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Research article

Biogas and Biohythane Production from Anaerobic Co-digestion of Canned Sardine Wastewater with Glycerol Waste

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Abstract: A biochemical methane potential (BMP) test investigated the effect of glycerol waste (GW) concentration on anaerobic co-digestion with canned sardine wastewater (CSW). They were studied using the single-stage process at mesophilic (P1) and thermophilic (P2) conditions and two-stage mesophilic (P3) processes. The P3 process has provided the most significant potential for improving biogas production in the sardine canning industry. Using 4% GW (v/v), the optimal hydrogen and methane concentrations at P3 are 43.00 ml H_2/g CODr and 303.69 ml CH_4/g CODr, respectively. The P3 process was 11.33 m^3 biohytane/m^3 mixed substrate, and the biohytane composition contained 43.11% CH_4, 21.45% H_2, and 35.43% CO_2. The modified Gompertz model could simulate satisfactory hydrogen and methane yields, corresponding to high regression coefficients (R^2>0.90). Hydrogen-producing bacteria in the H_2 batch reactor were dominated by *Micrococcus sp*. and *Desulfovibrio sp*., while *Methanosaeta sp*., *Methanoculleus sp*., and *Methanosarcina sp*. are the major methanogens in the CH_4 batch reactor. A two-stage process of co-fermenting CSW and GW could be a potential option for simultaneous biofuel recovery and waste treatment.

Keywords: Anaerobic co-digestion; Biochemical methane potential (BMP); Canned Sardine wastewater; Glycerol waste; Biohytan

1. Introduction

Anaerobic digestion (AD) is a biological degradation process that converts organic compounds (carbohydrates, proteins, and fats) to produce methane and carbon dioxide without oxygen. The AD can be divided into four consecutive stages. Firstly, complex organic compounds are hydrolyzed by hydrolytic bacteria to form water-soluble simple organic compounds. In the second stage, soluble simple organic compounds are converted into organic acids, carbon dioxide, hydrogen, and alcohol by acidogenic bacteria (acidogens), which is called acidogenesis. The third stage is acetogenesis, in which the organic acids produced are broken down into acetic acid, carbon dioxide, and hydrogen. Finally, hydrogen and acetic acid are converted into methane by metanogenic bacteria (methanogens), which is methanogenesis [1]. Biogas is the fermentation into AD from organic waste fermentation [2]. Most biogas compositions contain 60-65% of methane, 34-39% of carbon dioxide, and

about 1% of other gas, such as nitrogen and hydrogen sulfide [3]. Additionally, the advantage of AD is highly removed organic, using less energy and excess sediment than aerobic digestion [4]. Likewise, the quantity and quality of biogas depend on the substrate used in the production. If the substrate contains highly toxic (ammonia, hydrogen sulfide, sodium, etc.) that affect methane production, the co-digestion strategy can solve this problem. The co-digestion process is an option to increase the efficiency of AD; one waste stream is mixed with other waste for sharing costs with the treatment advantages of this process can be to dilute the inhibitor, improve the balance of nutrients and synergistic effect of microorganisms resulting in a higher yield of methane [5]. The biochemical methane potential test (BMP) is the most commonly used method by academic and technical professionals to determine the maximal methane production of a given substrate. The BMP test can also be used to estimate rate constants of rate-limiting steps (e.g., the hydrolysis rate of high-particulate substrates) required for optimal design and operation of anaerobic digesters [6].

The canned sardine industry is one of the main industries in Thailand. Most factories are located in the coastal areas in southern and eastern Thailand. Canned sardine wastewater (CSW) contained 100-3,000 mg/l BOD, 1,000-18,000 mg/l COD, and 80-1,000 mg/l nitrogen [4]. CSW is protein-rich wastewater rapidly decomposed into ammonia nitrogen during anaerobic digestion. High concentrations of ammonia could seriously inhibit the activity of methanogens, resulting in less efficiency in the production of biogas and treatment [8]. Generally, total ammonia nitrogen (TAN), i.e., ammonium ion (NH_4^+); AI^+ free ammonia nitrogen (NH_3); FAN, which is generated from the breakdown of protein-based substrates, is generally known as an inhibitor in the AD process [9]. Adjusting the C/N ratio of the substrate by using a co-digestion strategy could potentially reduce the concentration of TAN in an anaerobic system. Yenigum and Demirel [9] reported that a C: N ratio between 25 – 35 is optimal for the AD process due to low and stable TAN and FAN. However, a C: N ratio lower than 15 could lead to a high TAN and FAN level in the AD process. Thus, optimization of the C: N ratio resulted in a stable co-digestion process. Due to these limitations, only a small amount of biogas is produced from CSW and is not worth investing in an anaerobic treatment system. Thus, the AD system for treating the canned sardine industry is unattractive.

Glycerol waste (GW), a by-product of transesterification for biodiesel production, is generated approx. 10 kg-GW for every 100 kg of biodiesel produced. GW contains 50-60% of glycerol, 12-16% of alkalis, 15-18% of methyl esters, 8-12% of methanol, and 2-3% of water [10]. Pure glycerol is used in many industries, such as cosmetics, food, pharmaceuticals, etc. However, the purification process of GW is too expensive. Therefore, AD is currently an alternative method to utilize GW, as GW is a cheap and easy-to-implement high-carbon source for anaerobic biogas production [11]. Rivero et al. [10] reported that the high C of GW could increase the C/N ratio in the mixed substrate, dilute inhibitors of the process through an excess of N, and enhance methane production by about 50-200% in the AD process. Thus, GW is an interesting substrate to be used as a co-substrate in the co-digestion process to solve the high nitrogen content in the AD system and to increase biogas production. Anaerobic co-digestion between animal manure and 3-6% glycerin could produce 570-680 l CH_4/g VS, a threefold enhancement over feeding only waste [12].

