Science and Technology Indonesia
e-ISSN:2580-4391 *p*-ISSN:2580-4405
Vol. 9, No. 1, January 2024

Research Paper

Enhanced Efficiency for Biogas Production from Distillery Wastewater as Mixed with Molasses and Glycerol Waste in the Anaerobic Co-Digestion

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Abstract

This experiment was conducted to decide the impact of molasses and glycerol waste on upgraded methane production in anaerobic co-digestion with distillery wastewater. Co-substrates used for biogas production in the anaerobic co-fermentation process of distillery wastewater (DW) were molasses (ML) and glycerol waste (GW). The co-substrate concentration in all batch experiments varied between 1% and 5% (v/v). To study the efficiency of biogas production, the optimal ratio was chosen for operation in the PFR continuous reactor. Optimization results indicated that anaerobic co-digestion of DW with 5% GW and 1% ML could improve biogas quality and quantity. HRT for 30 days allowed R2 (95% DW: 5% GW) to produce maximum methane production per 11 m^3 CH_4/m^3 mixed wastewater, followed by R1 (99% DW: 1%). ML) 6 m^3 CH_4/m^3 mixed wastewater and control (100% DW) could only produce 2.7 m^3 CH_4/m^3 mixed wastewater methane. As co-substrates, GW and ML can be balanced to coordinate the C/N ratio and pH of DW. In particular, the C/N ratio of the mixed sewage can be balanced, and the concentration of ammonia nitrogen within an anaerobic digestion tank can be diluted. Therefore, GW can be used as an optimal co-substrate as it improves the C/N ratio, dilutes toxic compounds within DW, and provides lower prices, thus increasing the potential for methanogenesis within DW affected to increase biogas production.

Keywords

Anaerobic Co-Digestion, Biogas Production, Distillery Wastewater, Glycerol Waste, Molasses

Received: 13 August 2023, Accepted: 6 December 2023
https://doi.org/10.26554/sti.2024.9.1.120-128

1. INTRODUCTION

Community Refined Distillery Factory is a traditional Thai distilled spirit made in Satun province in southern Thailand. The annual discharge of distilled liquor wastewater is about 30 m^3/month. Distillation wastewater generally contains high concentrations of easily biodegradable organic matter, so a biological treatment technology called anaerobic digestion is the most promising. Anaerobic digestion of industrial wastewater is widely used worldwide not only for pollution control but also for energy recovery in the form of methane. High-performance anaerobic digesters that retain microorganisms also have high throughput, resulting in a small area in treatment (Angelidaki and Sanders, 2004).

Glycerol waste (GW) was waste from biodiesel generation. Around 100 g of glycerol waste, speaking to roughly 10% of the feedstock was produced from 1 kg of biodiesel (Yazdani and Gonzalez, 2007; Viana et al., 2012). Currently, glycerol waste prices are low due to oversupply (Yazdani and Gonzalez, 2007). Molasses is a by-product of sugar production and is used as a feedstock in various industries such as bioethanol production, and baker's yeast fermentation (Kobya and Delipinar, 2008; Ersahin et al., 2011). The points of interest of glycerol waste are easy digestion assimilation, high COD, cheap price, and long-term storage at room temperature (Ma et al., 2008). Glycerol waste was utilized as a co-substrate to advance biogas generation in an anaerobic fermentation process (Fountoulakis and Manios, 2009). Waste glycerol balanced the C/N ratio and weaken poisonous, with C/N and COD/N ratio values of 20 and 70, respectively, showing values within the anaerobic digestion process (Álvarez et al., 2010). Astals et al. (2012) detailed that the co-fermentation of swine manure and 4% glycerol waste might increment biogas generation by roughly 400%. A mixing ratio of 95:5 (cow manure: molasses) was accomplished a greatest methane yield of 300 mL CH_4/g VS (Fang et al., 2011). The purpose of this study is to measure

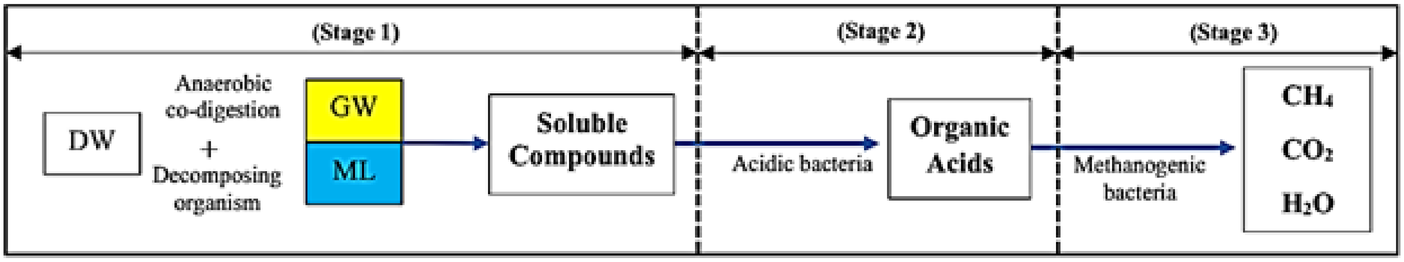

Figure 1. Chemical Reaction of Anaerobic Co-Digestion Process from Distillery Wastewater (DW)

the effect of molasses and glycerol waste on methane potential in anaerobic co-digestion with distilled wastewater. Shrestha et al. (2023) reported the co-digestion benefits were enhanced buffering capacity, enriched microbial diversity, dilution of toxic compounds, and process stability.

