# BIOHYTHANE PRODUCTION FROM PALM OIL MILL EFFLUENT USING TWO-STAGE ANAEROBIC DIGESTION – A PRELIMINARY STUDY

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## ABSTRACT

*The present study investigates the technical possibilities of biohythane (biohydrogen and biomethane) production from palm oil mill effluent (POME) using two-stage anaerobic digestion (AD). Biohydrogen was produced from a thermophilic dark fermenter (TDF) whereas biomethane was produced from a thermophilic anaerobic contact digester (TACD). The HRT applied in TDF and TACD was 3.75 and 6.25 day, respectively. The pH of TDF was adjusted to 5.5 by sludge recirculation from TACD. The biogas produced in TDF and TACD contain 26.20 % $H_2$ and 67.08 % $CH_4$, respectively. The $H_2$ and $CH_4$ yield was 0.261 L/g $COD_{removed}$ and 0.342 L/g $COD_{removed}$, respectively with total energy recovery equivalent to 665.29 MJ/$m^3$ POME. Only 2.07 % of this energy was contributed by $H_2$ and the remaining was dominated by $CH_4$.*

**Keywords:** Hydrogen, methane, two-stage, anaerobic digestion, palm oil mill effluent.

## INTRODUCTION

Anaerobic digestion (AD) is a feasible method for the treatment of effluent containing high concentration of organic matters such as palm oil mill effluent (POME) because it has the ability to produce renewable energy gain in the form of $CH_4$. The two-stage AD process has been reported as a practical biotechnology to produce biohythane (biohydrogen and biomethane) from a variety of organic materials [1]. Hydrogen is a clean alternative energy to get attention because it is environmentally friendly and has an end product of water after combustion.

Recently, investigation of two-stage AD of POME have been reporting using different bioreactor combinations and operational conditions [2-6]. These studies demonstrated varied treatment efficiency with promising biohythane production. However, these studies involved several practices to stimulate the two-stage AD of POME. Short hydraulic retention time (HRT) and high organic loading rate (OLR) was applied in two-stage AD using diluted POME [4]. Chemicals were used to adjust proper operating pH and alkalinity for biohydrogen production [3]. Furthermore, micronutrient was added and C:N:P of POME was adjusted to selected ration in previous study [2].

To our knowledge, such practices may be impractical in industrial scale treatment system. To overcome this issue, this study was conducted without chemicals or nutrients addition as well as dilution to avoid the modification of physicochemical characteristics of POME. This research aims to develop two-stage AD system using a thermophilic dark fermenter (TDF) and a thermophilic anaerobic contact digester (TACD) and investigate its technical possibilities for biohythane production from POME.

## MATERIALS AND METHODS

### Collection of POME and inoculum

POME was obtained from a palm oil mill located at Nibong Tebal, Pulau Pinang, Malaysia. POME was collected from a holding tank before discharge to the cooling pond then stored in refrigerator at 4 °C to minimize microbial activities. POME was preheated to 55 °C prior to experiment. Table 1. shows the typical physicochemical characteristics of POME. Inoculums for two-stage AD was collected from laboratory scale thermophilic anaerobic digester treating POME which have been operated for more than 12 months.

**Table 1.** Characteristics of POME used in this study.

| Parameter | Range | Mean ± SD |
|---|---|---|
| pH | 4.4 – 4.7 | 4.5 ± 0.1 |
| COD | 70600 – 77560 | 74820 ± 2312 |
| TSS | 16000 – 21500 | 18688 ± 1953 |
| VSS | 15600 – 21000 | 17567 ± 2084 |
| O & G | 3950 – 5600 | 4683 ± 565 |

* All parameters are in mg/L except pH.

### Equipment setup

Two 6 L water jacketed bioreactors were used as semi-continuous stirred tank reactor (SCSTR). One SCSTR with working volume of 3.75 L was worked as thermophilic dark fermenter (TDF) while another SCSTR with working volume of 6.25 L worked as thermophilic anaerobic contact digester (TACD). A 2 L settling tank was installed for liquid-solid separation. The operating temperature of 55 °C were maintained by heating bath circulator. The daily withdrawal and feeding of substrate were done by means of a peristaltic pump. Intermittent mixing at 120 rpm in 1 minutes for every 45 minutes was performed

by an overhead stirrer with preprogramed timers. A 40 L Tedlar gas sampling bag was connected to both SCSTR for biogas collection.