Presently, there are various modes of AD operation, such as one-stage mesophilic AD, one-stage thermophilic AD, two-stage mesophilic AD, and two-stage thermophilic AD. Each operating mode has different advantages and disadvantages. Mesophilic and thermophilic AD are operated at temperatures ranging from 30-40 ^{o}C and 45-65 ^{o}C, respectively [9]. Yenigum and Demirel [9] suggested that the advantages of thermophilic processes compared to mesophilic processes are higher digestion rates, higher methanogenesis rates, faster solid-liquid separation, and minimized accumulation of bacterial and viral pathogens. The disadvantage of the thermophilic process is that it is operated at high temperatures, causing a higher heating cost [13]. For two-stage AD, the first acidogenic stage produces volatile fatty acids (VFAs), hydrogen, and carbon dioxide. After that, solubilized effluent from the acidogenic stage is fed into the second (methanogenic stage) for methane and carbon dioxide production [14]. The advantages of two-stage AD compared with one-stage AD are increasing net energy balance, higher organic loading rates, enhancing the specific activity of methanogens, increasing methane production rate, and increasing overall COD and VS reduction efficiencies [15]. Researchers have generally found that AD systems operating in a two-stage configuration outperform conventional single-stage systems regarding methanogenesis and digestion stability [14].

The aim of this study is to evaluate the potential of hydrogen and methane production from batch anaerobic co-digestion of CSW and GW by using different operating modes (one-stage mesophilic, one-stage thermophilic, and two-stage mesophilic process) and using mixed anaerobic microflora at differences of GW

concentration. Experimental results are expected to be used for further development of biogas production by using the co-digestion strategy for wastewater generated from the canned sardine industry.

2. Materials and Methods

2.1 Inoculum, GW, and CSW

A mixed anaerobic microbiota was collected from a palm oil biogas plant in southern Thailand (Southern Palm (1978) Co., Ltd.) and used as an inoculum in this study. The inoculum was adapted with CSW to enhance mesophilic and thermophilic inoculum. CSW was mixed with the mixed anaerobic microflora in a 1:1 ratio, after which the pH of the broth was adjusted to the range of 6.8-7.2 by adding 1N NaOH and 1N HCl. The inoculum was then incubated at 37°C and 50°C in mesophilic and thermophilic inoculum incubators. If the inoculum exhibits a constant biogas production rate and composition, the acclimated inoculum can be further used in the experiment. GW was received from the biodiesel plant at Prince of Songkla University (Hat-Yai campus) in southern Thailand. The CSW was collected from Saim International Food Public Co., Ltd in southern Thailand. After collection, CSW was stored at 4 °C before use.

2.2 Biochemical methane potential (BMP)

Experiments were performed in a one-step mesophilic process (P1), a one-step mesophilic process (P2), and a two-step mesophilic process (P3) under anaerobic batch co-digestion (Figure 1). All experiments were performed under batch conditions using 120 mL glass serum bottles (60 mL working volume). P1, P2, and P3 were operated at 37°C, 50°C, and 37°C, respectively. For methanogenesis, 24 ml of inoculum and 36 ml of mixed substrate were placed in each glass serum bottle. Mixed substrates (CSW+GW) were tested at different GW concentrations ranging from 1% to 5% (v/v) and then adjusted to neutral pH. Nitrogen gas (1.5 MPa, 1 minute) was passed through the mixed substrate to replace oxygen, and the mixture was sealed with a silicone rubber and aluminum cap to create an anaerobic state. For the P3 process, the first stage was hydrogen production from the mixed substrate (CSW+GW), and the second stage was methane production from wastewater produced in the first stage. For the first hydrogen production, the inoculum was boiled at 105 °C for 60 min before being used to adjust the pH of the mixed substrate to approximately 5.5. The inoculum was not boiled for the second methanogenesis and was used under the same conditions as the P1 process. Hydrogen and methane production was measured by water displacement. Gas samples in the headspace of all experiments were then analyzed by gas chromatography. All Experiments used distilled water, sucrose, and 100%CSW as control experiments for comparison.

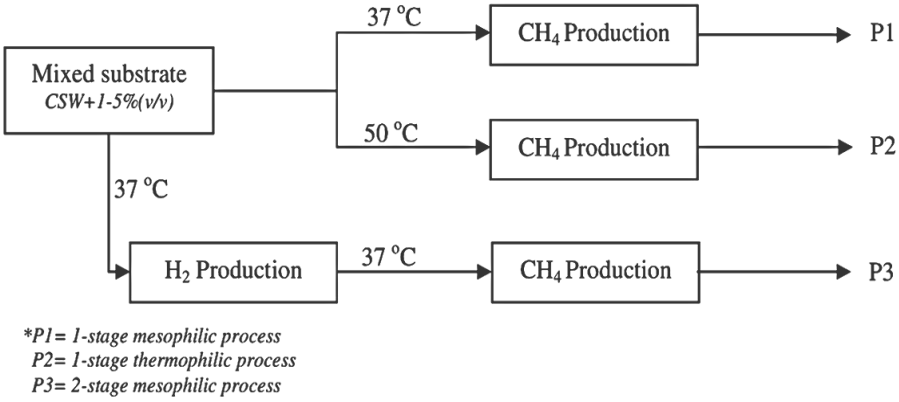

Figure 1. Research procedure

2.3 Kinetic of biogas analysis

In experiments, a modified Gompertz model was used to explain the biogas production from batch anaerobic co-digestion between CSW and GW as shown in Eq.(1) [16].

$$G(t) = G_0 . \exp\left\{-\exp\left[\frac{R_{\max} . e}{G_0}(\lambda - t) + 1\right]\right\} \quad (1)$$

Where G(t) is the cumulative hydrogen or methane yield (ml CH_4/g CODr or ml H_2/g CODr G_0 is the maximum hydrogen or methane yield (ml CH_4/g CODr or ml H_2/g CODr), R_{max} is the maximum hydrogen or methane production rate (ml CH_4/g CODr-day or ml H_2/g CODr-day), e is the exp(1) = 2.7183, λ is the lag phase time (day) and t is the cultivation time (day).