The composition of distilled wastewater included BOD5 (50,000 mg/L), COD (56,000 mg/L), and nitrogen content (2,180 mg/L) (Tang et al., 2007). Guerrero et al. (1997) detailed that the poisonous quality of free ammonia nitrogen (NH_3) ranges from 25 to 140 mg NH_3/L. The maximum yield of biogas was 0.63 m^3/kg COD with anaerobic filtration from a distillery effluent operated at 3 g/L-day TOC (Kida et al., 1994). At present, it is not cost-effective to invest in building a biogas production plant on wastewater from the distillery wastewater due to low biogas production and poor biogas quality. One way to increase biogas production in distillery wastewater is through using co-digestion processes.

2. EXPERIMENTAL SECTION

2.1 Materials

Distillery wastewater (DW), molasses (ML), and glycerol waste (GW) were used in this study. Medium-temperature methano genic sludge inoculum was collected from United Palm Oil Industry Public Company Limited, Krabi Province. The distillery effluent used as raw material was sourced from the Municipal Refinery Factory (Satun, Thailand). Approximately 50L of the sample was collected in PET bottles and transported to the laboratory for further analysis or processing. Samples were stored at 4°C before use. Molasses is purchased from Thai general farm stores (Phatthalung, Papayom). Glycerol waste was obtained from a pilot plant (Songkla University, Thailand) for the production of biodiesel from palm oil. The main properties of distillery wastewater, molasses, glycerin waste, and inoculum are shown in Table 1. Figure 1 showed chemical reaction chain of anaerobic co-digestion process from Distillery wastewater (DW).

2.2 Methods

2.2.1 Single Digestion Experiment

Anaerobic digestion of DW, ML, and GW was tested in 500 mL serum flasks with a working volume of 300 mL. All batch experiments were performed with different concentrations of substrate and co-substrate (ML and GW) ranging from 50-100% (v/v) and 1-5% (v/v), respectively. To determine appropriate concentrations were performed at medium temperature using substrate and co-substrate amounts for optimal methanogenesis. Biogas was obtained by water displacement. Biogas composition was routinely analyzed using GC-TCD. COD, VS, VFA, Alkalinity (American Public Health Association, 1998).

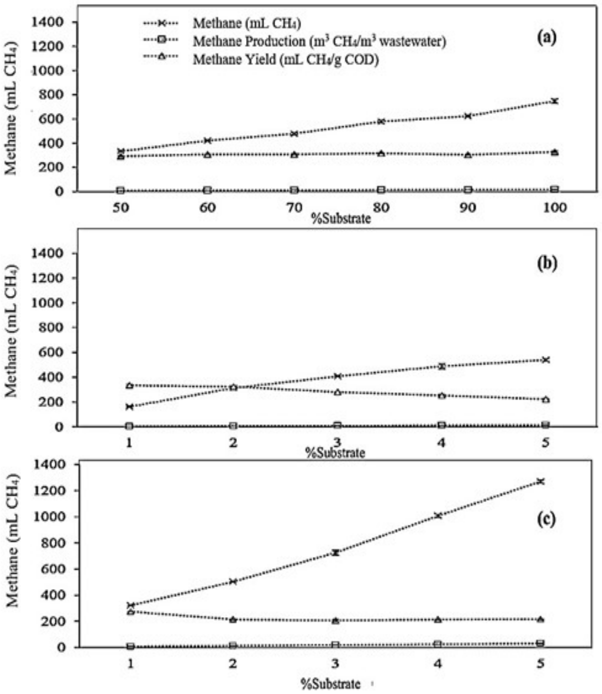

Figure 2. Methane, Methane Production and Methane Yield Profiles; (a) Anaerobic Digestion of DW, (b) Anaerobic Digestion of ML and (c) Anaerobic Digestion of GW

2.2.2 Co-Digestion Experiment

Anaerobic co-digestion of DW with ML and GW was evaluated using biochemical methane potential (BMP) tested in 500 mL serum flasks with a working volume of 300 mL. All batch experiments were performed under mesophilic conditions using 10 different concentrations (DW: ML and DW: GW) of the substrate; 99:1%, 98:2%, 97:3%, 96:4%, and 95:5% (v/v) range of co-substrates. to determine the appropriate amount of substrate and co-substrate for the best methane production.

Biogas was obtained by water displacement. Biogas composition was analyzed using GC-TCD. COD, VS, VFA, Alkalinity (American Public Health Association, 1998).

2.2.3 Continuous Reactor

The continuous system was operated using a Plug Flow Reactor (PFR) size of 2 L (Working volume 1 L). The optimal condition from the batch test was chosen for the continuous process which was operated at HRT for 20, 25, 30, and 35 days. During the fermentation, the raw materials were circulated so that the fermented raw materials were well mixed. A water displacement (gas counter) process was used to collect the biogas production every day. Biogas composition was analyzed by GC-TCD (Sompong et al., 2012). At steady state, samples were collected to analyze values such as pH, and volatile fatty acids and calculate the methane yield and efficiency of organic treatment.

