Study on biohydrogen production using different type of carrier materials in attached growth system

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Abstract. Renewable energy is known as clean energy with free from greenhouse gas emissions and global warming effects. It is generated from natural resources and one of the most promising renewable energy is biohydrogen. Biohydrogen production gets a great attention around the world because it could remove organic biomass and at the same time supplying clean hydrogen energy. In this study, three support carriers were used namely granular activated carbon (GAC), glass beads (GB) and moringa oleifera seeds (MOS). The main keys of this study was to identify the best support carrier that capable to enhance the biohydrogen production in attached growth system using Palm Oil Mill Effluent (POME) as feedstock. On the other hand, the physicochemical of the attached-biofilm were also investigated by using Scanning Electron Microscopy (SEM). Other parameter such as hydrogen concentration, volume of biogas, and kinetic study by using modified Gompertz equation has also been studied. At the end of the study, the best performance of biohydrogen production was performed by using GAC with hydrogen yield (HY) = 1.52 mol H₂/ mol glucose and the hydrogen production rate (HPR) = 58.50 mmol H₂/l.d, followed by GB which is HY = 1.43 mol H₂/ mol glucose and HPR = 54.840 mmol H₂/l.d and the last, MOS with HY = 1.08 mol H₂/ mol glucose and HPR = 41.44 mmol H₂/l.d. This study has shown that proper selection of support carrier could reflect the evolution of biohydrogen production.

1. Introduction

Renewable energy has gained a lot of attention because of it is free from greenhouse gas emissions and global warming effects. Renewable energy is energy that generated from natural resources. Nowadays, biogas is one of the most promising renewable energy which is sustainable and the combustion product poses no danger to the environment. Biohydrogen production is one of the attracting biogas in worldwide. This biohydrogen production process can remove organic biomass while simultaneously supplying clean hydrogen energy [1]. Palm oil mill effluent (POME) is one of the major sources of biohydrogen production in Malaysia. This is due to the high content of carbohydrates, protein, nitrogenous compounds, lipids and mineral that contains in POME [2].

Granular activated carbon (GAC) is made from organic materials with high carbon contents such as wood, lignite and coal. In biohydrogen production, GAC usually used as the support medium since it served excellently in microbial colonization [3][4]. Besides, glass beads (GB) are categorized by the method used to manipulate the glass - wound beads, drawn beads, and molded beads. In laboratory, it usually used as boiling stones, mixing beads or packing for distillation columns. However, in biohydrogen production, it is relatively good for hydrogen production performance in attached growth
system [5]. On the other hand, *moringa oleifera* is a tropical plant that is belong to the family *Moringaceae*. In Malaysia, it is known as Kelor or Merungcai plant. *Moringa oleifera seeds* (MOS) usually used as a natural coagulant in the wastewater treatment [6]. Due to high porosity characteristic in MOS, it has a high potential to be used as support carrier for biohydrogen production.

In this study, immobilized biohydrogen production using different support carriers were compared under mesophilic conditions in attached growth system. Support carriers that have been chosen for this study were GAC, GB and MOS. These support carriers were used to perform biohydrogen production through dark fermentation by utilizing raw POME as carbon source. Apart from the studies available in the literature, the aim of this study is (i) to identify the best support carrier that capable to enhance the biohydrogen production in the attached growth system, (ii) to characterize the mixed cultures biofilm formed on these support carriers for a better understanding of the attached growth cell for biohydrogen production, and (iii) to evaluate the effectiveness of different support carriers by using kinetic study (modified Gompertz equation) on biohydrogen production.

2. Materials and methodology

2.1. Feedstock and seed sludge

The raw Palm Oil Mill Effluent (POME) as a feedstock was obtained from the sludge pit of POME and seed sludge was collected from anaerobic pond at Malpom Industries, Sungai Bakap, Penang, Malaysia. These samples were preserved and refrigerated at 4 °C prior to use in the study, to decrease the biological degradation and acidification.

2.2. Support carriers

The carrier material is used as support matrix for microorganism and functions as host and attached media that offer a surface area for cell growth. In this study, three support carriers were used. First, granular activated carbon (GAC) with size in the range 2 – 3 mm. Second, is glass beads (GB) with size of 3 mm and the third, *Moringa Oleifera Seeds* (MOS) with size in the range of 0.5 – 1 cm. These support carriers were chosen due to physical and chemical inert characteristic.

2.3. Experimental setup and procedures

Batch fermentation was performed by using 100 ml serum bottle with working volume of 50 ml. The anaerobic sludge was carried out by adding support carriers (GAC/GB/MOS) in ratio of 1:1 of anaerobic sludge volume (ml) to support carriers weight (g) in the serum bottle. After the mixture is ready, the pH was adjusted to 6. Then, the serum bottle with different support carriers was purged with nitrogen for 2 minutes to formed anaerobic condition. After that, the serum bottles were incubated at 37 °C which was under mesophilic temperature (37 °C) for 24 hours in incubator. The fermentation was conducted for several repeated batches to ensure the biofilm formed on support carrier as immobilized cells are stable enough in terms of H2 production.

