BIOLOGICAL HYDROGEN PRODUCTION FROM PALM OIL MILL EFFLUENT (POME)

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BIOLOGICAL HYDROGEN PRODUCTION FROM PALM OIL MILL EFFLUENT (POME)

YEAP SHU YING

Thesis submitted in fulfilment of the requirements
for the award of the degree of
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STATEMENT OF AWARD FOR DEGREE

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SUPERVISOR'S DECLARATION

We hereby declare that we have checked this thesis and in our opinion, this thesis is adequate in terms of scope and quality for the award of degree of Bachelor of Engineering Technology in Energy and Environmental.

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ABSTRACT

In this study, we aim to produce biohydrogen production from POME by using indigenous hydrogen producing bacteria from POME, dark fermentation and continuously-stirred tank reactor (CSTR). Firstly, we successfully isolated four hydrogen producing bacteria, named Bacteria 1, 2, 3 and 4. We only selected Bacteria 2 as it yielded the highest percentage of biohydrogen gas. After that, the experiment was set up as shown in Figure 3.2. We also named the Bacterium 2 as JTY2017. JTY2017 was used to determine the optimum conditions to yield better hydrogen amount. The results showed the optimum condition was 35˚C and pH 5.5. The COD removal of Bacteria JTY2017 was 39%. Applications of nanoparticles (NPs) enhance bioactivity and metabolite recovery during dark fermentation and hence enhance biological hydrogen production from POME. The results obtained indicated that NPs can accelerate and increase the biohydrogen production yield in 48 hours. When the concentration of iron oxide NPs set at 4.0mg/l, the biohydrogen produced was the highest, at 76%. On the other hand, when the concentration of magnesium oxide NPs set at 4.0mg/l, the biohydrogen produced was the highest, at 71%. After that, the POME was sent to analyze and it showed that COD removal rate was increased too, compared to the non-NPs application. For Bacteria JTY2017 at 35˚C and pH 5.5, the POME's COD removal with addition of iron oxide and magnesium oxide NPs was 63% and 61%, respectively. Therefore, it is shown and proved that POME has the potential to produce renewable energy, and application of nanoparticles also help to enhance the results desired.

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LIST OF SYMBOLS

$CaCl_2 \cdot 2H_2O$	Calcium Chloride Dihydrate
Cl	Chloride
CO_2	Carbon dioxide
HCl	Hydrochloric acid
H_2	Hydrogen
Fe	Iron
$FeCl_3 \cdot 6H_2O$	Iron(III) Chloride Hexahydrate
$FeSO_4 \cdot 7H_2O$	Iron(II) Sulphate Heptahydrate
g	Gram
g/L	Gram per litre
KH_2PO_4	Monopotassium Phosphate
L	Litre
$L \cdot cysteine \cdot HCl \cdot H_2O$	L-Cysteine Hydrochloride Monohydrate
mg/L	Milligram per litre
$MgSO_4$	Magnesium Sulphate
$MgSO_4 \cdot 7H_2O$	Magnesium Sulphate heptahydrate
ml	Milli-Litre
$MnSO_4 \cdot 7H_2O$	Manganese Sulphate Heptahydrate
NaCl	Sodium Chloride
NaOH	Sodium hydroxide
Na_2MoO_4	Sodium Molybdate
$(NH_4)_2SO_4$	Ammonium Sulphate

NO_3	Nitrate
SO_4	Sulphate
Zn	Zinc

LIST OF ABBREVIATION

BOD	Biochemical Oxygen Demands
CPO	Crude Palm Oil
COD	Chemical Oxygen Demands
CSTR	Continuous Stirred-tank Reactor
FFB	Fresh Fruit Bunch
GC	Gas Chromatography
GHGs	Greenhouse Gases
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NPs	Nanoparticles
POME	Palm Oil Mill Effluent
OD	Oxygen Demand
XRD	X-ray Powder Diffraction

CHAPTER 1

INTRODUCTION

1.1 PROJECT BACKGROUND

Malaysia is the largest producer and exporter of palm oil (Ahmad, 2003). Over the past few years, Malaysian palm oil industry has grown rapidly to become the world's second largest producer of palm oil, accounted for 10.3% of the world's oils and fats production in year 2007 (Lam et al., 2009), contributed average crude palm oil production (CPO) of more than 13 million tonne per year. This industry can be counted as an important backbone to Malaysia's economy and it has remarkably increased the standard of living of its populations. The palm oil industry also provides a big employment opportunity to the rural families as agricultural workers in estates. Currently, palm oil is even intensively used as a source to produce biodiesel after the announcement of Fifth Fuel Policy under Eighth Malaysia Plan (2001–2005) (Lim and Teong, 2010).

Large quantities of water are used during the extraction of crude palm oil from the fresh fruit bunch, and about 50% of the water results in palm oil mill effluent (POME). Nevertheless, production of such huge amount of crude palm oil has consequently resulted to even larger amount of palm oil mill effluent (POME). The sum of all waste biomass emitted from palm oil industry of Malaysia, including POME, were amounted to 65.5 million ton in 2009 (Yacob et al,2005). POME generation is believed to continue rise in tonnes as production and processing of palm oil in Malaysia continue to rise to meet both domestic and global demand. In the process of palm oil milling, POME is generated through sterilization of fresh oil palm fruit bunches, clarification of palm oil and effluent from hydrocyclone operations (Borja et al., 1996a).

Palm oil mill effluent (POME) is a highly polluting wastewater that pollutes the environment if discharged directly due to its high chemical oxygen demand (COD) and

biochemical oxygen demand (BOD) concentration (Poh and Chong, 2009). POME consists of water soluble components of palm fruits as well as suspended materials like palm fiber and oil residues. Despite its biodegradability, POME cannot be discharged without first being treated because it is acidic and contains residual oil that cannot be easily separated using conventional gravity-based systems. Essentially, this oily mix needs a lot of oxygen before it can decompose completely, and this phenomenon is called having a high biochemical oxygen demand (BOD) (Madaki and Lau, 2013).

Although POME is a non-toxic thick brownish liquid waste with unpleasant smell, its COD and BOD values are high enough to cause serious pollution and environmental problem to the rivers. The palm oil industry is identified as one of the agricultural industries in Malaysia that generates the highest pollution load into rivers throughout the country have reported that POME contains a high concentration of organic matter, COD concentration in the range of 45,000–65,000 7mg/L and BOD of 18,000–48,000 mg/L. COD in the range of 80,100–95,000 mg/L and BOD of 23,400–52,100 mg/L has also been encountered (Soleimaninanadegani and Manshad 2014).

For alternative energy carriers, hydrogen could be the fuel of the future because of its high energy content, environmental friendliness of production, and also because it can give substantial social, economic and environmental credentials (Kotay and Das, 2008). Hydrogen is a carbonfree clean fuel, as the only final by-product of its combustion is water. Hydrogen can also be helpful in addressing global warming and increasing pollution problems. Furthermore, it is preferred over methane owing to its wider industrial applications, i.e. H_2 is used in the synthesis of ammonia and hydrogenation of edible oil, petroleum, coal and shale oil (Kothari et al., 2012). Also, hydrogen-using technologies play a major role in a substantial transformation toward a more flexible, less vulnerable, distributed energy system which meets energy needs in a cleaner, more efficient and cost-effective way (Barreto, 2003).

Hydrogen gas can be produced through dark fermentation from the POME sludge in a continuous stirred-tank reactor. A reactor where mixing is important is the tank flow or continuously stirred tank reactor (CSTR); it is also referred to as a" back-mix" reactor. This type of reactor is like the batch reactor (Theodor, 2012). CSTRs are open systems,

where material is free to enter or exit the system that operates on a steady-state basis, where the conditions in the reactor do not change with time. Reactants are continuously introduced into the reactor, while products are continuously removed. A continuous stirred-tank reactor (CSTR) was used to disclose the effective start-up conditions using potato starch as a substrate, but the maximum period of data was only 21 days and H_2 yields for two hydraulic retention times (HRTs) were shown at a glance (Hussy et al., 2003).

However, the hydrogen yield and specific hydrogen production rate during biological hydrogen production from POME are low, due to the poor biodegradability, bioactivity and substrate conversion efficiency of hydrogen producing microbes, and therefore, a technology needs further development (Gadhe et al., 2013). Although, complexity and poor biodegradability are one of the problems in dark fermentation of POME, the presence of high content of biodegradable organic compound can make POME an ideal candidate for biohydrogen production via amicrobial process. In real life application, by using hydrogen gas in internal combustion engines presently powered by natural gas, it can improve the combustion efficiency, decrease the fuel consumption, and reduce significantly the emissions of carbon monoxide, carbon dioxide, and nitrous oxides.

An application of nanoparticles (NPs) to enhance bioactivity and metabolite recovery during dark fermentation has gained enormous attention due to unique surface and quantum size effects of NPs (Han et al., n.d.). In this project, we are able to transform the status of POME from waste to resource by converting it into advantageous energy through dark fermentation process. Also we will be able to get accelerated hydrogen gas production from POME for the output, by adding suitable NPs.

1.2 PROBLEM STATEMENT

Palm Oil Mill Effluent (POME) is a highly polluting wastewater with high chemical oxygen demand (COD) and biochemical oxygen demand (BOD) from the discharges of palm oil industries. The direct discharge of POME to the water source such as river without following the laws and regulations, will lead to obvious and severe environment pollution typically water pollution. Therefore, it is compulsory to treat the POME before it is being released into the environment.

Recently, ponding system has been used to treat POME. The decision on whether or not to use this system depends on many factors. The main factors are land price, conditions of the surrounding area and the loss of biogas as a source of energy. The main problem faced by palm oil industry in Malaysia is requirement of large scale of land. Because of that factor, high cost of maintenance such as labor monitoring will be needed by the industry in order to treat the waste. Eventually, the treatment of POME often incurs high non-profitable cost in an industry that reduces the company's profit. Thus, the concept of transforming waste to energy, from POME to hydrogen gas, makes waste treatment seem more appealing and cost-effective.

1.3 OBJECTIVE

i. To isolate an indigenous hydrogen producing bacteria from palm oil mill effluent sludge.
ii. To investigate the hydrogen production efficiency from simple and complex carbon source using newly isolated bacterial strain.
iii. To determine the effect of metal oxide nanoparticles on hydrogen production.

1.4 SCOPE OF STUDY

The scope of this study is to isolate the hydrogen producing bacteria. We will identify and find only the hydrogen producing bacteria among bacteria found in the POME

Secondly, we will use standard and complex carbon sources for the bacterial isolate to produce hydrogen after isolating the bacteria. The results gained will be compared.

Lastly, we will use two samples of NPs nanoparticles to accelerate the hydrogen production and the results will be compared too.

