Heliyon 10 (2024) e34817

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Synergistic integration of hydrothermal pretreatment and co-digestion for enhanced biogas production from empty fruit bunches in high solids anaerobic digestion

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ARTICLE INFO

Keywords:
Palm oil mill waste
Empty fruit bunches
Decanter cake
Hydrothermal pretreatment
Anaerobic co-digestion

ABSTRACT

This study investigates the co-digestion of hydrothermally pretreated empty fruit bunches (EFB) at 190 °C for 5 min (HTP190-EFB) with decanter cake (DC) to improve biogas production in high solid anaerobic digestion (HSAD). The HTP190-EFB exhibited a 67.98 % reduction in total solids, along with the production of 0.89 g/L of sugar, 2.39 g/L of VFA, and 0.56 g/L of furfural in the liquid fraction. Co-digestion of HTP190-EFB with DC at mixing ratios of 5, 10, and 15 %w/v demonstrated improved methane yields and process stability compared to mono-digestion of HTP190-EFB. The highest methane yield of 372.69 mL CH_4/g-VS was achieved in the co-digestion with 5 %w/v DC, representing a 15 % increase compared to digestion of HTP190-EFB (324.30 mL CH_4/g-VS) alone. Synergistic effects were quantified, with the highest synergistic methane yield of 77.65 mL CH_4/g-VS observed in the co-digestion with 5 %w/v DC. Microbial community analysis revealed that co-digestion of hydrothermally pretreated EFB with decanter cake promoted the growth of *Clostridium* sp., *Lactobacillus* sp., *Fibrobacter* sp., *Methanoculleus* sp., and *Methanosarcina* sp., contributing to enhanced biogas production compared to mono-digestion of pretreated EFB. Energy balance analysis revealed that co-digestion of HTP190-EFB with DC resulted in a total net energy of 599.95 kW, 52 % higher than mono-digestion of HTP190-EFB (394.62 kW). Economic analysis showed a shorter return on investment for the co-digestion system (0.86 years) compared to the mono-digestion of HTP190-EFB (1.02 years) and raw EFB (2.69 years). The co-digestion of HTP190-EFB with 5 %w/v DC offers a promising approach to optimize methane yield, process stability, and economic feasibility, supporting the palm oil industry for producing renewable energy and sustainable waste management.

1. Introduction

Southeast Asian nations, especially Indonesia, Malaysia, and Thailand, rely heavily on the palm oil sector for their economy. However, the rapid expansion of palm oil production has led to significant waste, such as empty fruit bunches (EFB) and decanter cakes

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https://doi.org/10.1016/j.heliyon.2024.e34817
Received 3 April 2024; Received in revised form 16 July 2024; Accepted 17 July 2024
Available online 25 July 2024
2405-8440/

(DC) [1]. Improper management of these wastes can result in serious environmental problems such as soil erosion, water contamination, and greenhouse gas emissions [2]. Anaerobic digestion (AD) has been used to produce biogas from palm oil mill wastes, and this practice has gained popularity recently [3]. A proven method called anaerobic digestion turns organic waste into biogas, which is mainly made up of carbon dioxide (30–45 %) and methane (55–70 %) [4]. This procedure lessens the adverse environmental effects of disposing of organic waste and offers a sustainable substitute for fossil fuels, which supply 80 % of the world's energy [5]. High solids anaerobic digestion (HSAD), which involves the digestion of feedstocks with a total solids content above 15 %, has gained attention due to its advantages over traditional low-solids digesters (<10 % TS) [6]. HSAD offers benefits such as 20–30 % reduced digestate volume, 25–40 % lower energy requirements for heating, and a 30–50 % smaller reactor footprint, making it suitable for large-scale biogas production [7]. However, the complex lignocellulosic structure of EFB, which contains 38.3 % cellulose, 35.3 % hemicellulose, and 22.1 % lignin, hinders its biodegradability and limits its potential for biogas production [8].

Several pretreatment techniques have been looked into to improve the digestibility of EFB and get around this problem. The co-digesting pretreated EFB (1 % NaOH presoaking and hydrothermal treatment at 230 °C for 15 min) with POME resulted in a 98 % increase in methane yield compared to untreated EFB, with a maximum methane production of 82.7 m^3 CH_4/ton of mixed treated EFB and POME [9]. Nieves et al. [10] investigated the effects of NaOH and phosphoric acid pretreatments on biogas production from EFB. The best improvement was achieved when 8 % NaOH was used for 60 min, resulting in a 100 % increase in methane yield compared to untreated EFB. Suksong et al. [11] studied the effects of fungal pretreatment using *Trichoderma reesei* TISTR 3080 and *Pleurotus ostreatus* DSM 11191 on EFB biodegradability and methane production via solid-state anaerobic digestion (SS-AD). Pretreatment with *T. reesei* and *P. ostreatus* increased methane yield by 44–52 % compared to raw EFB, with maximum methane production of 75.8 and 64.9 m^3 CH_4/ton EFB, respectively. Purwandari et al. [12] investigated the effects of N-methylmorpholine-N-oxide (NMMO) pretreatment on EFB digestion. The best improvement in biogas production was achieved by a dissolution mode pretreatment using 85 % NMMO at 120 °C for 3 h, resulting in a 48 % increase in methane yield and a 167 % increase in initial methane production rate compared to untreated EFB. Among the various methods, hydrothermal pretreatment is efficacious in improving the methane yield of various lignocellulosic feedstocks by breaking down the complex lignocellulosic structure and increasing the ability of anaerobic microbes to access cellulose and hemicellulose [13]. However, there is limited research on the effects of hydrothermal pretreatment presoaking in water on EFB biodegradability and methane yield and its co-digestion with other palm oil mill wastes, such as POME and decanter cake (DC).

Co-digestion, the simultaneous digestion of two or more substrates, has emerged as a promising strategy to enhance biogas production and process stability [14]. By combining substrates with complementary characteristics, such as carbon-rich EFB (C/N ratio of 54.7) and nitrogen-rich decanter cake (C/N ratio of 20.1), co-digestion can lessen the inhibitory effects of hazardous substances, boost buffering capacity, and enhance nutritional balance [15]. Co-digestion allows for a more balanced nutrient mix, improves microbial synergy, and can overcome the limitations posed by the digestion of single substrates [16]. The co-digesting pretreated EFB with POME resulted in a 98 % increase in methane yield compared to untreated EFB, with a maximum methane production of 82.7 m^3 CH_4/ton of mixed-treated EFB and POME [9]. However, the beneficial effects of co-digestion hydrothermally pretreated EFB with decanter cake in high solids anaerobic digestion have not been thoroughly investigated. This study aims to examine the co-digestion of hydrothermally pretreated EFB at 190 °C for 5 min (HTP190-EFB) with decanter cake (DC) for enhanced biogas production in high solids anaerobic digestion. The specific objectives are to evaluate the impact of hydrothermal pretreatment on EFB composition and biodegradability, assess the synergistic methane yield of co-digestion HTP190-EFB with DC, and respond to microbial community of anaerobic co-digestion hydrothermal pretreatment EFB with decanter cake. The results of this study should help create effective and long-lasting plans for handling waste from palm oil mills and encouraging bioenergy generation.

