Effects of process, operational and environmental variables on biohydrogen production using palm oil mill effluent (POME)

Bidattul Syirat Zainal, Ali Akhbar Zinatizadeh, Ong Hwai Chyuan, Nurul Syuhadaa Mohd, Shaliza Ibrahim

A batch study for biohydrogen production was conducted using raw palm oil mill effluent (POME) and POME sludge as a feed and inoculum respectively. Response Surface Methodology (RSM) was used to design the experiments. Experiments were conducted at different reaction temperatures (30–50 °C), inoculum size to substrate ratios (I:S) and reaction times (8–24 h). An optimum condition of biohydrogen production was achieved with COD removal efficiency of 21.95% with hydrogen yield of 28.47 ml H2 g−1 COD removed. The I:S ratio was 40:60, with reaction temperature of 50 °C at 8 h of reaction time. The study showed that a lower substrate concentration (less than 20 g L−1) for biohydrogen production using pre-settled POME was achievable, with optimum HRT of 8 h under thermophilic condition (50 °C). This study also found that pre-settled POME is feasible to be used as a substrate for biohydrogen production under thermophilic condition.

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Introduction

In the past 15 years, lot of past research focussed on producing biohydrogen using different types of wastewater; namely municipal wastewater, agricultural wastewater, and beverages wastewater [1–4]. This is possibly due to the fuel crisis resulting from fossil fuel resource depletion [5]. Other than the fuel crisis, the combustion of fossil fuel will also lead to the emissions of toxic materials, which is responsible for many environmental problems [6]. In addition to that, it will indirectly contribute to other consequences such as the increase of greenhouse gases (GHGs), the rising of sea levels, the impact on climate change, and the diminishing biodiversity [7].

One of the plausible resources for the biohydrogen production in Malaysia is from the treatment of palm oil-based industry's wastewater as this industry is among the world's biggest palm oil exporter [8]. Regardless of its many useful products, there are some harmful aspects associated with the
production. Tons of wastewater that is produced every day, namely palm oil mill effluent (POME), can endanger the environment. The biogas that is produced from POME during anaerobic treatment is a thought ful challenge resulted from the current production processes [5,9].

POME is categorized as a very high contaminating wastewater that contains a biochemical oxygen demand (BOD) of 25,000 mg L\(^{-1}\) and chemical oxygen demand (COD) of 69, 500 mg L\(^{-1}\) [10]. Some other properties contain in POME that may harm the environment include glycerine, dissolved oil and fatty acids, crude oil solids and any other soluble material [11]. Therefore, direct discharge into the land is not encouraged. According to the Department of Environment Malaysia, in conjunction to the Environment Quality Act 1974, POME must be treated before it is directly released into the environment [12]. Since raw POME normally is discharged at 80–90 °C, therefore several researchers reported that treatment of POME can be done whether in mesophilic or thermophilic conditions [13–15]. Some other studies have been suggested for the treatment of POME. This includes by using evaporation ponds; applying thermal, physicochemical, and biological treatment. For that wastewater to be treated by biological means, its BOD/COD ratio needs to be greater than 0.5 [16] Moreover, biological treatment is also preferable due to its cost effectiveness as compared to chemical treatment.

Production of biogas from POME is widely known by using anaerobic digestion. Since its potential of treating wastes while producing renewable energy, it has become the most studied technology. Anaerobic digestion is a process where organic materials are decomposed in a condition where there is no oxygen present and useful biogas is produced, simultaneously. There are three stages of reactions involved in anaerobic digestion. The first stage is hydrolysis, followed by acidogenesis and lastly methanogenesis [17–19].

Hydrolysis is a process where complex organic compounds are converted (hydrolyse) by fermentative bacteria to simple monomers such as fatty acids, monosaccharides and amino acids. Next, in acidogenesis process, these simple monomers included sugars will be degraded further to acetate, hydrogen (H\(_2\)) and carbon dioxide (CO\(_2\)) [16]. Acetate, H\(_2\) and CO\(_2\) will be the precursors for methane production in methanogenesis process.