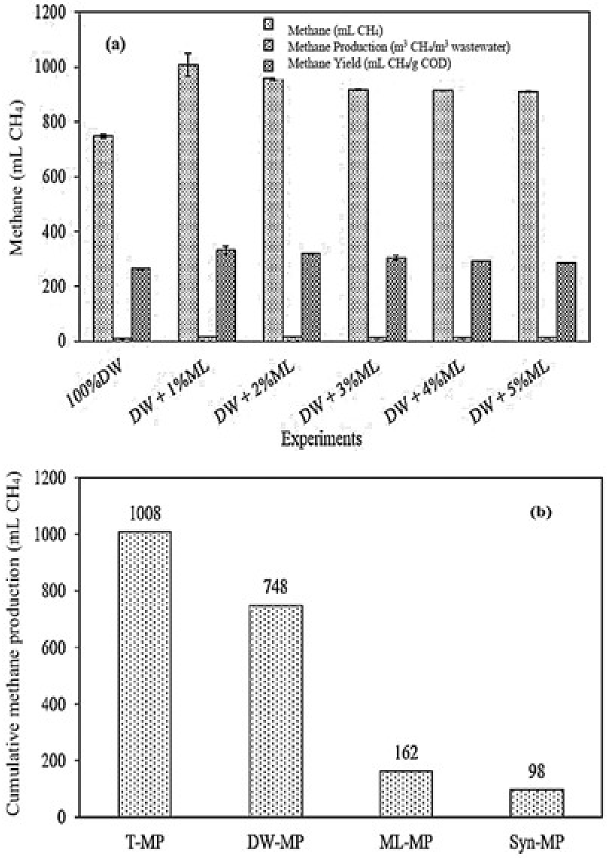

Figure 3. (a) Methane Yield Profiles from the Anaerobic Co-Digestion of Distillery Wastewater with Molasses (b) Cumulative Methane Production from Anaerobic Co-Digestion of DW with ML at the Ratio of 99:1; T-MP (Total Methane Production), DW-MP (Distillery Wastewater Methane (100%) Production), ML-MP (Molasses (1%) Methane Production, Syn-MP (Synergistic Methane Production)

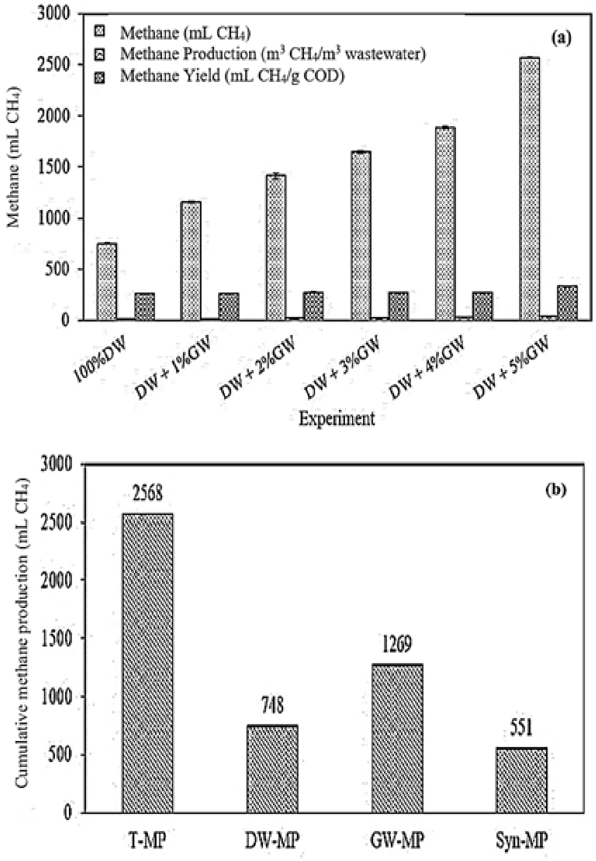

Figure 4. (a) Methane Yield Profiles from the Anaerobic Co-Digestion of Distillery Wastewater with Glycerol Waste; (b) Cumulative Methane Production from Anaerobic Co-Digestion of DW with GW at the Ratio of 95:5; T-MP (Total Methane Production), DW-MP (Distillery Wastewater (100%) Methane Production), GW-MP (Glycerol Waste 5%) Methane Production, Syn-MP (Synergistic Methane Production)

2.2.4 Microbial Community Analysis

As previously described Kongjan et al. (2011), polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) was used to study the structure of archaeal communities in single- and co-digestion of methanogenesis. PCR products from experiments were purified and sequenced by Macrogen Inc. (Seoul, Korea). The closest matches for partial 16S rRNA gene sequences were identified by Genbank database searches using BLAST (Altschul et al., 1997).