2. Materials and methods

2.1. Substrate preparation

The empty fruit bunches (EFB) and decanter cake (DC) were collected from a local palm oil mill in Southern Thailand. The EFB was air-dried to reduce moisture content and then shredded to a uniform size of approximately 2–5 cm to ensure consistency in pretreatment. The ground materials were kept in sealed containers to avoid microbiological contamination and moisture loss. The EFB and DC were determined for total solids (TS), volatile solids (VS), oil and grease, and total Kjeldahl nitrogen (TKN), following the standard methods for the examination of water and wastewater [17]. The total carbon, hydrogen, nitrogen, sulfur, and oxygen (CHNS/O) content in EFB and DC were analyzed using a CHNS/O analyzer (Flash EA 1112 Series, Thermo Scientific, Netherlands). The analysis involved dynamic flash combustion at temperatures of 900 °C for carbon, hydrogen, nitrogen, and sulfur and 1060 °C for oxygen [18]. The cellulose, hemicellulose, and lignin contents were determined following the National Renewable Energy Laboratory (NREL) protocol [19].

2.2. Inoculum preparation and acclimatization

Anaerobic digestion sludge was the source of the inoculum employed in the tests, which was collected from the biogas reactor at Bio Energy-Satun Co., Ltd., located at 6°51′42.0″N 99°52′15.2″E. An enrichment and acclimatization process was carried out to prepare the inoculum using 2 % w/v avicel (cellulose microcrystalline particle size 50 μm) as the substrate [20]. The acclimatization was performed in a 10.0 L reactor, incubated at a mesophilic temperature of 40 °C under anaerobic conditions. The reactor was monitored for biogas production over 14 days. Upon a noticeable decrease in biogas production, indicating the consumption of readily available substrates, 20 % of the working volume was removed and replaced with an equivalent volume of 2 % w/v avicel to sustain microbial activity and facilitate further acclimatization [21]. Before commencing the anaerobic digestion experiments, the inoculum was degassed to eliminate any background methane originating solely from the inoculum. The degassing process was conducted by incubating the inoculum in a sealed reactor at 40 °C for 7 days without adding substrate. During this period, the reactor was periodically vented to release any accumulated biogas. The degassed inoculum was then used for the subsequent anaerobic digestion experiments, ensuring the methane production could be attributed to the investigated substrates [22].

2.3. Hydrothermal pretreatment process

The air-dried EFB, which was approximately 2–5 cm in size, was mixed with water at a ratio of 1:10 based on the total solid content of the EFB to ensure adequate moisture content for the hydrothermal process [23]. A reactor withstanding high temperatures and pressures, equipped with temperature and pressure control systems, was used for the hydrothermal pretreatment. The hydrothermal pretreatment was conducted at a temperature of 190 °C for 5 min, based on previous studies indicating its effectiveness in breaking down lignocellulosic structures in biomass like EFB [13]. After the completion of the treatment, the reactor was cooled down to a safe handling temperature, and the pretreated EFB was removed, ready for use as a substrate in the subsequent anaerobic digestion experiments.

2.4. High solids anaerobic co-digestion

Anaerobic digestion with high solids was used to examine the impact of methane production on hydrothermally prepared EFB (EFB-HTP190) and decanter cake (DC) co-digesting. The co-digestion substrates were prepared by adding DC to EFB-HTP190 to achieve total solids (TS) contents ranging from 10 to 20 %. Anaerobic methane production was measured using a batch biochemical methane potential (BMP) assay under mesophilic conditions (40 °C). The experimental setup included 500 mL glass bottles with a working volume of 200 mL and fixed volatile solids (VS) loading of 7.5 % VS. Each bottle was sealed with a butyl rubber stopper to maintain anaerobic conditions. A negative control comprising solely of the substrate and a positive control utilizing 50 μm-sized microcrystalline cellulose particles were used as controls. The bottles were incubated in a temperature-controlled shaker incubator (SI500, Staurt, UK) at 40 °C and 100 rpm (revolutions per minute) to ensure proper mixing and maintain a homogeneous environment throughout the digestion process. Daily gas production was recorded using a graduated syringe, and the volume was corrected for standard temperature and pressure (STP) using the ideal gas law. The biogas composition in the headspace of the bottles was monitored every three days using gas chromatography with a thermal conductivity detector (GC-TCD) (GC-8A; Shimadzu Corp., Japan) connected with Shin Carbon Packed Column (60/80 mesh, 1 m × 3.20 mm). The gas chromatography operation condition was set according to Mamimin et al. [24]. The GC-TCD was calibrated using a standard gas mixture containing known methane (CH_4) and carbon dioxide (CO_2) concentrations. The operating conditions for the GC-TCD were as follows: injection temperature of 150 °C, column temperature of 50 °C, and detector temperature of 200 °C, with helium as the carrier gas at a flow rate of 14 mL/min. The pH of the digester contents was monitored every three days using a portable pH meter HANNA HI 5221–02 (Hanna Instruments, USA) to ensure that the digestion process remained within the optimal range for anaerobic microorganisms (6.5–7.5). If the pH deviated from this range, adjustments were made using 1M NaOH or 1M HCl solutions to maintain a stable environment for the anaerobic microorganisms. The volume of NaOH or HCl added to each bottle was recorded to account for any changes in the working volume and to ensure an accurate calculation of the methane yield.

2.5. PCR-DGGE analysis of the microbial community

The microbial community in various substrates anaerobic digesting processes was quantitatively analyzed by PCR-DGGE, as previously reported by Prasertsan et al. [25]. DNA was extracted from digester sludge samples, and the 16 S rRNA gene was amplified. 8 % polyacrylamide gels with a 30–60 % denaturing gradient were used to separate the PCR products for 16 h at 100 V. After staining, prominent bands were excised, re-amplified, and sequenced. On average, 15–20 distinct bands were observed per sample, representing the dominant microbial populations. Sequencing of these bands typically yielded 200 base pair reads with >97 % similarity to database sequences. Statistical analysis of banding patterns revealed 30–40 % similarity between samples from different substrates. Dominant groups like *Clostridium*, *Bacteroides*, and *Methanobacterium* were present in >80 % of samples. Less common groups appeared in 20–30 % of samples.

