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Research article

Enhancing thermophilic methane production from oil palm empty fruit bunches through various pretreatment methods: A comparative study

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ABSTRACT

This study investigated the effects of various pretreatment methods on the anaerobic digestibility of oil palm empty fruit bunches (EFB) for methane production. Pretreatment methods included weak alkaline (2 % $Ca(OH)_2$), weak acid (2 % acetic acid), acidified palm oil mill effluent (POME), biogas effluent, hydrothermal (180 °C, 190 °C, and 200 °C), and microwave pretreatments. All pretreatment methods enhanced methane yield compared to untreated EFB (189.45 mL-CH_4/g-VS), with weak alkaline pretreatment being the most effective (277.11 mL-CH_4/g-VS), followed by hydrothermal pretreatment at 180 °C (244.33 mL-CH_4/g-VS) and biogas effluent pretreatment (238.32 mL-CH_4/g-VS). The enhanced methane yield was attributed to increased cellulose content (45.5 % for weak alkaline pretreatment), reduced hemicellulose (18.0 % for hydrothermal pretreatment at 200 °C), and lignin contents (19.0 % for hydrothermal pretreatment at 200 °C), decreased crystallinity index (40.0 % for hydrothermal pretreatment at 200 °C), and increased surface area. Weak alkaline pretreatment also showed the highest net energy balance (8.73 kJ/g-VS) and a short break-even point (2 years). Microbial community analysis revealed that weak alkaline pretreatment favored the growth of syntrophic acetate-oxidizing bacteria and hydrogenotrophic methanogens, contributing to improved methane yield. This study demonstrates the potential of EFB pretreatment, particularly weak alkaline and biogas effluent pretreatment, for enhancing methane production and sustainable management of palm oil mill waste.

1. Introduction

The global palm oil industry has experienced rapid growth, with an annual production increase of 7.4 % between 2010 and 2020, reaching 72.9 million tonnes in 2021 [1]. Indonesia, Malaysia, and Thailand are the top producers, accounting for 84 % of the world

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palm oil supply [2]. However, this expansion has led to the generation of large quantities of lignocellulosic waste, particularly oil palm empty fruit bunches (EFB), which represent 22 % of the total fresh fruit bunch weight [3]. In Thailand, approximately 2.8 million tonnes of EFB were generated in 2020 [3], while in Malaysia, 22.4 million tonnes [4]. Suksong et al. [5] reported that EFB is often treated as waste material and is either incinerated or left to decompose in open fields, leading to environmental concerns such as air pollution and greenhouse gas emissions. Composting has also been explored as a management option, but it has limitations such as long processing times and the need for extensive land areas [6]. In contrast, anaerobic digestion (AD) offers a more sustainable and efficient approach to managing EFB waste while producing renewable energy in methane. AD can be conducted in closed systems, reducing the environmental impact, and the resulting digestate can be used as a nutrient-rich fertilizer [7]. EFB consists of 38–60 % cellulose, 20–38 % hemicellulose, and 10–25 % lignin [8], making it a potential feedstock for AD. However, the complex lignocellulosic structure of EFB can hinder its direct utilization in AD processes, necessitating effective pretreatment strategies to enhance methane production [3].

Various pretreatment methods investigated the potential of EFB as an AD feedstock, including chemical, physical, and biological techniques. According to research conducted by O-Thong and colleagues [9], the combined anaerobic digestion of POME and EFB that underwent a two-step pretreatment (initial 0.1 % NaOH presoaking followed by hydrothermal processing at 230 °C for 15 min) led to a significant enhancement in methane production. This method nearly doubled the methane yield, showing a 98 % increase compared to untreated EFB. The peak methane output achieved through this process was reported as 82.7 cubic meters of CH4 per ton of the pretreated EFB and POME mixture. In their study, Nieves and collaborators [10] observed that raw EFB produced 200 mL of methane per gram of volatile solids. Their research explored the impact of various chemical pretreatments, including sodium hydroxide and phosphoric acid. Notably, when EFB was subjected to an 8 % NaOH solution for 60 min, they recorded a twofold increase in methane generation compared to the untreated biomass. Research into EFB pretreatment methods has yielded promising results for enhancing methane production. Suksong et al. [11] explored the potential of fungal pretreatment, demonstrating a substantial increase in methane yield ranging from 44 % to 52 %. In a separate study, Purwandari et al. [12] investigated the efficacy of N-Methylmorpholine N-oxide (NMMO) pretreatment, which resulted in a noteworthy 48 % improvement in methane generation. Chanthong and Kongjan [13] took a different approach, utilizing acetic acid as a pretreatment agent, and observed a significant 55.21 % boost in methane output. Meanwhile, Sealor et al. [14] focused on physical pretreatment methods, specifically examining the effects of particle size reduction. Their findings revealed that reducing EFB particles to 0.5 cm in size led to a remarkable increase in methane yield, ranging from 54 % to 61 %. These diverse studies collectively highlight the potential of various pretreatment strategies to enhance methane production from EFB significantly. The research conducted by Venturin and colleagues [15] underscores the critical role of pretreatment in boosting methane yield from EFB. Their work highlights several key mechanisms by which pretreatment enhances biogas production, such as disrupting the complex structure of the lignocellulosic material, expanding the available surface area of the biomass, and facilitating better access for microbial enzymes to cellulose and hemicellulose components. Despite growing research on EFB pretreatment for AD, there is a lack of comprehensive studies comparing the effectiveness of different pretreatment methods under similar conditions.

This study aims to comprehensively analyze multiple pretreatment techniques for EFB under consistent experimental conditions. We evaluate a wide range of pretreatment methods (chemical, physical, and biological) side-by-side, assessing methane yields and changes in chemical composition, structural properties, and microbial communities. Furthermore, we conduct a thorough techno-economic analysis and energy balance assessment to determine the most promising methods for large-scale application. Investigating waste streams like acidified POME and biogas effluent as pretreatment agents promotes a circular economy approach. This research will facilitate the selection of the most efficient and economically viable pretreatment method for large-scale implementation, contributing to sustainable waste management strategies in the palm oil industry.

2. Materials and methods

2.1. Collection and characterization of EFB and POME

The study utilized two primary materials from the palm oil industry in Krabi province, Thailand: empty fruit bunches (EFB) and palm oil mill effluent (POME). The EFB underwent a preparation process involving three days of sun exposure for drying, followed by mechanical grinding to achieve a uniform particle size of 0.5 cm. Both EFB and POME were kept under refrigeration at 4 °C to preserve their properties until the commencement of anaerobic digestion experiments. To characterize the POME, researchers conducted a series of analyses following established protocols [16]. These assessments included measurements of pH levels, chemical oxygen demand (COD), biochemical oxygen demand (BOD), total solids (TS) content, volatile solids (VS) content, and oil and grease concentrations.

2.2. Pretreatment methods

Weak alkaline pretreatment, EFB samples were mixed with 2 % (w/v) $Ca(OH)_2$ solution at a solid-to-liquid ratio of 1:10 (w/v) in sealed glass bottles and incubated at room temperature for 24 h. As informed by previous studies, the selection of 2 % concentration for $Ca(OH)_2$ pretreatment was based on a balance between pretreatment effectiveness and economic feasibility [17]. Lower concentrations of base (<1 %) have been reported to be less effective in lignin removal and cellulose accessibility enhancement. In comparison, higher concentrations (>5 %) can lead to increased reagent costs and potential degradation of cellulose and hemicellulose [17,18]. Pretreated EFB was neutralized with 1 M HCl to achieve a neutral pH. In weak acid pretreatment, EFB samples were mixed with 2 %

(v/v) acetic acid at a solid-to-liquid ratio of 1:10 (w/v) in sealed glass bottles and incubated at room temperature for 24 h [13]. The selection of a 2 % concentration for acetic acid pretreatment was based on previous studies demonstrating its effectiveness in hemicellulose solubilization and cellulose accessibility enhancement [19]. Concentrations below 1 % have shown limited efficacy in hemicellulose removal, while concentrations above 5 % can form inhibitory compounds and increase equipment corrosion [20]. Pretreated EFB was neutralized with 1 M NaOH to achieve a neutral pH. In acidified POME, POME was acidified to pH 4.0 by microbial fermentation following the protocol of Mamimin et al. [21]. EFB samples were mixed with acidified POME at a solid-to-liquid ratio of 1:10 (w/v) in sealed glass bottles and incubated at room temperature for 24 h. Pretreated EFB was neutralized with 1 M NaOH to achieve a neutral pH. Biogas effluent pretreatment was collected from an anaerobic digester treating palm oil mill effluent (POME) at a local palm oil mill. EFB samples were mixed with biogas effluent in sealed glass bottles at a 1:10 (w/v) solid-to-liquid ratio. The mixture was incubated at room temperature (30 ± 2 °C) for 24 h with occasional manual shaking [22]. The pH of the pretreated EFB was measured and adjusted to 7.0 ± 0.2 using 1 M HCl or 1 M NaOH as needed. For hydrothermal pretreatment, EFB samples were mixed with distilled water at a solid-to-liquid ratio of 1:10 (w/v) in a high-pressure reactor (Parr Instrument Company, USA) and heated to 180 °C, 190 °C, or 200 °C for 30 min [23]. Hydrothermal pretreatment relies on high-temperature water (150–230 °C) to solubilize hemicellulose and disrupt the lignocellulosic structure, leading to faster reaction rates and shorter pretreatment times (5–60 min) [23]. In microwave pretreatment, EFB samples were mixed with distilled water at a solid-to-liquid ratio of 1:10 (w/v) in a microwave-safe container for microwave pretreatment. The container was loosely covered to prevent pressure buildup while allowing for steam release during pretreatment. The mixture was then treated in a domestic microwave oven (Samsung, Thailand) with a frequency of 2450 MHz and a power output of 600 W for 5 min. The short pretreatment time of 5 min was selected based on previous studies by Nomanbhay et al. [24] and Zhu et al. [25] that demonstrated the effectiveness of microwave pretreatment in enhancing the digestibility of lignocellulosic biomass within a short duration. After the microwave pretreatment, the EFB samples were cooled to room temperature before further processing. To achieve a neutral pH, pretreated EFB was neutralized with 1 M NaOH at ambient temperature. The whole pretreatment mixture was then used for anaerobic digestion experiments.

