THE PRODUCTION OF HYDROGEN FROM THE RESIDUE OF PALM OIL

*Maryam Ghanbarian1 and Mohd Firdaus Abdul Wahab2
1Department of Microbiology, Falavarjan Branch, Islamic Azad University, Falavarjan, Iran
2University Technology Malaysia (UTM)
*Author for Correspondence

ABSTRACT
Currently, fossil fuels are the basis of the global energy requirements which lead to the foreseeable depletion of limited fossil energy resources. Because of the production of pollutants like NOx, SOx, COx, C,Hx, ash, soot, and the droplets of tars, the use of fossil fuels causes the change of global climate. Furthermore, based on the growth of urbanization and industrialization, environmental pollution is a very important issue to be tackled. Therefore, the search for clean energy alternatives to satisfy growing energy demand is crucial. The main purpose of doing the present study is to perform isolation of bacteria from waste of palm oil, to conduct bacterial characterization (microbiology and biochemical tests), and to perform hydrogen production assay. For this purpose, 2 samples (raw and sludge) were collected to isolate the bacteria of interest including facultative anaerobic and strict anaerobic bacteria. The isolated bacteria were characterized by microbial and biochemical tests in order to identify them and determine the characteristics to use them for hydrogen production. This study proves that the isolated bacteria from raw and sludge samples of POME grown under anaerobic condition are able to produce hydrogen, and the bacteria isolated of strict anaerobic from sludge samples (Sludge1) produced highest amount of hydrogen gas from starch fermentation.

Keywords: Hydrogen, Isolated Bacteria, Hydrogen Production,

INTRODUCTION
Nowadays, through the rapid growth of world energy consumption, the focus of carbon and sustainable neutral energy sources has attracted more attention for future needs. One of the most important alternatives in existing petroleum-based fuels is biofuels that can be used as transportation fuels. It is capable to advance sustainability and decrease the greenhouse gas emissions by little change to current technologies. Organic materials such as oilseeds, starch, cellulose and animal fats can be used as biofuel sources and are divided into gaseous or liquid biofuels (Carere et al., 2008). Over the past few decades, the hydrogen gas and its usage in electricity generation and transportation has attracted more attention because it possesses high-energy yields (122 kJ.g⁻¹), it is a renewable energy source, and does not contribute to the greenhouse effect. Moreover, it easy to achieved, it means that it can be produced by various methods, through fermentation of biomass using microorganism, coal gasification, reforming of hydrocarbons, photochemical process, electrolysis, and biological routes. In order to generating the hydrogen, a wide variety of methods are presented in biological systems that comprise photo fermentations, direct bio photolysis, indirect bio photolysis, and dark fermentations. Among them the light fermentation has been considered by many investigators because of its economic viability and high profit (Agrawal et al., 2012).

Acidogenic waste treatment process produces biohydrogen in nature where acid forming bacteria yields hydrogen, organic acid compound and carbon dioxide (Angenent et al., 2004). Dark fermentation or light driven photosynthesis can produce biohydrogen (Tao et al., 2007) and compared to photosynthetic routes, it is achieved by dark fermentation of organic waste materials (Levin et al., 2004). Dark fermentation has many advantages such as no light energy required, high rate of cell growth, no oxygen limitation problems and it is able to work on low capital cost (Hallenbeck, and Benemann, 2002). Nowadays, many researches focus on the possibility of hydrogen production from different industries by applying wastewater treatment strategy with the organic wastes (Yu et al., 2008). For instance, 15.2...
millions of tons of wastewater is generated annually by the palm oil industry in Malaysia, which is known as Palm Oil Mill Effluent (POME) with high lignocellulose and cellulose material. To degrade the organic substances, it is very time consuming. The previous studies have reported on utilizing the POME sludge as an inoculum, and have reported a promising level of hydrogen production (Atif et al., 2005). Palm oil is actually one of the most multipurpose crops in the tropical countries such as Indonesia and Malaysia. For processing 1 ton of fresh fruit bunches (FFB), approximately 1.5 m³ water are used, and about half of this would be considered as POME. Because of its high chemical and biological oxygen demands, it is a great threat to nature. The incomplete and raw treated POME contains high content of degradable organic materials. Therefore, due to oxygen depletion, this causes serious pollution of waterways. At present, in Malaysia 265 active palm oil mills exist with annual Crude Palm Oil (CPO) production capacity of 13 million tones. To treat POME, several techniques have been proposed, such as flotation, crop irrigation, ultra filtration adsorption, and various biodegradation processes (Rasdi, 2009).

