The Co-Production of Hydrogen and Methane from Dark Fermentation of Mixed Palm Oil Mill Effluent and Aquaculture Wastewater: Gompertz Modelling and Sludge Recovery

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The potentials for non-stop hydrogen and methane production employing an ideal loading mixture of palm oil mill effluent (POME) and aquaculture wastewater (AWW) in a double-phase digester at a thermophilic state are presented. Different organic loadings were studied such as 31, 41, 51 and 61 Kg COD/(m^3 d) for the generation of hydrogen; 9, 11, 14 and 16 kg COD/(m^3 d) for the synthesis of methane. In a UASB reactor, hydrogen production was kept under control with a constant HRT of 12 h. At the loading of 51 kg COD/h, the maximal H_2 content, volumetric H_2 generation rate and H_2 yield were observed as 46%, 6 L H_2/d and 34 mL H_2/g COD, respectively (m^3 d). After an HRT of 6 days, the substrate from the hydrogen digester was further fermented into methane in the CSTR digester. At an organic loading rate of 14 kg COD/h, the highest volumetric CH_4 generation rate and yield were 11 L CH_4/d and 0.13 m^3 CH_4/kg COD, respectively (m^3 d). This two-stage procedure removed 92% of the chemical oxygen requirement overall. Based on the findings, the Gompertz modeling was a good fit for the cumulative methane generation patterns, with a strong correlation coefficient (> 0.994). Sludge recovery was 0.07 m^3 sludge/m^3 wastewater and water recovery was 0.82 m^3/m^3 wastewater. This double-phase technique has the potential to contribute greatly to the development of a comprehensive waste management plan, including the digestion of palm oil mill effluent and aquaculture wastewater.

Keywords: Aquaculture wastewater, Dark fermentation, Hydrogen production, Methane production, Fertilizer recovery.

INTRODUCTION

Carbon molecules are converted into energy products (biogas) through the complex series of complex events known as anaerobic digestion under anaerobic conditions [1]. Smaller amounts of oxygen, water and hydrogen sulfide (50-2000 ppm) as well as methane (55-70%) and carbon dioxide (30-45%), make up the majority of the anaerobic digestion biogas [2]. Methane and carbon dioxide also make up the majority of the biogas produced by anaerobic digestion. Creating biogas, a renewable energy source, by anaerobic digestion is an economical procedure [3]. Anaerobic digestion can provide sustainable methane gas, lessening the world's reliance on fossil fuels, while getting rid of odour and hazardous microbes found in sewage sludge [4]. Renewable methane energy may help offset some of the energy requirements of the sewage sludge anaerobic digestion equipment, allowing for the achievement of carbon neutrality sooner [5].

A fresh and renewable energy source with no carbon emissions is hydrogen. During combustion, all that is produced as a byproduct is water [5]. In comparison to thermochemical approaches, fermentation is proven to be more energy efficient, practical and environmentally beneficial [6]. The total chemical oxygen demand of the 16 million tons of POME produced by Malaysian palm oil mills each year is about 94,000 mg/L, making it high-strength complex wastewater [7]. Straight release of POME into the environment can harm the physico-chemical properties of the soil, contaminate the groundwater and possibly even have an impact on the air quality. Due to effective digestion of substrates and stable energy production, biohydrogen synthesis utilizing POME as a substrate has garnered a lot of interest [4]. Hydraulic retention time, organic loading rates, pH and temperatures are just a few of the operational factors that affect how quickly organic matter is hydrolyzed during dark fermentation. Acidogenic bacteria convert organic substrate

into hydrogen using a process known as dark fermentation that doesn't require light energy. A biological process called anaerobic digestion may be applied to clean substrate and capture CH_4 from the latter [5]. Organic loading rate (OLR) appears to be the key parameter that may influence H_2 generation, microbes and other aspects of the digester's operation, which is an interesting finding [6]. Given the higher level of a volatile fatty acid residue at a higher organic loading rate, a single-phase digester has difficulty achieving energy production and higher organic elimination, which could result in reactor failure [7]. The two-stage process, on the other hand, gets around this issue and boosts process efficiency generally in terms of degradation rate, gas production, simple waste management and energy productivity [8].