1.5 EXPECTED OUTCOMES

The expected outcomes of this study are:

i. Isolating an indigenous hydrogen producing bacteria from palm oil mill effluent sludge.
ii. Investigating the hydrogen production efficiency from simple and complex carbon source using newly isolated bacterial strain.
iii. Determining the effect of metal oxide nanoparticles on hydrogen production.

CHAPTER 2

LITERATURE REVIEW

2.1 BACKGROUND ON PALM OILL MILL EFFLUENT (POME)

2.1.1 Introduction of POME

Palm oil mill effluent (POME) is a wastewater generated from palm oil milling activities which requires effective treatment before discharge into watercourses due to its highly polluting properties. Palm oil mill effluent is the brownish liquid waste that results from the sterilisation and clarification processes in milling oil palm. Untreated or partially treated POME contains an extremely high degradable organic matter (Nwoko & Ogumyemi, 2010). These result in the high value of BOD and COD which shown in Table 2.1, is the main cause of environmental pollution.

Table 2.1: Characteristics of untreated POME

***Parameter**	**Mean**	**Range**
pH	4.2	3.4-5.2
Biological Oxygen Demand (BOD)	25000	10250-43750
Chemical Oxygen Demand (COD)	51000	15000-100000
Total Solids	40000	11500-79000
Suspended Solids	18000	5000-54000
Volatile Solids	34000	9000-72000
Oil and Grease	6000	130-18000
Ammoniacal Nitrogen	35	4-80
Total Nitrogen	750	180-1400

*All parameter in mg/l except pH and temperature (˚C)

Source: (Malaysian Palm Oil Board, 2012).

2.1.2 Environmental Regulations of POME Discharge

Palm oil industry in Malaysia has shown a rapid growth from the past few decades. According to Malaysian Palm oil Board (MPOB, 2011), the oil palm planted area in 2011 reached 5.00 million hectares, an increase of 3.0% against 4.85 million hectares recorded the previous year. Despite the increase in production of CPO, there is also significant increase in the amount of waste produced by the industry. The standard of POME discharge (Table 2.2) has been set since 1978 in order to protect the environment from pollution.

Table 2.2: Palm Oil Mill Effluent Discharge Standards.

***Parameter**	**Std. A**	**Std. B**	**Std. C**	**Std. D**	**Std. E**	**Std. F**
	1978	1979	1980	1981	1982	1984
pH	5-9	5-9	5-9	5-9	5-9	5-9
Biological Oxygen Demand (BOD)	5000	2000	1000	500	250	100
Chemical Oxygen Demand (COD)	10000	4000	2000	1000	1000	1000
Total Solids	4000	2500	2000	1500	1500	1500
Suspended Solids	1200	800	600	400	400	400
Oil and Grease	150	100	75	50	50	50
Ammoniacal Nitrogen	25	15	15	10	150	100
Total Nitrogen	200	100	75	50	50	50
Temperature (^{o}C)	45	45	45	45	45	45

*Units in mg/L except pH and temperature

Source: (MPOB, 2012).

2.1.3 Chemical characteristic

Chemical of POME 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 (Cl) and a wide variety of compound, for example nitrate (NO_3) and Sulphate (SO_4).

2.2 HYDROGEN GAS

Hydrogen (H_2) is hydrogen has been considered as a “clean” energy source and carrier and discussed in comparison with present day energy sources mainly based on fossil fuels and nuclear energy (Bicelli, 1986). Especially it is an ideal substituent for fossil fuel due to its high energy content (1220/g), recyclability and non-polluting character. Some important advantages of hydrogen such as hydrogen is a non-toxic, clean energy carrier that has a high specific energy on a mass basis, hydrogen can be stored over relatively long periods of time compared to electricity and hydrogen can be utilized in all parts of the economy (Midilli, A., et al,2005). 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).

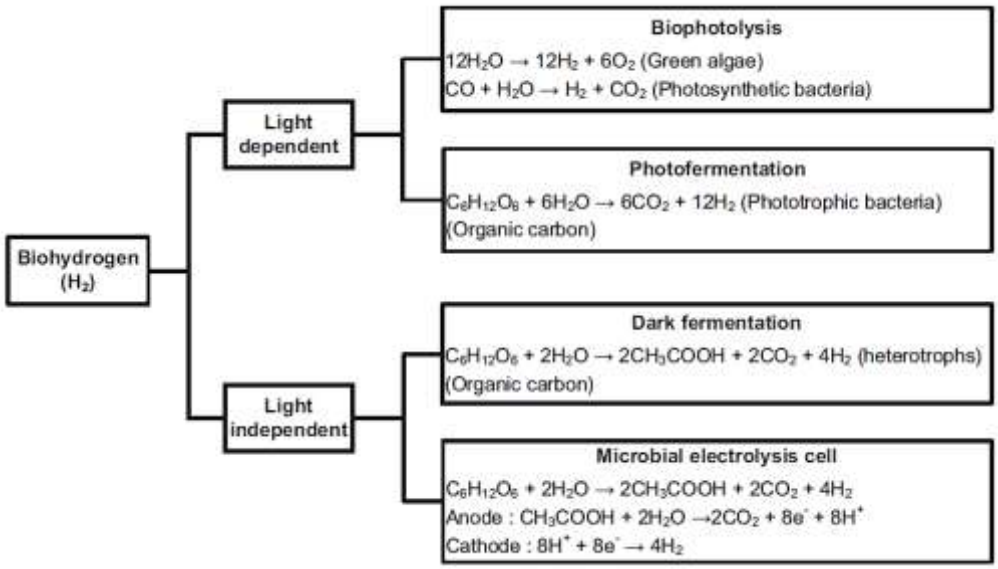
In Malaysia, there are many types of biomass generated by palm oil mill processing, such as of empty fruit bunches, palm press fiber, palm kernel cake, palm kernel shell, sludge cake Sand palm oil mill effluent (POME), and it annually generates about 15.2 million tons of waste water. Existing treatment 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 greenhouse 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.

Hydrogen can be produced from biological processes which are less energy intensive and more environmental friendly in terms of global reduction of CO_2. These

renewable biohydrogen producing technologies have potential to become cost competitive as they can use low value waste biomass as feedstock (Kotay et al., 2008), e.g. municipal, agricultural and industrial organic waste and wastewater. Biohydrogen can be produced by both autotrophic and heterotrophic microorganisms, as shown in Figure 2.1 (Das and Veziroglu, 2008).

In autotrophic conversions (also known as direct or indirect biophotolysis), solar energy is directly converted to hydrogen via photosynthetic reactions mediated by photosynthetic microorganisms, i.e. microalgae, protists and photosynthetic bacteria. Under heterotrophic conditions, the organic substrates are transformed into simpler organic compounds with simultaneous production of molecular hydrogen (Li and Fang, 2007). There are two types of heterotrophic conversions, photo-fermentation carried out by photosynthetic bacteria and dark fermentation carried out by anaerobic bacteria that convert carbohydrates into biohydrogen.

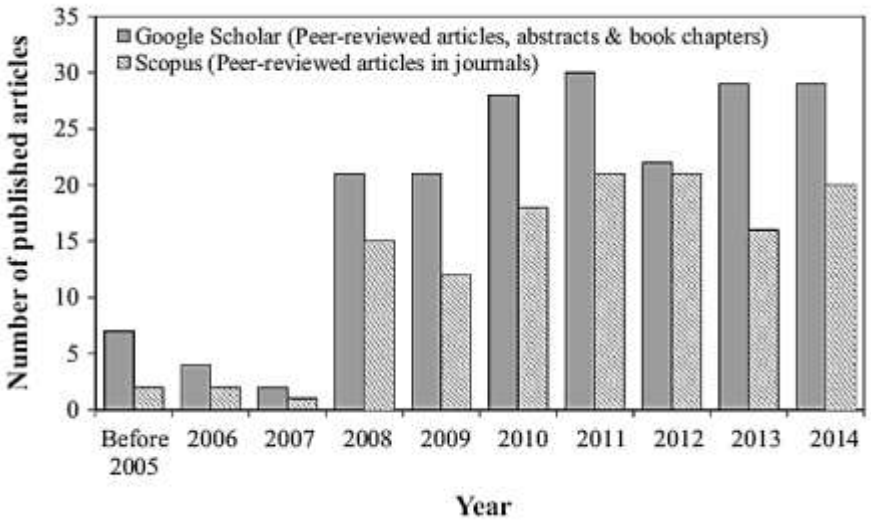
Figure 2.1: Biological Pathway to produce hydrogen.



2.3 DARK FERMENTATION

Dark fermentation is the most studied and promising technology for biohydrogen production owing to its higher production rates and treatment capacity for organic wastes. Several substrates rich in carbohydrates are also usable, such as first generation fuel crops (e.g. sugar cane, wheat, corn, and sugar beets) as well as second generation biomass like agricultural residues as well as industrial waste and wastewater (Debabrata and Veziroglu, 2008). In recent years, there are increasing research activities in this domain, as shown in Figure 2.1, by the increasing number of peer reviewed articles with ‘‘dark fermentation’’ in the title.

Figure 2.1: Number of peer reviewed publications on DF published in the last decade. (Scopus and Google Scholar, 2014)



The improvement of fermentation process for POME is important because POME is one of relatively potential as a substrate for generation of hydrogen. POME is produced with an average value of 25,000 mg/L biochemical oxygen demand (BOD) and 50,000mg/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. 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.

2.4 CONTINUOUS STIRRED TANK REACTOR (CSTR)

CSTR is equivalent to a closed-tank digester with mixer. The mechanical agitator provides more area of contact with the biomass thus improving gas production. In POME treatment, CSTR has been applied by a mill under Keck Seng (Malaysia) Berhad in Masai, Johor and it is apparently the only one which has been operating continuously since early 1980s (Tong and Jaafar, 2006). Other applications of CSTR on wastewater treatment include dilute dairy wastewater (Chen and Shyu, 1996); jam wastewater (Mohan and Sunny, 2008) and coke wastewater (Vázquez et al., 2006) where coke wastewater was treated in aerobic conditions.

The CSTR in Keck Seng's palm oil mill has COD removal efficiency of approximately 83% and CSTR treating dairy wastewater has COD removal efficiency of 60%. In terms of methane composition in generated biogas, it was found to be 62.5% for POME treatment and 22.5–76.9% for dairy wastewater treatment. Another study on POME treatment using CSTR has been investigated by Ugoji (1997) where results indicated that COD removal efficiency is between 93.6–97.7%. The difference of COD removal efficiency between the two published results by Keck Seng and Ugoji is due to the different operating conditions where the latter study was done in laboratory scale. In the plant scale POME treatment at Keck Seng's palm oil mill, the treated wastewater could not be assumed to be well mixed due to the large volume of feed which might affect the overall efficiency of the COD removal.