2.6. Analytical methods

Biogas production was measured using the water displacement method, where the produced biogas was captured in an inverted graduated cylinder filled with water. The volume of water displaced indicated the volume of gas produced. Gas volume measurements were recorded daily and adjusted for standard temperature and pressure (STP) to ensure consistency and comparability of data. The biogas composition, specifically the percentages of methane (CH_4) and carbon dioxide (CO_2), was analyzed using gas chromatography with a thermal conductivity detector (GC-TCD). For precise determination of the amounts of CH_4 and CO_2, the GC-TCD was calibrated using reference gas mixtures. Using a gas-tight syringe, gas samples were taken from the headspace of the digestion bottles for GC analysis. Equation (1) illustrates how the hydrolysis constant (kh) was calculated using the first-order kinetic process and the cumulative methane generation from this batch experiment.

Table 1
Composition of empty fruit bunch (EFB, palm decanter cake (DC), and hydrothermally pretreated empty fruit bunch at 190 °C (HTP190-EFB).

Parameters	EFB	DC	HTP190-EFB
Total solids (%)	37.50 ± 1.05	75.00 ± 2.25	12.00 ± 0.96
Total volatile solids (% TS)	94.64 ± 2.84	86.00 ± 1.75	96.35 ± 0.92
Solid yield (g-TS/g-TS)	–	–	0.32 ± 0.01
Hydrolysis efficiency (%)	–	–	67.98 ± 2.04
Solid fraction composition			
Cellulose (% TS)	36.70 ± 1.10	23.90 ± 0.72	48.41 ± 1.45
Hemicellulose (% TS)	38.10 ± 1.14	20.60 ± 0.62	21.94 ± 0.66
Lignin (% TS)	18.46 ± 0.55	13.98 ± 0.42	26.01 ± 0.78
Ash (% TS)	6.74 ± 0.20	8.76 ± 0.26	3.65 ± 0.11
Protein (%TS)	4.87 ± 0.15	17.7 ± 0.53	nd
lipids (% TS)	0.20 ± 0.01	13.78 ± 0.41	nd
*nd = not detected			

Table 2
Composition of the liquid hydrolysate obtained from the hydrothermal pretreatment of empty fruit bunch at 190 °C.

Component	Concentration (g/L)
Cellobiose	0.22 ± 0.01
Glucose	0.27 ± 0.01
Xylose	0.31 ± 0.01
Arabinose	0.31 ± 0.01
Lactic acid	0.46 ± 0.01
Acetic acid	1.69 ± 0.05
Propionic acid	0.21 ± 0.01
Ethanol	0.03 ± 0.00
HMF	0.00 ± 0.00
Furfural	0.56 ± 0.02

$$ln = \frac{B_\infty - B}{B} \tag{1}$$

Equation (2) illustrates how the kinetics of methane production during batch fermentation were fitted using a modified Gompertz model.

$$B_t = B_\infty \times exp\left\{ - exp\left[\frac{R_{max} \times e}{B_\infty}(\lambda - t) + 1\right]\right\} \tag{2}$$

Where t is the time (d), B_t is the cumulative methane production at time t, and B_∞ is the methane production at the end of the trial period. R_{max} denotes the highest methane production rate (mL-CH_4/g-VS/d), λ is the lag phase period (d), and e = exp(1) = 2.7183.

3. Results and discussion

3.1. Composition of raw and pretreated substrates

The composition of empty fruit bunch (EFB), palm decanter cake (DC), and hydrothermally pretreated empty fruit bunch at 190 °C (HTP190-EFB) is presented in Table 1 The total solids (TS) content of EFB and DC was 37.5 % and 75.0 %, respectively, while HTP190-EFB had a significantly lower TS content of 12.0 %. The reduction in TS content of HTP190-EFB can be attributed to the solubilization of some components during the hydrothermal pretreatment [26]. The total volatile solids (VS) content expressed as a percentage of TS, was highest in HTP190-EFB (96.35 %), followed by EFB (94.64 %) and DC (86.00 %). EFB, DC, and HTP190-EFB cellulose content was 36.70 %, 23.90 %, and 48.41 %, respectively. The higher cellulose content in HTP190-EFB compared to raw EFB indicates that the hydrothermal pretreatment effectively concentrated the cellulose percentage in the substrate previously processed by removing some hemicellulose and lignin [27]. The hemicellulose content of EFB (38.10 %) was higher than that of DC (20.60 %) and HTP190-EFB (21.94 %). The lower hemicellulose content in HTP190-EFB compared to raw EFB suggests that a significant portion of the hemicellulose was solubilized during the hydrothermal pretreatment, which is consistent with the findings of previous studies [23]. The lignin content of EFB, DC, and HTP190-EFB was 18.46 %, 13.98 %, and 26.01 %, respectively. The higher lignin content in HTP190-EFB compared to raw EFB can be attributed to removing hemicellulose and other soluble components during the hydrothermal pretreatment, resulting in a relative increase in the lignin fraction [28]. The ash content, representing the inorganic fraction, was lowest in HTP190-EFB (3.65 %), followed by DC (4.90 %) and EFB (6.74 %). The lower ash content in HTP190-EFB suggests that some inorganic components may have been solubilized during hydrothermal pretreatment. The compositional analysis of the substrates highlights the effects of hydrothermal pretreatment on the structure and composition of EFB. The pretreatment process led to a

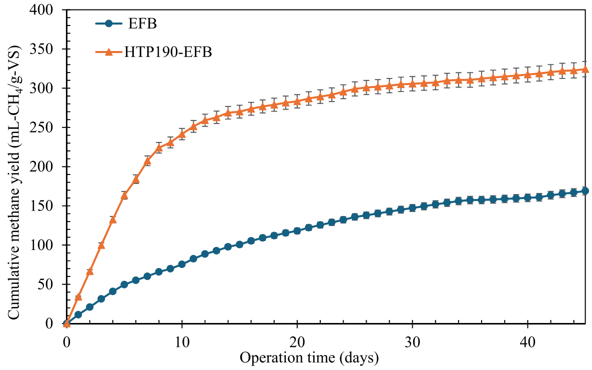

Fig. 1. Cumulative methane yield from the empty fruity bunch (EFB) and hydrothermal pretreated EFB (HTP190-EFB).