2.4 Microoganism community analysis by DGGE

Sludge from the optimal condition was collected for community analysis using the DGGE technique. Polymerase chain reactor gradient gel electrophoresis (PCR-DGGE) was used to study microbial community structure in this experiment, the procedure of which was explained by Kongjan et al. [17]. The PCR products from the experiment were purified and sequenced by Macrogen Inc. (Seoul, Korea). The closest matches for partial16S rRNA gene sequences were identified by database searches in Gene Bank using BLAST [18].

2.5 Analytical methods

pH was measured by using a pH meter (Horiba, Japan). Chemical oxygen demand (COD), total solid (TS), volatile solid (VS), total nitrogen (TN), protein, carbohydrate, and fat were analyzed following the procedures explained in the Standard Method [19]. The volume of biogas was measured by water replacement and biogas composition was monitored by gas chromatograph GC-8APT with thermal conductivity detector (TCD), Shimazu, Japan [13]. Gas chromatograph GC-8APF analyzed the VFA with a flame ionization detector (FID), Shimazu, Japan [13].

3. Results and Discussion

3.1 GW and CSW property

The main composition of the CSW was: pH 6.8, total chemical oxygen demand (COD) 12.00 g/l, total solids 8.5 g/l, volatile solids 6.4 g/l, total nitrogen 1.50 g/l, 3.90 g/l protein, 1.91 g/l carbohydrate, 0.13 g/L fat, and C: N ratio 11. The main composition of GW was: pH 8.8, total chemical oxygen demand (COD) 1760 g/l, total solids 969 g/l, volatile solids 910 g/l, total nitrogen 1.7 g/l, protein 1.28 g/l, carbohydrate 845 g/l, fat 63.76 g/l, C: N ratio 949. Table 1 shows the chemical composition of the mixed substrates after co-digestion with GW (1–5% (v/v)). After co-digestion, the concentrations of COD and TN increased from 28 to 82 g/l and from 0.887 to 1.047 g/l. In addition, pH and C: N ratios were improved in the range of 7.28-8.17 and 27-73.

Table 1. The chemical property of the mixed substrate after co-digested with GW

Samples	pH	COD (g/L)	TN (g/L)	C/N ratio
CSW+1%GW	7.28	28.00	0.887	27
CSW+2%GW	7.52	48.00	0.993	43
CSW+3%GW	7.76	56.00	1.004	51
CSW+4%GW	8.01	70.00	1.027	63
CSW+5%GW	8.17	82.00	1.047	73

3.2 Biochemical Methane Potential

For P1, the maximum cumulative methane production was 293.82 ml, with 73.15% methane in biogas during digestion at 1% GW, corresponding to a 244.85 ml CH4/g CODr methane yield. Methane production was increased by approximately 8.27 times compared to a single digestion of CSW, producing 35.52 mL of methane, corresponding to 62.15% of the methane in biogas. As shown in Figure 2A, adding 2-5% GW to CSW increased methane production by 1.94-3.83 fold, increasing GW concentration and potentially significantly reducing methane production. Moreover, the COD removal efficiency with co-fermentation at 1% GW for 25 25-day fermentation period is about 97.29%, which is consistent with the increased methane production. The P2 process was carried out under thermophilic conditions (^oC). The results showed that adding 2% of GW into

CSW could result in the maximum methane production with a methane yield of 255.21 ml CH_4/g CODr (68.48% of methane in biogas) for 14 days of fermentation, as shown in Figure 2B. The methane production increased by about 40.76 fold compared to a single-digestion of CSW, which gave methane 16.29 ml CH4/g CODr (37.28% of methane in biogas). In addition, P2 introduced higher COD concentrations (28-48 g/l) into the system than P1, allowing methanogens to adapt to optimal conditions in P2 and resulting in higher levels of methane production. So, the efficiency of COD removal was high, about 97.50%, which was similar to P1. Furthermore, increasing the concentration of GW higher than 3% (v/v) could lower methane production but still higher than a single-digestion of CSW (3.06-7.81 fold). Finally, the P3 process was separated into 2 reactors consisting of the reactor for hydrogen production (1-stage) and methane production (2-stage) and operated at mesophilic conditions. P3 could get a high COD compared with P1 and P2 processes. The system can take a high organic loading rate (OLR). The optimum condition for P3 was using 4% GW as a co-substrate, leading to an initial concentration of 70 g-COD/l. The production of maximum hydrogen and methane was 45.40 ml (39.04% of H_2) and 634.11 ml (78.48% of CH_4), corresponding to hydrogen and methane yields of 43.00 ml H_2/g CODr and 303.69 ml CH_4/g CODr, respectively. (Figure 2C). Rivero et al. [10] reported that hydrogen and methane yields of 26 mL H_2/g CODr and 290 mL CH_4/g CODr can be achieved by anaerobic mesophilic co-digestion of sewage sludge at 1% (v/v) GW. Hydrogen and methane production was 648.57 and 7.75 fold of those from the single-digestion of CSW, which could barely generate hydrogen and methane of 0.07 and 81.87 ml, respectively. After 45 days of fermentation, the COD removal efficiencies in 1-stage- and 2-stage fermentation were 31.43% and 93.33%, respectively. The result showed that using GW as a co-substrate in co-digesting with CSW could significantly increase the potential of hydrogen and methane production. As a result, the maximum hydrogen and methane production was obtained in the P3 process. GW can improve the carbon source of CSW and reduce the production of toxic ammoniacal nitrogen, leading to an increase in the C: N ratio, as shown in Table 1. Thus, the proper C/N ratio could enhance microorganism adaptation and increase hydrogen and methane production. Additionally, adjusting the C/N ratio could effectively reduce the inhibition of organic acids [20].