Following the synthesis of hydrogen, the effluent is highly concentrated in volatile fatty acids. Anaerobic fermentation is regarded as an appropriate treatment for producing methane, which can be used to produce extra energy from metabolites produced during the hydrogen generation phase. In two-stage fermentation, the first reactor's acidity is controlled, while the second digester's methanogenic consortia are controlled to produce methane [9]. The UASB process, which keeps higher biomass level, higher flexibility and requires no use of a distinct clarifier, has become the primary alternative for creating biogas [10]. Due to more favourable thermodynamic conditions, a smaller diversity of digested byproducts and less H_2 fractional pressure inhibitions, high temperatures represent an intriguing method of converting POME into hydrogen [11].

The Gompertz model became popular in the biogas generation to describe growth and product development data because it is simple and well-suited to batch data [11]. The Gompertz model was used to match the digestion of Typha and found that the hydrolysis process is the main barrier preventing the anaerobic fermentation of Typha [12]. This model was applied to fit the biogas digestion of municipal trash [11]. They found that it correlates well with changes in total gas production and can be used to gauge the effects of changing the ratio of digestion to digestion and biogas digestion. When using the Gompertz model to evaluate the impact of saline sludge on co-fermentation with kitchen waste, feasibility and subsequent energy recovery, there is a significant knowledge gap. The co-fermentation of organic substrates has the potential to yield agricultural irrigation fluid and fertilizer.

Previously, pure substrates including food and farming wastes were used in extensive studies of dark fermentation by single-stage fermentation [13]. But up till now, there hasn't been any investigation into how to optimize the organic loading rates in a double-phase digester using mixed palm oil mill effluent and aquaculture wastewater as substrates at thermophilic states. To evaluate the highest H_2 and CH_4 generation and sludge recovery and digestion of mixed palm oil mill effluent and aquaculture wastewater with different organic loadings, present work aims at the double-phase digester.

EXPERIMENTAL

Sample and seeding source and characteristics: A palm oil mill effluent sample was taken from a commercial waste processing company's receiving tank in Lepar Hilir, Malaysia.

Using $CaCO_3$, the pH and alkalinity of sample were brought down to 5.4 and 1351-1601 mg/L, respectively. Aquaculture wastewater was taken from a fish processing firm in Terengganu province, Malaysia. Ammonium chloride and potassium hydrogen phosphate were used to keep the COD:N:P ratio at 250:7:1. Table-1 lists the properties of raw POME and AWW and displays the mean values of triplicates for each. The POME and AWW were mixed in equal proportion. The inoculum of UASB-H2 reactor was made up of anaerobic sludge that had been heated at 100 °C for 1 h. Without any heat treatment, the industrial-scale anaerobic reactor sludge from a similar plant was utilized as the seeding sludge in the continuously stirred tank reactor for the manufacture of methane.

TABLE-1 QUALITIES OF UNPROCESSED MIXED POME & AWW (50%:50%)		
Parameters	Mixture	Inoculum
Biochemical oxygen demand	3498 ± 300	3502 ± 400
pH	5 ± 0.01	5.2 ± 0.01
Chemical oxygen demand	56499 ± 300	56502 ± 200
Total nitrogen	958 ± 100	961 ± 200
Total carbohydrate	16400 ± 100	16402 ± 100
Total phosphorus	110 ± 3	112 ± 2
NH_4–N	800 ± 100	811 ± 200
Oil	108998 ± 10	109001 ± 10
Phosphate	20 ± 5	22 ± 2
Total solids	31998 ± 200	32001 ± 100
Volatile solids	25998 ± 200	26001 ± 300
Ash	4498 ± 100	4501 ± 100
Iron	1.95 ± 0.1	1.99 ± 0.1
Suspended solids	8298 ± 100	8301 ± 100
*All in mg/L except pH		

Setup and operation of reactor: Hydrogen was produced using a cylindrical 5-L stainless steel UASB reactor. The pH, temperature, organic loading rate and hydraulic retention time during the starting phase were kept at pH 5.4-5.6, 50 °C, 21 kg COD/(m^3 d) and time 12 h, respectively, for 21 days. The organic loading rate range was investigated in increasing increments of 11 kg COD/(m^3 d) from the starting 30 kg COD/(m^3 d) to the final 61 kg COD/(m^3 d). For the manufacture of methane, an 11 L cylindrical, 10 L working-volume continuous stirred tank reactor (CSTR) made of borosilicate glass was employed (Fig. 1). The pH and temperature of the mixed liquid in CSTR were kept under control at 7.4 pH and 60 °C, respectively. The effluent from the UASB was used to fill the CSTR, which ran for 75 days at 120 rpm and a constant HRT of 5 days. Throughout the experimental period, the efficiency of UASB and CSTR in terms of gas output, VFA output and COD elimination effectiveness were tracked.