reduction in TS content, an increase in cellulose and lignin content, and a decrease in hemicellulose and ash content compared to raw EFB. These changes in composition are expected to improve the biodegradability and methane production potential of HTP190-EFB during anaerobic digestion [29]. The pretreated substrate, HTP190-EFB, exhibits favorable characteristics for enhanced biogas production through anaerobic digestion. Table 2 presents the composition of the liquid hydrolysate obtained from the hydrothermal pretreatment of empty fruit bunch (EFB) at 190 °C. The liquid hydrolysate contains various compounds formed during pretreatment, including sugars, organic acids, ethanol, and furan derivatives. The presence of cellobiose (0.22 g/L), glucose (0.27 g/L), xylose (0.31 g/L), and arabinose (0.31 g/L) in the liquid hydrolysate indicates the solubilization of hemicellulose and partial hydrolysis of cellulose during the hydrothermal pretreatment [30]. The solubilized sugars can be readily biodegradable substrates for anaerobic microorganisms, potentially enhancing methane production during anaerobic digestion [31]. Organic acids, such as lactic acid (0.46 g/L), acetic acid (1.69 g/L), and propionic acid (0.21 g/L), were also detected in the liquid hydrolysate. The breakdown of sugars and the deacetylation of hemicellulose during hydrothermal pretreatment are responsible for the existence of these organic acids [13]. Acetic acid, the most abundant organic acid in hydrolysate, is known to be a key intermediate in the anaerobic digestion process and can be directly converted to methane by acetoclastic methanogens [32]. The liquid hydrolysate also contained a small amount of ethanol (0.03 g/L), which may have been derived from the degradation of sugars [33]. Furfural, a furan derivative, was detected in the liquid hydrolysate at a concentration of 0.56 g/L. Furfural is formed from the dehydration of pentoses (e.g., xylose and arabinose) under high temperatures and acidic conditions during hydrothermal pretreatment [34]. Although furfural is known to be a potential inhibitor of anaerobic digestion at high concentrations, the low concentration detected in the hydrolysate is unlikely to have a significant inhibitory effect on the methane production process [35]. Interestingly, 5-hydroxymethylfurfural (HMF), another common furan derivative formed from the dehydration of hexoses (e.g., glucose), was not detected in the liquid hydrolysate. This suggests that the hydrothermal pretreatment conditions (190 °C) were not severe enough to cause significant glucose degradation to HMF [36]. The composition of the liquid hydrolysate highlights the effectiveness of hydrothermal pretreatment in solubilizing and partially hydrolyzing the hemicellulose and cellulose fractions of EFB. The hydrolysate containing sugar and organic acid might increase the methane produced during anaerobic digestion by giving anaerobic microbes easily biodegradable substrates.

3.2. Methane production from EFB and pretreated EFB

The methane yield of raw empty fruit bunch (EFB) and hydrothermally pretreated empty fruit bunch at 190 °C (HTP190-EFB) over a 45-day anaerobic digestion period is presented in Fig. 1. The final methane yield of EFB and HTP190-EFB at the end of the 45-day digestion period was 169.04 mL CH_4/g-VS and 324.30 mL CH_4/g-VS, respectively. HTP190-EFB achieved a 91.8 % higher methane yield than raw EFB, confirming that hydrothermal pretreatment at 190 °C significantly enhanced the methane production potential of EFB. The methane yield curves for both substrates exhibited a similar pattern, with a rapid increase in the early stages of digestion, followed by a gradual decrease in the production rate. However, HTP190-EFB showed a higher initial rate of increase and maintained a higher methane yield throughout the digestion period than EFB. This can be attributed to the increased accessibility of fermentable sugars and improved biodegradability of the pretreated substrate [37]. The methane yield curve of HTP190-EFB began to plateau around day 30, indicating that most readily biodegradable components had been converted to methane by this time. In contrast, the curve for EFB showed a more gradual increase until the end of the digestion period, suggesting a slower rate of substrate conversion and a higher proportion of recalcitrant components [38]. The enhanced methane yield from HTP190-EFB can be explained by the structural and compositional changes in the lignocellulosic biomass during hydrothermal pretreatment. The pretreatment process disrupts the lignin-carbohydrate complex, reducing the crystallinity of cellulose and increasing the accessible surface area for enzymatic hydrolysis. Additionally, hydrothermal pretreatment solubilizes a portion of the hemicellulose, producing fermentable sugars readily converted to methane by anaerobic microorganisms [37]. Comparable to or greater than those reported in earlier investigations on anaerobic digestion of pretreated lignocellulosic biomass, HTP190-EFB produced a methane yield of 324.30 mL CH_4/g-VS. With mechanical processing and EFB size reduction to 0.5 cm, Saelor et al. [39] observed a methane production of 178.33–199.32 mL CH_4/g-VS while using integrated straw mushroom (*Volvariella volvacea*) culture as a bio-pretreatment for EFB,

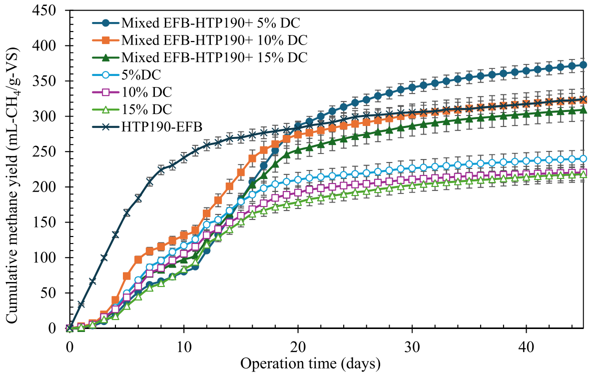

Fig. 2. Cumulative methane yield from co-digestion hydrothermal pretreated EFB with decanter cake and mono digestion of decanter cake (DC).

Table 3
Presents the cumulative methane production, methane yield, hydrolysis constant (kh), methane production rate, lag phase, and digestion time from mono and co-digestion of empty fruit bunch (EFB), hydrothermally pretreated EFB at 190 °C (HTP190-EFB), decanter cake (DC).

