



Production of Methane and Hydrogen from Mild-Strength Industrial Wastewater

MD. NURUL ISLAM SIDDIQUE^{1,*}, AZ ZAHIRAH¹, MD. ZAMRI BIN IBRAHIM¹, NORAAINI BINTI ALI^{1,2}, SHAHRUL BIN ISMAIL¹, WAN SANI WAN NIK¹ and M. REZAUL KARIM CHOWDHURY¹

¹Faculty of Marine Engineering Technology & Informatics, University Malaysia Terengganu (UMT), 21030 Kuala Nerus, Terengganu, Malaysia

²Institute of Tropical Aquaculture & Fisheries, University Malaysia Terengganu (UMT), Kuala Nerus, Malaysia

*Corresponding author: Fax: +60 96 683991; Tel: +60 96 683614; E-mail: m.nurul@umt.edu.my

Received: 30 September 2023;

Accepted: 7 March 2024;

Published online: 30 March 2024;

AJC-21594

Over the past two decades, fossil fuel reserves have sharply shrunk, which has resulted in a global fall in energy sources. Alternative carbon-neutral renewable energy sources have caught the interest of experts due to the erratic nature of energy prices and their severe ecological effects. The treatment plant's mild-strength effluent from an equalization tank at an industrial factory was used in this study to generate hydrogen and methane. This mild-strength effluent had a chemical oxygen demand (COD) content of 2.8 ± 1.0 g COD/L. A double stage anaerobic digestion system's hydrogen-generating digester was run at a hydraulic retention time of 8 h while the methane-generating digester was run at a hydraulic retention time of 24 h. The highest methane production rate (MPR), methane yield (MY), methane content and COD elimination were, in order, 71 ± 30 mL/L-d, 57 ± 10 mL/g COD, $90 \pm 1\%$ and 77% , respectively. In this experiment, energy efficiency was assessed using the following criteria: Maximum heating was at a value of 2.1×10^8 kcal/y. Utilizing energy-equivalent coal, natural gas, or fuel reduced annual carbon emissions by 8.7×10^4 kg CO₂/y, 5.1×10^4 kg CO₂/y and 7.1×10^4 kg CO₂/y, respectively.

Keywords: Two-phase, Hydrogen generation from mild-strength industrial effluent, Methane generation.

INTRODUCTION

Several studies have been conducted to create sustainable alternative energy sources in response to the major reliance on fossil fuels, to meet out the rising need for worldwide energy usage, the expanding need to overcome ecological challenges and other factors [1]. Fuel cell engines, which will eventually replace combustion engines, employ hydrogen, a great clean energy source, to produce an electric current that powers an engine [2].

Unsteadiness in a waste treatment system is usually brought on by variations in food processing features. It is generally accepted that the concentration of contaminants and the characteristics of the wastewater play a major role in determining the optimum treatment method. For medium-to-high strength wastewaters, COD >2000 mg/L and for mild strength wastewaters, COD 2000 mg/L, respectively, are used to determine the strength of the wastewater [3]. Biomass, which is derived from organic matter and ecological sources and may be transformed into energy-rich gasoline like hydrogen and methane by anaerobic process [4], is one of the most important latent

energy resources. Since mild-strength wastewater performs poorly, anaerobic digestion normally uses high-strength wastewater [5]. However, recently anaerobic systems have become a feasible alternative to traditional aerobic processes for treating mild-strength substrates, for example, municipal effluent [6].

Numerous bacteria in anaerobic systems biotransform organic substances, typically biomass and methane, in four basic phases *viz.* hydrolysis, acetogenesis, acidogenesis and methanogenesis. Exoenzymes are used in the hydrolysis stage to convert complex organic molecules into the simple organic matter. The hydrolyzed monomers next move onto the acidogenesis phase, where they are broken down by microbes and transformed into intermediary substances like acetic acids, hydrogen and volatile fatty acids (VFA). A subsequent conversion of VFA into acetic acid occurs at the acetogenesis phase. Those intermediates are eventually transformed into CH₄, CO₂ and H₂O by microbes called methanogens during methanogenesis [7].

Traditionally, the single-phase anaerobic fermentation involves carrying out each of these procedures in a single pot. On the other hand, operating in a double-stage system that

separates acidogenesis from methanogenesis is known to offer additional benefits including hydrogen build-up, reduced hydraulic retention time and higher digestion capacity. Anaerobic double-stage digestion techniques can be used to generate bio-energy such as biomethane, biohydrogen and biomethane.

Bio-H₂ might be used as a starting point in the explanation of biomethane because bio-H₂ and bio-CH₄ are the two main components in its formation. Furthermore, the produced bio-CH₄ and bio-H₂ may be combined in the ideal ratios to create green biomethane, that should theoretically have a greater combustion capacity [8]. There may be significant benefits to using a double-stage system rather than a one-stage process for treating these effluent [9]. It is possible to choose and raise the bacterial quantity in each digester as well as intermediary chemicals thanks to the disintegration of the key reactions in the anaerobic process. This allows for the optimization of each community's activity and improves the conditions in which it can thrive. In addition, the double-stage fermentation has potential to boost energy recovery by an additional 8 to 43% [10].

The microbes are typically eroded during lower retention times run in the digester [11], the immobilized-cell technique provides an effective and reliable method for continuous H₂ generation, enabling the active utilization of carbon compounds. Contrarily, steady immobilized H₂ generation is frequently a lengthy system that might be affected by several variables, including pH, inoculum, temperature and substrate concentration [12]. Recent advancements in the immobilization technology have been made to combat the theory of cell loss as well as boost productivity and output [13].

This study treated mild-strength wastewater with a double-stage anaerobic system. The main objective of this study is to assess the potential for methane and hydrogen generation as well as contaminant elimination for a double-stage anaerobic system on the mild-strength substrate, using an immobilization technique to increase total energy output.