Palm oil industry generates residues or wastes in two forms. The first one is liquid waste, mainly Palm Oil Mill Effluent (POME), which is highly polluting at an average of 50,000 mg/L chemical oxygen demand (COD) and 25,000 mg/L biochemical oxygen demand (BOD). The second waste comprises of Empty Fruit Bunch (EFB), trunks, shell, and fronds in the solid form. The cheapest technologies, open digester pond and tanks or lagoon systems are used to treat the POME. POME should be treated first before it is disposed to appropriate places based on the rules of wastewater disposal amendment of the Department of Environment (DOE) Malaysia. In general, the operations of these systems require a wide space that uncontrollably releases GHG particularly CH₄ and CO₂ to the atmosphere (Zakaria, 2007).

Statement of Problem

Currently, fossil fuels are the basis of the global energy requirements which lead to the foreseeable depletion of limited fossil energy resources. Because of the production of pollutants like NOₓ, SOₓ, CO₂, C₃H₆, ash, soot, and the droplets of tars, the use of fossil fuels causes the change of global climate. Furthermore, based on the growth of urbanization and industrialization, environmental pollution is a very important issue to be tackled. Therefore, the search for clean energy alternatives to satisfy growing energy demand is crucial (Das, and Veziròglu, 2001).

Environment become increasingly unhealthy and polluted for living organisms since the global industrial revolution that include deforestation, release the pollutants into lands, rivers and air. Greenhouse gases (GHG) contains some dangerous combinations such as nitrous oxide, carbon dioxide (CO₂), chlorofluorocarbon (CFC), carbon monoxide (CO) and methane (CH₄) that trap a majority of the thermal radiation emitted from the earth’s surface and have strong electromagnetic absorption capacity. These cause negative effects to the world such as global warming, depletion of ozone layer and significant raise of ocean level (Zakaria, 2007).

On the other hand, fossil fuels hydrogen that are from fuel cells or burned directly is a clean energy with zero carbon emissions. One of the main methods of producing hydrogen is the steam reforming of methane that leads to the release of large amounts of greenhouse gases. In addition, in spite of the wide adoption of hydrogen and accounts for around 2% of world consumption of energy as a fuel, it is still limited by several challenges (Das, and Veziròglu, 2001; Lens, 2005).

These challenges can be overcome by the production of hydrogen from plant or waste material in a biological process (Weiland,2003; Angelidaki and Ellegaard, 2003). Therefore, many studies have been done on the investigation of new sustainable energy sources to substitute fossil fuels. In conclusion, hydrogen is a viable alternative fuel and “energy carrier” of future due to the cleanliness with no CO₂ emissions and its ease of use in electricity generation (Kapdan and Kargi, 2006). The procedure of the hydrogen production and its application has been shown in Figure 1.1.

Significance and Objective of the Study

Hydrogen is one of the most powerful and clean and energy carriers that can be converted to electricity by using a fuel cell, which in the developed countries is modified as a main energy carrier (Nakada et al., 1999).
This study aims to generate hydrogen as a final product by evaluating the possibilities of the bacteria as a biofuel producer. Additionally, biogas can be made in a huge amount by identifying the bacteria and culture them to use in industrial units. Therefore, the main purpose of doing the present study is to perform isolation of bacteria from waste of palm oil, to conduct bacterial characterization (microbiology and biochemical tests), and to perform hydrogen production assay.

**Literature Review**

**Biohydrogen Production:** A study has been conducted on the explorations of the volumetric biohydrogen creation capacity of a consortium comprising of two types of mesospheric facultative anaerobic bacteria in an anaerobic fluidized bed bioreactor which is involves carrier induced granular particles comprising of *Citrobacter freundii* and *Enterobacter cloacae* (Thompson et al., 2008). On the other hand, according to Ismail et al. (2011), the producing of average hydrogen rate per unit volume of POME was 2.1 mL/L/day at hydraulic retention time (HRT) 2 days. Hydrogen is established from the 43% of the total gas and methane was not discovered during the 150 days of constant operation. Throughout HRT 4 days, the soluble carbohydrate degradation efficiency was highest at 81.2%. In the other words, by following the process of acetic >butyric >ethanol >propionic acid, the soluble metabolites was produced. The Denaturing Gradient Gel Electrophoresis (DGGE) determine the microbial diversity of the immobilized consortia which is changed by growing dominant species phylogenetically related to *Clostridaceae* at different HRTs (Ismail and Soon, 2011).