Feedstocks	Cumulative methane (mL CH_4)	Methane yield (mL CH_4/g-VS)	kh (d^{-1})	Methane production rate (mL CH_4/L/d)	Lag phase (d)	Digestion time (d)
EFB	676.17 ± 20.19	169.04 ± 5.07	0.05 ± 0.0022	4.53 ± 0.14	1.00 ± 0.00	>45
HTP190-EFB	1297.20 ± 38.92	324.30 ± 9.73	0.18 ± 0.0024	23.18 ± 0.70	0.00 ± 0.00	20.00 ± 0.60
5 % DC	960.04 ± 28.80	240.01 ± 7.20	0.11 ± 0.0034	16.08 ± 0.48	1.98 ± 0.0	18.33 ± 1.36
10 % DC	882.32 ± 26.47	220.58 ± 6.62	0.13 ± 0.0038	17.10 ± 0.51	1.97 ± 0.06	19.65 ± 1.70
15 % DC	872.23 ± 26.14	218.06 ± 6.54	0.12 ± 0.0035	19.17 ± 0.5	2.87 ± 0.09	22.50 ± 1.28
Mixed HTP190-EFB with 5 % DC	1490.78 ± 44.72	372.69 ± 11.18	0.13 ± 0.0034	20.00 ± 0.61	1.74 ± 0.05	25.60 ± 1.01
Mixed HTP190-EFB with 10 % DC	1292.03 ± 38.76	323.01 ± 9.69	0.11 ± 0.0034	17.74 ± 0.53	2.34 ± 0.10	23.97 ± 0.96
Mixed HTP190-EFB with 15 % DC	1236.48 ± 37.09	309.12 ± 9.27	0.11 ± 0.0034	16.30 ± 0.49	3.44 ± 0.16	24.51 ± 1.04

Mamimin et al. [24] achieved a methane output of 281 mL CH_4/g-VS. The methane yield results demonstrate that hydrothermal pretreatment at 190 °C is an effective method for enhancing the anaerobic biodegradability and methane production potential of EFB.

3.3. Methane production from co-digestion pretreated EFB with decanter cake

The methane yield of co-digestion of hydrothermally pretreated EFB at 190 °C (HTP190-EFB) with decanter cake (DC) at different mixing ratios (5, 10, and 15 %w/v) is presented in Fig. 2. The highest methane yield of 372.69 mL-CH_4/g-VS was achieved by the co-digestion of HTP190-EFB with 5 %w/v of DC, followed by 323.01 mL CH_4/gVS for the co-digestion with 10 %w/v of DC and 309.12 mL CH_4/g-VS for the co-digestion with 15 %w/v of DC. These findings suggest that, in comparison to HTP190-EFB mono-digestion (324.30 mL-CH_4/g-VS), adding DC to HTP190-EFB increases methane production. The co-beneficial effects of combining the two substrates are responsible for the increased methane output in the co-digestion systems. Because of its strong buffering capacity and nutritional richness, DC can help keep a steady pH and provide a balanced nutrient environment for anaerobic microorganisms [40]. Additionally, readily biodegradable organic matter in DC can stimulate the growth and activity of anaerobic microorganisms, leading to enhanced methane production [41]. The methane yield curves for all co-digestion systems exhibited a similar trend, with a rapid increase in the early stages of digestion followed by a gradual plateauing towards the end of the digestion period. This trend suggests that the readily biodegradable components of the substrates were quickly converted to methane, while the more recalcitrant fractions were slowly degraded over time [42]. Comparing the co-digestion systems, the highest methane yield was achieved with the lowest DC mixing ratio (5 %w/v), indicating that a higher proportion of HTP190-EFB in the substrate mixture favors methane production. This can be explained by the increased accessibility of cellulose and hemicellulose in HTP190-EFB due to the hydrothermal pretreatment, which facilitates their conversion to methane. The mono-digestion of DC at different total solids (TS) contents (5, 10, and 15 %w/v) resulted in lower methane yields than the co-digestion systems. The highest methane yield among the mono-digestion systems was 240.01 mL-CH_4/g-VS for 5 %w/v of DC, 220.58 mL-CH_4/g-VS for 10 %w/v of DC, and 218.06 mL mL-CH_4/g-VS for 15 %w/v of DC. These results suggest that the mono-digestion of DC is less efficient than the co-digestion with HTP190-EFB in methane production. Table 3 presents the cumulative methane production, methane yield, hydrolysis constant (kh), methane production rate, lag phase, and

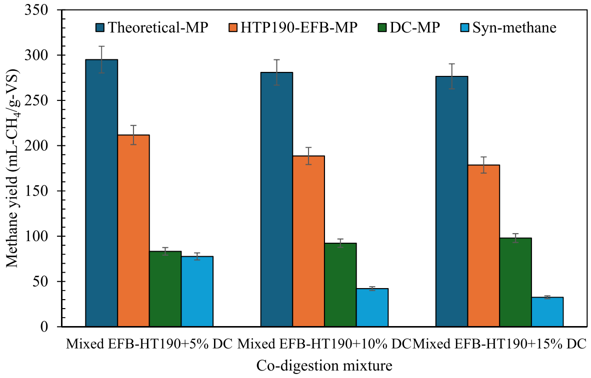

Fig. 3. Synergistic effects in co-digestion of hydrothermal pretreated EFB with decanter cake at various concentrations.

digestion time for EFB, hydrothermally pretreated EFB at 190 °C (HTP190-EFB), decanter cake (DC), and co-digestion of HTP190-EFB with DC at various mixing ratios. The highest cumulative methane production and methane yield were achieved by the co-digestion of HTP190-EFB with 5 % DC, reaching 1490.78 mL-CH_4 and 372.69 mL-CH_4/g-VS, respectively. This was followed by the mono-digestion of HTP190-EFB, with a cumulative methane production of 1297.20 mL-CH_4 and a methane yield of 324.30 mL-CH_4/g-VS. The highest kh value of 0.18 d^{-1} was observed for HTP190-EFB, indicating that the hydrothermal pretreatment enhanced the hydrolysis rate of EFB. The co-digestion systems and mono-digestion of DC exhibited lower kh values, ranging from 0.11 to 0.13 d^{-1}. The methane production rate was highest for HTP190-EFB (23.18 mL-CH_4/L/d), followed by the co-digestion of HTP190-EFB with 5 %w/v of DC (20.00 mL-CH_4/L/d). The enhanced biodegradability of the substrates as a result of hydrothermal pretreatment and the cooperative effects of co-digestion are responsible for the high methane production rates in these systems [43]. The lag phase, which represents the time required for the anaerobic microorganisms to adapt to the substrate and produce methane, varied among the feedstocks. EFB had the most prolonged lag phase of 1 day, while HTP190-EFB had no lag phase, indicating that the hydrothermal pretreatment enhanced the readily biodegradable components in the substrate. The co-digestion systems and mono-digestion of DC had lag phases ranging from 1.74 to 3.44 days. The digestion time, which is the time required to reach the maximum methane yield, was the longest for EFB (>45 days), while HTP190-EFB had the shortest digestion time of 20 days. The co-digestion systems and mono-digestion of DC had digestion times ranging from 18.33 to 25.60 days. Because of their increased biodegradability and synergistic effects, the pretreatment and co-digested substrates had reduced digestion durations [44]. With a mixing ratio of 5 % w/v of DC, the co-digestion of hydrothermally pretreated EFB with decanter cake produced the highest cumulative methane output and yield. Compared to raw EFB, the hydrothermal pretreatment increased the hydrolysis rate and decreased the lag phase and digesting time.

