ASEAN
Journal of Scientific and Technological Reports
Online ISSN:2773-8752

Research article

Optimization of Hydrogen and Methane Co-production from Co-digestion of Canned Seafood Wastewater with Glycerol Waste in a Two-stage Continuous System: Comparing CSTR-PFR and CSTR-CSTR Reactors

Tussanee Srimachai[1, 6, 7, 8], Mathavee Thipmunee[2], Yakob Longsoh[2], Peemanas Manaswin[4], and Kiattisak Rattanadilok Na Phuket[5, 6, 7, 8*]

[1] College of Innovation and Management, Songkhla Rajabhat University, Songkhla, 90000, Thailand
[2] Biofuel and Biocatalysis Innovation Research Unit, Mahidol University, Nakhonsawan Campus, 60130, Thailand;
[3] La-ngu Industrial and Community Education College, Satun, 91100, Thailand
[4] College of Innovation and Management, Songkhla Rajabhat University, Songkhla, 90000, Thailand
[5] College of Innovation and Management, Songkhla Rajabhat University, Songkhla, 90000, Thailand
[6] Faculty of Science and Technology, Songkhla Rajabhat University, Songkhla, 90000, Thailand
[7] Microbial Resources and Utilization Center SKRU, Songkhla Rajabhat University, Songkhla, 90000, Thailand
[8] Community Innovation Learning and Transfer Center "Thung Yai Sarapee Model" Songkhla Rajabhat University, Satun, 91100, Thailand
* Correspondence: kiattisak.pa@skru.ac.th

Citation:
Srimachai, T.; Thipmunee, M., Longsoh, Y.; Manaswin, P., Rattanadilok Na Phuket, K. Optimization of hydrogen and methane Co-production from Co-digestion of canned seafood wastewater with glycerol waste in a two-stage continuous wystem: comparing CSTR-PFR and CSTR-CSTR reactors. *ASEAN J. Sci. Tech. Report.* **2025**, *28*(6), e259743. https://doi.org/10.55164/ajstr.v28i6.259743.

Article history:
Received: July 26, 2025
Revised: September 20, 2025
Accepted: September 27, 2025
Available online: October 19, 2025

Publisher's Note:
This article is published and distributed under the terms of the Thaksin University.

Abstract: The challenge posed by canned seafood wastewater (CSW) involves a low COD of 6.80 g/L and a high protein concentration of 3.56 g/L, making it unsuitable for hydrogen and methane production. Consequently, the potential return on investment for establishing a commercial system remains inadequate. To address this issue, a two-stage anaerobic digestion system incorporating co-digestion with glycerol waste (GW) was implemented. The two-stage co-digestion of CSW with GW, at various mixing ratios of 99:1, 98:2, 97:3, 96:4, and 95:5% (v/v), resulted in hydrogen yields of 15.6, 33.6, 38.7, 65.0, and 6.3 ml H_2/g COD, respectively, while methane yields were measured at 311, 320, 326, 345, and 99 ml CH_4/g COD, correspondingly. The ideal conditions for achieving the highest yields of hydrogen and methane from the anaerobic co-digestion of CSW with GW were found to be at a mixing ratio of 96:4% (v/v). The ongoing production of hydrogen and methane in a two-stage process utilizing CSTR-PFR and CSTR-CSTR reactors can yield hydrogen and methane at rates of 27.44 and 163.61 L/L of wastewater, and 20.41 and 145.35 L/L of wastewater, respectively. Anaerobic co-digestion of CSW with GW could enhance the production of hydrogen and methane from a two-stage anaerobic digestion system.

Keywords: Hydrogen; methane; canned seafood wastewater; glycerol waste; two-stage anaerobic digestion system

1. Introduction

Hydrogen and methane are types of renewable energy from the decomposition of organic waste, such as sewage and industrial waste, used in the production of hydrogen (Hydrolysis, Acidogenesis) and methane production (Methanogenesis). The microorganism was divided into two phases. Acidogenic bacteria produce a pH of 5-6 and a hydraulic retention time (HRT) of 2 days in the system. As part of the methane production process, the methanogen requires different conditions from the acid-producing bacteria. The pH value in this stage ranges from 7 to 8 and takes about 15–20 days. Separating the two microorganisms will help to degrade the substance. Single digestion and co-digestion to balance nutrients. It allows the degradation process to occur completely, and it could harvest hydrogen and methane [1]. Bertasini et al. [2] reported that hydrogen-methane co-production is achieved through anaerobic fermentation using a two-stage biological process. In the first stage, known as dark fermentation, microorganisms break down biomass feedstocks to produce hydrogen and volatile fatty acids (VFAs). In the second stage, methanogenic microorganisms are added to the effluent from the first stage, which utilizes anaerobic fermentation to convert the VFAs produced during hydrogen production into methane [3].

Sillero et al. [4] reported that the agri-food industry generates numerous waste streams of different origins and compositions, which are susceptible to pollution sources. Canned seafood wastewater represents a significant industry that provides advantages for Thailand. The process of seafood canning consumes a substantial amount of water, resulting in high levels of effluent ranging from 14 to 20 cubic meters per ton of material [5]. The chemical makeup of canned seafood wastewater (CSW) includes nitrogen concentrations between 80 and 1,000 mg/l, COD levels from 1,000 to 18,000 mg/l, and BOD values ranging from 100 to 3,000 mg/l [6]. Anaerobic digestion is less popular than the problem of organic nitrogen and high sodium concentrations. This substance inhibits microbial activity in anaerobic systems [7]. Canned seafood processing wastewater contains high levels of protein and fat, which tend to degrade ammonia quickly in anaerobic conditions. The tiny amount of biogas generated as a result of these limitations makes the expense of building an anaerobic treatment system unjustified. Most seafood processing plants do not use an anaerobic digestion system for wastewater treatment. Most factories prefer to use aeration systems instead because they are easier to manage, but they also have higher energy costs. However, the problem can be solved by using a common fermentation technology. Co-digestion technology is the use of wastewater from fermented canned seafood processing plants in combination with other organic carbon sources. The advantage of joint fermentation technology is the concentration of organic matter in the effluent, expressed as COD. Dissolved toxins reduce the effect of methane-producing microorganisms in wastewater, resulting in higher methane yield [8].