3.3 Effect of volatile fatty acid (VFA)

In all experiments, VFAs detected in the P1 process on 7 days of fermentation were acetic acid, propionic acid, I-butyric acid, and N-butyric acid. Under P1 optimum conditions, adding 1%GW generated total VFAs 801.17 mg/l, containing 91.48 mg/l acetic acid, 619.04 mg/l propionic acid, and 90.65 mg/l N-butyric acids (Figure 3A). Adding GW into CSW in a 2-5% range resulted in higher total VFA accumulation in the AD system. Although VFAs were the main substrate for producing methane by methanogens, their accumulation in the AD system with high concentration could directly inhibit methanogens, consequently causing the final pH to decrease (Figure 4). Decreasing pH in the AD system could majorly reduce methanogen activity. GW as a co-substrate at the concentration of more than 1% (v/v) in P1 resulted in a significant decrease in final pH. (Figure 4A). Using 1%GW as co-substrate, the final pH was 7.78, but the concentration of GW was more than 1% (v/v), and the pH was decreased to a range of 5.6-6.4. Generally, at lower pH, VFA is turned into an undissociated form. This undissociated VFA becomes more toxic to methanogens due to its ability to free cross-membrane cells, leading to dissociated and consequently lower internal pH and, finally, the cause of disruption of homeostasis [21]. Normally, the optimal pH range in the AD system for methane generation is between 6.00 and 8.00 [3]. Franke-Whittle et al. [22] reported that the accumulation of most situations reflected an imbalance between acid producers and consumers, resulting in a pH drop in the system, which could inhibit the growth of methanogens.

For the P2 process, methanogens under thermophilic conditions could take GW up to 2% (v/v) concentration for the highest methane production. The total VFAs detected on 5 days was 2,031.14 mg/l, consisting of 495.35 mg/l acetic acid, 257.80 mg/l propionic acid, 43.11mg/l I-butyric acid, and 1,234 mg/l N-butyric acids (Figure 3B). Adding GW higher than 3% (v/v) resulted in higher VFAs accumulation and pH decrease (Figure. 4B). The final pH of 7.46 was detected under the AD system with 2% (v/v) GW), while Anaerobic co-digesting CSW with GW higher than 2% (v/v) in P2 resulting in pH lower than 6.10. The high performance of P2 thermophilic AD compared to P1 mesophilic AD is definitely due to the advantages of operating under thermophilic conditions.

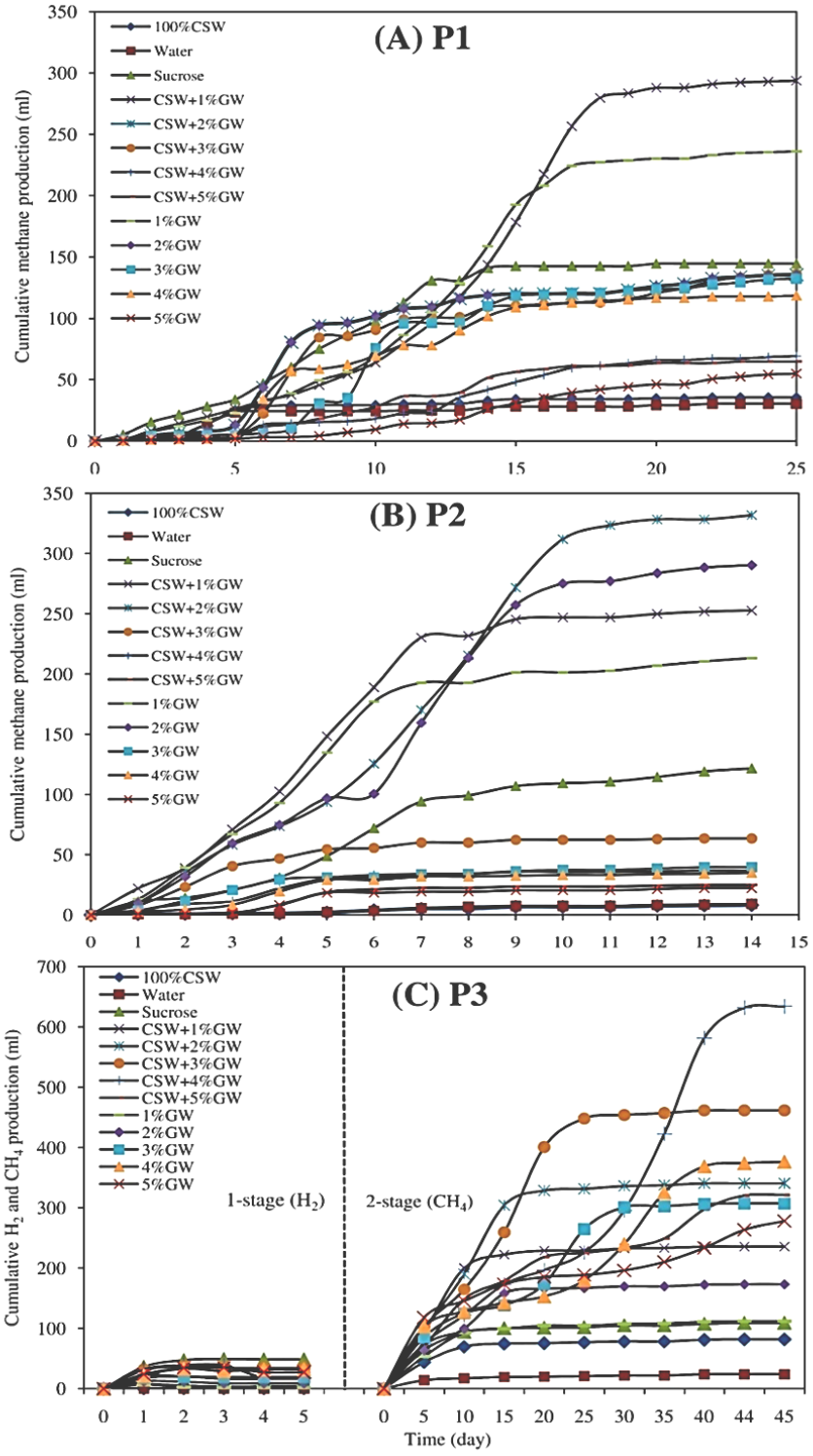

Figure 2. Hydrogen and methane production profile; (A) P1 process, (B) P2 process and (C) P3 process