2.3. *Anaerobic digestion setup*

The inoculum for the anaerobic digestion experiments was obtained from a thermophilic anaerobic digester operating at 55 °C ± 2 °C, which treated palm oil mill effluent (POME) at a local palm oil mill in Thailand. This ensured that the microbial community was already adapted to thermophilic conditions. The inoculum was characterized by its total solids (TS), volatile solids (VS), pH, and chemical oxygen demand (COD) following standard methods [16]. The inoculum was acclimated to lignocellulosic biomass degradation for one week before the start of the batch digestion experiments [26]. The inoculum was fed with increasing concentrations of microcrystalline cellulose (avicel) as follows day 1–2 of 0.5 g/L avicel, day 3–4 of 1.0 g/L avicel, and day 5–7 of 2.0 g/L avicel. Biogas production, methane content, volatile fatty acid (VFA) concentrations, and cellulase activity were monitored throughout the acclimation period. Batch anaerobic digestion experiments were conducted in triplicate using 500 mL glass bottles with a working volume of 200 mL. Pretreated EFB samples were added to the bottles at a substrate-to-inoculum ratio of 1:2 (VS basis) [26]. The bottles were filled with inoculum sealed with rubber stoppers and aluminum caps, and the headspace was flushed with N_2: CO_2 gas (80 %:20 %) for 5 min to ensure anaerobic conditions. The bottles were incubated at 55 °C in a water bath for 45 days. Negative and positive controls were prepared using water and avicel, respectively. Biogas production was measured daily using the water displacement method and corrected for background gas production by subtracting the gas produced in the negative control. Throughout the study duration, which spanned 45 days, regular assessments were conducted to determine the evolving composition of the produced biogas.

2.4. *Analytical methods*

The study employed various techniques to characterize the feedstock materials. Empty fruit bunches (EFB) underwent compositional analysis for cellulose, hemicellulose, and lignin content using the protocol described by Sluiter et al. [27]. For palm oil mill effluent (POME), carbohydrate content was determined following Morris's methodology [28]. EFB and POME were subjected to elemental analysis (C, H, N, O) using the procedure outlined by Lesteur et al. [29]. These analyses provided comprehensive data on the feedstocks structural, carbohydrate, and elemental composition, essential for understanding their potential in biogas production. Key parameters (alkalinity, COD, pH, TS, and VS) were measured following standard methods [16]. Daily biogas volume was quantified using the water displacement technique [30]. These analyses provided crucial data on the digestion process and its outputs. Biogas composition was analyzed using gas chromatography (GC-8A Shimadzu) with thermal conductivity detectors (GC-TCD) and a Shin-Carbon ST 100/120 Restek column. Argon served as the carrier gas. The GC program involved temperature ramping from 70 °C to 180 °C. Duplicate 0.5 mL gas samples were injected. Gas measurements were normalized to STP conditions. Volatile fatty acids (VFAs) were quantified using an Agilent 7890A gas chromatograph equipped with a flame ionization detector (FID) and a DB-FFAP capillary column. This setup allowed for precise measurement of VFA concentrations in the digestion samples. Microbial community dynamics were analyzed using PCR-DGGE, adapting the method from Prasertsan et al. [31]. Genomic DNA was extracted from digester sludge, and the 16S rRNA gene was amplified with universal bacterial primers. PCR products were separated into 8 % polyacrylamide gels with a 30–60 % denaturing gradient. Prominent bands were sequenced, typically yielding 15–20 distinct species per sample with ~200 bp reads showing >97 % similarity to known sequences. To determine the most closely related bacterial species, the acquired genetic sequences were analyzed using the BLAST algorithm, comparing them against the repository of sequences in the GenBank database.

2.5. Analysis of biogas production kinetics

To analyze the kinetics of biogas generation, we employed two mathematical models: a first-order kinetic model and the modified Gompertz equation. We utilized a first-order kinetic model (Eq. (1)) to describe the biogas production rate, adapting the approach from Ref. [32]. The model is expressed as:

$$\ln(B\infty - B) = -Kh * t + \ln(B\infty) \quad \text{Eq. 1}$$

B = cumulative methane produced at time t, B∞ = ultimate methane yield, Kh = hydrolysis rate constant (d^{-1}), and t = time (days). To calculate the hydrolysis constant (Kh), we plotted ln(B∞ - B) against t. The slope of this linear relationship represents Kh. To account for the lag phase in methane production, we applied the modified Gompertz equation (Eq. (2)), following the method outlined in Ref. [33]:

$$M = P * \exp\{-\exp[(Rmax * e / P) * (\lambda - t) + 1]\} \quad \text{Eq. 2}$$

Where M = cumulative methane yield at time t, P = maximum methane production potential, Rmax = peak methane production rate, λ = lag phase duration, t = time, and e = Euler's number (approximately 2.7183). For the first-order model, the experimental data was fitted to models by linear regression on the transformed data. For the modified Gompertz model, the experimental data was fitted to models by non-linear regression analysis in SigmaPlot® 11.0. The Gompertz model parameters (P, Rmax, and λ) were estimated by minimizing the sum of squared errors between observed and predicted values. The coefficient of determination (R^2) and the root mean square error (RMSE) were calculated to assess the goodness of fit for both models.

2.6. Energy balance and economic evaluation

An energy balance analysis was conducted to assess the energy efficiency of each pretreatment method. The energy input for pretreatment (Ep) was calculated based on the specific energy consumption of the equipment used (e.g., autoclave, microwave, or heating system) and the pretreatment duration. The energy output from methane production (Em) was determined using the experimental methane yield data and the lower heating value of methane (35.8 MJ/m^3). The energy efficiency (Ee) of each pretreatment method was calculated using Equation (3).

$$Ee = (Em - Ep) / Ep \times 100\% \quad \text{Eq. 3}$$

Where Ee is the energy efficiency (%), Em is the energy output from methane production (MJ/kg-VS), and Ep is the energy input for pretreatment (MJ/kg-VS). A break-even analysis was performed to assess the economic viability of each pretreatment method. The capital cost (CC) of the pretreatment equipment was estimated based on vendor quotes and literature data. The operating cost (OC) was calculated considering the cost of chemicals, energy consumption, labor, and maintenance. The annual revenue (AR) from methane production was determined using the experimental methane yield data, a methane selling price of \$0.5/m^3, and an annual operating time of 8000 h. The annual net cash flow (NCF) was calculated using Equation (4).

$$NCF = AR - OC \quad \text{Eq. 4}$$

Where NCF is the annual net cash flow (\$/year), AR is the annual revenue from methane production (\$/year), and OC is the annual operating cost (\$/year). The break-even point (BP) was determined by calculating the cumulative net cash flow (CNCF) over a 10-year project lifetime, considering a 10 % discount rate. The CNCF for each year was calculated using Equation (5).

$$CNCFn = CNCFn - 1 + NCFn / (1 + r)^n \quad \text{Eq. 5}$$

Where CNCFn is the cumulative net cash flow in year n (\$), CNCFn-1 is the cumulative net cash flow in the previous year (\$), NCFn is the net cash flow in year n (\$), r is the discount rate (%), and n is the year number (1-10). The break-even point was when the CNCF became positive, indicating that the total revenue exceeded the pretreatment method's total cost. A sensitivity analysis was conducted to evaluate the impact of key parameters on the economic viability of the most promising pretreatment method. The parameters

Table 1
Chemical composition of untreated and pretreated oil palm empty fruit bunches (EFB) with various methods.

