Biohydrogen production from palm oil mill effluent with *Moringa Oleifera* seeds as support carrier in attached growth system

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Abstract. Biohydrogen production by dark fermentation is one of the attracting alternatives for renewable energy in worldwide. By employing immobilized cells, hydrogen production and cell density could be improved. This study aimed to investigate the efficiency of Moringa Oleifera Seeds (MOS) immobilized cells in enhancing the biohydrogen production using repeated batch fermentation under mesophilic condition, 37°C. The efficiency of MOS as support carrier, effect of the initial pH (5.0-7.0) and performance of raw and diluted Palm Oil Mill Effluent (POME) using MOS immobilized cells were investigated using anaerobic sludge as inoculums. The cumulative hydrogen production results were fitted into a modified Gompertz equation to find the maximum hydrogen production. MOS immobilized cells was more efficient in producing hydrogen compare to suspended cells (without MOS). The optimal pH obtained using MOS immobilized cells was found to be at pH 6 using raw POME with the maximum hydrogen production (Hₘ) of 122 mL, the maximum hydrogen production rate (Rₘ) of 39.0 mL/h, and 560 ppm of hydrogen concentration.

1. Introduction

In recent years, there has been an increasing interest in renewable energy. This phenomenon occurs due to its advantages as it is free from greenhouse gas emissions and related global warming effects [1]. Renewable energy is the energy that is generated from natural resources such as sunlight, wind, water, biogas and various forms of biomass. Biohydrogen production is one of the attracting renewable energy in worldwide. Through biohydrogen production process, clean hydrogen energy could be produced [2] and at the same time wastewater could be treated if it were used as the feedstock in the process. There are various forms of hydrogen production processes, which are steam reforming, electrolysis of water, dark fermentation and photo fermentation. The dark fermentation process is more preferred in terms of environmental friendly which produce ‘green energy’ as product. Palm oil mill effluent (POME) is a thick brownish liquid with a distinct odor from the process of oil extraction, washing and cleaning process in mill [3]. POME is one of the potential feedstock for biohydrogen production in Malaysia. This is due to the high content of carbohydrates, protein, nitrogenous compounds, lipids and minerals that contains in POME [4]. Raw POME contains about 92-94% of water, 6-7% total solids, higher chemical oxygen demand (COD) which in the range of 75-78 g/L and biochemical oxygen demand (BOD) of about 23-55 g/L [5]. On the other hand, *Moringa Oleifera* is a tropical plant that belongs to the family *Moringaceae*. This *M. Oleifera* has multiple uses...
such as natural coagulant and act as a part of traditional diets in many countries [6]. *Moringa Oleifera* Seeds (MOS) also contain active coagulating agents characterized [8]. This MOS produce 34-80% of oil yield and also consists of 31.65% protein, 7.54% fiber, 8.90% moisture and 6.53% ash [8]. In addition to the advantages, due to the porosity characteristics in MOS, it has a high potential to be used as support carrier in attached growth system.

2. Materials and experimental procedures

2.1. Feedstock and anaerobic sludge

Raw POME and the anaerobic sludge were collected directly from Malpom Industries, Sungai Bakap, Pulau Pinang and were used as feedstock and seed sludge (inoculum) respectively for this study. These samples were collected in clean containers and stored in the laboratory cold room.

2.2. Support carrier

*Moringa Oleifera* Seeds (MOS) was used as carrier in this attached system growth for biohydrogen production. The size range of the MOS is from 0.7-1.3 cm. The seeds were obtained from local supplier.

2.3. Experimental setup and procedure

Batch fermentation were conducted in 100 mL serum bottle that filled with 50 mL working volume and adjusted to a specific pH. This batch of fermentation was conducted into five different range of pH which are from 5.0 to 7.0. The immobilization was carried out by adding 5% of MOS (in weight to total working volume) as support carrier. To provide an anaerobic conditions in the serum bottle, nitrogen were purged for a few minutes in each of the serum bottle. The batch fermentation was conducted under mesophilic temperature (37°C) for 24 hours in incubator.