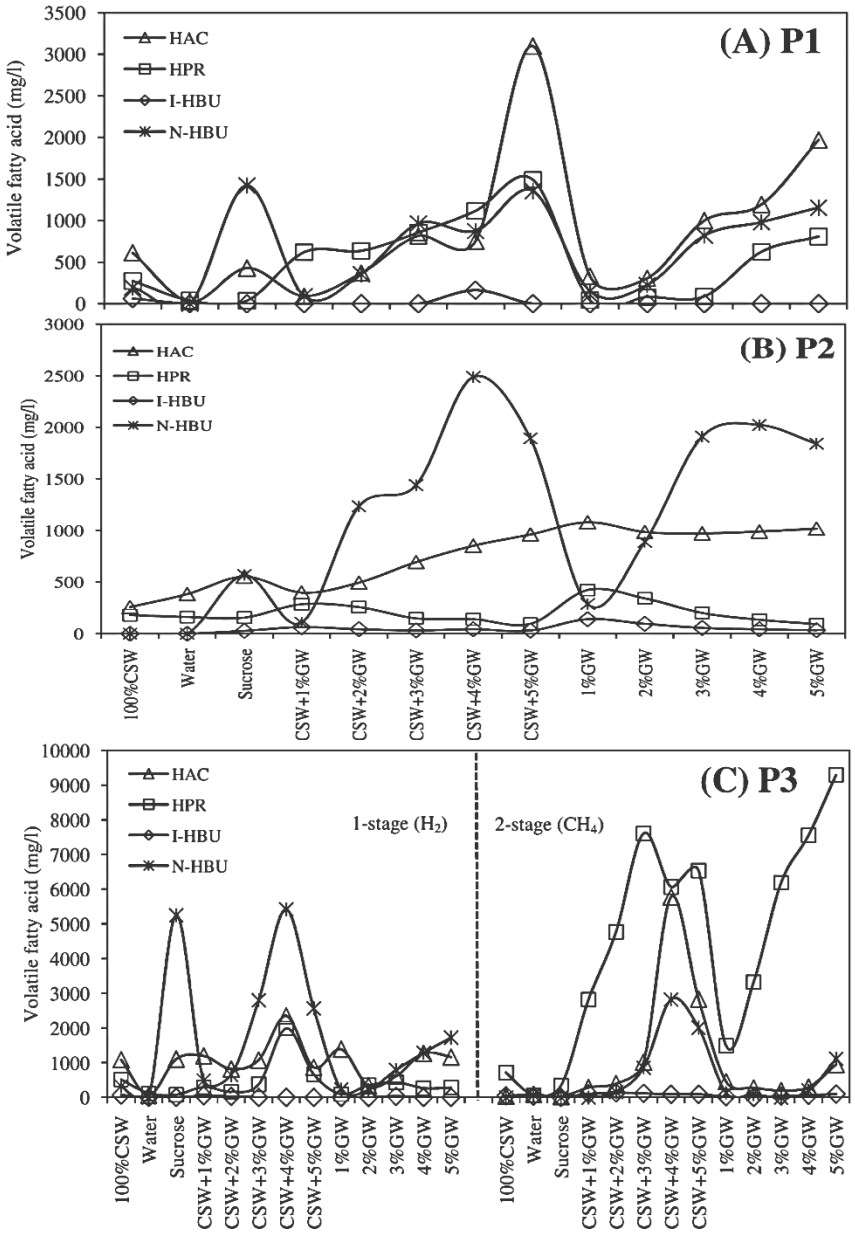

Figure 3. Volatile fatty acid profile; (A) P1 process, (B) P2 process and (C) P3 process

For the P3 two-stage AD process, the increase in the concentration of VFAs followed the increase in the concentration of COD by adding the GW ranging from 1-5% (v/v). In the first fermentation stage with 4% of GW co-digested with CSW having the maximum hydrogen production yield of 43.00 ml-H_2/g-CODr, the main composition of VFA at 3 days was contained 5,420.53 mg/l N-butyric acid, 2,360.54 mg/l acetic acid and 1,990.05 mg/l propionic acid (Figure 3C). Using the GW as a co-substrate in anaerobic co-digestion for hydrogen production was suitable since hydrogen was generated along with only the formation of N-butyric acid and acetic acid. Other metabolites generated could reduce hydrogen production. Furthermore, the formation of propionic acid could cause a severe reduction in biohydrogen production because propionic acid formation consumes hydrogen previously produced [1]. Sreethawong et al. [23] reported that propionic acid formation should be avoided to improve biohydrogen production. Additionally, the final pH in all co-digestions had pH values ranging from 5.20-5.90 (Figure 4(C)), which corresponded to the experiment of Lue et al. [24] reporting hydrogen production decreased with an increase in pH of more than 6. The effluent from the 1-stage was subsequently digested in the second stage process for methane production. The total VFA at

5 days of fermentation with 4% of GW was 14,756.93 mg/l, which contained 5,779.65 mg/l acetic acid, 6,061.11 mg/l propionic acids, 92.39 mg/l I-butyric acid and 2,823.85 mg/l N-butyric acid (Figure 3C, 5C). The VFA in P3 was generated in a higher concentration than in the P1 and P2 processes; however, the VFA accumulation in the P3 process was lower, corresponding to the final neutral pH (7.60-7.80) in all experiments. This is due to the system having good buffering capacity indicating a balance between acid producers and consumers (Figure 4C). Thus, methanogens in the second stage of P3 could adapt well to hydrogen effluent mainly containing VFA to produce methane higher than P1 and P2. Therefore, high VFA concentration (acetic, propionic, and butyric acid) is not problematic for producing methane for the P3 process.