Pretreatment	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Ash (%)
Untreated	38.0 ± 1.1	22.5 ± 0.7	25.0 ± 0.8	4.5 ± 0.1
Weak alkaline	43.5 ± 1.3	20.0 ± 0.6	21.5 ± 0.6	4.0 ± 0.1
Weak acid	42.0 ± 1.3	21.0 ± 0.6	23.0 ± 0.7	4.2 ± 0.1
Acidified POME	40.5 ± 1.2	21.5 ± 0.6	24.0 ± 0.7	4.4 ± 0.1
Biogas effluent	41.0 ± 1.2	21.0 ± 0.6	23.5 ± 0.7	4.3 ± 0.1
Hydrothermal 180 °C	44.5 ± 1.3	19.0 ± 0.6	20.0 ± 0.6	3.8 ± 0.1
Hydrothermal 190 °C	45.0 ± 1.4	18.5 ± 0.6	19.5 ± 0.6	3.7 ± 0.1
Hydrothermal 200 °C	45.5 ± 1.4	18.0 ± 0.5	19.0 ± 0.6	3.6 ± 0.1
Microwave	43.0 ± 1.3	20.5 ± 0.6	22.0 ± 0.7	4.1 ± 0.1

assessed included the methane selling price (\$0.3–0.7/m^3), the pretreatment cost (±20 %), and the methane yield (±20 %). The break-even point was recalculated for each scenario to identify the most critical parameters affecting the economic feasibility of the pretreatment method.

3. Results and discussion

3.1. Effect of pretreatment methods on EFB characteristics

The chemical composition of untreated and pretreated EFB is presented in Table 1. The results show that all pretreatment methods increased the cellulose content and decreased the hemicellulose and lignin content compared to untreated EFB. Hydrothermal pretreatment at 200 °C exhibited the highest cellulose content (45.5 ± 1.4 %) and the lowest hemicellulose (18.0 ± 0.5 %) and lignin (19.0 ± 0.6 %) contents. Weak alkaline and microwave pretreatments also showed significant improvements in the chemical composition of EFB, with cellulose contents of 43.5 ± 1.3 % and 43.0 ± 1.3 %, respectively. Acidified POME and biogas effluent pretreatments had a relatively lower impact on the chemical composition than other pretreatment methods. The observed rise in cellulose proportion, coupled with a reduction in hemicellulose and lignin levels, can be linked to the dissolution and extraction of these constituents during the pretreatment stage [24,34]. Removing hemicellulose and lignin exposes the cellulose fibers, making them more accessible for enzymatic hydrolysis during anaerobic digestion [35]. The effectiveness of hydrothermal pretreatment at higher temperatures can be explained by the increased severity of the pretreatment conditions, which leads to greater solubilization of hemicellulose and lignin [36].

The crystallinity index and degree of polymerization of untreated and pretreated EFB are presented in Table 2. All pretreatment methods decreased the crystallinity index and degree of polymerization compared to untreated EFB. Hydrothermal pretreatment at 200 °C showed the most significant reduction in both crystallinity index (40.0 ± 1.2 %) and degree of polymerization (920 ± 28). Weak alkaline and microwave pretreatments also resulted in notable decreases in crystallinity index (44.0 ± 1.3 % and 45.0 ± 1.4 %, respectively) and degree of polymerization (1020 ± 31 and 1040 ± 31, respectively). Acidified POME and biogas effluent pretreatments had a relatively lower impact on the crystallinity index and degree of polymerization than other pretreatment methods. The reduction in crystallinity index and degree of polymerization can be attributed to the disruption of the highly ordered crystalline structure of cellulose and the cleavage of the long cellulose chains during the pretreatment process [37]. The decreased crystallinity and degree of polymerization enhance cellulose accessibility to microbial enzymes during anaerobic digestion, leading to improved methane yields [38]. The effectiveness of hydrothermal pretreatment at higher temperatures can be explained by the increased severity of the pretreatment conditions, which results in more significant disruption of the crystalline structure and cleavage of cellulose chains [36]. The characteristics of liquid compounds after pretreatment are presented in Table 3. The presence of glucose, xylose, and diverse organic acids indicates the solubilization of hemicellulose and lignin during pretreatment. Notably, weak acid pretreatment resulted in the highest concentration of acetic acid (4.56 g/L) and propionic acid (2.06 g/L), suggesting effective hemicellulose hydrolysis. Biogas effluent pretreatment also produced a high concentration of acetic acid (5.67 g/L), which could contribute to enhanced methane production. Hydrothermal pretreatments showed a temperature-dependent increase in glucose and xylose concentrations, with the 200 °C treatment yielding the highest glucose content (1.3 g/L). This trend indicates more severe biomass breakdown at higher temperatures. The presence of furfural, particularly in hydrothermal pretreatments (0.13–0.8 g/L), suggests the occurrence of sugar degradation reactions, which increase with temperature [37]. Weak alkaline pretreatment, while not producing the highest concentrations of individual compounds, generated a balanced profile of glucose (0.42 g/L), lactic acid (0.81 g/L), and acetic acid (3.71 g/L). This combination of readily biodegradable compounds could explain its effectiveness in enhancing methane yields. The variations in chemical composition across pretreatment methods can be attributed to differences in their mechanisms of action on the EFB structure. These changes, including the solubilization of hemicellulose and lignin, disruption of cellulose crystallinity, and chain cleavage, likely enhance cellulose accessibility to microbial enzymes during anaerobic digestion, thereby improving methane yields. Based on these results, hydrothermal pretreatment at higher temperatures and weak alkaline pretreatment appear to be the most effective in modifying the chemical composition of EFB, making them promising methods for enhancing its anaerobic digestibility.

Table 2
Crystallinity index and degree of polymerization of untreated and pretreated oil palm empty fruit bunches (EFB) with various methods.

Pretreatment	Crystallinity index (%)	Degree of polymerization
Untreated	50.0 ± 1.5	1200 ± 36
Weak alkaline	44.0 ± 1.3	1020 ± 31
Weak acid	46.0 ± 1.4	1060 ± 32
Acidified POME	48.0 ± 1.4	1120 ± 34
Biogas effluent	49.0 ± 1.5	1150 ± 35
Hydrothermal 180 °C	42.0 ± 1.3	980 ± 29
Hydrothermal 190 °C	41.0 ± 1.2	950 ± 29
Hydrothermal 200 °C	40.0 ± 1.2	920 ± 28
Microwave	45.0 ± 1.4	1040 ± 31

Table 3
Characteristics of liquid compounds after pretreatment of various pretreatment methods.

Pretreatment	Characteristics of liquid compounds after pretreatment (g/L)									
	Cellobiose	Glucose	Xylose	Succinic	Lactic acid	Acetic acid	Propionic acid	Methanol	Ethanol	Furfural
Weak alkaline	–	0.42	–	–	0.81	3.71	0.04	–	0.01	<0.05
Weak acid	–	0.47	–	0.05	1.72	4.56	2.06	–	–	<0.05
Acidified POME	–	–	–	0.03	1.8	4.17	0.04	0.31	0.01	<0.05
Biogas effluent	–	0.42	–	0.02	0.27	5.67	0.15	0.06	0.02	<0.05
Hydrothermal 180 °C	0.24	0.7	0.04	0.83	1.04	4.21	0.60	–	0.05	0.13
Hydrothermal 190 °C	0.31	0.8	1.2	0.11	1.12	4.13	0.10	–	0.12	0.37
Hydrothermal 200 °C	0.42	1.3	0.08	0.06	0.31	4.03	0.02	–	0.09	0.80
Microwave	–	0.49	0.02	0.01	1.36	3.17	0.04	–	0.08	0.20

3.2. Impact of pretreatment on the anaerobic digestion process

The initial and final pH values, as well as the volatile fatty acid (VFA) concentrations at the beginning and end of the anaerobic digestion process, were monitored to assess the impact of various pretreatment methods on the stability and performance of the AD process (Table 4). The initial pH values for all pretreatment methods and the untreated control ranged from 7.03 to 7.89, within the optimal range for anaerobic digestion (6.8–7.2) [20]. The final pH values increased slightly in all cases, ranging from 7.55 to 8.41, indicating stable operation of the AD process without acidification. The highest final pH was observed in the biogas effluent pretreatment (8.41 ± 0.25), which can be attributed to the high buffering capacity of biogas effluent [39]. The initial VFA concentrations varied among the pretreatment methods, with the highest value observed in the weak alkaline pretreatment (6.2 ± 0.2 g/L) and the lowest in the untreated EFB (1.5 ± 0.2 g/L). The elevated levels of VFAs observed initially in the pretreated specimens can be explained by the intensified breakdown and dissolution of lignocellulosic materials during the pretreatment phase [7]. The final VFA concentrations decreased significantly in all cases, with the lowest value observed in the untreated EFB (0.1 ± 0.1 g/L) and the highest in the acidified POME pretreatment (0.8 ± 0.1 g/L). The reduction in VFA concentrations during the AD process indicates the efficient conversion of VFAs to methane by the methanogenic archaea [26,40]. Alkalinity and ammonia nitrogen are essential parameters that influence the stability and performance of the anaerobic digestion process. Alkalinity provides buffering capacity to the system, while ammonia nitrogen can act as a nutrient for the microorganisms but can also be inhibitory at high concentrations [41]. The alkalinity values at the end of the AD process varied among the pretreatment methods, with the highest value observed in the biogas effluent pretreatment (9.08 ± 0.27 $gCaCO_3/L$) and the lowest in the acidified POME pretreatment (3.35 ± 0.10 $gCaCO_3/L$). The high alkalinity in the biogas effluent pretreatment can be attributed to the buffering capacity of the biogas effluent, which contains high levels of organic acids and bicarbonates [39]. The alkalinity values in all cases were within the optimal range for anaerobic digestion (2–5 $gCaCO_3/L$) [42], indicating that the pretreatment methods did not adversely affect the buffering capacity of the AD system. The final ammonia nitrogen concentrations ranged from 5.1 ± 0.15 mg/L in the acidified POME pretreatment to 7.5 ± 0.23 mg/L in the biogas effluent pretreatment. These values are well below the inhibitory threshold for ammonia nitrogen in anaerobic digestion (1500–3000 mg/L) [43], suggesting that ammonia inhibition was not a concern in the studied AD systems. The slightly higher ammonia nitrogen concentration in the biogas effluent pretreatment can be attributed to the nitrogen content of the biogas effluent itself [21].