**Operation of two-stage AD**

First, POME was fed into TDF for biohydrogen production (Figure 1.). After that, the dark-fermented POME (DF-POME) was fed into TACD for biomethane production. The anaerobically digested (AD-POME) mixture was allowed to settling for 1 hour. The sludge recirculation ratio was fixed to 1 where 45 % returned to TDF and 55 % returned to TACD. Excess sludge combined with supernatant to become AD-POME. The TDF operated at organic loading rate (OLR) of 19.95 ± 0.39 g COD/L.d which equivalent to hydraulic retention time (HRT) of 3.75 days. The HRT of TACD was 6.25 days.

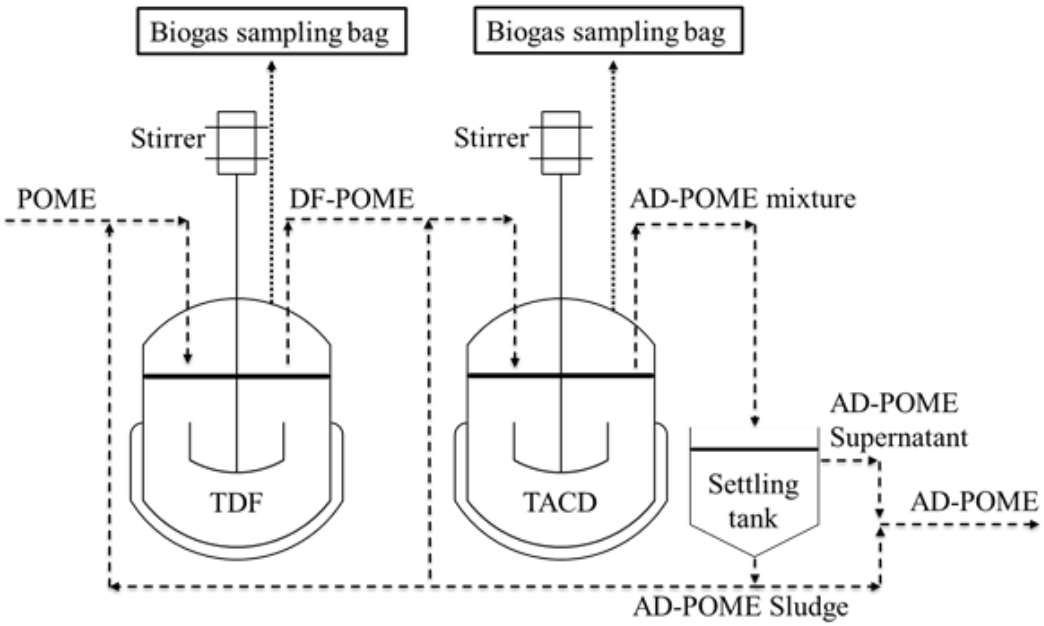


**Figure 1.** Schematic diagram of two-stage AD of POME.

**Analysis of samples**

The measurement of pH, total alkalinity (TA), chemical oxygen demand (COD), total suspended solid (TSS), mixed liquor volatile suspended solid (MLVSS) were according to APHA Standard Method [7]. Free fatty acid (FFA) was analyzed using GC system (Shimadzu GC-2010 Plus) with a flame ionization detector (FID) and equipped a BP 21, 25 m × 0.22 mm ID × 0.25 µm (SGE). Biogas volumes were recorded using a graduated gas tight syringe. Biogas compositions were analyzed using Clarus 500 Gas Chromatography (Perkin Elmer).

**RESULTS AND DISCUSSION**

Sludge recirculation effectively stabilized TACD at pH value of 8.1 and TA of 7165 mg $CaCO_3$/L along the experimental period (Table 2.). By recirculating AD-POME sludge from TACD, the pH in TDF was daily adjusted to 5.5 for biohydrogen production. The pH in TDF was slightly dropped to 5.2 due to high TA of 2134 mg $CaCO_3$/L which buffering the dark fermentation process in a stable pH conditions.