In order to process inside the biodigester, any biodegradable organic material can be applied as inputs. On the other hand, some materials are also more desired as inputs than others for technical and economic reasons and many attentions is considered to the treatment of hydrogen (H₂) as eco-friendly fuel and alternative all over the world these days. Currently, fossil fuels, water and biomass mainly produces the H₂. The reactions of light oil fractions or natural gas with steam at high temperatures produce approximate 90% of H₂. Energy source are consumed as fossil fuels by these methods which are considered to be energy intensive, although they are not always environmental friendly. The current use of H₂ is equivalent to 3% of the energy consumption and increasing rapidly in the coming year. Bioaugmentation, it means the dominant bacterium which is selected from the nature is added to the...
Research Article

wastewater treatment system directly to reach the target and to develop the capacity of the mixed treatment system to improve hydrogen production yield (Atif et al., 2005).

The accessibility of capable strains is very important to save cost of H₂ production process. Therefore, the H₂ producers’ isolation is related to the H₂ production capability of each strain that need laborious experiments for measuring their H₂ production activity and improving the many individual cells under well-controlled conditions. The production of microbial H₂ can be non-photosynthetic or photosynthetic, or photosynthetic bacteria (Miyake and Kawamura, 1987).

Far away from the availability of the light, in general, in compare to the photosynthetic process, the fermentative H₂ process has a faster production rate. On the other hand, the productions that use photosynthetic process is higher than the H₂ conversion yield (mol H₂/mol substrate). According to Kumar and Das (2001) Enterobacter cloacae IIT-BT 08 has a high H₂ conversion yield of 2.2 mol H₂/mol glucose and a high specific H₂ production rate of 29.6 mmol H₂ (g cell)⁻¹ h⁻¹ [13]. Moreover, Taguchi et al. (1992) stated the H₂ production yields of Clostridium beijerinckii AM21B isolated from termites ranged from 1.3 to 2.0 mol H₂/mol glucose (Taguchi et al., 1992).

According to the study done by Oh et al (2002), Rhodopseudomonas palustris P4 is a new chemoheterotrophic bacterium that could make H₂ from CO and water was isolated from a sludge digester and could be as a facultative anaerobe with a CO-dependent H₂ production activity (20.7 mmol/g cell h) and a high-specific growth rate (0.347 h⁻¹). The majority of R. palustris P4 are capable to perform fermentative H₂ production because it belongs to non-sulfur purple bacteria and knowing the potential of R. palustris P4 as a fermentative H₂ producer might be interesting. Therefore, the R. palustris P4 growth and H₂ production from various sugars in batch culture was investigated in this study. Moreover, the essential parameters such as temperature, pH, H₂=CO₂ pressure, concentrations of phosphate and glucose, and the kind of sugars was studied. In addition, H₂ conversion yield and carbon mass balance were evaluated by glucose as a model substrate. Lastly, in order to estimate its potential, R. palustris P4 was compared to other fermentative H₂ producers (Oh et al., 2005).

Yokoyama et al. (2007) conducted a study on the community structures of anaerobic microflora hydrogen production were evaluated under extreme thermophilic conditions based on the two culture-independent methods such as clone library analyses and denaturing gradient gel electrophoresis (DGGE) and their results were verified and reliable. On the other hand, the four hexose based substrates such as lactose, glucose, soluble and cellobiose starch enrich the community structures of ETM are based on the hydrogen-producing extreme thermophile, Caldoanaerobacter subterraneous, with diversity at subspecies levels. The xylose enrich the ETM was more varied than those enriched with the other substrates, and comprised of the bacterium based on an unclassified bacterium, the C. subterraneus and distantly related to a hydrogen-producing extreme thermophile, xylan-degrading and Caloramator fervidus (Yokoyama et al., 2007).