2.5 OPTIMUM CONDITIONS FOR PRODUCTION OF HYDROGEN

In recent years the production of hydrogen has attracted much research interest because it enables using waste materials compared to conventional electrolysis and thermo-catalytic reformation. An anaerobic system could be designed to produce hydrogen as the major product (Abbasi and Abbasi, 2011). Dark and photo-fermentation processes are the two major options for producing hydrogen through the anaerobic method (Rittmann and Herwig, 2012). The dark fermentation process involves the

production of hydrogen and VFA through the stage of acidogenesis by acidogenic bacteria such as Clostridium spp. Photo-fermentation process enables the hydrogen production from VFA with the presence of light, the predominant microbial community is photosynthetic bacteria such as Rhodobacter or Rhodopseudomonas spp. (Lee et al., 2012).

Unfortunately, the yield of hydrogen from experiments has been significantly less than the expected theoretical yield; the difference is being that some of the raw materials are converted into by-products. During acidogenesis, butyrate and ethanol are produced that are termed as fermentation barriers to limit the hydrogen production. In connection, during anaerobic digestion, only one third of the electron potential is transferred to produce hydrogen, leaving the remaining two thirds being transferred to fermentation by-products (Abdallah et al., 2016).

2.5.1 pH

For hydrogen production, the growth rate microorganisms and dynamics of fermentation largely depend on the initial pH of the reactor. A change in pH triggers a microbial shift that eventually defines the metabolic pathway of the microorganisms. A variation of the hydrogen ion concentration causes a change in pH that eventually leads to the variation of discharges detected by the redox potential. Research has shown that activities of the fermentation products largely rely on the pH and it is an important ecological factor for hydrogen producing bacteria (Ruggeri et al., 2015).

Although the optimum value of pH in a reactor varies according to the substrates' composition, research findings have indicated a favorable range that is common for all hydrogen production processes through anaerobic digestion. Results from one experiment indicated the initial increase of pH in the acidic range favored hydrogen production. This particular study concluded a pH value of 6.9 for maximum yield of hydrogen and a value of 7.2 for maximum average production rate for hydrogen (Wang and Wan, 2011).

Another experiment involved the production of hydrogen in batch reactor using an initial concentration of 6000 mg/L glucose as a substrate (Liu et al., 2011). Their findings showed a pH value equal to 4 could discourage microbial growth. In addition, they reported that at pH 7.0 the hydrogenase activity was low, which finally resulted in a low hydrogen yield (ranged from 0.12 to 0.64 mmol/mmol glucose). They concluded that pH values from 5.5 to 6.8 are the most favorable for biohydrogen production. Ruggeri et al. (2015) performed a research study aiming to produce hydrogen from noodle manufacturing wastewater. By analyzing Clostridium butyricum CGS5, the results included a pH value of 5.5 for maximum hydrogen production where a pH of 4.5 could have inhibitory effects.

Hence, controlling the pH in a lab scale experiment may not reflect the real costs when the experiment is conducted in an industry context. However, the type of waste material and bioreactor type should be defined for more precise tuning of pH value in an anaerobic process.

2.5.2 Temperature

Not many studies have compared the productivity of hydrogen when using thermophilic, mesophilic and psychrophilic processes. Results for research data show that the overall production of hydrogen did increase during thermophilic operation compared to the mesophilic strategy (Jariyaboon and Kongjan, 2015). The findings included a faster acclimatization rate of thermophilic inoculum compared to the mesophilic inoculum. Another analysis considered hydrogen production using two-stage induced bed reactors (IBR) from dairy waste processing (Zhong et al., 2015). The results indicated a value of 131.5 ml H2/g-COD removed at 60°C compared to 116.5 ml H2/g-COD removed at 40°C.

2.6 NANOPARTICLES (NPS)

Nanotechnology is recognized by the European Commission as one of its six ‘‘Key Enabling Technologies’’ that contribute to sustainable competitiveness and growth in several industrial sectors. The current challenges of sustainability, food security and climate change are engaging researchers in exploring the field of nanotechnology as new source of key improvements for the agricultural sector (Claudia Parisi, 2015). Nanotechnology is the engineering and art of manipulating matter at the nanoscale (1–100 nm); that considered as one of the most important advancements in science and technology of the last decades. It is also expected to revolutionize our ability to improve the environment. Moreover, it offers the potential of new functional materials, processes and devices with unique activity toward obstinate contaminants, enhanced mobility in environmental media. Particles in nanometric size range are termed nanoparticles (NPs). The size greatly depends on the process used for their synthesis. They can be obtained by bottom-up assembly of atoms through chemical process or, on the contrary, from top-down fragmentation of bulk material. The latter allows the synthesis of smaller particles (Yvette Meissner, 2008).

Nano-size is the cardinal property for interaction with biological systems since it determines the ability to penetrate cell membranes, thus facilitating the passage across biological barriers, interaction with immune system, uptake, absorption, distribution and metabolism (Mario Luna-delRisco, 2011). NPs have unique properties such as large surface area, high reactivity due to high surface area to volume ratio, high specificity, self-assembly and dispersibility (Nassar, 2010).

Application of nanoparticles (NP) to enhance bioactivity and metabolite recovery during dark fermentation has gained enormous attention due to unique surface and quantum size effects of NP's (Abhijit Gadhe, 2015). Hydrogenase, a key enzyme of the hydrogen production pathways, catalyzes reduction of proton to hydrogen during Dark Fermentation. Based on the metal content present at the active site, the hydrogenase enzyme is classified into [Fe-Fe] and [Ni-Fe] hydrogenase. The presence of Fe and Ni at the active site of enzyme implies that both the metals have an important effect on fermentative hydrogen production. To date, a number of studies have reported an

enhancement of biohydrogen production by addition of iron and nickel salts using pure carbon source (Karadag D, 2010).

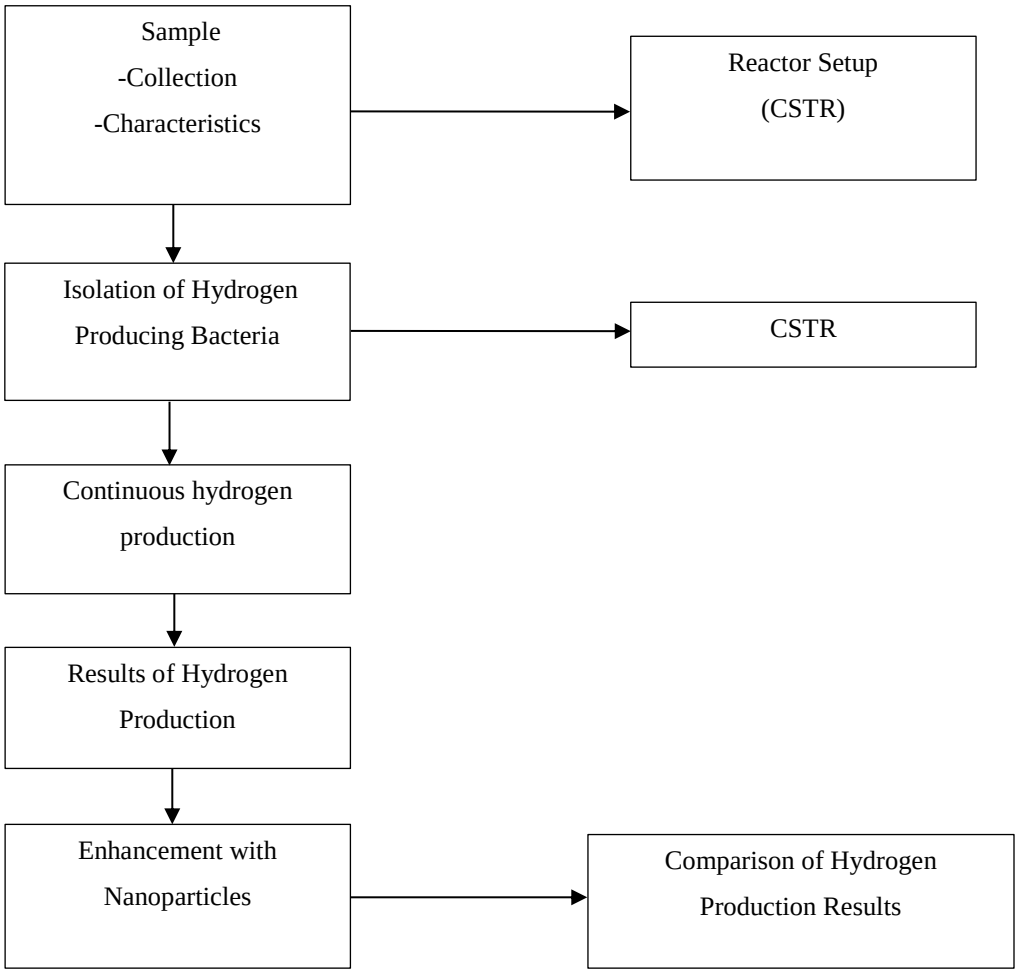
In dark fermentation, the NADPH or ferredoxin based hydrogen production process uses NADPH as an electron donor. In this pathway, hydrogen is directly generated from NADPH by NADPH dependent [Fe Fe] hydrogenase or [Ni Fe] hydrogenase or indirectly by ferredoxin dependent hydrogenase via the intermediate electron carrier ferredoxin. In principle, the nanoparticles have been reported to enhance ferredoxin oxidoreductase activity by increasing electron transfer rate owing to an enhanced surface and quantum size effects. Therefore, an enhancement of ferredoxin oxidoreductase activity in response to NPs addition has been considered to be important to increase the hydrogen production yield during dark fermentation (Mohanraj S, 2014). To date an application of iron and nickel NP's to enhance biohydrogen production from pure carbon source via glucose, sucrose, etc., have sporadically been reported.

CHAPTER 3

METHODOLOGY

There are five important elements for the process flow, which are the sample POME, isolation of bacteria, reactor operation, hydrogen gas production and nanoparticles enhancement.

Figure 3: Process flow of hydrogen production



3.1 INOCULUM COLLECTION AND PREPARATION – POME

The POME and sludge samples were collected from FELDA Palm Oil Industry, located at Lepar Hilir 3, Gambang, Pahang. The POME was filtered and then kept in the refrigerator, together with the sludge sample at temperature of 4°C. The POME was then fully characterised, as presented in Table 4.1.

3.2 HEAT TREATED SLUDGE

First of all, the sludge sample was poured and spread on an aluminium foil in a container, then put into the oven. The temperature of the oven was set to 70°C and heated for 10 minutes, then repeated the step with temperature of 85°C and also 100°C. The sludge was then dried and heat-treated in oven overnight.

The heat-treated sludge was filtered into powders in the next day, and wrapped in new aluminium foil before it was kept in the refrigerator at temperature of 4°C.