EXPERIMENTAL

Wastewater and seeding: An industrial producer situated at Terengganu, Malaysia, collected mild-strength wastewater from the equalization container of the effluent treatment plant. The wastewater's pH was 7 ± 1 , its total COD was 2.8 ± 1.0 g/L, its total carbohydrate content was 1.8 ± 1.5 g/L and its NH₃-N content was 3.0 ± 2.2 g/L. The seeding of the hydrogen digester was done by activated sludge that was acquired from a nearby effluent treatment facility. The seeding sludge was heated at 95 °C for 1 h to inhibit methanogenesis. The volatile suspended solids (VSS), pH and T-COD levels of the seeding sludge were 11.3, 6.2 and 13.8 g/L, respectively. The digester used to produce methane was inoculated with granular sludge that was obtained from a business in central Malaysia that produced fructose. The VSS, pH and T-COD levels in this sludge were 35 g/L, 7.8 and 69 g/L, respectively.

At longer hydraulic retention times (8 and 12 h) and shorter hydraulic retention times (2, 4 and 6 h), respectively, the hydrogen generation digester used suspended and immobilized seed inoculum. Pre-treated activated sludge was enhanced with

industrial wastewater containing 10 g COD/L and a preliminary pH of 6.1 for 2 days in a glass tank to produce hydrogen-producing bacteria. Also added with 400 mL of NaC₆H₇O₆ solution (20 g/L) was 100 mL of enhanced hydrogen producer. The blended liquid was then injected into a syringe and immersed in a calcium chloride solution (40 g/L) to generate an immobilized cell (diameter around 3 mm).

Fermentation system for methane and hydrogen

Considering the impact of temperature and nitrogen:

To evaluate the effect of temperature and nitrogen source on the production of methane and hydrogen, several experiments were conducted using NH₄Cl and urea on three unlike seeding sources (C/N proportion 10). The methane, hydrogen and batch tests were done sequentially with three inoculums: Sewage sludge 1, cow dung and sewage sludge 2, with NH₄Cl and urea as nitrogen supplements (NH₃-N₃ \pm 2 g/L).

In first test, mesophilic hydrogen generation was examined about various nitrogen sources. Two hydrogen reactor trials (using NH₄Cl and urea) were run for 60 h (a starting pH of 6 and a wastewater level of 3 g COD/L). The liquid H₂ metabolite was then gathered and used right away to ferment methane. The initial pH was changed to 7 and four runs of the methane digester were carried out in two separate mesophilic and thermophilic environments (35 °C and 55 °C) to evaluate the 45-day inhibitory effects of temperature on methane generation.

A system of methane and hydrogen is built in a steady system. The double-phase anaerobic digestion procedure involved a digester that produced methane and hydrogen in series. Two up-flow digesters with a working capacity of 1 L were used to generate anaerobic methane and hydrogen. The hydrogen digester was grown for various hydraulic retention durations (HRTs) of 12, 8, 4 and 2 h at a pH of 5.3 ± 0.1 and a temperature of 35 ± 1 °C. In the hydrogen digester, immobilized inoculum was substituted for suspended seeding at shorter HRTs (2 and 4 h) to reduce potential cell loss.

A nutrition solution containing the inorganic additives indicated below (mg/L) was added to the mild-strength wastewater *viz.* NH₄Cl 5350, Na₂HPO₄ 3490, NaH₂PO₄ 21040, MgCl₂·6H₂O 100, K₂HPO₄ 125, FeSO₄·7H₂O 25, MnSO₄·6H₂O 15, CoCl₂·5H₂O, 0.125 and CuSO₄·5H₂O 5. Anaerobic granular sludge from a facility in central Malaysia that produces fructose was initially seeded into the digester for methane synthesis (working capacity 1 L). The granular sludge had a pH of 8.0, a VSS of 37.7 g/L and a T-COD of 70.2 g/L, respectively. At HRT 24 h, the methane production digester used the digested wastewater from the H₂-producing digester as its wastewater without the addition of any additional food sources.

Analytical methods: A gas chromatograph (GC-TCD) outfitted with a thermal conductivity detector (Chromatograph 8700T) was used to determine the composition of the biogas and a wet gas meter (Ritter, Germany) was utilized to monitor the production of gas. The generated gas's composition and the quantities of ethanol and volatile fatty acid were assessed as previously mentioned [14]. The broth under test was examined for carbohydrate content, pH, volatile suspended solid and oxidation-reduction potential level, per the prior study [15].

Total energy efficiency: Hydrogen/methane production capacity (methane yield (MY) and hydrogen yield (HY)) and hydrogen/methane generation rate (HPR/MPR) were used to evaluate the effectiveness of hydrogen generation. The entire value of heating might be computed by eqn. 1:

$$\text{Total energy (kJ)} = \Sigma \text{HPR} \times \frac{P}{RT} \times \text{HH}_2 \text{MPR} \times \frac{P}{RT} \times \text{HCH}_4 \quad (1)$$

HPR and MPR, respectively, stand for the hydrogen and methane generation rates (L/L-d); P stands for the measurement pressure of R for gas constant (0.082 L atm/mol K); gas (1 atm); T for the measurement temperature of gas (273 + 25 K); and H for the heating value (kJ/mol).

RESULTS AND DISCUSSION

Effects of temperature and ammonia level on biogas generation efficiency: Before running under fixed conditions, this trial evaluated the ultimate values of parameters such as ammonia sources, inoculum source and temperature. Additionally, it looked at the biogas production effectiveness of enhanced immobilized hydrogen and granular methane producers.