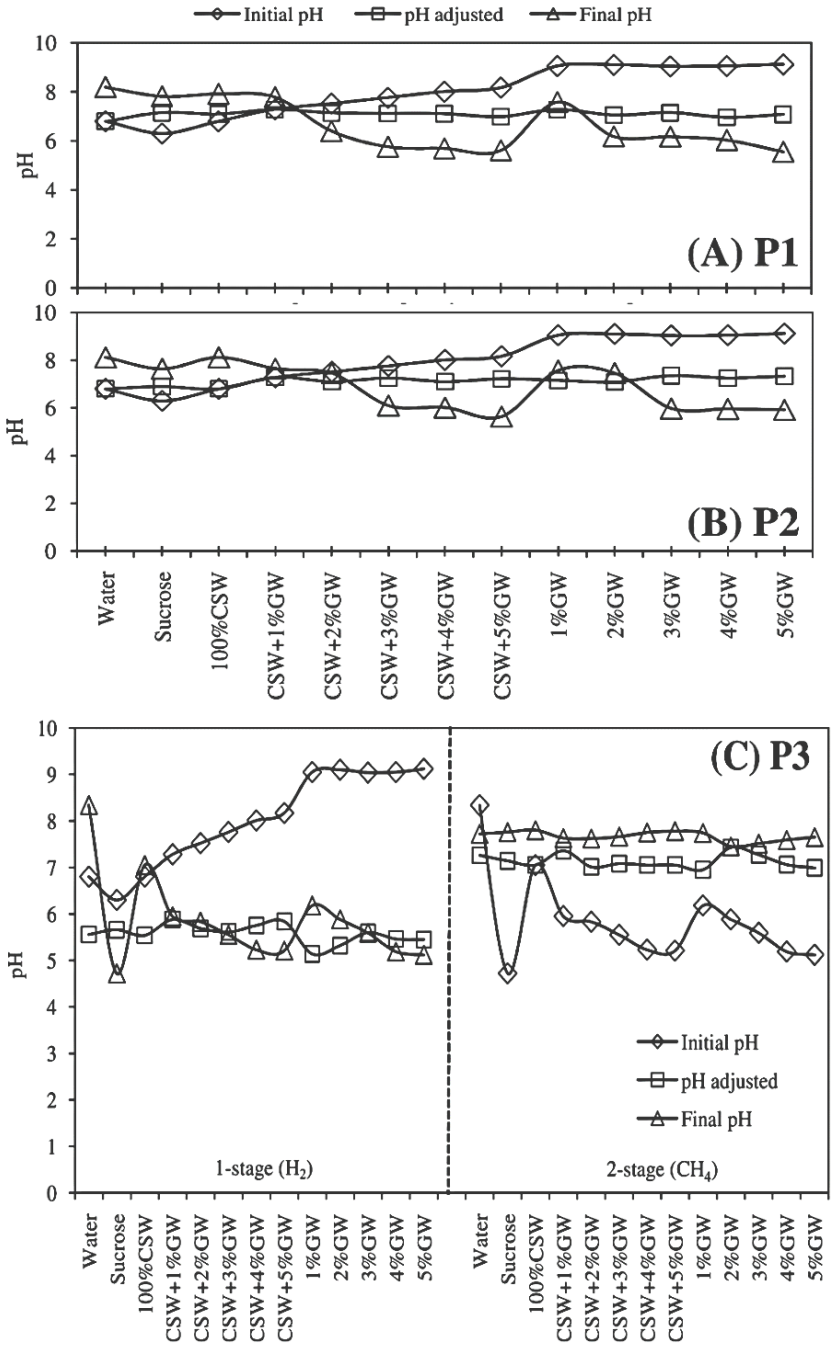

Figure 4. pH profile; (A) P1 process, (B) P2 process and (C) P3 process

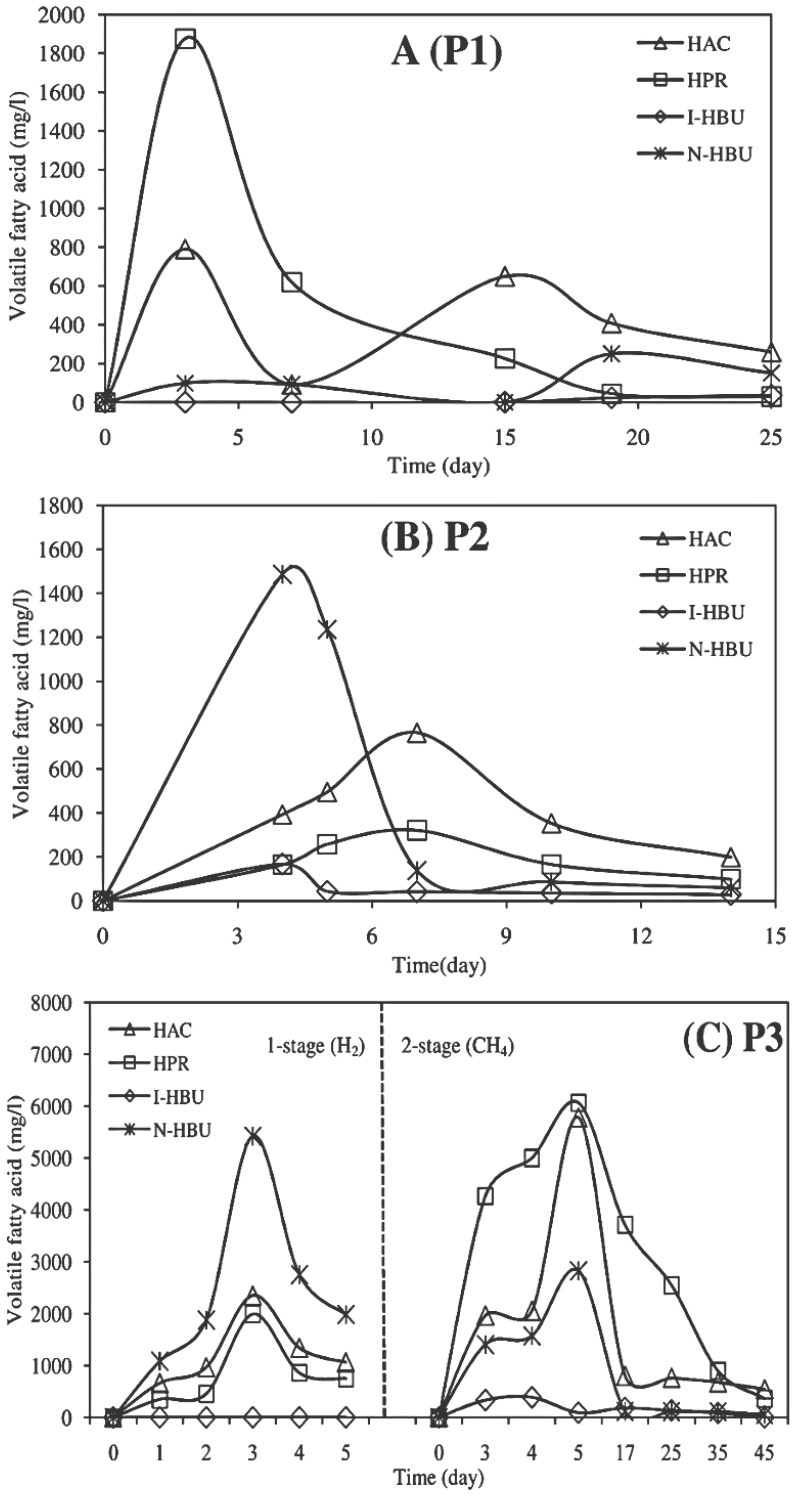

Figure 5. Comparison of the volatile profile under the optimal condition by co-digestion of CSW and GW at different operating processes; (A) P1 process (CSW+1%GW), (B) P2 process (CSW+2%GW), and (C) P3 process (CSW+4%GW)

3.4 Kinetic analysis under the optimal condition

Among three different process configurations, P3 is the best, having the maximum concentration of COD (70 g/l), resulting in maximum methane production. In the optimal condition of 3 processes, the methane

yield of P3 (CSW+4%GW) is 1.19 and 1.24 fold of P2 (CSW+2%GW) and P1 (CSW+1%GW), respectively. The maximum VFA was generated from 3 to 5 days in all processes during the methane production and on 3 days for fermentation to produce hydrogen. VFA generated is afterward used by methanogenic microorganisms to produce methane, resulting in continued VFA reduction in the systems, as shown in Figure 5. For P3, total VFA existing in the first stage was 3,794.21 mg/l consisting of 1,058.55 mg/l acetic acid, 750 mg/l propionic acid, and 1,985.66 mg/l N-butyric acids at 5 days of fermentation, while the second stage had total VFA 960.04 mg/l, containing 543.42 mg/l acetic acid, 360.51 mg/l propionic acid and 51.10 mg/l N-butyric acid at the end of batch operation day 45 as shown in Figure 5C. Additionally, single-digestion of CSW and co-digestion with GW could prove a synergistic effect is generated by adding GW for co-digestion to produce biogas in all processes. P3 could produce maximum methane production. The total energy yield from the P3 process was 11.33 m^3 biohytanes/m^3 mixed substrate. The biohytane composition in this study contained 43.11% CH_4, 21.45% H_2, and 35.43% CO_2. Mamin et al. [15] and Khongkliang et al. [25] reported that the composition of biohythane (hydrogen and methane) obtained from two-stage anaerobic digestion of palm oil mill effluent (POME) and starch processing effluent is 51% CH_4, 14% H_2, 35% CO_2, CH_4 is reported to be 55%, 10% H_2, and 35% CO_2. This is very similar to his H2 composition results from this study, indicating that it is significantly lower. As a result, GW could increase the potential in hydrogen and methane production from CSW by using the P3 process. The result indicates that 2-stage anaerobic co-digestion with GW under mesophilic condition (P3) could enable it to operate under high VFA levels compared to the thermophilic condition, which has a higher cost in operation. So, P3 is one of the attractive choices for increasing the potential to produce hydrogen and methane in the canned sardine industry. Furthermore, the initial temperature of CSW is in the mid-temperature range, so operation in thermophilic conditions is not suitable for CSW.