The repeated batch procedure was similar to the batch fermentation as aforementioned. However, the repeated batch procedure was carried out repeatedly in series for a stable hydrogen production. At the end of each batch, the medium broth was evacuated and the MOS were incubated with new fresh medium. This procedure was repeated for ten batches at different initial pH to examine the stability and durability of the MOS as support carrier.

The method for batch profile was the same as the batch fermentation, as aforementioned. The specialty on the batch profiling was that the method was only applied for the highest hydrogen production from raw and diluted POME results. The samplings were done every 3 hours interval for 24 hours.

2.4. Modified Gompertz equation

Modified Gompertz equation was used to determine the cumulative hydrogen production by batch dark fermentation [7]. The modified Gompertz equation defined as follow:

\[
H_t = H_m \exp \left( \frac{R_m \cdot e^{\lambda - t}}{H_m (\lambda - t) + 1} \right)
\]

Where, \(H_t\) is the cumulative hydrogen production (mL) at culture time \(t\), \(H_m\) is the maximum amount hydrogen production (mL), \(R_m\) is the maximum hydrogen production rate (mL/L), were adjusted to measure the hydrogen evolution data, \(\lambda\) is the lag time (h) and the value of \(e\) is 2.71828.

3. Results and discussion

3.1. The efficiency of MOS as support carrier

In this work, the aim was to investigate the efficiency of MOS as support carrier in producing biohydrogen. For this purpose, a batch fermentation using raw POME, as feedstock; and anaerobic
sludge, as inoculum was conducted at mesophilic condition, 37°C. This batch fermentation was carried
out for 24 hours in 100 mL serum bottles experiment with 50 mL working volume. Figure 1 shows the
biohydrogen production of batch fermentation with five different initial pH values. Two types of
samples were compared to examine the efficiency of the support carrier as immobilized cell, which are
immobilized cell using MOS as support carrier, and suspended cell without MOS (namely control
sample).

As shown in Figure 1, the sample with MOS is more efficient in producing hydrogen compare to
the sample without MOS. The highest biohydrogen production using MOS was recorded at pH 6 with
137 mL and 568 ppm of hydrogen concentration. A possible explanation for this might be that, the
porosity characteristics in MOS aid in enhancing the hydrogen production rate. Table 1 shows the
hydrogen concentration that was obtained using Gas Analyser Model GA 5000. It is apparent from this
table that initial pH 6 recorded the highest gas concentration with 568 ppm. The pH range of 6.0–6.5 is
considered weakly acidic, which encourages microorganisms to release express protons from
cytoplasm for the enhancement of hydrogen production and resumption of cell growth [9]. On the
other hand, at initial pH of 5.0 seems that substantial sup-pression was noted in the hydrogen
production rate [10], which recorded the lowest gas concentration which is 231.5 ppm at 38.5 mL of
hydrogen production. These results illustrate that secretion of acid in the medium can cause
accumulation and a sharp drop in the pH value of the culture broth [11]. It is clearly shown that the
experiment in the presence of MOS even under non-favorable pH conditions exhibited better hydrogen
production than the suspended cells in the control experiments in all different initial pH values studied.
The immobilization system enabled the cells to withstand considerable shear force and stay active
towards a stressful environment.

![Figure 1. Hydrogen Production with MOS and without MOS (Control)](image_url)
Table 1. Concentration of biohydrogen with MOS and without MOS (Control) using raw POME as feedstock

| pH  | Biohydrogen Concentration (ppm) |
|-----|-------------------------------|
|     | MOS cells | Control |
| 5.0 | 231.5      | 9.5     |
| 5.5 | 235        | 11.5    |
| 6.0 | 568        | 22      |
| 6.5 | 428        | 14.5    |
| 7.0 | 310.5      | 12.5    |

3.2. The optimum pH using MOS immobilized cells by repeated batch fermentation

Environmental parameters can exert their influences during the immobilisation process in a repeated batch, therefore it is crucial to study the effect of the pH at the initial stage of development of the MOS immobilised cells. In addition, initial pH value is one of the most essential factor that affecting the microbial activity in order to produce hydrogen. The results obtained for series of batch fermentation in five different initial pH using MOS immobilized cells are shown in Figure 2.