Approximately 10% of the raw materials used in biodiesel manufacturing are glycerol wastes, which are byproducts of the process [9]. In 2011, the world's total glycerol waste was about 3,000,000 metric tons. It is expected to increase to 4,600,000 tons by 2020, based on the expansion of biodiesel production [10]. The advantages of using glycerol waste include an easy-to-digest fermentation process during the decomposition process, which enhances the C: ratio due to its high carbon content, and dilutes the poison in the system [11]. The use of glycerol waste as a co-fermentation medium has been reported to increase methane production by 50-200% due to the optimal use of suitable fermentation media, which promotes the fermentation process to yield positive synergies. Therefore, it is possible to utilize fermentation technology to develop and apply it to canned seafood processing factories. This is a waste treatment process that can add value to waste by converting it into energy. It is interesting because it has the potential to develop into a sustainable source of energy in the future. The study focused on optimizing the production of hydrogen and methane through the single digestion of canned seafood wastewater and glycerol waste, as well as the co-digestion of these two waste streams. Additionally, it examined continuous hydrogen and methane production by comparing the reactor's performance in methane processes using a two-stage anaerobic method.

2. Materials and Methods

2.1 Feedstock and inocula

Canned seafood wastewater from Siam International Food Company, Songkhla, Thailand. Inocula from the biogas system of Chotiwat Hatyai, trading frozen food, Songkhla, Thailand. Characteristics of canned seafood wastewater and glycerol waste are shown in Table 1. Anaerobic sludge was collected from the biogas system. The sludge was treated by heating, where it was boiled at 100 °C for 1 h [12] to remove methanogenic bioactivity from the hydrogen inoculum. Before using the hydrogen, the inoculum was starved for 1 week to minimize the effects of organic materials contained in the microbial sludge before starting the system. The methane inoculum was incubated at pH 7 under mesophilic conditions (37°C).

2.2 Batch reactor

Hydrogen and methane production from single digestion of canned seafood wastewater (CSW 50, 60, 70, 80, 90 and 100% (v/v)), glycerol waste (GW 1, 2, 3, 4, and 5% (v/v)) and co-digestion canned seafood wastewater with glycerol waste was tested at different mixing ratios were determined in batch assays under the mesophilic condition as described previously. The first stage was operated in a batch test under an initial pH of 5.5. Hydrogen effluent was investigated for methane production in the second stage under an initial pH of 7. A two-stage batch fermentation system, comprising hydrogen fermentation in the first stage and methane fermentation in the second stage, was established in 500 mL serum bottles with a working volume of 200 mL. Two-stage anaerobic co-digestion of CSW with GW at concentrations of 99:1, 98:2, 97:3, 96:4, and 95:5% (v/v). The system was flushed with nitrogen gas to generate anaerobic conditions. During the fermentation experiment, total gas volume and composition were periodically monitored by gas counters and gas chromatography, respectively.

2.3 Continuous reactor

Continuous anaerobic co-digestion of CSW with GW was operated by comparing the continuous stirred-tank reactor (CSTR) to the plug-flow reactor (PFR), R1, and the continuous stirred-tank reactor (CSTR) to the continuous stirred-tank reactor (CSTR), R2. Working volumes are 1 L (1-stage) and 5 L(2-stage), respectively. The continuous experiment was operated under the optimal conditions determined from the batch test. Experiment reactors were operated at HRTs of 2, 3, 4, and 5 days for the hydrogen stage (1-stage) and HRTs of 10, 15, 20, and 25 days for the methane stage (2-stage) under mesophilic conditions. The biogas production was measured by water displacement (using a gas counter) every day. Biogas composition analysis by GC-TCD.

2.4 Microorganism community analysis by DGGE

Polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) was employed in this study to analyze the microbial community structure, following the methodology described by Kongjan et al. [13]. The PCR products obtained from the experiment were purified and sequenced by Macrogen Inc. (Seoul, Korea). The closest matches for the partial 16S rRNA gene sequences were determined by performing database searches in GenBank using BLAST [14].

2.5 Analytical methods

The protein, carbohydrate, lipid content, pH, volatile fatty acids (VFAs), and alkalinity of CSW and GW were assessed. Standard procedures for examining wastewater were applied to single digestion of canned seafood wastewater, glycerol waste, and co-digestion of wastewater with glycerol waste. The amount of biogas produced daily for each test was noted using the water displacement technique [15]. Gas chromatography with thermal conductivity detectors (TCD) was employed to analyze the composition of the biogas. Methane, carbon dioxide, hydrogen, and nitrogen were evaluated using a GC-TCD equipped with a 3.3 ft stainless steel column packed with Shin Carbon (60/80 mesh). Argon served as the carrier gas at a flow rate of 14 mL/min. The temperatures for the injection port, oven, and detector were set at 120 °C, 40 °C, and 100 °C, respectively [16]. A 1 mL gas sample was injected in two separate trials for H_2 (1-stage) and CH_4 (2-stage). The theoretical methane potential was calculated based on the elemental composition of carbon, hydrogen, nitrogen, and oxygen using Bushwell's formula, which is derived from the stoichiometric conversion of the compound to methane [17]. The energy yield from biogas was estimated using energy factors of 12.9 J per mL of H_2 and 40.1 J per mL of CH_4 [18].