3.3 PRE-EXPERIMENTAL SETUP

3.3.1 Preparation of Trace Elements Solution

For sludge cultivation, the trace elements solution is first prepared. For the trace solution, 1 litre of distilled water was filtered using microfiber papers and added in 0.2g/L of $MgSO_4 \cdot 7H_2O$, 25mg/L of $FeSO_4 \cdot 7H_2O$, 0.2g/L of L•crystaine•HCl•H_2O, 10mg/L of NaCl, 10mg/L of $Na_2MoO_4 \cdot 2H_2O$, 10mg/L of $CaCl_2 \cdot 2H_2O$, and 15mg/L of $MnSO_4 \cdot 7H_2O$. The trace solution was then poured in a 1 litre Schott bottle and stirred with magnetic stirrer. After that, the solution was kept in the refrigerator at temperature of 4°C.

3.3.2 Sludge Activation

For sludge activation, a medium is needed. 500ml distilled water was filtered with microfiber papers and poured into a 1 litre schott bottle. By following the recommended nutrients ratio which COD:N:P ratio of 100:2:0.5, 5.0g of Glucose, 0.1g of $(NH_4)_2SO_4$ and 0.025g of KH_2PO_4 were added into the filtered distilled water. Next, 5 drops of trace elements solution were added into solution by using a micropipette and then the schott

bottle was wrapped with aluminium foil. Then, 10g of heat-treated sludge powders was added into the bottle to activate the sludge. The schott bottle was labelled as Mixture A and put aside for 48 hours.

3.3.3 Preparation for isolation of bacterial strain

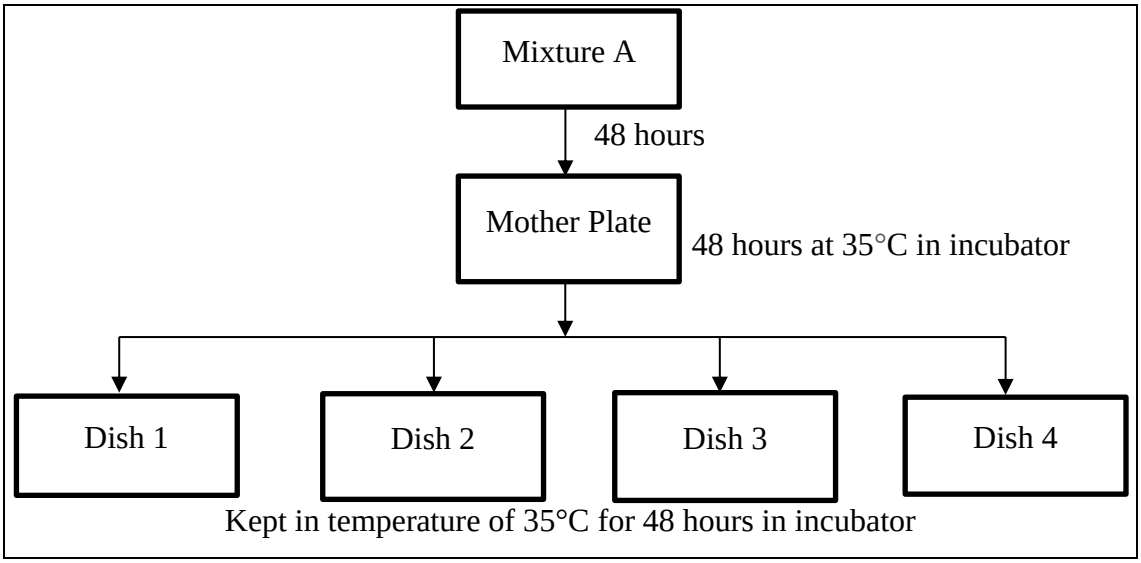
For isolation of bacterial strain, Agar Plate must be prepared. 100ml of trace elements solution, 10.0g of Glucose, 0.25g of $(NH_4)_2SO_4$ and 0.05g of KH_2PO_4 and 2.5g of Agar powder were added into a 250ml conical flask and loosen the closed cap of the conical flask. Next, the flask was put inside the autoclave machine at 121°C for 5 hours. After that, the Agar mixture was transferred to a Petri Dish before the mixture cooled down.

After the Agar Plate is cooled down and ready, a few drops of Mixture A was added onto the Agar Plate using a micropipette and spread gently using L-shape glass rode in a biological safety cabinet. Note that before that, the cabinet already cleaned using ethanol. The Petri Dish was labelled as Mother Plate and then covered and sealed with the Parafilm M (plastic paraffin film). Then, the Petri Dish was put inside an anaerobic jar. The anaerobic jar was ensured to be closed tightly before it was transferred to an incubator at temperature of 35°C for 48 hours.

3.3.4 Isolation of bacterial strain

For isolated bacteria samples, the steps of preparing Agar Plate were repeated and another 4 Agar Plates are prepared. From the Mother Plate, 4 most obvious unknown bacteria colonies were transferred onto the 4 new Petri Dishes, which are labelled as Dish 1, Dish 2, Dish 3 and Dish 4. They were also covered and sealed with Parafilm M. All of the 4 Dishes were put inside the anaerobic jar. The anaerobic jar was ensured to be closed tightly before it was kept in an incubator at temperature of 35°C for 48 hours. The schematic diagram of this step is as shown in Figure 3.1.

Figure 3.1: Schematic Diagram of Preparation for isolation of bacterial strain



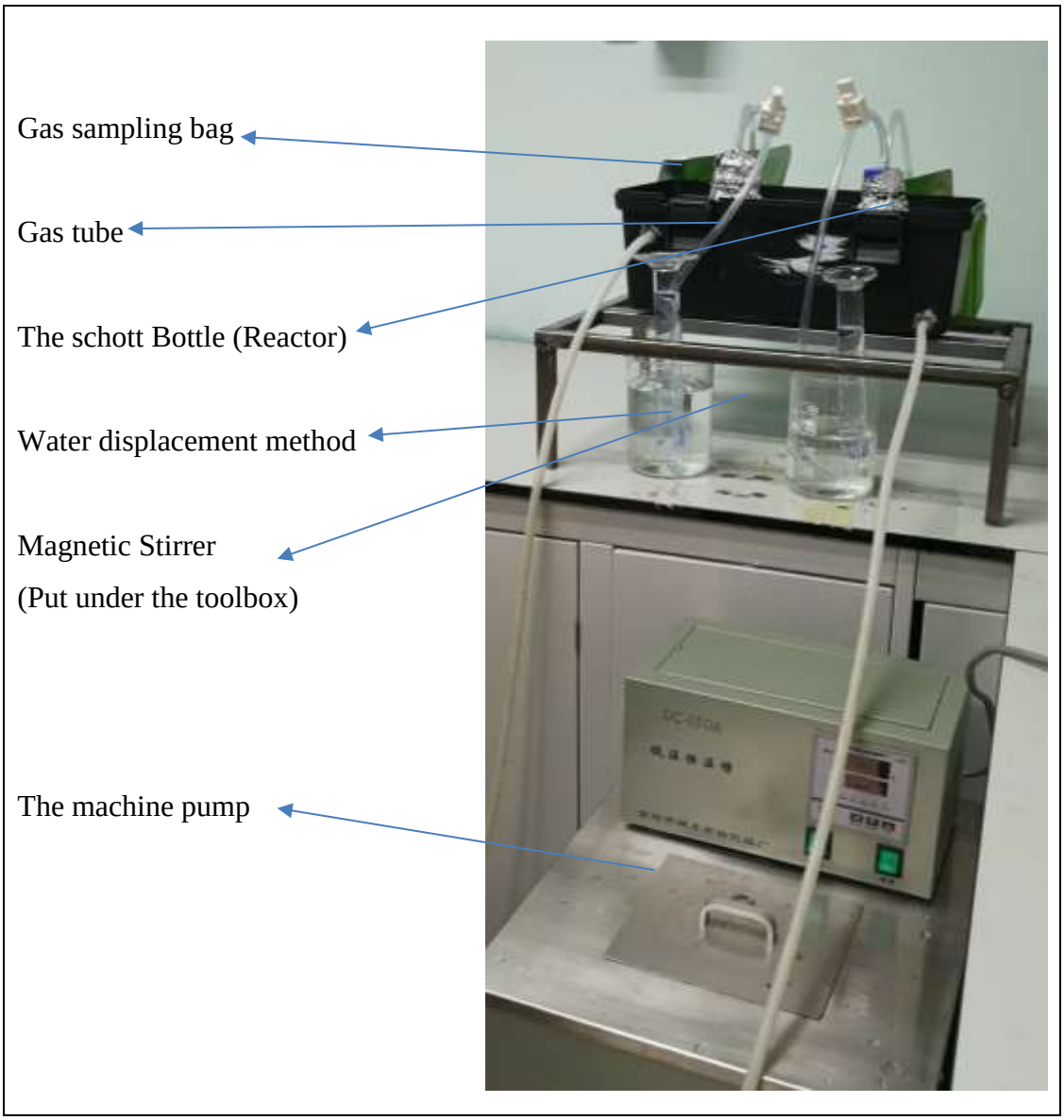
3.4 EXPERIMENTAL SET UP

3.4.1 Customized CSTR

A broth medium was prepared by mixing 400ml of trace elements solution with 40.0g of Glucose, 1.0g of $(NH_4)_2SO_4$ and 0.2g of KH_2PO_4 and stirred with magnetic stirrer. The broth medium was then put into autoclave machine at 121°C for 5 hours. After that, the COD of the broth medium was determined by Dissolved Oxygen (DO) meter before it was transferred to 4 different schott bottles, 100ml each.

After the 48 hours in incubator, 4 of the Petri Dish were took out. The bacteria on Dish 1, 2, 3 and 4 were then transferred into the broth medium, which the process was taken out inside biological safety cabinet. The bacteria were labelled as Bacteria A, B, C and D from Dish 1, 2, 3 and 4 respectively. The 4 bottles were then wrapped with aluminum foil and closed with a cap with 2 gas tubes and they were put inside the customized tool box that was connected to the water machine pump with controlled temperature. Argon gas was flowed in both bottles at the rate of $5cm^3$/min to ensure no other gases inside the bottles besides acting as carrier gas. Also, one of the gas tubes was connected to gas sampling bag and another was for water displacement method to measure the volume of gas produced. The magnetic stirrers were put under the tool box so the mixtures inside the 2 bottles were continuously stirred. A customized CSTR was completed and the batch experiment was set up as shown in Figure 3.2.

Figure 3.2: The customized CSTR set



The temperature of the water flowing was set at 25°C for 9 days. The experiment was done twice, at which 2 bottles each time, first bacteria 1 and 2, followed by bacteria 3 and 4 for the second time. The experiments were repeated under temperature of 30°C, 35°C, 40°C, 45°C and 50°C.