For a normal process of microbial fermentation, Hallenback and Ghosh (2009) reported that organic wastes only have about 7.5–15% of energy to be converted to hydrogen, whilst the rest will remain in volatile fatty acids (VFA), i.e. acetate acids (AA), butyric acid (BA), propionic acid (PA) and lactic acid (LA) [20]. VFA will then be converted to methane or any other suitable by-products through a process called methanogenesis [21]. A basic dark fermentation process can be simplified using equation below [22]:

\[
C_6H_{12}O_6 + 4H_2O -> 2CH_3COO^- + 2HCO_3^- + 4H^+ + 4H_2 \quad (1)
\]

Biohydrogen production from POME is not a new study in this field. O’Thong et al. (2008) in their study showed that under thermophilic condition (60 °C), a hydrogen yield of 4.2 L H\(_2\) L\(^{-1}\)waste and COD reduction of 37% was achieved when using POME of 85 g COD L\(^{-1}\) [23]. Other studies reported a hydrogen yield of 4.5 L H\(_2\)/Lwaste and a COD reduction of 40% using high concentration of POME (10–59.3 g L\(^{-1}\) COD) as substrate [24], a hydrogen yield of 0.27 L H\(_2\) g\(^{-1}\) COD and 57% of a COD reduction under thermophilic condition [25] and 51.5 ml H\(_2\) g\(^{-1}\) COD of hydrogen yield with 15.1% of a COD reduction using initial COD of 5–35 g L\(^{-1}\) COD at 55 °C [26], respectively.

Whether it is mesophilic and thermophilic in anaerobic treatment, both conditions give different impacts on the COD removal, biohydrogen production yield and rate of POME. Oh et al. (2003) in their study of effects of temperature (25–40 °C) on hydrogen production concluded the increasing of temperature from 25 to 36 °C also improved the cell growth rate and hydrogen production rate. They also reported that at 36 °C, maximum hydrogen yield was achieved (i.e. 2.49 mol H\(_2\) mol\(^{-1}\) glucose) [27]. Chong et al. (2009) in their study using Clostridium sp. that extracted from the mixed cultures of POME anaerobic sludge reported that at 37 °C, total accumulated hydrogen gas was higher (i.e 3 L H\(_2\) day\(^{-1}\)) [28]. Other study done by Lee et al. (2006) reported that temperature above 35 °C may inhibit the growth of the granular sludge [29]. Meanwhile, with thermophilic condition, Mamimin et al. (2015) reported that this condition is good for POME to be converted to H\(_2\) as it has less variety of end-products, thermodynamic condition as well as has low inhibition of hydrogen partial pressure [30].

High production rate of hydrogen was produced in dark fermentation process but with low hydrogen yield [31,32]. The most recent batch study done by Norfadilah et al. (2016) used high concentration of raw POME as a substrate (initial COD concentrations were 32 g L\(^{-1}\) to 86 g L\(^{-1}\)), and they reported a COD removal of approximately 37% with the maximum hydrogen yield of 5.98 L H\(_2\) L\(^{-1}\) COD at 10% POME sludge [33]. Additionally, a study done byMohammadi et al. (2012) using pre-settled POME as substrate reported that the highest hydrogen yield calculated was 124.48 mmol H\(_2\) g\(^{-1}\) COD removed with COD removal of 54.2% [34]. However, the study was done at low COD influent of 3 g L\(^{-1}\) COD – 10 g L\(^{-1}\) COD.

Based on the presented studies, most of the researchers were done at high substrate concentrations (>20 g L\(^{-1}\) COD). It is also understood that using high strength wastewater as a substrate in a mesophilic or thermophilic condition could increase the COD removal efficiency by increasing the rate of degradation along with the biogas production [15,35]. However, Poh et al. (2009) reported that in Malaysia, only mesophilic temperature conditions are conducted for anaerobic POME treatments [36].