The sampling was done every day which involved the samples of biogas and liquid samples of the fermentation. The gas obtained was being analyzed by using a gas analyzer (GA 5000).

![Figure 1. Schematic diagram of experimental setup.](image-url)
2.4. Microbial morphology

The attached-biofilm on support carriers was observed by using Scanning electron microscopy (SEM) to discover the attachment of bacterial cell’s shape on the support carriers. The samples were prepared prior to visualize it using SEM. First, the samples were gently washed with 0.1 M phosphate buffer solution and allowed to settle naturally to remove the unwanted particles. Then the samples were fixed with 4 % paraformaldehyde and left for 4 hours. The fixed samples were dehydrated by successive passages through 40 %, 60 %, 80 % and 100 % ethanol. Lastly, the dried samples were observed by using Scanning electron microscopy (SEM).

2.5. Analysis of H₂ production by using Modified Gompertz equation

A modified Gompertz equation was used to correlate experiment data in order to quantify hydrogen production. The correlation fitting was done using SigmaPlot v10.0 (Systat Software Inc, USA). Theoretically, modified Gompertz equation is given in the equation below [7]:

\[
H = \frac{H_{\text{max}} \cdot \exp\left(-\exp\left[\frac{R_{\text{max}} \cdot e}{H_{\text{max}}} (\lambda - t) + 1\right]\right)}{p}
\]  

(1)

Where, \(H\) is the cumulative hydrogen production (ml), \(H_{\text{max}}\) is maximum hydrogen production (ml), \(R_{\text{max}}\) is maximum hydrogen production rate (ml.h⁻¹), \(e\) is Euler's number (\(e = 2.73\)), \(\lambda\) is lag phase time (h) and \(t\) is incubation time (h).

3. Results and discussion

3.1. Biohydrogen production

The results shown in Figure 2 and 3 are the data of the volume of biogas and the hydrogen concentration for the repeated batch fermentation. From the experiment, the biogas was composed of hydrogen and carbon dioxide and free of methane gas. The biogas also free from oxygen gas, indicating the system was performed fully in anaerobic condition. This study also showed that the volume of biogas comprises of hydrogen gas in the range of 52 to 56 % by using GC gas analysis. Besides, Figure 2 represents that the volume of biogas in parallel to the hydrogen concentration (Figure 3) for each day.

![Figure 2](image-url)

**Figure 2.** The volume of biogas production against time.
Figure 3. The concentration of hydrogen production against time.

Table 1. Comparison of volume of biogas and hydrogen concentration at the end of fermentation process.

| Samples                          | Volume of biogas (ml) | Hydrogen Conc. (ppm) |
|----------------------------------|-----------------------|----------------------|
| Control (without support carrier)| 85                    | 468                  |
| Granular activated carbon (GAC)  | 95                    | 1000                 |
| Glass beads (GB)                 | 90                    | 564                  |
| *Moringa oleifera seeds* (MOS)   | 68                    | 122.5                |

At the end of the repeated batch fermentation, the result shows the highest hydrogen produced is by using GAC with 95 ml of biogas and 1000 ppm of hydrogen concentration, followed by GB which is 90 ml and 564 ppm and the last is *moringa oleifera seeds* (MOS) with 68 ml and 122.5 ppm. On the other hand, the volume of biogas produced for batch control was 85 ml with 468 ppm of hydrogen concentration. The results demonstrated that the performances of GAC to produce biogas is more effective compared to GB and MOS.

This study found that the use of GAC immobilized cells leads the microbes to meet the porous area of GAC to promote optimal biological activity for biohydrogen production [8]. Besides, the results supported by the statement of [3][4] where GAC was used as the support medium for biohydrogen production which can serve excellently in microbial colonization. Other than that, large area of the GAC particles helps to enhance cell density during the fermentation process which contributes to higher hydrogen production [9].

On the other hand, this study also showed that GB immobilized cells performed well in producing hydrogen. This is due to their good porous structure and homogenous composition [10]. In addition, [10] also mentioned that cell immobilization by using GB as a support carrier resulting 92% of the biomass were attached to GB. It indicates that GB was also a good support carrier to enhance the performance of biohydrogen production.

In this study, *MOS* shows the lowest hydrogen production at the end of repeated batch fermentation. To the extent of my knowledge, this study was the first using *MOS* as carrier to immobilized cells for biohydrogen production. By referring to the previous research, *MOS* usually used as a natural coagulant or adsorbent in wastewater treatment [6]. The results shows the hydrogen production drastically increased at the beginning of the fermentation. The highest hydrogen produced by MOS was 88.5 ml and 554 ppm on 6th day. However, starting 7th day, it was undergoing a decline process until the end of fermentation process. This study found that *MOS* has a potential to produce hydrogen
gas rapidly at the beginning of the fermentation process. This is because MOS has a high porosity that leads the microorganisms’ easily attached on MOS and increase the metabolic activity in a short time. In addition, the high metabolic activity increased the population of microbes which contributes to the high hydrogen produced. It showed that MOS can act as a natural support carrier to produce hydrogen gas for a batch system. However, the use of MOS immobilized cells is not suitable for the continuous system. This is because MOS is a natural organic where it has reached the limit to perform well on biohydrogen production. Therefore, the use of MOS as support carrier at the initial stage of fermentation process is a good option because it could initiate a good hydrogen production for startup.