In terms of the kind of nitrogen supply and temperature, unlike waste characteristics were watched for each trial stage. Table-1 shows the effect of nitrogen supply type on hydrogen generation. In the NH_4Cl experiment utilizing sewage sludge 1, the hydrogen fermentation halted after 60 h. The total generation of hydrogen (HP) and hythane (HY) was 33 mL and 1.3 L/g COD 0.31 L/L. d, respectively and the greatest rate of hydrogen production (HPR_{max}) was 0.32 L/L-d. Even though both cow dung and sewage sludge 2 had higher HPR_{max} values, the quantities of HP in each substance were somewhat milder than in sewage sludge1 at 33 mL and 32 mL, respectively. All inoculums that used NH_4Cl as their nitrogen source often exhibited elevated HP and HY. Sewage sludge 2 produced maximal HP and HY, which were approximately twice as high as the same amount of urea treated with inoculum. Cow dung had the highest HP and HY in the urea study when compared to other seeds in the same test and it stayed steady, unlike ammonium supplementation. Two more operational factors with a big effect on H_2 generation are the starting and pH.

Numerous works on the impact of pH on different parts of a hydrogen-generating process have been conducted, the bulk of which concentrated on the impact of pH during inoculum treatment. Since keeping pH levels between 5 and 6 increased H_2 generation and yields, this test began at the optimum pH of 6

[16]. After completion, the pH of NH_4Cl experiment significantly decreased from 6 to around 4, whereas the pH of the other experiments increased to about 6.5 (Table-1). Additionally, the metabolites produced helped to milder the pH and the TVFA generated by NH_4Cl dosing was higher (1905-2009 mg COD/L) than the values produced by the urea experiment (1310-1516 mg COD/L). Therefore, the use of NH_4Cl as a nitrogen source was sufficient for H_2 formation.

Methane production (MP), methane yield (MY) and highest methane production rate (MPR_{max}) for all seeding sources were in the range of 56 to 66 mL, 0.27 to 0.33 L/g COD and 0.93 to 1.67 L/L-d after 45 days, correspondingly, according to Table-1. These values were higher than in the thermophilic environment. The development and enzymatic activities of several significant methanogens have been observed to slow down at higher temperatures [17]. Mesophilic anaerobic digestion (MAD) has been shown in numerous studies to function better than thermophilic anaerobic digestion (TAD), since TAD is more sensitive to inhibitors [18]. Similar to 55 °C experiment, no methane signal was detected in the biogas samples obtained from that experiment. The MYs for all the examined inoculums, especially cow dung, which produced the most methane (0.33-0.34 L/g COD), were reasonably comparable for methane fermentation at 35 °C. Although methane output varied non-significantly across the various nitrogen sources for biological fermentation, methane production was shown to be partially regulated by the nitrogen supply, even though ammonia is a crucial ingredient for bacterial development. Additionally, methane production (MP) is influenced by the ammonium content at various temperatures. According to research, the ammonia/ammonium ratio will vary depending on temperature and pH even with the same additional ammonium quantity [19]. It was found that the thermophilic digester had higher free ammonia concentrations than the mesophilic digestion. Free ammonia nitrogen (FAN) makes up less than 1% of total ammonia in reactors operating at pH 7 and 37 °C [20]. In mesophilic conditions, a pH increase from 7 to 8 more than doubles free ammonia levels and at thermophilic temperatures, the increase is even greater. Under the mesophilic conditions, immobilized anaerobic biomass is used to speed up reactions and boost yields [21]. The findings demonstrate that the supplementation of ammonium sources enhances the bioactivity of microorganisms that produce methane and hydrogen in mesophilic environments.

TABLE-1
BATCH CULTURE OF HYDROGEN AND METHANE UTILIZING DIVERSE SEED INOCULUM AND NITROGEN SOURCES

Sludge	H_2 synthesis					CH_4 formation			
	Ultimate pH	H_2 (mL)	HPR_{max} (L/L-d)	HY (L/gCOD)	TVFA (mg COD/L)	Ultimate pH	CH_4 (mL)	MPR_{max} (L/L-d)	MY (L/gCOD)
NH_4Cl source of nitrogen									
Cow waste	4.7	32	0.39	1.36	1905	7.13	66	1.28	0.33
Sewage sludge 1	4.8	33	0.32	1.41	2009	7.18	60	0.93	0.31
Sewage sludge 2	4.5	31	0.42	1.32	1971	7.25	56	0.95	0.25
Urea nitrogen source									
Cow waste	6.4	20	0.37	1.36	1455	7.26	57	1.27	0.32
Sewage sludge 1	6.2	18.2	0.77	0.70	1309	7.38	59	1.60	0.25
Sewage sludge2	6.3	17.8	0.62	0.66	1516	7.34	56	1.67	0.31

H₂ and CH₄ generation in a continuous system

Effectiveness of methane and hydrogen generation:

Anaerobic digestion hypothesis posits that the process can be separated into four parts, with acidogenesis as the second phase and methanogenesis as the third phase, both producing biohydrogen [22]. The ideal operational conditions for each step are inimical to one another because of the variations in micro-bial requirements [23]. With sugars being the chosen major carbon source for anaerobic bacteria like *Clostridium* spp., *Enterobacter* spp., *Thermoanaerobacterium* spp., etc. the optimum pH during acidogenesis was typically between 5.3 and 6.4 [24]. On the other hand, methanogenesis typically occurs at a pH of around 7, with archaea playing a significant role and feeding on simple organic acids such as acetic acid [25]. Therefore, from the standpoint of process optimization, it makes sense to split these two processes into two different pots, with the VFA-rich waste from the preliminary container acting as input for the subsequent container [26].