Modelling: The accumulative methane generation during anaerobic digestion was described using the Gompertz modeling [14]:

$$y(t) = a.\exp[-\exp(b-ct)]$$

where y(t) presents per gram of volatile solids methane (CH_4) accumulation at time t.

The values of the model parameters with its correlation coefficient for generating hydrogen from POME and AWW are given in Table-2.
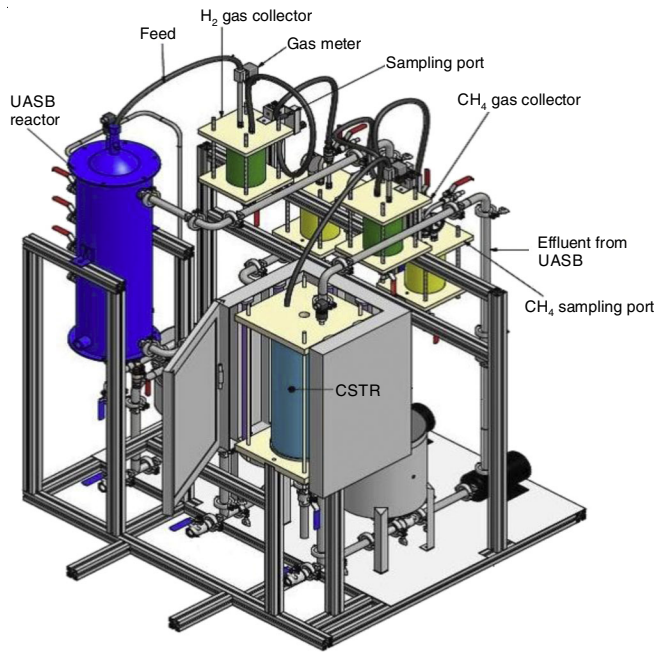
Fig. 1. Schematic diagram of the double-phase digester [Ref. 19]

TABLE-2
GOMPERTZ MODEL'S KINETIC PARAMETERS
FOR GENERATING HYDROGEN FROM
POME AND AWW (50%:50%)

OLR [kg COD/(m³ d)]	Model parameter			Accumulative methane generation (mL)	Correlation coefficient (%)
	a	b	c		
9	15	1.03	0.092	15	0.998
11	29	1.16	0.092	29	0.998
14	34	1.03	0.082	34	0.996
16	18	1.02	0.112	18	0.997

Analytical methods for basic parameters: According to APHA standards, the concentrations of the physico-chemical parameters were measured. Wastewater needs to be adequately described before the anaerobic process [15]. So, the features of substrates were determined by analysis. Following the guidelines of APHA, 2015 [15], the features of the substrates were identified at the wastewater processing Lab of the University Malaysia Terengganu.

RESULTS AND DISCUSSION

The volumetric hydrogen production rate and hydrogen yield were both positively impacted by the gradual rise in the organic loading rate from 31 to 51 kg COD/(m³ d) (Figs. 2