The methane yields from co-digestion, mono-digestion of HTP190-EFB and DC, theoretical methane yields, and synergistic methane yields are shown in Fig. 3. The theoretical methane yield for each co-digestion system was calculated based on the average of the methane yields from the mono-digestion of HTP190-EFB and DC. The synergistic methane yield, which represents the additional methane production achieved through co-digestion compared to the theoretical yield, was determined by subtracting the theoretical methane yield from the actual co-digestion methane yield [9]. The highest synergistic methane yield of 77.65 mL-CH_4/g-VS was observed in the co-digestion of HTP190-EFB with 5 %w/v of DC, followed by 42.11 mL-CH_4/g-VS for co-digestion with 10 %w/v of DC and 32.54 mL-CH_4/g-VS for co-digestion with 15 %w/v of DC. These results demonstrate that co-digestion enhances methane production beyond the simple additive effect of the individual substrates. The complementary qualities of the substrates (high buffering capacity and nutrient content of DC) can assist in maintaining a stable anaerobic digestion process and encourage the proliferation and activity of anaerobic bacteria, which can be responsible for the synergistic benefits of co-digestion [7]. The overall methane production in HTP190-EFB can be enhanced by promoting the hydrolysis and fermentation of the more resistant components with readily biodegradable organic matter in DC [41]. The decreasing trend in synergistic methane yield with increasing DC mixing ratios (from 5 to 15 %w/v) suggests that a lower proportion of DC in the co-digestion system is more favorable for achieving synergistic effects. This may be due to the optimal balance between the readily biodegradable components in DC and the slowly degradable components in HTP190-EFB at lower DC mixing ratios [45]. When hydrothermally pretreated empty fruit bunch (HTP190-EFB) and decanter cake (DC) are digested together, notable synergistic effects lead to a higher methane production than when each substrate is digested separately. The co-digestion of HTP190-EFB with 5 % DC produced the maximum synergistic methane output, demonstrating the significance of substrate mixing ratios in maximizing the anaerobic digestion process.

3.4. Microbial community of anaerobic co-digestion pretreated EFB with decanter cake

Significant changes were found in the bacterial (Fig. S1a) and archaeal (Fig.S1b) communities between the hydrothermally processed EFB at 190 °C (HTP190C) and the co-digestion of HTP190C with 5%w/v decanter cake (HTP190C + 5%w/v DC) according to the microbial community analysis. The co-digestion of HTP190C with 5 %w/v DC increased the relative abundance of several key bacterial genera compared to the mono-digestion of HTP190C. *Clostridium* sp. and *Lactobacillus* sp. showed a substantial increase in the co-digestion system (Fig. 4a). This can be attributed to their ability to ferment various substrates and produce volatile fatty acids

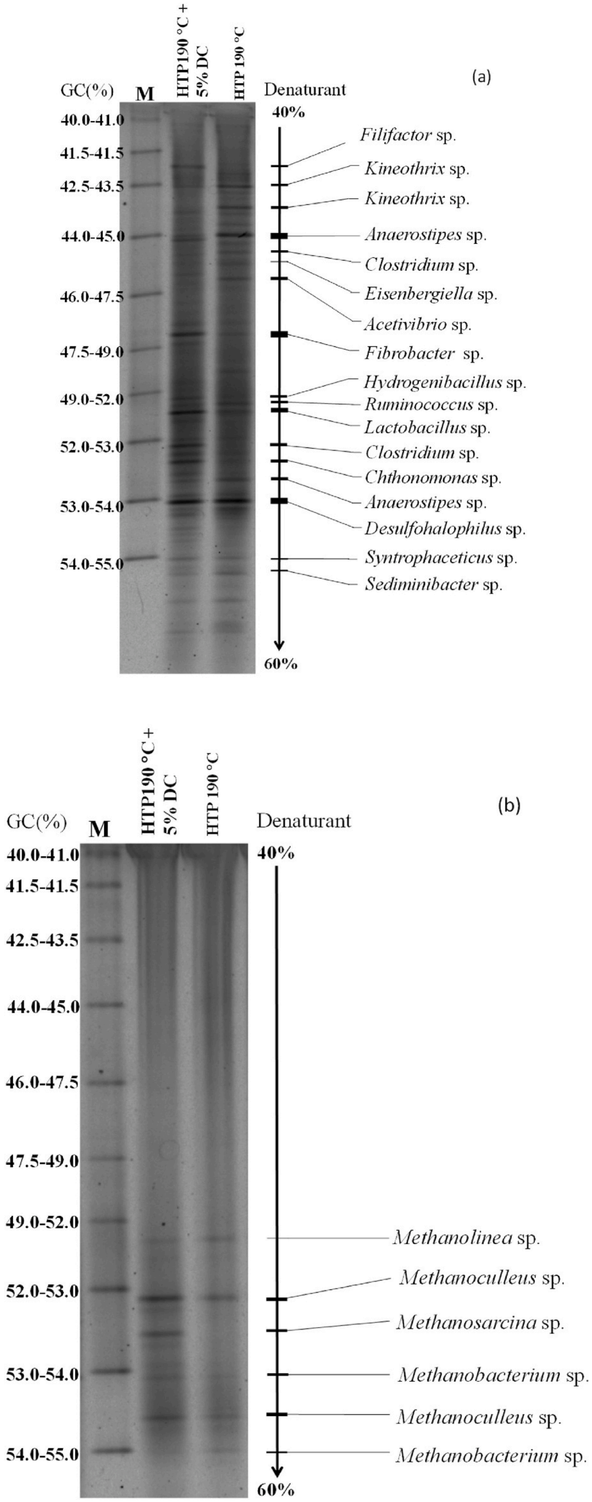

Fig. 4. The shifts in the bacterial (a) and archaeal (b) communities during the mono-digestion of hydrothermally pretreatment EFB at 190 °C and the co-digestion of hydrothermally pretreatment EFB at 190 °C with a decanter cake.

(VFAs) [46]. The increased abundance of *Fibrobacter* sp., a well-known cellulolytic bacterium, suggests enhanced cellulose degradation in the co-digestion system [21]. Other bacterial genera that showed an increase in the co-digestion system include *Kineothrix* sp., *Anaerostipes* sp., *Acetivibrio* sp., *Hydrogenibacillus* sp., *Ruminococcus* sp., *Chthonomonas* sp., *Desulfohalophilus* sp., and *Sediminibacter* sp.

Table 4
Mass balance, energy balance, and economic evaluation of empty fruit bunches with single and co-digestion.