The production of methane and hydrogen at different HRTs throughout the double-stage anaerobic digestion system is shown in Table-2. The hydrogen digester's HRTs were gradually decreased from 12 to 2 h, while the digester's HRT was kept at 24 h. As a result of reducing the HRT for the hydrogen digester from 12 to 2 h, rates of generating methane and hydrogen were greatly improved. The hydrogen production rate (HPR) was zero during HRT 12 h (run 1) but increased to 115.42 mL/L-d during HRT 2 h (run 4). From 0.74 0.46% in Run 2 to 9.8 8.9% in run 3 and 46.0 14.0 in run 4, the biogas' hydrogen concentration increased. In contrast to the findings of other studies that have been reported, the HPR value in this experiment was rather low.

Though the hydrogen yield (HY) value was higher than some of the data that had previously been published, such as the HY of 3.21 mmol/g COD from cheese-processing effluent at 7.0 g COD/L, HRT 24 h in a continuous stirred tank reactor [27], the HY value was still lower than some of the data. The use of mild-strength manufacturing substrate provided this unpredicted detection in the HY and HPR profiles of HRT changes. Mild-strength effluent causes a milder organic loading rate (OLR) during extended hydraulic retention time. When given simple sugar-rich wastes like glucose, sucrose, starch and others, the majority of known hydrogen-producing microbes may utilize substrate at a high OLR and high HPR [28]. Complex saccharide containing high-strength wastewater, such as palm

oil mill effluent (POME), is also frequently found to have an ideal HRT greater than 6 h [29].

The mild organic loading rate at a longer hydraulic retention time (H₂ generation run 1) may have caused a rapid depletion of sugars lower than the necessary level to support hydrogen generating microbes, ultimately depleting the microorganisms that could have aided in the progression of anaerobic digestion from the second to the third and/or fourth stages. Acetogens, which are the microbes in charge of methanogens and the third phase of anaerobic digestion, which produce methane, are expected to flourish in microorganism voids created by the disappearance of bacteria that produce hydrogen. Due to the considerable acetate accumulation (Table-2) in run 1, the hypothesis of acetogens blooming was created. The presence of methane in run 1 and the mild hydrogen proportion indicate that methanogens have been activated. Additionally, methanogens have been demonstrated to be able to use hydrogen as a fuel while still creating methane [30]. Consequently, run 1 (mild OLR with extended HRT) effectively revives the effectiveness of single-pot anaerobic digestion.

Eventually, lower HRT increases OLR, but it also increases substrate flow rate, which has an impact on the concentration of volatile suspended solids (VSS) as observed in Table-2. The majority of studies show that VSS concentration peaks at the right HRT. But in present case, the VSS was consistently declining relative to the HRT decrement. The loss of VSS was rather substantial while the suspended cell was being used in the hydrogen digester at HRT for 4 h (data not shown). Extreme VSS loss is referred described as "cell wash-out" [16]. Finally, the digester failed due to insufficient VSS maintenance. In this system, bacterial immobilization was applied to deal with this problem. The identical digester was restarted after being (at HRT 4 h) seeded with an immobilized hydrogen producer. When suspended cells continuously create biohydrogen under milder HRT operation, immobilization techniques can be used to combat the theory of cell loss [17]. The change in approach worked since the HY and HPR improved following microbial immobilization. In connection with the shift in HRT, immobilization also altered the VSS accumulation pattern. In this study, it was found that for the suspended system, the VSS decreased concurrently with the HRT. However, this tendency was countered by the immobilized mechanism. The highest HPR and HY were recorded during a 2 h HRT (run 4) that used an immobilized hydrogen producer.

TABLE-2
PERFORMANCE OF BIOHYDROGEN AND METHANE SYNTHESIS

Period of cultivation		HRT (h)	Biogas (mL/Ld)	Hydrogen (%)	Methane (%)	VSS (mg/L)	HPR (mL/Ld)	HY (mL/g COD)	MPR (mL/Ld)	MY (mL/g COD)
Run 1	H ₂	12 ^a	28 ± 24 ^c	0.1 ± 0.1	28.0 ± 3	1749 ± 323	0.03 ± 0.02	0.5 ± 0.1	–	–
	CH ₄	24	10 ± 1	N. D	64.0 ± 3.0	1500 ± 364	–	–	3.6 ± 0.3	4.6 ± 0.4
Run 2	H ₂	8 ^a	135 ± 15	0.6 ± 0.5	4.5 ± 3.0	943 ± 393	1.0 ± 0.99	1.2 ± 0.7	–	–
	CH ₄	24	159 ± 80	0.1 ± 0.0	64.0 ± 2.0	926 ± 260	–	–	71.0 ± 31.0	57.0 ± 12.0
Run 3	H ₂	4 ^b	361 ± 10	9.6 ± 8.9	N. D	671 ± 277	3.10 ± 0.7	17.0 ± 17.0	–	–
	CH ₄	24	19 ± 7	6.7 ± 11.0	25.0 ± 11.0	882 ± 302	–	–	2.3 ± 0.7	7.0 ± 2.0
Run 4	H ₂	2 ^b	249 ± 21	45.0 ± 14.0	3.5 ± 0.9	943 ± 318	114.00 ± 42	171.0 ± 65.0	–	–
	CH ₄	24	10 ± 2	5.7 ± 4.0	2.1 ± 1.8	800 ± 233	–	–	0.1 ± 0.1	0.6 ± 0.2

^aSystem of suspended cell; ^bSystem of immobilized cell; ^cn ≥ 3

To have a clear understanding of the impact of changing the HRT on the H₂ digester, the hydraulic retention time of the methane digester was maintained fixed for 24 h. The maximal MPR and MY for run 2 were 71 ± 30 mL/L-d and 57 ± 11 mL/g COD, respectively (hydrogen digester HRT at 8 h). This was caused by a higher methane content and quicker biogas development. The MPR and MY results on the other trials were awful. Thus, it is proposed that the effluent at 8 h HRT may be particularly advantageous to methanogens in the methane digester.