and 3). The quantity observed was 6 L H₂/d and 34 mL H₂/g COD, respectively. At 51 kg COD/(m³ d), the highest VHPR and H₂ yields were found. But when OLR was raised to 61 kg COD/(m³ d), the efficiency of UASB digester suffered. The hydrogen generation also fell, reaching an average of 3.52 L H₂/d, but it was discovered to be greater than the quantities seen at the lowermost organic loading of 31 kg COD/d (m³ d) (Table-3). At 51 kg COD, the highest H₂ concentration of whole biogas was 45% (m³ d) and the average H₂ concentration found at 61 kg COD/(m³ d) was 35%, which was 11% more than the H₂ levels observed at 31 kg COD/(m³ d). The H₂ generation utilizing the UASB digester was more advantageous for higher OLR and low HRT, according to these results. Due to active substrate metabolism, the microbes were able to adapt to greater substrate availability, which led to an early and high H₂ output [14,15]. Since, no methane was found for the used OLR, methanogenic activity was probably repressed during acidogenic activity. It resulted from the pH being maintained at 5.2 to 5.5, which was
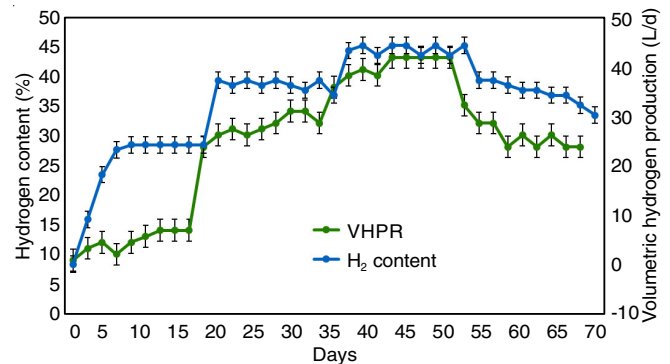

Fig. 2. Hydrogen concentration and volumetric hydrogen production rate (VHPR) in the UASB reactor
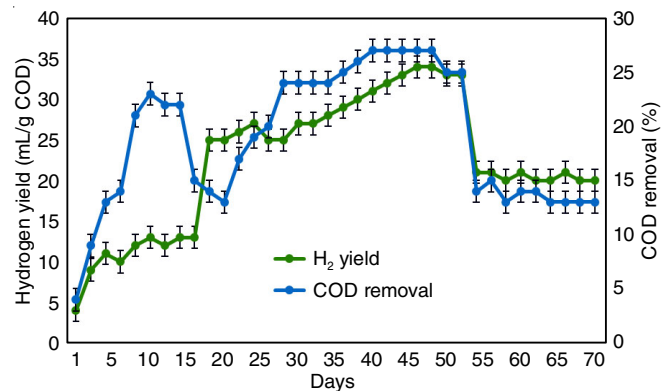

Fig. 3. COD elimination and hydrogen production in the UASB reactor

TABLE-3
UASB HYDROGEN REACTOR'S OLR EFFECTS WITH POME & AWW (50%:50%)

Operating circumstances				
OLR [kg COD/(m³ d)]	31	41	51	61
MLVSS (g/L)	25	36	47	53
HRT (h)	12	12	12	12
pH	4.4 ± 0.01	4.4 ± 0.01	4.4 ± 0.01	4.4 ± 0.01
COD removal (%)	29 ± 5	38 ± 5	46 ± 5	35 ± 5
Hydrogen content (%)	27 ± 3	39 ± 3	46 ± 5	34 ± 5
VHPR (L H₂/d)	1.53 ± 0.01	3.84 ± 0.01	6 ± 0.01	3.5 ± 0.01
Hydrogen yield (mL H₂/g COD)	15 ± 1	29 ± 2	34 ± 1	18 ± 2

unfavourable for methanogens but permitted the growth of acidogenic [16]. Comparatively, POME was used as wastewater in a batch digester at a hydraulic retention time of 3 d and reported 6.6 L H_2/d volumetric hydrogen production and 57% H_2 concentration [17]. The UASB digester showed a maximum COD elimination of 34% at an organic loading of 51 kg COD/(m^3 d), with mean COD removal rates of 26% at 41 kg COD/(m^3 d) and 21% at the lowermost organic loading. With loading conditions, the wastewater breakdown rate rose from 31 to 51 kg COD/(m^3 d) (Fig. 4).
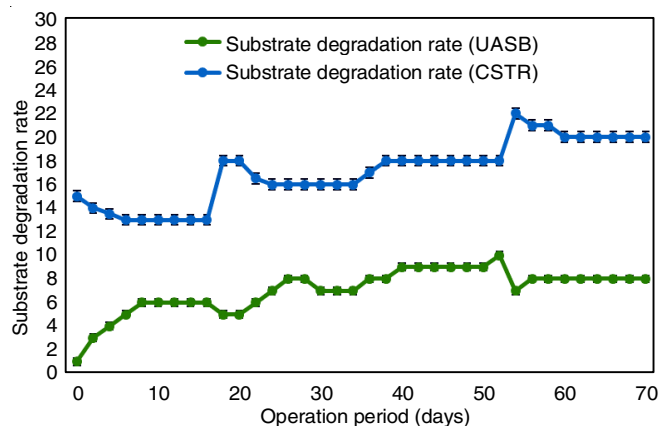