Palm Oil Biohydrogen

Badiei et al. (2011) conducted study to explore the microbial community of an Anaerobic Sequencing Batch Reactor (ASBR) function at mesophilic temperature under changing Hydraulic Retention Times (HRTs) for estimating the optimal production of hydrogen by applying Palm oil Mill Effluent (POME) as substrate. Heat treatment enriched the POME sludge with hydrogen-producing bacteria that was applied as acclimated and inoculum with the POME. At each operating HRT determine the microbial community to first isolating cultivable bacteria and after that implementing Polymerase Chain Reaction (PCR). The products of PCR were in order and the order identification was done by applying the Genbank database and BLAST algorithm. The results showed that about 30% of the isolates were Lactobacillus species, 50% were members of the genus Streptococcus and around 20% were species of genus Clostridium. The presence of spherical and rod-shaped microbial morphologies was confirmed by the Scanning Electron Microscopy (SEM) analysis in the sludge samples of bioreactor throughout the prolonged cultivation (Badiei et al., 2007).
According to Kamal et al. (2011) study, after pre-treatment, POME was directly applied without standardize the initial concentration of TC content and to regulate the initial pH, only small number of buffer were added. Table 2.1 showed the production hydrogen at different initial pH (5.5 and 7) by applying local isolate, and Clostridium butyricum. In non-sterile condition, the fermentation was done. The result revealed that the maximum production of hydrogen for initial pH 7 that was specified by alkaline-heat pretreatment, however, the higher production of hydrogen was given by acid-heat pretreatment for initial pH 5.5. In order to produce a majority of the gas, initial pH 7 was higher than initial pH 5.5 and also at initial pH 7, production of hydrogen was higher than pH 5.5. Finally, the fermentation will be stopped at dissociated form of acid and lower pH inhibits the production of hydrogen (Kamal et al., 2011).

Atif et al. (2005) carried out the study on the production of anaerobic in hydrogen from Palm Oil Mill Effluent (POME) by microflora 5-L bioreactor at 60°C and pH 5.5. POME sludge was used as a source of inoculate and gathered from the anaerobic pond of a POME treatment plant at a palm oil mill. Kelly-Yong et al. (2007) aimed to show the accessibility of oil palm biomass to hydrogen through gasification reaction in supercritical water, as a renewable energy for policy-makers. In 2007, the oil palm ranked as number 1 fruit crops with 35.90% of the total edible oil or 36.90 million tons production in the world. In fact, oil constitutes only produce about 10% of the palm, and the rest 90% was biomass. In total, the production of oil palm biomass is about 184.6 million tons annually, and the maximum result of hydrogen production via this method is 2.16×1010 kg H\textsubscript{2}/y ear with an energy content of 2.59 EJ/year for the current worldwide hydrogen demand is almost 50% (Kelly-Yong et al., 2007).

**MATERIALS AND METHODS**

**Palm Oil Sample**
The sludge sample and raw sample taken from the first and third pool of Sedenak Palm Oil Production Factory in Johor Bahru respectively, were brought to microbiology lab in UTM in a short time (1 h), and were put in the fridge to keep them fresh to begin the experiments.

**Material preparation**

*Isolation Media Preparation:* In order to isolate the bacteria from the samples, definite media was prepared to cultivate the bacteria and obtain the pure colonies. Nutrient broth (NB) and Nutrient agar (NA) are two kinds of media that were used in this current study for isolation step of facultative anaerobic and strict anaerobic bacteria.

*Facultative Anaerobic Media Preparation:* Nutrient Broth (NB) media was used for isolation and growth of the facultative anaerobic bacteria. This media was made according to manufacturer’s protocol. In order to prepare this media precise amount of powder was dissolved in accurate amount of distilled water and finally put in autoclave at 121°C for 15 min.

NA (Nutrient Agar) was made according to manufacturer’s protocol and then heated and autoclaved. It was then poured into Petri plates and left solidify. Then, the prepared media was divided into 10 plates to prepare the serial dilution.

Then 4 mL of NB was poured into two Universal bottles and then 4 mL of each sample was poured into the NB and incubated at 37°C for 24 h. At the next step, for making dilution, 9 mL of NB was poured into another 10 tubes.

*Anaerobic Media Preparation:* NB weighted according to the instruction was dissolved in 1 L of water. Serum bottle should be used in order to make the anaerobic media. Then 200 mL of NB was mixed with 0.2 mL Resazurin (blue). Then the media was heated in order to remove O\textsubscript{2}. For the complete elimination of O\textsubscript{2}, the media was put under nitrogen pump (sparged) (Figure 3.3) by gas distribution unit. After 1 hour, the color changed from blue to pink, then 40 mL of media was poured into 2 serum bottles by pipette and sealed.

Precise amount of NA was dissolved in 1 L distilled water. In order to remove O\textsubscript{2} from media, 200 mL of prepared NB was mixed with 0.2 mL resazurin and heated for 45 min at 40 °C. Removal of O\textsubscript{2} and dissolving of N\textsubscript{2} in media caused the color change of the media to pink (Figure 3.4). Then the media was
divided into serum bottles (15 mL each) and were sealed. They were autoclaved and kept at 70 ºC in order to prevent solidifying.