Thus, this study was initiated to study biohydrogen production of POME in a dark fermentation process. Raw POME was used as a substrate and POME anaerobic sludge as an inoculum. The inoculum sizes (10:90\% inoculum), reaction times (8–24 h) and mesophilic to thermophilic conditions (30–50 °C) were varied to study their effects on biohydrogen production and its COD removal efficiency. For the optimization study, (RSM) was applied for hydrogen yield and COD removal efficiency. In this study, the substrate concentrations were varied from a low concentration of <20 g L\(^{-1}\) to high concentration of >20 g L\(^{-1}\) CODin.
Materials and methods

Inoculum preparation

The POME sludge was taken from an anaerobic pond and was obtained from Jugra Palm Oil Mill, Banting, Selangor, Malaysia and used as inoculum. The inoculum was prepared at 5.4 g MLVSS L⁻¹. Prior to its use, 100 °C for an hour was applied to the sludge using water bath to promote hydrogen-producing bacteria (HPB) and to suppress the hydrogenotrophic methanogens [37]. The chemical parameters of the seed sludge were presented in Table 1.

Pre-settled POME (substrate) preparation

The substrate was the POME collected from Jugra Palm Oil Mill, Banting, Selangor, Malaysia after acidification process. Prior to its use, raw POME was allowed to settle before the supernatant was extracted and used as a substrate. The supernatant was called pre-settled POME. The characteristics of the substrate were presented in Table 1.

Batch study

Using a working volume of 100 ml, a batch study was conducted using 156 ml serum bottles [38]. The reaction starts right after the substrate was mixed with inoculum with initial COD of 32,320 mg L⁻¹ for 10:90 (I:S; v/v), initial COD of 28,720 mg L⁻¹ for 20:80 (I:S; v/v) and initial COD of 16,720 mg L⁻¹ for 40:60 (I:S; v/v), without any pH adjustment. Then, the pH mixture was adjusted to pH 5.5 ± 0.2 with 1 N HCl or 1 N NaOH. Prior to the test, the serum bottles were purged with nitrogen gas for 10 min at 10 ml min⁻¹ in order to create anaerobic conditions. All batch tests were conducted based on the RSM data using Design Expert® Software (StatEase Inc., version 7.0.0) shown in Table 2. The total biogas composition was monitored by gas chromatography. The liquid samples were analysed for effluent COD at 8, 16 and 24 h intervals.

Analytical methods

Every 8 h interval, chemical oxygen demand (COD) were measured. Meanwhile, volatile suspended solids (VSS) and total suspended solids (TSS) were analysed at initial experiment. All the tests were conducted according to APHA Standard Methods 2540 D, 2540 G, and 5220 D, respectively [39].

By using GC (Perkin Elmer, AutoSystem Gas Chromatograph, 600 Series LINK), the composition of the biogas was analysed using a pack GC column Supelco, 40/80 carboxen 1000, MR2924D, 10’ × 1/8’ and thermal conductivity detector (TCD). At flowrate of 30 ml min⁻¹, carrier gas used was high purity argon. Oven, injector and detector's temperature were set to 100 °C, 150 °C and 200 °C respectively. A 0.5 ml gas tight syringe 2500 μ Hamilton, USA was used for gas sampling for injection purposes.

Kinetic study

In this batch mode study, H₂ production from POME was analysed and optimized by applying central composite design (CCD) in RSM. The suggested design mode was quadratic, with 20 runs (six replicates at central points). The three factors, namely I:S, reaction temperature and fermentation time were used. Table 2 showed the experimental conditions based on CCD design for biohydrogen production from POME. Each factor in the design was assessed at low, high and central levels in order to estimate the experimental variability, design and corresponding experimental results. A three-dimensional (3D) plots were presented in Figs. 1 and 2 to interpreted the effects of the variables studied and results were analysed by using ANOVA. Meanwhile, by using Eq. (2), the coefficients of the polynomial model were obtained [40];

\[ Y = \beta_0 + \beta_iX_i + \beta_jX_j + \beta_{ij}X_iX_j + \beta_{i2}X_i^2 + \beta_{j2}X_j^2 + \beta_{ij}X_iX_j + \ldots \]  \( (2) \)

where \( \beta \) is the regression, \( i \) is linear and \( j \) is a quadratic coefficient, respectively. Based on Figs. 1 and 2, the effects of relation between both variables and responses were generated. The values were presented using ANOVA are shown in Table 3.