The VS reduction during the AD process was highest in the weak alkaline pretreatment (64 ± 1.92 %) followed by hydrothermal pretreatment at 190 °C (56.05 ± 1.68 %), the hydrothermal pretreatment at 180 °C (54.67 ± 1.64 %) and the biogas effluent

Table 4
The parameters at the beginning and end of the anaerobic digestion period of various pretreatment methods.

Treatment	Initial pH	Final pH	Initial VS (g/L)	Final VS (g/L)	VS reduction (%)	Initial VFA (g/L)	Final VFA (g/L)	Alkalinity ($gCaCO_3/L$)	Final NH_4^+ (mg/L)
Weak alkaline	7.79 ± 0.23	8.08 ± 0.24	19.49 ± 0.58	7.02 ± 0.21	64 ± 1.92	6.2 ± 0.2	0.25 ± 0.02	6.62 ± 0.20	6.5 ± 0.20
Weak acid	7.23 ± 0.22	7.72 ± 0.23	19.49 ± 0.58	9.44 ± 0.28	51.60 ± 1.55	4.8 ± 0.2	0.45 ± 0.05	3.75 ± 0.11	5.2 ± 0.16
Acidified POME	7.31 ± 0.22	7.55 ± 0.25	19.49 ± 0.58	10.43 ± 0.31	46.52 ± 1.40	5.1 ± 0.2	0.8 ± 0.05	3.55 ± 0.27	5.1 ± 0.23
Biogas effluent	7.03 ± 0.21	8.41 ± 0.23	20 ± 0.58	9.46 ± 0.28	52.69 ± 1.58	5.5 ± 0.2	0.3 ± 0.05	9.08 ± 0.10	7.5 ± 0.15
Hydrothermal 180 °C	7.32 ± 0.22	7.77 ± 0.23	19.49 ± 0.58	8.84 ± 0.27	54.67 ± 1.64	5.8 ± 0.2	0.3 ± 0.03	3.75 ± 0.11	5.9 ± 0.18
Hydrothermal 190 °C	7.41 ± 0.22	7.73 ± 0.23	19.49 ± 0.58	8.57 ± 0.26	56.05 ± 1.68	5.6 ± 0.2	0.5 ± 0.05	4.2 ± 0.13	5.2 ± 0.16
Hydrothermal 200 °C	7.6 ± 0.23	7.73 ± 0.23	19.49 ± 0.58	9.56 ± 0.29	50.98 ± 1.53	5.4 ± 0.2	0.7 ± 0.04	3.95 ± 0.12	6.6 ± 0.20
Microwave	7.83 ± 0.23	8.03 ± 0.24	19.49 ± 0.58	9.72 ± 0.29	50.15 ± 1.50	5.3 ± 0.2	0.2 ± 0.05	5.3 ± 0.16	6 ± 0.18
Untreated	7.89 ± 0.23	8 ± 0.24	19.49 ± 0.58	11.04 ± 0.33	43.36 ± 1.30	1.5 ± 0.2	0.1 ± 0.02	6 ± 0.18	5.8 ± 0.17

pretreatment (52.69 ± 1.58 %). These pretreatment methods also resulted in high methane yields, indicating that the efficient degradation of the organic matter contributed to enhanced methane production. The compositional analysis of the digested material reveals that the cellulose content decreased significantly in all pretreatment cases compared to the untreated control, indicating the effective degradation of cellulose during anaerobic digestion (Table 5). The lowest cellulose content in the digested material was observed for the alkaline pretreatment (10.2 %), followed by the hydrothermal pretreatment at 180 °C (12.5 %) and the biogas effluent pretreatment (14.1 %). These results are consistent with the higher methane yields obtained. The hemicellulose content in the digested material was also lower in all pretreatment cases compared to the untreated EFB, suggesting the solubilization and degradation of hemicellulose during pretreatment and anaerobic digestion. The lowest hemicellulose content was observed for the hydrothermal pretreatment at 200 °C (2.1 %), followed by the alkaline pretreatment (3.5 %) and the acidic pretreatment (4.2 %). These findings align with the known mechanisms of hemicellulose solubilization by high-temperature water, alkaline, and acidic treatments [18,20]. The lignin content in the digested material was reduced in all pretreatment cases compared to the untreated EFB, indicating the partial degradation and removal of lignin during pretreatment and anaerobic digestion. The lowest lignin content was observed for the alkaline pretreatment (8.3 %), followed by the hydrothermal pretreatment at 200 °C (9.1 %) and the microwave pretreatment (10.5 %). The effectiveness of alkaline and hydrothermal pretreatments in lignin removal has been well-documented in previous studies [44,45]. The crystallinity index of the digested material was also lower in all pretreatment cases compared to the untreated EFB, suggesting the disruption of the crystalline structure of cellulose during pretreatment and anaerobic digestion. The lowest crystallinity index was observed for the hydrothermal pretreatment at 200 °C (25.2 %), followed by the weak alkaline pretreatment (28.1 %) and the microwave pretreatment (30.4 %). The reduction in crystallinity index enhances cellulose accessibility to microbial enzymes during anaerobic digestion [46]. These results demonstrate that the various pretreatment methods employed in this study effectively degraded the lignocellulosic components of EFB, leading to improved methane yields during anaerobic digestion.

Fig. 1 illustrates the transformations in the exterior structure of EFB, comparing its appearance before and following mild alkaline pretreatment. The untreated EFB (Fig. 1a and b) exhibits a smooth and compact surface, while the pretreated EFB (Fig. 1c and d) shows a more porous and disrupted surface with the exposure of cellulose fibers. The alterations observed in the material exterior structure can be linked to the extraction of hemicellulose and lignin components during pretreatment. This process results in improved access for microbial enzymes to the cellulose fibers [24]. The FTIR spectra of untreated and weak alkaline pretreated EFB are presented in Fig. 2, and the characteristics and variation of bands in the spectra are summarized in Table 6. The pretreated EFB shows a decrease in the intensity of the bands associated with hemicellulose (771 and 1029 cm^{-1}) and lignin (1236, 1327, 1460, 1510, and 1593 cm^{-1}), indicating the removal of these components during the pretreatment process. The increase in the intensity of the cellulose bands associated with cellulose (897, 1162, 1370, 1421, 2920, and 3543 cm^{-1}) suggests the exposure of cellulose fibers after removing hemicellulose and lignin [24]. The impact of pretreatment on the anaerobic digestion process was evaluated regarding VFA production, pH, alkalinity, and surface morphology of EFB. Weak alkaline pretreatments resulted in higher total VFA concentrations, indicating enhanced hydrolysis of the pretreated EFB. All pretreatment methods increased pH and alkalinity during the anaerobic digestion process, suggesting stable operation of the systems. The changes in surface morphology and FTIR spectra of EFB after weak alkaline pretreatment confirmed the removal of hemicellulose and lignin, which enhances the accessibility of cellulose to microbial enzymes.

3.3. Methane production from pretreated EFB

The methane yields from untreated and pretreated EFB are presented in Table 7. All pretreatment methods resulted in higher methane yields than untreated EFB (189.45 ± 5.68 mL CH_4/gVS). Weak alkaline pretreatment showed the highest methane yield (277.11 ± 8.31 mL CH_4/gVS), followed by hydrothermal pretreatment at 180 °C (244.33 ± 7.33 mL CH_4/gVS) and biogas effluent pretreatment (238.32 ± 7.17 mL CH_4/gVS) (Fig. 3). The methane yields from microwave, hydrothermal pretreatment at 190 °C, 200 °C, and acidified POME pretreatment were relatively lower, ranging from 218.60 ± 6.56 to 229.69 ± 6.89 mL CH_4/gVS. Weak acid pretreatment exhibited the lowest methane yield among the pretreatment methods (202.77 ± 6.08 mL CH_4/gVS) (Fig. 3a). The higher methane yields from weak alkaline pretreated EFB can be attributed to the enhanced accessibility of cellulose to microbial enzymes due to the changes in chemical composition and structural modifications induced by the pretreatment process [9]. The removal of hemicellulose and lignin, reduction in crystallinity index, and decrease in the degree of polymerization of cellulose contribute to the improved anaerobic digestibility of pretreated EFB [34]. The effectiveness of weak alkaline pretreatment in

Table 5
Compositional analysis and crystallinity index of the digested material after anaerobic digestion.