2.2.5 Analytical Methods

pH was measured with electrodes (Aqwa, AD11). Total (TS) and volatile solids (VS), chemical oxygen demand (COD), total nitrogen (TN), alkalinity, protein, carbohydrates, and, fat were measured using standard methods (American Public Health Association, 1998). Volatile fatty acids (VFA) were analyzed using a gas chromatograph (GC-FID, Shimadzu GC-17A Gas

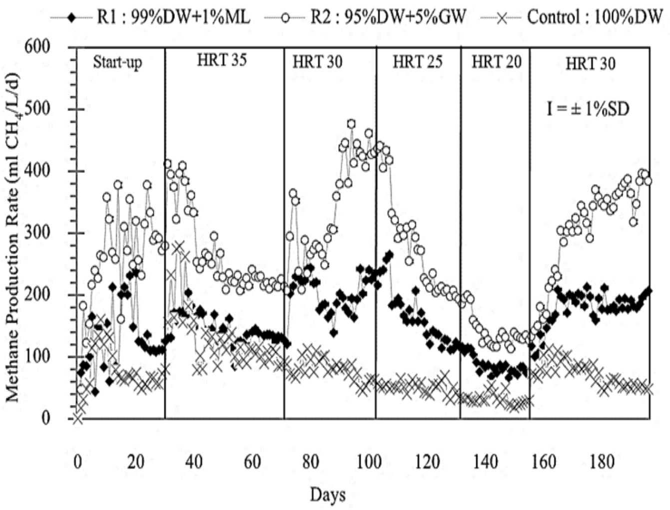

Figure 5. Methane Production Rate from Continuous Anaerobic Co-Digestion of Distillery Wastewater in R1, R2 and Control at Different HRT

Chromatograph, Japan). Biogas composition was analyzed using a Shimadzu gas chromatograph equipped with a thermal conductivity detector (GC-TCD, Shimadzu GC-BA Gas Chromatograph, Japan). The parameters are oxygen, hydrogen, carbon, and nitrogen using a CHN analyzer (LECO CHNS-932 and VTF-900). The synergistic effect was calculated from the methane production (%) of the optimal ratio of DW, GW, and ML of the batch system compared to the methane production (%) of single DW, GW, and ML (Sompong et al., 2012).

3. RESULTS AND DISCUSSION

3.1 Characteristics of Distillery Wastewater, Molasses, and Glycerol Waste

Tables 1 and 2 showed the properties of DW, ML, and GW before and after mixing. DW had a high COD and a C/N ratio of 25.03, much lower than those of ML and GW. ML and GW are comprised primarily of COD and have a very high C/N ratio of 37.77. Furthermore, the addition of ML and GW at concentrations of 1–5% (v/v) to DW can increase the C/N ratio to 25.10–27.02. Li et al. (2011) detailed that appropriate C/N ratios for anaerobic digestion between 20 and 30. As co-substrates, GW and ML can be tailored to the C/N ratio and pH of DW. In particular, the C/N ratio of mixed wastewater can be balanced and the ammonia-nitrogen concentration can be diluted in the anaerobic digester (Astals et al., 2012). The COD concentration of DW was found to be 57.5 g/L, which does not correspond to the economic viability of biogas production. However, the COD concentrations in GW and ML were very high, 2,925 g/L and 1,210 g/L, respectively. By digesting GW and ML together with DW, the COD concentration can be increased.

3.2 Methane Potential of Distillery Wastewater, Molasses, and Glycerol Waste

Analytical results of DW in Table 1 showed that DW was a concentrated substrate with high total nitrogen content that could potentially inhibit the process. Cumulative methane, methane production, and methane yield from DW, ML, and GW under mesophilic conditions are shown in Figure 1. Methane yields from anaerobic digestion of DW at 50, 60, 70, 80, 90 and 100% (v/v) were 292, 308, 308, 318, 304, and 328 mL CH_4/g COD, respectively (Figure 2a). Maximum methanogenesis was achieved at 100% DW with 12 m^3 CH_4/m^3 wastewater. Figure 2c: Methane yields from GW anaerobic digestion at 1, 2, 3, 4, and 5% (v/v) were 274, 215, 206, 215, and 216 mL CH_4/g COD, respectively. On the other hand, anaerobic digestion of GW at 5% GW yielded a maximum methane content of 31 m^3 CH_4/m^3 wastewater. Therefore, DW and GW had no toxic effects on the anaerobic digestion process. On the other hand, 1% ML anaerobic digestion was the best experiment for methanogenesis, with a maximum methane yield of 334 mL CH_4/g COD (Figure 2b). As the initial substrate loading rate increased, the methane potential of ML decreased. Low methane yields at high substrate concentrations indicated that they could inhibit the process upon overload (Sompong et al., 2012). The anaerobic digestion process of molasses is regularly restricted by the high content of non-biodegradable organic matter within the wastewater, and anaerobic digestion only results in partial degradation of the organic fraction. (Mischopoulou et al., 2016).

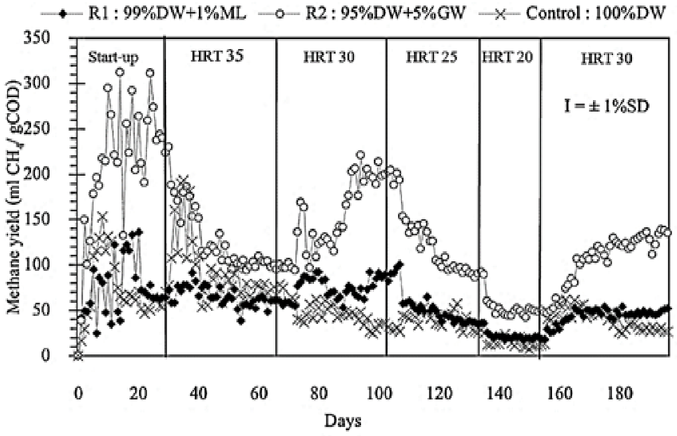

Figure 6. Methane Yield from Continuous Anaerobic Co-Digestion of Distillery Wastewater in R1, R2 and Control at Different HRT