Methods of Isolation

Two kinds of media were prepared for isolation of bacteria in the samples. Two kinds of media were prepared for isolation of bacteria in the samples. Nutrient broth and nutrient agar were two kinds of media that were prepared for facultative anaerobic and anaerobic bacteria.

Selection of Isolated Bacteria

Streak Plate Method: The grown colonies of bacteria under facultative anaerobic condition might be different morphologically. In order to cultivate each colony, different plates containing NA were prepared and the colonies were transferred to them and cultivation was done by streak plating method and was incubated at 37°C for 16 h. The streak plating technique isolates individual bacterial cells (colony-forming units) on the surface of an agar plate using a wire loop. Once again, the idea is to obtain isolated colonies after incubation of the plate.

In anaerobic condition the tube lids were not fully open, oxygen was removed by flame, and the grown colonies on the tubes wall were taken by needle and transferred to 10 mL of NB and incubated at 37°C for 24h. Then the bacteria were subjected to chemical and morphological tests for bacterial characterization.

Storage of Isolated Bacteria: To store the isolated bacteria for future usage, the glycerol stock of bacteria was prepared. Firstly, 100 mL of glycerol 80% was prepared. In order to prepare an appropriate solution to store the bacteria, 0.3 mL of the glycerol was mixed with 0.7 mL of each sample and transferred to the centrifuge tubes and shaked. Then the centrifuge tubes were put in cryostorage box and stored in -80°C freezer.

Characterization

Characterization of bacteria based on the microbial and biochemical characters have been the traditional method of identification of bacteria. In fact, determination of shape, gram reaction by doing microbiological and biochemical tests and technique is referred to as general characterization of bacteria. Similar methods of characterization were used for both facultative anaerobic and strict anaerobic bacteria.

Hydrogen Production Assay of Isolated Bacteria by RGA

The suitable condition for this purpose is fermentation, and the media used was synthetic starch waste water. Therefore, according to the Klatt et al (2003) this media was prepared.

RESULTS AND DISCUSSION

Isolation of Bacteria

There were two samples that were taken from palm oil production factories in Johor Bahro in the (sludge and raw waste). Total of 13 single colonies were isolated from facultative anaerobic condition from the $10^6$ and $10^3$ concentrations and were kept at 4°C. While for strict anaerobic isolation, 8 single colonies were isolated from the $10^3$ and $10^6$ concentrations. Then, the colonies were transferred to NB media and were kept at 4°C.

Determination of Hydrogen Production

RGA (Residual Gas Analysis): Firstly, some steps were done to prepare the sample. As mentioned before the Buchner flasks was connected to the container, containing mixture of synthetic starch media and bacteria and were put at 37°C for 24 h to trap the produced gas from fermentation. During this step the R4 from facultative anaerobic and 8 samples of strict anaerobic was analysed to determine the amount of the H$_2$ production. After 24 h the connectors of buchner and container were removed and then the buchner flask were connected to the RGA in order to measure the H$_2$. Figure 2 shows the differences in the % partial pressure obtained from the biogases (H$_2$, CO$_2$ and CH$_4$) from raw strict anaerobic samples. As illustrated in this Figure 2, the amount of gas production is different. It seems that the amount of CH$_4$ gas is the same in all samples while the amount of another gases are different. Figure 3 shows the different amount of gas production from sludge samples (strict anaerobic isolates). As shown in this Figure, the amount of H$_2$ production is not lower compared to the other gases.
The amount of H₂ production from raw and sludge isolates is shown in Figure 4 that illustrates that all raw samples are able to produce H₂ but comparatively the amount of H₂ in raw 1 is slightly more than the raw 2 an and raw 4 whereas in raw 3 the amount of H₂ is highest in comparison with all raw samples.

Among the sludge strict anaerobic isolates the amount of H₂ is highest for sludge 1 in comparison with another sludge isolates (Figure 5).
The main purpose of the determination of hydrogen in this current study is identification of bacteria capable to produce $H_2$ and to compare the amount of $H_2$ production among the all samples of both types of bacteria (facultative anaerobic and strict anaerobic). Figure 6 shows the comparison between all capable samples to produce $H_2$. Besides, the results of $H_2$% production in strict anaerobic and facultative anaerobic tabulated in Table 1. Hence, amount of $H_2$ production is highest in sludge 1 of anaerobic samples.