On the first batch of fermentation, the highest hydrogen production was obtained at pH 7 with 100 mL while the lowest hydrogen was recorded at pH 5 with 25 mL. However, the repeated batch seemed to exhibit a stable hydrogen production starting from the third cycle of repeated batches. The repeated batch fermentation had found that the initial pH 6 provided the most consistent hydrogen production compare to other initial pHs with an average of 120 mL of hydrogen production. The repeated batch approach shows promising results and rapidly allows the microorganisms in the culture to adapt to their environment. The successive batches were in the range of 200 ppm to almost 600 ppm of hydrogen concentration, depending on initial pH of fermentation broth. Results in Figure 2 has shown that the screening of efficient hydrogen-producing bacteria from a mixed culture by repeated batch cultivation has become a suitable strategy to avoid variation in the inoculum, thus maintaining high microorganism growth rates [12,13].

![Figure 2. Hydrogen Production using MOS Immobilized Cells at Various Initial pH Values](image-url)
3.3. Performance of diluted POME using MOS immobilized cells

Generally, the attached growth system is not suitable for substrates with high solid contents. Therefore, this study had attempted to use the MOS immobilised cells with diluted POME to study the best dilution rate for the enhancement of biohydrogen production. Batch fermentation using diluted POME at different dilution rate of 20%, 40%, 60% and 80% were conducted at 37°C with the optimal initial pH value of 6 that was obtained from previous batch fermentation study. Two types of samples were conducted for this batch fermentation, which are immobilized cell using MOS (support carrier) and suspended cell (without MOS). Figure 3 shows the experimental data of hydrogen production from various dilution rate of POME with MOS immobilised cells.

The highest hydrogen production volume of 52 mL was recorded using MOS immobilized cells at 40% dilution rate with hydrogen concentration of 286 ppm. However, at dilution rate of 60% without MOS (control), the hydrogen production recorded was the lowest with only 9.5 mL. If compare to the results in Figure 1 for pH 6 using raw POME (without dilution), the performance of 40% dilution rate (52 mL) is almost three fold lower than the raw POME (137 mL). The strong justification might be due to the reduction of carbohydrate content when dilution is conducted. Hence, less carbohydrate could be converted to hydrogen production and this will effect the performance from the diluted POME. Further study should be conducted by considering equal carbohydrate content as in raw POME by supplementation of additional synthetic sugars (eg: sucrose, glucose) to examine the feasibility of the MOS in different dilution rate of POME.

3.4. Kinetic study using Modified Gompertz equation

The kinetic study using modified Gompertz equation was performed only for pH 6 using raw POME (RP) and 40% dilution rate of POME (DP) due to the highest hydrogen production was recorded by MOS immobilised cells in previous experiments. Figure 4 shows the cumulative hydrogen production results that were fitted using modified Gompertz equation. There are three important parameters that were obtained from the data fitted using the modified Gompertz equation, which are; maximum hydrogen production ($H_m$), maximum hydrogen rate ($R_m$) and lag phase ($\lambda$). The kinetic parameters that were obtained for raw POME at pH 6 were $H_m = 122$ mL, $R_m = 39.0$ mL/h and $\lambda = 3.58$ h while kinetic parameters recorded for diluted POME at dilution rate of 40% were $H_m = 41$ mL, $R_m = 5.8$ mL/h and $\lambda = 9.50$ h. As can be seen from Table 2, raw POME at pH 6 depicted better results compared to diluted POME at 40% dilution rate.