Results and discussion

COD removal efficiency

Based on Table 3, Model F-value of 22.32 indicates that the model is significant. Values of Prob > F less than 0.05 represents that the model terms are significant. For Response 1 on COD removal efficiency, only variable A is a significant model term. Some of the insignificant model terms were removed to make the model simplified. The reduced quadratic model equation for the response is reported below:

\[ \text{COD rem. eff. } = 40.24 - 9.87A - 1.42B + 1.11C + 2.07AB + 0.89AC - 7.08C^2 \]  \( (3) \)

From the 3D response surface plots (Fig. 1), COD removal efficiency was increased as the I:S increased at 30 °C for 8
Table 2 – Central Composite Design Experimental Conditions and Results (with 6 replicates at central point).

<table>
<thead>
<tr>
<th>Run</th>
<th>I:S</th>
<th>Variables</th>
<th>COD removal efficiency (%)</th>
<th>H₂ Yield (mL H₂/g CODrem.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Temperature (°C)</td>
<td>Reaction time (hrs)</td>
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<tr>
<td>2</td>
<td>20:80</td>
<td>40</td>
<td>16</td>
<td>42.25</td>
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<tr>
<td>9</td>
<td>10:90</td>
<td>50</td>
<td>24</td>
<td>41.67</td>
</tr>
<tr>
<td>7</td>
<td>10:90</td>
<td>40</td>
<td>16</td>
<td>49.09</td>
</tr>
<tr>
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<td>40</td>
<td>8</td>
<td>33.43</td>
</tr>
<tr>
<td>3</td>
<td>40:60</td>
<td>40</td>
<td>16</td>
<td>27.91</td>
</tr>
<tr>
<td>11</td>
<td>10:90</td>
<td>30</td>
<td>8</td>
<td>49.50</td>
</tr>
<tr>
<td>12</td>
<td>20:80</td>
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<tr>
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<td>42.71</td>
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<td>8</td>
<td>35.07</td>
</tr>
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<td>40</td>
<td>16</td>
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<td>41.32</td>
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<td>24</td>
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<td>20:80</td>
<td>40</td>
<td>16</td>
<td>44.57</td>
</tr>
<tr>
<td>10</td>
<td>40:60</td>
<td>50</td>
<td>24</td>
<td>24.72</td>
</tr>
</tbody>
</table>

Fig. 1 – 3D image of effects of I:S and reaction temperature (°C) on COD removal efficiency (%) at 8 h reaction time, 16 h reaction time and 24 h reaction time.
reaction time. However, COD removal efficiency decreases when reaction temperature was increased to thermophilic condition (50 °C). This trend can also be seen for reaction times of 16 h and 24 h. These interactions imply that temperature (B) and reaction time (C) were not significantly affecting COD removal efficiency. Fig. 1 also showed that the higher the I:S ratio (<20 g L⁻¹ COD), the lower the COD removal efficiency was. The highest COD removal efficiency of 49.50% reported to be at 30 °C for 8 h reaction time at the influent COD concentration of 32,320 mg L⁻¹ with I:S of 10:90.

Other similar findings also have been reported by Khemkhao (2011) by using high strength wastewaters as a substrate [35]. The COD removal efficiency was increased when organic loading rate (OLR) was increased. However, they reported that sudden increase of OLR might have an impact on the process stability. Therefore, the reactor needs to be adapted to the new condition before resulting in more than 80% COD removal efficiency.