![Figure 6: The comparison of $H_2$ production among facultative anaerobic (fan) and strict anaerobic isolates (an)](image)

Table 1: The summary results of $H_2$ production by strict anaerobic and facultative anaerobic bacteria

<table>
<thead>
<tr>
<th>Strict anaerobic</th>
<th>% $H_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw 1</td>
<td>0.000626</td>
</tr>
<tr>
<td>Raw 2</td>
<td>0.000133</td>
</tr>
<tr>
<td>Raw 3</td>
<td>0.00228</td>
</tr>
<tr>
<td>Raw 4</td>
<td>0.000134</td>
</tr>
<tr>
<td>Sludge 1</td>
<td>0.048428</td>
</tr>
<tr>
<td>Sludge 2</td>
<td>6.15989</td>
</tr>
<tr>
<td>Sludge 3</td>
<td>3.03196</td>
</tr>
<tr>
<td>Sludge 4</td>
<td>0.001419</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Facultative anaerobic</th>
<th>% $H_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw 4</td>
<td>3.1025105</td>
</tr>
</tbody>
</table>

Pathway of Bacterial $H_2$ Generation

Hydrogen is most commonly produce by algae, bacteria and archaea. Biohydrogen is a potential biofuel obtainable from both cultivation and from waste organic material. Hydrogen is an ideal, clean, and potentially sustainable energy carrier for the future due to its abundance and non-polluting nature. Numerous bacteria, cyanobacteria, and algae are capable of producing hydrogen from water, solar energy, and a variety of organic substrates. Improvement of these diverse biochemical pathways is needed in order to make biohydrogen competitive with current production method.

If hydrogen by fermentation is to be introduced as an industry, the fermentation process will be dependent on organic acids as substrate for photo-fermentation. The organic acids are necessary for high hydrogen production rates (Mathews and Wang, 2009).

The organic acids can be derived from any organic material source such as sewage waste waters or agricultural wastes. The most important organic acids are acetic acid (HAc), butyric acid (HBe) and propionic acid (HPc). A huge advantage is that production of hydrogen by fermentation does not require glucose as substrate. The fermentation of hydrogen has to be a continuous fermentation process, in order sustain high production rates, since the amount of time for the fermentation to enter high production rates are in days. Figure 7 illustrates the anaerobic metabolic pathway of hydrogen production in clostridium (Abo-Hashesh et al., 2007).
Conclusion
The fossil fuels are basis of the global energy requirements which lead to the foreseeable depletion of limited fossil energy resources. Because of the production of pollutants like NO\(_x\), SO\(_x\), CO\(_x\), C\(_x\)H\(_x\), ash, soot, and the droplets of tars, the use of fossil fuels causes the change of global climate. Furthermore, based on the growth of urbanization and industrialization, the environmental pollution is imminent. Therefore, searching for clean energy alternatives is crucial to satisfy growing energy demand. This study investigates the generation of hydrogen by evaluating the possibilities of bacteria as agent.

The main objective of this study was to isolate the bacteria capable to produce hydrogen. For this purpose, 2 samples (raw and sludge) were collected and to isolate the bacteria of interest. Two types of bacteria were isolated (facultative anaerobic and strict anaerobic bacteria).

The isolated bacteria were characterized by microbial and biochemical tests in order to identify them and determine the characteristics to use them for hydrogen production. The properties of the bacteria were different and it means that different kinds of bacteria exist in POME samples. Finally, the isolated and identified bacteria were used to produce hydrogen and the amount of produced hydrogen was determined by RGA, which is a sophisticated mass spectrometry-based gas detector. Facultative anaerobic bacteria samples were able to produce hydrogen except the sample Raw4. On the other hand, the strict anaerobic bacteria samples were able to produce hydrogen but among these samples [Raw3 an], [Sludge1 an], and [Sludge4 an] produced gas more than another samples. The hydrogen production in sample [Sludge 1 an] was relatively high (0.048428%). This sample is the gram negative bacillus, and all its biochemical tests were negative.

This study proves that the isolated bacteria from raw and sludge samples of POME grown under anaerobic condition are able to produce hydrogen, and the bacteria isolated of strict anaerobic from sludge samples (Sludge1) produced highest amount of hydrogen gas from starch fermentation.

REFERENCES
Research Article


Rasdi Z (2009). Optimization of Biohydrogen Production from Palm Oil Mill Effluent by Natural Microflora. The Open Biotechnology Journal 3 79-86.