3.5 ANALYTICAL METHOD

The gas contents collected in the gas sampling bags were analyzed every 24 hours, and the results were recorded for further analysis. The analysis was carried out using Gas Chromatography (GC) with a thermal conductivity detector. After 9 days, the volume of hydrogen produced can be determined by water displacement method from the set up. The final COD of the broth medium were analyzed by DO meter. The initial screening results obtained were recorded in Table 4.2.1 and its graphical method was shown in Figure 4.2.

3.6 POME AS INOCULUM

3.6.1 Different pH at 37°C

From the results obtained from GC analysis, Bacteria 2 was chosen as it produced most hydrogen gas per g COD removal. This time, POME was the inoculum. Firstly, the 1200ml of fresh filtered POME was needed to put inside the autoclave machine at 121°C for 5 hours. After it was cooled down, the characteristics of the POME was analyzed and recorded for further comparison. Next, 1200ml of the POME, 120.0g of Glucose, 3.0g of $(NH_4)_2SO_4$, 0.6g of KH_2PO_4 and a few drops of trace elements solution were mixed together and stirred with magnetic stirrer. After that, POME was transferred to 6 different schott bottles, 100ml each, which were labelled as Bottle 1, 2, 3, 4, 5 and 6. The 600ml left was kept in refrigerator at 4°C.

The pH of each schott bottles was adjusted and set to be pH 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0 by adding hydrochloric acid (HCl) or sodium hydroxide (NaOH) for Bottle 1, 2, 3, 4, 5 and 6 respectively. After that, the Bacteria 2 was transferred into Bottle 1 and 2 only. The experiment was set up in the customized CSTR, as shown in Figure 3.2. The bottles were then wrapped with aluminum foil and closed with a cap with 2 gas tubes and they were put inside the customized tool box that was connected to the water machine pump under controlled temperature. Argon gas was flowed in both bottles at the rate of $5cm^3$/min to ensure no other gases inside the bottles besides acting as carrier gas. Also, one of the gas tubes was connected to gas sampling bag and another was for water

displacement method to measure the volume of gas produced. The magnetic stirrers were put under the tool box so the mixtures inside the 2 bottles were continuously stirred. The experiment also been carried out which 2 bottles at each time, as batch experiment. The controlled temperature of the flowing water was set at temperature of 37°C for 9 days.

3.6.2 Analytical Method

The gas contents collected in the gas sampling bags were analyzed every 24 hours and the results were recorded for further analysis. The analysis was carried out using Gas Chromatography (GC) with a thermal conductivity detector. After 9 days, the volume of hydrogen produced can be determined by the water displacement method from the set up. Lastly, the final COD of the POME will be analyzed by DO meter. The steps were repeated for Bottle 3 and 4, followed by Bottle 5 and 6. The 6 gas sampling bags for Bacteria 2 were then sent to Central Laboratory for analysis and the hydrogen gas yielded for Bacteria 2 was recorded in Table 4.3.1.

3.6.3 Different temperature at pH 5.5

The same experimental steps were carried out as in Methodology 3.6.1 but the pH was fixed at 5.5. This time, different temperature was set for each bottle in the water bath, which 25°C, 30°C, 35°C, 40°C, 45°C and 50°C for Bottle 1, 2, 3, 4, 5 and 6 respectively. For this batch experiment, only 1 bottle was carried out at one time. Bacteria JTY2017 will be used for this experiment.

3.6.4 Analytical Method

After 48 hours, the gas sampling bags were collected and the steps were repeated for Bottle 2, 3, 4, 5 and 6. The 6 gas sampling bags for Bacteria JTY2017 and were then sent to Central Laboratory for analysis. The analysis was carried out using Gas Chromatography (GC) with a thermal conductivity detector. The percentage of hydrogen gas obtained for both Bacteria 2 was recorded in Table 4.3.2.

3.7 APPLICATION OF NANOPARTICLES (NPS) ON HYDROGEN PRODUCTION FROM POME

3.7.1 Nanoparticles (NPs) Preparation – Hydrothermal Approach

For nanoparticles, 3.75g of glucose is dissolved in 20 mL of deionized distilled water and then, 0.75g of Iron(III) Chloride Hexahydrate, $FeCl_3 \bullet 6H_2O$ was mixed with the deionized distilled water and stirred with magnetic stirrer. The carbohydrate: metal molar ratio was 5:1. The mixture was then poured into the Teflon-lined stainless steel autoclave as shown in Figure 3.7.1. After that, the autoclave was transferred into an oven at temperature of 180°C for 24 hours.

The products were filtered off after 24 hours, then washed several times, first with distilled water and then ethanol, and finally dried in a vacuum oven at temperature of 60 °C for 5 hours. After synthesis, the metal oxide-carbon composites were undergo calcination in air at 550 °C for 4 hours to remove the carbon core, leading to hollow metal oxide particles. Lastly, the powder was collected properly on aluminum foil inside sealed Petri Dish. The above steps were repeated on second NPs sample, Magnesium Sulphate heptahydrate, (MgSO4•7H2O). Some of the particle size and morphology were visualized using a Gemini scanning electronic microscope (SEM). The X-ray Powder Diffraction (XRD) pattern were also obtained.

Figure 3.7.1: Teflon-lined stainless steel Autoclave

3.7.2 Effect of NPs on hydrogen production from POME

A total amount of 1200ml fresh filtered POME was put inside the autoclave at temperature of 121°C for 5 hours. After it was cooled down, 1000ml of the filtered POME, 120.0g of Glucose, 2.4g of $(NH_4)_2SO_4$, 0.6g of KH_2PO_4 and few drops of trace elements solution were mixed together and stirred with magnetic stirrer. The pH value of the POME was adjusted to 5.5 by adding NaOH and the POME mixture was then separated into 2 schott bottles, with labelled Bottle A1 and B at 100ml each. After that, Bacteria 2 were added into the POME followed by adding iron oxide powder of concentration of 0.5mg/l and 2.0mg/l in both Bottle A and B respectively. The experiment was set up as shown in Figure 3.2.

The bottles were then wrapped with aluminum foils and closed tightly with a cap with 2 gas tubes and they were put inside the customized tool box that was connected to the water machine pump under controlled temperature. Argon gas was flowed in both bottles at the rate of $5cm^3$/min to ensure no other gases inside the bottles besides acting as carrier gas. Also, one of the gas tubes was connected to gas sampling bag and another was for water displacement method to measure the volume of gas produced. The magnetic stirrers were put under the tool box so the mixtures inside the 2 bottles were continuously stirred. The controlled temperature was set at 35°C for optimum rate of hydrogen production. The experiment was carried out as batch experiment for 9 days. The steps were repeated for concentration of iron oxide at 4mg/l and 6mg/l, 8mg/l and 10mg/l.

The gas contents in the gas sampling bags were analyzed every 24 hours, the results were recorded for further analysis. The analysis was carried out using Gas Chromatography (GC) with a thermal conductivity detector. After 9 days, the volume of hydrogen produced can be determined by the water displacement method from the set up. Lastly, the final COD of the POME from will be analyzed by DO meter. All of the steps were repeated again but this time is for magnesium oxide NPs. The results obtained were recorded in Table 4.4.1 and in graphical method in Figure 4.4.3.

The characteristics of the final POME parameters in the bottle that yielded the highest amount of hydrogen for iron oxide NPs and magnesium oxide NPs were also recorded. The results were showed in Table 4.4.2.

CHAPTER 4

RESULT AND DISCUSSION

4.1 CHARACTERISTICS OF THE UNTREATED POME SAMPLE

The POME sample was sent to the Central Laboratory for analysis, the Table 4.1 shows the characteristics of both reference POME and studied POME sample.

Table 4.1: Characteristics of Reference POME and Studied POME sample

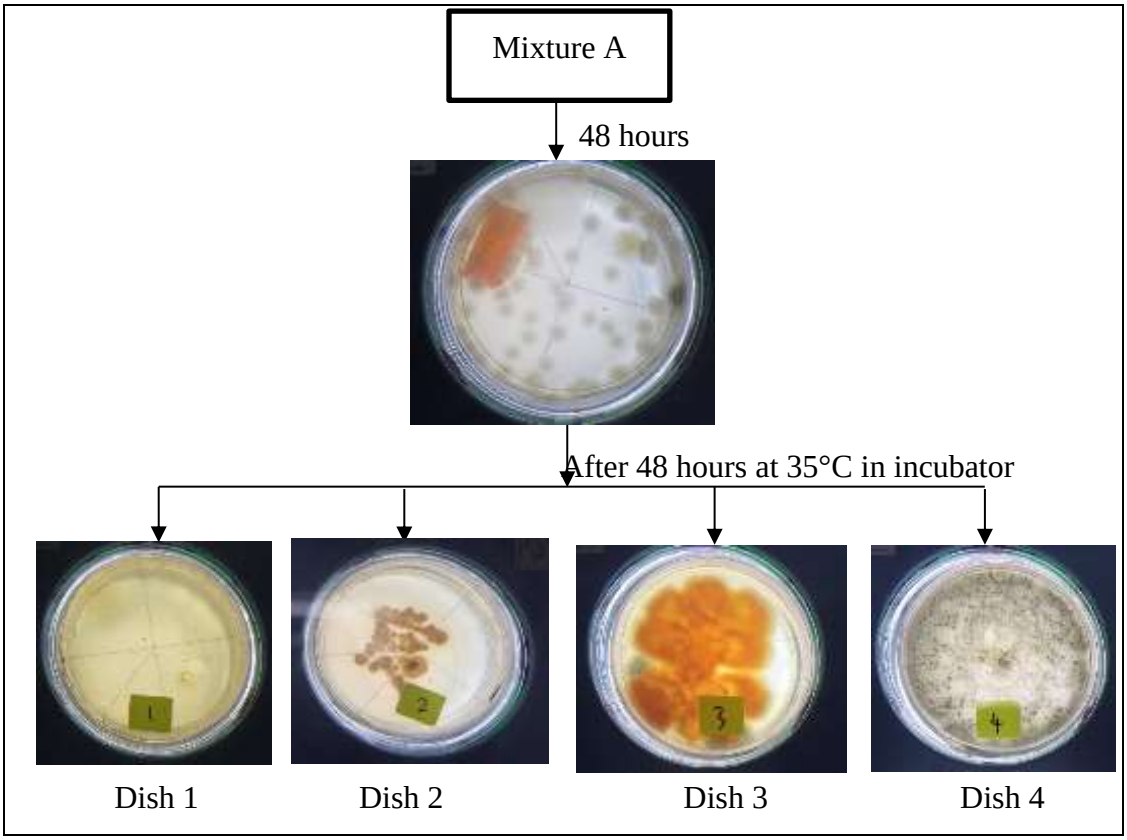
***Parameter**	**Range** (Malaysian Palm Oil Board, 2012)	**Studied Sample**
pH	3.4-5.2	4.7
BOD	10250-43750	25000
COD	15000-100000	50000
Total Solids	11500-79000	40500
Suspended Solids	5000-54000	18000
Volatile Solids	9000-72000	34000
Oil and Grease	130-18000	4000
Ammoniacal Nitrogen	4-80	35
Total Nitrogen	180-1400	750

*All parameter in mg/l except pH and temperature (˚C)

4.2 PICTURES OF BACTRIAL STRAIN AND INITIAL SCREENING

The photos of bacteria obtained in Petri Dish 1, 2, 3 and 4 were taken before proceed to the CSTR method. A schematic diagram of the process was shown in Figure 4.1.