3. Results and Discussion

3.1 Substrate and co-substrate characterization

The CSW was sourced from Siam International Food Company, located in Songkhla, Thailand. The primary components of CSW included a pH of 7.3, 6.8 g/l of total chemical oxygen demand (COD), 2.48 g/l of total solids, 1.23 g/l of volatile solids, 0.57 g/l of total nitrogen, 3.56 g/l of protein, 0.19 g/l of carbohydrate, and 1.55 g/l of fat. Following its collection, the CSW was kept at -20 ºC until needed. The glycerol waste (GW) was obtained from the biodiesel facility at Prince of Songkhla University's Hat-Yai campus in southern Thailand. The key components of GW were as follows: a pH of 8.7, 1,082 g/l of total chemical oxygen demand (COD), 279.53 g/l of total solids, 254.96 g/l of volatile solids, 0.26 g/l of total nitrogen, 1.65 g/l of protein, 845 g/l of carbohydrate, and 63.76 g/l of fat. The details regarding the composition of canned seafood wastewater and glycerol waste are presented in Table 1.

Table 1. Characteristics of canned seafood wastewater, glycerol waste, and inoculum composition

Compositions	Substrates		
	CSW	**GW**	**Inoculum**
pH	7.3	8.7	7.2
TS (g/L)	2.48	309.41	77.45
VS (g/L)	1.23	284.25	67.06
Ash (g/L)	1.24	24.57	10.39
COD (g/L)	6.80	1,082	ND
VFA (mg/L)	1,216	2,080	7.140
Carbohydrate (g/L)	0.89	20.82	0.25
Protein (g/L)	3.56	1.65	16.87
Nitrogen (g/L)	0.57	0.26	2.7
Lipid (g/L)	0.11	88.65	9.24
C: N	11.93	416.00	ND

ND: Not Determined

3.2 Hydrogen and methane production from single digestion of CSW and GW

Two-stage anaerobic single digestion of CSW at a concentration of 50, 60, 70, 80, 90, and 100% (v/v) has H_2 yield of 5.6, 8.3, 8.8, 9.5, 10.7, and 18.0 mL H_2/g COD, respectively (Figure 1) and methane yield was 274, 323, 345, 333, 328 and 321 mL CH_4/g COD, respectively (Figure 2). Glycerol waste (GW) had a high COD of 1,082 g/L. Two-stage anaerobic single digestion of GW at a concentration of 1, 2, 3, 4, and 5% (v/v) has H_2 yield of 10.9, 24.3, 37.2, 23.6, and 2.5 ml H_2/g COD, respectively (Figure 3) and methane yield was 179, 299, 336, 228, and 147 CH_4/g COD, respectively (Figure 4). The single digestion at 3% GW yields high hydrogen, and 70% CSW yields high methane. Results agreed with [6] CSW is a type of wastewater protein that rapidly decomposes into ammonia nitrogen, particularly during anaerobic digestion. High concentrations of such compounds can directly inhibit the activity of methanogens [19]. The anaerobic breakdown of these types of waste during the acidification stage occurs more quickly than the methanogenic stage, resulting in the accumulation of volatile fatty acids (VFAs) in the reactor. This accumulation results in a gradual decrease in pH, which subsequently hinders the performance of methanogenic archaea. Therefore, co-digestion contributes to balancing the C: N ratio in the system.