Pretreatment Method	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Crystallinity Index (%)
Untreated	20.8 ± 0.6	10.2 ± 0.3	15.5 ± 0.5	45.0 ± 1.4
Weak alkaline	10.2 ± 0.3	3.5 ± 0.1	8.3 ± 0.2	28.1 ± 0.8
Weak acid	15.3 ± 0.5	4.2 ± 0.1	12.4 ± 0.4	36.2 ± 1.1
Acidified POME	16.1 ± 0.5	5.6 ± 0.2	13.1 ± 0.4	38.5 ± 1.2
Biogas effluent	14.1 ± 0.4	4.8 ± 0.1	11.8 ± 0.4	34.7 ± 1.0
Hydrothermal 180 °C	12.5 ± 0.4	3.1 ± 0.1	10.2 ± 0.3	31.8 ± 1.0
Hydrothermal 190 °C	11.9 ± 0.4	2.6 ± 0.1	9.7 ± 0.3	29.5 ± 0.9
Hydrothermal 200 °C	11.2 ± 0.3	2.1 ± 0.1	9.1 ± 0.3	25.2 ± 0.8
Microwave	13.6 ± 0.4	4.5 ± 0.1	10.5 ± 0.3	30.4 ± 0.9

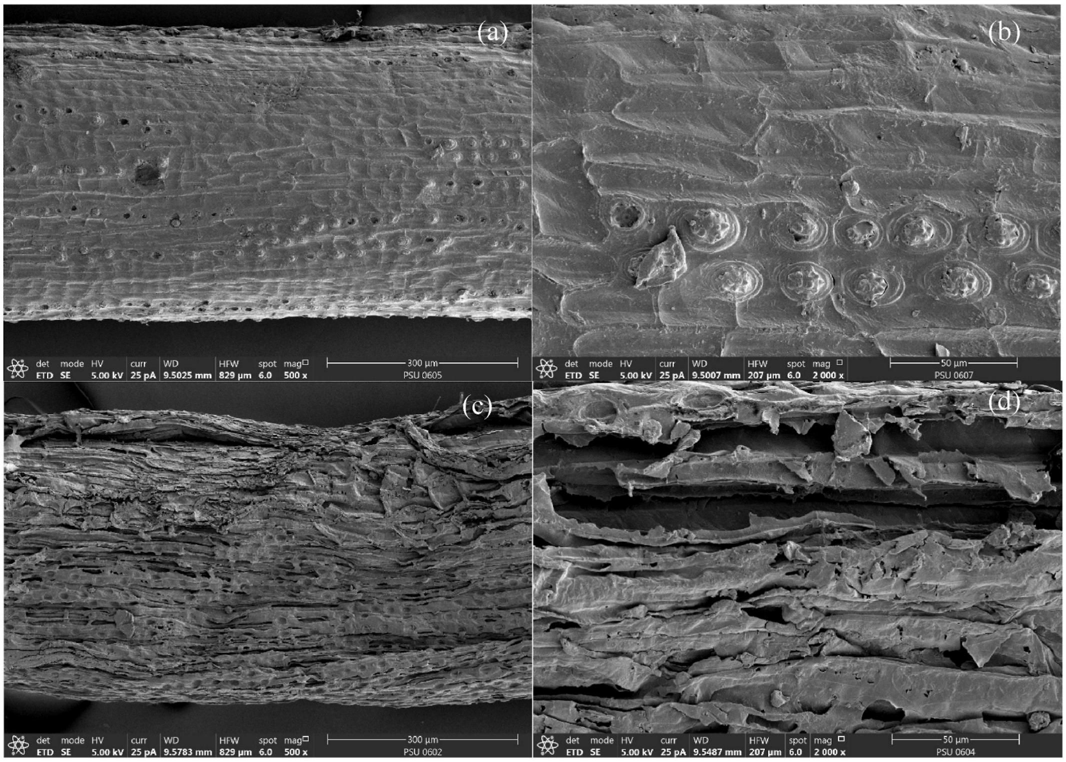

Fig. 1. Scanning electron microscopy (SEM) images revealed empty fruit bunches (EFB) surface morphology before and after weak alkaline pretreatment. (a) Raw EFB at 500x magnification. (b) Raw EFB at 2000x magnification. (c) Weak alkaline-pretreated EFB at 500x magnification. (d) Weak alkaline-pretreated EFB at 2000x magnification.

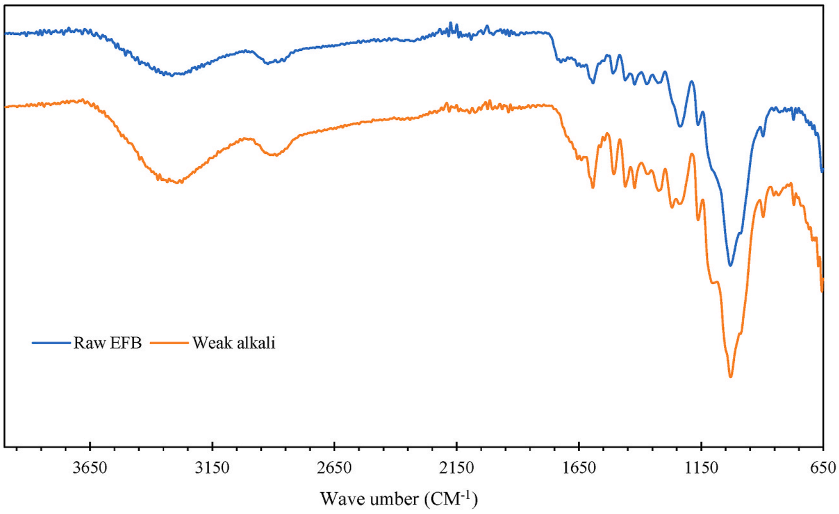

Fig. 2. FTIR spectra of untreated empty fruit bunch and pretreated empty fruit bunch with weak alkaline.

enhancing methane yield can be explained by its ability to solubilize lignin and hemicellulose, as well as its delignification effect, which increases the porosity of the biomass and facilitates microbial access to cellulose [35]. The methane production per tonne of EFB followed a similar trend as the methane yields, with weak alkaline pretreatment resulting in the highest methane production (96.99 ± 1.62 m^3/tonne-EFB) and weak acid pretreatment showing the lowest (70.97 ± 1.19 m^3/tonne-EFB). The biodegradability of EFB, which represents the fraction of organic matter converted to methane, also increased with pretreatment. Weak alkaline pretreatment resulted in the highest biodegradability (63.57 ± 1.91 %), while untreated EFB showed the lowest (43.46 ± 1.30 %). The improved digestibility of EFB following pretreatment can be explained by two primary factors: first, the heightened susceptibility of cellulose to

Table 6

Characteristics and variation of bands in the fourier transform infrared spectrometer spectra of raw empty fruit bruches and weak alkaline treated empty fruit bruches.

Wavenumber (cm^{-1})	Functional group	Assignment
771	C=O Bending	Hemicellulose
897	C-H deformation in cellulose	Cellulose
1029	C-O stretching	Hemicellulose
1162	C-O-C asymmetric stretching	Cellulose
1236	C-O stretching	Lignin
1327	C-O Stretching	Lignin
1370	C-H bending	Cellulose
1421	C-H2 symmetric bending	Cellulose
1460	C=C stretching of the aromatic ring	Lignin
1510	C=C stretching of the aromatic ring	Lignin
1593	C=C stretching	Lignin
2920	C-H stretching	Cellulose I
3543	O-H stretching	Cellulose II

Table 7

Kinetic parameters for methane production from untreated and pretreated empty fruit bruches.