A high amount of organic acids, carbohydrates, proteins and lipid in POME can make POME as a good source of substrate for the mixed cultures in the POME sludge. A different study done on the start-up of 2.5 L UASFF bioreactor using acclimated POME sludge as inoculum and pre-settled POME as a substrate showed that an acclimated POME sludge can remove the substrate COD by more than 20% as well as a higher hydrogen yield. A pre-treatment of heat-treated sludge also contributed to suppress the methanogenic bacteria and enhanced hydrogen producing bacteria. COD removal efficiency of 49.50% by using dark fermentation was slightly similar with other studies reported by using the same substrate [41]. The complex organic compounds found in POME is an indicator of the low COD removal efficiency recorded at higher I:S (40:60), as shown in Fig. 1.

**Hydrogen yield**

From Table 3, as the model F value is equal to 14.24, hydrogen yield regression model is significant. The R-squared (R²) value was reported at 0.84. After simplifying the quadratic model, the regression equation for the response is as follows:

![Fig. 2 – 3D image of effects of I:S and reaction time (h) on hydrogen yield (ml H₂ g⁻¹ COD removed) at reaction temperature of 30°C, 40°C and 50°C.](image_url)
ANOVA for response surface reduced quadratic model.

Table 3 - ANOVA for response surface reduced quadratic model.

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Prob &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Response COD removal efficiency</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model</td>
<td>1297.78</td>
<td>216.30</td>
<td>22.32</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>A-I:S</td>
<td>974.13</td>
<td>194.13</td>
<td>100.51</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>B-Temperature</td>
<td>20.10</td>
<td>20.10</td>
<td>2.07</td>
<td>0.1735</td>
</tr>
<tr>
<td>C-Reaction time</td>
<td>12.35</td>
<td>12.35</td>
<td>1.27</td>
<td>0.2793</td>
</tr>
<tr>
<td>AB</td>
<td>34.26</td>
<td>34.26</td>
<td>3.53</td>
<td>0.0827</td>
</tr>
<tr>
<td>AC</td>
<td>6.29</td>
<td>6.29</td>
<td>0.65</td>
<td>0.4351</td>
</tr>
<tr>
<td>C²</td>
<td>250.65</td>
<td>250.65</td>
<td>25.86</td>
<td>0.0002</td>
</tr>
<tr>
<td>R-Squared</td>
<td>0.9115</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adj R-Squared</td>
<td>0.8707</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack of Fit</td>
<td>102.97</td>
<td>12.87</td>
<td>2.79</td>
<td>0.1363</td>
</tr>
</tbody>
</table>

2. Response H₂ Yield (ml H₂/g COD removed)                      |                |             |         |          |
| Model                | 17.25          | 3.45        | 14.24   | <0.0001  |
| A-I:S                | 10.43          | 10.43       | 43.02   | <0.0001  |
| B-Temperature        | 0.83           | 0.83        | 3.41    | 0.0861   |
| C-Reaction time      | 2.36           | 2.36        | 9.74    | 0.0075   |
| AC                   | 1.06           | 1.06        | 4.36    | 0.0555   |
| B²                   | 2.58           | 2.58        | 10.65   | 0.0057   |
| R-Squared            | 0.8357         |             |         |          |
| Adj R-Squared        | 0.7770         |             |         |          |
| Lack of Fit          | 2.61           | 0.29        | 1.85    | 0.2577   |

H₂ Yield = 2.39 + 1.02A + 0.29B + 0.29B² - 0.49C - 0.36AC + 0.72B² (4)

The 3D response surface as shown in Fig. 2 are based on Eq. (4). The coded value for A, B and C represents I:S (A), temperature (B) and reaction time (C). From the response surface plots (Fig. 2), the increment of hydrogen yield has been observed as the I:S decreased from 32,320 mg L⁻¹ (10:90) to 16,720 mg L⁻¹ (40:60) at initial reaction time of 8 h. However, as the reaction time increased to 24 h, hydrogen yield decreased. These interactions implied that I:S and reaction time may significantly affect the hydrogen yield. The findings of this study are similar with the study done by Kotsopoulos and colleagues. They reported that optimum hydraulic retention time (HRT) of 8–14 h were believed to produce optimum hydrogen production [42].