**Table 2.** pH, TA and MLVSS of two-stage AD.

| Two-stage AD | pH | TA, mg $CaCO_3$/L | MLVSS, mg/L |
|---|---|---|---|
| **TDF** | 5.2 ± 0.1 | 2134 ± 156 | 18319 ± 736 |
| **TACD** | 8.1 ± 0.1 | 7165 ± 140 | 12429 ± 742 |

The decline of pH in TDF was due to remarkable FFA production, as shown in Table 3. Accumulation of FFA in DF-POME was observed, especially for acetic acid (9444 mg/L), propionic acid (818 mg/L) and butyric acid (4518 mg/L). The butyric acid and acetic acid ratio (B/A) in TDF 0.326, which is relatively closed to the results obtained from O-Thong et al. [6]. Previous research on biohydrogen production from POME by thermophilic fermentation process had recorded B/A ratio ranged from 0.30 to 1.20 [8]. However, high concentration of FFA either in dissociated or undissociated form may inhibit biohydrogen production [9]. Only trace amount of FFA remained in AD-POME which is palmitic acid (195 mg/L), stearic acid (46 mg/L) and oleic acid (160 mg/L). This indicating most of the FFA were rapidly degraded and utilized by anaerobic microorganisms. The degradation of COD and TSS in two-stage AD of POME was 78.5 and 62.0 %, respectively.

**Table 3.** FFA of POME, DF-POME and AD-POME in this study.

| FFA, mg/L | POME | DF-POME | AD-POME |
|---|---|---|---|
| Acetic acid | 2546 ± 250 | 9444 ± 865 | < MQL |
| Propionic acid | 162 ± 46 | 818 ± 65 | < MQL |
| Butyric acid | 287 ± 77 | 4518 ± 939 | < MQL |
| Valeric acid | 79 ± 12 | 65 ± 7 | < MQL |
| Caproic acid | 129 ± 11 | 419 ± 15 | < MQL |
| Enanthic acid | 126 ± 12 | < MQL | < MQL |
| Myristic acid | 47 ± 16 | < MQL | < MQL |
| Palmitic acid | 1790 ± 151 | 1500 ± 126 | 195 ± 43 |
| Stearic acid | 131 ± 21 | 114 ± 15 | 46 ± 8 |
| Oleic acid | 1381 ± 156 | 1032 ± 70 | 160 ± 56 |
| Linoleic acid | 141 ± 19 | 157 ± 28 | < MQL |

MQL = Method detection limit

The biogas in TDF contained 26.20 % of $H_2$ whit production rate of 1.4 $m^3/m^3$ POME (Figure 2.). In TDF, methanogenic activity was inhibited because $CH_4$ was not detected. Whereas the biogas in TACD contained 67.08 % of $CH_4$ with production rate of 20.07 $m^3/m^3$ POME. This study shows that a two-stage AD of POME able to produce biogas of 35.213 $m^3/m^3$ POME containing 3.97 % of $H_2$ and 56.92 % of $CH_4$. The total energy of approximately 665.29 MJ /$m^3$ POME can be recovered from it. Among this, the energy was dominated by $CH_4$ because $H_2$ only contributed to 2.07 % of it. These energy recoveries were calculated based on density of $H_2$ and $CH_4$ of 0.0816 and 0.6492 kg/m3, respectively, at 28 °C and 1 atm and lower heating value of $H_2$ and $CH_4$ of 121 and 50 MJ/kg, respectively.