3.3 Methane Potential of Co-Digestion Raw Distillery Wastewater with Molasses

Co-digested fermentation of distillery wastewater and molasses further improved biodegradability and methanogenesis. Cumulative methane production from the co-digestion of DW and ML is shown in Figure 3. The methane yields for co-digestion of DW with 1, 2, 3, 4, and 5% (v/v) ML were 334,

Table 1. Characteristics of DW, ML, and GW, and Inoculum

Characteristics	DW	ML	GW	Inoculum
pH	3.52±0.02	4.95±0.01	8.86±0.01	7.61±0.02
TS (g/l)	44.08±0.60	914.50±2.17	279.53±3.34	81.26±1.41
VS (g/l)	40.67±0.58	671.97±4.96	254.96±2.94	48.48±0.00
Ash (g/l)	0.34±0.02	24.25±2.79	2.46±0.40	3.29±1.56
COD (g/l)	57±0.71	1210±2.83	2925±7.07	73±0.14
Alkalinity (mg/L)	6.67±0.02	583.33±0.01	3083.33±0.01	33.33±0.00
VFA (mg/L)	2214.26±0.01	9470.62±0.01	4521.50±0.00	280.39±0.00
Carbohydrate (g/l)	28.68±0.33	1355.56±5.23	22.47±0.22	11.32±1.11
Reducing sugar (g/l)	3.41±0.96	267.92±0.00	7.99±1.94	9.98±8.56
Lipid (g/l)	11.90±0.97	46.78±2.79	87.39±2.82	1.33±0.97
Hydrogen (%)	7.71±0.09	5.83±0.02	10.65±0.01	ND
Oxygen (%)	43.35±0.04	37.55±0.22	17.32±0.27	ND
Carbon (%)	47.06±0.54	55.15±0.04	720.03±0.27	ND
Nitrogen (%)	1.88±0.02	1.46±0.03	1.76±0.01	ND
C/N ratio	25.03±0.07	37.77±0.01	409±0.03	ND

ND: Not Determine

Table 2. Introductory Conditions Utilized within the Tests

Experiments	pH	Carbon (%)	TN (%)	C/N ratio
100%DW	3.52	47.06	1.88	25.03
1%ML	7.87	0.22	0.01	37.77
5%GW	7.91	1.44	0.00	-
DW+1%ML	7.64	20.04	0.47	25.10
DW+2%ML	7.67	17.70	0.46	25.20
DW+3%ML	7.68	15.85	0.45	25.30
DW+4%ML	7.79	14.36	0.44	25.40
DW+5%ML	7.74	13.12	0.44	25.50
DW+1%GW	7.73	22.07	0.48	25.40
DW+2%GW	7.75	21.13	0.47	25.78
DW+3%GW	7.80	20.28	0.47	26.18
DW+4%GW	7.81	19.50	0.46	25.60
DW+5%GW	7.85	18.78	0.46	27.02

321, 303, 291, and 287 mL CH_4/g COD, respectively. Co-digestion of 99% DW and 1% ML was the optimal mixing ratio for methanogenesis, showing cumulative methanogenesis of 1,008 mL CH_4 and methane recovery of 334 mL CH_4/g COD at 96% biodegradability (Table 3). Maximum methane recovery from 100% DW was 265 mL CH_4/g COD with a biodegradability of 75%. On the other hand, the maximum methane yield from 1% ML was 334 mL CH_4/g COD with 95% biodegradability. The synergistic effect of co-digested DW and 1% ML resulted in a 29% increase in methane yield compared to DW alone (Figure 3). In this study, we found that increasing the proportion of molasses in the co-fermentation decreased the methane yield. However, the high content of non-biodegradable organic matter in the molasses and the anaerobic digestion resulting in only partial decomposition of the organic fraction limit the use of anaerobic processes for treating molasses ware common (Mischopoulou et al., 2016).

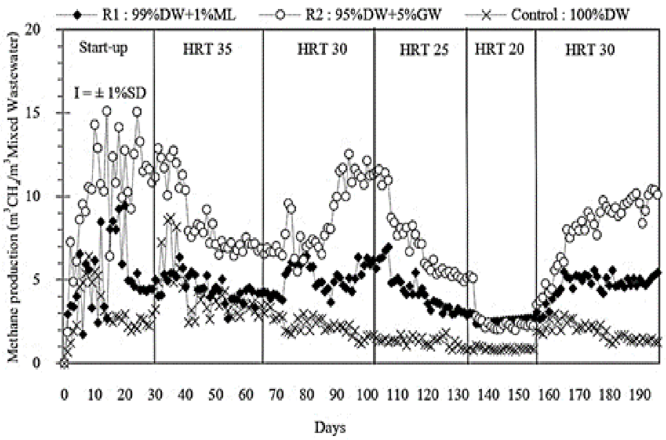

Figure 7. Methane Production Per Mixed Wastewater from Continuous Anaerobic Co-Digestion of Distillery Wastewater in R1, R2 and Control at Different HRT

3.4 Methane Potential of Co-Digestion Raw Distillery Wastewater with Glycerol Waste

The rate of methane production of DW was low and slow due to its high organic nitrogen content, which inhibits anaerobic processes. Therefore, co-digestion of DW and readily biodegradable organic matter (GW) is required to enhance the biodegradability of DW. Distillery wastewater (DW) was tested for the feasibility of co-fermentation with readily biodegradable organic matter (GW). Cumulative methane production and methane yield from the co-fermentation of DW and GW have appeared in Figure 4. The methane yields for co-fermentation