Pretreatment	Methane production rate (mL CH_4/d)	Lag time (days)	k_h (d^{-1})	Methane yield (mL CH_4/g-VS)	Methane production (m^3/ton-EFB)	Improvement (%)	Biodegradability (%)
Weak alkaline	13.91 ± 0.42	4.02 ± 0.30	0.1074 ± 0.00	277.11 ± 8.31	96.99 ± 1.62	46.27 ± 1.39	63.57 ± 1.91
Weak acid	9.1 ± 0.27	3.58 ± 0.20	0.1053 ± 0.00	202.77 ± 6.08	70.97 ± 1.19	18.72 ± 0.56	46.52 ± 1.40
Acidified POME	9.39 ± 0.28	4.24 ± 0.25	0.109 ± 0.00	218.60 ± 6.56	76.51 ± 1.28	7.02 ± 0.21	50.15 ± 1.50
Biogas effluent	12.76 ± 0.38	5.1 ± 0.15	0.154 ± 0.00	238.32 ± 7.17	83.41 ± 1.33	21.24 ± 0.64	54.67 ± 1.64
Hydrothermal 180 °C	14.79 ± 0.44	5.84 ± 0.18	0.157 ± 0.00	244.33 ± 7.33	85.51 ± 1.38	25.79 ± 0.64	56.05 ± 1.68
Hydrothermal 190 °C	11.78 ± 0.35	4.94 ± 0.15	0.1298 ± 0.00	222.20 ± 6.67	77.77 ± 1.24	28.96 ± 0.87	50.98 ± 1.53
Hydrothermal 200 °C	12.65 ± 0.38	7.54 ± 0.23	0.1577 ± 0.00	229.69 ± 6.89	80.39 ± 1.07	17.28 ± 0.52	52.69 ± 1.58
Microwave	14.35 ± 0.43	6.04 ± 0.18	0.1772 ± 0.00	224.92 ± 6.75	78.72 ± 1.26	15.39 ± 0.46	51.60 ± 1.55
Untreated	6.9 ± 0.21	4.73 ± 0.14	0.1403 ± 0.00	189.45 ± 5.68	66.30 ± 1.11	0 ± 0.00	43.46 ± 1.30

enzymatic action by microbes, and second, the dissolution of hemicellulose and lignin components during the pretreatment process [23].

The kinetic parameters for methane production from untreated and pretreated EFB are presented in Table 7. The methane production rate was highest for weak alkaline pretreatment (13.91 ± 0.42 mL CH_4/d), followed by hydrothermal pretreatment at 180 °C (14.79 ± 0.44 mL CH_4/d) and microwave pretreatment (14.35 ± 0.43 mL CH_4/d) (Fig. 3b). Untreated EFB exhibited the lowest methane production rate (6.9 ± 0.21 mL CH_4/d). The higher methane production rates from pretreated EFB can be attributed to the enhanced accessibility of cellulose to microbial enzymes, which facilitates faster hydrolysis and fermentation of the substrate [9]. The initial delay period, indicating the duration necessary for microbial populations to acclimate to the feedstock and commence methane generation, differed across the various pretreatment techniques. Hydrothermal pretreatment at 200 °C showed the longest lag time (7.54 ± 0.23 days), while weak acid pretreatment exhibited the shortest lag time (3.58 ± 0.20 days). Extended initial delay periods noted in certain pretreatment approaches may be linked to the emergence of substances that hinder microbial activity. Compounds such as furfural and 5-hydroxymethylfurfural (HMF) can develop during pretreatment, potentially causing these prolonged lag phases [34]. Such substances can potentially impede anaerobic microbes proliferation and metabolic processes, resulting in a prolonged phase of microbial adjustment [47]. The hydrolysis constant (K_h), which represents the rate of hydrolysis of the substrate, was highest for microwave pretreatment (0.1772 ± 0.00 d^{-1}) and lowest for weak acid pretreatment (0.1053 ± 0.00 d^{-1}). The higher hydrolysis constants observed in some pretreatment methods can be attributed to the enhanced accessibility of cellulose to microbial enzymes, which facilitates faster hydrolysis of the substrate [23].

The improvement in methane yield for each pretreatment method compared to untreated EFB is presented in the given data. Weak alkaline pretreatment showed the highest improvement in methane yield (46.27 ± 1.39 %), followed by hydrothermal pretreatment at 190 °C (28.96 ± 0.87 %) and 180 °C (25.79 ± 0.64 %). Acidified POME pretreatment and biogas effluent pretreatment also significantly improved methane yield, with values of 21.24 ± 0.64 % and 18.72 ± 0.56 %, respectively. Hydrothermal pretreatment at 200 °C and microwave pretreatment showed relatively lower improvements in methane yield (17.28 ± 0.52 % and 15.39 ± 0.46 %,

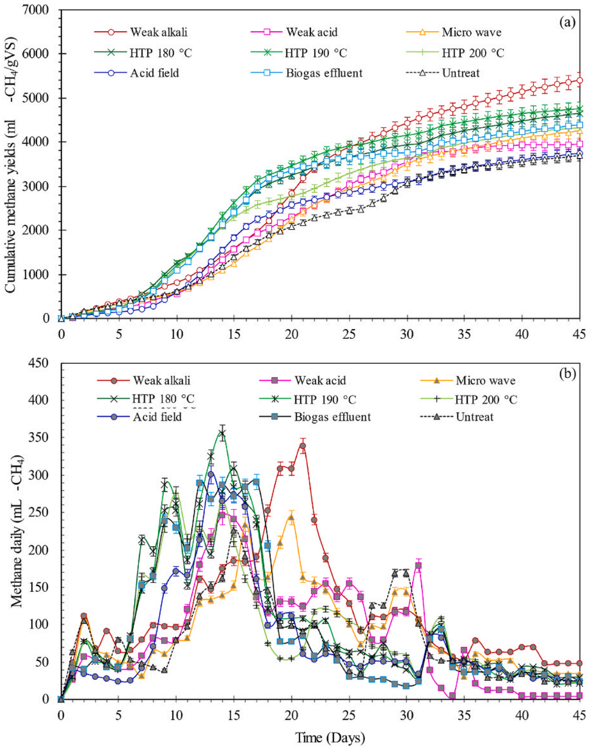

Fig. 3. Methane production profiles from untreated and pretreated empty fruit bunches during anaerobic digestion, (a) cumulative methane yield over the 45-day experimental period, and (b) daily methane production rate throughout the digestion process.

Table 8

Comparison of pretreatment methods for empty fruit bruches and similar lignocellulosic biomass for methane production, VS reduction, and pretreatment effectiveness.

Pretreatment Method	Substrate	Methane Yield (mL-CH$_4$/g-VS)	Methane Production (m^3/ton)	VS Reduction (%)	Pretreatment Effectiveness (% improvement)	References
Weak alkaline (2 % Ca (OH)$_2$)	EFB	277.11	96.77	64.00	46.27	This study
Weak alkaline (8 % NaOH)	EFB	400.00	140.00	NR	100.00	Nieves et al. [10]
Weak alkaline (0.1 % NaOH)	EFB	220.10	82.7	52.10	8.92	O-Thog et al. [9]
Hydrothermal (180 °C)	EFB	244.33	85.51	54.67	25.79	This study
Hydrothermal (230 °C, 15 min)	EFB	208.05	81.54	49.20	24.15	O-Thog et al. [9]
Biogas effluent	EFB	238.32	83.41	52.69	18.72	This study
Anaerobic digestate	Wheat straw	246.53	78.06	NR	51.850	Liu et al. [48]
Weak acid (2 % acetic acid)	EFB	202.77	70.97	51.60	7.02	This study
Weak acid (4 % acetic acid)	EFB	265.77	73.70	53.20	13.80	Chanthong and Kongjan [13]
Microwave (600 w, 5 min)	EFB	224.92	78.72	50.15	15.39	This study
Microwave (3000W, 5 min)	Agricultural straw	223.17	166.58	NR	2.13	Sapci [49]
Fungal (*T. reesei*)	EFB	216.70	75.80	49.30	44.00	Suksong et al. [11]
Untreated	EFB	189.45	66.30	43.36	–	This study
Untreated	EFB	185.20	64.8	42.10	–	Suksong et al. [11]

* NR = not reported.

respectively). Weak acid pretreatment exhibited the lowest improvement in methane yield (7.02 ± 0.21 %) among the pretreatment methods. The higher improvements in methane yield observed in weak alkaline and hydrothermal pretreatments can be attributed to their effectiveness in modifying the chemical composition and structure of EFB. The solubilization of hemicellulose and lignin, reduction in crystallinity index, and decrease in the degree of polymerization of cellulose during these pretreatments lead to enhanced accessibility of cellulose to microbial enzymes, resulting in higher methane yields [23,46]. The modest enhancements in methane production observed with hydrothermal pretreatment at 200 °C and microwave pretreatment can be explained by generating inhibitory substances. Specifically, compounds like furfural and 5-hydroxymethylfurfural (HMF) tend to form under more intense pretreatment conditions [47]. Such substances can potentially suppress the development and metabolic functions of anaerobic microbes, resulting in diminished methane production [46]. The lowest improvement in methane yield observed in weak acid pretreatment can be attributed to the limited effectiveness of this pretreatment method in modifying the chemical composition and structure of EFB. Weak acid pretreatment has been reported to be less effective in removing lignin and reducing cellulose crystallinity than other pretreatment methods [35], which may explain the lower improvement in methane yield. Compared to untreated EFB, all applied pretreatment techniques led to improvements in three key areas: methane output, the rate at which methane was generated, and the overall biodegradability of the material. Weak alkaline pretreatment was the most effective in enhancing methane production, followed by hydrothermal pretreatment at 180 °C and biogas effluent pretreatment. The enhanced methane production from pretreated EFB can be attributed to the changes in chemical composition and structural modifications induced by the pretreatment process, which increase cellulose accessibility to microbial enzymes during anaerobic digestion.