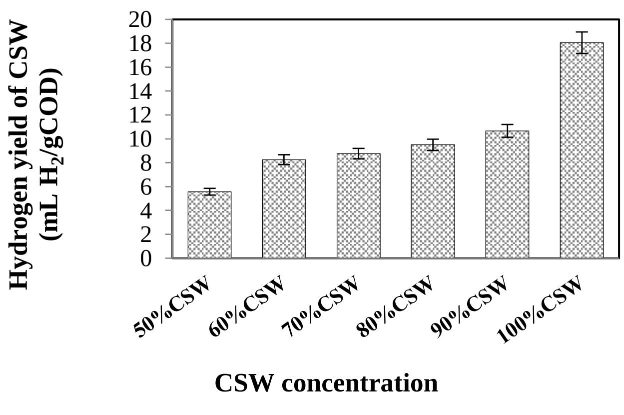

Figure 1. Hydrogen yield of CSW

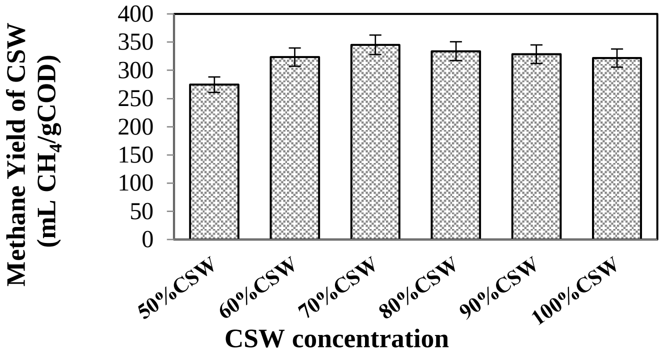

Figure 2. Methane yield of CSW

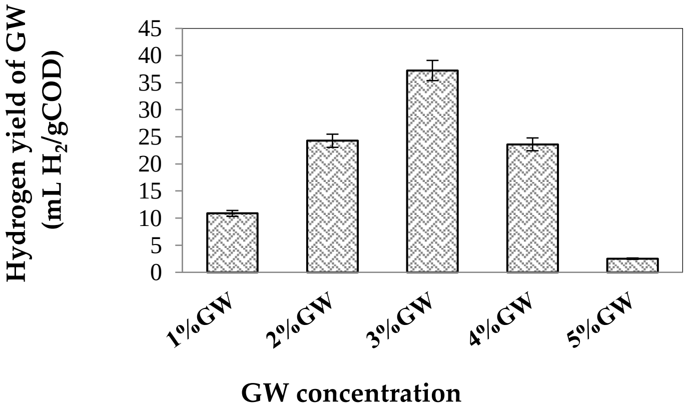

Figure 3. Hydrogen yield of GW

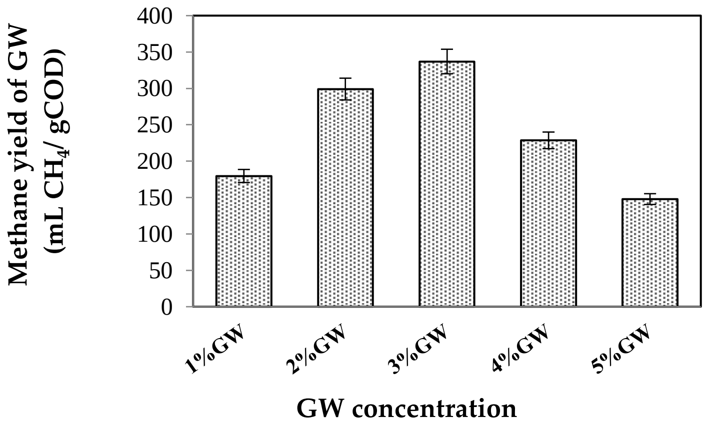

Figure 4. Methane yield of GW

3.3 Hydrogen and methane production from co-digestion of CSW and GW

Two-stage anaerobic co-digestion of CSW with GW at mixing ratios of 99:1, 98:2, 97:3, 96:4, and 95:5% (v/v) has H_2 yield of 15.63, 33.66, 38.79, 65.00, and 6.33 ml H_2/g COD, respectively (Figure 5) and methane yield was 311.57, 320.18, 340.26, 345.08 and 99.39 ml CH_4/g COD respectively (Figure 6). The maximum hydrogen and methane yields from anaerobic co-digestion of CSW with GW were achieved at a mixing ratio of 96:4% (v/v). The C: N ratio of 34.32 was more effective in biodegradation, resulting in higher hydrogen and methane yields (Table 2). The C: N ratio is a primary factor that indicates the presence of appropriate nutrients in the anaerobic system [20]. Additionally, optimizing the C: N ratio between 20 and 35 is practical for creating synergies during co-substrate digestion [21]. The degradation efficiency of co-digestion CSW with GW was higher than 10% in the hydrogen stage (1-stage) and 90% in the methane stage (2-stage), as shown in Table 2. Increasing the C:N ratio resulted in biodegradation rates higher than 14.44% and 98.59%, respectively. For biodegradation efficiency calculated by 1 - C/C_0 * 100, where C_0 is the initial COD concentration and C is the COD concentration after a specific time.

Table 2. The effect of the ratio CSW to GW on the C: N ratio, hydrogen and methane yield, and biodegradation

Condition	C: N ratio	Yield (mL/g COD)		Biodegradation (%)	
		H_2	CH_4	H_2	CH_4
99% CSW+1%GW	21.10	15.63	311.57	3.47	89.02
98% CSW+2%GW	25.20	33.66	320.18	7.48	91.48
97% CSW+3%GW	30.65	38.79	340.06	8.62	97.15
96%CSW+4%CW	34.32	65.00	345.08	14.44	98.59
95%CSW+5%CW	40.21	6.33	99.39	1.40	28.39

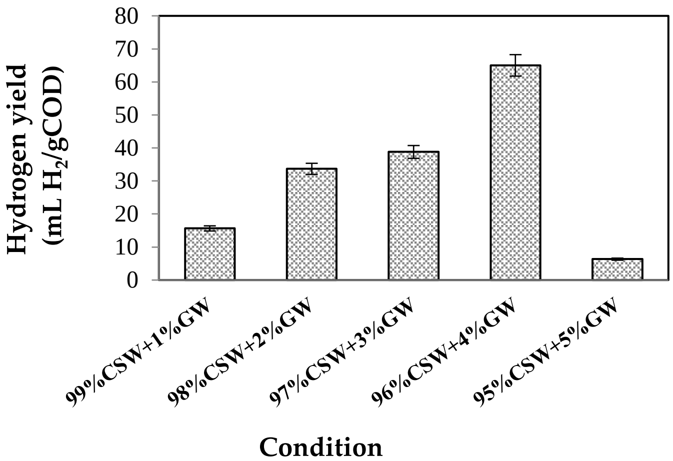

Figure 5. Hydrogen yield of CSW: GW

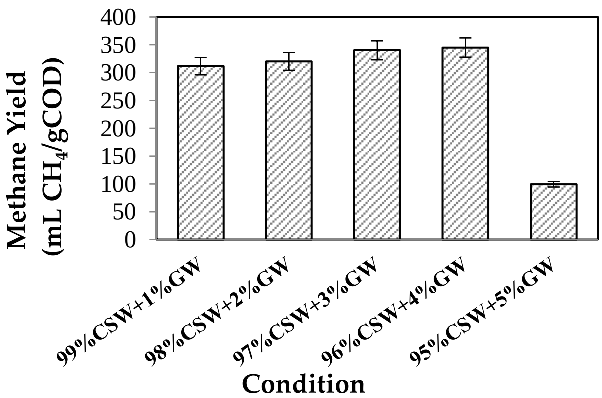

Figure 6. Methane yield of CSW: GW

The hydrogen energy of 0.018 kWh/kg COD in the first stage and 3.03 kWh/kg COD in the second stage is shown in Figure 7. Biodegradation resulted in a significant decrease at 5% GW, leading to reduced hydrogen and methane production. Results agreed with. Panpong et al. [22] demonstrated that incorporating GW as a co-substrate alongside CSW could significantly boost biogas production potential. GW can improve the carbon supply from CSW, thereby reducing toxicity. The concentration of ammonia nitrogen increased, as indicated by the rise in the C:N ratio. The system demonstrated good adaptability to the mixed substrate, resulting in increased production of hydrogen and methane.