Figure 4.1: Schematic diagram of Photos of bacterial strain and flow

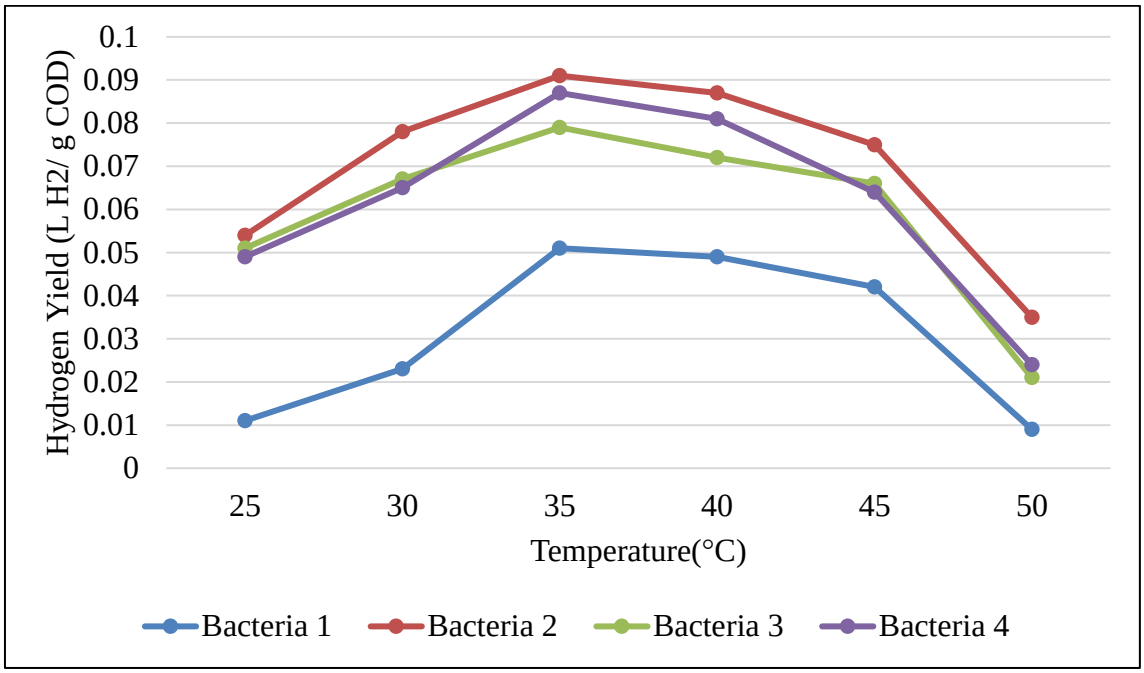


The initial screening results of the hydrogen yielded from Bacteria 1, 2, 3 and 4 were recorded in Table 4.2.1 and showed in graphical method in Figure 4.2.

Table 4.2.1: Initial Screening of Gas Obtained from Bacterial Strains

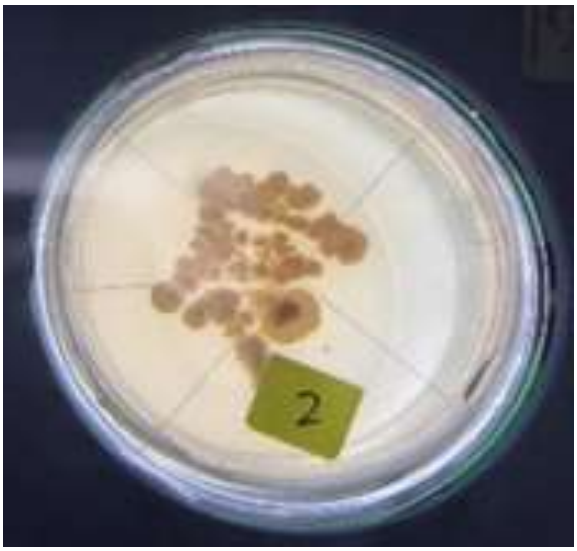
	Hydrogen Yield (L H_2/ g COD)			
Temperature(°C)	Bacteria 1	Bacteria 2	Bacteria 3	Bacteria 4
25	0.011	0.054	0.051	0.049
30	0.023	0.078	0.067	0.065
35	0.051	0.091	0.079	0.087
40	0.049	0.087	0.072	0.081
45	0.042	0.075	0.066	0.064
50	0.009	0.035	0.021	0.024

Figure 4.2: Graph of effects of temperature on hydrogen yield



Sample/Bacteria 2 was chosen for further experiment, as it had the highest production of hydrogen gas. The graph of effects of temperature on hydrogen yield was shown in Figure 4.2. The image of Sample/Bacteria 2 was shown in Figure 4.2.1 and the culture characteristics of Bacteria 2 in Table 4.2.2 below.

Figure 4.2.1: Sample/Bacteria 2



The Bacteria 2 was named as JTY2017.

Table 4.2.2: Culture Characteristics of Bacterial Strain JTY2017

Culture Characteristics of Bacterial Strain JTY2017	
Configuration	Filamentous
Margin	Wavy
Elevation	Raised
Surface	Smooth
Pigment	Brownish
Opacity	Opaque
Gram's reaction	-ve
Cell shape	Cocci

4.3 POME AS INOCULUM

4.3.1 Analysis of Hydrogen gas collected

The gas sampling bags were sent to GC for analysis. The hydrogen yield litre/g COD removal in different pH (temperature was fixed) and temperature (pH was fixed) were recorded in Table 4.3.1 and Table 4.3.2 respectively.

Table 4.3.1: Hydrogen yielded at different pH

Sample/Bacteria	Temperature (°C)	pH	Hydrogen Yield (L H_2/ g COD)
JTY2017	37	4.5	0.059
	37	5.0	0.089
	37	5.5	0.11
	37	6.0	0.093
	37	6.5	0.086
	37	7.0	0.047

The results obtained were then plotted in graphical form, as shown in Figure 4.3.1 for JTY2017 of different pH condition at 37°C. From the table, the optimum pH to produce highest hydrogen yield at 37°C was at pH 5.5, which 0.11 L H_2/ g COD was determined.

Figure 4.3.1: Graph of percentage of H_2 versus pH at 37°C of JTY2017

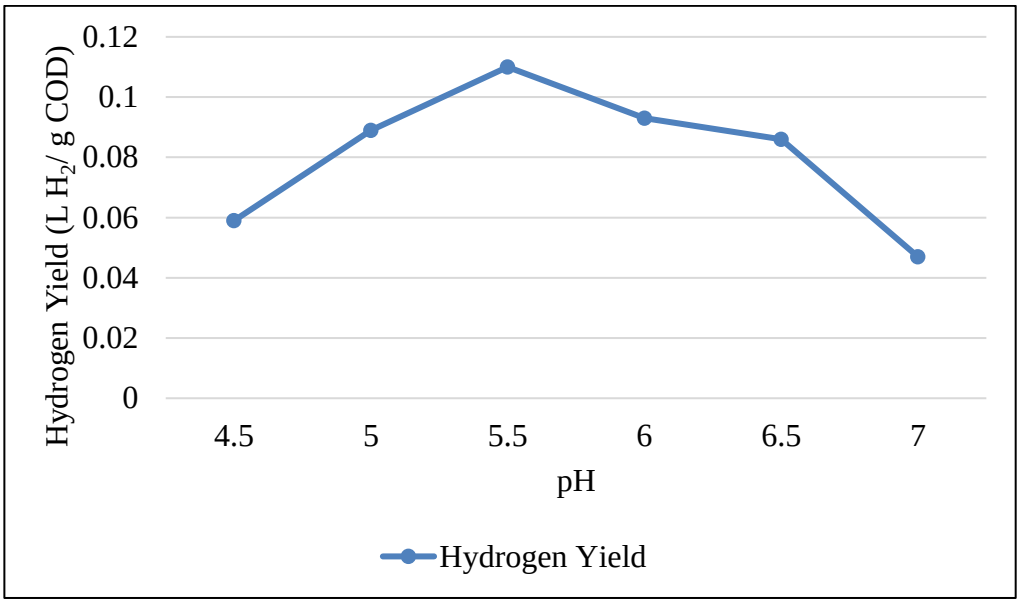
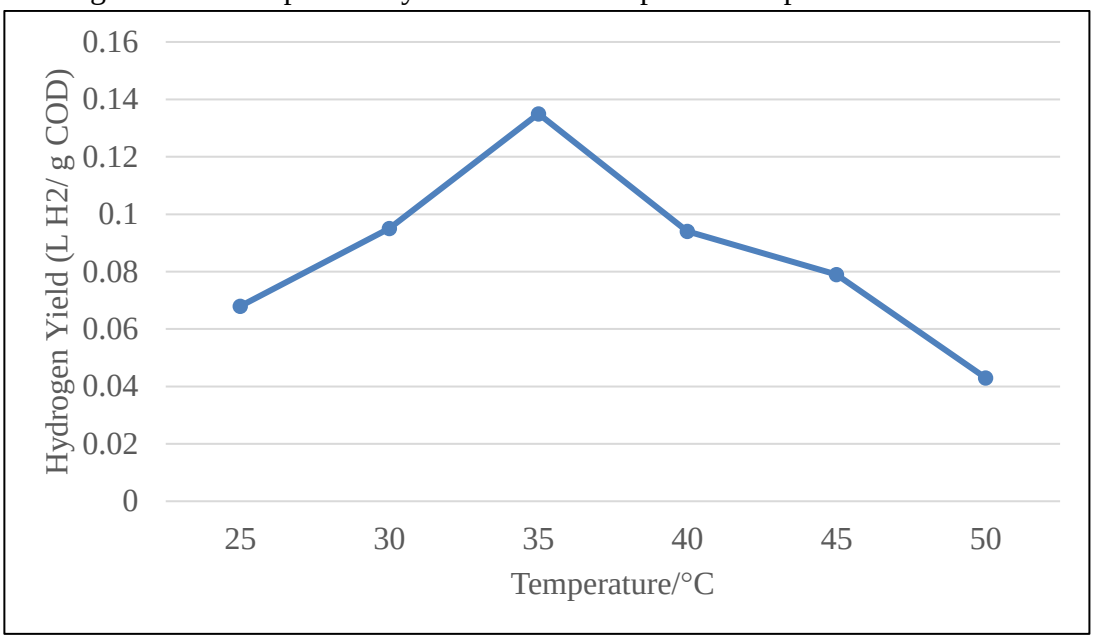


Table 4.3.2: Hydrogen yielded at different temperature.