To contextualize our findings within the broader field of lignocellulosic biomass pretreatment for anaerobic digestion, we compared our results with those reported in the literature for similar pretreatment methods on EFB and other lignocellulosic substrates (Table 8). Our weak alkaline pretreatment using 2 % $Ca(OH)_2$ yielded a high methane production (277.11 mL-CH_4/g-VS), which is significantly higher than the 220.10 mL-CH_4/g-VS reported by O-Thong et al. [9] for 0.1 % NaOH pretreatment. However, it falls short of the 400 mL-CH_4/g-VS achieved by Nieves et al. [10] using 8 % NaOH. This suggests that while our lower $Ca(OH)_2$ concentration was more effective than deficient NaOH concentrations, higher NaOH concentrations can achieve even greater methane yields, albeit potentially at higher economic and environmental costs. The hydrothermal pretreatment at 180 °C in our study (244.33 mL-CH_4/g-VS) outperformed the 208.05 mL-CH_4/g-VS reported by O-Thong et al. [9] for hydrothermal pretreatment at 230 °C for 15 min. This suggests lower temperature treatment may be more effective and energy efficient. Our biogas effluent pretreatment (238.32 mL-CH_4/g-VS) performed comparably to the anaerobic digestate pretreatment on wheat straw (246.53 mL-CH_4/g-VS) reported by Liu et al. [48]. This highlights the potential of using waste streams as effective pretreatment agents across different lignocellulosic substrates. The weak acid pretreatment in our study using 2 % acetic acid (202.77 mL-CH_4/g-VS) showed lower methane yield compared to the 4 % acetic acid pretreatment (265.77 mL-CH4/g-VS) reported by Chanthong and Kongjan [13]. Higher acid concentrations may lead to more extensive hemicellulose hydrolysis and improved methane yields. Our microwave pretreatment (224.92 mL-CH_4/g-VS)

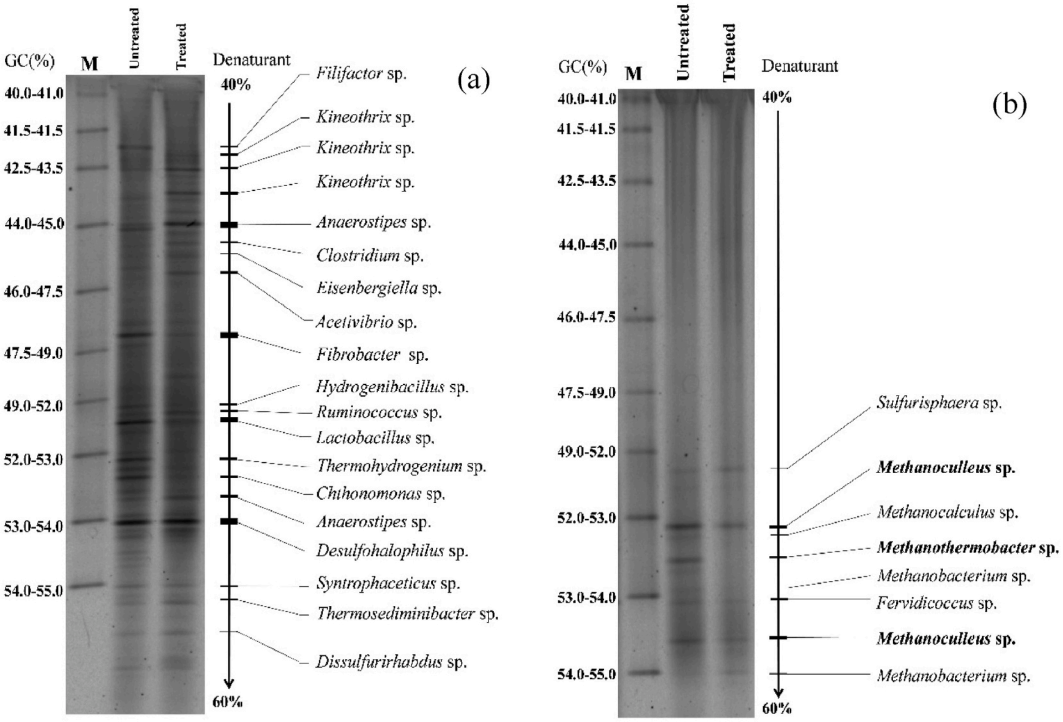

Fig. 4. DGGE profile of bacteria community (a) and archaea community (b) from raw EFB and weak alkaline pretreated EFB for methane production under thermophilic condition.

performed similarly to the 223.17 mL-CH_4/g-VS reported by Sapci [49] for agricultural straw despite the differences in substrate and microwave power. Regarding VS reduction, our weak alkaline pretreatment achieved the highest percentage (64.0 %) among the reported studies, correlating well with its high methane yield. The pretreatment effectiveness in our study ranged from 7.02 % (weak acid) to 46.27 % (weak alkaline). Notably, the 8 % NaOH pretreatment by Nieves et al. [10] showed a 100 % improvement, indicating the potential for further optimization of alkaline pretreatment. This comparative analysis demonstrates that pretreatment methods, particularly weak alkaline and hydrothermal pretreatments, perform well against similar methods reported in the literature. The high performance of our biogas effluent pretreatment is particularly noteworthy, as it represents a cost-effective and environmentally friendly option for enhancing methane production from EFB.

3.4. Microbial community analysis

The microbial community structure of untreated EFB and weak alkaline-treated EFB during anaerobic digestion under thermophilic conditions was analyzed using PCR-DGGE. The PCR-DGGE profiles of the bacterial and archaeal communities are presented in Fig. 4A and B, respectively. In the untreated EFB, the bacterial population was predominantly composed of species from the genera *Desulfohalophilus*, *Lactobacillus*, *Ruminococcus*, *Fibrobacter*, and *Anaerostipes*. Notably, *Desulfohalophilus* sp. is classified as a sulfate-reducing bacterium capable of oxidizing a range of organic compounds, such as lactate, pyruvate, and ethanol [50]. *Lactobacillus* sp. is a lactic acid bacterium that can ferment carbohydrates to produce lactic acid [51]. *Ruminococcus* sp. and *Fibrobacter* sp. are known for designing cellulose and hemicellulose [52]. *Anaerostip* sp. is an anaerobic bacterium that can ferment carbohydrates to produce acetate, butyrate, and hydrogen [51]. In weak alkaline-treated EFB, the bacterial community was dominated by *Desulfohalophilus* sp., *Syntrophaceticus* sp., *Anaerostip* sp., *Ruminococcus* sp., and *Clostridium* sp. *Syntrophaceticus* sp. is a syntrophic acetate-oxidizing bacterium that can convert acetate to hydrogen and carbon dioxide in the presence of hydrogen-utilizing methanogens [53]. *Clostridium* sp. is a versatile genus that ferments various carbohydrates and produces acetate, butyrate, and hydrogen [51]. The shift in the bacterial community composition after weak alkaline pretreatment suggests that the pretreatment process favored the growth of bacteria involved in the degradation of complex organic compounds and syntrophic acetate oxidation. The increased abundance of *Syntrophaceticus* sp. in weak alkaline-treated EFB indicates enhanced acetate oxidation, which is crucial for efficient methane production [53].

The archaeal community in untreated EFB was dominated by *Methanoculleus* sp. and *Methanothermobacter* sp., while weak alkaline-treated EFB was dominated by *Methanoculleus* sp. The microbial genus *Methanoculleus* includes hydrogenotrophic methanogens capable of converting hydrogen and carbon dioxide into methane through their metabolic processes [54]. *Methanothermobacter* sp. is also a hydrogenotrophic methanogen that can thrive in thermophilic conditions [55]. The prevalence of *Methanoculleus* sp. in EFB subjected to mild alkaline pretreatment indicates that this process created favorable conditions for the proliferation of these hydrogenotrophic methanogens. This abundance is likely explained by the increased presence of hydrogen and carbon dioxide, byproducts of syntrophic acetate oxidation, which serve as substrates for *Methanoculleus* sp [53]. The elevated presence of *Methanoculleus* sp. in the pretreated EFB correlates with the observed increase in methane production. This aligns with the understanding that hydrogenotrophic methanogens, such as Methanoculleus sp., primarily generate methane within anaerobic digestion processes [54]. The microbial community analysis using DGGE revealed that weak alkaline pretreatment altered the bacterial community composition in EFB, favoring the growth of bacteria involved in the degradation of complex organic compounds and syntrophic acetate oxidation. The pretreatment process also enhanced the growth of the hydrogenotrophic methanogen *Methanoculleus* sp., consistent with the higher methane yield observed in pretreated EFB. These findings highlight the importance of pretreatment in shaping the microbial community structure and improving the efficiency of anaerobic digestion systems.