Efficiency of contaminant removal and soluble metabolic products: COD removal using a double-stage anaerobic approach is shown in Fig. 1a. For all runs, the COD reduction in the hydrogen digester was about 30%. Similar COD removal rates in the H₂ reactor were reported in other literature sources. Bacteria that generate hydrogen (SMP) transform creating soluble metabolite products from carbohydrates. Typically, SMP is made up of a combination of organic acids and organic solvents. The microbial community and digester operating settings have a significant impact on the composition of SMP [30]. As a result, changes in HRT during hydrogen fermentation had no appreciable impact on the elimination of COD. The COD elimination process in the methane digester was also found to be higher when the H₂ digester was run at lengthier HRT (12 h and 8 h), despite being milder at longer HRT (run 3 and 4). The biggest COD reduction was observed in run 1. As a result, the two-stage anaerobic digester's total COD removal trend and the methane digester's COD removal trend eventually matched up.

Fig. 1b reveals the two-stage anaerobic system's entire sugar utilization. Typically, the hydrogen digester consumed 70% or more of the original sugar. Run 4 had the highest overall sugar consumption (98%). The hydrogen digester uses sugar far more efficiently overall than the methane digester does across all runs. As a result, the entire two-stage sugar consumption process follows a similar pattern to the hydrogen digester. The higher concentration of soluble metabolic products in the

hydrogen digester in Table-3 shows that the longer HRT can have a better hydrolysis rate. The COD removal in the double-stage anaerobic system obtained 91% for mild-strength wastewater treatment at 35 °C, which may be lowered by the drop in temperature. With a reduced feedstock content or a cellulosic substrate, the results were comparable to previous work [30], suggesting that acetate would be the primary SMP.

According to the results, a longer HRT increases hydrolysis efficiency during the acidogenesis stage and a lengthier hydraulic retention time is also needed in the methanogenic phase to maximize biogas generation in a double-stage anaerobic system. It should be observed that the overall COD removal trend matched the tendency for COD elimination in the CH₄ digester, but the total sugar utilization trend matched the tendency in total sugar consumption in the hydrogen digester. This study indirectly focuses on the diversity of the microbial population and the selection of carbon sources for metabolism. Sugar-eating bacteria dominated the hydrogen digester while archaea, which were in charge of removing COD, controlled the methane digester. The dynamic microbial community of the two-stage anaerobic system aligned with much of the existing research in the literature [29].

Potential energy and reduced CO₂ emissions for a large scale system simulation: During dark wastewater fermentation, the culture temperature affects the net energy gain. As shown in Table-4, the majority of dark fermentation experiments reported HY and MY based on the amount of biohydrogen and biomethane that is produced for each mole of feedstock used. Since mild-strength wastewater was frequently deemed inadequate for two-stage biogas fermentation, high-strength wastewater was used in the majority of the published investigations [9]. The HY achieved for an immobilized process at 2 h retention time was consistent with many previously reported investigations, while the MY obtained across all HRTs was significantly milder. When compared to other forms of typical wastewater, it was mostly for the reduced total COD concentration that

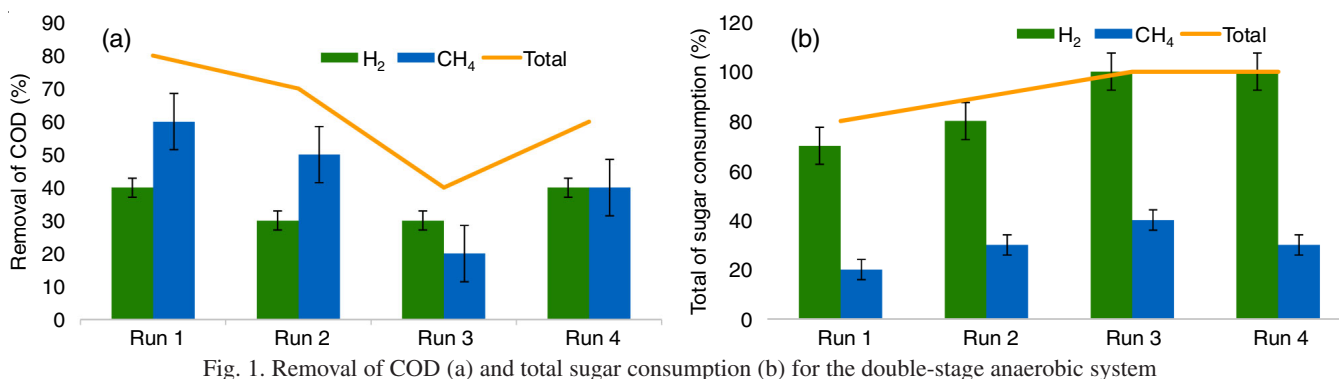


Fig. 1. Removal of COD (a) and total sugar consumption (b) for the double-stage anaerobic system

Period of cultivation	HRT (h)	Ethanol	Acetate	Propionate	Butyrate	Valerate	TVFA	SMP
		(mg COD/L)						
Run 1	12	143 ± 111	1106 ± 444	423 ± 253	398 ± 278	16 ± 7	1187 ± 964	2715 ± 416
Run 2	8	N.D.	230 ± 64	33 ± 10	96 ± 66	4 ± 2	367 ± 95	365 ± 95
Run 3	4	N.D.	310 ± 113	66 ± 51	26 ± 23	6 ± 2	409 ± 163	409 ± 163
Run 4	2	N.D.	346 ± 84	68 ± 28	46 ± 15	5 ± 4	468 ± 115	469 ± 115