Fig. 4. Degradation of the substrate in the CSTR and UASB

The rate of wastewater decomposition was measured in kilograms of COD reduced, or kg COD (rem). Mean values were 5 kg COD (rem) at 31 kg COD/(m^3 d) in steadystate circumstances, 7 kg COD (rem) at 41 kg COD/(m^3 d) and 8.5 kg COD (rem) at organic loading of 51 kg COD/(m^3 d). The substrate degradation was reduced to 8 kg COD with a further rise in the organic loading to 61 kg COD/(m^3 d) (rem). In contrast, at 61 kg COD/L, the wastewater elimination effectiveness was observed to be at its minimum level (18%). Enhanced organic loading is thought to be a sign of intense biomass concentration circumstances that can make the system hazardous. Khalid *et al.* [18] reported that the fermentation of food wastes at organic loading of 3.1 gVS/L/d generated volatile fatty acid accumulations, confirmed the observed trends. The continuously stirred tank reactor that was kept at a fixed hydraulic retention time of 6 days, received the effluent from the UASB reactor directly. Based on the COD contents of the effluents from UASBR, the organic loading in the continuous stirred tank reactor varied from 8.4 to 16 kg COD/(m^3 d). The continuously stirred tank reactor was effectively run throughout the experiment and the highest VMPR at the organic loading range of 41 and 51 kg COD/(m^3 d) in the UASBR was 11 L CH_4/d (Fig. 5).
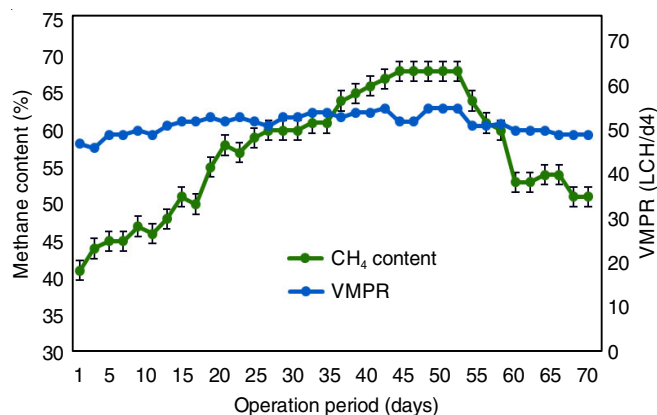

Fig. 5. Methane concentration in CSTR and the volumetric methane production rate (VMPR)

The methane yield was unchanged throughout the trial (0.13 m^3 CH_4/kg COD), however, it decreased to 0.04 m^3 CH_4/kg COD as the organic loading in UASBR was raised to 61 kg COD/(m^3 d) (Fig. 6). This happened because the digested wastewater properties of UASB reactor at 61 kg COD/(m^3 d), made it difficult for methanogenic bacteria to grow because of the accumulation of VFAs. Furthermore, short-chain VFAs were a crucial step in the fermentation process and can limit CH_4 generation in high quantities. Consequently, a significant decrease in pH and a rise in VFAs build-up offset operational failure [19]. Under the specified OLR ranges, the methane level in the continuous stirred tank reactor during methanogenesis varied from 42-68% (Table-4).
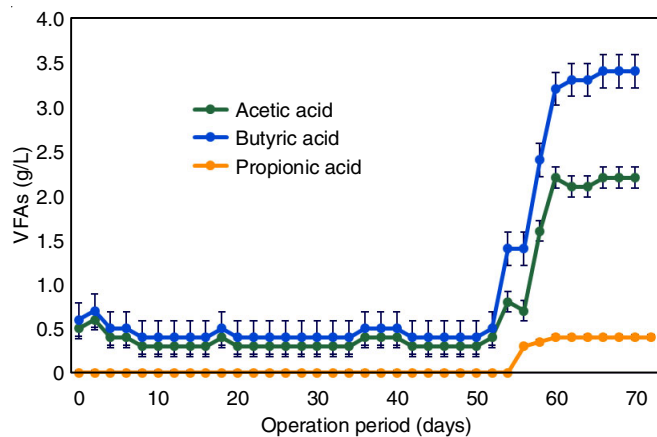