3.5. Energy balance and economic evaluation of pretreatment methods

The energy consumption, energy output from methane, and net energy balance of various pretreatment methods for EFB are presented in Table 9. Untreated EFB has no energy consumption for pretreatment but has the lowest energy output from methane production (6.82 kJ/gVS). Among the pretreatment methods, weak alkaline pretreatment has the highest net energy balance (8.73 kJ/gVS), indicating that the increased energy output from methane production (9.98 kJ/g-VS) outweighs the energy consumed during pretreatment (1.25 kJ/gVS). Hydrothermal pretreatments have the highest energy consumption among the pretreatment methods,

Table 9
Energy consumption and energy output of pretreatment methods.

Pretreatment	Energy consumption (kJ/g-VS)	Energy output from methane (kJ/g-VS)	Net energy balance (kJ/g-VS)
Untreated	0	6.82	6.82
Weak alkaline	1.25	9.98	8.73
Weak acid	1.15	7.30	6.15
Acidified POME	0.80	7.87	7.07
Biogas effluent	0.60	8.58	7.98
Hydrothermal 180 °C	2.50	8.80	6.30
Hydrothermal 190 °C	2.75	8.00	5.25
Hydrothermal 200 °C	3.00	8.27	5.27
Microwave	2.00	8.10	6.10

ranging from 2.50 to 3.00 kJ/gVS. This results in lower net energy balances than other pretreatments, with 6.30, 5.25, and 5.27 kJ/gVS values for hydrothermal pretreatment at 180 °C, 190 °C, and 200 °C, respectively. The high energy consumption of hydrothermal pretreatments can be attributed to the high temperature and pressure conditions required for the pretreatment process [56]. Acidified POME and biogas effluent pretreatments have relatively low energy consumption (0.80 and 0.60 kJ/gVS, respectively) and high energy output from methane (7.87 and 8.58 kJ/gVS, respectively), leading to favorable net energy balances of 7.07 and 7.98 kJ/gVS, respectively. The low energy consumption of these pretreatments can be attributed to using waste streams (POME and biogas effluent) as pretreatment agents, reducing the need for external energy input [39]. Microwave pretreatment has a moderate energy consumption (2.00 kJ/g-VS) and energy output from methane (8.10 kJ/gVS), resulting in a net energy balance of 6.10 kJ/g-VS, which is comparable to weak acid pretreatment (6.15 kJ/gVS). The energy consumption of microwave pretreatment can be attributed to the high power requirements for microwave heating [56]. The results highlight the trade-offs between energy consumption during pretreatment and the improved methane production from pretreated EFB. While hydrothermal pretreatments result in high methane production, their high energy consumption leads to lower net energy balances than other pretreatment methods.

On the other hand, acidified POME and biogas effluent pretreatments achieve high net energy balances due to their low energy consumption and high methane production. Economic feasibility and environmental sustainability should be considered when considering the large-scale application of EFB pretreatment in anaerobic digestion systems. Pretreatment methods with high net energy balances, such as weak alkaline, acidified POME, and biogas effluent pretreatments, may be more suitable for large-scale application due to their lower energy requirements and higher energy output from methane production. However, the availability and cost of the pretreatment agents (e.g., NaOH, POME, biogas effluent) should also be considered [39]. Furthermore, the environmental impact of the pretreatment methods should be evaluated, considering factors such as greenhouse gas emissions, water usage, and the generation of waste streams [56]. Pretreatment methods that utilize waste streams (e.g., acidified POME and biogas effluent) may have a lower environmental impact than methods that require external chemicals (e.g., weak alkaline and weak acid pretreatments). The energy balance analysis of EFB pretreatment methods reveals that weak alkaline, acidified POME, and biogas effluent pretreatments have the highest net energy balances, indicating their potential for large-scale application in anaerobic digestion systems. However, selecting the most suitable pretreatment method should consider the energy balance, the process economic feasibility, and environmental sustainability. Further research is needed to optimize the pretreatment conditions and assess the long-term performance of the selected pretreatment methods in large-scale anaerobic digestion systems.

The pretreatment methods investigated include weak alkaline, weak acid, acidified palm oil mill effluent (POME), biogas effluent, hydrothermal treatment at different temperatures (180 °C, 190 °C, and 200 °C), and microwave pretreatment. The untreated EFB serves as a control for comparison. The break-even analysis considers the capital cost, operating cost, and revenue from methane production over 10 years. The capital cost for all pretreatment methods and the untreated control is assumed to be $50 per ton of EFB in Year 0. The operating cost is estimated at $25 per ton of EFB per year, while the revenue from methane production is projected to be $55 per ton of EFB per year. Based on these assumptions, the annual net cash flow for all scenarios is calculated to be $30 per ton of EFB. The cumulative net cash flow is negative in Year 0 due to the initial capital investment and remains negative in Year 1. However, from Year 2 onwards, the cumulative net cash flow becomes positive. It continues to increase steadily, reaching $250 per ton of EFB by Year 10 (Fig. 5). Interestingly, the break-even analysis reveals that all pretreatment methods and the untreated control exhibit the same financial performance under the given assumptions. This suggests that the increased methane yield achieved through pretreatment does not significantly offset the additional costs of implementing these methods. It is important to note that this break-even analysis is based on specific assumptions and may not reflect the actual costs and revenues associated with each pretreatment method. Factors such as the cost of chemicals, energy consumption, and the efficiency of methane recovery can vary depending on the specific process conditions and scale of operation. Furthermore, the analysis does not consider the potential environmental benefits of enhanced methane production, such as reducing greenhouse gas emissions and generating renewable energy. These factors may influence the decision-making process when selecting a pretreatment method, alongside the financial considerations. The break-even analysis suggests that, under the given assumptions, all pretreatment methods and the untreated control exhibit similar financial performance over 10 years. However, a more comprehensive analysis incorporating variable costs, environmental benefits, and process-specific factors is necessary to decide on the most suitable pretreatment method for enhancing methane production from EFB feedstock.

4. Conclusions

This study investigated the effects of various pretreatment methods, including weak alkaline, weak acid, acidified POME, biogas effluent, hydrothermal, and microwave pretreatments, on the anaerobic digestibility of oil palm empty fruit bunches (EFB) for methane production. The results showed that all pretreatment methods improved the methane yield compared to untreated EFB, with weak alkaline pretreatment being the most effective, followed by hydrothermal pretreatment at 180 °C and biogas effluent pretreatment. Weak alkaline pretreatment enhanced the methane yield by 46.27 %, achieving a methane production of 277.11 mL-CH_4/g-VS. This pretreatment method also resulted in the highest net energy balance (8.73 kJ/g-VS) and a relatively short break-even point (2 years), indicating its potential for cost-effective application in anaerobic digestion systems. Hydrothermal pretreatment at 180 °C and biogas effluent pretreatment increased the methane yield by 25.79 % and 18.72 %, respectively. These pretreatment methods also showed favorable energy balances and economic parameters, suggesting their potential for large-scale application. The effectiveness of these pretreatment methods can be attributed to the changes in the chemical composition and structural properties of EFB, including increased cellulose content, reduced hemicellulose and lignin contents, decreased crystallinity index, and increased surface area. These changes enhanced cellulose accessibility to microbial enzymes, leading to improved hydrolysis and fermentation of EFB during anaerobic digestion. Moreover, the microbial community analysis revealed that weak alkaline pretreatment altered the bacterial

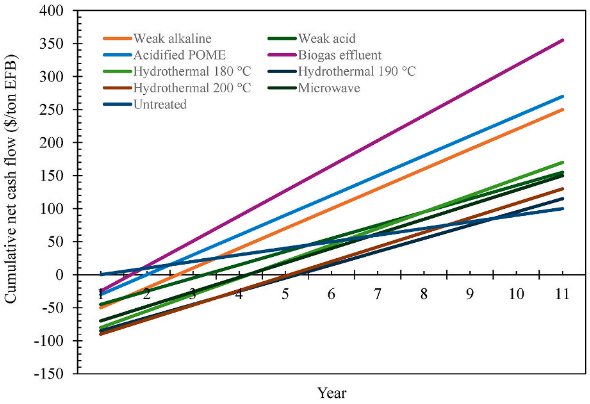

Fig. 5. Cumulative net cash flow of various pretreatment methods over 10 years.

community composition, favoring the growth of bacteria involved in the degradation of complex organic compounds and syntrophic acetate oxidation. The pretreatment process also enhanced the growth of the hydrogenotrophic methanogen *Methanoculleus* sp., contributing to the higher methane yield observed in pretreated EFB. Pilot-scale studies should assess the performance and feasibility of the most effective pretreatment methods (weak alkaline, hydrothermal at 180 °C, and biogas effluent) in large-scale anaerobic digestion systems.

CRediT authorship contribution statement

Sittikorn Saelor: Writing – original draft, Visualization, Methodology, Data curation. **Prawit Kongjan:** Writing – review & editing, Project administration, Data curation. **Poonsuk Prasertsan:** Writing – review & editing, Data curation. **Chonticha Mamimin:** Visualization, Data curation. **Sompong O-Thong:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Data availability

The data associated with this study have not been deposited into a publicly available repository. The data are included in the article, as well as supplementary material and references in the article.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e39668.

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