TABLE-4
COMPARING THE TWO-STAGE FERMENTATION METHOD TO EARLIER RESEARCH

Source	Digester	HRT (h)	Run temp. (°C)	HY (mL/gCOD)	MY (mL/gCOD)	Ref.	
POME	H ₂	ASBR	47	54	210.0	–	[26]
	CH ₄	UASB	360	37	–	315.0	
POME	H ₂	UASB	48	55	215.0	–	[27]
	CH ₄	CSTR	120	37	–	320.0	
Skim Latex	H ₂	UASB	36	55	63.0	–	[28]
	CH ₄	UASB	192	55	–	179.4	
Sugar cane syrup	H ₂	CSTR	72	37	88.0	–	[29]
	CH ₄	UASB	72	30	–	271.4	
Mild-strength industrial wastewater	H ₂	UASB	8 ^a	25	1.3 ± 0.7	–	This study
	CH ₄	UASB	24	25	–	58.0 ± 12.0	
Mild-strength industrial wastewater	H ₂	UASB	2 ^b	25	172.0 ± 65.0	–	This study
	CH ₄	UASB	24	25	–	0.7 ± 0.2	

^aSystem of suspended cell; ^bSystem of immobilized cell: ASBR, UASB, CSTR

was readily available. Furthermore, high-strength wastewater is often operated at longer HRT for both biohydrogen and biomethane, increasing the production of HY and MY.

The yields given above can be used in the following equation [27] to get the aforementioned net energy gain:

$$E_N = \frac{CkV(Y_H \times LHV_H + Y_M \times LHV_M) \times 10^{-8} - V_{\text{poc}_p} (T_f - T_a)}{VC} \quad (2)$$

where k is the COD equivalent of the wastewater feedstock (g feedstock/g COD), Y_H and Y_M are the H₂ and CH₄ generation rates (mL/g COD effluent), C is the substrate level (g COD/L), V is the reactor liquid volume (L) and LHV_M and LHV_H represent the mildest methane (50,000 kJ/kg) and heating values of hydrogen (120,000 kJ/kg). Water has a density of 1 kg/L (specific heat of 4.2 kJ/kg K) at 35 °C for fermentation (T_f). For our calculation, T_a was fixed to the usual ambient temperature of 25 °C.

The net energy gain for each run was summarized in Table-5. It appears that more energy was consumed throughout the process in numerous instances where the net energy gain was negative. The milder initial COD level of industrial effluent was primarily to blame for this undesirable result. As a result, MY and HY significantly drop and run 3 yielded the highest MY ever seen, 57, which was only a small portion of the 350 mL/g COD maximum theoretical MY. Although the majority of MY for high-strength wastewater documented varies between 180 and 320 mL/g COD [25]. An ideal two-stage biohydrogen

and biomethane system can result in more positive energy gain by increasing the initial COD [24]. Even though there was a negative net energy gain, this method is still better than additional techniques for treating wastewater that do not recover biogas in terms of overall energy use. By integrating moderate wastewater treatment with another type of renewable energy source, this negative energy gain could be balanced.

Table-5 outlines two options for reducing CO₂ emissions and energy potential if this system were to be used to treat industrial wastewater with a 1000 CMD capacity. A peak MPR of 0.072 L/L-d at HRT 24 h and an HPR of 0.001 L/L-d at retention time 8 h were used for case 1 to compute annual biogas output. The combined heating value of the 116 m³ of hydrogen and 2.4 × 10⁴ m³ of methane produced each year is 2.2 × 10⁸ kcal/y. By substituting hydrogen and methane for coal, natural gas and petrol, CO₂ emissions might be reduced from 5.2 × 10⁴ to 8.8 × 10⁴ kg CO₂/y (Table-6). In case 2, a mild-strength industrial effluent (1000 CMD) with a maximal HPR of 0.115 L/L-d at HRT 2 h and an MPR of 0.0001 L/L-d at HRT 24 h, could generate 32 m³ of CH₄ and 3794 m³ of H₂ every year.

Case 2 might decrease CO₂ emissions by 4706, 2797 and 3855 kg CO₂/y, correspondingly, by using natural gas, coal and petrol instead of the three fuels as listed in Table-6. According to research, it is possible to generate biogas energy by using mild strength industrial wastewater. The recommended retention times for methane and hydrogen digesters might be 8 h and 24 h, respectively.

TABLE-5
GAIN IN NET ENERGY IN THE CONTINUOUS DOUBLE-STAGE PROCESS

Cultivation period	HRT (h)	HPR (mL/L-d)	HY (mL/g COD)	MPR (mL/L-d)	MY (mL/g COD)	Gain in net energy (kJ/g COD)	
Run 1	H ₂	12 ^a	0.03 ± 0.02	0.5 ± 0.1	–	–	-14.2
	CH ₄	24	–	–	3.6 ± 0.3	4.6 ± 0.4	
Run 2	H ₂	8 ^a	1.0 ± 0.99	1.2 ± 0.7	–	–	-12.5
	CH ₄	24	–	–	72.0 ± 31.0	58.0 ± 12.0	
Run 3	H ₂	4 ^b	3.20 ± 0.7	18.0 ± 17.0	–	–	-14.0
	CH ₄	24	–	–	2.3 ± 0.7	7.0 ± 2.0	
Run 4	H ₂	2 ^b	114.00 ± 42	171.0 ± 65.0	–	–	-12.7
	CH ₄	24	–	–	0.1 ± 0.1	0.6 ± 0.2	

^aSystem of suspended cell; ^bSystem of immobilized cell

TABLE-6
POTENTIAL ENERGY AND CO₂ EMISSIONS

Items	Case 1	Case 2	
Mild-strength industrial wastewater (CMD)	1000	1000	
Power of the reactor	Hydrogen digester (m ³)	331	82
	Methane digester (m ³)	1000	1000
Hydraulic retention time	Hydrogen digester (h)	7	2
	Methane digester (h)	22	23
Rate of biogas production	Hydrogen digester (L/L-d)	0.001	0.115
	Methane digester (L/L-d)	0.071	0.0001
Output of annual biogas	H ₂ digester (m ³ /y) ^a	115	3794
	CH ₄ digester (m ³ /y)	23760	32
Combined heating value	(kcal/y) ^b	221, 551, 115	11, 906, 960
Reduction of CO ₂ emissions	Replacing coal (kg CO ₂ /y)	87565	4705
	Replacing natural gas (kg CO ₂ /y)	52017	2795
	Replacing fuel (kg CO ₂ /y)	71748	3857