For kinetic analysis, a modified Gompertz model was used to explain the biogas production. The parameters from the optimum process are summarized and compared with experimental and simulation results using the modified Gompertz model. The parameters from the optimum process are outlined in Table 2. Experimental and simulation results of the modified Gompertz model are shown in Figure 6. For the P3 process, the lag phase time (λ) is 3 days, lower than the 6.65 days of P1. The λ value in P3 is lower than P1, indicating the methanogen could adapt in the 2-stage AD process faster than that in the one-stage AD process under mesophilic conditions, resulting in a maximum methane yield (G0) was high (303.40 ml CH_4/g CODr), compared to P1(244.85). Additionally, the modified Gompertz model parameter for hydrogen production in P3 was 1.33 days of λ value and 42.83 ml H_2/g CODr of G_0 value. In all cases, the maximum production rate (R_{max}) is 21.65, 30.58, and 5.80 ml CH_4/g CODr-day for P1, P2, and P3, respectively. Although the R_{max} of P3 is significantly lower, the difference in COD loading and Rmax could not be compared. However, although the P2 process has the smallest λ value (2.55 days), the advantage of P3 is that it could get COD loading higher, generate H_2 gas, and have less cost for heating compared to the P2 process. Thus, biogas production co-digested with GW and CSW under P3 mesophilic AD process is more interesting than P1 and P2 in improving biogas production potential from wastewater from the canned sardine industry. The coefficient of determination (R^2) was higher for the modified Gompertz model during 0.918-0.984 for methane production and 0.976 for hydrogen production, indicating that the modified Gompertz model could simulate satisfactorily for P1, P2, and P3 processes. Figure 7 shows the DGGE profiles of sludge from the 1-stage and 2-stage of the P3 process. The bacteria community structure in the first-stage reactor (H_2) is dominated by *Micrococcus sp*. and *Desulfovibrio sp.* (Figure 7A). Meanwhile, the archaea community in the 2-stage reactor (CH_4) is dominated by *Methanosaeta sp*., *Methanoculleus sp*., and *Methanosarcina sp*., as shown in Figure 7B.