Table 3. Summary of a Resulting Parameter from Anaerobic Co-Digestion Experiments Between DW, GW, and ML

Experiments	Initial loading (g COD/L)	CH_4 yield (mL CH_4/g COD)	Biodegradability (%)	pH after digestion	VFA/Alk ratio
100%DW	11.40	265	75.43	7.64	0.15
1%ML	2.42	334	95.43	7.77	0.26
5%GW	29.25	217	62.00	7.70	0.25
DW+1%ML	9.72	334	96.23	7.67	0.13
DW+2%ML	10.01	321	92.32	7.68	0.15
DW+3%ML	10.24	303	87.12	7.79	0.16
DW+4%ML	10.43	291	83.47	7.74	0.12
DW+5%ML	10.58	287	81.87	7.72	0.26
DW+1%GW	13.34	289	82.63	7.73	0.26
DW+2%GW	16.96	277	79.28	7.75	0.24
DW+3%GW	20.25	271	77.43	7.80	0.27
DW+4%GW	23.25	270	77.10	7.81	0.25
DW+5%GW	26.00	339	94.05	7.85	0.30

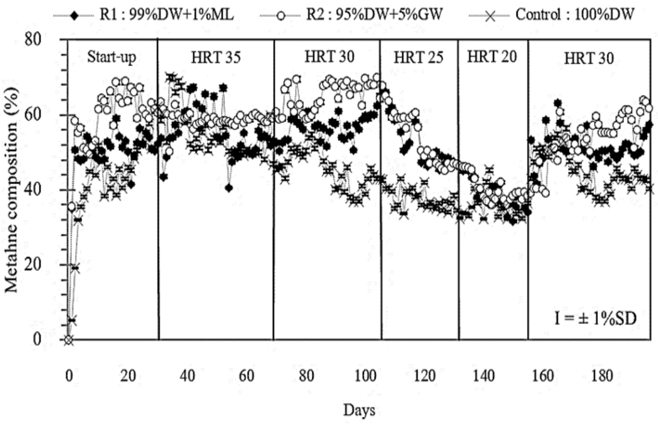

Figure 8. Methane Composition (%) from Continuous Anaerobic Co-Digestion of Distillery Wastewater in R1, R2 and Control at Different HRT

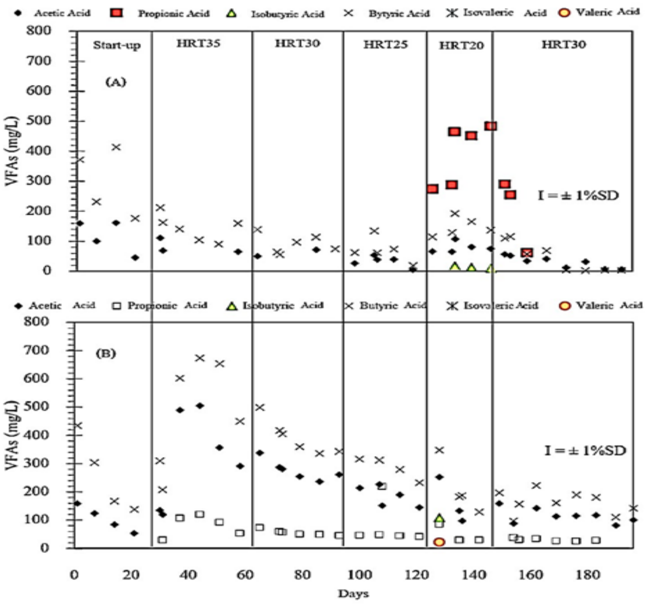

Figure 9. VFAs Concentration from Continuous Anaerobic Co-Digestion of Distillery Wastewater in R2 (A) and R1 (B) at Different HRT

of DW containing 1, 2, 3, 4 and 5% (v/v) GW were 262, 276, 270, 269, and 339 mL CH_4/g COD, respectively. The best methanogenesis results were obtained when DW and GW had a fermentation ratio of 95:5% (v/v) (Figure 4).

The mixing ratio of 95:5 of DW and GW resulted in a synergistic methane potential of 551 mL CH_4 (Figure 4), a cumulative methane production of 2,568 mL CH_4, and a methane yield of 339 mL CH_4/g COD, 94% biodegradable (Table 3). Maximum methane recovery from 100% DW was 265 mL CH_4/g COD with a biodegradability of 75%. On the other hand, the maximum methane recovery from 5% GW was 216 mL CH_4/g COD with a biodegradability of 62%. The yield of methane enhanced by 29% compared to fermenting of a single DW. These results indicated that adding 5% GW to DW could increase the production of biogas. Nuchdang and Phalakornkule (2012) detailed that co-fermentation of pig manure and GW yielded a methane of 320 mL CH_4/g COD. Alves et al. (2022) detailed adding 1% and 3% of crude glycerol (GL) to anaerobic co-digestion (AcoD) of primary sludge (PS) and food waste (FW) expanding the methane yields by 45.4% (343.3 mL CH_4/gVS) and 122.7% (525.7 mL CH_4/gVS). Furthermore, adding 5% GW to DW expanded the C/N ratio from 25.03 to 27.02 (Table 2). Using GW as co-substrates in co-digestion with DW resulted in the initial pH-enhancing between 7.73 -7.85, which was more reasonable for the methanogenesis process than a single DW that had an initial pH of 3.52 only (Table