Additionally, the designed test was monitored for a fermentation time of 24 h in order to understand the variation during the fermentation period in producing maximum hydrogen yield without shifting the process towards methanogenesis. Other than that, this study also showed that the reaction (fermentation) time have significant effects on the hydrogen yield. Based on Fig. 2, the maximum amount of hydrogen yield of 28.47 ml H₂ g⁻¹ COD removed was detected at I:S of 40:60 (initial COD concentration of 16,720 mg L⁻¹) at 8 h reaction time and 50 °C of reaction temperature (Table 2 and Fig. 2). Subsequently, at reaction temperature of 50 °C for I:S of 20:80 with reaction time of 8 h, hydrogen yield of 15.84 ml H₂ g⁻¹ COD removed was detected. In pre-settled POME, the concentration of organic acids might be high, thus a low hydrogen yield detected at a longer reaction time in the dark fermentation process. This might also inhibit the metabolism of specific hydrogen producing bacteria which then lower the responses.

Optimization

A numerical optimization was chosen with the criteria of A (I:S), B (temperature) and C (reaction time) was set in range, and the goal was to maximize hydrogen yield. Based on the desirability of 0.98, I:S ratio at 40:60 with 50 °C and 8 h reaction time was the optimum condition to maximize hydrogen yield, with COD removal efficiency of 21.95%. The 3D plot of the optimization is shown in Fig. 3.

Based on Fig. 3, the highest desirability (0.98) was achieved when I:S ratio was 40:60 under thermophilic condition (50 °C) with lowest HRT of 8 h. The 3D surface showed the higher the I:S ratio, the lowest the desirability under 50 °C. Meanwhile, the counter plot of I:S ratio versus temperature shows the highest COD removal efficiency (45.78%) can be achieved at I:S ratio of 10:90 under mesophilic condition (i.e 30 °C). The trend in its 3D plot can be distinguished by the highest the I:S ratio, the lowest the COD removal efficiency. In 3D surface response of hydrogen yield, at 50 °C, the highest hydrogen yield (i.e. 3.69 ml H₂ g⁻¹ COD rem) reached at I:S ratio of 40:60. 3D plot showed that at 50 °C, the maximum hydrogen yield shall be obtained when I:S ratio increases.

In dark fermentation process, as HPB in POME sludge may tolerate at high temperatures, therefore the role of temperature is very important. A study done by Mansor et al. (2016) shows that high value of total volatile fatty acids (TVA) was reported at 55 °C during fermentation of using POME [43]. This finding was concluded by them that at this temperature, it is considered as a good condition for biohydrogen production. Other studies also has been proven by Kotsopoulos et al. (2006)
using extra thermophilic condition (70 °C) for biohydrogen production [42]. All these findings using high temperature for biohydrogen production also shows that either the metabolic pathway or the microbial community in the fermentation are shifted during the process.

Conclusions

Based on the experimental study, it can be concluded that POME sludge as inoculum and raw POME as a substrate are feasible for biohydrogen production. The main findings found in this study are:

i) Inoculum size and reaction time does have a significant effect on the hydrogen yield;

ii) Temperature and reaction time are not significant factors on the COD removal efficiency; however, it was found that inoculum size has significant effect on it;

iii) The highest desirability (0.981) was achieved at constant reaction time of 8 h. This is paralleled to a 21.95% of COD removal efficiency and highest hydrogen yield of 28.47 ml H₂ g⁻¹ CODremoved at 50 °C with 40:60 I:S ratio.

For a future study, a scale-up of biohydrogen reactor using raw POME and POME anaerobic sludge need to be examined under thermophilic condition with a short HRT. The performance of the bioreactor can be distinguished by its hydrogen production rate, hydrogen yield as well as its COD removal efficiency.

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