^aRun day: 330 d; ^bH₂ heating value 3.4×10^4 kcal/kg and CH₄ heating value 1.3×10^4 kcal/kg

Diverse nitrogen sources yield different types of nitrogen: Ammonia is a vital ingredient for bacterial growth during anaerobic digestion, although it can hinder methanogenesis if present in large doses. The biological breakdown of nitrogenous materials produces ammonia. Ammonium ions (NH₄⁺) and free ammonia (NH₃) are the two main forms of inorganic nitrogen that contain ammonia. An anaerobic digestion system may experience inhibition from either type directly or indirectly. Free ammonia (FAN), when present in excess amounts, was a strong inhibitor of the anaerobic system. Features of the anaerobically digestible substrate, pH and process temperature (thermophilic or mesophilic) are all directly related to how the anaerobic digestion process is inhibited [22]. Methane production rate (MPR) that are milder could signify ammonia-nitrogen concentration inhibition during the anaerobic digestion process. The methane reactor operating at NH₃-N 3 ± 2.2 g/L produced the greatest MPR ever recorded, 72 mL/L/d. While some have hypothesized that TAN concentrations as high as 1500-7000 mg/L can lead to AD instability, others have found that concentrations 1700-1800 mg/L were completely suppressive when combined with an unacclimated inoculum [19]. This broad range of inhibitory ammonia concentrations was most likely caused by changes to the substrates, inoculums, ambient factors (temperature, pH) and acclimation durations [20]. In contrast, municipal wastes were degraded in pilot digesters with steady increments in total ammonia nitrogen (TAN) concentrations from 800 to 6900 mg/L, at a pH of 7.9 and at a level of 3300 mg TAN/L [21].

Since free ammonia can cross cell membranes, its concentration was originally believed to be the main factor leading to methanogenic microflora; hence, the inhibition of anaerobic digestion should be correlated with both the TAN concentration and the free ammonia level. They also showed that at pH 7 and 35 °C in an anaerobic digester, whereas at the same temperature but pH 8, the free ammonia climbs to 9%, the free ammonia signifies lower than 1% of total ammonia [22]. The three variables TAN, pH and temperature are the main determinants of the free ammonia concentration. Especially when the FAN concentration was greater than 1000-1200 mg/L (6000 mg NH₄-N/L) at 38 °C and 8.1 pH, ammonia inhibition was observed when digesting the abattoir wastes [23].

Optimizing the C to N proportion of the substrate by combining effluent streams for digestion is one of the many ammonia inhibitor management strategies and it's also likely the most practical and affordable. By modifying the feedstock's C:N ratio, the overall ammonia level of the anaerobic system may be managed. By adjusting the C to N proportions, ammonia accumulations in anaerobic systems are prevented. Carbon-rich substrates are used to increase COD and hence widen the COD:TKN ratio [24]. It is essential to achieve an ideal biogas generation while balancing a sufficient food supply and ammonia inhibition and numerous works have described several different C to N proportions. The effects of C:N ratios between 3.2 and 30 on the elimination of ammonia, in particular for waste from the fleshing of leather [25]. They found that anaerobic digestion of wastes from the leather industry yielded more cumulative biogas and a higher free ammonia concentration (817 mg/L) at pH 6.5, reducing the level of NH₃ emitted while digestion by 79% in comparison to raw leather fleshing waste with a pH as high as 11.3 pH.

Conclusion

A continuous operating strategy for the treatment of mild-strength industrial wastewater in double-stage anaerobic digesters with an immobilized hydrogen generator and methane generator was highlighted in this work. By using an immobilized hydrogen generator for a short hydraulic retention time (HRT) of about a couple of h, hydrogen generation may be boosted. When the hydrogen-sand methane digesters were grown for, respectively, 8 and 24 h of hydraulic retention time, for a commercial scale operation, the highest heating value from hydrogen and methane generation would be realized. An additional advantage is that this results in lower levels of carbon dioxide emissions.

ACKNOWLEDGEMENTS

The Faculty of Marine Engineering Technology & Informatics at the University Malaysia Terengganu (UMT), which provided lab space, is gratefully acknowledged by the authors. Only due to TAPERG-55368 was this task capable of being completed.