Sample/Bacteria	pH	Temperature/°C	Hydrogen Yield (L H_2/ g COD)
JTY2017	5.5	25	0.068
	5.5	30	0.095
	5.5	35	0.135
	5.5	40	0.094
	5.5	45	0.079
	5.5	50	0.043

The results obtained were then plotted in graphical form, as shown in Figure 4.3.2 for JTY2017 of different temperature condition at pH 5.5. From the table, the optimum temperature to yield the highest hydrogen at pH 5.5 was 35°C, which 0.135 L H_2/ g COD was determined.

Figure 4.3.2: Graph of H_2 yielded versus temperature at pH 5.5 of JTY2017



4.3.2 Optimum Conditions for Production of Hydrogen

From the analysis and results obtained, we found the optimum conditions of temperature and pH at which the highest yielded hydrogen gas was determined. The optimum pH to produce highest hydrogen yield at 37°C was at pH 5.5, which 0.11 L H2/ g COD was determined. On the other hand, the optimum temperature to yield the highest hydrogen at pH 5.5 was 35°C, which 0.135 L H_2/ g COD was determined.

Hence, the optimum condition is at temperature of 35°C and pH 5.5 for Bacteria JTY2017.

4.4 ACCELERATED HYDROGEN PRODUCTION USING NANOPARTICLES

However, the hydrogen yield and specific hydrogen production rate during biological hydrogen production from POME are low, due to the poor biodegradability, bioactivity and substrate conversion efficiency of hydrogen producing microbes, and therefore, a technology needs further development (Gadhe et al., 2013). Although, complexity and poor biodegradability are one of the problems in dark fermentation of POME, the presence of high content of biodegradable organic compound can make POME an ideal candidate for biological hydrogen production via amicrobial process.

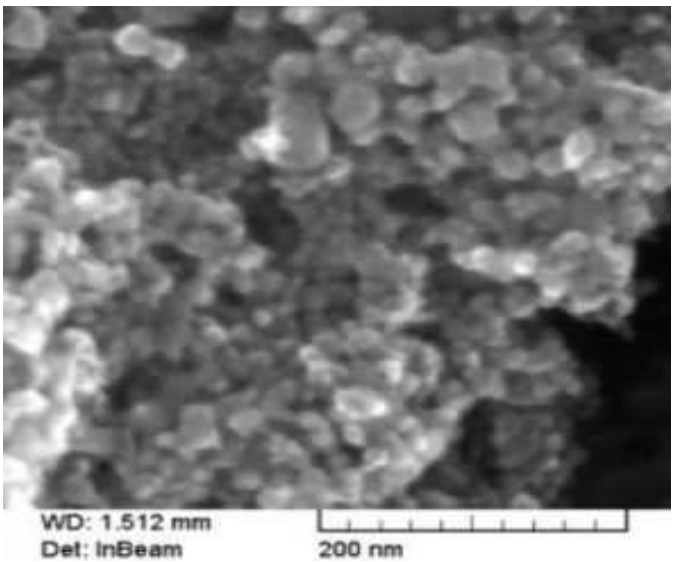
Therefore, an application of nanoparticles (NPs) to enhance bioactivity and metabolite recovery during dark fermentation has gained enormous attention due to unique surface and quantum size effects of NPs (Han et al., n.d.).

In this project, we will be using nanoparticles of iron (Fe) and magnesium (Mg) as our enhancement samples.

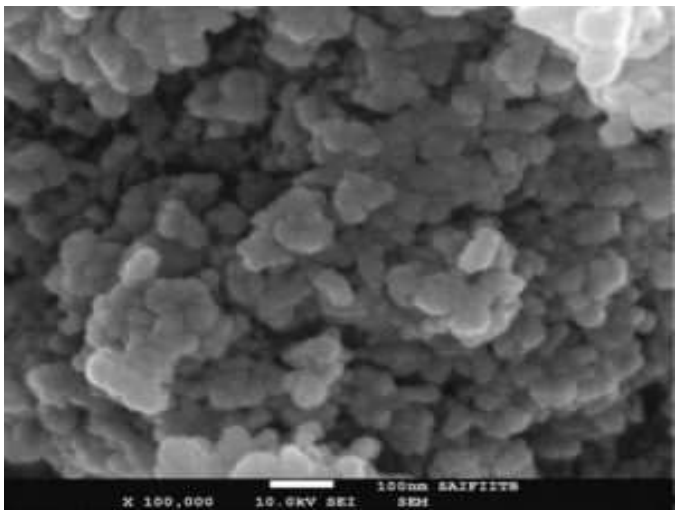
4.4.1 SEM image of NPs of iron oxide and magnesium oxide

Figure 4.4.1: SEM image of NPs of (i) iron oxide and (ii) magnesium oxide

(i)



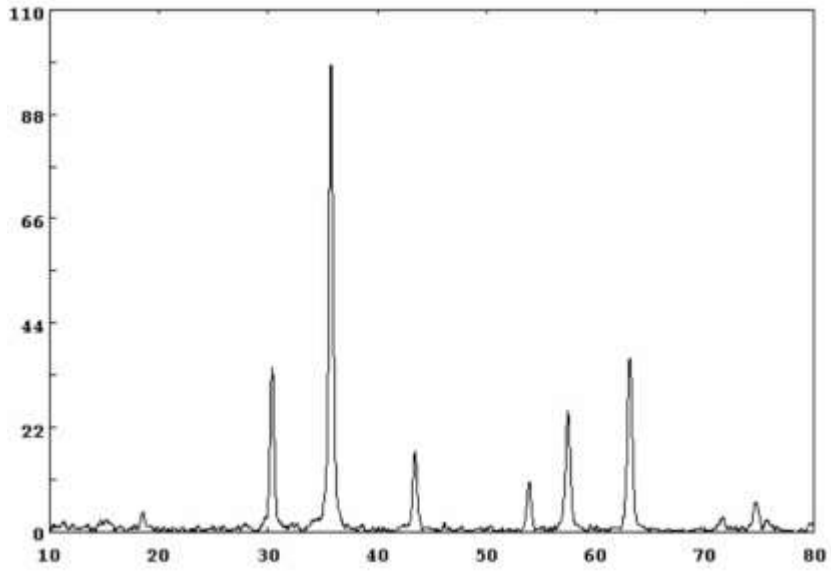
(ii)



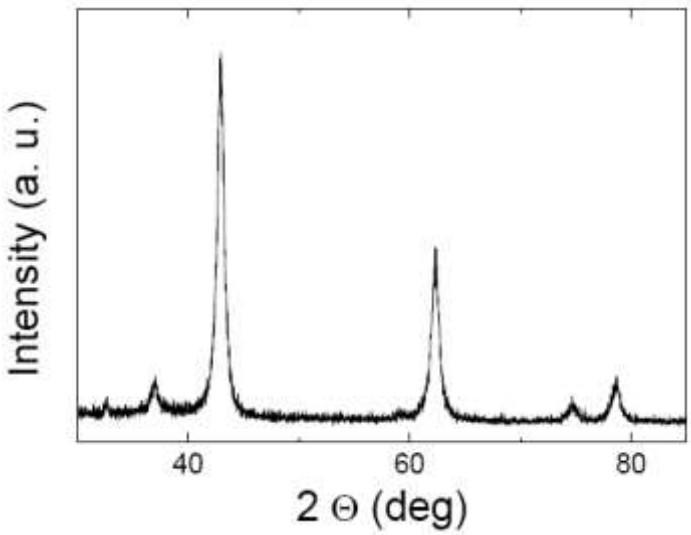
4.4.2 XRD of NPs of iron oxide and magnesium oxide

Figure 4.4.2: XRD Pattern of (i) iron oxide NPs and (ii) magnesium oxide NPs

(i)



(ii)



4.4.3 Percentage of Hydrogen Produced after Application of NPs

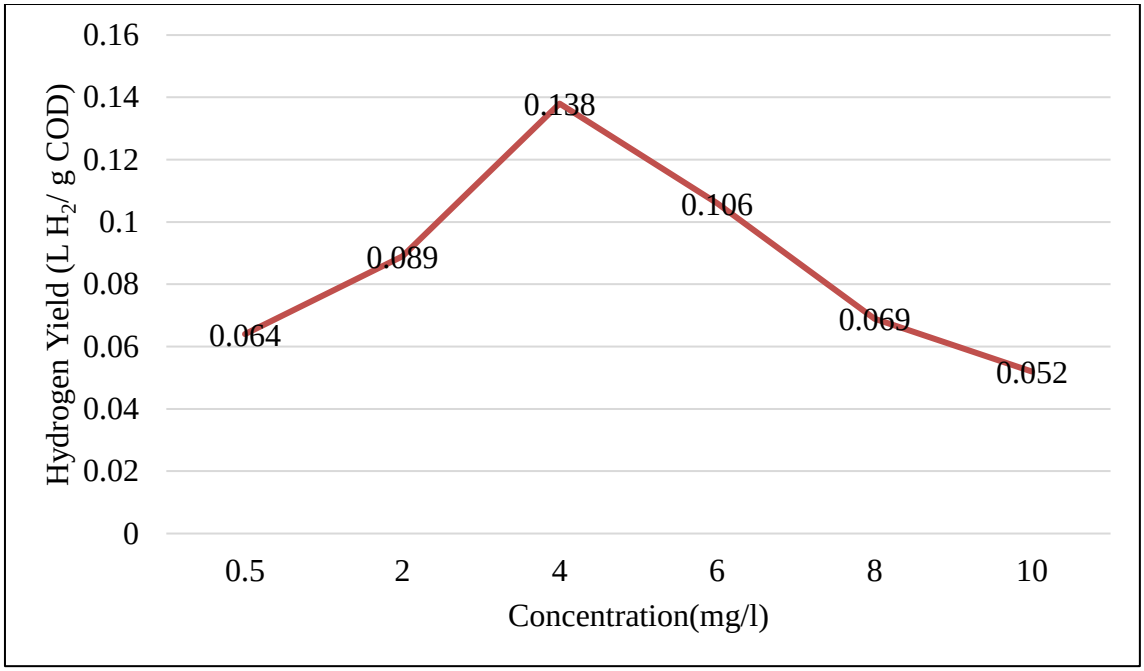
Table 4.4.1: Hydrogen Production in application of NPs at different concentration