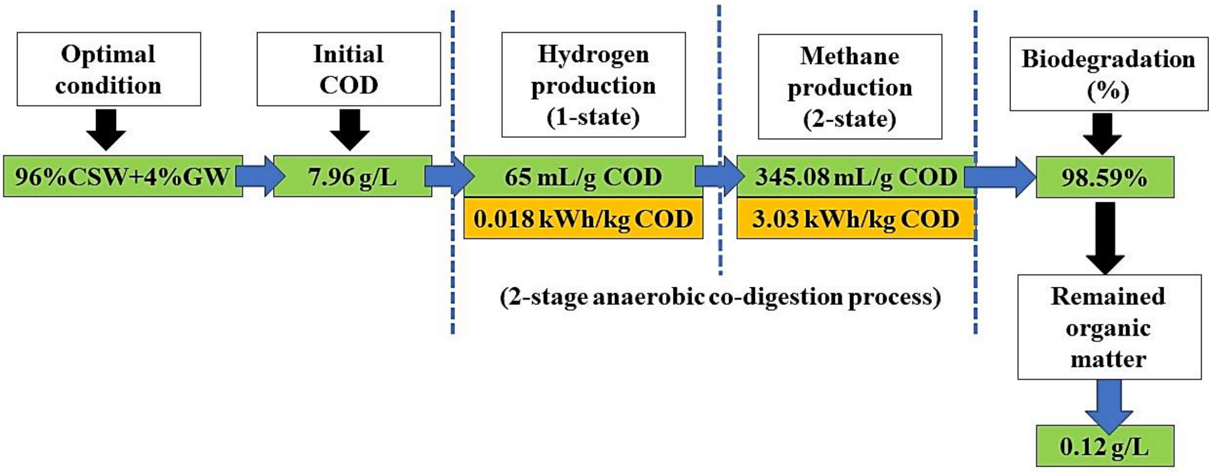

Figure 7. Mass and energy balance

3.4 Continuous hydrogen and methane production of co-digestion CSW with GW, and comparing between R1 and R2 reactors

A mixing ratio of 96:4% (v/v) was determined to be the optimal condition from a batch test; it will continue to be operated in a continuous system. Continuous hydrogen and methane production from co-digestion of CSW with GW of mixing ratio of 96:4% (v/v) by two-stage CSTR-PFR (R1) and CSTR-CSTR (R2) was investigated. R1 and R2 reactors had maximum hydrogen and methane yield (Hydrogen and methane production rate) of 25.2 mL H_2/L/day, 150 mL CH_4/L/day, 18.76 mL H_2/L/day, and 133.60 mL CH_4/L/day, respectively. Wongarmat et al. [23] reported that a hydrogen production rate of 193.6 mL H_2/L/day at an optimal hydraulic retention time (HRT) of 3 days and a methane production rate of 422.0 mL CH_4/L/day with an HRT of 20 days from co-digesting filter cake (FC), biogas effluent (BE), and anaerobic sludge (AS) from the sugar and ethanol industry. The reason why the hydrogen and methane production rate in co-digesting FC, BE, and AS from the sugar and ethanol industry is higher when compared to the co-digested canned seafood industry with glycerol waste due to the high COD value of FC, BE, and AS (290.03, 58.56, and 78.85 g/L) [23]. The canned seafood industry had 6.80 g/L of COD, resulting in a higher hydrogen and methane production rate than wastewater from the canned seafood industry. Hydrogen and methane production from the Two-stage R1 and R2 reactors were 27.44 and 163.61 L/L wastewater, 20.41 and 145.35 L/L wastewater, respectively. Hydrogen reactor production increased when the HRT was increased to 4 days, decreased after the HRT was reduced to 3 days, and then recovered to 4 days of HRT, indicating that 4 days of HRT is the maximum HRT (Figure 8). While reactor methane production increased when the HRT was extended to 20 days, the trend decreased after reducing the HRT to 15 and 10 days, and then recovered within 20 days of the original HRT, indicating that 20 days of HRT was the optimal HRT for methane production (Figure 9). Sillero et al. [4] reported that a maximum hydrogen yield was obtained in the acidogenic phase and mesophilic methanogenic phase at 5 and 12 days of HRT, respectively, from sewage sludge and waste from the agri-food sector (poultry manure and vinasse). The results from the experiment showed that methane production from the PFR reactor had a higher yield than the CSTR reactor. Corresponding to the result of Ting Sun et al. [24], the increased organic loading rate brought more substrate into the anaerobic digestion system and enhanced the concentration of substrate. When the organic loading rate increased to a high level, the overloading substrate for biogas/methane production was inhibited. The advantage of the PFR reactor was that it was fed slowly, due to its long pipe system, resulting in the PFR being able to obtain a higher organic loading rate compared to the CSTR reactor [25]. Consequently, it results in the microorganisms in the system being more stable and resistant to chemicals and changes than those in the CSTR reactor. PFR reactors provided flexibility to the system even with increased feed solid concentration, and therefore, are more stable than CSTR systems. Therefore, the R1 reactor (CSTR+PFR) was the optimal continuous system for producing hydrogen and methane from CSW through co-digestion with GW in a two-stage anaerobic digestion system. In addition, co-digestion results in controlling the C/N ratio, enhancing the elimination of organic compounds, decreasing inhibitors, and preserving moisture content [26] to make the Hydrogen and Methane Co-production system balanced and efficient. The optimal results showed that the 1-stage (H_2) in R1 contained a microorganism community of a total of 14 dominant species, which were dominated by *Pseudofulvimonas sp., Clostridium sp., Burkholderia sp., Thermanaerovibrio sp., Sphingomicrobium sp., and Thermococcoides sp.* at HRT 4 days (HRT 4), is the best organic retention period for hydrogen production for the CSTR-PFR reactor (R1) (Figure 10). Meanwhile, the archaea community in the 2-stage reactor (CH_4) was dominated by *Methanobacterium sp., Methanoculleus sp., Methanosarcina sp., and Methanosaeta sp.* at HRT 20 days (Figure 10). Most archaea communities form a methyl group in the acetate molecule, from which methane is formed in more than 70 percent of cases.