Fig. 6. Production of VFA over time in CSTR

TABLE-4
OLR's IMPACT ON THE CSTR's METHANE REACTOR WITH POME & AWW (50%:50%)

Operating circumstances				
OLR [kg COD/(m^3 d)]	9	11	14	16
MLVSS (g/L)	6	8	12	14
HRT (h)	6	6	6	6
pH	7.3 ± 0.01	7.3 ± 0.01	7.3 ± 0.01	7.3 ± 0.01
COD removal (%)	90 ± 5	92 ± 3	93 ± 3	87 ± 5
Methane content (%)	44 ± 1	56 ± 1	68 ± 2	60 ± 1
VHPR (L H_2/d)	5.5 ± 0.01	9 ± 0.01	11 ± 0.01	7 ± 0.01
Methane yield (mL H_2/g COD)	0.12 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.05 ± 0.01

The loading rates in the UASBR were kept at 51 kg COD/ (m³ d, resulting in an average CH_4 concentration of 67.74%. Similar results were reported by Khalid *et al.* [20] utilizing POME at an HRT of 3 d, with a methane generation rate and methane content of 6 CH_4/L/d and 66%, respectively. The highest OLR of 15.8 kg COD/(m³ d), where the average substrates digestion rate was 22 kg COD (rem), was followed by OLRs of 14, 19 and 11 kg COD/(m³ d), where the average substrate degradation rate was 17 kg COD (rem) and 9 kg COD/(m³ d), where the average substrate degradation rate was 14, on average (Fig. 5). The rate of substrate breakdown was shown to increase with a rise in the organic loading limit, however, COD elimination was observed to decline from 79% to 70%. This behaviour was caused by the effluent properties from UASBR at the greatest organic loading, which contained high levels of acetic acid, butyric acid and propionic acid, generating adverse conditions for methanogenic bacteria [21,22].

Figs. 6 and 7 depict the fluctuations in VFA concentrations throughout the double-phase system. Butyric and acetic acids were the two VFAs that predominated throughout the first stage, which was consistent with the findings of Yang *et al.* [23]. The content of butyric acid (organic loading rate 31-51 kg COD/ (m³ d)) ranged from 2.4 to 6.9 g/L. The average rise in butyric acid content under the maximum loading of 61 kg COD/(m³ d) was 13.5 g/L. Isobutyric and acetic acid levels were 2.6 g/L and 6.1-14.1 g/L, respectively, during the entire experimentation. Between 0.57 and 0.95 g/L of propionic acid were detected in organic loading of 51 kg COD/(m³ d) and 61 kg COD/(m³ d), respectively. The concentrations of valeric and isovaleric acids were not found at all. In the subsequent phase, acetic acid and *n*-butyric acid were removed at high rates across all runs. The VFA level was in the limit of 0.51-0.98 g/L at COD concentrations of 8.5, 10.5 and 13.4 kg/(m³ d), with acetic acids predominating with a mean value of 75.1% of the total VFA. This happened as a result of the autotrophic methanogenesis pathway's subsequent conversion of butyric, isobutyric and propionic acids into acetates and methane [24,25]. Furthermore, a small amount of total VFA indicates that methanogens are operating effectively. In CSTR, VFA was reduced on average by almost 96%. The VFA level grew to a mean concentration of 2.17 g/L for the maximum organic loading of 16 kg COD/ (m³ d), which caused the reactor performance to degrade. Under steadystate conditions, the mean VFA decrease in continuously stirred tank reactor reduced to 85.4%. Additionally, the effluent contained acetic acid, butyric acid (which accounted for an average of 8.2% of the total VFA) and propionic acid (which accounted for an average of 29.03% of the total VFAs). It was thought that methanogen washout and substrate loading shock were to blame for the acidity. A visible imbalance in anaerobic digestion was caused by the abrupt rise in the OLR, which led to an excess of acid generation. Additionally, the methanogenic actions tended to wane and finally build up acid [26,27]. Lebuhn *et al.* [28] reported a similar pattern, noting that a rise in OLR led to the formation of propionic and butyric acids while the methanogenic phase. Thermoanaerobacterium species may grow at temperatures of 55-60 ºC and pH values of 5-6 [29,30]. The species is primarily a producer of hydrogen and may use
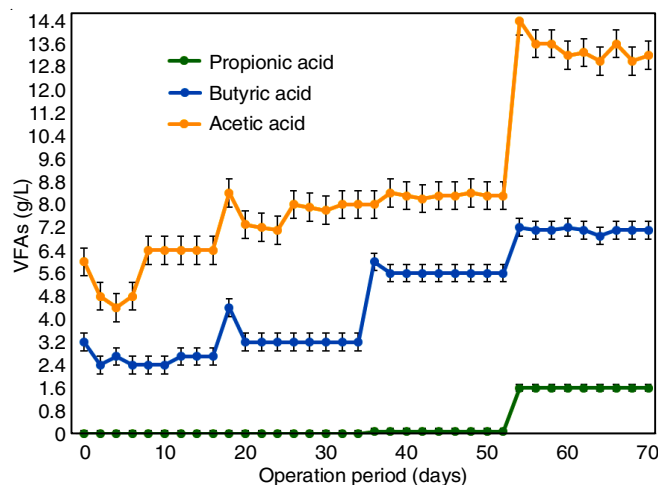