Bacteria JTY2017	Hydrogen Yield (L H_2/ g COD)	
Concentration(mg/l)	Iron oxide	Magnesium oxide
0.5	0.064	0.07
2	0.089	0.094
4	0.138	0.145
6	0.106	0.12
8	0.069	0.078
10	0.052	0.059

4.4.4 Graph of Percentage of Hydrogen Produced with effect of NPs

Figure 4.4.3: Graph of Hydrogen Production in application of (i) iron oxide NPs and (ii) magnesium oxide NPs at different concentration

(i)



(ii)

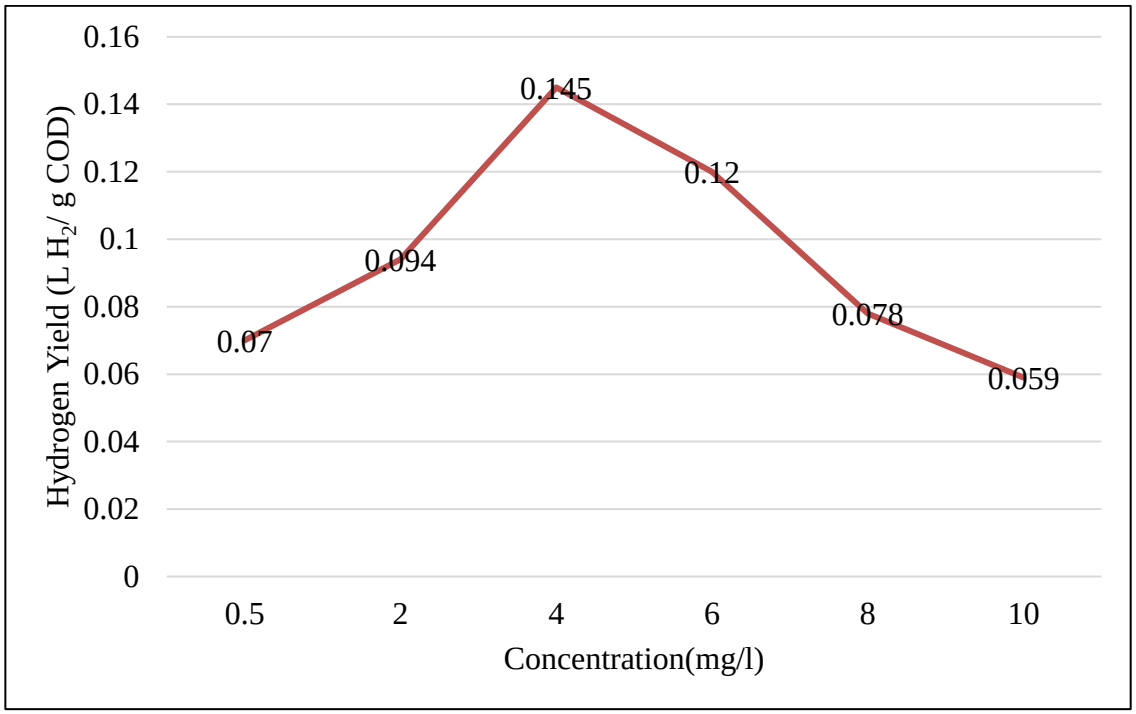
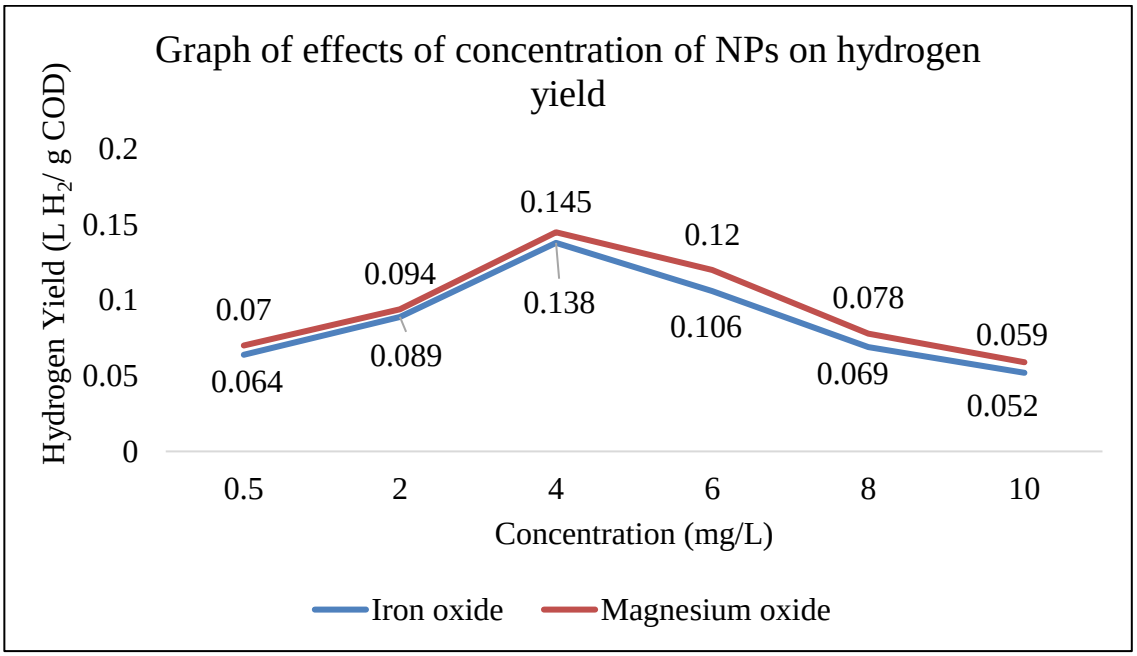


Figure 4.4.4: Comparison between the effects of 2 NPs on Hydrogen yielded



4.4.5 Characteristics of POME for Bacteria 2 after application of NPs

Table 4.4.2: The parameters on initial and final POME for JTY2017

	JTY2017					
NPs	**Iron oxide**			**Magnesium oxide**		
***Parameter**	**Initial**	**Final**	**Change (%)**	**Initial**	**Final**	**Change (%)**
pH	4.7	4.7	0	4.7	4.7	0
COD	50000	19500	61	50000	18500	63

*All parameter in mg/l except pH and temperature (˚C)

From the results shown in the table, MgO and FeO NPs were able to accelerate the hydrogen yield. Highest hydrogen yield was observed at concentration of 4 mg/L, which yield 0.145 L H_2/ g COD and 0.138 L H_2/ g COD. At the same time, the COD removals for MgO and FeO NPs were recorded at 63% and 61% respectively.

4.5 PROJECT MANAGEMENT

4.5.1 Budget and Cost Analyses

Table 4.5.1: List of Cost Chemicals

	Chemicals	**Quantities**	**Cost (RM)**	**Total Cost (RM)**
1.	$FeCl_3 \cdot 6H_2O$	1	15	15
2.	$FeSO_4 \bullet 7H_2O$	1	6	6
3.	$MgSO_4 \bullet 7H_2O$	1	1040	1040
4.	$(NH_4)_2SO_4$	1	5	5
		TOTAL	**(including GST)**	1066.00

Table 4.5.2: List of Cost Materials for Project Set Up

No.	**Materials**	**Quantities**	**Cost (RM)**	**Total Cost (RM)**
1.	Tool Box	1	17.50	17.50
2.	Hot Glue Gun	1	8.50	8.50
3.	Glue Stick 8pcs	1	2.70	2.70
4.	PE Tubing 5m	1	4.00	4.00
5.	OXOID Anaerobic Jar	1	1300.00	1300.00
		TOTAL	**(including GST)**	1332.70

4.5.2 Workflow

Table 4.5.3: Project Workflow

ACTIVITY	Week													
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Short briefing from Dr. Che Ku														
Discussion on the project about the reactor and hydrogen production process														
Describe all the methodology i. Materials ii. Chemicals needed iii. Apparatus needed														
Preparation the application form of workshop, training chemical hazardous and risk assessment														
Start the experiment and follow the planned methodology														
Send the bacteria, gases and NPs for further analysis in Central Lab.														
Poster preparation														
Thesis write-up														
Submission of first draft thesis report, extended abstract and logbook														
Mock presentation of project														
Improvisation of the final individual thesis report														
submission of Presentation Approval Form and poster														
Submission of the final individual thesis report, extended abstract, peer evaluation form and general handling form														
Presentation of the project														

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

POME is always regarded as a highly polluting wastewater generated from palm oil mills. However, reutilization of POME to generate renewable energies in commercial scale has a great potential especially when coupled with wastewater treatment process. POME can be treated anaerobically to breakdown organic matters while releasing biomethane and sometimes, biohydrogen gases.

In this project, we will only take biohydrogen production into account. Firstly, we successfully isolated the hydrogen producing bacteria, named Bacteria 1, 2, 3 and 4. We only selected Bacteria 2 as it yielded the highest biohydrogen gas. After that, we used Bacteria 2 to determine the optimum conditions to yield the best hydrogen in biogas. At the same time, we also named the Bacteria 2 as JTY2017. When at pH 5.5 and 37˚C, it yielded 0.11 L H_2/ g COD and when at 35˚C and pH 5.5, it yielded 0.135 L H_2/ g COD Therefore, the results showed the optimum condition was 35˚C and pH 5.5.

However, the hydrogen yield and specific hydrogen production rate during biological hydrogen production from POME are low. The results obtained with application of nanoparticles (NPs) in the project experiments indicated that NPs can accelerate and increase the biohydrogen production yield in batch experiment for 9 days due to unique surface and quantum size effects of NPs. When the concentration of iron oxide NPs set at 4.0mg/l, the biohydrogen yielded was the highest, at 0.138 L H_2/ g COD. On the other hand, when the concentration of magnesium oxide NPs set at 4.0mg/l, the biohydrogen produced was the highest, at 0.145 L H_2/ g COD. After that, the POME was sent to analyze and it showed that COD removal rate was increased too, compared to the non-NPs application. For Bacteria JTY2017 at 35˚C and pH 5.5, the POME's COD

removal with addition of iron oxide NPs and magnesium oxide NPs was 61% and 63% respectively.

Therefore, it is an undeniable fact that POME has the potential to produce various renewable energies. Such win–win strategies are in-line with the current zero waste sustainability concept and can make substantial contribution towards better environmental protection.

5.2 Recommendation

It is recommended that the project can be further proceeding for analysis or enhancement by using two-stage UASB hydrogen and CSTR methane reactor system. From there, we can produce two biogases as the experimental output, which are methane and hydrogen gases and at the same time increase the efficiency of the COD removal in POME while producing more useful energy. However, this two-stage reactor might take longer time and set up cost.

It is recommended that the project can be further proceeding for analysis or enhancement by using different nanoparticles such as nickel oxide NPs and cobalt oxide NPs. Nanoparticles have the ability to accelerate and enhance the useful gas production yield and increase COD removal rate as well.

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