CONFLICT OF INTEREST

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

REFERENCES

- J. Malinauskaite, H. Jouhara, D. Czajczyńska, P. Stanchev, E. Katsou, P. Rostkowski, R.J. Thorne, J. Colón, S. Ponsá, F. Al-Mansour, L. Anguilano, R. Krzyzyska, I.C. López, A. Vlasopoulos and N. Spencer, *Energy*, **141**, 2013 (2017); <https://doi.org/10.1016/j.energy.2017.11.128>
- M.N.I. Siddique and Z.A. Wahid, *J. Clean. Prod.*, **194**, 359 (2018); <https://doi.org/10.1016/j.jclepro.2018.05.155>
- M.N.I. Siddique, M.S. Abdul Munaim and A.W. Zularisam, *J. Clean. Prod.*, **106**, 380 (2015); <https://doi.org/10.1016/j.jclepro.2014.08.003>
- B.K. Zaied, M. Nasrullah, M.N.I. Siddique, A.W. Zularisam, L. Singh and S. Krishnan, *Sci. Total Environ.*, **706**, 136095 (2020); <https://doi.org/10.1016/j.scitotenv.2019.136095>
- B.K. Zaied, M. Nasrullah, M.N. Islam Siddique, A.W. Zularisam, L. Singh and S. Krishnan, *J. Environ. Chem. Eng.*, **8**, 103551 (2020); <https://doi.org/10.1016/j.jece.2019.103551>
- F.M. Liew, M.E. Martin, R.C. Tappel, B.D. Heijstra, C. Mihalcea and M. Köpke, *Front. Microbiol.*, **7**, 694 (2016); <https://doi.org/10.3389/fmicb.2016.00694>
- T. Nevzorova and V. Kutcherov, *Energy Strategy Rev.*, **26**, 100414 (2019); <https://doi.org/10.1016/j.esr.2019.100414>
- M.N. Islam Siddique, Z.B. Khalid and M.Z.B. Ibrahim, *J. Environ. Chem. Eng.*, **8**, 103569 (2020); <https://doi.org/10.1016/j.jece.2019.103569>
- M.A. Dareioti, A.I. Vavouraki, K. Tsigkou and M. Kornaros, *Energies*, **14**, 5423 (2021); <https://doi.org/10.3390/en14175423>
- A. Schievano, A. Tenca, S. Lonati, E. Manzini and F. Adani, *Appl. Energy*, **124**, 335 (2014); <https://doi.org/10.1016/j.apenergy.2014.03.024>
- M.N.I. Siddique, M.S.A. Munaim and A.W. Zularisam, *J. Taiwan Inst. Chem. Eng.*, **58**, 451 (2016); <https://doi.org/10.1016/j.jtice.2015.06.038>
- T.J. Tse, D.J. Wiens and M.J.T. Reaney, *Fermentation*, **7**, 268 (2021); <https://doi.org/10.3390/fermentation7040268>
- N.A. Mohidem, M. Mohamad, M.U. Rashid, M.N. Norizan, F. Hamzah and H.N. Mat, *J. Compos. Sci.*, **7**, 488 (2023); <https://doi.org/10.3390/jcs7120488>
- M.N.I. Siddique and Z.B.A. Wahid, *Water Environ. Res.*, **90**, 835 (2018); <https://doi.org/10.2175/106143017X15131012153031>
- N.I. Siddique and Z.A. Wahid, *J. Environ. Sci. Technol.*, **5**, 155 (2012); <https://doi.org/10.3923/jest.2012.155.167>
- B.K. Zaied, M.N.I. Siddique, A.W. Zularisam, M.F. Ahmad and Y.M. Salih, *Asian J. Chem.*, **31**, 2413 (2019); <https://doi.org/10.14233/ajchem.2019.22196>
- M.N.I. Siddique, M.S.A. Munaim and Z.B.A. Wahid, *J. Clean. Prod.*, **145**, 303 (2017); <https://doi.org/10.1016/j.jclepro.2017.01.061>
- Z.B. Khalid, M.N.I. Siddique, A. Nayeem, T.M. Adyel, S.B. Ismail and M.Z. Ibrahim, *J. Environ. Chem. Eng.*, **9**, 105489 (2021); <https://doi.org/10.1016/j.jece.2021.105489>
- S. Krishnan, L. Singh, P. Mishra, M. Nasrullah, M. Sakinah, S. Thakur, N.I. Siddique and Z.A. Wahid, *Environ. Technol. Innov.*, **8**, 360 (2017); <https://doi.org/10.1016/j.eti.2017.08.005>
- Z.B. Khalid, M.N.I. Siddique, M. Nasrullah, L. Singh, Z.B.A. Wahid and M.F. Ahmad, *Environ. Technol. Innov.*, **16**, 100446 (2019); <https://doi.org/10.1016/j.eti.2019.100446>
- M.N.I. Siddique, M. Sakinah Abd Munaim and A.W. Zularisam, *J. Ind. Eng. Chem.*, **20**, 331 (2014); <https://doi.org/10.1016/j.jiec.2013.03.030>
- P.N.Y. Yek, C. Li, W. Peng, C.S. Wong, R.K. Liew, W.A. Wan Mahari, C. Sonne and S.S. Lam, *Chem. Eng. J.*, **425**, 131886 (2021); <https://doi.org/10.1016/j.cej.2021.131886>
- Q. Yang, S. Ravnskov, J.W.M. Pullens and M.N. Andersen, *Sci. Total Environ.*, **816**, 151649 (2022); <https://doi.org/10.1016/j.scitotenv.2021.151649>
- Z. Shi, M. Usman, J. He, H. Chen, S. Zhang and G. Luo, *Water Res.*, **205**, 117679 (2021); <https://doi.org/10.1016/j.watres.2021.117679>
- Y. Li, X. Chen, L. Liu, P. Liu, Z. Zhou, Y. Huhetaoli, Y. Wu and T. Lei, *J. Anal. Appl. Pyrolysis*, **162**, 105449 (2022); <https://doi.org/10.1016/j.jaap.2022.105449>
- A. Pant and J.P.N. Rai, *Environ. Challenges*, **5**, 100262 (2021); <https://doi.org/10.1016/j.envc.2021.100262>
- G. Kaur, D. Johnravindar and J.W.C. Wong, *Bioresour. Technol.*, **308**, 123250 (2020); <https://doi.org/10.1016/j.biortech.2020.123250>
- D. Johnravindar, J.W.C. Wong, D. Chakraborty, G. Bodedla and G. Kaur, *J. Environ. Manage.*, **290**, 112457 (2021); <https://doi.org/10.1016/j.jenvman.2021.112457>
- Z. Yang, Z. Wang, G. Liang, X. Zhang and X. Xie, *Chem. Eng. J.*, **426**, 131777 (2021); <https://doi.org/10.1016/j.cej.2021.131777>
- F. Zheng, J. Fang, F. Guo, X. Yang, T. Liu, M. Chen, M. Nie and Y.C. Chen, *Eng. J. (N.Y.)*, **432**, 134377 (2022); <https://doi.org/10.1016/j.cej.2021.134377>