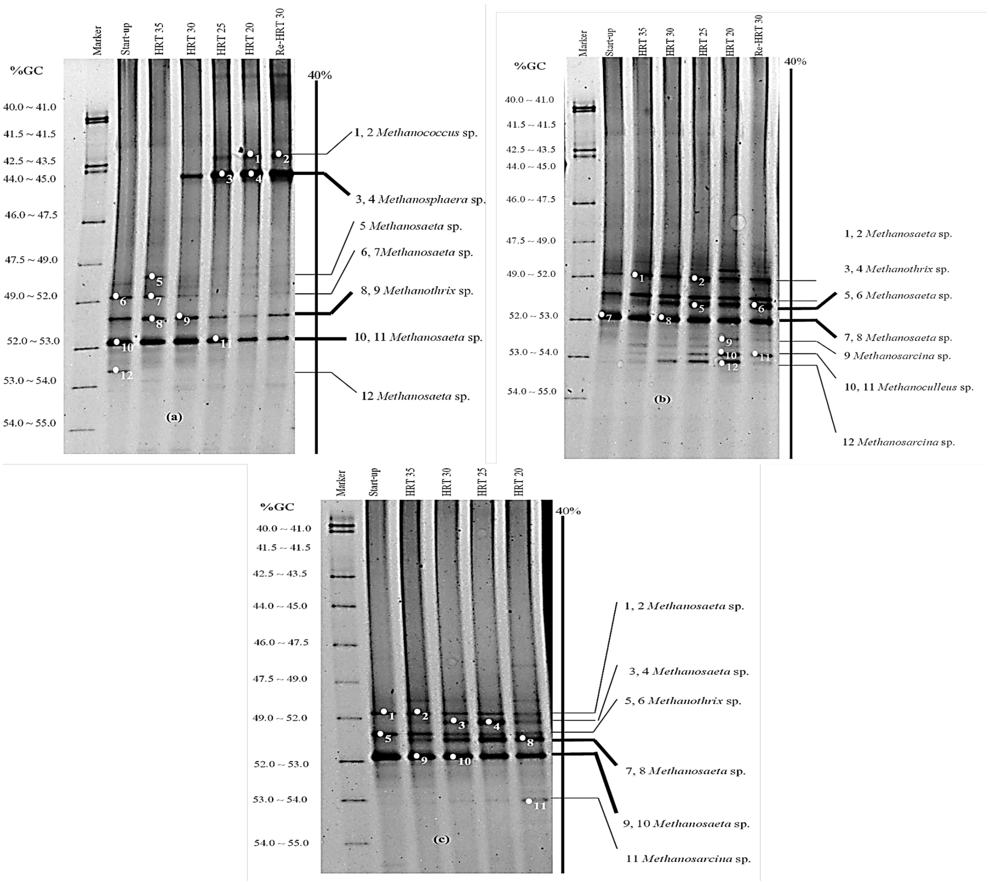

Figure 10. (a) R2 (95: 5) (b) R2 (99: 1) (c) Control (100: 0); DGGE Profiles (Archaeal Community) of 16S rRNA Gene Fragments from Continuous Anaerobic Co-Digestion of Distillery Wastewater at Different HRT

2). So, the advantage using of GW as a co-substrate could be adjusting the pH value of DW, potentially saving the cost of chemicals for pH adjustment. Final volatile fatty acids and alkalinity ranged from 1.23 to 1.62 g/L and 5.09 to 5.48 g/L, respectively. The resulting VFA/Alk ratios ranged from 0.24 to 0.30, indicating the effectiveness of anaerobic fermentation (Table 3). The optimal ratio of VFA/Alk for an anaerobic process ought to not surpass 0.4 (Souza, 1986). This suggests that co-fermentation of glycerol and stillage resulted in increased amounts of methane recovered, resulting in energy benefits. Similar to the results detailed by Rodríguez et al. (2006), which showed that digesting winery effluent or waste-activated sludge alone produced less methane and removed less COD than digesting mixed waste. This means that co-fermentation reduces the negative impact of some substrates on both wastes.