3.2. Microbial SEM image observation
Figure 4 shows the microbial SEM images of the attachment of bacterial cell’s shape on the support carriers. As shown in Figure 4 (a),(b) and (c), a closer view of 10 000x magnification illustrates that the images of the individual attached cells on the surfaces of these three support carriers. Besides, the SEM images in Figure 4 (d), (e) and (f) represents the bacteria colonization with rod-shaped were dominated on the biofilm and fully covered on the porous surface of the support carriers. However, the colonization of bacteria on GAC is more than GB and MOS resulting in highest biohydrogen produced by using GAC immobilized cells. The same attachment behavior was also observed by Lutpi et al., (2015), in their work using synthetic media containing sucrose. Therefore, these SEM images showed that a stable and successful immobilization was achieved under mesophilic conditions.

![Figure 4](image)

**Figure 4.** Close – up view of rod – shaped bacteria (magnification 10 000x) during biohydrogen production under mesophilic conditions (37 °C), on a) GAC, b) GB, c) MOS. Bacterial colonization after 13 days of the repeated batch fermentation onto d) GAC, e) GB, f) MOS.

3.3. Kinetic analysis by using Modified Gompertz equation
The kinetic analysis was investigated for GAC and GB immobilized cells due to the stability of hydrogen performance at the end of the repeated batch fermentation. Therefore, kinetic study for MOS immobilized cells is not investigated due to the reduction of the performance biohydrogen production in the repeated batch fermentation. There are three important parameters that were obtained from the data fitted using modified Gompertz equation which are, maximum hydrogen production (Hm), maximum hydrogen production rate (Rm) and lag phase time (λ).
Table 2 shows the summary results obtained by using modified Gompertz equation. Meanwhile, Figure 5 shows the details of Gompertz curve – fitting graph of cumulative gas production. For GAC immobilized cells, the kinetic parameters obtained for hydrogen production based on data fitted by the modified Gompertz model are $H_m = 88.2$ ml, $R_m = 9.9$ ml/h and $\lambda = 0.45$ hr. Meanwhile, the kinetic parameters obtained for hydrogen production by using GB immobilized cells based on data fitted by the modified Gompertz model are $H_m = 84.3$ ml, $R_m = 10.1$ ml/h and $\lambda = 0.71$ hr.

Both performances by using GAC and GB as support carriers seems effective due to the stable of hydrogen production. From the results obtained, it showed that the best maximum hydrogen production was by using GAC immobilized cells, followed by GB immobilized cells. On the other hand, the shorter lag phase time revealed that the time taken by microorganisms to adapt to the environment was short which contributes to produce hydrogen gas rapidly [11]. It indicated that GAC as a support carrier has a shorter lag phase compared to GB immobilized cells.

Based on Table 2, the highest hydrogen yield (HY) by using support carrier is by using GAC with 1.52 mol H$_2$/mol glucose and the hydrogen production rate (HPR) was 58.50 mmol H$_2$/l.d, followed by GB which was HY = 1.43 mol H$_2$/mol glucose with HPR = 54.84 mmol H$_2$/l.d and the last, MOS with HY = 1.08 mol H$_2$/mol glucose and HPR = 41.14 mmol H$_2$/l.d. It showed that the performance of biohydrogen production by using GAC is more effective compared to GB and MOS. Therefore, it indicated that the proper selection of support carrier in producing hydrogen can influence the performance of biohydrogen production.

**Table 2.** Modified Gompertz equation parameter values for biohydrogen production.

| Samples | Hm (ml) | Rm (ml/h) | $\lambda$ (h) | Yield (HY) mol H$_2$/mol glucose | HPR mmol H$_2$/l.d |
|---------|---------|-----------|---------------|----------------------------------|--------------------|
| Control | 79.03   | 10.28     | 0.89          | 1.24                             | 51.80              |
| GAC     | 88.24   | 9.87      | 0.45          | 1.52                             | 58.50              |
| GB      | 84.25   | 10.09     | 0.71          | 1.43                             | 54.84              |
| MOS     | -       | -         | -             | 1.08                             | 41.44              |

4. Conclusion

The biohydrogen production performance of immobilized cell system with GAC, GB and MOS were investigated in this study. It indicated that the best support carriers for biohydrogen production in this study is by using GAC. Adding GAC resulted in prompt microbial colonization and biofilm development with a hydrogen yield of 1.52 mol H$_2$/mol glucose and hydrogen production rate of 58.50 mmol H$_2$/l.d. This may be due to the protective effect of the GAC carrier of the attached growth biofilm against acidic environment. However, it can be seen that by using these support carriers, they have potential and contributes to enhance the biohydrogen production. Last but not least, it is obvious that dark fermentative biohydrogen production with immobilized cell provides promising advantages for a sustainable hydrogen economy.
Figure 5. Gompertz curve – fitting graph of cumulative gas production for a) GAC; b) GB; c) control sample.
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