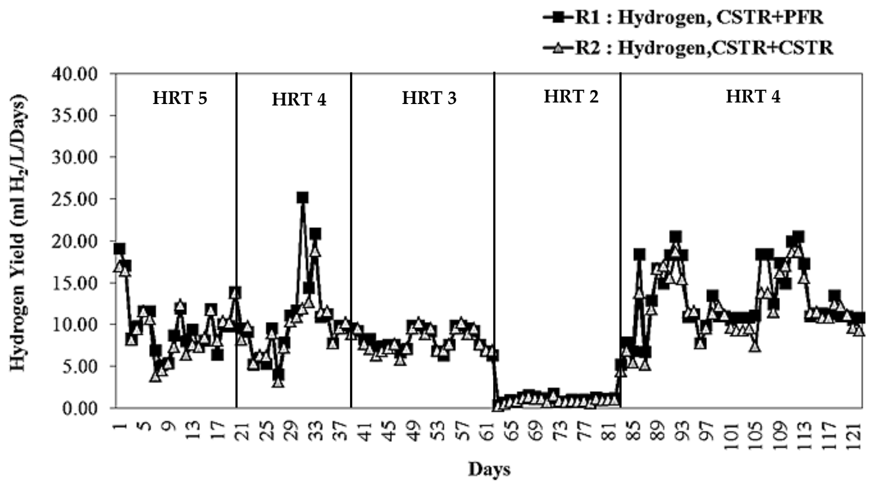

Figure 8. Hydrogen yield of different reactors and different HRTs

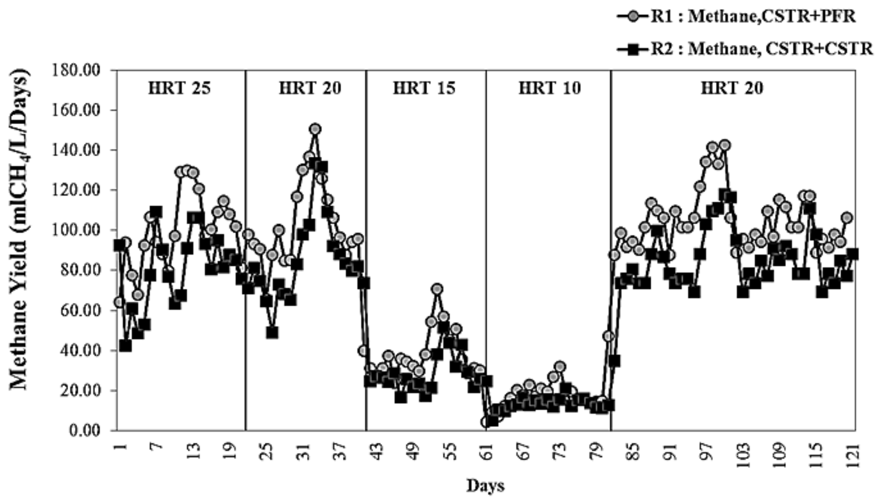

Figure 9. Methane yield of different reactors and different reactor HRTs

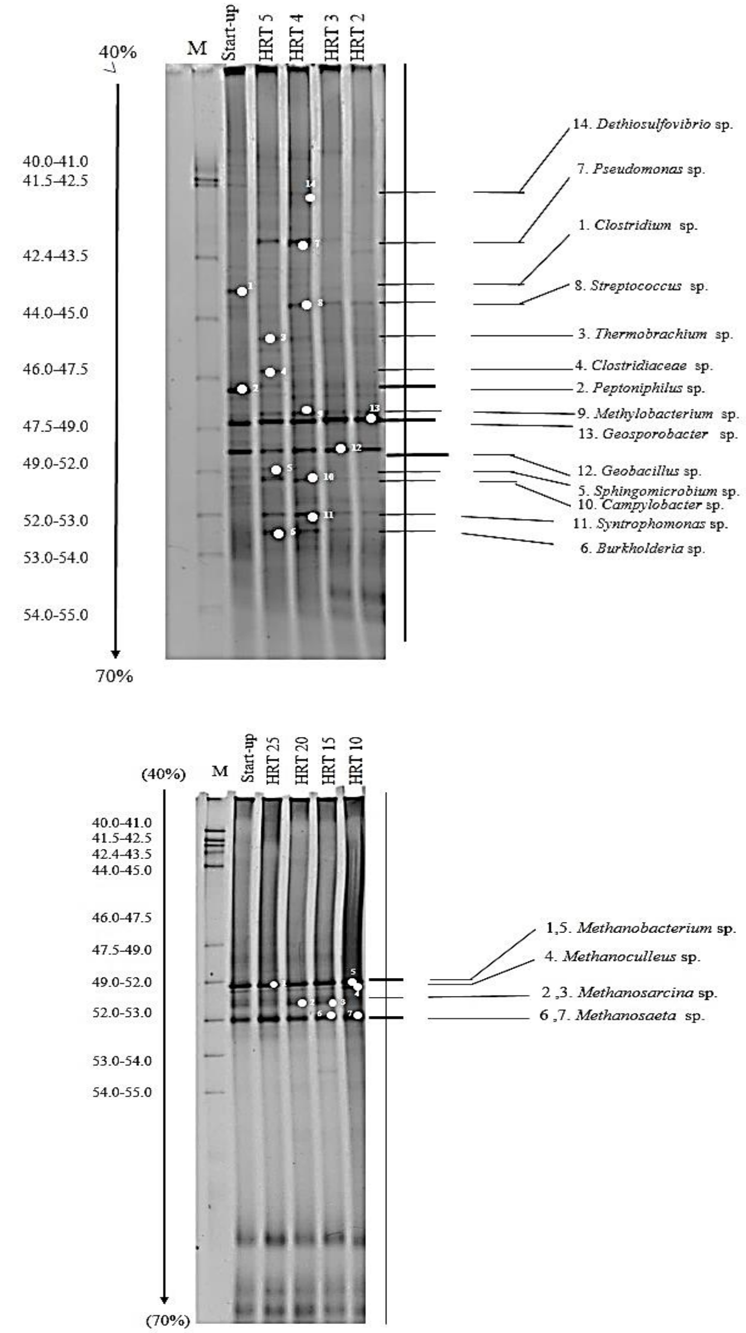

Figure 9. DGGE profiles of 16S rRNA gene fragments for sludge samples from 1-stage (H_2 production) (A), and 2-stage (CH_4 production) of CSW+4%GW co-digestion by two-stage CSTR-PFR (R1)

4. Conclusions

Optimization of conditions to achieve maximum hydrogen and methane yields from two-stage anaerobic co-digestion of CSW with GW was achieved at a mixing ratio of 96:4% (v/v), resulting in yields of 65.00 mL H_2/g COD and 345.08 mL CH_4/g COD. 4% GW was the optimal co-substrate for CSW upgrading, enhancing hydrogen and methane quality and yielding positive synergies in effective biodegradation for a two-stage digestion process. Additionally, GW can dilute toxic compounds within CSW and offers low prices due to the waste generated from biodiesel production. Continuous systems of CSW with a GW of mixing ratio of 96:4% (v/v) by two-stage CSTR-PFR (R1) and CSTR-CSTR (R2) were investigated. R1 and R2 had maximum hydrogen and methane production of 27.44 and 163.61 L/L wastewater and 20.41 and 145.35 L/L wastewater at HRT 20 days. CSTR-PFR (R1) reactor was an optimal continuous process for productive hydrogen and methane from CSW because PFR is a long pipe system, resulting in a higher organic loading rate when compared with the CSTR reactor, resulting in the microorganisms in the system being stable, more resistant to chemicals and changes than the CSTR reactor. Pseudofulvimonas sp., Clostridium sp., Burkholderia sp., *Thermanaerovibrio* sp., *Sphingomicrobium* sp., and *Thermococcoides* sp dominated the microorganism community of hydrogen production. Meanwhile, the archaea community in methane production was dominated by *Methanobacterium* sp., *Methanoculleus* sp., Methanosarcina sp., and *Methanosaeta* sp.

5. Acknowledgments

We are thankful to Siam International Food Company. Songkhla, Thailand, for providing the inoculum and wastewater in this study. Moreover, thank you to Songkhla Rajabhat University and Thaksin University for their support in workplace research.