Table 2. The modified Gompertz model parameter values at various process

Process	modified Gompertz model parameter (H_2)				modified Gompertz model parameter (CH_4)			
	G_0 (ml H_2/g CODr)	R_{max} (ml H_2/g CODr-day)	λ (day)	R^2	G_0 (ml CH_4/g CODr)	R_{max} (ml CH_4/g CODr-day)	λ (day)	R^2
P1	-	-	-	-	244.85	21.65	6.65	0.957
P2	-	-	-	-	255.21	30.58	2.55	0.984
P3	42.83	1.25	1.33	0.976	303.40	5.80	3.00	0.918

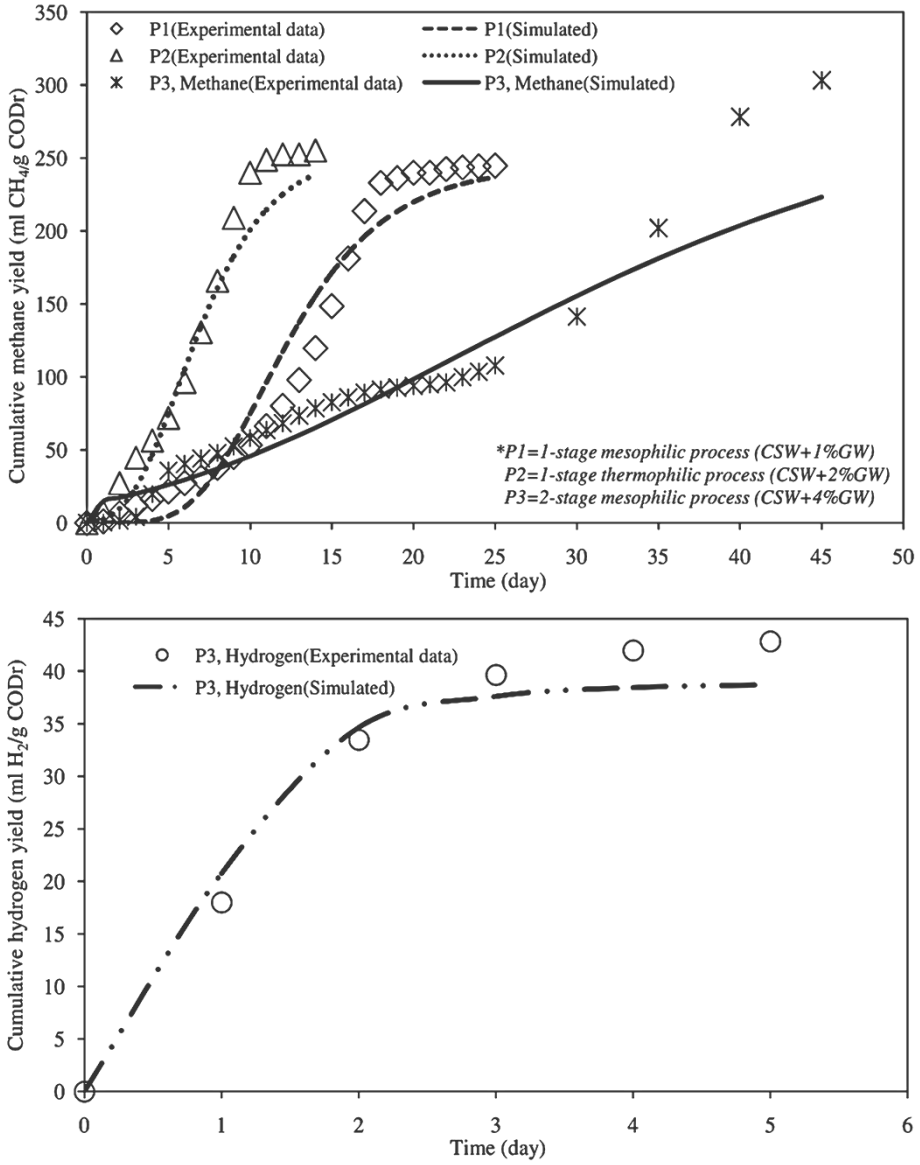

Figure 6. Comparison of the Cumulative methane and hydrogen yield between experimental data and simulation by modified Gompertz model

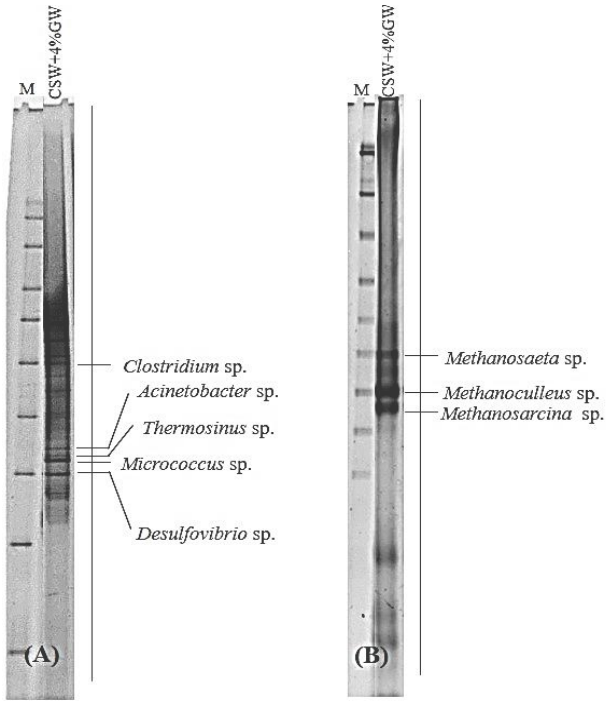

Figure 7. DGGE profiles of 16S rRNA gene fragments for sludge samples from the P3 process of CSW+4%GW, (A) 1- 1-stage fermentation and (B) 2-stage fermentation

4. Conclusions

The results show that two-stage anaerobic co-digestion (P3) with GW under mesophilic conditions can increase hydrogen and methane production by 648.57-fold and 7.75-fold, respectively, compared to CSW-only digestion. The optimal concentration of GW using co-substrate in the P3 process was 4%(v/v). The P3 can handle higher COD concentrations than processes P1 and P2, resulting in a higher organic loading rate (OLR) in the continuous system. So, the P3 process is one of the interesting choices for increasing the potential of hydrogen and methane production from canned sardine wastewater.

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