerobic conditions at room temperature and will be used for hydrogen production.

1.2 PROBLEM STATEMENT

1. Hydrogen production from POME wastewater is low.
2. 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 OBJECTIVES

1. To compare the performance between suspended and immobilized cell reactor for hydrogen production at different Hydraulic Retention Time (HRT).
2. To study the effect of beads size on hydrogen production.

1.4 SCOPE OF STUDY

The scope of this study is to compare between suspended and immobilized cell reactor for hydrogen production at different Hydraulic Retention Time (HRT) using palm oil mill effluent. Immobilized-cell systems will be used in production of hydrogen and higher production of hydrogen at different HRT will be analyzed.

This study also studies about the effect of immobilized beads size on hydrogen production. The immobilized sludge bead used is in the range of 3mm beads. These beads will be allowed to perform under anaerobic conditions at room temperature.

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 laboratories experimental. First stage is to determine the characteristic of the POME base on the experimental results. Second stage is to determine the amount of hydrogen production by using immobilized cell reactor and the effects of beads size.

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 OUTCOME

The expected outcomes of this study are:

1. There are differences of performance between suspended and immobilized cell reactor for hydrogen production at different hydraulic retention time (HRT).
2. The beads size has effects on hydrogen production.

1.6 SIGNIFICANT OF STUDY

The significant 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 (H_2) is considered as a clean source of energy and an ideal substituent for fossil fuel due to its high energy content (122kJ/g), recyclability and non-polluting character. Nowadays, due to demand of the energy is expending by the industries, power plants, offices and households as well as individual life, depletion of non-renewable energy such as coal, oil, gasoline and metal cores occur. Many researchers have been carried out to use biomass as alternative renewable resources due to this problem (Lay *et al.*, 1999, Levin *et al.*, 2004, Prasertsan and Prasertsan, 1996, Vijayaraghavan *et al.*, 2007). Thus, production of hydrogen is important to replace conventional fossil fuels.

Due to the characteristic which is reliable and sustainable energy for the future, the production of biological hydrogen (biohydrogen) from biomass has received wide attentions (Debabrata and Veziroglu, 2001, Levin *et al.*, 2004). In Malaysia, there are many types of biomass generated by palm oil mill processing, such as of empty fruit bunches, palm press fiber, palm kernel cake, palm kernel shell, sludge cake and palm oil mill effluent (POME) (Prasertsan and Prasertsan, 1996). The palm oil industry in Malaysia annually generates about 15.2 million tons of waste water. Existing treatment of POME in a series of open lagoons is quite unpractical because it gives bad impact towards environment due to its high ambient temperatures which results in the uncontrolled production green house gases (GHGs) such as of methane and carbon dioxide. Thus, it is very important to develop an alternative method for POME treatment to save our environment besides it can increase hydrogen production.

The improvement of fermentation process for POME is important because POME is one of relatively potential as a substrate for generation of hydrogen (O Thong *et al.*, 2007, Vijayaraghavan and Ahmad, 2006, Atif *et al.*, 2005). POME is produced with an average values of 25 000 mg/L biochemical oxygen demand (BOD) and 50 000 mg/L chemical oxygen demand (COD), respectively (Yacob *et al.*, 2005). Due to its characteristic with high organic content, biohydrogen production could be achieved via dark fermentation (Yusoff *et al.*, 2009). Dark fermentation has many advantages such as high rate of cell growth, operation without light source and no limitation oxygen problems. Thus, dark fermentation is much better for POME to increase generation of hydrogen.

In addition, hydraulic retention time (HRT) become a vital parameters to control the anaerobic process. HRT also play an important role in increasing biohydrogen production as biomass is maintained at certain density (Levin *et al.*, 2004, Wu *et al.*, 2008). Many researchers investigated the effects of HRTs on the biohydrogen production rate, biogas composition and biomass concentration in the liquid effluent and the experimental results showed that different HRTs influenced the biohydrogen production (Zhang *et al.*, 2006, Wu *et al.*, 2008).

During the fermentation of biohydrogen from different HRT, biohydrogen concentration increased gradually. A suggestion has been introduced that the facultative microbial flora as well as anaerobic microbe were competitive in the absence of methanogenic bacteria because the biogas produced in all set of experiments contained H, CO and no methane gas was detected. It was being observed that the fastest steady state is at HRT 2 days. Compared to HRT 5 and 3 days, the biohydrogen concentration attained was at the range of 27-30% after 14 days operation time, which is highest. Furthermore, with longer HRT, availability of CO in the fermentation might be increased. According to Nath and Das, (2004) by removing the CO from the culture medium can increase the yield of biohydrogen. In this study, it seems that biohydrogen concentration reduced at longer HRT operation due to mixing up with CO. Due to short HRT, the accumulation of CO could be avoided and biohydrogen concentration could be increased.

The PEG-immobilized beads had a porous microstructure that facilitated the transfer of nutrients and substrates, which ensuring the growth of microorganisms for hydrogen production. Thus, the resulting PEG immobilized cells displayed a very stable performance in continuous generation of hydrogen from POME. In addition, the PEG immobilized cells able to achieve a stable and high production of hydrogen at a relatively high dilution rate (low HRT) without cell washout.

Immobilized cell systems have become common alternatives to suspended cell systems in continuous operations. It also can be operated at high dilution rates (or low retention times) without encountering washout of cells. The immobilization-cell system also is gifted with a feature which it has an ability of creating a local anaerobic environment and it is well suited to oxygen-sensitive dark hydrogen fermentation. Furthermore, immobilized cells are more tolerant to environment distraction, process stability, reusable and higher biological activity (Singh *et al.,* 2012).

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 generation of hydrogen using immobilized cell system is different between HRT. The production of hydrogen also may affected by the beads size.

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 O_2. 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)

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; over 50.0% 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
2. Clarification of the extracted crude palm oil-clarification wastewater is about 60% of total POME
3. 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).

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

Parameter	Average	Metal	Average
pH	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

All in mg/l except ph

BOD_5=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 aggregate 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 (NO_3) and Sulphate (SO_4), (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 used to measure the amount of organic compounds in water. Usually, COD test is used to determine the amount of organic pollutants found in surface water such as lakes and rivers or waste water. COD test plays an important role in order to measure water quality. It is measured in milligrams per litre (mg/L), which indicates the mass of oxygen consumed per litre of solution. Other references may express the units as parts per million (ppm) (Wikipedia 2012).

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)