Author Contributions: Conceptualization, T.S. and K.R.; methodology, M.T., Y.L., T.S., and K.R.; formal analysis, M.T., T.S., and P.M.; investigation, Y.L., and T.S.; writing—original draft preparation, K.R.; writing—review and editing, K.R.

Funding: This research was financially supported by the Energy Policy and Planning Office (EPPO).

Conflicts of Interest: The authors declare no conflict of interest.

References

[1] Liu, X.; Li, R.; Ji, M.; Han, L. Hydrogen and methane production by co-digestion of waste activated sludge and food waste in the two-stage fermentation process substrate conversion and energy yield. *Bioresource Technol.* **2013**, *146*, 317–323. https://doi.org/10.1016/j.biortech.2013.07.096

[2] Bertasini, D.; Battista, F.; Mancini, R.; Frison, N.; Bolzonella, D. Hydrogen and methane production through two stage anaerobic digestion of straw residues. *Environ. Res.* **2024**, *247*, 118101. https://doi.org/10.1016/j.envres.2024.118101

[3] Dong, Z.; Cao, S.; Zhao, B.; Wang, Y.; Wang, L.; Li, N. Optimization of hydrogen-methane co-production from corn stover via enzymatic hydrolysis: Process intensification, microbial community dynamics, and life cycle assessment. *Bioresource Technol.* **2025**, *426*, 132367. https://doi.org/10.1016/j.biortech.2025.132367

[4] Sillero, L.; Perez, M.; Solera, R. Optimisation of anaerobic co-digestion in two-stage systems for hydrogen, methane and biofertiliser production. *Fuel* **2024**, *365*, 131186. https://doi.org/10.1016/j.fuel.2024.131186

[5] Palenzuela-Rollon, A. *Anaerobic Digestion of Fish Processing Wastewater with Special Emphasis on Hydrolysis of Suspended Solids*; Taylor and Francis: London, **1999**.

[6] Chowdhury, P.; Viraraghavan, T.; Srinivasan, A. Biological treatment processes for fish processing wastewater: A review. *Bioresource Technol.* **2010**, *101*(2), 439–449. https://doi.org/10.1016/j.biortech.2009.08.065

[7] Chen, Y.; Cheng, J. J.; Creamer, K. S. Inhibition of anaerobic digestion process: A review. *Bioresource Technol.* **2008**, *99* (10), 4044–4064. https://doi.org/10.1016/j.biortech.2007.01.057

[8] Kangle, K. M.; Kore, S. V.; Kore, V. S.; Kulkarni, G. S. Recent trends in anaerobic co-digestion. *Environ. Res. Technol.* **2012**, *2*(4), 210–219.