3.5 Continuous Anaerobic Co-Digestion System

To investigate biogas production efficiency, the optimal ratio from the batch reactor to operation in PFR continuous flow reactor was selected. Results were examined in terms of organic retention time by adjusting the pH of the methanogenesis reactor to 7.0–7.5 throughout the experiment. Wastewater should be conditioned with sodium bicarbonate ($NaHCO_3$) before entering the system. Sequential anaerobic co-digestion of DW with a 99:1 mixing ratio of DW and ML (R1), DW with GW mixing ratio of 95:5 (R2), and single DW (100%) were performed by the PFR reactor. A single DW (100%) was used for control in a continuous system. The results show that the maximum methane production rate for the experimental set of DW and GW ratios of 95:5 at 30 days of hydraulic retention time (HRT) is 425 mL CH_4/L/d, followed by HRTs of 35, 25, and 20 days, methane production rates were 220, 205, and 120 mL CH_4/L/day, respectively. In a replicate study of HRT for 30 days, the production rate of methane was 395 mL CH_4/L/day. In the experimental set of DW and ML in a 99:1 ratio, 30 days of HRT resulted in a maximum methane production rate of 225 mL CH_4/L/d, followed by 35, 25, and 20 days of HRT with methane production rates of it was 135, 125, and 75 mL CH_4/L/day, respectively. Comparing the individual DWs (100%) showed relatively low maximal rates of methanogenesis compared to R1 and R2 which could only produce 85 mL CH_4/L/d of methane at 35 days of HRT, followed by HRTs of 30, 25, and 20 days with methane production rates of 50, 35, and 20 mL CH_4/L/d, respectively (Figure 5). Corresponding to the maximum methane yield of R2, the HRT of 30 days was 162 mL CH_4/g COD, which was calculated from the organic loading rate (OLR) of 2.62 g COD/L/d, followed by HRTs of 35, 25, and 20 days, resulting in methane yields of 99, 60 and 30 mL CH_4/g COD at OLR of 1.74, 2.63, and 2.84 g COD/L/d, respectively. A methane yield was 97 mL CH_4/g COD from repeating experiments at HRT 30 days of the DW and GW at the ratio of 95: 5 (R2). R1 had a maximum methane yield of 110 mL CH_4/g COD at HRT 30 days for OLR 2.15 g COD/L/d, followed by HRTs of 35, 25, and 20 days, resulting in methane yields of 57, 50, and 28 mL CH_4/g COD at OLR of 2.21, 3.17, and 3.93 g COD/L/d, respectively. From repeated experiments of R1, HRT of 30 days yielded methane equal to 97 mL CH_4/g COD. Compared with a single DW (control) had maximum methane yield was 55 mL CH_4/g COD at HRT 35 days for OLR 1.44 g COD/L/d, followed by HRTs of 30, 25, and 20 days, resulting in methane yields of 35, 25, and 8 mL CH_4/g COD at OLR of 1.79, 1.95, and 2.28 g COD/L/d, respectively which is less than that of R2 and R1 experiments (Figure 6). Additionally, the HRT of 30 days was the optimal condition for methane production by R2 could produce maximum methane production per mixed wastewater of 11 m^3 CH_4/m^3 Mixed Wastewater, followed by R1 of 6 m^3 CH_4/m^3 Mixed Wastewater. Compared with a single DW (control) could produce only 2.7 m^3 CH_4/m^3 Mixed Wastewater at HRT 35 days which showed that the co-digestion process takes less time to decompose than no co-digestion (Figure 7). Because, the co-digestion process could improve the C/N ratio and weaken the harmful components in DW, resulting in enhanced efficiency for biogas production from distillery wastewater (DW). The maximum methane composition of R2 could be produced in the range of 60-70%, followed by R1 in the range of 62-65% at HRT 30 days and a single DW (control) had in the range of 30-50% only (Figure 8).

Furthermore, inhibition of methanogens limited the conversion of available VFAs, resulting in more VFA effects accumulating until the pH decreased. However, different acids have different decomposition rates such as propionic acid decomposes more slowly than other acids (Izumi et al., 2010). Corresponding with this experiment, the volume of VFAs in R2 generated propionic acid higher specifically at 20-day HRT of the trial when operation at HRT was reduced (Figure 9), resulting in a pH drop. All experiments (R1, R2, and control) showed *Methanosarcina* and *Methanothrix* species as dominant in the archaeal community in anaerobic co-digestion of DW with ML and GW (Figure 10). Qin et al. (2022) reported *Methanobacteriaceae* were the dominant methanogen in co-digestion of cattle manure (CM) and molasses vinasse (MV).

4. CONCLUSION

Anaerobic co-digestion of DW with 5% GW and 1% ML had the potential to upgrade biogas quality and amount. Cumulative methane production and methane yield were 2,568 mL CH_4 and 339 mL CH_4/g COD from DW containing 5% GW and 1,008 mL CH_4 and 334 mL CH_4/g COD from DW containing 1% ML, respectively. Cumulative methane production and methane recovery from 5% GW DW increased by 29% compared to fermentation DW and 5% GW alone. Adding GW to DW as a carbon source increased the C/N ratio from 25.03 to 27.02 and also diminished the poisonous quality of ammonia in an anaerobic system. To investigate the efficiency of biogas production, the optimal ratio from the batch reactor to operation in the PFR continuous flow reactor was selected. In addition, the HRT of 30 days was the optimal condition for methane production by R2 (95%DW: 5%GW) could produce maximum methane production per mixed wastewater of 11 m^3 CH_4/m^3 Mixed Wastewater, followed by R1(99%DW: 1%ML) of 6 m^3 CH_4/m^3 Mixed Wastewater and control (100%DW) could produce methane only 2.7 m^3 CH_4/m^3 Mixed Wastewater. Therefore, GW can be used as a co-substrate because it will increase the potential for methanogenesis in DW. DGGE profiling of sludge from the methane reactor indicated that *Methanothrix sp.* and *Methanosaeta sp.* were dominant archaeal communities and played an important role in methanogenesis.

5. ACKNOWLEDGEMENT

The authors thank the Research and Development Institute Thaksin University (RDITSU) RTA572995035, Agricultural Research Development Agency (ARDA), and the Thailand Research Fund, through grant number MSD58I0120. We would also like to express our deep gratitude to Songkhla Rajabhat University and Thaksin University for their cooperation in research facility.

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