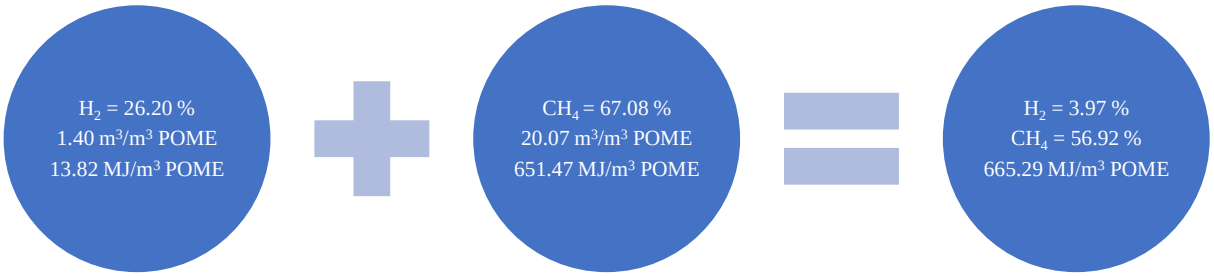


**Figure. 2** Biohythane and energy production from two-stage AD of POME.

The performance of current research of two-stage AD of POME for biohythane production was summarized in Table 4. These studies demonstrated promising biohythane production using different bioreactor combinations and operating conditions. However, it should be noted that, current research involved the use of diluted POME, enriched inoculum, adjusting pH and alkalinity using chemicals, adding micro nutrient as well as balancing the C:N:P to a selected ratio [2-6]. These practices may stimulate biohythane production but its practicality and economic feasibility remain questionable in industrial scale application. Instead of focus in treatability study, future research should consider the operational conditions in laboratory scale investigation that able to mimic performance of industrial scale application.

**Table 4.** Current research of two-stage AD of POME for biohythane production

| Bioreactor (T, °C) | HRT, d | OLR, g COD/L.d | Biogas content, % | Biogas production rate, L/L.d | Biogas yield, L/g $COD_{removed}$ | Ref. |
|---|---|---|---|---|---|---|
| ASBR (55) | 2 | 60 | 55, $H_2$ | 1.8 | 0.210 | [5] |
| UASB (28 - 34) | 15 | 6 | 73, $CH_4$ | 2.6 | 0.315 | |
| UASB (55) | 2 | 75 | 55, $H_2$ | 1.92 | 0.215 | [2] |
| CSTR (37) | 5 | - | 70 – 80, $CH_4$ | 3.2 | 0.320 | |
| CSTR (55) | 2 | 14.3[a] | 55, $H_2$ | 1.31 | 0.180 | [6] |
| UASB (37) | 15 | 1.58[a] | 73, $CH_4$ | 1.18 | 0.271 | |
| UASB (55) | 0.375 | 75 | 35, $H_2$ | 2.1[b] | 0.075 | [3] |
| CSTR (55) | 12 | 12 | 65, $CH_4$ | 13[b] | 0.156 | |
| UASB (55) | 0.5 | 50 | 45.08, $H_2$ | 2.5[b] | 0.033 | [4] |
| CSTR (60) | 5 | 13.1 | 67.74, $CH_4$, | 10.58[b] | 0.11 | |
| TDF (55) | 3.75 | 19.95 | 26.20, $H_2$ | 0.37 | 0.261 | This |

| | | | | | | |
|---|---|---|---|---|---|---|
| TACD (55) | 6.25 | - | 67.08, $CH_4$ | 3.21 | 0.342 | study |

[a] Self-estimated; [b] Unit in L/d.

In this study, the $H_2$ and $CH_4$ yield was 0.261 and 0.342 L/g $COD_{removed}$, respectively, the highest among the published reports (Table 4). These observations also implied high efficient of mixed inoculum for biohythane production. Besides providing TA to buffer stable pH conditions in TDF, the sludge recirculation returned indigenous hydrogen producing bacteria to replace those already wash out. Yet, the recorded $H_2$ production rate was much lower than current research. The experimental results indicated TDF was operated in suboptimal condition which may be linked to the comparatively long HRT that caused accumulation of FFA. Operational control such as HRT and sludge recirculation ratio should be modified to optimize biohythane production as well as treatment efficiency.

## CONCLUSION

Biohythane production using two-stage AD of POME was demonstrated in this study. Total energy of 665.29 $MJ/m^3$ POME could be recovery in biogas which containing 3.97 % of $H_2$ and 56.92 % of $CH_4$. The degradation of COD and TSS in two-stage AD of POME was 78.5 and 62.0 %, respectively. The sludge recirculation from TACD able to adjust the pH in TDF to optimum value of 5.5. Low $H_2$ production in TDF was observed which may be due to its suboptimal operating conditions.

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