![Figure 3. Hydrogen production at different dilution rate conducted at initial pH 6](image-url)
Figure 4. Kinetic study based on Modified Gompertz equation for pH 6 using raw POME (RP) and 40% dilution rate of POME (DP) by MOS immobilised cells

Table 2. Modified Gompertz Equation parameters obtained using pH 6 using raw POME (RP) and 40% dilution rate of POME (DP) by MOS immobilised cells

| Sample                        | pH  | Modified Gompertz Equation Parameter Values for hydrogen production | Hydrogen concentration using MOS cells (ppm) |
|-------------------------------|-----|---------------------------------------------------------------------|---------------------------------------------|
|                               |     | Initial | Final | $H_m$ (mL) | $R_m$ (mL/h) | $\lambda$ (h) |  |
| Raw POME                      | 6.0 | 4.801   | 122   | 39.0        | 3.58          | 560            |  |
| Diluted POME (40% Dilution Rate) | 6.0 | 5.173   | 41    | 5.8         | 9.50          | 280            |  |

3.5. Total alkalinity and sugar consumption

The results obtained for total alkalinity from batch profiling fermentation using MOS immobilized cells are presented in Figure 5 for raw POME and Figure 6 for diluted POME at 40% dilution rate. Based on the graph shown in Figure 5 and 6, the total alkalinity were increased as the time increases during the fermentation. This results were reflected to the previous results that obtained for batch profiling of hydrogen production in Figure 4 and Table 2. Both figures (Figure 5 and Figure 6) also show the percentage of sugar (%) that were recorded during the batch profiling. It is a clear trend of decreasing in the sugar percentage against the time of fermentation. The initial percentage of sugar that obtained for both batch profiling fermentation are assume to be 100%, whereby the total carbohydrates that contain in the feedstock were not yet be consumed by the microorganisms. Interestingly, after three hours of fermentation, the bacteria start to consumed the sugar until 18% and 40% for raw and diluted POME, respectively. This conditions show that the consumption of carbohydrate were converted to produce hydrogen gas, and at the same time producing the fatty acids which were measured as total alkalinity. The consumption of the carbohydrate from the diluted POME is more rapid compare to the raw POME might be due to less complex carbohydrate and constituents that ease the conversion of sugar by microorganisms from the diluted POME. Future study on the
inhibition effect should be conducted to validate whether the dilution rate ease the process of biohydrogen conversion due to less inhibitor in the fermentation broth.

![Graph of total alkalinity and sugar consumption using MOS immobilized cells at using raw POME](image1.png)

**Figure 5.** Graph of total alkalinity and sugar consumption using MOS immobilized cells at using raw POME

![Graph of total alkalinity and percentage of sugar consumption using MOS immobilized cells at 40% dilution rate](image2.png)

**Figure 6.** Graph of total alkalinity and percentage of sugar consumption using MOS immobilized cells at 40% dilution rate

### 4. Conclusion

This study set out to determine biohydrogen production from palm oil mill effluent (POME) with Moringa Oleifera Seeds (MOS) as support carrier in attached growth system. This study has shown that MOS immobilized cells help in improving hydrogen production. The efficiency of MOS as support carrier in producing biohydrogen has been proved by comparing the MOS immobilized cells with suspended cells sample (control). From this comparison, MOS immobilized cells obtained better performance of hydrogen production compare to the suspended cells. The most obvious finding to emerge from this study is the optimal pH obtained was found to be pH 6 with the maximum hydrogen production ($H_m$) is 122 mL, the maximum hydrogen production rate ($R_m$) is 39.0 mL/h, the lag phase ($\lambda$) is 3.58 h and the hydrogen concentration is 560 ppm. The modified Gompertz equation clearly shown that the performance of raw POME with MOS as support carrier is more effective in producing biohydrogen compare to the diluted POME with MOS. Overall, this study was successfully achieved and all of the objectives has been set out.
Acknowledgement
The authors would like to acknowledge financial support from Dana Bantuan Khas Penerbitan Saintifik UniMAP, from Universiti Malaysia Perlis which makes this important research viable and effective.

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