	Description	EFB	DC	HTP190-EFB	HTP190-EFB-DC
Mass balance	Total solids (kg/ton)	375.00 ± 8.35	750.00 ± 22.50	120 ± 9.61	170.00 ± 1.11
	Total volatile solids (kg/ton)	354.90 ± 7.80	645.00 ± 25.80	115.2.00 ± 9.25	150.00 ± 10.45
	Sugar (kg/ton)	–	–	8.90 ± 0.27	8.90 ± 0.27
	VFA (kg/ton)	–	–	23.90 ± 0.72	23.90 ± 0.72
	Furfural (kg/ton)	–	–	5.60 ± 0.17	5.60 ± 0.17
Energy balance	Methane volume (m^3/ton-substrate)	60.75 ± 1.47	135.45 ± 9.00	37.32 ± 2.11	55.8 ± 3.90
	Methane content (kg)	43.55 ± 0.98	197.09 ± 6.01	26.75 ± 1.41	40.00 ± 2.60
	Energy from CH_4 (kW)	675.03 ± 15.11	1504.90 ± 92.72	414.62 ± 21.71	619.95 ± 40.15
	Energy input (kW)	–	–	20.00 ± 0.60	20.00 ± 0.60
	Total NET energy	675.03 ± 15.11	1504.90 ± 92.72	394.62 ± 21.11	599.95 ± 39.55
	Retention time (day)	45.00 ± 1.20	22.50 ± 1.28	20.00 ± 0.60	25.60 ± 0.60
Economic analysis	Investment biogas reactor (m^3)	1000.00 ± 30.00	500.00 ± 15.00	500.00 ± 15.00	500.00 ± 15.00
	Energy generated per day (kWh)	15.00 ± 0.34	66.88 ± 4.12	19.73 ± 1.06	23.44 ± 1.55
	Capital cost (USD/m^3)	75.00 ± 2.25	75.00 ± 2.25	75.00 ± 2.25	75.00 ± 2.25
	Investment biogas reactor (USD)	75,000.00 ± 2250.00	37,500.00 ± 1125.00	37,500.00 ± 1125.00	37,500.00 ± 1125.00
	Income (USD/day)	102.78 ± 2.30	458.27 ± 28.21	135.17 ± 7.23	160.58 ± 10.59
	Return on Investment (Year)	2.69 ± 0.08	0.30 ± 0.01	1.02 ± 0.03	0.86 ± 0.03

These microorganisms break complex organic matter and create volatile fatty acids (VFAs) and other intermediates during the hydrolysis, acidogenesis, and acetogenesis phases of anaerobic digestion [45]. Since *Syntrophaceticus* sp. is recognized for its syntrophic acetate-oxidizing abilities, which can improve methane production by providing substrates for hydrogenotrophic methanogens, its enhanced abundance in the co-digestion system is especially interesting [47]. *Methanoculleus* sp. and *Methanosarcina* sp. were found to be more abundant in the co-digestion system than in the mono-digestion of HTP190C, according to the examination of the archaeal community (Fig. 4b). *Methanosarcina* sp. is a versatile methanogen that can perform both acetoclastic and hydrogenotrophic methanogenesis. In contrast, *Methanoculleus* sp. is a hydrogenotrophic methanogen that produces methane using hydrogen and carbon dioxide [47]. The higher abundance of these methanogens in the co-digestion system suggests a more effective conversion of VFAs and hydrogen to methane. This may contribute to the higher biogas yield observed in the co-digestion of HTP190C with 5 %w/v DC. *Methanobacterium* sp. and *Methanolinea* sp. demonstrate the significance of hydrogenotrophic methanogenesis in the anaerobic digestion of pretreated EFB in both systems [9]. The microbial community analysis reveals that the co-digestion of hydrothermally pretreated EFB with decanter cake promotes the growth of key bacterial and archaeal populations involved in the anaerobic digestion. The increased abundance of cellulolytic bacteria, syntrophic acetate-oxidizing bacteria, and versatile methanogens in the co-digestion system may contribute to the enhanced biogas production observed compared to the mono-digestion of pretreated EFB.

3.5. Mass balance, energy balance, and economic analysis

The total solids (TS) and total volatile solids (VS) were highest for DC (750.00 kg/ton and 645.00 kg/ton, respectively), followed by EFB (375.00 kg/ton and 354.90 kg/ton), HTP190-EFB-DC (170.00 kg/ton and 150.00 kg/ton), and HTP190-EFB (120 kg/ton and 115.2 kg/ton). The lower TS and VS content in HTP190-EFB can be attributed to the solubilization of organic matter during hydrothermal pretreatment [48]. The pretreatment also resulted in the production of sugar (8.90 kg/ton), VFA (23.90 kg/ton), and furfural (5.60 kg/ton) in the liquid fraction of HTP190-EFB and HTP190-EFB-DC. The methane volume and energy from CH_4 were highest for DC (135.45 m^3/ton-substrate and 1504.90 kW, respectively), followed by HTP190-EFB-DC (55.8 m^3/ton-substrate and 619.95 kW), EFB (60.75 m^3/ton-substrate and 675.03 kW), and HTP190-EFB (37.32 m^3/ton-substrate and 414.62 kW). The energy input for HTP190-EFB and HTP190-EFB-DC was 20.00 kW, used for hydrothermal pretreatment [49]. The total net energy was highest for DC (1504.90 kW), followed by HTP190-EFB-DC (599.95 kW), EFB (675.03 kW), and HTP190-EFB (394.62 kW). The retention time was shortest for HTP190-EFB (20 days) and longest for EFB (45 days), with DC and HTP190-EFB-DC having retention times of 22.5 and 25.6 days, respectively. The investment cost for the biogas reactor was considered to be USD 75,000 for a 1000 m^3 reactor (EFB) and USD 37,500 for a 500 m^3 reactor (DC, HTP190-EFB, and HTP190-EFB-DC). The energy generated per day was highest for DC (66.88 kWh), followed by HTP190-EFB-DC (23.44 kWh), HTP190-EFB (19.73 kWh), and EFB (15.00 kWh). The income per day followed a similar trend, with DC having the highest income (USD 458.27), followed by HTP190-EFB-DC (USD 160.58), HTP190-EFB (USD 135.17), and EFB (USD 102.78). The return on investment (ROI) was the shortest for DC (0.30 years), followed by HTP190-EFB-DC (0.86 years), HTP190-EFB (1.02 years), and EFB (2.69 years). The mass, energy, and economic analyses of empty fruit bunch (EFB), decanter cake (DC), hydrothermally processed EFB at 190 °C (HTP190-EFB), and co-digestion of HTP190-EFB with DC are shown in Table 4. The outcomes suggest that HTP190-EFB co-digestion with DC (HTP190-EFB-DC) performs better than HTP190-EFB mono-digestion, demonstrating the co-digestion synergistic effects [45]. The hydrothermal pretreatment of EFB enhances its biodegradability but requires additional energy input, resulting in lower net energy and ROI than untreated EFB.