Fig. 7. Production of VFA over time in UASBR

a variety of carbohydrates, including glucose, cellulose, dextran and cellobiose. The major species in the CSTR were methanosarcina and methanoculleus, which also contributed significantly to methane generation. When acetate and butyrate concentrations are high (> 1.3 mM), methanosarcina species are known to perform the methanogenic activity and to be leading [31].

The co-fermentation of the organic substrates has the potential to produce fertilizer and irrigation fluid for agriculture. Table-5 displayed the characteristics of the fermented waste. The sludge and water were recovered at a ratio of 0.07 (m³ sludge/m³ wastewater) and 0.82 (m³/m³ wastewater), respectively. To assess their possible use, the characteristics of sludge were compared to the standards outlined in the current Malaysian recommendations. Sludge may be used as farming cement if the quantity of heavy metals is within the thresholds listed in Appendix K3 of the Environmental Quality Regulations 2009 (PU (A) 433) (Environmental Requirements, 2010) [3]. The produced liquid can be used for irrigation, while the fermenting slurry can be used as fertilizer.

TABLE-5
PROPERTIES OF DECOMPOSED SLURRY
TREATED WITH POME & AWW (50%:50%)

	Solid portion
Recovery of sludge (m³ sludge m⁻³ substrate)	0.07
Moisture (%)	95
Zn (g/kg dry weight)	0.57
Ni (g/kg dry weight)	0.18
Cu (g/kg dry weight)	0.18
Cr (g/kg dry weight)	0.03
Hg (g/kg dry weight)	0.002
Pb (g/kg dry weight)	7.3×10^{-3}
Cd (g/kg dry weight)	2.6×10^{-4}
	Water portion
Recovery of water (m³ water m⁻³ substrate)	0.82
COD (g/L)	0.30
Turbidity (unfiltered turbidity, UNF)	1290
Suspended solids (g/L)	0.07

Conclusion

The present approach emphasizes the effectiveness assessment of double-phase hydrogen and methane digestion utilizing

palm oil mill effluent and aquaculture wastewater. The procedure proved steady throughout hydrogen production up to 51 kg COD/h (m^3 d). At 51 kg COD/g, the maximal hydrogen yield, VHPR and content were 45%, 2.6 L H$_2$/d and 33.5 mL H$_2$/g, respectively (m^3 d). At an organic loading of 13.1 kg COD/(m^3 d), the highest volumetric methane production rate and CH$_4$ production of subsequent phase were 10.6 L CH$_4$/d. and 0.12 m^3 CH$_4$/kg COD, respectively. Under ideal conditions, 91% of COD was removed overall by the two-stage method. The performance, which was shaky at the start of stage, was worsened by an additional rise in organic loading to 61 kg COD/m^3 d. Water and sludge recovery rates were 0.82 (m^3/m^3 wastewater) and 0.07 (m^3 sludge/m^3 wastewater), respectively. According to these findings, mixed palm oil mill effluent and aquaculture wastewater was effective wastewater for the effective synthesis of hydrogen and methane in a double-phase digester. For a high-rate digester system with immobilized biomass employing polymers in a double-phase system, this approach could be further enhanced and scaled up.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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