[9] Yazdani, S. S.; Gonzalez, R. Anaerobic fermentation of glycerol: A path to economic viability for the biofuels industry. *Curr. Opin. Biotechnol.* **2007**, *18*(3), 213–219. https://doi.org/10.1016/j.copbio.2007.05.002

[10] Viana, M. M.; Freitas, A. V.; Leitao, R. C.; Pinto, G. A. S.; Santaella, S. T. Anaerobic digestion of crude glycerol a review. *Environ. Technol. Rev.* **2012**, *1*(1), 81–92. https://doi.org/10.1080/09593330.2012.692723

[11] Fountoulakis, M. S.; Manios, T. Enhanced methane and hydrogen production from municipal solid waste and agro-industrial by-products co-digested with crude glycerol. *Bioresource Technol.* **2009**, *100*(11), 3043–3047. https://doi.org/10.1016/j.biortech.2009.01.016

[12] Ginkel, S.; Sung, S. Biohydrogen production as a function of pH and substrate concentration. *Environ. Sci. Technol.* **2001**, *35*(23), 4726–4730. https://doi.org/10.1021/es001979r

[13] Kongjan, P.; O-Thong, S.; Angelidaki, I. Performance and microbial community analysis of two-stage process with extreame thermophiliclic hydrogen and thermophilic methane production from hydrolysate in UASB reactors. *Bioresource Technol.* **2012**, *102*(4), 4028–4035. https://doi.org/10.1016/j.biortech.2010.12.009

[14] Altschul, S. F.; Madden, T. L.; Schäffer, A. A.; Zhang, J.; Zhang, Z.; Miller, W.; David, J.; Lipman, D. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* **1997**, *25*(17), 3389–3402. https://doi.org/10.1093/nar/25.17.3389

[15] Yan, Z.; Son, Z.; Li, D.; Yuan, Y.; Liu, X.; Zheng, T. The effects of initial substrate concentration, C/N ratio, and temperature on solid-state anaerobic digestion from composting rice straw. *Bioresource Technol.* **2015**, *177*, 266–273. https://doi.org/10.1016/j.biortech.2014.11.089

[16] Mamimin, C.; Thongdumyu, P.; Hniman, A.; Prasertsan, P.; Imai, T.; O-Thong, S. Simultaneous thermophilic hydrogen production and phenol removal from palm oil mill effluent by *Thermoanaerobacterium*-rich sludge. *Int. J. Hydrogen Energy* **2012**, *37*(20), 15598–15606. https://doi.org/10.1016/j.ijhydene.2012.04.062

[17] Symons, G. E.; Buswell, A. M. The methane fermentation of carbohydrates. *J. Am. Chem. Soc.* **1933**, *55* (5), 2028–2036. https://doi.org/10.1021/ja01332a039

[18] Kongjan, K.; O-Thong, S.; Angelidaki, I. Biohydrogen production from desugared molasses (DM) using thermophilic mixed cultures immobilized on heat treated anaerobic sludge granules. *Int. J. Hydrogen Energy* **2011**, *36*(22), 14261–14269. https://doi.org/10.1016/j.ijhydene.2011.06.130

[19] Siegert, I.; Banks, C. The effect of volatile fatty acid additions on the anaerobic digestion of cellulose and glucose in batch reactors. *Process Biochem.* **2005**, *40*(10), 3412–3418. https://doi.org/10.1016/j.procbio.2005.01.025

[20] Sani, K.; Jariyaboon, R.; O-Thong, S.; Cheirsilp, B.; Kaparaju, P.; Raketh, M.; Kongjan, P. Deploying two-stage anaerobic process to co-digest greasy sludge and waste activated sludge for effective waste treatment and biogas recovery. *J. Environ. Manag.* **2022**, *316*, 115307. https://doi.org/10.1016/j.jenvman.2022.115307

[21] Cremonez, P. A.; Teleken, J. G.; Weiser Meier, T. R.; Alves, H. J. Two-stage anaerobic digestion in agroindustrial waste treatment: A review. *J. Environ. Manag.* **2021**, *281*, 111854. https://doi.org/10.1016/j.jenvman.2020.111854

[22] Panpong, K.; Srimachai, T.; Nuithitikul, K.; Kongjan, P.; O-Thong, S.; Mai, T.; Kaewthong, N. Anaerobic co-digestion between canned sardine wastewater and glycerol waste for biogas production: Effect of different operating processes. *Energy Procedia* **2017**, *138*, 260–266. https://doi.org/10.1016/j.egypro.2017.10.050

[23] Wongarmat, W.; Sittijunda, S.; Imai, T.; Reungsang, A. Co-digestion of filter cake, biogas effluent, and anaerobic sludge for hydrogen and methane production: Optimizing energy recovery through two-stage anaerobic digestion. *Carbon Resour. Convers.* **2025**, *8*, 100248. https://doi.org/10.1016/j.crcon.2024.100248

[24] Ting Sun, M.; Lei Fan, X.; Zhao, X.; Fu, S. F.; He, S.; Manasa, M. R. K.; Guo, B. Effects of organic loading rate on biogas production from microalgae: Performance and microbial community structure. *Bioresource Technol.* **2017**, *235*, 292–300. https://doi.org/10.1016/j.biortech.2017.03.075

[25] Namsree, P.; Suvajittanont, W.; Puttanlek, C.; Uttapap, D.; Rungsardthong, V. Anaerobic digestion of pineapple pulp and peel in a plug-flow reactor. *J. Environ. Manag.* **2012**, *110*, 40–47. https://doi.org/10.1016/j.jenvman.2012.05.017

[26] Rabii, A.; Aldin, S.; Dahman, Y.; Elbeshbishy, E. A review on anaerobic co-digestion with a focus on the microbial populations and the effect of multi-stage digester configuration. *Energies* **2019**, *12*(6), 1106. https://doi.org/10.3390/en12061106.