The current study is contrasted with earlier research on the anaerobic digestion of empty fruit bunches (EFB) under varied anaerobic digestion settings, co-digestion tactics, and pretreatment techniques in Table 5. In this work, mesophilic (40 °C) high-solid anaerobic digestion (HSAD) conditions are investigated for the co-digestion of hydrothermally prepared EFB at 190 °C for 5 min with decanter cake (DC) at a mixing ratio of 5 % (w/v). Except for Suksong et al. [2], who reported a methane yield of 0.438 m^3/kg-VS using

Table 5
Anaerobic digestion of empty fruit bunches: a comparison of the current investigation with previously published reports.

Pretreatment condition	Anaerobic digestion condition	Methane yield (m^3/kg-VS)	Methane production (m^3/ton-EFB)	Co-digestion	Mixing ratio	References
Mechanical size reduction to 2 mm	Thermophilic (55 °C)	0.340	82.7	POME	0.4:1 (VS basis)	[9]
NaOH (8 %), 60 min	Mesophilic (55 °C)	0.24	21.12	–	–	[10]
T. reesei and *P. ostreatus* cultivation	Mesophilic (40 °C), SS-AD and initial TS 16 %	0.263, 0.315	75.8, 64.9	–	–	[11]
Clostridiaceae and Lachnospiraceae fermentation	Thermophilic (55 °C), SS-AD, and initial TS 12.8 %	0.217, 0.0852	27.7, 70.6	–	–	[47]
–	Thermophilic (55 °C), L-AD + SS-AD	0.438	60.9	L-AD effluent recycling		[2]
V. volvacea cultivation	Thermophilic (55 °C), SS-AD	0.281	50.6	POME	5 % (v/w)	[24]
Particle size reduction to 0.5 cm	Thermophilic (55 °C), HS-AD	0.288	86.9	POME	31:1 (VS basis)	[39]
HTP190 °C 5 min	Mesophilic (40 °C), HS-AD	0.372	55.8	DC	5 % (w/v)	Present study

a coupled liquid and solid-state anaerobic digestion (L-AD + SS-AD) system with effluent recycling, the methane yield of 0.372 m^3/kg-VS achieved in the current study is higher than that of the majority of previous studies. The high methane yield in the present study can be attributed to the combined effects of hydrothermal pretreatment and co-digestion with DC, which likely improved the biodegradability of EFB and provided a more balanced nutrient composition for anaerobic microorganisms. Compared to mechanical size reduction pretreatment [9] and alkaline pretreatment [10], the hydrothermal pretreatment in the present study resulted in a higher methane yield, demonstrating its effectiveness in enhancing EFB biodegradability. The present study also outperformed fungal pretreatment using *T. reesei* and *P. ostreatus* [11] and biological pretreatment using *V. volvacea* cultivation [24] regarding methane yield. The methane production per ton of EFB in the present study (55.8 m^3/ton-EFB) is lower than some of the previous studies, such as O-Thong et al. [9] (82.7 m^3/ton-EFB) and Saelor et al. [39] (86.9 m^3/ton-EFB). This difference can be attributed to the varying pretreatment methods, co-digestion strategies, and anaerobic digestion conditions employed in these studies. Co-digestion with POME has been reported to enhance methane production from EFB in several studies [9,24,39]. The present study demonstrates that co-digestion with DC can also improve methane yield and production from hydrothermally pretreated EFB, highlighting the potential of DC as a co-substrate for the anaerobic digestion of EFB. The mixing ratio of co-substrates is another important factor influencing the performance of anaerobic co-digestion. The present study used a mixing ratio of 5 % (w/v) DC, which resulted in a high methane yield. In comparison, O-Thong et al. [9] and Saelor et al. [39] used mixing ratios based on VS content, while Mamimin et al. [24] used a mixing ratio based on volume. These differences in mixing ratio expression make direct comparisons challenging. Still, the present study's results indicate that a 5%w/v DC mixing ratio effectively enhances methane production from hydrothermally pretreated EFB. The methane yield and production achieved in this study are comparable to or higher than those reported in previous studies, highlighting the potential of this approach for improving the efficiency and sustainability of biogas production from palm oil mill wastes.

4. Conclusion

In this study, the integration of hydrothermal pretreatment (HTP) and co-digestion strategies for enhancing biogas production from empty fruit bunch (EFB) and decanter cake (DC) was investigated. The hydrothermal pretreatment of EFB at 190 °C (HTP190-EFB) resulted in significant changes in substrate composition, including a 67.98 % reduction in total solids and a 67.5 % reduction in total volatile solids compared to raw EFB. The pretreatment also produced 0.89 g/L of sugar, 2.39 g/L of VFA, and 0.56 g/L of furfural in the liquid fraction. The co-digestion of HTP190-EFB with DC at different mixing ratios (5, 10, and 15 %w/v DC) demonstrated improved methane yields and process stability compared to the mono-digestion of HTP190-EFB. The co-digestion of HTP190-EFB with 5 % DC produced the most significant methane yield of 372.69 mL-CH_4/g-VS, a 15 % w/v increase over the mono-digestion of HTP190-EFB (324.30 mL-CH_4/g-VS). Quantifying co-digestion synergistic effects revealed that co-digestion with 5 % w/v DC produced the maximum synergistic methane output, measuring 77.65 mL-CH_4/g-VS. A total net energy of 599.95 kW was obtained from the co-digestion of HTP190-EFB with DC (HTP190-EFB-DC), which was 52 % more than from the mono-digestion of HTP190-EFB (394.62 kW), according to the energy balance analysis. The results of the economic analysis indicated that the co-digestion system had a worse return on investment (0.86 years) than raw EFB (2.69 years) and HTP190-EFB mono-digestion (1.02 years). The co-digestion of hydrothermally pretreated EFB with decanter cake, especially at a mixing ratio of 5 % w/vDC, presents a viable method to maximize methane yield, process stability, and economic viability, supporting the production of renewable energy and sustainable waste management in the palm oil sector.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Sukonlarat Chanthong: Writing – review & editing, Writing – original draft, Project administration, Methodology, Data curation. **Prawit Kongjan:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Data curation. **Rattana Jariyaboon:** Validation, Resources, Data curation. **Sompong O-Thong:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to thank the National Research Council of Thailand (NRCT) for the grant under the Research and Researcher for PhD Industry (RRI) program (grant. No. NRCT5-RRI 63017-P20) and the Talented Mid-Career Research Grant (Grant No. N41A640088) for financial support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e34817.

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